Microbubbles remove *Listeria monocytogenes* from the surface of stainless steel, cucumber, and avocado

Pengyu Chen

Thesis submitted to the faculty of Virginia Polytechnic Institute and States in partial fulfillment of the requirement for the degree of

Master of Science

In

Food Science and Technology

Joseph D. Eifert, Chair

Sunghwan Jung

Laura K. Strawn

December 2021

Blacksburg Virginia

Keywords: Foodborne illness, *Listeria monocytogenes,* Listeriosis, Stainless steel, Cucumber, Avocado, Cavitation bubbles, Cross-contamination

Abstract

Fresh produce may be contaminated by bacterial pathogens including *Listeria monocytogenes* during harvesting, packaging, or transporting. Consumers may be at risk of foodborne illness if produce become contaminated. In this project, a cavitation process (formation of bubbles in water) was studied to determine the efficacy of microbubbles at inactivating the pathogen *L. monocytogenes* on stainless steel and the surface of fresh cucumber and avocado. Stainless steel coupons (1”×2’’), cucumber, and avocado surfaces were inoculated with *L. monocytogenes* (LCDC strain). After 1, 24 or 48 h, loosely attached cells were washed off, and inoculated areas were targeted by microbubbles (~0.5 mm dia.) through an air stone (1.0 L air/min) for 1, 2, 5, or 10 min. After treatment, samples were transferred to sterile containers and serial diluted in peptone water and plated on Oxford agar. Plates were incubated for 48 h at 35℃. For stainless steel, the mean log reduction of *L. monocytogenes* (48 h drying) peaked at 2.95 after 10 min of microbubbles when compared to a no bubble treatment. After 48 h pathogen drying, cucumbers treated for 10 min resulted in a 1.78 mean log reduction of *L. monocytogenes*. For avocados, the mean log reduction of *L. monocytogenes* (24 hr drying) peaked at 1.65 after 10 min of microbubbles. This cavitation treatment (10 mins) reduced over 95% of *L. monocytogenes* on the surface of stainless steel, cucumber, and avocado. Microbubble applications may be an effective, economical, and environmental-friendly way to remove *L. monocytogenes* and possibly other bacterial pathogens from food impact surfaces and the surface of whole, intact fresh produce.

Public Abstract

Fresh produce may be contaminated by bacterial pathogens like *Listeria monocytogenes* during the process of harvesting, packaging, or transport. Consuming contaminated fresh produce without enough and proper decontaminated measures, consumers are in risk of being infected with foodborne illness. A cavitation process (formation of bubbles in water) was conducted to determine the efficacy of microbubbles at inactivating the pathogen *Listeria monocytogenes* on the impact surface of fresh produce (stainless steel), fresh cucumber, and fresh avocado.

Stainless steel coupons (1”×2’’), fresh cucumbers, and fresh avocados were inoculated respectively with *Listeria monocytogenes* (LCDC strain). After removing loosely attached pathogen cells on the surface of inoculated steel coupons, cucumbers, and avocados, their inoculated areas were targeted by microbubbles (~0.5 mm dia.) for 1, 2, or 10 minutes, with a constant air flow rate of 1.0 L/min through an air stone.

The *L. monocytogenes* on stainless steel and cucumber, and avocado surfaces was reduced by 95% to 99%. This study suggests that microbubbles may remove, and possibly inactivate, *L. monocytogenes* effectively from the surface of fresh produce and their impact surfaces. Microbubbles thus could be an effective, economical, and environmental-friendly tool for minimizing produce contamination.

Acknowledgements

First and foremost, I want to appreciate my advisor, Dr. Joseph Eifert for all the help in my undergraduate and graduate career. You gave me valuable opportunities, advice, patience, and everything that I needed in the past 4 years. You always allowed me work at my own pace in the lab and writings, and always gave me many constructive suggestions for my study. You were always willing to spend your own time helping me and advising me in all aspects of my education at Virginia Tech. You offered me a chance to the graduate school and gave me impressive and excellent topics to work on. Thank you so much for everything.

I also want to express my sincere gratitude to my committee members, Dr. Sunny Jung and Dr. Laura Strawn, for their great help and support. You both gave me very valuable suggestions and support for my research study. Thank you very much for making my research study surprisingly productive and enjoyable.

I would thank my undergraduate lab partner, Haofu Li for every effort you made to help me in my research study in the lab. Thank you very much for spending much your own valuable time in the lab with me. You made my research study more effective and meaningful.

Lastly, I would like to thank every member of the Food Science department at Virginia Tech, especially Kim Waterman, who gave me so much help in the lab. I could not have asked for better faculties, staff, classmates, and roommates in my life. I will treasure these wonderful memories forever.

Table of Contents

List of Tables and Figures

I. Introduction:

Fresh produce is a significant part of a healthy diet. However, foodborne illness outbreaks have occurred frequently and been recognized increasingly among all the world nowadays. This reflects a growing concern of safety and hygiene problems of fresh produce. A large number of foodborne outbreaks derive from fresh fruits and vegetables. There is a report that the proportion of foodborne outbreaks that relate to fresh fruits and vegetables increased from <1% in the1970s to 6% in the 1990s in the United States (Lynch et al., 2009).

An important bacterial pathogen that can contaminate fresh produce is *Listeria monocytogenes*, which can lead to listeriosis. Unfortunately, there are about 1600 people getting listeriosis each year based on the estimated report from the U.S. Centers for Disease Control and Prevention (CDC). The hospitalization rate of listeriosis can reach up to 94%. Also, listeriosis results in about 260 deaths every year. Pregnant women and newborns are high-risk groups for listeriosis. (FDA, 2020). Economically, the annual cost of *L. monocytogenes* control can reach up to approximately 2.4 billion US dollars (Ivanek, 2004).

L. monocytogenes can be found on many fresh fruits and vegetables. Also, the U.S. Food and Drug Administration states that *L. monocytogenes* has been found in raw, unpasteurized milk and cheeses, ice cream, raw or processed vegetables, raw or processed fruits, raw or undercooked poultry, hotdogs, sausages, deli meats, and raw or smoked fish and even in raw pet food (FDA, 2019). Additionally, *L. monocytogene*s has been recovered in water and soil. Fresh produce like raw vegetables can be contaminated by *L. monocytogenes* from manure used as soil amendments. Meats and

dairy products can also contain *L. monocytogenes* because animals may carry the organism without appearing ill or environmental contamination can occur during meat processing (USDA, 2019).

To reduce potential *L. monocytogenes* contaminations, the U.S. Food and Drug Administration advises that all fruits and vegetables should be washed under running water just before eating, cutting, cooking and peeling. For firm produce like melons and cucumbers, consumers may need to scrub the surfaces using a clean brush. In addition to using water to remove *L. monocytogenes*, the FDA recommends keeping refrigerated foods under 40°F or less to reduce risk of *Listeria* infection by slowing down or halting the growth of *L. monocytogenes*. Other prevention measures are cleaning refrigerators frequently to remove food spills which may harbor *L. monocytogenes*, and washing hands and kitchen surfaces regularly to avoid cross-contamination of *L. monocytogenes* among different food impact surfaces, such as stainless steel surfaces used by food processing industries.

During the past three decades, cavitation has become a more common treatment methodology to remove bacteria from object surfaces. The definition of cavitation is the formation of vapor cavities, such as bubbles in a liquid, caused by forces acting on the liquid. Cavitation will occur, when a rapid change of pressure acts on a liquid to form cavities (Lee, 2018). Historically, the word “cavitation” was first found in English scientific literature in the late 19th century. Researchers carried out many experiments to study the physical aspects of the cavitation phenomenon and examine the effects of cavitation on industrial systems. The common approaches used to research cavitation were theoretical and numerical approaches at that time and equations have been

developed to describe the dynamics of bubbles in a liquid under a variety of conditions. Prior scientists to study bubble dynamics are Rayleigh (1917), Lamb (1923), Cole (1948), Blake (1949), Plesset (1949). It was not difficult for them to study bubble dynamics, because the spherical shape of bubbles is simple in both theoretical and experimental ways (Franc and Michel, 2010).

Cavitation and bubble dynamics have a very wide range of practical application in many fields, that include hydraulic, mechanical and naval engineering, oil exploration, clinical medicine, sonochemistry, and ultrasonic cleaning for electrical and medical microdevices (Blake et al., 2015). For example, a new rotation generator that can induce super cavitation works economically, and is more effective than conventional methods to produce cavitation bubbles that can effectively remove the bacteria *Legionella pneumophila*, *Escherichia coli*, and *Bacillus subtilis* (Šarc, 2018).

The application of physical cavitation bubbles was found to remove and inactivate *Listeria* on the surface of fresh Roma tomatoes and cantaloupes. Lee et al., (2018) reported that *Listeria* on the surface of fresh Roma tomatoes and cantaloupe was detached and inactivated using a physical continuous bubble stream. The efficacy of the cavitation bubble stream per min can be up to a 2.89 and 2.63 log pathogen reduction for Roma tomatoes and cantaloupe, respectively.

In this research reported here, cavitation bubbles were applied to the surface of stainless steel, cucumber, and avocado to determine their effectiveness for removing loosely attached and firmly attached (biofilm) cells of *Listeria monocytogenes*. Moreover, cross-contamination from inoculate to uninoculated cucumbers and avocados was tested to determine likelihood of cross-contamination. Finally, a quality

evaluation was performed on treated cucumber and avocado after bubble treatment to ensure consumer acceptability.

II. Literature Review

1. Listeriosis

Listeriosis is a foodborne illness caused by *Listeria monocytogenes* and it can lead to stillbirths and meningoencephalitis in a broad range of animals in the environment. It was confirmed that *L. monocytogenes* in food causes human disease after investigations of some large epidemics of listeriosis during the 1980s. The susceptible human groups for listeriosis are pregnant females, newborns, and elders (McLauchlin, 1990). High risked human groups also include people with weakened immune systems. There is a very low possibility for other group of people infected with listeriosis to develop a severe illness (CDC, 2016).

To diagnose listeriosis effectively, when *L. monocytogenes* is cultured from blood, spinal fluid, or some normally sterile site, listeriosis can be diagnosed. Listeriosis can be diagnosed if isolation of *Listeria spp.* from nonsterile sites, for example, placenta and amniotic fluid, occurs with clinical symptoms. It is significant that *L. monocytogenes* can be inhibited by a variety of antibiotics, such as penicillin G, ampicillin, erythromycin, trimethoprim–sulfamethoxazole, chloramphenicol, rifampin, tetracyclines, and aminoglycosides. Among these antibiotics, ampicillin and penicillin are both recommended to treat invasive listeriosis. And *L. monocytogenes* is more susceptible to ampicillin than to penicillin. Additionally, the combination of an aminoglycoside and a beta-lactam antibiotic produces synergy in vitro can also be a treatment for listeriosis. For patients who are allergic to penicillin, trimethoprim and sulfamethoxazole can be a therapy instead. There is not a standardized therapy duration for treating listeriosis. The recommendation is that therapy duration of uncomplicated sepsis or meningitis is two

weeks, therapy duration of endocarditis or nonmeningitic disease in the immunocompromised host is 4 to 6 weeks (McLauchlin, 1990). It is also important to avoid infecting with listeriosis. The recommendations and guidelines are still developing. Some high-risk group like pregnant females, immunosuppressed adults, elders, and newborns should not eat unpasteurized milk products, not well-cooked meat, and raw eggs in some human groups. Moreover, prevention of cross-contamination between raw food and cooked food is necessary. Cooking guidelines should be well developed for consumers to reduce the possibility of infection with listeriosis from contaminated food products (McLauchlin, 1990).

2. *Listeria monocytogenes*

Listeria monocytogenes is a gram-positive intracellular bacterium which has commonly caused several outbreaks of foodborne diseases since 1970s. And the foodborne illness that attributes to *L. monocytogenes* is named as listeriosis. *L. monocytogenes,* as an organism, has a unique virulence system with multifactor, including the thiol-activated hemolysin, listeriolysin O. This character and ability are vital for multiplication of *L. monocytogenes* in host phagocytic cells and spreading among cells (Farber, 1991). Moreover, the sophisticated relationship between the *L. monocytogenes* and its host cells has been revealed, and the invasive and replicative capacities of *L. monocytogenes* acting in various cell types have been studied. The pathogen *L. monocytogenes* can be found in a variety of environments, including soil, water, various food products, humans, and animals (Hamon, 2006).

L. monocytogenes can be found in different area, such as soil, water, decaying vegetation and animals, which contain high level of moisture. This pathogen can survive

strong and still grow under refrigerating temperature and other food preservation methods. Moreover, *L. monocytogenes* can be transmitted easily during the process of harvesting, preparing, packaging, transportation and storage in the environment. Raw produces, water, soil, and air can be their vehicle to transmit the contamination of *L. monocytogenes* (FDA, n.d.).

3. Foodborne illness associated with fresh produce

Fresh produce products take up around 46% of foodborne illness, 38% of hospitalization due to food poisoning associated with pathogens, and 23% of death due to pathogens in food in the United States. *Salmonella* and *L. monocytogenes* both account for most food poisoning cases each year. 459 illness cases due to *Salmonella* outbreak, related to tomatoes, occurred in more than 20 states in the United States in 2005, and 1,500 illness cases due to *Salmonella* outbreak, related to jalapeno peppers in United States and Canada. In 2011, a *L. monocytogenes* outbreak, associated with cantaloupe, ended up with 147 people sickened and 33 people dead. Therefore, both *Salmonella* and *L. monocytogenes* can contaminate the fresh produce products and risk the health of consumers during the process from pre-harvest step to consumer's table (Strawn et al., 2013).

L. monocytogenes can be found in fresh produce. Several severe *Listeria monocytogenes* outbreaks related to fresh produce have occurred during the last decades among the world. In 1977, an outbreak of *Listeria* attributing to contaminated canned corn happened in two elementary schools and one college in Italy, which caused 2930 people, who were from elementary schools and colleges. An outbreak of

listeriosis associated with chopped celery was reported in the state of Texas in 2010. As a result, 10 patients got hospitalized and 5 patients passed away in next three months. In 2011, another listeriosis break attacked 28 different states in the United States attributing to the consumption of contaminated melons. During this listeriosis break, 147 people were infected and 33 of them passed away. Scientists used pulsed-field gel electrophoresis (PFGE) to analyze the *L. monocytogenes* from samples of patients' blood and from shredded parts of cantaloupes, and consequently found the subtypes of both samples match each other. In the same year, Romaine lettuce was contaminated by *L. monocytogenes* and caused another serious outbreak in 19 states in the United States. The samples of romaine lettuce in California was tested by the Food and Drug Administration (FDA) and was shown to be positive for *L. monocytogenes*. Consequently, industries and government recalled a large amount of processed and packaged lettuce (Zhu et al., 2017). Therefore, preventing *L. monocytogenes* is important to protect the health of consumers.

4. *L. monocytogenes* association with cucumber or avocado

The prevalence of *L. monocytogenes* in cucumber is around 21.9% in fresh produce (Zhu et al., 2017). Whole and sliced cucumbers can be contaminated by *L. monocytogenes* at different temperatures during storage. A research study found that *L. monocytogenes* was able to grow on both whole and sliced cucumbers at various environment temperatures, including 23 ± 2, 4 ± 2, and −18 ± 2°C for different storage days. The results show that the populations of *L. monocytogenes* are not significantly different on both whole and sliced cucumbers for 5 hours at 4 ± 2°C, but increased significantly at 23 ± 2°C instead (2.3 log CFU per cucumber on whole cucumbers, and

1.7 log CFU per cucumber on sliced cucumbers). For 21 days, *L. monocytogenes* grows and increases significantly on both whole (2.8 log CFU per cucumber) and sliced cucumbers (2.9 log CFU per cucumber) at 4 ± 2°C. On frozen whole and sliced cucumbers, the *L. monocytogenes* can survive for more than 120 d. Thus, it is necessary to evaluate cucumber conditions at each step of the supply chain to reduce potential *Listeria* contamination (Bardsley, 2019).

Although there has not been any incident of *L. monocytogenes* on fresh whole avocados reported in recent years, avocados have become a new concern for contamination of *Listeria monocytogenes* today. *L. monocytogenes* can enter the avocado pulp from the stem or stem scar. When exposed to the hydrocooled water with *Listeria monocytogenes*, the pathogen inside the edible parts of avocados can range high from 5.90 to 7.19 log CFU/g due to the bacteria transmission from the bottom end to the inside parts of fresh whole avocados (Chen et al., 2016). Different temperatures storage treatments have been given to avocado pulp (AP) and processed guacamole (PG) to study the growth rates of *L. monocytogenes* under different conditions. After 24- and 48-h storage at 22°C, cell counts for *L. monocytogenes* increased from 2 to 6 and 9 log CFU/g respectively for avocado pulp (AP). And, the growth rate of *L. monocytogenes* on AP decreased significantly at 4 to 7°C for both 24- and 48-hours storage time. Distinctively, processed guacamole did not show significant increased growth rates for *L. monocytogenes* at 22°C for 48 h and at 4 to 7°C for 15 d. The potential reason for bacteriostatic effect in the processed guacamole might be inclusion of citric acid and disodium dihydrogen pyrophosphate into the PG products. Although the growth rate of *Listeria monocytogenes* on avocado pulp and processed guacamole

can be controlled and inhibited under refrigeration temperature, this pathogen can still survive more than 58 weeks at -18°C on avocado fruit (Iturriaga, 2002).

5. Control of pathogens on fresh produce and food impact surfaces

5.1. Cleaning of fresh produce

Using wash water disinfectants can effectively avoid the transfer of pathogens from contaminated fresh produce products to clean fresh produces. The disinfectants that can be used during the process of fresh produce washing include chlorine, ClO_2, O_3, and PAA. All these disinfectants have different efficacy and limitations of use when being applied as washing chemicals. It is significant to optimize the amount of using different disinfectants to clean fresh produce, since negative impacts of using these disinfectants on environment, such as water contamination, cross-contamination, are undesirable (Banach et al., 2015).

Chlorine is applied to wash and clean fresh produce due to its bactericidal capacity and economical cost. When chlorine contacts wash water during washing, it yields an oxidizer, HOCI, which can inactivate pathogens effectively (Banach et al., 2015). Commonly, the dosage of commercial chlorine use in industry is around 50 to 200 mg/L. 100mg/L is the most common dosage used in industry (Baur *et al.*, 2004). And the application of this certain dosage of chlorine requires a short impact time, which is about 1 to 2 minutes, and a certain pH value range from 6.0 to 7.5 to sustain the stabilization of HOCI as a mean to prevent chemicals from corroding the processing equipment. However, when stabilizing the HOCI form during a washing process, the organic load with components of soil, exudates, and debris will rise lead to

contaminating the clean water and water tanks. To minimize the possibility of cross-contamination during washing with chlorine, the chlorine level in fresh produce has to be optimized to maintain water quality. Scientists are investigating the use of chemical combinations to increase the safety level of chlorine use. T128 is determined that can help stabilize HOCI when contacting wash water and kill the pathogen *E. coli* from the wash water. T128 can inhibit cross-contaminations as well. Therefore, T128 may help chlorine application maintain a safer level in washing fresh produce (Banach et al., 2015).

Chlorine dioxide (ClO_2) can be used as disinfectant for washing fresh produce in the form of gas or in the form of dissolved solution. It has been shown that chlorine dioxide can inactivate a wide range of microorganisms effectively, including bacteria spores, amoebal cysts, Giardia cysts, *Cryptosporidium*, *Mycobacterium tuberculosis*, *Legionella*, *E. coli*, *Salmonella*, and *Listeria*. ClO_2 acts to inhibit metabolism of the cell membrane of microorganisms against its permeable capacity. Moreover, it is determined that ClO_2 can suppress the re-growth of biofilm formations efficiently. Since ClO_2 is highly oxidative and penetrable, it can effectively work on disinfecting at a broad range of pH value. When the pH value of ClO_2 rises, the ClO_2 disinfectant is able to inactivate more microorganisms. Gaseous ClO_2 is more penetrable into microorganisms than aqueous ClO_2, which shows more efficiency in inactivating microorganisms. Moreover, unlike, aqueous ClO_2, gaseous ClO_2 has less impact on increasing spoilage micro flora in the food produce. Thus, compared with aqueous ClO_2, gaseous ClO_2 can extend the storage time of food produce. However, the efficiency of gaseous ClO_2 is not stable when its concentration and applied time vary. One of the significant limitations of

gaseous ClO_2 as disinfectant is date. In addition, gaseous ClO_2 can potentially harm the health of workers. Thus, it is necessary to control and monitor the concentration level of ClO_2 in the workplace to ensure safety of workers (Banach et al.,2015).

Besides gaseous form of ClO_2, aqueous ClO_2 is applied to disinfect fresh produce. In 2004, Researchers compared the efficiency of different chemical sanitizers (aqueous form) in the inactivation of *E. coli* O157:H7 and *Listeria monocytogenes* on fresh produces including lettuce, apples, cantaloupes, and strawberries. They found that certain amount of aqueous ClO_2 (3ppm or 5 ppm) can effectively decrease the number of *E. coli* O157:H7 and *Listeria monocytogenes* on the fresh produce from detected to nondetectable levels. Moreover, Rodgers et al. (2004) showed that both *E. coli* O157:H7 and *Listeria monocytogenes* can be inactivated over 5 logs when exposed to their applied aqueous chemical sanitizers, including ozone, ClO_2, chlorine, and PAA, for 2 to 5 minutes exposure time. The result shows that ClO_2 is the second most effective disinfectant to inactivate both pathogens to over 5 log reductions, which only take 19 to 21 seconds exposure time (Rodgers et al., 2004). Van Haute et al. (2017) has compared the efficiency of aqueous ClO_2 and chlorine as disinfectants on washing fresh-cut lettuce. The scientists detect the number of log reduction of molds and *E. coli* before and after applying aqueous 4 to 10 mg/L of ClO_2 and 40 to 70 mg/L of free chlorine on washing fresh-cut lettuce. In their study, the applied dose of free chlorine cannot successfully decrease the detected number of molds to undetectable levels in 2 minutes' exposure time. The applied dose of ClO_2 can inactivate molds for over 3 log reduction for 2 minutes and can significantly decrease the number of *E. coli* to undetectable levels (>5 log reduction) in 3 minutes' exposure time. Aqueous ClO_2 is

determined to reduce more molds and *E. coli* than free chlorine does, yet approximately 77% of the aqueous ClO_2 is reduced to ClO_2^- and chlorate is generated during washing process of fresh-cut lettuce. In the future, the levels of residues of ClO_2^- and chlorate on the fresh-cut lettuce are required to be in a certain range to ensure safety of aqueous ClO_2 disinfectant (Van Haute et al, 2017). In addition, Banach et al., (2015) points out the limitation of industrial application of aqueous ClO_2. The dissolved ClO_2 in wash water of fresh produce can have some residuals in the washing tank to re-contaminate the fresh produce while washing, as a result of the decrease of efficiency.

Ozonation can be an alternative of chlorine as an effective anti-microbial method in washing fresh produce. It is determined that ozonation can be a clean way to sanitize the fresh produce to maintain the safety and conserve the water used. A study conducted in 2012 applies an ozone washing system on cleaning *Bacillus subtilis* on fresh lettuce for 10 minutes to measure the efficiency and compare with chlorination. In the study, scientists conducted the ozone washing system to clean the lettuce with inoculated *Bacillus subtilis* for 10 minutes and then measured the *Bacillus subtilis* spores left in the water used in the experiment. Then, the concentration of ozone was measured before and after each treatment and water quality was measured as well. At last, chlorine treatment was done with same conditions as ozonation treatment to compare. The results show that both ozonation and chlorine treatments can effectively inactivate *Bacillus subtilis* on fresh lettuce, and their log reductions are similar (0.95 to 2.08 log CFU for ozone, and 0.86 to 1.61 log CFU for chlorine). However, the ozone concentration remains stable when repeating ozone treatments up to 5 repetitions; the chlorine concentration decreases about 10% over 5 repetitions of treatments. Moreover,

the quality of wastewater in the ozone experiment is much better than the condition of wastewater used in the chlorine treatment. Therefore, ozonation can be an excellent alternative for chlorine as an economical and environmental-friendly disinfectant to be used in industry (Rosenblum et al., 2012)

Ultraviolet light can be applied to clean fresh produce as an alternative to traditional thermal processing. Yaun et al. (2004) reported using ultraviolet energy with a wavelength of 253.7 nm to remove *Salmonella* spp. or *Escherichia coli* on the surface of fresh produce, including red apples, leaf lettuce, and tomatoes. In the result, *Salmonella* on red apples can be reduced up to 3.3 log using UV light energy at 24 mW/cm^2. Moreover, both *Salmonella* (2.65 log reduction) and E. coli (2.79 log reduction) can be inactivated to a similar level. Therefore, it is determined that UV light energy could be an effective measure for cleaning fresh produce by industry (Yaun et al., 2004).

With the increasing concern of food safety, some methodologies have been developed to control the growth of *Listeria monocytogenes* on fresh produce. Ultrasound and essential oils can have some inhibitions on the growth of *Listeria monocytogenes* on fresh produce. The essential oil, including 1% lemon essential oil and 2% cinnamon essential oil, can reduce the pathogen by 1 log for one day. However, the combined effect of both essential oils and ultrasound on inhibiting *Listeria* growth is weakened, because the combined effect can only reduce 0.8 log of the pathogen for one day. Thus, it works better to use essential oils and ultrasound independently as effective and feasible antimicrobial methodologies (Özcan et al., 2015). Additionally, some antimicrobial chemicals are added into the treatment water of fresh produce to kill

and reduce pathogens, including *Listeria monocytogenes*. Chlorine, peracetic acid (PAA), chlorine dioxide (ClO_2), ozone, and electrolyzed oxidizing water (EOW) are some of the most common antimicrobial agents added (Pietrysiak et al., 2019). Microbiologically, the combination of bacteriophages and a bacteriocin can inhibit the growth of *L. monocytogenes* on fresh-cut produce like melon and apple slices. The combination of bacteriophages and nisin (a bacteriocin) can have 5.7 log and 2.3 log maximum *Listeria* reduction on honeydew melon slices and apple slices, respectively. However, the effectiveness of this bacteriophages and bacteriocin treatment is subject to the initial concentration of *L. monocytogenes* on the fresh produce (Leverentz et al., 2003).

5.2. Cleaning of stainless steel:

Besides contaminating the fresh produce, *Listeria monocytogenes* can adhere to food impact surfaces, especially stainless steel, which is the most common material in food industries. *Listeria monocytogenes* will adhere to the stainless steel and can form biofilms on the surface of the stainless steel in food processing plants. Different strains of *L. monocytogenes* have different survival and adhesion time on the impact surface of the stainless steel in food processing plants. Persistent *L. monocytogenes* strains show the highest adherence level on the stainless steel surface at 1- and 2-hour time periods. Persistent stains of this pathogen from both poultry and ice cream plants adhere with higher number of cell counts than all non-persistent strains adhere to the impact surface of stainless steel after short impact time. The study of adhesion of different strains of *Listeria monocytogenes* is significant for cleaning and disinfection of food impact

surface, because high numbers of bacteria adhesion on impact surface will facilitate the cleaning and disinfection procedures (Lundén et al., 2000). The stainless steel surface adhered bacteria, including *Listeria* can be effectively controlled or removed by existing cleaners, including chlorinated alkaline detergent (1.6% and 10%), alkaline detergent (1.6% and 10%), alkaline peroxide (3%), Enzyme Blend, detergent blend + ClO_2, and anionic detergent (1.6%). In most food processing factories, cleaners can be effective applied to remove the attached pathogens on etched stainless steel surfaces (Krysinski et al., 1992).

6. Controlling risk factors of *Listeria monocytogenes* contamination

To reduce the *L. monocytogenes* contamination of fresh produce, some researchers come up with the idea of controlling the environmental risk factors of pathogenic contaminations. The study detects and compares the level of *L. monocytogenes* contamination based on six field management practices, which are manure application, reporting of wildlife, worker activity, irrigation, soil cultivation, and reporting of a buffer zone. The results show that most of these field management practices are associated with the time when they are conducted. The environment that are used manure one year ahead has seven times more *L. monocytogenes* contaminations than the environment without manure. Higher levels of contamination are found in the environment with wildlife observation reported 3 days previously, than the environment without wildlife observation reported for 7 days. Also, more *L. monocytogenes* contamination is detected in fields with more recent cultivated soil and with more recent worker activity, than the fields with less recent practices. Thus, people

can ameliorate the existing practices mentioned above to decrease the possibility of *L. monocytogenes* contaminations in grower's fields (Strawn et al., 2013).

7. Cavitation as an antimicrobial process

Cavitation is the formation, growth and rapid collapse of bubbles in a liquid (water) when exposed to pressure variations. There are two types of cavitation commonly used, which are acoustic cavitation and hydrodynamic cavitation. Acoustic cavitation can be applied to clean water and wastewater for inactivating bacteria and yeast. High frequency (850 kHz) ultrasound can inhibit the growth of bacteria and yeast under controlled temperature conditions. Yeasts were found more resistant to the high frequency ultrasound than some bacteria, including *Enterobacter aerogenes*, *Bacillus subtilis* and *Staphylococcus epidermidis.* Both yeasts and these bacteria are found to be inactivated effectively using high frequency ultrasound (Gao et al., 2014).

Besides acoustic cavitation, hydrodynamic cavitation can also be used to clean wastewater. In wastewater, hydrodynamic cavitation can successfully remove pharmaceuticals, cyanobacteria, algae, *Legionella* and Rotavirus in an energy-efficient way (Dular, 2016). *Listeria* and *Salmonella* on the surface of fresh produce, like raw Roma tomatoes and cantaloupes can be removed and inactivated by hydrodynamic cavitation bubbles. Applying cavitation bubbles at 14 liters/min for 1 min can reduce both *Listeria* and *Salmonella* by 1.2 and 0.9 log for each fresh fruit. Moreover, the combination of this 1 minute, 14 liter/min cavitation bubble treatment and chlorine can

have a higher efficacy in reducing the number of pathogens than only applying cavitation bubbles or only applying chlorine (Lee et al., 2018).

Additionally, Lee et al. (2018) reported that cavitation bubbles could reduce 0.6 to 1.3 log per Roma tomato or per cantaloupe more than the level of no bubble treatments. Moreover, higher air flow rate of air bubble applications could have a better level of inactivating *Listeria monocytogenes* and *S.* Newport as well potentially. From the research result, higher air flow rates could also reduce *Listeria* and *Salmonella* on the surface of uninoculated tomatoes after cross contamination from inoculated tomatoes. Since the pathogens can be detached from the surface of inoculated Roma tomatoes and then attach to the surface of the uninoculated ones in a short time (less than 30 seconds), which is shorter than the bubble treatment time (30 or 60 seconds), the ongoing bubble streams can inactivate the pathogen attached on the surface of uninoculated Roma tomatoes.

Beside only applying bubble streams on the surface of fruit, Lee et al. (2018) used bubbles and chlorine simultaneously to remove the pathogen on the fruit surface. From his research, a higher level of chlorine concentration and higher air flow rate of air bubbles can have a better efficacy than other combinations on inactivating the pathogen *Salmonella* on cantaloupe surfaces and cantaloupe rinse water. The pathogen reduction for both cantaloupe surface and cantaloupe rinse water was over 2.5 log reduction per cantaloupe. The combination of two disinfecting measures has a better efficacy over bubble treatment or chlorine treatment alone. Therefore, the combination of air bubbles and chlorine is determined to be an effective measure to kill *Salmonella* Newport on the surface of Roma tomatoes and cantaloupe.

III. Materials and Methods

1. Inoculation of test surfaces with *Listeria monocytogenes*

Listeria monocytogenes (strain LCDC, serotype 4b) were transferred from frozen (-80°C) storage to Tryptic Soy Broth (TSB) and incubated at 35±2°C for 24 hours. Then, the culture was transferred to fresh TSB and incubated at 35±2°C for 24 hours. Then, this culture was used to inoculate stainless steel, cucumbers and avocados. Culture identity and purity was confirmed by colony appearance on Rapid L'mono agar (Bio-Rad Laboratories, Richmond CA) and with the Microgen *Listeria*-ID biochemical identification kit (Microbiology International, Frederick, MD).

Stainless steel sheet (type 304, #2 Finish; speedy Metals, Milwaukee, WI) was cut into 1 x 2-inch coupons. Slicing cucumbers (*Cucumis sativas*) and avocados (*Persea americana* cultivar Hass) were purchased from local supermarkets. The Hass avocado is the most common cultivar of commercial avocados. Before the bacterial inoculation onto cucumbers, wax was removed from the cucumber surfaces by washing them using peptone water and drying them gently by paper wipes. Additionally, cucumbers and avocados were sprayed with 70% ethanol and then wiped and air-dried to reduce background microflora.

Stainless steel coupons were spot inoculated with 0.1 mL of a *L. monocytogenes* culture (~2 x 10^8 CFU/mL). The spots of inoculation were marked before inoculation. Inoculums were allowed to dry for 1, 24, or 48 hours at room temperature (22°C) in a sealed box with two small beakers of a sodium chloride solution to maintain the humidity from 50% to 60%. Cucumbers and avocados were also spot inoculated with 0.1 mL of a

L. monocytogenes culture (~2 x 10^8 CFU/mL) and stored at 10°C. Inoculums were allowed to dry for 1, 24, or 48 hours.

2. Cavitation treatments for stainless steel

After the test materials were dried (1, 24, or 48 hours), but before application of bubble treatments, the inoculated steel coupons were rinsed with 10 mL peptone water to remove loosely attached cells. These rinsates were collected and further analyzed as described in part 5.

2.1. Heavy bubble application:

Stainless steel coupons were individually placed into a 3.5-gallon plastic tank containing 4.5 L distilled water. Plastic forceps were used to hold the rectangle stainless steel coupon when applying bubbles to the inoculated surface. An air compressor (Campbell Hausfeld, 120V, 60Hz, 2A, 1 gallon tank, Maximum rated 110 psi) was used to deliver air through an air stone (MBD75, Diffuser Area 6-1/8'' x 1-1/8'', Range up to 0.75 LPM, Flow Rate @ 50 PSI 2.2 LPM, Gas Inlet Connection 1/4'' Hose Barb; Pentair, Golden Hills, MN) submerged in the bottom of the tank. A Masterflex flow meter (Cole-Parmer, Vernon Hills, IL) was used to regulate air flow to 1.0 L/min. The air stone produced ~0.5-1.0 mm diameter bubbles under water during the bubble treatment. After drying under room temperature and from 50% to 60% humidity, these stainless steel coupons were treated for 0, 1, 2, 5 or 10 minutes using the air stone with 1.0 L/min air flow rate. Three steel samples were tested for each combination of inoculum drying time and bubble treatment time. After treatment with a stream of heavy bubbles, stainless steel coupons were collected in a sterile bag with peptone water and sonicated

(Aquasonic Ultrasonic Cleaner, Volts: 117/120, 50/60 Hz) for 2 minutes at room temperature. Solutions were then diluted with peptone water and plated onto Oxford agar (Neogen, Lansing, MI) plates. Plates were incubated at 35±2°C for 48 hours. Three replications for the process above were conducted for a total of 108 samples.

2.2. Single bubble stream application:

L. monocytogenes culture (0.1 mL) was inoculated on to stainless steel coupons (75 x 25 mm) and dried for 1 hour or 24 hours under room temperature with 50% to 60% humidity. Coupons were individually clamped onto a support frame with the inoculated side facing down. The frame allowed the angle of the steel to be adjusted so that bubbles can impact the steel at different angles (0°, 15°, 22.5°, 30°, 45°, or 60°). These are the angles formed between the inoculated surface of the stainless steel coupon and the bottom of the water tank.

After drying, individual steel coupons were submerged into a 3.5-gallon plastic tank containing 4.5 L distilled water. A syringe pump (New Era Pump System, Inc, Model NO.300, Volts/Hz 12VDC, Serial NO. 306522) was used to deliver a very small volume of air (0.2 mL/min) using a small syringe (1.0 cc/mL) through a hose and a small diameter glass pipet under water. Air bubbles (~1.0 mm. diameter) exited individually through the glass pipet at rate of ~1 bubble/sec and traveled a distance of ~10-12 cm. Coupons were treated separately with single bubbles directed at the inoculated area with a flow rate of 0.2 mL/min for either 0, 1, or 2 min. Three steel samples were tested for each combination of inoculum drying time and bubble treatment time and impact angle. After treatment, coupons were removed and placed into a sterile bag with

peptone water and sonicated for 2 minutes at room temperature. Solutions were diluted and spread plated onto Oxford agar, and incubated at 35±2°C for 48 hours.

3. Cavitation treatment of cucumber surfaces

After inoculated cucumbers were dried (1, 24, or 48 hours), but before application of bubble treatments, they were rinsed with 10 mL peptone water to remove loosely attached cells. These rinsates were collected and further analyzed as described in part 5. Rinsed cucumbers were individually placed into a 3.5-gallon plastic tank containing 4.5 L distilled water. Two elastic rubber wires on the top of both opposite edges of the tank were used to hold the cucumbers in place when applying bubbles to their surface (down side). A compressed air supply was used to deliver 1.0 L/min of air through an air stone as described in part 2. A stream of bubbles (~0.5-1.0 mm diameter) was applied for either 0, 1, 2, 5 or 10 minutes. Three samples of cucumbers were tested for each combination of inoculum drying time and bubble treatment time for each of three replications.

4. Cavitation treatment of avocado surfaces

After inoculated avocados were dried (1, 24, or 48 hours), but before application of bubble treatments, they were rinsed with 10 mL peptone water to remove loosely attached cells. These rinsates were collected and further analyzed as described in part 5. The air compressor and air stone used for cavitation treatment on avocados were the same as used for cavitation treatment on cucumbers. Rinsed avocados were individually placed into a 3.5-gallon plastic tank containing 4.5 L distilled water. A

stream of bubbles (~0.5-1.0 mm diameter) was applied for either 0, 1, 2, 5 or 10 minutes. Three samples of avocados were tested for each combination of inoculum drying time and bubble treatment time for each of three replications.

5. Recovery of *L. monocytogenes* from inoculated surfaces

Before application of bubble treatments, inoculated steel coupons, cucumbers and avocados were rinsed with 10 mL distilled water to remove loosely attached cells. The rinses were collected, diluted with peptone water and plated on to Oxford agar plates. Plates were incubated at 35±2°C for 48 hours and enumerated.

Then, the rinsed test materials were treated with a bubble application (0, 1, 2, 5, or 10 min). Firmly attached cells (biofilm) were removed from the steel coupons, cucumbers and avocados by placing the sample in a sterile bag or cup with added peptone water. Containers were then sonicated for 2 minutes at room temperature (22°C). Solutions were then diluted and plated onto Oxford plates and incubated at 35±2°C for 48 hours. Finally, cell counts of the solutions after sonication of bubble-treated samples were compared to the cells counts of the solutions after sonication where samples were not treated with bubbles.

6. Cross-contamination from inoculated to un-inoculated produce

To simulate cross contamination of produce with *L. monocytogenes*, one inoculated cucumber and one un-inoculated cucumber were added to a water tank. Cucumber pairs were similar in size and shape. All cucumbers were wiped to remove wax and reduce background microflora as described previously. Inoculated cucumbers

received 0.1 mL of a *Listeria monocytogenes* (LCDC) culture and allowed to dry for 1, 24 or 48 hours. Then, an inoculated cucumber was placed in a water tank with one un-inoculated cucumber. A heavy bubble stream from the air stone were applied for 2 or 10 minutes with an airflow rate of 1.0 L/min. As a control, pairs of inoculated and uninoculated cucumbers were placed in the water tank for 1 min, but without a bubble application. Then, each sample cucumber was placed into separate sterile bags, and *L. monocytogenes* were recovered from the sample surface as described previously. The spread plates with *L. monocytogenes* were incubated at 35$\pm$2°C for 48 hours. A similar inoculation, treatment and recovery protocol were done for avocados. Three sample pairs of cucumbers and of avocados were tested for each combination of inoculum drying time and bubble treatment time. These experiments were replicated 3 times for both cucumbers and avocados. The proportion of cells that transferred to the uninoculated samples was determined.

7. Microbial quality evaluation of treated cucumbers and avocados after refrigerated storage (10°C)

Fresh cucumbers and avocados were stored in the refrigerator at 10 °C for up to 7 days. After storage for 0, 1, 4, or 7 days, three cucumbers and three avocados were individually placed in a sterile bag, diluted with peptone water and solutions were plated onto Tryptic Soy Agar plates. Plates were incubated at 35$\pm$2°C for 24 hours, then enumerated. Additional cucumbers and avocados were treated to a heavy bubble stream, as described previously, for 2 or 10 minutes. These samples were stored for either 0, 1, 4 or 7 days at 10°C. After storage, three cucumbers and three avocados

(from each storage time), were individually placed in a sterile bag, diluted with peptone water and solutions were plated onto Tryptic Soy Agar plates. Plates were incubated at 35±2°C for 24 hours, then enumerated. The process above was repeated for 3 replications.

8. Dye penetration of treated cucumbers and avocados

Cavitation bubble applications may cause the infiltration of *L. monocytogenes* inside produce if breaks in the skin of treated produce are created. A dye penetration test was conducted to determine the potential for bacteria to become internalized after exposure to cavitation bubbles.

Two cucumbers were put in the water tank (13.0 L) used above with a 0.1% solution of FD&C Red #40 dye (Spectrum Chemical Mfg. Corp.) for 2 or 10 minutes. Then, these cucumbers were pulled out from the dye solution and cut vertically and horizontally to observe any penetration of the dye into the stem and pulp of cucumbers. Then, two other fresh cucumbers were treated with a 1 L/min air flow rate of heavy bubbles for 2 or 10 min in the water tank used above with the 0.1% FD&C red dye solution. Then, these cucumbers were pulled out from the dye solution and cut vertically and horizontally to observe any penetration of dye into the stem and pulp of cucumbers. The observations of dye penetrations in both untreated and treated cucumbers were compared. The process above was repeated for avocado samples. All experiments were performed 3 times (n=9; 3 samples x 3 replications).

Statistical Analysis

Listeria Monocytogenes cell counts recovered from fresh produce and stainless steel coupons were compared to the cell counts of original inoculation on produce and stainless steel to determine the pathogen detachment from the surface of fresh produce and stainless steel after microbubble treatment.

A one-way analysis of variance (ANOVA) was used to determine significant differences between means for each variable tested at a statistical significance of $\alpha = 0.05$.

All calculations were performed with Jump 16.1 Statistical Software (Jump, 2021)

IV. Results

1. Cavitation treatments of stainless steel coupons

1.1. Heavy bubble application

After rinsing off loosely attached cells, approximately 6.65, 6.30, and 6.08 log mean CFU of *Listeria monocytogenes* per stainless steel coupon could be recovered after 1, 24, or 48 hours drying time, respectively. As the bubble treatment time increased from 0 to 1, 2, 5, or 10 min, the total mean CFU of *L. monocytogenes* recovered from stainless steel decreased for each set of coupons with different drying times (1, 24, or 48 hours). Thus, extended bubble treatment time appears to remove more bacteria, since less could be recovered from the steel coupons. Figure 1 shows The mean log CFU listeria monocytogenes recovered per avocado after different bubble treatment times from 0 to 10 minutes, the log CFU difference for stainless steel with 48 hours drying time is the highest (1.55 log CFU after 1 min treatment and 2.95 log CFU after 10 min treatment). Also, the log CFU difference recovered from stainless steel with 1 hour's drying time is the lowest (0.31 log CFU after 1 minute's bubble treatment and 2.08 log CFU after 10 minutes' bubble treatment). The effect of bubble treatment time was significant for each of the steel inoculum drying times ($p<0.001$).

1.2. Single bubble stream application

Figure 2, displays the difference in log CFU recovered between steel treated with bubbles and steel not treated with bubbles. The mean log CFU recovered under 1- and 2-minute bubble treatment times are similar. For 1-minute bubble treatments, the difference in mean log CFU recovered from stainless steel from recovery with no bubble treatment decreases from 0 degree at 1.51 log CFU to 30 degrees at 0.85 log CFU, and then increases to 1.53 log CFU at the 60° angle. The greater log difference recovery (from the 0 min bubble treatment samples) for steel positioned at 0° and 60° implies results from lower recovery from the steel at these angles. This implies that more bacteria may have been removed by the single bubble stream for the steel at these angles. Similarly, a relatively lower number of bacteria may have been removed from the steel held at a 30° angle. The trends for recovery are similar for samples exposed to 2 min of the single bubble stream.

2. Cavitation treatment of cucumber surfaces

After rinsing off loosely attached cells, approximately 7.15, 6.48, and 6.79 log mean CFU of *Listeria monocytogenes* per cucumber could be recovered after 1, 24, or 48 hours drying time, respectively. Figure 3 shows the mean log CFU listeria monocytogenes recovered per cucumber after different bubble treatment times from 0 to 10 minutes. The difference in *Listeria* recovery between no bubble treatments (0 min) and 10 min treatments was 1.09, 1.39 or 1.78 mean log CFU per cucumber for cucumbers dried for 1, 24 or 48 hr, respectively. For each of the drying times, extending bubble treatment times up to 10 min appear to remove more bacteria since bacterial

recovery from treated cucumbers was reduced. The log CFU recovered from cucumber with 48 hours' drying time is the highest at 1.02 log CFU after 1 minute's bubble treatment and at 1.78 log CFU after 10 minutes' bubble treatment, and the log CFU recovered from cucumber with 1 hour's drying time is the lowest at 1.09 log CFU after 10 minutes' bubble treatment. However, the log CFU recovered from cucumber after 1 minute's bubble treatment with 24 hours' drying time is the lowest value (0.24 log CFU) of figure 3 and lower than the value of the log CFU recovered (0.43 log CFU) after 1 minute's bubble treatment time with 1 hour's drying time. The effect of bubble treatment time was significant for each of the cucumber drying times ($p<0.001$). When cucumbers were dried for 24 or 48 hours, bacterial recovery of firmly attached cells was significantly different (lowered) between each of the bubble treatment times ($p<0.001$).

3. Cavitation treatment of avocado surfaces

After rinsing off loosely attached cells, approximately 8.13, 7.52, and 7.79 log mean CFU of *Listeria monocytogenes* per avocado could be recovered after 1, 24, or 48 hours drying time, respectively. As the bubble treatment time increased from 0 to 1, 2, 5, or 10 min, the total mean CFU of *L. monocytogenes* recovered from avocados decreased for each set of fruit with different drying times (1, 24, or 48 hours). Therefore, extended bubble treatment times may lead to greater bacterial removal or detachment. Figure 4 shows the mean log CFU listeria monocytogenes recovered per avocado after different bubble treatment times from 0 to 10 minutes. After a 1-min treatment, the mean log CFU recovered from avocado with 24 hours' drying time was the highest at 1.07 log CFU and lowest after 1 or 48 hours of drying (~0.4 log cfu/cucumber). The maximum

difference occurred with 10 minutes of bubbles for avocadoes where the inoculum dried for 24 hours (mean 1.65 log CFU per avocado). Similar to cucumbers, the effect of bubble treatment time was significant for each of the cucumber drying times ($p<0.001$). When avocados were dried for 1 hours, bacterial recovery of firmly attached cells was significantly different between each of the bubble treatment times ($p<0.001$). After 24 or 48 hours of drying, bacterial recovery of firmly attached cells was significantly different (lower) between 1 and 5 min bubble treatment times and between 2 and 10 min treatment times ($p<0.001$).

4. Inoculated to un-inoculated produce cross-contamination simulations

4.1. Cucumber

From table 1, which shows the percent transfer from cross-contaminated cucumbers at different bubble treatment times and the three different drying times, the percent transfer from contaminated cucumbers to uninoculated cucumbers increases when bubble treatment time increases. When *Listeria monocytogenes* are dried on cucumbers for 24 hours, the percent transfer is the highest, at which transfer percentage is 0.203% after 2 minutes' treatment time and 0.548% after 10 minutes' bubble treatment time. When *Listeria monocytogenes* are dried on cucumbers for 1 hour, the percent transfer is the lowest, at which transfer percentage is 0.059% after 2 minutes' treatment time and 0.306% after 10 minutes' bubble treatment time.

4.2. Avocado

Table 1 also shows that the percent transfer from cross-contaminated avocados increases when bubble treatment time increases. And, when *Listeria monocytogenes* are dried on avocados for 24 hours, the percent transfer is the highest, at which transfer percentage is 0.017% after 2 minutes' treatment time and 0.115% after 10 minutes' bubble treatment time. When *Listeria monocytogenes* are dried on avocados for 1 hour, the percent transfer is the lowest, at which transfer percentage is 0.006% after 2 minutes' treatment time and 0.092% after 10 minutes' bubble treatment time.

5. Quality evaluation of produce during refrigerated storage

5.1. Cucumber

Figure 5 displays the log mean CFU per cucumber from different storage times at different bubble treatment times. For both , for bubble treatment time: both 0 minute and 2 minutes, log mean CFU per cucumber increases from day 0 to day 7. For 10 minutes' bubble treatment, log mean CFU per cucumber increases from day 0 at 6.59 log CFU, to storage day 4 at 7.06 log CFU, and decreases from day 4 to day 7 at 6.57 log CFU. Among 3 different bubble treatment times, log mean CFU per cucumber after 0, 4, 7 days storage is the lowest after 10 minutes' bubble treatment time.

5.2. Avocado

According to the figure 6, the log mean CFU per avocado increases during storage from day 0 to day 7 when avocados receive no bubble treatment or when they received a 10 minute bubble treatment. For the 2 minutes' bubble treatment, the log mean CFU per avocado decreased from 4.96 log CFU to 3.89 log CFU after 7 days storage. The mean log CFU recovered immediately after a 2-minute bubble treatment

(log 4.96 for day 0) was higher than the initial counts for the no bubble treatment or 10-minute treatment (4.45 or 3.85 log CFU per avocado, respectively).

6. Dye penetration of treated cucumbers and avocados

Table 2 displays the number of cucumbers and avocados where dye penetration occurred through the skin surface or the stem scars at different bubble treatment times in room temperature water at 22°C. No dye penetration appears in the pulp and stem of cucumbers and avocados after 2 or 10 minutes and without a bubble treatment. However, dye penetrated all cucumbers and avocados through the stems after 2 minutes and 10 minutes bubble treatment. When cucumbers and avocados were placed in cool water (10°C) the results are the same as seen when the samples were held in room temperature water.

V. Discussion

1. Cavitation treatments of stainless steel, cucumber, and avocado surfaces

Based on the results, extended bubble treatment time promotes more removal of *L. monocytogene*s from stainless steel coupons, since significantly fewer *L. monocytogenes* were recovered from stainless steel after 10 min bubble treatments compared with no bubble treatment. The bubble application used in this study may reduce *L. monocytogenes* on stainless by nearly 3 logs (2.95 log CFU per sample) after inoculum dried for 48 hours and loosely attached cells were removed. For cucumber

and avocado, a 5-min application of the heavy bubble stream removed at least 1.0 log more of the firmly attached bacteria than submerging the produce in water without cavitation.

Lee et al. (2018) applied cavitating bubbles to inoculated fresh Roma tomatoes and cantaloupes using larger bubble sizes (~1-3 mm diameter) and higher air flows (3.5 - 14 L/min) than this study. And, Lee et al. (2018) found, *L. monocytogenes* significantly decreased on Roma tomatoes (~1.0 log CFU after 30 seconds and ~1.2 log CFU after 60 seconds) with a 14 L/min airflow rate compared to no cavitating bubbles. Also, *L. monocytogenes* significantly decreased on cantaloupes (0.65 log CFU for 30 seconds and ~0.74 log CFU for 60 s when the maximum flow rate was used. In this study reported here, the log reduction of *L. monocytogenes* on cucumber and avocado surfaces also increased with increasing bubble treatment time even though the airflow rate was lower, the treatment times longer, and the bubble diameters smaller. Another important difference in these studies is that Lee et al. (2018) did not remove loosely attached cells prior to any bubble treatments. Here, in our study with cucumbers, avocados and stainless steel coupons, the number of loosely attached cells enumerated after 1, 24 or 48 h drying was always higher than the quantity that could be recovered from the samples that were not treated with bubbles (treatment time = 0 min).

2. *Listeria* removal with chlorine sanitizer

As a common measure to clean fresh produce, chlorine is applied to wash and clean fresh produce due to its bactericidal capacity and economical cost. When chlorine contacts wash water during washing, it yields an oxidizer, HOCI, which can inactivate

pathogens effectively (Banach et al., 2015). Commonly, the dosage of commercial chlorine use in industry is around 50 to 200 mg/L, but 100mg/L is the most common dosage used in industry (Baur et al., 2004). And the application of this dosage of chlorine requires a short impact time, which is about 1 to 2 minutes, and a preferred pH of 6.0 to 7.5 to maintain the stabilization of HOCI in order to prevent chemicals from corroding the processing equipment. Banach, et al. (2015) shows that 5 log CFU of *E. coli* on fresh spinach can be removed completely with wash water containing a level of 7 mg/L free chlorine. Although the chlorine cleaning measure can inactivate more pathogens than streams of microbubbles, it can cause chlorine residuals on applied area and cross-contamination during washing. The present study was designed to evaluate microbubbles for removal or detachment of *L. monocytogenes* on surfaces, including stainless steel, avocado and cucumber surfaces. Even though microbubbles may reduce the concentration of *L. monocytogenes* on steel and some produce surfaces, they may or may not enhance microbial inactivation. Nevertheless, microbubbles can be an environmental-friendly alternative to chlorine for reducing microbial surface contamination of fresh produce that will have less chlorine odor and be less corrosive to equipment.

3. Single bubble treatment on stainless steel

From the results, the recovery rate of *L. monocytogenes* varied when the impact angle of the bubble treatment area (stainless steel surface) was adjusted between 0 and 60 degrees. Esmaili, et al. (2019) concluded that an inclined surface of cavitation impact surface can help produce a shear force to inactivate bacteria attached on the

surface. Since the microbubbles impact the stainless steel coupons surface in a certain distance (~ 100 mm vertically), the microbubbles variably bounce and slide along the surface of stainless steel coupons and generate a shear force that can remove attached *Listeria monocytogenes* on the surface (Esmaili et al., 2019). Therefore, based on the results, the shear force generated by varying angles appears to increase as the impact increases from 0 to 30 degrees, and decrease as the impact angle further increases from 30 to 60 degrees.

4. Inoculated to uninoculated produce to simulate cross-contamination

Based on the results, cross contamination of *Listeria monocytogenes* to clean cucumbers and avocados increased with extended bubble treatment time. The transfer rate to cucumbers and avocados was also higher when inoculum dried for 24 hours versus 1 or 48 hours. This result could be explained by differences in the total number of firmly or loosely attached cells that occurred with each drying time. Variable numbers of attached cells on inoculated cucumbers and avocados influence the number of cells that detach and adhere to uninoculated produce. The percentage of cells that transferred to cucumbers was always higher than the percentage transfer to avocados even though initial inoculums were similar. Compared with cucumbers, cross-contamination of avocados by *Listeria monocytogenes* is less affected by cavitation treatments. In Lee et al. (2018), cross-contamination of *L. monocytogenes* on Roma tomatoes was not significantly different with bubble treatment time in the transfer between inoculated to uninoculated Roma tomatoes (30 or 60 seconds), but

significantly different with airflow rates (0, 7 or 14 SLPM). Conversely, in this research, extended cavitation treatment time lead to a higher transfer percentage of *L. monocytogenes* on the cucumbers (0.548% after 10 min and and 0.203% after 2 min treatment time) and avocados (0.115% after 10 min and 0.017% after 2 min). Therefore, extended time over 2 minutes can lead to more transfer of pathogens from inoculated fruits to uninoculated ones.

5. Quality evaluation of produce during refrigerated storage (10°C)

Fresh cucumbers usually last within one week, and fresh avocados last for 7 to 10 days, respectively (Cindy, 2015). Based on the test results, longer storage times can lead to more bacteria growth and worsening quality of treated cucumbers for different bubble treatment times. Also, extended bubble treatment time appears to have higher effectiveness on removing bacteria from cucumbers, but surface aerobic plate counts did not decrease over time. Similarly, for avocados, surface aerobic plate counts increased with longer storage times, and surface concentrations could be initially reduced by a cavitating bubble treatment by less than one log CFU per unit.

No dye penetration occurred in the pulp and stem of the cucumbers and avocados after immersing the fruits into the FD&C Red 40 dye solutions. Only dye penetration was found on the stems of cucumbers and avocados after 2 minutes and 10 minutes bubble treatments in both 22°C and 4°C temperature dye solutions. Other researchers have found that bacteria (and dye solutions) can be pulled into a fruit or vegetable when the temperature of a fruit or vegetable is warmer (~10C warmer) than the water it is placed in. Some infiltration can still happen when fruit and the water are at

similar warm temperatures. In one study, the level of dye uptake by oranges and grapefruits was increased substantially when warm fruits (21°C) are immersed in the cool temperature water (4°C) (Center for Food Safety and Applied Nutrition, 1999). Therefore, unlike oranges and grapefruits, the temperature difference between the avocados and cucumbers did not show any effect on the level of dye uptake or penetration on cucumbers and avocados. Further studies are needed to determine if the cavitation bubble treatments used in this research would lead to bacterial infiltration into the stem or stem scar of raw produce.

6. Future Work

For our future work, different treatment time may be done for microbubble application. We could extend the bubble time to 20 or 30 minutes. Different pathogens found on the fruits or vegetables can be done as well in the future, for example, different strains of Listeria, like serotype ½ a or ½ b. Different storage times for fresh cucumbers and avocados can be done as well. we did 0, 4, 7 days storage. In the future, over 7 days storage can be done for both cucumbers and avocados

And different fruits like cantaloupe and papaya can be tried in the future for the listeria monocytogenes bubble treatment. The storage temperature for fresh produce can be adjusted to lower temperatures at around 4℃ or less. Lastly, different bubble treatment distances and different sizes of bubbles can be applied in the future. In this experiment, the bubble distance was 10-12 cm and the bubble size was 0.5 -1.0 mm diameter. In the future, the tinier bubbles and the closer distance between bubbles and fresh produce can be tried to avoid impact of water flow between bubbles and impact surface.

VI. References:

Banach, J. L., Sampers, I., Van Haute, S., & Van der Fels-Klerx, H. J. (2015). Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. *Int. J. Environ. Res. Public Health*, 2(8), 8658-8677. https://doi.org/10.3390/ijerph120808658.

Baur, S., Klaiber, R.G., Kobli, B. and Carle, R. (2004) Effect of different washing procedures on phenolic metabolism of shredded, packaged iceberg lettuce during storage. *J Agric Food Chem*, 52, 7017–7025.

Bardsley, C. A., Truitt, L. N., Pfuntner, R. C., Danyluk, M. D., Rideout, S. L., & Strawn, L. K. (2019). Growth and survival of *Listeria monocytogenes* and *Salmonella* on whole and sliced cucumbers. *Journal of Food Protection, 82*(2), 301-309. doi:10.4315/0362-028x.jfp-18-341.

CDC. (2016). People at risk. Retrieved February 22, 2021, from https://www.cdc.gov/*Listeria*/risk.html#:~:text=*Listeria*%20is%20most%20likely%20to,they%20rarely%20become%20seriously%20ill.

Center for Food Safety and Applied Nutrition. (n.d.). *Listeria (listeriosis)*. U.S. Food and Drug Administration. Retrieved November 9, 2021, from https://www.fda.gov/food/foodborne-pathogens/Listeria-listeriosis.

Center for Food Safety and Applied Nutrition. (1999). *Temperature differences on dye uptake by oranges and grapefruit*. U.S. Food and Drug Administration. Retrieved October 20, 2021, from https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/preliminary-experiments-effect-temperature-differences-dye-uptake-oranges-and-grapefruit.

Chen, Y., Evans, P., Hammack, T. S., Brown, E. W., & Macarisin, D. (2016). Internalization of *Listeria monocytogenes* in whole avocado. *Journal of Food Protection, 79*(8), 1440-1445. doi:10.4315/0362-028x.jfp-16-075.

Cindy. (2015, April 21). *Cucumbers - how long do cucumbers last? shelf life expiration date*. Eat By Date. Retrieved November 15, 2021, from https://www.eatbydate.com/vegetables/fresh-vegetables/how-long-do-cucumbers-last-shelf-life-expiration-date/.

Dular, M., Griessler-Bulc, T., Gutierrez-Aguirre, I., Heath, E., Kosjek, T., Krivograd Klemenčič, A., & Kompare, B. (2016). Use of hydrodynamic cavitation in (waste)water treatment. *Ultrasonics Sonochemistry, 29*, 577-588. doi:10.1016/j.ultsonch.2015.10.010.

Esmaili, E., Shukla, P., Eifert, J. D., & Jung, S. (2019). Bubble impact on a tilted wall: Removing bacteria using bubbles. *Physical Review Fluids*, *4*(4), 043603. https://doi.org/10.1103/physrevfluids.4.043603

Farber, J.M., Peterkin, P.I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiological Reviews, 55*(4), 752-752. doi:10.1128/mmbr.55.4.752-752.1991.

Gao, S., Hemar, Y., Ashokkumar, M., Paturel, S., & Lewis, G. D. (2014). Inactivation of bacteria and yeast using high-frequency ultrasound treatment. *Water Research, 60*, 93-104. doi:10.1016/j.watres.2014.04.038

Hamon, M., Bierne, H. & Cossart, P. (2006). *Listeria monocytogenes*: A multifaceted model. *Nature Reviews Microbiology, 4*(6), 423-434. doi:10.1038/nrmicro1413.

Iturriaga, M. H., Arvizu-Medrano, S. M. & Escartín, E. F. (2002). Behavior of *Listeria monocytogenes* in avocado pulp and processed guacamole. *Journal of Food Protection, 65*(11), 1745-1749. doi:10.4315/0362-028x-65.11.1745.

Krysinski, E. P., Brown, L. J., & Marchisello, T. J. (1992). Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. *Journal of Food Protection, 55*(4), 246-251. doi:10.4315/0362-028x-55.4.246.

Lee, J., Eifert, J., Jung, S., & Strawn, L. (2018). Cavitation bubbles remove and inactivate *Listeria* and *Salmonella* on the surface of fresh Roma tomatoes and cantaloupes. Front. Sustain. Food Syst. 2:61. doi: 10.3389/fsufs.2018.00061.

Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., Sulakvelidze, A. (2003). Biocontrol of *Listeria monocytogenes* on fresh-Cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied and Environmental Microbiology, 69*(8), 4519-4526. doi:10.1128/aem.69.8.4519-4526.2003.

Lundén, J. M., Miettinen, M. K., Autio, T. J., & Korkeala, H. J. (2000). Persistent *Listeria monocytogenes* strains show enhanced adherence to food contact surface after short contact times. *Journal of Food Protection, 63*(9), 1204-1207. doi:10.4315/0362-028x-63.9.1204.

McLauchlin, J. (1990). Human listeriosis in Britain, 1967–85, a summary of 722 cases: 1. Listeriosis during pregnancy and in the newborn. *Epidemiology and Infection, 104*(2), 169-183. doi:10.1017/s0950268800059343.

Pietrysiak, E., Smith, S., & Ganjyal, G. M. (2019). Food safety interventions to control *Listeria monocytogenes* in the fresh apple packing industry: A review.

Comprehensive Reviews in Food Science and Food Safety, 18(6), 1705-1726. doi:10.1111/1541-4337.12496.

Rodgers, S.L., Cash, J.N., Siddiq, M., Ryser, E.T. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J. Food Prot.* 2004, *67*, 721–731.

Rosenblum, J., Ge, C., Bohrerova, Z., Yousef, A., & Lee, J. (2012). Ozonation as a clean technology for fresh produce industry and environment: Sanitizer efficiency and wastewater quality. *Journal of Applied Microbiology*. *113*(4), 837-845. https://doi.org/10.1111/j.1365-2672.2012.05393.x

Strawn, L. K., Gröhn, Y. T., Warchocki, S., Worobo, R. W., Bihn, E. A., & Wiedmann, M. (2013). Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Applied and Environmental Microbiology*, *79*(24), 7618–7627. https://doi.org/10.1128/aem.02831-13

Yaun, B. R., Sumner, S. S., Eifert, J. D., & Marcy, J. E. (2004). Inhibition of pathogens on fresh produce by ultraviolet energy. *International Journal of Food Microbiology*, *90*(1), 1–8. https://doi.org/10.1016/s0168-1605(03)00158-2.

Van Haute, S., Tryland, I., Escudero, C., Vanneste, M., & Sampers, I. (2017). Chlorine dioxide as water disinfectant during fresh-cut iceberg lettuce washing: Disinfectant demand, disinfection efficiency, and chlorite Formation. *LWT*, *75*, 301–304. https://doi.org/10.1016/j.lwt.2016.09.002

Zhu, Q., Gooneratne, R., & Hussain, M. (2017). *Listeria monocytogenes* in fresh produce: Outbreaks, prevalence and contamination levels. *Foods, 6*(3), 21. doi:10.3390/foods6030021

VII. Tables and Figures

Drying time (h)	Air Flow for Bubble Application (L/min)	Treatment time (min)	Percent transfer Cucumbers	Percent transfer Avocados
1	0	1	0.001%	0.0002%
24	0	1	0.002%	0.001%
48	0	1	0.002%	0.001%
1	1	2	0.059%	0.006%
24	1	2	0.203%	0.017%
48	1	2	0.107%	0.012%
1	1	10	0.306%	0.046%
24	1	10	0.548%	0.115%
48	1	10	0.436%	0.092%

Table 1. *Listeria monocytogenes* transfer from inoculated, dried and rinsed cucumbers or avocados to uninoculated fruit (n=3).

Room Temperature Water (22°C)					
Air flow rate (liter/min)	Treatment time (min)	Cucumbers (~22°C)		Avocados (~22°C)	
		Surface	Stem	Surface	Stem
0 (no bubbles)	2	0	0	0	0
0 (no bubbles)	10	0	0	0	0
1	2	0	6	0	6
1	10	0	6	0	6

Table 2. Dye penetration into cucumbers and avocados placed into room temperature water (22 °C). (n=6, 3 replications x 2 samples)

Cool Temperature Water (10°C)					
Air flow rate (liter/min)	Treatment time (min)	Cucumbers (~22°C)		Avocados (~22°C)	
		Surface	Stem	Surface	Stem
0 (no bubbles)	2	0	0	0	0
0 (no bubbles)	10	0	0	0	0
1	2	0	6	0	6
1	10	0	6	0	6

Table 3. Dye penetration into cucumbers and avocados placed into cool water (10° C). (n=6, 3 replications x 2 samples)

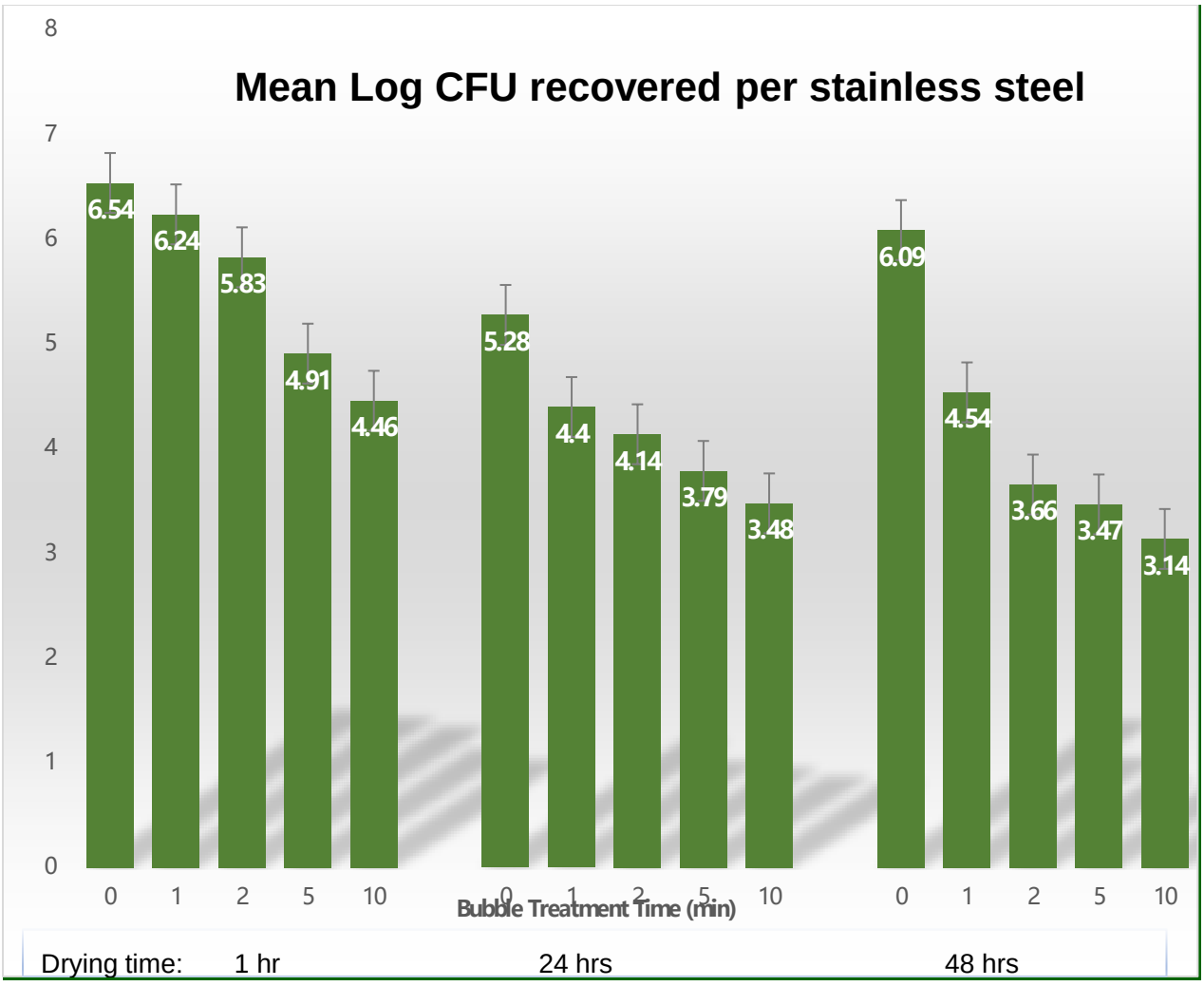


Figure 1. The mean log CFU listeria monocytogenes recovered per avocado after different bubble treatment times from 0 to 10 minutes (Mean of: 3 replications x 3 samples).

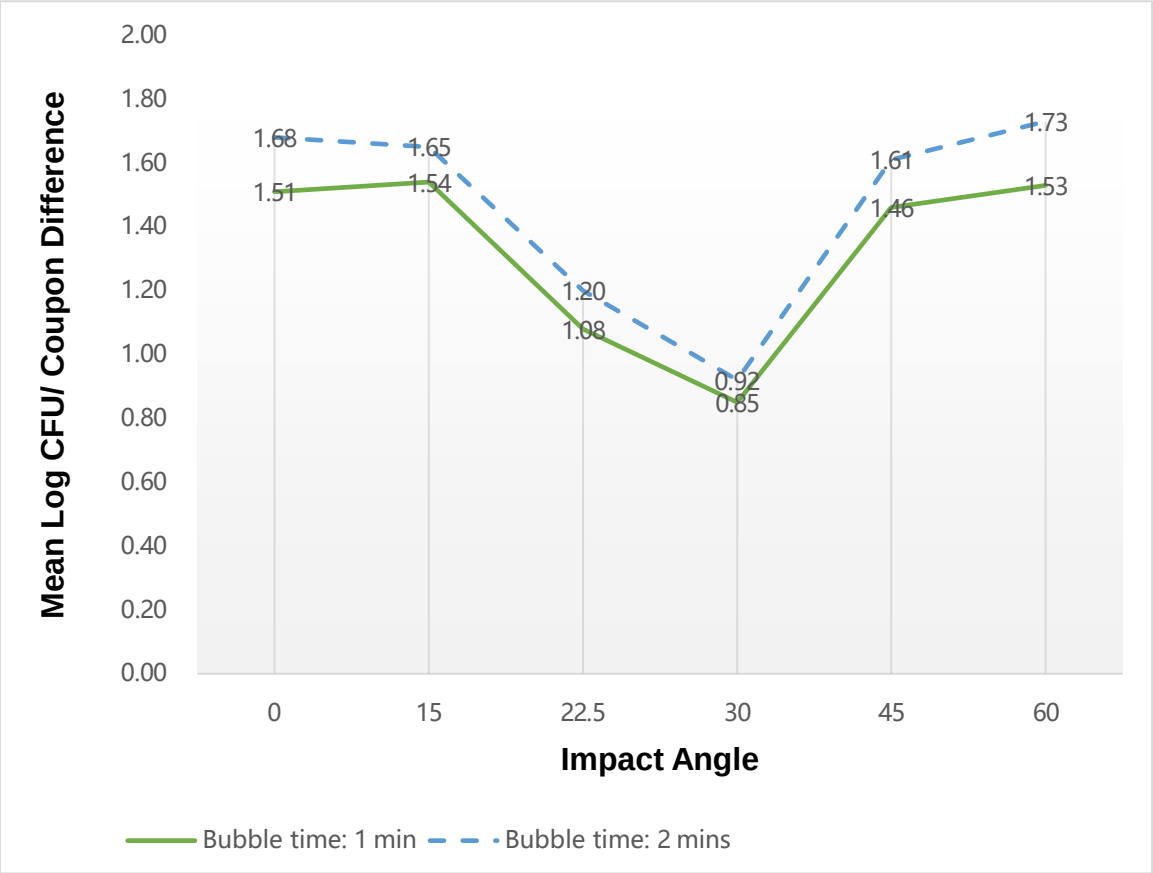


Figure 2. Difference in log CFU recovered from stainless steel from Time 0 at different angles.

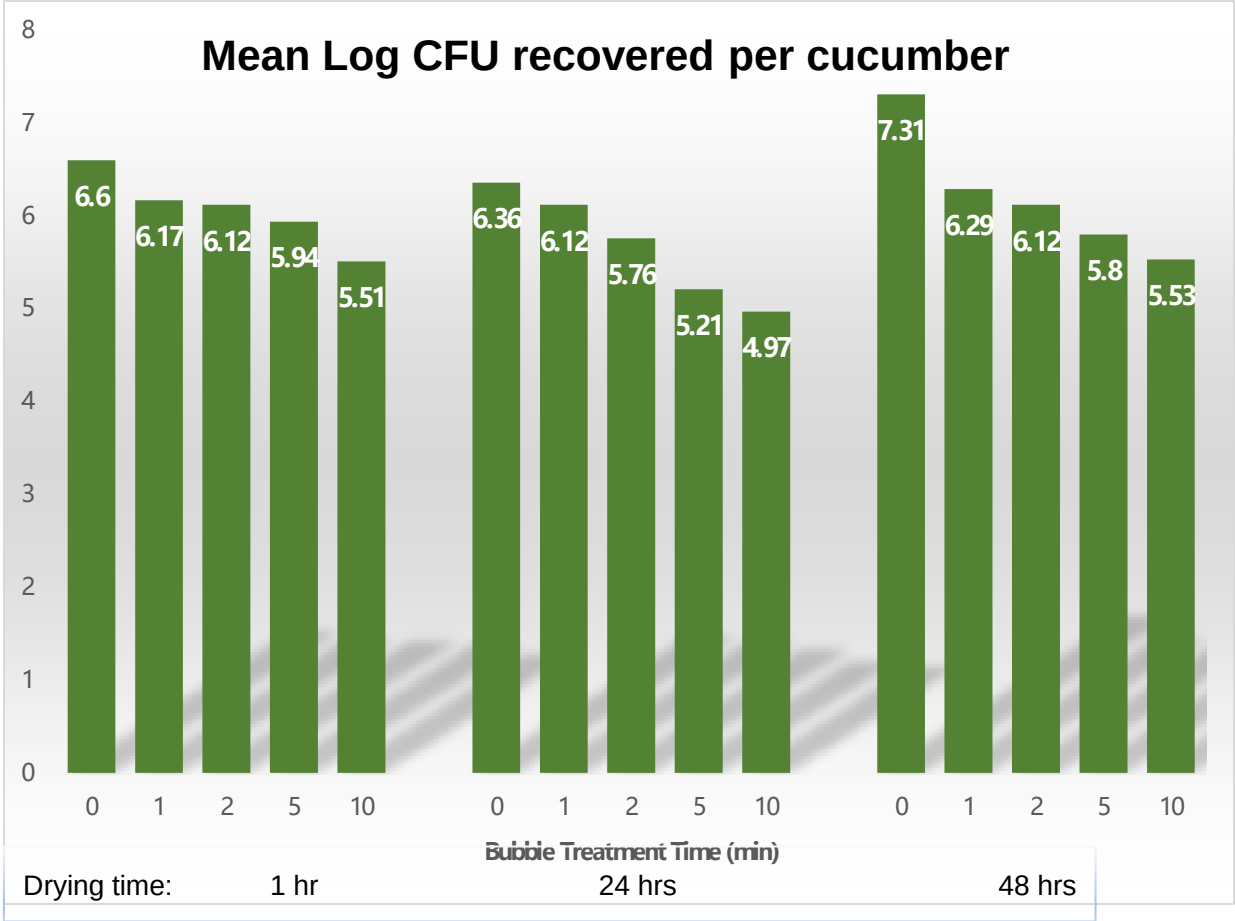


Figure 3. The mean log CFU listeria monocytogenes recovered per cucumber after different bubble treatment times from 0 to 10 minutes (Mean of 3 replications x 3 samples).

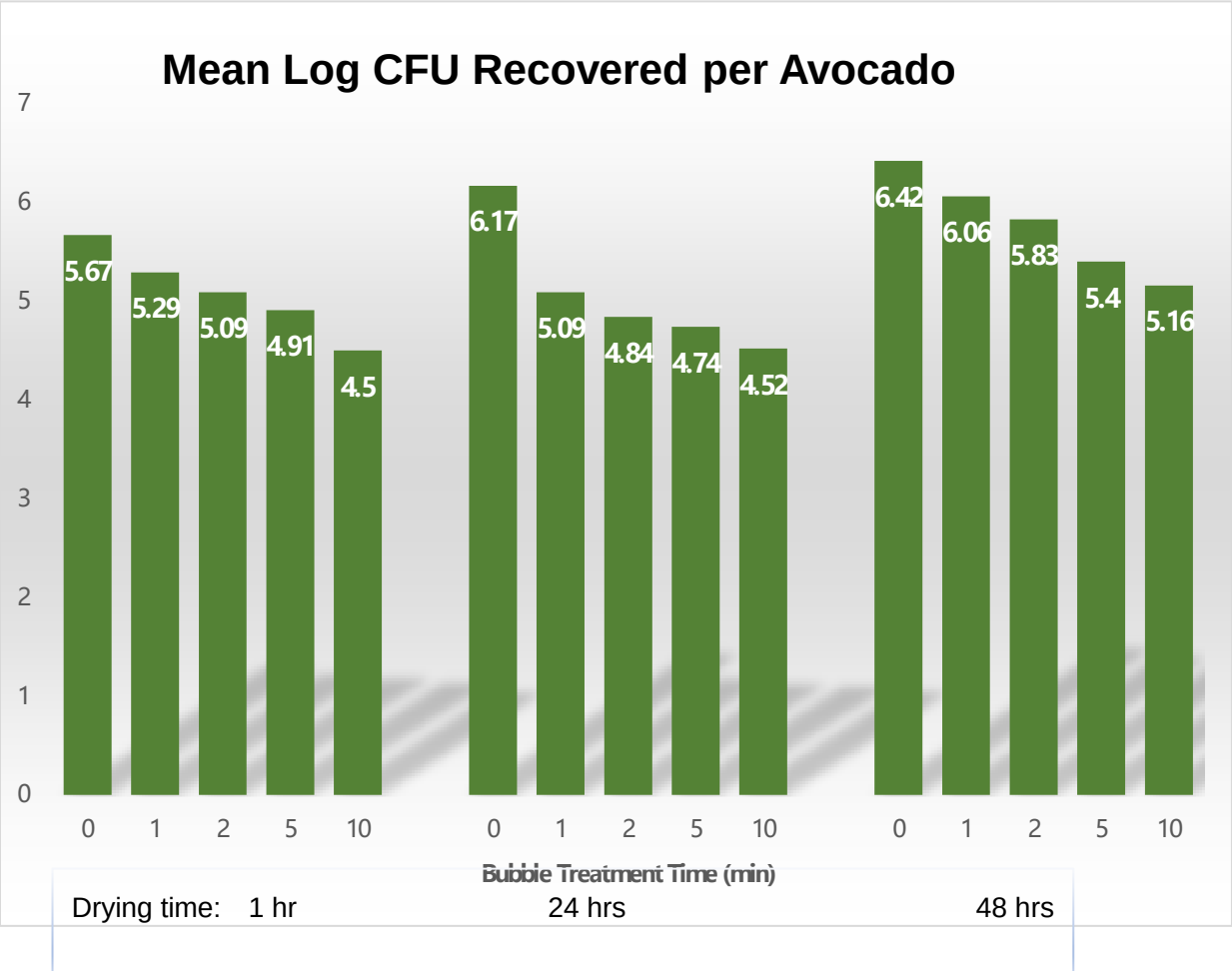


Figure 4. The mean log CFU listeria monocytogenes recovered per avocado after different bubble treatment times from 0 to 10 minutes (Mean of 3 replications x 3 samples).

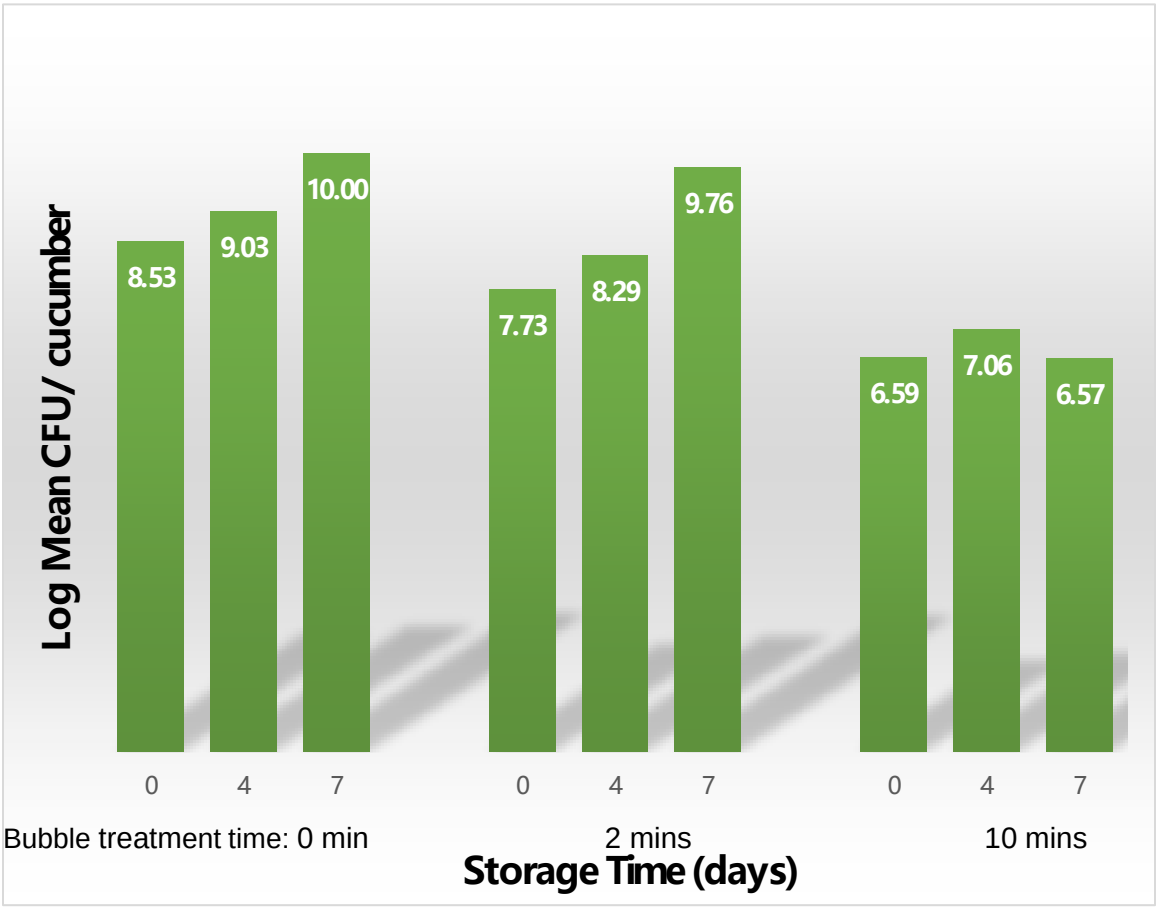


Figure 5. Log Mean CFU per cucumber from different storage times at different bubble treatment times (Mean of 3 replications x 3 samples).

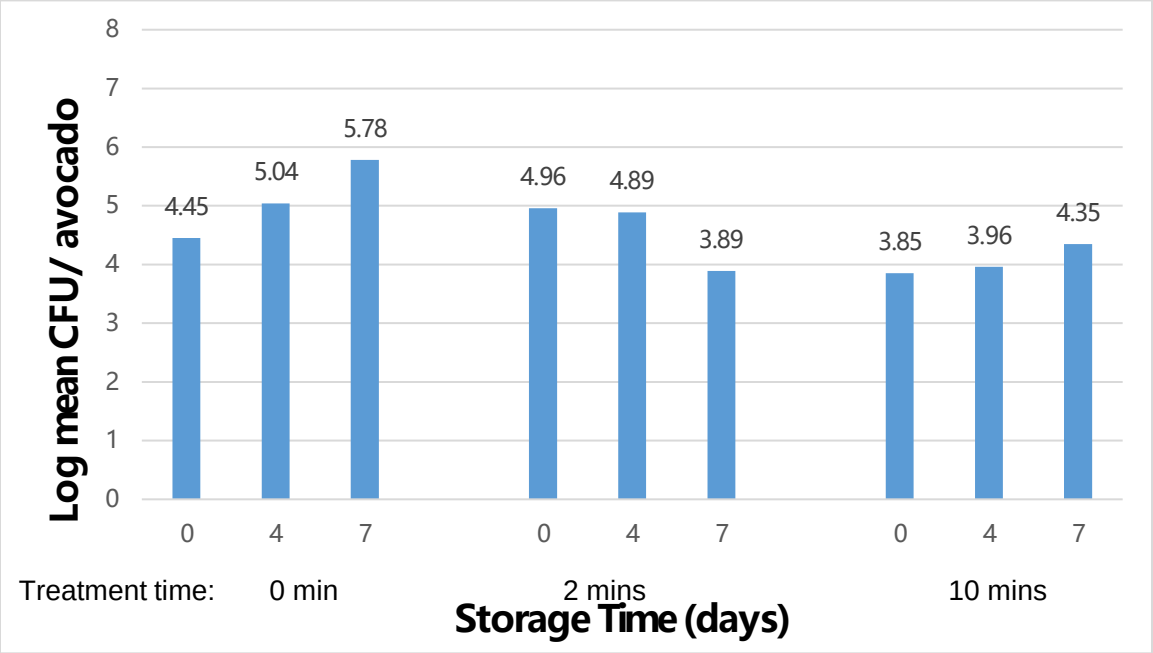


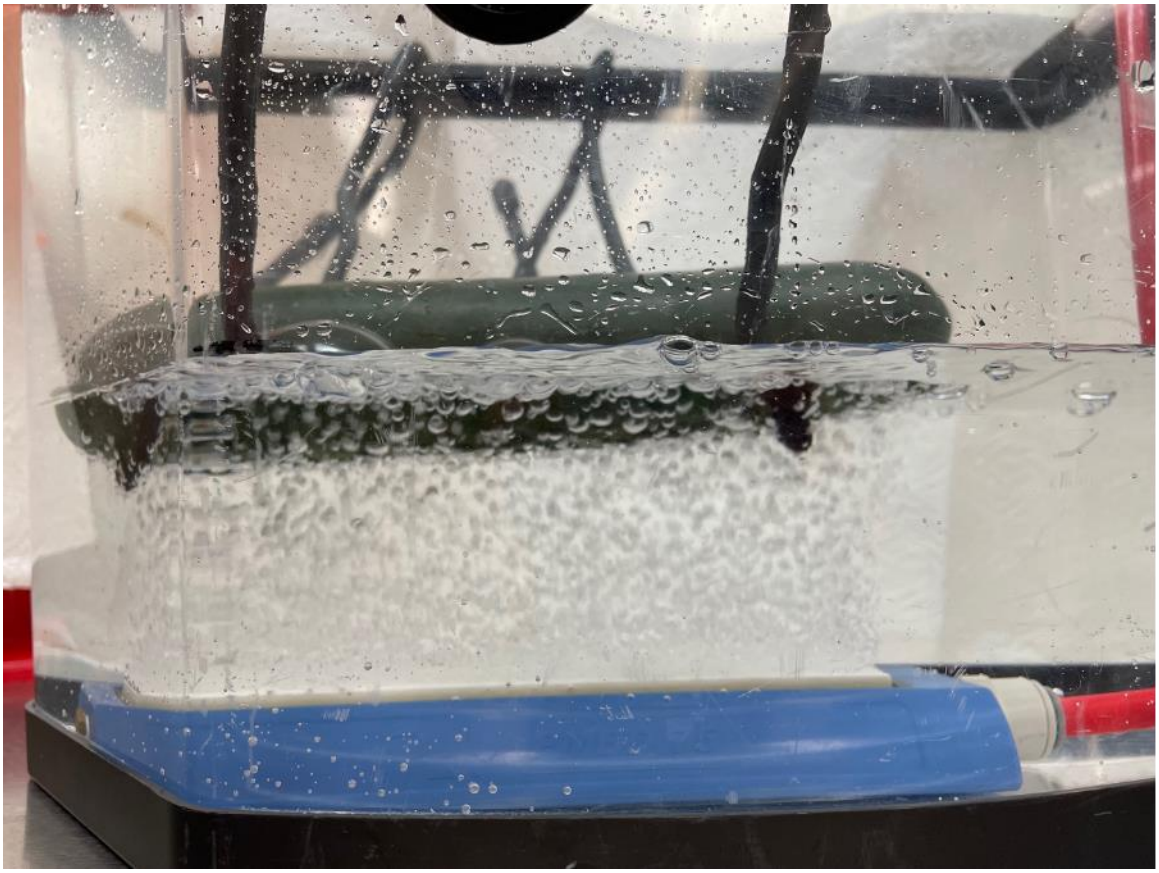
Figure 6. Log Mean CFU per avocado from different storage times at different bubble treatment times (Mean of 3 replications x 3 samples).

VIII. Appendices:

Appendix A. Dye penetration of cucumbers and avocados inside the water tank filled with FD&C red #40 solution.

Appendix B. Dye penetration of avocados after cavitation treatment.

Appendix C. Cavitation treatment (1 L/min air flow rate) on the surface of inoculated cucumber.



Appendix D. Set-up of cavitation treatments for cucumbers, avocados, and steel coupons.