PELARGONIC ACID EMULSION AS A POSTHARVEST SANITIZER TO REDUCE *ESCHERICHIA COLI* O157:H7 CONTAMINATION ON ROMAINE LETTUCE

By

SAMUEL PETER CIMOWSKY

(Under the Direction of Laurel L. Dunn)

ABSTRACT

Contamination by *Escherichia coli* O157:H7 is a critical concern for the leafy greens industry. For this reason, sanitizers are used to reduce pathogen cross-contamination post-harvest. A novel sanitizer containing pelargonic acid was examined on romaine lettuce inoculated with *E. coli* O157:H7; pathogen reduction and cross-contamination were evaluated post-wash. Three concentrations of pelargonic acid (7.5 mM, 30 mM, and 50 mM) were examined, as well as deionized water, 200 ppm chlorine, 80 ppm peroxyacetic acid, and a no rinse treatment. The impact of organic matter in wash waters was also evaluated; all wash waters were also spiked with lettuce puree (0.5% w/v) and compared to treatments without organic matter. Treatments were examined after storage durations of 0 h, 24 h, and 7 days. Pelargonic acid sanitizers significantly reduced *E. coli* O157:H7 and effectively controlled cross-contamination over the three storage durations; however, these treatments resulted in significant color changes to samples.

INDEX WORDS: Romaine lettuce, *Escherichia coli* O157:H7, Pelargonic acid, Sanitizer, Produce wash

PELARGONIC ACID EMULSION AS A POSTHARVEST SANITIZER TO REDUCE *ESCHERICHIA COLI* O157:H7 CONTAMINATION ON ROMAINE LETTUCE

By

SAMUEL PETER CIMOWSKY

B.S., The University of Georgia, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2020

© 2020

Samuel Peter Cimowsky

All Rights Reserved

PELARGONIC ACID EMULSION AS A POSTHARVEST SANITIZER TO REDUCE *ESCHERICHIA COLI* O157:H7 CONTAMINATION ON ROMAINE LETTUCE

by

SAMUEL PETER CIMOWSKY

Major Professor:	Laurel L. Dunn
Committee:	Govindaraj Dev Kumar Andre Luiz Biscaia Ribeiro da Silva

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
August 2020

DEDICATION

LaFerne Mills Workman

October 21, 1930 - October 18, 2019

I would like to dedicate my thesis to my grandmother. She was one of my most passionate supporters, always being the first attendee at graduations and sporting events. During childhood, my parents always threw my sister and me into the car to drive to a tiny town in Arkansas to see my grandmother. While sometimes those trips were boring, she was always the highlight and I relished the opportunity to just sit around watching movies and be spoiled with homemade waffles, frozen apple fritters, and cheap, soft-served ice cream.

As she and I spent time together, I learned a lot from her and developed many traits over the years… some traits were good, but others not so much (like a serious love of Sonic milkshakes!) One of the best being to openly grumble when the situation allows, but always knowing that sometimes you just need to suck it up and work hard and be thankful for your comforts because often those comforts are more than other people have.

Graduate school is tough, and it gets even tougher when you lose one of your major cheerleaders. While she is no longer here supporting me in person, I know she will always be in my corner cheering me on.

ACKNOWLEDGEMENTS

First and foremost, I must thank Dr. Laurel Dunn for her advice and support throughout my graduate studies. Being one of her first students I feel like sometimes we were learning together, but I am thankful for that because of her flexibility and fresh excitement for the process. I am certain that this experience will influence me throughout my career. I would also like to thank Dr. Andre da Silva and Dr. Govindaraj Dev Kumar for serving on my committee and providing guidance during the completion of my thesis project.

I would also like to thank my lab partner, Lizzy White, for all her support through thick and thin during this graduate school endeavor. Thank you to Dr. Mark Harrison, Gwen Hirsch, Dr. William Kerr, and Danny Morris for their guidance throughout the planning and execution of my project. Additionally, thank you to Chase Golden, Andrew Widmer, Anna Townsend, and Brittany Magdovitz for miscellaneous pieces of advice and helping me out when in a bind.

Finally, a big thanks to all my friends and family. I know that I would not have been able to complete this journey without their unyielding encouragement and support.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 3

Escherichia coli O157:H7 3

E. coli O157:H7 Attachment and Survival 5

Leafy Greens Industry 9

Antimicrobial Methods 10

Pelargonic Acid 16

Romaine Lettuce Texture and Color 20

3 MATERIALS AND METHODS 24

Bacterial strains used 24

Antibiotic adaptation 24

Leaf sample preparation 25

Background microflora 25

Inoculum preparation 25

Inoculation procedure 26

Attachment time for *E. coli* to romaine lettuce 26
Sanitizer treatment preparation 28
Treatment with sanitizers 29
Microbiological analyses 29
Statistical analyses for microbiological data 30
Texture and color analyses 31
Statistical analysis for texture and color analyses 31
4 RESULTS 33
Effect of treatments on inoculated samples 33
Effect of treatments on cross-contamination 34
Effect of treatments on romaine lettuce color 34
Effect of treatments on romaine lettuce texture 35
5 DISCUSSION 36
Wash water 36
Chlorine and PAA 37
Pelargonic acid treatments reduce *E. coli* O157:H7 on the lettuce surface and cross-contamination 38
Impact of organic load on sanitizers 38
Pelargonic acid effect on plant tissue 39
Storage implications 41
Potential uses of pelargonic acid 42
Conclusion 43
REFERENCES 44

LIST OF TABLES

Page

Table 1: Effect of the interaction of sanitizer and storage duration, average of DI water and no rinse per storage duration, and orthogonal contrasts for inoculated samples on TSARN .57

Table 2: Effect of the interaction of sanitizer and storage duration, average of water and no rinse per storage duration, and orthogonal contrasts for inoculated samples on TSA 58

Table 3: Effect of the interaction of sanitizer and storage duration, average of water per storage duration, and orthogonal contrasts for SUS on TSARN...59

Table 4: Effect of the interaction of sanitizer and storage duration, average of water per storage duration, and orthogonal contrasts for SUS on TSA 60

Table 5: Effect of the interaction of sanitizer treatments plus water and no rinse and storage duration on L* and a* 61

Table 6: Main effect of treatment and storage duration on b*..62

Table 7: Main effect of treatment and storage duration on skin elasticity (mm)...........................63

LIST OF FIGURES

Page

Figure 1: Romaine lettuce samples post-treatment of a) 0 h no rinse, b) 0 h DI water, c) 0 h 200 ppm chlorine, d) 0 h 80 ppm PAA, e) 0 h 7.5 mM pelargonic acid, f) 0 h 30 mM pelargonic acid, g) 0 h 50 mM pelargonic acid, h) 24 h no rinse, i) 24 h DI water, j) 24 h 200 ppm chlorine, k) 24 h 80 ppm PAA, l) 24 h 7.5 mM pelargonic acid, m) 24 h 30 mM pelargonic acid, n) 24 h 50 mM pelargonic acid, o) 7 d no rinse, p) 7 d DI water, q) 7 d 200 ppm chlorine, r) 80 ppm PAA, s) 7.5 mM pelargonic acid, t) 30 mM pelargonic acid, u) 50 mM pelargonic acid 64

CHAPTER 1

INTRODUCTION

Escherichia coli O157:H7 is one of the leading foodborne disease agents in the United States and is a critical pathogen to control (Crowe, Mahon, Vieira, & Gould, 2015). Consumption of leafy greens, such as romaine lettuce, has increased in recent decades, and several large *E. coli* O157:H7 outbreaks associated with leafy greens have made them a key commodity of concern for food safety professionals (Crowe et al., 2015; U. S. Department of Agriculture - Economic Research Service (USDA-ERS), 2019). During the postharvest washing process, contamination on small quantities of produce can result in large-scale cross-contamination in the absence of antimicrobial sanitizer. Even in the presence of the most frequently used sanitizers in the produce industry, little biocidal activity occurs against attached bacterial pathogens on contaminated produce entering a wash system (Brackett, 1999; Franz & van Bruggen, 2008). Currently, the primary objective of sanitizers in postharvest wash systems is to limit cross-contamination (Brackett, 1999; Franz & van Bruggen, 2008; Gomez-Lopez, Lannoo, Gil, & Allende, 2014; U.S. Food and Drug Administration (FDA), 2008).

Chlorine and peroxyacetic acid (PAA) are two of the most commonly used sanitizers in the produce industry and effectively control cross-contamination when used correctly, but due to cost, dangerous byproducts, and reduced efficacy due to the presence of organic load, alternative sanitizers are desired (Centers for Disease Control and Prevention (CDC), 2016; Gomez-Lopez et al., 2014; Kitis, 2004; Shen, Luo, Nou, Wang, & Millner, 2013; Sofos, Beuchat, Davidson, &

Johnson, 1998; FDA, 2008; Y. Zhang, Ma, Critzer, Davidson, & Zhong, 2016). One such alternative currently being examined is pelargonic acid. While commonly found as an active ingredient in herbicides, fungicides, and pesticides, pelargonic acid is approved for use as a food additive by the Food and Drug Administration (FDA, 2019, 2020). Previous work with pelargonic acid has determined a minimum inhibitory concentration of 31.25 mM after 1 hour against *Salmonella enterica* (Micallef & Kumar, 2017). White (2020) also found that pelargonic acid was an effective sanitizer for removing attached *S. enterica* and reducing cross-contamination on tomatoes.

The current study was performed to evaluate the efficacy of pelargonic acid against *E. coli* O157:H7 in postharvest wash systems for fresh produce. Romaine lettuce was selected due to its frequent association with outbreaks of *E. coli* O157:H7 in recent years. Pelargonic acid emulsified with quillaja saponin was tested at three concentrations (7.5, 30, and 50 mM) for its ability to reduce attached *E. coli* O157:H7 from the lettuce surface and prevent cross-contamination in a simulated wash system. The impact of organic material in the wash system was also evaluated using 0.5% w/v organic load (OL). Treated samples were immediately enumerated (0 h), or stored at 4 °C for up to 7 days to determine if continued antimicrobial activity existed through storage. Total aerobic plate count, color, and texture were also examined immediately post-treatment and after 24 h and 7 days of storage.

CHAPTER 2

LITERATURE REVIEW

***Escherichia coli* O157:H7**

The Centers for Disease Control and Prevention (CDC) reported that in 2017 841 documented foodborne disease outbreaks caused approximately 15,000 illnesses in the United States (CDC, 2017). Pathogens such as *Salmonella, Listeria monocytogenes* and Shiga toxin-producing (STEC) *Escherichia coli* (*E. coli*) were the leading pathogenic agents causing multi-state foodborne illnesses in the United States from 2010-2014. Of the 34 reported STEC outbreaks, 41% of those were connected to produce crops, including leafy greens, and 59% of those outbreaks were caused by the serogroup O157, indicating that *E. coli* O157:H7 is a critical pathogen requiring control (Crowe et al., 2015).

Most *E. coli* strains do not cause disease in healthy hosts and are endemic to the gut microbiome in humans and animals; however, some strains are harmful. These pathogenic strains include O157, O104, O145, O26, O111, and O103 (CDC, 2014; Yang, Lin, Aljuffali, & Fang, 2017). *E. coli* O157:H7 is a Gram-negative, enteric pathogen that inhabits and infects the intestinal tracts of warm-blooded species and has a particular affinity for beef and dairy cattle. Due to its preferential colonization of the intestinal tract, *E. coli* O157:H7 can be spread to produce fields when contaminated feces is directly applied to fields, or is introduced via water, soil, and wind (Franz & van Bruggen, 2008; FDA, 2008). STEC-containing feces can also be spread by infected workers, animals, tools, and equipment in the field or packing facility (Franz

& van Bruggen, 2008; Kumar, Libin, et al., 2019; FDA, 2008). Due to its robust nature *E. coli* O157:H7 is able to persist on crops even though the plant surface is not considered a hospitable environment for enteric pathogens (Dunn, 2017).

When produce contaminated by *E. coli* O157:H7 is consumed, the pathogen colonizes the intestinal tract of the new host; once there, the bacteria cause watery diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (Franz & van Bruggen, 2008; Gomez-Lopez et al., 2014). *E. coli* O157:H7 infections typically cause 1-3 days of non-bloody diarrhea, which can then become bloody, indicating colon damage; in severe infections, this can progress to further complications, including HUS. HUS, a form of acute kidney failure, is a result of Shiga toxin-mediated damage and is characterized by microvascular thrombi and swollen endothelial cells (Tarr, Gordon, & Chandler, 2005). Individuals with weakened or developing immune systems, such as young children or immuno-compromised individuals, are most susceptible to HUS (National Institute of Diabetes and Digestive and Kidney Diseases, 2015). Children are at a higher risk of initial illness due to their less rigorous hygiene skills than most adults and are more susceptible due to their underdeveloped immune systems, notably, their lack of protective antibodies to Shiga toxins (Doyle & Beuchat, 2007). The infectious dose of STEC is low, with fewer than 100 to 1000 bacterial cells necessary to cause infection. Diarrhea associated with *E. coli* O157:H7 is infrequently treated with antibiotics, and most patients instead receive supportive care including oral rehydration and electrolyte supplementation (Doyle & Beuchat, 2007; Yang et al., 2017).

While the use of antibiotics to treat *E. coli* O157:H7 infection is effective, there is no shortage of controversy over risk versus reward based upon the population in question. A prospective cohort study by Wong, Jelacic, Habeeb, Watkins, and Tarr (2000) suggests that

antibiotic treatment of young children with *E. coli* O157:H7 infection increases the risk of HUS and that antibiotic therapy should be withheld until it is established that causative agent is not likely to cause HUS. In a response to Wong et al. (2000), O'Ryan et al. (2000) worry that the message by Wong et al. (2000) to withhold antibiotic therapy until the cause of diarrhea is known presents more risks than benefits for places where the prevalence of *Shigella* spp. and *E. coli* infection is similar. O'Ryan et al. (2000) note that because the most widely accepted recommendation to treat diarrhea is to obtain a stool sample and immediately initiate antibiotic treatment for shigellosis in order to shorten the duration of diarrhea, decrease the risk of further complications, and reduce the risk of transmission, waiting to administer antibiotic therapy until after the results of the stool samples are received presents more risk than the potential risk of developing HUS. Additionally, a meta-analysis by Safdar et al. (2002) concluded that there is no higher risk of HUS after antibiotic administration for the treatment of *E. coli* O157:H7 infection.

***E. coli* O157:H7 Attachment and Survival**

E. coli firmly attaches to the produce surface and resists removal even with the aid of chlorine and other antimicrobial treatments. This resistance is due in part to rapid cell attachment and the initiation of biofilm formation on the produce or other surfaces (Beuchat, 2002; Costerton, Stweart, & Greenberg, 1999; Doyle & Beuchat, 2007; Ölmez & Temur, 2010). Prior to biofilm formation, either passive or active adhesion occurs. Passive adhesion is driven by gravity, diffusion, and fluid dynamics, while during active adhesion the bacterial cells initially attach using bacterial surface structures such as fimbriae, flagella, or pili. After contact is initiated, the attachment can be either reversible or irreversible. Reversible attachment involves van der Waals, electrostatic, and hydrophobic interactions, but since these are weak forces the bacteria can be easily removed by mild shear. Irreversible attachment occurs when biopolymers

anchor the cells to the surface (Chmielewski & Frank, 2003; Kumar, Ravi, Micallef, Brown, & Macarisin, 2018). Biopolymers are composed of extracellular polysaccharides, proteins, phospholipids, nucleic acids, and other polymeric substances (Chmielewski & Frank, 2003; Ölmez & Temur, 2010). A structured matrix of biopolymer is developed and bacterial cells begin to aggregate. A diverse microbial population, including bacteria, yeasts, and molds, exist within the formed matrix (Beuchat, 2002). When there is a sufficiently high concentration of bacterial cells, quorum sensing occurs. When quorum sensing occurs, a signaling molecule is secreted by the cells until a threshold is attained, at which point a community cellular response is triggered. This allows individual cells to make a coordinated response with the remainder of the population (Czárán & Hoekstra, 2009). This coordinated response can stimulate actions including continued biopolymer secretion and further cell aggregation. A microcolony with defined boundaries and transportation channels can eventually develop, which facilitate nutrient transport to the interior of the biomatrix (Costerton et al., 1999). This highly structured biomatrix establishes a homeostasis and cells contained within become increasingly resistant to heat, chemicals, and sanitizers (Beuchat, 2002; Costerton et al., 1999; Doyle & Beuchat, 2007; Lapidot, Romling, & Yaron, 2006; Ölmez & Temur, 2010). Biofilms effectively protect enclosed bacteria from disinfection, but structural maturity must occur before this benefit is realized as less developed biofilms remain susceptible (Kumar et al., 2017; Lapidot et al., 2006).

Antimicrobial solutes interact with biofilms in many ways. Often, antimicrobial agents fail to penetrate the full depth of a biofilm and the solutes of antimicrobials often diffuse at slower rates through a biofilm than through water. Additionally, reactive oxidants, such as hypochlorite and hydrogen peroxide, can be deactivated in the outer layer of a biofilm thus

protecting bacteria within the inner portions of the biofilm (Brown, Allison, & Gilbert, 1988; Costerton et al., 1999; De Beer, Srinivasan, & Stewart, 1994).

Romaine lettuce and other fresh produce crops are highly perishable foods by nature and care must be taken during transportation from harvest to consumer. For example, head lettuce can be kept in good condition in normal atmospheric conditions for 2 to 3 weeks at 0 ºC; enough time for microorganisms to proliferate prior to reaching the consumer (Burg, 2004). If produce is irresponsibly stored at higher temperatures, then the bacterial load present on that produce will be higher than if that produce was stored under ideal conditions. For example, Luo et al. (2010) found a 2.0 log CFU/g increase in *E. coli* O157:H7 cells when storing inoculated lettuce samples at 12 ºC as compared to 5 ºC. At 5 ºC, *E. coli* O157:H7 cells were able to survive, but their growth was limited.

There are multiple factors that make it extremely difficult to control *E. coli* O157:H7 and other pathogenic bacteria after they have already attached to fresh produce. In addition to biofilm formation, another factor is the internalization of the bacterial cells within the plant via damaged plant tissues, blossom scars, and stem scars (Burnett, Chen, & Beuchat, 2000; Kumar, Patel, & Ravishankar, 2020; Ölmez & Temur, 2010). Because bacteria in a biofilm are protected, foodborne pathogens are effectively out of reach of many surface-cleansing techniques commonly used in industry. Similarly, bacteria that become internalized and infiltrate the tissues of fresh produce are also protected from surface sanitation methods (Burnett et al., 2000; Doyle & Beuchat, 2007; Kumar et al., 2017; Ölmez & Temur, 2010).

E. coli O157:H7 reacts to changes in the environmental pH of foods and in the human body. Outbreaks have been linked to acidic foods such as yogurt, apple cider and other fruit beverages, mayonnaise and mayonnaise-based dressings and sauces, and fermented meats

(Cheville, Arnold, Buchrieser, Cheng, & Kaspar, 1996; Doyle & Beuchat, 2007; Hilborn et al., 2000; Morgan et al., 1993; Weagant, Bryant, & Bark, 1994). While the minimum pH for growth for *E. coli* O157:H7 is 4.0 to 4.5, the pathogenic bacteria must endure pH fluctuations within the upper portion of the gastrointestinal tract prior to entering the intestines of a new host (Doyle & Beuchat, 2007). The infectious dose of each pathogen is unique and is often reflected in a bacteria's ability to pass through and survive acidic gastric barriers in humans that can reach pHs as low as 1.5 to 2.5 (Doyle & Beuchat, 2007; Lin et al., 1996). The bacteria will enter a state of acid resistance and induce the production of protective proteins that drive resistance to physical and chemical challenges (Cheville et al., 1996; Doyle & Beuchat, 2007). *E. coli* acid tolerance has been studied extensively and three primary acid resistance systems have been noted: an acid-induced oxidative system, an acid-induced arginine-dependent system, and a glutamate-dependent system (Abdul-Raouf, Beuchat, & Ammar, 1993; Doyle & Beuchat, 2007). RpoS is an alternate sigma factor that regulates the production of stress response genes. RpoS is a key driver for the acid-induced oxidative system, but only plays a partial role in both the acid-induced arginine-dependent and glutamate-dependent systems (Cheville et al., 1996; Doyle & Beuchat, 2007; Lin et al., 1996). Acid resistance adaptation can heighten tolerance to heating, irradiation, and antimicrobials, but that resistance is lost when conditions that promote regular growth are resumed (Cheville et al., 1996; Doyle & Beuchat, 2007; Jordan, Oxford, & O'Byrne, 1999; Leyer, Wang, & Johnson, 1995).

E. coli O157:H7 can persist in the environment for long periods of time, but die-off over time is anticipated. Die-off rate is based upon multiple factors, including temperature, relative humidity, soil composition, and initial contact time (Chhetri et al., 2019; Vidovic, Block, & Korber, 2007). In a study analyzing well-characterized *E. coli* O157:H7 surrogates and coliforms

on the surface of watermelons, Chhetri et al. (2019) found that different parts of the melons were more likely to harbor bacteria, and that time affected bacterial attachment strength. No difference existed in coliform levels between the bottom half of watermelons that came in contact with the soil and the top half of watermelons that received more exposure to sunlight and other weather elements. They also determined that the *E. coli* O157:H7 surrogates' attachment strengths increased over time on the watermelon surface. The study concluded that *E. coli* robustness must be accounted for when developing pre-harvest and post-harvest risk management strategies.

Leafy Greens Industry

From 1985 to 2018, the per capita availability of fresh romaine lettuce rose from 3.0 pounds to 11.4 pounds in the United States (USDA-ERS, 2019). Food trends are constantly shifting in the United States and producers must stay ahead of those shifts. In the 1950's, a typical grocery store might have only carried a few hundred items, but now grocery stores carry thousands of different items to meet consumer demand (Hedberg, MacDonald, & Osterholm, 1994). Most consumers are no longer growing and preparing their own foods, but are going to the store or a commercial food service establishment expecting to find every type of product imaginable, regardless of season. One major trend is the increasing desire for fresh foods. The increased consumption of uncooked and unprocessed fruits and vegetables has emphasized the need for growers to ensure that the food they distribute is microbiologically safe (Brackett, 1999; DuPont, 2007; World Health Organization & Food and Agriculture Organization of the United Nations, 2008). To adapt to this increased demand, produce is no longer just distributed regionally, but now includes national and foreign markets. These global markets require more rigorous harvesting, storage, transportation, and production strategies to ensure food safety. The expanded distribution and supply chain might contribute to a higher proportion of consumers

contracting a foodborne disease as the need for an increased number of production steps opens the door to increased risk of contamination with pathogenic bacteria via temperature abuse, cross-contamination, etc. Additionally, a contributing factor to the increased risk is that growing, handling, equipment, and labeling standards are not universal worldwide (Chang & Schneider, 2012; Franz & van Bruggen, 2008; Kumar, Libin, et al., 2019; Manning & Monaghan, 2019; Sapers & Doyle, 2014; Yaron, 2014). The FDA suggests becoming aware of the practices of one's suppliers and using verification mechanisms, such as on-site audits, to ensure the use of food safety practices with that supplier (FDA, 2008). Until recently, there was a disconnect between government agencies working to monitor and control foodborne diseases and the complex, ever-growing chain of food production and preparation establishments (DuPont, 2007). To cope, actions such as the passing of the Food Safety Modernization Act (FSMA) in 2011 and the launching of the Lettuce Safety Initiative in 2006 were made to ensure that safety measures are included in the production of fresh produce (FDA, 2018). The Produce Safety Rule within FSMA gave the FDA regulatory authority over the growing, harvesting, packing, and holding of fresh produce and created universal standards regarding worker hygiene, microbial levels in agricultural water, use of animal by-products as soil amendments, and equipment cleaning and sanitation on farms in an effort to mitigate contamination (Crowe et al., 2015). To help growers produce safe food, the FDA has released guidance that contains information regarding areas to focus on for improving food safety practices (FDA, 1998, 2008).

Antimicrobial Methods

Prevention of initial contamination is the most important step to reduce the likelihood of produce-associated foodborne illness. Thus, accountability at all levels of agricultural systems is crucial to establish a successful food safety culture. The adoption of good agricultural and good

manufacturing practices (GAPs and GMPs) is integral to establish an effective food safety operation, but corrective actions may sometimes be necessary when these systems fail (FDA, 1998). In order to appeal to consumers, produce is washed to remove dirt and other debris. This introduces the potential for a small quantity of contamination to contaminate the remaining contents of the wash system. To combat this, sanitizers are frequently used during post-harvest washing to reduce pathogenic bacteria in the wash water. Producers must ensure that water temperature, turbidity and sanitizer concentrations are maintained throughout the washing process. Currently, most sanitizers do not effectively reduce pathogenic bacteria attached to the produce surface, so their main function is to maintain the bacteriological quality of the water and limit cross-contamination within the wash system (Brackett, 1999; Franz & van Bruggen, 2008; Gomez-Lopez et al., 2014; FDA, 2008).

Chlorine is the most commonly used sanitizer for the washing of fresh produce (Franz & van Bruggen, 2008; Gomez-Lopez et al., 2014; Sapers & Doyle, 2014; Teng et al., 2018). Chlorine compounds are primarily used as bleaching agents or disinfectants, and in industry are used primarily in two forms: sodium hypochlorite and calcium hypochlorite. While both compounds can dissolve in water, calcium hypochlorite is typically used as a solid in the form of white powder, pellets, or flakes. Sodium hypochlorite is typically aqueous. Both forms have a strong chlorine odor and can be hazardous at large concentrations (Agency for Toxic Substances & Disease Registry, 2014; PubChem, 2020a, 2020c). The extensive use of chlorine is due to its effectiveness at killing bacteria while in solution, its minimal impact on product quality at labeled concentrations, and its relatively low cost in comparison to other available methods. When monitoring chlorine in wash water, free chlorine is the most important form to measure. In a neutral solution, free chlorine is present as hypochlorous acid, which has the greatest

antimicrobial activity; thus the inactivation of pathogens during washing is directly related to the concentration of hypochlorous acid in solution and the time of exposure to the pathogen (Shen et al., 2013; FDA, 2008). A study was conducted to establish the ideal balance between the minimum amount of chlorine necessary to control cross-contamination in a washing system, while avoiding adverse effects including reduction in organoleptic quality or bleaching (Gomez-Lopez et al., 2014). As washing occurs, organic matter builds up in a wash system. This organic matter, or organic load, reacts with chlorine in the system and reduces its efficacy. Reduced efficacy results in longer required exposure times for the chlorine to exert its antimicrobial characteristics. While all systems require a base level of monitoring, a system requiring extra monitoring invites more opportunity for human error and introduces a risk of insufficient exposure of the chlorine to harmful bacteria in the wash system. In addition to reducing the efficacy of chlorine, the reaction between chlorine and organic matter can produce by-products that are undesirable and dangerous. When chlorine is added to water in the presence of bromine, hypobromous acid will also be formed and play an additional role in the production of by-products. These include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. Of these trihalomethanes, chloroform and bromodichloromethane are potential human carcinogens. There is currently insufficient evidence to classify dibromochloromethane and bromoform as carcinogens, but there is also not enough research available to classify them as non-carcinogenic (CDC, 2016; Gomez-Lopez et al., 2014; FDA, 2008; Y. Zhang et al., 2016). In addition, the continued replacement of chlorine in a wash system, along with the accumulation of by-products, can lead to the wash solution being unable to dissolve excess chlorine in solution, which can cause a release of noxious chlorine fumes (Shen et al., 2013). By determining the minimum level necessary to inhibit pathogenic bacteria and the maximum organic load before

water must be replaced, both operating costs and the quantity of dangerous by-products from production can be reduced. In order to ensure the continued safe use of chlorine in produce wash systems, legislation has been passed to ensure levels being used are safe for consumers. While the Environmental Protection Agency is the governing body that regulates chlorine levels, in a guidance document published by the FDA (1998), it was recommended to use chlorine levels of 50 - 200 ppm at a pH of 6.0 - 7.5 with a contact time of 1 - 2 minutes.

Peroxyacetic acid (PAA) is a widely used alternative to chlorine in agriculture and the food industry because it is a highly effective antimicrobial agent. It is described as having a vinegar-like smell. PAA, a strong oxidizing agent, is composed of peracetic acid and hydrogen peroxide. PAA inactivates cells by producing reactive oxygen species. These reactive oxygen species cause disruption and rupture the cell membrane by denaturing proteins and blocking enzymatic and transport systems, leading to damage of cellular DNA and lipids. In addition, as an oxidizing agent, it will oxidize the sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites (Block, 1991; CDC, 2008a; Huang, de Vries, & Chen, 2018; Kitis, 2004). There are multiple benefits to utilizing PAA as a sanitizer in a wash system. One benefit is that PAA has been shown to be effective at lower concentrations than chlorine, with regulations allowing food products to be treated with up to 80 ppm (Bang, Park, Kim, Md Furkanur Rahaman, & Ha, 2017; Block, 1991; U.S. Environmental Protection Agency (EPA), 2000). A second benefit, in contrast with chlorine, is that the usage of PAA results in no formation of harmful residuals and by-products (Kitis, 2004). Due to its high reactivity, it quickly decomposes to acetic acid, oxygen, and water (Warburton, 2014). A third benefit is that PAA does not require the constant monitoring of pH, in contrast to chlorine which does (Sánchez, Elizaquível, Aznar, & Selma, 2015). However, drawbacks of PAA include it’s negative impact on the quality of equipment and

cost, which is higher than that of chlorine due in part to limited production; which can result in overall increased operating costs during production (Kitis, 2004). Contrarily, in a test completed at a water pollution control plant in Berkeley Heights, NJ it was determined that, while the initial cost of materials for a peracetic acid-based procedure was roughly 10% greater than that for a chlorine-based procedure, there was a potential financial advantage of the peracetic acid-based procedure because the initial cost of equipment required was lower (Jepson & McAndrew, 2018). More research comparing the operating cost of PAA to traditional antimicrobials should be performed.

There are also many antimicrobial methods that are being tested as potential replacements for chlorine and PAA. One of the potential replacement methods is the use of essential oils. While many essential oils have been tested, some have more potential than others. The results of a study by Hyun, Bae, Song, Yoon, and Lee (2015) indicate that both cinnamon and oregano oils have strong antimicrobial properties against *E. coli* O157:H7, *Salmonella Typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. Thyme oil and clove bud oil were also shown to have comparable effectiveness to chlorine against *Salmonella* on contaminated snacking sweet peppers and against cross-contamination while also being less inhibited by organic load in wash water systems (Dunn et al., 2019). Additionally, Kang and Song (2018) found that nanoemulsions with essential oils could prove an alternative washing treatment against pathogenic bacteria.

High power ultrasound is frequently used on recycled water, which is water that has been previously been used as process water but is treated in a way that it can be reused. While methods used for water recycling have the potential to reduce microbial populations in process water, microorganisms must be rapidly inactivated in order to reduce the risk of cross-

contamination within the wash system. The use of high-power ultrasound combined with a chemical sanitizer has proven to be an effective method to reduce contamination. A downside to using high power ultrasound is the cost, which is higher than chemical sanitizers (Sánchez et al., 2015). Further studies investigating the effectiveness of high-power ultrasound as well as combination strategies should also be tested on pathogenic bacteria, such as *E. coli* O157:H7.

Irradiation is another potential bacterial inactivation method. The FDA has approved the use of irradiation on many food products, including lettuce and spinach. Food can be irradiated multiple ways: with gamma rays, with X-rays, and by electron beam. Some benefits include preservation of nutritional quality with no noticeable changes in taste, texture, and appearance. Irradiation doses of 0.5 kilograys (kGy) have the potential to reduce *E. coli* O157:H7 populations on lettuce by 3.6 to 3.8 logs without having any negative impact on texture (B. A. Niemira, Sommers, & Fan, 2002). While there is concern about the acceptability by consumers, the FDA has evaluated the safety of irradiated foods for more than 30 years. Foods that have been irradiated must include the FDA's Radura symbol accompanied by the statement "Treated with radiation" or "Treated by irradiation" (Doyle & Beuchat, 2007; DuPont, 2007; FDA, 2016). Finten, Garrido, Agüero, and Jagus (2017) performed a survey-based study where a majority of consumers expressed uncertainty in food irradiation, but there was an increase of acceptance by 90% after being provided with additional information. Additionally, irradiation is able to penetrate plant tissue to target internalized bacteria. Brendan A. Niemira (2007) compared chlorine at 300 ppm and 600 ppm to irradiation treatments with *E. coli* O157:H7 on both romaine lettuce and spinach finding that irradiation at 1.5 kGy resulted in a 4-log and 3-log reduction on *E. coli* O157:H7 populations on romaine lettuce and spinach samples, respectively, while chlorine treatments resulted in less than 1-log reductions for both types of leafy green.

Pelargonic Acid

Pelargonic acid, also known as nonanoic acid due to its nine-carbon long chain, is an organic fatty acid and is hydrophobic by nature. Fatty acid structures are characterized by a carboxyl group at one end and a methyl group at the other, both of which are connected by a carbon chain; chain length and degree of saturation determine fatty acid identity (Ciriminna, Fidalgo, Ilharco, & Pagliaro, 2019; PubChem, 2020b). Fatty acids are of interest as they have demonstrated antimicrobial properties. The mechanism behind their antimicrobial activity is primarily due to their insertion into and subsequent disruption of the lipid bi-layer of cellular membranes (Ciriminna et al., 2019; Dev Kumar, Mis Solval, Mishra, & Macarisin, 2020; Pohl, Kock, & Thibane, 2011). This insertion results in increased membrane fluidity, inducing disorganization and conformational changes within the membrane. In a brief period of time, this results in membrane leakage, collapse of intracellular components, and eventual cell lysis (Ciriminna et al., 2019; Dev Kumar et al., 2020; Fukuda, Tsujino, Fujimori, Wakabayashi, & Böger, 2004; Kumar, Macarisin, & Micallef, 2019; Micallef & Kumar, 2017; Pohl et al., 2011).

Research has shown that pelargonic acid can be used as an ingredient against bacterial cells (Ciriminna et al., 2019; Dev Kumar et al., 2020; Fukuda et al., 2004; Kumar, Macarisin, et al., 2019; Micallef & Kumar, 2017). Bacterial targets of fatty acids include the cell wall, cytoplasmic membrane, and specific metabolic functions associated with replication, protein synthesis, and function (Dev Kumar et al., 2020; Ricke, 2003). Additionally, the acidity of fatty acids can disrupt proton gradients across cellular membranes. While most fatty acids are weak acids, there is still a resulting fluctuation in the concentration of undissociated acid formed in the cell on either side of the membrane. Fatty acids accomplish this by penetrating the lipid membrane and then dissociating into anions and protons, thus shifting the anion and proton

balance and in turn, the pH. Because bacteria must maintain a near neutral pH to function, the cells work to reestablish that pH, which requires consumption of cellular adenosine triphosphate (ATP); sustained consumption of ATP eventually results in the depletion of available cellular energy and death (Ricke, 2003).

The basic mechanisms behind the biocidal activity of pelargonic acid are evident in both bacterial and plant cells. While the use of pelargonic acid against bacteria is a novel approach, current agricultural practices use the compound as an herbicide and fungicide. As an herbicide, pelargonic acid-based products are used to eliminate weeds from gardens, lawns, golf courses, parks, walkways, roads, and industrial sites (Ciriminna et al., 2019). Additionally, pelargonic acid-based products are used as blossom thinners, especially in tree fruit orchards (Ciriminna et al., 2019; Nichols, Embree, Cline, & Ju, 2004). Pelargonic acid herbicides are quick, non-selective, broad spectrum herbicides that are foliar-applied. Pelargonic acid does not translocate through woody tissues, does not attack the roots of plants, and has no soil activity. The herbicidal action against plant material is rapid, with damage evident after 15-60 minutes and plant collapse within 1-3 hours (Ciriminna et al., 2019). The primary targets of fatty acids in plant cells are plasma membranes, thylakoid membranes, and plant cuticles (Fukuda et al., 2004; U.S. Department of Agriculture (USDA), 2015).

Lederer, Fujimori, Tsujino, Wakabayashi, and Böger (2004) noted a two-fold herbicidal action for the phytotoxic properties of pelargonic acid. The first stage involved the light driven intercalation of the acid into cellular membranes with the second stage involving peroxidation of the membranes and other organelles. Fukuda et al. (2004) found that C9-C11 fatty acids caused strong, non-selective damage to plants such as crabgrass, cucumber, velvetleaf, and tobacco while C6 and C14 fatty acids showed no effect. These C9-C11 middle chain length fatty acids

have a good balance between intermediate levels of affinity for the target membranes and mismatch capability. Long chains have a high affinity for lipid bilayers, but a low degree of mismatch while short chains have a high degree of mismatch, but a low affinity for lipid bilayers (Ciriminna et al., 2019).

In addition to the herbicidal mechanisms of pelargonic acid, pelargonic acid is used as an anti-fungal agent because it inhibits the swelling and germination of spores. Organisms naturally self-inhibit their own spores to ensure their survival by preventing the germination of all their spores at the same time and place. Pelargonic acid is one of these self-inhibiting substances used by fungi. The swelling and germination of spores is associated with an increase in intracellular pH, but pelargonic acid and other weak acids dissipate pH gradients, thus preventing an increase in pH (Breeuwer, De Reu, Drocourt, Rombouts, & Abee, 1997; Sahin, Kula, & Erdogan, 2006).

Pelargonic acid is also used as an ingredient in pesticides. The U.S. Environmental Protection Agency licensed the sale of pesticide products containing ammonium nonanoate, labeling them as “biochemical herbicides.” They concluded that there was no risk to human health when properly handled based upon the low toxicity, and the fact that residues from their use are not likely to exceed the normal levels of exposure from commonly eaten foods (EPA, 2008). Because it is a skin and eye irritant, product labels describe precautions that users should follow to protect applicator health (Wahlberg & Lindberg, 2003). Users are also advised to take precautions to reduce unintentional contact, such as avoiding windy days and not using large spray droplets when applying pelargonic acid-based products (Ciriminna et al., 2019). Toxicity tests on non-target organisms, including birds, fish and honeybees, revealed little or no toxicity (European Food Safety Authority, 2013). Pelargonic acid decomposes rapidly when used on land and near water, resulting in minimal to no accumulation (Ciriminna et al., 2019).

Other uses for pelargonic acid exist, including within the food industry. The FDA has approved it for use as a food additive (FDA, 2019; 2020) as there are no expected risks to humans or the environment when used properly. Because it is found at low levels in many foods, people are already exposed to small amounts of the chemical (Ciriminna et al., 2019; Flavor and Extract Manufacturers Association, 2018; EPA, 2000). It has also been approved as a safe food-flavoring agent, as an ingredient in solutions for the peeling of fruits and vegetables, and can be used in a solution up to 1% in antimicrobial compounds on foods (Flavor and Extract Manufacturers Association, 2018).

There is a high desirability for naturally-derived antimicrobials. While the development of naturally occurring antimicrobial formulations can be a complex and expensive process, their use can make products more marketable to consumers who question the toxicological safety of synthetic antimicrobials (Sofos et al., 1998). Some preliminary studies have demonstrated that pelargonic acid may be a suitable alternative to synthetic antimicrobials against foodborne pathogens. Han and Micallef (2014) showed that *S. enterica* growth could be affected by the presence of certain fatty acids in tomato fruit exudates. Based upon the fatty acid present, the concentration in the soluble phase of that fatty acid, and its bioavailability in the cuticular matrix of a fruit, fatty acids exerted antimicrobial properties. In previous work with a novel pelargonic acid based sanitizer, Micallef and Kumar (2017) performed a study using fatty acids derived from tomato fruit exudates in combination with multiple solvents. They determined a concentration of 125 mM pelargonic acid in water was sufficient to inhibit *Salmonella* growth, while a minimum inhibitory concentration of 31.25 mM after 1 hour of exposure was determined for both pelargonic acid in quillaja saponin and pelargonic acid in dimethyl sulfoxide.

While many fatty acids exhibit antimicrobial properties, they often have poor solubility in water and undergo phase separation in aqueous mediums, thus their antimicrobial efficacy is inhibited. Surfactants act as emulsification agents and aide in the generation of fatty acid emulsions. Additionally, surfactants influence both the particle size and the amount of antimicrobial compound that comes in contact with bacterial targets (Dev Kumar et al., 2020). Quillaja saponin is a natural phytochemical that can be extracted and used as an surfactant (Böttcher & Drusch, 2017). Because pelargonic acid is a fatty acid and is natively hydrophobic, the addition of a natural emulsification agent, such as quillaja saponin, aids in making pelargonic acid a viable treatment when dispersed in water (Böttcher & Drusch, 2017; Kumar, Macarisin, et al., 2019; Micallef & Kumar, 2017).

Romaine Lettuce Texture and Color

Fruits and vegetables qualities are comprised of four different attributes: color and appearance, flavor, nutritional value, and texture. Acceptance of a piece of fruit or vegetable by the consumer is directly related to these four qualities. Consumers experience only three of these four attributes when they consume the product and they experience those three attributes in a specific order. The first attribute to be experienced is color and appearance. The second attribute is flavor, which is broken into both flavor and aroma. Aroma is the smell of a product, while overall flavor is composed of both aroma and taste of the product. The third and final attribute is texture. If one of these attributes is found objectionable by the consumer, then the overall quality of the product will be impacted. Notably, because color and appearance is the first attribute to be experienced by a consumer, if the color of that fruit or vegetable is rejected then the remaining attributes are irrelevant (Barrett, Beaulieu, & Shewfelt, 2010).

The color of a fruit or vegetable is determined by natural pigments on the surface. Different combinations of these pigments result in the unique colors associated with specific produce varieties. The primary pigments include chlorophylls (green), carotenoids (yellow, orange, and red), anthocyanins (red and blue), flavonoids (yellow), and betalains (red). Enzymatic and non-enzymatic browning negatively impact the implied quality of a fruit or vegetable, and the stability and final color of pigments is influenced by environmental conditions. Oxygen, heat, and pH can all have deleterious impact on the pigments, and therefore color, of a fruit or vegetable (Barrett et al., 2010). During harvesting, transportation, and processing of fruits and vegetables, these factors must be controlled to ensure high quality produce reaches the consumer.

Color can be quantified using the Lab color space. The Lab color space is a three-dimensional real number system that was designed to approximate human vision. It includes all colors in the spectrum, some of which are outside of human perception. There are three different components in the Lab color space: L*, a*, and b*. The L* component represents the human perception of lightness with L* = 0 representing black and L* = 100 representing white. The a* component represents red/green colors with negative values for greens and positive values for reds. The b* component represents yellow/blue colors with negative values for blues and positive values for yellows. For both the a* and b* components the values a* = 0 and b* = 0 depict true gray values (Panigrahy, Seal, & Mahato, 2020).

According to Bourne (1982), the textural properties of a food are the "group of physical characteristics that arise from the structural elements of the food, are sensed by the feeling of touch, are related to the deformation, disintegration and flow of food under a force, and are measured objectively by functions of mass, time, and distance." Fruit and vegetable texture is

generated by the combination of the individual plant cell walls and the middle lamella, which holds the individual cells walls together. Texture is typically used when referring to solid or semi-solid foods and can be further defined by various perceptions within the mouth. These perceptions include smoothness, thickness, firmness, hardness, crispness, and crunchiness (Barrett et al., 2010). Textures, such as crispness or crunchiness, are vital to the fresh commodity industry, as consumers perceive texture as an indicator of freshness. While using a pressure probe is useful to determine crispness or crunchiness, acoustic measurements can also be measured to add a second dimension to the overall textural measurements (Taniwaki & Sakurai, 2008).

Consumers have certain standards and expectations when selecting a fruit or vegetable in a grocery store. Ideally, bananas should be yellow with no brown spots, tomatoes red rather than orange, and lettuce should be green without having begun to turn brown. When a fruit or vegetable is not the desired color, consumers will reject it. A similar expectation is present for the texture of vegetables and fruits. Some vegetables and fruits, such as lettuce, carrots, or apples, should all have a crisp and rigid texture, while some fruits, such as cantaloupes and peaches should be soft and yield when consumed. Fruit that is excessively hard or vegetables, such as lettuce, that are wilted will be rejected (Barrett et al., 2010).

Consumer acceptability is a priority when stocking grocery shelves. If a fruit or vegetable is not evaluated as desirable and purchased, then safety hazards to consumers are irrelevant. Antimicrobial treatments may need to be selected to accommodate commodity differences in order to ensure that the quality of that produce will satisfy consumer standards. If an antimicrobial treatment is detrimental to the quality of a type of produce and results in an

undesirable product, the process should be re-evaluated to ensure an acceptable product is available to consumers.

CHAPTER 3

MATERIALS AND METHODS

Bacterial strains used

Escherichia coli O157:H7 cultures were obtained from the Department of Food Science and Technology, University of Georgia, Athens, GA via glycerol stocks stored at -80 ºC. Five serotypes of *E. coli* O157:H7 isolated from foodborne outbreaks were used in the study; outbreak strains were associated with sprouts, lettuce, cantaloupe (CDC 658), unpasteurized apple juice (SEA13B88), and spinach (K3995).

Antibiotic adaptation

Frozen cultures were revived from glycerol stocks and grown in 10 mL tryptic soy broth (TSB; BD Bacto, Sparks, MD) for 24 h at 37 ºC. A 10 µL loopful of culture was subsequently transferred to a fresh 10 mL TSB tube supplemented with 10 ppm nalidixic acid (Alfa Aesar, Ward Hill, MA) and incubated for 24 h at 37 ºC. The concentration of nalidixic acid in subsequent TSB transfers increased in 10 ppm increments until all cultures grew at a final concentration of 40 ppm nalidixic acid. This process was repeated with rifampicin (Research Products International, Mt. Prospect, IL) until the cultures grew in TSB supplemented with both 40 ppm nalidixic acid and 40 ppm rifampicin (TSBRN). Glycerol stocks were made using TSBRN and glycerol (Fisher Chemical) in a 50:50 ratio and stored at -80 ºC.

Leaf sample preparation

Heads of organic lettuce were stored for up to three days prior to use, and samples were prepared on the day prior to use. The outside leaves of each head of lettuce were removed and discarded. Uniformly sized and colored leaves were rinsed in deionized water to remove debris and dried. Leaf samples were treated with 254 nm of UV light for 10 min on each side to reduce natural microflora (Allende, McEvoy, Luo, Artes, & Wang, 2006) before being stored at 4 ºC in sterile sampling bags (VWR International, Radnor, PA).

Background microflora

To ensure the antibiotics in supplemented TSBRN effectively reduced lettuce background microflora, media was tested with each romaine lettuce trial. Three replicates with triplicate samples (n=9) were diluted with 1:1 w/v of 0.1% peptone (BD Bacto, Sparks, MD), hand massaged for 30 s in sterile sample bags, and spiral plated (Eddy Jet 2, Neutec Group Inc, Farmingdale, NY) in duplicate onto tryptic soy agar (TSA; BD Difco, Sparks, MD) supplemented with 40 ppm nalidixic acid and 40 ppm rifampicin (TSARN). Plates were incubated at 37 ºC for 24 h.

Inoculum preparation

Strains were transferred from frozen glycerol stocks into TSBRN and incubated at 37 °C for 24 h. A 10 µL loopful was transferred into fresh TSBRN, incubated for 20 h at 37 °C, after which a 300 µL aliquot of pure culture was spread plated onto individual TSARN plates and incubated for 24 h at 37 °C. To collect the culture, plates were then flushed with 3 mL of phosphate buffered saline (PBS; Fisher BioReagents, Fair Lawn, NJ), scraped using a sterile spreader, flushed with another 2 mL of PBS, and poured into a sterile centrifuge tube. Equal

aliquots of the individual cultures were combined in a single 50 mL centrifuge tube (Thermo Scientific, Rochester, NY) to make the *E. coli* O157:H7 cocktail. The cultures were vortexed for 20 s and then pelleted by centrifugation for 15 min at 2300 rotations per minute (RPM; Eppendorf centrifuge 5810, Hamburg, Germany). Supernatant was decanted and the pellet was resuspended with a volume of fresh PBS that was identical to the volume decanted. The culture was re-centrifuged for 15 min at 2300 RPM, the supernatant decanted, and the culture resuspended with the decanted volume of fresh PBS, and then vortexed for 20 s. The final concentration of the cocktail was approximately 10^{10} CFU *E. coli*/mL. Inoculum was diluted with 475 mL of PBS and poured into a sterile, rectangular, glass dish.

Inoculation procedure

Whole leaf samples were submerged in the inoculum using sterile tweezers for two min without agitation. Excess inoculum on the leaf sample was removed via a salad spinner (Progressive International, Kent, WA) in the biosafety cabinet. Four spins total were conducted, with a spin consisting of a full pull of the salad spinner cord and allowing the spinner to fully stop before initiating the subsequent spin. Samples were removed and allowed to air dry in a biosafety cabinet for 2 h.

Attachment time for *E. coli* to romaine lettuce

A 2 h drying interval after inoculation was determined in a preliminary experiment to establish the time required for *E. coli* to fully attach to the surface of romaine lettuce. Six heads of romaine lettuce were selected and prepared. Leaf coupons were generated and stored as described by Ells and Hansen (2006) with some modifications. Coupons were made by first removing and disposing of the outer leaves of each head of romaine lettuce. Inner leaves were

then removed from the head. A sterile, 1.6-inch diameter cookie cutter was used to create a coupon from the leaf. Coupons were made from the area directly to the left or right of the midriff (primary center vein). Multiple coupons were generated from each leaf, but each coupon was fully intact with no holes, and did not include the outside edge of the leaf. Four coupons (one for each attachment duration: 0 h, 4 h, 12 h, and 24 h) were made from each head of romaine lettuce. Coupons were stored in sterile sample bags with DI water-soaked Kim wipes and kept at 4 °C until use. Coupons were used within 48 h. Prior to use, coupons were rinsed with DI water, dried, and exposed to UV for 10 min on each side.

E. coli attachment strength was determined as described by Patel and Sharma (2010) and Ells and Hansen (2006) with some modifications. Twenty mL of cocktail was pipetted into a sterile petri plate. Using sterile tweezers, a single coupon was submerged in the cocktail for 2 min without agitation. The coupon was removed using sterile tweezers and placed into the salad spinner. All four coupons from the same head of lettuce were evenly spaced in the salad spinner prior to spinning. Four spins total were conducted as previously described. Coupons were removed from the salad spinner, placed into individual petri plates, and covered with the petri plate lid.

One coupon from each head of lettuce was designated an attachment duration, with six coupons per duration. All coupons remained in the biosafety cabinet at 25 °C for 2 h after inoculation prior to being stored at 4 °C in closed petri plates for the appropriate attachment duration.

When designated time had elapsed, coupons were submerged in 15 mL PBS for 3 s to remove any unattached cells. To reduce variable populations of cells that were concentrated around the cut, exposed edge of the coupons, a sterile 1.25-inch cookie cutter was used to further

reduce the diameter of each coupon. Using sterile tweezers, single coupons were put into sterile 50 mL centrifuge tubes with 25 mL of PBS with 0.1% Tween 80. Loosely attached cells were quantified by vortexing the coupon 20 s and enumerating *E. coli* from PBS. The coupon was then aseptically removed and placed into a new 50 mL centrifuge tube with 25 mL PBS. To quantify strongly attached cells, samples were then sonicated for 1 min with a Branson M1800 sonicating water bath (Branson Ultrasonics, Danbury, CT). PBS was serially diluted with 0.1% peptone and spiral plated in duplicate onto TSARN. TSARN plates were incubated for 24 h at 37 °C.

Attachment strength (S_r) was calculated as described by Dickson and Koohmaraie (Dickson & Koohmaraie, 1989). The S_r represents the ratio of strongly attached cells to strongly attached cells plus loosely attached cells. S_r = (strongly attached/(strongly attached + loosely attached)). Based upon the results of the attachment study, we chose an attachment time of 2 h.

Each attachment duration was replicated six times (n=6). All statistical analyses were performed using JMP software (SAS, 2018). The S_r were averaged across the six total samples for each attachment duration. Standard deviations were calculated for each set of six samples. The S_r were analyzed using the Tukey-Kramer multiple comparisons method with $p < 0.05$.

Sanitizer treatment preparation

Eleven treatments were examined: no rinse, sterile deionized (DI) water, 200 ppm chlorine, 200 ppm chlorine with 0.5% w/v organic load, 80 ppm peroxyacetic acid (PAA), 80 ppm PAA with 0.5% w/v organic load, 7.5 mM pelargonic acid (Pel 7.5), Pel 7.5 with 0.5% w/v organic load, 30 mM pelargonic acid (Pel 30), Pel 30 with 0.5% w/v organic load, 50 mM pelargonic acid (Pel 50), and Pel 50 with 0.5% w/v with organic load. Organic load (OL) was made by making a puree of romaine lettuce leaves prepared as described above.

Two-hundred ppm chlorine treatments were made using bleach (Clorox Regular Bleach EPA Reg. No 5813, Oakland, CA). Parts per million was measured using a Free Chlorine and Chlorine UHR portable photometer (Hanna Instruments, Woonsocket, RI). Eighty ppm PAA treatments were made using Sanidate 5.0 (BioSafe Systems, Hartford, CT). Parts per million was measured using a Peracetic Acid Test Kit (Thomas Scientific, Swedesboro, NJ). A 1M pelargonic acid stock, derived from coconut and quillaja saponin emulsion, was used to make the pelargonic acid treatments. Pelargonic acid was placed on a stir plate and vortexed for 15 min at approximately 750 RPM. The pH of all treatments was measured using an Accumet AB 250 pH probe (Fisher Scientific, Waltham, MA).

Treatment with sanitizers

Three intact leaves were used for each sample: one inoculated leaf and two uninoculated leaves. Five hundred mL of treatment wash was poured into a rectangular, autoclavable container. Sterile tweezers submerged the inoculated leaf for 2 min without agitation, after which the leaf was removed and placed into a sterile stomacher bag. One uninoculated leaf (Subsequently Uninoculated Sample; SUS 1) was submerged without agitation for 2 min in the same treatment wash, removed and placed in a new stomacher bag. The next uninoculated leaf (SUS 2) was immediately submerged in the same wash as the previous two leaves for 2 min, then placed in a new stomacher bag. Samples for 0 h analysis were immediately plated or filtered, while 24 h and 7 day treated leaves were stored at 4 ℃ prior to enumeration.

Microbiological analyses

Leaf-containing stomacher bags were diluted 1:5 (w/v) with a Smart Dilutor (Neutec Group Inc, Farmingdale, NY) in PBS with 0.2% Tween 80 (Sigma Life Science, St. Louis, MO)

and 0.1% sodium thiosulfate (Sigma Aldrich, St. Louis, MO). Samples were hand massaged for 20 s, then the rinsate was serially diluted in buffered peptone water (BPW; BD Difco, Sparks, MD) and spiral plated in duplicate onto TSA (to determine aerobic plate count) and TSARN (to enumerate antibiotic resistant *E. coli* O157:H7) plates. To lower the limit of detection (spiral plated sample limit of detection was 100 CFU/g), a 10-mL aliquot of rinsate was filtered through a 0.45-µm mixed cellulose ester membrane filter type HA (Whatman, GE Healthcare Life Sciences) with a Glassco filter system (Glassco Laboratory Equipments PVT. LTD.). The filter was then plated on TSARN. Treatment wash solution was also filtered and enumerated; both plate types were incubated at 37 °C for 24 h.

Statistical analyses for microbiological data

Each wash treatment at all three storage durations was replicated four times, with samples in duplicate (n=8). The limit of detection for the 0.45-µm mixed cellulose ester membrane filter is 1 log CFU/g. *E. coli* counts were averaged across the eight total samples for each combination of treatment, storage duration, and sample type. Statistical analyses were performed using generalized linear mixed techniques in the R Studio (RStudio Team, 2016). Inoculated samples were analyzed with sanitizer (i.e., Chlorine, PAA, Pel 7.5, Pel 30, and Pel 50), OL (i.e., presence of OL or not), storage duration (i.e., 0 h, 24 h, and 7 d), and their interactions as fixed effects. Subsequentially Uninoculated Samples (SUS) were analyzed with sanitizer, OL, storage duration, SUS (1 and 2), and their interactions as fixed effects. In both models, storage duration was treated as a repeated measurement and the heterogenous compound symmetry was used as the covariance structure due its smallest Akaike's information. Treatment wash solution was analyzed with sanitizer, OL, and their interactions as fixed effects. For all analyzes, when the F value was significant least square means comparisons were performed using the Tukey adjusted

probability value of 0.05, and means were portioned as needed. Additionally, orthogonal contrasts were used to evaluate the effect of sanitizer (i.e., Chlorine, PAA, Pel 7.5, Pel 30, and Pel 50) versus water and versus no rinse, and water versus no rinse for inoculated, as well as sanitizer versus water for SUS and treatment wash solution.

Texture and color analyses

An analysis of the impact on texture and color of all sanitizer treatments used in the study was performed on romaine lettuce. The outer leaves were removed from each lettuce head and discarded. Treatments examined included: no rinse, deionized water, 200 ppm chlorine, 80 ppm PAA, Pel 7.5, Pel 30, and Pel 50; no treatments contained OL. Romaine lettuce leaves were submerged in each treatment for 2 min without agitation. Samples were then removed from the treatment and blotted gently with paper towels to remove excess liquid. A MSEZ-4500L portable spectrophotometer (HunterLab, Reston, VA) was used to measure the L*, a*, and b* of each sample. A TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, United Kingdom) was used to measure the skin strength in Newtons (N) and the skin elasticity in mm of the treated leaf samples. The settings for the TA-XT2 Texture Analyzer were a trigger force of 3.0 g, a test speed of 5 mm/s, and a penetration distance of 15 mm. A metal, spherical probe with a diameter of 7 mm was used to puncture the treated samples. Zero hour samples were immediately analyzed, and 24 h and 7 d samples were stored at 4 ºC prior to texture and color analysis.

Statistical analysis for texture and color analyses

Each wash treatment at all three storage durations was replicated four times, with samples in duplicate (n=8). Statistical analyses were performed using generalized linear mixed techniques

in the R Studio (RStudio Team, 2016). L*, a*, b*, skin strength, and skin elasticity were analyzed with sanitizer (i.e., Chlorine, PAA, Pel 7.5, Pel 30, and Pel 50) plus water and no rinse, storage duration (i.e., 0 h, 24 h, and 7 d), and their interactions as fixed effects. Once again, storage duration was treated as a repeated measurement and the heterogeneous compound symmetry was used as the covariance structure due its smallest Akaike's information. When the F value was significant least square means comparisons were performed using the Tukey adjusted probability value of 0.05, and means were portioned as needed.

CHAPTER 4

RESULTS

Effect of treatments on inoculated samples

The effects of sanitizer treatments on *E. coli* O157:H7 on inoculated romaine lettuce were compared against the no rinse and DI water treatments (Table 1). There was no significant interaction of OL on treatment effect. All sanitizer-containing treatments performed significantly better than the no rinse and DI water treatments at all three storage durations. Chlorine and PAA resulted in reductions ranging from 1.79 (chlorine, 0 h) to 2.81 (chlorine, 7 d; Table 1). The Pel 30 and Pel 50 treatments performed significantly better than chlorine and PAA at all three storage durations, resulting in 2 to 3 log reductions at 0 h, over 4 log reductions at 24 h, and greater than 6 log reductions at 7 d. While the Pel 7.5 treatment performed similarly to the chlorine and PAA treatments at 0 h (1.8 log reduction) and 24 h (2.7 log reduction), it resulted in significantly greater *E. coli* O157:H7 reductions ($p \leq 0.05$) than chlorine and PAA after 7 d of storage (4.1 log reduction).

Sanitizer treatment effect on aerobic bacteria recovered on TSA (without antibiotics) from treated romaine lettuce samples that had been inoculated with antibiotic resistant *E. coli* O157:H7 strains were compared against the no rinse and DI water treatments (Table 2). There was no significant interaction of OL on treatment effect. All sanitizer treatments performed significantly better than both the no rinse and DI water treatments at all three storage durations. The chlorine, PAA, and Pel 7.5 treatments resulted in statistically similar reductions ranging

from 1.76 (chlorine, 0 h) to 2.83 (chlorine, 7 d; Table 2). The Pel 30 and Pel 50 treatments showed significantly greater reductions at 0 h and 24 h compared to chlorine, PAA, and Pel 7.5. After 7 d of storage the bacterial populations for samples treated with Pel 30 and Pel 50 increased by at least 1.5 log CFU/g whereas the bacterial populations after 7d for chlorine, PAA, and Pel 7.5 were not statistically different from the 24 h samples.

Effect of treatments on cross-contamination

Sanitizer treatment effects on *E. coli* O157:H7 SUS romaine lettuce samples, an indicator of efficacy against cross-contamination, were compared against sterile, DI water (Table 3). All sanitizer treatments were significantly different from the DI water treatment. No significant interactions existed due to OL and SUS. There was no significant change in effectiveness over time for the Pel 30 and Pel 50 treatments when examined over the three subsequent storage durations. However, both the chlorine and Pel 7.5 treatments were significantly more effective at the 7 d storage duration than at both the 0 h and 24 h storage durations.

Recovery of aerobic bacteria on TSA from SUS romaine lettuce samples was compared to the DI water treatment (Table 4). There were no significant interactions due to OL and SUS on treatment effect. All sanitizer treatments other than chlorine performed significantly worse from 0 h to 7 d. While the log reductions for Pel 30 and Pel 50 were at least 1.08 log CFU/g greater than chlorine, PAA, and Pel 7.5 at 0 h, after 7 d of storage the bacterial populations on samples treated with Pel 30 and Pel 50 increased to a level statistically similar to DI water .

Effect of treatments on romaine lettuce color

Leaf color change due to sanitizer treatment was compared to leaves treated with no rinse and DI water (Tables 5 and 6; Figure 1). Eight samples were examined for each treatment. For

b* (blueness/yellowness), there was no significant difference between the no rinse and all other sanitizer treatments at each storage duration, but overall samples after 24 h and 7 d were less yellow than samples at 0 h. L* (light/dark) and a* (red/green) were impacted by the various sanitizer treatments. From 0 h to 24 h, samples treated with the three pelargonic acid treatments dropped an average of 8.84 L* units more than DI water, chlorine, and PAA, which dropped an average of only 2.00 L* units. For a* between 0 h to 24 h, samples treated with the three pelargonic acid treatments became significantly redder than samples treated with DI water, chlorine, and PAA. The Pel 7.5 treated samples were less red after 24 h of storage when compared to the Pel 30 and Pel 50 samples, but after 7 d there was no difference among the redness of samples treated with pelargonic acid at any concentration.

Effect of treatments on romaine lettuce texture

There was no interaction due to any of the sanitizer treatments or storage durations on the skin strength (N) of treated lettuce samples. The effect of sanitizer treatments on the skin elasticity (mm) of treated romaine lettuce samples were compared against the no rinse and DI water treatments (Table 7). Eight samples were examined for each sanitizer treatment (n=8). While there are varying differences in skin elasticity between the samples of the different sanitizer treatments, the pelargonic acid sanitizers resulted in more skin elasticity than the non-pelargonic acid sanitizer treatments (excluding the no rinse). Overall, the 7 d samples showed less elasticity that samples from 0 h and 24 h with skin elasticity decreasing by an average of 1.4 mm from 24 h to 7 d of storage.

CHAPTER 5

DISCUSSION

Wash water

Harvest and packing practices vary substantially depending on lettuce variety, growing region, buyer requirements, operation, and other factors (FDA, 2006). Many operations field pack heads of lettuce, cool using forced air, and ship directly to the consumer without washing. The no rinse lettuce samples in the current study represented lettuce handled in this manner, where a contamination event (i.e. wildlife intrusion, contaminated irrigation water, etc.) may introduce pathogens to the produce surface, which in turn reaches the consumer due to no intervention step (Coleman & Maynard, 2014; Penn State Extension, 2019). Other operations that include packinghouses or fresh cut facilities, may process lettuce heads or chopped lettuce by washing using recirculated water from a spray bar system or flume (Coleman & Maynard, 2014; Penn State Extension, 2019). This recirculating system was demonstrated by introducing the SUS lettuce to the used water and examining cross-contamination.

The SUS lettuce washed in DI water alone experienced greater cross-contamination than SUS lettuce washed in sanitizer treatments. The findings of Rana et al. (2010) reflect our results as they demonstrated that washing tomatoes contaminated with *Salmonella* with water lacking sanitizer led to uniform contamination of uninoculated tomatoes in a wash system. Additionally, Aixia, Pahl, Buchanan, and Micallef (2015) found that washing leafy greens in dump tanks without sanitizer led to increases in microbial indicator organisms. In further discussions with

farmers, they determined that not using sanitizers was due to perceptions that using good quality water in their operation was sufficient, and was not a result of cost-saving decisions. While using high quality water with undetectable levels of *E. coli* is mandated by law (Penn State Extension, 2019), these studies demonstrate the importance of utilizing effective sanitizers and maintaining adequate sanitizer levels in recirculating wash water.

Chlorine and PAA

Chlorine and PAA are used by growers as key components of good agricultural practices, and both have been extensively studied in postharvest wash water against foodborne pathogens on different commodities (Allwood, Malik, Hedberg, & Goyal, 2004; Beuchat, 1999; Brackett, 1987; Delaquis, Stewart, Cazaux, & Toivonen, 2002; Lapidot et al., 2006; Lukasik et al., 2003; Oh, Dancer, & Kang, 2005; Rodgers, Cash, Siddiq, & Ryser, 2004; Sapers, Miller, & Mattrazzo, 1999; Shirron et al., 2009; Wisniewsky, Glatz, Gleason, & Reitmeier, 2000; Yuk, Bartz, & Schneider, 2005, 2006; S. Zhang & Farher, 1996; Zhuang, Beuchat, & Angulo, 1995). The chlorine and PAA findings in the current study support results of previous studies and provided a baseline against which the effectiveness of the novel pelargonic acid treatments could be compared. In the current study, *E. coli* O157:H7 cross-contamination was effectively reduced by chlorine and PAA, as recovery of only 1.00 and 1.27 log CFU/g occurred from the SUS samples across the three storage durations. However, neither chlorine nor PAA effectively removed attached *E. coli* O157:H7 from the lettuce surface. The results from this study are comparable to the findings of Beuchat, Adler, and Lang (2004) that determined a spray treatment of 200 ppm chlorine was not effective in eliminating viable *E. coli* O157:H7 cells. Additionally, Rodgers et al. (2004) found that after treatment with 200 ppm chlorine, 1 log *E. coli* O157:H7 was found on

shredded lettuce up to 9 days of storage at refrigeration temperature. They found similar results using an 80 ppm PAA solution with 1.5 log *E. coli* remaining on shredded lettuce.

Pelargonic acid treatments reduce *E. coli* O157:H7 on the lettuce surface and cross-contamination

Of the three pelargonic acid treatments examined in this study, Pel 30 and Pel 50 performed similarly to chlorine and PAA for controlling cross-contamination but performed better for reducing *E. coli* O157:H7 on inoculated lettuce samples. This is in agreement with previous work done by White (2020) that found that pelargonic acid treatments at 30 mM and 50 mM were more effective than both chlorine and PAA at removing *Salmonella* from inoculated tomatoes. Similarly to this study, White (2020) also found that the 30 mM and 50 mM pelargonic acid-based sanitizers tested controlled cross-contamination comparably to chlorine and PAA at 200 ppm and 80 ppm, respectively. While White (2020) suggested that a concentration of pelargonic acid lower than 30 mM could provide benefit for use on fresh produce, the lower pelargonic acid concentration treatment in this study, Pel 7.5, did not exhibit benefits surpassing chlorine and PAA until after 7 d of storage where there was a reduction of *E. coli* O157:H7 that was greater than both chlorine and PAA.

Impact of organic load on sanitizers

There was no interaction of OL with any of the sanitizer treatments tested in this study. These findings are contradictory to many sources as chlorine is known to be highly susceptible to organic matter (Fukuzaki, 2006; Pizzi, 2005; CDC, 2008a; USDA, 2011). Additionally, a study by Dunn et al. (2019) examining essential oil-based sanitizers found that the effectiveness of both chlorine and clove-bud oil sanitizers were reduced by the presence of 1% OL, but that

thyme oil was not impacted by OL presence. Y. Zhang et al. (2016) found that neither 2% nor 5% OL had an impact on thyme oil. One potential reason that the OL in our study had no interaction with any of the sanitizer treatments tested is that the level of OL used may have been insufficient. Based upon the OL concentrations used by Dunn et al. (2019) and Y. Zhang et al. (2016) raising the OL level from 0.5% to 1.0% or potentially higher (2-5%) could result in a notable impact. A second potential reason is the source of the OL used as this study, pureed romaine lettuce. Pureed produce is commonly used in studies aiming to introduce OL to a system (Dunn et al., 2019; Y. Zhang et al., 2016). Additionally, in an experiment comparing 200, 500, and 2000 ppm chlorine against *Salmonella*-inoculated cantaloupe, Beuchat and Ryu (1997) suggested that the high level of organic matter in the juice released from cut cantaloupe tissue was able to neutralize chlorine before it could affect *Salmonella*.. Gomez-Lopez et al. (2014) observed in a study using spinach that organic matter increased the chlorine demand, but the results by Luo et al. (2011) suggest that organic matter from dust and soil (spinach study) quenched chlorine more than organic matter from tissue exudates (lettuce study).

Pelargonic acid effect on plant tissue

As seen in Figure 1, the pelargonic acid-based sanitizer treatments induced significant color changes on the plant tissues while the chlorine and PAA did not. These changes reflect the foliar, herbicidal action of pelargonic acid as portions of the lettuce samples exposed to the pelargonic acid-based sanitizer treatments were altered while parts untouched by the sanitizer treatments remained unchanged (Ciriminna et al., 2019; Fukuda et al., 2004; Lederer et al., 2004; Nichols et al., 2004; USDA, 2015). These observations are similar to those found by Lederer et al. (2004) and Fukuda et al. (2004). Lederer et al. (2004) measured membrane leakage in cress and tobacco seedlings that were treated with pelargonic acid and after testing the effects of

pelargonic acid on crabgrass, cucumber, velvetleat, and tobacco plants Fukuda et al. (2004) suggested that middle chain fatty acids caused severe damage to cell membranes and thylakoid membranes of treated leaves. These studies highlight the structural breakdown caused by pelargonic acid on plant tissues.

Samples treated with pelargonic acid generally exhibited more skin elasticity than the other sanitizer treatments (Table 7), with the exception the no rinse treatment. The no rinse treatment used in this study was not an effective baseline of skin elasticity because the no rinse samples were not submerged in water whereas all other sanitizer treatment samples were, thus there was no benefit of increased turgor pressure (Vogler, Burri, Nelson, & Grossniklaus, 2020; D. Zhang & Zhang, 2020). The skin elasticities of the samples treated with pelargonic acid was greater than the other sanitizer treatments because the pelargonic acid caused tissue breakdown and less rigid structure of the lettuce leaves.

Beuchat (2002) found that bruised or cut plant tissues release nutrient-rich fluid that can enhance the growth of naturally occurring microflora or pathogens. This supports the TSA SUS results (Table 4) that indicated an approximately 4 log CFU/g increase in aerobic bacteria between 0 h and 7 d for Pel 30 and Pel 50 samples, while aerobic bacterial populations recovered from water and chlorine treated samples were not significantly different between the two storage durations. Additionally, the populations recovered from Pel 30 and Pel 50 treated samples are ≤ 1.5 log CFU/g at 0 h, indicating that the bacterial populations are severely decreased initially by the pelargonic acid, but over time the degraded tissues appear to promote bacterial growth.

Storage implications

The incorporation of antimicrobials into the formulations of edible films and coatings allows for continuous antimicrobial action or a gradual release of antimicrobial substances (Campos, Gerschenson, & Flores, 2011). Based upon the target pathogen, commodity, and other components in the film there are a variety of potential options available for the development of edible films with antimicrobial properties, a couple of which are mentioned here. Beverlya, Janes, Prinyawiwatkula, and No (2008) achieved 2-3 log CFU/g reductions more of *Listeria monocytogenes* than control samples after 14 days by utilizing a chitosan edible film dissolved with acetic acid on ready to eat roast beef. Additionally, Zhu et al. (2014) measured effective, while varying, log reductions of *Salmonella Newport* on multiple leafy greens by utilizing apple, carrot, and hibiscus edible films containing the antimicrobials carvacrol and cinnamaldehyde. Like some edible coatings, the pelargonic acid sanitizer treatments used in this study showed continuous action against *E. coli* O157:H7 over the three storage durations. Prior to adopting a coating as a viable solution, Campos et al. (2011) suggest that producers consider the effectiveness against the target microorganism against possible interactions with the food and its components. The continuous antimicrobial action against *E. coli* O157:H7 is promising, but the pelargonic acid sanitizer treatments did cause extensive color changes and increased aerobic microbial growth on 7 d samples. Pelargonic acid could be a valuable ingredient in the formulation of edible films. The inclusion of an additional ingredient to neutralize the phytotoxic effects of pelargonic acid on plant tissue could support an effective antimicrobial film without the negative impact on plant tissue.

Potential uses for pelargonic acid

Sofos et al. (1998) note that naturally occurring antimicrobials should be economical, effective, and not cause sensory changes in food. Sensory changes in a food can deter consumers from the initial purchase of an item. Over the three storage durations, pelargonic acid-based sanitizer treatments used in this study performed better against *E. coli* O157:H7 than both chlorine and PAA, but caused significant sensory changes to the romaine lettuce tested. White (2020) found similar results; where pelargonic acid-based sanitizer treatments were more effective than chlorine and PAA against *Salmonella*, but negatively impacted tomato quality.

Pelargonic acid-based sanitizers show potential against pathogens, and use on less fragile commodities, non-produce foods, or surfaces may be particularly viable. One such food is dry pet food. Multiple outbreaks of Salmonellosis have been linked to dry pet food (CDC, 2008b, 2012). Because *Salmonella* does not survive the extrusion process during the production of dry pet food, contamination typically occurs post-extrusion. In a study by Chen, Yin, Upadhayay, Brown, and Venkitanarayanan (2019) post-extrusion spray treatments with plant-derived antimicrobials showed potential to reduce *S. Scharzengrund* on dry pet food. Pelargonic acid could be examined similarly, or potentially be included in formation. Additionally, Dev Kumar et al. (2020) found that surfactant type and concentration play an important role in the antimicrobial efficacy of pelargonic acid emulsions, indicating that if pelargonic acid with one tested surfactant is not optimal on a particular commodity or surface the testing of an another surfactant on the same commodity or surface could result in improved efficacy.

Conclusion

Sanitizers are a vital component to ensure the safety of the food supply, especially in today's complex supply chain. While commonly used sanitizers are effective for cross-contamination control, they are insufficient in inactivating *E. coli* O157:H7 attached to the surface of produce. The pelargonic acid sanitizers tested in this study were both effective against *E. coli* O157:H7 attached to the surface of romaine lettuce and reduced cross-contamination within the simulated wash system. Additionally, treatment with the pelargonic acid sanitizers showed continuous antimicrobial action during storage. These results indicate that the tested sanitizers are promising and have many potential applications in industry such as being an ingredient in postharvest washes or sprays and being incorporated into edible coatings as an antimicrobial. Future work is necessary to elucidate the potential uses of pelargonic acid.

REFERENCES

Abdul-Raouf, U. M., Beuchat, L. R., & Ammar, M. S. (1993). Survival and growth of Escherichia coli O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. *Appl Environ Microbiol, 59*(8), 2364-2368.

Agency for Toxic Substances & Disease Registry. (2014). Calcium Hypochlorite (CaCl2O2)/Sodium Hypochlorite (NaOCl). Retrieved from https://www.atsdr.cdc.gov/MHMI/mmg184.pdf

Aixia, X. U., Pahl, D. M., Buchanan, R. L., & Micallef, S. A. (2015). Comparing the Microbiological Status of Pre- and Postharvest Produce from Small Organic Production. *Journal of Food Protection, 78*(6), 1072-1080. doi:10.4315/0362-028X.JFP-14-548

Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed 'Red Oak Leaf' lettuce. *Food microbiology, 23*(3), 241-249. doi:10.1016/j.fm.2005.04.009

Allwood, P. B., Malik, Y. S., Hedberg, C. W., & Goyal, S. M. (2004). Effect of Temperature and Sanitizers on the Survival of Feline Calicivirus, Escherichia coli, and F-Specific Coliphage MS2 on Leafy Salad Vegetables*(7),* 1451. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN152781335&site=eds-live

Bang, H., Park, S., Kim, S., Md Furkanur Rahaman, M., & Ha, S. (2017). Synergistic effects of combined ultrasound and peroxyacetic acid treatments against Cronobacter sakazakii biofilms on fresh cucumber. *Food science & technology, 84*, 91-98. doi:10.1016/j.lwt.2017.05.037

Barrett, D., Beaulieu, J., & Shewfelt, R. (2010). Color, Flavor, Texture, and Nutritional Quality of Fresh-Cut Fruits and Vegetables: Desirable Levels, Instrumental and Sensory Measurement, and the Effects of Processing*(5),* 369. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN271248776&site=eds-live

Beuchat, L. R. (1999). Survival of Enterohemorrhagic Escherichia coli O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant. *Journal of Food Protection, 62*(8), 845-849. doi:10.4315/0362-028x-62.8.845

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

Beuchat, L. R., Adler, B. B., & Lang, M. M. (2004). Efficacy of chlorine and a peroxyacetic acid sanitizer in killing Listeria monocytogenes on iceberg and Romaine lettuce using simulated commercial processing conditions. In (Vol. 67, pp. 1238-1242).

Beuchat, L. R., & Ryu, J. (1997). Produce Handling and Processing Practices. *Emerging Infectious Diseases, 3*(4), 459-465. doi:10.3201/eid0304.970407

Beverlya, R. L., Janes, M. E., Prinyawiwatkula, W., & No, H. K. (2008). Edible chitosan films on ready-to-eat roast beef for the control of Listeria monocytogenes. *Food microbiology, 25*(3), 534-537. doi:10.1016/j.fm.2007.11.002

Block, S. S. (1991). *Disinfection, sterilization, and preservation* (4th ed. ed.): Lea & Febiger.

Böttcher, S., & Drusch, S. (2017). Saponins — Self-assembly and behavior at aqueous interfaces. *Advances in Colloid and Interface Science, 243*, 105-113. doi:https://doi.org/10.1016/j.cis.2017.02.008

Bourne, M. C. (1982). *Food texture and viscosity : concept and measurement*: Academic Press.

Brackett, R. E. (1987). Antimicrobial Effect of Chlorine on Listeria monocytogenes. *Journal of Food Protection, 50*(12), 999-1003. doi:10.4315/0362-028X-50.12.999

Brackett, R. E. (1999). Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biology and Technology, 15*(3), 305-311. doi:https://doi.org/10.1016/S0925-5214(98)00096-9

Breeuwer, P., De Reu, J. C., Drocourt, J., Rombouts, F. M., & Abee, T. (1997). Nonanoic Acid, a Fungal Self-Inhibitor, Prevents Germination of Rhizopus oligosporus Sporangiospores by Dissipation of the pH Gradient. *Applied And Environmental Microbiology, 63*(1), 178. Retrieved from http://aem.asm.org/content/63/1/178.abstract

Brown, M. R. W., Allison, D. G., & Gilbert, P. (1988). Resistance of bacterial biofilms to antibiotics a growth-rate related effect? *Journal of Antimicrobial Chemotherapy, 22*(6), 777-780. doi:10.1093/jac/22.6.777

Burg, S. P. (2004). *Postharvest physiology and hypobaric storage of fresh produce. [electronic resource]*: CABI Pub.

Burnett, S. L., Chen, J., & Beuchat, L. R. (2000). Attachment of Escherichia coli O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Applied And Environmental Microbiology, 66*(11), 4679-4687. doi:10.1128/aem.66.11.4679-4687.2000

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. *Food and Bioprocess Technology*(6), 849. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsgao&AN=edsgcl.258601778&site=eds-live

Centers for Disease Control and Prevention. (2008a). Guideline for Disinfection and Sterilization in Healthcare Facilities. Retrieved from https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html#PeraceticAcidAnd

Centers for Disease Control and Prevention. (2008b). Multistate outbreak of human Salmonella infections caused by contaminated dry dog food--United States, 2006-2007. *MMWR. Morbidity and mortality weekly report, 57*(19), 521-524. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=mnh&AN=18480745&site=eds-live

Centers for Disease Control and Prevention. (2012). Multistate Outbreak of Human Salmonella Infantis Infections Linked to Dry Dog Food (Final Update). Retrieved from https://www.cdc.gov/salmonella/dog-food-05-12/index.html

Centers for Disease Control and Prevention. (2014). Shiga toxin-producing E. coli (STEC).

Centers for Disease Control and Prevention. (2016). Safe Water System - Disinfection By-Products. Retrieved from https://www.cdc.gov/safewater/chlorination-byproducts.html

Centers for Disease Control and Prevention. (2017). *Surveillance for Foodborne Disease Outbreaks, United States, 2017*. Retrieved from Atlanta, GA:

Chang, A. S., & Schneider, K. R. (2012). Evaluation of Overhead Spray-Applied Sanitizers for the Reduction of-Salmonella-on Tomato Surfaces*(1),* M65. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN305724589&site=eds-live

Chen, C., Yin, H., Upadhayay, A., Brown, S., & Venkitanarayanan, K. (2019). Efficacy of plant-derived antimicrobials for controlling Salmonella Schwarzengrund on dry pet food. *International Journal of Food Microbiology, 296*, 1-7. doi:https://doi.org/10.1016/j.ijfoodmicro.2019.02.007

Cheville, A. M., Arnold, K. W., Buchrieser, C., Cheng, C. M., & Kaspar, C. W. (1996). rpoS regulation of acid, heat, and salt tolerance in Escherichia coli O157:H7. *Appl Environ Microbiol, 62*(5), 1822-1824.

Chhetri, V. S., Fontenot, K., Strahan, R., Yemmireddy, V. K., Cason, C., Kharel, K., & Adhikari, A. (2019). Attachment strength and on-farm die-off rate of Escherichia coli on watermelon surfaces. *PLoS ONE, 14*(01), 1-14. doi:10.1371/journal.pone.0210115

Chmielewski, R. A. N., & Frank, J. F. (2003). Biofilm Formation and Control in Food Processing Facilities. *Comprehensive Reviews in Food Science and Food Safety, 2*(1), 22-32. doi:10.1111/j.1541-4337.2003.tb00012.x

Ciriminna, R., Fidalgo, A., Ilharco, L. M., & Pagliaro, M. (2019). Herbicides based on pelargonic acid: Herbicides of the bioeconomy. In.

Coleman, J., & Maynard, E. (2014). Food Safety for Fruit and Vegetable Farms. In: Purdue Extension.

Costerton, J. W., Stweart, P. S., & Greenberg, E. P. (1999). Bacterial Biofilms: A Common Cause of Persistent Infections. *Science, 284*(5418), 1318. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.2899085&site=eds-live

Crowe, S. J., Mahon, B. E., Vieira, A. R., & Gould, L. H. (2015). Vital Signs : Multistate Foodborne Outbreaks — United States, 2010–2014. *Morbidity and Mortality Weekly Report, 64*(43), 1221. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.24856869&site=eds-live

Czárán, T., & Hoekstra, R. F. (2009). Microbial Communication, Cooperation and Cheating: Quorum Sensing Drives the Evolution of Cooperation in Bacteria. *PLoS ONE, 4*(8), 1-10. doi:10.1371/journal.pone.0006655

De Beer, D., Srinivasan, R., & Stewart, P. S. (1994). Direct measurement of chlorine penetration into biofilms during disinfection. *Applied And Environmental Microbiology, 60*(12), 4339-4344. Retrieved from https://aem.asm.org/content/aem/60/12/4339.full.pdf

Delaquis, P., Stewart, S., Cazaux, S., & Toivonen, P. (2002). Survival and Growth of Listeria monocytogenes and Escherichia coli O157:H7 in Ready-to-Eat Iceberg Lettuce Washed in Warm Chlorinated Water. *Journal of Food Protection, 65*(3), 459-464. doi:10.4315/0362-028x-65.3.459

Dev Kumar, G., Mis Solval, K., Mishra, A., & Macarisin, D. (2020). Antimicrobial Efficacy of Pelargonic Acid Micelles against Salmonella varies by Surfactant, Serotype and Stress Response. *Scientific Reports, 10*(1), 1-13. doi:10.1038/s41598-020-67223-y

Dickson, J. S., & Koohmaraie, M. (1989). Cell-Surface Charge Characteristics and Their Relationship to Bacterial Attachment to Meat Surfaces. In (Vol. 55, pp. 832-836).

Doyle, M. P., & Beuchat, L. R. (2007). *Food microbiology : fundamentals and frontiers* (3rd ed. ed.): ASM Press.

Dunn, L. L. (2017). A Transcriptomic Analysis of Salmonella enterica Newport In Planta and after Postharvest Sanitization. *Trace: Tennessee Research and Creative Exchange*.

Dunn, L. L., Harness, M. L., Smith, D. M., Gorman, S. J., Zhong, Q., Davidson, P. M., & Critzer, F. J. (2019). Essential Oil Emulsions as Postharvest Sanitizers To Mitigate Salmonella Cross-Contamination on Peppers. *Journal of Food Protection, 82*(1), 159-163. doi:10.4315/0362-028X.JFP-18-190

DuPont, H. L. (2007). The Growing Threat of Foodborne Bacterial Enteropathogens of Animal Origin. *Clinical Infectious Diseases, 45*(10), 1353. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.4485708&site=eds-live

Ells, T. C., & Truelstrup Hansen, L. (2006). Strain and growth temperature influence Listeria spp. attachment to intact and cut cabbage. *International Journal of Food Microbiology, 111*(1), 34-42. doi:10.1016/j.ijfoodmicro.2006.04.033

European Food Safety Authority. (2013). Conclusion on the peer review of the pesticide risk assessment of the active substance Fatty acids C7 to C18 (approved under Regulation (EC) No 1107/2009 as Fatty acids C7 to C20). *EFSA Journal, 11*(1). doi:10.2903/j.efsa.2013.3023

The FDA: fresh leafy greens grown in the United States are safe. (2006). *FDA Consumer, 40*(6), 11-11. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=mnh&AN=17333559&site=eds-live

Finten, G., Garrido, J. I., Agüero, M. V., & Jagus, R. J. (2017). Irradiated ready-to-eat spinach leaves: How information influences awareness towards irradiation treatment and consumer's purchase intention. *Radiation Physics & Chemistry, 130*, 247-251. doi:10.1016/j.radphyschem.2016.09.004

Flavor and Extract Manufacturers Association. (2018). Gras Reference 2784 Nonanoic Acid. Retrieved from https://www.femaflavor.org/flavor-library/nonanoic-acid. https://www.femaflavor.org/flavor-library/nonanoic-acid

Franz, E., & van Bruggen, A. H. C. (2008). Ecology of E. coli O157:H7 and Salmonella enterica in the primary vegetable production chain. *Critical reviews in microbiology, 34*(3-4), 143-161. doi:10.1080/10408410802357432

Fukuda, M., Tsujino, Y., Fujimori, T., Wakabayashi, K., & Böger, P. (2004). Phytotoxic activity of middle-chain fatty acids I: effects on cell constituents. *Pesticide Biochemistry and Physiology, 80*(3), 143-150. doi:10.1016/j.pestbp.2004.06.011

Fukuzaki, S. (2006). Mechanisms of Actions of Sodium Hypochlorite in Cleaning and Disinfection Processes*(4),* 147. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN202673556&site=eds-live

Gomez-Lopez, V. M., Lannoo, A., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of Escherichia coli 0157:H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. In (Vol. 42, pp. 132-138).

Han, S., & Micallef, S. A. (2014). Salmonella Newport and Typhimurium Colonization of Fruit Differs from Leaves in Various Tomato Cultivars. *Journal of Food Protection, 77*(11), 1844-1850. doi:10.4315/0362-028X.JFP-13-562

Hedberg, C. W., MacDonald, K. L., & Osterholm, M. T. (1994). Changing Epidemiology of Food-Borne Disease: A Minnesota Perspective. *Clinical Infectious Diseases, 18*(5), 671. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsjsr&AN=edsjsr.4457788&site=eds-live

Hilborn, E. D., Mshar, P. A., Fiorentino, T. R., Dembek, Z. F., Barrett, T. J., Howard, R. T., & Cartter, M. L. (2000). An Outbreak of Escherichia coli O157:H7 Infections and Haemolytic Uraemic Syndrome Associated with Consumption of Unpasteurized Apple Cider. *Epidemiology and Infection, 124*(1), 31-36. Retrieved from www.jstor.org/stable/3865015

Huang, R., de Vries, D., & Chen, H. (2018). Strategies to enhance fresh produce decontamination using combined treatments of ultraviolet, washing and disinfectants. In (Vol. 283, pp. 37-44).

Hyun, J., Bae, Y., Song, H., Yoon, J., & Lee, S. (2015). Antibacterial Effect of Various Essential Oils against Pathogens and Spoilage Microorganisms in Fresh Produce. *Journal of Food Safety, 35*(2), 206-219. doi:10.1111/jfs.12162

Jepson, C., & McAndrew, T. (2018). *Peracetic Acid Disinfection - Demonstration of PAA as Sole Wastewater Disinfectant at the Berkeley Heights WPCP.* Paper presented at the Association of Environmental Authorities Spring Conference, Caesars's, Atlantic City, NJ.

Jordan, K. N., Oxford, L., & O'Byrne, C. P. (1999). Survival of low-pH stress by Escherichia coli O157:H7: correlation between alterations in the cell envelope and increased acid tolerance. *Applied And Environmental Microbiology, 65*(7), 3048-3055. Retrieved from https://pubmed.ncbi.nlm.nih.gov/10388702

Kang, J., & Song, K. B. (2018). Inhibitory effect of plant essential oil nanoemulsions against Listeria monocytogenes, Escherichia coli O157:H7, and Salmonella Typhimurium on red mustard leaves. *Innovative Food Science and Emerging Technologies, 45*, 447-454. doi:10.1016/j.ifset.2017.09.019

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: a review. *Environment International, 30*(1), 47. doi:10.1016/S0160-4120(03)00147-8

Kumar, G. D., Libin, Z. H. U., Siemens, M. C., Nolte, K., Brassill, N., Rios, D. V., . . . Ravishankar, S. (2019). Modified Coring Tool Designs Reduce Iceberg Lettuce Cross-Contamination. *Journal of Food Protection, 82*(3), 454-462. doi:10.4315/0362-028X.JFP-18-317

Kumar, G. D., Macarisin, D., & Micallef, S. A. (2019). Salmonella enterica Filamentation Induced by Pelargonic Acid Is a Transient Morphotype. In (Vol. 85).

Kumar, G. D., Patel, J., & Ravishankar, S. (2020). Contamination of spinach at germination: A route to persistence and environmental reintroduction by Salmonella. *International Journal of Food Microbiology, 326*, 108646. doi:https://doi.org/10.1016/j.ijfoodmicro.2020.108646

Kumar, G. D., Ravi, S., Micallef, S. A., Brown, E. W., & Macarisin, D. (2018). Aeolian contamination of fruits by enteric pathogens: an unexplored paradigm. *Current Opinion in Food Science, 19*, 138-144. doi:https://doi.org/10.1016/j.cofs.2017.12.003

Kumar, G. D., Williams, R. C., Al Qublan, H. M., Sriranganathan, N., Boyer, R. R., & Eifert, J. D. (2017). Airborne soil particulates as vehicles for Salmonella contamination of tomatoes. *International Journal of Food Microbiology, 243*, 90-95. doi:https://doi.org/10.1016/j.ijfoodmicro.2016.12.006

Lapidot, A., Romling, U., & Yaron, S. (2006). Biofilm formation and the survival of Salmonella Typhimurium on parsley. *International Journal of Food Microbiology, 109*(3), 229-233. doi:https://doi.org/10.1016/j.ijfoodmicro.2006.01.012

Lederer, B., Fujimori, T., Tsujino, Y., Wakabayashi, K., & Böger, P. (2004). Phytotoxic activity of middle-chain fatty acids II: peroxidation and membrane effects. *Pesticide Biochemistry and Physiology, 80*(3), 151-156. doi:10.1016/j.pestbp.2004.06.010

Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid adaptation of Escherichia coli O157:H7 increases survival in acidic foods. *Appl Environ Microbiol, 61*(10), 3752-3755.

Lin, J., Smith, M. P., Chapin, K. C., Baik, H. S., Bennett, G. N., & Foster, J. W. (1996). Mechanisms of acid resistance in enterohemorrhagic Escherichia coli. *Appl Environ Microbiol, 62*(9), 3094-3100.

Lukasik, J., Bradley, M. L., Scott, T. M., Dea, M., Koo, A., Hsu, W. Y., . . . Farrah, S. R. (2003). Reduction of Poliovirus 1, Bacteriophages, Salmonella Montevideo, and Escherichia coli O157:H7 on Strawberries by Physical and Disinfectant Washes*(2),* 188. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN127174787&site=eds-live

Luo, Y., He, Q., & McEvoy, J. L. (2010). Effect of Storage Temperature and Duration on the Behavior of Escherichia coli O157:H7 on Packaged Fresh-Cut Salad Containing Romaine and Iceberg Lettuce. *Journal of Food Science, 75*(7), M390-M397. doi:10.1111/j.1750-3841.2010.01722.x

Luo, Y., Nou, X., Yang, Y., Alegre, I., Turner, E., Feng, H., . . . Conway, W. (2011). Determination of Free Chlorine Concentrations Needed To Prevent Escherichia coli O157:H7 Cross-Contamination during Fresh-Cut Produce Wash*(3),* 352. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN287300360&site=eds-live

Manning, L., & Monaghan, J. (2019). Integrity in the fresh produce supply chain: solutions and approaches to an emerging issue. In (Vol. 94, pp. 413-421).

Micallef, S. A., & Kumar, G. D. (2017). Susceptibility of Salmonella enterica isolates from tomato farm environments to fatty acids naturally found on tomato fruit. *Foodborne Pathogens and Disease*(5), 293. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsgao&AN=edsgcl.501996023&site=eds-live

Morgan, D., Newman, C. P., Hutchinson, D. N., Walker, A. M., Rowe, B., & Majid, F. (1993). Verotoxin Producing Escherichia coli O 157 Infections Associated with the Consumption of Yoghurt. *Epidemiology and Infection, 111*(2), 181-187. Retrieved from www.jstor.org/stable/3863789

National Institute of Diabetes and Digestive and Kidney Diseases. (2015). Hemolytic Uremic Syndrome in Children. Retrieved from https://www.niddk.nih.gov/health-information/kidney-disease/children/hemolytic-uremic-syndrome

Nichols, D., Embree, C., Cline, J., & Ju, H. Y. (2004). Blossom and fruitlet thinners affect crop load, fruit weight, seed number, and return bloom of 'northern spy' apple. In (Vol. 39, pp. 1309-1312).

Niemira, B. A. (2007). Relative Efficacy of Sodium Hypochlorite Wash Versus Irradiation To Inactivate Escherichia coli O157:H7 Internalized in Leaves of Romaine Lettuce and Baby Spinach†. *Journal of Food Protection, 70*(11), 2526-2532. doi:10.4315/0362-028x-70.11.2526

Niemira, B. A., Sommers, C. H., & Fan, X. (2002). Suspending Lettuce Type Influences Recoverability and Radiation Sensitivity of Escherichia coli O157:H7†. *Journal of Food Protection, 65*(9), 1388-1393. doi:10.4315/0362-028x-65.9.1388

O'Ryan, M., Prado, V., Aragon, T., Fernyak, S., Reiter, R., Wong, C. S., . . . Tarr, P. I. (2000). Risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. *The New England Journal of Medicine*(17), 1271. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsgih&AN=edsgcl.66971990&site=eds-live

Oh, S. W., Dancer, G. I., & Kang, D. H. (2005). Efficacy of Aerosolized Peroxyacetic Acid as a Sanitizer of Lettuce Leaves*(8),* 1743. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN173198976&site=eds-live

Ölmez, H., & Temur, S. D. (2010). Effects of different sanitizing treatments on biofilms and attachment of Escherichia coli and Listeria monocytogenes on green leaf lettuce. *LWT - Food Science and Technology, 43*, 964-970. doi:10.1016/j.lwt.2010.02.005

Panigrahy, C., Seal, A., & Mahato, N. K. (2020). Fractal dimension of synthesized and natural color images in Lab space. *Pattern Analysis & Applications, 23*(2), 819-836. doi:10.1007/s10044-019-00839-7

Patel, J., & Sharma, M. (2010). Differences in attachment of Salmonella enterica serovars to cabbage and lettuce leaves. *International Journal of Food Microbiology, 139*(1), 41-47. doi:10.1016/j.ijfoodmicro.2010.02.005

Penn State Extension. (2019). Best Practices for Fresh Produce Food Safety. In.

Pizzi, N. G. (2005). *Water treatment operator handbook*. United States: American Water Works Association.

Pohl, C. H., Kock, J. L. F., & Thibane, V. S. (2011). Antifungal free fatty acids: a review. *Science against microbial pathogens: communicating current research and technological advances, 3*, 61-71.

PubChem. (2020a). Calcium hypochlorite. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/24504. https://pubchem.ncbi.nlm.nih.gov/compound/24504

PubChem. (2020b). Nonanoic acid. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/nonanoic_acid#section=Top

PubChem. (2020c). Sodium hypochlorite. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/23665760. https://pubchem.ncbi.nlm.nih.gov/compound/23665760

Rana, S., Parisi, B., Reineke, K., Stewart, D., Schlesser, J., Tortorello, M., & Fu, T. (2010). *Factors affecting Salmonella cross-contamination during postharvest washing of tomatoes.* Illinois Institute of Technology,

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 82*(4), 632-639. doi:10.1093/ps/82.4.632

Rodgers, S. L., Cash, J. N., Siddiq, M., & Ryser, E. T. (2004). A comparison of different chemical sanitizers for inactivating Escherichia coli O157 : H7 and Listeria monocytogenes in solution and on apples, lettuce, strawberries, and cantaloupe. In (Vol. 67, pp. 721-731).

RStudio: Integrated Development for R. (2016). RStudio Team [Mobile application software]. Retrieved from http://www.rstudio.com

Safdar, N., Said, A., Gangnon, R. E., Maki, D. G., Safdar, N., Said, A., . . . Maki, D. G. (2002). Risk of hemolytic uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 enteritis: a meta-analysis. *JAMA: Journal of the American Medical Association, 288*(8), 996-1001. doi:10.1001/jama.288.8.996

Sahin, N., Kula, I., & Erdogan, Y. (2006). Investigation of antimicrobial activities of nonanoic acid derivatives. *Fresenius Environmental Bulletin, 15*(2), 141-143.

Sánchez, G., Elizaquível, P., Aznar, R., & Selma, M. V. (2015). Virucidal effect of high power ultrasound combined with a chemical sanitizer containing peroxyacetic acid for water reconditioning in the fresh-cut industry. *Food Control, 52*, 126-131. doi:https://doi.org/10.1016/j.foodcont.2014.12.021

Sapers, G. M., & Doyle, M. P. (2014). Chapter 1 - Scope of the Produce Contamination Problem. In K. R. Matthews, G. M. Sapers, & C. P. Gerba (Eds.), *The Produce Contamination Problem (Second Edition)* (pp. 3-20). San Diego: Academic Press.

Sapers, G. M., Miller, R. L., & Mattrazzo, A. M. (1999). Effectiveness of Sanitizing Agents in Inactivating Escherichia coli in Golden Delicious Apples. *Journal of Food Science, 64*(4), 734-737. doi:10.1111/j.1365-2621.1999.tb15121.x

JMP by SAS. (2018). SAS [Mobile application software]. Retrieved from http://www.jmp.com

Shen, C., Luo, Y., Nou, X., Wang, Q., & Millner, P. (2013). Dynamic Effects of Free Chlorine Concentration, Organic Load, and Exposure Time on the Inactivation of Salmonella, Escherichia coli O157:H7, and Non-O157 Shiga Toxin-Producing E. coli. *Journal of Food Protection, 76*(3), 386-393. doi:10.4315/0362-028X.JFP-12-320

Shirron, N., Kisluk, G., Zelikovich, Y., Eivin, I., Shimoni, E., & Yaron, S. (2009). A Comparative Study Assaying Commonly Used Sanitizers for Antimicrobial Activity against Indicator Bacteria and a Salmonella Typhimurium Strain on Fresh Produce*(11),* 2413. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN260660911&site=eds-live

Sofos, J. N., Beuchat, L. R., Davidson, P. M., & Johnson, E. A. (1998). Naturally occurring antimicrobials in food. *Regulatory toxicology and pharmacology : RTP, 28*(2), 71-72. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=9927555&site=eds-live

Taniwaki, M., & Sakurai, N. (2008). Texture measurement of cabbages using an acoustical vibration method. *Postharvest Biology and Technology, 50*(2), 176-181. doi:10.1016/j.postharvbio.2008.05.010

Tarr, P. I., Gordon, C. A., & Chandler, W. L. (2005). Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *The Lancet, 365*(9464), 1073-1086. doi:https://doi.org/10.1016/S0140-6736(05)71144-2

Teng, Z., Luo, Y., Alborzi, S., Zhou, B., Chen, L., Zhang, J., . . . Wang, Q. (2018). Investigation on chlorine-based sanitization under stabilized conditions in the presence of organic load. *International Journal of Food Microbiology, 266*, 150-157. doi:https://doi.org/10.1016/j.ijfoodmicro.2017.11.027

U. S. Environmental Protection Agency. (2000). *Pelargonic Acid (217500) Fact Sheet* Office of Pesticide Programs

U.S. Department of Agriculture - Economic Research Service. (2019). *Food Availibility (Per Capita) Data System*. Retrieved from: https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/

U.S. Department of Agriculture. (2011). Guidance The Use of Chlorine Materials in Organic Production and Handling Retrieved from file:///C:/Users/samci/OneDrive/Desktop/USDA%202011.pdf

U.S. Department of Agriculture. (2015). Technical Evaluation Report - Soap Based Algicide/Demossers.

U.S. Environmental Protection Agency. (2000). *Peroxyacetic Acid; Exemption From the Requirement of a Tolerance*.

U.S. Environmental Protection Agency. (2008). *Ammonium Nonanoate*.

U.S. Food and Drug Administration. (1998). Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. Retrieved from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-fruits-and-vegetables#x

U.S. Food and Drug Administration. (2006). Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain. In.

U.S. Food and Drug Administration. (2008). *Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables*. College Park, MD 20740

U.S. Food and Drug Administration. (2016). Food Irradiation: What You Need to Know. Retrieved from https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know

U.S. Food and Drug Administration. (2018). FDA Food Safety Modernization Act (FSMA). Retrieved from https://www.fda.gov/Food/GuidanceRegulation/FSMA/default.htm

U.S. Food and Drug Administration. (2019). Food Additive Status List. Retrieved from https://www.fda.gov/food/food-additives-petitions/food-additive-status-list

U.S. Food and Drug Administration. (2020). Substances Added to Food: Nonanoic Acid. Retrieved from https://www.accessdata.fda.gov/scripts/fdcc/?set=FoodSubstances&id=NONANOICACID&sort=Sortterm&order=ASC&startrow=1&type=basic&search=pelar. https://www.accessdata.fda.gov/scripts/fdcc/?set=FoodSubstances&id=NONANOICACID&sort=Sortterm&order=ASC&startrow=1&type=basic&search=pelar

Vidovic, S., Block, H. C., & Korber, D. R. (2007). Effect of soil composition, temperature, indigenous microflora, and environmental conditions on the survival of Escherichia coli O157:H7. *Canadian Journal of Microbiology, 53*(7), 822-829. doi:10.1139/W07-041

Vogler, H., Burri, J. T., Nelson, B. J., & Grossniklaus, U. (2020). Simultaneous measurement of turgor pressure and cell wall elasticity in growing pollen tubes. In *Methods in Cell Biology*: Academic Press.

Wahlberg, J. E., & Lindberg, M. (2003). Nonanoic acid - an experimental irritant. In (Vol. 49, pp. 117-123).

Warburton, R. (2014). Peracetic Acid in the Fresh Food Industry. *Food Safety Magazine*.

Weagant, S. D., Bryant, J. L., & Bark, D. H. (1994). Survival of Escherichia coli O157:H7 in Mayonnaise and Mayonnaise-Based Sauces at Room and Refrigerated Temperatures. *Journal of Food Protection, 57*(7), 629-631. doi:10.4315/0362-028x-57.7.629

White, E. (2020). *Pelargonic Acid Emulsion as a Postharvest Sanitizer to Reduce Salmonella Contamination on Tomatoes.* (Masters of Science). University of Georgia, Athens, GA.

Wisniewsky, M. A., Glatz, B. A., Gleason, M. L., & Reitmeier, C. A. (2000). Reduction of Escherichia coli O157 : H7 counts on whole fresh apples by treatment with sanitizers. In (Vol. 63, pp. 703-708).

Wong, C. S., Jelacic, S., Habeeb, R. L., Watkins, S. L., & Tarr, P. I. (2000). The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. *New England Journal of Medicine, 342*(26), 1930-1936. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=lhh&AN=20002014465&site=eds-live

World Health Organization, & Food and Agriculture Organization of the United Nations. (2008). Microbiological hazards in fresh leafy vegetables and herbs : meeting report. In. Geneva: World Health Organization.

Yang, S., Lin, C., Aljuffali, I. A., & Fang, J. (2017). Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. *Archives of Microbiology, 199*(6), 811-825.

Yaron, S. (2014). Chapter 2 - Microbial Attachment and Persistence on Plants. In K. R. Matthews, G. M. Sapers, & C. P. Gerba (Eds.), *The Produce Contamination Problem (Second Edition)* (pp. 21-57). San Diego: Academic Press.

Yuk, H. G., Bartz, J. A., & Schneider, K. R. (2005). Effectiveness of Individual or Combined Sanitizer Treatments for Inactivating Salmonella spp. on Smooth Surface, Stem Scar, and Wounds of Tomatoes*(9),* M409. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN181621320&site=eds-live

Yuk, H. G., Bartz, J. A., & Schneider, K. R. (2006). The effectiveness of sanitizer treatments in inactivation of Salmonella spp. from bell pepper, cucumber, and strawberry. In (Vol. 71, pp. M95-M99).

Zhang, D., & Zhang, B. (2020). Pectin Drives Cell Wall Morphogenesis without Turgor Pressure. *Trends in Plant Science, 25*(8), 719-722. doi:https://doi.org/10.1016/j.tplants.2020.05.007

Zhang, S., & Farher, J. M. (1996). The effects of various disinfectants against Listeria monocytogenes on fresh-cut vegetables*(4),* 311. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edsbl&AN=RN012415916&site=eds-live

Zhang, Y., Ma, Q., Critzer, F., Davidson, P. M., & Zhong, Q. (2016). Organic thyme oil emulsion as an alternative washing solution to enhance the microbial safety of organic cantaloupes. *Food Control, 67*, 31-38. doi:10.1016/j.foodcont.2016.02.032

Zhu, L., Olsen, C., McHugh, T., Friedman, M., Jaroni, D., & Ravishankar, S. (2014). Apple, Carrot, and Hibiscus Edible Films Containing the Plant Antimicrobials Carvacrol and Cinnamaldehyde Inactivate Salmonella Newport on Organic Leafy Greens in Sealed Plastic Bags. *Journal of Food Science (John Wiley & Sons, Inc.), 79*(1), M61-M66. doi:10.1111/1750-3841.12318

Zhuang, R. Y., Beuchat, L. R., & Angulo, F. J. (1995). Fate of Salmonella montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied And Environmental Microbiology, 61*(6), 2127-2131. Retrieved from http://proxy-remote.galib.uga.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=7793934&site=eds-live

Table 1: Effect of the interaction of sanitizer and storage duration, average of DI water and no rinse per storage duration, and orthogonal contrasts for inoculated samples on TSARN.

	Storage duration		
	0 h	24 h	7 d
Sanitizer		log CFU/g	
200 ppm Chlorine	5.6 a[†] A[‡]	5.0 a B	4.4 b C
80 ppm PAA	5.5 a A	5.1 a AB	4.9 a B
Pel 7.5	5.6 a A	4.7 a B	3.1 c C
Pel 30	5.0 b A	3.1 b B	1.1 d C
Pel 50	4.2 c A	3.1 b B	1.1 d C
No treatment			
DI Water	6.93	6.96	6.64
No rinse	7.39	7.36	7.21
Contrasts			
Sanitizer vs DI Water			
200 ppm Chlorine x DI Water	**	**	**
80 ppm PAA x DI Water	**	**	**
Pel 7.5 x DI Water	**	**	***
Pel 30 x DI Water	***	***	***
Pel 50 x DI Water	***	***	***
Sanitizer vs No rinse			
200 ppm Chlorine x No rinse	**	**	**
80 ppm PAA x No rinse	**	**	**
Pel 7.5 x No rinse	**	**	***
Pel 30 x No rinse	***	***	***
Pel 50 x No rinse	***	***	***
No treatment			
DI Water x No rinse	ns	ns	ns

[†] Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among sanitizer treatments (rows) within storage duration (columns).

[‡] Values followed by similar uppercase letter indicate no significant differences ($p \leq 0.05$) among storage duration (columns) within sanitizer treatments (rows).

ns, *, **, *** nonsignificant, or significant at $p \leq 0.05$, 0.01, 0.001, respectively.

Table 2: Effect of the interaction of sanitizer and storage duration, average of water and no rinse per storage duration, and orthogonal contrasts for inoculated samples on TSA.

	Storage duration		
	0 h	24 h	7 d
Sanitizer		log CFU/g	
200 ppm Chlorine	5.63 a[†] A[‡]	4.92 a B	4.37 c B
80 ppm PAA	5.57 a A	5.21 a A	5.24 ab A
Pel7.5	5.68 a A	4.72 a B	4.91 bc B
Pel30	4.90 b B	3.75 b C	5.83 a A
Pel50	4.25 b B	3.77 b B	5.30 ab A
No treatment			
DI Water	6.99	6.92	6.93
No rinse	7.39	7.34	7.20
Contrasts			
Sanitizer vs Water			
200 ppm Chlorine x DI Water	**	**	***
80 ppm PAA x DI Water	*	*	***
Pel 7.5 x DI Water	***	***	***
Pel 30 x DI Water	***	***	**
Pel 50 x DI Water	***	***	**
Sanitizer vs No rinse			
200 ppm Chlorine x No rinse	**	**	***
80 ppm PAA x No rinse	**	**	***
Pel 7.5 x No rinse	***	***	**
Pel 30 x No rinse	***	***	**
Pel 50 x No rinse	***	***	**
No treatment			
DI Water x No rinse	ns	ns	ns

[†] Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among sanitizer treatments (rows) within storage duration (columns).
[‡] Values followed by similar uppercase letter indicate no significant differences ($p \leq 0.05$) among storage duration (columns) within sanitizer treatments (rows).
ns, *, **, *** nonsignificant, or significant at $p \leq 0.05$, 0.01, 0.001, respectively.

Table 3: Effect of the interaction of sanitizer and storage duration, average of water per storage duration, and orthogonal contrasts for SUS on TSARN.

	Storage duration		
	0 h	24 h	7 d
Sanitizer	log CFU/g		
200 ppm Chlorine	1.25 ab† A‡	1.27 ab A	1.06 a B
80 ppm PAA	1.01 b A	1.00 b A	1.03 a A
Pel 7.5	1.51 a A	1.54 a A	1.00 a B
Pel 30	1.00 b A	1.20 b A	1.02 a A
Pel 50	1.00 b A	1.00 b A	1.06 a A
No treatment			
DI Water	4.97	4.93	4.56
Contrasts			
Sanitizer vs Water			
200 ppm Chlorine x DI Water	***	***	***
80 ppm PAA x DI Water	***	***	***
Pel 7.5 x DI Water	***	***	***
Pel 30 x DI Water	***	***	***
Pel 50 x DI Water	***	***	***

† Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among sanitizer treatments (rows) within storage duration (columns).

‡ Values followed by similar uppercase letter indicate no significant differences ($p \leq 0.05$) among storage duration (columns) within sanitizer treatments (rows).

ns, *, **, *** nonsignificant, or significant at $p \leq 0.05$, 0.01, 0.001, respectively.

Table 4: Effect of the interaction of sanitizer and storage duration, average of water per storage duration, and orthogonal contrasts for SUS on TSA.

	Storage duration		
	0 h	24 h	7 d
Sanitizer		log CFU/g	
200 ppm Chlorine	2.61 ab† AB‡	2.94 b A	2.42 c B
80 ppm PAA	2.29 b B	3.50 ab AB	4.01 b A
Pel 7.5	3.05 a B	3.24 ab B	4.44 b A
Pel 30	1.53 c C	3.62 a B	5.66 a A
Pel 50	1.10 c C	3.31 ab B	5.25 a A
No treatment			
DI Water	4.98	5.13	5.7
Contrasts			
Sanitizer vs Water			
200 ppm Chlorine x DI Water	***	***	***
80 ppm PAA x DI Water	***	***	*
Pel 7.5 x DI Water	***	***	*
Pel 30 x DI Water	***	***	ns
Pel 50 x DI Water	***	***	ns

† Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among sanitizer treatments (rows) within storage duration (columns).

‡ Values followed by similar uppercase letter indicate no significant differences ($p \leq 0.05$) among storage duration (columns) within sanitizer treatments (rows).

ns, *, **, *** nonsignificant, or significant at $p \leq 0.05$, 0.01, 0.001, respectively.

Table 5: Effect of the interaction of sanitizer treatments plus water and no rinse and storage duration on L* and a*.

Treatment	Storage duration 0 h		24 h		7 d	
			L*			
200 ppm Chlorine	46.5	bc† A‡	46.1	ab A	47.0	a A
80 ppm PAA	50.1	b A	44.7	b B	47.7	a AB
Pel 7.5	46.3	bc A	36.9	c B	37.5	b B
Pel 30	44.8	c A	36.9	c B	36.9	b B
Pel 50	44.4	c A	35.2	c B	35.9	b B
DI Water	49.2	bc A	48.99	ab A	50.3	a A
No Rinse	55.5	a A	50.0	a B	50.9	a B
			a*			
200 ppm Chlorine	-8.22	b† B‡	-6.71	c A	-6.49	b A
80 ppm PAA	-7.83	ab A	-6.85	c A	-6.83	b A
Pel 7.5	-7.90	ab C	1.22	b B	2.47	a A
Pel 30	-6.87	a B	2.54	ab A	2.66	a A
Pel 50	-6.66	a B	2.84	a A	2.42	a A
DI Water	-7.65	ab A	-6.77	c A	-6.86	b A
No Rinse	-6.97	ab A	-6.35	c A	-6.33	b A

† Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among treatments (rows) within storage duration (columns).

‡ Values followed by similar uppercase letter indicate no significant differences ($p \leq 0.05$) among storage duration (columns) within treatments (rows).

Table 6: Main effect of treatment and storage duration on b*.

Effect	b*	
Treatment		
200 ppm Chlorine	14.9	ab†
80 ppm PAA	16.2	a
Pel 7.5	12.7	b
Pel 30	14.3	ab
Pel 50	14.6	ab
DI Water	15.5	a
No Rinse	15.1	ab
Storage duration		
0 h	16.9	a
24 h	13.8	b
7 d	13.5	b

† Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among treatments.

Table 7: Main effect of treatment and storage duration on skin elasticity (mm).

Effect	Skin elasticity (mm)	
Treatment		
200 ppm Chlorine	6.9	d[†]
80 ppm PAA	7.9	bcd
Pel 7.5	10.0	ab
Pel 30	10.8	a
Pel 50	9.7	abc
DI Water	7.5	cd
No Rinse	9.6	abc
Storage duration		
0 h	10.2	a
24 h	9.0	a
7 d	7.6	b

[†] Values followed by similar lowercase letter indicate no significant differences ($p \leq 0.05$) among treatments.

Figure 1: Romaine lettuce samples post-treatment of a) 0 h no rinse, b) 0 h DI water, c) 0 h 200 ppm chlorine, d) 0 h 80 ppm PAA, e) 0 h 7.5 mM pelargonic acid, f) 0 h 30 mM pelargonic acid, g) 0 h 50 mM pelargonic acid, h) 24 h no rinse, i) 24 h DI water, j) 24 h 200 ppm chlorine, k) 24 h 80 ppm PAA, l) 24 h 7.5 mM pelargonic acid, m) 24 h 30 mM pelargonic acid, n) 24 h 50 mM pelargonic acid, o) 7 d no rinse, p) 7 d DI water, q) 7 d 200 ppm chlorine, r) 80 ppm PAA, s) 7.5 mM pelargonic acid, t) 30 mM pelargonic acid, u) 50 mM pelargonic acid