The Effects of Cavitation on the Removal and Inactivation of *Listeria* and *Salmonella* on the Surface of Fresh Roma Tomatoes and Cantaloupes

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ABSTRACT

Raw produce has frequently been identified as the source of bacterial pathogens that can cause human illnesses, including listeriosis and salmonellosis. Microbial pathogens may attach and form biofilms on raw fruit surfaces and can be difficult to remove. A cavitation process (formation of bubbles in water) was studied for its effectiveness for removal and inactivation of *Listeria monocytogenes* and *Salmonella* Newport from the surfaces of fresh Roma tomatoes and cantaloupes.

Individual fruit were separately inoculated with each pathogen, then submerged in a water tank and treated with a bubble flow through an air stone using one airflow rate (0 – 14 liters/min.) for up to 60 sec. As air flow increased, pathogen reduction increased up to 1.2 log CFU/fruit greater than with water alone (no bubbles). Additional pathogen reduction in the tank water (organisms detached from the fruit) was observed with the bubble treatments. Therefore, these bubble streams can be used to enhance the detachment of bacteria from fruit surfaces and to inactivate a proportion of these detached microorganisms.

Additionally, recoveries of *Salmonella* from inoculated Roma tomatoes and cantaloupe were determined for treatment water that contained 50 or 150 ppm sodium

hypochlorite. The combination of cavitation and chlorine resulted in greater efficacy of inactivating the pathogen in treatment water, but not in removing this organism from the fruit surfaces. The physical force of a bubble stream on raw produce can effectively reduce and inactivate surface bacteria, and has the potential to reduce antimicrobial chemical and water use in post-harvest packing operations.

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PUBLIC ABSTRACT

Every year, one in six Americans will have been affected by a foodborne illness, many of which are caused by bacteria found on the surface of fresh fruits and vegetables. Most of these bacteria are removed with the help of a water wash with or without chlorine added. Nevertheless, microorganisms, including bacterial pathogens, may attach and form biofilms on raw fruit surfaces and can be difficult to remove. For this research, a cavitation process (formation of bubbles in water) was studied for its effectiveness for removal and inactivation of *Listeria monocytogenes* and *Salmonella* Newport from the surfaces of fresh Roma tomatoes and cantaloupes.

Individual fruit were separately spiked with each pathogen, then submerged in a water tank and treated with a bubble flow through an air stone using one airflow rate (up to 14 liters air per minute) for 30 or 60 seconds. As air flow increased, the number of bacteria was reduced by up to 94% more bacteria per fruit than when using water alone (no bubbles). Additional bacteria reduction in the tank water (organisms detached from the fruit) was observed with the bubble treatments. Therefore, these bubble streams can be used to enhance the detachment of bacteria from fruit surfaces and to kill or injure some of these detached microorganisms.

Additionally, recoveries of *Salmonella* from inoculated Roma tomatoes and cantaloupe were determined for treatment water that contained 50 or 150 parts per million sodium hypochlorite (chlorine solution). The combination of cavitation bubbles and chlorine showed a greater ability for inactivating these bacteria in the tank water, but not in removing this organism from the fruit surfaces. The physical force of a bubble stream on raw produce can effectively reduce and inactivate surface bacteria, and this process could reduce the amount of water or chemicals used to process fresh fruits and vegetables, while ensuring that these foods will not cause people to get sick upon eating.

To Lisa:

For all of your support, patience, and love.

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I. **Introduction**

Listeria monocytogenes and *Salmonella* account for over one million reported cases of foodborne illnesses every year (Scallan, et al., 2011). The resultant foodborne illnesses, listeriosis and salmonellosis, include vomiting, diarrhea, and possibly death (CDC, 2015). Moreover, these illness cases result in high medical costs, including 365 million dollars a year for *Salmonella* alone (CDC, 2010). Research pertaining to these pathogens is pertinent for the reduction in health care costs, illnesses, and safety of consumers.

A highly common produce item affected by these pathogens is raw tomatoes, due to their high volume of consumption (Asplund, 1991). Tomatoes are one of the biggest agricultural crops in America, with over 500,000 acres utilized solely for tomatoes, consisting of a 2-billion-dollar industry (ERS, 2010). Another produce item, cantaloupe, enact an equally challenging problem, not only due to raw consumption, but their uniquely irregular surface which may enhance biofilm formation and foodborne pathogen survival. Cantaloupes have presented a consistent problem with foodborne illnesses, particularly in the past five years, where multiple outbreaks of *Salmonella* have resulted in recalled product (CDC, 2012).

Foodborne illness caused by *L. monocytogenes* and *Salmonella* microorganisms could be reduced through additional efforts to prevent these organisms from adhering to or surviving on raw food surfaces. In some cases, the physical removal of surface bacteria can damage surface textures leading to loss of quality and structural integrity. This project will explore both the removal and inactivation of pathogenic microorganisms on fruit surfaces through non-inertial cavitation.

Within the past three decades, cavitation as a treatment methodology for the removal of bacteria has become more common, but more so in the fields of dentistry and orthodontics. Cavitation processes include ultrasound and sonication methods to destroy microbial populations on teeth (Marotti, 2013). However, the effects of cavitation have been studied for many known and studied for many years including where the collapse of rapidly-moving bubbles in water led to the disintegration of rocks in waterfalls, and metal propellers in ships and submarines (Rayleigh, 1917; Birkhoff, 1957).

While ultrasound and sonication processes have shown promise for pathogen reduction on foods or in food processes, the generation of excess heat and negative effects on sensory quality has rendered the research difficult to apply in the food industry (Jambrak, et al., 2008). This project will use of an airflow passed through a porous air stone to generate bubbles via non-inertial cavitation.

The objectives of this research are 1) To determine if cavitation bubbles enhance the removal or inactivation of inoculated *Listeria monocytogenes* and *Salmonella* from the surface of fresh Roma tomatoes or cantaloupe; 2) To determine if cavitation bubbles enhance the transfer of *Listeria monocytogenes* and *Salmonella* from the surface of inoculated fresh Roma tomatoes or cantaloupe to the surface of uninoculated fruit; and 3) To compare the efficacy of a chlorine sanitizer solution to cavitation bubbles for the removal or inactivation of inoculated *Listeria monocytogenes* and *Salmonella* from the surface of fresh Roma tomatoes or cantaloupe. This comparison can provides a safe benchmark for sanitation efficacy, while minimizing an effective concentration of chlorine.

II. Literature Review

1. *Microbial Pathogens on Raw Produce*

Recently, there is a clear trend towards eating more fresh produce items, such as fruits and vegetables, primarily due to a greater knowledge of nutrition and health (Produce for Better Health Foundation, 2015). However, the consumption of fruits and vegetables are often raw, which have higher risk of consuming foodborne pathogens. (Burnett et al., 2000). Heat is considered to be the most effective method of pathogen inactivation, and certainly the most common in history through pasteurization or sterilization (Lado et al., 2002). But, due to consumer demands for fresh fruits and vegetables, alternatives to heat processes must be determined. Moreover, recent research showing that excessive heat treatment can reduce nutrition, as well as cause undesirable sensory changes, further propagate non-thermal methods for pathogenic removal or inactivation on industrial scales (Meng, 2002). Since consumer expect visually appealing products, cleaning processes must be gentle and minimal. Thus, this minimized cleaning leads to higher pathogenic populations on the surface of fresh produce. Two of the highest pathogenic risks include *Listeria* and *Salmonella* and contamination can take place at any step of food processing, including planting, growing, and harvesting (Beuchat, 2002).

2. Listeria monocytogenes

Though *Listeria monocytogenes* were first discovered in 1890 in patients afflicted by listeriosis, the first comprehensive study of *Listeria monocytogenes* was published

in1966 by Mitchell Gray and Arden Killinger (Gray and Killinger, 1996). Since then, *Listeria monocytogenes* have received much more in-depth research of its characteristics, effects, and varying serotypes. *L. monocytogenes* is a gram-positive, non-sporeforming organism, which is facultatively anaerobic, allowing growth both in the presence and absence of oxygen (Farber, 1991). A particularly dangerous aspect of *L. monocytogenes* is its ability to grow in a wide range of temperatures, particularly refrigeration temperatures. *L. monocytogenes* have been documented to grow in temperatures as low as -2°C (Walker et al., 1990). On the other extreme, *L. monocytogenes* have been found to grow at temperatures of 35°C (Tienungoon et al., 2000).

Other characteristics that allow *L. monocytogenes* greater survival are its ability to grow in lower pH conditions than other major foodborne pathogens. *L. monocytogenes* have been documented to survive in pH of 4.1 in otherwise ideal conditions. Moreover, *L. monocytogenes* have been noted to grow especially well in environments with high salt concentrations (Cole et al., 1990). Lastly, *L. monocytogenes* are intensely competitive organisms in microflora communities. This is theorized to be due to *L. monocytogenes'* ability to acquire and delete multiple genes depending on the environment it is established (Glaser et al., 2001). Once present, *L. monocytogenes* have the ability to outcompete other organisms for nutrients and space, allowing *L. monocytogenes* populations to flourish on the surface of foods or food-contact surfaces (Leverentz et al., 2006).

These characteristics make *L. monocytogenes* extremely ubiquitous in the natural environment, as well as processing facilities, food packages, and food surfaces.

On farming environments, *L. monocytogenes* are commonly found in fecal matter, animal feed, soil, and water. A large-scale project testing over 40 samples in each of 50 different farms in the United States showed that 20.1% of all samples were contaminated with *L. monocytogenes*. In farms with animals, such as cattle, goats, and sheep, the percent positive samples reached 38.5% (Nightingale et al., 2004). This organism is also prevalent on the hide, mouth, and skin of animals, such as cattle or poultry. A study by Skovgaard and Morgen (1988) found *L. monocytogenes* contamination of feed and fecal samples of cattle farms of 61% and 51%, respectively.

In food processing plants, *L. monocytogenes* often thrive, despite consistent cleaning, due to its ability to grow in so many varied environments. Most commonly, *L. monocytogenes* were discovered in standing water, drains, factory residues, and food-contact surfaces (Cox et al., 1989). Since it is difficult to clean every surface from floor to ceiling in a large food-processing plant, small populations of *L. monocytogenes* are often detected through sampling. In food processing plants, these surfaces are frequently stainless steel, which afford the ability for variegated cleaning measures. However, *L. monocytogenes* have been consistently discovered on stainless steel surfaces despite varying temperatures and sanitizer treatments of differing pH values (Herald and Zottola, 1988). Lastly, in packaged foods, *L. monocytogenes* have been found to survive in a variety of packaged foods, including meats, poultries, ready-to-eat foods, and raw fruits and vegetables packaged for consumer purchase (Ming et al., 2006).

The prevalence and virulence of *L. monocytogenes* in a variety of environments, ultimately lead to ingestion by humans or animals, which can cause listeriosis. In

humans, listeriosis can cause a number of symptoms, including diarrhea, vomiting, meningitis, and encephalitis. Moreover, listeriosis has an extremely high mortality rate in humans, documented at 30-50% (Low, 1997). Lastly, listeriosis is perhaps most dangerous in pregnant women, where spontaneous abortion, stillbirth, or infection of the newborn is possible (Seeliger and Finger, 1983).

3. *Salmonella*

Salmonella is a motile, non-sporeforming, gram-negative bacterium, which is facultatively anaerobic (Food and Drug Administration, 2013). Similar to *L. monocytogenes, Salmonella* can survive in a wide range of temperatures, roughly 2°C to 47°C, as well as in a wide range of salinity in its environment (Pui et al., 2011). *Salmonella* have been observed to employ an acid tolerance response, where *Salmonella* grown at pH 7, then placed into a mildly acidic environment of pH 5.5, were found to be 100 to 1000 times more resistant to extremely acidic environments of pH 3.3 than *Salmonella* organisms transferred directly from pH 7 to pH 3.3 environments (Foster and Hall, 1990). This ability to adapt to differing levels of acidity adds an effective facet to *Salmonella* organisms' ability to remain ubiquitous in starkly different environments.

Another unique aspect of *Salmonella* is its ability to survive in very low moisture environments. Most notably, outbreaks of *Salmonella* have been documented in low-moisture foods, such as peanut butter, chocolate, and infant formula (Podolak et al., 2010). One theory suggests that *Salmonella* are able to survive in low-moisture foods due to fat content, of which foods like peanut butter and chocolates contain high

amounts of fat. This may also protect *Salmonella* from heat treatments (Burnett et al., 2000). Lastly, *Salmonella* have been discovered to contain genes that enhance the invasion and propagation of animal cells, which, in turn, also provide resistance against the host's microbiocidal products (Winfield and Groisman, 2003).

Salmonella spend the majority of their life in the intestines of animals, as part of the indigenous microflora community (Lawley et al., 2008). Animals, such as cattle, then secrete *Salmonella* through its feces, which can enter and survive in soil for many weeks (Zibilske and Weaver, 1978). Once in the soil, cross contamination occurs, whether through food consumption or transport via animals, humans, or machinery. Like *L. monocytogenes, Salmonella* can survive up to a week on dry, stainless steel surfaces, most commonly found in food-processing plants (Kusumaningrum et al., 2003). It is often in these plants where cross contamination occurs, particularly in meat industries, namely poultry. In study sampling 60 different raw poultry products in butcher shops, 60% were found to be contaminated with *Salmonella* (Antunes et al., 2003).

Upon ingestion of *Salmonella* contaminated products, salmonellosis afflicts the host. While certainly not as fatal as listeriosis, salmonellosis can cause gastroenteritis, bacteremia, and infection (Acheson and Hohmann, 2001). However, in immune-compromised individuals, the elderly, and infant populations, the mortality rate is significantly higher (Gomez et al., 1996).

4. *Steps of Possible Contamination*

The harvest, processing and packing of raw fruits and vegetables exposes these foods to the risk of contamination from pathogens. According to the United States Food

and Drug Administration's Good Agricultural Practices, there are five relevant areas to be especially mindful (FDA, 1998). First, is high water quality, as contaminated water can spread to all of the fields. The FDA recommends water from deep sources, like wells, and regular testing of the water supply for microorganisms (FDA, 1998). Second, manure management must be planned, as untreated manure harbors high loads of pathogens. Third, workers in the field must be aware of common infectious signs, practice good hygiene, and be trained in proper handling of foods (Michaels et al., 2004). Fourth, sanitation stations must be provided by the plant in accordance with the Occupational Safety and Health Act (FDA, 1998). This includes the proper number of well-stocked facilities over distance and number of workers, and training for proper use. Lastly, field sanitation practices are strongly recommended. This includes cleaning and sanitation of transportation bins, equipment, packing and storage areas, and boundaries preventing pests or wildlife from contaminating fields. A survey of farms across the United States showed that farms employing consistent sanitation processes were found to have lower incidences of contaminated samples than farms that did not (Rodriguez et al., 2006). However, a primary reason the complete removal or inactivation of foodborne pathogens on fresh produce remains a distinct challenge is due to the nature of pathogens becoming embedded in biofilms on surfaces (Carpentier, 1993).

Biofilms are characterized by formation via aggregated microorganism colonies, which can eventually form to protect bacteria, while also providing nutrients for continuous growth (Chmielewski, 2003). Moreover, biofilm can spread onto food processing equipment, storage surfaces, and transport containers, further contaminating other produce items (Blackman, 1996). Many well-known pathogens,

such as *Listeria* and *Salmonella* have been known to thrive in biofilms on the surface of fresh produce. In addition, some studies show that both *Listeria* and *Salmonella* biofilms are especially resistant to sanitation treatments effective against other pathogens (Pan, 2006; Joseph, 2001).

5. *Tomatoes*

Roma Tomato consumption in the United States has been increasing over the past few decades, recently reaching 80 tons, as it is utilized in a variety of cuisines, as well as providing nutrients and vitamins to consumers (Arizona University, 2010). In the United States, tomatoes are second most consumed vegetable, and the state of Virginia is the fourth highest producer of tomatoes (Dan, 2016). The Roma tomato (*Solanum lycopersicum* syn. *Lycopersicon esculentum*) is an oval shaped fruit, with a smooth surface and red color when ripe.

In the past few decades, tomatoes have consistently been associated with contamination by *Salmonella*, often resulting in multi-state outbreaks, and numerous illnesses. Tomatoes can be contaminated by pathogens through a variety of ways. In a study of tomatoes and *Salmonella* contamination, it has been shown that *Salmonella enterica* can contaminate the tomato fruit through direct root contact or direct flower inoculation (Barak and Liang, 2008). Moreover, Guo et al. (2001) have shown that contamination can occur through the stem scar post-harvest.

In 2004, one of the largest outbreaks of *Salmonella* through tomatoes took place in the United States, ultimately spreading to Canada as well. The outbreak affected 18 states, 1 Canadian province, and 561 illnesses (CDC, 2005). In a comprehensive study

from 1990-2010, researchers found that outbreaks associated with raw tomatoes resulted in 1959 illnesses in the United States, with 384 hospitalizations, and 3 deaths. Roma tomatoes were the second most common produce item, accounting for 23% of outbreaks (Bennett et al., 2015). Most recently, a burrito restaurant chain in Minnesota was found to have been the source of an outbreak of *Salmonella* Newport contaminated tomatoes, resulting in 115 cases reported of salmonellosis (Eikmeier, 2016).

Tomatoes undergo a washing process in the processing plant once delivered from the field. Most often, tomatoes pass through a flume system, which wash away large amounts of debris, such as soil or rocks, followed by treatment with a commercial sanitizer. Most commercial sanitizers utilize a chemical cleaning agent, such as chlorine or organic acids. The process may or may not include brushes or rollers for more efficacious cleaning (Wang and Ryser, 2014).

6. *Cantaloupe*

Cantaloupes (*Cucumis melo* var. *reticulus*) are the second leading melon for consumption in the United States (Boriss et al., 2014). Unlike tomatoes, cantaloupes are always eaten raw, and characterized by a rough netted surface. Moreover, the cantaloupe produces a natural, waxy substance on its surface, making it more hydrophobic, and thus more difficult for water and sanitizer to effectively clean the surface of the cantaloupe (Bastos et al., 2005). These factors are critical to biofilm formation and have made the topography of the cantaloupe historically difficult to clean. The deep grooves and pockets of the cantaloupe surface are visibly clear by the naked eye, and especially through topographical modeling (Wang et al., 2009).

As with tomatoes, cantaloupe remains a consistent culprit for pathogen outbreak, particularly with *L. monocytogenes.* Researchers have shown that *L. monocytogenes* are particularly well suited for survival on the surface of cantaloupe, whereas the flesh does not aid pathogenic survival as effectively (Martinez et al., 2016). Though consumers effectively do not eat the rind, the act of cutting the melon into pieces can cause spread of pathogens from the surface into the fruit. Pathogens adhere themselves onto the knife, which are then dragged through the flesh, contaminating the edible portions (Shearer et al., 2016).

Large illness outbreaks due to cantaloupe have occurred in the past decade. In 2011, Jensen Farms was the origin of a 28-state *L. monocytogenes* outbreak beginning in Colorado, resulting in 147 cases, 143 hospitalizations, and 33 deaths (CDC, 2012). The following year, another outbreak took place in Kentucky, this time concerning *Salmonella,* where 261 cases were reported, as well as three deaths (Lopez et al., 2016).

Similar to tomatoes, cantaloupes are often placed through a washing system, consisting of varying steps, such as a water wash to remove large debris, followed by a chemical sanitation wash, most often chlorine or hydrogen peroxide (Ukuku and Fett, 2002). Despite these treatments, outbreaks still remain common in cantaloupe.

7. *Antimicrobial Processes and Chemicals*

Alternatives to heat treatment are important to ensure safety and maintain quality of raw, fresh fruits and vegetables. Several non-thermal processes and antimicrobial chemicals are used in the food industry to reduce or prevent pathogens on raw foods.

Most commonly, sanitizers such as chlorine, are utilized in the industry to remove or inactive pathogens. Other sanitizers, such as organic acids, hydrogen peroxide, and ethanol are also utilized in food industries (Beuchat, 1997).

Chlorine is one of the most commonly used sanitizers in the food industry today. Its efficacy has been noted and utilized for a number of decades. Both chlorine as a liquid sanitizer and a gas as chlorine dioxide, have been found to be effective. Chlorine as a liquid sanitizer has been found to reduce the population of *L. monocytogenes* at $\log_{10}$ reductions of 1.5 - 2 in fresh cut produce at 200ppm (Zhang, 1996). Other research shows higher population reductions of *L. monocytogenes* at higher concentrations, such as 2000ppm, and greater agitation methods (Beuchat et al., 1998). Similar results have been found for *Salmonella* inactivation, where chlorine treatment of fresh produce resulted in 1-2 log reduction of *Salmonella* populations (Weissinger, 2000; Zhuang, 1995; Wei, 1995). Many projects involving the research of tomatoes, have also treated produce with chlorine at concentrations of 100 – 200 ppm, which follows common industry practice (Bhowmik, 2006; Zhang, 1996; Weissinger, 2000; Cliffe-Byrnes, 2005). Further research has been completed on other pathogens, such as *E. coli* O157:H7*,* with similar results (Singh, 2002). Thus, chlorine serves as an effective sanitation method for a variety of pathogens.

However, the use of chlorine is becoming more and more criticized. As consumers become more interested in chemical-free fruits and vegetables, the use of chlorine is becoming less marketable. Moreover, the growing health concerns of chlorine, namely the possible formation of chlorinated organic compounds such as

chloramines and trihalomethanes, have left consumers wary (Issa-Zacharia et al., 2010).

Essential oils also have antimicrobial properties, however, their use may be suitable only for certain foods, which either hide the sensory changes, or improve sensory aspects in some way (Burt, 2004). Lastly, bacteriocins, or antimicrobial peptides or proteins derived from diverse bacterial species, have gained more attention due to their efficacy. However, a great number of factors affect the efficacy of bacteriocins, including food type, temperature, pH, and oxygen levels. In certain conditions, bacteriocins may proliferate pathogens naturally on food surfaces (Galvez et al., 2008).

One increasingly popular antimicrobial process method is high-pressure processing. Since 2000, products undergoing high-pressure processing have increased 10-fold, mainly used in fruit and vegetable juices, packaged meat products, and cooked vegetable products (Huang et al., 2017). However, high-pressure processing harms the organoleptic properties of fresh fruits and vegetables, and thus, is not widely used in processing raw fruits and vegetables. Finally, irradiation processes for foods are highly effective for inactivating microorganisms, but are used on a very limited basis. Though reviewed by all major international health organizations as safe, irradiation continues to have a consumer hurdle, where the idea of consuming irradiated foods is not widely accepted (Farkas and Farkas, 2011). Thus, apart from chemical sanitizers, most non-thermal methods have limited scope for fresh fruits and vegetables. However, cavitation, as a non-thermal sanitation process for raw fruits and vegetables has not been studied extensively.

8. *Cavitation Treatment*

Cavitation refers to the formation of vapor cavities (e.g. bubbles) in a liquid due to forces acting upon the liquid. Cavitation usually occurs when a liquid is subjected to rapid changes in pressure that cause the formation of cavities. Cavitation was originally discovered after metal boat propellers were found to be disintegrated by the bubbles generated by the propellers. Undoubtedly, this would be far too powerful for use as surface cleaners on fresh produce. However, cavitation can be comprised in two forms: non-injected and injected (also known as stable cavitation). The first forms cavitating bubbles through a input of high energy, such as high pressure differentials, as shown by the destroyed metal boat propellers. Another example is ultrasound treatment, employed in dentistry applications, as well as in the food industry. Injected cavitation does not require a pressure differential or other high energy inputs, and relies on bubble production through injected air (Feng, 2011). Injected cavitation can still produce impressive amounts of force, and high-intensity injected cavitation is currently used in the food industry to generate emulsions, disrupt cells, promote chemical reactions, inhibit enzymes, tenderize meat and modify crystallization processes (McClements, 1995).

The difficulties of removing biofilm and lack of non-thermal treatment options continue to encourage new sanitation methods, such as cavitation. Ultrasound technology utilizes cavitation to mechanically remove microorganisms from surfaces. Ultrasound has been well reviewed and used in dentistry and orthodontic applications for many years. The production of bubbles by ultrasound occurs through sound waves

at high frequencies, above that which humans can hear (McClements, 1995). Cavitation from ultrasound has been ubiquitous in standard dentistry cleaning equipment, and is highly effective at removing microbial populations in biofilms formed on teeth, in the form of plaque (Parini, 2006). More importantly, however, is the efficacy of the cavitation of these bubbles on oral biofilm. Similar to biofilm on the surface of produce, oral biofilms present the same challenges of difficult removal, and the ability to harbor and provide nutrients to harmful microbes. One such microbe is *Streptococcus,* which can cause cavities and illness, such as pneumonia (Patterson, 1994). Research has shown that the cavitation of bubbles can remove up to 81% more of *Streptococcus* populations in oral biofilms, when compared to washing with water (Parini, 2006). Thus, cavitation as a treatment method for the removal of foodborne pathogens proves promising.

Lower intensity injected cavitation is currently used as a pasteurization technique in many drink industries, such as juice and diary. In the dairy industry, microbial populations are inactivated by bubble generation (Chandrapala, 2012). In addition, industry members use similar methods to deactivate enzymes in apple and carrot juice (O'Donnell, 2010). Cavitation as a surfactant is still in the early stages of research. Promising results have been shown in reducing *E. coli* on alfalfa seeds (Scouten, 2001). Removal of *Salmonella* on fresh lettuce leaves was extremely effective when ultrasound was utilized (Seymour, 2002). This project aims to show the efficacy of low intensity injected cavitation as a treatment method to reduce and inactivate surface pathogens on fresh produce.

9. *Chlorine and Cavitation Treatments*

As effective as chlorine is as a sanitizer, it is repeatedly shown to be much more effective when coupled with cavitation. The pathogen removal potential of cavitation, along with the added agitation, make cavitation and chlorine an extremely powerful agent in removing microbial populations. In an experiment removing *Salmonella* Typhimurium from lettuce leaves, combining chlorine and cavitation was found to be roughly twice as effective as using either chlorine or cavitation alone. Treatment with chlorine and cavitation removed an average of 2.7 log of *Salmonella* Typhimurium as compared to 1.5 log reduction from cavitation treatment alone and 1.7 log reduction from using chlorine alone (Seymour, 2002). Similar results were achieved in removing *E. coli* on fresh lettuce, which reduced pathogenic populations by 2 logs (Piysaena, 2003). Chlorine wash and cavitation treatment were both used in removing *E. coli* on organic spinach leaves, which increased efficacy by 1.1 log reduction of *E. coli*, more than chlorine alone (Zhou, 2009). Lastly, an experiment using chlorine and cavitation to remove *Salmonella* and *E. coli* on the surface of fresh apples and lettuce resulted in unrecoverable levels of bacteria (Huang, 2006).

III. References for Literature Review

Acheson, D. and Hohmann, E.I. (2001). Nontyphoidal salmonellosis. *Clinical Infectious Diseases* 32.2: 263-269.

Antunes, P., Réu, C., Sousa, J.C., Peixe, L. and Pestana, N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology* 82.2: 97-103.

Arizona University. (2010). Roma Tomato. http://cals.arizona.edu/fps/sites/cals.arizona.edu.fps/files/cotw/Roma_Tomato.pdf (Accessed Aug. 8, 2016).

Asplund, K. and Nurmi, E. (1991). The growth of *Salmonella* in tomatoes. *International Journal of Food Microbiology, 13(2):177-181*.

Barak, J.D., and Liang, A.S. (2008). Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. *PLoS One* 3.2: e1657.

Bastos, M.D.S.R., Soares, N.D.F.F., de Andrade, N.J., Arruda, A.C. and Alves, R.E. (2005). The effect of the association of sanitizers and surfactant in the microbiota of the cantaloupe (*Cucumis melo* L.) melon surface. *Food Control* 16.4: 369-373.

Bermúdez-Aguirre, D., Mobbs, T. and Barbosa-Cánovas, G.V. (2011). Ultrasound applications in food processing. *Ultrasound technologies for food and bioprocessing* (pp. 65-105). Springer New York.

Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection*, 61(10): 1305-1311.

Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and infection*, *4*(4): 413-423.

Birkhoff, G. (1957). Jets, Wakes and Cavities, *Journal of Fluid Mechanics*, 3: 437-440.

Bhowmik, S. and Pan, J.C. (2006). Shelf life of mature green tomatoes stored in controlled atmosphere and high humidity. *Journal of Food Science*, 57(4): 948-953.

Bennett, S.D., Littrell, K.W., Hill, T.A., Mahovic, M. and Behravesh, C.B. (2015). Multistate foodborne disease outbreaks associated with raw tomatoes, United States, 1990–2010: a recurring public health problem. *Epidemiology and infection* 143.07: 1352-1359.

Blackman, I., and Frank, J.F. (1996). Growth of *Listeria monocytogenes* as a biofilm on various food-processing surfaces. *Journal of Food Protection* 59(8): 827-831.

Boriss, H. (2014). "Melon Profile." *Agricultural Marketing Service*. Agricultural Marketing Service. http://www.agmrc.org/commodities-products/vegetables/melon-profile/. (Accessed Aug. 8, 2016).

Burnett, S. L., and Beuchat, L.R. (2000). Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *Journal of Industrial Microbiology and Biotechnology* 25(6): 281-287

Burnett, S.L., Gehm, E.R., Weissinger, W.R. and Beuchat, L.R. (2000). Survival of *Salmonella* in peanut butter and peanut butter spread. *Journal of Applied Microbiology* 89.3: 472-477.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology* 94.3: 223-253.

Carpentier, B., and Cerf, O. (1993). Biofilms and their consequences, with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology,* 75(6): 499-511.

Center for Disease Control and Prevention. Foodborne Germs and Illnesses. *http://www.cdc.gov/foodsafety/foodborne-germs.html* (accessed Dec. 15, 2015)

Center for Disease Control and Prevention. (2012). Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado (Final uipdate). http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/ . (Accessed Aug. 8, 2016).

Center for Disease Control. (2005). "Outbreaks of *Salmonella* infections associated with eating Roma tomatoes--United States and Canada, 2004." https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5413a1.htm . (Accessed Aug. 8, 2016).

Center for Diseases Control and Prevention. (2010). *Salmonella*- technical. http://www.cdc.gov/salmonella/ (Accessed Dec. 19, 2015).

Chandrapala, J., Oliver, C., Kentish, S. and Ashokkumar, M. (2012). Ultrasonics in food processing–food quality assurance and food safety. *Trends in Food Science & Technology*, *26*(2), pp.88-98.

Chmielewski, R. A. N., and Frank, J.F. (2003). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety* 2(1): 22-32.

Cliffe-Byrnes, V., and O'Beirne, D. (2005). Effects of chlorine treatment and packaging on the quality and shelf-life of modified atmosphere (MA) packaged coleslaw mix. *Food Control* 16(8): 707-716.

Cole, M. B., Jones, M.V. and Holyoak, C. (1990). The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *Journal of Applied Bacteriology* 69.1: 63-72.

Cox, L.J., Kleiss, T., Cordier, J.L., Cordellana, C., Konkel, P., Pedrazzini, C., Beumer, R. and Siebenga, A. (1989). *Listeria* spp. in food processing, non-food and domestic environments. *Food Microbiology* 6.1: 49-61.

Dan, Y. (2016). "The Nutritional Benefits of Tomatoes." *The Nutritional Benefits of Tomatoes*. The Institute for Advanced Learning and Research. http://www.ialr.org/index.php/about-us/news-articles/216-the-nutritional-benefits-of-tomatoes. (Accessed Aug. 8, 2016).

Eikmeier, D. (2016). *Salmonella* Newport *outbreak associated with tomatoes consumed at burrito chain a: Lessons learned for investigating chain a outbreaks*. Council of State and Territorial Epidemiologists. https://cste.confex.com/cste/2016/webprogram/Paper6618.html. (Accessed Aug. 8, 2016).

Farber, J.M. and Peterkin, P.I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, *55*(3): 476-511.

Farkas, J. and Mohácsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science & Technology* 22.2: 121-126.

Food and Drug Administration. (2013). Bad Bug Book: Foodborne Pathogenic microorganisms and natural toxins handbook. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/default.htm (Accessed Mar. 9, 2016).

Food and Drug Administration. (1998). "Good Agricultural Practices (GAP) & Good Handling Practices (GHP)." *Agricultural Marketing Service*. http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169112.pdf (Accessed Aug. 8, 2016).

Foster, J.W. and Hall, H.K. (1990). Adaptive acidification tolerance response of *Salmonella* Typhimurium. *Journal of Bacteriology* 172.2: 771-778.

Gálvez, A., López, R.L., Abriouel, H., Valdivia, E. and Omar, N.B. (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical Reviews in Biotechnology* 28.2: 125-152.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T. and Charbit, A. (2001). Comparative genomics of *Listeria* species. *Science* 294.5543: 849-852.

Gomez, T.M., Motarjemi, Y., Miyagawa, S., Käferstein, F.K. and Stöhr, K. (1996). Foodborne salmonellosis. *World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales* 50.1-2: 81-89.

Gray, Mitchell L., and Killinger, A.H. (1966). *Listeria monocytogenes* and listeric infections. *Bacteriological reviews* 30.2: 309.

Guo, X., Chen, J., Brackett, R.E. and Beuchat, L.R. (2001). Survival of *Salmonella* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied and Environmental Microbiology* 67.10: 4760-4764.

Herald, P.J., and Zottola, E.A. (1988). Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. *Journal of Food Science* 53.5: 1549-1562.

Huang, H.W., Wu, S.J., Lu, J.K., Shyu, Y.T. and Wang, C.Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control* 72: 1-8.

Huang, T.S., Xu, C., Walker, K., West, P., Zhang, S. and Weese, J. (2006) Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. *Journal of Food Science* 71(4): M134-M139.

Issa-Zacharia, A., Kamitani, Y., Muhimbula, H.S. and Ndabikunze, B.K. (2010). A Review of microbiological safety of fruits and vegetables and the introduction of electrolyzed water as an alternative to sodium hypochlorite solution. *African Journal of Food Science*, *4*(13): 778-789.

Jambrak, A.R., Mason, T.J., Lelas, V., Herceg, Z. and Herceg, I.L. "Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions." *Journal of Food Engineering* 86.2 (2008): 281-287.

Joseph, B., Otta, S.K., Karunasagar, I. and Karunasagar, I. (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology*, *64*(3): 367-372.

Lado, Beatrice H., and Yousef, A. (2002). Alternative food-preservation technologies: efficacy and mechanisms. *Microbes and Infection,* 4.4: 433-440.

Lawley, T.D., Bouley, D.M., Hoy, Y.E., Gerke, C., Relman, D.A. and Monack, D.M.

(2008). Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. *Infection and immunity* 76.1: 403-416.

Leverentz, B., Conway, W.S., Janisiewicz, W., Abadias, M., Kurtzman, C.P. and Camp, M.J. (2006). Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Applied and Environmental Microbiology* 72.2: 1135-1140.

Lopez, K., Phalen, K., Vahl, C.I., Roberts, K.R. and Getty, K.J. (2016). Investigation of handling practices for fresh produce and the efficacy of commercially available produce washes on removal of pathogens and natural microflora on whole cantaloupe surfaces. *Food Control* 68: 251-259.

Low, J. C. and Donachie, W. (1997). A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal* 153.1: 9-29.

Marotti, J., Heger, S., Tinschert, J., Tortamano, P., Chuembou, F., Radermacher, K. and Wolfart, S. (2013). Recent advances of ultrasound imaging in dentistry–a review of the literature. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *115*(6): 819-832.

Martinez, M.R., Osborne, J., Jayeola, V.O., Katic, V. and Kathariou, S. (2016). Capacity of *Listeria monocytogenes* strains from the 2011 cantaloupe outbreak to adhere, survive, and grow on cantaloupe. *Journal of Food Protection* 79.5: 757-763.

McClements, D.J. (1995) Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science & Technology* 6(9): 293-299.

Meng, JH, and Doyle, M. (2002). Introduction: Microbiological food safety. *Microbes and Infection,* 4.4: 395-397.

Michaels, B., Keller, C., Blevins, M., Paoli, G., Ruthman, T., Todd, E. and Griffith, C.J. (2004). Prevention of food worker transmission of foodborne pathogens: risk assessment and evaluation of effective hygiene intervention strategies. *Food Service Technology* 4.1: 31-49.

Ming, X., Weber, G.H., Ayres, J.W. and Sandine, W.E. (1997). Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *Journal of Food Science* 62.2: 413-415.

Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L. and Wiedmann, M. (2004). Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied and Environmental Microbiology* 70.8: 4458-4467.

O'Donnell, C. P., Tiwari, B.K., Bourke, P., and Cullen, P.J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Science & Technology.* 21(7): 358-367.

Pan, Y., Breidt, F., and Kathariou, S. (2006). Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology* 72.12: 7711-7717.

Parini, M.R., and Pitt, W.G. (2006). Dynamic removal of oral biofilms by bubbles. *Colloids and Surfaces B: Biointerfaces* 52(1): 39-46.

Parini, M.R., Eggett, D.L., and Pitt, W.G. (2005). Removal of *Streptococcus mutans* biofilm by bubbles. *Journal of Clinical Periodontology* 32(11): 1151-1156.

Patterson MJ. (1996). Streptococcus. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 13. http://www.ncbi.nlm.nih.gov/books/NBK7611/ (Accessed Dec. 17, 2015).

Piyasena, P., Mohareb, E., and McKellar, R.C. (2003). Inactivation of microbes using ultrasound: a review. *International Journal of Food Microbiology* 87(3): 207-216.

Podolak, R., Enache, E., Stone, W., Black, D.G. and Elliott, P.H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection* 73.10: 1919-1936.

Produce for Better Health Foundation. *State of the Plate, 2015 Study on America's Consumption of Fruit and Vegetables*, Produce for Better Health Foundation, 2015. http://pbhfoundation.org/about/donor_updates/ed/020516 (Accessed Feb. 29, 2016).

Pui, C.F., Wong, W.C., Chai, L.C., Robin, T., Ponniah, J., Sahroni, M., Hidayah, N., Anyi, U., Mohamad Ghazali, F., Cheah, Y.K. and Radu, S. (2011). *Salmonella*: A foodborne pathogen. *International Food Research Journal* 18.2: 465-473.

Rayleigh, L. (1917), On the pressure developed in a liquid during the collapse of a spherical cavity, *Philosophical Magazine* 34(6): 94-98.

Ritenour, M., Sargent, S.A. and Bartz, J.A. (2002). *Chlorine use in produce packing lines*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

Rodriguez, A., Pangloli, P., Richards, H.A., Mount, J.R. and Draughon, F.A.N.N. (2006). Prevalence of *Salmonella* in diverse environmental farm samples. *Journal of Food Protection* 69.11: 2576-2580.

Sagong, H.G., Lee, S.Y., Chang, P.S., Heu, S., Ryu, S., Choi, Y.J. and Kang, D.H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology*, 145(1): 287-292.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17*(1): 1-21.

Seeliger, H.P.R. and Finger, H. (1983). Listeriosis. *Infectious Diseases of the Fetus and the Newborn Infant*, pp. 333-365, eds. J.S. Remington and J.K. Saunders.

Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A. and Lockwood, A. (2002). Ultrasound decontamination of minimally processed fruits and vegetables. *International Journal of Food Science & Technology*, *37*(5): 547-557.

Shearer, A.E., LeStrange, K., Castañeda Saldaña, R. and Kniel, K.E. (2016). Transfer of pathogens from cantaloupe rind to preparation surfaces and edible tissue as a function of cutting method. *Journal of Food Protection* 79.5: 764-770.

Singh, N., Singh, R.K., Bhunia, A.K. and Stroshine, R.L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157: H7 on lettuce and baby carrots. *LWT-Food Science and Technology*, *35*(8): 720-729.

Skovgaard, N. and Morgen, C.A. (1988). Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. *International Journal of Food Microbiology* 6.3: 229-242.

Tienungoon, S., Ratkowsky, D.A., McMeekin, T.A. and Ross, T. (2000). Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology* 66.11: 4979-4987.

Ukuku, D.O. and Fett, W. (2002). Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection* 65.6: 924-930.

United States Department of Agriculture. (2010). *ERS - Vegetables & Pulses: Tomatoes*. http://www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes.aspx (Accessed Dec. 19, 2015).

Walker, S. J., Archer, P. and Banks, J.G. (1990). Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology* 68.2: 157-162.

Wang, H., Feng, H., Liang, W., Luo, Y. and Malyarchuk, V. (2009). Effect of surface

roughness on retention and removal of *Escherichia coli* O157: H7 on surfaces of selected fruits. *Journal of food science* 74.1: E8-E15.

Wang, H. and Ryser, E.T. (2014). *Salmonella* transfer during pilot plant scale washing and roller conveying of tomatoes. *Journal of Food Protection* 77.3: 380-387.

Wang, Y., Somers, E.B., Manolache, S., Denes F.S. and Wong, A.C.L. (2003). Cold plasma synthesis of poly(ethylene glycol)-like layers on stainless steel surfaces to reduce attachment and biofilm formation by *Listeria monocytogenes*. *Journal of Food Science*. 68(1): 2772-2779.

Wei, C.I., Cook, D.L. and Kirk, J.R. (1985). Use of chlorine compounds in the food industry. *Food Technology, 39*:107-115.

Wei, C.I., Huang, T.S., Kim, J.M., Lin, W.F., Tamplin, M.L. and Bartz, J.A. (1995). Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. *Journal of Food Protection*, *58*(8): 829-836.

Weissinger, W.R., Chantarapanont, W. and Beuchat, L.R. (2000). Survival and growth of *Salmonella* Baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. *International Journal of Food Microbiology*, *62*(1): 123-131.

Winfield, M.D., and Groisman, E.A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* 69.7: 3687-3694.

Zhang, S. and Farber, J.M. (1996). The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology*, *13*(4): 311-321.

Zhou, B., Feng, H. and Luo, Y. (2009). Ultrasound enhanced sanitizer efficacy in reduction of *Escherichia coli* O157: H7 population on spinach leaves. *Journal of Food Science*, *74*(6): M308-M313.

Zhuang, R.Y., Beuchat, L.R. and Angulo, F.J. (1995). Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, *61*(6): 2127-2131.

Zibilske, L. M., and Weaver, R.W. (1978). Effect of environmental factors on survival of *Salmonella* Typhimurium in soil. *Journal of Environmental Quality* 7.4: 593-597.

IV. Materials and Methods

1. *Introduction*

The purpose of this study was to determine the efficacy of cavitation treatment in removing or inactivating *Listeria monocytogenes* and *Salmonella* Newport from the surface of fresh cantaloupe and Roma tomatoes. First, this project examines the air-flow rate and time ranges over which an optimum treatment for the removal and inactivation of *L. monocytogenes* and *Salmonella* Newport from the surface of fresh cantaloupe and Roma tomatoes is found. Second, this project determines the potential for cross-contamination during treatment among fresh Roma tomatoes. Thirdly, this project compares cavitation treatments with and without the presence of sodium hypochlorite solutions. Applications of chlorine solutions are the most common method of treating fresh produce today (Waters and Hung, 2014).

2. *Pathogen Cultivation*

2.1 *Listeria monocytogenes*

The selection of the strains of the foodborne pathogen *Listeria monocytogenes* correlates to those most frequently implicated in foodborne illnesses. Four strains used in this study (subtypes 1/2a, 1/2b, and 4b) were combined into a cocktail in equivalent proportions. and used in the study, specifically (FDA/CFSAN, 2003).

Listeria monocytogenes strains (serotypes Scott A, 4b-J1815, R2-503, and LM 0042) were obtained from the culture collection of the Department of Food Science and Technology, Virginia Polytechnic Institute and State University. Each strain was

separately cultivated in trypticase soy broth supplemented with 0.6% yeast extract (TSB-YE) for 36±2 hours at 36±2°C. The culture was transferred twice by a single loop inoculation to new tubes of TSB-YE after a 24±2 hour interval. Once each strain was subcultured, each strain was plated onto Oxford agar to confirm morphology and concentration. Following confirmation, broth cultures of each strain were combined to form a cocktail with equivalent concentrations of each strain.

2.2 *Salmonella*

Salmonella Newport has been recognized in a number of illness outbreaks, particularly in tomato crops (Greene et al., 2005). *Salmonella* Newport starter cultures with resistance to 50ppm Nalidixic acid were obtained from the culture collection of the Department of Food Science and Technology, Virginia Polytechnic Institute and State University. Nalidixic acid resistance was used to as a way to detect inoculated organisms in the presence of naturally occurring organisms. The *Salmonella* Newport culture was cultivated in trypticase soy broth supplemented with 30 ppm Nalidixic acid for 36±2 hours and incubated at 36±2°C. At 24±2 hour intervals, the culture was transferred by a single loop inoculation to new tubes of TSB-YE with 50ppm Nalidixic acid. This was done to assure growth in the presence of Nalidixic acid. Once cultured at the 50ppm Nalidixic acid resistance, the *Salmonella* Newport culture was plated onto Tryptic Soy Agar (TSA) plates supplemented with 50ppm Nalidixic acid. The concentration of cells, as well as culture purity were confirmed through the TSA plating.

3. *Fruit Inoculation*

Ripe, Roma tomatoes were purchased from local grocery markets, and stored at 4°C. Each Roma tomato was placed onto a sterile holding plate, and 100 µl of the *L. monocytogenes* cocktail culture (10^9 CFU/ml) was spot inoculated into 5 equal sized, 20 µl spots onto each tomato individually. Then each tomato remained on the sterile holding plate inside a biosafety lab cabinet, and air-dried for one hour. This follows the procedures of Zhou et al. (2009) and Hun-Gu et al. (2011), and is commonly used as an effective method of inoculation. The same procedure was used for *Salmonella* Newport inoculation, with five equivalent 20 µl spot inoculations placed on separate Roma tomatoes.

Ripe cantaloupes (*Cucumis melo* var. *reticulus*) were purchased from a local grocery market, and stored in their original box without modified atmosphere at 4°C. Inoculation of the cantaloupes was similar to that of the tomatoes, however, a higher number of spot inoculations were used to accommodate the larger size of the fruit. Instead of 100 µl spot treatment, five 100 µl spots were placed around the surface of the cantaloupe, for a total of 500 µl of *L. monocytogenes* cocktail per cantaloupe. The cantaloupes remained on sterile holding plates to air-dry in a biosafety lab cabinet for one hour. The same procedure was used for *Salmonella* Newport inoculation, with five-100 µl spot inoculations placed around the surface of the cantaloupe.

4. *Cavitation Treatment*

Cavitation bubbles were generated using a motorized air pump with adjustable air-flow outputs (Active Aqua (Hydrofarm) model AAPA70L). The motorized air pump

was connected to an air stone (Uxcell #B5014), which was placed at the bottom of a water chamber. The air stone was held at the bottom of the water chamber by water-proof tape. Tomatoes were treated in smaller chambers (TopFin 1 gallon (3.8 liter) plastic aquarium), as seen in Appendix Figure A, while cantaloupes were treated in larger chambers (TopFin 2.6 gallon (9.8 liter) glass aquarium).

4.1 *Roma Tomatoes*

After the one-hour drying period, each inoculated Roma tomato was placed individually into the smaller water chamber, which was filled with 2000mL of distilled water. The lid of each water chamber had a small camera tripod drilled into it, which acted as a barrier for the Roma tomato. This helped assure that the Roma tomato remained directly above the stream of bubbles, while also allowing it to naturally turn and move within the bubbles. The placement of the Roma tomato directly above the air stone allowed for maximum and consistent contact with the bubble stream. Bubbles were generated using one of five different air-flow settings: 0 (control), 3.5, 7, 10.5 and 14 Standard Liters Per Minute (SLPM). The air-flow rate was measured by an air-flow meter (Omega FMA-LP1600A). Treatment times were either 30 or 60 sec. Each Roma tomato was inoculated and treated for each combination of airflow and treatment time, for each pathogen. Each combination was then repeated for three trials.

4.2 *Cantaloupe*

Cavitation treatment for cantaloupes followed the same general procedure. After the one-hour drying period, each inoculated cantaloupe was placed individually into the

larger water chamber, which was filled with 5000mL of distilled water. The lid of each water chamber did not have a small camera tripod drilled into it, since the size of the cantaloupe was sufficient for it to be directly above the bubble stream. Bubbles were generated using one of five different air-flow settings: 0 (control), 3.5, 7, 10.5 and 14 Standard Liters Per Minute (SLPM). The air-flow rate was measured by an air-flow meter (Omega FMA-LP1600A).

Treatment times were either 30 or 60 sec. Each cantaloupe was inoculated and treated for each combination of airflow and treatment time, for each pathogen. Each combination was then repeated for three trials.

4.3 *Determination of treatment times*

The effects of varying airflow rates were observed when *Salmonella* Newport was added to a water tank without any fruit or chlorine. This was done to determine if the bubbles had any effect on pathogens when in the water without a surface to attach. A small difference (~0.2 log) in recovery was observed between all three treatment times, ranging from 1 to 5 minutes (Appendix Figure B). This suggested that longer treatment times (> 1 min) did not significantly reduce pathogen recovery from the fruit at the observed airflow rates, thus, 30 or 60 seconds were used throughout the experiments.

5. *Quantitative Recovery of Listeria monocytogenes from Product*

After the 30 or 60 second cavitation treatment, each fruit was aseptically placed into a sterile stomacher bag with buffered peptone water (99 mL per Roma

tomato, 500 mL for cantaloupe). Each fruit was then agitated inside the stomacher bag by hand for two minutes by moving back and forth at a constant arc (FDA, 2015). This solution was serially diluted onto two Oxford agar plates. Plates were incubated at 36±2°C for 36±2 hours. After incubation, plates were counted using an automated colony counter (ProtoCol; Microbiology International).

6. *Quantitative Recovery of Salmonella* Newport *from Product*

The process of *Salmonella* Newport recovery from the surface of inoculated Roma tomatoes and cantaloupes followed the same procedure, with the only difference in the plating. For *Salmonella* Newport recovery, the solution from each stomacher bag was serially diluted and spread plated onto two Tryptic Soy Agar (TSA) supplemented with 50ppm Nalidixic acid. Plates were incubated at 36±2°C for 36±2 hours. After incubation, plates were counted using an automated colony counter (ProtoCol; Microbiology International).

7. *Recovery and Quantification from Water*

After inoculated fruit were removed from the treatment tanks, 100 µl of the remaining water in the chamber was removed from the tank and directly plated onto the appropriate selective agars (Oxford agar for *L. monocytogenes* and TSA with 50ppm Nalidixic acid for *Salmonella* Newport recovery). Additional ten-fold dilutions of the tank water were plated, in duplicate, onto appropriate plates. Plates were then incubated at 36±2°C for 36±2 hours and enumerated as described previously.

8. *Cross-contamination from Inoculated to Uninoculated Roma Tomatoes*

To evaluate pathogen transfer to uncontaminated fruit in the presence of a bubble stream, one Roma tomato was inoculated with 100 µl of *L. monocytogenes* culture and placed into the 3.8 L chamber with 2000 mL water and one uninoculated Roma tomato. Detection of the pathogen on the uninoculated tomato could indicate cross-contamination from the inoculated tomato. The two tomatoes in the tank were treated for either 30 or 60 seconds with three air-flow rates of 0 (control), 7 or 14 SLPM. Three pairs of tomatoes (inoculated and uninoculated) were tested with each combination of bubble rate and treatment time. After each treatment, the tomatoes were removed and placed into individual stomacher bags with 99mL of buffered peptone water. *L. monocytogenes* were recovered from each tomato as previously described in the quantitative recovery step. The water remaining in the treatment chamber was also recovered following the procedure outlined in the water quantification step. These tests for cross contamination between inoculated and uninoculated tomatoes were replicated with Roma tomatoes inoculated or uninoculated with *Salmonella* Newport.

9. *Chlorine Solution Treatment of Roma Tomatoes and Cantaloupe*

9.1 *Comparison to Chlorine*

To determine the efficacy of cavitation as a treatment method, chlorine washes were used as an industry standard benchmark. The proportion of viable *Salmonella* Newport recovered from each fruit and the treatment water was used to compare fruit treated with and without chlorine. Individual Roma tomatoes were inoculated with 100 µl of the *Salmonella* Newport culture as described above, and placed into 2000 ml water.

The water inside the chamber was supplemented with sodium hypochlorite at a concentration of either 0 (control), 50 or 150 ppm (Ritenour et al. 2002). After 30 or 60 sec, the Roma tomatoes were removed and quantified for *Salmonella* Newport as outlined above. The remaining water in the treatment tank was also enumerated for *Salmonella* Newport as outlined above. Three trials were repeated for each combination of time and chlorine concentration.

These tests were replicated with cantaloupes inoculated with *Salmonella* Newport (500 ul) and submerged in 5000 mL water. The proportion of viable *Salmonella* recovered from each fruit and the tank water were compared for fruit treated with and without chlorine in the tank water.

For both fruits, a neutralizing agent (BD Difco D/E neutralizing broth) was used to determine if residual chlorine on the fruits on in the tank water reduced pathogen recovery. Use of the neutralizing agent did not present any difference in pathogen recovery when compared to pathogen recovery numbers without the neutralizing agent (data not shown).

9.2 *Efficacy of Combining Chlorine and Cavitation*

While chlorine is often considered an industry standard practice, tests to determine any difference in efficacy when combined with cavitation were completed. Individual Roma tomatoes were inoculated with 100 µl of the *Salmonella* Newport culture, and placed into 2000 ml water. Sodium hypochlorite concentrations in the water were either 0 (control), 100 or 200 ppm. Each Roma tomato was treated for either 30 or 60 seconds. For each combination of chlorine concentration and treatment time,

bubbles were applied using an air-flow rate of 14 SLPM. Three trials were tested for each combination of chlorine concentration, cavitation, and treatment time. After treatment, the Roma tomatoes were removed and quantified for *Salmonella* Newport as outlined above. The remaining water in the treatment tank was also enumerated for *Salmonella* Newport as outlined above.

These tests were replicated with cantaloupes inoculated with *Salmonella* Newport (500 µl) and submerged in 5000 mL water. The proportion of viable *Salmonella* recovered from each fruit and the tank water were compared for fruit treated with and without cavitation for chlorinated water (0, 50 or 150 ppm NaOCl).

10. *Data Analysis*

Organism counts enumerated from produce surfaces and the organism counts recovered from tank water were compared to the original inoculum level to determine the proportion of viable organisms that were attached to the fruit after treatment, the proportion removed in the water, and the proportion that could not be recovered. The analysis of the recovery of pathogens on the surface of produce items and in the water include the differences between specific flow rates, times, pathogens, and mean of recovered organisms.

A one-way analysis of variance (ANOVA) were used to determine significant differences between means for each variable tested at a statistical significance of α = 0.05. When the ANOVA indicates a difference between means, Tukey's multiple range test will be used to assess significant differences between means. All calculations were performed with R® 3.2.5 Statistical Software (R Core Team, 2016).

V. Results and Discussion

Objective 1: Effects of Cavitation on the Removal and Inactivation of *L. monocytogenes* and *Salmonella* Newport on the Surface of Roma Tomatoes and Cantaloupes

1.1 Recovery of Pathogens from the Surface of Fruits

1.1.1 *Tomatoes*

Five different levels of airflow rates were utilized to determine the efficacy of cavitation on the removal or inactivation of pathogens on the surface of Roma tomatoes. Figure 1 shows the log recovery of both *L. monocytogenes* and *Salmonella* Newport as a function of airflow rate. As the airflow rate increases, a steady decrease in log recovery is seen for both pathogens. Airflow rate was statistically significant ($P<.05$) in the removal or inactivation of pathogen populations.

Figure 2 shows the log recovery of *L. monocytogenes* as a function of time, either 30 or 60 seconds. At each airflow rate, the difference in log recovery between 30 and 60 seconds of treatment are statistically insignificant ($P > .05$) in the removal of pathogen populations. The recovery of *Salmonella* Newport from Roma tomato surfaces was not significantly different when bubbles were applied for 30 vs. 60 sec. (Appendix Figure D).

1.1.2 *Cantaloupes*

As with the Roma tomatoes, inoculated cantaloupes were treated at five different levels of airflow rates to determine the efficacy of cavitation on the removal or inactivation of pathogens on the surface of cantaloupes. Figure 3 shows the log

recovery of both *L. monocytogenes* and *Salmonella* Newport as a function of airflow rate. Similar to the Roma tomatoes, as the airflow rate increases, a steady decrease in log recovery is seen for both pathogens on the surface of cantaloupes. Airflow rate was statistically significant (P<.05) in the removal or inactivation of pathogen populations.

Figure 4 shows the log recovery of *Salmonella* Newport as a function of time, either 30 or 60 seconds. At each airflow rate, the difference between 30 seconds and 60 seconds of treatment are statistically insignificant (P > .05) in the removal of pathogen populations. The recovery of *L. monocytogenes* from cantaloupe surfaces also showed insignificant difference, as seen in Appendix Figure E.

1.2 Recovery of Pathogens from Treatment Water

1.2.1 *Roma Tomatoes*

After recovery of the pathogens on the surface of Roma tomatoes, the water remaining in the treatment chamber was enumerated at all five different levels of airflow rates to determine any effect of cavitation on pathogens in the water. Figure 3 shows the log recovery of both *L. monocytogenes* and *Salmonella* Newport as a function of airflow rate in the treatment water. While there is a strong overall trend of decreased pathogen recovery with higher airflow rates, with the most effective at 14 SLPM. Airflow rate was statistically significant (P<.05) in the inactivation of pathogen populations in the treatment water.

Figure 2 shows the log recovery of *L. monocytogenes* in the treatment water, as a function of time, either 30 or 60 seconds. Similar to the recovery of pathogens from the surface of Roma tomatoes, pathogen recovery from the treatment water showed

insignificant difference (P > .05) between 30 seconds or 60 seconds of treatment time. The recovery of *Salmonella* Newport in Roma tomato treatment water also showed insignificant difference, as seen in Appendix Figure D.

1.2.2 *Cantaloupes*

After recovery of the pathogens on the surface of cantaloupes, the water remaining in the treatment chamber was enumerated at all five different levels of airflow rates to determine any effect of cavitation on pathogens in the water. Figure 3 shows the log recovery of both *L. monocytogenes* and *Salmonella* Newport as a function of airflow rate in the treatment water. Similar to the recovery of pathogens from the surface of cantaloupes, recovery of pathogens from the water followed decreasing numbers as airflow rate increased. Airflow rate was statistically significant (P<.05) in the inactivation of pathogen populations in the treatment water.

Figure 4 shows the log recovery of *Salmonella* Newport in the treatment water, as a function of time, either 30 or 60 seconds. Similar to the recovery of pathogens from Roma tomato treatment water, treatment times were statistically insignificant (P > .05) for recovery of pathogens in cantaloupe treatment water. Differences in the recovery of *L. monocytogenes* in cantaloupe treatment water was not statistically significant with respect to treatment time (Appendix Figure E).

1.3 Discussion

Cavitation treatment for the removal or inactivation of both *Salmonella* Newport and *L. monocytogenes* on the surface of Roma tomatoes were similar to that of other research (Scouten, 2001; Seymour et al., 2002). An approximate 1-log decrease in the

pathogen population on fruit surfaces was the maximum observed, which was statistically significant (P<.05). As the airflow rate increased, the recovery of pathogens followed the general trend of decreasing for both fruits and both pathogens. However, the type of fruit also made a statistically significant difference (P<.05). On the surface of Roma tomatoes, *Salmonella* Newport was observed to have higher initial concentrations and higher adherence. Conversely, for cantaloupe, *L. monocytogenes* had higher initial populations and adherence. Most likely, the different pathogens' biochemistry and morphology adapted better to different environments. The cantaloupes' netted, rough texture may be better suited for *L. monocytogenes,* whereas the smooth texture of the Roma tomato may be better for *Salmonella* Newport*.* This coincides with the types of outbreaks commonly seen in the United States. Tomatoes are consistently associated with *Salmonella* outbreaks, whereas cantaloupes have been commonly associated with *Listeria* outbreaks. There was so significant difference in the treatment times between 30 and 60 seconds.

In the tank water post-treatment, an increase in pathogen recovery was observed consistently when airflow rate increased from 0 to 3.5 SPLM. This suggests the removal of pathogens from the surface of the fruit remains in the water at viable levels. However, as the airflow rate increases, the pathogen population significantly decreases. This suggests further that higher airflow rates have the ability to inactivate both *Salmonella* Newport as well as *L. monocytogenes.* Moreover, the higher the airflow rate, the more effective the inactivation. The results of the enumeration of treatment water coincide with the observations that *L. monocytogenes* adhere better to cantaloupes. The recovery of *L. monocytogenes* post-treatment of inoculated cantaloupe were less than

that of *Salmonella* Newport. Similar to fruit surface enumeration, the treatment time did not have a significant difference between 30 and 60 seconds. However, the type of fruit and type of pathogen both had significant effects on the efficacy of cavitation in inactivating pathogens in the treatment water. *L. monocytogenes* had significantly less inactivation than *Salmonella* Newport. This may suggest greater resistance of *L. monocytogenes* to the effects of cavitation.

Objective 2: Cross Contamination from Inoculated Roma Tomatoes to Uninoculated Roma Tomatoes

2.1 *Tomatoes*

Three different levels of airflow rates were utilized to determine the possibility of cross contamination from inoculated Roma tomatoes to uninoculated Roma tomatoes during simultaneous cavitation treatment. Figure 5 shows the log recovery of the uninoculated Roma tomato for *L. monocytogenes* as a function of the three airflow rates. The inoculated tomato and recovery of pathogens in the treatment water follow previously observed, and expected trends of decreased recovery as the airflow rate increases. However, for the uninoculated Roma tomato, the largest decrease was seen from 0 SLPM to 7 SLPM, with little change from 7 SLPM to 14 SLPM. Transfer and recovery of *Salmonella* Newport followed extremely similar trends. Airflow rate was statistically significant ($P<.05$) in the removal or inactivation of pathogen populations.

Figure 5 shows the log transfer of *L. monocytogenes* as a function of treatment times. While the inoculated tomato and pathogen recovery in the treatment water show

no significant difference between treatment times (P > .05), there is a significant difference in treatment times (P<.05) in the transfer of pathogens to the uninoculated tomato, which is generally less with longer treatment times. The same trend follows for *Salmonella* Newport cross contamination enumeration, as seen in Figure 6.

2.2 *Discussion*

Cross contamination from inoculated Roma tomatoes to uninoculated Roma tomatoes was clearly observed. Since research on non-inertial cavitation cross contamination is sparse, the results could not be confirmed through other research. An approximate 1-log decrease in pathogen transfer was observed when airflow rate was increased from 0 SLPM to 14 SLPM. The decrease in pathogen population transfer on Roma tomato surfaces due to airflow rate was statistically significant (P<.05). However, even at the highest airflow rate, the amount of organisms transferred was significant.

Treatment times, either 30 or 60 seconds, were statistically significant (P<.05), with less organisms remaining on the uninoculated Roma tomato after the longer treatment time. This suggests that cross contamination occurs within 30 seconds. The pathogen may detach from the uninoculated Roma tomato and adhere to uninoculated Roma tomato, and once attached to the uninoculated tomato, may be removed or inactivated a second time during the second 30 seconds of cavitation treatment.

Objective 3: Comparisons with Chlorine Wash and Determining Efficacy of Combined Chlorine and Cavitation Treatment

3.1 Recovery of Pathogens from the Surface of Fruits

3.1.1 *Roma Tomatoes*

At each of the three chlorine concentrations of 0, 50, and 150ppm, two different levels of airflow rates were utilized to determine the efficacy of cavitation compared with a chlorine wash, as well as to observe any increased efficacy in combining chlorine and cavitation. Figure 7 shows the log reduction of *Salmonella* Newport for Roma tomatoes as a function of both airflow rates and chlorine concentration. The reduction of *Salmonella* Newport populations on the surface of Roma tomatoes was significantly higher ($P<.05$) at 150ppm of chlorine, compared to 50ppm chlorine. Moreover, at the higher airflow rate, log reduction of *Salmonella* Newport from the surface of Roma tomatoes was significantly higher ($P<.05$) compared to no airflow rate.

3.1.2 *Cantaloupes*

Similar to the Roma tomatoes, log recovery of *Salmonella* Newport from the surface of cantaloupes followed the same trend of significantly higher reduction ($P<.05$) at 150ppm chlorine concentration than at 50ppm, as seen in Figure 8. Furthermore, the higher airflow rate was significantly more effective ($P<.05$) at reducing *Salmonella* Newport populations than the no airflow rate.

3.2 *Recovery of Pathogens from Treatment Water*

3.2.1 *Tomatoes*

After treatment of each fruit, the water remaining in the treatment tank was enumerated to determine the effects of chlorine, cavitation, and the combination of chlorine and cavitation. Figure 7 shows the log recovery of *Salmonella* Newport in the

treatment tanks for Roma tomatoes as a function of both airflow rates and all three chlorine concentrations. There is a clear trend of higher reduction as chlorine concentration increases, for both airflow rates. Each increment of increased chlorine concentration was significantly higher in log reduction ($P<.05$). Similarly, the higher airflow rate was significantly higher in log reduction ($P<.05$) than no airflow rate.

3.2.2 *Cantaloupes*

Figure 8 shows the enumeration of treatment water at each combination of airflow rate and chlorine concentration for cantaloupes. The trend is exactly the same as the Roma tomato treatment water, with chlorine concentration being significantly higher in log reduction ($P<.05$), and the higher airflow rate significantly higher in log reduction ($P<.05$), for all chlorine concentrations.

3.3 *Discussion*

To compare the efficacy of cavitation, comparisons to an industry standard chlorine was were made. Three different chlorine concentrations were used, 0ppm, 50ppm and 150ppm. In addition, cavitation and chlorine were combined to determine any additional beneficial results from combining treatment. While the chlorine wash did not produce major differences in reducing *Salmonella* Newport organisms from the surface of fresh Roma tomatoes and cantaloupe, when compared with no chlorine, combining both treatments resulted in greater efficacy than in removing or inactivating *Salmonella* Newport from the surface of fresh Roma tomatoes and cantaloupe with cavitation or chlorine alone. This is consistent with research performed with a variety of pathogens and raw fruits or vegetables (Weissinger et al., 2000; Zhuang et al., 1995;

Wei et al., 1995; Singh et al., 2002). An approximate 0.5-log to 1-log decrease in the *Salmonella* Newport population on fruit surfaces was observed, which was statistically significant ($P<.05$). Similar to objective 1, the type of fruit also made a statistically significant difference. However, unlike objective 1 results, *Salmonella* Newport was found to have higher adherence to cantaloupe surfaces, rather than Roma tomatoes.

In the tank water post-treatment, there was consistently a statistically significant difference in pathogen log reduction ($P<.05$) when using chlorine, versus not using chlorine. Moreover, the higher chlorine concentration of 150ppm proved much more effective in removing *Salmonella* Newport populations in the water than the lower 50ppm concentration. This may be due to the available free chlorine in the water. The higher chlorine concentration would have much more free chlorine available to inactivate pathogens, resulting in lower enumeration of *Salmonella* Newport in treatment water.

Along with airflow rate, the type of fruit proved to be statistically significant ($P <.05$) in recovery of *Salmonella* Newport in treatment water. This maintains with the above observations with enumeration of *Salmonella* Newport from the surface of both fruits. Since *Salmonella* Newport was shown to be more adherent to cantaloupe in this phase of experiment, less organisms were recovered from treatment tanks post-treatment of cantaloupes. Lastly, treatment times were not statistically significant ($P<.05$) in enumeration of the treatment water.

VI. Conclusion

The purpose of this research was to show the effects of cavitation in the removal and inactivation of *Listeria monocytogenes* and *Salmonella* Newport on the surface of fresh Roma tomatoes and cantaloupes. In the first objective of the experiment, the effects of solely using cavitation were tested. Both the *L. monocytogenes* and *Salmonella* Newport populations were decreased by approximately 1-log when using the highest airflow rate of 14 SLPM for 60 sec when compared to no bubble application. Enumeration of the remaining treatment water post-cavitation also showed a 0.5-log to 1-log decrease in bacterial concentrations as airflow increased, proposing that cavitation has an effect on organisms remaining in the water, as well as on the surface on fruits.

In the second objective of this project, potential cross contamination was observed by treating an inoculated Roma tomato simultaneously with an uninoculated Roma tomato, to observe any pathogenic transfer. Results showed that statistically significant ($P<.05$) transfer of both *L. monocytogenes* and *Salmonella* Newport did occur. However, higher airflow rates resulted in less total adherence of pathogens onto the uninoculated Roma tomato. In other words, after pathogens transferred from the inoculated Roma tomato to the uninoculated Roma tomato, cavitation at the higher airflow rates removed or inactivated pathogens on the surface of the uninoculated tomato.

In the final objective of this experiment, cavitation as a treatment method was compared with an industry standard chlorine wash. In addition, additional efficacy was

observed when cavitation was combined with chlorine in the treatment tank. First, cavitation was found to be equally effective as a chlorine wash in removing or inactivating pathogens from the surface of fresh Roma tomatoes and cantaloupes. However, this was not true of the treatment water. Chlorine was more effective at reducing pathogenic populations in the water in the treatment tank. This is consistent with the use of chlorine in the food industry as a method to reduce pathogenic populations in wash waters (Wei et al., 1985). Second, combining cavitation and chlorine was found to be more effective than using either alone for removing *L. monocytogenes* and *Salmonella* Newport organisms from the surface of both fresh Roma tomatoes and cantaloupe. This remained true for reducing pathogenic loads in the treatment water as well.

Food safety can be enhanced through the use of physical processes, namely injected cavitation, to remove and inactivate common foodborne pathogens on the surface of fresh produce. This project observed efficient and effective reduction in pathogen populations using such non-thermal physical processes. This technology holds great promise in potentially providing widespread utility among a variety of food surfaces and applications in other antimicrobial treatments in the food industry, at low costs, low treatment times, and energetically sustainable manners.

Further research is strongly encouraged in a wide variety of areas regarding injected cavitation. Variegated pathogens and food types in necessary, particularly with fresh leafy greens, as its complicated topography may be better sanitized with injected cavitation bubbles. In addition, injected cavitation may hold potential in treating sprout seeds. Higher airflow rates are also another direction future research must tend, as this

project was limited to 14 SLPM via the air pump motor. Smaller bubble sizes, different types of gases, and industrial scales are additional pieces of data required to further determine the true efficacy of this method. Undoubtedly, this project only scratches the surface of injected cavitation as a sanitation method. Further data will allow valuable information on the efficacy of this treatment and will allow further exploration in the future for physical treatment to remove foodborne pathogens on produce surfaces.

VII. References

Acheson, D. and Hohmann, E.I. (2001). Nontyphoidal salmonellosis. *Clinical Infectious Diseases* 32.2: 263-269.

Antunes, P., Réu, C., Sousa, J.C., Peixe, L. and Pestana, N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology* 82.2: 97-103.

Asplund, K. and Nurmi, E. (1991). The growth of *Salmonella* in tomatoes. *International Journal of Food Microbiology, 13(2):177-181*.

Barak, J.D., and Liang, A.S. (2008). Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. *PLoS One* 3.2: e1657.

Bastos, M.D.S.R., Soares, N.D.F.F., de Andrade, N.J., Arruda, A.C. and Alves, R.E. (2005). The effect of the association of sanitizers and surfactant in the microbiota of the Cantaloupe (Cucumis melo L.) melon surface. *Food Control* 16.4: 369-373.

Bermúdez-Aguirre, D., Mobbs, T. and Barbosa-Cánovas, G.V. (2011). Ultrasound applications in food processing. *Ultrasound technologies for food and bioprocessing* (pp. 65-105). Springer New York.

Beuchat, L.R., Nail, B.V., Adler, B.B. and Clavero, M.R.S. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection*, 61(10): 1305-1311.

Beuchat, L.R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and infection*, *4*(4): 413-423.

Birkhoff, G. (1957). *Jets, Wakes and Cavities*, Journal of Fluid Mechanics, 3: 437-440.

Bhowmik, S. and Pan, J.C. (2006). Shelf life of mature green tomatoes stored in controlled atmosphere and high humidity. *Journal of Food Science*, 57(4): 948-953.

Bennett, S.D., Littrell, K.W., Hill, T.A., Mahovic, M. and Behravesh, C.B. (2015). Multistate foodborne disease outbreaks associated with raw tomatoes, United States, 1990–2010: a recurring public health problem. *Epidemiology and infection* 143.07: 1352-1359.

Blackman, I., and Frank, J.F. (1996). Growth of *Listeria monocytogenes* as a biofilm on various food-processing surfaces. *Journal of Food Protection* 59(8): 827-831.

Boriss, H. (2014). "Melon Profile." *Agricultural Marketing Service*. Agricultural Marketing Service. http://www.agmrc.org/commodities-products/vegetables/melon-profile/. (Accessed Aug. 8, 2016).

Burnett, S. L., and Beuchat, L.R. (2000). Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. *Journal of Industrial Microbiology and Biotechnology* 25(6): 281-287

Burnett, S.L., Gehm, E.R., Weissinger, W.R. and Beuchat, L.R. (2000). Survival of *Salmonella* in peanut butter and peanut butter spread. *Journal of Applied Microbiology* 89.3: 472-477.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology* 94.3: 223-253.

Carpentier, B., and Cerf, O. (1993). Biofilms and their consequences, with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology,* 75(6): 499-511.

Center for Disease Control and Prevention. Foodborne Germs and Illnesses. *http://www.cdc.gov/foodsafety/foodborne-germs.html* (accessed Dec. 15, 2015)

Center for Disease Control and Prevention. (2012). Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms, Colorado (FINAL UPDATE). http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/. (Accessed Aug. 8, 2016).

Center for Disease Control. (2005). "Outbreaks of *Salmonella* Infections Associated with Eating Roma Tomatoes--United States and Canada, 2004." https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5413a1.htm . (Accessed Aug. 8, 2016).

Center for Diseases Control and Prevention. (2010). *Salmonella*- technical. http://www.cdc.gov/salmonella/ (Accessed Dec. 19, 2015).

Center for Diseases Control and Prevention. (2010). *Salmonella*- technical. http://www.cdc.gov/salmonella/outbreaks (Accessed Dec. 19, 2015).

Chandrapala, J., Oliver, C., Kentish, S. and Ashokkumar, M. (2012). Ultrasonics in food processing–food quality assurance and food safety. *Trends in Food Science & Technology*, *26*(2), pp.88-98.

Chmielewski, R. A. N., and Frank, J.F. (2003). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety* 2(1): 22-32.

Cliffe-Byrnes, V., and O'Beirne, D. (2005). Effects of chlorine treatment and packaging on the quality and shelf-life of modified atmosphere (MA) packaged coleslaw mix. *Food Control* 16(8): 707-716.

Cole, M. B., Jones, M.V. and Holyoak, C. (1990). The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *Journal of Applied Bacteriology* 69.1: 63-72.

Cox, L.J., Kleiss, T., Cordier, J.L., Cordellana, C., Konkel, P., Pedrazzini, C., Beumer, R. and Siebenga, A. (1989). *Listeria* spp. in food processing, non-food and domestic environments. *Food Microbiology* 6.1: 49-61.

Dan, Y. (2016). "The Nutritional Benefits of Tomatoes." *The Nutritional Benefits of Tomatoes*. The Institute for Advanced Learning and Research. http://www.ialr.org/index.php/about-us/news-articles/216-the-nutritional-benefits-of-tomatoes (Accessed Aug. 8, 2016).

Eikmeier, D. (2016). *Salmonella* Newport *Outbreak Associated with Tomatoes Consumed at Burrito Chain A: Lessons Learned for Investigating Chain a Outbreaks*. Council of State and Territorial Epidemiologists. https://cste.confex.com/cste/2016/webprogram/Paper6618.html (Accessed Aug. 8, 2016).

Farber, J.M. and Peterkin, P.I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews*, *55*(3): 476-511.

Farkas, J. and Mohácsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science & Technology* 22.2: 121-126.

Food and Drug Administration. (2015). Bad Bug Book: Foodborne Pathogenic microorganisms and natural toxins handbook. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/default.htm (Accessed Mar. 9, 2016).

Food and Drug Administration. (2003). Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready to eat foods. http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM197330.pdf (Accessed Dec. 17, 2015).

Food and Drug Administration. Detection and Enumeration of *Listeria monocytogenes*. Bacteriological Analytical Manual, Ch. 10. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm (accessed Feb. 2, 2016).

Food and Drug Administration. (1998). "Good Agricultural Practices (GAP) & Good Handling Practices (GHP)." *Agricultural Marketing Service*. http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM169112.pdf (Accessed Aug. 8, 2016).

Foster, J.W. and Hall, H.K. (1990). Adaptive acidification tolerance response of *Salmonella* Typhimurium. *Journal of bacteriology* 172.2: 771-778.

Gálvez, A., López, R.L., Abriouel, H., Valdivia, E. and Omar, N.B. (2008). Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. *Critical reviews in biotechnology* 28.2: 125-152.

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T. and Charbit, A. (2001). Comparative genomics of *Listeria* species. *Science* 294.5543: 849-852.

Gomez, T.M., Motarjemi, Y., Miyagawa, S., Käferstein, F.K. and Stöhr, K. (1996). Foodborne salmonellosis. *World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales* 50.1-2: 81-89.

Gray, Mitchell L., and Killinger, A.H. (1966). *Listeria monocytogenes* and listeric infections. *Bacteriological reviews* 30.2: 309.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F. and Painter, J.A. (2005). Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields. *Epidemiology and Infection*, *136*(2):157-165.

Guo, X., Chen, J., Brackett, R.E. and Beuchat, L.R. (2001). Survival of *Salmonella* on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied and Environmental Microbiology* 67.10: 4760-4764.

Herald, P.J., and Zottola, E.A. (1988). Attachment of *Listeria monocytogenes* to stainless steel surfaces at various temperatures and pH values. *Journal of Food Science* 53.5: 1549-1562.

Huang, H.W., Wu, S.J., Lu, J.K., Shyu, Y.T. and Wang, C.Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control* 72: 1-8.

Huang, T.S., Xu, C., Walker, K., West, P., Zhang, S. and Weese, J. (2006) Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. *Journal of Food Science* 71(4): M134-M139.

Issa-Zacharia, A., Kamitani, Y., Muhimbula, H.S. and Ndabikunze, B.K. (2010). A Review of microbiological safety of fruits and vegetables and the introduction of electrolyzed water as an alternative to sodium hypochlorite solution. *African Journal of Food Science*, *4*(13): 778-789.

Joseph, B., Otta, S.K., Karunasagar, I. and Karunasagar, I. (2001). Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology*, *64*(3): 367-372.

Lado, Beatrice H., and Yousef, A. (2002). Alternative food-preservation technologies: efficacy and mechanisms. *Microbes and Infection,* 4.4: 433-440.

Lawley, T.D., Bouley, D.M., Hoy, Y.E., Gerke, C., Relman, D.A. and Monack, D.M. (2008). Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. *Infection and immunity* 76.1: 403-416.

Leverentz, B., Conway, W.S., Janisiewicz, W., Abadias, M., Kurtzman, C.P. and Camp, M.J. (2006). Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Applied and Environmental Microbiology* 72.2: 1135-1140.

Lopez, K., Phalen, K., Vahl, C.I., Roberts, K.R. and Getty, K.J. (2016). Investigation of handling practices for fresh produce and the efficacy of commercially available produce washes on removal of pathogens and natural microflora on whole cantaloupe surfaces. *Food Control* 68: 251-259.

Low, J. C. and Donachie, W. (1997). A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal* 153.1: 9-29.

Marotti, J., Heger, S., Tinschert, J., Tortamano, P., Chuembou, F., Radermacher, K. and Wolfart, S. (2013). Recent advances of ultrasound imaging in dentistry–a review of the literature. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, *115*(6): 819-832.

Martinez, M.R., Osborne, J., Jayeola, V.O., Katic, V. and Kathariou, S. (2016). Capacity of *Listeria monocytogenes* strains from the 2011 cantaloupe outbreak to adhere, survive, and grow on cantaloupe. *Journal of Food Protection* 79.5: 757-763.

McClements, D.J. (1995) Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science & Technology* 6(9): 293-299.

Meng, JH, and Doyle, M. (2002). Introduction: Microbiological food safety. *Microbes and Infection,* 4.4: 395-397.

Michaels, B., Keller, C., Blevins, M., Paoli, G., Ruthman, T., Todd, E. and Griffith, C.J. (2004). Prevention of food worker transmission of foodborne pathogens: risk assessment and evaluation of effective hygiene intervention strategies. *Food Service Technology* 4.1: 31-49.

Ming, X., Weber, G.H., Ayres, J.W. and Sandine, W.E. (1997). Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *Journal of Food Science* 62.2: 413-415.

Nightingale, K.K., Schukken, Y.H., Nightingale, C.R., Fortes, E.D., Ho, A.J., Her, Z., Grohn, Y.T., McDonough, P.L. and Wiedmann, M. (2004). Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment. *Applied and Environmental Microbiology* 70.8: 4458-4467.

O'Donnell, C. P., Tiwari, B.K., Bourke, P., and Cullen, P.J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in food science & technology.* 21(7): 358-367.

Pan, Y., Breidt, F., and Kathariou, S. (2006). Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Applied and Environmental Microbiology* 72.12: 7711-7717.

Parini, M.R., and Pitt, W.G. (2006). Dynamic removal of oral biofilms by bubbles. *Colloids and Surfaces B: Biointerfaces* 52(1): 39-46.

Parini, M.R., Eggett, D.L., and Pitt, W.G. (2005). Removal of *Streptococcus mutans* biofilm by bubbles. *Journal of Clinical Periodontology* 32(11): 1151-1156.

Patterson MJ. (1996). Streptococcus. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 13. http://www.ncbi.nlm.nih.gov/books/NBK7611/ (Accessed Dec. 17, 2015).

Piyasena, P., Mohareb, E., and McKellar, R.C. (2003). Inactivation of microbes using ultrasound: a review. *International Journal of Food Microbiology* 87(3): 207-216.

Podolak, R., Enache, E., Stone, W., Black, D.G. and Elliott, P.H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection* 73.10: 1919-1936.

Produce for Better Health Foundation. *State of the Plate, 2015 Study on America's Consumption of Fruit and Vegetables*, Produce for Better Health Foundation, 2015. http://pbhfoundation.org/about/donor_updates/ed/020516 (Accessed Feb. 29, 2016).

Pui, C.F., Wong, W.C., Chai, L.C., Robin, T., Ponniah, J., Sahroni, M., Hidayah, N., Anyi, U., Mohamad Ghazali, F., Cheah, Y.K. and Radu, S. (2011). *Salmonella*: A foodborne pathogen. *International Food Research Journal* 18.2: 465-473.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rayleigh, L. (1917), On the pressure developed in a liquid during the collapse of a spherical cavity, *Philosophical Magazine* 34(6): 94-98.

Ritenour, M., Sargent, S.A. and Bartz, J.A. (2002). *Chlorine use in produce packing lines*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

Rodriguez, A., Pangloli, P., Richards, H.A., Mount, J.R. and Draughon, F.A.N.N. (2006). Prevalence of *Salmonella* in diverse environmental farm samples. *Journal of Food Protection* 69.11: 2576-2580.

Sagong, H.G., Lee, S.Y., Chang, P.S., Heu, S., Ryu, S., Choi, Y.J. and Kang, D.H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology*, 145(1): 287-292.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases*, *17*(1): 1-21.

Seeliger, H.P.R. and Finger, H. (1983). Listeriosis. *Infectious Diseases of the Fetus and the Newborn Infant*, pp. 333-365, eds. J.S. Remington and J.K. Saunders.

Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A. and Lockwood, A. (2002). Ultrasound decontamination of minimally processed fruits and vegetables. *International Journal of Food Science & Technology*, *37*(5): 547-557.

Shearer, A.E., LeStrange, K., Castañeda Saldaña, R. and Kniel, K.E. (2016). Transfer of Pathogens from Cantaloupe Rind to Preparation Surfaces and Edible Tissue as a Function of Cutting Method. *Journal of Food Protection* 79.5: 764-770.

Singh, N., Singh, R.K., Bhunia, A.K. and Stroshine, R.L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157: H7 on lettuce and baby carrots. *LWT-Food Science and Technology*, *35*(8): 720-729.

Skovgaard, N. and Morgen, C.A. (1988). Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. *International Journal of Food Microbiology* 6.3: 229-242.

Tienungoon, S., Ratkowsky, D.A., McMeekin, T.A. and Ross, T. (2000). Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology* 66.11: 4979-4987.

Ukuku, D.O. and Fett, W. (2002). Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection* 65.6: 924-930.

United States Department of Agriculture. (2010). *ERS - Vegetables & Pulses: Tomatoes*. http://www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes.aspx (Accessed Dec. 19, 2015).

Walker, S. J., Archer, P. and Banks, J.G. (1990). Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology* 68.2: 157-162.

Wang, H., Feng, H., Liang, W., Luo, Y. and Malyarchuk, V. (2009). Effect of surface roughness on retention and removal of *Escherichia coli* O157: H7 on surfaces of selected fruits. *Journal of Food Science* 74.1: E8-E15.

Wang, H. and Ryser, E.T. (2014). *Salmonella* transfer during pilot plant scale washing and roller conveying of tomatoes. *Journal of Food Protection* 77.3: 380-387.

Wang, Y., Somers, E.B., Manolache, S., Denes F.S. and Wong, A.C.L. (2003). Cold plasma synthesis of poly(ethylene glycol)-like layers on stainless steel surfaces to reduce attachment and biofilm formation by *Listeria monocytogenes*. *Journal of Food Science*. 68(1): 2772-2779.

Waters, B. W. and Hung, Y.C. (2014). The effect of organic loads on stability of various chlorine-based sanitizers. *International Journal of Food Science & Technology*, 49(2): 867–875.

Wei, C.I., Cook, D.L. and Kirk, J.R. (1985). Use of chlorine compounds in the food industry. *Food Technology, 39*:107-115.

Wei, C.I., Huang, T.S., Kim, J.M., Lin, W.F., Tamplin, M.L. and Bartz, J.A. (1995). Growth and survival of *Salmonella* Montevideo on tomatoes and disinfection with chlorinated water. *Journal of Food Protection*, *58*(8): 829-836.

Weissinger, W.R., Chantarapanont, W. and Beuchat, L.R. (2000). Survival and growth of *Salmonella* baildon in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer. *International Journal of Food Microbiology*, *62*(1): 123-131.

Winfield, M.D., and Groisman, E.A. (2003). Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* 69.7: 3687-3694.

Zhang, S. and Farber, J.M. (1996). The effects of various disinfectants against *Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology*, *13*(4): 311-321.

Zhou, B., Feng, H. and Luo, Y. (2009). Ultrasound enhanced sanitizer efficacy in reduction of *Escherichia coli* O157: H7 population on spinach leaves. *Journal of Food Science*, *74*(6): M308-M313.

Zhuang, R.Y., Beuchat, L.R. and Angulo, F.J. (1995). Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, *61*(6): 2127-2131.

Zibilske, L. M., and Weaver, R.W. (1978). Effect of environmental factors on survival of *Salmonella* Typhimurium in soil. *Journal of Environmental Quality* 7.4: 593-597.

VIII. Tables and Figures

	Time	Airflow Rate (SLPM)				
	(sec)	0	3.5	7	10.5	14
Tomatoes						
L. monocytogenes	30	1.56[a]	1.69[b]	1.92[c]	2.23[d]	2.57[e]
	60	1.71[A]	1.82[B]	2.05[C]	2.34[d]	2.89[e]
Salmonella Newport	30	1.34[a]	1.63[b]	1.76[c]	1.88[c]	2.06[d]
	60	1.31[a]	1.54[B]	1.73[c]	1.92[d]	2.23[E]
Cantaloupes						
L. monocytogenes	30	1.90[a]	2.07[b]	2.28[c]	2.39[c]	2.55[d]
	60	1.86[a]	2.02[b]	2.19[C]	2.31[d]	2.63[E]
Salmonella Newport	30	2.10[a]	2.30[b]	2.43[c]	2.46[c]	2.65[d]
	60	2.23[A]	2.30[a]	2.48[b]	2.65[C]	2.88[D]

[a,b,c,d,e] Denotes significant differences (P<.05) from previous airflow rates

[A,B,C,D,E] Denotes significant differences (P<.05) in time

Table 1. Log reductions of pathogens on Roma tomatoes and cantaloupe for all airflow rates and treatment times. (n=6).

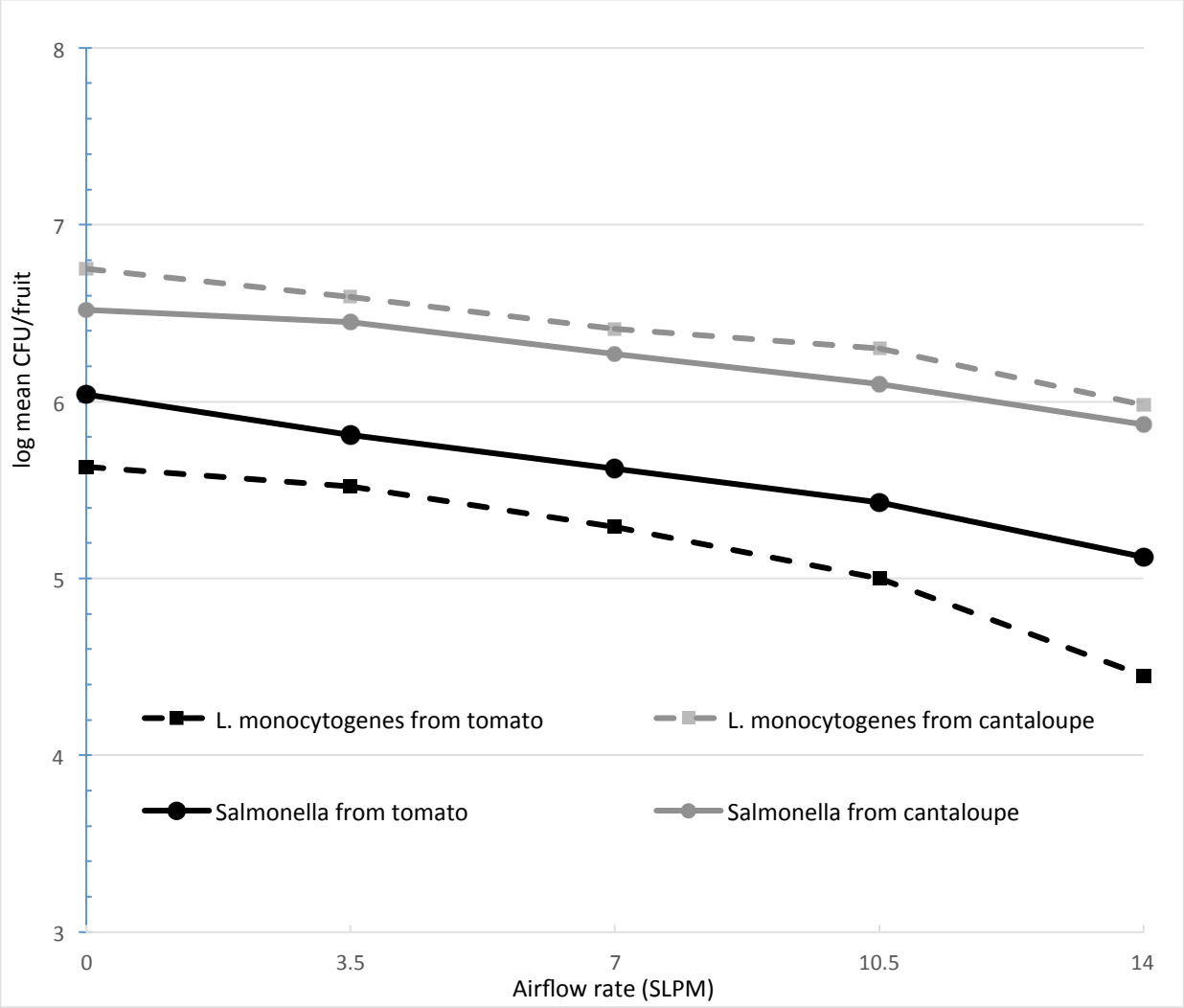


Figure 1. Recovery of *L. monocytogenes* and *Salmonella* Newport from the surfaces of Roma tomatoes and cantaloupe. (n=6)

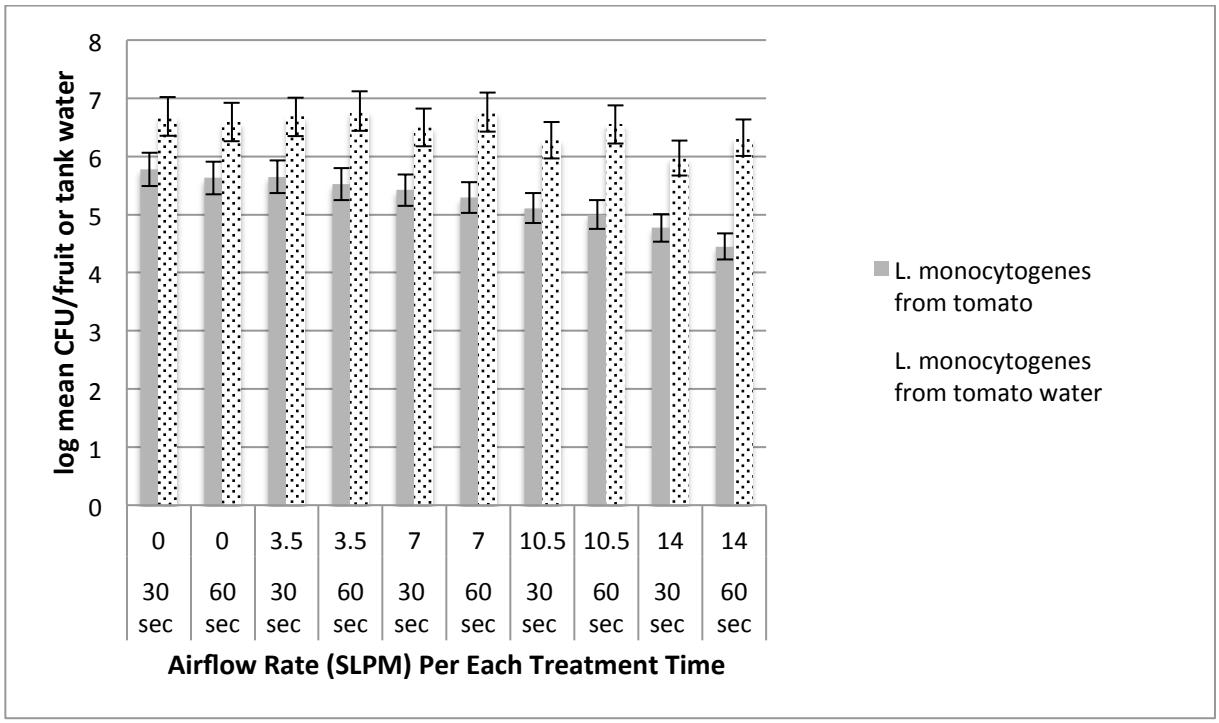


Figure 2. Recovery of *L. monocytogenes* from the surface of Roma tomatoes and recovered from the water post-treatment. (n=6)

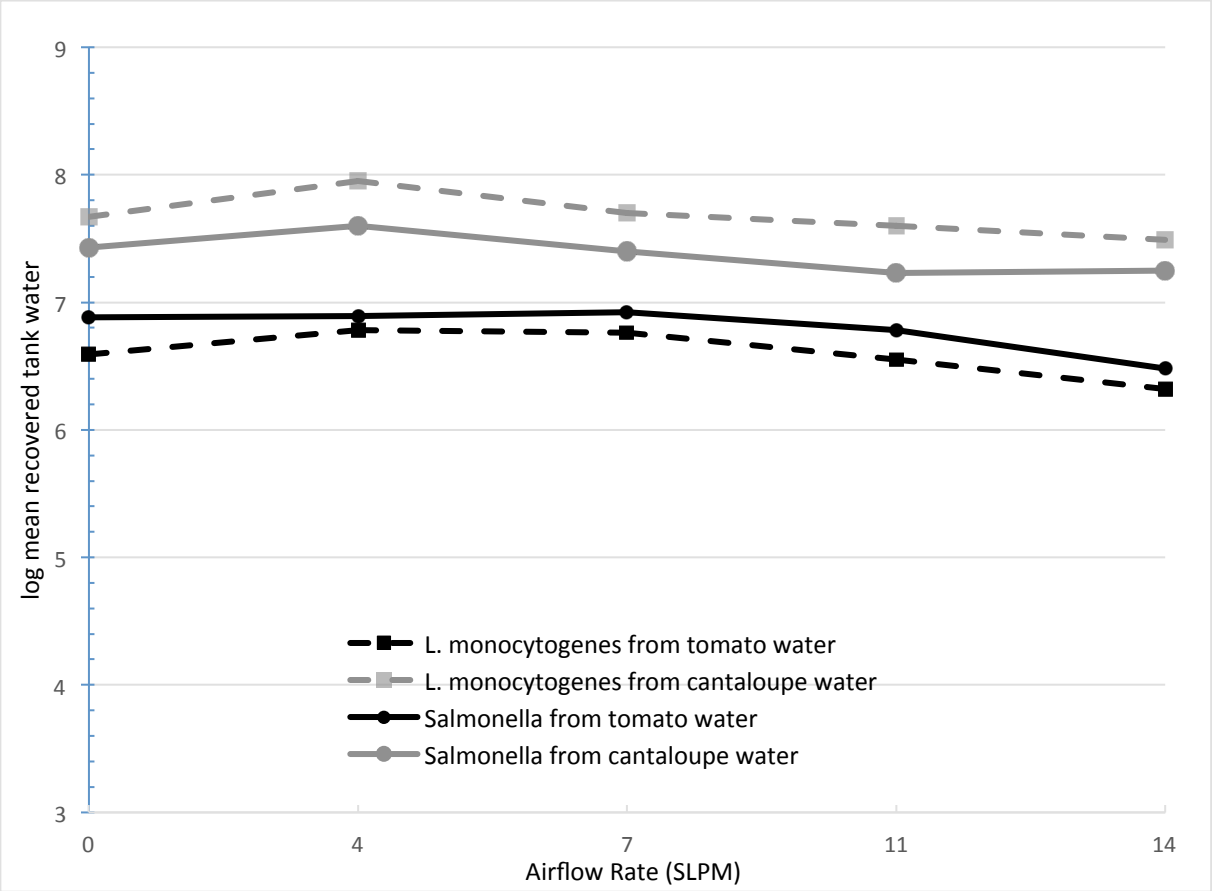


Figure 3. Recovery of *L. monocytogenes* and *Salmonella* Newport from tank water post-treatment for each fruit. (n=6)

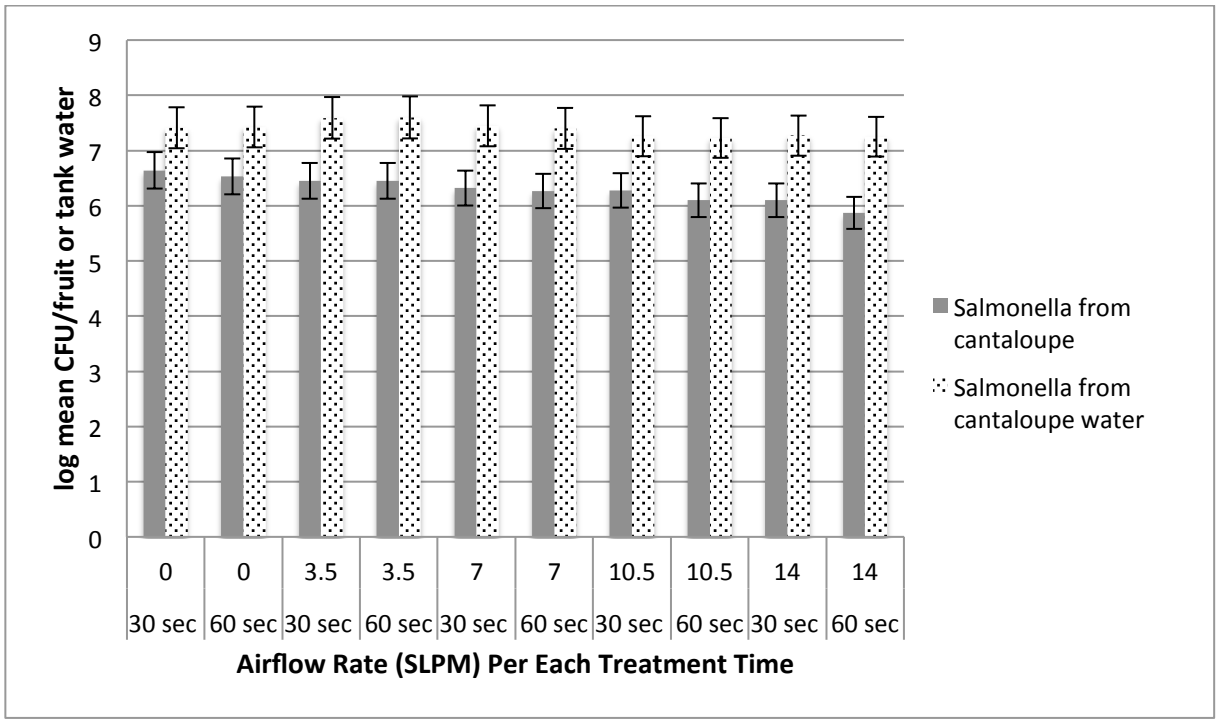


Figure 4. Recovery of *Salmonella* from the surface of cantaloupes and recovered from the water post-treatment, as a function of treatment time. (n=6)

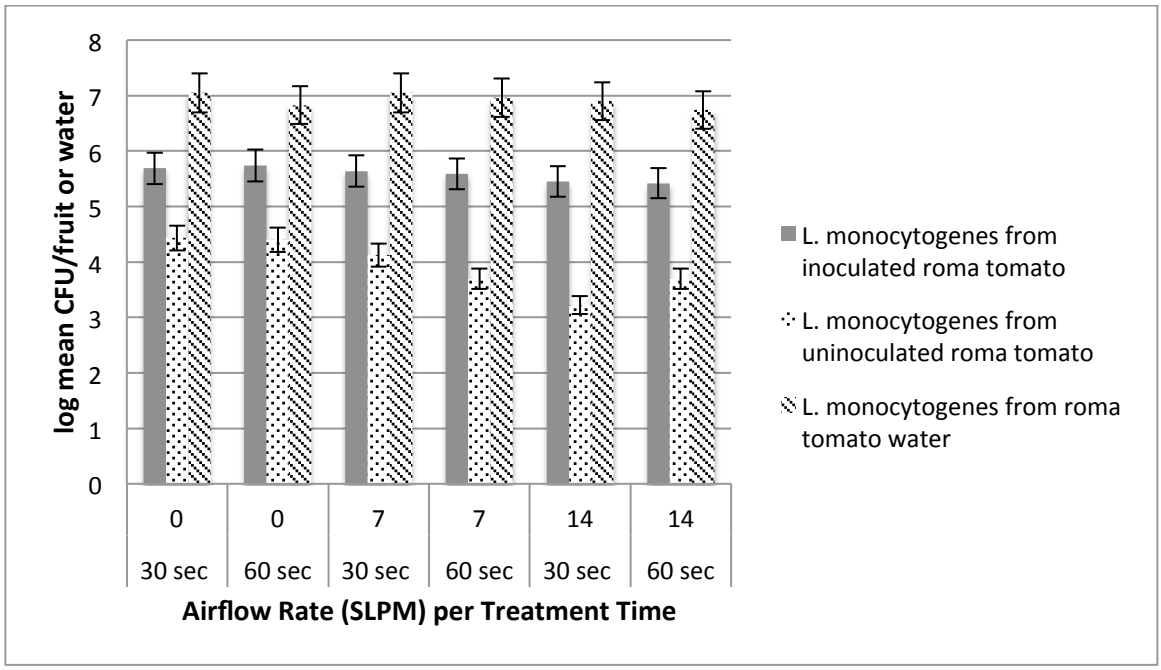


Figure 5. Recovery of *L. monocytogenes* from inoculated and uninoculated Roma tomatoes, as a function of treatment time. (n=6).

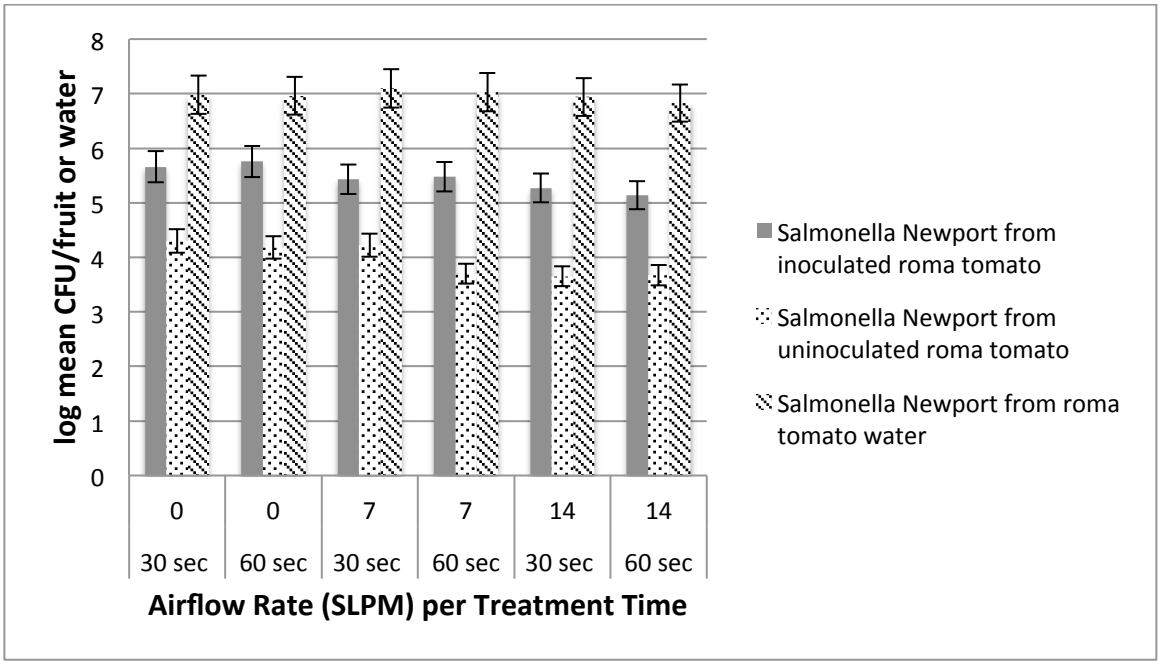


Figure 6. Recovery of *Salmonella* Newport from inoculated and uninoculated Roma tomatoes, as a function of treatment time. (n=6).

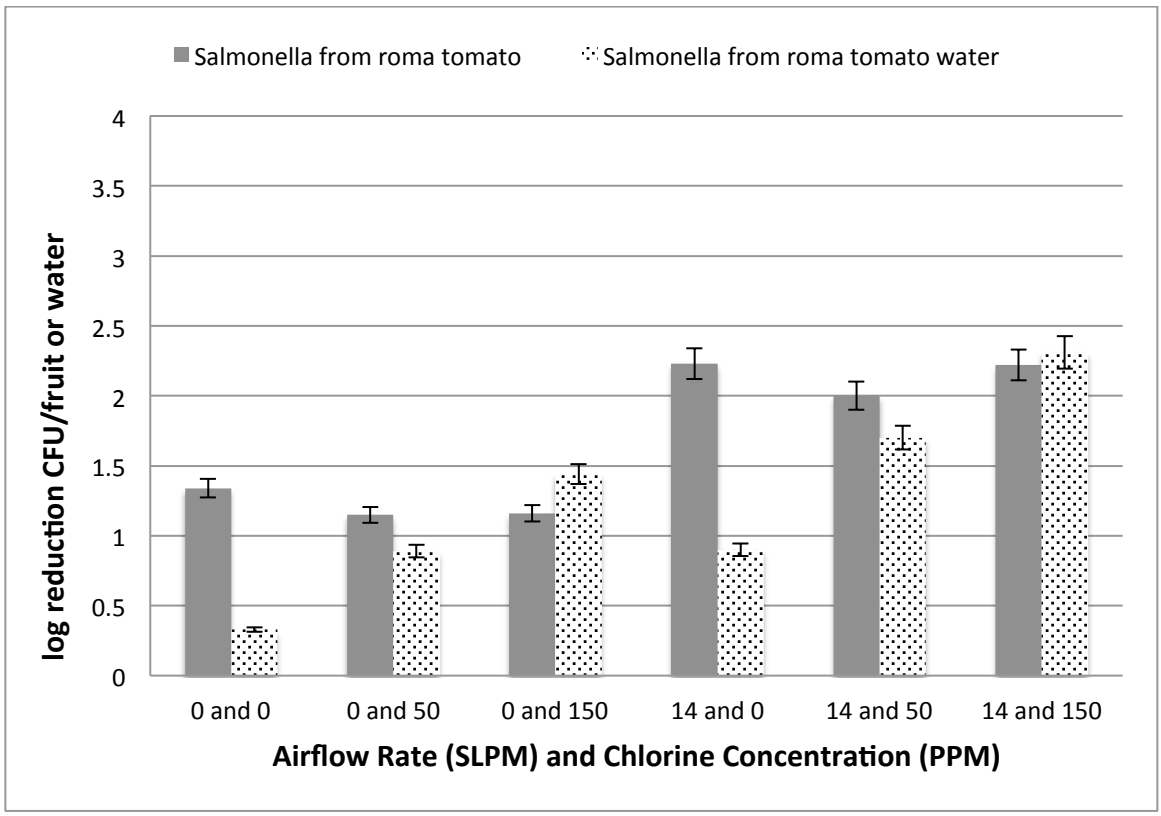


Figure 7. Log reduction of *Salmonella* on Roma tomatoes as a function of airflow rate and chlorine concentration. (n=6)

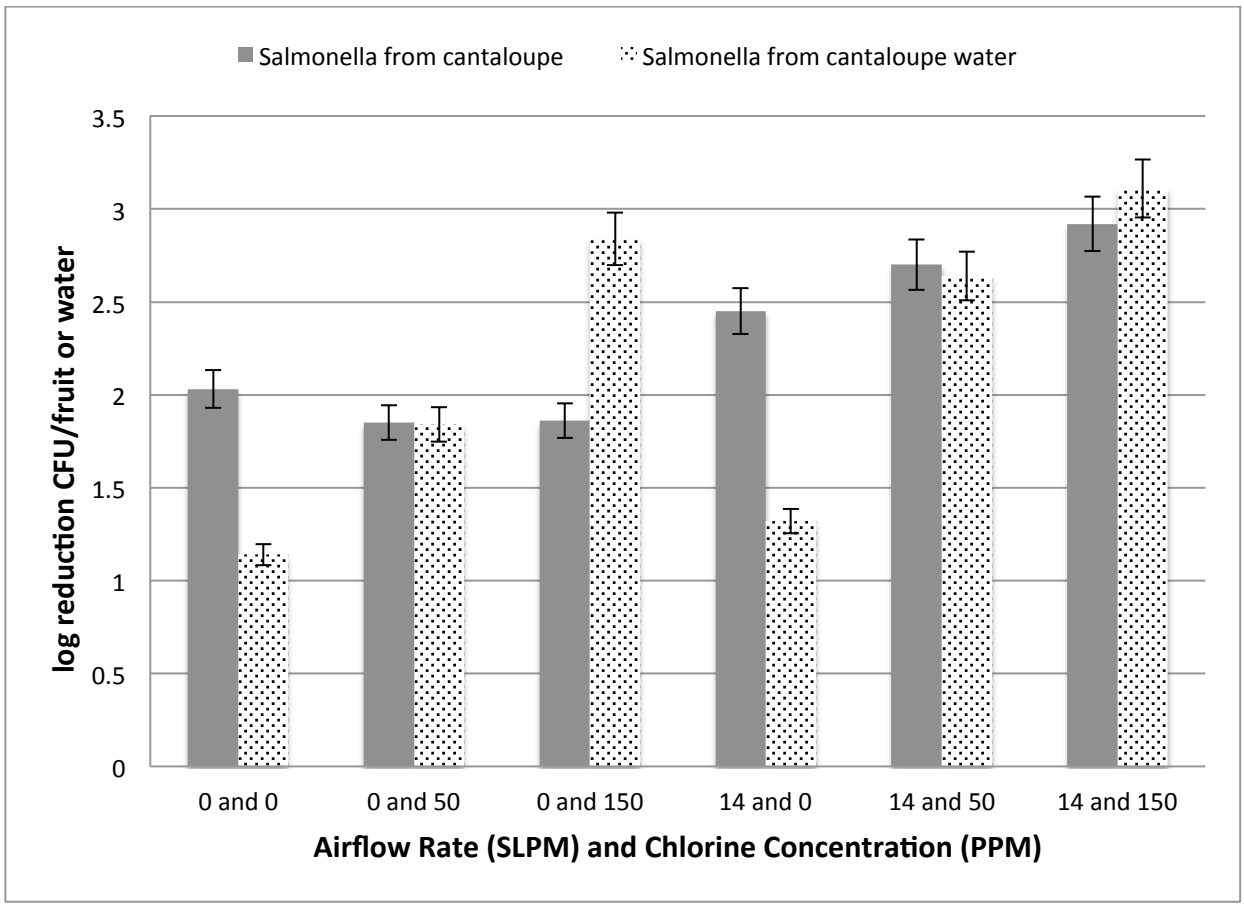
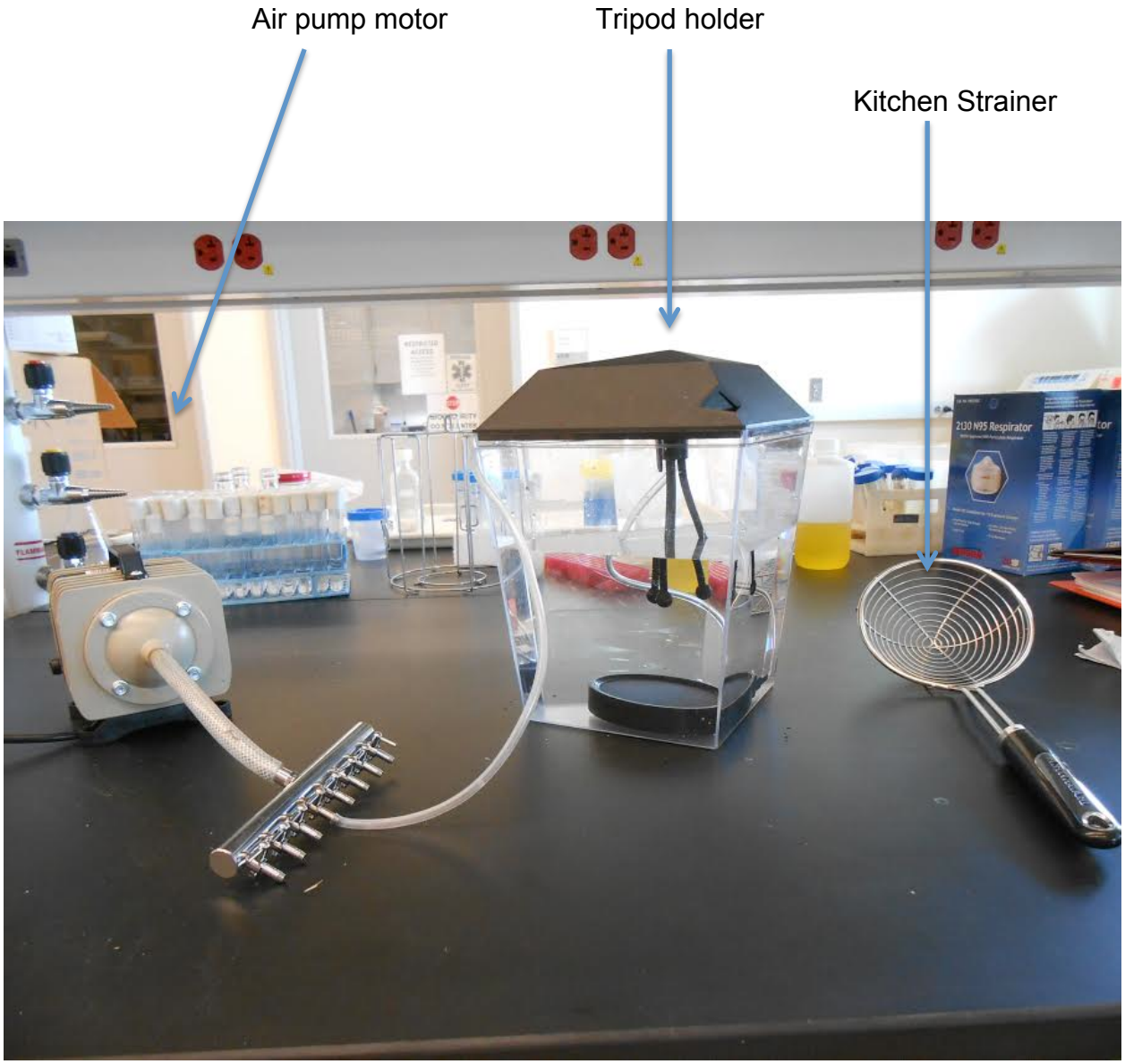


Figure 8. Log reduction of *Salmonella* on cantaloupes as a function of airflow rate and chlorine concentration. (n=6)

IX. Appendix

Appendix Figure A. Example of Procedure Material



Appendix Figure B. *Salmonella* Newport Recovery Post-Treatment in Water

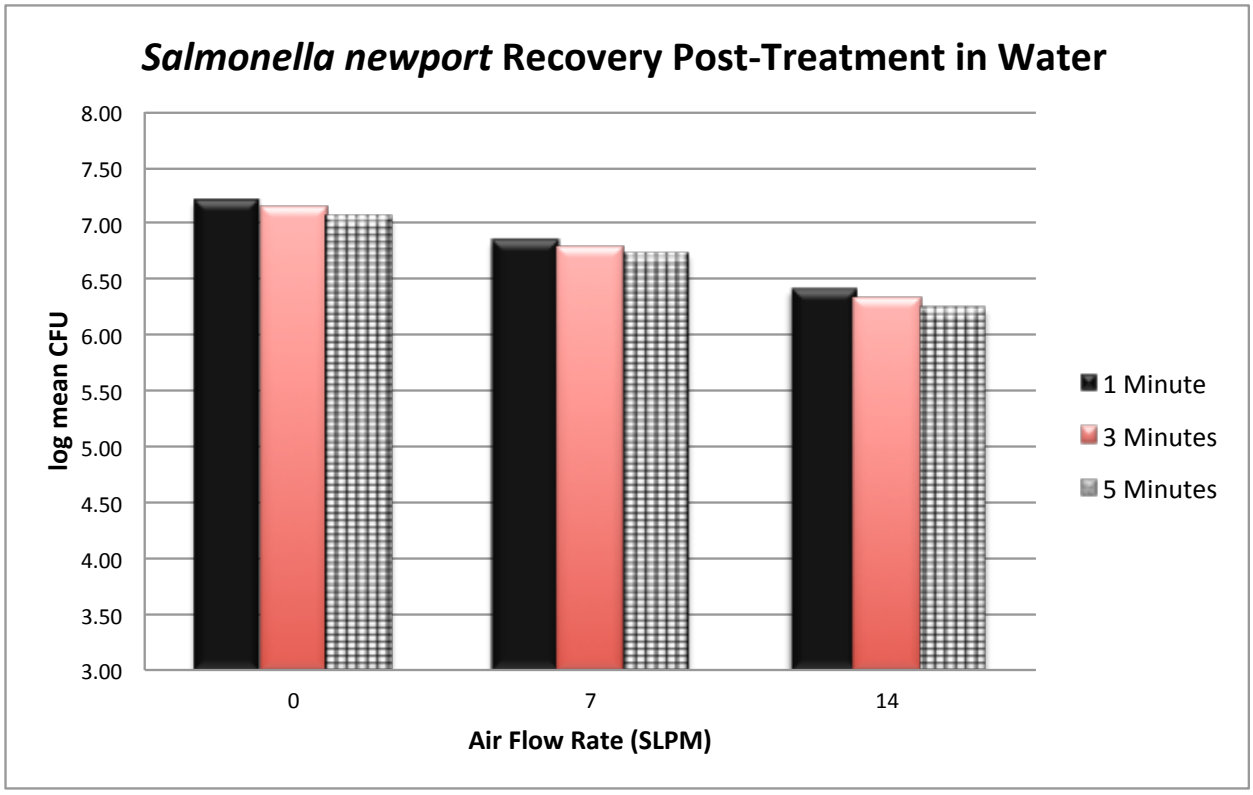


Figure B. Recovery of *Salmonella* Newport after treatment in water at three separate times and three separate airflow rates. (n=6)

Appendix C. Experimental Design Summary

Objective 1: Evaluation of cavitating bubbles for detachment and inactivation of pathogens on produce surfaces.

- Produce (2): Roma tomatoes and cantaloupe
- Pathogens (2): *Listeria monocytogenes* and *Salmonella* Newport
- Air flow for bubbles (5): 0, 3.5, 7, 10.5 and 14 SLPM.
- Treatment times (2): 30 and 60 seconds.
- Replicates (3): one fruit per replicate
- Total samples (fruit) = 120
- Analyses: quantitative recovery of pathogens from fruit and tank water

Objective 2: Evaluation of cross-contamination from inoculated to uninoculated Roma tomatoes treated with cavitating bubbles.

- Produce (1): Roma tomatoes
- Pathogens (2): *Listeria monocytogenes* and *Salmonella* Newport
- Air flow for bubbles (3): 0, 7, and 14 SLPM.
- Treatment times (2): 30 and 60 seconds.
- Replicates (3): two fruit per replicate (only one is inoculated)
- Total samples = 36
- Analyses: quantitative recovery of pathogens from each fruit separately (inoculated and uninoculated) per sample and from treatment chamber water

Objective 3: Evaluation of a chlorine sanitizer solution, with and without cavitation bubbles, for the removal or inactivation of *Salmonella* Newport from produce surfaces.

- Produce (2): Roma tomatoes and cantaloupe
- Pathogens (1): *Salmonella* Newport
- Air flow for bubbles (2): 0, and 14 SLPM.
- Chlorine concentration in water tank (3): 0, 100 or 200 ppm NaOCl
- Treatment times (2): 30 and 60 seconds.
- Replicates (3): one fruit per replicate
- Total samples (fruit) = 72
- Analyses: quantitative recovery of pathogens from fruit and tank water

Appendix Figure D. Recovery of *Salmonella* Newport from Roma Tomatoes

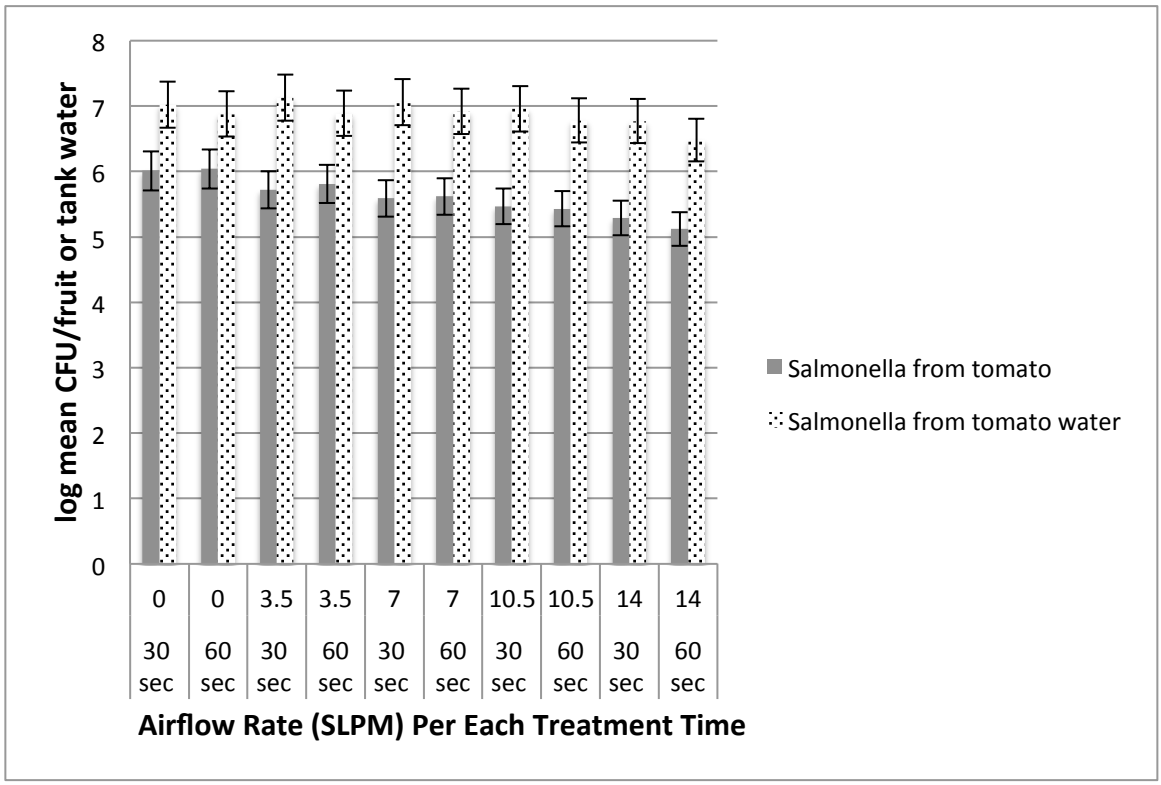


Figure D. Recovery of *Salmonella* Newport from the surface of Roma tomatoes and recovered from the water post-treatment, as a function of time. (n=6)

Appendix Figure E. Recovery of *L. monocytogenes* from Cantaloupe

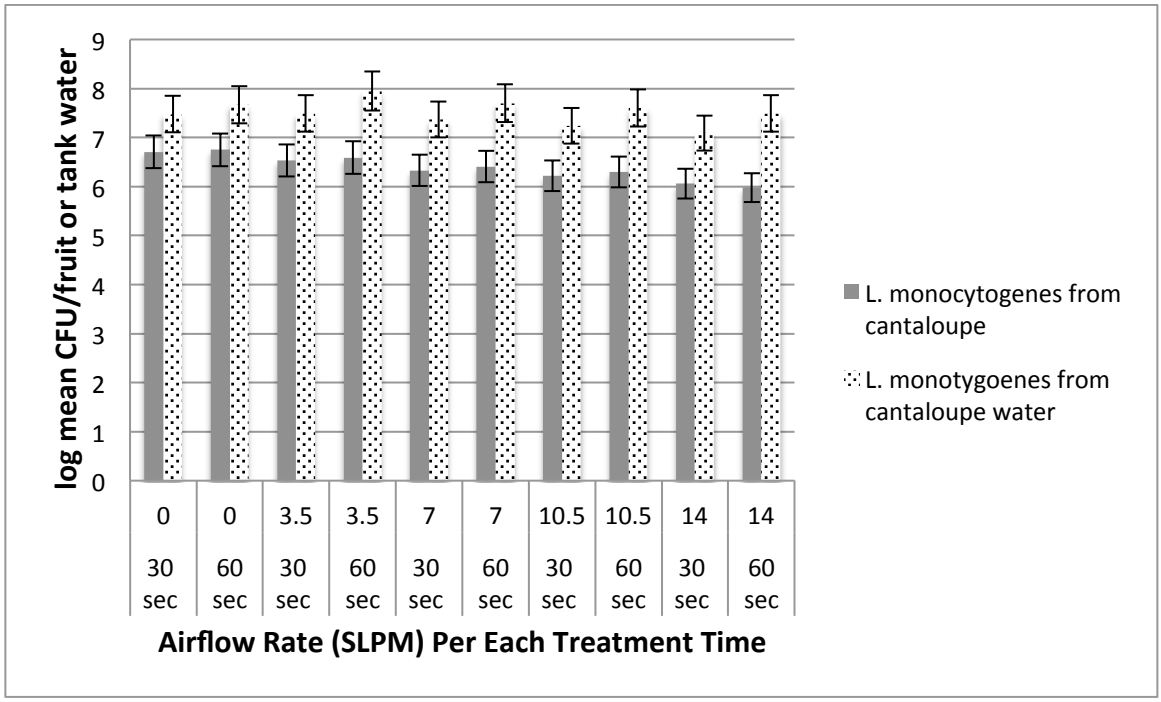


Figure E. Recovery of *L. monocytogenes* from the surface of cantaloupe and recovered from the water post-treatment, as a function of time. (n=6)