Efficacy of Alternative Sanitization Methods on Wild Blueberries and Fresh Cut Cantaloupe

Selena Callahan
University of Maine, selena.callahan@maine.edu

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EFFICACY OF ALTERNATIVE SANITIZATION METHODS ON WILD BLUBERRIES
AND FRESH CUT CANTALOupe

By
Selena Callahan
B.S., Oregon State University, 2016

A THESIS
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
(in Food Science and Human Nutrition)

The Graduate School
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August 2019

Advisory Committee:
Jennifer Perry, Assistant Professor of Food Microbiology, Advisor
Balunkeswar Nayak, Associate Professor of Food Processing
Brian Perkins, Research Assistant Professor of Food Science
Robson Machado, Assistant Extension Professor and Food Science Specialist
Efficacy of Alternative Sanitization Methods on Wild Blueberries and Fresh Cut Cantaloupe

By Selena Callahan

Thesis Advisor: Dr. Jennifer Perry

An Abstract of the Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science (in Food Science and Human Nutrition)
August 2019

In the last decade, outbreaks linked to the consumption of contaminated fresh produce have increased mitigation efforts to minimize the risks associated with these products. Due to the lack of a kill step during processing of ready-to-eat (RTE) foods, good manufacturing practices (GMPs), and sanitization are the key prevention areas most commonly used to control these risks. Many factors must be considered by processors for successful intervention such as sanitizer type, method of application, duration of application, and concentration. Additionally, product considerations such as water content, pH, and bacterial harborage sites can contribute greatly to how effective an applied treatment may be. The goal of this research was to evaluate alternative sanitization methods at the pilot scale to combat *Listeria innocua* and native microflora on fresh cut cantaloupe and wild blueberries. Parameters chosen for these studies aimed to mimic current processing conditions to provide a better understanding of these factors in a large-scale processing environment.

To carry out this research, a bench scale study was completed to determine which treatment combinations to use at the pilot scale. Sequential applications of chlorine (200 ppm), peracetic acid (PAA, 80 ppm), and electrolyzed water (200 ppm active chlorine constituents)
were compared to a water control. The combination of sanitizer treatments were applied via dip or spray singly, or sequential application of dip then spray, or duplicate spray.

At the pilot scale, sanitizer combination treatments were evaluated for 3 minutes of contact time followed by freezing for up to two weeks (wild blueberries) or refrigerated storage for 48 hours (fresh cut cantaloupe). Microbial populations were evaluated at each stage to determine the efficacy of each treatment. For fresh cut cantaloupe we found that none of the treatments investigated were effective at reducing *Listeria innocua* populations at the pilot scale and observed an increase in population following refrigerated storage. Similarly, for wild blueberries none of the treatments investigated significantly reduced *Listeria innocua* populations and after two weeks of freezing *Listeria innocua* was detected in the product. Our results demonstrate the complex interaction of the treatment applied and product type, volume of product, and application method. Understanding these interactions can help processors to make informed decisions for potential food safety interventions and the applicability of current research at the pilot scale. Further investigation into the effects of growing practices on microbial communities and other potential pathogen inactivation methods need to be evaluated.
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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Product safety is a driving force behind how we prepare and process food before it reaches consumers. Chemical sanitization methods are among the tools at the forefront of assuring this safety and are applicable to a vast product range. More specifically, chlorine has been the most widely accepted sanitizer for food product and processing facilities due to its strong germicidal properties and low cost.

Peracetic acid (PAA) has become another sanitizer used in the food industry and is known for its quick acting germicidal properties compared to chlorine. Recent concerns have come forth regarding the effectiveness of chlorine and its potential to be an environmental hazard (CDC, 2018b). Among these concerns is the safety of workers who are directly impacted by the way our food is produced. Interest in green technologies has become the center of discussions regarding these issues as they provide a promising alternative to conventional methods.

Electrolyzed water is a novel treatment that can be used as both a cleaner and sanitizer in a multitude of food industry applications (Abadias et al., 2008). This technology has been shown to be effective on an array of fresh produce, meat, seafood, and processing equipment (Abadias et al., 2008; Al-Holy & Rasco, 2015). It is easily accessible and affordable to a variety of processors and home consumers. The use of electrolyzed water is widely accepted due to the ease of application and preparation of the treatment.

Sanitation of fresh produce is a crucial intervention method against pathogenic and background microflora commonly found on these foods. Research has displayed a wide range of effectiveness using these solutions making it difficult to create more cohesive sanitation
protocols within the food industry. Here we discuss methods of pathogen contamination and intervention and evaluate current studies on produce sanitation.

1.2 Pathogens Associated with Fresh Produce

In the United States, foodborne pathogens account for approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually (CDC, 2018a). Several pathogens have been associated with outbreaks linked to fresh produce (Table 1) as a result of the various harvesting practices associated with these products. Specifically, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. are environmental pathogens that can contaminate produce via the soil, irrigation water, and fecal contamination from animals (FDA, 2008). Additionally, spoilage microorganisms such as yeast and mold are also found in these environments. Although spoilage microorganisms are less likely to threaten human health, they have been known to rapidly accelerate produce spoilage. Our global, industrialized system in which produce is cultivated, harvested, and processed greatly contributes to the likelihood of contamination. For example, produce is grown in large monocultures and transported over long distances before reaching the consumer (Wallinga, 2009). This process provides a reservoir for contamination via frequent human handling and various temperature fluctuations during transport and across processing facilities. Pathogenic bacteria have adopted mechanisms of survival in response to these changes allowing them to survive in various environments contributing to the outbreaks associated with these products (Madden, 1992). Proper harvesting and processing techniques are crucial to mitigating these risks and ensuring the safety of ready-to-eat products.

1.2.1 *Listeria monocytogenes*

Listeriosis, a foodborne disease caused by *Listeria monocytogenes*, infects approximately 1,600 people each year and kills approximately 260 people in the United States (CDC, 2019).
The high mortality rate (~ 20 percent) of this pathogen and unknown infectious dose make it a major concern to processors selling ready-to-eat (RTE) foods in which there is often no additional preparation before consumption (FDA, 2017; WHO, 2018). People with compromised immune systems, such as pregnant women and the elderly, are most susceptible to this disease (CDC, 2019). Onset of illness can take anywhere from 3-70 days which makes it especially difficult to identify the source of contamination (CDC, 2019). Listeriosis infection results in fever, stiff neck, confusion, weakness, vomiting, and in some cases diarrhea (CDC, 2019). Severe cases can result in spontaneous abortion, meningitis, and septicemia (CDC, 2019).

1.2.2 *Listeria monocytogenes* Characteristics

*Listeria* is a genus of Gram positive, facultative anaerobic rods that have flagella allowing for motility (Southwick et al., 1998). *Listeria monocytogenes*, the only human pathogen in the genus, is an intracellular pathogen and has many mechanisms for invading human cells. Due to its unknown infectious dose, the FDA has implemented a zero-tolerance policy for *Listeria monocytogenes* in RTE foods (FDA, 2018). *Listeria* spp. originates from the environment and has been isolated from soil, water, and animal feces (WHO, 2019). Therefore, effective post-harvest processing is crucial to reducing the potential for *Listeria* contamination.

*Listeria monocytogenes* has shown the ability to survive in a variety of environments. Its ability to form biofilms allows for survival and persistence on a variety of surfaces in the processing environment (Moretro and Langsrud, 2004). Biofilm formation occurs when bacteria secrete extracellular polysaccharides allowing for attachment to a variety of surfaces including glass, stainless steel, and plastic (Harvey et al., 2007). Once established, the biofilm can serve as a constant reservoir for contamination and poses increased resistance to sanitizer treatments (Harvey et al., 2007).
Listeria spp. typically grow at temperatures of 30-37°C, at a pH between 4.5-9.6, and water activity of greater than 0.97. However, it was observed that they were capable of surviving in refrigeration and frozen storage for prolonged periods of time (Fang et al., 2013; Flessa et al., 2005; Montville, 2012). If produce comes into the facility contaminated, the likelihood of survival and cross contamination are high due to the use of wash tanks, frequent rinsing, and low temperature maintained in the facility. Intervention methods are crucial to mitigating these risks and include compliance with sanitation protocols, proper employee hygienic practices, and environment testing. Zoning distinguishes high risk areas of pathogen contamination in the facility. Facilities are separated into zones 1 to 3 (FDA, 2017). Zone 1 areas are any food contact surfaces and are the focus to prevent cross contamination (FDA, 2017). Zone 2 (an area near equipment that come into contact with food) and zone 3 (warehouse where food is packaged and stored) are also important because contamination occurring at this stage is unlikely to be discovered before distribution (FDA, 2017).

### 1.3 Outbreaks Associated with Produce and Frozen Food Products

Several outbreaks have been linked to the consumption of fresh produce over the last decade (Table 1). Table 1 outlines outbreaks associated with fresh and frozen products in the United States.

<table>
<thead>
<tr>
<th>Product</th>
<th>Year</th>
<th>Pathogen</th>
<th>Illness/Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantaloupes</td>
<td>2008</td>
<td><em>Salmonella</em> Litchfield</td>
<td>51 illnesses</td>
</tr>
<tr>
<td>Frozen Mamey Fruit Pulp</td>
<td>2010</td>
<td><em>Salmonella</em> Typhi</td>
<td>9 illnesses</td>
</tr>
<tr>
<td>Cantaloupes</td>
<td>2011</td>
<td><em>Listeria monocytogenes</em></td>
<td>147 illnesses, 33 deaths</td>
</tr>
</tbody>
</table>
Table 1.1 Continued

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Year</th>
<th>Pathogen</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantaloupe</td>
<td>2011</td>
<td>Salmonella Panama</td>
<td>20 illnesses</td>
</tr>
<tr>
<td>Papaya</td>
<td>2011</td>
<td>Salmonella Agona</td>
<td>106 illnesses</td>
</tr>
<tr>
<td>Mangos</td>
<td>2012</td>
<td>Salmonella Braenderup</td>
<td>127 illnesses</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>2012</td>
<td>Salmonella Typhimium and Newport</td>
<td>261 illnesses, 3 deaths</td>
</tr>
<tr>
<td>Frozen food products</td>
<td>2013</td>
<td>Escherichia coli O0121</td>
<td>35 illnesses</td>
</tr>
<tr>
<td>Ice cream</td>
<td>2015</td>
<td>Listeria monocytogenes</td>
<td>10 illnesses, 3 deaths</td>
</tr>
<tr>
<td>Frozen Strawberries</td>
<td>2016</td>
<td>Hepatitis A</td>
<td>143 illnesses</td>
</tr>
<tr>
<td>Frozen Vegetables</td>
<td>2016</td>
<td>Listeria monocytogenes</td>
<td>9 illnesses, 3 deaths</td>
</tr>
<tr>
<td>Pre-cut Melon</td>
<td>2018</td>
<td>Salmonella Adelaide</td>
<td>77 illnesses</td>
</tr>
<tr>
<td>Pre-cut Melon*</td>
<td>2018</td>
<td>Listeria monocytogenes</td>
<td>20 illnesses, 7 deaths, 1 miscarriage</td>
</tr>
</tbody>
</table>

* Indicates outbreak in Australia, all other outbreaks were in the United States (CDC, 2008-2018 & WHO, 2018).

1.4 Blueberries

Blueberries are a popular food commodity amongst consumers for their nutritional profile and ability to aid in disease prevention (Michalska et al., 2015). Wild blueberries are a major commodity in Maine where the market is valued at 17.6 million dollars annually. Maine is responsible for producing the majority of American-grown wild blueberries; 67,800 pounds in 2018 (USDA, 2018). Blueberries are typically sold in markets as a fresh or frozen product and are popular in smoothie mixes and other frozen pastry products.

1.4.1 Lowbush versus Highbush Blueberries

In the United States, two distinct botanical varieties of blueberry are grown commercially. Vaccinium angustifolium, also known as the lowbush or wild blueberry, is a
native species to Canada and the New England region (Yarborough, 2015). *Vaccinium corymbosum*, also known as the highbush or cultivated blueberry, is native to the Eastern United States and is the most common commercially grown blueberry, currently produced in 38 states (U.S. Highbush Blueberry Council, 2019). Both lowbush and highbush blueberries are excellent sources of vitamin C, dietary fiber, and anthocyanins, which act as antioxidants (USDA, 2016).

Agricultural practices employed in growing and harvesting differ between the two varietals due to plant height, size differential, availability, and the environment in which they are grown. Unlike the highbush blueberries, lowbush blueberries produce much smaller fruit, resulting in a more nutrient dense, sweeter fruit (Wild Blueberries, 2019). Lowbush blueberries thrive in acidic soils with high organic matter and require a chilling period to produce fruit (Lord, 2016). Mature plants range in size from 4-15 inches high, making them readily susceptible to microbial contamination from the soil (Yarborough, 2015).

Burning and pruning are two techniques commonly used by growers to regenerate their crop after harvest. Pruning or burning is done during dormancy to ensure the regrowth of vegetative shoots which then produce flower buds that year (Lord, 2016). The following year, flower buds generate an edible crop. Lowbush blueberries are grown in a two-year growing cycle, which is performed by pruning or burning half of the crop each year, to ensure that a crop is available each harvest season.

Highbush blueberries are native to the Eastern United States. Unlike the lowbush blueberry that are grown typically in Northern Maine and Eastern Canada, they are grown as far south as Florida (U.S. Highbush Blueberry Council, 2019). Similar to lowbush blueberries, highbush blueberries require acidic soils with high organic matter. Highbush blueberries also require pruning to ensure the production of new vegetative shoots in the first year and fruit in the
second. A mature plant is 72 to 144 inches in height, making the fruit less susceptible to contamination via soil. These plants are harvested in July or August, and produce larger, less sweet fruits than their lowbush counterpart. Lowbush blueberries are typically sold as a frozen commodity (99 percent), while highbush blueberries are more likely to be sold fresh (Yarborough, 2015; Penn State Extension, 2017).

1.4.2 Harvesting and Processing of Blueberries

Exclusive to Maine and Eastern Canada, lowbush blueberries span 44,000 acres in Maine alone (New England Agricultural Statistics Service, 2017). Lowbush blueberries are harvested in July or August through mechanical or hand raking (Figure 1). Once raked, the blueberries are loaded into large plastic bins, then transported to a processing facility.

![Figure 1.1 Hand Raking of Wild Blueberries in the Field](image)

Photo credit: Ocean Point Inn

Once the blueberries arrive at the processing facility, they are subjected to winnowing to remove dirt and leaves from the product. Following winnowing, the product undergoes a series of rinsing and sanitizing steps in a float tank or by spray bars located above the conveyer belt to remove any remaining debris and to reduce the load of microorganisms on the blueberries. The cleaned and sanitized product then enters a fluidized bed freezer where each berry is individually quick frozen at −40°F. After freezing, product is sorted by size and further packaged based on customer specifications. Any remaining product is stored until distribution. Processing variations
such as sanitizer type and concentration, location and number of spray bars, and order of sanitizer application are each dependent on the specific production facility. From a quality perspective, minimal processing is preferred to maintain color and textural attributes of the product. From a microbiological perspective, it is important to ensure the safety of the product through adequate sanitizing. Food safety interventions can occur through many steps in the process and include the usage of the concentration and type of sanitizer, adequate exposure time to sanitizer, and by diminishing cross-contamination through proper containment of potential contaminants and debris from the sanitized product.

1.5 Fresh Cut Produce

The fresh cut produce market has steadily increased in recent years as a result of ease of access and consumer demand for healthy products. Fresh cut product accounts for 16% of fresh produce sales at retail and 60% of foodservice sales, totaling approximately $27 billion annually (Produce Marketing Association, 2014). Fresh cut products include a variety of fruits, but most often consist of cantaloupe, honeydew, watermelon, and pineapple. They are typically sold as mixed fruits in salad bars or single fruit packages in the refrigerated section of the grocery store. Although nutritious, fresh cut produce is especially susceptible to microbial contamination due to the added preparation of these products (Food and Drug Administration, 2008). Due to outbreaks (Table 1) associated with the consumption of contaminated fresh produce, sanitization methods require more exploration.

1.5.1 Harvest and Processing of Cantaloupe

Cantaloupe production in the United States accounts for approximately 51,600 acres with a market value of $261 million annually (Agriculture Marketing Resource Center, 2018). The US is one of the leading consumer markets of cantaloupe with the average American consuming
about 27 pounds of cantaloupe per year (Agriculture Marketing Resource Center, 2018). Outbreaks associated with the consumption of contaminated cantaloupe flesh have caused concerns regarding the way this product is processed and consumed. Cantaloupes are harvested from April to December with a growth cycle of up to 90 days (Agricultural Marketing Resource Center, 2018). They are primarily grown in California, Arizona, Texas, Georgia, and Florida as a result of the warmer climate in these areas (Agriculture Marketing Resource Center, 2018). Due to their direct contact with the soil, their risk for pathogen contamination increases (Orzolek, et al., 2006; Sinkel, et al.). As the fruit matures, the outer netting becomes rough and coarse, which creates an ideal matrix for bacterial attachment (Suslow, 2004). Cantaloupes are harvested by twisting the melon from the vine or by cutting of the stem. Harvested melons are then loaded onto pallets and wrapped in plastic before being transported to packinghouses (Suslow, 2004). Upon reaching the packinghouse, whole cantaloupes are cooled to 40°F or below (Suslow, 2004). Following cooling, cantaloupes are rinsed to remove any dirt or debris from the rind while a sanitizer is used to prevent cross contamination by microorganisms.

Despite the intended purpose of wash tanks, these vessels have been shown to be a source of cross contamination when compromised whole cantaloupes encounter the tank surfaces (Luo et al., 2011, 2012). Following rinsing, cantaloupes are distributed to be sold intact or for further processing onsite. Consumer or processor handling, such as cutting into a contaminated rind, can serve as a mechanism for contamination of the otherwise sterile cantaloupe flesh (Suslow, 2004). With a pH of ~ 6 and high water content, cantaloupe flesh provides an ideal reservoir for bacteria to survive and grow (Bowen et al., 2006).
1.6 Chlorine

Chlorine is the most widely accepted sanitizer used in the food industry. Despite its popularity, there has long been a stigma associated with chlorine usage due to its potential health and environmental implications (CDC, 2018b). Federal regulations allow chlorine to be used as a sanitizer on fresh produce with concentrations up to 200 ppm (FDA, 2018). Chlorine is typically used in wash baths as a dip for fresh produce but can also be applied as a spray. Contact times and concentrations vary based on the type of system in which it is applied. The effectiveness of chlorine can be attributed to the amount of free chlorine present in the form of HOCl in the solution, which is dependent on pH and presence of organic matter (Abadias et al., 2011). Although chlorine works best at an acidic pH due to the production of HOCl, it is typically applied at pH 6-7.5 to avoid corrosion of equipment (Rico et al., 2007).

1.6.1.1 Chlorine Production

Commercially, chlorine can be produced in a multitude of ways. Currently, there are three methods of production with membrane cell electrolysis being the most common.

1.6.1.2 Mercury Cell Electrolysis

To produce chlorine via this method, titanium anodes are placed in a sodium chloride solution which flows over a liquid mercury cathode. A current is then applied, and chlorine gas (Cl₂) is released at the anode while sodium dissolves into the cathode creating an amalgam (Eurochlor, 2018). The amalgam solution goes into a second reactor where it is converted back to mercury via a reaction with water. The reaction with water results in a 50% caustic soda solution of NaOH and H₂ gas (Lakshamanan et al., 2013). Hydroxyl radical formation via mercury cell electrolysis has slowly been phased out due to environmental and toxicity concerns.
and the use of mercury (Lakshamanan et al., 2013). Mercury electrolysis is also the most energy intensive method for chlorine production, which has greatly discouraged its use.

1.6.1.3 Diaphragm Cell Electrolysis

Diaphragm cell electrolysis is a method by which the cathode and anode are separated by a diaphragm. The use of a diaphragm prevents the cathode solution from mixing with the anode solution. A saturated brine solution containing water and NaCl is continuously passed through the anode where it then flows through the diaphragm to the cathode (Eurochlor, 2018). At the anode Cl₂ is produced, while at the cathode a caustic soda solution of NaOH (12%) and H₂ gas is produced (Lakshamanan et al., 2013).

1.6.1.4 Membrane Cell Electrolysis

Among the different methods of chlorine production, membrane cell electrolysis was found to be the most energy efficient method (Eurochlor, 2018). A saturated brine solution is passed from the anode and flows through a diaphragm only permeable to sodium (Na⁺) to the cathode (Paiidar et al., 2016). At the anode, Cl₂ is generated and at the cathode H₂ gas and a caustic soda solution containing 30% NaOH is produced. These products require further processing to reach 50% concentration of NaOH and H₂ (Eurochlor, 2018).

1.6.1.5 Sodium Hypochlorite (Bleach) Generation

Sodium hypochlorite (NaOCl), also known as bleach is produced using a caustic soda solution and Cl₂ gas or liquid. To accomplish this, the caustic soda solution is first diluted with water to 25% caustic soda (Oxychem, 2014). Chlorine gas or liquid is circulated through the diluted caustic soda solution producing sodium hypochlorite instantaneously. The final solution contains 5.25% sodium hypochlorite and is cooled, filtered, and bottled (Oxychem, 2014).
1.6.2 Chlorine Mechanism of Action

Chlorine can act on a variety of microorganisms and has shown to be effective against bacteria and viruses. Of the products, hypochlorous acid (HOCL) is thought to have the greatest antimicrobial effect (Odlaug, 1980). Although the exact mechanism of action on gram positive cells is not fully elucidated, it is thought that the compound’s neutrality permits easy diffusion through the bacterial cell membrane, which facilitates membrane pore formation and damages DNA; effects which inhibit the cells’ normal activities (Camper and McFeters, 1979). It has also been hypothesized that HOCL potentially inhibits essential enzyme activity and deteriorates membrane transport capacity of the cell, both of which are crucial to cell survival (Albrich et al., 1986).

1.6.3 Concerns with Chlorine Use

Concern regarding worker safety due to prolonged exposure to chlorine vapors has been an issue with this treatment (CDC, 2018b). Vapors can cause skin irritation and respiratory distress at concentrations as low as 60 ppm, which can potentially be mitigated by use of alternative treatments (EPA, 2016). Dependent on the type of matrix in which it is applied, chlorine has the potential to form concerning byproducts such as chlorophenols and trihalomethanes (THM) which are potentially carcinogenic. Studies have shown a link between increase of bladder and rectal cancer risk and the consumption of chlorinated water (EPA, 2016, Morris et al., 1992). In addition to the safety considerations, these compounds that have also been shown to cause off taste and odors (Pascual et al., 2007).

1.7 Peracetic Acid (PAA)

Peracetic acid (PAA) is a newer compound that is known for its strong sanitizing capability and is approved for use on USDA organic certified products (AMS-USDA, 2011). It
is currently used in a wide range of facilities and has been approved for use on many products such as fresh produce, meat, poultry, and seafood (USDA, 2016). It is applied in a manner similar to chlorine and can be used in a wash bath or as a spray. PAA has an oxidizing capacity of approximately 1.8V, compared to sodium hypochlorite which has an oxidizing capacity of 1.5V (Du et al., 2018). Its high oxidizing capacity contributes to how quickly it can kill microbial cells (USDA, 2000). PAA has been shown to be effective against a wide range of organisms, and is approved for use in wastewater, produce, and meat sanitation (USDA, 2016). It does not react with organic matter as readily as chlorine-based sanitizers, making it more stable in wash water. Due to the selective reactivity with biomolecules, it is less likely to form harmful byproducts compared to other sanitizers (Du et al., 2018). Additionally, PAA is considered a greener technology compared to chlorine because its decomposition in water produces non-hazardous oxygen and hydrogen peroxide (USDA, 2016).

1.7.1 Production of PAA

The production of PAA can be accomplished using hydrogen peroxide and acetaldehyde or acetic anhydride in the presence of sulfuric acid (Santoro et al., 2007). Due to the reversible nature of the reaction, an equilibrium is reached between reactants and products (Zhao et al., 2007). For commercial use this reaction takes place over ten days to obtain high yields of PAA resulting in a 10-15 % PAA solution which is diluted as appropriate by the end user (USDA, 2000).

1.7.2 Mechanism of Action

PAA can act on a wide range of cell types including bacteria, viruses, bacterial spores, and protozoan cysts (Bonetta et al., 2017; Park et al., 201; Vandekinderen et al., 2009). Its germicidal effect is due to the oxidation of sulfhydryl and sulfur bonds present in proteins,
enzymes, and metabolites (USDA, 2016). The ability to oxidize these compounds can impair cellular biochemical pathways including those associated with transport of extracellular nutrients across membranes (Vandekinderen, 2009). PAA has also been shown to potentially inactivate catalase, which detoxifies free hydroxyl radicals that are crucial in increasing germicidal capacity (Flores et al., 2014). As a result of its diverse antimicrobial properties, PAA can be used in a variety of systems such as wastewater disinfection and food processing.

1.7.3 Concerns with PAA Use

Due to the presence of acetic acid in PAA, when added to water, the organic matter in the effluent increases. This increase potentially leads to microbial survival in wash tanks, leading to diminished sanitizing capacity of this step (Kitis, 2003). At concentrations of 50-500ppm, PAA (liquid) poses threats to human health including mucous membrane damage and irritation to lungs, eyes, and skin (USDA, 2016).

1.8 Electrolyzed Water

Electrolyzed water has been used in a variety of food industry applications and has been shown to be effective as a cleaner and a germicidal agent (Abadias et al., 2008). Ease of preparation does not pose a substantial risk to workers, compared to chlorine, and only requires a salt solution, and a current. When a current is no longer applied, the salt solution returns to its normal state and can be readily disposed of (Jemni et al., 2014). Furthermore, the startup cost and inputs required for this type of system is relatively low making it available to a wide range of production systems (Al-Holy et al., 2015).

1.8.1 Production of Electrolyzed Water

Electrolyzed water is produced by adding sodium chloride (NaCl) and water to an electrolysis chamber containing a diaphragm which separates the cathode from the anode
A current is then applied to the salt solution, generating voltage between the cathode and anode. Electrolysis occurs and NaCl and H₂O (water) dissociate into Na⁺ and Cl⁻ and H⁺ and OH⁻ respectively (Rahman et al., 2016). The cations Na⁺ and H⁺ move towards the cathode generating sodium hydroxide (NaOH) and hydrogen gas (H₂) while the anions Cl⁻ and OH⁻ move toward the anode resulting in hypochlorous acid (HOCI), hypochlorite ion (OCI⁻) hydrochloric acid (HCl), oxygen (O₂) and chlorine (Cl₂) ; (Al-Haq et al., 2005).

Currently, there are four types of electrolyzed water treatments available which are reviewed by Hricova, et al., and Rahman, et al. (Table 2). In single chamber systems containing a membrane, basic electrolyzed water (BEW) and acidic electrolyzed water (AEW) are produced concurrently. BEW is produced near the cathode while AEW is produced near the anode. Single cell systems without a diaphragm create a mixture of AEW and BEW. The diversity of these treatments contributes to the multitude of applications in which this sanitizer can be used.

Table 1.2. Four Types of Electrolyzed Water Treatments Outlining their pH and ORP Values.

<table>
<thead>
<tr>
<th>Type</th>
<th>pH</th>
<th>ORP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic electrolyzed water (BEW)</td>
<td>10-13</td>
<td>800 to 900</td>
</tr>
<tr>
<td>Acidic electrolyzed water (AEW)</td>
<td>2.0-3.0</td>
<td>&gt;1100</td>
</tr>
<tr>
<td>Neutral electrolyzed water (NEW)</td>
<td>7.0-8.0</td>
<td>750 to 900</td>
</tr>
<tr>
<td>Slightly acidic electrolyzed water (SAEW)</td>
<td>5.0-6.5</td>
<td>850</td>
</tr>
</tbody>
</table>

Information adopted from Rahman et al., (2016)

1.8.2 Mechanism of Action

Electrolyzed water used as a sanitizer contains the active chlorine species HOCI, Cl₂ and ClO⁻ with the addition of hydrogen peroxide. The germicidal capability of EO is not fully
understood but has been modeled by Fuzuaki (2006) who attributed this activity to HOCl and ClO⁻. ClO⁻ is not capable of lipid bilayer penetration but is thought to oxidize the cell from the outside, inactivating functional proteins present in the plasma membrane (Fuzuaki, 2006).

Antimicrobial activity of electrolyzed water is largely due to the formation of HOCl (Hotta et al., 1995). Due to its polarity, HOCl penetrates bacterial membranes, inhibiting enzyme activity, damaging the membrane and DNA, and deteriorating transport capacity (Albrich et al., 1986; Fuzuaki, 2006).

1.8.3 Concerns with Electrolyzed Water Use

Although electrolyzed water is a promising technology, it has presented a few limitations for widespread application. When applied at a low pH, free chlorine can be released, which could cause corrosion of equipment and discomfort to workers (Rahman et al., 2010). Additionally, electrolyzed water can lose its antimicrobial activity quite rapidly if it is not used immediately (Rahman et al., 2016). Improper storage of solution will reduce chlorine concentration, further jeopardizing its antimicrobial properties. The implications of this treatment on the quality of a variety of food products are not well understood. Therefore, determining the effects of the treatment on texture, color, taste, and nutritional qualities of the food products is necessary.

1.9 Factors that Contribute to Sanitizer Efficacy

There are many factors that contribute to the germicidal capacity of aqueous sanitizer treatments. Organic matter, pH, and water temperature contribute to the overall effectiveness of chlorine (liquid), PAA, and electrolyzed water. It is well known that the formation of HOCl in electrolyzed water and chlorine is responsible for their germicidal properties (Estrela et al., 2002; Hotta et al., 1995; Kim et al., 2000; Odlaug, 1980). Compared to application at a high pH, application of these treatments at a neutral pH has been shown to result in the strongest
germicidal effects due to the increased concentration of chlorine compounds (Kim et al., 2000; Odlaug, 1980). A study conducted by Len and others demonstrated that as pH increases, the formation of HOCl decreases due to the increased dissociation of HOCl into OCl− and H+ (2000). In addition, other studies evaluating the relationship between pH and ORP suggest that at low pH, chlorine concentration increases, suggesting that a combination of pH and ORP play a role in the germicidal effect of electrolyzed water (Park and others, 2004). The presence of organic matter in wash water can greatly decrease the germicidal capacity of chlorine and electrolyzed water due to their tendency of interacting with organic matter before interacting with microbial populations present (Beuchat et al., 2004).

For electrolyzed water applications, flow rate and salt concentration are linearly correlated with the current generated (Hsu, 2005). Hsu demonstrated that as flow rate increases, current increases, due to greater electrolysis of salt. This contributes to the sanitizer’s effectiveness because the salt concentration is directly correlated with the concentration of HOCl generated. Water hardness has also been shown to affect pH, ORP, and overall free chlorine present in the solution (Forghani et al., 2015). Recent studies have confirmed the importance of understanding water hardness and its effect on the germicidal properties of this sanitizer.

Peracetic acid (PAA) has displayed a wide range of antimicrobial properties and has been used in several systems as a disinfectant. PAA was found to be less affected by organic matter and pH than chlorine and electrolyzed water, and more affected by high temperatures (Kunigk et al., 2001; Rodgers et al., 2004). This is because high temperatures promote instability and combustion of peroxide radicals in PAA solutions (Kunigk et al., 2001). Hence, this treatment is useful for produce disinfection due to the use of cold-water in these processing lines.
There are a multitude of factors that can greatly diminish the antimicrobial efficacy of aqueous sanitizer treatments. Thus, a deeper understanding of these factors can encourage more effective utilization of these treatments in large scale processing facilities, as compared to idealized, bench scale experiments.

### 1.10 Sanitizer Application on Fresh Produce

Sanitizing fresh produce is effective in diminishing the risk of cross contamination with pathogens when applied appropriately. Sanitizer application method, such as a dip or spray, and the type of sanitizer, are dependent on the commodity being sanitized. There are several considerations when sanitizing a product, including: potential effect on final product quality, surface conformation of the product, and the likelihood of pathogen survival in the product. Commodities with firm surfaces, such as avocados, apples, and cantaloupe may be less susceptible to loss of product quality from sanitizer application but are more difficult to decontaminate due to potential bacterial harborage sites present on the surface of these products. Conversely, berry products (blackberries, blueberries, raspberries), and fresh herbs may be more susceptible to loss of quality post-sanitization, making this methodology less feasible. The likelihood of successful decontamination of fresh produce depends on several factors such as product pH, water activity, and their susceptibility to breakage during harvest and processing (Beuchat et al., 2004; Lang et al., 2004; Ukuku and Fett, 2004). Current research on produce sanitation efficacy varies greatly in terms of sanitizer concentration applied, contact time of sanitizer, and inoculation protocol for test microorganism.

Gonzalez and others (2004) evaluated the effectiveness of PAA and chlorine sanitizer treatments against *Escherichia coli* 0157:H7 on fresh cut carrots and achieved reductions of ~1.5 log CFU/g. Beuchat and others (2004), evaluated the effectiveness of chlorine treatment against
Listeria monocytogenes on iceberg and romaine pieces, and shredded iceberg with inoculum levels ranging from 1.5 – 4.7 log CFU/g and observed varying results based on inoculum level and sample type. Specifically, a higher reduction (1.7 log CFU/g) was achieved on lettuce pieces with high inoculum, compared to shredded lettuce with low inoculum (0.2 log CFU/g) and high inoculum (1.3 log CFU/g). One study conducted by Vandekinderen and others (2009) evaluated the effectiveness of PAA (25, 150, and 250 ppm) against native microflora on grated carrot, fresh cut cabbage, iceberg lettuce, and leek. They found that PAA was most effective at reducing populations on carrots and cabbage (0.5-3.5 log CFU/g) followed by lettuce, and leek (0.4-2.4 log CFU/g and 0.4-1.4 log CFU/g) which can be explained by the differences in surface type of each product. PAA concentration and contact time were linearly correlated to the reductions achieved. Several other studies have been conducted to evaluate sanitizer efficacy on fresh produce but none have demonstrated the same results.

Although limited information is available, studies evaluating sanitation efficacy on wild blueberries have been conducted at the bench scale with high inoculum levels making it difficult to utilize these results in large scale production (Crowe et al., 2005; Sheng et al., 2019; Tadepalli et al., 2018, 2019). A recent study conducted on wild blueberries looked at several sequential sanitizer combinations (chlorine (100 or 200 ppm), lactic acid (2%), chlorine dioxide (15 ppm), ozone (5 ppm) for up to 3 minutes of contact time followed by freezing for one week. Authors observed reductions of 1.8-6.9 log CFU/g of L. monocytogenes with chlorine (100 ppm) followed by lactic acid (2%) being the most effective treatment (Tadepalli et al., 2019). In this study, treated product consisted of previously frozen wild blueberries, which may have given rise to inconsistencies in inoculation when compared to the majority of studies using fresh berries. Another study conducted by Tadepalli and others (2018), evaluated the reductions of E. coli
O157:H7, Salmonella, and L. monocytogenes with single spray treatments of chlorine dioxide and chlorine for up to 10 minutes followed by one week of freezing, and achieved reductions of 4.4 log CFU/g, 5.4 log CFU/g, and 6.1 log CFU/g respectively. Both studies evaluated sanitizer treatments on previously frozen wild blueberries and used high starting inoculum levels (~7 log CFU/g) which could have potentially affect applicability of results at commercial scale. Crowe and others (2005) evaluated the efficacy of sanitizer treatments (0.5% hydrogen peroxide, chlorine, and 0.5% citric acid) against background microflora on fresh wild blueberries and observed a microbial reduction of less than 2 log CFU/g. A recent study by Sheng and others (2019), evaluated the effects of chlorine (100 ppm) and Neo-Pure 0.4% (58% sodium percarbonate, 20% tetraacetyl-ethylenediamine, 18% citric acid, and 4% surfactant; generates PAA in water) treatments and cold storage against L. monocytogenes using dip and spot inoculation. Listeria populations that survived the sanitizer treatments were relatively stable during the frozen storage and the method of inoculation greatly affected Listeria reductions achieved from treatments. After treatment with chlorine, blueberries spot inoculated with high levels of Listeria (8.6 log CFU/g) were reduced by 5.7 log CFU/g compared to those spot inoculated with low levels of Listeria (3.8 log CFU/g) which achieved a reduction of 2.5 log CFU/g. Blueberries dip inoculated with 4.0 log CFU/g achieved reductions of 0.5 log CFU/g. All of the studies described above achieved different microbial reductions which could be explained by the lack of cohesiveness in methodology.

Similar to the studies conducted on wild blueberries, differences in experimental conditions on cantaloupe pose another challenge for applicability at commercial scale. Due to its rough surface, incidence of bacterial internalization, and flesh which promotes bacterial survival, sanitizing cantaloupe has been largely ineffective (Fang et al., 2013). Ukuku and others (2001,
evaluated the effects of sanitizer treatments on whole and cut cantaloupes with the addition of refrigerated storage against Salmonella and L. monocytogenes and the likelihood of bacterial transfer during cutting. The washing of contaminated cantaloupe rind with chlorine (1000 ppm) achieved up to a 3.5 log CFU/cm^2 reduction in the Listeria populations present and prevented the transfer of pathogen to the product flesh. They also found that Listeria inoculated on cantaloupe flesh stored at 4°C demonstrated survival for up to 15 days. Ukuku and others (2001) evaluated the survival of Salmonella (3.8 log CFU/cm^2) on chlorine (1000 ppm) treated cantaloupe rind and the incidence of transfer to the flesh upon cutting. They achieved 3.0 log CFU/cm^2 reductions after treatment, and Salmonella was undetected on the cut flesh. Following refrigerated storage, sanitizer treatment effectiveness was diminished. Fang and others (2013) evaluated the survival of L. monocytogenes and background microflora in cantaloupe flesh at temperatures ranging from 8-40°C. They found that for all storage temperatures all populations grew for up to eight days before reaching the stationary phase. Despite observing pathogen population reductions, all studies have failed to reach a 5-log reduction, which is the ideal threshold generally set for a “kill step”. A deeper understanding of pathogenic bacteria survival in cantaloupe flesh is necessary to ensure product safety for consumers and validate the effectiveness of processes applied by growers and manufacturers.

These studies demonstrate the variability in methodology which can greatly influence the overall effectiveness of sanitizer treatments. The studies evaluated demonstrate the challenge presented to processors and the importance of understanding the methodology used and the applicability of these results at the commercial scale.
1.11 Significance and Experimental Objectives

Due to outbreaks that have occurred in fresh cut cantaloupe and frozen food products, there has been a desire to evaluate the efficacy of current sanitization methods on processing lines. This study aimed to mimic a processing environment which included not controlling for the variability you typically see in large processing facilities. This includes variability within the product, uneven application of sanitizer, and uneven distribution of inoculum. Additionally, background microflora was not removed on the product prior to inoculation and treatment to mimic the complex interactions between bacteria and sanitizer. Sanitizer treatments were applied for three minutes (maximum feasible for commercial line configuration) and at a concentration currently approved under FDA regulations. Current research on these product types have evaluated sanitizer treatments at the bench scale using extended contact times and high sanitizer concentrations which diminishes the applicability of the results at the commercial scale. The results obtained in this study demonstrate the complex system in which sanitizers are applied and how variability can greatly alter your results. This data can be applied to the pilot scale and help processors to make more informed decisions regarding their sanitation protocol.

The goal of this study was to:

- Compare the sanitizing capacity of peracetic acid (PAA), electrolyzed oxidizing water (EO), and chlorine against *L. innocua* and native microflora on wild blueberries and fresh cut cantaloupe applied within commercial processing constraints, including:
  - To determine if current processes were meeting current goals for food safety
  - To determine if combination treatments are an effective method at controlling microbial populations
To evaluate the effects of contact time on sanitizer efficacy as well as the method of application (immersion verse spray, spray-spray, immersion-spray)

To validate the treatment protocol at the pilot scale

1.12 Conclusion

Current research on fresh produce is often centered around small-scale studies with varying levels of inoculum, concentration of sanitizers applied, and the method in which produce is treated. There have been no prior studies that have looked at the efficacy of sanitizer treatment against *Listeria* spp. at the pilot scale for either of the commodities discussed in this review. Here, we aimed to evaluate sanitization on high risk (cantaloupe) and low risk (wild blueberries) products at the bench and pilot scales and better understand relationships between the product, sanitizer, and pathogen survival in scenarios relevant to each commodity as scale increases. The evaluation of sanitizer treatments and product storage at the pilot scale can help producers to better understand their sanitizing process as well as the survival of pathogens in a retail setting.

This research can assist producers in making more informed decisions when harvesting and processing their product to mitigate any future food safety risks.

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CHAPTER 2

REDUCTION AND SURVIVAL OF *L. INNOCUA* AND NATIVE MICROFLORA IN WILD BLUEBERRIES

2.1 Abstract

*Listeria monocytogenes* outbreaks in frozen foods have caused concern regarding the way food is handled during processing. Increasing sanitizing capacity of post-harvest processing lines is crucial to ensure safety of frozen produce. The goal of this study was to compare the sanitizing capacity of peroxyacetic acid (PAA), electrolyzed oxidizing water (EO), and chlorine against *Listeria innocua* and native microflora on wild blueberries. To test the effectiveness of these sanitizers, PAA (80 ppm), EO (200 ppm active chlorine constituents), chlorine (200 ppm), and water (control) were applied to inoculated, fresh wild blueberries by immersion and/or spraying, singly and in combination (3 minutes total contact time). In addition to sanitizer application, treated product was individually blast frozen, stored at -20°C and analyzed for surviving bacteria for up to two weeks. Data were analyzed by multiway ANOVA in conjunction with Tukey HSD in R Studio. To investigate the effectiveness of sanitization treatments, application sequence and method at reducing *L. innocua* populations, a benchtop study followed by a scale up study were completed. For the benchtop study we found that immersion then spraying significantly reduced *L. innocua* populations (p < 0.05), compared to the untreated control. For yeast populations, all methods of sanitizer application significantly reduced these populations. The spray application of EO followed by chlorine was significantly (p < 0.05) more effective at reducing *L. innocua* populations compared to the untreated control. For immersion followed by spraying, all treatments except for water followed by water significantly (p < 0.05) reduced *L. innocua* populations compared to the untreated control. Sequential sprays with chlorine followed by PAA and EO followed by chlorine reduced yeast
populations to a significantly (p < 0.05) greater extent than the application of water followed by water. In our pilot scale study, we found that immersion in PAA followed by spraying with PAA achieved a reduction of 2.2 log CFU/g against *L. innocua* and was the most effective application sequence tested. None of the treatments investigated significantly (p > 0.05) reduced the total aerobic population compared to the untreated control. Regardless of contact time, immersion in PAA followed by spraying with PAA was significantly more effective than the application of water followed by water (p =0.03) and chlorine followed by PAA (p= 0.03) at reducing the total aerobic population, resulting in a population decrease of 1.9 log CFU/g, versus 0.6 log CFU/g for water followed by water and 0.5 log CFU/g for chlorine followed by PAA. After freezing for two weeks, viable *L. innocua* cells were still present in the frozen product (73% positive). Yeast populations on incoming fruit was high (~7.0 log CFU/g) and none of the treatments investigated reduced these populations significantly (p > 0.05) compared to the untreated control. Maximum sanitizer concentration and application by immersion then spraying are recommended to achieve the largest possible population reduction on wild blueberries.

2.2 Introduction

In recent years, outbreaks linked to *Listeria monocytogenes* have provided validity for concern regarding survival of this pathogen in frozen foods. In 2015, Blue Bell Creamery products contaminated with *L. monocytogenes* caused 10 illnesses and three deaths (CDC, 2015). Additionally, in 2016, a multistate outbreak occurred in frozen vegetables contaminated with *L. monocytogenes*. Products produced by CRF Frozen Foods were associated with nine illnesses and three deaths (CDC, 2016).

Wild blueberries are native to Maine and Eastern Canada. There are many potential health benefits of consuming blueberries which is one reason for the increase in consumption is
observed in recent years (USDA-ARS, 2016). Although there are many known health benefits from consuming this product, the way in which it is processed and consumed, can contribute to the likelihood for microbial contamination (Kniel and Shearer, 2014; Tefera et al., 2018). Wild blueberries are grown closely to the ground making them susceptible to microbial contamination via the soil (Tefera et al., 2018; Yarborough, 2015). Wild blueberries are harvested in late summer through a process of mechanized raking. The majority of the crop (approximately 99%) is processed for frozen storage and sale. This process commonly includes a winnowing step followed by sanitization through a process of immersion or spraying in a wash tank/conveyer system. Wash tanks can serve as a reservoir for contamination if contaminated product is brought through the facility (Luo et al., 2010, 2012). Further, locations within the processing environment may serve as harborage points for pathogens, as demonstrated in the 2016 CRF outbreak.

Due to cooler temperatures and the presence of moisture, processing facilities provide a suitable environment for survival of *Listeria* spp. (Hill et al., 2001). Additionally, outbreaks which have occurred in frozen foods contaminated with *L. monocytogenes* validate concern with this product type due to the ability of *Listeria* to survive during frozen storage (Flessa et al., 2005; Palumbo and Williams, 1991). The lack of a “kill step” in the processing of wild blueberries and other frozen fruits increases the risk of contamination as they may not be cooked before consumption.

To ensure the safety and prevent microbial contamination of produce that is sold ready-to-eat (RTE), post-harvest processing practices are crucial. Current industry standards include use of chlorine (up to 200 ppm) as the main source of decontamination of fresh produce (FDA-CFSAN, 1998). The use of alternative sanitizers has been of interest to producers and consumers
due to the potential microbial contamination risk and the environmental and human health risks associated with the use of chlorine (Ruiz-Cruz et al., 2007). While alternative treatments may be effective at the bench scale, it is imperative to consider the constraints of existing equipment configuration at the commercial scale. Economic feasibility of treatment application, heterogeneity of product, inoculum distribution, and sanitizer application at larger scale should also be taken into account. This study aimed to evaluate the use of alternative sanitization methods on wild blueberries and the survival of \textit{L. innocua} after two weeks of frozen storage. Specific attention was given to scale by reproducing a pilot scale version of the common processing line configuration.

2.3 Materials and Methods

2.3.1 Bacterial Strains and Inoculum Preparation

\textit{Listeria innocua} strain ATCC # 33090 (American Type Culture Collection, Manassas, VA) was grown in tryptic soy broth (TSB, Alpha Biosciences, Baltimore, MD) at 30°C for 24 hours. Overnight cultures were spread onto tryptic soy agar (TSA, Alpha Biosciences, Baltimore, MD) and grown for 24 hours at 30°C to promote increased resistance to treatment (Gandhi and Chikindas, 2007). \textit{L. innocua} lawns were harvested with 0.1% peptone (Difco, Sparks, MD) and transferred to 5 mL of 0.1% peptone. The resulting solution was used to inoculate fresh wild blueberries to achieve a final concentration of ~4 log CFU/g. In this study \textit{L. innocua} was used as a surrogate for \textit{L. monocytogenes} due to high potential for environmental contamination during pilot scale work.
2.3.2 Fresh Wild Blueberries

Fresh wild blueberries were obtained from Allen’s Freezer Incorporated (Ellsworth, ME) for four consecutive weeks during August 2018. Blueberries were transported in perforated plastic produce bins and stored at 4°C for up to 3 days before treatment.

2.3.3 Inoculation of Wild Blueberries

Fresh wild blueberries were rinsed with cold tap water and laid out on sterile plastic trays for manual removal of large debris. Blueberries were stored at ambient temperature in a biological safety cabinet (SterilGARD Hood, Sanford, ME) for 24 hours to dry before inoculation. Rinsed wild blueberries were weighed into sterilized smooth-bottomed aluminum tins for inoculation. Each tin of blueberries was inoculated with prepared *L. innocua* cell suspension (as described above) and shaken for 45 seconds to ensure even distribution of the inoculum solution. Inoculated wild blueberries were left to dry at ambient temperature for 24 hours in a biological safety cabinet.

2.3.4 Microbiological Analysis- Enumeration of Native Microflora on Unwashed Fresh Wild Blueberries

Duplicate samples of fresh, unwashed and washed wild blueberries were taken combined with 0.1% peptone and agitated for 2 minutes. After agitation, the samples were serially diluted in 0.1% peptone and spread plated onto TSA and acidified potato dextrose agar (APDA, Alpha Biosciences, Baltimore, MD). Plates were incubated at 37°C for 48 hours (TSA), and room temperature for 5 days (APDA). Microbial populations were determined (log CFU/g) for total aerobic bacteria and yeast.
2.3.5 Microbiological Analysis- Enumeration of *L. innocua* and Native Microflora on Inoculated Wild Blueberries

After inoculation, triplicate samples were taken to determine *L. innocua* and native microflora population levels on the wild blueberries. Samples were homogenized and plated as described above with the addition of modified Oxford agar (MOX, Alpha Biosciences, Baltimore, MD), incubated at 30°C for 48 hours.

2.3.6 Sanitizer Treatment Preparation

Individual sanitizer treatments were prepared using sterilized tap water. Concentration of electrolyzed water (stock solution concentration of 500 ppm, pH 6.5; JDP- Clarentis®, Palm Beach Gardens, FL) was determined using a HACH digital titrator kit (HACH, Model 16900, Loveland, CO) and diluted to achieve a final concentration of 200 ppm active chlorine constituents. A 5% Sodium Hypochlorite solution (Lab Chem, Zelienople, PA) was diluted to yield a final concentration of 200 ppm and verified using commercial test strips (Micro Essential Lab, Brooklyn, NY). Concentrated peroxyacetic acid (15% vol/vol) was diluted to achieve a final concentration of 80 ppm and was verified using a commercial peracetic acid test kit (Alpha Chemical, Stoughton, MA). Diluted solutions were stored for no longer than 24 hours before use.

2.3.7 Benchtop Study

2.3.7.1 Sanitizer Treatment of Inoculated Wild Blueberries

Inoculated wild blueberries were subjected to a variety of sanitizer treatments to determine the efficacy of the application method, and sequence of application on *L. innocua* and native microflora. Inoculated blueberries were distributed into sterile steam pans for treatment. For sanitizer treatments, the blueberries were subjected to spraying, immersion, or combination treatments of multiple sprays or immersion followed by spraying. For spraying and immersion
contact time varied between 30 seconds and 5 minutes with duplicate samples taken at predetermined time points. For combination treatments, blueberries were treated for up to 3 minutes total contact time and samples were taken in duplicate at 90 seconds and 3 minutes. Post-treatment microbiological analysis was carried out as previously described.

2.3.8 Pilot Scale Study

2.3.8.1 Microbiological Analysis- Enumeration of *L. innocua* and Native Microflora on Unwashed, Washed, Inoculated, and Treated Fresh Wild Blueberries

Enumeration of bacterial populations were the same as described above with the addition of coliforms population enumeration. To determine the coliform population, samples were plated on 3M petrifilm (3M, Maplewood, MN) and incubated at 35°C for up to 48 hours.

2.3.8.2 Sanitizer Treatment Preparation

Sanitizers were prepared as described above. Tap water was used in place of sterile water.

2.3.8.3 Pilot Scale Setup

Pilot scale work was completed on a conveyer consisting of three spray bars and a mechanized perforated belt (Figure 1). The system was controlled with a DirectSoft5 (Automation Direct, Cumming, GA) programmable logic controller (PLC) interface. Three peristaltic pumps were run at 30 Hz with a flow rate of 0.5 GPM. Two nozzles discharging a flat cone spray pattern were mounted to each spray bar. Parameters for the conveyer were chosen based on equipment capabilities as well as current industry processing practices.
Figure 2.1a-c. Custom Built Mock Processing Line and Setup for Treatment of Inoculated Wild Blueberries. 1a. Sanitizer immersion of inoculated wild blueberries in sterilized steam pan, 1b. perforated tray with inoculated wild blueberries after immersion, 1c. mock processing line showing spray bars (a) used to spray sanitizer treatments and perforated conveyor belt (b)

2.3.8.4 Sanitizer Treatment and Freezing of Inoculated Wild Blueberries

Wild blueberries were immersed in then sprayed with chosen sanitizer combinations. Prior to treatment, the conveyor lines were purged for 1 minute with the corresponding sanitizer for each line. Wild blueberries were immersed in sanitizer for 1.5 minutes followed by spraying for 1.5 minutes. Samples were taken in duplicate after each step to determine the efficacy of individual steps. After treatment, blueberries were individually blast frozen for 4 hours, packaged and stored at -20°C for up to two weeks. Upon completion of sanitization, the lines were purged with water for 30 seconds to remove residual sanitizer.
2.3.8.5 Microbiological Analysis-Enumeration of *L. innocua* and Native Microflora Treated Inoculated Wild Blueberries after 24 Hours of Freezing

At 24 hours after freezing, *L. innocua* and native microflora populations were determined as described previously.

2.3.8.6 Microbiological Analysis-Enumeration of *L. innocua* and Native Microflora on Frozen Treated Inoculated Wild Blueberries after 2 Weeks of Frozen Storage

After two weeks of frozen storage, samples were taken to assess survival of *L. innocua* and native microflora on wild blueberries. For enrichment, 10g of blueberries were diluted 1:10 in Listeria Enrichment Broth (LEB, Alpha Biosciences, Baltimore, MD) which was incubated at 30°C for 24 hours. Incubated enrichment was spread onto MOX and incubated at 30°C for 48 hours.

2.3.8.7 Statistical Analysis

Data was analyzed for to determine statistical significance (p <0.05) between sanitizer, application method and contact time using a multiway ANOVA and Tukey HSD in R studio.

2.4 Results

2.4.1 Benchtop Study

2.4.1.1 Efficacy of Sanitizer Treatments and Method of Application on Inoculated Wild Blueberries

2.4.1.2 Immersion

Pre-treatment *L. innocua* populations on berries ranged from 4.2-4.5 log CFU/g with an average of 4.3 log CFU/g. Pre-treatment levels of native yeast ranged from 6.9-7.1 log CFU/g with an average of 7.0 log CFU/g. Inoculation procedures were the same for each trial and samples were taken in triplicate to account for any variability.
For immersion treatments, *L. innocua* population reductions ranged from 0.3-1.1 log CFU/g (Figure 2). Immersion in EO was the most effective at reducing *L. innocua* populations with a reduction of 1.1 log CFU/g. However, due to variability, none of the treatments investigated significantly (p > 0.05) reduced *L. innocua* populations compared to the untreated control. Initial yeast populations were high and reductions were relatively low, ranging from 0.1-0.6 log CFU/g (Figure 3). Regardless of contact time, immersion in chlorine (p = 0.01), and PAA (p = 0.01) were more effective against yeast than immersion in water. The application of chlorine and PAA reduced yeast populations by 0.6 log CFU/g compared to water which reduced yeast populations by 0.2 log CFU/g. The application of EO for 5 minutes and PAA for 3 minutes reduced yeast populations by 0.7 log CFU/g and significantly (p < 0.05) reduced yeast populations compared to the untreated control.

**Figure 2.2 Reduction of *L. innocua* on Wild Blueberries Immersed in Sanitizer Treatments for Increasing Time Intervals**

Data represents mean reduction of *L. innocua* population after immersion treatment with sanitizer (n = 3), error bars represent standard deviation, black (30 sec), light gray (1.5 min), dark grey (3 min), striped (5 min).
Figure 2.3. Reduction of Yeast on Wild Blueberries Immersed in Sanitizer Treatments for Increasing Time Intervals

Data represents mean reduction of yeast population after immersion treatment with sanitizer (n = 3), error bars represent standard deviation, black (30 sec), light gray (1.5 min), dark grey (3 min), striped (5 min), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water.

### 2.4.1.3 Spray

Spraying alone resulted in \textit{L. innocua} reductions of 0.1-2.5 log CFU/g (Figure 4) and yeast population reductions of 0.1-1.0 log CFU/g (Figure 5). None of the treatments investigated significantly (p > 0.05) reduced \textit{L. innocua} populations compared to the untreated control. Spraying with PAA for three minutes significantly (p < 0.05) reduced yeast populations compared to the untreated control. For all contact times, spraying with chlorine, PAA or EO resulted in yeast population reductions of 0.6-0.7 log CFU/g, and was significantly (p < 0.05) more effective than spraying with water (0.2 log CFU/g reduction).
Figure 2.4. Reduction of *L. innocua* on Wild Blueberries Sprayed with Sanitizer for Increasing Time Intervals
Data represents mean reduction of *L. innocua* population after spray treatment with sanitizer (n = 3), error bars represent standard deviation, black (30 sec), light gray (1.5 min), dark grey (3 min), striped (5 min).

Figure 2.5. Reduction of Yeast on Wild Blueberries Spray Treated with Sanitizer for Increasing Time Intervals
Data represents mean reduction of yeast population after spray treatment with sanitizer (n = 3), error bars represent standard deviation, black (30 sec), light gray (1.5 min), dark grey (3 min), striped (5 min), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water.
2.4.1.4 Combination Spray

Sequential sprays of sanitizer reduced \textit{L. innocua} populations by 0.5-2.1 log CFU/g (Figure 6). Except for EO followed by chlorine, none of the treatments applied significantly (p < 0.05) reduced \textit{L. innocua} populations compared to the untreated control or treatment with water alone. Yeast population reductions ranged from 0.0-0.8 log CFU/g (Figure 7) and with the exception of water followed by water, and EO followed by chlorine, were significantly (p = 0.0) reduced when compared to the untreated control. The application of chlorine followed by PAA resulted in a population reduction of 0.8 log CFU/g and significantly (p = 0.0) reduced yeast populations to a greater extent compared to water alone, which resulted in a reduction of 0.5 log CFU/g.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure26}
\caption{Reduction of \textit{L. innocua} on Wild Blueberries Subsequently Sprayed with Sanitizer for Increasing Time Intervals}
\begin{flushleft}
Data represents mean reduction of \textit{L. innocua} population after subsequent spray treatment with sanitizer (n = 3), light grey (spray 1), black (spray 2), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water
\end{flushleft}
\end{figure}
Data represents mean reduction of yeast population after subsequent spray treatment with sanitizer (n = 3), light grey (spray 1), black (spray 2), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water.

2.4.1.5 Immersion followed by Spray

Immersing followed by spraying was the most effective method of application at reducing \( L. \, innocua \) populations. \( L. \, innocua \) population reductions ranged from 1.3- 3.2 log CFU/g (Figure 8). All treatments investigated, excluding water followed by water, significantly (p < 0.05) reduced \( Listeria \) populations compared to the untreated control. Immersion in EO followed by spraying with PAA was the most effective treatment against \( L. \, innocua \), and significantly (p=0.03) reduced \( L. \, innocua \) populations to a greater extent than immersion in water followed by spraying with water. Immersion in PAA followed by spraying with chlorine and water followed by chlorine was the most effective treatment against yeast, and significantly (p=0.02) reduced yeast populations compared to the untreated control (Figure 9).
Figure 2.8. Reduction of *L. innocua* on Wild Blueberries Subsequently Immersed then Spray with Sanitizer for Increasing Time Intervals

Data represents mean reduction of *L. innocua* population after subsequent spray treatment with sanitizer (n = 3), light grey (immersion), black (spray). Superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water.

Figure 2.9. Reduction of Yeast on Wild Blueberries Subsequently Immersed then Spray with Sanitizer for Increasing Time Intervals

Data represents mean reduction of yeast population after immersion-spray treatment with sanitizer (n = 3), error bars represent standard deviation, light grey (immersion), black (spray). Superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water.
2.4.2 Pilot Scale Study

Pre-treatment *L. innocua* populations ranged from 4.0-5.2 log CFU/g with an average of 4.7 log CFU/g. Pre-treatment levels of native yeast ranged from 7.0-7.1 log CFU/g with an average of 7.0 log CFU/g. Total aerobic bacteria ranged from 6.0-7.2 log CFU/g with an average of 6.7 log CFU/g. Coliforms ranged from 1.0-1.2 log CFU/g with an average of 0.52 log CFU/g. Inoculation procedures were the same for each trial and samples were taken in triplicate to account for any variability.

2.4.2.1 Efficacy of Sanitizer Treatments and Frozen Storage for Two Weeks on Inoculated Wild Blueberries

Immersion followed by spraying with sanitizer reduced *L. innocua* populations by up to 2.2 log CFU/g (Figure 10). Statistically, none of the treatment combinations, significantly (p > 0.05) reduced *L. innocua* populations compared to the untreated control. The immersion of inoculated berries in PAA followed by spraying with PAA resulted in the largest reduction of *L. innocua*. Sanitizer treatments investigated reduced total aerobic bacteria populations up to 1.9 log CFU/g (Figure 11). None of the treatments applied significantly (p < 0.05) reduced the total aerobic bacteria population compared to the untreated control. Regardless of contact time, the application of PAA followed by PAA significantly reduced the total aerobic bacteria population to a greater extent compared to the application of water followed by water (p=0.03) and chlorine followed by PAA (p = 0.03). Similar to the benchtop study, yeast population reductions remained low and reached a maximum reduction of only 0.5 log CFU/g (Figure 12). While none of the treatments investigated significantly (p > 0.05) reduced yeast populations when compared to the untreated control or to each other, the application of chlorine followed by chlorine was most effective combination against these organisms. Coliform presence appeared to be unaffected by treatment but was reduced by subsequent freezing (Table 2.1). Although we
observed slight variability in coliform positive samples, we observed a downward trend in population post freezing which resulted in 4 out of 30 positive samples (13%).

Figure 2.10. Reduction of *L. innocua* on Wild Blueberries Immersed then Spray Treated with Sanitizer followed by Frozen Storage for 24 Hours
Data represents mean reduction of *L. innocua* population after immersion-spray treatment with sanitizer and frozen storage for 48 hours (n = 3), light grey, (immersion), white (spray), black (24 hours after freezing), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water

Figure 2.11. Reduction of Total Aerobic Bacteria on Wild Blueberries Immersed then Spray Treated with Sanitizer followed by Frozen Storage for 24 Hours
Data represents mean reduction of *L. innocua* population after immersion-spray treatment with sanitizer and frozen storage for 48 hours (n = 3), light grey, (immersion), white (spray), black (24 hours after freezing), superscripts indicate significant differences (p < 0.05) between sanitizer treatments and water
Figure 2.12. Reduction of Yeast on Wild Blueberries Immersed then Spray Treated with Sanitizer followed by Frozen Storage for 24 Hours
Data represents mean reduction of *L. innocua* population after immersion-spray treatment with sanitizer and frozen storage for 48 hours (n = 3), light grey, (immersion), white (spray), black (24 hours after freezing).

Table 2.1. Positive Coliforms Detected in Treated Wild Blueberries and 24 hours after Freezing. Table indicates positive coliform samples across three trials before and after treatment:

<table>
<thead>
<tr>
<th>Coliforms</th>
<th>Pre-Treatment</th>
<th>Post-Treatment</th>
<th>24 Hours After Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Water</td>
<td>4/6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Water-Chlorine</td>
<td>1/6</td>
<td>3/6</td>
<td>1/6</td>
</tr>
<tr>
<td>PAA-PAA</td>
<td>0/6</td>
<td>4/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Chlorine-Chlorine</td>
<td>2/6</td>
<td>3/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Chlorine-PAA</td>
<td>2/6</td>
<td>3/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

2.4.2.2 *L. innocua* Survival after Two Weeks of Frozen Storage

After two weeks of frozen storage samples were analyzed for the presence of *L. innocua*.

Out of 15 samples that were tested, 11 were positive for *L. innocua* (Table 2.4).
Table 2.2. Survival of *L. innocua* in Treated Wild Blueberries after Two Weeks of Freezing.  
10g samples were analyzed for the presence of *L. innocua*. Survival column indicates samples positive for *L. innocua* for each of the three trials tested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (10g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Water</td>
<td>2/3</td>
</tr>
<tr>
<td>Water-Chlorine</td>
<td>2/3</td>
</tr>
<tr>
<td>PAA-PAA</td>
<td>3/3</td>
</tr>
<tr>
<td>Chlorine-Chlorine</td>
<td>2/3</td>
</tr>
<tr>
<td>Chlorine-PAA</td>
<td>2/3</td>
</tr>
</tbody>
</table>

2.5 Discussion

The goal of this study was to explore alternative sanitization methods for wild blueberries that could be integrated into current processing practices with minimal capital expense. Treatment of inoculated wild blueberries at the pilot scale was conducted for realistic evaluation of sanitizer efficacy more representative of a large-scale processing environment. Additionally, frozen storage for up to two weeks allowed for exploration of the synergy between sanitization and freezing against *L. innocua*. Our results can be used as a tool for producers to choose proper processing interventions to mitigate food safety risks and quality concerns associated with this product type.

Due to wild blueberries being produced solely in the Northeastern United States, little research on aqueous sanitization has been done. Those studies that have been published have not taken into account equipment limitations of existing processing lines and have been conducted at bench scale. As demonstrated by the results of this study, the introduction of confounding factors unavoidably introduced as scale increases is expected to result in reduced treatment efficacy. The majority of research related to this commodity has been focused on the nutritional profile of wild blueberries. The lack of research on wild blueberries is likely due to the absence of historical outbreaks associated with this product in addition to the small market size. A similar study on wild blueberries previously conducted at the University of Maine investigated the efficacy of
chlorine, 0.5% citric acid, and 0.5% hydrogen peroxide against native yeast, mold, coliforms, and total aerobic bacteria with the addition of frozen storage for up to 5 weeks (Crowe et al., 2002). Investigators found that chlorine was most effective at reducing populations of interest followed by hydrogen peroxide, and citric acid (Crowe et al., 2002). In this study, none of the treatments investigated significantly (p > 0.05) reduced *L. innocua*, yeast, coliforms, total aerobic bacteria, or mold populations which may be attributed to inherent variability in the product and present microflora on incoming fruit.

Application of aqueous sanitizers reduced *L. innocua* by up to 2.2 log CFU/g but variability was too great to observe any significant differences between treatments. This variability can be attributed to many factors which must be considered by producers selling this product. Wild blueberries were inoculated via swirling in the inoculum solution which led to purposeful heterogenous distribution of bacterial cells. Additionally, propagation of bacterial cells on solid, as opposed to liquid medium, and an extended post-inoculation drying step were both employed to increase resistance of inoculum to treatment. The purpose of this type of inoculation was to mimic the uneven distribution and desiccation of microbial populations in the field. Uneven distribution of bacterial cells in addition to the location of bacterial cells can affect how the sanitizer interacts with the product and ultimately how effective the sanitizer is against microbial populations (Allende et al., 2008). In addition to uneven distribution of bacterial cells, yeast populations on incoming fruit were approximately 7.0 log CFU/g. Presence of competing microorganisms, such as yeast, has been shown to interfere with the efficacy of sanitizer treatments due to an increase in the organic load (Allende et al., 2008). Yeast is found in the environment and populations change year to year due to the current conditions in the field. Weather conditions, produce type, soil amendments, and geographic location can contribute to
native microflora growth and survival which are factors that are difficult to control (Beuchat, 2002). Field mitigation strategies to minimize the growth and survival of other competing microorganisms can help to increase the efficacy of post-harvest mitigation strategies against pathogenic bacteria.

Similar to other research on L. monocytogenes survival in frozen products, we found viable L. innocua cells after two weeks of frozen storage. Other studies have assessed the survival of L. monocytogenes in freezer temperatures and have recorded survival for up to twelve weeks of frozen storage (Flessa et al., 2005; Oyarzábal et al., 2003;). This is a concern for processors due to outbreaks that have been associated with frozen products (CDC 2015, 2016). Post-harvest processing should focus on mitigating food safety risks due to what is known about the survival of L. monocytogenes in frozen foods.

Sanitizer treatments investigated reduced L. innocua up to 2.2 log CFU/g at the pilot scale. To be considered a “kill step”, post-harvest treatments must reduce pathogenic bacteria by 5 log CFU/g (FDA Preventive Controls, 2018). Our results indicate that sanitization cannot be used as a kill step in this product type due to not reaching a 5-log reduction as well as demonstration of considerable variability. Wild blueberries are considered a low risk food product and our results suggest that if contamination occurs it is likely that a sanitization step would reduce the risk, but not eliminate it. Additionally, finding viable L. innocua cells after two weeks of frozen further corroborates that freezing is not a reliably effective strategy for pathogen inactivation. The combination of sanitization and freezing was, however, more effective against coliforms and can likely be used an effective strategy to reduce coliform populations below the detectable limit.
The parameters used in this study were chosen to mimic current industry processing lines and is one of the first studies to investigate sanitization of wild blueberries at the pilot scale. Wild blueberries were inoculated to mimic worst case scenario field contamination and treated in crowded conditions they would encounter in the processing environment. Aqueous sanitizers were applied through a dip tank followed by a spray which is the method of application current processors use to sanitize wild blueberries. The addition of freezing for up to two weeks was meant to mimic the storage of a frozen food product before consumption and evaluate *L. innocua*’s ability to survive for an extended period in freezer temperatures. Treatment of wild blueberries at the pilot scale with the addition of frozen storage mimics the interactions between microbial communities, the product, and the effect of sanitizers. The results found in this study demonstrate the need for pre-harvest interventions to control for microorganisms that could potentially be diminishing the effect of post-harvest processing sanitization efforts.

### 2.6 Conclusion

This study aimed to assess the efficacy of alternative sanitization treatments and freezing against *L. innocua* and native microflora on wild blueberries. None of the treatments investigated significantly reduced *L. innocua* populations when compared to the untreated control. Application of sanitizer by immersion then spraying and using maximum sanitizer concentration is recommended to achieve the largest possible population reductions on wild blueberries. Current processes should focus on reducing yeast populations as well as further investigation into cell adherence and sanitizer interference will be crucial for production of a safe product. Additionally, freezing may be an effective strategy to reducing coliform populations below the detectable limit. Producers interested in alternatives to chlorine should not see significant differences in microbial quality.
2.7 Acknowledgements

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3.1 Abstract

Outbreaks that have occurred in fresh cut produce over the last 10 years have caused increasing concern regarding the way these commodities are produced and consumed. Increasing the antimicrobial efficacy of post-harvest and retail processing is a crucial step toward ensuring final product safety. Much is known about the survival of *Listeria* spp. in fresh cut cantaloupe, but current research has shown inconsistent efficacy of sanitization processes. The goal of this study was to investigate alternative sanitization methods against *Listeria* on fresh cut cantaloupe and the survival of *L. innocua* during post-treatment refrigerated storage for 48 hours. Our research consisted of a benchtop study and a pilot scale study which mimicked a fresh cut processing line. Inoculated fresh cut cantaloupe pieces were treated with peroxyacetic acid (PAA, 80 ppm), chlorine (200 ppm), or electrolyzed water (EO, 200 ppm active chlorine constituents), compared to a water control. Sanitizer treatments consisted of combinations of a single sanitizer or the application of two sanitizers sequentially. The benchtop study was used to compare the efficacy of sanitizer treatment and method of application to determine which treatments should be explored in the pilot study. In the benchtop study, immersion with EO for 5 minutes and PAA for 1.5 minutes and 3 minutes, significantly (*p* < 0.05) reduced *L. innocua* populations compared to the untreated control. Immersion with PAA reduced *L. innocua* populations to a significantly greater extent than treating the fresh cut cubes with chlorine (*p* = 0.02) or water (*p* = 0.0). Spraying singly and in combination with immersion bidirectionally did not significantly reduce *L. innocua* populations for any treatment combination compared to the untreated control (*p* > 0.05). For the pilot scale study, inoculated cantaloupe pieces were treated
by spray application of water or aqueous sanitizer (chlorine, PAA, or sequential applications of these). Following treatment, the samples were subjected to refrigerated storage for 48 hours. Using cultural techniques, *L. innocua*, yeast and mold, and total aerobic mesophiles were monitored throughout storage. We found that all treatments resulted in minimal population reductions for all organisms investigated. *L. innocua* was reduced by up to 1.5 log CFU/cm² with the application of chlorine as a spray followed by PAA as a spray. This decrease was followed by a subsequent population increase of up to 1.1 log CFU/cm² after 48 hours of refrigeration. Regardless of sanitation treatment applied, *L. innocua* populations were not reduced significantly (p > 0.05) by treatment when compared to each other. Between 6 and 48 hours of refrigerated storage, *L. innocua* populations increased significantly (p < 0.05) with the application of all treatments except water followed by water, chlorine followed by chlorine, and PAA followed by PAA. Yeast populations post-treatment were not significantly (p < 0.05) reduced when compared to each other. During refrigerated storage, yeast populations did not significantly increase except in cantaloupe that had been treated with chlorine followed by PAA. Neither treatment nor refrigerated storage for 48 hours significantly affected levels of total aerobic bacteria or mold (p > 0.05). Scanning electron microscopy allowed visualization of the porous nature of cut cantaloupe and the internalization of bacterial cells. Results suggest that surface characteristics of cut melon discourage the effectiveness of aqueous sanitization due to bacterial attachment occurring inside the pores of the cantaloupe flesh. In order to provide safety assurance of fresh cut produce produced at large scale, alternative methods for pathogen inactivation should be investigated.
3.2 Introduction

Recent outbreaks associated with fresh cut produce have caused increasing concern for the consumption of these products. Specifically, outbreaks linked to *Salmonella* spp. and *Listeria monocytogenes* provide validity for this concern. From 2008-2018, melon has been implicated in several foodborne outbreaks. In 2018, pre-cut melon supplied by Caito Foods LLC. to grocery stores, was contaminated with *Salmonella* resulting in 77 illnesses (CDC, 2018). The same year, fresh cut cantaloupe from a single grower in Australia was contaminated with *L. monocytogenes* and resulted in 19 illnesses, 7 deaths, and 1 miscarriage (World Health Organization, 2018). Cantaloupe has also been the source of additional outbreaks of *Salmonella* spp. and *L. monocytogenes* in 2012, 2011, 2008 and 2011(CDC, 2008, 2011, 2012).

Consumption of fresh cut fruit has increased in recent years due to ease of access and affordability. Specifically, fresh cut cantaloupe appeals to consumers due to nutritional benefits as well as availability in salad bars, mixed fruit cups, and pre-cut packages in the refrigerated section of grocery stores. However, the growth and harvest practices of cantaloupes contribute to the ease of contamination of the product from the environment (Suslow, 2004). Cantaloupes are grown in direct contact with the soil and have a growth cycle of up to 90 days depending on the variety (Orzolek, et al., 2006; Sinkel, et al.). Contact with the soil contributes to the possibility for contamination with *L. monocytogenes*. As the fruit matures, the outer netting becomes rough and coarse, which creates an ideal matrix for bacterial attachment (Suslow, 2004). Harvested cantaloupes are transported to packinghouses, where they are sanitized and rinsed in wash tanks prior to cooling and distribution. Wash tanks can be a second source of cross contamination if compromised whole cantaloupes encounter the tank surfaces (Luo et al., 2011, 2012). The use of wash tanks has been shown to be ineffective at reducing microbial loads
on fresh produce and in some cases, produce has shown an increase in bacterial load after washing (Akins et al., 2008; Duffy et al., 2004). The incidence of passive internalization of *Listeria* from the cantaloupe rind into the flesh during washing has been attributed to temperature differentials between the fruit and the water (Macarisin et al., 2017). Once the product reaches a store, it is then sold whole or may be further processed onsite to be sold as fresh cut. Consumer handling, such as cutting into a contaminated rind, can serve as a mechanism for contamination of the otherwise sterile cantaloupe flesh (Suslow, 2004).

Post-harvest processing practices are crucial to ensuring the safety of produce that is further processed into fresh cut product. Current industry standards include use of chlorine (up to 200 ppm) as the main source of decontamination of fresh produce (FDA-CFSAN, 1998). The use of alternative sanitizers has been of interest due to the reoccurrence of outbreaks and illnesses associated with this type of product and the environmental and human health risks associated with the use of chlorine (Ruiz-Cruz et al., 2007). Cantaloupe flesh provides an ideal reservoir for bacteria to grow and survive due to pH, water content, organic matter present, and the psychotrophic nature of *Listeria* (Bowen et al., 2006). Although the survival of *Listeria* in cantaloupe is well understood, the variation of sanitizer concentrations used in current research have produced inconsistent results on sanitization due to inoculation protocol and product form (Rodgers et al., 2004; Svoboda et al., 2016; Ukuku et al., 2002). The goal of this study was to explore alternative methods of sanitization of fresh cut cantaloupe as well as to investigate the survival of *L. innocua* for up to 48 hours of refrigerated storage.
3.3 Materials and Methods

3.3.1 Bacterial Strains and Inoculum Preparation

*L. innocua* ATCC 33090 (American Type Culture Collection, Manassas, VA) was grown in tryptic soy broth (TSB, Alpha Biosciences, Baltimore, MD) at 30°C for 24 hours. Overnight cultures were spread onto tryptic soy agar (TSA, Alpha Biosciences, Baltimore, MD) and grown for 24 hours at 30°C to promote increased resistance to treatment (Gandhi and Chikindas, 2007). *L. innocua* lawns were harvested with 0.1% peptone (Difco, Sparks, MD) and diluted in 9mL of 0.1% peptone. The resulting solution was used to spot inoculate fresh cut cantaloupe to achieve a final concentration of ~5 log CFU/cm². When inoculum solutions were not being used, they were stored at 4°C for up to 30 minutes to prevent the growth of *L. innocua*. In this study *L. innocua* was used as a surrogate for *L. monocytogenes*.

3.3.2 Fresh Cut Cantaloupe

Fresh whole cantaloupes were obtained from local supermarkets (Bangor, ME area) and used within 1 week of purchase. Whole cantaloupes were stored in the refrigerator prior to cutting. To prepare for inoculation, the rind was removed with a sterile knife and the flesh was cut with a sterile knife and cutting board into ~2 cm x 2 cm cubes. Cubes were aseptically placed in sterile foil pans, covered with foil, and refrigerated for up to 2 hours until inoculation with *L. innocua*.

3.3.3 Inoculation of Fresh Cut Cantaloupe

Fresh cut cantaloupe was spot inoculated on one exposed side using 100µL of the inoculum solution (as described above). The inoculum solution was vortexed several times throughout inoculation to ensure even cell distribution and pipette tips were changed frequently. Inoculated cantaloupe pieces were kept at 4°C for 24 hours to ensure adherence of bacteria to the product.
3.3.4 Microbiological Analysis-\textit{L. innocua} and Native Microflora Enumeration of Inoculated Fresh Cut Cantaloupe

To determine the initial level of inoculum, five pieces of cantaloupe (total surface area of 5 pieces; 20 cm$^2$) were sampled with sterilized tongs immediately after inoculation. The sample was diluted with 0.1\% peptone agitated for 2 minutes, serially diluted, and plated on modified Oxford agar (MOX, Alpha Biosciences, Baltimore, MD). After 24 hours of refrigeration, all inoculated cantaloupe cubes were combined into a single sterile container. With a sterile spoon, 3 samples of inoculated cantaloupe containing 5 pieces each (20 cm$^2$ inoculated area, 120 cm$^2$ total surface area), were analyzed to determine the starting level of inoculum before treatment. Samples were diluted with 0.1\% peptone, agitated, serially diluted and plated on MOX. Plates were incubated at 30$^\circ$C for 48 hours.

3.3.5 Sanitizer Treatment Preparation

Individual sanitizer treatments were prepared to achieve the desired volume and concentration using sterilized water. Concentration of electrolyzed water (stock solution concentration of 500 ppm, pH 6.5; JDP- Clarentis®, Palm Beach Gardens, FL) determined using a HACH digital titrator kit (HACH, Model 16900, Loveland, CO) and diluted to make a final solution of 200 ppm active chlorine constituents. A 5\% Sodium Hypochlorite (Lab Chem, Zelienople, PA) solution was diluted to yield a final concentration of 200 ppm and verified using commercial test strips (Micro Essential Lab, Brooklyn, NY). According to manufacturer’s instructions, 15\% Peroxyacetic acid (15\% vol/vol) was diluted to achieve a final concentration of 80 ppm and was verified using a commercial peracetic acid test kit (Alpha Chemical, Stoughton, MA).
3.3.6 Benchtop Study

3.3.6.1 Sanitizer Treatment of Inoculated Fresh Cut Cantaloupe

Inoculated cantaloupe pieces were subjected to a variety of sanitizer treatments to determine the efficacy of the application method, the sequence of application and contact time on *L. innocua* survival. Cantaloupe pieces in sterile steam pans were subjected to sanitizer spray or immersion for up to 5 minutes and samples were taken in duplicate with a sterilized spoon at predetermined time points. Combination treatments of sequential spraying or immersion followed by spraying were also applied. For combination treatments, cantaloupe pieces were treated for up to 3 minutes total and samples were taken in duplicate at 90 seconds and 3 minutes.

3.3.6.2 Microbiological Analysis-*L. innocua* and Native Microflora Enumeration of Treated Inoculated Fresh Cut Cantaloupe

Immediately after treatment, duplicate cantaloupe samples were taken with a sterile spoon. Each sample was diluted as appropriate in 0.1% peptone and agitated with a Bagmixer 400 stomacher (Interscience Laboratories Inc. Weymouth, MA). After agitation, the samples were serially diluted in 0.1% peptone and spread plated onto MOX. MOX plates were overlaid with soft TSA to allow the injured cells to recover and to obtain an accurate population count (Wesche and Ryser, 2015). Thin layer method Plates were incubated at 30°C for 48 hours and *L. innocua* population was determined (log CFU/cm²).

3.3.7 Pilot Scale Study

3.3.7.1 Microbiological Analysis-*L. innocua* and Native Microflora Enumeration of Inoculated Fresh Cut Cantaloupe

Enumeration of microflora on inoculated fresh cut cantaloupe was identical to the benchtop study with the exception of media selection. Microbial populations (log CFU/cm²) were
determined for total aerobic bacteria, yeast and mold, and *L. innocua* and samples were plated on TSA, acidified potato dextrose agar (APDA, Alpha Biosciences, Baltimore, MD), and MOX. Plates were incubated at 37°C for 48 hours, room temperature for 5 days, or 30°C for 48 hours, respectively.

### 3.3.7.2 Sanitizer Treatment Preparation
Sanitizers were prepared as described above. Tap water was used in place of sterile water.

### 3.3.7.3 Pilot Scale Setup
Pilot scale work was completed on a conveyer consisting of three spray bars and a mechanized perforated belt (Figure 1). The system was controlled with a DirectSoft5 (Automation Direct, Cumming, GA) programmable logic controller (PLC) interface. Three peristaltic pumps were run at 30 Hz with a flow rate of 0.5 GPM. Two nozzles discharging a flat cone spray pattern were mounted to each spray bar. Parameters for the conveyer were chosen based on equipment capabilities as well as current industry processing practices.

![Figure 3.1](image)

**Figure 3.1.** Custom-built Pilot Scale Processing Line and Programmable Logic Controller Used to Treat Fresh Cut Cantaloupe in the Pilot Scale Study. 1a. (a) spray bars used to spray sanitizer treatments (b) perforated conveyer belt. 1b. (a) emergency stop button (b) conveyer belt start button (c) conveyer belt stop button (d) pump 1, 2, and 3 power buttons.
3.3.7.4 Sanitizer Treatment and Refrigerated Storage of Treated Fresh Cut Cantaloupe

Inoculated cantaloupe cubes were sprayed sequentially with chosen sanitizer treatments. Prior to treatment, conveyer lines were purged for 1 minute with the appropriate sanitizer to ensure proper priming. Cantaloupe cubes were sprayed twice with a 90 second contact time period between each spray treatment. Samples were taken in duplicate after each step to determine the efficacy of individual steps. After treatment, cantaloupe cubes were stored in flat-bottomed plastic produce containers at 4°C for up to 48 hours. Upon completion of sanitization, the lines were purged with water for 30 seconds to remove any residual sanitizer.

3.3.7.5 Microbiological Analysis—L. innocua and Native Microflora Enumeration of Treated Fresh Cut Cantaloupe

Treated cantaloupe was aseptically sampled after each spray and at 6, 12, 24, and 48 hours of refrigeration. Each sample was added to the appropriate amount of 0.1% peptone to create a 1:10 dilution and agitated for 2 minutes. After agitation, the samples were serially diluted in 0.1% peptone and spread plated onto TSA, MOX, and APDA. MOX plates were overlaid with TSA to allow the injured cells to recover and obtain an accurate population count. Microbial populations were determined (log CFU/cm²) for total aerobic bacteria, yeast and mold, and L. innocua. Plates were incubated as previously described.

3.3.7.6 Scanning Electron Microscopy of Fresh Cut Cantaloupe

Inoculated cut cantaloupe pieces were visualized using scanning electron microscopy to investigate surface topography and cellular attachment. Cantaloupe flesh was cut into 2 cm x 2 cm cubes and immersed in L. innocua overnight culture for 24 hours. L. innocua culture was enumerated on MOX as a comparison to what was visible on the cantaloupe flesh. Inoculated cantaloupe flesh was fixed in 3% glutaraldehyde and rinsed in 0.1M cacodylate buffer. The
samples were post fixed in 1% osmium tetroxide (OSO₄) and dehydrated in ethanol. Following dehydration, samples were critical point dried in CO₂ and mounted and sputter coated in gold palladium. Cantaloupe pieces were imaged using an Amray 1820 SEM (University of Maine, Electron Microscopy Lab, Orono, ME).

3.3.7.7 Statistical Analysis

Data was analyzed to determine statistical significance (p <0.05) among treatments and contact time using a multiway ANOVA and Tukey HSD in R studio.

3.4 Results

3.4.1 Benchtop Study

3.4.1.1 Efficacy of Sanitizer Treatments and Method of Application on Inoculated Fresh Cut Cantaloupe

3.4.1.2 Immersion

Pre-treatment inoculum levels between trials were consistent and ranged from 3.7-4.0 log CFU/cm². Inoculation procedures were the same for each trial and samples were taken in triplicate to account for any variability.

For immersion treatments, *L. innocua* population reductions ranged from 0.1-0.6 log CFU/cm² and was significantly (p = 0.0) reduced from the application of PAA for 90 seconds (p = 0.01), 3 minutes (p = 0.04), and 5 minutes (p = 0.0), and EO for 5 minutes (p = 0.0) compared to the untreated control. Across all treatments there was a greater reduction with increasing sanitizer contact time. For all treatments, *L. innocua* population reductions peaked at 5 minutes and followed a downward trend at each sampling point (Figure 2). Regardless of contact time, the application of PAA was significantly more effective at reducing *L. innocua* populations than applying chlorine (p = 0.03) or water (p = 0.01).
Figure 3.2. Population of *L. innocua* on Fresh Cut Cantaloupe Immersed in Sanitizer for 3 minutes
Data represents the mean population of *L. innocua* after 3 minutes of contact with sanitizer treatment (n = 3), error bars represent standard error, superscripts indicate significant differences (p < 0.05) between treatments and the untreated control.

### 3.4.1.3 Spraying

The pre-treatment inoculum level of *L. innocua* ranged from 3.6-4.5 log CFU/cm² with an average inoculum of 4.0 log CFU/cm². Spray treatments reduced *L. innocua* population by up to 0.4 log CFU/cm² which was not significantly different than the untreated control. With the exception of the water, *L. innocua* population reductions peaked at 3 minutes (Figure 3). No significant effect (p > 0.05) of contact time was observed regardless of sanitizer applied.
Figure 3.3. Population of *L. innocua* on Fresh Cut Cantaloupe Sprayed with Sanitizer for Increasing Time Intervals
Data represents the mean population of *L. innocua* at given time intervals (n = 3), error bars represent standard error

### 3.4.1.4 Sequential Spray

Pre-treatment *L. innocua* levels ranged from 3.6-4.1 log CFU/cm² with an average of 3.9 log CFU/cm². Combination of sequential spray treatments resulted in similar reductions compared to immersing or spraying alone. *L. innocua* population reductions ranged from 0.0-0.2 log CFU/cm² across all treatments. With the exception of the application of PAA followed by EO, there was no additive *L. innocua* reduction with the application of a second spray (Figure 4). In comparison to the untreated control, we found that spraying in combination did not significantly (p > 0.05) reduce the *L. innocua* population.
Figure 3.4. Population of *L. innocua* on Fresh Cut Cantaloupe Sequentially Sprayed with Sanitizer for 3 minutes
Data represents the mean population of *L. innocua* after 3 minutes of contact with combination sanitizer treatments (n = 3), error bars represent standard error.

Table 3.1. Reduction of *L. innocua* on Fresh Cut Cantaloupe Subsequently Sprayed with Sanitizer for Increasing Contact Time Intervals
Data represents the mean reduction of *L. innocua* after treatment with combination sanitizer application (n = 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contact Time</th>
<th>Log Reduction (CFU/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Water</td>
<td>1:30 minutes</td>
<td>0.02 +/- 0.01</td>
</tr>
<tr>
<td>Water-Water</td>
<td>3 minutes</td>
<td>0.03 +/- 0.02</td>
</tr>
<tr>
<td>PAA-EO</td>
<td>1:30 minutes</td>
<td>0.06 +/- 0.04</td>
</tr>
<tr>
<td>PAA-EO</td>
<td>3 minutes</td>
<td>0.20 +/- 0.12</td>
</tr>
<tr>
<td>PAA-Chlorine</td>
<td>1:30 minutes</td>
<td>0.16 +/- 0.09</td>
</tr>
<tr>
<td>PAA-Chlorine</td>
<td>3 minutes</td>
<td>0.08 +/- 0.05</td>
</tr>
<tr>
<td>EO-Chlorine</td>
<td>1:30 minutes</td>
<td>0.11 +/- 0.06</td>
</tr>
<tr>
<td>EO-Chlorine</td>
<td>3 minutes</td>
<td>0.16 +/- 0.09</td>
</tr>
</tbody>
</table>

3.4.1.5 Sequential Immersion-Spray Treatment
Sanitizer immersion followed by spraying resulted in *L. innocua* population reductions ranging from 0-0.3 log CFU/cm² across treatments (Table 3.2). A multiway ANOVA analysis revealed no significant differences (p > 0.05) in *L. innocua* populations compared to the untreated control with the application of chlorine followed by EO achieving the highest reduction of 0.3 log CFU/cm².
Figure 3.5. Population of *L. innocua* on Fresh Cut Cantaloupe Subsequently Immersed then Sprayed with Sanitizer for 3 minutes
Data represents the mean population of *L. innocua* after 3 minutes of contact time with sanitizer treatments (n = 3), error bars represent standard error.

Table 3.2. Reduction of *L. innocua* on Fresh Cut Cantaloupe Immersed then Sprayed with Sanitizer for Increasing Contact Time Intervals
Data represents the mean reduction of *L. innocua* after treatment with combination sanitizer application (n = 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contact Time</th>
<th>Log Reduction (CFU/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-Water</td>
<td>1:30 minutes</td>
<td>0.0 +/- 0.0</td>
</tr>
<tr>
<td>Water-Water</td>
<td>3 minutes</td>
<td>0.0 +/- 0.0</td>
</tr>
<tr>
<td>Water-EO</td>
<td>1:30 minutes</td>
<td>0.12 +/- 0.07</td>
</tr>
<tr>
<td>Water-EO</td>
<td>3 minutes</td>
<td>0.09 +/- 0.05</td>
</tr>
<tr>
<td>Water-Chlorine</td>
<td>1:30 minutes</td>
<td>0.16 +/- 0.07</td>
</tr>
<tr>
<td>Water-Chlorine</td>
<td>3 minutes</td>
<td>0.13 +/- 0.08</td>
</tr>
<tr>
<td>Water-PAA</td>
<td>1:30 minutes</td>
<td>0.0 +/- 0.0</td>
</tr>
<tr>
<td>Water-PAA</td>
<td>3 minutes</td>
<td>0.0 +/- 0.0</td>
</tr>
<tr>
<td>EO-Chlorine</td>
<td>1:30 minutes</td>
<td>0.04 +/- 0.02</td>
</tr>
<tr>
<td>EO-Chlorine</td>
<td>3 minutes</td>
<td>0.0 +/- 0.0</td>
</tr>
<tr>
<td>EO-PAA</td>
<td>1:30 minutes</td>
<td>0.04 +/- 0.02</td>
</tr>
<tr>
<td>EO-PAA</td>
<td>3 minutes</td>
<td>0.07 +/- 0.04</td>
</tr>
<tr>
<td>Chlorine-EO</td>
<td>1:30 minutes</td>
<td>0.07 +/- 0.04</td>
</tr>
<tr>
<td>Chlorine-EO</td>
<td>3 minutes</td>
<td>0.27 +/- 0.15</td>
</tr>
<tr>
<td>Chlorine-PAA</td>
<td>1:30 minutes</td>
<td>0.07 +/- 0.04</td>
</tr>
<tr>
<td>Chlorine-PAA</td>
<td>3 minutes</td>
<td>0.22 +/- 0.12</td>
</tr>
<tr>
<td>PAA-EO</td>
<td>1:30 minutes</td>
<td>0.01 +/- 0.01</td>
</tr>
<tr>
<td>PAA-EO</td>
<td>3 minutes</td>
<td>0.13 +/- 0.07</td>
</tr>
<tr>
<td>PAA-Chlorine</td>
<td>1:30 minutes</td>
<td>0.01 +/- 0.01</td>
</tr>
<tr>
<td>PAA-Chlorine</td>
<td>3 minutes</td>
<td>0.20 +/- 0.11</td>
</tr>
</tbody>
</table>

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3.4.2. Pilot Scale Study

3.4.2.1 Efficacy of Sanitizer Treatments and Refrigerated Storage for up to 48 hours on Inoculated Fresh Cut Cantaloupe

3.4.2.2 Sanitizer Treatments and Refrigerated Storage

Starting population levels for yeast, mold, total aerobic bacteria, and *L. innocua* varied across trials. This was expected as the total number of inoculated cantaloupe pieces greatly increased compared to the benchtop study. Variability was accounted for by averaging initial and pre-treatment populations and by sampling in triplicate. Pre-treatment *L. innocua* inoculum levels ranged from 4.1-6.2 log CFU/cm² with an average of 5.3 log CFU/cm². Pre-treatment levels of total aerobic bacteria ranged from 0-1.2 log CFU/cm² and averaged 0.4 log CFU/cm². Pre-treatment yeast and mold populations ranged from 0.7-1.0 log CFU/cm² with an average of 0.9 log CFU/cm² (yeast) and 0.7 log CFU/cm² (mold).

At pilot scale, combinations of spray sanitizing treatments resulted in larger population reductions across treatments than those observed in benchtop studies. Refrigerated storage resulted in an initial population decrease followed by a subsequent recovery of *L. innocua* and yeast. *L. innocua* populations present after sanitizer treatments ranged from 3.8-4.7 log CFU/cm² across all treatments representing an average population of 4.3 log CFU/cm² (Figure 6). Reductions of *L. innocua* were low with a range of 0.5-1.5 log CFU/cm² for all treatments. After 48 hours of refrigerated storage, *L. innocua* populations increased by up to 1.1 log CFU/cm² compared to lowest, post-treatment level with samples subjected to chlorine followed by PAA showing the largest population increase. *L. innocua* populations were lowest at 6 hours post-treatment followed by a significant (p < 0.05) increase with the application of chlorine followed by PAA and PAA followed by chlorine.
Figure 3.6. *L. innocua* in Sanitizer Treated Fresh Cut Cantaloupe During Refrigerated Storage for 48 Hours
Data represents mean *L. innocua* population after subsequent spray treatment with sanitizer and refrigerated storage for 48 hours (n= 3)

Similar to *L. innocua*, the total aerobic population was minimally affected by treatment with populations ranging from 0.6-2.3 log CFU/cm² after treatment (Figure 7). In all treatments, except for the application of chlorine followed by PAA, and chlorine followed by chlorine, there was an increase in population after 48 hours of refrigeration compared to the population post-treatment (p > 0.05).

Figure 3.7. Total Aerobic Bacteria Population in Sanitizer Treated Fresh Cut Cantaloupe During Refrigerated Storage for 48 Hours
Data represents mean total aerobic bacteria population after subsequent spray treatment with sanitizer and refrigerated storage for 48 hours (n= 3)
As expected, yeast and mold populations remained low after treatment and refrigerated storage. Yeast populations ranged from 0.8-0.9 log CFU/cm² and mold ranged from 0.7-0.8 log CFU/cm² across treatments (Figure 8,9). Mold populations generally decreased over time while yeast populations demonstrated a slight decrease followed by an increase during storage. Yeast populations were not significantly (p > 0.05) reduced by any of the treatments applied when compared to each other. Between 6 and 48 hours of refrigerated storage, yeast populations did not grow significantly except for cantaloupe treated with chlorine followed by PAA.

**Figure 3.8. Yeast Population in Sanitizer Treated Fresh Cut Cantaloupe During Refrigerated Storage for 48 Hours**

Data represents mean yeast population after subsequent spray treatment with sanitizer and refrigerated storage for 48 hours (n= 3)
Figure 3.9. Mold Population in Sanitizer Treated Fresh Cut Cantaloupe During Refrigerated Storage for 48 Hours
Data represents mean mold population after subsequent spray treatment with sanitizer and refrigerated storage for 48 hours (n= 3)

3.4.2.3 Scanning Electron Microscopy (SEM)

Inoculated and uninoculated cantaloupe pieces were imaged using scanning electron microscopy to look at cantaloupe flesh surface structure and *L. innocua* attachment. Figure 10 demonstrates the highly porous surface of the cantaloupe flesh which creates an ideal structure for bacterial attachment. Pores in the cantaloupe flesh range from approximately 100-500µm compared to bacterial cells which are less than 10 µm in diameter (Figure 10). In Figure 11, the method of flesh attachment is evident with the greatest number of bacterial cells visible inside of the surface pores and absent on the flesh outer surface. The plated overnight culture used to inoculate the cantaloupe pieces contained 9.2 log CFU/ml.
Figure 3.10. Scanning Electron Microscopy Image of Uninoculated Cantaloupe Flesh
Image of surface of inoculated cantaloupe flesh. Fresh cut cantaloupe cubes were inoculated with *L. innocua* and analyzed for bacterial attachment

Figure 3.11. Scanning Electron Microscope Image of Inoculated Cantaloupe Flesh
Images show bacterial attachment in the pores (a) of the cantaloupe flesh. String-like material are extracellular polysaccharides (b) secreted by *L. innocua*. Also shown are other cell types which are likely yeast based on size (c)

3.5 Discussion

The aim of this study was to investigate the efficacy of sanitizer treatments (EO, chlorine, and PAA) and refrigerated storage against *L. innocua*, yeast and mold, and total aerobic population on fresh cut cantaloupe at the pilot scale. Treating fresh cut cantaloupe on a pilot scale processing line allowed for evaluation of sanitizer efficacy representative of a more
realistic scenario as compared to bench-scale studies. Furthermore, refrigeration for 48 hours allowed for determination of \( L.\ innocua \) survival and growth in a retail-like setting. Our results can be used as a tool for producers to choose the proper sanitation protocol to mitigate food safety risks associated with this type of product.

Previous studies analyzing cantaloupe (whole or cut), vary in inoculation method, sanitizer application, and tend to focus on evaluating pathogen survival in refrigerated storage and temperature abuse conditions (Table 3.3). Additionally, research has focused on evaluating the incidence of \( Listeria \) contamination during cutting and the potential to sanitize cantaloupe rinds (Table 3.3) in order to prevent transfer to flesh. This study focused on the potential of sanitization to reduce microbial populations on flesh post-contamination. In our study, all sanitizer combinations failed to significantly reduce (\( p > 0.05 \)) \( L.\ innocua \) populations due to surface topography of fresh cut cantaloupe.

When comparing this study to others, differences in inoculation protocol, study design, and the use of whole or cut cantaloupe can account for variability in reduction with sanitizers and population data. The surface structure of whole cantaloupes and the flesh vary greatly and therefore can account for such variability in sanitation and storage results (Table 3.2). This study was the first to assess the efficacy of sanitizers on fresh cut cantaloupe with bacterial cells grown on solid vs. liquid media. With such a long growing period, whole cantaloupes may harbor bacterial cells in a desiccated state which can then be transferred to the cantaloupe flesh upon cutting (Ukuku and Sapers, 2001). It is known that bacterial cells in a desiccated state are more resistant to lysis via sanitizer application than planktonic cells grown under laboratory conditions (Møretrø and Langsrud, 2004). The use of this method in many benchtop experiments may result in artificially favorable results from sanitization.
Reductions achieved after sanitizer treatments were very minimal which can be attributed to many factors. Small reductions in *L. innocua* were likely contributed to by the complex nature of the cantaloupe flesh. Cantaloupe pieces were inoculated on one side to mimic cross contamination upon cutting which purposefully led to heterogenous distribution of cells. Refrigerating cantaloupe pieces for 24 hours allowed for bacterial attachment to the cantaloupe flesh. Representative of large-scale production, sanitizer application was inconsistent across individual cantaloupe surfaces. Inconsistency in sanitizer application with the addition of inoculation on one surface can potentially explain minimal reduction of *L. innocua*, but we feel that this is representative of the low frequency, incidental contamination that occurs in such a product. With the exception of the final trial, all samples were agitated using a stomacher. Due to equipment failure, the final trial was stomached by hand. Using a mechanical method of agitation could potentially result greater fluid movement and liberation of attached cells. Stomaching by hand is limited by the breakage of the cantaloupe as some pieces were easier to break than others. Population recovery and reduction results can also be supported by SEM imaging which confirmed bacterial attachment in the pores rather than on the surface, which is expected to minimize cell sanitizer contact. Due to inherent variability in fresh cut cantaloupe and the lack of efficacy of sanitization treatments it is not advised to document theoretical capabilities of treatments for enhancing safety in such a high-risk product.

Population data was obtained and tracked before treatment through 48 hours of refrigerated storage. *L. innocua* populations gradually decreased after treatment followed by a subsequent increase of up to 1.1 log CFU/cm² after refrigerated storage. This data is consistent with other studies that have tracked *L. innocua* populations in fresh cut cantaloupe under refrigerated conditions (Danyluk et al., 2014; Fang et al., 2013). The initial decrease in
population is likely attributed to the sanitizer’s interaction with the bacteria and the changes in
temperature experienced leading up to refrigerated storage. The increase in *L. innocua*
populations can be explained by optimal growth conditions in the cantaloupe flesh and an ideal
surface for attachment (Svoboda et al., 2016).

The total aerobic population varied amongst sanitizer treatments and based on
observations mainly were representative of the *L. innocua* population. Very few bacterial
morphotypes were observed, which may have been due to the population of *Listeria* present on
the product. Similar to *L. innocua*, yeast populations increased slightly during refrigerated
storage. Yeast are very adaptable to a wide range of environments and can grow in foods with
high water activity and sugar content (USDA, 2012). The mid-range pH, sugar content, and high
water activity present in the cantaloupe flesh could have contributed to the recovery of yeast.
Unlike *L. innocua* and yeast, almost all mold populations decreased, almost reaching the low
threshold of the detectable limit of $1 \times 10^2$ log CFU/cm$^2$. Mold populations observed were
consistently very low. Variation in yeast, mold, and total aerobic populations may be attributed
the handling of the cantaloupe before cutting and treatment. Additionally, with such a small
amount of these populations being present the likelihood that we could detect them consistently
or discern statistically relevant treatment effects is low.

The parameters used in this study were designed to mimic industry-relevant processing
conditions and this is one of the only studies to evaluate sanitizer efficacy on fresh cut
cantaloupe at the pilot scale. For all studies, sanitizer was applied to the inoculated fresh cut
cantaloupe under crowded conditions. Additionally, combination sanitizer treatments were
applied with subsequent sprays since immersion would likely diminish product quality and
increase the likelihood of bacterial cross-contamination and is unlikely to be employed in an
industrial setting. Following sanitizer application, treated cantaloupe pieces were monitored under refrigerated storage for 48 hours. The monitoring of bacterial populations for up to 48 hours was done to mimic a retail environment and quantitatively evaluate the interaction between sanitizer application and bacterial populations during refrigerated storage. Application of sanitizer under pilot scale conditions followed by refrigerated storage reflects the complex interactions between sanitizers, bacteria, storage conditions, and the inherent nature of the The most universal trend observed was the increase in *L. innocua* population during refrigerated storage. The results found in this study illustrate that if cantaloupe flesh becomes contaminated it is a serious threat to human health. The environment in which cut melon is stored provides optimal conditions for the pathogen of interest and should be considered by retailers before selling this product. Based on the data found, further research is warranted for alternative methods to reduce microbial populations in fresh cut cantaloupe.

**Table 3.3. Previous Studies Investigating the Efficacy of Sanitizers against Pathogenic Microorganisms on Cantaloupe**

<table>
<thead>
<tr>
<th>Author</th>
<th>Inoculation</th>
<th>Produce</th>
<th>Pathogen</th>
<th>Sanitizer</th>
<th>Log Reduction</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ukuku et. al, 2002</td>
<td>Immersion 4.2log CFU/cm² (treated rind) 3.5log CFU/cm² (untreated rind)</td>
<td>Whole cantaloupe Fresh cut cantaloupe pieces</td>
<td><em>Listeria monocytogenes</em></td>
<td>1000 ppm chlorine for 2 minutes</td>
<td>2-3.5log CFU/cm² on rind, transfer to flesh was evaluated</td>
<td>4°C and 20°C for 15 days</td>
</tr>
<tr>
<td>Ukuku et. al, 2001</td>
<td>Immersion 3.8log CFU/cm²</td>
<td>Whole Cantaloupes Fresh Cut Cantaloupe pieces</td>
<td><em>Salmonella stanley</em></td>
<td>1000 ppm chlorine for 5 minutes</td>
<td>3.4-0.8log CFU/cm² Over 6 days of storage at 4°C</td>
<td>4°C and 20°C for 5 days</td>
</tr>
</tbody>
</table>
3.6 Conclusion

The goal of this study was to assess the efficacy of aqueous sanitization methods and refrigerated storage on fresh cut cantaloupe. We found that *Listeria innocua* populations were not significantly reduced by any of the combination treatments applied. Due to the nature of the product and what is known about the potential of *Listeria* spp. to survive in changing environmental conditions, producers should explore alternative methods for pathogen control and/or inactivation.

3.7 Acknowledgements

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BIOGRAPHY OF AUTHOR

Selena Callahan was born in Sonora, CA on January 17, 1993. Selena was raised in Angels Camp, CA where she lived for 18 years and graduated from Bret Harte High School in 2011. Following high school, Selena attended Butte College in Chico, CA for three years and then moved to Corvallis, OR to complete her degree. She graduated from Oregon State University in December of 2016 with a Bachelor of Science in Microbiology and a minor in Chemistry. Upon completion of her master’s, Selena plans to move back to the west coast to be closer to family and friends and hopes to pursue a career in Food Microbiology. Selena is a candidate for the Master of Science Degree in Food Science and Nutrition from the University of Maine in August 2019.