

EFFICACY OF MULTIPLE CONSUMER USE WASHING TECHNIQUES IN REDUCING
ESCHERICHIA COLI O157:H7, *SALMONELLA ENTERICA*, AND *LISTERIA*
MONOCYTOGENES CONTAMINATION ON PRODUCE

by

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(Under the Direction of Joseph F. Frank)

ABSTRACT

Consumption of fresh produce is on the rise. With the increased consumption of fresh produce items comes an increase in chances of a foodborne outbreak. This study evaluated the efficacy of home washing technologies (ozonated water, electrolyzed oxidizing (EO) water, Veggie Wash[®], dilute chlorine bleach, and running tap water) against *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* on tomatoes, cantaloupe, and broccoli. Dilute chlorine bleach was the most effective on all produce, providing log reductions up to 3.89 log. EO water and ozonated water were moderately effective on all produce. Running tap water was effective on tomatoes, but not on broccoli or cantaloupes. Veggie Wash provided the lowest reductions for tomatoes and was only moderately effective on broccoli and cantaloupes. Reductions were higher for tomatoes (1.14 to 3.89 log) than for broccoli (0.63 to 1.57 log) or cantaloupe (0.55 to 2.48 log) when subjected to the same washing technologies.

INDEX WORDS: Decontamination, Tomatoes, Broccoli, Cantaloupe, *Salmonella*, *Escherichia Coli* O157:H7, *Listeria monocytogenes*, Chlorine bleach, Electrolyzed Oxidizing water, Ozone, Veggie Wash, Produce, Fruit, Vegetables

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CHAPTER 1

INTRODUCTION

Fruits and vegetables are an important part of the everyday diet. Fruits and vegetables are considered “nutrient-dense foods,” meaning that they are minimally processed, do not contain solid fats and added sugars, and provide a full range of essential nutrients, including important vitamins, dietary fiber, and minerals (103). According to MyPyramid.gov the recommended serving size of fruits for the average individual is between 1½ and 2 cups per day, while for vegetables the serving size for the average individual is between 2½ and 3 cups per day (102). While Americans are nowhere near consuming the recommended 5 servings a day of fruits and vegetables, there does appear to be an overall increase in consumption. Between 1994 and 2004 the average percent of Americans who ate the recommended servings increased from 39% to 40% (30). This may be due to the increase of available produce items via international trade, the inclination to add desirable tastes and textures to the diet, or even to better comply with government recommendations.

With improvements in transportation and infrastructure, and to global increases in per capita populations and income levels, the global food trade is very likely to continue to increase. Consumers benefit from this worldwide trade by having items with lower prices, a greater quantity of choices, and year-round selection. For example, in order to be able to have cantaloupe whenever we wish, we must import them from climates that are warmer year round, such as Mexico and Honduras. According to the United States Department of Agriculture, 33% of tomatoes, 37% of cantaloupes, and 6% of broccoli that was consumed in the United States was

imported in the year 2000 (101). Of the total amount of food and agricultural imports from less developed countries, fresh food products, including fruits and vegetables, account for half.

Another issue comes when the less developed countries that these items are originating from do not have the same sanitation standards or good manufacturing practices as the developed countries they are exporting to (104).

While regulatory agencies and food industries attempt to make our food as safe as possible, there is only so much they can do, as sometimes there is no adequate kill step in processing. This combination of increased consumption plus the increased availability of foods produced under inadequate conditions in the global food market also increases our chances of receiving contaminated food. The consuming public needs to be more diligent in washing fresh produce items prior to consumption. While there are many different products on the market and different approaches about what will work best, the average consumer does not have the ability to evaluate. This research focuses on promising techniques available for use in the home.

A water wash is the recommended method and is the most commonly used technique for washing produce in the home (19, 70, 82). Water is generally known to reduce bacterial counts by 1 log CFU/g, but the question is whether or not this reduction is enough (87, 89). Chlorine washes are used in industry and produce a greater reduction than water, but are not recommended for use in the home. Other novel sanitizing technologies, such as aqueous ozone or electrolyzed oxidizing water, have the potential to replace the traditional water or chlorine wash, and are now available at the household level. Ozone is a molecule that is able to destroy a broad spectrum of organisms, while also decomposing to pure oxygen without leaving any toxic residues behind. Electrolyzed oxidizing water is made from a dilute salt solution which is passed through an electric current. This technology is also able to destroy a broad spectrum of

organisms and will revert to normal water after use. Other options include commercial produce washes which are typically made from natural oils, organic acids, and/or surfactants. These produce washes may have antimicrobial activity, but typically just wash the pathogen off of the produce item, making the possibility of cross-contamination an issue. While the majority of these technologies have been tested in a laboratory for industry settings, it is important to look at these washes from a consumer stand point.

This study aims to evaluate and compare the efficacy of novel home washing technologies, including ozonated water, electrolyzed oxidizing water, a commercial produce wash (Veggie Wash[®]), dilute chlorine bleach, and running tap water against *E. coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* on tomatoes, cantaloupe, and broccoli. Because the quality of the water used in these technologies will affect the performance, and to determine whether these technologies would be appropriate for all homes, different incoming water qualities (degree of hardness and pH) will also be evaluated.

CHAPTER 2

LITERATURE REVIEW

Outbreaks and Recalls

In the last five years there have been several foodborne outbreaks involving *Salmonella enterica*, *Listeria monocytogenes*, and enterohemorrhagic *Escherichia coli*. There have also been numerous food recalls involving these same pathogens. So far in 2010 there have been three notable outbreaks, one involving *Salmonella* Newport, one involving *E. coli* O145, and one involving *Listeria monocytogenes*. In May 2010, there were 26 confirmed and 7 probable cases of *E. coli* O145 in 5 states. This outbreak was associated with prepackaged shredded romaine lettuce. Twelve patients of the 30 with available information developed a type of kidney failure known as hemolytic uremic syndrome (HUS) (31). In June 2010, there were 44 confirmed cases of *Salmonella* Newport in 11 states, 7 of which resulted in hospitalizations. This outbreak was associated with raw alfalfa sprouts (32). In October 2010, there were 10 confirmed cases of *Listeria monocytogenes* in Texas, 5 of which resulted in deaths. This outbreak was associated with fresh celery (73). In 2009 there was one notable outbreak involving *Salmonella* Saintpaul in which there were 228 cases in 13 states. This outbreak was also associated with raw alfalfa sprouts (29). In 2008 there were two notable outbreaks, one involving *Salmonella* Litchfield and the other involving *Salmonella* Saintpaul. In April 2008, there were 51 confirmed cases of *Salmonella* Litchfield in 16 states, at least 16 of which resulted in hospitalizations. This outbreak was associated with cantaloupe from Honduras (28). In August 2008, there were 1442 confirmed cases of *Salmonella* Saintpaul in 43 states, at least 286 of which resulted in

hospitalizations, and the infection may have contributed to two deaths. This outbreak was associated with jalapeño and serrano peppers (27). In 2006 there were two notable outbreaks, one involving *Salmonella* Typhimurium and the other involving *E. coli* O157:H7. In October 2006, there were 199 confirmed cases of *E. coli* O157:H7 in 26 states. 102 patients were hospitalized and 31 developed HUS. This outbreak was associated with prepackaged raw spinach and contributed to three deaths (25). In November 2006, there were 183 confirmed cases of *Salmonella* Typhimurium in 21 states plus two patients in Canada, 22 of which resulted in hospitalizations. This outbreak was associated with tomatoes (26).

In addition to tainted produce items that make it to someone's table, there are a multitude of products that are contaminated, but caught before they get a chance to make someone ill. So far in 2010 alone there have been 10 recalls associated with fresh produce. Five were associated with *Salmonella* including chile peppers, spinach, prepackaged romaine lettuce, and two incidences of raw alfalfa sprouts (37, 40, 43-45). One was associated with *E. coli* O145 and romaine lettuce (41). The other four recalls were associated with *L. monocytogenes* and included prepackaged salad, raw alfalfa sprouts, prepackaged sliced apples, and fresh spinach (38-39, 42, 47).

Pathogens of Concern

As seen by these outbreaks and recalls, the most common bacterial foodborne pathogens on produce are *Salmonella enterica*, enterohemorrhagic *E. coli*, and *Listeria monocytogenes*. Members of the genus *Salmonella* are facultatively anaerobic gram-negative rods. There are over 2,500 different serovars within the two species (*S. bongori* and *S. enterica*) of the genus *Salmonella*. This is a bacterium that is versatile enough to survive in multiple extreme environmental conditions such as extreme high ($\leq 54^{\circ}\text{C}$) and low (4°C) temperatures, making it a

pathogen of great concern. The typical growth range is between 35°C and 37°C, neutral pH (~7), and high water activity ($a_w < 0.95$). It can take as few as 1-10 cells to infect a human host and can cause enterocolitis. Enterocolitis involves nonbloody diarrheal stools and abdominal pain and tends to be self limiting. Symptoms generally occur within 8 to 72 hours after ingestion of the pathogen and can last up to 5 days. Symptoms can be more severe in the young and elderly (36).

Salmonella species are abundantly found in the natural environment, particularly in the fecal material of animals. The bacterium has been isolated from the feces of cattle, swine, poultry, fish, shellfish, birds, reptiles, amphibians, and insects (36, 48). When this contaminated fecal material is introduced to the environment, the bacteria can remain viable for months (48). This is a problem when produce is grown in these contaminated environments, since food is the primary vector for *Salmonella* transmission to humans. As noted earlier, *Salmonella* can cause outbreaks from a variety of produce sources, but the most notable ones appear to be alfalfa sprouts, cantaloupe, and tomatoes (36).

Whereas all of the many different serotypes of *Salmonella* are associated with disease, there are a rare number of *Escherichia coli* that are pathogenic, as the majority of the species are normal members of the commensal microflora (75). There are many different diarrheagenic *E. coli* isolates, but enterohemorrhagic *E. coli* (EHEC) tend to be the greatest cause of *E. coli* foodborne illness in North America based on frequency and severity of the illness. These bacteria are facultatively anaerobic gram-negative rods with the optimal growth ranges of 35°C – 37°C, neutral pH, and high water activity, although they can resist extremely low pH (as low as 1.5) and refrigeration temperatures (4°C). The most common EHEC pathogen is *E. coli* O157:H7, but *E. coli* O145 appears to be on the rise. *E. coli* O157:H7 symptoms begin with

nonbloody diarrhea and severe abdominal cramps over the first day or two. It can then progress into bloody diarrhea, hemorrhagic colitis, and, in extreme cases, hemolytic uremic syndrome (HUS), a severe, and sometimes fatal, kidney infection. Symptoms generally occur after 3 to 4 days following ingestion of the bacterium, and can last up to 10 days (36). There is an estimated 73,480 cases of *E. coli* O157:H7 illness per year in the United States (84). Symptoms are more severe for the young and elderly, especially since HUS largely affects children. There is a 1% fatality rate from *E. coli* O157:H7 infection (36).

Cattle is the main source of EHEC, but the pathogens have also been isolated from sheep, goats, swine, deer, dogs, poultry, horses, cats, wild birds, and rodents (36, 48). *E. coli* O157:H7 is transmitted by the fecal-oral route and is transmitted via food, water, or person-to-person. The person-to-person route is crucial because the infectious dose is possibly as few as 10 cells, so when there is minimal personal hygiene, secondary transmission can become a problem. *E. coli* O157:H7 mainly causes outbreaks from beef and dairy products, but also contaminates leafy greens, among other produce items (36). In August 1993, *E. coli* O157:H7 was implicated in an outbreak associated with cantaloupe (34).

Listeria monocytogenes is a facultatively anaerobic gram-positive rod shaped bacterium (75). This bacterium is extremely common, found in soil, feces, plants, water (13, 36, 48, 75), and, as Dr. Larry Beuchat says, “in or on a large number of species representative of every class of living things that swim, crawl, jump, walk or fly (12).” While the optimal growth ranges are 30°C – 37°C, neutral pH, and high water activity, this is a bacterium that can persist in low pH and high salt environments, and is able to grow at refrigeration temperatures (4°C) (36). When growth at refrigeration temperatures is coupled with the fact that this is a bacterium that is readily found in nature, contamination becomes a big issue. Heisick et al (53) conducted a test to

determine if *Listeria* species were present on fresh produce from local supermarkets. Ten different types of produce were tested: broccoli, cabbage, carrots, cauliflower, cucumbers, lettuce, mushrooms, potatoes, radishes, and tomatoes. Out of these 10 types of produce tested, *Listeria* species were isolated from 6. The produce that tested positive tended to be the produce that was grown directly in or on soil. Forty-eight percent of the *Listeria* isolates found were determined to be *L. monocytogenes*.

Since *L. monocytogenes* is readily found in soil, raw plants, and animal products, it is easy for a food worker to accidentally bring the pathogen into a food processing facility. This pathogen forms biofilms on a variety of surfaces such as stainless steel, rubber, or glass, making it a concern for ready-to-eat products including raw produce (36). In 1981 there was an outbreak in Canada involving *L. monocytogenes* which was associated with locally prepared coleslaw that included cabbage and carrots. This outbreak caused 34 cases of perinatal listeriosis and 7 cases of adult disease. The cause was thought to be cabbage that had been fertilized with contaminated sheep manure and then held in cold storage for an extended period (12-13, 36).

L. monocytogenes is different in how it causes disease because, unless an extremely high dose, such as 10^6 , is ingested, the average healthy individual will most likely not be affected. If the average healthy individual is affected, the individual will typically suffer from febrile gastroenteritis or flu-like symptoms. The high risk groups for this disease include pregnant women, neonates, elderly, and persons with a compromised immune system. This invasive form of listeriosis can cause septicemia, meningitis, or meningoencephalitis. In pregnant women the risk is for the fetus, as the infection can cause stillbirths or spontaneous abortions. In neonates less than 7 days old the disease manifests as sepsis and pneumonia while in neonates older than 7 days the infection can result in sepsis or meningitis. Gastroenteritis usually occurs within a day

after ingestion of the pathogen while invasive listeriosis requires several weeks of incubation (36, 75).

Produce of Concern

While there are many different produce items that could be chosen, tomatoes, broccoli, and cantaloupe represent valid produce items of concern along with varying surface areas. With consumer demand increasing, it has become necessary to grow and import produce from around the globe. As we begin to receive produce items from further away, the chance for contamination increases. Tomatoes have been linked to several outbreaks and have been previously studied. Between 1996 and 2008 there were 14 outbreaks associated with fresh tomatoes. These tomato associated outbreaks caused 1,927 illnesses and 3 deaths. All 14 of these outbreaks were determined to be caused by bacterial pathogens (24).

Cantaloupes are another commodity that is often imported. Between 1996 and 2008 there were 13 outbreaks associated with melons, 10 of which were cantaloupes. These melon associated outbreaks caused 507 illnesses and 2 deaths. Again, all 13 of these outbreaks were determined to be caused by bacterial pathogens (23). Between 1990 and 1996 there were 2 outbreaks associated with cantaloupes and *Salmonella* causing 645 illnesses (5). Castillo et al (22) performed a study looking at *Salmonella* contamination during production of cantaloupe and found that of the 1,735 samples collected from the environment of packing sheds, water, and cantaloupes themselves, 31 samples tested positive. These samples were taken from two farms, one in Texas and one in Mexico, to show a representation of the different sources of cantaloupes. There was no statistical difference in *Salmonella* contamination between the two countries, but there were significantly higher *E. coli* levels found on the melons sampled in Mexico. This is an

indication of a higher frequency of fecal contamination which was attributed to poor hygienic conditions in the packing shed. Cantaloupes are more of a concern than other melons because of the netted rind surface that allows a greater surface area for bacterial attachment, plus this meshwork of raised tissue on the surface protects the pathogens, making them more difficult to eliminate (23, 97). This netting is also highly hydrophobic to reduce water loss, but, according to Ukuku and Fett (97), this surface hydrophobicity strongly correlates with the strength of attachment of *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7.

Broccoli, while not implicated in any outbreaks, has a complex surface structure that could potentially harbor and protect pathogens. Broccoli is a fresh-cut product, as the florets are cut from the plant prior to sale, so contamination could be an issue during the cutting process. Broccoli is also commonly eaten raw, which eliminates any microbiological killing step (57).

Methods of Contamination

Produce items can become contaminated at many different points in the farm-to-fork continuum. The main points for contamination include pre-harvest in the field, post-harvest and processing, and storage and distribution. In the field the produce item can become contaminated by fecal droppings from the local wildlife, contaminated irrigation water, application of inadequately processed manure, dispersal by air or biological vectors such as insects, or contamination of soil. It is important to ensure only potable water is used for irrigation, that there is a limited amount of local wildlife infiltration into the fields, and that the field is not located downhill of or near any facility that may pose a food safety risk, such as livestock fields, chemical plants, landfills, or sewage treatment facilities (13, 16, 23-24, 35, 48, 84).

The harvest and processing stages can introduce a greater chance of contamination. Harvest can injure the produce item, allowing pathogens to invade the tissues. Other issues

include cross-contamination via contaminated harvesting equipment, sorting equipment, transport containers, rinse water or ice, biological vectors such as insects, or improper storage temperature (13, 16, 23-24, 35, 48, 84). When produce is grown in a less developed country, for example tomatoes from Mexico or cantaloupes from Honduras, the hygiene of workers, water quality issues/standards, and the enforcement of good manufacturing practices can become an issue (23-24). During processing, these same cross-contamination sites are an issue. One major problem during processing can occur during the cool down step, where if the water is not microbiologically sound and is cooler than the produce item, the water will actually infiltrate the produce item at the stem scar or at injured points (23-24, 35). *L. monocytogenes* can form biofilms in processing facilities which can then contaminate numerous amounts of produce (48). Johnston et al (58) found that the packing process greatly increased the microbial load for cantaloupes. This is most likely due to the combination of cross-contamination on the conveyor belt and the netting surface of the cantaloupe harboring microorganisms. The study looked at packing sheds and suggested field packing would be less problematic in that it could decrease exposure to dirty rinse water, contact with dirty equipment, additional human handling, and other post-harvest sources of contamination. It is important to ensure worker hygiene, equipment sanitation, the use of potable water, possibly containing an antimicrobial, and to focus on temperature regulations.

The final points for contamination are at the storage and distribution stages of the farm-to-fork continuum. Many of the same issues from post-harvest and processing stages are a concern at the storage and distribution stage such as cross-contamination via water and ice, storage bins, distribution equipment, transport containers, display areas, or biological vectors. Improper storage temperature during transport, display, or final storage, or improper handling

after purchase, such as bagging raw produce with raw meat, can also result in produce contamination (13). Preparation can also become an issue if prepared with contaminated equipment or, in the case of cantaloupe, if the rind is inadequately cleaned (10, 16, 100). Ukuku and Sapers (100) found *Salmonella* that was attached to the rind of cantaloupes could transfer to the edible surface through cutting of the melon. They also determined that *Salmonella* was able to grow on the edible surface and that *Salmonella* could survive on inoculated cantaloupes at 4°C for 5 days. Behrsing et al (10) determined that *Listeria innocua* could actually grow on the surface of cantaloupes during storage. They stored inoculated cantaloupes at 8°C for 7 days and found an increase in populations of *L. innocua*. They also tested storage viability of *Salmonella* and *E. coli* and found that while the pathogens did not grow during storage, the counts did not significantly change over 7 days at 8°C.

Government Recommendations vs. Consumer Habits

While there are various guidelines and regulations for the growth and distribution of fresh produce including the Food and Drug Administration's Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables, there are no rules that the public has to follow prior to consuming these products. And while the industry does as much as possible to keep produce free of contamination, as evidenced by the numerous outbreaks and recalls, it does not appear to be enough and the public needs to take matters into their own hands. According to Fight BAC!, a website from the Partnership for Food Safety Education, the USDA recommends a three step process prior to consuming fresh fruits and vegetables: Prepare the kitchen, Use water, and Scrub thoroughly. To prepare the kitchen begin by washing hands with warm water and soap for at least 20 seconds, use hot water and soap to thoroughly clean cutting boards, countertops, and utensils, and always make sure to keep fresh produce separated from raw meat.

The use water step refers to rinsing the produce under running tap water, ensuring all produce to consume raw, including those with inedible rinds or skins (such as cantaloupe) are rinsed. The final step is to scrub thoroughly by rubbing the surface of firm-skin fruits and vegetables while rinsing with running tap water or by scrubbing produce with a rind, such as cantaloupe, with a clean vegetable brush also while rinsing under running tap water. Produce also follows the overall mantra of safe food handling which includes clean, separate produce from meat, cook thoroughly (which does not apply to fruits and vegetables eaten raw), and chill, meaning to refrigerate promptly (82).

Even though these recommendations are simple to follow, consumer surveys show that a large percentage of consumers practice risky produce handling methods. According to a survey done in 1993 by Bruhn and Schutz (19) around 63% of respondents considered their personal and family health to be very good to excellent, but about 40% admitted to experiencing gastrointestinal troubles personally or within their family within the previous six months. These gastrointestinal troubles included upset stomach, diarrhea, flu-like symptoms, nausea, fever, and/or vomiting and suggest that respondents and/or family members have experienced foodborne illness. This is in complete contradiction with the 85% of respondents that claimed to be very or somewhat informed about food safety. A more recent survey was done in 2002 by Li-Cohen and Bruhn (70). This survey showed that consumers practice unsafe produce handling methods beginning at the market and following through all the way to consumption. Beginning at the market, more than 50% of respondents indicated that they practiced no special requirements for packing and separating fresh produce while only 28% indicated separating fresh produce from meat, poultry, and fish. This “separate” step is one of the four key steps of reducing microbial contamination, meaning that since 50% of respondents do not separate, they

are more likely to contaminate fresh produce. In the home, while 81% of respondents indicated that they wash fresh produce just prior to consumption, 6% indicated that they never or seldom wash fresh produce. Although only 6% overall indicated never or seldom washing fresh produce, melons were the exception, as more than 35% of the respondents indicated never washing melons prior to preparation. The most common method of washing indicated was washing under running tap water, which is the recommended method. These results indicate that consumers are not educated enough about food safety. If consumers were better educated, and had a better means of keeping themselves and their families safe, they might be more likely to practice safe food handling.

In Home Produce Washing Technologies

There are many different methods that could be utilized for in home use, including ozonation, electrolyzed oxidizing water, chlorinated water, commercial fruit and vegetable washing solutions, lactic/acetic/ascorbic/citric acid solutions, ultraviolet radiation, hydrogen peroxide, or a multitude of other antimicrobial compounds. Studies have also been done using pediocin and nisin, bacteriocidal compounds produced by lactic acid bacteria (8). It is beyond the scope of this review to discuss every possible treatment, but will focus on the following treatment options: ozonation, electrolyzed oxidizing water, chlorinated water, commercial fruit and vegetable washing solutions, and the recommended water wash.

Water. While the USDA recommends a rinse under running tap water to cleanse produce items, the question is whether or not it is enough. It is generally recognized that water washes will remove approximately 1 log CFU. Most studies use some form of water wash as a control, but very few studies wash produce in a similar manner as would be performed in the home. Most studies test dip washing methods, where the produce item is submerged in the water for a given

amount of time. Lettuce is a commonly contaminated commodity and is frequently used as a test produce. Singh et al (91) found no significant difference in *E. coli* O157:H7 counts after washing lettuce in deionized water for 10 minutes, while Koseki et al (67) found no significant difference in background microflora counts after washing lettuce in tap water for 10 minutes. Kim et al (65) used a sterile distilled water bath with agitation to reduce the native microflora counts by 0.85 log units. Park et al (78) also used a deionized water bath to wash lettuce inoculated with *E. coli* O157:H7 or *L. monocytogenes*. There was no difference between washing the lettuce for 1 minute or 3 minutes or between the two pathogens; this method reduced the bacterial load by approximately 1.5 log units. Rodgers et al (87) also tested *E. coli* O157:H7 and *L. monocytogenes*, but tested multiple produce items including lettuce, cantaloupe, and apples. This study included a 5 minute tap water wash and found similar results for each produce item and each pathogen: 0.8 log units reduction of *L. monocytogenes* and 0.7 log units reduction of *E. coli* O157:H7 on lettuce, 0.9 log units reduction of *L. monocytogenes* and 0.8 log units reduction of *E. coli* O157:H7 on cantaloupes, 0.8 log units reduction of *L. monocytogenes* and 0.7 log units reduction of *E. coli* O157:H7 on apples.

The submersion technique is extremely common for cantaloupes since the melons are difficult to work with. Annous et al (3) tested *Salmonella* Poona and *E. coli* ATCC 25922 (an *E. coli* O157:H7 surrogate) on cantaloupes using a tap water wash where the cantaloupes were immersed for 3 minutes. This study found no significant difference between the bacterial counts found on unwashed cantaloupes and the cantaloupes washed with tap water. Solomon et al (92) also found no significant difference in *Salmonella* counts when immersing inoculated cantaloupes in a tap water bath for 10 minutes. Ukuku found no significant difference in bacterial counts using a tap water bath on cantaloupe in three different studies: in 2001 with

Sapers (100) testing *Salmonella*, in 2002 with Fett (96) testing *L. monocytogenes*, and in 2006 (95) looking at native microflora. While Ukuku found water to make no significant difference in these three studies, in 2006, in a study done with Fett (98), found that a 5 minute tap water bath reduced *Salmonella* on cantaloupe by 1.8 log units. Parnell et al (81) also found a 0.7 log units reduction of *Salmonella* on cantaloupe when immersed in a sterile distilled water bath for 60 seconds.

Broccoli is a difficult commodity to wash because of the high surface area and the hydrophobic nature of the commodity. Bari et al (8) found that when broccoli was added to distilled water and agitated for 1 minute there was a 0.48 log units reduction in *L. monocytogenes*. Albrecht et al (2) tested broccoli inoculated with *E. coli* ATCC 25742 by submerging in a sterile distilled water bath for 2 minutes and found a reduction of 1.1 log units. Behrsing et al (11) dipped broccoli inoculated with *E. coli* TGI (another *E. coli* O157:H7 surrogate) into deionized water for 30 seconds which reduced the bacteria by 1.8 log units.

Tomatoes are another commodity commonly treated by the submersion method. Because of the smooth surface of tomatoes, it is easier to remove bacteria and thus get a greater log reduction than other produce items. Wei et al (106) dipped tomatoes inoculated with *Salmonella* in tap water for 30 seconds and found a 2.75 log units reduction. Bari et al (7) tested *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on tomatoes. This study found that when the tomatoes were covered with sterile distilled water and rubbed for 40 seconds, each pathogen was reduced by approximately 2.1 log units.

These studies washed produce by a submersion method because it is difficult to replicate rinsing produce under running tap water, but since this is what usually occurs in the home it is important to test this method. Parnell and Harris (80) developed a method to simulate running

tap in a way that was reproducible by utilizing what they called the pour and rinse method. This method involved spraying the produce item with 5 mL of sterile distilled water, rubbing for 30 seconds, and then pouring 200 mL of sterile distilled water over the produce item in a strainer. This resulted in a 2.6 log units reduction in *Salmonella* on apples. Apples may not be the focus of this review, but their surface morphology is very similar to tomatoes. Beuchat et al (15) also evaluated a spray and rinse method to simulate at home use which was very similar to the Parnell and Harris method. Each tomato was sprayed 5 or 6 times with a commercial spray bottle, taking care to spray the entire surface. After a 30 second hold period, each tomato was placed in a sterile bag and rubbed for 30 seconds. Sterile distilled water (200 mL) was then added to the bag and vigorously agitated for 30 seconds. This technique resulted in a 2.7 log unit reduction in *Salmonella* and a 3.8 log unit reduction in *L. monocytogenes*. The study of Baur et al (9) used actual running tap water, though in a commercial setting, to reduce native microflora by 0.5 log units on lettuce. Barak et al (6) came closer to the home setting by rinsing cantaloupes under running tap water for 1 minute. This study used *P. agglomerans* as a *Salmonella* Poona surrogate and found a 15% reduction in recoverable bacteria. Although this study used a technique that is closer to what a consumer would use in the home, it is impractical to think that any consumer actually washes produce for 1 minute. The study most applicable to practical home use was done by Kilonzo-Nthenge et al (61). This study used running tap water to reduce numbers of *L. innocua* (a *L. monocytogenes* surrogate) on lettuce, broccoli, and tomatoes. These researchers used a more practical time of a 15 second rinse. The bacterium was reduced by 1.4 log units on lettuce and broccoli, and 2.1 log units on tomatoes.

Ozone. Ozone is a generally recognized as safe (GRAS) molecule comprised of three oxygen atoms. It is synthetically formed when oxygen is passed through an electrical current which

splits the diatomic oxygen molecules into atoms and forces recombination into the ozone molecule. This process is known as the corona discharge method. This method required two electrodes separated by a ceramic dielectric medium and a narrow discharge gap. One electrode is the high tension electrode while the other is the ground electrode. As air, or pure oxygen gas, passes through the discharge gap and a high voltage alternating current is applied across the discharge gap, if there is sufficient kinetic energy for the electrons, the oxygen molecule dissociates. As collisions occur between the oxygen atoms, ozone is formed (50, 66). A diagram of ozone generation is found in Figure 1. As a gas, ozone is blue at room temperature with a pungent odor, and at high concentrations is toxic to humans (60).

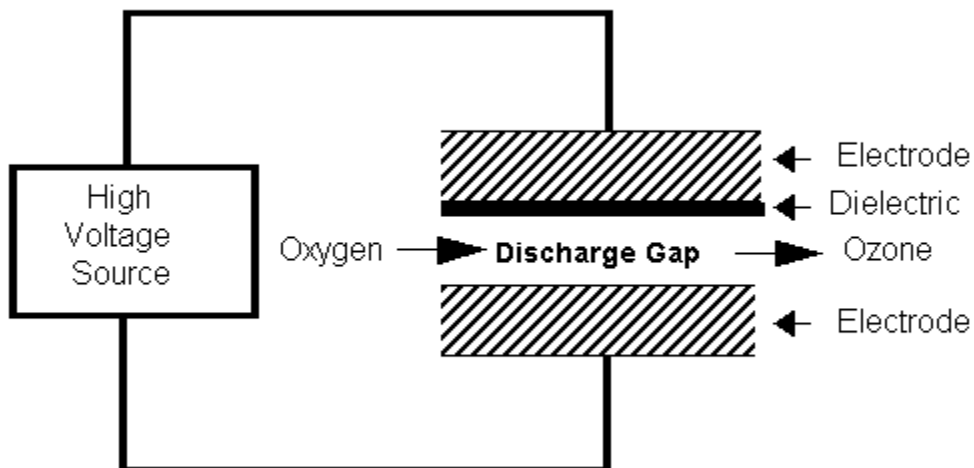


Figure 1. Basic design of a general corona discharge generator. Adapted from (66).

Ozone is the second most powerful common oxidizing agent, is not stable at normal atmospheric conditions, and will decompose rapidly in a stepwise fashion (50, 66). It first produces hydroperoxyl ($\cdot\text{HO}_2$) and hydroxyl ($\cdot\text{OH}$) radicals. The hydroxyl radical is the key

transient species because it is a chain-propagating radical, meaning as it decomposes to a nonreactive species, it creates more radicals. The final radical formed is the superoxide ($\cdot\text{O}_2^-$) radical which then decomposes to diatomic oxygen (66). Because ozone decomposes rapidly to oxygen, it leaves no residues behind, making it ideal for use in the food industry. Ozone degrades more rapidly in the aqueous state than in the gaseous state, but is more safe to work with (50). The rate of decomposition in aqueous solution depends on many different factors including temperature, pH, and organic material. Ozone gas is unable to fully react with water, thus forming a true physical solution. As the temperature of the water decreases, the solubility of ozone increases. Another way to increase solubility is the method of preparing the aqueous ozone. The most common method is to bubble the ozone into the water. The greatest solubility occurs when the bubbles are small, giving a greater surface area to contact with the water. While solubility is important to achieve a high initial concentration of ozone, stability is just as important because if the ozone degrades before it has the chance to kill the bacteria it has defeated the purpose. There are two main factors contributing to the stability of ozone in an aqueous solution: the pH of the water and the purity of the water. The pH factor states that the stability of ozone is inversely related to the pH of the solution, meaning that as the pH decreases, the stability of ozone increases. The purity factor states that the more pure the water, the greater the stability of ozone. This is explained by the fact that ozone is highly reactive, meaning that if there are readily oxidizable compounds in the water, the ozone is going to degrade faster than if the water is more pure (60, 66).

Ozone is a powerful oxidizing agent, and it is this oxidation capacity that makes ozone extremely effective in destroying a wide spectrum of microorganisms, including gram-positive bacteria, gram-negative bacteria, spores, fungi, viruses, and parasites. Many different theories

exist about the exact mechanism of antimicrobial action because ozone attacks numerous cellular components, but the cell envelope is the main theory as the primary target of bacterial ozone oxidation. As ozone degrades the cell envelope, particularly the double bonds of the unsaturated lipids and the sulfhydryl groups, the cell envelope loses its integrity, leading to leakage of the cell contents and cell lysis (50, 60, 66). Other theories involve the fact that ozone does not selectively destroy certain intracellular systems, but will cause widespread oxidation of internal cellular proteins, including nucleic acids (50).

Many different studies have been done on the efficacy of ozone on pathogenic bacteria, both *in vitro* and in a food system. Restaino et al (85) determined the antimicrobial efficacy of ozonated deionized water against 11 different organisms of both pathogenic and spoilage concern, including *Pseudomonas aeruginosa*, *E. coli* ATCC 25922, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, *L. monocytogenes*, *Enterococcus faecalis*, *Yersinia enterocolitica*, *Candida albicans*, *Zygosaccharomyces bailii*, and *Aspergillus niger*. Another factor tested was the efficacy of ozone against these organisms with and without the presence of organic material. Two different organic materials were tested, soluble starch (SS) and bovine serum albumin (BSA). The results showed that ozonated water was highly effective in killing foodborne organisms, especially *L. monocytogenes* and gram-negative bacteria both in the presence and absence of organic material. The results also showed that the type of organic material present was more influential than the amount of organic material present. When comparing residual ozone levels between 20 ppm of BSA and 20 ppm of SS, the ozone levels were significantly reduced in the presence of BSA and showed no effect in the presence of SS. Another *in vitro* study was done by Kim and Yousef (64) on *E. coli* O157:H7 and *L. monocytogenes*. This study took a known level of pathogen and added it to a known level of

ozone in aqueous solution. They found that *E. coli* O157:H7 decreased by 1.3 log units when exposed to 0.3 ppm ozone and decreased by 3.8 log units when exposed to 1.0 ppm ozone. *L. monocytogenes* had a greater rate of inactivation, with a decrease of 4.6 log units when exposed to 0.4 ppm ozone and a decrease of 5.7 log units when exposed to 0.8 ppm ozone. This study showed that the inactivation process continues until either the level of ozone is undetectable or there are no survivors.

These *in vitro* studies are important to show that ozone is successful at inactivating pathogens of concern, but are not practical. It is important to look at studies where actual food products were used because the inactivation rate will be less as the ozone will also react with the organic material. Singh et al (91) submerged lettuce inoculated with *E. coli* O157:H7 in 200 mL of aqueous ozone and agitated the mixture at 120 rpm for 10 minutes. They also tested three different inoculation methods: the dip method, where the entire lettuce leaf is dipped in the inoculum, the drop method, where 10 µL of the bacterial cocktail was placed in four spots on the leaf, and the sprinkle method, where leaves were added to a bag containing 1 mL of the cocktail and shaken for 5 minutes. The reduction for the dip method was 1.21 log units, the drop method was 2.81 log units, and the sprinkle method was 1.60 log units. The drop method had the greatest reduction, and is the method generally accepted because it most represents actual contamination in the field. Koseki et al (67) also looked at lettuce soaked in aqueous ozone for 10 minutes. This study found that 5 ppm aqueous ozone reduced background yeasts and molds by about 1 log unit, aerobic bacteria by 1.5 log units, and coliform bacteria by about 1 log unit. Kim et al (65) also tested lettuce, but looked at the differences in reducing background microflora between aqueous ozone and bubbling ozone. They found that bubbling ozone was more effective, reducing ~2 log units of the natural microflora in 3 minutes and ~4 log units in 5

minutes. Baur et al (9) tested washing lettuce with ozone in a commercial setting. This study prewashed cut lettuce heads in aqueous ozone for 120 seconds and then rinsed with tap water for 90 seconds. This resulted in approximately 1 log unit reduction. They also found that washing trimmed lettuce in ozone was not practical because of the higher ozone concentrations needed resulting from the high organic load from the release of cellular fluids. Rodgers et al (87) tested lettuce, apples, strawberries, and cantaloupe for reductions of *E. coli* O157:H7 and *L. monocytogenes* in 3 ppm aqueous ozone for log reduction times (LRT). The LRT is defined as the time it takes a sanitizer at room temperature to reduce the population of an organism by 1 log. They found that the LRT for *L. monocytogenes* in 3 ppm ozone was approximately 30 seconds for lettuce, 22 seconds for apples, 20 seconds for strawberries, and 40 seconds for cantaloupe. The LRT values for *E. coli* O157:H7 in 3 ppm ozone was approximately 30 seconds for lettuce, 21 seconds for apples, 19 seconds for strawberries, and 41 seconds for cantaloupes. When compared to the LRT values for peracetic acid, chlorine dioxide, and chlorinated trisodium phosphate, ozone had the shortest LRT values.

Although ozone is a powerful oxidizing agent and degrades to oxygen, leaving no residues behind, it is unstable and cannot be generated then stored. It is also difficult to predict how the sanitizer will behave in the presence of different organic materials. Other issues may include causing injury to the produce if excessive ozone is used (66, 77). On the positives side, ozone is inexpensive to produce, after the initial investment the only things required are water and electricity, is safe in aqueous solution, and degrades without chemical residues.

Electrolyzed Oxidizing Water. Electrolyzed oxidizing (EO) water, also known as acidic electrolyzed water, is an antimicrobial agent that is produced without added chemicals except sodium chloride (NaCl). It is produced by taking an electrolytic cell, which is a chamber

containing a positively charged electrode and a negatively charged electrode which are separated by a membrane, adding the dilute salt solution, and subjecting the system to direct current voltage (Figure 3). This process causes the negatively charged ions in the dilute salt solution, which includes chloride and hydroxide, to move to the side of the membrane with the positively charged electrode, give up electrons, and become hydrochloric acid, hypochlorous acid, hypochlorite ion, oxygen gas, and chlorine gas. This becomes the EO water with a low pH (2.3-3.0), high oxidation reduction potential (ORP, >1000mV), and containing free chlorine. The ORP of a solution indicates the ability of that solution to oxidize or reduce anything present in the solution. The higher the positive ORP value of a solution corresponds to a higher oxidizing strength (1). The amount of free chlorine depends on the amount of NaCl that was added to the solution and the type of generator used, but is on average approximately between 10 and 100 ppm (55, 63, 89). Another type of water is simultaneously produced when the positively charged ions in the dilute salt solution, which include hydrogen and sodium, move to the side of the membrane with the negatively charged electrode, accept electrons, and become hydrogen gas and sodium hydroxide. This water is referred to as electrolyzed reduced (ER) water, or alkaline electrolyzed water, and has a high pH (10.0-11.5) and low ORP (-800 to -900 mV) (55, 63).

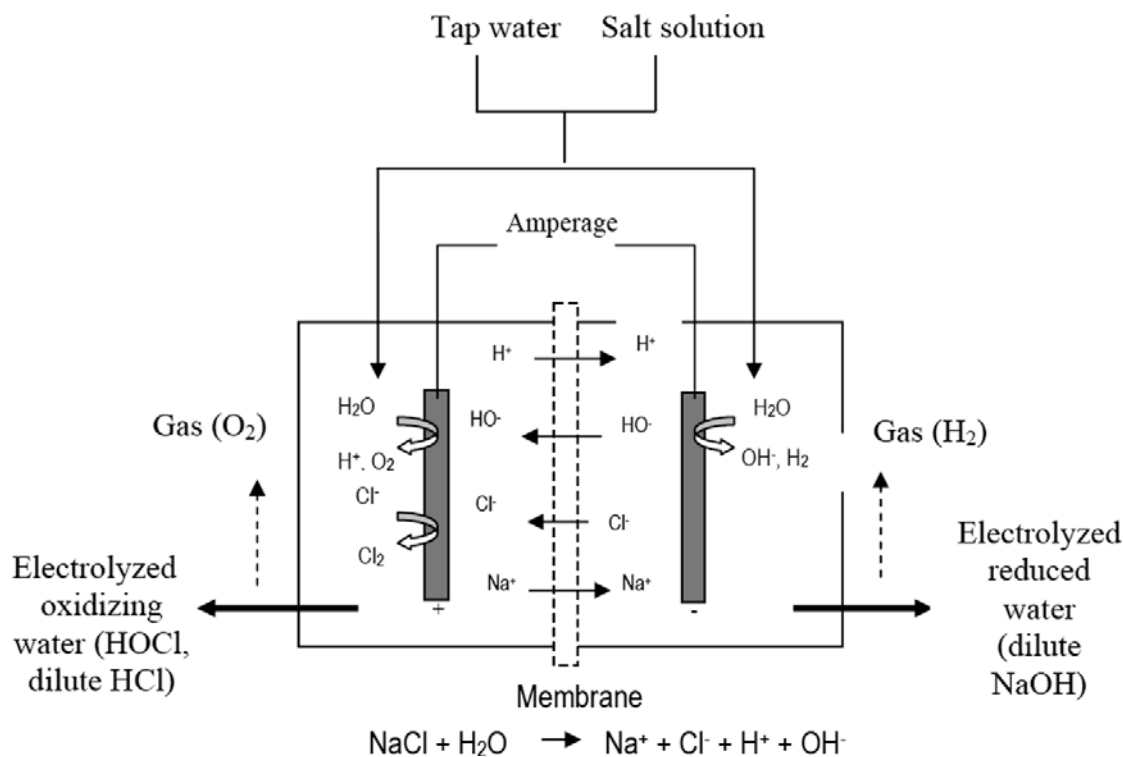


Figure 2. Basic design of an electrolyzed water generator and produced compounds. Adapted from (55).

EO water is relatively stable under most storage conditions, but will lose antimicrobial activity over time. While pH and ORP appear to remain constant, chlorine levels will drop as dissolved chlorine gas evaporates and hypochlorous acid decomposes (1). Even though EO water has both an extremely high ORP and extremely low pH, it is thought that the chlorine is the main bactericidal property. Koseki et al (67) showed that EO water was more effective than ozonated water in disinfecting lettuce even though ozonated water showed a higher ORP. They concluded that available chlorine was the main factor of antimicrobial activity, particularly hypochlorous acid. Hypochlorous acid produces hydroxyl ($\cdot\text{OH}$) radicals which act on

microorganisms. As previously stated ozone also produces hydroxyl radicals, though at a lower level than from hypochlorous acid in EO water.

EO water possesses antimicrobial activity against a wide variety of microorganisms including gram-negative bacteria, gram-positive bacteria, fungi, viruses, algae, protozoans, and nematodes. It is not yet fully understood how EO water destroys microorganisms, but it appears to be most likely due to the low pH, high ORP, free chlorine concentration, or a combination of all three. Most bacteria grow in the pH range of 4-9. The low pH of EO water may sensitize the cell envelope to the entry of hypochlorous acid into the cell. Hypochlorous acid appears to kill microorganisms by inhibiting glucose oxidation via oxidizing the sulfhydryl groups of the enzymes responsible for carbohydrate metabolism. The high ORP of EO water could also affect microorganisms since most aerobic bacteria grow at the ORP range of 200 to 800 mV while most anaerobic bacteria grow at -700 to +200 mV. The high ORP (>1000 mV) of EO water could modify ATP production and metabolic functions. Overall, oxidation can damage cell membranes leading to cell lysis and disrupt cellular metabolic processes. Liao et al (71) tested the role of ORP in how EO water destroys microorganisms. This study inoculated 100 mL of EO water with 10^{10} *E. coli* O157:H7 and then studied the bacteria under an electron microscope and fluorescence. The results showed that the ORP of EO water damages the outer and inner membranes of *E. coli* O157:H7 which will inevitably lead to the inactivation of the pathogen. Chlorine is a well known sanitizer, though the mode of action for destroying microorganisms is yet unknown. Some theories for the mode of action of chlorine include disruption of protein synthesis, oxidative decarboxylation of amino acids, inhibition of oxygen uptake, leakage of macromolecules, reactions with nucleic acids, or creation of chromosomal abnormalities. Other

redox reactions can also occur, creating toxic compounds and highly reactive radicals such as O^- , Cl^- , or OH^- which can contribute to the sanitizing effect of EO water (1, 55).

Many different studies have been done on the efficacy of EO water on pathogenic bacteria, both *in vitro* and in a food system. Kim et al (63) determined the antimicrobial efficacy of EO water against a five strain cocktail of *E. coli* O157:H7. One mL of the cocktail was added to 100 mL of EO water and left to react for 30 seconds. This study found that the EO water decreased the populations of *E. coli* O157:H7 to below the detection limit, reducing the populations by over 9 log units. Kim et al (62) repeated the same procedure in another study, but looked at the inactivation of not only *E. coli* O157:H7, but also *L. monocytogenes* and *Bacillus cereus*. Again 1 mL of the cocktail of one of the three organisms was added to 100 mL of EO water, but this time was left between 10 and 60 seconds, depending on the level of chlorine in the treatment water (10 ppm vs. 56 ppm). For *E. coli* O157:H7, populations were reduced to below the detection limit at all treatment lengths for both levels of chlorine. For *L. monocytogenes*, populations were decreased by approximately 8 log units in 30 seconds in the treatment containing 10 ppm chlorine, and was reduced to below the detection limit in all other treatment lengths for both levels of chlorine. For *B. cereus*, populations were decreased by 3.5 log units in 30 seconds and 2.6 log units in 60 seconds in the treatment containing 10 ppm chlorine, and 3.3 log units in 10 seconds and was reduced to below the detection limit in 30 seconds in the treatment containing 56 ppm chlorine. This study showed that higher concentrations of chlorine and longer treatment times were more effective in reducing the population of bacterial pathogens. Venkitanarayanan et al (105) performed a similar experiment by testing *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* against 9 mL of EO water. Again, 1 mL of each cocktail was combined with the EO water, but was then incubated in a water bath at either 4°C,

23°C, 35°C, or 45°C for varying lengths of time. This study found that the pathogens were more readily inactivated at the higher temperatures (35°C and 45°C) than at the lower temperatures (4°C and 23°C). Within 2 minutes at 35°C, all three pathogens were reduced to below detection limits (approximately 8 log units reduction), and within 1 minute at 45°C both *E. coli* O157:H7 and *L. monocytogenes* were reduced to below detection limits (approximately 8 log units reduction) while *Salmonella* was reduced by approximately 7 log units.

Again, while these *in vitro* studies show how effective EO water is at inactivating pathogens, it is a very different scenario when fresh produce is introduced. Bari et al (7) tested EO water against *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* on tomatoes. Inoculated tomatoes were added to a bag containing 20 mL of EO water, thoroughly rubbed by hand for 20 seconds, 200 mL of sterile distilled water was then added to the bag, and then rubbed for an additional 20 seconds. The EO water produced in this study had a moderate level of chlorine, approximately 30 ppm. This study found approximately a 7.7 log units reduction in *E. coli* O157:H7, a 7.4 log units reduction in *Salmonella*, and a 7.6 log units reduction in *L. monocytogenes*. Koseki et al (67) tested EO water also containing approximately 30 ppm chlorine against native microflora on lettuce. This study found that that EO water reduced viable aerobic bacteria by 2 log units in 10 minutes, reduced coliform bacteria to a level below 10^2 , and reduced yeast and mold populations by 1.5 log units. They also took scanning electron microscope pictures of the treated lettuce and found that the quality of the lettuce is not affected by treatment with EO water. Park et al (78) looked at inactivating *E. coli* O157:H7 and *L. monocytogenes* on lettuce using EO water with 45 ppm chlorine. The inoculated leaves were treated by immersing the leaf in 1.5 L of EO water and agitating at 100 rpm for either 1 or 3 minutes. The results were a 5 log units reduction of *E. coli* O157:H7 at both time periods, and a

5.1 log units reduction in *L. monocytogenes* after 1 minute and a 5.6 log units reduction after 3 minutes, with no significant difference between the two times. This study also tested lettuce quality characteristics via three expert evaluators and found that treatment with EO water containing 45 ppm chlorine did not significantly affect quality characteristics. Most recently, Hung et al (56) tested the efficacy of EO water on the inactivation of *E. coli* O157:H7 on strawberries and broccoli. This study treated the produce by immersing strawberries in 125 mL of EO water and broccoli in 90 mL of EO water at either 4°C or 24°C, for 1 or 5 minutes, and at one of two different chlorine concentrations (23 vs. 54 for strawberries and 54 vs. 98 for broccoli). The greatest reduction for strawberries was at 54 ppm chlorine, 4°C, and 5 minutes, which had a reduction of 1.28 log units. Broccoli had the greatest reduction in the solution containing 98 ppm chlorine, at 4°C for 5 minutes. This combination gave a reduction of 1.78 log units.

There are few downsides to using EO water. The main disadvantage is the high initial cost. EO water can also rust some metals and will degrade over time. The many advantages for using EO water include easy generation, no added chemicals except common table salt, the fact that EO water is environmentally friendly since it reverts to normal water after use, has low chlorine while still being highly effective, and has a relatively low cost after initial investment (1).

Commercial Produce Washes. There are numerous commercial produce washes on the market, ranging from all natural, to organic, to surfactant solutions, to mineral acids, to alkaline washes, to combinations of the above (89). The ingredients of these washes tend to be GRAS, incorporating plant extracts with surfactants. Some of the common washes on the market include Fit[®] Fruit and Vegetable Wash (HealthPro Inc), Veggie Wash[®] Fruit and Vegetable

Wash (Beaumont Products), Fresh Produce Wash (Vegetec International Ltd), Eat Cleaner™ Fresh Produce Wash (Grow Green Industries Inc), Environne™ Fruit and Vegetable Wash (Consumer Health Research Inc), and Ultimate Fruit and Vegetable Wash (Punati Corp). Fit® Fruit and Vegetable Wash is made from 100% all natural ingredients including purified water, oleic acid, glycerol, ethyl alcohol, potassium hydrate, baking soda, citric acid, and distilled grapefruit oil. Veggie Wash® Fruit and Vegetable Wash contains water, natural cleaners made from corn and coconut, lemon oil, sodium citrate, and glycerin. Fresh Produce Wash contains fatty acid saccharose esters, glutamate, monosodium citrate, and glycerol.

While these commercial produce washes are not universally used, it is important to note that these are commonly applied in the home. Beuchat et al (15) tested Fit against tomatoes inoculated with *Salmonella* or *L. monocytogenes*. The researchers attempted a technique that is similar to home use; they sprayed the inoculated tomato with the sanitizer, rubbed by hand for 30 seconds, then rinsed. The results showed that for *Salmonella*, the Fit treatment reduced counts by over 6.7 log units. For *L. monocytogenes*, the Fit treatment reduced counts by 5 log units. This study showed that Fit has an extremely high antimicrobial activity against both of these pathogens. Harris et al (52) also tested the efficacy of Fit on tomatoes inoculated with *Salmonella*. This study also utilized the spray technique to wash the produce. The results showed that Fit was more effective than washing in water alone, as the Fit wash removed approximately 3 log units more pathogens than the water wash. Park et al (79) tested liquid and powdered Fit in flume water for a 5 minute wash on the native microflora, inoculated *E. coli* O157:H7, and inoculated *Salmonella* counts on potatoes. The powdered Fit reduced *E. coli* O157:H7 levels by 1.44 log units, while the liquid Fit reduced the population by 1.52 log units. The powdered Fit reduced *Salmonella* levels by 1.36 log units, while the liquid Fit reduced the

population by 1.83 log units. The powdered Fit reduced aerobic bacteria levels by 1.03 log units, while the liquid Fit reduced the population by 1.20 log units. This study showed that Fit was effective in reducing microbial populations, and showed a significantly greater reduction rate than water. Beuchat et al (17) tested the efficacy of Fit on alfalfa seeds inoculated with *Salmonella* or *E. coli* O157:H7. This study soaked the seeds in 40 mL of the solution for 15 or 30 minutes. The results showed that Fit reduced the population of the *Salmonella* by 2.3 log units, and of *E. coli* O157:H7 by more than 5.4 log units. Burnett et al (20) also tested Fit, but focused on *L. monocytogenes* on lettuce. For the washing treatment, the lettuce was submerged in 50 mL of solution and allowed to soak for 5 minutes prior to analysis. The results of this study showed that there was a 1.51 log units reduction in *L. monocytogenes* on lettuce treated with Fit. Kilonzo-Nthenge et al (61) tested the efficacy of Veggie Wash against *L. innocua* (a *L. monocytogenes* surrogate) on tomatoes, broccoli, lettuce, and apples. The produce was soaked in the Veggie Wash solution for 2 minutes and then rinsed with tap water for 15 seconds. Results showed a reduction of 2.89 log units for tomatoes, 1.50 log units for broccoli, 1.73 log units for lettuce, and 2.28 log units for apples. While the Veggie Wash treatment was significantly different than a 15 second water rinse for tomatoes, there was no significant difference for broccoli, lettuce, or apples. Hellstrom et al (54) tested the efficacy of Fresh Produce Wash against lettuce inoculated with *L. monocytogenes*. The lettuce in this study was first pre-rinsed with tap water for 30 seconds and then soaked in the treatment for 60 seconds. The results showed that the Fresh Produce Wash treatment reduced the bacterial load by approximately 1 log unit. While this is not a very large reduction, the study did find that it was significantly different from the water wash, which only reduced the bacterial load by approximately 0.5 log units.

The results these commercial produce washes give in the home will be slightly different than what is found in a laboratory. One main issue to point out is that the majority of these washes are primarily surfactants, meaning that they wash the pathogen off rather than killing the pathogen. This is a key factor when using in the home, as the residual water has the potential to cross-contaminate other surfaces or foods if not disposed of properly. While these washes tend to be more on the expensive side, they are also usually made from natural ingredients, making them safe for human consumption and the environment.

Chlorine-Based Sanitizers. Chlorine bleach is the most commonly used sanitizer in the food industry (77, 89). Common chlorine-based sanitizers include liquid chlorine, hypochlorites, and both organic and inorganic chloramines. The main antimicrobial compound in chlorine-based sanitizers is hypochlorous acid, which, as stated before, produces hydroxyl ($\cdot\text{OH}$) radicals. These hydroxyl radicals act on a broad spectrum of microorganisms, acting on microbial membranes, inhibiting cellular enzymes, and oxidizing cellular proteins and nucleic acids. The antimicrobial activity of chlorine depends on the amount of hypochlorous acid that is free to come in contact with microorganisms. The amount of hypochlorous acid present is a function of pH, where the lower the pH, the greater the hypochlorous acid concentration. At pH 5 the vast majority of chlorine in the solution is in the form of hypochlorous acid, while at pH 7 only approximately 75% is hypochlorous acid. Hypochlorous acid concentration is also affected by temperature, where maximum solubility in water is around 4°C. While this is the temperature where there is the highest concentration of hypochlorous acid, it is not recommended to wash produce at this low of a temperature. When the temperature of the wash water is lower than the temperature of the produce item, there is a temperature-generated pressure differential and water will be sucked into the produce item, along with any contaminants in the water. Organic

material is another factor that will affect the hypochlorous acid concentration. Organic material, such as materials leaching from cut produce surfaces, will react with the hypochlorous acid, decreasing the available chlorine and the overall antimicrobial activity. The maximum allowable level for washing applications without a rinse step is 200 ppm available chlorine, and chlorine products are generally used in the 50 to 200 ppm range. The average exposure time is 1 to 2 minutes (77, 90).

Many different studies have tested the effectiveness of chlorine washes, and compared chlorine washes with newer technologies. Pirovani et al (83) looked at the reduction of native microflora on fresh-cut spinach at three different chlorine concentrations and for three different soak times. The three different chlorine concentrations were 125, 75, and 25 ppm, while the three different soak times were 8, 5, and 2 minutes. This study found that the greatest reduction was the combination of 75 ppm for 8 minutes, with a reduction of 3.68 log units. The overall conclusion of this study showed that initial chlorine concentration does not affect the population reduction when washing times are long, but for short washing times the higher the chlorine concentration, the greater the reduction. Behrsing et al (11) tested the efficacy of two different initial chlorine concentrations and three different soak times in reducing *E. coli* TGI (an *E. coli* O157:H7 surrogate) on cos lettuce and broccoli. The chlorine concentrations used were approximately 50 and 100 ppm while the three different soak times were 30 seconds, 2, and 5 minutes. For lettuce, the greatest reduction of the bacterium was during the 5 minute dip in both 50 and 100 ppm chlorine solutions, where the reduction was 2.67 log units. The researchers showed that for cos lettuce, when dipped in a chlorine solution of 50 ppm or greater for 30 seconds or longer would reduce *E. coli* populations by between 1.9 and 2.8 log units. When looking at broccoli, this study found that broccoli was much more difficult to clean, as there was

no significant difference between either of the chlorine solutions or water after washing for 30 seconds. The greatest reduction of the bacterium for broccoli was during the 5 minute dip in the 100 ppm chlorine solution, where the reduction was 2.54 log units. The researchers noted that this difference between the two commodities was due to broccoli's thick waxy cuticle which repels water. Albrecht et al (2) also looked at washing broccoli in a 50 ppm chlorine solution for 2 minutes. This study found that the chlorine wash reduced coliform populations by approximately 1 log unit, but only slightly reduced the aerobic microorganism populations. Wei et al (106) tested rifampicin resistant *Salmonella* Montevideo against chlorine solutions both *in vitro* and on tomatoes. The *in vitro* study looked at the effect of organic material on the efficacy of the chlorine solution. The organic material was either tryptic soy broth (TSB) or Butterfield's buffer. This section of the study looked at three different chlorine concentrations (50, 75, and 100 ppm), and three different incubation times (30 seconds, 1, and 2 minutes). This part found that with increasing inoculation concentrations, a higher chlorine concentration and longer soak time is required to eliminate all the bacteria, no matter what the organic material. The second part of this study looked at the efficacy of a 100 ppm chlorine solution on inoculated tomatoes at three different soak periods (30 seconds, 1, and 2 minutes). This part of the study also looked at the effect of organic material, testing a culture in water vs. a culture in TSB. The culture in water appeared to be eliminated more easily than the culture in TSB, since the culture in TSB survived and/or grew during storage (20 hours). While the culture in water showed significantly lower counts than the culture in TSB, none of the bacteria was completely eliminated. For the culture in water, the bacterial counts following the chlorine treatment were significantly lower than the counts following a similar tap water treatment; while for the culture in TSB, there was no significant difference between the chlorine wash and the water wash. Zhuang et al (107) also

tested the efficacy of a chlorine treatment on tomatoes inoculated with *Salmonella* Montevideo. This study compared 2 minute chlorine treatments at 50, 100, 200, and 300 ppm, and found a significant reduction in bacterial populations after the 50 and 100 ppm treatments which reduced bacterial populations by approximately 0.8 and 1.4 log units, respectively, but the further increased concentrations of chlorine did not result in a further reduction. The 100, 200, and 300 ppm treatments were not significantly different, and resulted in an average reduction of approximately 1.5 log units. Parnell and Harris (80) tested apples inoculated with *Salmonella* in a similar manner to those tests performed on tomatoes. This study treated the apples by placing them in a sterile bag, adding 5 mL of 200 ppm chlorine solution, rubbing for 5 seconds, rinsing with 200 mL of water, and then drying with a paper towel. This resulted in a reduction of approximately 5.2 to 6.2 log units. They did note that the steps involving rinsing with 200 mL of water then drying with a paper towel increased the reduction of the pathogen by 0.5 log units. This is a small, but significant reduction.

Cantaloupes are a commonly contaminated commodity, thus there are numerous studies performed to optimize cantaloupe sanitation. Parnell et al (81) tested cantaloupes inoculated with an avirulent strain of *Salmonella* Typhimurium against a 200 ppm chlorine solution. After inoculation, the melons were placed in a sterile bucket and 1500 mL of the chlorine solution was poured over the melon and allowed to soak for 60 seconds. The results showed a reduction of the *Salmonella* population by 1.8 log units, with an additional 1 log unit reduction when scrubbing was added. The researchers determined that the small reduction in bacterial populations was due to the protection provided by the netting of the rind, plus the organic matter on the rind contributed to a decrease in concentration of chlorine, which decreased overall effectiveness of the wash. Barak et al (6) also tested cantaloupes inoculated with *Salmonella*,

washing with a 150 ppm chlorine solution for 20 seconds with a 2 minute tap water rinse. This study found that the chlorine treatment removed over 92% of the pathogen. While this study had a high reduction rate, it should be pointed out that the initial inoculum was low, approximately 10^4 CFU/mL and used an impractically long rinse step.

The Eastern Regional Research Center in Pennsylvania has performed numerous evaluations on cantaloupe-among other produce items. In 2006, Ukuku and Fett (98) tested cantaloupes inoculated with 16 different serovars of *Salmonella*, washing with a 200 ppm chlorine solution. The melons were washed by completely submerging the melon in the treatment for 5 minutes. This study found approximately a 3 log units reduction in melons treated within 40 minutes of inoculation, while melons held longer after inoculation showed reductions of *Salmonella* populations of approximately 2.4 log units. Ukuku and Sapers (100) also tested cantaloupes inoculated with *Salmonella*, but used a commercial level of 1,000 ppm for the 5 minute chlorine wash. This study found that cantaloupes that were recently inoculated (0 to 24 hours) had a reduction of 3.4 log units, while, as in the previous study, the efficacy of the chlorine treatment was less for the inoculated cantaloupes that had been stored for longer periods of time (72, 120, and 144 hours). Two other points this study showed are that *Salmonella* will survive attached to cantaloupe surfaces for at least 6 days, and that *Salmonella* will transfer from contaminated rinds to the edible portion of the melon. In 2001, Ukuku et al (99) also looked at testing inoculated cantaloupes with the commercial level of 1,000 ppm chlorine wash for 5 minutes. This study focused on *E. coli* ATCC 25922 (an *E. coli* O157:H7 surrogate) and also looked at results of storage length. When washed 24 hours after inoculation, the chlorine treatment resulted in a 3.2 log units reduction in the inoculated bacterium. Just as in the other studies, the longer the washing treatments were after inoculation, the more difficult it

became to remove the bacterium. After the maximum hold time for this study, 120 hours, the chlorine treatment was ineffective. These results show that the longer the inoculum has to attach to the surface, the stronger that attachment will be, possibly even resulting in biofilm formation. Another study done by Ukuku and Fett (96) focused on *L. monocytogenes* inoculated cantaloupes. While this study also looked at the commercial chlorine level of 1,000 ppm, the wash was for only 2 minutes. When washed after 24 hours after inoculation, the chlorine treatment resulted in approximately a 3 log units reduction of the pathogen. The main difference between this study and the previous studies was, when the inoculated cantaloupe was held longer prior to treatment for *Salmonella* and *E. coli* it became more difficult to remove the pathogens, but with *L. monocytogenes* it actually became easier.

Chlorine treatments are the most accepted method of sanitizing produce, so when a new treatment is undergoing testing, it is common to compare its efficacy to that of a similar chlorine treatment. Baur et al (9) compared rinsing lettuce in two different chlorine treatments with an ozone treatment. Chlorine treatment #1 was with a 100 ppm rinse for 90 seconds, chlorine treatment #2 was with a 200 ppm rinse for 120 seconds followed by rinsing with tap water for 90 seconds. The ozone treatment was with 1 mg/L ozone for 120 seconds followed by rinsing with tap water for 90 seconds. The simple chlorine treatment (#1) resulted in a reduction of background microflora of approximately 0.7 to 1.5 log units. The second chlorine treatment resulted in a greater reduction than the simple chlorine treatment, but the ozone treatment was not as effective as either of the chlorine treatments. The samples treated with ozone showed similar reduction rates to those rinsed in only water, which reduced counts by approximately 0.5 log units. One other item this study showed was that the lettuce that was rinsed with chlorine led to the lowest bacterial counts throughout the 9 days of storage. Bari et al (7) compared the

effectiveness of chlorine to EO water. This study looked at *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on tomatoes. An inoculated tomato was placed in a sterile bag containing either 20 mL of EO water (with a chlorine concentration of 30 ppm) or 20 mL of a 200 ppm chlorine solution, rubbed by hand for 20 seconds, then rinsed by adding 200 mL of sterile distilled water and again rubbing for 20 seconds. For *E. coli* O157:H7, the reduction of the pathogen from the chlorine treatment was approximately 4.6 log units while the reduction from the EO treatment was approximately 7.7 log units. For *Salmonella*, the reduction of the pathogen from the chlorine treatment was also approximately 4.6 log units while the reduction from the EO treatment was approximately 7.4 log units. For *L. monocytogenes*, the reduction of the pathogen from the chlorine treatment was again approximately 4.6 log units while the reduction from the EO treatment was approximately 7.6 log units. The results of this study showed that for all three of the main foodborne pathogens of concern, EO water was more successful at reducing bacterial numbers than the classic chlorine wash. Park et al (78) also looked at comparing the efficacy of EO water to a chlorine treatment. This study used lettuce inoculated with *L. monocytogenes* or *E. coli* O157:H7. The treatments involved immersing the inoculated leaves into the treatment solution for 1 or 3 minutes while shaking at 100 rpm. The chlorine concentration used was 45 ppm while the EO water also contained 45 ppm available chlorine. While both treatments significantly reduced the bacterial pathogen numbers, there was no significant difference between chlorinated water and EO water for either of the wash times. This study showed that EO water is at least equally effective in reducing numbers of *L. monocytogenes* and *E. coli* O157:H7 on lettuce. Koseki et al (67) tested the efficacy of EO water and ozone compared to chlorine on the background microflora of lettuce. The lettuce was soaked in 2,000 mL of treatment solution for either 1, 5, or 10 minutes. The available chlorine in the chlorine treatment

was 155 ppm, while only 30 ppm for EO water. The ozone concentration was 5 ppm for the ozone treatment. The results of this study showed that the EO water and the chlorine treatment reduced the aerobic bacteria population by 2 log units after treatment for 10 minutes, while for the same treatment time ozone reduced the aerobic bacteria population by only 1.5 log units. All treatments reduced coliform bacteria populations to below the detection limit. Yeast and mold populations were decreased by 1.5 log units for both the EO water and chlorine treatments, while only by 1 log unit for the ozone treatment. This study showed again that an EO water treatment is comparable to a chlorine treatment in reducing background microflora on lettuce. In 2001, Beuchat et al (15, 17) completed two different studies to compare a commercial produce wash against a chlorine treatment. The first study looked at reducing the counts of *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. The inoculated alfalfa seeds were added to 40 mL of either the commercial produce wash (Fit) or a 200 ppm chlorine treatment and placed on a rotary shaker (100 rpm) for either 15 or 30 minutes. *Salmonella* populations were reduced by 1.9 log units after treatment in a 200 ppm chlorine solution for 30 minutes while the populations were reduced by 2.3 log units when washed in Fit for either 15 or 30 minutes. *E. coli* O157:H7 populations were reduced by 1.3 log units after treatment in a 200 ppm chlorine solution for 30 minutes while the populations were reduced by more than 5 log units when washed in Fit for either 15 or 30 minutes. This study showed that washing alfalfa seeds in Fit was equivalent to washing in a 2,000 ppm chlorine solution (17). The second study looked at reducing the counts of *Salmonella* or *L. monocytogenes* on tomatoes. This study used a spray method of washing; each inoculated tomato was sprayed five or six times with either a commercial produce wash (Fit) or a 200 ppm chlorine solution. The tomato was sprayed over the entire surface, placed in a sterile bag, rubbed by hand for 30 seconds, and then rinsed by adding 200 mL of sterile distilled water and agitating

for an additional 30 seconds. The results of this study found that for *Salmonella*, the chlorine treatment reduced counts by 3.1 log units while Fit reduced counts by over 6.7 log units. For *L. monocytogenes*, the chlorine treatment reduced counts by 4.2 log units while Fit reduced counts by 5 log units. This showed that Fit appeared to exceed antimicrobial activity over a 200 ppm chlorine solution. Another point this study found was that overall, *L. monocytogenes* appeared to be more tolerant than *Salmonella* to both treatments (15).

While chlorine is a commonly used and effective sanitizer, there are many disadvantages to using this chemical. Chlorine can irritate skin and damage mucous membranes if not properly used. When chlorine combines with organic compounds in food, toxic by-products are formed. These by-products include trihalomethanes and haloacetic acids. These compounds are mutagenic and carcinogenic, and can be released in drinking water which can adversely affect the public as well as the environment. Also, low levels of chlorine, such as the levels safe for consumption, may not be effective against certain bacteria or other foodborne pathogens. Another issue can occur as chlorine may cause an altered taste and/or odor in treated products. While these concerns are major and valid, it is also important to note that the lack of a suitable sanitizer would result in increased cases of foodborne illness and possibly loss of lives. For that reason it is important to continue chlorine use until a more suitable and effective sanitizer is realized (50, 65, 77, 90).

CHAPTER 3

INFLUENCE OF WATER HARDNESS AND PH ON THE EFFECTIVENESS OF HOME WASHING TECHNOLOGIES

Introduction

Hard water levels vary across the entire nation as shown in Figure 3. Water hardness is defined as the concentration of minerals in water. The higher hard water level corresponds to higher mineral concentrations (94). These minerals are impurities in the water, and will change the way sanitizers and other washing treatments behave (90). These changes could include inactivating the chemicals in the sanitizer and buffering the pH, both of which may diminish the effectiveness of the sanitizer (74). Calcium and magnesium tend to be the most common minerals in water, and also have the greatest adverse effect on certain sanitizers (68, 86). When beginning to test the efficacy of home washing technologies, it is important to determine whether differences in hard water will have a deleterious effect or not.

Ozone solubility is dependent on certain factors, such as temperature, water quality, and pH (65). Ozone degrades very rapidly in impure solutions, thus it is crucial to test the efficacy of ozone in hard water such as found in homes around the country. Ozone has a greater stability when the pH is lower, and the stability will decrease as the pH increases. Ozone is also less soluble and decomposes faster the higher the temperature of the water (50, 60, 66). It is important to determine the effect of the testing solution because the greater the solubility of the ozone and the longer the ozone remains stable, the greater the opportunity to inactivate the

pathogen. The objective of this study is to determine whether the quality of the incoming water (degree of hardness and pH) has an effect on the efficacy of various home washing technologies.

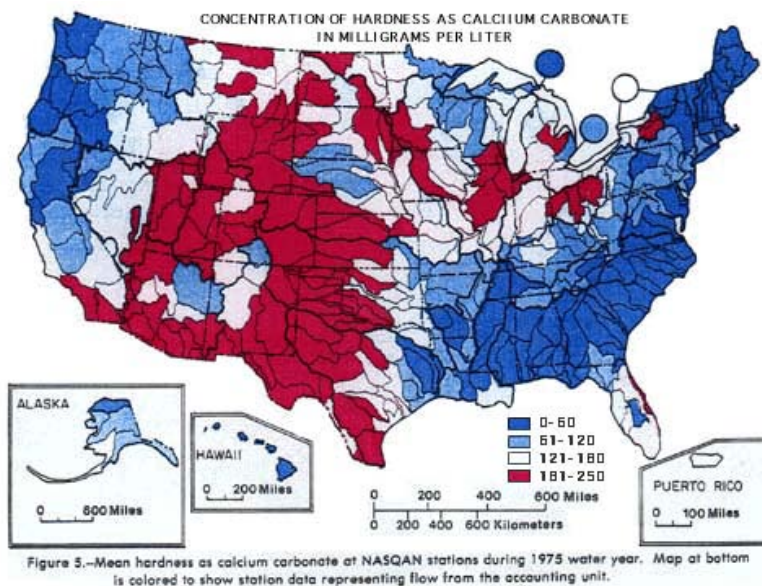


Figure 3. Map of national mean hardness levels in 1975. Adapted from (18).

Materials and Methods

Bacterial Strains. Five strains of *Salmonella enterica* were used in this study. A list of all five strains, reference numbers, and sources can be found in Table 1. Prior to use, each strain was made resistant to 100 µg/mL rifampicin (Sigma-Aldrich Chemical Co., St. Louis, MO).

Rifampicin is an antibiotic that kills bacteria by inhibiting the bacterial RNA polymerase (21).

By using antibiotic resistant strains of bacteria, this allowed for consistent recovery of the

inoculated pathogens rather than competing microflora (14, 33). The concentration of 100

µg/mL rifampicin was selected because this was the lowest concentration that produced

spontaneous mutations while inhibiting the background microflora (76, 88). The stock

rifampicin was prepared by dissolving 5 grams of the initial powder form of the antibiotic into

66.66 mL of dimethylsulfoxide (DMSO; Sigma-Aldrich). This created a solution with a concentration of 75 mg/ml rifampicin. This solution was then further diluted by adding 27 μ L to 500 mL of tryptic soy agar (TSA; Difco Laboratories, Becton Dickinson, Sparks, MD) producing plates containing 100 μ g/mL rifampicin. The stability of rifampicin in DMSO is at least eight months, and at least 6 days with no loss of activity when mixed with agar and stored at 15°C (59). Initial stock wild-type cultures were streaked onto TSA containing 100 μ g/mL rifampicin (TSA-R100), incubated at 37°C for 24 h, and then checked for spontaneous mutations. These mutated colonies were then tested to ensure correct strain, similar growth as wild-type strain, and lack of antibiotic dependence (14, 93). Stock cultures were maintained at -80°C on Microbank™ Bacterial and Fungal Preservation System beads (Pro-Lab Diagnostics, Round Rock, TX).

TABLE 1. List of *Salmonella enterica* strains used in this study.

Strain	Reference Number	Source
<i>S. Bailden</i>	Not Available	Tomato associated outbreak
<i>S. Montevideo</i>	G4639	Tomato associated outbreak
<i>S. Poona</i>	01A3923	Cantaloupe associated outbreak
<i>S. Stanley</i>	H1256	Alfalfa sprouts associated outbreak
<i>S. Typhimurium</i>	DT 104:H3380	Clinical Isolate

Preparation of Inoculum. Prior to use, each strain was subcultured 3 times in tryptic soy broth (TSB; Difco) containing 100 μ g/mL rifampicin (TSB-R100) at 37°C for 24 h. Each strain was centrifuged at 6,500 rpm and 15°C for 15 minutes (Beckman Coulter Allegra 21R Refrigerated High Speed Table Top Centrifuge). The pellet was then washed once in 0.1% peptone water (PW; Difco) and resuspended in 3 mL of PW to obtain approximately 10¹⁰ CFU/mL. This high

of concentration of the bacterial cocktail is necessary to be able to determine the expected log reduction (14). Equal volumes (1 mL) of each strain were combined to form a 5 strain cocktail.

Produce. Unwaxed tomatoes (*Solanum lycopersicum*; 142 g – 265 g, mean weight 198 g) were purchased from a local grocery store and stored at 4°C for no longer than 7 days after purchase. Each piece of produce was inspected to ensure no rotten or damaged spots. Prior to inoculation each tomato was rinsed under running deionized water (DW) for 15 seconds to remove residual organic material. Three tomatoes were treated together and considered one replication.

Produce Inoculation. Each tomato was placed end side up on a sterile holder for inoculation. Each tomato was inoculated with 50 µL of the bacterial cocktail in 10 spots around, but carefully avoiding, the blossom scar. The fruit was then dried in a laminar flow class II biosafety hood at 22°C for 1 h to allow for attachment (14-15, 69, 78). The fruit was inoculated no earlier than 2 hours prior to use. The bacterial cocktail was held at 4°C between uses.

Hardness Levels. Two different hardness levels were tested. Soft water (0 parts per million [ppm] hardness) was represented by deionized water. Hard water (200 ppm hardness) was made by combining calcium carbonate (CaCO₃; J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, NJ) with magnesium carbonate (MgCO₃; Fisher Scientific, Fair Lawn, NJ) in a 3:1 ratio. 150.0 mg of CaCO₃ plus 50.0 mg of MgCO₃ were added to 1 L of DW, mixed thoroughly, and kept at 15°C for at least two days prior to use. To assist the dissolving of the minerals, the pH was first dropped to 5 by adding distilled white vinegar (0.5% acidity, Publix[®], Lakeland, FL) and then returned to pH 8 by adding 0.1 N sodium hydroxide (NaOH; J.T. Baker).

An additional test to determine the effect of combined hardness and pH was performed for ozone. The hardness levels were the same as previously described, while the pH levels were pH 5 and 8. Both pH values were achieved as previously described.

Treatments. Four treatments were tested to determine the effect of hardness: ozonated water, electrolyzed oxidizing water, dilute chlorine bleach water, and Veggie Wash[®] fruit and vegetable wash. All treatments were run at $15^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Ozonated water was produced by using the Lotus Sanitizing System (Model LSR 100, Tersano Int., Buffalo, NY). Initial ozone concentrations were determined via the Hach Indigo Colorimeter method (Hach Co., Loveland, CO). pH was determined by using a Thermo Scientific Orion combination pH electrode on an Orion 3-Star Plus Benchtop pH/mV Meter (Thermo Scientific, Beverly, MA). Temperature was determined by using a Fisher Scientific Traceable[®] Memory/Waterproof Thermometer (Fisher Scientific).

Electrolyzed oxidizing (EO) water was produced by using a Bion-Tech EO generator (BTM-3000, Bion-Tech Co., Seoul, South Korea). The company provided a standardized scoop which held approximately 0.85 g of table salt (NaCl; Publix[®] table salt) which is required in order to properly produce acidic EO water. Treatment water and one level scoop of salt were added to both chambers of the unit. After a generation time of 20 min, 2 L of water was collected from the acidic chamber. Acidic EO water (4 L) was generated on each test day, no earlier than 1 h prior to treatment. pH and temperature were determined as previously described. Oxidation reduction potential was determined by using an Epoxy Sure-Flow Combination Redox/ORP Electrode also with the Orion meter (Fisher Scientific). Free chlorine was determined by using an Iodine-Chlorine Kit (#101; Ecolab Center, St. Paul, MN).

Dilute chlorine bleach water was prepared by combining 4 to 6 mL of household bleach containing 6.0% sodium hypochlorite (Clorox Co., Oakland, CA) with 3.785 L (one gallon) of treatment water to obtain approximately 65 ppm free chlorine. Temperature, ORP, and free chlorine were determined as previously described.

Veggie Wash[®] fruit and vegetable wash was prepared according to the manufacturer's directions. 60 mL (approximately ¼ cup) of the Veggie Wash solution was added to 3.785 L of treatment water and thoroughly mixed to ensure homogeneity. Temperature was determined as previously described.

Treatment Exposure. Ozonated water was prepared just prior to use to ensure maximum contact time between the ozone and the pathogens. 3 L of treatment water was added to the sterilized bowl and veggie retainer system provided with the equipment. The three inoculated tomatoes were added to the water, submerged via the veggie retainer, and the unit was started. The cycle took approximately 3 min until the digital display reached 100%, and then was followed by a 2 min hold period. The tomatoes were removed from the water using sterilized tongs within 30 s of the end of the hold period and immediately placed into a 55 oz Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 50 mL of sterile neutralizing buffer. The neutralizing buffer used in this study was formulated to provide the concentration of chlorine neutralizing agents in Dey-Engley broth: 1 g of sodium thioglycolate (Sigma Aldrich), 6 g of sodium thiosulfate (Acros Organics, Beverly, MA), and 2.5 g DW of sodium bisulfite (Acros Organics) were added to 1 L of DW.

EO water was prepared no earlier than 1 hour prior to use. 3.785 L of treatment water were added to a sterilized 1.5 gallon stainless steel bowl, the three inoculated tomatoes added, submerged via a sterile Ziploc[®] freezer bag (Double zipper freezer bags, S.C. Johnson, Racine, WI) containing DW, and left to soak for 2 min. After the 2 min hold time the tomatoes were removed and placed in the neutralizing buffer as previously described.

Dilute chlorine bleach water was prepared immediately prior to use to ensure maximum free chlorine levels. Tomatoes were treated as previously described.

Veggie Wash[®] was prepared immediately prior to use. Tomatoes were treated as previously described.

Microbial Analysis. Uninoculated and treated tomatoes were hand rubbed for 2 min prior to analysis (14, 69). A single person was responsible for rubbing all tomatoes in every experiment in order to reduce variability of the pressure and intensity of the rub. The rinse solution was then tested for residual pathogens. This solution was serially diluted (1:10) in PW and then surface plated in duplicate on TSA-R100 using the spread plate technique. Plates were incubated at 37°C for 24 h prior to enumeration. Presumptive colonies were randomly selected using Harrison's disc method (51), streaked on Xylose Lysine Desoxycholate (XLD) agar, and incubated at 37°C for 24 h to confirm presence of *Salmonella*.

Residual Treatment Water. Residual treatment water was tested for the presence of the pathogen. Treatment water (1 mL) was serially diluted (1:10) in PW and surface plated in duplicate on TSA-R100 using the spread plate technique. For treatments resulting in low bacterial counts, 1 mL of treatment water was added to 9 mL of TSB-R100 and incubated at 37°C for 48 h for enrichment. Positive enrichments were confirmed by streaking on TSA-R100.

Statistical Analysis. Three tomatoes were treated together to generate one replication. Three replications were performed per treatment. Final counts (CFU/sample) were analyzed after log transformation. Significant differences between means were determined by Analysis of Variance and Tukey's comparison method in Minitab[®] Statistical Software (Minitab Inc., State College, PA) and reported at a significant level of $\alpha = 0.05$.

Results/Discussion

Rifampicin-resistant colonies recovered after treatments were attributed to the *Salmonella* inoculation, as background counts from uninoculated samples (negative controls) were tested

against TSA-R100 to ensure no background microflora was rifampicin resistant. Reductions in *Salmonella* counts after treatments were relative to the populations of inoculated pathogens on untreated samples (positive controls). The inoculation level used in this study exceeds the typical natural contamination level in order to ensure valid observation of bacterial reductions after the different washing techniques. All treatments produced a significant pathogen reduction when compared to the untreated inoculated controls ($P < 0.05$).

While it is well known that water hardness differs in homes around the country, it is not well researched whether these differences in water hardness will impact the efficacy of home use produce washing treatments. The calcium and magnesium in hard water can react with chemicals in the washing treatments, leaving a lower concentration of the chemicals available to react with the microorganisms. The calcium and magnesium in hard water can also buffer the pH of the sanitizer. This is a problem for treatments such as aqueous ozone, electrolyzed oxidizing (EO) water, and dilute chlorine bleach as all of these treatments have a greater effectiveness at lower pH values (74). The influence of hardness and pH on the effectiveness of home washing technologies is shown in Table 2. This experiment showed that water hardness did not impact the efficacy of ozonated water (with a reduction in *Salmonella* populations for the 0 ppm hardness treatment of 1.73 log units while the reduction for the 200 ppm hardness treatment was 1.02 log units), EO water (with a reduction for the 0 ppm hardness treatment of 1.16 log units while the reduction for the 200 ppm hardness treatment was 1.32 log units), dilute chlorine bleach water (with a reduction for the 0 ppm hardness treatment of 2.21 log units while the reduction for the 200 ppm hardness treatment was 2.50 log units), or treatment with Veggie Wash (with a reduction for the 0 ppm hardness treatment of 1.14 log units while the reduction for the 200 ppm hardness treatment was 1.32 log units). This is in agreement with Lopes (72) who

showed that 100 ppm sodium hypochlorite and 100 ppm organic chlorine sanitizers reduced bacterial numbers of *Salmonella* Typhimurium and *Listeria monocytogenes* by 5 log units in 30 seconds of 500 ppm hard water.

The effect of pH on the efficacy of ozonated water was also evaluated. Treatment with ozonated water at pH 5 reduced *Salmonella* populations by 1.92 (0 ppm hardness) or 1.22 (200 ppm hardness) log units, while ozonated water at pH 8 only reduced *Salmonella* populations by 1.53 (0 ppm hardness) or 0.84 (200 ppm hardness) log units. While none of these treatments were significantly ($P > 0.05$) different from one another, the treatments at pH 5 showed a higher reduction than the treatments at pH 8. This agrees with Khadre et al (60) and Kim et al (66) who said that the stability of ozone increases as the pH decreases.

The results of this study indicate that these produce washing techniques, including ozonated water, EO water, Veggie Wash, and dilute chlorine bleach, are effective over a range of hardness levels. The results also showed that the ozone water treatment is more effective at lower pH values which can be achieved by adding a small amount of vinegar to the treatment water. Future studies can focus on these technologies without adjusting the hardness level of the treatment water, and with an adjustment of the pH for ozone.

TABLE 2. Influence of hardness and pH on the effectiveness of home washing technologies on tomatoes inoculated with *Salmonella*.

Treatment	Recovered (log CFU/tomato) ^{1,2}		pH
	Soft Water (0 ppm) ³	Hard Water (200 ppm)	
Ozone pH 5	5.62 a	6.33 a	5.11 ± 0.40
Ozone pH 8	6.01 a	6.71 a	8.45 ± 0.55
Bleach	5.33 a	5.04 a	
EO Water	6.38 a	6.22 a	
Veggie Wash	6.40 a	6.45 a	

¹Initial inoculum levels were 7.54 log CFU/tomato

²n = three replicates containing three tomatoes per replicate

³Mean values followed by the same letter are not significantly different (P ≤ 0.05)

CHAPTER 4

EFFICACY OF HOME WASHING TECHNOLOGIES

Introduction

There is a current rise in the amount of fresh fruits and vegetables eaten in the United States (30). With the increased consumption of fresh produce items comes an increased risk of a foodborne outbreak. Pathogen contamination of fresh produce can occur over numerous points in the farm-to-fork continuum, including in the field, during packing and shipping, or during storage (13, 16, 23-24, 35, 48, 84). Over the past five years the FDA has reported eight bacterial outbreaks associated with fresh produce, and in 2010 alone there have been ten recalls of fresh produce due to bacterial contamination (46). The main bacterial pathogens of concern remain *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. These pathogens have been linked to outbreaks involving lettuce, spinach, cabbage, peppers, apples, alfalfa sprouts, tomatoes, and cantaloupes.

As these outbreaks continue to occur, the adequacy of consumer washing fresh produce in running tap water has come into question. This has led to the development of numerous home use sanitizing treatments. While the most common method of washing fresh produce in the home remains rinsing under running tap water, other sanitizing treatments, including aqueous ozone, electrolyzed oxidizing water, commercial produce washes, and dilute chlorine bleach are being evaluated for their efficacy against pathogenic microorganisms in the home setting.

Aqueous ozone is considered generally recognized as safe (GRAS) and involves infusing water with ozone, a molecule containing three oxygen atoms. This molecule is able to destroy a

broad spectrum of microorganisms, including gram-negative bacteria, gram-positive bacteria, spores, fungi, viruses, and parasites. Ozone is a naturally occurring molecule that decomposes into oxygen, leaving no residues behind (50, 66). There are ozone generators specifically designed for home use, that are compact, easy to use, and safe. These generators are a viable possibility for consumers who are looking for a way to be more active in sanitizing produce.

Electrolyzed oxidizing (EO) water is created by passing a dilute salt solution through an electric current. This produces water with a high oxidation reduction potential (ORP), low pH, and containing free chlorine. The low pH of EO water is not harmful to the skin, but, in combination with the high ORP and free chlorine, is effective in inactivating a wide range of microorganisms, including gram-negative bacteria, gram-positive bacteria, fungi, viruses, algae, and protozoans (55, 63, 89). There also are EO generators specifically designed for home use, but are not currently available domestically, although are still a viable sanitation method.

Commercial produce washes are widely available at a multitude of stores nationwide. These washes are typically made from natural oils and surfactants. These produce washes may have antimicrobial activity, but typically just rinse the pathogen off of the produce item, making the possibility of cross-contamination an issue (89).

Chlorine-based sanitizers are the most commonly used method of sanitizing produce in industry. The maximum allowable level for washing applications without a rinse step is 200 ppm available chlorine. The average treatment is 1 to 2 minutes in a dilute chlorine bleach solution in the 50 to 200 ppm range (77, 90). While chlorine-based sanitizers are more effective than water washes, they are not recommended for use in the home, as this chemical is not intended for consumption.

In order to give consumers more practical information about how to reduce bacterial contamination on produce, multiple sanitation techniques must be tested in the same manner as they would be used in the home setting. The objective of this study is to determine and compare the efficacy of multiple home use washing technologies against *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* on tomatoes, broccoli, and cantaloupe.

Materials and Methods

Bacterial Strains. Five strains each of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* were used in this study. A list of all fifteen strains, reference numbers, and sources can be found in Table 3. Prior to use, each strain was made resistant to 100 µg/mL rifampicin as described in Chapter 3. Stock cultures were maintained at -80°C on Microbank™ Bacterial and Fungal Preservation System beads.

TABLE 3. List of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica* strains used in this study.

Strain	Reference Number	Source
<i>L. monocytogenes</i>	LCDC	Cabbage associated outbreak
<i>L. monocytogenes</i>	G3982	Clinical isolate from a Jalisco cheese outbreak
<i>L. monocytogenes</i>	Scott A	Human isolate
<i>L. monocytogenes</i>	LM254	From drain of chicken processing plant
<i>L. monocytogenes</i>	LM311	From raw chicken product
<i>E. coli</i> O157:H7	H1730	Lettuce associated outbreak
<i>E. coli</i> O157:H7	F4556	Alfalfa sprouts associated outbreak
<i>E. coli</i> O157:H7	#994	Salami isolate
<i>E. coli</i> O157:H7	SEA13B88	Apple juice associated outbreak
<i>E. coli</i> O157:H7	CDC-658	Cantaloupe associated outbreak
<i>S. Baildon</i>	Not Available	Tomato associated outbreak
<i>S. Montevideo</i>	G4639	Tomato associated outbreak
<i>S. Poona</i>	01A3923	Cantaloupe associated outbreak
<i>S. Stanley</i>	H1256	Alfalfa sprouts associated outbreak
<i>S. Typhimurium</i>	DT 104:H3380	Clinical Isolate

Preparation of Inoculum. The inoculum was prepared as described in Chapter 3.

Produce. Tomatoes were prepared as described in Chapter 3. Broccoli (*Brassica oleracea*; 50 g – 129 g; mean weight 92 g) and cantaloupes (*Cucumis melo* var *cantalupensis*; 1324 g – 1947 g; mean weight 1558 g) were purchased from a local grocery store and stored at 4°C for no longer than 7 days after purchase. Each piece of produce was inspected to ensure no rotten or damaged spots. Prior to inoculation each piece of produce was rinsed under running deionized water (DW) for 15 seconds to remove residual organic material. Three pieces of one type of produce were treated together and considered one replication.

Produce Inoculation. Each tomato was inoculated as described in Chapter 3. Each broccoli or cantaloupe was placed on a sterile holder for inoculation. Broccoli was inoculated with 50 μ L of the bacterial cocktail in 10 spots on the floret. A 5 cm by 5 cm square was marked on the cantaloupe using a permanent marker (Sharpie[®], Newell Rubbermaid, Freeport, IL) and was then inoculated as described previously. The fruit was then left to dry in a laminar flow class II biosafety hood at 22°C for 1 h to allow for attachment. The produce was inoculated no earlier than 2 hours prior to use. The bacterial cocktail was held at 4°C between uses.

Treatments. Five treatments were tested to determine efficacy: ozonated water, electrolyzed oxidizing water, dilute chlorine bleach water, Veggie Wash[®] fruit and vegetable wash, and running tap water. All treatments were run at 15°C \pm 2°C. Ozone, EO water, diluted bleach water, and Veggie Wash were all prepared as described in Chapter 3. Water for ozonation use was dropped to pH 5 as described in Chapter 3.

Treatment Exposure. Tomatoes were treated as described in Chapter 3 for ozone, EO water, dilute chlorine bleach water, and Veggie Wash. Broccoli and cantaloupe were treated as described in Chapter 3 for EO water, dilute chlorine bleach water, and Veggie Wash. For ozone, since broccoli does not displace as much water as tomatoes or cantaloupe, 3.785 L DW was used instead of 3 L DW. The ozone apparatus would not contain three cantaloupes at once, thus each cantaloupe was run separately. The same water was used, but the unit was rerun for each cantaloupe. Prior to each run, pH was determined as described in Chapter 3 to ensure that the target pH of 5 was obtained.

For the tap water treatment, tap water was run continuously over the produce at a rate of approximately 2 L/min for 15 s. The produce was positioned under the running tap water so that

the inoculated section was neither directly hit by the water nor directly where the water ran off the produce item. Following treatment, the produce was neutralized as described in Chapter 3.

Microbial Analysis. Tomatoes were analyzed for pathogens as described in Chapter 3.

Uninoculated and treated broccoli was stomached for 2 min in a Stomacher 400 (Tekmar Co., Cincinnati, OH) at the normal speed setting prior to analysis (8, 61). The solution was then analyzed as described in Chapter 3. Uninoculated and treated cantaloupes had the marked portion of the rind excised with a flame sterilized scalpel prior to analysis (4-5, 10). The excised rind was checked to ensure all remaining meat was removed, placed in a Whirl-Pak bag, and rubbed by hand for 2 min (4). A single person was responsible for rubbing all pieces of cantaloupe skin in every experiment in order to reduce variability of the pressure and intensity of the rub. The solution was then analyzed as previously described in Chapter 3. *Salmonella* and *E. coli* O157:H7 treatment plates were incubated at 37°C for 24 h prior to enumeration, while *L. monocytogenes* treatment plates were incubated at 37°C for 48 h prior to enumeration.

Presumptive colonies were randomly selected by the Harrison's disc method (51) and streaked on Xylose Lysine Desoxycholate (XLD; Difco) agar to confirm presence of *Salmonella*, MacConkey II Agar with Sorbital (SMAC; Difco) to confirm presence of *E. coli* O157:H7, or Oxford (OX; Difco) agar to confirm presence of *L. monocytogenes*. All selective media were incubated at 37°C for 24 h prior to confirmation.

Residual Treatment Water. Residual treatment water was analyzed as described in Chapter 3.

Statistical Analysis. Three produce items were treated together to generate one replication. Three replications were performed per treatment. Final counts (CFU/sample) were analyzed after log transformation. Significant differences between means were determined by Analysis of

Variance and Tukey's comparison method in Minitab[®] Statistical Software (Minitab Inc.) and reported at a significant level of $\alpha = 0.05$.

Results

Rifampicin-resistant colonies recovered after treatments were attributed to the pathogen inoculation, as background counts from uninoculated samples (negative controls) were tested against TSA-R100 to ensure no background microflora was rifampicin resistant. Reductions in pathogen counts after washing were relative to the populations of inoculated pathogens on untreated samples (positive controls). The inoculation level used in this study exceeds the typical natural contamination level in order to ensure valid observation of bacterial reductions after the different washing techniques.

Tomatoes. All treatments on tomatoes produced a significant ($P < 0.05$) reduction in pathogen levels from the untreated inoculated samples (positive controls). For the treatments involving *Salmonella*, the Veggie Wash and EO water treatments showed the lowest significant bacterial CFU reductions of 1.14 and 1.16 log units, respectively, among all other treatments (Table 5). The dilute chlorine bleach and running tap water treatments inactivated a greater number of pathogens, 2.21 and 2.13 log units respectively, among all other treatments. The ozone treatment showed a moderate reduction of 1.74 log units, and was not significantly different from any other treatment.

For the treatments involving *E. coli* O157:H7, the Veggie Wash and EO treatments also showed the lowest significant bacterial CFU reductions of 1.24 and 1.31 log units, respectively, among all other treatments (Table 6). The ozone treatment gave a reduction of 2.04 log units and was significantly greater than the EO and Veggie Wash treatments. The running tap water treatment gave a reduction of 2.62 log units and was more effective than the ozone, EO, and

Veggie Wash treatments. The combination of the dilute chlorine bleach, *E. coli* O157:H7, and tomatoes cannot be compared to the other treatments because of the lack of consistency in the results. Out of 7 repetitions, which included 21 tomatoes, 6 tomatoes were below the detection limit (2 log CFU/tomato), 12 tomatoes recovered between 2 and 4.5 log CFU/tomato, and 3 tomatoes recovered above 4.5 log CFU/tomato (Table 7).

For the treatments involving *L. monocytogenes*, the dilute chlorine bleach and EO treatments were more effective than the running tap water, ozone, and Veggie Wash treatments (Table 8). The dilute chlorine bleach and EO treatments gave bacterial CFU reductions of 3.89 and 3.21 log units, respectively, while the running tap water, ozone, and Veggie Wash treatments gave reductions of 2.44, 2.30, and 1.56 log units, respectively.

Broccoli. All treatments on broccoli produced a significant ($P < 0.05$) reduction in pathogen levels from the untreated inoculated samples (positive controls). For the treatments involving *Salmonella*, the dilute chlorine bleach and EO treatments were significantly greater than the Veggie Wash and running tap water treatments (Table 9). The dilute chlorine bleach and EO treatments reduced bacterial counts by 1.57 and 1.39 log units, respectively. The Veggie Wash and running tap water treatments reduced bacterial counts by 0.77 and 0.63 log units, respectively. The ozone treatment showed a moderate reduction of 1.11 log units, and was not significantly different from any other treatment.

For the treatments involving *E. coli* O157:H7, the EO and dilute chlorine bleach treatments gave bacterial CFU reductions of 1.14 and 1.04 log units, respectively, and were significantly greater than the running tap water treatment, which showed a reduction of 0.67 log units (Table 10). The Veggie Wash and ozone treatments showed moderate reductions of 0.98 and 0.83 log units, respectively, and were not significantly different from any other treatment.

For the treatments involving *L. monocytogenes*, again, the dilute chlorine bleach and EO treatments were significantly greater than the Veggie Wash and running tap water treatments (Table 11). The dilute chlorine bleach and EO treatments reduced bacterial counts by 1.27 and 1.18 log units, respectively, while the Veggie Wash and running tap water treatments reduced bacterial counts by 0.69 and 0.66 log units, respectively. The ozone treatment showed a moderate reduction of 0.99 log units, and was not significantly different from any other treatment.

Cantaloupe. All treatments on cantaloupes involving *Salmonella* and *E. coli* O157:H7 produced a significant ($P < 0.05$) reduction in pathogen levels from the untreated inoculated samples (positive controls). For the treatments involving *Salmonella*, the dilute chlorine bleach treatment was significantly more effective than all other treatments, showing a reduction in bacterial counts of 2.48 log units (Table 12). The Veggie Wash treatment was significantly greater than the ozone treatment, showing a bacterial CFU reduction of 1.66 log units compared to the ozone treatment reduction of 0.96 log units. The EO and running tap water treatments showed a moderate reduction of 1.35 and 1.30 log units, respectively, and were not significantly different from either the Veggie Wash treatment or the ozone treatment.

For the treatments involving *E. coli* O157:H7, the dilute chlorine bleach treatment was more effective than the Veggie Wash, ozone, or EO water treatments, with a reduction in bacterial counts of 1.94 log units (Table 13). The Veggie Wash, ozone, and EO water treatments showed bacterial CFU reductions of 1.02, 0.84, and 0.68 log units, respectively. The running tap water showed a moderate reduction of 1.09 log units, and was not significantly different from any other treatment.

For the treatments involving *L. monocytogenes*, again, the dilute chlorine bleach treatment was more effective than the running tap water treatment, with a bacterial CFU reduction of 1.43 log units (Table 14). The running tap water treatment reduced bacterial counts by 0.55 log units, and did not produce a significant reduction from the untreated inoculated controls. The Veggie Wash, EO water, and ozone treatments showed moderate reductions of 0.86, 0.76, and 0.72 log units, respectively.

Residual Treatment Water. For tomatoes, no *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* was detected by either direct plating (detection limit 2 log CFU/mL) or enrichment in the residual treatment water of the ozone, EO, or dilute chlorine bleach treatments. The Veggie Wash treatment did have viable pathogens in the residual treatment water, with counts of 3.38, 3.79, and 2.47 log CFU/mL for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. For broccoli, no *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* was detected by either direct plating or enrichment in the residual treatment water of the EO or dilute chlorine bleach treatments. The residual pathogens in the ozone treatment were undetectable by direct plating, but the presence of the pathogen was detected by enrichment procedures in 1 of 3 replicates for each pathogen. The Veggie Wash treatment did have viable pathogens in the residual treatment water, with counts of 5.25, 5.19, and 4.97 log CFU/mL for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively. For cantaloupe, no *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes* was detected by either direct plating or enrichment in the residual treatment water of the EO or dilute chlorine bleach treatments. The residual pathogens in the ozone treatment were undetectable by direct plating, but the presence of the pathogen was detected by enrichment procedures in 1 of 3 replicates for both *Salmonella* and *L. monocytogenes*. No *E. coli* O157:H7 was detected by enrichment in the residual

treatment water of ozone during treatments with cantaloupe. The Veggie Wash treatment did have viable pathogens in the residual treatment water, with counts of 1.59, 2.97, and 1.86 log CFU/mL for *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*, respectively.

Discussion

This study tested the efficacy of multiple washing technologies on different types of produce in a home use setting. While many of these technologies have been thoroughly studied for the industry setting, most of these studies do not focus on the home setting.

The efficacy of the ozone treatment differed depending on the pathogen and produce item combination. For tomatoes inoculated with *Salmonella*, all broccoli treatments, and cantaloupes inoculated with *L. monocytogenes*, the ozone treatment was not significantly ($P < 0.05$) different from any other treatment. The reductions for these treatments ranged from 1.74 to 0.72 log units. The ozone treatment provided the lowest reductions for tomatoes inoculated with *L. monocytogenes*, cantaloupes inoculated with *Salmonella*, and cantaloupes inoculated with *E. coli* O157:H7. The reductions for these treatments ranged from 2.30 to 0.84 log units. For the treatment involving tomatoes inoculated with *E. coli* O157:H7, the reduction from the ozone was significantly different from both the treatment with the greatest reduction and the treatments with the lowest reductions. This treatment reduced bacterial populations by 2.04 log units. These results are similar to those of Singh et al (91), who treated *E. coli* O157:H7 inoculated lettuce in 200 mL of 10 mg/L aqueous ozone for 10 minutes and found a reduction of 2.81 log units. Another study by Koseki et al (67) also found between 1 and 1.5 log units reduction in aerobic and coliform bacteria on lettuce that was soaked in 5 mg/L aqueous ozone for 10 minutes.

The EO water treatment performed with irregular efficacy, depending on the produce item and the pathogen. Although the EO water treatment had the highest reductions for broccoli,

it had the lowest reductions for cantaloupes and tomatoes. For tomatoes inoculated with *L. monocytogenes* and all broccoli treatments, the EO water treatment showed the greatest reductions, ranging from 3.21 to 1.14 log units. For cantaloupes inoculated with *Salmonella* or *L. monocytogenes*, the EO water treatment showed moderate reductions, ranging from 1.35 to 0.76 log units. For tomatoes inoculated with *Salmonella* or *E. coli* O157:H7, and cantaloupes inoculated with *E. coli* O157:H7, the EO water treatment showed the lowest reductions, ranging from 1.31 to 0.68. These results are similar to Hung et al (56) who tested *E. coli* O157:H7 on strawberries and broccoli, and found a reduction of 1.28 log units on strawberries and a reduction of 1.78 log units on broccoli. These results are in contrast to Bari et al (7) who found a 7.7 log units reduction in *E. coli* O157:H7, a 7.4 log units reduction in *Salmonella*, and a 7.6 log units reduction in *L. monocytogenes* on tomatoes after washing in EO water for 20 seconds. Park et al (78) also found a high reduction in *E. coli* O157:H7 and *L. monocytogenes* on lettuce. The results showed a 5 log unit reduction of *E. coli* O157:H7, and a 5.1 to 5.6 log unit reduction in *L. monocytogenes* after washing up to 3 minutes. The EO water used in the Bari et al (7) and Park et al (78) studies had free chlorine levels of 30 to 45 ppm while the EO water used in this study had a free chlorine level of 9 ± 3 ppm. This indicates that the chlorine concentration of the EO water may be important in the effectiveness of the treatment.

The efficacy of the Veggie Wash treatment differed, depending on the pathogen and produce item combination. For all tomato treatments, broccoli inoculated with *Salmonella* or *L. monocytogenes*, and cantaloupes inoculated with *E. coli* O157:H7, the Veggie Wash treatment provided the lowest reductions. These reductions ranged from 1.56 to 0.69 log units. For broccoli inoculated with *E. coli* O157:H7 and cantaloupes inoculated with *L. monocytogenes*, the Veggie Wash treatment was not significantly different from any other treatment. The reductions

for these treatments ranged from 0.98 to 0.80 log units. For the treatment involving cantaloupes inoculated with *Salmonella*, the reduction from the Veggie Wash was significantly different from both the treatment with the greatest reduction and the treatments with the lowest reductions. This treatment reduced bacterial populations by 1.66 log units. The combination of Veggie Wash with a running tap water rinse should produce a greater reduction in pathogens than a Veggie Wash treatment alone. These results are similar to Kilonzo-Nthenge et al (61) who used a 2 minute Veggie Wash treatment to reduce *L. innocua* counts on tomatoes by 2.89 log units, on broccoli by 1.50 log units, on lettuce by 1.73 log units, and on apples by 2.28 log units. It should be noted that Veggie Wash is not marketed as an antimicrobial treatment.

The dilute chlorine bleach treatment showed the greatest reductions for all pathogen and produce item combinations. The reductions for the tomato treatments ranged from 3.89 to 2.21 log units, for the broccoli treatments ranged from 1.57 to 1.04 log units, while the cantaloupe treatments ranged from 2.48 to 1.43 log units. These results are similar to those of Behrsing et al (11) who treated broccoli with a 100 ppm chlorine solution and found a reduction of 2.54 log units. Albrecht et al (2) also looked at broccoli in a 50 ppm chlorine solution and found a reduction in coliform populations of approximately 1 log unit. Pirovani et al (83) investigated background populations on fresh-cut spinach and found a reduction of 2.39 log units when soaked in a 75 ppm chlorine solution for 2 minutes. Zhuang et al (107) determined the reduction of *Salmonella* on tomatoes which were soaked in a 50 ppm or 100 ppm chlorine solution for 2 minutes and found a reduction of 0.8 and 1.4 log units, respectively. Baur et al (9) investigated washing lettuce in a 100 ppm chlorine solution and found a reduction in the background microflora of approximately 0.7 to 1.5 log units.

The treatment involving tomatoes inoculated with *E. coli* O157:H7 that was washed in the dilute chlorine bleach has a high standard deviation because of the 6 out of 21 tomatoes that resulted in counts that were below the detection limit. The overall average reduction for this treatment was 4.37 log units.

The efficacy of the running tap water treatment differed, depending on the produce item and the pathogen combination. Although the running tap water treatment had the among highest reductions for tomatoes, it had the lowest reductions for broccoli and cantaloupes. For tomatoes inoculated with *Salmonella* or *E. coli* O157:H7, the running tap water treatment showed the greatest reductions, ranging from 2.63 to 2.13 log units. For cantaloupes inoculated with *Salmonella* or *E. coli* O157:H7, the running tap water treatment showed moderate reductions, ranging from 1.30 to 1.09 log units. For tomatoes inoculated with *L. monocytogenes*, all broccoli treatments, and cantaloupes inoculated with *L. monocytogenes*, the running tap water treatment showed the lowest reductions, ranging from 2.44 to 0.55 log units. The inefficacy of running tap water on broccoli can be attributed to the running water being unable to penetrate the high surface area of the broccoli, plus the hydrophobic characteristics of the vegetable. The treatments requiring submersion of the broccoli were much more effective. The treatment involving cantaloupes inoculated with *L. monocytogenes* that was rinsed with running tap water did not produce a reduction large enough to show a significant reduction compared to the untreated inoculated controls. These results agree with Kilonzo-Nthenge et al (61) who also used a 15 second running tap water treatment to reduce *L. innocua* counts on produce. They found that the bacterium was reduced by 1.4 log units on lettuce and broccoli, and 2.1 units on tomatoes.

The microbial populations recovered from the residual treatment water are important because in a home setting, these pathogens can potentially cross-contaminate other produce items or food preparation surfaces. No pathogens were detected (detection limit of 1 CFU/mL) in the residual treatment solutions of any treatment involving dilute chlorine bleach or EO water. This shows that these treatments kill the pathogens they remove, preventing the potential of cross-contamination. Pathogens were detected in approximately one-third of the ozone residual treatment solutions by enrichment. This shows that ozone is partially ineffective at killing the pathogens removed from the produce item, and has potential for cross-contamination. Pathogens were recovered from all Veggie Wash residual treatment solutions, showing that Veggie Wash has a high potential for cross-contamination.

The efficacy of each washing treatment was dependent on both the pathogen and the produce item involved. Reductions were greater for tomatoes than for broccoli or cantaloupe. This can be attributed to the smoother surface of the tomatoes compared to the highly complex surfaces of broccoli and cantaloupe. These highly complex surfaces include the thousands of florets on the head of a piece of broccoli and the roughness, pits, and crevices that comprise the netting of cantaloupes. These surfaces can protect bacteria that become entrapped within the surface structure, thus limiting their exposure to the sanitizer (49). These irregularities can account for the variability in enumeration of pathogens on cantaloupes using plate count procedures. Ukuku et al (99) and Ukuku (95) also reported variability in total plate counts after washing cantaloupes.

The results from this study suggest that all of these washing treatments have the potential to reduce surface bacterial contamination. While the dilute chlorine bleach treatment showed the greatest reduction of all pathogens for all produce items, the U.S. Department of Agriculture

does not recommend the use of this chemical on items that will be ingested. Future studies should look into these treatments with an added rubbing or brushing step.

TABLE 4. Physicochemical properties of washing solutions prior to treatment.

Treatment	Temperature (°C) ¹	ORP (mV)	Free Chlorine Concentration (ppm)	pH
Ozone ²	14.6 ± 1.0	NA ³	NA	4.27 ± 0.19
Bleach	14.5 ± 0.6	657.2 ± 13.5	70 ± 7	NA
EO Water	16.0 ± 1.0	1093.5 ± 28.0	9 ± 3	2.92 ± 0.20
Veggie Wash	14.3 ± 0.8	NA	NA	NA
Running Tap	23 ± 1.0	NA	0-1	NA

¹n = three replications

²Initial ozone concentration: 0.75 ± 0.4 mg/L

³NA, not analyzed

TABLE 5. Populations of *Salmonella* recovered from tomatoes and treatment solutions after treatment.

Treatment	Mean <i>Salmonella</i> Population			Properties of Treatment Solution (After Treatment) ²	
	On Tomatoes (log CFU/Sample) ¹		In Solution (log CFU/mL)	ORP (mV)	Free Chlorine (ppm)
	Recovered ³	Reduction			
None	7.54 a	--	--	--	--
Ozone	5.80 bc	1.74	ND ⁴	NA	NA
Bleach	5.33 b	2.21	ND	689.2 ± 2.0	75 ± 4
EO Water	6.38 c	1.16	ND	1117.5 ± 10.5	10 ± 1
Veggie Wash	6.40 c	1.14	3.38	NA	NA
Running Tap	5.41 b	2.13	NA ⁵	NA	NA

¹n = three replicates containing three tomatoes per replicate

²Includes properties for *Salmonella*, *E. coli* O157:H7, and *Listeria* treatments

³Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

⁴ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁵NA, not analyzed

TABLE 6. Populations of *E. coli* O157:H7 recovered from tomatoes and treatment solutions after treatment.

Treatment	Mean <i>E. coli</i> O157:H7 Population		
	On Tomatoes (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	7.62 a	--	--
Ozone	5.58 b	2.04	ND ³
EO Water	6.31 c	1.31	ND
Veggie Wash	6.38 c	1.24	3.79
Running Tap	5.00 d	2.62	NA ⁴

¹n = three replicates containing three tomatoes per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁴NA, not analyzed

TABLE 7. Frequency of *E. coli* O157:H7 recovered from tomatoes after dilute chlorine bleach treatment.¹

Mean <i>E. coli</i> O157:H7 Population		
Number of Tomatoes ²		
Below Detection Limit (2 log CFU/sample)	Between 2 and 4.5 log CFU/sample	Above 4.5 log CFU/sample
6	12	3

¹No pathogens detected in any residual treatment solution by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

²n = seven replicates containing three tomatoes per replicate

TABLE 8. Populations of *Listeria monocytogenes* recovered from tomatoes and treatment solutions after treatment.

Treatment	Mean <i>Listeria monocytogenes</i> Population		
	On Tomatoes (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	8.04 a	--	--
Ozone	5.74 b	2.30	ND ³
Bleach	4.15 c	3.89	ND
EO Water	4.83 c	3.21	ND
Veggie Wash	6.48 b	1.56	2.47
Running Tap	5.60 b	2.44	NA ⁴

¹n = three replicates containing three tomatoes per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁴NA, not analyzed

TABLE 9. Populations of *Salmonella* recovered from broccoli and treatment solutions after treatment.

Treatment	Mean <i>Salmonella</i> Population			Properties of Treatment Solution (After Treatment) ²	
	On Broccoli (log CFU/Sample) ¹		In Solution (log CFU/mL)	ORP (mV)	Free Chlorine (ppm)
	Recovered ³	Reduction			
None	8.61 a	--	--	--	--
Ozone	7.50 bc	1.11	1/3 ⁴	NA	NA
Bleach	7.04 b	1.57	ND ⁵	678.6 ± 9.7	72 ± 8
EO Water	7.22 b	1.39	ND	1099.4 ± 28.6	8 ± 2
Veggie Wash	7.84 c	0.77	5.25	NA	NA
Running Tap	7.98 c	0.63	NA ⁶	NA	NA

¹n = three replicates containing three broccoli per replicate

²Includes properties for *Salmonella*, *E. coli* O157:H7, and *Listeria* treatments

³Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

⁴1 out of 3 samples were positive for *Salmonella* by enrichment

⁵ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁶NA, not analyzed

TABLE 10. Populations of *E. coli* O157:H7 recovered from broccoli and treatment solutions after treatment.

Treatment	Mean <i>E. coli</i> O157:H7 Population		
	On Broccoli (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	8.74 a	--	--
Ozone	7.91 bc	0.83	1/3 ³
Bleach	7.70 b	1.04	ND ⁴
EO Water	7.60 b	1.14	ND
Veggie Wash	7.76 bc	0.98	5.19
Running Tap	8.07 c	0.67	NA ⁵

¹n = three replicates containing three broccoli per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³1 out of 3 samples were positive for *E. coli* O157:H7 by enrichment

⁴ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁵NA, not analyzed

TABLE 11. Populations of *Listeria monocytogenes* recovered from broccoli and treatment solutions after treatment.

Treatment	Mean <i>Listeria monocytogenes</i> Population		
	On Broccoli (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	8.61 a	--	--
Ozone	7.62 bc	0.99	1/3 ³
Bleach	7.34 b	1.27	ND ⁴
EO Water	7.43 b	1.18	ND
Veggie Wash	7.92 c	0.69	4.97
Running Tap	7.95 c	0.66	NA ⁵

¹n = three replicates containing three broccoli per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³1 out of 3 samples were positive for *L. monocytogenes* by enrichment

⁴ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁵NA, not analyzed

TABLE 12. Populations of *Salmonella* recovered from cantaloupe rinds and treatment solutions after treatment.

Treatment	Mean <i>Salmonella</i> Population			Properties of Treatment Solution (After Treatment) ²	
	On Cantaloupe (log CFU/Sample) ¹		In Solution (log CFU/mL)	ORP (mV)	Free Chlorine (ppm)
	Recovered ³	Reduction			
None	7.39 a	--	--	--	--
Ozone	6.43 b	0.96	1/3 ⁴	NA	NA
Bleach	4.91 c	2.48	ND ⁵	681.3 ± 7.8	73 ± 5
EO Water	6.04 bd	1.35	ND	1102.7 ± 14.2	8 ± 2
Veggie Wash	5.73 d	1.66	1.59	NA	NA
Running Tap	6.09 bd	1.30	NA ⁶	NA	NA

¹n = three replicates containing three cantaloupe per replicate

²Includes properties for *Salmonella*, *E. coli* O157:H7, and *Listeria* treatments

³Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

⁴1 out of 3 samples were positive for *Salmonella* by enrichment

⁵ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁶NA, not analyzed

TABLE 13. Populations of *E. coli* O157:H7 recovered from cantaloupe rinds and treatment solutions after treatment.

Treatment	Mean <i>E. coli</i> O157:H7 Population		
	On Cantaloupe (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	7.95 a	--	--
Ozone	7.11 b	0.84	ND ³
Bleach	6.01 c	1.94	ND
EO Water	7.27 b	0.68	ND
Veggie Wash	6.93 b	1.02	2.97
Running Tap	6.86 bc	1.09	NA ⁴

¹n = three replicates containing three cantaloupe per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁴NA, not analyzed

TABLE 14. Populations of *Listeria monocytogenes* recovered from cantaloupe rinds and treatment solutions after treatment.

Treatment	Mean <i>Listeria monocytogenes</i> Population		
	On Cantaloupe (log CFU/Sample) ¹		In Solution (log CFU/mL)
	Recovered ²	Reduction	
None	7.89 a	--	--
Ozone	7.17 bc	0.72	1/3 ³
Bleach	6.46 c	1.43	ND ⁴
EO Water	7.13 bc	0.76	ND
Veggie Wash	7.09 bc	0.80	1.86
Running Tap	7.34 ab	0.55	NA ⁵

¹n = three replicates containing three cantaloupe per replicate

²Mean values followed by the same letter are not significantly different ($P \leq 0.05$)

³1 out of 3 samples were positive for *L. monocytogenes* by enrichment

⁴ND, not detected by direct plating (2 CFU/mL detection limit) or by enrichment (1 CFU/mL detection limit)

⁵NA, not analyzed

CHAPTER 5

SUMMARY AND CONCLUSIONS

Consumption of fresh fruits and vegetables is on the rise. With the increased consumption of fresh produce items comes an increase in risk of a foodborne outbreak. The consuming public needs to be more diligent in washing fresh produce items prior to consumption. There are various products on the market and different approaches to consumer washing of fresh produce, however, consumers do not have the ability to evaluate them. This research focused on promising techniques available for use in the home, including aqueous ozone, electrolyzed oxidizing water, Veggie Wash[®] fruit and vegetable wash, dilute chlorine bleach, and running tap water. Because the quality of the water used in these technologies has the potential to impact their performance, different incoming water qualities (degree of hardness and pH) were also evaluated.

Treatments prepared with hard water (200 ppm hardness) were equal in efficacy to treatments prepared with soft water (0 ppm hardness) in reducing the numbers of pathogens on fresh produce. This shows that these treatments are effective over a wide range of hardness levels, and thus can be effectively utilized in every home. The ozone treatment was slightly more effective at lower pH values, which can be easily achieved by adding a small amount of vinegar to the treatment water prior to use.

Overall, the ozone treatment was moderately effective, reducing average bacterial counts between 2.30 and 0.72 log units. This limited effectiveness can be contributed to the ozone molecules failing to contact the bacteria, possibly because of the produce surface characteristics.

This technology is more effective on smooth surface produce items, such as tomatoes, than on items with a complex surface morphology. This technology has a moderate initial investment, followed by minimal investments to run, is safe to use in the home, and has a lack of toxic residues, making this a suitable candidate for in home produce washing.

The electrolyzed oxidizing water treatment worked well for broccoli, but was only moderately effective for both tomatoes and cantaloupes, reducing average bacterial counts between 3.21 and 0.68 log units. EO water was more effective on broccoli presumably because it was able to penetrate into the complex surface structures. While EO water has a high initial investment, there is a minimal investment to run, as water and table salt are the only materials required. This technology is a prime candidate for in home produce washing as there are no added chemicals and no toxic residues left behind.

The Veggie Wash treatment was only moderately effective, reducing average bacterial counts between 1.66 and 0.69 log units. While the Veggie Wash treatment reduced bacterial counts by approximately 1.3 log units on tomatoes, it was the treatment with the lowest reductions. The Veggie Wash treatment is not a pathogen killing treatment, as pathogens recovered in the residual treatment water ranged from 1.59 to 5.29 log units. This shows that Veggie Wash has the potential to cross-contaminate other produce items and/or food preparation areas in the home.

The dilute chlorine bleach treatment was the most effective treatment, reducing average bacterial counts between 3.89 and 1.14 log units. While this technology was effective against these pathogens, it is not recommended for washing fresh produce in the home as it would be difficult for consumers to obtain proper concentrations and it is not a food grade solution.

The running tap water treatment worked better for tomatoes than for broccoli or cantaloupes, reducing bacterial counts between 2.62 and 0.55 log units. This can be attributed to the smooth surface of the tomatoes allowing the running water to remove the pathogens while the complex surfaces of the broccoli and cantaloupes will protect the pathogens. Future studies should determine the efficacy of scrubbing these complex surface items while rinsing under running tap water.

Overall, this study showed the potential of multiple home washing technologies against bacterial pathogens on produce items with varying surface morphologies. These results will provide consumers with the scientific evidence behind these technologies, and will assist them in making a decision concerning the selection of produce washing methods for home use.

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