EVALUATING THE REUSABILITY OF ORGANIC WASH TREATMENTS IN REDUCING *ESCHERICHIA COLI* O157:H7 ON ORGANIC LEAFY GREENS

By

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EVALUATING THE REUSABILITY OF ORGANIC WASH TREATMENTS IN REDUCING *ESCHERICHIA COLI* O157:H7 ON ORGANIC LEAFY GREENS

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Sincerely,
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Abstract:

In recent years, organic leafy-greens have been associated with *Escherichia coli* O157:H7 related outbreaks. Approved antimicrobials for organic produce are limited, resulting in investigations into plant-derived alternatives. Oregano and cinnamon essential oil (EO) and their primary constituents, carvacrol and cinnamaldehyde, respectively, have proven to be effective against *E. coli* O157:H7. Flume-tank washing of organic greens, prior to packaging, is common practice where wash water is re-used multiple times before being discarded. It is therefore important to evaluate the re-usability of antimicrobials during flume-tank wash**.** The purpose of this study was to evaluate the re-usability of essential oils and their primary constituents for flume-tank washing of organic leafy-greens to reduce *E. coli* O157:H7. Oregano and cinnamon EO and carvacrol and cinnamaldehyde were tested at 0.5% concentration. Additionally a Fulvic Acid III formulation was tested at 3% concentration. Hydrogen peroxide, water and phosphate buffered saline were used as controls. Organic leafy greens, baby and mature spinach and romaine and iceberg lettuce, were inoculated with *E. coli* O157:H7 (10^6 CFU/g). Each antimicrobial was re-used five times to wash (for 1 min) five separate batches of inoculated leafy greens that were stored at 4°C and surviving bacteria enumerated on days 0, 1, and 3. Wash water was enumerated for *E. coli* O157:H7 after each use and pH and turbidity measured. Tested antimicrobials showed significant ($P < 0.05$) reduction of *E. coli* O157:H7 over five washes. Carvacrol and oregano EO were the most effective, reducing pathogen populations to undetectable levels on day 0 in all leafy greens except mature spinach where undetectable levels were achieved on day 3 with carvacrol. Cinnamon EO and cinnamaldehyde were able to reduce pathogen populations to undetectable levels in all leafy greens by day 1. Wash water resulting from the antimicrobial washes did not show any growth of *E.*coli O157:H7.This study provides evidence that plant-derived compounds could serve as effective sanitizers that retain their antimicrobial activity with continued use.

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CHAPTER I

INTRODUCTION

In recent years, minimally processed leafy-greens have been associated with *Escherichia coli* O157:H7 related outbreaks. Each year in the United States (U.S.), it is estimated that 48 million Americans will become ill as a result of foodborne illness. Of these 48 million, there are an expected 128,000 hospitalizations and 3,000 deaths. This organism is ranked among the top five-foodborne pathogens causing illness that result in hospitalization. (Centers for Disease Control and Prevention, CDC; 2012; Scallen et al., 2011). The CDC reports that between the years 2003-2012 there were 255 foodborne disease outbreaks involving *E. coli* O157:H7, of which, 29.21% were associated with leafy greens (CDC, 2012). Additionally, in the last two decades Americans are including more fresh produce in their diet. People are generally becoming more concerned with their physical health, the state of the environment, as well as perception of novelty. This concern has been coupled with an increased interest in organic fresh fruits and vegetables (Tregear et al., 1994; Pollack, S.L. 2001). In the year 2000, the U.S. organic food industry reported that more organic produce had been purchased from supermarkets than from anywhere else (Dimitri and Greene, 2002). This increased interest has been influenced by several factors including product convenience, technological advances allowing improved quality of

produce and longer shelf life, as well as widespread availability of the products (Pollack, S.L. 2001) In a final attempt for producers to eliminate pathogen contamination, fresh fruits and vegetables undergo a washing step prior to packaging. Antimicrobials are routinely used in the wash water before packaging. However, even with these preventative measures in place outbreaks continue to occur. Organic producers of fresh fruits and vegetables must follow the guidelines laid out by the USDA National Organic Program (USDA-NOP). These producers are encouraged to utilize Good Agricultural Practices (GAP) to reduce the risk of any contamination that may occur. These practices include emphasis on the use of naturally derived fertilizers, insecticides, and pesticides. Composted animal manure is frequently used in organic farming and could be an attributing factor to the contamination of produce as a result of fecal runoff (Jay et al., 2007) The NOP prohibits the use of synthetic fertilizers, insecticides, pesticides and produce wash antimicrobials to prevent pathogen contamination. Some of the approved sanitizers for organic produce include ozone, peracetic acid, and hydrogen peroxide (USDA, 2011). Due to a limited number of approved treatments for organic produce, there is a need to investigate alternatives. Plant-derived compounds have been historically utilized for their flavor, aroma, bactericidal, and preservative properties. Recent studies have demonstrated the antimicrobial effects of essential oils and plant extracts against *Salmonella enterica* and *E. coli* O157:H7 on lettuce and organic leafy greens (Moore-Neibel et al., 2012; Moore et al. 2011). However, few studies have investigated the reusability of these antimicrobials for washing organic leafy greens. Flume-tank washing of organic greens, prior to packaging, is a common practice where wash water is re-used

multiple times before being discarded. It is therefore important to evaluate the re-usability of antimicrobials during flume-tank washing.

CHAPTER II

REVIEW OF LITERATURE

A. Organic Leafy Greens

1. Organic Produce

Over the course of the last four decades, there has been a growing interest in individuals becoming more environmentally conscious. This trend has resulted in increased production of organically grown products and has grown from a minor concern to an increasingly popular area of concern in the United States (Grant, 2007). In organic as well as conventional produce industry, sanitizers are routinely incorporated in order to eliminate pathogens and prevent cross contamination in the wash water. However, even with these preventative measures in place, foodborne outbreaks and recalls associated with foodborne pathogens continue to occur.

2. *E. coli* O157:H7 outbreaks associated with leafy greens

Minimally processed leafy greens have been associated with several *E. coli* O157:H7 related outbreaks in recent years. The CDC reports that between the years 2003-2012 there were 255 foodborne disease outbreaks involving *E. coli* O157:H7, of which, 29.21% were associated with leafy greens (CDC, 2012a). Additionally, in the last two decades Americans have started including more fresh produce in their diet. People are generally becoming more concerned with their physical health, the state of the environment, as well as perception of novelty. This concern has been coupled with an increased interest in organic fresh fruits and vegetables (Tregear et al., 1994; Pollack, 2001). In the year 2000, the US organic food industry reported that more organic produce had been purchased from supermarkets than from anywhere else (Dimitri and Greene, 2002). This increased interest has been influenced by several factors including product convenience, technological advances allowing improved quality of produce for greater periods, and widespread availability of the products (Pollack, 2001). However, improper washing techniques of produce in the consumer kitchen, and the lack of cooking the produce at high enough temperatures to destroy pathogens have increased the risk of ingestion of dangerous pathogenic organisms, like *E. coli* O157:H7 (Lynch, Tauxe, & Hedberg, 2009). Produce commodities that have commonly been associated with outbreaks include pre-packaged salad, lettuce, juice, melon, and sprouts.

Two mutli-state outbreaks involving *E. coli* O157:H7 occurred in 2006 involving baby spinach and iceberg lettuce. The outbreak involving baby spinach resulted in 205 illnesses and involved 26 states. The Utah and New Mexico health departments investigated a multistate cluster of *E. coli* O157:H7, the outbreak strain was sourced to three bags of a single brand of spinach (Grant et al., 2008). Furthermore, Grant et al.,

(2008) found that washing spinach before consumption did not affect the odds of contracting illness. They believe that the reason for this could be that the pathogen could have internalized to the edible portion of the plant though the root system, or the affinity of pathogens to adhere to the cut surfaces of leafy greens (Warriner, Ibrahim, Dininson, Wright & Waites, 2003; Hassan and Frank, 2003). In the second *E. coli* O157:H7 outbreak involving iceberg lettuce 71 people contracted foodborne illness across five states. Of the 71 infected persons, 53 were hospitalized with eight cases of hemolytic uremic syndrome. The outbreak strain was isolated from iceberg lettuce eaten at the major U.S. restaurant chain Taco Bell (CDC, 2006). Oftentimes, outbreaks cannot be traced back to the original source of contamination. In 2012 an outbreak of *E. coli* O157:H7 involving an organic bagged spinach blend resulted in 33 illnesses. Investigation of the source of the outbreak proposed that the strain could have originated from a single producer in Massachusetts, although, no evidence has been provided to confirm the true source (CDC, 2012b).

3. Processing of organic leafy greens

There are several factors that can contribute to the survival of pathogens in the processing environment. Identification and manipulation of these factors is important in preventing persistence and spread of pathogens. Key factors include temperature, water and nutrient availability, moisture content and oxygen. These factors are important when considering storage temperature, package atmosphere, type of product, and bacterial strain. Interestingly, many species of pathogenic bacteria have mechanisms of resistance to these factors allowing them to survive and thrive (Francics and O'Beirne, 2001).

Temperature control is one of the most important factors in maintaining microbial growth. The use of high and low temperatures to manage microbial populations is a concept widespread in food production. The foodborne pathogens *Listeria monocytogenes* and *E. coli* O157:H7 have been shown to survive at refrigerated temperatures (4 ^{o}C) with *E. coli* O157:H7 decreasing slightly over time (Francis and O'Beirne, 2001). A study conducted by Li, Brackett, Chen, and Beuchat (2001) demonstrated that *E. coli* O157:H7 inoculated iceberg lettuce held in storage conditions of 15 ^{o}C showed significant growth (3-log CFU g^{-1}) of *E. coli* O157:H7 over a period of 18 days. This study demonstrates the role that temperature abuse can play in the growth of microbes.

In a final attempt to eliminate pathogen contamination, fresh fruits and vegetables, undergo a washing step prior to packaging. There are a number of different washing practices that can include multi-rinse dips that can consist of 2 to 3 rinses with either water or with a sanitizer that is included in the wash water. This process is termed flume-tank washing and is accomplished with a belt-driven machine with long and narrow water tanks where fresh produce is ushered through shallow troughs of turbulent water. This process will aid in the removal of any soil, organic matter, or pathogens from harvest, prior to drying and packaging. In organic as well as conventional produce industry, sanitizers are routinely incorporated in order to eliminate pathogens and prevent cross contamination in the wash water. A common compound that is incorporated is hydrogen peroxide. This compound is popular as it is fast acting and has been shown to be effective against a range of foodborne pathogens. The concentration of this compound as far as food grade standards are concerned can range from 1-5%, although 3 % is most

commonly used with a contact time of no more than 2 minutes. The compound is unfortunately quite unstable and therefore storage is important in regards to temperature and sunlight, both of which affect the shelf life of this sanitizer. In this research project, we tested 3% concentration of hydrogen peroxide against *E. coli* O157:H7 on organic leafy greens however, it is noteworthy to mention that this compound was purchased from a local grocery store and we did not conduct any tests to validate the concentration of this compound. Even with a washing step, that incorporates a sanitizer, still foodborne outbreaks and recalls associated with foodborne pathogens continue to occur. A recent example includes the multistate outbreak of shiga-toxin producing *E. coli* O157:H7 infections linked to organic spinach and spring mix blend (CDC, 2012). Organic producers of fresh fruits and vegetables must follow the guidelines laid out by the US Department of Agriculture-National Organic Program (USDA-NOP). All producers are encouraged to follow Good Agricultural Practices (GAP) to reduce the risk of any contamination that may occur. These practices include the use of naturally derived fertilizers, insecticides, sanitizers, antimicrobials or pesticides. The NOP prohibits the use of synthetic antimicrobials to prevent pathogen contamination during the processing of organic fresh produce.

B. *Escherichia coli* O157:H7

1. Classification

*Escherichia co*li O157:H7 is a Gram-negative rod and a pathogenic strain of the bacterium, *E. coli*. The O157:H7 strain is named as such because of its expression of the 157th somatic (O) antigen, and the 7th flagellar (H) antigen (Mead and Griffin, 1998).

The bacteria *E. coli* O157:H7 was first implicated in outbreaks in 1982 in Oregon and Michigan, USA, after it was isolated from individuals who developed abdominal cramps and bloody diarrhea as a result of eating hamburgers at a restaurant chain (CDC, 1982). Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and are collectively referred to as diarrheagenic *E. coli*.(CDC, 2012): Enterohemorrhagic *E. coli* (EHEC), Shiga-toxin producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), and Diffusely adherent *E. coli* (DAEC) (CDC, 2012b). *E. coli* O157:H7 has been associated with the EHEC and STEC pathogroups. The EHEC pathogroup is characterized by a variety of symptoms that can include abdominal cramps, non-bloody diarrhea and hemorrhagic colitis which could develop into hemolytic-uremic syndrome (HUS) (Karmali *et al.* 1983). The classification STEC as stated earlier refers to the ability of *E. coli* to produce one of two Shiga toxins (also known as verocytotoxins). The Shiga toxin is primary source of virulence in *E. coli* O157:H7. The primary target of the Stx toxin is the endothelia cell; however, platelets, monocytes, and meningeal cells can also be bound by these toxins (Karmali, 2004).

2. Epidemiology

A study conducted that analyzed 90 confirmed *E. coli* O157:H7 outbreaks that occurred in the UK, Ireland, Denmark, Norway, Finland USA, Canada, and Japan, between the years of 1982 and 2006, (Snedeker et al 2009) found that the source of transmission for food and dairy products accounted for 54.4% of the outbreaks, 12.2% resulted from animal contact, 7.8% from water and 2.2% from environmental sources. The transmission source

of the remaining 28.9% of outbreaks was unknown (Snedeker et al., 2009). Another study that reviewed *E. coli* O157:H7 outbreaks reported to the Centers for Disease Control and Prevention in the United States from the year 1982 to 2002. This particular study reviewed 350 reported outbreaks constituting 8598 cases including 1,493 (17%) hospitalizations 354 (4%)hemolytic uremic syndrome cases, and 40 (0.5%) deaths (Rangel et al., 2005). Additionally this study found that the transmission route of 52% of outbreaks was foodborne. Of the foodborne outbreaks, 41% were associated with ground beef and 21% with produce (Rangel et al., 2005). Although ground beef and produce substantially contribute to *E. coli* O157:H7 transmission many types of food products have been implicated in outbreaks. In the USA in 2009, prepackaged raw cookie dough was strongly associated with a multistate outbreak resulting in 72 cases of *E. coli* O157:H7 infection, ten of those cases developing hemolytic uremic syndrome (CDC, 2009).

3. Pathogenicity

Strains of *E. coli* that produce the shiga-toxin are considered dangerous enteropathogens as they can result in food or waterborne diahhrea. The shiga-toxin producing strain *E. coli* O157:H7 is among the most well known of the STECs in the scientific community as well as the general community. This organism began to gain national attention in 1982 resulting in an outbreak of haemorrhagic colitis affecting 47 people in Oregon and Michigan (Riley *et al*. 1983). Those afflicted reported symptoms including cramping and abdominal pain, watery diarrhea followed by bloody diarrhea and little to no fever (Riley *et al*. 1983).

One of the primary virulence factors of the *E. coli* O157 is its capacity to produce Shiga-toxins. Shiga toxins are classified as either Shiga toxin 1 (Stx1), or Shiga toxin 2 (Stx2). Of the two, it has been reported that Stx2 demonstrates a superior cytotoxic effect on human glomerular endothelial cells compared to Stx1 (Karmali, 2004). The toxin contains two domains, an A and B subunit. The B subunit is a pentamer that binds to specific glycolipids in the host cell. Following binding of the B subunit, the A subunit is internalized into the cell and acts on the ribosome to disrupt protein synthesis (Sandvig, Bergan, Dyve, Skotland, & Torgersen, 2010). Interestingly, these toxins have varying effects between species due to highly specific receptors necessary for entry in the cell. Species such as cattle, deer, and swine can be carriers of these toxigenic bacteria and show no symptoms, shedding them in their feces where they could spread to humans (Asakura et al., 2001).

Like many Gram-negative pathogenic bacteria, *E. coli* O157:H7 harbors lipopolysaccharides (LPS) in its outer membrane. This outer membrane complex known as LPS (also known as lipoglycans and endotoxins) is comprised of three main components. The components are the O antigen, the core oligosaccharide, and lipid A. The O antigen consists of a repeating glycan polymer attached to the core oligosaccharide. Though not all strains possess the O antigen, the composition varies between strains (Raetz and Whitfield, 2002). Presence and absence of the O antigen give the designation of a rough or smooth LPS (Rittig et al., 2003). Full length O-chains indicate a smooth LPS while the absence of those chains renders the LPS rough. A rough LPS is more hydrophobic and as a result, have more penetrable cell membranes to hydrophobic antibiotics (Tsujimoto, Gotoh, & Nishino, 1999). The hydrophobic fatty acid chains of the lipid A region anchor the LPS

into the bacterial membrane. Upon lysis of the cell, fragments of the lipid A domain are released into the circulatory systems and cause fever, diarrhea, and possibly fatal septic shock. (Tidswell et al., 2010)

4. Prevalence and Survival on Farm

Contamination of agriculture products with *E. coli* O157:H7 can occur though a number of reservoirs including manure, soil, and water; along with other vectors like wildlife and insects. While *E. coli* O157:H7 has been isolated from many animal types including sheep, pigs, horses, chickens, and wildlife, the primary reservoir is considered to be cattle (Beutin, Geier, Seinruck, Zimmermann, & Scheutz, 1993; Schoeni and Doyle, 1994; Armstrong, Hollingsworth, & Morris, 1996). Increased contamination from *E. coli* O157:H7 has been linked with intensive agriculture such as the introduction the pathogen from its original reservoir to an unexposed area, such as a produce farm. Contamination can occur either through direct contact with *E. coli* O157:H7, or indirectly by the consumption of contaminated water or food. Cattle have been identified as the primary reservoir of *E. coli* O157. A study by Laegreid, Elder and Keen (1999) found that most bovine animals have been exposed to this organism. Studies show that reported prevalence in herds of cattle range from 10% to 28% with seasonal fluctuation (Karmali, Gannon, & Saregeant, 2010). This organism is disseminated into the environment by multiplying in the gastrointestinal tract and are shed through the feces. Survival of these organisms in the farm environment is dependent on bacterial concentration, temperature, pH, and competing microflora (O'Neill, Bolton, & Fanning, 2011). A study conducted by Mukherjee, Speh, Dyck, and Diez-Gonzalez (2004) evaluated the prevalence of coliforms, *E. coli*, and

Salmonella in organic and conventional produce. Produce types included tomatoes, leafy greens, lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples, and seven other types of produce. Of the produce types tested organic lettuce had the largest prevalence of *E. coli* with 22.4% positive samples. All organic farms tested (32) used either aged or composted animal manure as fertilizer. Percentages of positive samples of *E. coli* for conventional and organic produce were 1.6 and 9.7% respectively. Additionally, samples of manure or compost that was aged for less than 12 months showed levels of *E. coli* that were 19 times greater than farms that aged compost for longer than 12 months (Mukherjee, Speh, Dyck, & Diez-Gonzalez (2004).

5. Contamination of leafy greens in processing

Lynch, Tauxe, and Hedberg (2009) suggested that there are three specific points in the food production chain at which *E. coli* O157:H7 can enter: in the field, during industrial processing, and preparation in the kitchen. Risk factors associated with contamination in the field include the presence of wild animals, irrigation water, inadequately composted manure or fertilizer, and cross contamination from contact with humans (Delaquis, Bach, & Dinu, 2007). Among these risk factors, contaminated irrigation water and animal manure are considered to be the major contamination sources. In the investigation of a nationwide outbreak involving *E. coli* O157:H7 and spinach, the outbreak strain was isolated from feral swine and other environmental samples in close proximity to the produce fields (Jay et al., 2008). A study by Solomon, Yaron, and Matthews (2002) demonstrated that *E. coli* O157:H7 could be transmitted from manure-contaminated soil and irrigation water to lettuce plants. Researcher found that *E. coli*O157:H7 can be internalized and migrate

though the root system to the edible portion of the plant (Solomon, Yaron, & Matthews, 2002).

Another point at which leafy green contamination can occur is during industrial processing. A number of environmental conditions in the processing environment can influence the survival of pathogenic bacteria. Important conditions include nutrient and water availability and temperature. Some steps during the processing of leafy greens can attribute to the contamination of leafy greens. Ice can be used to chill and maintain relative humidity levels in leafy greens during processing. A study by Kim and Harrison (2008) demonstrated that romaine lettuce chilled with ice inoculated with *E. coli* O157:H7 could be transferred onto other produce layers in containers with melted ice made of contaminated water. Furthermore, research has shown that transmission of pathogens can be increase when leafy greens are exposed to cold water. The contraction of internal airspaces on the surface can draw in water and potential contaminates as demonstrated by Penteado, Eblen, and Miler (2004), with *Salmonella* on fresh mangos. Additional processing steps include cutting and shredding of the produce which can attribute to the cross contamination of other products if pathogens are present (Delaquis, Bach, & Dinu, 2007)

The third major point at which *E. coli* O157:H7 can enter the food production chain is preparation in the kitchen. This type of contamination can occur by the handling of an infected person(s) at the retail market or in the kitchen by the food handler. Proper hygiene and cooking/handling techniques such as thoroughly washing the leafy greens before

preparing should be implemented to curb pathogenic exposure (Fransz and van Bruggen, 2008).

C. Antimicrobial treatments.

1. Chemical composition of antimicrobials

Literature has shown that depending the location of the plant being grown, the area from the plant which the oils is used are extracted and the extraction method can all play an important role on the chemical composition of various essential oils (McGimpsy et al., 1994; Friedman et al., 2002). The essential oils of plants are generally extracted via distillation from aromatic plants. These compounds are naturally produced by the plants as secondary metabolites and contain a variety of volatile molecules such as terpenes and terpenoids that contribute to their strong aroma (Bakkali, Averbeck, Averbeck, & Idaomar, 2007). These compounds can be synthesized from all the organs of the plant such as buds, flowers, fruits, roots and bark. Another important factor worthy of note in the in the extraction of these compounds is that the section or area in which the oil is extracted can play a significant role in the composition on the oil. Cinnamon essential oil that is derived from cinnamon bark can contain up to as much 81% of the primary antimicrobial agent trans-cinnamaldehyde, with trace amounts of the phenylpropene compound eugenol. However, when oil is extracted directly from the cinnamon leaf the percentage of eugenol can be as high as 70% while levels of trans-cinnamaldehye are found in trace amounts. (Friedman et al., 2000).

In general, pure essential oils can be subdivided into two distinct groups of chemical constituents; the hydrocarbons which are made up almost exclusively of terpenes (monoterpenes, sesquiterpenes, and diterpenes), and the oxygenated compounds

which are mainly esters, aldehydes, ketones, alcohols, phenols, and oxides. Essential oils are unique and can contain some 20-60 of these components at a wide range of concentrations depending on which species of plant they are derived from. (Bakkali, Averbeck, Averbeck, & Idaomar, 2007). In most cases, there are two to three primary chemical constituents that constitute a significant percentage (20–70%) of their composition compared to the other compounds present. In the case of oregano essential oil, carvacrol and thymol can constitute up to 30% and 27% respectively (Bakkali, Averbeck, Averbeck, & Idaomar, 2007).

Plant-derived compounds have historically been utilized for their flavor, aroma, bactericidal, and preservative properties. A number of studies conducted previously in our lab have assessed the efficacy of a variety of plant-derived antimicrobial treatments including the essential oils of oregano, cinnamon, and lemongrass and their primary constituents' carvacrol, cinnamaldehyde, and citral, respectively (Denton et al., 2015;Budhini et al., 2014). Efficacy of plant extracts including olive, apple, and grape seed have also been examined (Budhini et al., 2014), along with various fulvic and organic acid formulations. Additional studies have demonstrated the antimicrobial effects of several essential oils and plant extracts against *Salmonella enterica* and *E. coli* O157:H7 on lettuce and organic leafy greens (Moore et al. 2011; Moore-Neibel et al., 2012;).

2. Mechanisms of Antimicrobial Activity

a. Activity of Phenolic Compounds

Carvacrol is a phenolic compound and along with thymol, they exist as the primary chemical constituents of oregano essential oil (Veldhuizen et al., 2006). The phenols are characterized by two key features including an aromatic benzene ring, and a hydroxyl group that is bonded to the carbon ring. (Dorman and Deans, 2000; Arfa et al., 2006). A study by Burt and Reinders (2003) hypothesized that these molecules act upon the cell wall. They believed that these compounds would sensitize the cell membrane causing the cell wall to be saturated with these molecules increasing the permeability and compromising the integrity of the cell wall. This sensitization of the cytoplasmic membrane will result in collapse of the structure and trigger the leakage of intracellular constituents (Joven *et al.,* 1994). Essentially, phenolic compounds exert an irreversible process within the cell that compromises the ability of the cytoplasmic membrane to function properly by altering the ratios of protein to lipid in the membrane. (Sikkema *et.al.,* 1995; Kisko and Roller, 2005). The hydrophobicity of carvacrol appears to be a key factor in allowing the compound to be accumulated into the cell membrane. After accumulation of the compound within the cell, alteration of the membrane structure as a result of hydrogen bonding and proton releasing ability may be responsible for inhibition or death of the cell (Arfa *et al*., 2006).

A study conducted by Dorman and Deans (2000) found that the hydroxyl group component of the phenolic structure may have an effect on the antimicrobial activity of carvacrol. This was confirmed when they compared carvacrol to its methyl ether. They found that not only the presence of the hydroxyl group but its position on the benzene ring could affect its efficiency (Dorman and Deans, 2000). A study by Afra et al. (2006) expanded on the role of the hydroxyl group by hypothesizing that when these compounds are in

the presence of a system of delocalized electrons the gradient across the cytoplasmic membrane was reduced, allowing for a collapse of the proton motive force and depletion of the ATP pool.

b. Activity of Aldehyde Compounds

Cinnamaldehyde, the primary chemical constituent of cinnamon essential oil, is a volatile, aldehyde compound that has been found to affect the protein synthesis in the cell wall's surface causing alteration of the cell wall structure and eventually leading to penetration into the cell cytoplasm (Di Pasque et al., 2007; Somolinos et al., 2009). It has been proposed that the interaction of these volatile aldehyde-containing compounds with the cell wall is due to the highly electronegative nature of an aldehyde group that is conjugated to a carbon-carbon double bond Moyleyar and Narasimham (1986). This increased electronegativity is believed to enhance the antibacterial activity of these compounds (Kurita *et al.* 1979, 1981). The reason for this is these compounds may interfere with certain biological processes such as electron transfer and can react with the critical nitrogen components of the cell wall surface, such as proteins or nucleic acids. These cell surface nitrogen interactions can therefore inhibit the growth of bacteria. In a study conducted by Gill and Holley (2006), researchers treated *E. coli* and *Listeria monocytogenes* with cinnamaldehyde and observed a significant reduction in cellular ATP, providing support to their hypothesis that interaction of volatile aldehyde compounds with bacterial cells can interrupt critical cellular function such as ATP synthesis and membrane stability

c. Activity of Fulvic Acid

Similar to the other antimicrobials tested in this study, these compounds target the

cell wall of the bacteria. Several cellular process can be interrupted with this compound including the integrity of the cytoplasmic membrane as well as other metabolic functions within the cytoplasm including replication and protein synthesis (Denyer and Stewart, 1998; Davidson, 2001). As these compounds are acids, one of the most critical factors in determination of their effectiveness is pH. Acids to be used as sanitizers for food are generally weak acids therefore pH plays a critical role in the concentration of undissociated acid that is formed The mechanisms by which organic acids inhibit microbes are not fully understood however they are capable of bacteriostatic and bactericidal activity. These characteristics are largely dependent on the physiochemical characteristics of the surrounding environment (Davidson, 2001). Traditionally it has been believed that undissociated forms of organic acids can penetrate the lipid membrane of bacterial cells. When the acid is internalized into the cytoplasm the pH is neutral, and the acid can then dissociate into anions and protons (Eklund, 1983, 1985; Salmond *et al.,* 1984; Cherrington *et al.,* 1990, 1991; Davidson, 2001). The presence of anions and protons within the cytoplasm can present problems for bacteria where pH in the cytoplasm is important for the sustainability of functional macromolecules. The energy requirements to export the excess protons depletes cellular energy pools of ATP affecting cellular viability (Davidson, 2001). The mechanisms by which organic acids are bactericidal or bacteriostatic are difficult to establish due to the complex strategies cells have for generating energy and their ability to maintain their internal pH as a result of proteins and DNA that are acid sensitive (Thompson and Hinton, 1996).

d. Bacterial and Bacteriostatic Effects on Bacterial Cells

Due to the diversity of the molecules present in essential oils, it would appear that these compounds do not attack any specific cellular target. The compounds found in these oils are lipophilic and therefore tend to combine with or dissolve in lipids. This allows these components to easily pass through the cell wall and into the cytoplasmic membrane (Knobloch et al., 1989). Once within the membrane they can disrupt multiple cellular components critical to cell viability including layers of polysaccharides, fatty acids, and phospholipids. The disruption of these cellular components affects the cells viability. (Sikkeme et al., 1994). This internal disruption leads to a reduction of membrane potential due to loss of ions, collapsing the proton pump, and eventually depletion of the ATP pool (Turina et al., 2006).

The physical characteristics of some bacteria can have a significant impact on an antimicrobials effectiveness. In particular, the type of cell wall can play a role. Bacteria can be divided into either Gram-negative or a Gram-positive cell wall. Gram-positive bacteria possess a thick layer of peptidoglycan on the outer layer with a singular phospholipid inner bilayer while Gram-negatives possess two lipid bilayers (Bakkali et al., 2008). In addition to the lipid bilayers on Gram-negative bacteria, they also possess a hydrophobic lipopolysaccharide (LPS) component on the exterior of their cell wall. This LPS can play a role in bacterial resistance to hydrophobic drugs (Andra, 2004).

The presence of the LPS component of Gram-negative bacteria can assist in the prevention of hydrophobic components accumulating within the cytoplasm of bacteria (Bezic et al., 2003). However, this can be circumvented by some of the hydrophobic constituents found in essential oils. This is accomplished as they are able to penetrate the outer membrane through porin proteins (Helendar et al., 1998). Once inside the

cytoplasm of the cell the antimicrobials are able to disrupt cellular functions, permeablizing essential cellular components (Bakkali et al., 2008).

CHAPTER III

METHODOLGY

A. Bacterial Culture Preparation.

A cocktail of two *Escherichia coli* O157:H7 strains (ATCC 43895, 43888) were used to prepare the dip inoculation. Each strain was maintained as a frozen stock culture at -80 ^{o}C. Prior to an experiment, swabs of frozen culture were taken and placed into tryptic soy broth (TSB; Bacto^{TM,} Becton Dickinson, Sparks, MD), and incubated at 37 ^{o}C for 18-24hrs. The revived bacteria were further transferred into 9 ml TSB and allowed to grow at 37 ^{o}C for 18-24 h. Overnight cultures of the two strains were then prepared by adding 100ul to 9.9 ml TSB to obtain a 9-$\log_{10}$ CFU/ml population. A cocktail was prepared by mixing equal volumes of the overnight cultures of the two strains. A dip inoculum was then prepared by further diluting the cocktail in buffered peptone water (BPW; BBL^{TM}, Difco, BD) to obtain approximately 6-$\log_{10}$ CFU/ml bacterial population.

B. Preparation of Organic Leafy Greens

Organic leafy greens used in this study were baby spinach, mature bunched spinach, romaine lettuce, and iceberg lettuce. The greens were obtained from local grocery stores in Stillwater, OK. Leafy greens were purchased on the day of the experiment and stored at refrigeration temperature (4 ^{o}C) until use. Prior to weighing, all leafy greens were washed thoroughly for 2 minutes under running tap water (room temperature (RT); 23-25 ^{o}C) to remove soil and organic matter. Samples of mature bunched spinach leaves were prepared by separating the leaves from their stalks while whole leaves of baby spinach were used. The heads of romaine and iceberg lettuce were purchased as whole heads and had had the individual outer leaves removed along with the core for the lettuce. The lettuce leaves were further cut into smaller pieces (approximately 1.5 sq inches) with a pair of sterile scissors, using aseptic techniques. Appropriate sample sizes were then weighed out and placed in a plastic bin for washing. The attained leaves were washed three times using sterilized distilled water to remove any soil or other organic material present on the leaves. Washed leaves were then exposed to ultraviolet (UV) radiation (254 nm) for 30 minutes; 15 minutes on each side of the leafy green, to eliminate any potential remaining background micro flora accumulated on the leafy green surface. Following the preparation of the dip inoculum, leafy greens were dip inoculated for 2 minutes, then allowed to dry in a biosafety hood for 30 minutes allowing *E. coli* O157:H7 to adhere to leafy green surface. Leafy green samples were set aside before the inoculation, and after time allowed for adherence for negative and positive controls, respectively.

C. Preparation of Antimicrobial Treatments

The antimicrobial treatments selected for this study were plant-derived essential oils: oregano and cinnamon, and their primary constituents: carvacrol, and cinnamaldehyde, respectively. The concentrations of the essential oils, as well as their primary constituents was 0.5%. This concentration was selected because previous experiments conducted in our lab have demonstrated that these were the most effective concentrations tested against foodborne pathogens. For this reason, these particular concentrations were selected for the reusability study. In addition to the essential oils, compounds, and fulvic acid formulation, experimental controls of sterile distilled water, phosphate buffered saline (PBS) and 3% hydrogen peroxide were tested as well. Both hydrogen peroxide and sterile water are commonly used washing solutions used in the organic produce industry* and were used to compare efficacy of compound and essential oil treatments to industry standard washes. PBS was also tested as a control due to its use in the wash solutions to help disperse essential oil and compound treatments.

D. Re-usability of essential oils of oregano and cinnamon, carvacrol and cinnamaldehyde, and fulvic Acid (III) against *Escherichia coli* O157:H7 on organic leafy greens

Samples of 220 g leafy greens and antimicrobial treatments were prepared as described above for each experiment. In order to evaluate the reusability of the antimicrobials the same wash water was used to wash 5 separate 10g batches of inoculated leafy greens. Each 10 g sample was washed in the appropriate antimicrobial treatment solution for 1 minute with gentile agitation, using a horizontal back-and -forth

motion. Following each wash, the leaves were removed from the Whirl-Pak™ bag using sterilized forceps. Any remaining liquid was shaken off and the treated leaves were transferred to a new sterile Whirl-Pak™ bag and stored at 4 °C over a period of 3 days. The pH of the wash water was recorded for each organic sanitizer before and after every wash using a calibrated pH meter. Additionally, after each wash, the wash-water for each antimicrobial was tested for turbidity using a spectrophotometer and wash water samples were collected to enumerate for bacterial survivors. Populations of *E. coli* O157:H7 were observed on days 0, 1, and 3 of storage. To enumerate the surviving *E. coli* O157:H7 populations a 2 g sample was taken from each stored sample and transferred into a sterile Whirl-Pak™ bag and stomached with 18 ml sterile-buffered peptone water (BPW;BBLTM, Difco, BD) at 230 rpm for 1 minute. Samples were then serially diluted in BPW and appropriate dilutions plated in duplicates on Sorbitol MacConkey (SMAC, Remel, Thermo Fisher Scientific, Lenexa, Kansas, USA) agar. Colonies of *E. coli* O157:H7 were counted after incubation at 37 °C for 18-24 hours.

E. Statistical Analysis

Bacterial populations of *E. coli* O157:H7 were converted to $\log_{10}$ CFU g^{-1}. Statistical analysis was conducted using PROC GLM under SAS v9.4 to determine significant difference ($P<0.05$) among treatments. Means were separated using Duncan's multiple range test. All experiments were repeated 3 times.

CHAPTER IV

FINDINGS

Results of the study for each of the leafy greens treated with five organic sanitizers, along with the controls, are shown in tables (1.1-5.4). The mean values represent the $\log_{10}$ CFU/g population of *E. coli* O157:H7 recovered on SMAC agar. Log reductions compared to the positive control are also shown. Data from the negative control is not shown as no bacterial growth was detected for any of the samples Results from the enumeration of wash water from the sanitizers are not shown as no bacterial survivors were enumerated in any of the organic sanitizer washes tested. Of the controls used in this study only sterile distilled water, and PBS exhibited bacterial survivors in the wash water and can be seen in Figures (1-4). Additionally, pH and turbidity readings were taken for each of the five washes of the sanitizers and controls. However, no significant statistically differences were observed over the course of 5 washes (Tables 6.1-6.4 and 7.1-7.4).

A. Iceberg Lettuce

1. *Reusability of Essential Oil Treatments*

Essential Oils The oregano and cinnamon essential oil treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 3.1, 4.1). Oregano EO at 0.5% concentration displayed the highest potential as a reusable antimicrobial sanitizer evaluated in this study. In iceberg lettuce, oregano essential oil exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 3.1). Additionally, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. On organic iceberg lettuce, oregano EO demonstrated log reduction of *E. coli* O157:H7 populations of 4.5 to 4.6 $\log_{10}$ CFU/g^{-1} (Table 3.1) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 2.0 to 2.4 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0) and maintained similar reduction levels throughout the three day storage period with log reductions of 2.4 to 2.8 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 3.1). Cinnamon EO was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all washes. In iceberg lettuce, bacterial populations were not detected after the first two washes on day 0, whereas populations of 0.3 $\log_{10}$ CFU/g^{-1} (Table 4.1) were observed after the third wash with further increasing to 1.7 $\log_{10}$ CFU/g^{-1} by the fifth wash. In iceberg lettuce, *E. coli* O157:H7 colonies were only seen on samples from washes four and five on day 1 and no growth was detected from any of the leaf samples by day 3. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamon EO. The pH and turbidity of each wash water was recorded (Tables

**) however, no statistically significant differences were observed throughout the five washes.

2. *Reusability of Compound Treatments*

Compounds On organic iceberg lettuce both compound treatments carvacrol and cinnamaldehyde treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 1.1, 2.1). In iceberg lettuce carvacrol at 0.5% concentration, exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 1.1). Furthermore, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. On iceberg lettuce, carvacrol demonstrated complete pathogen reduction of *E. coli* O157:H7 populations ranging from 4.0 to 4.6 $\log_{10}$ CFU/g^{-1} (Table 1.1) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 2.0 to 2.4 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0), maintaining consistent reduction levels throughout the three day storage period with log reductions of 2.4 to 2.8 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 1.1). Cinnamaldehyde was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all leafy greens. In iceberg lettuce, bacterial populations were not detected in the first wash on day 0, whereas populations ranging from 1.4 to 2.5 $\log_{10}$ CFU/g^{-1} (Table 2.1) were observed in the subsequent washes 2 to 5

(Table 2.1). Sampling on day 1 revealed *E. coli* O157:H7 populations of 0.3 and 0.5 $\log_{10}$ CFU/g^{-1} on washes 3 and 5 respectively (Table 2.1). No *E. coli* O157:H7 colonies were detected in any of the leaf samples on day 3 of storage. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamaldehyde. The pH and turbidity of each wash water was recorded (Tables **) however, no statistically significant differences were observed throughout the five washes.

3. *Reusability of Fulvic Acid Treatments*

Fulvic Acid Iceberg lettuce samples treated with fulvic acid significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the distilled water and positive controls. In iceberg lettuce fulvic acid at 3.0% concentration exhibited log reductions of 2.0-2.3 $\log_{10}$ CFU/g^{-1} (Table 5.1) on day 0 for five washes. Iceberg lettuce treated with fulvic acid showed a linear reduction of *E. Coli* O157:H7 populations over the three day storage period with log reductions of 2.9-3.5 and 3.0 to 4.1 $\log_{10}$ CFU/g^{-1} on days 1 and 3 respectively (Table 5.1). No *E. coli* O157:H7 colonies were detected in samples from the first wash on day 3 of storage (Table 5.1). The distilled water control significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the positive control. Distilled water washes on iceberg lettuce showed log reductions of 0.9-1.4 $\log_{10}$ CFU/g^{-1} on day 0 and by day 3 of storage showed log reductions 1.0-1.8 $\log_{10}$ CFU/g^{-1} (Table 5.1). No *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. The pH and turbidity of each wash water was recorded (Tables 6.1-7.4) however, no statistically significant differences were observed throughout the five washes.

B. Romaine Lettuce

1. *Reusability of Essential Oil Treatments*

Essential Oils The oregano and cinnamon essential oil treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 3.2, 4.2). Oregano EO at 0.5% concentration displayed the highest potential as a reusable antimicrobial sanitizer evaluated in this study. In romaine lettuce, oregano essential oil exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 3.2). Additionally, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes with essential oil treatment. On romaine lettuce, oregano EO demonstrated log reductions of *E. coli* O157:H7 population of 4.1 to 4.6 $\log_{10}$ CFU/g^{-1} (Table 3.2) over the storage period of three days. Hydrogen peroxide 3% and PBS were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 2.3 to 2.5 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0) and maintained similar reduction levels throughout the three day storage period with log reductions of 2.4 to 2.9 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 3.2). Leaf samples treated with cinnamon EO reduced *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all washes with the exception of wash three. Bacterial populations were detected in all washes on day 0 ranging from 0.1 to 1.9 $\log_{10}$ CFU/g^{-1} (Table 4.2). No *E. coli* O157:H7 was detected in the first two washes of romaine

lettuce on day 1, populations of 0.4 $\log_{10}$ CFU/g^{-1} (Table 4.2) were observed after the second wash, further increasing to 0.8 $\log_{10}$ CFU/g^{-1} by the fifth wash. Colonies of *E. coli* O157:H7 were only seen on samples from washes three to five on day 1 and no growth was detected from any of the leaf samples by day 3 (Table 4.2). No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamon EO. The pH and turbidity of each wash water was recorded (Tables 6.1-7.4) however, no statistically significant differences were observed throughout the five washes.

2. *Reusability of Compound Treatments*

Compounds On organic romaine lettuce both compound treatments carvacrol and cinnamaldehyde treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 1.2, 2.2). In romaine lettuce carvacrol at 0.5% concentration, exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 1.2). Furthermore, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. On romaine lettuce, carvacrol demonstrated complete pathogen reduction of *E. coli* O157:H7 populations ranging from 4.0 to 4.7 $\log_{10}$ CFU/g^{-1} (Table 1.1) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 2.0 to 2.5 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0), increased reduction levels were observed throughout the three day storage

period with log reductions of 2.2 to 3.0 $\log_{10}$ CFU/g^{-1} observed by day 3 of storage (Table 1.2). Cinnamaldehyde was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for romaine lettuce. Bacterial populations of 1.1 to 2.4 $\log_{10}$ CFU/g^{-1} were detected on day 0 (Table 2.2). Sampling on day 1 revealed no bacterial growth in washes 1 to 3, *E. coli* O157:H7 populations of 0.5 and 0.3 $\log_{10}$ CFU/g^{-1} were observed on washes 4 and 5 respectively (Table 2.2). No *E. coli* O157:H7 colonies were detected in any of the leaf samples on day 3 of storage. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamaldehyde. The pH and turbidity of each wash water was recorded (Tables 6.1-7.4) however, no statistically significant differences were observed throughout the five washes.

3. *Reusability of Fulvic Acid Treatments*

Fulvic Acid Romaine lettuce samples treated with fulvic acid significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the distilled water and positive controls. In romaine lettuce, fulvic acid at 3.0% concentration showed log reductions of 2.3-2.7 $\log_{10}$ CFU/g^{-1} (Table 5.2) on day 0 for five washes. Romaine lettuce treated with fulvic acid showed a linear reduction trend of *E. Coli* O157:H7 populations over the three day storage period with log reductions of 2.7-3.0 and 3.0 to 3.8 $\log_{10}$ CFU/g^{-1} on days 1 and 3 respectively (Table 5.2). No *E. coli* O157:H7 colonies were detected in samples from the first wash on day 3 of storage (Table 5.2). The distilled water control significantly reduced ($P<0.05$) *E. coli* O157:H7

populations for all five consecutive wash treatments compared to the positive control. Distilled water washes on iceberg lettuce showed log reductions of 1.1-1.4 $\log_{10}$ CFU/g^{-1} on day 0 and by day 3 of storage showed log reductions 1.3-1.7 $\log_{10}$ CFU/g^{-1} (Table 5.2). No *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. The pH and turbidity of each wash water was recorded (Tables 6.1-7.4) however, no statistically significant differences were observed throughout the five washes.

C. Baby Spinach

1. *Reusability of Essential Oil Treatments*

Essential Oils The oregano and cinnamon essential oil treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 3.3, 4.3). In baby spinach, oregano essential oil exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 3.3). Additionally, no *E. coli* O157:H7 colonies were detected in the enumeration of antimicrobial wash water for all five washes. On organic baby spinach, oregano EO demonstrated log reductions of *E. coli* O157:H7 populations of 4.5 to 4.7 $\log_{10}$ CFU/g^{-1} (Table 3.3) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 2.0 to 2.8 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0) and demonstrated increased levels of reduction

levels throughout the three day storage period with log reductions of 2.1 to 3.1 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 3.3). Cinnamon EO was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all washes. In baby spinach, bacterial populations were detected in all washes on day 0, with populations of 0.6 to 1.9 $\log_{10}$ CFU/g^{-1} (Table 4.3). Sampling on day 1 revealed no *E. coli* O157:H7 in the first wash, populations of 0.1 $\log_{10}$ CFU/g^{-1} were observed in the second wash, further increasing to 0.6 $\log_{10}$ CFU/g^{-1} by the fifth wash (Table 4.3). In baby spinach, no growth was detected from any of the leaf samples by day 3. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamon EO. The pH and turbidity of each wash water was recorded (Tables 6.3, 7.3) however, no statistically significant differences were observed throughout the five washes.

2. *Reusability of Compound Treatments*

Compounds On organic baby spinach both compound treatments carvacrol and cinnamaldehyde treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 1.3, 2.3). In iceberg lettuce carvacrol at 0.5% concentration, exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 1.3). Furthermore, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. On baby spinach, carvacrol demonstrated complete pathogen reduction of *E. coli* O157:H7 populations ranging from 4.5 to 4.7 $\log_{10}$ CFU/g^{-1} (Table 1.3) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this

experiment. Hydrogen peroxide significantly reduced (P<0.05) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 1.6 to 2.4 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0), maintaining consistent reduction levels throughout the three day storage period with log reductions of 1.8 to 2.2 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 1.3). Cinnamaldehyde was effective at reducing *E. coli* O157:H7 populations to undetectable limits by day 1 of storage for all leafy greens. In baby spinach, bacterial populations were not detected in the first wash on day 0, whereas populations ranging from 0.9 to 1.7 $\log_{10}$ CFU/g^{-1} (Table 2.1) were observed in the subsequent washes 2 to 5 (Table 2.3). Sampling on day 1 revealed no growth of *E. coli* O157:H7. (Table 2.3). Additionally, no *E. coli* O157:H7 colonies were detected in any of the leaf samples on day 3 of storage. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamaldehyde. The pH and turbidity of each wash water was recorded (Tables 6.3-7.3) however, no statistically significant differences were observed throughout the five washes.

3. *Reusability of Fulvic Acid Treatments*

Fulvic Acid Baby spinach samples treated with fulvic acid significantly reduced (P<0.05) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the distilled water and positive controls. In baby spinach fulvic acid at 3.0% concentration exhibited log reductions of 2.6-3.6 $\log_{10}$ CFU/g^{-1} (Table 5.3) on day 0 for all five washes. Baby spinach leaf samples showed a linear trend of reduction against *E.*

Coli O157:H7 populations over the three day storage period with log reductions of 3.2-3.6 and 3.0 to 4.0 $\log_{10}$ CFU/g^{-1} on days 1 and 3 respectively (Table 5.3). No *E. coli* O157:H7 colonies were detected in samples from the first wash on day 3 of storage (Table 5.3). The distilled water control significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the positive control. Distilled water washes on iceberg lettuce showed log reductions of 1.3-1.6 $\log_{10}$ CFU/g^{-1} on day 0 and by day 3 of storage showed log reductions 1.0-1.5 $\log_{10}$ CFU/g^{-1} (Table 5.1). No *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. The pH and turbidity of each wash water was recorded (Tables 6.3-7.3) however, no statistically significant differences were observed throughout the five washes.

Mature Spinach

1. Reusability of Essential Oil Treatments

Essential Oils The oregano and cinnamon essential oil treatments both significantly reduced ($P<0.05$) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 3.4, 4.4). In organic mature spinach, oregano essential oil exhibited complete reduction of pathogen populations after initial application (Day 0) for all five washes (Table 3.3). Additionally, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. Oregano EO demonstrated log reduction of *E. coli* O157:H7 populations of 4.0 to 4.1 $\log_{10}$ CFU/g^{-1} (Table 3.1) over the storage period of three days. PBS and hydrogen peroxide 3% were used as control treatments in this

experiment. Hydrogen peroxide significantly reduced (P<0.05) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 1.3 to 2.0 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0) and maintained similar reduction levels throughout the three day storage period with log reductions of 1.3 to 1.9 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 3.3). Cinnamon EO was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all washes. In mature spinach, bacterial populations were not detected after the first wash on day 0, whereas populations of 0.1 $\log_{10}$ CFU/g^{-1} (Table 4.4) were observed after the first wash, further increasing to 1.5 $\log_{10}$ CFU/g^{-1} by the fifth wash. In mature spinach, *E. coli* O157:H7 colonies were only seen on samples from wash five on day 1 at 0.5 $\log_{10}$ CFU/g^{-1} , and no growth was detected from any of the leaf samples by day 3 (Table 4.4). No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamon EO. The pH and turbidity of each wash water was recorded (Tables 6.1-7.4) however, no statistically significant differences were observed throughout the five washes.

2. Reusability of Compound Treatments

Compounds On organic iceberg lettuce both compound treatments carvacrol and cinnamaldehyde treatments both significantly reduced (P<0.05) *E. coli* O157:H7 for all five consecutive wash treatments (Tables 1.1, 2.1). In mature spinach carvacrol at 0.5% concentration, exhibited log reduction of pathogen populations by 2.7 to 3.6 $\log_{10}$ CFU/g^{-1} after initial application (Day 0) for all five washes (Table 1.4). Populations of 0.5 to 0.6

$\log_{10}$ CFU/g^{-1} were observed on day 1 of sampling for washes 2 to 5. No growth was detected from any of the leaf samples by day 3 (Table 4.4). Furthermore, no *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. PBS and hydrogen peroxide 3% were used as control treatments in this experiment. Hydrogen peroxide significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five washes compared to the positive control. Log reductions of 0.9 to 1.3 $\log_{10}$ CFU/g^{-1} were observed on hydrogen peroxide washes after initial application (Day 0), maintaining consistent reduction levels throughout the three day storage period with log reductions of 1.2 to 1.3 $\log_{10}$ CFU/g^{-1} observed on day 3 of storage (Table 1.4). Cinnamaldehyde was effective at reducing *E. coli* O157:H7 populations to undetectable limits by the third day of storage for all leafy greens. In mature spinach, bacterial populations were not detected in the first wash on day 0, whereas populations ranging from 1.6 to 2.1 $\log_{10}$ CFU/g^{-1} (Table 2.4) were observed in the subsequent washes 2 to 5 (Table 2.4). Sampling on day 1 revealed *E. coli* O157:H7 populations of 0.1 and 0.5 $\log_{10}$ CFU/g^{-1} on washes 4 and 5 respectively (Table 2.4). No *E. coli* O157:H7 colonies were detected in any of the leaf samples on day 3 of storage. No *E. coli* O157:H7 colonies were recovered from the enumeration of wash water from cinnamaldehyde. The pH and turbidity of each wash water was recorded (Tables 6.4-7.4) however, no statistically significant differences were observed throughout the five washes.

3. Reusability of Fulvic Acid Treatments

Fulvic Acid Mature spinach samples treated with fulvic acid showed significant reduction ($P<0.05$) of *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the distilled water and positive controls (Table 5.4). In mature spinach, fulvic acid at 3.0% concentration exhibited log reductions of 1.4-2.5 $\log_{10}$ CFU/g^{-1} (Table 5.4) on day 0 for all five washes. Mature spinach treated with fulvic acid showed a linear reduction of *E. Coli* O157:H7 populations over the three day storage period with log reductions of 2.0 to 2.7 and 2.3 to 3.0 $\log_{10}$ CFU/g^{-1} on days 1 and 3 respectively (Table 5.4). No *E. coli* O157:H7 colonies were detected in samples from the first wash on day 3 of storage (Table 5.4). The distilled water control significantly reduced ($P<0.05$) *E. coli* O157:H7 populations for all five consecutive wash treatments compared to the positive control. Distilled water washes on iceberg lettuce showed log reductions of 0.9-1.4 $\log_{10}$ CFU/g^{-1} on day 0 and by day 3 of storage showed log reductions 1.0-1.8 $\log_{10}$ CFU/g^{-1} (Table 5.4). No *E. coli* O157:H7 colonies were detected in the enumeration of wash water for all five washes. The pH and turbidity of each wash water was recorded (Tables 6.4-7.4) however, no statistically significant differences were observed throughout the five washes.

CHAPTER V

CONCLUSION

Note: Please read the directions in the following paragraph very carefully before proceeding.

A. Reusability of Plant-Derived Essential Oils and Compounds

In our current study, the reusability of a select group of plant-derived antimicrobials were evaluated. The results of this study demonstrated that all antimicrobials tested showed high potential for re-usability. The essential oils of oregano and cinnamon (0.5% v/v), their primary chemical constituents carvacrol and cinnamaldehyde respectively (0.5% v/v), and Fulvic Acid-III (3.0% v/v) were able to continually show significant reduction ($P<0.05$) of *Escherichia coli* O157:H7 populations over the course of 5 washes on all leafy greens tested. A number of studies have investigated the effectiveness essential oils against foodborne pathogens (Friedman, Henika, and Mandrell, 2002; Gutierrez, Rodriguez, Barry-Ryan, and Bourke, 2008), in addition to the primary chemical constituents of essential oils (Burt, Vlielander, Haagsman, and Veldhuizen, 2005). A study conducted by Van Rensburg et al. (2000) found that fulvic acid demonstrated antimicrobial activity against many pathogenic bacteria in vitro, including *Enterococcus*

faecalis, Staphylococcus aureus,, E. coli, Streptococcus pyogenes, Klebsiella pneumonia, Proteus mirabilis and *Candida albicans* . Fulvic acid has also shown to be effective against *Listeria monocytogenes, Salmonella* Typhimurium and, *P. aeruginosa*. Additionally, Zhu et al. (2014) found fulvic acid to be inhibitory against foodborne pathogens *Listeria monocytogenes, Salmonella* Typhimurium and, *P. aeruginosa* on food contact surfaces. There are a plethora of studies that have evaluated the effectiveness of these antimicrobials, but to date there are limited studies available that evaluate the potential for reusability of these sanitizers in a practical setting. The purpose of this study was to evaluate the potential for reusability of natural sanitizer treatments including oregano and cinnamon essential oils (0.5% v/v), their primary chemical constituents carvacrol and cinnamaldehyde respectively (0.5% v/v), and Fulvic Acid-III (3.0% v/v). The results of the study demonstrated that these treatments were able to continually show significantly reduction ($P<0.05$) of *Escherichia coli* O157:H7 populations over the course of 5 washes on all leafy greens compared to the positive control.

For all the fresh produce types, significant reduction ($P<0.05$) was observed with all the antimicrobials tested when compared to the positive control. Of the organic sanitizers evaluated in this study, oregano EO proved to be the most effective at continually reducing *E. coli* O157:H7 populations. It is noteworthy to mention that wash water samples were enriched in non-selective media to ensure that bacteria were not in a viable but non-culturable state. However, individual leaf samples from treatments that showed no bacterial growth were not enriched to verify that all bacteria had been eliminated. Bacteria from leaf samples were only enumerated on days 0, 1, & 3 of the

experiment on selective media (SMAC). A number of studies that have investigated the efficacy of essential oils have observed similar results. A study carried out by Burt and Reinders (2003) demonstrated oregano essential oil to be bactericidal against *E. coli* O157:H7 (no viable cells detected; $>10^4$ log reduction).

.From the results of this study, fulvic acid showed inhibition similar to the industry control, hydrogen peroxide. However, while pathogen populations on leafy greens treated with hydrogen peroxide remained static over three days of storage, those treated with fulvic acid declined steadily over the storage period demonstrating a delayed antimicrobial effect. Additionally, while fulvic acid was unable to reduce microbial populations to undetectable levels on day 0, greens treated with this compound showed a linear reduction trend of *E. Coli* O157:H7 populations throughout the three-day storage period

B. Effects of Refrigerated Storage Temperature and Duration

Previous studies that have been carried out in our lab have investigated the effects of refrigerated storage at various temperatures (4 ^{o}C, 8 ^{o}C). Results showed that there was either no significant change ($P<0.05$) in surviving *E. coli* O157:H7 populations for each of the three consecutive days of storage at 4 ^{o}C.or only slight decrease in bacterial survivors. In a study conducted by Francis and O'Beirne (2001) researchers found that *E. coli* O157:H7 activity was limited when held at refrigerated temperatures (4 ^{o}C) although another study conducted by Delaquiz, Bach, and Dinu (2007) demonstrated that temperatures from 6-8^{o}C still allowed for bacterial growth. For the purposes of this study, the leafy greens were stored at 4 ^{o}C during storage. The Food and Drug

Administration has put forth guidance regulations that processed leafy greens, including those that have been chopped, cut, or torn are required to be held at refrigerated temperatures at 41 ^{o}F (5 ^{o}C) or less during post-harvest processing (FDA 2009).

C. Control Treatments

In the current study, three control treatments were used as standards to compare the reusability of industry standard washes with the plant-derived essential oil and primary compound's antimicrobial efficacy against *E. coli* O157:H7 on organic leafy greens. Overall, with the exception of the fulvic acid treatments, the essential oils and compound treatments at concentrations of 0.5% showed statistically significant ($P<0.05$) reduction than the control treatments throughout all five consecutive washes. Fulvic acid treatment reuse at a concentration of 3.0% showed similar levels in reduction when compared to hydrogen peroxide treatments. However, unlike the essential oil and compound treatments in this study, hydrogen peroxide did not show a trend of continuing reduction over time. It is hypothesized that hydrogen peroxide may only have short-term effects towards the bacteria. Additionally, due to the unstable nature of hydrogen peroxide, it may not have the ability to exert a continuing trend in reduction. This could be a reason that the bacteria *E. coli* O157:H7 is adaptable to the repeated hydrogen peroxide antimicrobial treatment exposure.

D. Conclusion

The findings of the present study provide evidence that the essential oils of cinnamon and oregano, as well as their primary constituents' carvacrol and cinnamaldehyde, demonstrate significant antimicrobial effects against *E. coli* O157:H7 with continued use during simulated small scale flume tank washing. Fulvic acid III was able to significantly reduce ($P<0.05$) populations of *E. coli* O157:H7 ranging from 1.0 to 2.9 $\log_{10}$ amongst all leafy greens tested after initial application (Day 0) and demonstrated results similar to hydrogen peroxide. Additionally while fulvic acid was unable to reduce microbial populations to undetectable levels on day 0, leafy greens treated with this compound showed a linear reduction trend of *E. coli* 0157:H7 populations over the three day storage period. All tested antimicrobials in this study continued to significantly reduce ($P<0.05$) *E. coli* O157:H7 populations on all leafy green types. This study clearly demonstrates that plant-derived compounds could serve as effective sanitizers to inactivated E. coli O157:H7 and retain their antimicrobial activity with continued use. Future areas of research include sensory analysis of the tested leafy greens to analyze consumer acceptability.

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APPENDICES

Table 1.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 5 washes with 1-minute Carvacrol Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6^{a}	4.3^{a}	4.0^{a}
PBS 1	-	$3.5^{a,b}$	$2.9^{b,c}$	$2.3^{b,c}$
PBS 2	-	$3.4^{a,b,c}$	$3.7^{a,b}$	$3.2^{a,b}$
PBS 3	-	$3.7^{a,b}$	$3.9^{a,b}$	$3.2^{a,b}$
PBS 4	-	$3.6^{a,b}$	$4.0^{a,b}$	3.3^{a}
PBS 5	-	$3.7^{a,b,c}$	$3.8^{a,b}$	$3.1^{a,b}$
HP 1	3.0	$2.2^{b,c,d}$	2.2^{c}	$1.5^{c,d}$
HP 2	3.0	$2.6^{b,c,d}$	1.9^{c}	1.2^{d}
HP 3	3.0	$2.4^{b,c,d}$	2.3^{c}	$1.6^{c,d}$
HP 4	3.0	$2.7^{b,c,d}$	2.3^{c}	$1.9^{c,d}$
HP 5	3.0	$2.8^{b,c,d}$	2.7^{c}	$1.6^{c,d}$
CAR 1	0.5	ND^{e}	ND^{d}	ND^{e}
CAR 2	0.5	ND^{e}	ND^{d}	ND^{e}
CAR 3	0.5	ND^{e}	ND^{d}	ND^{e}
CAR 4	0.5	ND^{e}	ND^{d}	ND^{e}
CAR 5	0.5	ND^{e}	ND^{d}	ND^{e}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 1.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 5 washes with 1-minute Carvacrol Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.0^{a}	4.7^{a}	4.0^{a}
PBS 1	-	$3.0^{b,c}$	3.4^{b}	2.6^{c}
PBS 2	-	$3.4^{a,b}$	3.5^{b}	$3.0^{b,c}$
PBS 3	-	$3.5^{a,b}$	$3.6^{b,c}$	3.1^{b}
PBS 4	-	$3.3^{a,b}$	$3.7^{b,c}$	$3.0^{b,c}$
PBS 5	-	$3.4^{a,b}$	$3.8^{b,c}$	3.1^{b}
HP 1	3.0	$1.5^{e,f}$	1.6^{c}	$1.4^{d,e}$
HP 2	3.0	$1.5^{e,f}$	2.1^{c}	1.0^{e}
HP 3	3.0	$2.1^{d,e}$	1.9^{c}	1.8^{d}
HP 4	3.0	$2.0^{d,e}$	2.3^{c}	1.8^{d}
HP 5	3.0	$1.6^{e,f}$	1.8^{c}	1.8^{d}
CAR 1	0.5	ND^{g}	ND^{d}	ND^{e}
CAR 2	0.5	ND^{g}	ND^{d}	ND^{e}
CAR 3	0.5	ND^{g}	ND^{d}	ND^{e}
CAR 4	0.5	ND^{g}	ND^{d}	ND^{e}
CAR 5	0.5	ND^{g}	ND^{d}	ND^{e}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 1.3 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 5 washes with 1-minute Carvacrol Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.5[a]	4.7[a]	4.5[a]
PBS 1	-	4.1[a]	3.4[c,d]	3.8[b]
PBS 2	-	4.2[a]	3.9[b,c]	3.8[b]
PBS 3	-	4.0[a]	3.8[b,c]	3.8[b]
PBS 4	-	4.0[a]	3.8[b,c]	3.6[b]
PBS 5	-	4.6[a]	4.2[a,b]	3.9[a,b]
HP 1	3.0	2.1[b,c]	2.6[e]	2.3[c]
HP 2	3.0	2.7[b]	2.9[d,e]	2.4[c]
HP 3	3.0	2.2[b,c]	2.6[e]	2.3[c]
HP 4	3.0	2.8[b]	2.8[d,e]	2.4[c]
HP 5	3.0	2.9[b]	2.9[d,e]	2.7[c]
CAR 1	0.5	ND[d]	ND[f]	ND[d]
CAR 2	0.5	ND[d]	ND[f]	ND[d]
CAR 3	0.5	ND[d]	ND[f]	ND[d]
CAR 4	0.5	ND[d]	ND[f]	ND[d]
CAR 5	0.5	ND[d]	ND[f]	ND[d]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 1.4 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 5 washes with 1-minute Carvacrol Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.0[a]	4.2[a]	3.9[a]
PBS 1	-	3.4[a,b]	3.6[a,b,c]	3.5[b]
PBS 2	-	3.1[a,b,c]	3.7[a,b,c]	3.6[a,b]
PBS 3	-	3.4[a,b]	3.8[a,b,c]	3.6[a,b]
PBS 4	-	3.5[a,b]	3.7[a,b,c]	3.5[b]
PBS 5	-	3.4[a,b]	3.8[a,b]	3.7[a,b]
HP 1	3.0	2.7[b,c,d]	2.8[c]	2.6[c]
HP 2	3.0	3.1[a,b,c]	2.8[b,c]	2.5[c]
HP 3	3.0	2.9[a,b,c,d]	3.1[b,c]	2.5[c]
HP 4	3.0	3.1[a,b,c]	2.8[b,c]	2.7[c]
HP 5	3.0	3.0[a,b,c,d]	3.1[b,c]	2.7[c]
CAR 1	0.5	0.4[e]	ND[d]	ND[d]
CAR 2	0.5	0.6[f]	0.5[d]	ND[d]
CAR 3	0.5	0.6[f]	0.6[d]	ND[d]
CAR 4	0.5	0.5[f]	0.6[d]	ND[d]
CAR 5	0.5	1.3[f]	0.6[d]	ND[d]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 2.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 5 washes with 1-minute Cinnamaldehyde Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6[a]	4.3[a]	4.0[a]
PBS 1	-	3.5[a,b]	2.9[b,c]	2.3[b,c]
PBS 2	-	3.4[a,b,c]	3.7[a,b]	3.2[a,b]
PBS 3	-	3.7[a,b]	3.9[a,b]	3.2[a,b]
PBS 4	-	3.6[a,b]	4.0[a,b]	3.3[a]
PBS 5	-	3.7[a,b,c]	3.8[a,b]	3.1[a,b]
HP 1	3.0	2.2[b,c,d]	2.2[c]	1.5[c,d]
HP 2	3.0	2.6[b,c,d]	1.9[c]	1.2[d]
HP 3	3.0	2.4[b,c,d]	2.3[c]	1.6[c,d]
HP 4	3.0	2.7[b,c,d]	2.3[c]	1.9[c,d]
HP 5	3.0	2.8[b,c,d]	2.7[c]	1.6[c,d]
CIN 1	0.5	ND[e]	ND[d]	ND[e]
CIN 2	0.5	2.5[b,c,d]	ND[d]	ND[e]
CIN 3	0.5	1.7[c,d,e]	0.3[d]	ND[e]
CIN 4	0.5	1.4[d,e]	ND[d]	ND[e]
CIN 5	0.5	1.7[c,d]	0.5[d]	ND[e]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CIN: Cinnamaldehyde; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 2.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 5 washes with 1-minute Cinnamaldehyde Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.0^{a}	4.7^{a}	4.0^{a}
PBS 1	-	$3.0^{b,c}$	3.4^{b}	2.6^{c}
PBS 2	-	$3.4^{a,b}$	3.5^{b}	$3.0^{b,c}$
PBS 3	-	$3.5^{a,b}$	$3.6^{b,c}$	3.1^{b}
PBS 4	-	$3.3^{a,b}$	$3.7^{b,c}$	$3.0^{b,c}$
PBS 5	-	$3.4^{a,b}$	$3.8^{b,c}$	3.1^{b}
HP 1	3.0	$1.5^{e,f}$	1.6^{c}	$1.4^{d,e}$
HP 2	3.0	$1.5^{e,f}$	2.1^{c}	1.0^{e}
HP 3	3.0	$2.1^{d,e}$	1.9^{c}	1.8^{d}
HP 4	3.0	$2.0^{d,e}$	2.3^{c}	1.8^{d}
HP 5	3.0	$1.6^{e,f}$	1.8^{c}	1.8^{d}
CIN 1	0.5	1.1^{f}	ND^{d}	ND^{f}
CIN 2	0.5	$1.7^{d,e,f}$	ND^{d}	ND^{f}
CIN 3	0.5	$2.4^{d,e}$	ND^{d}	ND^{f}
CIN 4	0.5	$1.3^{e,f}$	0.5^{d}	ND^{f}
CIN 5	0.5	$2.1^{d,e}$	0.3^{d}	ND^{f}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CIN: Cinnamaldehyde; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 2.3 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 5 washes with 1-minute Cinnamaldehyde Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.5[a]	4.7[a]	4.5[a]
PBS 1	-	4.1[a]	3.4[c,d]	3.8[b]
PBS 2	-	4.2[a]	3.9[b,c]	3.8[b]
PBS 3	-	4.0[a]	3.8[b,c]	3.8[b]
PBS 4	-	4.0[a]	3.8[b,c]	3.6[b]
PBS 5	-	4.6[a]	4.2[a,b]	3.9[a,b]
HP 1	3.0	2.1[b,c]	2.6[e]	2.3[c]
HP 2	3.0	2.7[b]	2.9[d,e]	2.4[c]
HP 3	3.0	2.2[b,c]	2.6[e]	2.3[c]
HP 4	3.0	2.8[b]	2.8[d,e]	2.4[c]
HP 5	3.0	2.9[b]	2.9[d,e]	2.7[c]
CIN 1	0.5	ND[f]	ND[f]	ND[d]
CIN 2	0.5	1.0[d,e]	ND[f]	ND[d]
CIN 3	0.5	1.7[c,d]	ND[f]	ND[d]
CIN 4	0.5	0.8[e,f]	ND[f]	ND[d]
CIN 5	0.5	0.9[e]	ND[f]	ND[d]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CIN: Cinnamaldehyde; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 2.4 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 5 washes with 1-minute Cinnamaldehyde Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.0^{a}	4.2^{a}	3.9^{a}
PBS 1	-	$3.4^{a,b}$	$3.6^{a,b,c}$	3.5^{b}
PBS 2	-	$3.1^{a,b,c}$	$3.7^{a,b,c}$	$3.6^{a,b}$
PBS 3	-	$3.4^{a,b}$	$3.8^{a,b,c}$	$3.6^{a,b}$
PBS 4	-	$3.5^{a,b}$	$3.7^{a,b,c}$	3.5^{b}
PBS 5	-	$3.4^{a,b}$	$3.8^{a,b}$	$3.7^{a,b}$
HP 1	3.0	$2.7^{b,c,d}$	2.8^{c}	2.6^{c}
HP 2	3.0	$3.1^{a,b,c}$	$2.8^{b,c}$	2.5^{c}
HP 3	3.0	$2.9^{a,b,c,d}$	$3.1^{b,c}$	2.5^{c}
HP 4	3.0	$3.1^{a,b,c}$	$2.8^{b,c}$	2.7^{c}
HP 5	3.0	$3.0^{a,b,c,d}$	$3.1^{b,c}$	2.7^{c}
CIN 1	0.5	ND^{h}	ND^{d}	ND^{d}
CIN 2	0.5	$1.9^{e,d}$	ND^{d}	ND^{d}
CIN 3	0.5	$1.6^{e,f}$	ND^{d}	ND^{d}
CIN 4	0.5	$2.1^{c,d,e}$	0.1^{d}	ND^{d}
CIN 5	0.5	$2.0^{c,d,e}$	0.5^{d}	ND^{d}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CIN: Cinnamaldehyde; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 3.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 5 washes with 1-minute Oregano Essential Oil Plant-Derived Compound Treatment Held at 4ºC

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6[a]	4.5[a]	4.5[a]
PBS 1	-	3.6[b]	3.1[b]	3.3[b]
PBS 2	-	3.7[b]	3.6[b]	3.6[b]
PBS 3	-	3.6[b]	3.6[b]	3.5[b]
PBS 4	-	3.9[a,b]	3.5[b]	3.5[b]
PBS 5	-	4.0[a,b]	3.5[b]	3.4[b]
HP 1	3.0	2.2[c]	2.0[c]	2.1[c]
HP 2	3.0	2.3[c]	2.1[c]	1.8[c]
HP 3	3.0	2.1[c]	2.2[c]	1.9[c]
HP 4	3.0	2.2[c]	2.2[c]	1.9[c]
HP 5	3.0	2.6[c]	2.2[c]	2.1[c]
OEO 1	0.5	ND[d]	ND[d]	ND[d]
OEO 2	0.5	ND[d]	ND[d]	ND[d]
OEO 3	0.5	ND[d]	ND[d]	ND[d]
OEO 4	0.5	ND[d]	ND[d]	ND[d]
OEO 5	0.5	ND[d]	ND[d]	ND[d]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; OEO: Oregano Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 3.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 5 washes with 1-minute Oregano Essential Oil Plant-Derived Compound Treatment Held at 4ºC

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6^{a}	4.6^{a}	4.1^{a}
PBS 1	-	3.3^{b}	3.1^{b}	2.9^{d}
PBS 2	-	3.2^{b}	3.3^{b}	$3.0^{c,d}$
PBS 3	-	3.4^{b}	3.4^{b}	$3.2^{b,c}$
PBS 4	-	3.5^{b}	3.6^{b}	3.3^{b}
PBS 5	-	3.7^{b}	3.7^{b}	$3.2^{b,c,d}$
HP 1	3.0	$2.2^{c,d}$	$1.3^{d,e}$	1.2^{f}
HP 2	3.0	$2.1^{c,d}$	$1.8^{c,d}$	1.6^{e}
HP 3	3.0	$2.1^{c,d}$	1.9^{c}	1.7^{e}
HP 4	3.0	2.3^{c}	2.0^{c}	1.7^{e}
HP 5	3.0	$2.2^{c,d}$	1.9^{c}	1.7^{e}
OEO 1	0.5	ND^{e}	ND^{f}	ND^{f}
OEO 2	0.5	ND^{e}	ND^{f}	ND^{f}
OEO 3	0.5	ND^{e}	ND^{f}	ND^{f}
OEO 4	0.5	ND^{e}	ND^{f}	ND^{f}
OEO 5	0.5	ND^{e}	ND^{f}	ND^{f}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; OEO: Oregano Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 3.3 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 5 washes with 1-minute Oregano Essential Oil Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.7[a]	4.7[a]	4.5[a]
PBS 1	-	3.6[b]	3.5[b]	3.4[b]
PBS 2	-	3.8[b]	3.8[b]	3.6[b]
PBS 3	-	3.9[b]	3.9[b]	3.8[b]
PBS 4	-	4.0[a,b]	3.9[b]	3.6[b]
PBS 5	-	4.0[a,b]	4.1[a,b]	3.6[b]
HP 1	3.0	1.9[d,e,f]	1.8[c]	1.4[e]
HP 2	3.0	2.1[c,d,e]	2.3[c]	1.7[d,e]
HP 3	3.0	2.7[c]	2.3[c]	1.7[d,e]
HP 4	3.0	2.6[c,d]	1.7[c]	2.1[c,d]
HP 5	3.0	2.7[c]	2.3[c]	2.4[c]
OEO 1	0.5	ND[g]	ND[d]	ND [f]
OEO 2	0.5	ND[g]	ND[d]	ND[f]
OEO 3	0.5	ND[g]	ND[d]	ND[f]
OEO 4	0.5	ND[g]	ND[d]	ND[f]
OEO 5	0.5	ND[g]	ND[d]	ND[f]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; OEO: Oregano Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 3.4 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 5 washes with 1-minute Oregano Essential Oil Plant-Derived Compound Treatment Held at 4ºC

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.1^{a}	4.1^{a}	4.0^{a}
PBS 1	-	$3.4^{b,c}$	$3.1^{b,c}$	$3.1^{b,c}$
PBS 2	-	$3.4^{b,c}$	$3.3^{b,c}$	$3.3^{b,c}$
PBS 3	-	$3.4^{b,c}$	$3.3^{b,c}$	$3.5^{a,b}$
PBS 4	-	$3.2^{c,d}$	3.4^{b}	$3.2^{b,c}$
PBS 5	-	3.9^{b}	3.5^{b}	$3.2^{b,c}$
HP 1	3.0	2.1^{g}	2.4^{e}	2.1^{e}
HP 2	3.0	$2.6^{e,f}$	$2.9^{c,d}$	$2.4^{d,e}$
HP 3	3.0	$2.8^{d,e}$	2.3^{e}	$2.5^{d,e}$
HP 4	3.0	$2.6^{e,f}$	$2.4^{d,e}$	$2.4^{d,e}$
HP 5	3.0	$2.6^{e,f}$	$2.6^{d,e}$	$2.7^{c,d}$
OEO 1	0.5	ND^{g}	ND^{f}	ND^{f}
OEO 2	0.5	ND^{g}	ND^{f}	ND^{f}
OEO 3	0.5	ND^{g}	ND^{f}	ND^{f}
OEO 4	0.5	ND^{g}	ND^{f}	ND^{f}
OEO 5	0.5	ND^{g}	ND^{f}	ND^{f}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; OEO: Oregano Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 4.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 5 washes with 1-minute Cinnamon Essential Oil Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6[a]	4.5[a]	4.5[a]
PBS 1	-	3.6[b]	3.1[b]	3.3[b]
PBS 2	-	3.7[b]	3.6[b]	3.6[b]
PBS 3	-	3.6[b]	3.6[b]	3.5[b]
PBS 4	-	3.9[a,b]	3.5[b]	3.5[b]
PBS 5	-	4.0[a,b]	3.5[b]	3.4[b]
HP 1	3.0	2.2[c]	2.0[c]	2.1[c]
HP 2	3.0	2.3[c]	2.1[c]	1.8[c]
HP 3	3.0	2.1[c]	2.2[c]	1.9[c]
HP 4	3.0	2.2[c]	2.2[c]	1.9[c]
HP 5	3.0	2.6[c]	2.2[c]	2.1[c]
CEO 1	0.5	ND[d]	ND[d]	ND[d]
CEO 2	0.5	ND[d]	ND[d]	ND[d]
CEO 3	0.5	0.3[d]	ND[d]	ND[d]
CEO 4	0.5	1.7[d]	0.1[d]	ND[d]
CEO 5	0.5	1.7[d]	0.5[d]	ND[d]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CEO: Cinnamon Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 4.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 5 washes with 1-minute Cinnamon Essential Oil Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.6[a]	4.6[a]	4.1[a]
PBS 1	-	3.3[b]	3.1[b]	2.9[d]
PBS 2	-	3.2[b]	3.3[b]	3.0[c,d]
PBS 3	-	3.4[b]	3.4[b]	3.2[b,c]
PBS 4	-	3.5[b]	3.6[b]	3.3[b]
PBS 5	-	3.7[b]	3.7[b]	3.2[b,c,d]
HP 1	3.0	2.2[c,d]	1.3[d,e]	1.2[f]
HP 2	3.0	2.1[c,d]	1.8[c,d]	1.6[e]
HP 3	3.0	2.1[c,d]	1.9[c]	1.7[e]
HP 4	3.0	2.3[c]	2.0[c]	1.7[e]
HP 5	3.0	2.2[c,d]	1.9[c]	1.7[e]
CEO 1	0.5	0.1[f]	ND[g]	ND[g]
CEO 2	0.5	1.0[e]	ND[g]	ND[g]
CEO 3	0.5	1.6[d,e]	0.4[f,g]	ND[g]
CEO 4	0.5	1.9[c,d]	0.4[f,g]	ND[g]
CEO 5	0.5	1.9[c,d]	0.8[e,f]	ND[g]

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CEO: Cinnamon Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 4.3 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 5 washes with 1-minute Cinnamon Essential Oil Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.7^{a}	4.7^{a}	4.5^{a}
PBS 1	-	3.6^{b}	3.5^{b}	3.4^{b}
PBS 2	-	3.8^{b}	3.8^{b}	3.6^{b}
PBS 3	-	3.9^{b}	3.9^{b}	3.8^{b}
PBS 4	-	$4.0^{a,b}$	3.9^{b}	3.6^{b}
PBS 5	-	$4.0^{a,b}$	$4.1^{a,b}$	3.6^{b}
HP 1	3.0	$1.9^{d,e,f}$	1.8^{c}	1.4^{e}
HP 2	3.0	$2.1^{c,d,e}$	2.3^{c}	$1.7^{d,e}$
HP 3	3.0	2.7^{c}	2.3^{c}	$1.7^{d,e}$
HP 4	3.0	$2.6^{c,d}$	1.7^{c}	$2.1^{c,d}$
HP 5	3.0	2.7^{c}	2.3^{c}	2.4^{c}
CEO 1	0.5	0.6^{h}	ND^{d}	ND^{f}
CEO 2	0.5	$1.2^{f,g}$	0.1^{d}	ND^{f}
CEO 3	0.5	$1.7^{e,f}$	0.1^{d}	ND^{f}
CEO 4	0.5	$1.9^{d,e,f}$	0.2^{d}	ND^{f}
CEO 5	0.5	$1.9^{d,e,f}$	0.6^{d}	ND^{f}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CEO: Cinnamon Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 4.4 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 5 washes with 1-minute Cinnamon Essential Oil Plant-Derived Compound Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.1^{a}	4.1^{a}	4.0^{a}
PBS 1	-	$3.4^{b,c}$	$3.1^{b,c}$	$3.1^{b,c}$
PBS 2	-	$3.4^{b,c}$	$3.3^{b,c}$	$3.3^{b,c}$
PBS 3	-	$3.4^{b,c}$	$3.3^{b,c}$	$3.5^{a,b}$
PBS 4	-	$3.2^{c,d}$	3.4^{b}	$3.2^{b,c}$
PBS 5	-	3.9^{b}	3.5^{b}	$3.2^{b,c}$
HP 1	3.0	2.1^{g}	2.4^{e}	2.1^{e}
HP 2	3.0	$2.6^{e,f}$	$2.9^{c,d}$	$2.4^{d,e}$
HP 3	3.0	$2.8^{d,e}$	2.3^{e}	$2.5^{d,e}$
HP 4	3.0	$2.6^{e,f}$	$2.4^{d,e}$	$2.4^{d,e}$
HP 5	3.0	$2.6^{e,f}$	$2.6^{d,e}$	$2.7^{c,d}$
CEO 1	0.5	ND^{i}	ND^{f}	ND^{f}
CEO 2	0.5	0.1^{i}	ND^{f}	ND^{f}
CEO 3	0.5	1.1^{h}	ND^{f}	ND^{f}
CEO 4	0.5	$1.6^{f,g}$	ND^{f}	ND^{f}
CEO 5	0.5	1.5^{h}	0.5^{f}	ND^{f}

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CEO: Cinnamon Essential Oil; 1-5: Reuse Washes 1-5

[2]Values represent average mean of three replications.

[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance

[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)

[5] ND signifies no bacterial survivors were detected.

Table 5.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 5 washes with 1-minute Fulvic Acid Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.3[a]	4.3[a]	4.1[a]
DW 1	-	2.9[b]	2.6[b]	2.3[c]
DW 2	-	3.0[b]	2.3[b]	2.5[b,c]
DW 3	-	3.1[b]	2.9[b]	2.7[b,c]
DW 4	-	3.1[b]	3.0[b]	3.1[b]
DW 5	-	3.4[b]	3.1[b]	2.9[b,c]
FA 1	3.0	2.0[c]	1.0[c]	ND[e]
FA 2	3.0	2.1[c]	1.1[c]	0.5[e]
FA 3	3.0	2.1[c]	1.4[c]	1.1[d]
FA 4	3.0	2.1[c]	0.8[c]	0.4[e]
FA 5	3.0	2.3[c]	1.4[c]	0.3[e]

[1]DW: Distilled Water; FA: Fulvic Acid III; 1-5: Reuse Washes 1-5
[2]Values represent average mean of three replications.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance
[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)
[5] ND signifies no bacterial survivors were detected.

Table 5.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 5 washes with 1-minute Fulvic Acid Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.5[a]	4.1[a]	4.0[a]
DW 1	-	3.0[b]	2.8[b]	2.3[b]
DW 2	-	3.1[b]	2.8[b]	2.3[b]
DW 3	-	3.3[b]	3.1[b]	2.5[b]
DW 4	-	3.2[b]	2.9[b]	2.7[b]
DW 5	-	3.4[b]	3.0[b]	2.7[b]
FA 1	3.0	1.8[c]	1.1[c]	0.5[c,d]
FA 2	3.0	1.9[c]	1.4[c]	0.2[d]
FA 3	3.0	2.1[c]	1.3[c]	1.0[c]
FA 4	3.0	2.2[c]	1.3[c]	0.7[c,d]
FA 5	3.0	1.9[c]	1.1[c]	0.3[d]

[1]DW: Distilled Water; FA: Fulvic Acid III; 1-5: Reuse Washes 1-5
[2]Values represent average mean of three replications.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance
[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)
[5] ND signifies no bacterial survivors were detected.

Table 5.3 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 5 washes with 1-minute Fulvic Acid Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.5[a]	4.1[a]	4.0[a]
DW 1	-	2.9[b]	2.7[b]	2.5[b]
DW 2	-	3.0[b]	2.9[b]	2.6[b]
DW 3	-	3.2[b]	3.2[b]	2.9[b]
DW 4	-	3.1[b]	3.0[b]	3.0[b]
DW 5	-	3.2[b]	3.1[b]	2.9[b]
FA 1	3.0	0.9[d]	0.5[c]	0.6[b,c]
FA 2	3.0	1.5[c,d]	0.9[c]	0.3[c]
FA 3	3.0	1.6[c,d]	0.8[c]	1.0[b]
FA 4	3.0	1.8[c]	0.8[c]	ND[c]
FA 5	3.0	1.9[c]	0.5[c]	0.1[c]

[1]DW: Distilled Water; FA: Fulvic Acid III; 1-5: Reuse Washes 1-5
[2]Values represent average mean of three replications.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance
[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)
[5] ND signifies no bacterial survivors were detected.

Table 5.4 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 5 washes with 1-minute Fulvic Acid Treatment Held at 4°C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1}) Day 0	Day 1	Day 3
Control	-	4.3[a]	4.1[a]	4.0[a]
DW 1	-	2.7[b,c]	3.0[b]	2.7[b]
DW 2	-	2.7[b,c]	3.0[b]	3.0[b]
DW 3	-	2.7[b,c]	3.0[b]	2.6[b]
DW 4	-	3.0[b,c]	3.2[b]	2.9[b]
DW 5	-	3.2[b]	3.2[b]	3.0[b]
FA 1	3.0	2.3[c,d]	2.0[c,d]	1.3[c,d]
FA 2	3.0	2.5[b,c]	1.4[e]	1.3[c,d]
FA 3	3.0	2.6[b,c]	2.1[c]	1.7[c]
FA 4	3.0	2.9[b,c]	1.8[c,d,e]	1.5[c,d]
FA 5	3.0	1.8[d]	1.6[d,e]	1.0[d]

[1]DW: Distilled Water; FA: Fulvic Acid III; 1-5: Reuse Washes 1-5
[2]Values represent average mean of three replications.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance
[4]Statistical groups are separated by column for each day of sampling (0, 1, & 3)
[5] ND signifies no bacterial survivors were detected.

Table 6.1 pH of Antimicrobial and Control Wash Waters on Organic Iceberg Lettuce

Samples		pH of Wash Water from Inoculated Leaf Samples				
Treatments		WW1	WW2	WW3	WW4	WW5
HP	4.71	5.62	6.12	6.41	6.57	6.67
PBS	7.13	7.11	7.09	7.11	7.10	7.11
DW	7.71	7.28	7.32	7.34	7.35	7.35
CAR	7.13	7.13	7.10	7.10	7.08	7.08
CIN	7.02	6.99	6.99	6.99	6.99	6.99
OEO	7.05	7.04	7.02	7.01	6.99	6.98
CEO	6.99	6.96	6.96	6.95	6.94	6.95
FA	2.63	2.80	2.84	2.88	2.91	2.94

1 HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5

[2]Values represent average mean of three replications.

Table 6.2 pH of Antimicrobial and Control Wash Waters on Organic Romaine Lettuce

Samples	pH of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	4.56	5.67	6.14	6.39	6.52	6.64
PBS	7.02	7.02	7.00	7.02	7.01	7.02
DW	7.92	7.45	7.47	7.49	7.47	7.45
CAR	7.04	7.02	7.01	7.01	7.00	7.01
CIN	6.95	6.92	6.93	6.92	6.90	6.92
OEO	6.98	6.99	6.99	6.98	6.98	6.98
CEO	6.92	6.92	6.91	6.92	6.92	6.91
FA	2.92	2.91	2.93	2.95	2.95	2.97

[1] HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

Table 6.3 pH of Antimicrobial and Control Wash Waters on Organic Baby Spinach

Samples	pH of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	4.59	5.52	5.95	6.20	6.37	6.50
PBS	7.04	7.02	7.02	7.02	7.01	7.01
DW	7.38	7.40	7.44	7.43	7.43	7.40
CAR	7.04	7.02	7.01	7.00	7.00	6.99
CIN	6.98	6.96	6.95	6.94	6.93	6.94
OEO	6.96	6.95	6.94	6.94	6.93	6.94
CEO	6.93	6.90	6.91	6.91	6.89	6.89
FA	2.89	2.90	2.90	2.92	2.94	2.95

1 HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

Table 6.4 pH of Antimicrobial and Control Wash Waters on Organic Mature Spinach

Samples	pH of Wash Water from Inoculated Leaf Samples					
Treatments		WW1	WW2	WW3	WW4	WW5
HP	4.71	5.62	6.12	6.41	6.57	6.67
PBS	7.13	7.11	7.09	7.11	7.10	7.11
DW	7.71	7.28	7.32	7.34	7.35	7.35
CAR	7.13	7.13	7.10	7.10	7.08	7.08
CIN	7.02	6.99	6.99	6.99	6.99	6.99
OEO	7.05	7.04	7.02	7.01	6.99	6.98
CEO	6.99	6.96	6.96	6.95	6.94	6.95
FA	2.63	2.80	2.84	2.88	2.91	2.94

1 HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

Table 7.1 Turbidity of Antimicrobial and Control Wash Waters on Organic Iceberg Lettuce

Samples	Turbidity of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	0.02	0.03	0.04	0.05	0.05
PBS	0.02	0.15	0.19	0.23	0.28
DW	0.13	0.09	0.09	0.11	0.12
CAR	0.28	0.32	0.30	0.25	0.29
CIN	0.39	0.57	0.93	1.03	1.20
OEO	0.35	0.40	0.44	0.43	0.41
CEO	1.04	1.44	1.76	1.95	2.14
FA	0.03	0.04	0.05	0.27	0.18

1 HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications

Table 7.2 Turbidity of Antimicrobial and Control Wash Waters on Organic Romaine Lettuce

Samples	Turbidity of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	0.18	0.15	0.41	0.39	0.49
PBS	0.09	0.11	0.31	0.62	0.60
DW	0.01	0.01	0.01	0.03	0.02
CAR	0.24	0.28	0.33	0.40	0.32
CIN	0.42	0.65	1.07	1.28	1.41
OEO	0.38	0.45	0.52	0.58	0.57
CEO	0.51	0.87	1.15	1.31	1.53
FA	0.01	0.02	0.02	0.03	0.03

TMTS Treatments; HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

Table 7.3 Turbidity of Antimicrobial and Control Wash Waters on Organic Baby Spinach

Samples	Turbidity of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	0.02	0.01	0.02	0.02	0.03
PBS	0.00	0.01	0.03	0.02	0.03
DW	0.01	0.01	0.03	0.02	0.03
CAR	0.33	0.48	0.52	0.44	0.42
CIN	0.47	0.64	1.05	1.28	1.48
OEO	0.36	0.55	0.61	0.66	0.71
CEO	0.93	1.16	1.47	1.66	1.77
FA	0.01	0.01	0.01	0.02	0.02

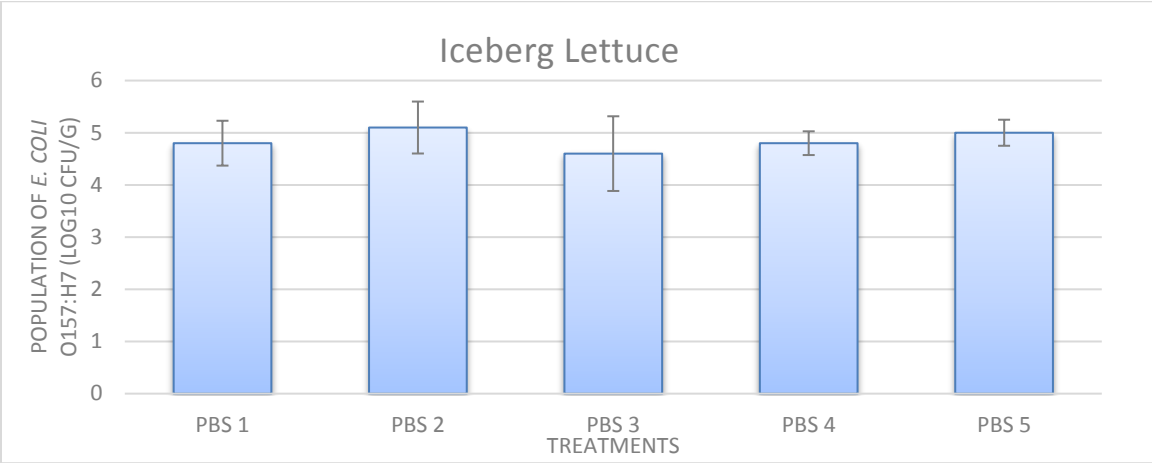
TMTS Treatments; HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

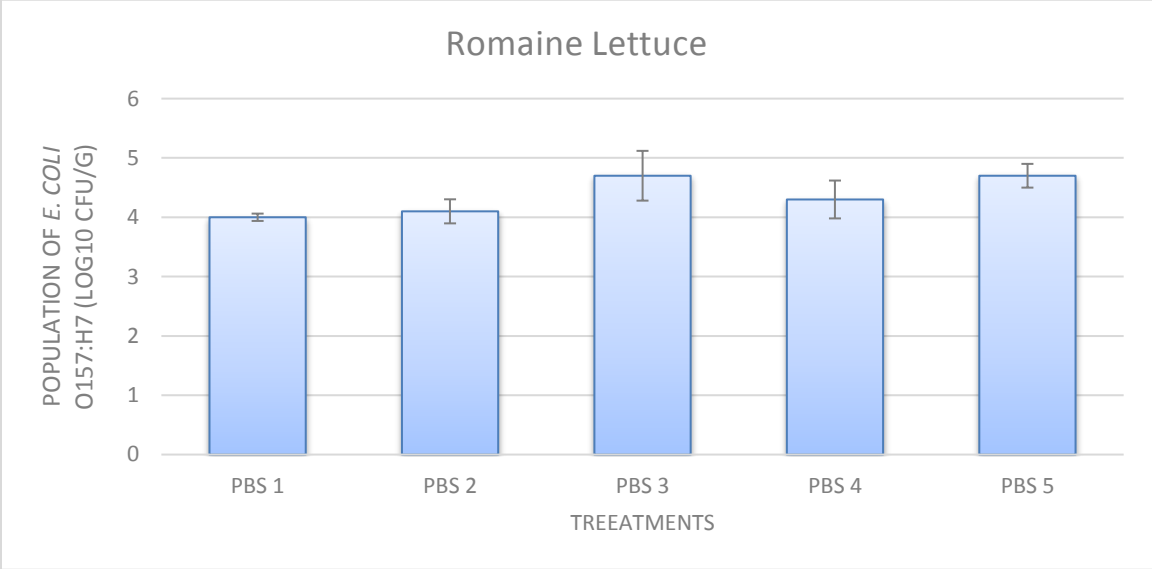
Table 7.4 Turbidity of Antimicrobial and Control Wash Waters on Organic Mature Spinach

Samples	Turbidity of Wash Water from Inoculated Leaf Samples				
Treatments	WW1	WW2	WW3	WW4	WW5
HP	0.02	0.03	0.04	0.05	0.05
PBS	0.02	0.15	0.19	0.23	0.28
DW	0.13	0.09	0.09	0.11	0.12
CAR	0.28	0.32	0.30	0.25	0.29
CIN	0.39	0.57	0.93	1.03	1.20
OEO	0.35	0.40	0.44	0.43	0.41
CEO	1.04	1.44	1.76	1.95	2.14
FA	0.03	0.04	0.05	0.27	0.18

TMTS Treatments; HP Hydrogen Peroxide; PBS: Phosphate Buffered Saline; DW: Distilled Water; CAR: Carvacrol; CIN: Cinnamaldehyde; OEO: Oregano Essential Oil; CEO Cinnamon Essential Oil FA: Fulvic Acid III; WW1: Wash Water 1; WW2: Wash Water 2; WW3: Wash Water 3; WW4: Wash Water 4; WW5: Wash Water 5
[2]Values represent average mean of three replications.

Figure 1. *Escherichia coli* O157:H7 Bacterial Survivors from Enumeration of Wash Water on Organic Iceberg and Romaine Lettuce in Phosphate Buffered Saline

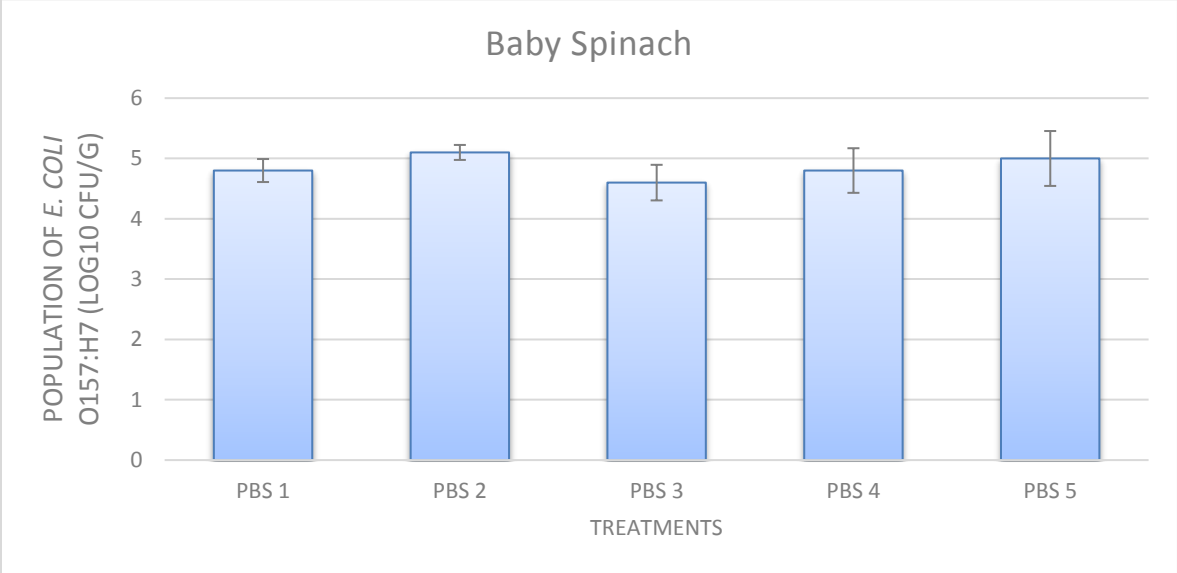


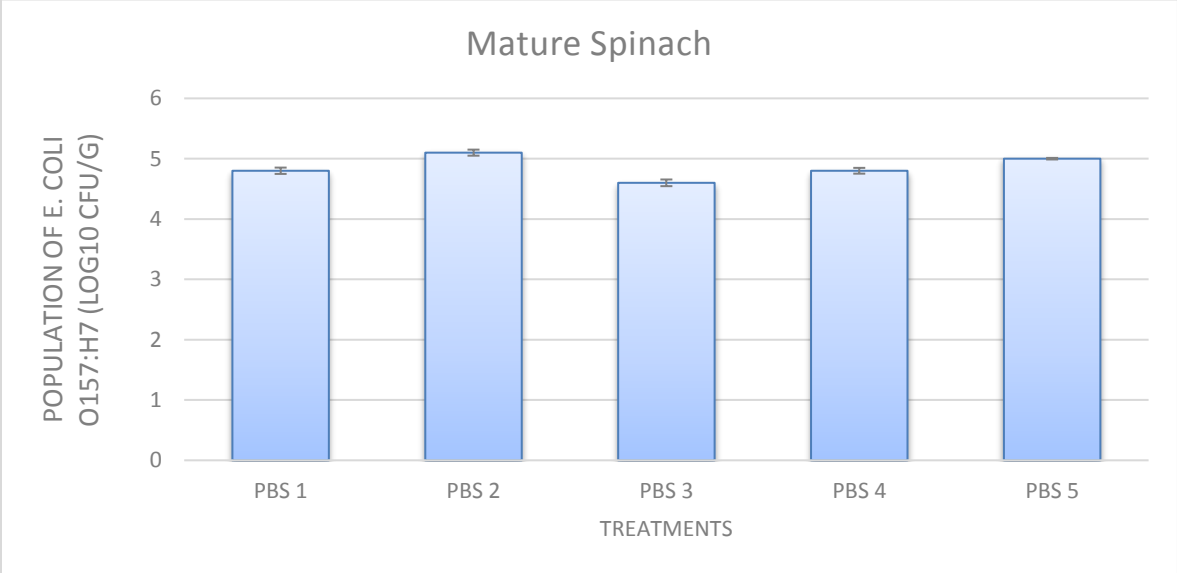


[a]PBS: Phosphate Buffered Saline

[b]Values represent average mean of three replications.

Figure 2. *Escherichia coli* O157:H7 Bacterial Survivors from Enumeration of Wash Water on Organic Baby and Mature Spinach in Phosphate Buffered Saline

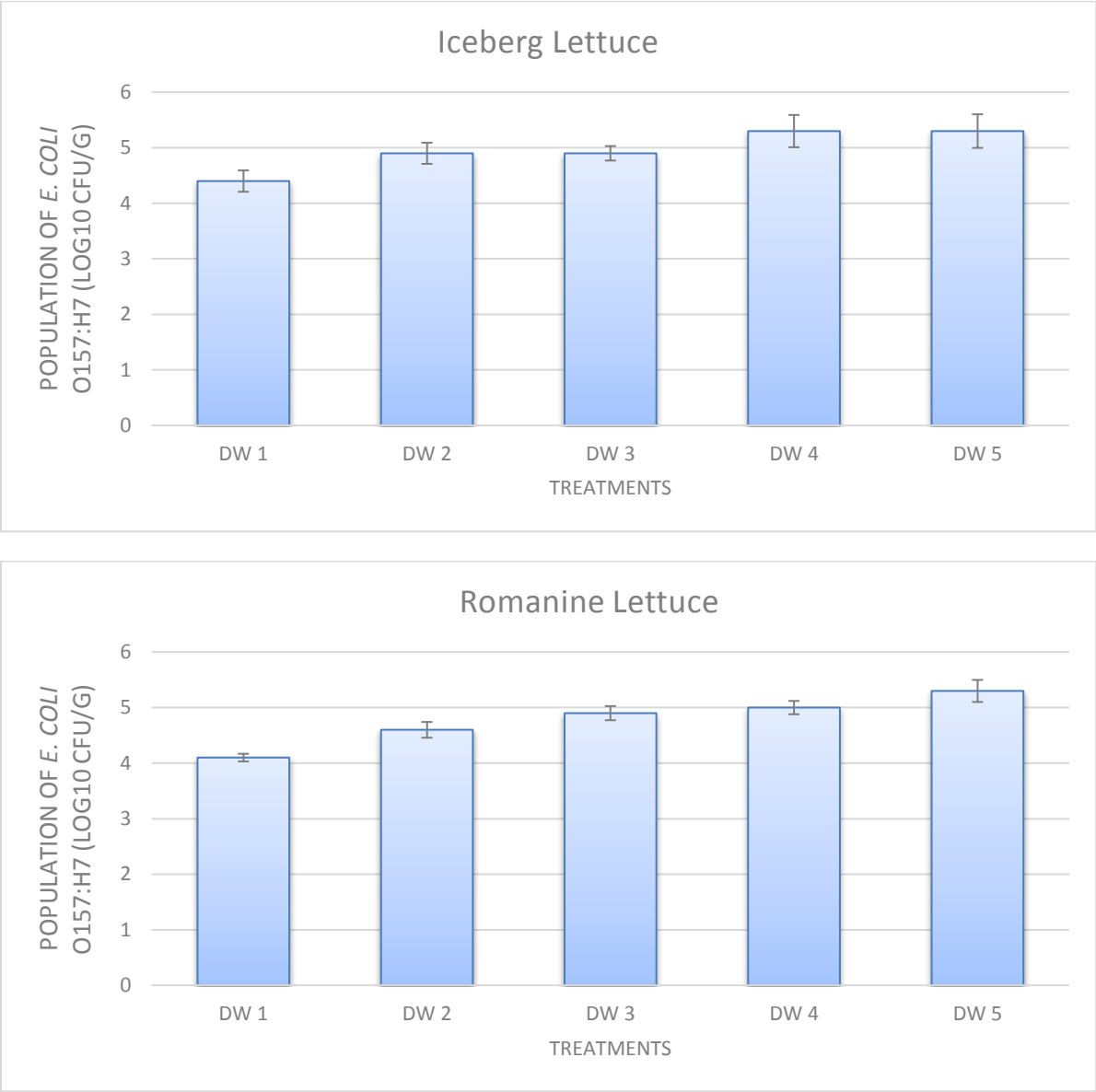




[a]PBS: Phosphate Buffered Saline

[b]Values represent average mean of three replications.

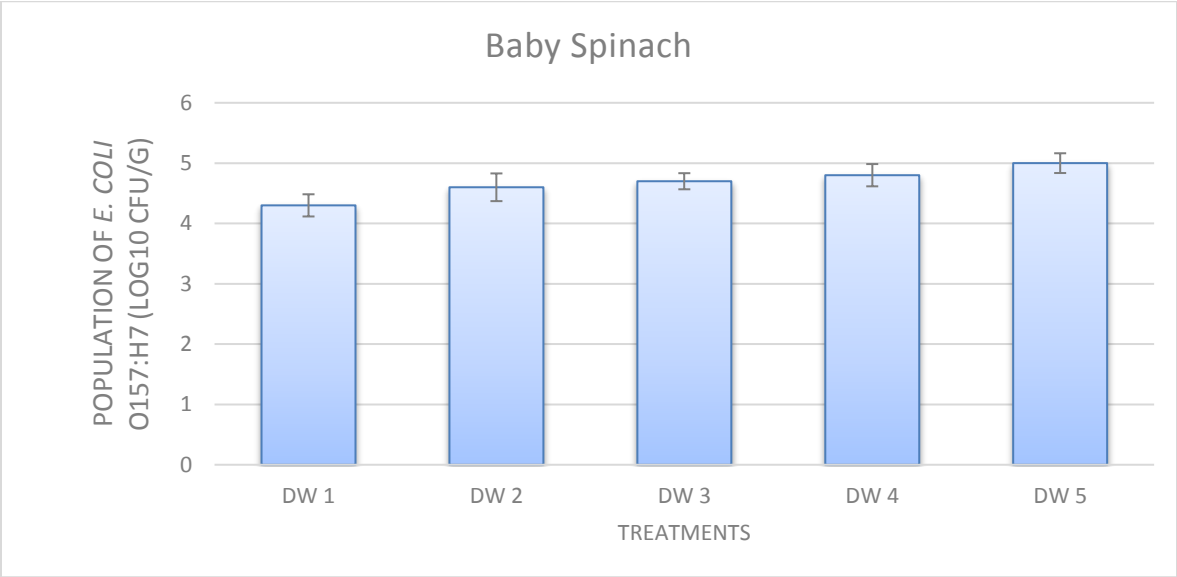
Figure 3. *Escherichia coli* O157:H7 Bacterial Survivors from Enumeration of Wash Water on Organic Iceberg and Romaine Lettuce in Distilled Water

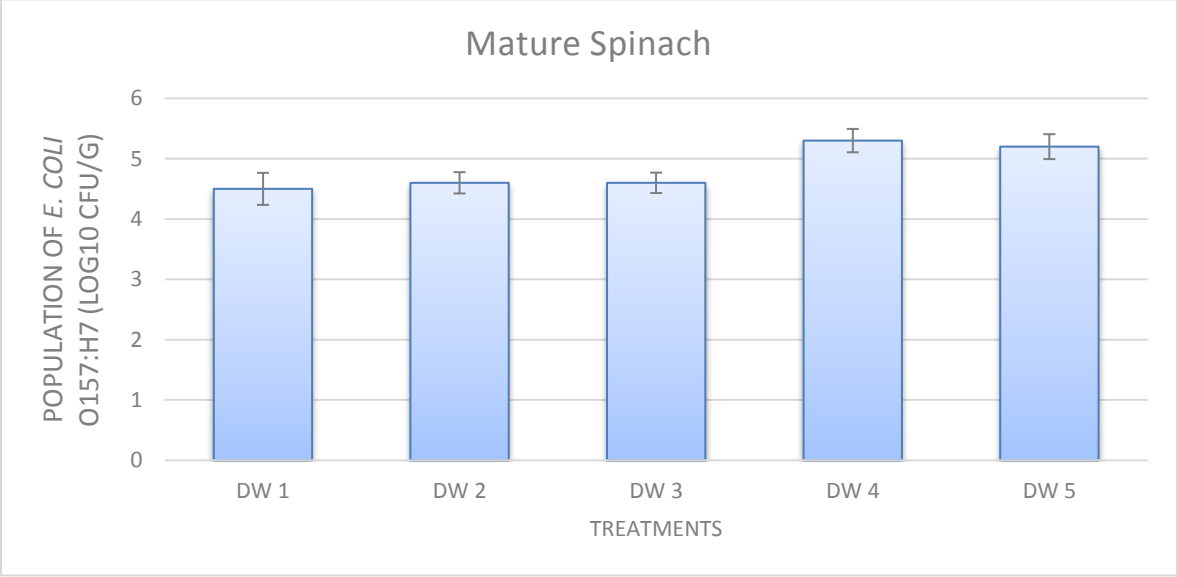


[a]DW: Distilled Water

[b]Values represent average mean of three replications.

Figure 4. *Escherichia coli* O157:H7 Bacterial Survivors from Enumeration of Wash Water on Organic Baby and Mature Spinach in Distilled Water





[a]DW: Distilled Water

[b]Values represent average mean of three replications.

VITA

Justin Wade Brooks

Candidate for the Degree of

Master of Science

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