EVALUATING THE EFFECTS OF ORGANIC SANITIZERS AND PLANT-ANTIMICROBIALS ON HARVESTING EQUIPMENT AND SENSORY PROPERTIES OF ORGANIC LEAFY GREENS

by
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STATEMENT BY AUTHOR

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ABSTRACT

Foodborne outbreaks associated with leafy greens are attributed to many factors including cross-contamination between harvesting equipment and leafy greens. The objectives of the first study were a) to evaluate the efficacy of organic sanitizers and plant antimicrobials on these tools, and b) to determine if modified designs of coring knives are easier to decontaminate in comparison to the original design.

Recently plant extracts and essential oils are gaining popularity due to their antimicrobial properties and being viewed as natural compounds. Studies have shown that plants compounds have antimicrobial activities both *in vitro* and in foods. However, studies regarding the effects of these antimicrobials on the organoleptic properties of foods are limited. The objectives of the second study were to a) perform sensory analysis to identify plant extracts or essential oils with highest preference liking by consumers in organic leafy greens; b) identify the effects of these compounds on sensory attributes of treated organic leafy greens; and c) determine changes in firmness and color properties of treated organic leafy greens.

In order to reduce the strong aroma and flavor characteristics associated with essentials oils and plants extracts, these compounds can be incorporated into edible films. Edible films are thin layer of films made using fruit or vegetable pulp containing plant antimicrobials. The main objective of the last study was to determine preference liking by consumers and changes in physical properties of organic baby spinach treated with antimicrobial edible films.

Three different designs of coring tools were evaluated. Coring tools were inoculated with *S*. Newport and treated with one of the following: deionized water, 50 ppm bleach, 3% hydrogen peroxide, 5% Chico wash™, 0.1% Oregano oil, 0.4% SaniDate 5.0®, 3% fulvic acid, or 0.1% oregano oil for 5 min. The surviving *Salmonella* populations on the tools were determined by

swabbing four different locations on the tools and plating onto xylose lysine desoxycholate (XLD) agar. After inoculation with *Salmonella* overnight culture (8 log CFU/ml), an average of 6.35 log CFU/cm^2 attached onto the original coring tool, 6.31 log CFU/cm^2 on modified design 1, and 6.26 log CFU/cm^2 on modified design 2 coring tools. When comparing the efficacy of sanitizers, 3% H_2O_2 had the highest reductions of 5.98±0.56-6.22±0.29 log CFU/cm^2 in *Salmonella* population. Treatments with 0.39% SaniDate 5.0® and 0.1% oregano oil were comparable (to hydrogen peroxide) which yielded reductions of 5.89±0.80-6.19±0.22 log CFU/cm^2 and 5.51±0.58-5.90±0.46 log CFU/cm^2, respectively. When comparing the four locations on these tools, the greatest reduction was seen at location 2/3 on all three designs of coring tools.

Organic iceberg lettuce and baby spinach were washed with various essential oils, plant extracts, and their combinations in tap water for 2 min. After wash treatment, each sample was stored at 4°C for 20-24 h before performing sensory evaluation and measuring changes in physical properties (color and texture) of leafy greens. A randomized block design with an affective test was used and 60 panelists were asked to evaluate each sample for preference liking based on a 9-point hedonic scale where 9 was extremely liked and 1 not liked at all. Preference liking was evaluated for the following parameters: aroma, color, freshness, mouthfeel, flavor, and overall acceptability. Additionally, panelists quantified each sample using a 5-point hedonic scale for the following attributes: pungency, browning, bitterness, off-odor, and sourness. Changes in firmness values and color of leafy greens were measured using Texture Analyzer and Chroma meter, respectively.

Similar procedure was followed for sensory analysis of baby spinach treated with antimicrobial edible films wherein the edible films were added to bagged spinach. Edible films were made from hibiscus, apple, or carrot pulp which included 0.5%, 1.5%, or 3% of carvacrol or cinnamaldehyde. Forty panelists were asked to evaluate each sample based on preference liking and identify intensity of sensory attributes (pungency, browning, bitterness, off-odor, and sourness). Changes in color and firmness values were measured for organic baby spinach treated with edible films in plastic bags.

Sensory analysis showed that washing organic iceberg lettuce and baby spinach with 0.1% cinnamon oil had the highest preference liking (7-moderately liked) and the least impact on sensory attributes (1-not affected at all) of these leafy greens. Similar results were observed for spinach leaves treated with cinnamaldehyde containing edible films showing higher preference liking values in comparison to those treated with carvacrol containing edible films. Our results also indicated that essential oils had higher impact on the firmness values and plant extracts had higher impact on the color properties of leafy greens.

For textural analysis, washing iceberg lettuce with 0.1% oregano oil in combination with 10% olive extract yielded the highest firmness value ($F=783.1\pm53.8$). For spinach, samples washed with 0.1% lemongrass oil in combination with 1% apple extract yielded the highest firmness value ($F=939.30\pm35.2$). Additionally, no significant difference ($p\leq0.05$) was found in firmness or color values of baby spinach treated with edible films containing plant antimicrobials. Results from the coring tool study will provide alternative organic sanitizer options for washing these tools which are more effective than currently used chemical sanitizers such as bleach. Findings from the sensory study will help in identifying appropriate antimicrobial treatments for washing organic leafy greens. Additionally, use of edible films with essential oils may prevent the adverse effects due to the direct application of essential oils on organic leafy greens.

CHAPTER 1: LITERATURE REVIEW

1.1 An Overview of *Salmonella*

Salmonella is a Gram-negative rod shaped bacterium, which belongs to the family *Enterobacteriaceae*. *Salmonella* is motile with peritrichous flagella (Yan et al., 2004). This bacterium is non-spore forming with size from 0.7-1.5 μm in diameter and 2.0-5.0 μm in length (Bronze, 2005). *Salmonella* is a facultative anaerobe which can reduce nitrate to nitrite, and ferment glucose (Yan et al., 2004). The members of the family *Enterobacteriaceae* are generally non-lactose fermenting, oxidase and urease-negative, citrate-utilizing, acetylmethyl carbinol-negative and potassium cyanide-negative (Yan et al., 2004). The optimum growth of *Salmonella* usually occurs at 37°C, pH levels of 4-8 and water activity conditions above 0.93 (Baird-Parker, 1990). This bacterium has three major antigens: H or flagellar antigen, O or somatic antigen, and Vi antigen, which is only present in some serovars (Ralph, 1996).

Evolutionary evidence indicated that based on phylogenetic linkage, *S*. Typhi strains existed 15,000 to 150,000 years ago (Sánchez-Vargas et al., 2011). With improved sanitary practices, it may seem that the number of illnesses may be reduced; however, with the rise of antibiotic resistant strains, the number of foodborne illnesses are on the rise. Over the past two decades there has been a 25% decrease in the number of foodborne illnesses caused by *Escherichia coli* O157:H7 and *Campylobacter;* however, there is no reduction seen in salmonellosis (Osterholm, 2011).

Most serotypes of *Salmonella* are zoonotic; however, there are certain serotypes such as *S.* Typhi and *S.* Paratyphi which only cause infection in humans (Yan et al., 2004). Clinical symptoms vary widely from common gastrointestinal disease such as diarrhea, vomiting to enteric fever such as typhoid fever. Treatment for *Salmonella* infection also varies from fluid-intakes to various antibiotic therapies and currently there is a vaccine available for typhoid fever (Ralph, 1996). One of the main ways to prevent the spread of *Salmonella* outbreaks is to ensure that proper hygiene is practiced at food service establishments and food is cooked and handled properly. *Salmonella* is associated with many food commodities from chicken to dried nuts; however, it is becoming a major concern in leafy greens. Leafy greens are generally consumed raw; therefore, they pose a higher risk for the survival of foodborne pathogens. Also, chemicals and pesticides are not permitted for use by the organic produce industry, thus increasing the risk of survival of other foodborne pathogens as well.

Salmonella is widely distributed in nature and is a major concern for both human and animal disease. *Salmonella* is a zoonotic organism which is present in the intestinal tract of most farm animals, wild animals, reptiles, amphibians, and arthropods (Baird-Parker, 1990). Even though *Salmonella* is ubiquitous in the environment, there are certain host-adapted serovars specific to animals. For example, it has been known that *S. pullorum* and *S. gallinarum* cause severe disease in poultry but are rare in humans (Baird-Parker, 1990). In animals, *Salmonella* is manifested in four major forms; enteritis, septicemia, abortion, and asymptomatic carriage (Agbaje et al., 2011). *Salmonella* infection in farm animals and its transmission to humans, especially the multi-resistant antibiotic strains, are becoming a major health concern and there is a huge economic loss. *Salmonella* are excreted in high numbers in farm animal feces and can be transferred to various food sources. Another major way for the transfer of *Salmonella* to meat products is via evisceration. *Salmonella* is naturally present in the gut of farm animals, and when they are eviscerated after harvest, contamination can easily be transferred throughout the meat.

Following this chain of contamination, when meat is improperly cooked this allows for the survival of bacterial cells, which can be consumed by humans.

1.1.1 Nomenclature of Salmonella

The nomenclature of *Salmonella* is complex and is periodically being changed. Currently, the nomenclature of *Salmonella* genus followed by the Center for Disease Control and Prevention (CDC) is based on the recommendation of World Health Organization (WHO). There are 2,463 different serotypes which belong to the genus *Salmonella* (Brenner et al., 2000). Currently, *Salmonella* is divided into two species: *S. bongori* and *S. enterica* (Popoff et al., 1998). *Salmonella. enterica* is further divided into six subspecies: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *Salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI) (Yan et al., 2004). Serovars belonging to *S. enterica* subsp. *enterica* are generally named after the geographical location from where they were first isolated (Popoff et al., 1998). Members of each subspecies are identified based on different biochemical tests and their close resemblance is based on genomics. Majority of the *Salmonella* serotypes belong to *S. enterica* subsp. *enterica* (Brenner et al., 2000). The simplified antigenic formulation of *Salmonella* serovars is based on the Kauffmann-White scheme and is updated by the WHO as new serovars are identified (Agbaje et al., 2011). The Kauffmann white scheme is based on the serological identification of O (somatic) and H (flagellar) antigens. The subspecies of *Salmonella* are further subdivided into serovars that are differentiated by flagella, carbohydrates, and lipopolysaccharide (LPS) structure (Coburn et al., 2007).

1.1.2 Outbreaks of Salmonella

It is estimated that 93.8 million cases of gastroenteritis are due to *Salmonella* spp., which lead to 155,000 deaths per year worldwide (Majowicz et al., 2010). More than 95% of cases of *Salmonella* are related to foodborne outbreaks, and in the United States, salmonellosis is accounting for 30% of deaths resulting from foodborne illnesses (Hohmann, 2001). In the United States non-typhoidal *Salmonella* causes an estimated 1.4 million illnesses each year, which accounts for 11% of all foodborne illnesses (Scallan et al., 2011). In the United States, an estimated 1.2 million illnesses of salmonellosis are reported which lead to 19,000 hospitalization and 380 deaths annually (CDC, 2015). The leading cause of hospitalizations has been linked to non-typhoidal species, which account for 35% of all hospitalizations from foodborne infections (Scallan et al., 2011). In recent years, the following food commodities have been associated with *Salmonella* outbreaks: cucumber, frozen chicken, pork, live poultry, and nut butter spreads (CDC, 2015).

Specifically, *Salmonella enterica* subspecies *enterica* serotype Newport (*S.* Newport) causes 100,000 infections annually, which is ranked the top third among the serotypes causing foodborne illnesses from multistate outbreaks (Cao et al., 2014). *S.* Newport is a multi-drug resistant foodborne bacterium that is increasingly becoming common over the last 15 years (Todd, et al., 2013). *S*. Newport is becoming a major public health concern. In the United States, National Antimicrobial Resistance Monitoring System (NARMS) showed that in 1998, only 1% of *S*. Newport isolates were MDR-AmpC phenotype, however, that number increased to 26% by 2001 (Switt et al., 2009). In addition, there is an increase in outbreaks associated with leafy greens and *Salmonella* has been known to be one of the main bacterial causative agents involved in these outbreaks.

The United States probably has one of the best measures in terms of food safety, yet there are billions of dollars lost each year due to foodborne pathogens. The cost associated with *Salmonella* incidences is reaching up to several billion dollars annually and the cost may be much higher as only 2% of all cases are reported to the CDC (Lee et al., 2015). It is estimated that the total economic burden due to *Salmonella* in the United States is around $2.8 billion annually. The majority of this cost is associated with premature death in infants, which is followed by the average cost of hospitalizations. With the increasing problem of foodborne illnesses and the cost associated with these illnesses, there needs to be a stronger enforcement in controlling these outbreaks, and more research needs to be conducted in order to combat the problem of foodborne illnesses.

Salmonella outbreaks are associated with multiple food commodities; however, they are commonly linked to high-risk foods such as eggs, meat, poultry, fruits, and vegetables. The CDC reviewed all outbreaks of *Salmonella* from 1998-2008 and calculated the frequency and percentage of outbreaks associated with each food commodity. In this study, it was found that there were 1,491 outbreaks of *Salmonella* from 1998-2008, and of those outbreaks, only 595 outbreaks were linked to specific food commodities. In these outbreaks, the most common serovars included *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, and *S.* Heidelberg. The main finding from this study was that eggs (112 outbreaks, 28%) were the most common food commodity implicated in *Salmonella* outbreaks. These outbreaks were further associated with chicken (64 outbreaks, 16%), pork (37 outbreaks, 9%), beef (33 outbreaks, 8%), fruits (33 outbreaks, 8%), and turkey (28 outbreaks, 7%).

Many large outbreaks during the past decade have been associated with fresh food commodities such as apple cider, cantaloupe, raspberries, bagged lettuce and spinach, tomatoes, green onions, and sprouts (Brackett, 1999; Beuchat, 2002). Among fresh produce, leafy greens are the major commodities involved in outbreaks.

1.1.3 Symptoms of salmonellosis

Salmonella infection can occur in any individual; however, immunocompromised individuals are at higher risk. Non-typhoidal *Salmonella* generally causes common gastrointestinal disease such as vomiting, diarrhea, and cramps with or without fever. Other symptoms include anorexia, abdominal pain, headache, and blood in the stool (D'Aoust, 1991). The infection in the patients may initiate in one location, however, it can spread rapidly throughout the body if not treated with proper antibiotics. A small percentage of patients (<5%) may also develop extra-gastrointestinal infection, including bacteremia (Yan et al., 2004). There are several strains of *Salmonella*, which can cause bacteremia, and usually invasive infection arises when *Salmonella* cells are able to gain entry through the epithelial layer. *Salmonella* can also invade non-phagocytic cells of the intestinal epithelial tissues (Peyer's patch), causing gastroenteritis. The infectious dose of *Salmonella* is generally 10^5 cells in a healthy individual and lower among children and immunocompromised individuals. Epidemiological evidence has shown that 10-100 bacterial cells can cause illness in young children and elderly individuals, especially if the organism is present in a high-fat food such as cheese or hamburgers (Baird-Parker, 1990). The incubation period of *Salmonella* depends on the dose of bacteria, but symptoms generally develop 6 to 48 hours after ingestion of contaminated food or water (Ralph, 1996). The symptoms can last anywhere from two to seven days. The initial symptoms are self-limiting, however, in 5% of the infected patients, there is a chance for the development of bacteremia and other invasive manifestations which are generally associated with certain serotypes, patient's age, and immunosuppression (Ram et al., 2008). In about 15% of all

Salmonella infections, patients are considered to be asymptomatic carriers of the bacterial cells for two months (D'Aoust, 1991).

Human salmonellosis is generally self-limiting with primary treatment being fluid and electrolyte replacement. In severe cases, antibiotics are used, however, one drawback with antibiotics is that they have little clinical improvement and the carrier state is prolonged. Commonly used antibiotics to combat salmonellosis include Chloramphenicol and Trimethoprim- sulfamethoxazole (TMX).

It is estimated that enteric fever causes 200,000 deaths and 22 million illnesses each year worldwide, predominantly in developing nations (Crump et al., 2004). Enteric fever is the severe form of salmonellosis with the most common being typhoid fever which is caused by *S.typhi* (Ralph 1996). Generally, infection from *S. typhi* and *S. paratyphi* results in invasion of extra-intestinal tissue, which leads to the systemic spread of the pathogen. The symptoms of enteric fever are nonspecific and include fever, anorexia, headache, myalgias, and constipation (Ralph, 1996). The incubation period for enteric fever ranges from 6-30 days with an onset of illness around 4 days with fever at 102°F-104°F (38°C-40°C) (Newton, 2015). During enteric fever, there is an initial low-grade fever, which is generally followed by a high-sustained fever in the second week. *Salmonella typhi* is generally spread by consumption of contaminated food and water; therefore, it is more prevalent in developing nations. The best method for prevention of typhoid fever is ensuring proper sanitation, and in the United States, there is a vaccine available for *S. typhi* mainly for travelers. With the rise of multi-drug resistant strains, it is becoming challenging to treat enteric fever, especially in immunocompromised individuals such as children and the elderly. Currently, ciprofloxacin is the first line therapy, however, due to rise in antibiotic resistance, fluoroquinolone for 5-7 days is the secondary treatment (Sánchez-Vargas et al., 2011).

1.1.4 Pathogenicity and virulence factors of Salmonella

The initial step in *Salmonella* infection is gaining entry into the host cell and this is done by membrane ruffling. Ruffling in the host membrane is done by *Salmonella* proteins which will modify the actin cytoskeleton of the host cell and thus allow the entry of bacterial cells (Bourdet-sicard & Nhieu, 1999). One of the mechanisms by which *Salmonella* is able to persist in the host cell is by escaping the host immune system and these virulence factors, along with other virulence traits have been known to transfer via horizontal gene transfer and integrate into the bacterial chromosomes. *Salmonella* is able to cross the small intestine by invading the M cells of the Peyers' patch and destroying the M cells and the adjacent epithelium cells (Groisman & Mouslim, 2000).

Another virulence factor of *Salmonella* is the pathogenicity island (SPI), and so far, five islands have been identified. The *S.* Newport genome contained SPI-I through SPI-4 sequence and there was a vast diversity at the region around *mutS* downstream of SPI-I (Cao et al., 2014) The main factor for *Salmonella* to be considered pathogenic is that it must be able to gain entry into non-phagocytic host cells and be able to quickly adapt to a wide host range.

The type III secretion system (TTSS-1) was initially identified as the virulence factor which was required for the entry of *Salmonella* into the host cells (Zhang et al., 2003). The main function of TTSS-1 is to translocate effector proteins into the cytosol of host cells (Galan, 2001). Some other specific features of TTSS are a) absence of cleavable proteins secreted via *sec*-dependent pathways (secretion dependent), b) requirement of host cell for activation of secretion pathways, and c) presence of customized accessory proteins for secretion and translocation

(Galan, 2001). *Salmonella* has two types of TTSS; the first one is located on centromere 63 of the chromosome pathogenicity island I (SPI-1), and the second one at centromere 31 of the pathogenicity island II (SPI-2) (Hensel, 2000). Type III secretion system II (TTSS-2), encoded by *Salmonella* pathogenicity island-1 (SPI-1) and pathogenicity island-2 (SPI-2) is also used to inject bacterial effector proteins into the host cells (Lahiri et al., 2010). Both SPI-1 and SPI-2 are encoded by type III secretion systems, which consist of multiple protein complexes resulting in translocation of effector proteins across the host cell and directly into the epithelium of the cell's cytoplasm (Sánchez-Vargas et al., 2011). Furthermore, SPI-1 is present in all serotypes of *Salmonella* and is required for invasion of non-phagocytic host cells (Lahiri et al., 2010). There are four main proteins which are injected through SPI-1 type III secretion system which allows proteins to be exported through the host plasma membranes: SopE, SipA, SipC, and StpP (Groisman & Mouslim, 2000). SPI-2 is required for intracellular survival and systemic pathogenesis (Lahiri et al., 2010). Furthermore, the ability of *Salmonella* to sustain inside the macrophages is mediated by SPI-2. In conclusion, *Salmonella* is the only organism that uses two distinct type III secretion systems, first by destroying the host cell, and second by invading the macrophages to hide from the host's immune system (Groisman & Mouslim, 2000). In order for *Salmonella* to be considered virulent, it must have the following traits: a) ability to invade host cells, b) lipopolysaccharide coat, c) ability to replicate inside the host cell, and d) elaboration of toxins (Ralph, 1996).

1.1.5 Detection of Salmonella

Salmonella are detected using common microbiology lab techniques such as Gram staining, growing onto selective and differential media, and molecular techniques such as polymerase chain reactions (PCR). Additionally, biochemical tests can be used for the identification of *Salmonella* as they only ferment certain sugars. A commonly used differential medium for the identification of *Salmonella* is xylose lysine desoxycholate (XLD) agar in which *Salmonella* ferments xylose and produces hydrogen sulfide, which gives rise to black colonies indicating the presence of *Salmonella.* Detection methods for *Salmonella* have been standardized by many regulatory agencies such as the FDA, International Organization for Standardization (ISO), Association of Official Analytical Chemists (AOAC) and the Food Safety and Inspection Service (FSIS) of the USDA. The current ISO standards for *Salmonella* identification consist of a pre-enrichment of samples in buffered peptone water (BPW) followed by a selective enrichment in Rappaport–Vassiliadis (soya base) (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) media (Lee et al., 2015). Commonly used media for the identification of *Salmonella* include *Salmonella-Shigella* agar (SS), brilliant green agar (BGA), bismuth sulfite agar (BSA), Hektoen enteric (HE) agar, and XLD agar (Lee et al., 2015). Change in the color of the media and colony themselves can be used for the identification of *Salmonella* (Mallinson et al., 2000). Conventional methods are useful in food microbiology as they are easy to use and economically feasible; however, to avoid false positives and for rapid results, researchers are now using molecular techniques for rapid *Salmonella* detection. Using molecular cloning and recombinant DNA techniques, immunology-based assays and nucleic acid-based assays have been developed (Alakomi et al., 2009). Of all the new rapid detection methods, PCR and Enzyme-linked immunosorbent assay (ELISA) have shown comparable specificity and sensitivity to conventional methods (Lee et al., 2015). Latex agglutination assay is another commonly used assay for the identification of *Salmonella.* The principle of this assay is based on the antibodies which will react with the antigen present on the surface of *Salmonella* to form visible aggregates (Thorns et al., 1994). Currently, there are numerous detection methods, which are available, based on many criteria such as sensitivity, specificity, and cost effectiveness. However, due to an increase in foodborne illnesses and complex food models, other rapid and accurate detection methods are needed which can be utilized on the site of outbreak for a rapid response.

1.1.6 Antibiotic Resistance in Salmonella

Antibiotic resistance has become a major challenge worldwide, as even the new drugs are not effective against some pathogens. Antibiotic resistance in foodborne pathogens is especially a major concern because; the resistance could spread rapidly due to the extensive and prolonged usage of antibiotics in livestock food animals. Antibiotic resistance is not only becoming a concern for pathogenic organisms, but also for the endogenous flora of humans and animals. There have been numerous studies showing evidence of the transfer of resistance genes from zoonotic infection to human pathogens (Chen et al., 2013). Many of the enteric pathogens such as *Salmonella* and *E. coli* O157:H7 are naturally present in the gut of farm animals and use of antibiotics on these animals could pose a risk for increasing the resistance among human pathogens. Use of antibiotics as a growth promoter has become a major issue for the animal industry due to the ongoing controversy to ban use of growth promoters on food animals. Antibiotic resistance in *Salmonella* has increased from 20-30% in 1990 to almost 70% at the beginning of 21st century (Chen et al., 2013).

Due to an increase in the spread of resistance, extended-spectrum antibiotics are used to treat *Salmonella* infections, however, many developing nations are reporting *Salmonella* strains that are resistant to even these broad-spectrum antibiotics (Su et al., 2004). In the United States, a national survey conducted in 1994-1995 found that, of 4008 isolates of *Salmonella* tested, 21 (0.5%) were resistant to nalidixic acid and by 2000 there was a 5-fold increase in resistance to nalidixic acid (Su et al., 2004). Mechanism of resistance to fluoroquinolones in *Salmonella* is mostly due to mutations in the quinolone-resistance determining-region (QRDR) of the DNA gyrase genes (Cloeckaert & Chaslus-Dancla, 2001).

1.2 Sources of Contamination

Cross-contamination of produce is generally divided into two categories: pre-harvest and post-harvest. Transfer of contamination on to produce can occur via multiple routes. Pre-harvest contamination generally results via contact of produce with the following: soil, feces, irrigation water, insecticides, dust, or even human handling (Beuchat, 2002). It has been known that foodborne pathogens are shed in the environment through feces of animals and some organisms such as *Listeria monocytogenes* are naturally found in the environment (Ivanek et al., 2006). In the organic industry manure may be used as a fertilizer which could pose a potential risk as a source of pathogens to contaminate fruits and vegetables (Beuchat, 2002). Studies conducted on the bovine feces samples indicated that *E .coli* O157:H7 is able to survive for 42-49 d at 37˚C and 49-56 d at 22˚C (Wang et al., 1996). Prolonged survival of foodborne pathogens in the environment increases the risk of cross-contamination between these pathogens and produce.

Foodborne outbreak investigations can be characterized into five major phases: surveillance/detection, epidemiology, environmental/trace back, regulatory/enforcement, and prevention/research (Matthews et al., 2009). Even with these strategic investigations, it is much harder to pinpoint what exactly causes an outbreak on the field. The World Health Organization (WHO) conducted some field studies, which show that crop production, location, packaging house, and processing facilities have been linked to produce contamination (WHO, 2008).

1.3 Transfer of Contamination to Leafy Greens in the harvesting field

As the consumption of organic leafy greens increases, there is also a potential for a rise in foodborne illnesses, as organic growers are limited to the usage of only certain chemicals to reduce the bacterial loads on leafy greens. The limitation of chemical sanitization in organic crop production during pre-harvest may provide an opportunity for foodborne pathogens to survive and cross-contaminate food products post-harvest. There are several sources of cross-contamination to leafy greens including, manure from domesticated animals, application of irrigation water from contaminated water source, contact with humans, or fecal-contamination from wild life. Additionally, pathogens can be transferred from contact with a contaminated field and harvesting equipment, and contact with bio-aerosols. Studies have shown that current sanitizing practices on the field only result in about 1-2 logs reduction of microbial load due to pathogen survival in biofilms or due to neutralization of sanitizer in the presence of organic matter (WHO, 2008; UGA 2011; Holvoet et al., 2012).

Many investigations have suggested that the point of contamination for most produce-associated outbreaks originates in the farm during growing and harvesting (Matthews et al., 2009). In addition, irrigation water plays a crucial role in transferring pathogens on to produce. According to the 2008 Farm and Ranch Irrigation Survey, the vast majority of water to horticultural crops in the US is applied by sprinkler-irrigation (USDA, 2008). A study showed that surface drip irrigation system had 1 log higher reduction of *E. coli* on lettuce compared to flood-irrigation system (Song et al., 2006). Generally, lettuce heads are harvested by hand, cored, trimmed, sprayed with sanitizing wash, bagged, and boxed in the field (Matthews et al., 2009). Therefore, it is crucial that each of these practices is studied in detail in order to determine prevention strategies of cross-contamination from field on to produce.

1.4 What is coring tool?

Coring tool is a harvesting equipment used by farmers to core lettuce heads on the field before they are packaged for retail. Generally, whole lettuce heads that not destined for the fresh-cut market are not sanitized after being cored. Thus, coring tool is one of the last equipment to which leafy greens come into contact with prior to being packaged and sent to retail stores. Coring in the field (CIF) is relatively a recent concept which was initiated in the 1990s to increase processing plant yields, decrease non-marketable tissue, and reduce shipping costs (Zhou et al., 2012). Removing the outer leaves and coring in the field has increased processing plant production from 60-70% to almost 100% (Yang et al., 2012). However, coring increases the risk of cross-contamination since leaves come in direct contact with the knives. There is also significant amount of tissue damage, and additional human handling poses an additional risk. Barker-Reid et al. (2009) showed that *E. coli* had much greater persistence on damaged iceberg lettuce plant tissues than on undamaged plants. Thus, it is crucial that coring tool is disinfected regularly and is kept free from contamination to prevent further outbreaks associated with leafy greens.

Coring knives can be contaminated via multiple routes such as direct contact with soils, plants, or workers gloves; therefore, they can easily transfer microbes onto lettuce leaves which may be used for human consumption (Suslow et al., 2003). Another major concern with coring tools is that they are constantly used in the field for up to 3-4 hours before they are sanitized, thus, providing a niche for microbes to grow, especially in the presence of organic matter and in temperature abuse conditions. Several studies have shown that if coring knives are contaminated, then pathogens can be transferred to multiple lettuce heads, as workers use them for hours before sanitizing them. A study conducted by researchers at the Yuma Agricultural Center in Yuma, AZ showed that contamination could be transferred to as many as 75 heads of lettuce with a single contaminated coring knife (Fallon et al., 2011).

1.5 Usage of chlorine and the need for alternative organic sanitizers

The traditional methods of cleaning produce and equipment in order to reduce the microbial load is via physical or chemical treatments. Cleaning and sanitizing methods generally involve application of water, use of chemicals (bleach/detergents), and mechanical treatments by brush or scrub (Parish et al., 2003). One of the most frequently used sanitizers include chlorine-based sanitizers such as bleach due to their low cost compared to other sanitizers. However, studies have shown that chlorinated wash water has <2 logs reduction on leafy greens (Park et al., 2011). In addition, there is a potential for the formation of carcinogenic by-products when chlorine reacts with the organic matter, thus, increasing the risk of workers being exposed to high toxicity (Sapers, 2001).

Studies have shown that in the presence of organic matter, chlorinated water produces carcinogenic by-products. Trihalomethanes cause liver and renal tumor in mice (Komulainen, 2004). Additionally, the pH of chlorine-based sanitizers must be kept between 6.5-7.5 in order to be effective against microbial loads; therefore, bleach must be constantly added in the wash water as it can quickly loose the efficacy in the presence of organic matter. Maintaining the pH of chlorine-based sanitizers is a difficult task at the farm since generally, the sanitizing buckets are only being replaced every 4-5 h, despite severe weather conditions. In addition, chlorine-based sanitizers have been shown to be corrosive to metals, so it poses another problem for harvesting equipment to be properly sanitized. Additionally, the concentration of hypochlorous acid (HOCl) is significantly impacted by temperature, presence of organic matter, light, air, and metals (Parish et al., 2003). It has been suggested that the temperature of chlorinated water must be maintained at least 10°C higher than produce item in order to reduce microbial infiltration which can be casued by pressure differential (Parish et al., 2003). With all these concerns, organic growers are looking for alternative options for organic sanitizers.

1.5.1 Alternative organic Sanitizers

Currently in the organic industry, options for available organic sanitizers are limited and this industry is further seeking alternative options. The National Organic Standards Board (NOSB) and the National Organic Program (NOP) have approved some organic sanitizers, but more sanitizers need to be evaluated to show their efficacy against foodborne pathogens. Commonly used organic sanitizers in the industry include: hydrogen peroxide, chlorine with maximum final residual at 4 mg/L, peroxyacetic acid, acetic acid, alcohol, ammonia-based sanitizers, and ozonated water.

There is not one specific sanitizer, which is more effective compared to others. There are multiple factors that are involved in determining the efficacy of sanitizers including; disinfectant type, dosage, residual concentration, contact time, temperature, pH, presence of organic matter, and other physicochemical properties (Sapers, 2001).

Organic acids have been generally regarded as safe (GRAS) to be used as food ingredients and some studies have shown antimicrobial activity of these organic acids against foodborne pathogens (Park et al., 2011).On iceberg lettuce, the efficacy of lactic, acetic, citric, and ascorbic acids was tested against *E. coli* and *L. monocytogenes* (Akbas and Olmez, 20007). Also, the combination effect of lactic and acetic acids with chlorine was tested against *L. monocytogenes* on shredded lettuce (Zhang & Farber, 1996). The maximum log reductions of *L. monocytogenes* using chlorine were 1.3 logs (Zhang & Farber, 1996). In addition, they showed that lactic acid was more effective against *L. monocytogenes* than acetic acid (Zhang & Farber, 1996). The efficacy of propionic, acetic, lactic, malic, and citric acids against *E. coli* O157:H7, *S*. Typhimurium, and *L. monocytogenes* has been evaluated on organic fresh apples and lettuce (Park et al., 2011). All three organic acids showed significant ($P<0.05$) differences in pathogen reduction on lettuce contaminated with *Salmonella, E.coli,* and *L. monocytogenes* compared to the control (distilled water) (Park et al., 2011).

At higher concentrations, organic acids can be used to decontaminate food products (Virto et al., 2005). Since organic acids are weak acids, they can exist in pH dependent equilibrium between dissociated and undissociated stage (Van Haute et al., 2013). Exact mechanisms of how these acids inactivate foodborne pathogens are not known, but a common belief is that there will be a membrane disruption of bacterial cells. Citric acid has been known to be effective against both Gram positive and negative organisms and the acid acts as both an acidifying and a sequestering agent. It has been demonstrated that the sequestering agents will bind to insoluble metal complexes and make them water soluble (Lee at al., 2007)

Another type of organic acid which is recently gaining popularity is fulvic acid. Fulvic acid is a type of humic acid with low molecular weight that is generally used as a soil supplement (Sherry et al., 2012). Carbohydrate-derived fulvic acid is a heat stable, low molecular weight, water-soluble, cationic, colloidal material with proposed therapeutic properties (Sherry et al., 2012). Fulvic acid has been known to have antibacterial and antifungal activity (Van Rensburg et al., 2000). Additionally, fulvic acid was shown to be non-toxic in a rat model with anti-inflammatory properties (Sabi et al., 2011). Fulvic acid is a microbicidal compound that acts nonspecifically on the bacterial cell membrane (Sherry et al., 2012). The membrane permeability assay has shown that the bacterial cellular ATP content was released in the presence of fulvic acid (Sherry et al., 2012).

Another commonly used sanitizer approved for use by the organic industry includes peroxyacetic or peracetic acid (PAA). Peracetic acid is produced from a reaction of acetic acid with hydrogen peroxide in the presence of sulfuric acid (Kitis, 2004). The exact mechanism of how peroxyacetic acid is effective against foodborne pathogens in model food systems is unknown; however, it could act as an oxidizing compound. PAA oxidizes the sulfhydryl bonds in proteins, enzymes, and other metabolites, which leads to an increase in cell permeability (Herdt et al., 2009). Some advantages of PAA include efficacy at low concentrations and in the presence of organic matter (Vandekinderen et al., 2009). Another major advantage of PAA is that it is effective under a wide range of pH and temperature conditions. Even with all these advantages, since PAA is relatively a new sanitizer in the market, it is more expensive in comparison to other commonly used sanitizers, and additional studies need to be further conducted to show its efficacy against various foodborne pathogens. The cost of PAA is a major disadvantage for its widespread application in the USA, as it is generally four to five times more expensive compared to hypochlorite (Kitis, 2004). Another concern about PAA is the allowed concentration for use by the industry. FDA has determined that the maximum allowed concentration of PAA in wash water should be 80 ppm (actual concentration in water) for washing fruits and vegetables (FDA code title 21).

1.6 Antimicrobial activity of plant extracts and essential oils

Essential oils (EOs) also referred to as volatile compounds are aromatic oily liquids which are obtained from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, woods, fruits, and roots (Burt, 2004). These essential oils are extracted from the plant material via expression, fermentation, effleurage, and steam distillation (VanWelie, 1997). The method used to extract essential oils may have an impact on antimicrobial properties as the organoleptic profiling could be different depending on the extraction method (Burt, 2004). It has been shown that essential oils extracted using hexane, have better antimicrobial activity than those extracted using steam distillation (Packiyasothy and Kyle, 2002). Essential oils consist of numerous components and the analysis of these components can be done by mass spectroscopy or gas chromatography. The phenolic compounds present in these EOs are the primary factors, which are responsible for antibacterial properties (Cosentino et al., 1999).

EOs have been known to have antimicrobial activity against various foodborne pathogens and they are GRAS-listed by the FDA; therefore, they are gaining popularity as natural sanitizers or preservatives in the food industry. EOs are plant secondary metabolites which are biosynthesized in the granular structures of plant cells (Bajpai et al., 2011). Numerous studies have been conducted which show that these EOs have antimicrobial activity against foodborne pathogens (Burt & Reinders, 2003). To some extent all EOs have been known to exert antimicrobial activity; however, there is variation in their chemical structure due to the abundance or lack of mineral content, changes at genetic levels, and the impact of environmental changes (Salgueiro et al., 1997). Numerous studies have also shown that these EOs, which are present in low quantities, have synergistic effects against bacteria (Paster et al., 1995).

Essential oils have been known to have antibacterial, antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties (Burt, 2004). Usage of EOs varies from flavoring agents to perfumes and in aromatherapy. Due to their antibacterial activity, the essential oils have been applied in the following: dental root canal sealer, antiseptics, feed supplements for lactating cows, and more. Also, research has shown that they can be used as food preservatives. Essential oils of *Origanum majorana* L., *Coriandrum sativum* L., *Hedychium spicatum*, *Commiphora myrrha* and *Cananga odorata* showed above 50% protection of chickpea seed from *Aspergillus* *flavus* infestation (Prakash et al., 2012). Additionally, carvacrol and cinnamaldehyde were effective against antibiotic resistant *S. enterica in vitro* and on celery and oysters (Ravishankar et al., 2010).

Recently there has been a trend showing an increase in the consumptions of organic food, as consumers are becoming aware of the impact of pesticides and synthetic products on their health and the environment. Therefore, consumers and organic industry are further seeking alternative treatment options, which may be effective against biological pathogens. *In vitro studies* have suggested that EOs have antibacterial activity against *L. monocytogenes*, *S. typhimurium*, *E. coli* O157:H7, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus* at levels between 0.2 and 10 µl ml $^{-1}$ (Burt, 2004). Previous studies have evaluated the effectiveness of oregano and cinnamon essential oils and their respective active components carvacrol and cinnamaldehyde *in vitro* and in foods. Carvacrol and cinnamaldehyde exhibited antimicrobial activity against C. *jejuni in vitro* (Ravishankar et al., 2008). During refrigerated storage at day 3, 0.5% oregano oil had 4.8 log (CFU/ml) reduction in *S*. Newport population on iceberg/romaine/baby/mature spinach (Moore-Neibel et al., 2013). Other researchers have tested antimicrobial activities of these compounds in liquid foods such as milk. The efficacy of cinnamon and clove essential oils against *L. monocytogenes* in pasteurized milk was evaluated and it was found that cinnamon bark oil (300-4000 ppm) and clove oil (250-2000 ppm) had reduction of *L. monocytogenes* by 1.5-3.5 logs and 2.5-4 logs, respectively (Cava et al., 2007).

Studies have shown that higher concentrations of EOs are required to obtain similar effects (as *in vitro*) in foods. This could be due to the complexity of food, presence of nutrients and moisture to support bacterial growth and extrinsic properties such as food packaging (Gill et al., 2002; Tassou et al., 1995). Mint oil had limited antibacterial activity in high fat foods (pate, and fish) against *L. monocytogenes and S.* Enteritidis compared to low fat foods (cucumber and yogurt) at the same concentration (Tassou et al., 1995).

1.7 Mechanism of Action of Plant-Based Antimicrobials

Generally, EOs are more effective against Gram+ compared to Gram- bacteria due to the lack of outer membrane. The effectiveness of essential oils depends on the amount present; at low concentration they can interfere with the bacterial enzymes and at higher concentration they can denature the proteins (Tiwari et al., 2009). Additionally, the mechanism of action (MOA) of essential oils/their active components depends on their chemical composition (Nazzaro et al., 2013). Thymol and carvacrol have analogous antimicrobial activity; however, they have different MOA against Gram+ and Gram – bacteria (Nazzaro et al., 2013). The hydroxyl group on carvacrol and thymol is at different locations, yet this difference does not affect the antimicrobial activity of either compound (Nazzaro et al., 2013). Mechanism of EOs is not linked to one specific change, but as a cascade reaction which affects the entire bacterial cell (Burt, 2004). Due to having multiple components affecting different parts of a bacterial cell, it is difficult to suggest the exact mechanism of action of these compounds.

EOs are hydrophobic which allows them to interact well with the lipids in the membrane of a bacterial cell and cause disruption of the mitochondria, leakage of inner cell contents, and disruption of the potassium ion influx which leads to cell death (Knobloch et al, 1989; Sikkema et al., 1994). Among the plant compounds, the most antibacterial activity against foodborne pathogens is associated with EOs that contain high concentrations of phenolic compounds such as carvacrol or eugenol (Farag et al., 1989). Studies have indicated that EOs, which contain phenolic compounds, may have similar MOA- disruption of the cytoplasmic membrane, proton motive force (PMF), electron flow, active transport, and coagulation of the cell contents (Denyer and Hugo, 1991b; Sikkema et al., 1995; Davidson, 1997). Alternative mechanism of action of these compounds includes cyclic hydrocarbons distorting the lipid-protein interaction or these hydrocarbons directly interacting with the hydrophobic portion of proteins (Juven et al., 1994; Sikkem et al., 1995). Additionally, these compounds can also interfere with the motility of bacterial cells. For example, treatment with p-cymene, which is a precursor of carvacrol, resulted in a decrease in cellular motility due to damage to the PMF required for flagellar movement (Nazzaro et al., 2013). Eugenol alters the membrane, affects the transportation of ions via the membrane, and changes the fatty acid composition of bacterial cell membrane (Nazzaro et al., 2013). In addition, eugenol acts against different bacterial enzymes such as ATPase, histidine carboxylase, amylase, and protease (Thoroski, 1989; Wendakoon et al., 1995). MOA of these compounds could occur via multiple routes and not all compounds have been studied in detail yet; therefore, this topic will remain an interest for future research along with an understanding of the synergistic effects of combinations of antimicrobials against foodborne pathogens in order to improve food safety.

1.8 Edible Films

One of the main ways in which essential oils can be applied on produce is via dip treatment since it will allow for an even contact with and a uniform treatment of produce. However, there may be a loss in crispiness or firmness of produce from essential oils, and plant extracts may cause browning to the leaves. Therefore, edible films containing essential oils, are gaining popularity. Edible films are thin layers of plant material which can be consumed and provide a barrier to moisture, oxygen, and solute movement of food (Bourtoom, 2008). Edible films are made from renewable ingredients of plants, therefore, they can be easily degraded thus, reducing environmental pollution (Bourtoom, 2008). Another major advantage of edible films is that they may enhance the organoleptic properties of a food product, since they could contain various components (flavoring, coloring, and sweeteners) (Bourtoom, 2008). The major food commodities with potential use of edible films include meat, fish, poultry, bread, cheese, fruits and vegetables (Labuza & Breene, 1989).

Edible films are generally categorized in the active packaging area which is a relative new concept to extend the shelf-life of a product, improve safety and sensory properties, and maintain product quality (Vermeiren et al., 2000). Antimicrobial packaging is useful for surface decontamination, particularly for meat products. Edible films containing antimicrobials could be efficient in allowing slower migration of the antimicrobial agents from the packaging material onto the surface of food; there can be extended contact during food storage and transportation; and also direct contact with food surface could help reduce cross-contamination (Quintavalla & Vicini, 2002).

The effectiveness of apple puree film containing cinnamon, lemongrass, and oregano oil was tested against *E. coli* O157:H7 *in vitro* (Rojas-Graü et al., 2006). Antimicrobial effectiveness was determined as follows: oregano oil> lemongrass>cinnamon oil (Rojas-Graü et al., 2006). Results indicated that incorporation of essential oils in the edible films decreases water permeability and increases vapor oxygen permeability; however, there is no effect on the tensile properties of the film (Rojas-Graü et al., 2006). Antimicrobial activity of apple edible films that contained either allspice, cinnamon, or clove bud essential oils was evaluated against *E. coli* O157:H7, *L. monocytogenes,* and *S. enterica* (Du et al., 2009). Results indicated that cinnamon oil containing films (0.3-0.5% w/w) were the most effective at inactivating foodborne pathogens with direct contact followed by clove bud oil and then allspice oil (Du et al., 2009). This study also suggested that antimicrobial edible films are more effective against *L. monocytogenes* than against *S. enterica* (Du et al., 2009). Another study showed complete reduction of *E. coli* O157:H7 by apple film containing 1.0% carvacrol within 24 h *in vitro* (Du et al., 2008).

There is a concentration dependency on the effectiveness of edible films containing essential oils against foodborne pathogens. Apple-based edible films containing 3% cinnamaldehyde or carvacrol showed the highest reduction against *S. enterica* (4.3 log CFU/g) and *E. coli* O157:H7 (6.8 log CFU/g) on poultry (Ravishankar et al., 2009). Edible films made from apple, carrot and hibiscus containing 3% carvacrol films reduced *Salmonella* by 5 log CFU/g at day 0 and 1.5% carvacrol films reduced *Salmonella* by 1 to 4 log CFU/g at day 7 in organic leafy green salad bags (Zhu et al., 2014). Edible films containing essential oils may be more effective on certain food type. For example, edible films containing carvacrol or cinnamaldehyde were more effective on iceberg lettuce in comparison to other leafy greens (romaine lettuce, baby/mature spinach) (Zhu et al., 2014). Therefore, further studies are required to determine efficacy of these films on food and on sensory acceptance.

1.9 Sensory Evaluation Overview

If plant-based antimicrobials are used as potential anti-bacterial or preservation agents in food, then their impact on the organoleptic properties of food needs to be further evaluated. Multiple plant-based antimicrobials have been shown to have antibacterial activities against foodborne pathogens and spoilage organisms. However, studies on the impact of these plant-antimicrobials on the sensory properties of leafy greens have been limited. Performing sensory evaluation will allow for screening of EOs that may be most preferred by consumer panelists. Additionally, careful selection of EOs will reduce the impact of essential oils on organoleptic properties especially in certain food types.

Sensory evaluation has been defined as “a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the sense of light, smell, touch, taste, and hearing” (Stone and Sidel, 2004). A sensory study is a quantitative study where a link can be formed between various products characteristics based on human sensory stimuli. Performing sensory analysis will allow us to make predictions about the products and help guide product development, as the ultimate target audience for food products will be consumers. In addition, many of the instrumental devices are not capable of responding to sensory attributes in a manner similar to humans, since they lack biological fluids such as saliva or the ability of smell. A sensory study is the only possible way to generate human response to samples being tested. The main reason for sensory evaluation is to identify consumers’ preference liking, which ultimately leads to product development and reduces uncertainty in marketing process.

There are three basic types of tests for sensory evaluation: difference test, quantitative descriptive analysis (QDA), and affective test. Difference test is a type of discrimination test where simply the difference among two or three similar products will be identified (Lawless and Heymann, 2010). Generally, difference test or discrimination test will be conducted with 25-40 individuals who have been screened based on their sensory perception to samples being evaluated (Lawless and Heymann, 2010). The QDA test quantifies the perceived intensities of sensory characteristics of a product (Lawless and Heymann, 2010). QDA test is widely used, as it requires panelists who are highly trained to judge specific characteristics of the product being tested, and thereby, it reduces variability in the data that is generated. For QDA studies, sample size of the study will be from 10 to 20 individuals. The third type of test is known as affective test, this test quantifies the degree of liking or disliking of a particular sample (Lawless and Heymann, 2010). In an affective test, untrained panelists are used, therefore, sample size is much higher- anywhere from 75-150 panelists may be required to test one product (Lawless and Heymann, 2010). Affective test is generally used when quantification of product is required based on preference liking. From affective testing, segments of people who preferred one sample over the other could be identified based on various parameters such as gender, age, ethnic groups, or even social-economical status.

1.9.1 Influence of plant antimicrobials on sensory properties of foods

Several studies have shown antimicrobial properties of plant compounds on leafy greens against foodborne pathogen; however, to our knowledge studies concerning their impact on sensory properties are limited. In many studies, sensory analysis has been conducted on food items which are cooked or to which spices are added as naturally flavoring agents. Addition of 0.8% (v/w) oregano oil to beef meat fillets had an initial reduction of *L. monocytogenes* by 2-3 logs and was acceptable after storage at 5°C and after cooking (Tsigarida et al., 2000). With 1% (v/w) oregano oil added to minced meat, the acceptability of meat improved based on flavor, odor, and color for samples stored under modified atmospheric packaging (Skandamis et al., 2001).

Essential oils and natural extracts have been used to improve the shelf-life of a food product and improve lipid stability (Mohamed and Mansour, 2012). Therefore, essential oils can be used as a part of ingredients in many food items to improve the flavoring aspects and at the same time can be used as preservatives since they have antimicrobial properties. Sensory analysis conducted on salad dressing containing 1.22% of salt and 0.4% of oregano oil showed that the product was preferred by panelists, thus allowing use of oregano oil in the salad dressing (Cattelan et al., 2015). Additionally, oregano oil (0.05% w/w) added to extra virgin olive oil exhibited positive attributes during 21 days storage in comparison to the control (Asensio et al., 2012). Consumers do not accept addition of some EOs in some foods. For example, addition of thyme, rosemary, carvacrol, and p-cymene into tomato juice, vegetable soup, and poultry (burgers) had adverse impact on the taste acceptance by panelists (Espina at al., 2014). Incorporation of EOs in a food product can affect the acceptance of that product by the consumers either positively or negatively; therefore, a sensory study is important to determine the preference liking of essential oils by consumers during new product development.

1.9.2 Influence of plant antimicrobials on texture of food products

Texture has been defined as the "sensory and functional manifestation of the structural, mechanical and surface properties of food detected through the senses of vision, hearing, touch, and kinesthetic" (Szczesniak, 2002). Textural properties can be described as visual or tactile attributes and both have significant impact on ways consumers will perceive the product. Texture properties are generally an indication of food quality rather than food safety. The crispiness values of a sample can be determined by their textural properties, which are measured using texture analyzers or Instron. One of the first groups to correlate instrumental texture parameters with sensory texture attributes was Friedman et al. (1963). The General Food Texture profile group developed a piece of equipment to translate the textural properties of food samples (Lawless and Heymann, 2010). The Texturometer has a probe or plunger, which penetrates through the food samples and the penetration force, indicates the crispiness value of the sample (Lawless and Heymann, 2010). Various versions of the texture analyzers have been designed; however, most analyzers are based on the force measurements. Correlations can be made between mouthfeel (data obtained from sensory analysis) and textural measurements (data obtained from Texture Analyzer) to determine how the sample can be modified to increase the preference liking.

Textural changes among food commodities are one of the main influencing factors, which can determine the quality of the product. Textural changes of food commodities can be affected by multiple factors such as ingredients. Changes in texture of fresh vegetables are usually associated with loss of moisture which decreases the turgidity in the plant cells (Severino et al., 2014). Water loss is another factor, which can lead to change in texture, water loss of 5%, or more can cause texture breakdown in fresh lettuce (Ryall and Lipton, 1972). Several studies have shown that there will be improvement in firmness as a result of use of chitosan-based coatings on fruits and vegetables (Devlieghere et al., 2004). Green bean samples treated with palmitoylated (covalently linked fatty acids) chitosan and nanoemulsion of mandarin essential oils showed reduction in the firmness 12 days after treatment (Severino et al., 2014). Different essential oils may have different impact on the texture and organoleptic properties of leafy greens; therefore, additional research is required to see how they will enhance or reduce the crispiness value of leafy greens.

Many studies have shown that the addition of EOs influences the tensile strength of food products. There was a significant decrease in tensile strength and elastic modulus when EOs were added to apple films (Du et al., 2009). Incorporation of clove bud oil had a greater impact on the texture of apple films than allspice or cinnamon oils (Du et al., 2009). Other studies have shown that washing fresh-cut lettuce with calcium lactate had higher significant crispiness values than those washed with chlorinated water (Rico et al., 2006). Using Cryo-SEM micrographs, these investigators were able to show that there was loss of turgor (shrinkage) in tissue cells, of iceberg lettuce, washed with chlorine in comparison to calcium lactate which showed no evident difference (Rico et al., 2006).

1.9.3 Influence of plant antimicrobials on color of food products

Plant antimicrobials could greatly influence the color properties of food especially leafy greens, as they may retain the color of extracts. Color is one of the main attributes, influencing the organoleptic properties of leafy greens. In addition, consumers have a strong preference for the color of leafy greens in terms of purchasing that product. The shelf-life of leafy green vegetables appeal to consumers based on crispiness, color, and wetness of these products (Zhou et al., 2004). Sensory properties such as color and texture play a crucial role in consumer's choice for fresh produces such as leafy greens (Gutierrez et al., 2009). No significant difference was noted between vegetables washed with EOs or chlorine after storage based on color, texture, and water activity of the product as well as the gas composition in the package (Gutierrez et al., 2009).

Visual representation of a food product has a significant impact on consumers' choice. Many studies have shown that foods that are deep in color will receive a higher rating for flavors (DuBose et al., 1980). Studies have shown that individuals are able to differentiate between skim milk and whole milk based on appearance, flavor, and mouthfeel (Lawless and Heymann, 2010). However, the perception of fat content is mainly differentiated by the appearance of milk (Pangborn et al., 1964).

Color can be measured using various color schemes; however, in the food industry CIELAB coordinate system is one of the common ways to identify color intensities of samples being evaluated. Previously used XYZ color coordinate system was not sufficient to differentiate between colors because the distance between individual colors did not correspond to the actual perceived color difference. Therefore, the Commission of Illumination developed the CIE LAB coordinate system in 1976. Both Hunter L, a, b and CIE 1976 (LAB) coordinate systems are based on the opponent theory that receptors in human eyes perceive color as opposite pairs. In the CIE LAB system, L* is the measurement of lightness where L values range from 0-100; values close to 100 indicate light color and values close to 0 will indicate dark color. A* is the measurement of green-red which ranges from negative to positive values. B* values are measurements of blue-yellow which also range from negative to positive values (Gutierrez et al., 2009). CIE LAB coordinate system is more commonly used than other color schemes, as it is more recent in comparison to others. Therefore, this system is able to differentiate between two closely related colors and may be comparable to those colors perceived by humans.

Color measurements are useful in determining if consumers will accept a new product based on the appearance. Use of plant-antimicrobials could have different impact on the color properties of the samples being evaluated, which may be perceived as positive or negative by the consumers. Siripatrawan and Harte (2010) reported that adding green tea extract into chitosan films significantly affected ($P < 0.05$) the $L*$, $a*$, and $b*$ values of the film surface. The edible films were darker in color after the addition of apple skin polyphenols and there was an increase in a* and b* values as the concentration of apple skin polyphenol was increased (Du et al., 2011).

1.10 Goals and Objectives

The consumption of organic leafy greens has increased in recent years along with foodborne illnesses associated with leafy greens. The organic industry is limited to using the available sanitizers, as strict rules and regulations are applied by the USDA-NOP. Commonly used sanitizers have many disadvantages ranging from losing efficacy in the presence of organic matter to having negative health concerns to workers. Therefore, there is a critical need to evaluate the efficacy of other natural products, which may be effective in eliminating foodborne pathogens. In this study, we tested several organic sanitizers and plant-based antimicrobials against *S*. Newport on harvesting equipment to determine their efficacy. Several studies have shown that plant-based extracts and essential oils have antimicrobial properties against foodborne pathogen both *in vitro* and in foods. However, studies on the effects of these plant antimicrobials on sensory and organoleptic properties of food are limited. Therefore, the goal of the second project was to evaluate if these antimicrobials have any impact on the organoleptic properties of leafy greens and identify specific compounds, which would have the highest preference liking among consumers of organic leafy greens. The third project was a continuation of the second project in which we tested the sensory attributes and preference liking of spinach samples treated with antimicrobial edible films in salad bags. The texture and color properties of the leafy greens may be impacted by the addition of essentials oils, plant extracts, or edible films; therefore, changes in color and texture properties of treated leafy greens were measured using a Chroma meter and Texture Analyzer, respectively. This thesis is divided into three different projects, which are presented in the following chapters.

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CHAPTER 2: EVALUATING THE DECONTAMINATION POTENTIAL OF *SALMONELLA* NEWPORT ON ICEBERG LETTUCE CORING TOOLS USING PLANT ANTIMICROBIALS AND ORGANIC SANITIZERS

2.1 Abstract

The objectives of this study were a) to determine if the modified designs of coring knives are easier to decontaminate in comparison to the original design and b) to evaluate the efficacy of organic sanitizers and plant-antimicrobials against *Salmonella* Newport on these tools. Coring knives were inoculated with *S*. Newport and washed with: deionized water (DI water), 50 ppm bleach, 3% hydrogen peroxide (H_2O_2), 5% Chico wash™, 0.1% Oregano oil, 0.39% SaniDate 5.0®, or 3% fulvic acid for 5 min. Before treatment, *Salmonella* populations of 6.4±0.3, 6.3±0.1, and 6.4±0.3 log CFU/cm^2 were recovered from the original, modified design 1, and modified design 2 coring knives, respectively. When comparing the efficacy of sanitizers, 3% H_2O_2 had the highest *Salmonella* reductions of 6.0±0.6-6.2±0.3 log CFU/cm^2. Treatments with 0.39% SaniDate 5.0® and 0.1% oregano oil were comparable which yielded reductions of 6.2±0.2 and 5.9±0.5 log CFU/cm^2, respectively. The average reductions due to 50 ppm bleach were 2.8±0.8-3.5±1.3 log CFU/cm^2. The wash water after treatment with each sanitizer was sampled after enrichment to check for *Salmonella* survivors; however, no survivors were detected (except deionized water and 3% fulvic acid) suggesting that these organic sanitizers and plant antimicrobials have bactericidal effects on *Salmonella*. The results from this study indicate that SaniDate 5.0® and oregano oil are more effective at inactivating *Salmonella* compared to 50 ppm bleach. The results from this study could provide guidelines regarding the areas on the coring tools that may require rigorous sanitization and suggest potential alternative organic sanitizers that could be more effective than 50 ppm bleach against *Salmonella* for decontaminating coring knives.

2.2 Introduction

In recent years, there has been an increase in the consumption of leafy greens, particularly organic produce, as people are changing their eating habits. In 2000, more organic food was purchased in comparison to conventional food and organic fresh-produce ranked the highest among the organic food commodities purchased (Dimitri et al., 2002). However, during the last two decades there has also been an increase in leafy greens outbreaks due to contamination by foodborne pathogens (Taban and Halkman, 2011). One particular concern is non-typhoidal *Salmonella,* specifically the multi-drug resistant isolates. In the United States, *Salmonella* causes 1.2 million illnesses each year, of which 100,000 are linked to multi-drug resistant *S.* Newport (CDC, 2012). Furthermore, CDC reports that 46% of all foodborne illnesses and 23% of related deaths are associated with contaminated produce (Painter et al., 2013).

Increased consumption cannot be the sole factor for a rise in these outbreaks; therefore, other risks must also be considered. The location and history of the land where the crop is grown, presence of domestic or wild animals, types of irrigation water used, and human or mechanical contact with harvesting equipment are factors commonly attributed to these outbreaks (Brackett, 1999; Tauxe, 1997).

What is coring tool?

A coring tool is a harvesting equipment used by farmers to core lettuce heads in the field before they are packaged for retail. Coring in the field (CIF) is a relatively recent concept which was initiated in the 1990s to increase processing plant yields, decrease nonmarketable tissue, and reduce shipping costs (Zhou et al., 2012). Removing the outer leaves and coring in the field has increased processing plant production from 60-70% to almost 100% (Yang et al., 2012). Additionally, CIF reduces waste disposal costs while maintaining the market quality of lettuce

(Zhou et al., 2012). However, coring increases the risk of cross contamination since the leaves come in direct contact with the knives. While coring, there is also a significant amount of tissue damage as well as additional human handling (Yang et al., 2012). It has been shown that *E. coli* had a much greater presence on damaged iceberg lettuce than undamaged plants (Barker-Reid et al., 2009). Thus, it is crucial that the coring tool is disinfected regularly and is free from contamination to prevent further outbreaks associated with leafy greens.

Coring knives can be contaminated via multiple routes: direct contact with soils, plants, or workers gloves (Suslow et al., 2003). Another major concern with coring tools is that they are constantly used in the field for up to 2-4 hours before they are sanitized. A study conducted by researchers at the Yuma Agricultural Center showed that from a single contaminated coring knife, the bacterial contamination could be transferred to as many as 75 heads of lettuce (Fallon, 2011). A coring knife harboring 5 log of *E. coli* on its blade surface is able to transfer 3 log CFU/g of *E .coli* on to the cored region of the lettuce immediately after coring (McEvoy et al., 2009). These authors further showed that once *E. coli* is present in the cored region then there is an increase in the *E. coli* population after 4 h at 30°C storage and the population continues to increase over time (McEvoy et al., 2009). To combat this problem, research has been focused on the development of modified versions of coring tools that will remove or modify areas which may be more prone to adherence by microorganisms (such as the welded region). Researchers at the University of Illinois showed that modified design without the welding region harbors significantly fewer *E. coli* cells than the commercially used design (Zhou et al., 2012).

Another challenge that the organic industry faces is limitation of available organic sanitizers. One of the most frequently used sanitizers include chlorine-based sanitizers such as bleach due to their lower cost in comparison to other sanitizers. However, studies have shown that chlorinated wash water has <2 logs reduction on leafy greens (Park et al., 2011). Also, there is potential for the formation of carcinogenic by-products when chlorine reacts with the organic matter, thus increasing the risk of workers being exposed to high toxicity (Sapers, 2001).

Many sanitizers, such as chlorine dioxide (ClO_2) (Beuchat, 1998), trisodium phosphate (Zhuang and Beuchat, 1996), hydrogen peroxide (H_2O_2) (Lin et al., 2002), and ozonated water (Restaino et al., 1995) have already been evaluated for their effectiveness against pathogens. The antimicrobial activity of organic acids (lactic, citric, acetic, and ascorbic acid) against *E. coli* and *L. monocytogenes* on iceberg lettuce was compared (Akabas and Olmez, 2007). Zhang et al., (1996) evaluated the combination effect of lactic and acetic acids (AA) with chlorine to reduce *L. monocytogenes* on shredded lettuce. To our knowledge, the efficacy of organic sanitizers against *S.* Newport on coring knives has not been investigated.

Therefore, the objective of this study was a) evaluate if tools with modified designs are easier for decontamination; and b) test the efficacy of plant antimicrobials and organic sanitizers against *Salmonella* Newport on the coring tools.

2.3 Material and Methods

2.3.1 Bacterial Strains Used, Culture Preparation and Media

The strain used for this study was a multi-drug resistant *Salmonella enterica* serovar Newport *LAJ160311,* with the JJPX 01.0014 PulseNet PFGE profile. An overnight culture was prepared by inoculating cryopreserved cells in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubating for 20-24 h at 37ºC. For inoculation, *S.* Newport was grown in 300 ml of TSB overnight, ensuring that the culture was 8 log CFU/ml.

2.3.2 Coring tool designs Tested

Coring tools used in this study were designed and fabricated by our engineering faculty from the Department of Agriculture and Biosystems Engineering at The University of Arizona. Engineers fabricated several new modified versions of coring knives by modifying the coring ring portion and the length of coring tool. The original design included the welded region between the handle and the ring of the tool (Figure 1A). Three modified coring knives were designed to facilitate easier coring and improve food safety features with the following modifications: Modified Design 1 had a short front in the ring section of the tool compared to the original design: Modified design 2 had the original front for the ring portion, but no welding; Modified design 3 was similar to modified design 1 with the exception that the coring ring was at an angle to facilitate easier coring. In all the modified versions of the coring tools, the welded region was removed to ensure smoothness of the tool, which could help prevent attachment of bacterial cells compared to the rough welded region. Additionally, three different lengths of coring tool designs were made with length 1 at 7.5 cm, length 2 at 10.5 cm, and length 3 at 13.5 cm. Our engineers used combinations of different designs (D1-D3) and different lengths (L1-L3) to fabricate modified designs of tools with improved food safety features. For this study modified design 1 length 2 (D1L2- Figure 1B) and modified design 2 length 2 (D2L2- Figure 1C) were compared to the original design (with welding- Figure 1A) to test the efficacy of organic sanitizers and plant antimicrobials on these tools.

2.3.3 Preparation of Coring Tools before Treatments

Before inoculation, coring tools were rinsed with 20% bleach, and air dried for 10 min, followed by a wash with sterile deionized water to ensure the coring knives were free from contamination and chorine residues. After decontamination, tools were sampled from various locations and streaked on XLD (xylose lysine desoxycholate) to ensure no survival of *S.* Newport on these tools before inoculation for the next experiment.

2.3.4 Treatment of the Tools Using Various Sanitizers

Chlorine solution of 50 ppm was prepared from concentrated sodium hypochlorite (6% Clorox bleach, Clorox Co., Oakland, CA) and pH of the solution was measured to be around 9 before each treatment. Hydrogen peroxide (H_2O_2; 3% concentration) was purchased from local retail stores and used as such. Chico wash™ (E3 Organics, Inc., Orland, CA) , SaniDate 5.0® (BioSafe Systems LLC, Hartford, CT) and a fulvic acid based sanitizer (GTX Technologies LLC, Amarillo, TX) were obtained from the respective manufacturers. Oregano oil (made from 100% pure *Origanum vulgare*), was obtained from Lhasa Karnak Herb Company (Berkeley, CA, USA). All treatment solutions were prepared in deionized (DI) water right before the treatment at the following concentrations: 50 ppm bleach, 3% H_2O_2, 5% Chico wash™, 0.1% Oregano oil, 0.39% SaniDate 5.0®, or 3% fulvic acid. For all treatment solutions, pH values were measured and are indicated in Table 2.1.

All coring tools were washed with the respective treatments for 5 min and after treatment, four locations on the tools were swabbed using a three way-swabbing method (vertical, horizontal and diagonal swabbing). Dip treatment was used to stimulate commercial practices. The four locations swabbed on each coring tool are depicted in Figure 2. Location 1 was the area between the handle and the ring of the tool, location 2 was the outer back surface of the ring portion, location 3 was the inside region of the coring ring, and location 4 was the entire circumference of the coring ring. Each location on the coring knives was restricted to an area of 3cmx3cm when swabbing in order to ensure that the same surface area was swabbed for all locations, by having a template on coring tools. The swab samples were directly placed in 9 ml of Dey-Engley (DE) neutralizing broth, serially diluted further in 0.1% peptone water, and plated in duplicate on XLD agar. The plates were incubated at 37ºC for 24 h and counted. The experiment was conducted in three repeats and the data depicted is an average of three repeats.

2.3.5 Enrichment of Treatment Water (wash water)

Coring tool wash waters after each sanitizer treatment were plated on both selective (XLD) and non-selective (TSA-tryptic soy agar) agar media to determine if *S*. Newport cells are only being removed from tools or if these sanitizers have killing or injuring effect. Wash water was further enriched in TSB and plated on non-selective media to detect the presence of low numbers of survivors.

2.3.6 Statistical analysis

Mean log (CFU/cm^2) values of *S.* Newport recovered on XLD from three replicate experiments were calculated for control and each treatment. Comparisons among bacterial populations on different designs, locations, and treatments were made using One-way ANOVA Tukey's pairwise comparison at a level of significance of $P \leq 0.05$ using Minitab 17 (State College, PA, USA).

2.4 Results and Discussion

The primary objective of this study was to identify the coring tool design that would be easier to decontaminate in comparison to other designs tested in this study. Three different designs of coring tools were compared in this study: 1) original design (which included the welded region) 2) modified design 1 (with no welding region and smaller ring portion) 3) modified design 2 (with no welded region and original coring ring portion). Design 1 length 2 (D1L1) and design 2 length 2 (D2L2) were used in this study based on our previous work. Members of our lab tested various designs by coring 100 heads of iceberg lettuce using coring tools contaminated with non-pathogenic *E. coli*. Our results showed that D1L2 and D2L2 were the two designs that transferred the least amount of *E. coli* onto iceberg lettuce. Ninety percent of iceberg lettuce heads were found positive for *E. coli* when the original design was used, 70% were positive for D1L3, 66% positive for D1L2, and 44% were found positive using coring tool D2L2 (data not shown).

The initial experiment was set up to measure the surviving population of *S*. Newport on three coring tool designs when inoculated with 8 log CFU/cm^2. At each location on each design, there was consistently at least 6 log CFU/cm^2 of surviving population of *Salmonella*. As indicated in figures 3-5 there was no significant difference ($p \leq 0.05$) between the initial *Salmonella* population among various locations within the same design of coring tool.

No significant ($p \leq 0.05$) difference was found in the surviving population of *S.* Newport among various locations for the same design, yet looking at the numerical value, the highest number of bacterial cells (6.64±0.20 log CFU/cm^2) were recovered from the original design location 4 (the ring portion). The ring portion of the tool is the first area that comes in direct contact with iceberg lettuce; therefore, it is important that this area remain free of contamination. McEvory et al. (2009) showed that the coring ring harboring 5 logs CFU/g *E. coli* O157:H7 transferred 3 log CFU/g of *E. coli* onto the cored region of lettuce heads immediately after coring. When the ring portion comes in contact with iceberg lettuce, there may be significant tissue damage to lettuce (Yang et al., 2012). Tissue damage from mechanical or even enzymatic treatments may enhance the growth of pathogens on produce (McEvoy et al., 2009). A study has shown that cutting vegetables with a dull knife allows for more tissue damage and better growth for *E. coli* than cutting vegetables with a sharp knife (Seo and Frank 1999). Therefore, it is crucial that the ring portion of tools are maintained in good working condition with frequent sanitization. In considering the design of knives particularly for the ring portion, modified design 2 may be a better option for the workers as we have shown that at location 4 this design had the lowest surviving population (6.34±0.54 log CFU/cm^2) of *S*. Newport.

There is a theory in literature which suggests that the welded region on the original coring tool design may allow for better adherence of microorganism as this region is more rough in comparison to other areas. A study showed that there was higher surviving population of *E. coli* O157:H7 at the welded region (Zhou et al., 2012). However, in our study there was no significant difference ($p \leq 0.05$) between the original design and the modified coring knives at various locations for populations of *S.* Newport. The difference in the results of Zhou et al. and our study may be due to the use of different pathogens and the type of metal grade used in the knife portion of the coring tools. In our study, the knives of all three coring tool designs were made from the same grade of metal (304-stainless steel); therefore, it was shown that simply removing the welded region does not change/reduce the risk of adherence of *S.* Newport at various locations on coring tools. Alternative research needs to be conducted to ensure that coring tools remain free from contamination.

Use of organic sanitizers or plant antimicrobials may provide alternative options for organic industry to decontaminate coring tools. Efficacy of organic sanitizers on food contact surfaces has not been studied extensively, especially for harvesting equipment. In our studies, we have shown that potential organic sanitizers such as fulvic acid, SaniDate 5.0®, hydrogen peroxide, and oregano oil are more effective in washing coring tools, compared to bleach. Chlorine based sanitizers have been known to form carcinogenic by-products and are considered to be corrosive (Rodgers et al., 2004). Fulvic acid-based sanitizers effectively reduced *L. monocytogenes*, *S.* Typhimurium and *P. aeruginosa* populations on stainless steel, high-density polyethylene, polyvinyl chloride and polycarbonate coupon surfaces (Zhu et al., 2014). SaniDate 5.0® is a combination of peroxyacetic acid (PAA) and hydrogen peroxide, which had the highest reduction in population of *Salmonella* on these tools.

Peroxyacetic acid is produced from the reaction of acetic acid or acetic anhydride with hydrogen peroxide in the presence of sulfuric acid (Kitis, 2004). Hydrogen peroxide is widely used as a disinfectant at concentrations from 3%-90%, as this has been considered environmental friendly (Small et al., 2007). One disadvantage of hydrogen peroxide is its low disinfection efficacy and is considered slow disinfection (Koivunen and Heinonen – Tanski, 2005). However, our study has shown that when PAA and hydrogen peroxide are combined (SaniDate 5.0®), then efficacy of this sanitizer increases. Adding hydrogen peroxide to PAA induces a synergistic antimicrobial effect (Vandekinderen et al., 2009). Another advantage of PAA over hydrogen peroxide is that, PAA is effective at very low concentrations (<0.3% v/v). Similar results are seen in our study, where 0.39% SaniDate 5.0® was equally effective at reducing populations of *S.* Newport from coring tools compared to 3% hydrogen peroxide.

The main finding in this study was that it is easier to remove the *S.* Newport cells from locations 2 and 3 on these coring tools and that some sanitizers (SaniDate5.0®, oregano oil) are more effective than others against *S.* Newport. On the original design, treatment with DI water had the highest reduction at location 2 (2.1±0.4 log CFU/cm^2). Treatments that had the highest reduction at location 4 were 5% Chico Wash™ (4.4±0.3 log CFU/cm^2), 3% H_2O_2 (6.6±0.2 log CFU/cm^2), 0.1% oregano oil (6.6±0.2 CFU/cm^2), and 0.39% SaniDate 5.0® (6.5±0.5 log CFU/cm^2). The highest reduction at location 3 was observed with 50 ppm bleach (4.8±0.2 log CFU/cm^2) and 3% fulvic acid (6.0±0.3 log CFU/cm^2) (Fig 2.3).

On the modified design 1, the highest reduction from DI water (3.0±0.2 log CFU/cm^2), 3% H_2O_2 (6.4±0.2 log CFU/cm^2), and 0.39% SaniDate 5.0® (6.4±0.2 log CFU/cm^2) was observed at location 4. Treatments that resulted in the highest reduction at location 3 were 5% Chico Wash™ (6.1±0.1 log CFU/cm^2), 50 ppm bleach (5.4±0.2 log CFU/cm^2), and 0.1% oregano oil (6.3±0.1 log CFU/cm^2), while 3% fulvic acid (5.6±0.2 log CFU/cm^2) demonstrated the highest reduction at location 2 (Fig 2.4). On modified design 2, the highest reductions due to treatments with DI water (2.4±0.3 log CFU/cm^2), 3% H_2O_2 (6.4±0.1 log CFU/cm^2), 0.1% oregano oil (6.1±0.3 log CFU/cm^2), 0.39% SaniDate 5.0® (6.4±0.1 log CFU/cm^2) were observed at location 2. Treatments that showed the highest reductions at location 3 were 5% Chico Wash™ (6.1±0.2 log CFU/cm^2), 50 ppm bleach (3.5±0.2 log CFU/cm^2), and 3% fulvic acid (6.1±0.2 log CFU/cm^2) (Fig 2.5).

Overall, for all designs, it was much easier to remove the attached cells from location 2 (outer surface of back ring) and location 3 (inside of the ring) compared to other locations due to having a smoother surface with curvatures, where treatment solution may be able to hold for a longer time. Droplets of sanitizers were remaining for longer periods on these locations after the tools were removed from the sanitizing solutions, which were visually observed during the course of the experiment. Additionally, moisture adherence may be higher on these locations, which would require longer period for sanitizer droplets to evaporate thus, providing longer contact times with the surfaces at these two locations.

On the original design, treatment with 3% H_2O_2 and 0.1% oregano oil yielded the highest reduction of 6.6±0.2 log CFU/cm^2 in *Salmonella* population at location 4 (Figure 2.3). On modified design 1 length 2, 3% H_2O_2 and 0.39% SaniDate 5.0® had the highest reduction of 6.4±0.2 log CFU/cm^2 in *Salmonella* population at location 4 (Figure 2.4). Similarly, on modified design 2 length 2, 3% H_2O_2 and 0.39% SaniDate 5.0® had the highest reduction of 6.4±0.1 log CFU/cm^2 in *Salmonella* population at location 2 (Figure 2.5). Treatment with DI water showed reductions ranging from 0.7±0.2-2.1±0.4 log CFU/cm^2, 1.4±0.1-3.0±0.2 log CFU/cm^2, and 1.2±0.4-2.4±0.3 log CFU/cm^2 on the original, modified design 1 and modified design 2 tools, respectively.

On the original design location 1, 3% H_2O_2 was significantly ($p \geq 0.05$) different from all other treatments yielding the highest reduction (6.0±0.2 log CFU/cm^2) in surviving population of *Salmonella.* On location 2, treatments with 3% H_2O_2, 0.1% Oregano oil, and 0.39% SaniDate 5.0® were significantly ($p \geq 0.05$) different from control, DI water, 50 ppm bleach, 5% Chico Wash™, and 3% fulvic acid. Similar trend was seen on locations 3 and 4 where most effective treatments were 3% H_2O_2, 0.1% oregano oil, and 0.39% SaniDate 5.0® (Figure 2.3). For D1L2 (Figure 2.4), again similar results were seen where location 1 was much harder to remove the surviving population of *Salmonella* and the most effective sanitizers were 3% H_2O_2, 0.1% oregano oil, and 0.39% SaniDate 5.0® at all four locations. For D2L2 (Figure 2.5), location 1 again was much harder to remove *Salmonella;* however, at location 3, no survivors were detected after 5% Chico Wash™, 3% fulvic acid, 3% H_2O_2, and, 0.1% oregano oil treatments.

Overall, when comparing the wash treatment on the coring knives with each of the locations on the same design of coring knives, there was no significant difference ($p \leq 0.05$) between the reductions caused by the following sanitizers; SaniDate 5.0®, 0.1% oregano oil, and 3% H_2O_2, suggesting that they were equally effective against *Salmonella* on the coring tools. However, there was a significant ($p \geq 0.05$) difference in *Salmonella* reductions when tools were washed with 50 ppm bleach (compared to SaniDate5.0®, 0.1% oregano oil, and 3% H_2O_2), which only resulted in reductions of 2.8±0.8-3.5±1.3 log CFU/cm^2 among three coring knives.

No survivors were detected when wash water was directly plated on both XLD and TSA agars immediately after washing contaminated tools for all sanitizers, except DI water and 3% fulvic acid (Table 2.1). Enrichment of wash water was plated on both XLD and TSA agar to ensure there is not a presence of low number of injured cells. From enriched wash water samples, no survivors were detected except for DI water and 3% fulvic acid treatment, indicating that *Salmonella* cells were injured and not inactivated with these two treatments. From these results, it is evident that all treatment solutions including 0.1% oregano oil have bactericidal activity against *S*. Newport suggesting that these could serve as alternative sanitizer options for the organic industry.

2.5 Conclusions

In conclusion, this study provides a few potential alternative organic sanitizers and an essential oil, which are equally effective at inactivating *S.* Newport on coring tools compared to hydrogen peroxide or bleach. Additionally, it was much easier to remove contamination from certain part of the coring tool (location 3) due to shape of the tool; however, other areas on the tool may require rigorous cleaning procedures. In future, the efficacy of these sanitizers against other foodborne pathogens of concern in lettuce needs to be evaluated, which could lead to a wider and cost-effective usage of these sanitizers. Future studies also need to evaluate the efficacy of sanitizers after coring lettuce heads, which would indicate efficacy of these sanitizers in the presence of organic matter. In addition, time-dependent and concentration-dependent activities of these sanitizers need to be evaluated to determine the optimum concentration and time exposure required for inactivating foodborne pathogens.

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Table 2.1: pH values of treatment solution and surviving (log CFU/ml) population of S. Newport in wash water

Treatments	pH AVG± STD Dev	AVG± STD Dev Survival (log CFU/ml) of *S.* Newport in wash water
DI Water	6.7±0.3	10.0±0.1
50 ppm bleach	9.3±0.1	0
5% Chico Wash™	2.1±0.1	0
3% H2O2	4.4±0.1	0
0.1% Oregano oil	6.3±0.2	0
0.39% SaniDate 5.0®	3.4±0.02	0
3% Fulvic Acid	2.4±0.1	9.5±0.2

Figure 2.1: Different versions of coring knives used in this study. (A) Original coring tool design where welded region is located between the ring and the handle of the tool. (B) Modified design 1 with length 2 where welded region is removed and the coring ring is shortened. (C) Modified design 2 with length 2 where again welded region is removed and, the design of the coring ring is kept similar to the original design.

Figure 2.1 (A) (B) (C)

Figure 2.2: Image of coring tool indicating four different locations that were swabbed after treatment in order to determine the surviving population of S. Newport. Each location was limited to an area of 9 cm^2 (3X3 cm) when swabbing.

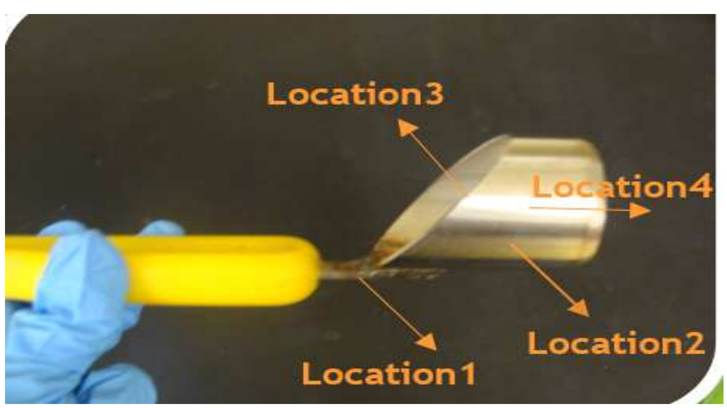


Figure 2.3: Survival of *Salmonella* Newport on original design coring tool after treatment with various sanitizers. Values plotted represent the average of three replicates. Error bars represent standard deviation from the means. Bars with different letters A, B, C, D, E, and F show significant difference (P≤0.05) among locations and treatments on each coring tool design.

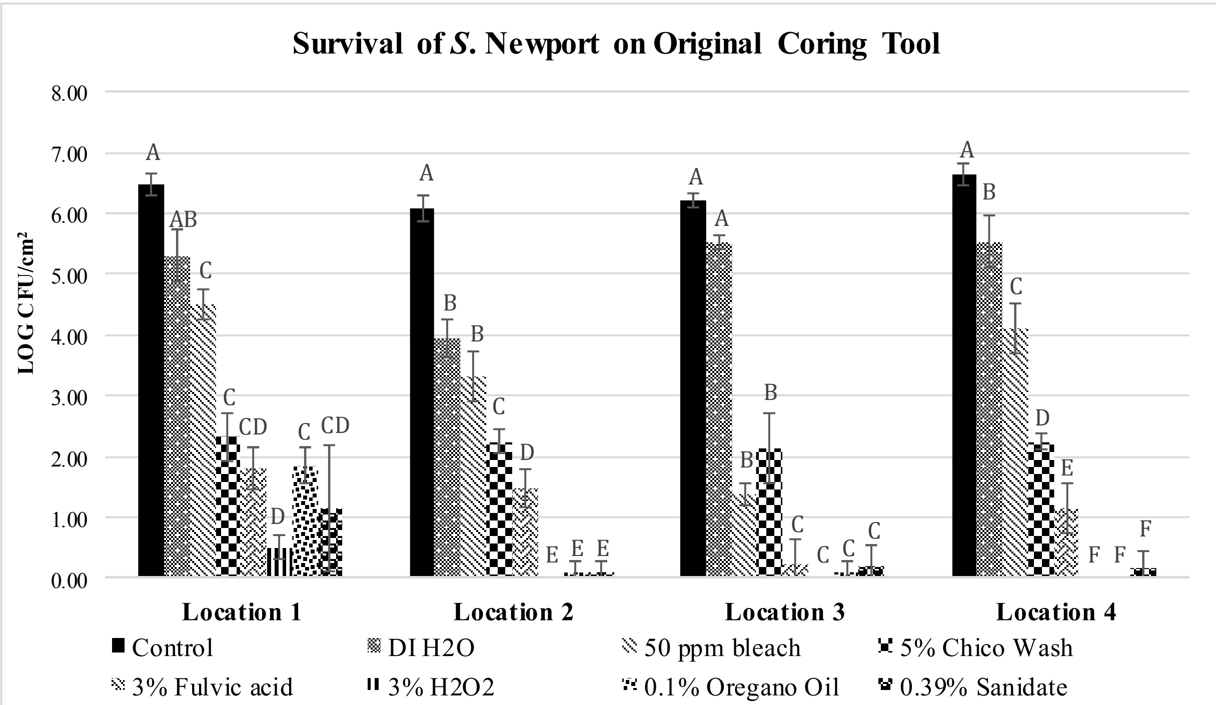


Figure 2.4: Survival of *Salmonella* Newport on modified design 1 length 2 coring tool after treatment with various sanitizers. Values plotted represent the average of three replicates. Error bars represent standard deviation from the means. Bars with different letters A, B, C, D, E, and F show significant difference (P≤0.05) among locations and treatments on each coring tool design.

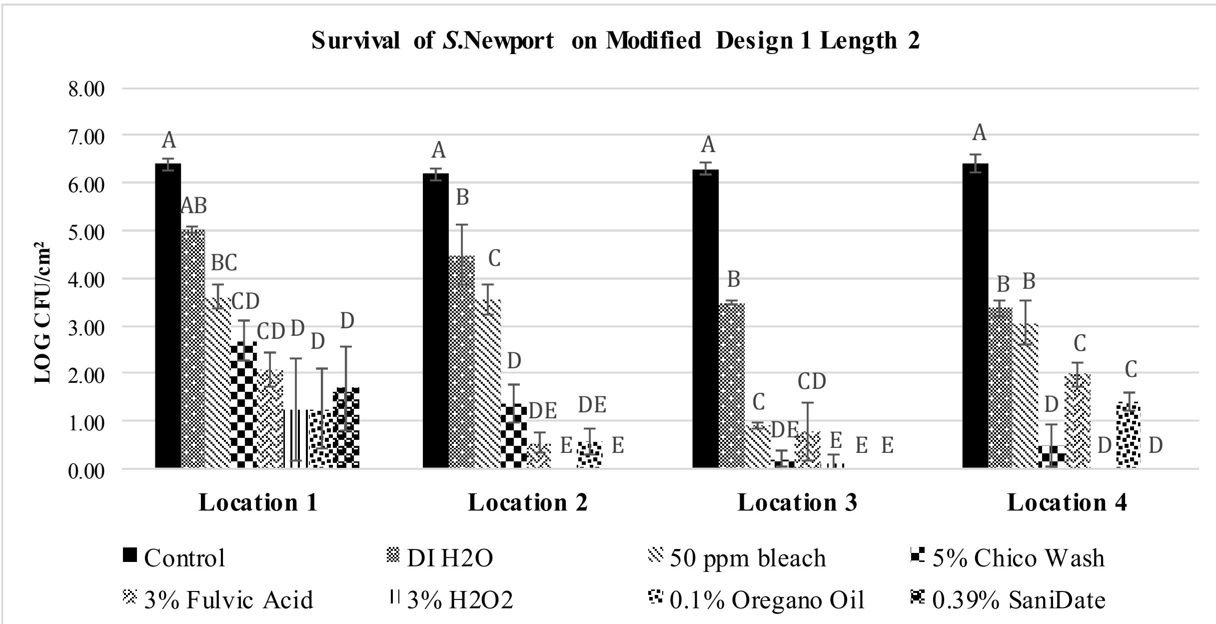
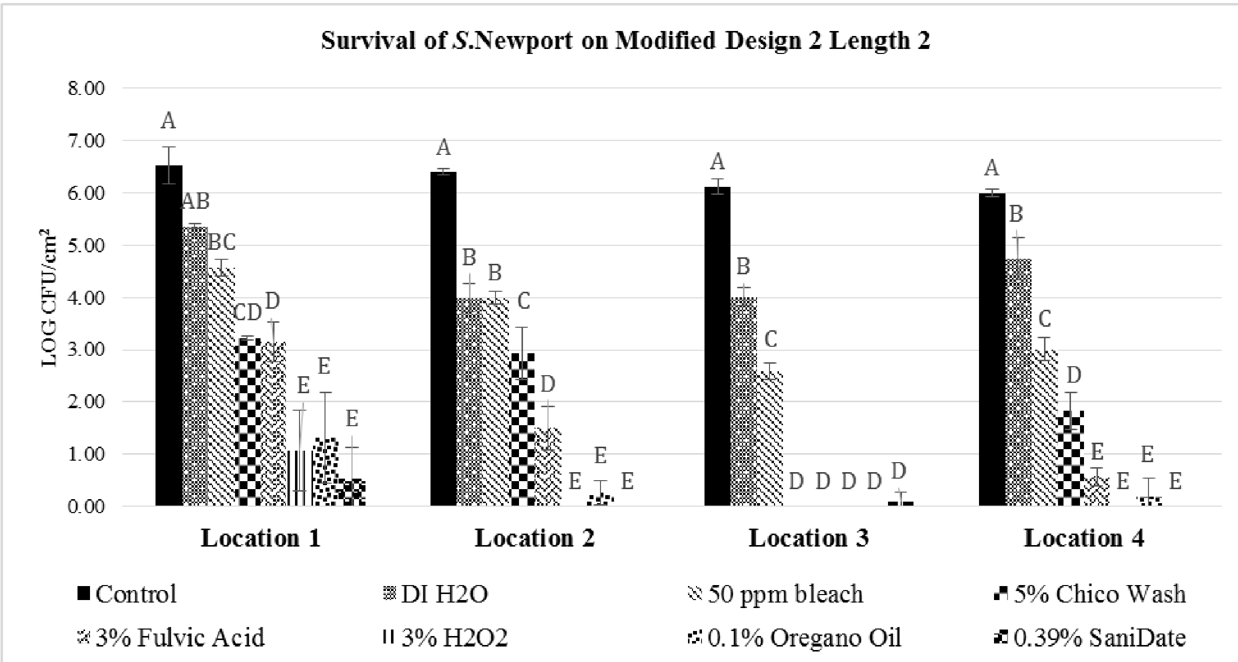


Figure 2.5: Survival of *Salmonella* Newport on modified design 2 length 2 coring tool after treatment with various sanitizers. Values plotted represent the average of three replicates. Error bars represent standard deviation from the means. Bars with different letters A, B, C, D, E, and F show significant difference (P≤0.05) among locations and treatments on each coring tool design.



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CHAPTER 3: IMPACT OF PLANT-BASED ANTIMICROBIAL WASHES ON SENSORY PROPERTIES OF ORGANIC LEAFY GREENS

3.1 Abstract

The objective was to study the sensory attributes of organic leafy greens treated with plant antimicrobials and identify treatments most accepted by panelists. Organic leafy greens were washed with antimicrobials and stored at 4°C for 24 h prior to serving panelists. Antimicrobials evaluated include: 0.1% clove bud, lemongrass, oregano, or cinnamon essential oils; 0.1% carvacrol or citral; 3% grapeseed, apple, or 10%/7% olive extract; combination of essential oils with extracts; 3% hydrogen peroxide; and untreated control. A randomized block design with an affective test was used and 60 panelists were asked to evaluate samples for preference liking based on a 9-point hedonic scale and for sensory attributes based on a 5-point hedonic scale. Changes in texture and color of leafy greens were measured using a Texture analyzer and a Chroma meter, respectively. On the basis of preference liking, overall acceptability of spinach and lettuce treated with 0.1% cinnamon oil was ranked the highest (7.5±1.4 and 7.1±1.7, moderately liked), respectively. For texture analysis, washing iceberg lettuce with 0.1% oregano oil+10% olive extract and spinach with 0.1% lemongrass oil+1% apple extract yielded the highest firmness values of F=783.1±53.8 Newtons and 939.30±35.2 Newtons, respectively. Based on the International Commission on Illumination CIE LAB color schemes, treatment with 0.1% oregano oil+10% olive extract had the greatest impact on color of iceberg lettuce with the lowest L*(44.5±6.2) indicating the darkest color. These results will help identify plant antimicrobials that have the least impact on sensory properties of organic leafy greens and are preferred by consumers.

3.2 Introduction

Organic foods have recently gained popularity among consumers because they are perceived to be more environmentally friendly (Goldman & Clancy, 1991), nutritious, and health promoting than conventionally grown foods (Makatouni 2002; Magnusson et al., 2003). As of 2012, the sales of organic food products in the United States have reached a market of $28.4 billion, which is 4% of the total food sales (USDA-ERS, 2016). The top selling category among organic food products is fruits and vegetables (USDA-ERS, 2016). As the consumption of organic food has increased, there is also a concern about increased risk of foodborne illnesses. Previous studies have shown that organically grown produce has higher microbial load than conventionally grown produce (Oliveira et al., 2010). The organic food industry is limited in its sanitation options, particularly in the usage of chemical-based sanitizers; therefore, alternative organic sanitization options such as plant antimicrobials in the wash water are being evaluated.

Essential oils (EOs) are gaining popularity as potential organic sanitizers or preservatives because they have broad-spectrum antimicrobial activity and are considered 'Generally Recognized as Safe' (GRAS) (FDA, 2005). There is an increased interest in natural antimicrobials compared to chemical preservatives due to many health concerns. Previous *in vitro* studies have shown that EOs have antibacterial activity against *Listeria monocytogenes, Salmonella* Typhimurium*, Escherichia coli* O157:H7*, Shigella dysenteriae, Bacillus cereus* and *Staphylococcus aureus* at concentrations of 0.2-10 $\mu l\ ml^{-1}$ (Burt, 2004). If these EOs are to be used as natural preservatives or disinfectants, their organoleptic properties on treated foods should be considered, because they can alter the taste or flavor of the food (Hsieh et al., 2001). A sensory appeal is one of the most important factors that will determine food choices by consumers (Steptoe et al., 1995). A study showed that EOs not only reduced the pathogen load during storage in meat samples, but also improved the organoleptic properties of meat (Karabagias et al., 2011).

It has been shown that treatment with 1mM carvacrol or cinnamic acid showed no significant adverse effects on the organoleptic attributes of kiwi fruits and honeydew melons (Roller & Seedhar, 2002). Chida et al. (2006) showed that citrus oil components such as limonene, and l-octanol had no effect on the aroma quality of some foods. Gutierrez et al. (2009) showed that there was no significant difference between vegetables washed with chlorine and EOs based on color, texture, and water activity of the samples as well as the gas composition in the package. No significant difference was found between lettuce washed with 250 ppm oregano oil versus that washed with chlorinated water, as evaluated by a sensory panel (Gutierrez et al., 2009). In vegetable soup, the acceptable concentration for rosemary, thyme, carvacrol, or *p*-cymene was 20 μL/L, whereas for lemon oil it was 200 μL/L (Espina et al., 2014).

The characteristics that impact the quality of fruits and vegetables are described by four attributes: 1) color and appearance; 2) flavor (taste and aroma); 3) texture; and 4) nutritional value (Barrett et al., 2010). In order to avoid the undesirable organoleptic effects of EOs on food, careful selection of these compounds is necessary. In this study, we therefore tested various essential oils, their active components, plant extracts, both alone and in combinations to determine both preference liking by consumers and effects on the sensory properties of organic leafy greens. Due to the increased popularity of natural treatments, especially in the organic industry, this study focused on investigating concerns related to sensory acceptability of plant-based antimicrobials on organic leafy greens.

3.3 Materials and Methods

3.3.1 Plant Antimicrobials Used

EOs used in this study included oregano oil, lemongrass oil, clove bud oil, and cinnamon oil; the active components used included carvacrol, and citral. The plant extracts evaluated were apple, grapeseed, and olive extract. Oregano oil (made (from 100% pure *Origanum vulgare*), and clove bud oil (100% pure *Eugenia caryophyllata*) were obtained from Lhasa Karnak Herb Company (Berkeley, CA, USA). Cinnamon oil (100% pure *Cinnamomum cassia*) and lemongrass oil (100% pure *Cymbopogon flexuosus*) were obtained from Now Foods (Bloomingdale, IL, USA). Natural citral (96%,), and carvacrol (99%) were obtained from Sigma Aldrich® (St. Louis, MO, USA). Olive extract *(Olea europaea)* HIDROX® 10x in liquid concentrate was obtained from CreAgri, Inc. (Hayward, CA, USA). Grapeseed extract was obtained from Swanson Health products (Fargo, ND, USA). Apple skin extract was obtained from Apple Poly LLC. (Morrill, NE). H_2O_2 (3%) was purchased from the local retail stores.

All treatment solutions were made in tap water in their respective chosen concentrations. The following plant antimicrobials were evaluated for preference liking and specific sensory attributes on organic baby spinach and iceberg lettuce samples: 0.1% oregano oil, 0.1% lemongrass oil, 0.1% clove bud oil, 0.1% cinnamon oil, 0.1% carvacrol, 0.1% citral, 3% apple extract, 3% grapeseed extract, 7% olive extract, 10% olive extract (the concentration is higher because this is a liquid extract as opposed to others in the powder form), and various combinations of EOs with plant extracts. H_2O_2 (3%) was used as a control.

3.3.2 Treatments of Organic Leafy Greens

Organic iceberg lettuce and baby spinach were purchased from a local retail store the same day of use. Organic leafy greens were thoroughly washed with tap water and air-dried.

Lettuce and spinach samples were washed in water (300 ml) containing the respective concentrations of plant antimicrobials for 2 min with gentle agitation. After treatment, the leafy green samples were stored at 4°C for 20-24 h. A control (washed in tap water without any antimicrobials) was included during each trial.

3.3.3 Sensory Analysis

Sensory analysis was performed using 60 untrained panelists during each trial. A total of 6 trials were conducted (3 for lettuce and 3 for spinach). A randomized block design with affective test was conducted to generate data for preference liking (Cattelan et al., 2015). Each panelist was provided with 1 g of sample for each treatment and was asked to drink water to clean his/her palette and wait at least 2 min prior to evaluating the next sample. Panelists were asked to evaluate each sample for preference liking based on the 9-point hedonic scale where, 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely. Sensory parameters for preference liking were: aroma, color, freshness, mouthfeel, flavor, and overall acceptability. Panelists were also asked to quantify each sample for sensory attributes including pungency, browning, bitterness, off-odor, and sourness based on a 5-point hedonic scale where 1 was rated the lowest and 5 the highest (1=not pungent, 2=slightly pungent, 3=moderately pungent, 4=very pungent 5=extremely pungent).

The sensory study was conducted at the sensory lab in the Department of Nutritional Sciences, University of Arizona. Constant yellow lights were used in the sensory booths allowing panelists to evaluate visual differences among samples. Panelists also answered questions about demographics such as age, gender, ethnicity, frequency of consumption of organic/conventionally grown leafy greens, and the types of leafy greens they would like to purchase (iceberg lettuce, romaine lettuce, spinach, or mixed greens).

3.3.4 Texture Analysis

The texture of each antimicrobial treated leafy green was measured using an Instron or Texture Analyzer (Texture Lab Pro, provided by Food Technology Corporation, Sterling, VA, USA). The leafy greens were treated as described earlier with the appropriate concentrations of each antimicrobial or their combinations and stored at $4^{o}C$ for 20-24 h prior to measurements. A 1000N load cell was attached with a Kramer shear cell with an 8-blade probe attached to the instrument with the speed set to 250 mm min^{-1} (Martin-Diana et al., 2006). A sample (15 g) of treated leafy greens was placed in the Kramer cell chamber to determine the crispiness/firmness value. Three repeats were conducted and an average of the highest peak force from three trials was indicated as its crispiness value.

3.3.5 Color Measurements

The color of the treated leafy greens was measured using a Minolta Chroma Meter (Model CR-400, Minolta, Inc., Tokyo, Japan). The color was measured using the International Commission on Illumination- CIE L*, a*, and b* coordinates. The L* value is a measurement of lightness from dark (L*=0) to absolute light (L*=100); a* axis ranges from green (-) to red (+); and b* axis ranges from blue (-) to yellow (+) (Du et al., 2011). The instrument was calibrated using Minolta standard white reflector plate. Four repeats of the experiment were conducted and three different readings were taken during each repeat (total readings 12) for each sample.

3.3.6 Statistical Analysis

For sensory analysis, each experiment was divided into three trials with new sets of 60 panelists at each trial. A randomized block design was used for sensory analysis. An average was calculated based on 60 responses for preference liking and sensory characteristics for each treatment. Data were analyzed using One-way Analysis of variance (ANOVA) Tukey's pairwise test at a level of significance of $p \leq 0.05$ using Minitab 17 (State College, PA, USA). A linear regression was used to determine the correlation between overall acceptability and other sensory parameters based on preference liking. Statistical analysis on texture and color measurements was also done using ANOVA, with statistical level of significance considered at $p \leq 0.05$.

3.4 Results and Discussion

Numerous studies have shown antimicrobial properties of plant-based compounds (Moore-Neibel et al., 2013; Todd et al., 2013; Ravishankar et al., 2010; 2008); however, studies concerning their effect on the sensory properties of leafy greens have been limited. One study showed that the addition of oregano essential oil preserved the intensity rating of positive attributes in extra virgin olive oil during storage (Asensio et al., 2012). To our knowledge, the impact of plant antimicrobial washes on the sensory attributes of organic leafy greens has not been extensively studied. Therefore, in this study, we identified plant antimicrobial washes for organic leafy greens that had the highest preference liking among consumers, and described how the color and firmness attributes of leafy greens are impacted by these compounds.

The concentration of each antimicrobial for washing organic leafy greens was chosen based on previous work indicating its antimicrobial properties. A total of 15 different treatments were evaluated for lettuce and 16 different treatments for spinach samples. Sensory analyses on lettuce samples were conducted first; therefore, treatments that were considerably disliked by panelists were not further tested on spinach samples. In addition, preliminary work was conducted using higher concentrations of plant antimicrobials for sensory analysis; however, due to majority of consumers' dislike preferences, lower concentrations were chosen for this study. Affective test is a sensory test commonly used to identify samples with the highest preference liking when comparing multiple samples using untrained panelists (Lawless and Heymann, 2003). Hence, we chose this type of sensory test for our study. Additionally, we asked the panelists to quantify the level of pungency, browning, bitterness, off-odor, and sourness noticed in an antimicrobial treated leafy green sample. To our knowledge extensive sensory studies have not been conducted on organic leafy greens washed with plant antimicrobials; this study can therefore be used as a baseline to identify sensory characteristics of these samples.

3.4.1 Demographics Information of the Panelists

The criteria for the panelists to participate in this study included the following: a) each panelist must be at least 18 years or older, b) must be a non-smoker, and c) be able to differentiate between colors. Results for all demographic information are shown in Figure 3.1. A total of 360 responses from panelists was obtained in this study (180 responses were obtained for a total of 15 lettuce samples and another 180 for a total of 16 spinach samples). Of the 360 participants, 75% were female, and 25% were male. The age of the panelists ranged from 18 to 78 years, with majority of them (65%) in the age range 18-25 years. On the basis of ethnicity, the panelists included 47% white, 24% Hispanic or Latino, 22% Asian, 3% African American, and 4% others. The highest preference concerning the types of leafy greens was for spinach (35%), followed by mixed greens (34%), romaine (22%), and iceberg lettuce (9%). Of the participants, 56% indicated that they consume conventionally grown leafy greens at least twice a week and 24% indicated that they consume organic leafy greens at least twice a week. Another similar study showed that overall, there were no significant differences between the consumer liking or consumer perceived sensory qualities of organically and conventionally grown vegetables including tomatoes, cucumbers, and onions (Zhao et al., 2007).

Figure 3.1: Demographic information on the panelists who participated in the sensory study for both iceberg lettuce and spinach washed with plant antimicrobials. Data depict responses from a total of 360 participants of whom 180 evaluated iceberg samples and 180 evaluated baby spinach samples. Figure 3.1 A) Gender ratio 3.1 B) Ethnicity ratio 3.1 C) Pattern for consumption of leafy greens 3.1 D) Prior preference for type of leafy greens.

Figure 3.1A

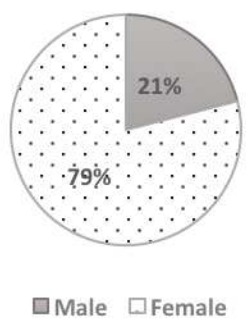


Figure 3.1B

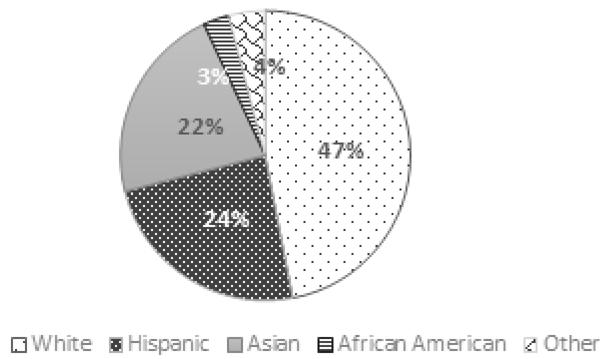


Figure 3.1C

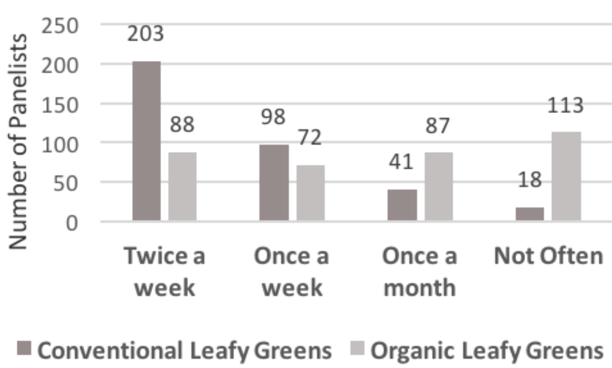
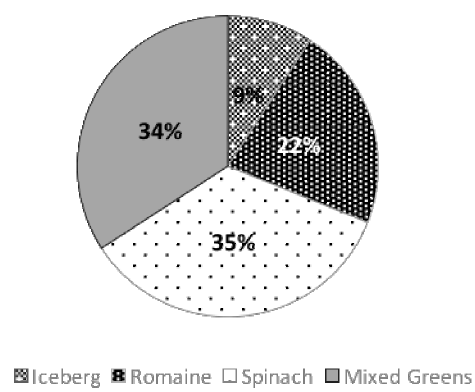


Figure 3.1D



3.4.2 Sensory Analysis of Organic Iceberg Lettuce Treated with Plant Antimicrobials

Among all plant antimicrobial treatments, iceberg lettuce washed with 0.1% cinnamon oil had the highest preference liking by panelists, ranging from 6 to 7 on the hedonic scale (slightly/moderately liked). Cinnamon oil may be preferred by panelists as there is a sweet taste associated with it. Another possible reason could be the familiarity of cinnamon among consumers. Majority of the population in this study were 18-25 years old white female which may also describe higher preference liking for cinnamon oil compared to others. Sweetness taste has higher hedonic appeal especially among children and young people (Drewnowski et al., 2012). A study conducted using 1005 participants from North America showed that females preferred comfort foods such as chocolate and ice cream compared to males (Wansink et al., 2003). For aroma, color, freshness, mouthfeel, flavor, and overall acceptability, there were no significant differences ($p \leq 0.05$) between control, 3%, H_2O_2, and 0.1% cinnamon oil treatments for iceberg lettuce samples (Table 3.1; Fig 3.2). Additionally, other treatments that were slightly or moderately preferred by panelists include: 0.1% clove bud oil, 0.1% citral, and 0.1% oregano oil. Treatment of organic iceberg lettuce with 0.1% cinnamon oil had the least impact on pungency, browning, bitterness, off-odor, and sourness of lettuce (Table 3.2; Fig 3.3). Other studies have indicated similar results where vegetables (iceberg lettuce, beet, and arugula) sanitized with *Origanum vulgare* and *Rosmarinus officinalis* essential oils alone and in combination were "liked slightly" and "neither liked nor disliked" based on the 5-point hedonic scale (De Azeredo et al., 2011).

Combination treatments and treatments with plant extracts had the highest influence on preference liking by consumers based on color, as these treatments imparted higher browning characteristics to iceberg lettuce. According to Barrett and others (2006), customers evaluate visual appearance and color first followed by taste, aroma, and texture. EOs used (individually) for washing iceberg lettuce did not have a significant ($p \leq 0.05$) impact on browning, since there were no significant differences between the control, 3% H_2O_2, 0.1% cinnamon oil, 0.1% citral, and 0.1% oregano oil, all of which had a rating level of 1 (not brown at all).

Washing iceberg lettuce with tap water (control), 3% H_2O_2, 3% grapeseed extract, or 3% apple extract did not have a significant impact ($p \leq 0.05$) on the pungency level, as all samples were rated at level 1-not pungent at all. The majority of the treatments evaluated did not increase the bitterness or sourness level of iceberg lettuce in comparison to the control or 3% H_2O_2. Again, samples that were not significantly ($p \leq 0.05$) different based on off-odor in comparison to the control or 3% H_2O_2 included those that were treated with 0.1% cinnamon oil.

Figure 3.2: Sensory profile of preference liking for organic iceberg lettuce after treatment with plant antimicrobials and storage for 24 h at 4°C. Preference liking was evaluated for: aroma, color, freshness, mouthfeel, flavor, and overall acceptability based on a 9-point hedonic scale where 9 is extremely liked, and 1 is not liked at all. Three separate trials were conducted during which 5-6 new plant antimicrobials were evaluated. Data depicted are averages of 60 responses during each trial.

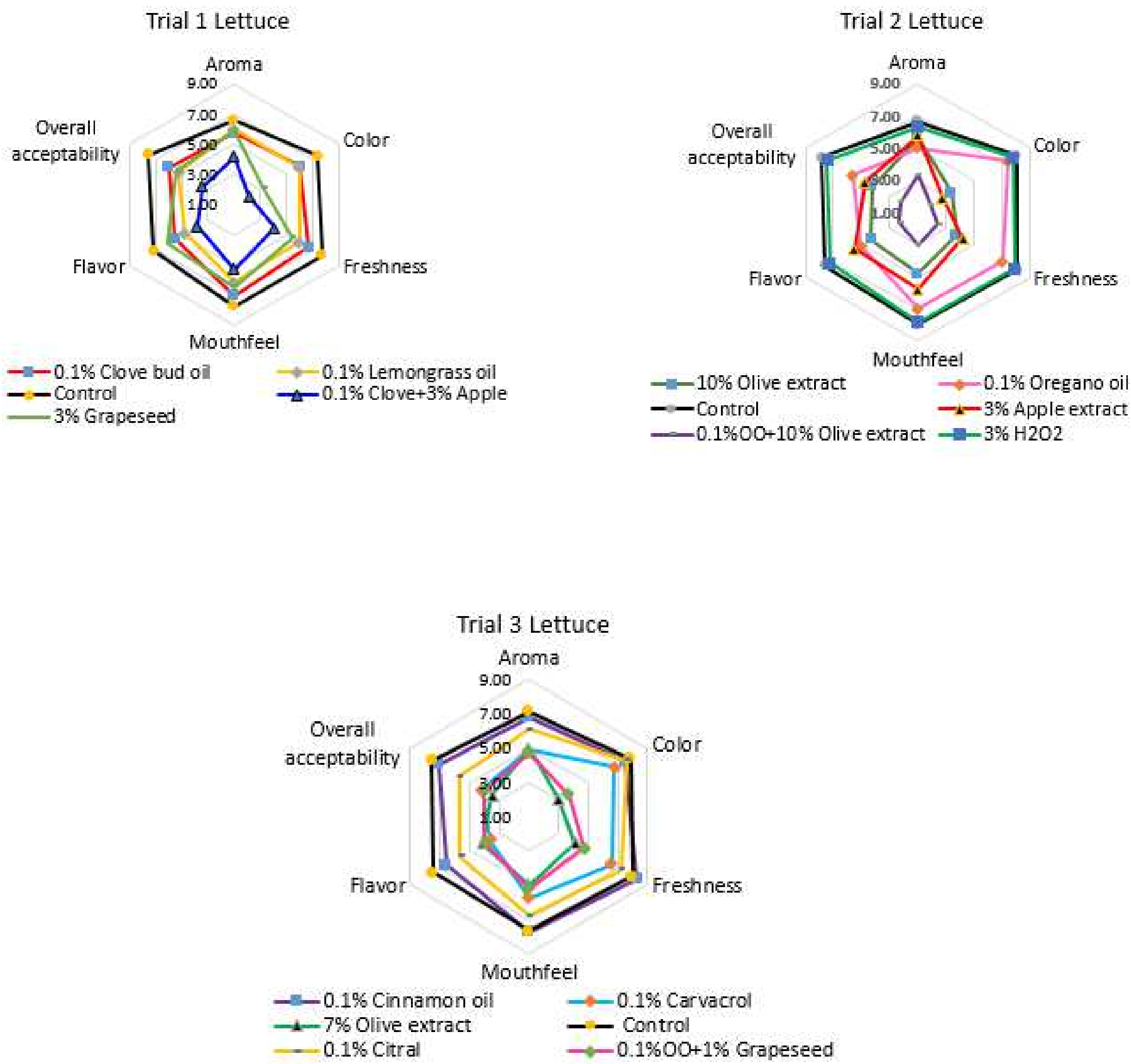
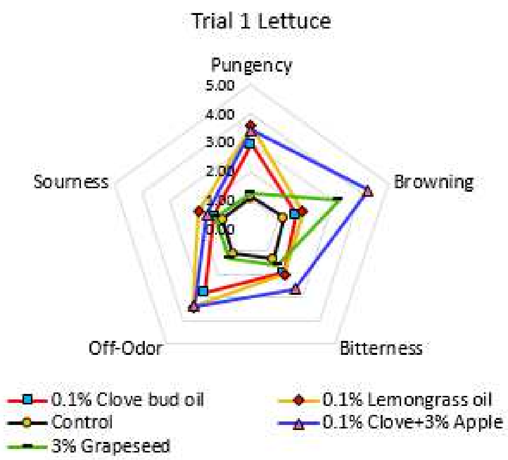
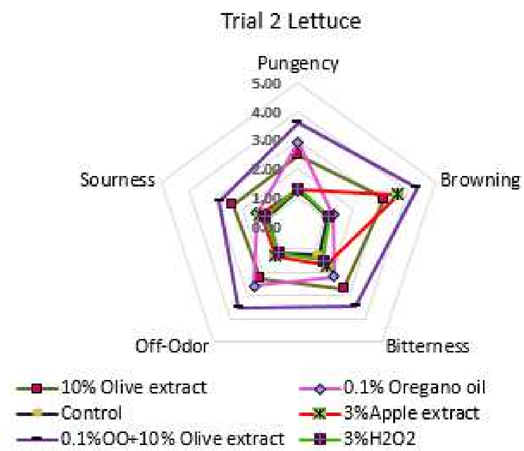


Figure 3.3: Impact of plant antimicrobials on sensory characteristics of treated iceberg lettuce stored at 4°C for 24 h during three separate trials evaluated by panelists. The impact on the sensory characteristics (pungency, browning, bitterness, off-odor, and sourness) was rated based on a 5-point hedonic scale with 5 being highest impact and 1 being no impact at all. Data depicted are an average response of 60 panelists during each trial.





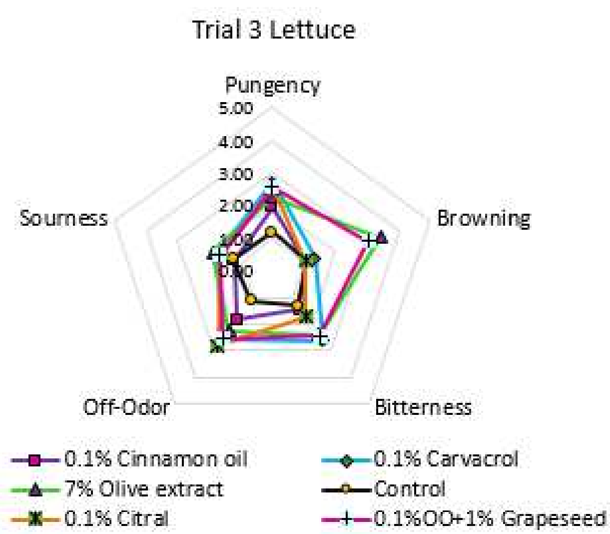


Table 3.1: Preference liking for lettuce samples after being washed with essential oils, plant extracts, or combination of both. Data depicted is an average of 60 responses for each treatment ± standard deviation. Different letters signify statistical difference between various treatments ($p \leq 0.05$) for each sensory parameter.

Treatments	Aroma	Color	Freshness	Mouthfeel	Flavor	Overall acceptability
Control	6.55 ± 1.56^{A}	7.40 ± 1.65^{A}	7.70 ± 1.60^{ABC}	7.73 ± 1.38^{A}	7.15 ± 1.98^{A}	7.53 ± 1.48^{A}
3% H_2O_2	6.35 ± 1.89^{A}	7.82 ± 1.69^{A}	7.97 ± 1.34^{AB}	7.78 ± 1.57^{A}	7.30 ± 1.67^{A}	7.48 ± 1.37^{A}
0.1% Cinnamon oil	6.83 ± 1.69^{A}	7.49 ± 1.60^{A}	8.25 ± 0.94^{A}	7.71 ± 1.45^{A}	6.59 ± 1.90^{AB}	7.12 ± 1.67^{AB}
0.1% Clove bud oil	5.73 ± 2.11^{ABC}	6.02 ± 1.96^{B}	6.75 ± 1.75^{BCD}	7.02 ± 1.70^{AB}	5.53 ± 2.46^{BCDE}	5.97 ± 1.96^{BC}
0.1% Citral oil	6.07 ± 2.62^{AB}	7.56 ± 1.76^{A}	7.22 ± 2.09^{ABC}	6.78 ± 2.35^{AB}	5.66 ± 2.58^{BCD}	5.70 ± 2.44^{C}
0.1% Oregano Oil	5.00 ± 2.46^{BCD}	7.43 ± 1.77^{A}	7.05 ± 1.79^{ABCD}	6.95 ± 1.96^{AB}	5.08 ± 2.53^{CDEF}	5.65 ± 2.29^{C}
3% Grapeseed extract	5.90 ± 1.63^{ABC}	3.23 ± 1.49^{CD}	5.40 ± 2.23^{EFG}	6.42 ± 1.84^{BC}	6.03 ± 2.21^{ABC}	5.28 ± 1.68^{CD}
0.1% Lemongrass oil	5.85 ± 2.70^{ABC}	6.00 ± 2.21^{B}	5.95 ± 2.26^{DEF}	6.13 ± 1.90^{BCD}	4.80 ± 2.56^{CDEF}	5.27 ± 2.24^{CDE}
3% Apple extract	5.80 ± 1.80^{ABC}	2.80 ± 1.86^{CDE}	4.25 ± 2.62^{GH}	5.77 ± 2.21^{BCDE}	5.53 ± 2.26^{BCDE}	4.78 ± 2.11^{CDE}
10% Olive extract	5.68 ± 1.92^{ABC}	3.45 ± 2.02^{C}	3.78 ± 2.15^{H}	4.80 ± 2.20^{E}	4.30 ± 2.49^{DEF}	4.25 ± 2.10^{DEF}
0.1% Carvacrol	4.88 ± 2.15^{BCD}	6.56 ± 2.03^{AB}	6.53 ± 2.27^{CDE}	5.75 ± 2.63^{BCDE}	3.65 ± 2.18^{FG}	4.20 ± 2.32^{DEF}
0.1 oregano oil +1% Grapeseed extract	4.73 ± 2.12^{CD}	3.64 ± 2.13^{C}	4.71 ± 2.23^{FGH}	5.21 ± 2.32^{CDE}	3.98 ± 2.48^{F}	4.01 ± 2.31^{EF}
7% Olive extract	5.00 ± 2.02^{BCD}	2.98 ± 1.93^{CDE}	4.07 ± 2.12^{H}	5.00 ± 2.41^{DE}	4.16 ± 2.46^{EF}	3.52 ± 2.06^{F}
0.1% Clove bud oil +3% Apple extract	4.17 ± 2.87^{DE}	2.13 ± 1.62^{DE}	4.09 ± 2.32^{H}	5.27 ± 2.20^{CDE}	3.83 ± 2.31^{F}	3.52 ± 1.99^{F}
0.1% oregano oil +10% Olive extract	3.35 ± 2.27^{E}	1.93 ± 1.44^{E}	2.47 ± 2.04^{I}	2.98 ± 2.01^{F}	2.27 ± 2.00^{G}	2.25 ± 1.73^{G}

Table 3.2: Impact of plant-antimicrobials on the sensory characteristics of organic iceberg lettuce evaluated by panelists. Data represent an average of 60 responses for each sample and values next to the average indicate standard deviation (±). Average values, which do not share the same letter, are significantly different (p≤0.05).

Treatments	Pungency	Browning	Bitterness	Off-Odor	Sourness
Control	1.11 ± 0.33^{G}	1.18 ± 0.50^{EFG}	1.31 ± 0.72^{GH}	1.08 ± 0.33^{F}	1.03 ± 0.18^{G}
3% H_2O_2	1.33 ± 0.68^{FG}	1.14 ± 0.51^{FG}	1.48 ± 0.77^{FGH}	1.15 ± 0.52^{F}	1.20 ± 0.51^{EFG}
0.1% Cinnamon oil	1.83 ± 0.94^{EF}	1.05 ± 0.22^{G}	1.28 ± 0.59^{H}	1.72 ± 0.90^{EF}	1.13 ± 0.39^{FG}
0.1% Clove bud oil	2.93 ± 1.10^{BCD}	1.62 ± 0.80^{DE}	1.90 ± 1.02^{DEFGH}	2.78 ± 1.19^{BCD}	1.34 ± 0.74^{DEFG}
0.1% Citral oil	2.73 ± 1.25^{D}	1.27 ± 0.58^{EFG}	1.62 ± 0.94^{EFGH}	2.85 ± 1.16^{BCD}	1.59 ± 0.93^{CDEFG}
0.1% Oregano oil	2.93 ± 1.35^{BCD}	1.30 ± 0.62^{EFG}	2.17 ± 1.38^{BCDE}	2.60 ± 1.37^{CD}	1.51 ± 0.89^{CDEFG}
3% Grapeseed extract	1.28 ± 0.55^{FG}	3.22 ± 0.96^{BC}	1.62 ± 0.87^{EFGH}	1.32 ± 0.57^{F}	1.35 ± 0.73^{DEFG}
0.1% Lemongrass oil	3.57 ± 1.27^{AB}	1.88 ± 0.96^{D}	1.98 ± 1.07^{CDEFG}	3.40 ± 1.22^{AB}	1.92 ± 1.03^{BCD}
3% Apple extract	1.25 ± 0.60^{FG}	3.65 ± 0.92^{B}	1.71 ± 1.00^{EFGH}	1.30 ± 0.53^{F}	1.37 ± 0.66^{DEFG}
10% Olive extract	2.48 ± 1.03^{D}	3.17 ± 0.83^{C}	2.70 ± 1.27^{B}	2.23 ± 1.10^{DE}	2.40 ± 1.17^{AB}
0.1% Carvacrol	2.73 ± 1.07^{D}	1.55 ± 0.81^{DEF}	2.53 ± 1.37^{BCD}	2.90 ± 1.07^{ABC}	1.75 ± 1.02^{CDE}
0.1% Oregano oil +1% Grapeseed extract	2.88 ± 1.19^{CD}	3.23 ± 0.85^{BC}	2.14 ± 1.18^{BCDEF}	2.98 ± 1.19^{ABC}	1.67 ± 1.02^{CDEF}
7% Olive extract	2.38 ± 1.17^{DE}	3.65 ± 0.95^{B}	2.46 ± 1.21^{BCD}	2.43 ± 1.14^{CD}	1.97 ± 1.16^{BC}
0.1% Clove bud oil +3% Apple extract	3.43 ± 1.20^{ABC}	4.27 ± 0.84^{A}	2.63 ± 1.24^{BC}	3.37 ± 1.22^{AB}	1.60 ± 1.03^{CDEFG}
0.1% oregano oil +10% Olive extract	3.62 ± 1.14^{A}	4.37 ± 0.76^{A}	3.45 ± 1.28^{A}	3.52 ± 1.21^{A}	2.87 ± 1.49^{A}

3.4.3 Sensory Analysis of Organic Baby Spinach Treated with Plant Antimicrobials

For spinach samples, the treatment with the highest preference liking based on aroma was 0.1% lemongrass, and based on all other parameters (color, freshness, mouthfeel, flavor, and overall acceptability) 0.1% cinnamon oil had the highest preference liking (Table 3.3; Fig 3.4). The treatments that had adverse impact on the color parameters based on preference liking were: 3% grapeseed extract, 0.1% clove bud oil+3% apple extract, 0.1% oregano oil+1% grapeseed extract, 0.1% carvacrol, and 0.1% oregano oil+7% olive extract. Treatments that gave slightly brown (2.0-2.3) color to baby spinach included: 3% grapeseed, 0.1% clovebud oil+3% apple extract, 0.1% oregano oil+1% grapeseed extract, and 0.1% oregano oil+7% olive extract. All other treatments were rated not brown at all (1.1-1.7). The following treatments did not have significantly different ($p \leq 0.05$) pungency or off-odor levels in comparison to the control and 3% H_2O_2: 0.1% clove bud oil, 3% apple extract, 3% grapeseed extract, and a combination of 0.1% clove bud oil+3% apple extract; they all had a rating of 1 (not pungent/off-odor at all). Treatments that were significantly different ($p \geq 0.05$) based on bitterness included: 0.1% oregano oil+1% grapeseed extract, 0.1% oregano oil, 7% olive extract, 0.1% carvacrol, and 0.1% oregano oil+7% olive extract. Treatments that significantly ($p \geq 0.05$) impacted the sourness value of spinach included: 0.1% citral, 0.1% lemongrass oil+1% apple extract, 0.1% oregano oil, 7% olive extract, 0.1% carvacrol, and combination of 0.1% oregano+7% olive extract which had ratings of 1.9±1.1-2.1±1.2 (slightly sour).

The least preferred treatment for spinach samples was a combination of 7% olive extract with 0.1% oregano oil based on aroma, flavor and overall acceptability. For color preference, spinach treated with 3% grapeseed extract had the lowest rating (6.0±2.4, slightly like), and for freshness, 0.1% clove bud oil+ 3% apple extract had the lowest rating (5.8±2.5, neither like or dislike); however, the difference was not significant ($p \leq 0.05$) between these treatments and 7% olive extract+0.1% oregano oil. The combination of olive extract with oregano oil was the least preferred based on overall acceptability (4.4±2.5, slightly dislike), with this treatment being ranked the highest for pungency (3.2±1.2), browning (2.0±0.9), and bitterness (3.0±1.3) (Table 3.4; Fig 3.5). There is a negative correlation between rating of preference liking and sensory characteristics of pungency, browning, bitterness, off-odor, and sourness (data not shown).

Figure 3.4*:* Sensory profile of preference liking for organic baby spinach after treatment with plant antimicrobials and storage for 24 h at 4°C. Preference liking was evaluated for: aroma, color, freshness, mouthfeel, flavor, and overall acceptability based on a 9-point hedonic scale where 9 is extremely liked, and 1 is not liked at all. Three separate trials were conducted during which 5-6 new plant antimicrobials were evaluated. Data depicted are an average of 60 responses during each trial.

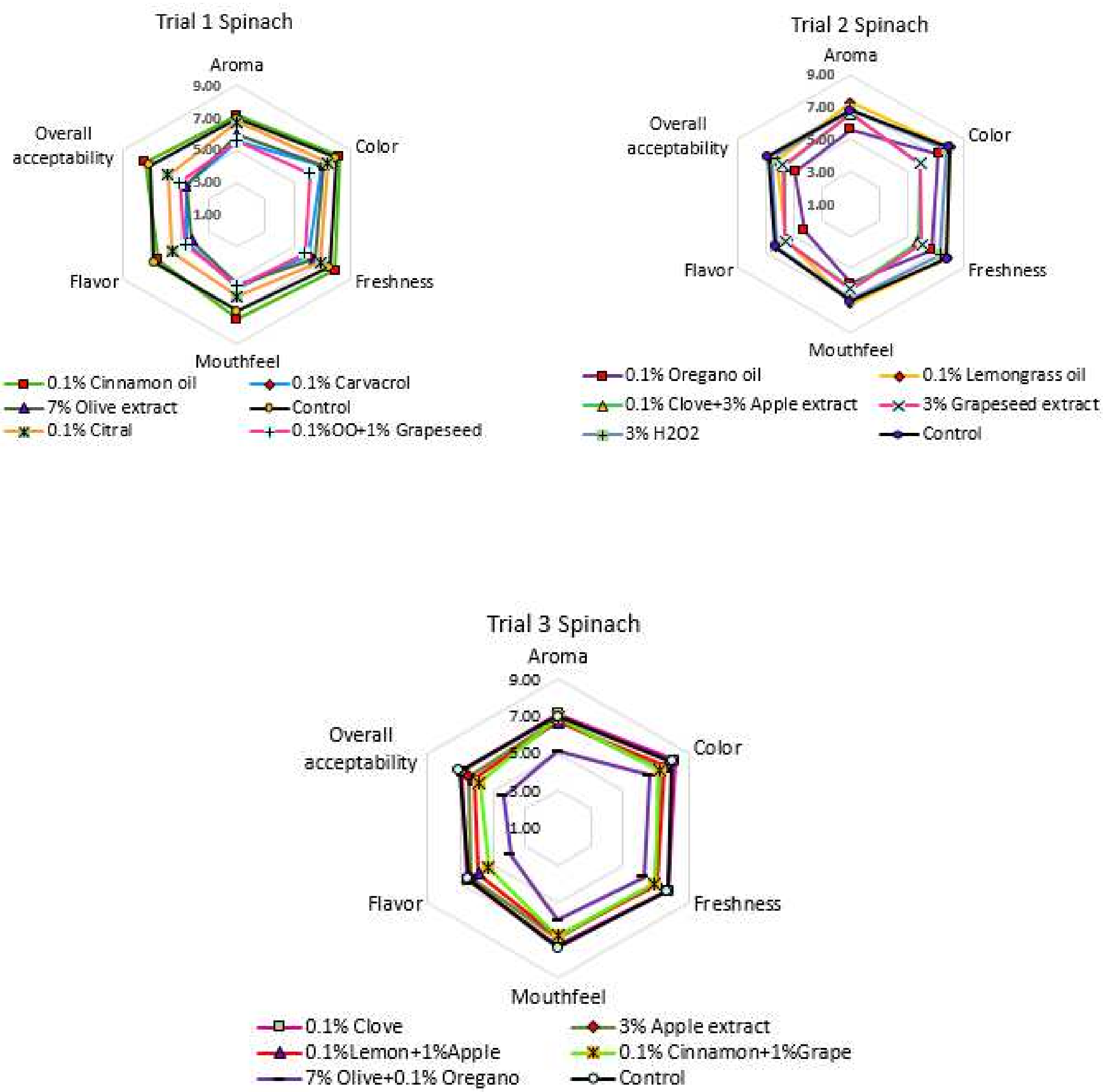


Figure 3.5: Impact of plant antimicrobials on the sensory characteristics of treated baby spinach stored at 4°C for 24 h during three separate trials evaluated by panelists. The impact on sensory characteristics (pungency, browning, bitterness, off-odor, and sourness) was rated based on a 5-point hedonic scale with 5 being highest impact and 1 being no impact at all. Data depicted are an average response of 60 panelists during each trial.

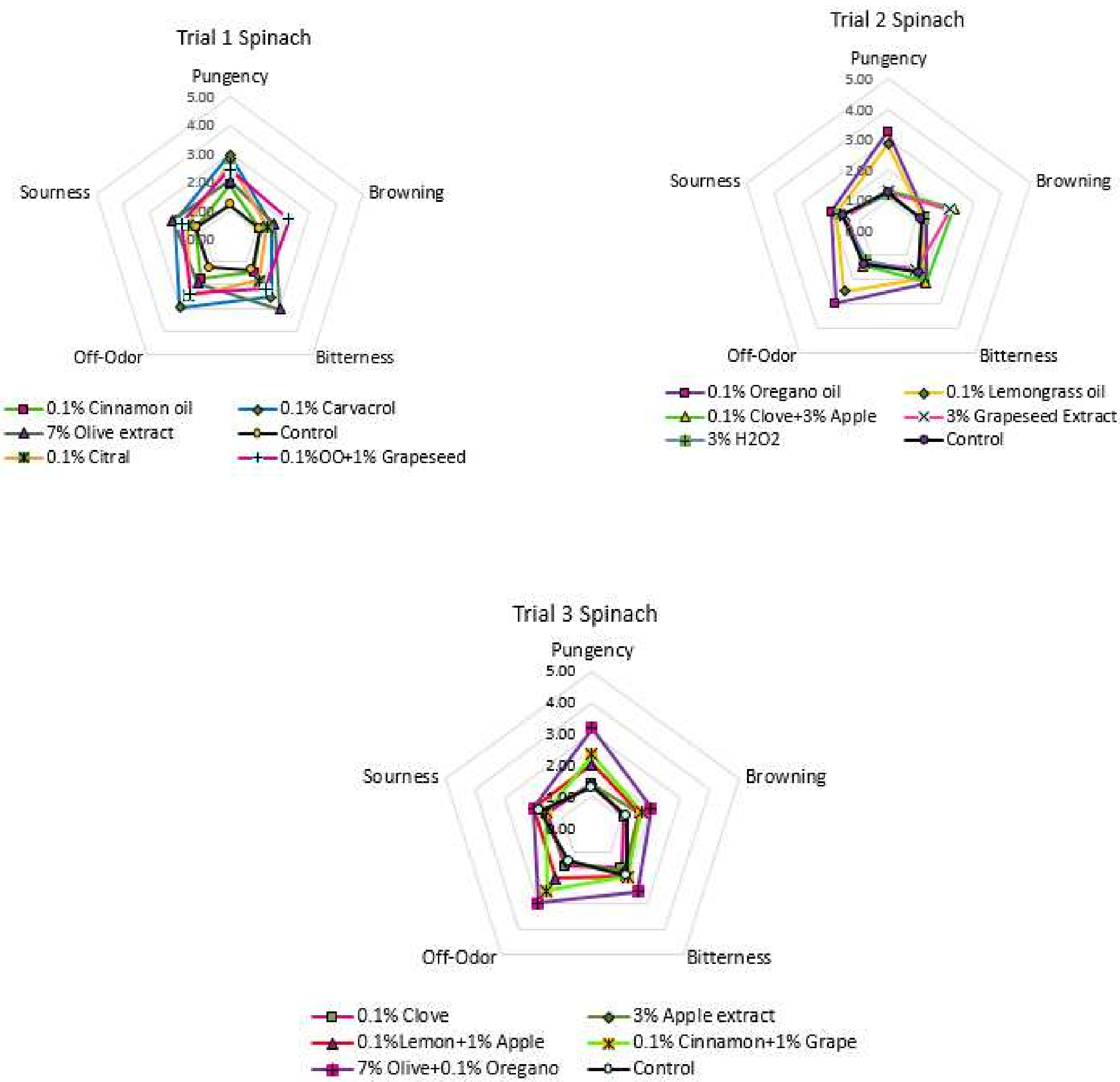


Table 3.3: Preference liking for spinach samples after being washed with essential oils, plant extracts, or combination of both. The data depicted is an average of 60 responses for each treatment ± standard deviation. Different letters signify statistical difference between various treatments ($p \leq 0.05$) for each sensory parameter.

Treatments	Aroma	Color	Freshness	Mouthfeel	Flavor	Overall acceptability
0.1% Cinnamon oil	7.12 ± 1.77^{AB}	8.18 ± 1.10^{A}	7.92 ± 1.51^{A}	7.47 ± 1.33^{A}	6.57 ± 2.09^{AB}	7.48 ± 1.37^{A}
Control	6.93 ± 2.02^{ABC}	8.00 ± 1.35^{AB}	7.50 ± 1.77^{AB}	6.93 ± 1.78^{ABC}	6.86 ± 1.76^{A}	7.14 ± 1.46^{AB}
0.1% Clove bud oil	7.10 ± 1.79^{AB}	8.17 ± 1.14^{A}	7.80 ± 1.35^{A}	7.27 ± 1.75^{AB}	6.58 ± 2.14^{AB}	6.95 ± 1.89^{ABC}
3% H_2O_2	6.83 ± 1.92^{ABCD}	7.78 ± 1.70^{AB}	7.32 ± 2.06^{ABC}	6.97 ± 1.85^{ABC}	6.29 ± 2.42^{AB}	6.65 ± 2.05^{ABC}
3% Apple extract	6.77 ± 1.89^{ABCDE}	7.30 ± 1.94^{ABC}	7.00 ± 1.90^{ABCDE}	6.88 ± 1.80^{ABC}	6.35 ± 1.98^{AB}	6.50 ± 2.19^{ABC}
0.1% Lemongrass oil	7.30 ± 1.80^{A}	8.03 ± 1.05^{AB}	7.78 ± 1.39^{A}	7.19 ± 1.54^{ABC}	5.51 ± 2.18^{ABCD}	6.24 ± 1.83^{ABCD}
0.1% Lemongrass+ 1% Apple extract	6.65 ± 2.22^{ABCDE}	7.57 ± 1.58^{ABC}	7.08 ± 1.92^{ABCD}	6.83 ± 2.00^{ABC}	5.90 ± 2.50^{ABC}	6.17 ± 2.42^{ABCD}
0.1% Citral oil	6.72 ± 2.36^{ABCDE}	7.42 ± 1.96^{ABC}	6.85 ± 2.02^{ABCDEF}	6.02 ± 2.20^{BCDE}	5.58 ± 2.14^{ABCD}	5.88 ± 2.12^{BCDE}
0.1% Cinnamon oil +1% Grapeseed extract	6.88 ± 2.00^{ABC}	7.22 ± 1.70^{ABCD}	6.95 ± 1.83^{ABCDEF}	$6.77 \pm 1.78A^{BCD}$	5.28 ± 2.34^{BCDE}	5.82 ± 2.01^{BCDE}
3% Grapeseed extract	6.60 ± 1.88^{ABCDE}	5.95 ± 2.38^{E}	6.05 ± 2.20^{DEF}	6.29 ± 2.04^{ABCDE}	5.63 ± 2.36^{ABCD}	5.80 ± 2.15^{BCDE}
0.1% Clove bud oil +3% Apple extract	6.55 ± 1.87^{ABCDE}	5.93 ± 2.27^{E}	5.77 ± 2.48^{F}	6.20 ± 2.21^{BCDE}	5.62 ± 2.51^{ABCD}	5.68 ± 2.40^{CDEF}
0.1% Oregano oil +1% Grapeseed	5.53 ± 2.13^{DEF}	6.13 ± 2.18^{DE}	5.78 ± 2.28^{EF}	5.40 ± 2.21^{E}	4.63 ± 2.23^{CDE}	5.03 ± 2.12^{DEF}
0.1% Oregano	5.63 ± 2.69^{CDEF}	7.20 ± 1.74^{ABCD}	6.70 ± 1.78^{ABCDEF}	5.93 ± 2.18^{CDE}	4.30 ± 2.52^{DE}	4.93 ± 2.37^{DEF}
7% Olive extract	5.93 ± 2.32^{BCDEF}	7.12 ± 1.72^{ABCD}	6.41 ± 2.08^{BCDEF}	5.32 ± 2.41^{E}	4.19 ± 2.48^{DE}	4.59 ± 2.27^{EF}
0.1% Carvacrol	5.48 ± 2.56^{EF}	7.00 ± 2.11^{BCDE}	6.05 ± 2.28^{DEF}	$5.50 \pm 2.50D^{E}$	4.50 ± 2.68^{CDE}	4.58 ± 2.44^{EF}
7% Olive extract +0.1% Oregano oil	5.10 ± 2.31^{F}	6.62 ± 2.00^{CDE}	6.27 ± 2.19^{CDEF}	5.93 ± 2.22^{CDE}	3.92 ± 2.44^{E}	4.36 ± 2.48^{F}

Table 3.4: Impact of plant-antimicrobials on sensory characteristics of organic baby spinach evaluated by panelists. Data represent an average of 60 responses for each sample and values next to the averages indicate standard deviation (±). Average values, which do not share the same letter, are significantly different ($p \leq 0.05$).

Treatments	Pungency	Browning	Bitterness	Off-Odor	Sourness
0.1% Cinnamon oil	1.90 ± 1.12^{EF}	1.13 ± 0.50^{DE}	1.51 ± 0.88^{DE}	1.75 ± 1.04^{CDEF}	1.27 ± 0.61^{C}
Control	1.21 ± 0.52^{G}	1.13 ± 0.39^{DE}	1.43 ± 0.67^{E}	1.23 ± 0.59^{F}	1.27 ± 0.58^{C}
0.1% Clove bud oil	1.40 ± 0.69^{FG}	1.08 ± 0.28^{E}	1.60 ± 0.83^{DE}	1.52 ± 0.85^{DEF}	1.52 ± 0.81^{ABC}
3% H_2O_2	1.18 ± 0.39^{G}	1.25 ± 0.51^{CDE}	1.72 ± 0.83^{DE}	1.23 ± 0.56^{F}	1.48 ± 0.77^{BC}
3% Apple extract	1.38 ± 0.85^{FG}	1.60 ± 0.72^{BCD}	1.58 ± 0.83^{DE}	1.37 ± 0.81^{DEF}	1.72 ± 1.01^{ABC}
0.1% Lemongrass oil	2.85 ± 1.19^{ABC}	$1.23 \pm 0.50C^{DE}$	1.95 ± 0.86^{CDE}	2.50 ± 1.16^{AB}	1.82 ± 1.07^{ABC}
0.1% Lemongrass oil +1% Apple extract	2.03 ± 1.14^{E}	1.60 ± 0.74^{BCD}	1.92 ± 1.02^{CDE}	2.00 ± 1.15^{BCD}	1.98 ± 1.21^{AB}
0.1% Citral oil	2.67 ± 1.32^{ABCD}	1.38 ± 0.76^{CDE}	1.80 ± 0.98^{DE}	2.48 ± 1.36^{AB}	1.93 ± 1.09^{AB}
0.1% Cinnamon oil +1% Grapeseed extract	2.33 ± 1.15^{CDE}	1.67 ± 0.77^{BC}	1.98 ± 1.05^{BCDE}	2.50 ± 1.24^{AB}	1.58 ± 0.87^{ABC}
3% Grapeseed extract	1.28 ± 0.61^{FG}	2.18 ± 0.89^{A}	1.58 ± 0.83^{DE}	1.33 ± 0.60^{EF}	1.52 ± 0.81^{ABC}
0.1% Clove bud oil +3% Apple extract	1.31 ± 0.59^{FG}	2.33 ± 1.02^{A}	1.95 ± 1.07^{CDE}	1.45 ± 0.77^{DEF}	1.52 ± 0.79^{ABC}
0.1% Oregano oil +1% Grapeseed extract	2.45 ± 1.08^{BCDE}	2.20 ± 1.04^{A}	2.15 ± 1.20^{BCD}	2.39 ± 1.10^{ABC}	1.80 ± 0.99^{ABC}
0.1% Oregano	3.25 ± 1.19^{A}	1.33 ± 0.60^{CDE}	2.60 ± 1.28^{AB}	2.98 ± 1.29^{A}	1.98 ± 1.19^{AB}
7% Olive extract	2.05 ± 1.14^{DE}	1.68 ± 0.83^{BC}	3.03 ± 1.26^{A}	1.92 ± 1.00^{BCDE}	2.12 ± 1.21^{A}
0.1% Carvacrol	2.97 ± 1.16^{AB}	1.58 ± 1.02^{BCD}	2.51 ± 1.12^{ABC}	2.98 ± 1.18^{A}	2.05 ± 1.17^{AB}
0.1% oregano oil + 7% olive extract	3.18 ± 1.19^{A}	1.98 ± 0.85^{AB}	3.00 ± 1.34^{A}	2.97 ± 1.26^{A}	2.00 ± 1.20^{AB}

3.4.4 Comparison of Spinach and Iceberg Lettuce Samples

Overall, most of the treatments for spinach samples had a higher preference liking by consumers in comparison to those for lettuce samples. When panelists were asked which leafy greens was preferred/consumed the most, spinach was also rated the highest and this could have influenced the preference liking for spinach samples after treatment. Additionally, plant antimicrobials had a lower impact on the color and texture properties of organic baby spinach in comparison to iceberg lettuce; therefore, this could be a factor contributing to the lower preference rating for iceberg lettuce samples. Combination treatments such as 0.1% clove bud oil+3% apple extract had a rating of 3.5±1.99 (dislike moderately) for iceberg lettuce; however, the same treatment had a rating of 5.7±2.4 (neither like nor dislike) for baby spinach. A similar trend was seen with the combination treatment of 0.1% oregano oil+1% grapeseed extract and the individual treatment of 7% olive extract, where the preference rating based on the overall acceptability was increased by 1-unit scale on spinach samples in comparison to iceberg lettuce.

It was evident that the combination treatments did not increase the preference liking among consumers for both iceberg lettuce and spinach samples. In general, a concentration-dependent effect on the preference liking was observed. For example, in case of iceberg lettuce samples, the treatment with 3% apple extract was rated 4.8±2.1 (dislike slightly) and when 3% apple extract was combined with 0.1% clove bud oil, the overall acceptability dropped by about 1 unit (3.5±2.0, dislike moderately). However, when lower concentrations of extracts were used in combination with essential oils, then the preference liking slightly increased. Treatment with 0.1% oregano oil in combination with 1% grapeseed extract had a preference liking of 4.0±2.3 (slightly dislike), which was better than that of 0.1% clove bud oil+ 3% apple extract; however, using plant extracts or essential oils alone in general had much higher preference liking.

Treatment of 0.1% clove bud oil alone for iceberg lettuce showed a preference rating of 6.0±2.0 (slightly like) and 3% apple extract had a rating of 4.8±2.1 (slightly dislike).

Linear regression (Figure 3.6) was conducted to compare the data on the ranking of overall acceptability with aroma, color, freshness, mouthfeel, and flavor. For iceberg lettuce samples, flavor (R^2=0.91) and mouthfeel (R^2=0.92) parameters were closely related to the rating of overall acceptability (Figure 3.6 A) for all treatments. For spinach samples, flavor (R^2=0.94) was the most influencing factor in determining the overall acceptability of a sample suggested by the strong correlation value (Figure 3.6 B). A similar trend was seen where a correlation of R^2=0.91 was found between flavor and overall acceptability of a salad dressing incorporated with 0.2% oregano EO and 1.14% salt (Cattelan et al., 2015). In some cases, a strong correlation was seen between color attributes and overall acceptability for lettuce samples washed with plant extracts. For example, iceberg lettuce leaf washed with a combination of 0.1% oregano oil+10% olive extract had the highest correlation of R^2=0.80 between color and overall acceptability (data not shown). In general, for all treatments, upon comparing overall acceptability with other sensory parameters (aroma, color, freshness, mouthfeel, and flavor), flavor was the most influencing factor determining the overall acceptability of samples as indicated by Figure 3.6 A and 3.6 B.

Additionally, each panelist was asked about the likelihood of purchasing leafy greens washed with a specific treatment. In response to this question, iceberg lettuce or spinach treated with 0.1% cinnamon oil was very/extremely likely (25-26/60) to be purchased by panelists (data not shown). A strong correlation was seen between the rating of overall acceptability and likelihood of purchasing for both spinach (R^2=0.95) and iceberg lettuce (R^2=0.96) after washing with plant antimicrobials (data not shown).

Figure 3.6: Linear correlation between values of average overall acceptability rating and other sensory parameters (aroma, freshness, mouthfeel, color, and flavor) after treatment with plant antimicrobials for iceberg lettuce (3.6 A) and baby spinach (3.6 B) samples.

Figure 3.6 (A)

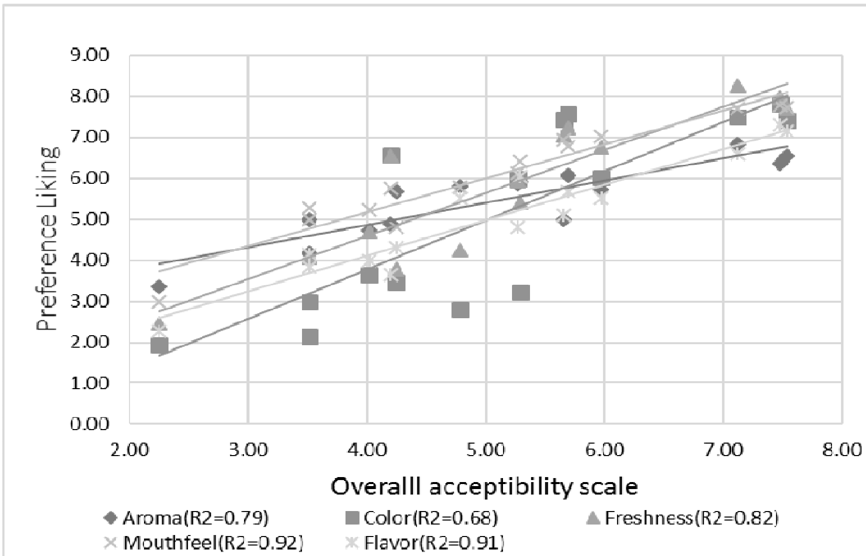
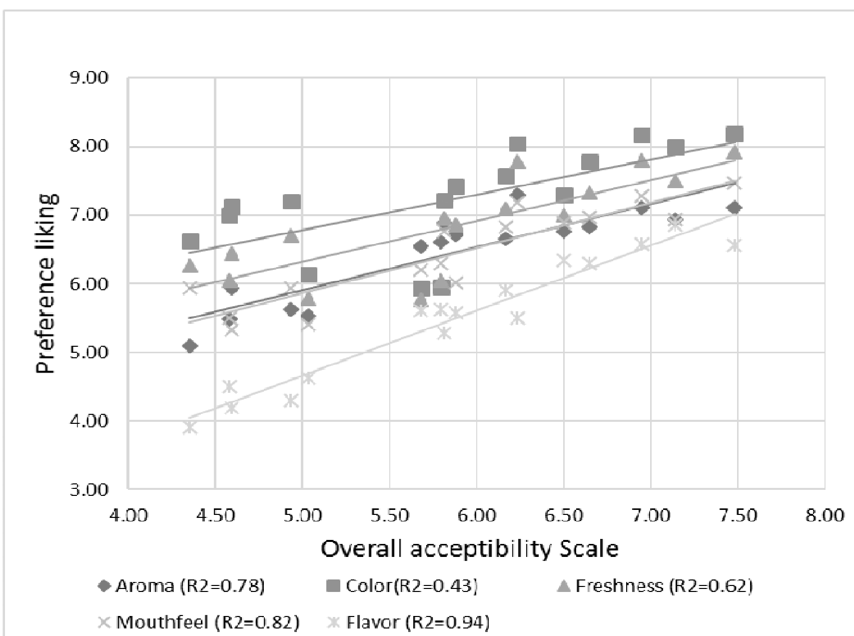


Figure 3.6 (B)



3.4.5 Effects of Plant Antimicrobial Treatments on the Texture of Organic Leafy Greens

Textural changes are one of the main causes of quality loss in a food product (Martin-Diana et al., 2006). It is therefore crucial to evaluate the impact of plant antimicrobial treatments on organic leafy greens. It is difficult however, to obtain texture measurements on iceberg lettuce because of the heterogeneity of lettuce leaves. Iceberg lettuce has two types of tissues, vascular and photosynthetic, which are relatively different in textural properties (Toole et al., 2000). In the present study, we have shown that overall, plant antimicrobials had a higher impact on the textural properties of iceberg lettuce in comparison to spinach, due to the softer tissues and non-uniformity in the texture of iceberg lettuce.

Texturometer analysis showed that iceberg lettuce washed with calcium lactate had significantly higher ($p>0.05$) crispiness value than samples washed with chlorine (Martin-Diana et al., 2006). All plant antimicrobial treatments for iceberg lettuce had higher firmness values (Table 3.5) than control, except the individual treatments of 0.1% lemongrass oil and 3% apple extract. These results indicate that some plant antimicrobials are able to improve the textural properties of iceberg lettuce in comparison to the control (tap water wash). Our results showed that treatments with cinnamon oil, clove bud oil, citral, grapeseed extract, and carvacrol may enhance the textural properties of organic leafy greens because greater force was required to crush these samples, and also panelists rated these treatments higher for freshness quality.

For iceberg lettuce, treatment with 0.1% oregano oil +10% olive extract combination yielded the highest force ($F=783.1\pm53.8$ N). This treatment significantly ($p\geq0.05$) influenced the texture of iceberg lettuce by making the sample softer and increasing its elasticity. By visualization and tactile feel, this did not indicate crispiness; however, due to an increase in elasticity, this sample might have needed a higher force to be crushed in comparison to other treatments. The treatment of iceberg lettuce with 0.1% oregano oil in combination with 10%

olive extract was also rated the lowest by consumers for mouthfeel and freshness (Table 3.1; Fig 3.2), indicating that this treatment significantly influenced the textural properties of iceberg lettuce. The firmness of spinach was not very much affected by plant antimicrobial treatments in comparison to iceberg lettuce, since spinach is thicker and more uniform in textural properties than iceberg lettuce. Additionally, the weight of iceberg lettuce is more water-based than spinach. The treatment with 0.1% oregano oil on spinach had the greatest loss in firmness ($F=774.7\pm42.1$ N) as indicated by the least force required to crush the samples in comparison to other treatments. Cinnamon oil may improve the textural properties of iceberg lettuce as shown by our results and it had higher crispiness value ($F=607.9\pm26.5$ N) compared to the control ($F=575.8\pm82.1$ N) (Table 3.5).

For spinach samples, in general, plant extracts had less impact on the firmness quality than EOs, even though no significant ($p\leq0.05$) difference was observed. Additionally, combination treatments of plant extracts and EOs improved the firmness of spinach leaves, as greater force was required to crush these samples. Combination treatments such as 0.1% lemongrass oil+1% apple extract ($F=939\pm35.2$ N), and 0.1% cinnamon oil+1% grapeseed extract ($F=904.23\pm3.09$ N) enhanced the firmness quality of spinach samples. Plant extracts in combination with EOs may form a complex that perhaps may prevent EOs from coming directly into contact with the leaf, thus preventing any adverse effect on the organoleptic properties. One way to minimize the organoleptic effects of EOs is via the formation of nanoemulsions that could help improve EO stability and antimicrobial activity (Donsi et al., 2011). Studies have shown that a combination of essential oils with other treatments may act synergistically to improve their antimicrobial activity and thus may help reduce the concentrations of EOs to prevent any adverse impact on the sensory properties of the food product (Gutierrez et al., 2009).

Table 3.5: Crispiness/Peak Force values of organic iceberg lettuce and spinach samples after washing with plant-antimicrobials and storage at 4° C for 24 hr. Crispiness values were measured by taking an average of the highest peak force (N) required to crush the samples from three separate trials. Different letters signify statistical difference ($p \leq 0.05$) between force values of various treatments.

Treatment	Peak Force Lettuce (N)	Peak Force Spinach (N)
Control	575.8 ± 82.1^{C}	845.00 ± 5.84^{ABCD}
3% H_2O_2	663.1 ± 53.9^{ABC}	893 ± 45.8^{ABC}
0.1% Cinnamon oil	607.9 ± 26.5^{BC}	933.5 ± 43.5^{AB}
0.1% Clove bud oil	663.6 ± 36.0^{ABC}	898.1 ± 65.9^{ABC}
0.1% Oregano oil	649.7 ± 60.1^{ABC}	774.7 ± 42.1^{D}
0.1% Lemongrass oil	574.4 ± 49.8^{C}	885.3 ± 51.7^{ABCD}
0.1% Citral	$631.1 \pm 19.1B^{C}$	891.8 ± 46.9^{ABC}
0.1% Carvacrol	665.1 ± 23.5^{ABC}	822.8 ± 22.1^{BCD}
3% Apple extract	571.6 ± 60.0^{C}	924.9 ± 35.6^{ABC}
3% Grapeseed extract	721.0 ± 29.2^{AB}	845 ± 10.43^{ABCD}
7% Olive extract	$630.3 \pm 51.9B^{C}$	893.4 ± 22.9^{ABC}
7% Olive extract +0.1% Oregano oil	NA	844.9 ± 19.2^{ABCD}
10% Olive Extract	718.5 ± 36.7^{AB}	NA
0.1% Oregano oil + 10% Olive extract	783.1 ± 53.8^{A}	NA
0.1% Oregano + 1% Grapeseed extract	657.7 ± 22.9^{ABC}	818.0 ± 18.0^{CD}
0.1% Clove+ 3% Apple extract	667.6 ± 50.3^{ABC}	854.8 ± 59.4^{ABCD}
0.1% Lemongrass oil +1% Apple extract	NA	939.0 ± 35.2^{A}
0.1% Cinnamon oil + 1% Grapeseed extract	NA	904.23 ± 3.09^{ABC}

3.4.6 Impact of Plant Antimicrobial Treatments on the Color of Organic Leafy Greens

The color properties of spinach samples were not significantly ($p \leq 0.05$) affected by plant antimicrobials because spinach is much darker in color compared to iceberg lettuce (Table 3.7). Our data have also indicated that spinach samples had more negative a* values in comparison to iceberg samples, indicating a darker green color for spinach (Table 3.7). When panelists evaluated these leafy greens, the preference liking rankings were much higher for spinach samples than iceberg lettuce for color attributes. For iceberg samples, the treatment that showed the greatest impact on color properties was a combination of 0.1% oregano oil with 10% olive extract. This treatment was the least preferred by panelists on the basis of color and rated the highest for browning on iceberg lettuce. The combination of 0.1% oregano oil with 10% olive extract had the lowest L* value of 44.5±6.2 (Table 3.6) among all treatments, indicating that sample had the darkest color among lettuce samples. Additionally, the treatment with 3% grapeseed extract on iceberg lettuce had a*=0.10±3.0 indicating a slight reddish color that was also seen visually by panelists. Higher impact on color change may be evident in combination treatments rather than on individual treatments. A higher impact on color was found when iceberg lettuce was washed in ozonated water containing calcium lactate in comparison to individual treatments (Rico et al., 2006). In the present study, all other plant antimicrobial treatments did not have a significant ($p \leq 0.05$) impact on the color properties of iceberg lettuce or spinach.

Table 3.6: CIE L*, a*, b* coordinates obtained using Chroma meter for antimicrobial treated iceberg lettuce. Average values, which do not share the same letter, are significantly different ($p \leq 0.05$).

Treatments	L*	a*	b*
0.1% Carvacrol oil	51.32 ± 5.69^{ABC}	-4.35 ± 5.80^{ABC}	16.08 ± 6.62^{A}
0.1% Cinnamon oil	57.06 ± 5.15^{AB}	-4.22 ± 3.04^{ABC}	9.69 ± 7.31^{A}
0.1% Citral oil	54.01 ± 9.21^{AB}	-6.19 ± 2.94^{BC}	14.79 ± 5.97^{A}
0.1% Clove bud oil	58.20 ± 3.95^{A}	-5.02 ± 4.07^{ABC}	11.75 ± 9.04^{A}
0.1% Lemongrass oil	48.39 ± 9.37^{BC}	-5.42 ± 3.96^{BC}	15.13 ± 7.37^{A}
0.1% Oregano oil	51.75 ± 8.38^{ABC}	-5.10 ± 2.87^{ABC}	14.28 ± 6.47^{A}
0.1% Oregano oil+1% Grapeseed extract	49.20 ± 6.31^{BC}	-2.92 ± 2.89^{ABC}	13.67 ± 5.34^{A}
0.1% Oregano oil +10% Olive extract	44.49 ± 6.21^{C}	-0.93 ± 3.32^{AB}	16.94 ± 4.38^{A}
0.1% Clove bud oil + 3% Apple extract	56.31 ± 3.56^{AB}	-4.86 ± 4.78^{ABC}	19.38 ± 6.17^{A}
10% Olive extract	55.05 ± 7.39^{AB}	-2.47 ± 2.10^{ABC}	17.41 ± 5.76^{A}
3% Apple extract	52.84 ± 5.17^{ABC}	-3.35 ± 4.26^{ABC}	16.29 ± 8.34^{A}
3% Grapeseed extract	55.63 ± 5.36^{AB}	0.10 ± 2.97^{A}	18.74 ± 6.21^{A}
7% Olive extract	54.40 ± 5.66^{AB}	-5.44 ± 3.97^{BC}	19.20 ± 8.29^{A}
Control	58.38 ± 4.58^{A}	-6.5 ± 3.47^{C}	14.94 ± 7.13^{A}
H_2O_2	59.03 ± 5.05^{A}	-8.15 ± 4.88^{C}	18.71 ± 7.83^{A}

Table 3.7: CIE L*, a*, b* coordinates obtained using Chroma meter for antimicrobial treated spinach. Average values, which do not share the same letter, are significantly different ($p \leq 0.05$).

Treatments	L*	a*	b*
0.1% Carvacrol oil	34.73 ± 3.81^{BC}	-14.29 ± 2.28^{B}	18.18 ± 2.58^{A}
0.1% Cinnamon oil	39.26 ± 1.63^{AB}	-13.61 ± 1.52^{AB}	19.37 ± 1.85^{A}
0.1% Citral oil	38.02 ± 3.49^{AB}	-14.13 ± 1.78^{B}	18.93 ± 4.01^{A}
0.1% Clove oil	35.87 ± 4.42^{ABC}	-13.92 ± 1.51^{AB}	18.91 ± 2.92^{A}
0.1% Lemongrass oil	34.63 ± 4.95^{BC}	-14.49 ± 2.13^{B}	18.40 ± 2.57^{A}
0.1% Oregano oil	32.87 ± 3.69^{C}	-14.36 ± 1.51^{B}	18.63 ± 2.34^{A}
0.1% Oregano oil + 7% Olive extract	37.34 ± 2.46^{ABC}	-13.70 ± 1.81^{AB}	18.87 ± 3.53^{A}
0.1% Cinnamon oil + 1% Grapeseed extract	37.45 ± 3.19^{ABC}	-10.60 ± 6.83^{A}	17.77 ± 4.25^{A}
0.1% Clove bud oil + 3% Apple extract	38.71 ± 1.42^{AB}	-12.56 ± 1.16^{AB}	18.22 ± 2.88^{A}
0.1% Lemongrass oil +1% Apple extract	38.27 ± 3.65^{AB}	-13.60 ± 1.20^{AB}	18.90 ± 3.88^{A}
0.1% Oregano oil +1% Grapeseed extract	37.17 ± 2.33^{ABC}	-12.42 ± 2.16^{AB}	20.22 ± 2.39^{A}
3% Apple extract	38.25 ± 2.64^{AB}	-12.88 ± 1.34^{AB}	17.61 ± 2.70^{A}
3% Grapeseed extract	37.47 ± 5.02^{ABC}	-12.16 ± 2.10^{AB}	20.97 ± 4.74^{A}
7% Olive extract	40.27 ± 4.32^{A}	-13.68 ± 1.71^{AB}	21.28 ± 4.79^{A}
Control	37.48 ± 2.90^{ABC}	-13.84 ± 1.73^{AB}	18.63 ± 3.38^{A}
3% H_2O_2	38.54 ± 1.65^{AB}	-14.26 ± 1.97^{AB}	19.84 ± 3.84^{A}

3.5 Conclusion

This study provides useful information about plant antimicrobials that could potentially be used as organic sanitizers, while having a low impact on the organoleptic properties of organic leafy greens. Organic leafy greens treated with 0.1% cinnamon oil had the highest preference liking by panelists. This treatment also had the least impact on the sensory properties of both spinach and lettuce; therefore, cinnamon oil may be a good alternative to chemical sanitizers. In general, treatments with EOs were rated high for pungency and off-odor, whereas treatments with plant extracts were rated high for browning. Combination treatments of plant extracts with essential oils were the least preferred; so, alternative treatments need to be evaluated. Our results have shown that certain types of leafy greens such as baby spinach may have higher preference liking after washing with plant antimicrobials; however, additional research needs to be conducted. Future studies will focus on conducting sensory analysis of organic leafy greens with trained panelists to reduce the variability in sensory data. The influence of plant antimicrobials on the sensory properties of other organic leafy greens such as romaine lettuce merits further investigation. At the end, practical application of effective plant antimicrobials will be highly influenced by their sensory effects on foods.

Acknowledgments

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CHAPTER 4: EFFECT OF ANTIMICROBIAL EDIBLE FILMS ON THE SENSORY AND PHYSICAL PROPERTIES OF ORGANIC SPINACH IN SALAD BAGS

4.1 Abstract

The effects of edible films containing plant antimicrobials on organic baby spinach were determined via sensory analysis and changes in physical properties. Edible films were made from pulp of hibiscus, apple, or carrot with cinnamaldehyde or carvacrol (0.5%, 1.5%, or 3%). Organic baby spinach salad bags containing antimicrobial edible films (3% w/w) were stored at 4°C for 20-24 h before performing sensory evaluation with 40 panelists and measuring changes in physical properties. Preference liking was evaluated based on 9-point hedonic scale for the following parameters: aroma, color, freshness, mouthfeel, flavor, and overall acceptability. Additionally, panelists quantified each sample using a 5-point hedonic scale for the following attributes: pungency, browning, bitterness, off-odor, and sourness. On the basis of sensory evaluation, spinach treated with hibiscus film without plant-antimicrobials (control) had the highest preference liking based on an overall acceptability rating of 7.1 (moderately liked). The treatment with carrot and hibiscus-based control edible film had the least impact on sensory attributes of spinach (pungency, browning, bitterness, off-odor, and sourness). The changes in firmness values in spinach samples were measured using a texture analyzer. Color measurements were made using a Chroma meter applying the CIE LAB coordinate system. There were no significant ($p \leq 0.05$) changes in firmness or color values between spinach treated with antimicrobial edible films and the controls. Results from this study will provide potential options for incorporating edible films into salad bags without influencing the physical or sensory properties of baby spinach.

4.2 Introduction

Numerous studies have suggested the use of plant-based antimicrobials to protect food from pathogenic bacteria. Burt (2004) has discussed the antimicrobial activities of the active components of various essential oils such as carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde and cinnamic acid *in vitro* and in food models. Additionally, essential oils (Eos) of oregano, thyme, cinnamon, clove bud, and allspice as well as their active components showed antimicrobial activities against *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella enterica in vitro* (Friedman and others 2002). However, some of these compounds may adversely affect the sensory properties of foods. Therefore, careful selection of food-compatible plant antimicrobials is needed, depending on food types to avoid undesirable organoleptic effects.

Several studies have been conducted on the effect of plant-derived antimicrobials on the properties of food. For example, treatment with 1mM carvacrol or cinnamic acid showed no significant adverse effects on the organoleptic attributes of kiwi fruits and honeydew melons (Roller and Seedhar 2002). The addition of oregano oil to extra virgin olive oil maintained the positive attributes (fruity, pungency, bitterness and off) during 21 days of storage at 23°C (Asensio and others 2012). Samples containing 0.1% of oregano oil or thyme oil gave desirable odor and taste characteristics of lamb meat (Karabagias and others 2011). Gutierrez and others (2009) have suggested that a combination of essential oils could help minimize the concentration of each and thus reduce the adverse sensory impacts in food.

Plant antimicrobials can be applied in foods in two ways: (a) adding antimicrobials directly to food; and (b) incorporating the antimicrobials in edible films which are then used to wrap the foods or added as ingredients in foods (Du and others 2008). Edible films are thin layers of food-based material which can be consumed and provide a barrier to moisture, oxygen, and solute movement of food (Bourtoom 2008). Another major advantage of edible films is that these films may enhance the organoleptic properties of a food product because they could have various components (flavoring, coloring, and sweeteners). Edible films can be used as a carrier for antioxidants, flavoring agents, coloring agents, and antimicrobials to extend the shelf-life and improve food quality (Vojdani and Torres 1990; Cuppet 1994; Yaman and Baymdirh others 2001; Coma and others 2002; Vargas and others 2006). Another advantage of edible films is that they can replace plastic packaging with biodegradable materials which will lead to a reduction in overall packaging and waste disposal (Valencia-Chamorro and others 2011). The major food commodities that could potentially be used with edible films include meat, fish, poultry, bread, cheese, fruits and vegetables (Labuza and Breene 1989).

Previous studies in our lab have indicated the efficacy of plant antimicrobials against foodborne pathogens in foods. Oregano oil at 0.1%-0.5% showed 1-4.7 log CFU/g reduction in *Salmonella* Newport population on organic baby spinach within 24 h (Moore-Neibel and others 2013). Treatment with 0.1-0.5% cinnamon oil showed 0.5-2.5 log (CFU/g) reduction in *S.* Newport population within 24 h on organic baby spinach (Todd and others 2013). Essential oils can improve the microbial safety of food; however they should not have a negative impact on sensory properties which may influence their acceptance by the consumers (De Azerêdo and others 2012).

Studies have shown that edible films can improve shelf-life and food quality serving as barriers for moisture loss, oxygen uptake, lipid oxidation, and enhancing aroma and flavors in food (Kester and others 1986). Other studies have indicated that films made from apple consisting of essential oils (oregano, cinnamon, lemongrass) and their active components (carvacrol, cinnamaldehyde, and citral) showed antimicrobial activity against *E. coli* O157:H7 *in vitro* (Rojas-Graü and others 2006, 2007). Ham and bologna wrapped with edible films made from apple, hibiscus, and carrot pulp containing 3% carvacrol showed 2-3 log CFU/g reduction of *Listeria monocytogenes* within 7 days of storage at 4°C (Ravishankar and others 2012). The use of edible films may be appropriate on fresh produce as higher reduction of foodborne pathogens is observed on leafy greens in comparison to meat products. Edible films containing 3% carvacrol showed 5 log CFU/g reduction by day 0 against *Salmonella* in organic leafy greens in plastic salad bags (Zhu and others 2014).

Higher concentrations of essential oils may be required for better antimicrobial activity; however, this may lead to unpleasant odor or flavor (Gutierrez and others 2009). Tomato juice and vegetable soup had higher preference liking than control when incorporated with 20 µL/L of pennyroyal mint essential oil or rosemary essential oil, respectively, but the preference liking decreased by 15-20% when the concentration of essential oils was increased to 100µL/L (Espina and others 2014b). A combination treatment can be used to maintain a balance between sensory acceptability and antimicrobial efficacy. Sub-lethal concentrations of carvacrol and 1,8-cineole (1/8 MIC+1/8 MIC) improved the majority of sensory attributes of vegetables (iceberg lettuce, chard, arugula) after wash and refrigeration storage compared with vegetables sanitized with either of these compounds alone (at the MIC) (De Sousa and others 2012). Innovative ways need to be investigated where essential oils may be used in food products without having adverse effects on the organoleptic properties. Therefore, in this study, we determined preference liking of baby spinach treated with antimicrobial edible films.

The use of essential oils and plants extracts directly may have adverse effects on the organoleptic properties of leafy greens. To minimize the impact of plant antimicrobials on sensory properties they can be incorporated into edible films. The objectives of this study were a) to determine the effect of antimicrobial edible films on the sensory properties of organic baby spinach by evaluating using a 40-member sensory panel and b) to evaluate the impact of these films on the color and texture of treated organic baby spinach in salad bags.

4.3 Materials and Methods

4.3.1 Edible Films Used

The following edible films were used in this study: hibiscus, carrot, and apple-based films. Each film type was incorporated with one of the following concentrations of essential oils: 0.5%, 1.5%, and 3% of carvacrol or cinnamaldehyde. In each type, control films without any antimicrobials were also included in each experiment.

4.3.2 Edible Films Preparation

Edible films were made from hibiscus, apple, or carrot pulp. The films contained vegetable or fruit puree, high methoxy pectin (3%), vegetable glycerin, citric acid, and ascorbic acid. The film-forming solution was made by blending high methoxy pectin solution, fruit or vegetable puree, and vegetable glycerin into a mixer for 15 min at low speed. The solution was mixed again for 45 min at low speed after adding citric and ascorbic acid with a total mixing time of 60 min. The film solution (325 g) was then equally divided into seven 600 ml stainless steel beakers and refrigerated. The following concentrations of carvacrol or cinnamaldehyde were added to beaker: 0.5% (1.63g), 1.5% (4.95g), and 3% (10.05g) one beaker for control (no antimicrobials added). The samples were homogenized at refrigerated temperature on the Brinkman Polytron PT3000 (Brinkman Instruments Inc.,Westbury, N.Y., U.S.A.) for 3.5 min between 20000 and 24000 rpm using a probe that was 20 mm in diameter. The film solution (58-61g) was then placed on a polyester (PET) film and casted using a draw down bar. Films were then allowed to dry on a lab bench at room temperature (23-25°C) for 14 h. Films were then cut into pieces by using a single edge razor. Edible films were made at the USDA-ARS-WRRC facility in Albany, California, USA. A detailed description for casting edible films has been described in our previous publication (Ravishankar and others 2012).

4.3.3 Treatment of Bagged Spinach with Edible Films

Organic baby spinach was purchased from retail store the same day the treatment was performed. Organic baby spinach was washed thoroughly with tap water and air-dried at room temperature. Between 50 and 70 g of spinach was packed in plastic Ziploc® bags. In each bag, one type of antimicrobial edible film or a control type of film cut into small pieces was added at 3% (w/w) and mixed well with the bag contents. Salad bags containing edible films were stored at 4°C for 20-24 h prior to performing sensory analysis and measuring changes in color or texture.

4.3.4 Sensory Analysis

Sensory analysis was performed using 40 untrained panelists during each trial. A total of 4 trials were conducted. At each trial, 40 panelists evaluated 5 or 6 different samples (including a control) at a time. A 1 g of each sample was given to the panelists and after each sample, panelists were asked to drink water and wait for at least 2 min prior to evaluating the next sample. Panelists were asked to evaluate each sample based on a 9-point hedonic scale where 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely. Sensory parameters were evaluated based on preference liking for aroma, color, freshness, mouthfeel, flavor, and overall acceptability. A randomized block design with affective test was conducted to generate data for preference liking. Panelists quantified each sample for sensory characteristics on the basis of pungency, browning, bitterness, off-odor, and sourness. Sensory characteristics were evaluated using a 5-point hedonic scale, where 1 would be rated the lowest and 5 would be rated the highest (1=not pungent, 2=slightly pungent, 3=moderately pungent, 4=very pungent 5=extremely pungent).

Sensory analysis was conducted at the University Of Arizona- Department Of Nutritional Sciences in sensory booths with constant yellow light. Panelists also answered questions about demographics such as age, gender, ethnicity, frequency of consumption of organic/conventionally grown leafy greens, and types of leafy greens preferred or purchased most prior to treatment (iceberg, romaine, spinach, or mixed greens).

4.3.5 Texture Analysis

Texture of each spinach sample was measured using a Texture Lab Pro, Food Technology Corporation (Sterling, VA, USA). A 1000 N load cell was attached to a Kramer shear cell with an 8-blade probe attached to the instrument (Martín-Diana and others 2006). The speed for the experiment was set to 250 mm min^{-1}. A sample of 15 g of baby spinach with edible films was placed in the Kramer cell and the 8-blade probe was used to crush the sample to measure firmness. Three readings of the same samples were taken and an average of the highest peak force was indicated as the crispiness value for each sample evaluated.

4.3.6 Color Measurements

Color of the spinach samples was measured using a Minolta Chroma Meter (Model CR-400, Minolta, Inc., Tokyo, Japan). The color was measured using the CIE L*, a*, and b* coordinates. Illuminant D65 and 10° observer angle was used (Du and others 2009). The instrument was calibrated using the Minolta standard white reflector plate. The L* value is the measurement of lightness from dark (L*=0) to absolute light (L*=100), the a* axis ranges from green (-) to red (+) and b* axis ranges from blue (-) to yellow (+) (Barrett and others 2010).

Three different readings were taken during each repeat and each experiment was repeated three times, providing a total of 9 readings for each sample.

4.3.7 Statistical Analysis

For sensory analysis, each experiment was divided into four trials with new sets of 40 panelists at each trial, who evaluated 5 to 6 samples. Randomized block design was used for sensory analysis. A linear regression was used to determine the correlation between overall acceptability and other sensory parameters based on preference liking by the panelists. Statistical analysis for each study (sensory, texture measurements, and color measurements) was conducted by One-way Analysis of variance (ANOVA) Tukey's pairwise test at level of significance of $p \leq 0.05$ using Minitab 17 (State College, PA, USA). Comparison of significance was made between the various types of films and between the various concentrations of antimicrobials within each type of film.

4.4 Results and Discussion

Edible films (3% w/w) were incorporated into bagged baby spinach and stored at 4°C for 20-24 h prior to evaluation by panelists. At this specific concentration and storage time, previous studies have indicated the antimicrobial properties of edible films in baby spinach bags. Zhu and others (2014) have shown that apple, carrot, and hibiscus films containing 3% carvacrol or cinnamaldehyde had 5-6 logs reduction in the population of *S*. Newport in bagged baby spinach soon after treatment. These edible films may enhance or reduce the acceptability of the product based on sensory attributes. Incorporating a high level of apple skin polyphenols in edible films has been known to adversely affect the palatability of edible films by inducing astringency or bitterness (Du and others 2011). On the other hand, coatings may provide a glossy appearance on the food surface which may help improve the visual quality of food (Trezza and others 2000).

Therefore, research needs to be conducted to identify formulations of the antimicrobials that will be preferred by panelists.

4.4.1 Demographic Information

The criteria for panelists to participate in this study included the following: a) each panelist must be at least 18 years or older, b) must be a non-smoker, and c) be able to differentiate between colors. Demographic results are depicted in Figure 4.1. A total of 160 panelists participated in this study of which 88% were female, and 12 % were male. The age of the panelists ranged from 18 to 55 years with majority of the population (89%) falling in the age range of 18-25 years. Based on ethnicity, panelists included 47% White, 29% Hispanic, 9% Asian, 3% African American, and 12% others. Each panelist was asked to indicate the type of leafy greens they consume/purchase the most and the following results were noted: 34% mixed greens, 32% spinach, 22% romaine, and 11% indicated liking for iceberg lettuce. Panelists also indicated the frequency of consumption of leafy greens and 45% indicated that they consume conventional leafy greens twice a week and 13% indicated consumption of organic leafy greens twice a week.

Figure 4.1: Demographic information regarding panelists who participated in the sensory study for evaluating spinach from salad bags containing antimicrobial edible films. Data depicts responses from a total of 160 participants. Figure 4.1A) Gender ratio B) Ethnicity ratio C) Pattern for consumption of leafy greens D) Preference for type of leafy greens.

Figure 1A

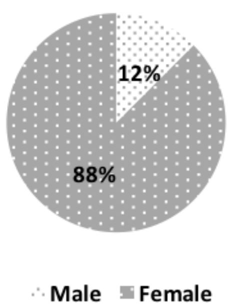


Figure 1B

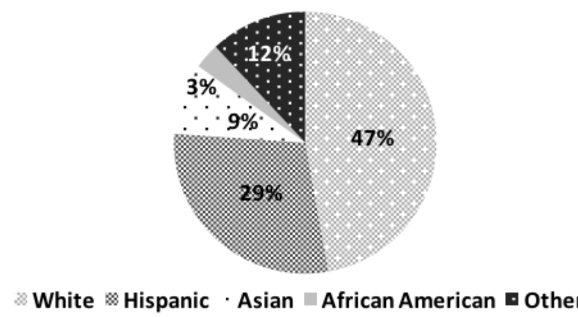


Figure 1C

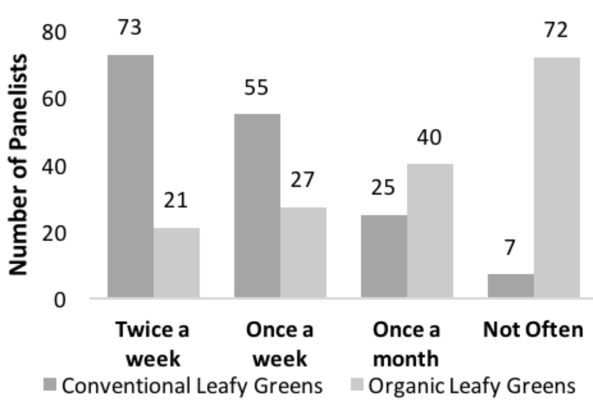
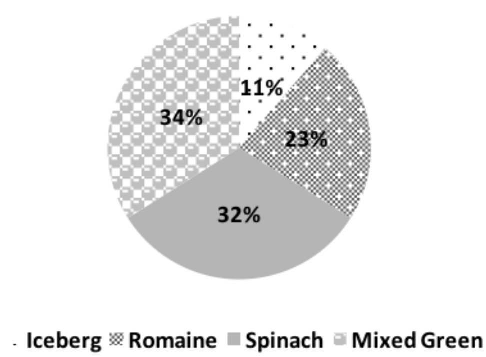


Figure 1D



4.4.2 Sensory Analysis

The results from sensory analysis of organic baby spinach treated with edible films are presented in Table 1 and Table 2. Previous studies conducted in our lab showed that spinach washed with essential oils had higher preference liking by consumers in comparison to iceberg lettuce. Additionally, demographic information indicated that panelists had higher preference liking for spinach than the lettuces (Figure 4.1D). Therefore, in this study we conducted sensory analysis of organic baby spinach treated with edible films. Our previous studies also indicated that leafy greens treated with cinnamon oil had the highest preference liking. Therefore, in this study we selected carvacrol and cinnamaldehyde, as previously these compounds have been known to be acceptable by consumers and have shown antimicrobial activity. Based on the preference liking by consumers, spinach treated with edible films had rating from 7.1±1.7 (moderately liked for apple-control film) to 3.6±2.5 (moderately dislike- apple-1.5% carvacrol).

Overall edible films incorporated with cinnamaldehyde had higher preference liking based on aroma, color, freshness, mouthfeel, flavor, and overall acceptability than those incorporated with carvacrol (Table 4.1; Fig 4.2). Our previous studies also support this result because spinach washed with 0.1% cinnamon oil had higher preference liking in comparison to that washed with 0.1% oregano oil. A concentration-dependent activity was observed: a higher preference liking was seen in edible films with lower concentration of either carvacrol or cinnamaldehyde. For example, carrot film with 0.5% cinnamaldehyde had a preference liking of 6.6±2.3 (slightly liked), whereas carrot film with 3% cinnamaldehyde had a rating of 5.9±2.5 (neither liked nor disliked).

The efficacy of essential oils against spoilage organisms and foodborne pathogens is concentration and time dependent (Moore-Neibel and others 2013); however, as shown in our studies, the preference liking decreases with the use of higher concentration of essential oils. Combination treatments can therefore be considered that could lead to a higher reduction in microbial counts and yet maintain sensory quality. A combination of oregano and rosemary oil at a sub-inhibitory concentration extended the shelf-life and improved safety of fresh vegetables (De Medeiros Barbosa and others 2016). There was no difference in preference liking between the three types of films. The types of essential oil incorporated into these films had a higher impact on the preference liking than the edible film material itself. Comparing the control films of apple, hibiscus, and carrot, the highest preference liking was for apple films; however, no significant difference ($p \leq 0.05$) was seen between the other two films.

The aroma rating ranged from 7.1±1.8 (moderately liked) to 4.8±2.7 (slightly dislike) and the majority of the sample were ranked slightly liked on the basis of the aroma property. There was no significant difference ($p \leq 0.05$) in preference liking of aroma in any samples except for carrot-3% carvacrol, hibiscus-1.5% carvacrol, and appple-1.5% carvacrol, all of which had the lowest rating for aroma from 5.2±2.4 (neither like nor dislike) to 4.8±2.7 (dislike slightly). The color of spinach was not impacted much by the addition of edible films as all the samples were ranked between 7.9±1.4 (moderately liked) and 6.1±2.3 (slightly like). The freshness quality of spinach was also ranked highly because the majority of the samples were rated between 6 and 7 (slightly to moderately liked). The effect on mouthfeel was observed in the spinach sample treated with carrot-3% carvacrol film because it had the lowest ranking of 4.7±2.8 (slightly disliked); however, most other samples ranked between slightly and moderately liked. Flavor was the most impacted by the addition of these edible films with the preference ranking ranging from 7.1±1.9 (moderately liked) to 3.5±2.5 (moderately disliked). Overall, spinach treated with edible films containing cinnamaldehyde had a higher preference for flavor compared to those that included carvacrol. The rating of overall acceptability ranged from 7.1±1.7 (moderately liked) to 3.6±2.5 (moderately disliked) and spinach treated with films containing cinnamaldehyde had higher preference liking compared to those treated with carvacrol films.

Panelists were also asked to quantity specific attributes noticed in each spinach sample based on pungency, browning, bitterness, off-odor, and sourness. Results for the impact of antimicrobial edible films on sensory attributes of spinach samples are presented in Table 4.2 and Figure 4.3. The pungency level ranked from 3.5±1.2 (moderately pungent) to 1.3±0.6 (not pungent at all). In addition, there was a concentration dependency for pungency level because samples treated with edible films containing higher concentrations of either cinnamaldehyde or carvacrol were ranked higher for pungency. For the majority of the samples, carvacrol was rated higher for pungency compared to cinnamaldehyde. All types of edible films did not have any adverse effect on the browning level on spinach because samples had a rating of 1 (not brown at all). Spinach treated with apple film-1.5% carvacrol was rated the highest for bitterness at a rating of 3.4±1.4 (moderately bitter) and the lowest bitterness rating was for spinach sample treated with apple-control films having a rating of 1.5±0.8 (not bitter at all). The highest off-odor was detected in spinach treated with hibiscus-3% cinnamaldehyde and apple-1.5% carvacrol films rated at 3.2±1.5 (moderately off). The sourness ranking ranged from 1.3 ±0.06 (not sour at all) for spinach treated with apple- 0.5% cinnamaldehyde and hibiscus-0.5% carvacrol films to 2.6±1.5 (slightly sour) for those treated with apple-1.5% carvacrol films.

Figure 4.2: Sensory profile of preference liking for organic baby spinach treated with antimicrobial edible films after storage for 24 h at 4°C. Preference liking was evaluated for: aroma, color, freshness, mouthfeel, flavor, and overall acceptability based on a 9-point hedonic scale where 9-extremely liked, and 1-not liked at all. A) Hibiscus Edible films B) Apple Edible Films C) Carrot Edible Films. All three film types contained various concentrations of carvacrol or cinnamaldehyde. Data depicted is average of 40 responses for each sample.

Figure 4.2A

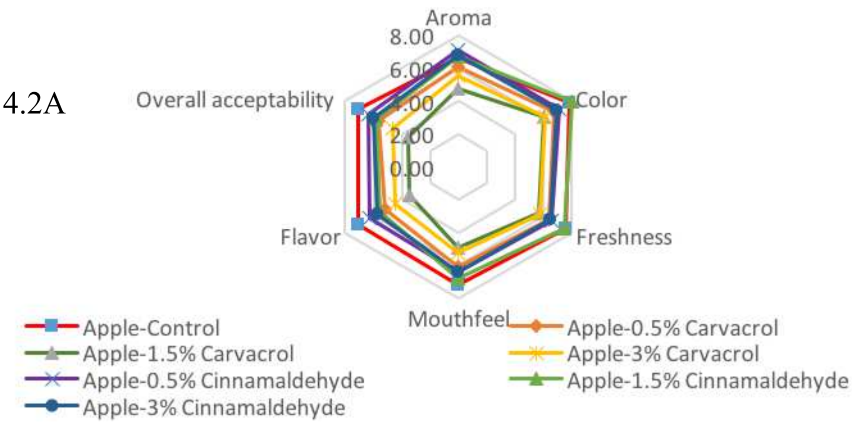


Figure 4.2B

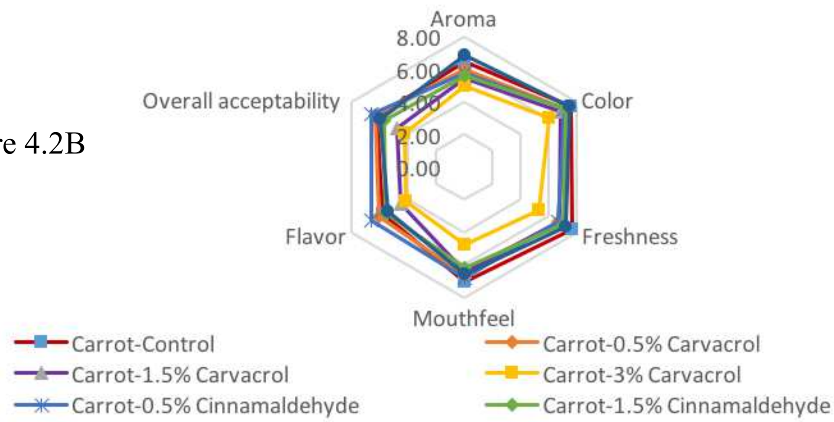


Figure 4.3C

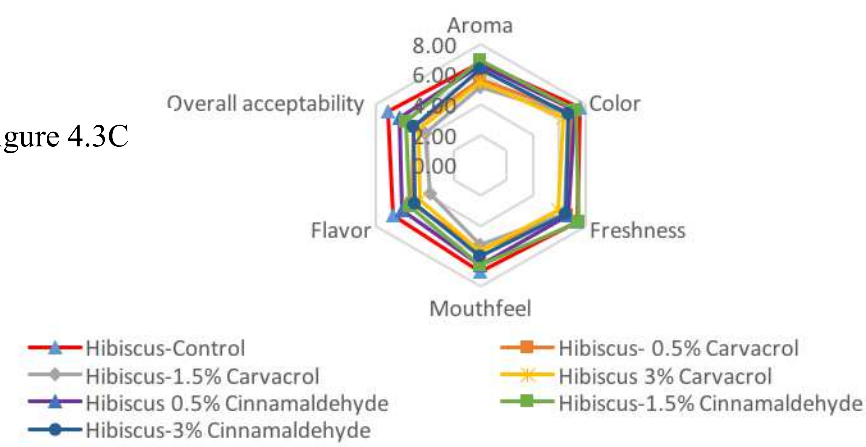


Figure 4.3: Impact of edible films containing plant antimicrobials on sensory characteristics of baby spinach stored at 4°C for 24 hr. Impact on sensory characteristics (pungency, browning, bitterness, off-odor, and sourness) were rated based on a 5-point hedonic scale with 5-highest impact and 1- no impact at all. Data depicted is an average response of 40 panelists for each sample. A) Hibiscus Edible Films B) Apple Edible Films C) Carrot Edible Films

Figure 4.3A

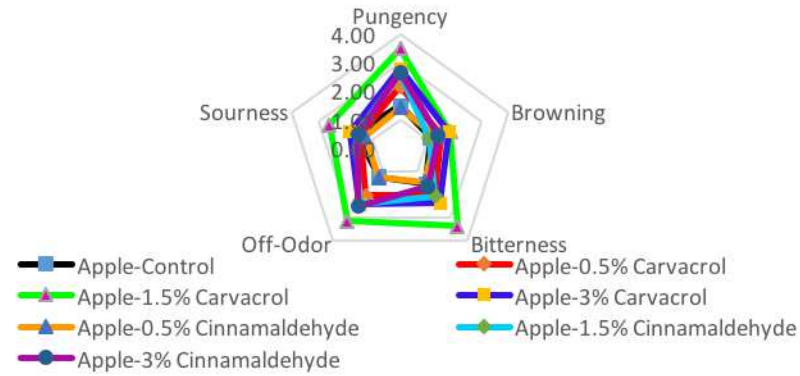


Figure 4.3B

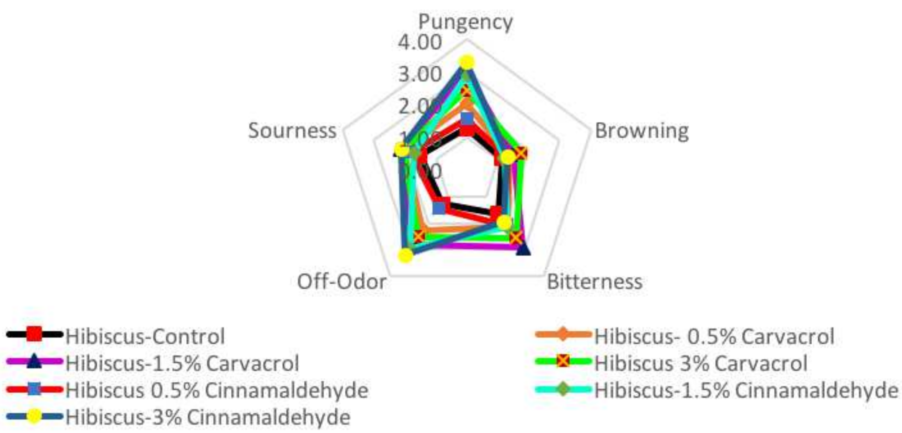


Figure 4.3C

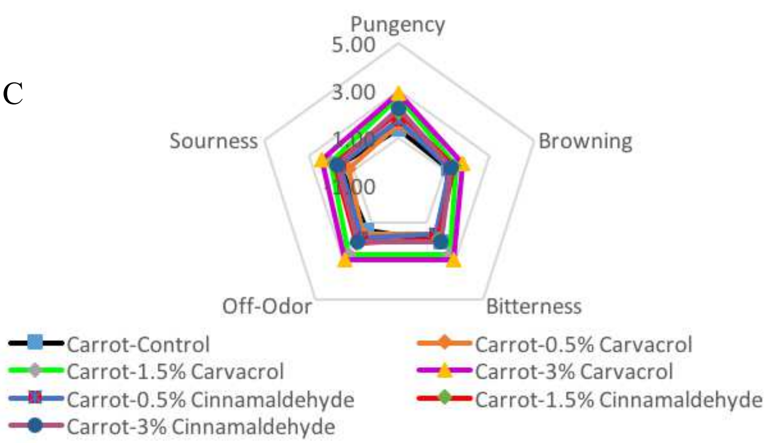


Table 4.1: Preference liking of spinach treated with antimicrobial edible films and stored at 4°C for 24 hrs. Data shown is an average of responses from 40 panelists for each sample ± standard deviation. Values that do not share common letters are significantly different (p≥0.05).

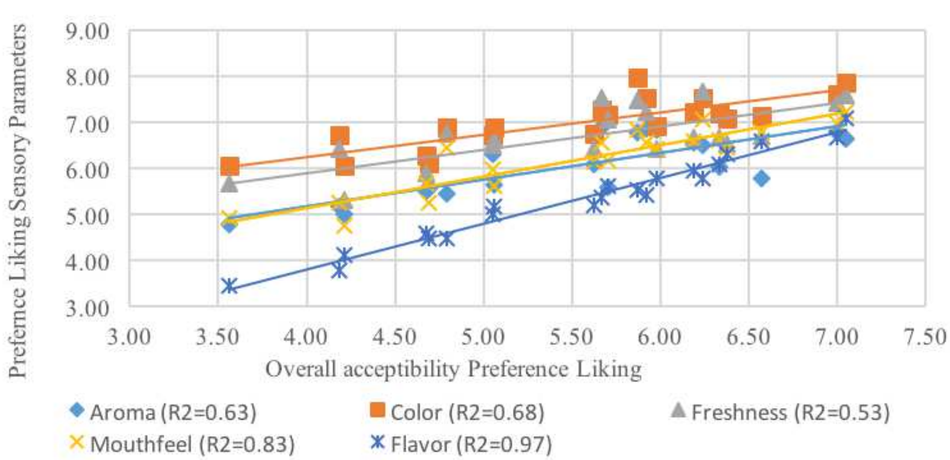
Sample#	Aroma	Color	Freshness	Mouthfeel	Flavor	Overall acceptability
Apple-Control	6.6±1.8^{ABC}	7.9±1.4^{A}	7.6±1.6^{A}	7.2±1.8^{A}	7.1±1.9^{A}	7.1±1.7^{A}
Hibiscus-Control	6.8±1.7^{AB}	7.6±1.5^{AB}	7.4±1.7^{AB}	7.0±1.9^{AB}	6.7±2.0^{AB}	7±1.8^{AB}
Carrot-0.5% Cinnamaldehyde	5.8±2.2^{ABCD}	7.1±1.8^{ABC}	6.7±2.1^{ABCD}	6.8±1.7^{AB}	6.6±2.0^{ABC}	6.6±2.3^{ABC}
Apple-0.5% Cinnamaldehyde	7.1±1.8^{A}	7.1±1.8^{ABC}	6.5±2.1^{ABCD}	6.5±2.2^{ABCD}	6.3±2.2^{ABCD}	6.4±2.1^{ABC}
Carrot-0.5% Carvacrol	6.0±2.0^{ABCD}	7.2±1.7^{ABC}	6.7±2.0^{ABCD}	6.6±1.9^{ABC}	6.1±2.2^{ABCDE}	6.3±2.1^{ABC}
Carrot-Control	6.5±1.8^{ABCD}	7.5±1.5^{ABC}	7.7±1.6^{A}	7.1±1.8^{AB}	5.8±2.5^{ABCDEF}	6.2±2.3^{ABC}
Hibiscus 0.5% Cinnamaldehyde	6.6±1.8^{ABC}	7.2±2.0^{ABC}	6.7±2.3^{ABCD}	6.6±2.0^{ABC}	6.0±2.3^{ABCDE}	6.2±2.2^{ABCD}
Apple-3% Cinnamaldehyde	6.8±2.2^{AB}	6.9±1.8^{ABC}	6.4±2.1^{ABCD}	6.4±2.1^{ABCD}	5.8±2.7^{ABCDEF}	6.0±2.3^{ABCDE}
Carrot-3% Cinnamaldehyde	6.8±1.8^{AB}	7.5±1.6^{ABC}	7.2±1.6^{ABC}	6.6±2.0^{ABCD}	5.4±2.5^{ABCDEFG}	5.9±2.5^{ABCDE}
Apple-1.5% Cinnamaldehyde	6.8±1.6^{AB}	8.0±1.3^{A}	7.5±1.3^{AB}	6.8±2.0^{AB}	5.5±2.7^{ABCDEF}	5.9±2.6^{ABCDE}
Carrot-1.5% Cinnamaldehyde	5.6±2.1^{ABCD}	7.2±2.0^{ABC}	7.1±2.1^{ABC}	6.2±2.4^{ABCD}	5.6±2.5^{ABCDEF}	5.7±2.2^{ABCDE}
Hibiscus-1.5% Cinnamaldehyde	6.9±1.8^{A}	7.3±2.0^{ABC}	7.5±1.5^{AB}	6.6±2.2^{ABC}	5.4±5.4^{ABCDEFG}	5.7±2.6^{ABCDE}
Apple-0.5% Carvacrol	6.1±2.3^{ABCD}	6.7±1.9^{ABC}	6.4±2.1^{ABCD}	6.1±2.3^{ABCD}	5.2±2.6^{ABCDEFG}	5.6±2.2^{ABCDE}
Hibiscus- 0.5% Carvacrol	5.6±2.0^{ABCD}	6.9±1.9^{ABC}	6.6±2.1^{ABCD}	5.6±2.3^{ABCD}	5.2±2.5^{ABCDEFG}	5.1±2.4^{BCDEF}
Hibiscus-3% Cinnamaldehyde	6.3±2.5^{ABCD}	6.7±2.2^{ABC}	6.5±2.3^{ABCD}	6±2.6^{ABCD}	5±3.0^{BCDEFG}	5.1±2.9^{CDEF}
Carrot-1.5% Carvacrol	5.5±5.5^{ABCD}	6.9±1.8^{ABC}	6.8±1.7^{ABCD}	6.4±1.9^{ABCD}	4.5±2.6^{DEFG}	4.8±2.5^{CDEF}
Apple-3% Carvacrol	5.6±2.5^{ABCD}	6.1±2.3^{BC}	5.7±2.5^{CD}	5.3±3.0^{BCD}	4.5±3.0^{DEFG}	4.7±2.8^{CDEF}
Hibiscus 3% Carvacrol	5.5±2.3^{ABCD}	6.3±2.6^{BC}	5.9±2.4^{BCD}	5.7±2.5^{ABCD}	4.6±2.7^{CDEFG}	4.7±2.8^{CDEF}
Carrot-3% Carvacrol	5.0±2.5^{CD}	6.1±2.3^{BC}	5.3±2.5^{D}	4.7±2.8^{D}	4.1±2.6^{EFG}	4.2±2.6^{DEF}
Hibiscus-1.5% Carvacrol	5.2±2.4^{BCD}	6.7±2.0^{ABC}	6.4±2.4^{ABCD}	5.3±2.6^{BCD}	3.8±2.6^{FG}	4.2±2.5^{EF}
Apple-1.5% Carvacrol	4.8±2.7^{D}	6.1±2.4^{C}	5.7±2.5^{CD}	4.9±2.4^{CD}	3.5±2.5^{G}	3.6±2.5^{F}

Table 4.2: Impact on the sensory characteristics of spinach treated with antimicrobial edible films and stored at 4°C for 24 hrs. Each treatment was evaluated by 40 panelists and data depicted is an average ± Std Dev. Same letters next to each value indicate that there is no significant difference (p≤0.05).

Sample#	Pungency	Browning	Bitterness	Off-Odor	Sourness
Hibiscus-Control	1.3 ± 0.6^{H}	1.1 ± 0.3^{D}	1.7 ± 0.8^{EF}	1.3 ± 0.7^{H}	1.5 ± 0.8^{C}
Carrot-Control	1.3 ± 0.6^{GH}	1.2 ± 0.6^{CD}	2 ± 1.1^{BCDEF}	1.4 ± 0.6^{GH}	1.7 ± 0.8^{BC}
Apple-0.5% Cinnamaldehyde	1.4 ± 0.7^{GH}	1.3 ± 0.5^{ABCD}	1.5±0.8F	1.3 ± 0.6^{H}	1.3 ± 0.6^{C}
Apple-Control	1.5 ± 0.6^{GH}	1.1 ± 0.4^{D}	1.6±1.0F	1.3 ± 0.5^{H}	1.6 ± 0.8^{BC}
Carrot-0.5% Carvacrol	1.6 ± 0.8^{FGH}	1.5 ± 0.7^{ABCD}	1.6 ± 0.9^{F}	1.6 ± 0.9^{FGH}	1.3 ± 0.5^{C}
Hibiscus 0.5% Cinnamaldehyde	1.6 ± 0.8^{FGH}	1.3 ± 0.6^{ABCD}	2.1 ± 1.2^{BCDEF}	1.5 ± 0.8^{GH}	1.8 ± 1.1^{ABC}
Carrot-0.5% Cinnamaldehyde	1.8 ± 1.1^{EFGH}	1.3 ± 0.5^{BCD}	1.6 ± 1.1^{F}	1.8 ± 1.1^{EFGH}	1.6 ± 1.1^{BC}
Carrot-1.5% Cinnamaldehyde	2.1 ± 1.1^{DEFGH}	1.5 ± 0.7^{ABCD}	1.8 ± 1.1^{DEF}	2.1 ± 1.2^{CDEFGH}	1.9 ± 1.2^{ABC}
Hibiscus- 0.5% Carvacrol	2.1 ± 1.0^{DEFGH}	1.4 ± 0.6^{ABCD}	2.1 ± 1.3^{BCDEF}	2.3 ± 12^{BCDEFG}	2.1 ± 1.4^{ABC}
Apple-0.5% Carvacrol	2.2 ± 1.3^{CDEFGH}	1.5 ± 0.8^{ABCD}	2.1 ± 1.1^{BCDEF}	2.1 ± 1.3^{CDEFGH}	1.5 ± 0.7^{C}
Carrot-3% Cinnamaldehyde	2.2 ± 1.2^{CDEFG}	1.3 ± 0.6^{ABCD}	2 ± 1.2^{BCDEF}	2 ± 1.1^{DEFGH}	1.8 ± 1.0^{ABC}
Hibiscus 3% Carvacrol	2.5 ± 1.3^{BCDEF}	1.8 ± 1.1^{ABC}	2.6 ± 1.3^{ABCDE}	2.5 ± 1.3^{ABCDEF}	2.1 ± 1.4^{ABC}
Apple-1.5% Cinnamaldehyde	2.6 ± 1.2^{ABCDE}	1.1 ± 0.3^{D}	2.1 ± 1.2^{BCDEF}	2.4 ± 1.3^{ABCDEF}	1.6 ± 0.9^{BC}
Apple-3% Cinnamaldehyde	2.7 ± 1.5^{ABCDE}	1.4 ± 0.7^{ABCD}	1.7 ± 1.1^{EF}	2.5 ± 1.4^{ABCDEF}	1.6 ± 1.0^{BC}
Carrot-1.5% Carvacrol	2.7 ± 1.2^{ABCD}	1.5 ± 0.7^{ABCD}	2.7 ± 1.4^{ABCD}	2.7 ± 1.3^{ABCDE}	2.1 ± 1.2^{ABC}
Apple-3% Carvacrol	2.8 ± 1.3^{ABCD}	1.8 ± 0.9^{A}	2.4 ± 1.3^{BCDEF}	2.5 ± 1.2^{ABCDEF}	1.9 ± 1.2^{ABC}
Carrot-3% Carvacrol	2.8 ± 1.2^{ABCD}	1.8 ± 1.0^{AB}	2.9 ± 1.5^{ABC}	2.9 ± 1.5^{ABCD}	2.4 ± 1.4^{AB}
Hibiscus-1.5% Cinnamaldehyde	2.9 ± 1.5^{ABCD}	1.2 ± 0.4^{CD}	2.2 ± 1.3^{BCDEF}	3.0 ± 1.4^{ABC}	1.7 ± 1.1^{BC}
Hibiscus-1.5%Carvacrol	3.0 ± 1.3^{ABC}	1.5 ± 0.8^{ABCD}	2.9 ± 1.5^{AB}	2.8 ± 1.4^{ABCD}	2.1 ± 1.2^{ABC}
Hibiscus-3% Cinnamaldehyde	3.3 ± 1.4^{AB}	1.3 ± 0.8^{ABCD}	2.0 ± 1.1^{CDEF}	3.2 ± 1.5^{A}	2.1 ± 1.4^{ABC}
Apple-1.5% Carvacrol	3.5 ± 1.2^{A}	1.8 ± 0.9^{A}	3.4 ± 1.4^{A}	3.2 ± 1.4^{AB}	2.6 ± 1.5^{A}

Linear regression (Figure 4.4) was conducted to compare the data on the ranking of overall acceptability with that of aroma, color, freshness, mouthfeel, and flavor. For edible film samples, the average rating for flavor (R^2=0.97) was closely related to the average rating of overall acceptability. This suggests that flavor was the most influencing factor in determining overall acceptability of any sample. A similar trend was seen where a correlation of R^2=0.91 was found between flavor and overall acceptability of a salad dressing incorporated with 0.2% oregano essential oils and 1.14% salt (Cattelan and others 2015). Each panelist was asked how likely they are to purchase a particular sample and 19/40 mentioned very or extremely likely to purchase spinach treated with of hibiscus-control and apple-control films (data not shown). The next highest rated sample for very or extremely likely to be purchased included spinach treated with carrot-0.5% cinnamaldehyde films (16/40) (data not shown). A strong correlation of R^2=0.94 was found between the rating of overall acceptability and likelihood of purchasing a sample (data not shown).

Figure 4.4: Linear correlation between values of average overall acceptability rating and other sensory parameters (aroma, freshness, mouthfeel, color, and flavor) for organic baby spinach treated with edible films containing plant antimicrobials.



A coating may not be an ideal use of edible films for leafy greens because a large surface area would need to be covered. Other studies have shown that coating applications could lead to a significant decrease in aroma and flavor of strawberries when edible films contained oleic acid and chitosan (Vargas and others 2006). Therefore, in this study we incorporated pieces of edible films into salad bags rather than coating the entire spinach leaf. Additionally, it is important to determine what type of essential oils or plant extracts and the concentration that may be incorporated into various types of food commodities. Some essential oils that are strong in flavor are used in meat or fish products. Concentrations of up to 100 µL/L of limonene (present in citrus EOs), 150 µL/L of carvacrol, or 200 µL/L of lemongrass essential oil, or other citrus essential oils did not affect the sensory attributes of some fruits juices such as orange juice (Raybaudi-Massilia and others 2009, Espina and others 2014a).

4.4.3 Textural and Color Analysis

The firmness value of baby spinach was measured using a Texture analyzer. Three separate trials were conducted for each sample and an average of the highest peak force required to completely crush the sample was indicated as its firmness or crispiness value (Table 4.3). There was no significant ($p \leq 0.05$) difference among all the edible films sampled based on firmness value. Overall, edible films that contained cinnamaldehyde had a lower impact on the firmness values as suggested by higher force required to crush these samples than those that included carvacrol. For example, the highest firmness/crispiness value was observed for spinach treated with apple-1.5% cinnamaldehyde film (F=889.8±52.5 N), whereas the lowest firmness value was observed for spinach treated with hibiscus-3% carvacrol film (F=643.3±60.7 N). A concentration-dependent activity due to antimicrobial edible films treatment on the firmness of spinach was not observed. However, for spinach samples, incorporating of 1.5% of either carvacrol or cinnamaldehyde resulted in higher firmness value in comparison to 0.5% or 3% concentrations.

The results presented here indicate that presence of certain concentrations of these compounds is extremely important for how they will affect the organoleptic properties of baby spinach. The type of films used such as apple, hibiscus, or carrot did not have a major impact on the firmness values, but the essential oil active components (carvacrol or cinnamaldehyde) incorporated in these films had a greater impact on the firmness values.

Table 4.3: Crispiness values of organic baby spinach after storage at 4°C for 24 h in salad bags containing edible films with plant-antimicrobials. Crispiness values were measured by taking an average of the highest peak force (N) required to crush the samples from three separate trials. Different letters signify statistical difference ($p \geq 0.05$) between force values of each treatment.

Treatments	AVG Force (N)
Carrot Control	799.8 ± 74.7^{A}
Carrot 0.5% Cinnamaldehyde	827.7 ± 65.6^{A}
Carrot 1.5% Cinnamaldehyde	875.5 ± 77.6^{A}
Carrot 3% Cinnamaldehyde	847.8 ± 25.8^{A}
Carrot 0.5% Carvacrol	734.8 ± 75.5^{A}
Carrot 1.5% Carvacrol	867.1 ± 75.3^{A}
Carrot 3% Carvacrol	734.78 ± 17.1^{A}
Hibiscus Control	749.2 ± 62.0^{A}
Hibiscus 0.5% Cinnamaldehyde	851.52 ± 4.55^{A}
Hibiscus 1.5% Cinnamaldehyde	777.1 ± 109.8^{A}
Hibiscus 3% Cinnamaldehyde	876.7 ± 121.1^{A}
Hibiscus 0.5% Carvacrol	703.7 ± 62.4^{A}
Hibiscus 1.5% Carvacrol	867.1 ± 75.3^{A}
Hibiscus 3% Carvacrol	643.3 ± 60.7^{A}
Apple Control	734.8 ± 75.5^{A}
Apple 0.5% Cinnamaldehyde	723.7 ± 123.4^{A}
Apple 1.5% Cinnamaldehyde	889.8 ± 52.5^{A}
Apple 3% Cinnamaldehyde	805.9 ± 98.8^{A}
Apple 0.5% Carvacrol	754.1 ± 145.3^{A}
Apple 1.5% Carvacrol	826.9 ± 48.5^{A}
Apple 3% Carvacrol	693.4 ± 76.7^{A}

Color properties of baby spinach were not adversely impacted by the addition of edible films, as no significant ($p \leq 0.05$) change was indicated in L*, a*, b* values compared to the controls (Table 4.4). Spinach samples are much darker in color than iceberg lettuce; color properties would therefore not be impacted as much compared to leafy greens which are lighter in color. The L* (light vs. dark) is the major value which may influence consumers' preference; however, for this value, the majority of the samples were not significantly ($p \leq 0.05$) different from the controls. The color properties of the films may be affected by the addition of essential oils as shown in other studies. For example, the addition of essential oils at various concentrations) to apple puree caused an increase in the L* value, b* value and whitish index of the resulting edible films (Du and others 2009). In our study there was no adverse effect on color properties of spinach leaf because the essential oils were incorporated into edible films, therefore oils did not directly come in contact with the spinach leaf.

Table 4.4: CIE L*, a*, b* coordinates measured using a Chroma meter for baby spinach treated with edible films containing plant antimicrobials and stored at 4°C for 24 h. Average values, which do not share the same letter, are significantly different ($p \leq 0.05$).

Treatments	L*	a*	b*
Apple Control	39.02±2.94^{BCD}	-14.06±1.73^{AB}	19.63±4.69^{AB}
Apple 0.5% Carvacrol	38.26±2.41^{BCD}	-14.1±1.67^{AB}	19.21±2.87^{AB}
Apple 1.5% Carvacrol	38.98±3.09^{BCD}	-15.48±1.16^{B}	20.99±1.93^{AB}
Apple 3% Carvacrol	36.30±3.78^{D}	-14.74±1.52^{AB}	18.46±3.38^{AB}
Apple 0.5% Cinnamaldehyde	39.78±1.69^{BCD}	-13.55±2.78^{AB}	18.72±5.01^{AB}
Apple 1.5% Cinnamaldehyde	39.19±2.96^{BCD}	-14.35±2.30^{AB}	20.89±5.89^{AB}
Apple 3% Cinnamaldehyde	38.36±2.90^{BCD}	-13.65±1.47^{AB}	18.34±4.00^{AB}
Carrot Control	39.79±3.28^{BCD}	-14.8±1.32^{AB}	19.98±2.27^{AB}
Carrot 0.5% Carvacrol	38.60±2.57^{BCD}	-15.05±1.55^{AB}	20.54±3.01^{AB}
Carrot 1.5% Carvacrol	40.52±3.70^{BCD}	-14.34±1.66^{AB}	19.96±3.46^{AB}
Carrot 3% Carvacrol	42.78±3.98^{AB}	-12.98±1.61^{AB}	18.82±4.92^{AB}
Carrot 0.5% Cinnamaldehyde	42.29±3.28^{AB}	-13.03±1.46^{AB}	17.95±2.79^{AB}
Carrot 1.5% Cinnamaldehyde	40.88±3.26^{BCD}	-15.08±2.13^{AB}	21.28±4.04^{AB}
Carrot 3% Cinnamaldehyde	39.75±2.71^{BCD}	-13.45±1.66^{AB}	18.23±2.75^{AB}
Hibiscus Control	40.45±1.54^{BCD}	-12.17±1.22^{A}	17.34±2.29^{B}
Hibiscus 0.5% Carvacrol	42.04±3.51^{ABC}	-13.65±2.27^{AB}	19.65±3.82^{AB}
Hibiscus 1.5% Carvacrol	38.70±3.44^{BCD}	-14.45±1.57^{AB}	20.70±3.40^{AB}
Hibiscus 3% Carvacrol	36.79±3.40^{CD}	-13.43±2.48^{AB}	21.75±2.51^{AB}
Hibiscus 0.5% Cinnamaldehyde	47.32±4.42^{A}	-13.96±1.45^{AB}	21.30±2.43^{AB}
Hibiscus 1.5% Cinnamaldehyde	41.42±3.49^{BCD}	-15.93±2.11^{B}	23.63±3.49^{A}
Hibiscus 3% Cinnamaldehyde	38.43±3.42^{BCD}	-15.46±0.93^{AB}	21.48±1.70^{AB}

4.5 Conclusions

Our previous studies had indicated that the acceptable level of essential oils in wash water was 0.1%; however, with use of edible films the acceptable concentrations of essential oils can be increased to 0.5% or even 1.5% as we have shown in this study that spinach was acceptable at these concentrations. Spinach treated with films that contained cinnamaldehyde had a higher preference liking and less adverse effects on sensory attributes (such as pungency, browning, off-odor, bitterness, and sourness) than those that included carvacrol. Spinach samples treated with films that included cinnamaldehyde were also more likely to be purchased than those samples treated with films containing carvacrol. Additionally, there was a concentration-dependent trend as lower concentrations of antimicrobials in films had higher preference liking. Use of essential oils or plant extracts in water may impact the firmness and color properties of leafy greens (as shown in our previous studies); however, with use of edible films, no significant change in firmness and color value was detected. This study provides an innovative way to incorporate plant antimicrobials in leafy greens salads without adversely impacting the sensory quality. Sensory studies on other types of leafy greens such as iceberg and romaine lettuces treated with antimicrobial edible films merit further investigations.

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CONCLUSIONS

The aim of the first project was to determine if certain types of coring tool designs are easier to decontaminate in comparison to others and evaluate the efficacy of organic sanitizers and plant antimicrobials against *S.* Newport on coring tools. The second project focused on identifying plant antimicrobial treatments for organic leafy greens that will have the least impact on sensory attributes and therefore, liked/preferred by consumers. Additionally, a sensory analysis was conducted on baby spinach treated with antimicrobial edible films within salad bags. Additionally, changes in physical properties (firmness, color) of leafy greens washed with plant antimicrobials or with edible films in salad bags were measured using texture analyzer and Chroma meter, respectively.

The coring tool study suggested that all designs of coring tools are similar with regard to the ease of decontamination process as all tools are made from the same grade of metal (stainless steel) as the original design. However, we were able to identify organic sanitizers and plant antimicrobials which were more effective at reducing *Salmonella* from coring tools than traditionally used chemicals such as 50 ppm bleach. Treatments with 5% Chico Wash™ (4.1±0.2-5.1±1.1), 3% fulvic acid (5.0±0.7-5.2±0.7), 3% H_2O_2 (6.0±0.6-6.2±0.3), 0.1% oregano oil (5.5±0.6-5.9±0.5 and 0.39% SaniDate 5.0® (6.0±0.8-6.2±0.2) had higher log (CFU/cm^2) reductions of *S*. Newport on coring tools compared to 50 ppm bleach (2.8±0.8-3.5±1.3). In conclusion, this study provides a few potential alternative organic sanitizers and an essential oil, which are equally effective at inactivating *S.* Newport on coring tools compared to hydrogen peroxide or bleach. Additionally, it was much easier to remove contamination from certain parts of the coring tool (location 3) possibly due to the shape of the tool; however, other areas on the tool may require rigorous cleaning.

Sensory analysis results showed that leafy greens treated with 0.1% cinnamon oil had the least impact on sensory attributes and hence, were the most liked by consumers. Additionally, washing leafy greens with cinnamon oil had the least impact on sensory attributes (pungency, browning, off-odor, bitterness, and sourness) as it was rated at scale of 1-not affected at all. Some essential oils (such as oregano oil) may impact the texture of leafy greens and to combat that we incorporated carvacrol (main active component of oregano oil) and cinnamaldehyde (main active component of cinnamon oil) into edible films which helped in reducing the impact on the texture of treated spinach. Sensory analysis also suggested that treatments with plant essential oils and plant extracts may be more appropriate to be used on spinach in comparison to other leafy greens, as consumers in general, have the highest preference liking for spinach and these compounds have the least adverse impact on organoleptic properties of spinach samples. Cinnamon oil may be used to wash organic leafy greens as this essential oil has antimicrobial properties and also had the highest preference liking and the least impact on the physical properties of leafy greens. In order to further improve the use of cinnamon oil it can be incorporated into edible films which could increase the preference liking by consumers and also reduce any adverse effects on organoleptic properties of leafy greens.

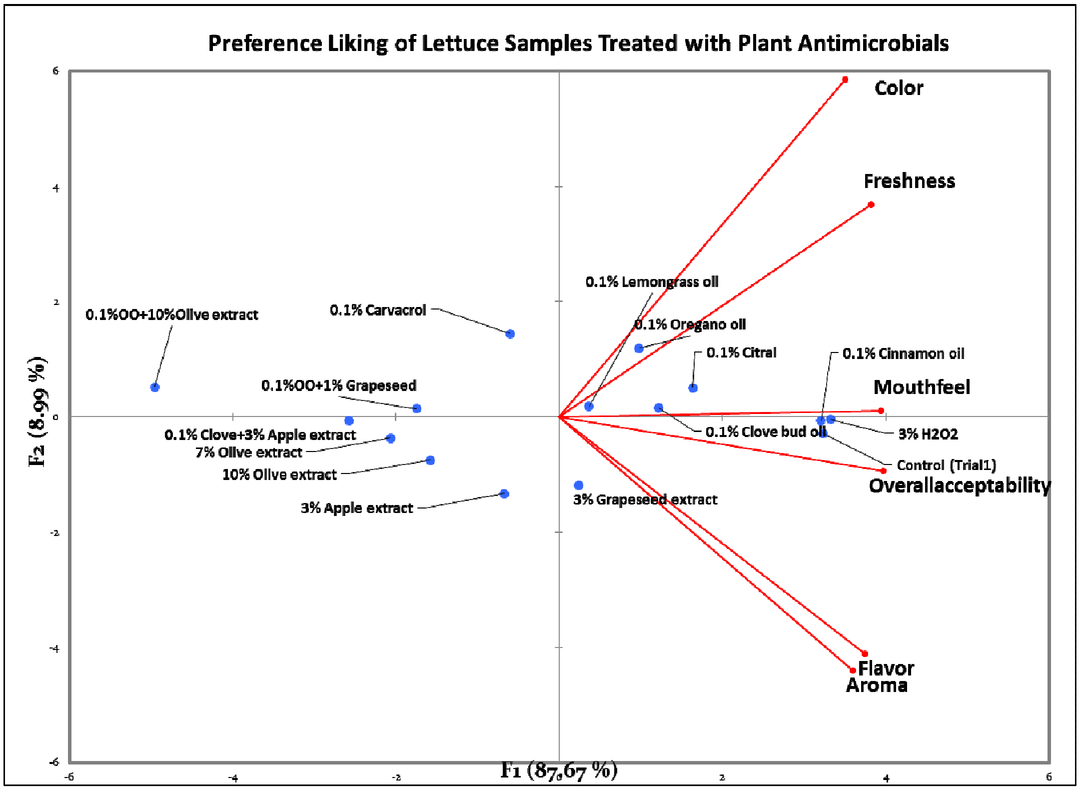
Overall, the texture and color properties of spinach were not adversely affected by plant antimicrobials washes compared to iceberg lettuce samples. Iceberg lettuce is lighter in color and topology of the iceberg leafy is higher for water weight which may have impacted the firmness and loss in color properties. Washing iceberg lettuce with plant extracts influenced the lightness of the leafy; whereas, essential oils impacted the firmness quality of lettuce leafs. To prevent loss in color attributes and firmness plant antimicrobials can be incorporated into edible films. Our results have shown that there was not a significant difference in color or firmness attributes of spinach samples in salad bags which contained edible films with pant antimicrobials.

Future work with coring tools could involve investigating the efficacy of organic sanitizers and plant antimicrobials in the presence of organic matter or on bacterial biofilms. Additionally, we can test these sanitizers against other foodborne pathogens which will lead to more cost-effective and broader applications of these sanitizers and plant antimicrobials. Sensory analysis of treated organic leafy greens can be conducted with trained panelists to reduce the variability in sensory data. Also sensory analysis needs to be conducted on different types of leafy greens (such as romaine) to see how these compounds will impact the firmness and color properties. Innovative research employing modified packaging in combination with essential oils can help reduce their effective concentrations and thereby, limit their impact on sensory properties, while enhancing the antimicrobial activity.

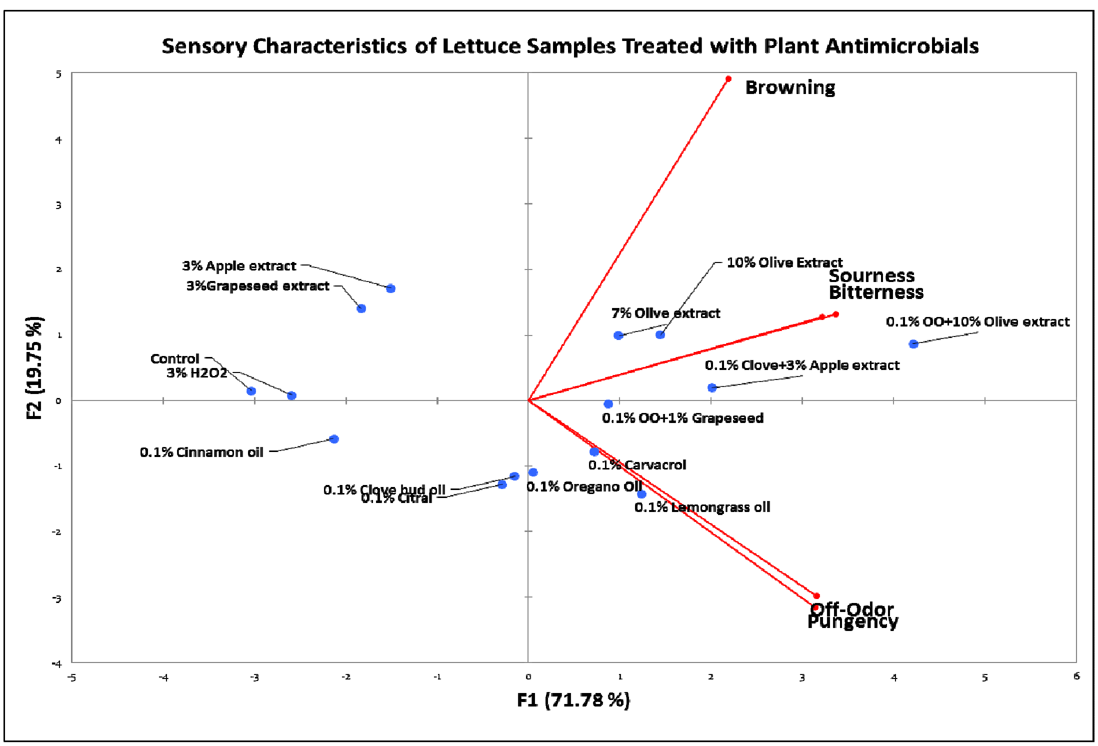
APPENDICES

APPENDIX A

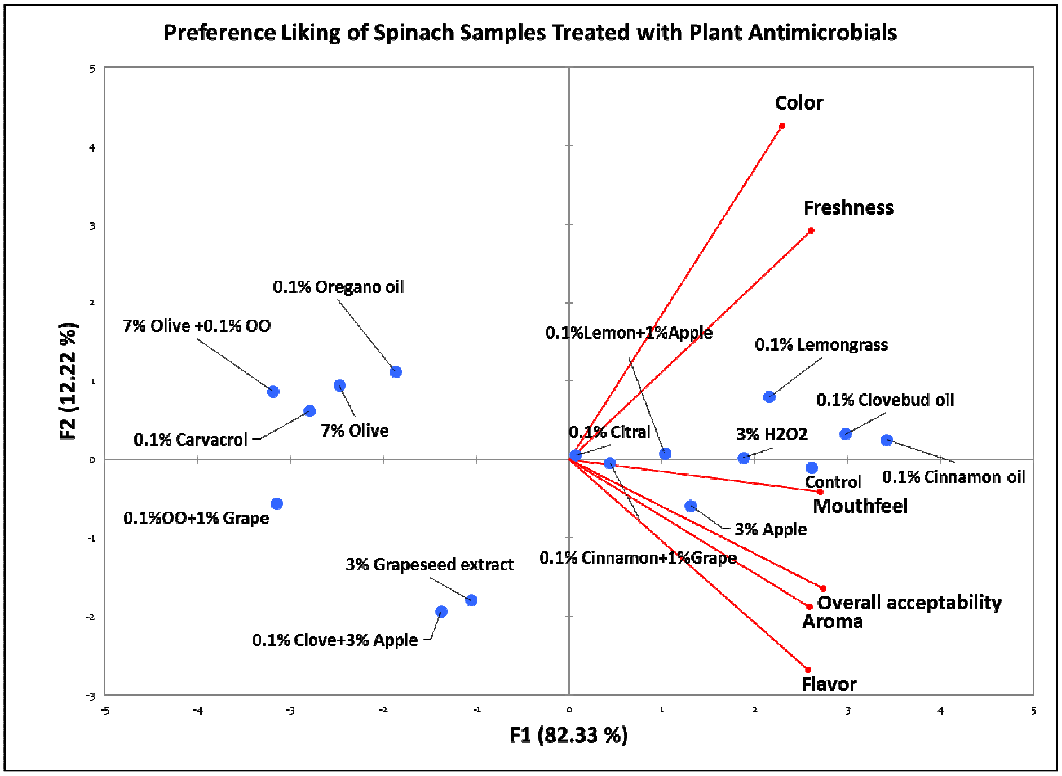
PRINCIPLE COMPONENT ANALYSIS (PCA) OF PREFERENCE LIKING AND SENSORY CHARACTERISTICS FOR ICEBERG/SPINACH SAMPLES TREATED WITH PLANT ANTIMICROBIALS AND WITH EDIBLE FILMS



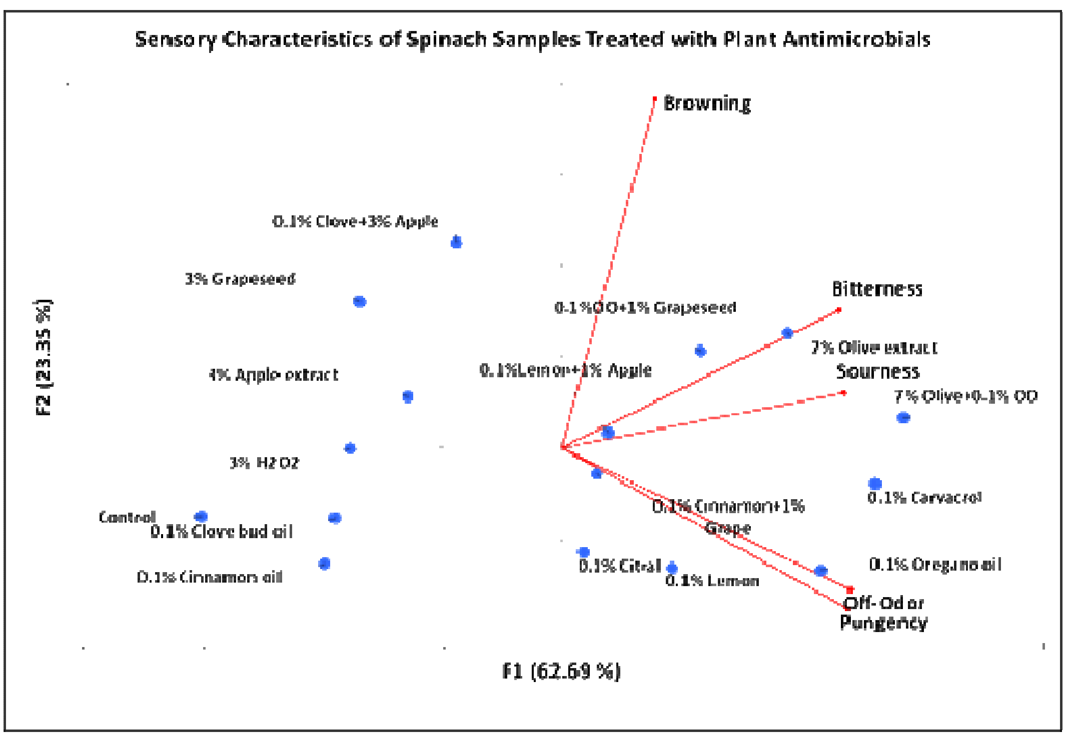
Preference Liking of Lettuce Samples Treated with Plant Antimicrobials
Color
Freshness
Mouthfeel
Overallacceptability
Flavor
Aroma
0.1%OO+10%Olive extract
0.1% Carvacrol
0.1% Lemongrass oil
0.1% Oregano oil
0.1% Citral
0.1% Cinnamon oil
0.1%OO+1% Grapeseed
0.1% Clove+3% Apple extract
7% Olive extract
10% Olive extract
3% Apple extract
3% Grapeseed extract
0.1% Clove bud oil
3% H2O2
Control (Trial1)
F2 (8.99 %)
F1 (87.67 %)



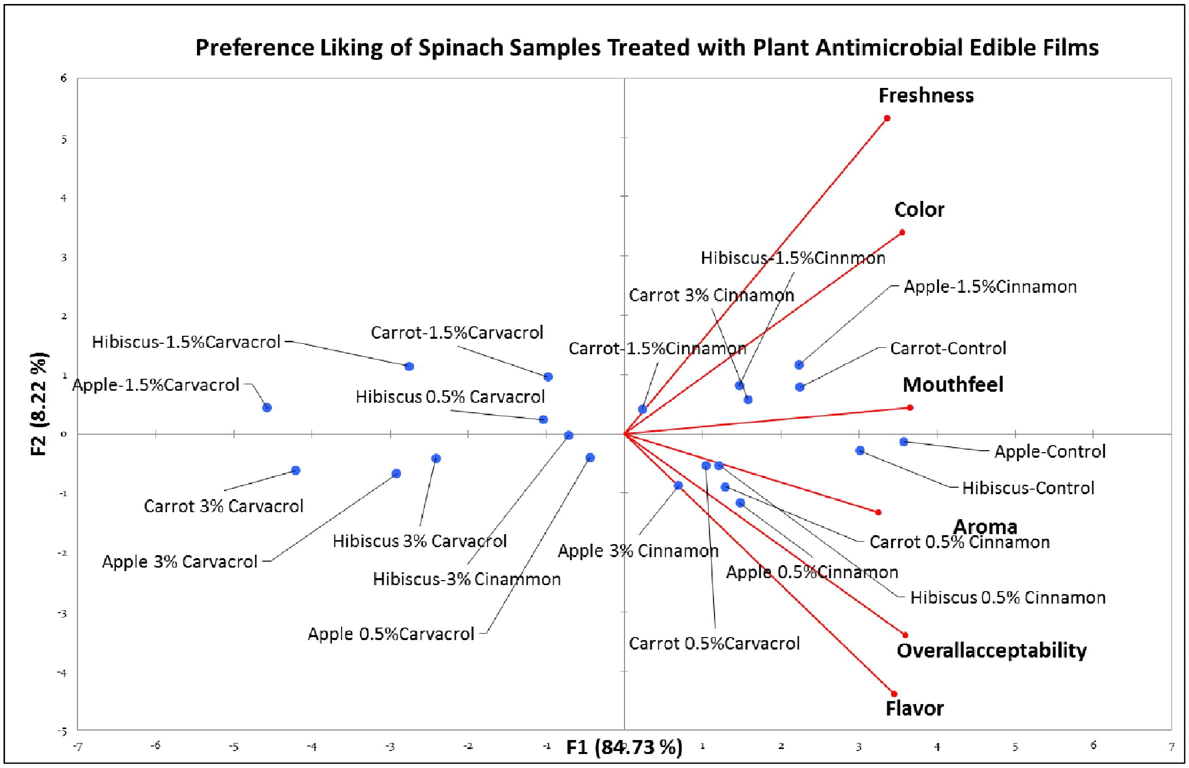
Sensory Characteristics of Lettuce Samples Treated with Plant Antimicrobials
Browning
Sourness
Bitterness
Off-Odor
Pungency
3% Apple extract
3%Grapeseed extract
10% Olive Extract
7% Olive extract
0.1% OO+10% Olive extract
Control
3% H2O2
0.1% Clove+3% Apple extract
0.1% OO+1% Grapeseed
0.1% Cinnamon oil
0.1% Carvacrol
0.1% Clove bud oil
0.1% Citral
0.1% Oregano Oil
0.1% Lemongrass oil
F2 (19.75 %)
F1 (71.78 %)

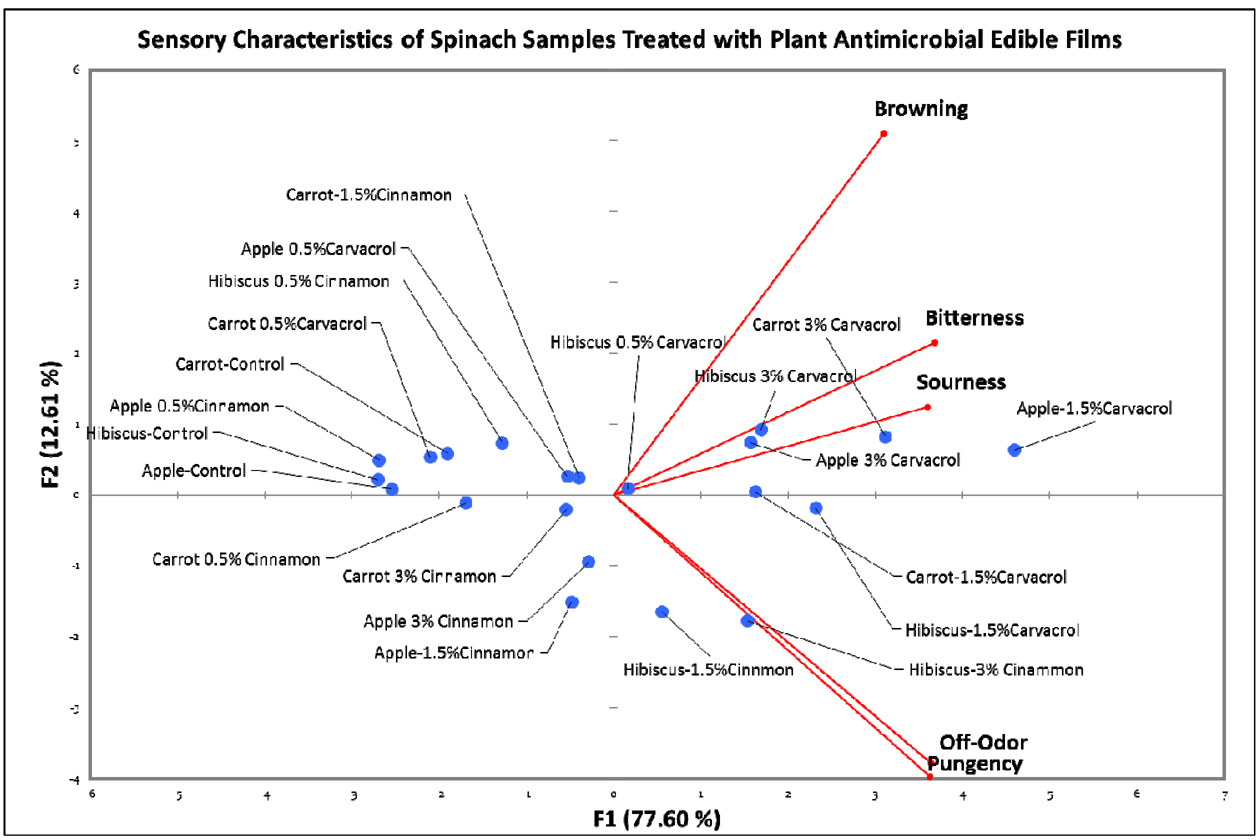


Preference Liking of Spinach Samples Treated with Plant Antimicrobials
F2 (12.22 %)
F1 (82.33 %)
Color
Freshness
Mouthfeel
Overall acceptability
Aroma
Flavor
0.1% Oregano oil
7% Olive +0.1% OO
0.1% Carvacrol
7% Olive
0.1%OO+1% Grape
3% Grapeseed extract
0.1% Clove+3% Apple
0.1%Lemon+1%Apple
0.1% Lemongrass
0.1% Clovebud oil
0.1% Citral
3% H2O2
Control
0.1% Cinnamon oil
3% Apple
0.1% Cinnamon+1%Grape



Sensory Characteristics of Spinach Samples Treated with Plant Antimicrobials
F2 (23.35 %)
F1 (62.69 %)
Browning
Bitterness
Sourness
Off-Odor
Pungency
0.1% Clove+3% Apple
3% Grapeseed
4% Apple extract
3% H2O2
Control
0.1% Clove bud oil
0.1% Cinnamon oil
0.1%OO+1% Grapeseed
0.1%Lemon+1% Apple
7% Olive extract
7% Olive+0.1% OO
0.1% Carvacrol
0.1% Cinnamon+1% Grape
0.1% Citral
0.1% Lemon
0.1% Oregano oil

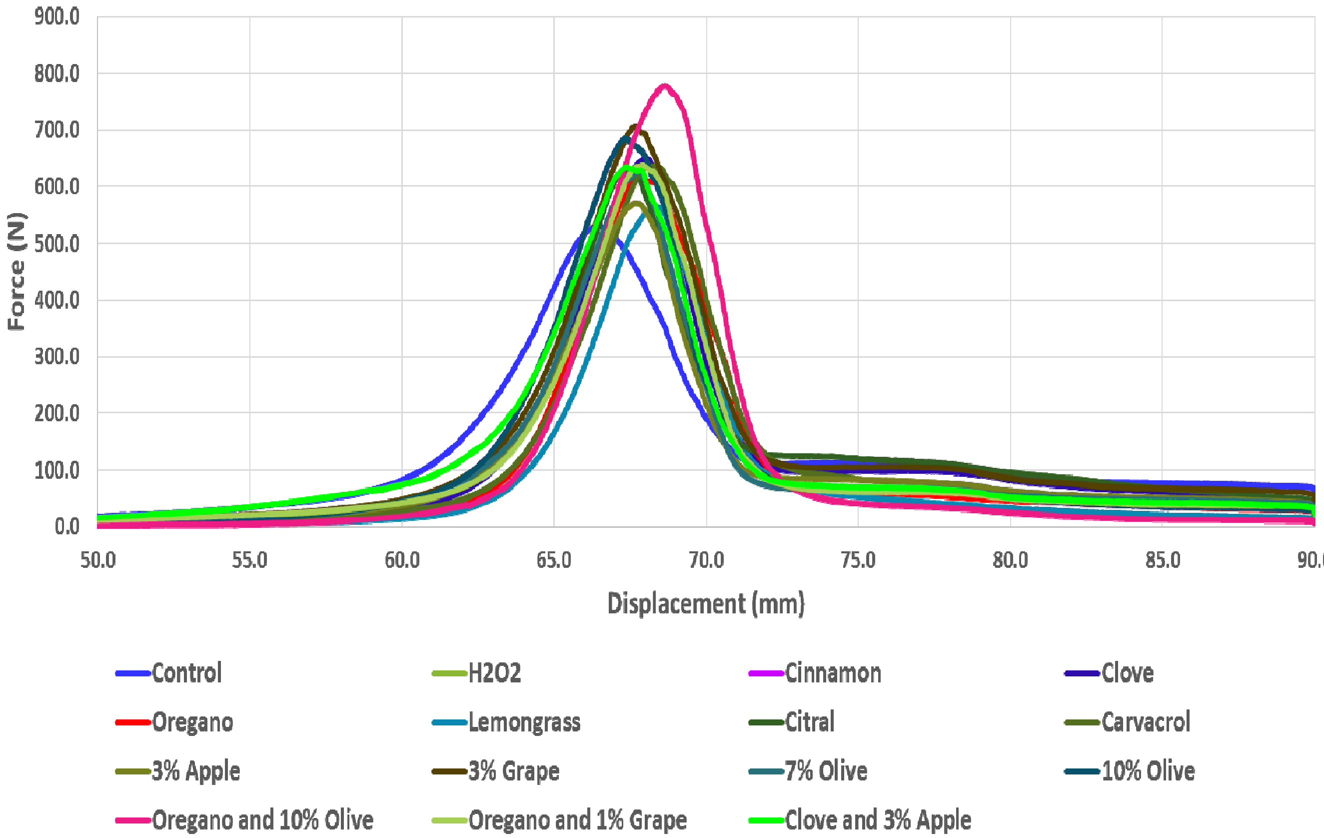

Preference Liking of Spinach Samples Treated with Plant Antimicrobial Edible Films
Freshness
Color
Mouthfeel
Aroma
Overallacceptability
Flavor
Hibiscus-1.5%Cinnmon
Carrot 3% Cinnamon
Apple-1.5%Cinnamon
Carrot-Control
Carrot-1.5%Cinnamon
Hibiscus-1.5%Carvacrol
Carrot-1.5%Carvacrol
Apple-1.5%Carvacrol
Hibiscus 0.5% Carvacrol
Apple-Control
Hibiscus-Control
Carrot 0.5% Cinnamon
Carrot 3% Carvacrol
Apple 3% Carvacrol
Hibiscus 3% Carvacrol
Hibiscus-3% Cinammon
Apple 3% Cinnamon
Apple 0.5%Cinnamon
Hibiscus 0.5% Cinnamon
Apple 0.5%Carvacrol
Carrot 0.5%Carvacrol
F1 (84.73 %)
F2 (8.22 %)


Sensory Characteristics of Spinach Samples Treated with Plant Antimicrobial Edible Films
Browning
Bitterness
Sourness
Off-Odor
Pungency
Carrot-1.5%Cinnamon
Apple 0.5%Carvacrol
Hibiscus 0.5% Cinnamon
Carrot 0.5%Carvacrol
Carrot-Control
Apple 0.5%Cinnamon
Hibiscus-Control
Apple-Control
Hibiscus 0.5% Carvacrol
Carrot 3% Carvacrol
Hibiscus 3% Carvacrol
Apple-1.5%Carvacrol
Apple 3% Carvacrol
Carrot 0.5% Cinnamon
Carrot 3% Cinnamon
Apple 3% Cinnamon
Apple-1.5%Cinnamon
Hibiscus-1.5%Cinnmon
Carrot-1.5%Carvacrol
Hibiscus-1.5%Carvacrol
Hibiscus-3% Cinammon
F1 (77.60 %)
F2 (12.61 %)

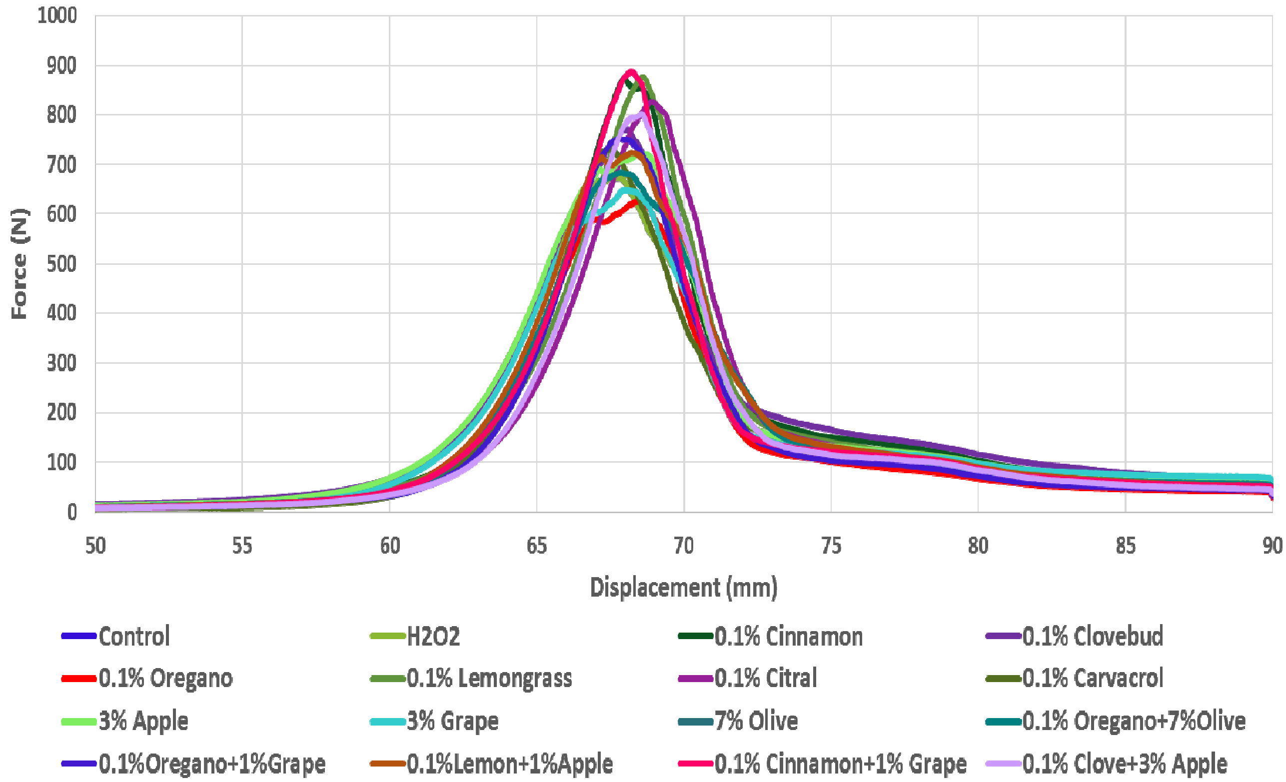
APPENDIX B

TEXTURE MEASUREMENT GRAPHS FOR ORGANIC ICEBERG LETTUCE AND BABY SPINACH WASHED WITH PLANT ANTIMICROBIALS

Graph 1: Texture Analysis of organic iceberg lettuce washed with plant antimicrobials after storage at 4°C for 20-24 h.



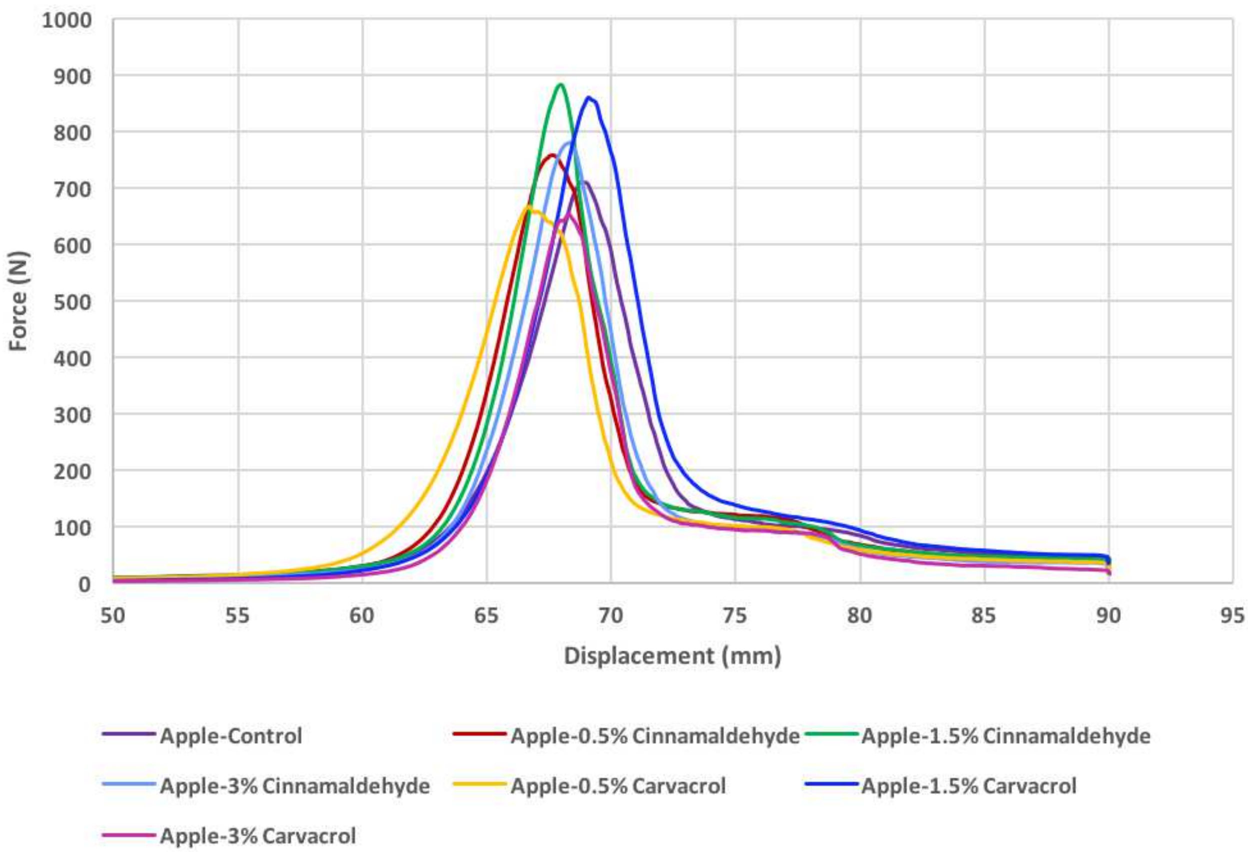
Graph 2: Texture Analysis of organic baby spinach washed with plant antimicrobials after storage at 4°C for 20-24 h.



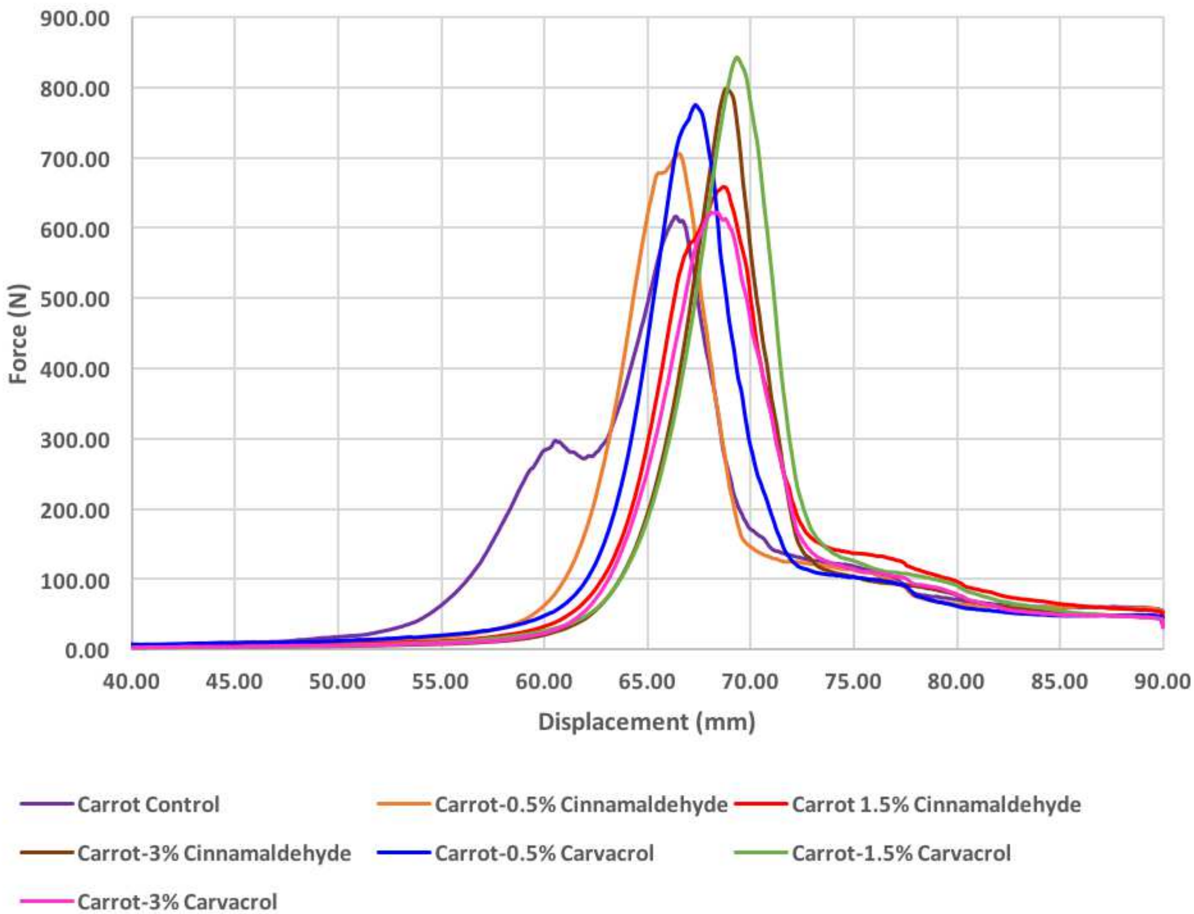
APPENDIX C

TEXTURE MEASUREMENT GRAPHS FOR ORGANIC BABY SPINACH TREATED WITH EDIBLE FILMS (APPLE, CARROT, HIBISCUS) CONTAINING PLANT ANTIMICROBIALS IN SALAD BAGS

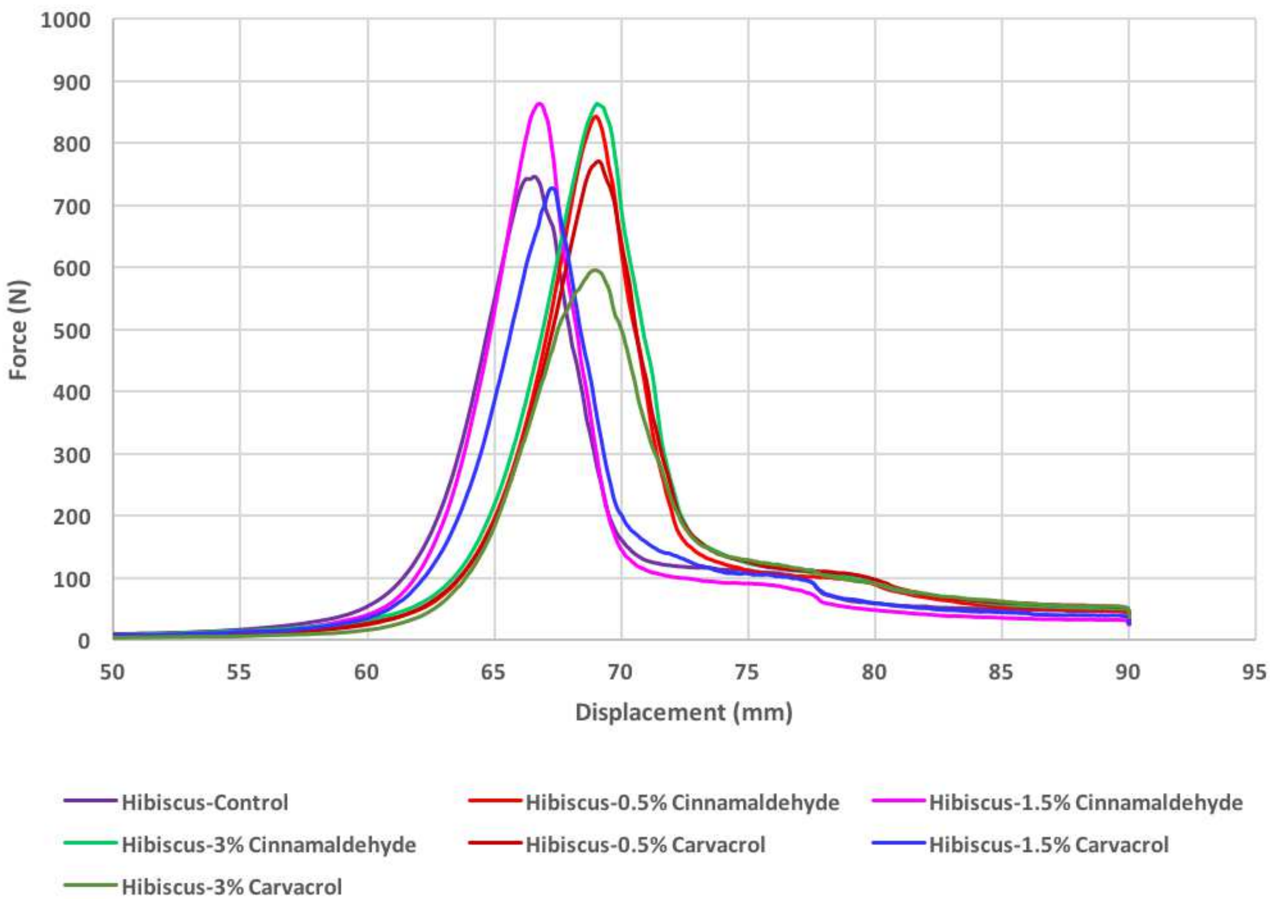
Graph 1: Texture Analysis of organic baby spinach with apple-edible films incorporated with plant antimicrobials in salad bags after storage at 4°C for 20-24 h.



Graph 2: Texture Analysis of organic baby spinach with carrot-edible films incorporated with plant antimicrobials in salad bags after storage at 4°C for 20-24 h.



Graph 3: Texture Analysis of organic baby spinach with Hibiscus-edible films incorporated with plant antimicrobials in salad bags after storage at 4°C for 20-24 h.



APPENDIX D

LIKELIHOOD OF PURCHASING ORGANIC ICEBERG LETTUCE, BABY SPINACH WASHED WITH PLANT ANTIMICROBIALS, OR BABY SPINACH TRATED WITH EDIBLE FILMS CONTAINING PLANT ANTIMICROBIALS IN SALAD BAGS

Table 1: Likelihood of purchasing organic iceberg lettuce washed with plant antimicrobials after storage at 4°C for 24 h. Each sample was evaluated by 60 panelists.

Treatments	Not at all	Slightly	Moderately	Very	Extremely
10% Olive extract	35	17	6	2	0
3% Apple extract	26	13	17	4	0
0.1% Oregano Oil + 10% Olive extract	53	4	0	3	0
0.1% Oregano Oil + 1% Grapeseed	35	13	8	3	0
0.1% Clove bud + 3% Apple extract	48	8	3	0	1
0.1% Carvacrol	34	13	9	2	1
7% Olive extract	41	10	7	1	1
0.1% clove bud oil	17	15	18	8	2
3% Grapeseed extract	22	21	7	7	3
0.1% Lemongrass oil	25	12	11	8	4
0.1% Citral	15	17	11	10	6
0.1% Oregano oil	21	12	9	11	7
0.1% Cinnamon oil	7	9	18	18	8
3% H2O2	1	6	20	11	22
Control	4	6	9	17	24

Table 2: Likelihood of purchasing organic iceberg lettuce washed with plant antimicrobials after storage at 4°C for 24 h. Each sample was evaluated by 60 panelists.

Treatments	Not at all	Slightly	Moderately	Very	Extremely
7% Olive extract	30	15	7	6	2
0.1% Oregano Oil +1% Grapeseed	24	16	14	3	3
0.1% Oregano oil	31	11	8	7	3
7% Olive extract + 0.1% Oregano oil	36	13	6	2	3
0.1% Carvacrol	30	11	9	4	4
0.1% Citral	15	15	13	13	4
0.1% Cinnamon oil + 1% Grapeseed	16	18	15	6	5
0.1% Lemongrass oil	14	15	16	8	7
3% Grapeseed extract	14	18	11	10	7
3% Apple extract	8	10	18	16	8
0.1% Lemongrass + 1% Apple extract	17	13	10	12	8
0.1% Clove bud oil	8	14	14	14	10
0.1% Clove bud oil +3% Apple extract	21	11	11	6	11
0.1% Cinnamon oil	6	10	19	12	13
Control	3	10	15	17	15
3% H2O2	12	11	10	9	18

Table 3: Likelihood of purchasing organic baby spinach treated with edible films containing plant antimicrobials in salad bags after storage at 4°C for 24 h. Each sample was evaluated by 40 panelists.

Treatments	Not at all	Slightly	Moderately	Very	Extremely
Carrot Control	7	8	10	7	6
Carrot 0.5% Cinnamaldehyde	7	10	7	6	10
Carrot 1.5% Cinnamaldehyde	12	9	8	6	5
Carrot 3% Cinnamaldehyde	14	8	3	6	9
Carrot 0.5% Carvacrol	6	11	9	7	7
Carrot 1.5% Carvacrol	20	8	5	3	4
Carrot 3% Carvacrol	19	7	9	2	3
Hibiscus Control	6	8	7	12	7
Hibiscus 0.5% Cinnamaldehyde	7	9	9	12	3
Hibiscus 1.5% Cinnamaldehyde	13	8	11	4	3
Hibiscus 3% cinnamaldehyde	19	7	3	7	4
Hibiscus 0.5% Carvacrol	9	14	7	8	2
Hibiscus 1.5% Carvacrol	27	5	4	2	1
Hibiscus 3% Carvacrol	18	4	8	6	4
Apple Control	2	8	11	6	13
Apple 0.5% Cinnamaldehyde	4	12	10	5	9
Apple 1.5% Cinnamaldehyde	17	8	7	3	5
Apple 3% Cinnamaldehyde	6	13	8	6	7
Apple 0.5% Carvacrol	12	7	10	7	4
Apple 1.5% Carvacrol	26	8	5	1	0
Apple 3% Carvacrol	18	6	6	7	3