ANTIMICROBIAL EFFICACY OF ESSENTIAL OILS AND THEIR PRIMARY CONSTITUENTS AGAINST *ESCHERCHIA COLI* O157:H7 ON ORGANIC LEAFY GREENS

By

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I dedicate this thesis to my son. He is, and will always be my rock. I could not ask for a more perfect son, and friend.

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Abstract:

The purpose of this study was to determine the effectiveness of plant-based essential oils and their primary constituents against *Escherichia coli* O157:H7 during the washing and short-term storage of organic leafy greens. Organic baby and mature spinach, and romaine and iceberg lettuce were inoculated with *E. coli* O157:H7 at 5-log_{10} CFU g^{-1}. Essential oils of cinnamon, oregano and lemongrass and their primary constituents cinnamaldehyde, carvacrol, and citral at 0.1, 0.3 and 0.5% (v/v) concentrations, along with controls of hydrogen peroxide and water, were used to wash the inoculated leafy greens for one or two minutes. The leafy greens were then stored at 4 or 8 ^{o}C and bacterial populations determined on day 0, 1, and 3 of storage. All essential oils and their primary constituent treatments showed significant ($P<0.05$) reduction of *E. coli* O157:H7 populations on all leafy greens. Oregano essential oil was the most effective essential oil with concentrations of 0.5% showing the greatest reduction, providing non-detectable growth after initial application (day 0). Similarly, carvacrol was the most effective compound providing non-detectable growth on all leafy greens on day 3 for all concentrations. There was no significant difference ($P<0.05$) between 1 and 2-minute treatment exposures on all leafy greens. Storage temperatures of 4 and 8 ^{o}C showed significant difference only in controls, with higher growth at 8 ^{o}C storage. Higher concentration (0.3 and 0.5%) of both essential oils and compounds exhibited no-detectable growth after 3 days in both 4 ^{o}C and 8 ^{o}C storage. This study provides evidence that plant-based essential oils, as well as their isolated compounds, can act as effective natural antimicrobials for washing organic leafy greens during short-term storage.

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INTRODUCTION

Escherichia coli O157:H7 has been a major contributor to food-borne diseases, with 19 reported outbreaks in the United States since 2006 (Centers for Disease Control and Prevention (CDC), 2013). This pathogen has been commonly associated with ground beef (Rangel, Sparling, Crowe, Griffin, and Swerdlow, 2005), but due to increased availability and demand of fresh produce, foodborne outbreaks associated with fresh produce have increased from less than 20 cases in 1970 to greater than 100 in 1990 (Sivapalasingam, Friedman, Cohen, and Tauxe, 2004). In a little over five year's span, the United States (US) has had *E. coli* O 157:H7 associated outbreaks occurring in organic baby spinach and spring mix blend in 2012 (CDC, 2012a), romaine lettuce in 2011 (CDC, 2012b), and two outbreaks of fresh spinach in 2006 (Grant et al., 2008).

Escherichia coli O157:H7 contamination in leafy greens can originate from various factors including contaminated irrigation water, animal manure run-off, and contamination during post-harvest processing (Steele & Odemeru, 2004; Oliveira et al., 2010). In particular, organic produce growers have been hinted to have a higher risk of contamination due to the limited control against microbial contamination, as well as the inability to utilize effective interventions used in conventional farming. However, there is little evidence that organic leafy greens have a significant increase in contamination when compared to conventional farming (Oliveira et al., 2010). Organic growers must follow guidelines set by the United States Department of Agriculture (USDA) in order maintain organic certification, which include composted manure or vegetable waste for fertilizer, in place of synthetic fertilizers, natural derived treatments for

pesticide uses, and hydrogen peroxide or treated water to wash produce during post-processing, instead of chlorine washes (USDA, 2013). Islam et al. (2004) found that while composting is effective at eliminating pathogen contamination, if the manure composts contain *E. coli* O157:H7, the pathogen can still remain viable in the amended soil for greater than 5 months, allowing for easy contamination on to the leafy greens. Research has been conducted to discover more natural, effective antimicrobial interventions to be used in both pre- and post-processing of foods. Among these, studies determining antimicrobial efficacy with plant-based essential oils have portrayed positive results with high log reductions against food-borne pathogens. Such studies found antimicrobial efficacy from lemongrass essential oil (Moore-Neibel et al., 2011), and oregano essential oil (Moore-Neibel et al., 2013) against *Salmonella enterica*, as well as *Thymus*, *Satureja*, and *Origanum* derived essential oils against *E. coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteridis*, and *Bacillus cereus* (Chorianopoulos et al., 2004).

Though studies have proven essential oils and their primary constituents are effective at reducing food-borne pathogens on leafy green surfaces, there have not been, to the best of our knowledge, studies examining the efficacy of multifactorial treatment effects common to those applied in an organic post-harvest leafy green production. In effort to find interactive antimicrobial behaviors of essential oils against *E. coli* O157:H7 in applications found in leafy green processing, this present study will examine interactions between essential oils (originating from oregano, cinnamon, and lemongrass) and their primary constituents (carvacrol, trans-cinnamaldehyde, and citral), storage temperature and duration, treatment wash exposure duration, and treatment concentration; tested on four types of organic leafy greens.

REVIEW OF LITERATURE

A. *Escherichia coli* O157:H7

1. Classification

*Escherichia co*li O157:H7 is a Gram-negative rod and a pathogenic strain of *E. coli*. It is classified by its expression of the 157th somatic (O) antigen, and the 7th flagellar (H) antigen (Mead and Griffin, 1998). Originating from the intestinal tracts of bovine species, *E. coli* O157:H7 was first identified in 1982 in relation to an outbreak of hemorrhagic colitis (National Institute of Health and Infectious Disease, NIH, 1997). *Escherichia coli* is divided into 6 pathoytpes (Centers for Disease Control and Prevention, CDC, 2012b): Enterohemorrhagic *E. coli* (EHEC) (also referred as Shiga-toxin Producing *E. coli*, STEC; and Verocytoxin-producing *E. coli*, VTEC), commonly associated with hemorrhagic colitis (NIH, 1997), and a major cause of hemolytic uremic syndrome (HUS) (Gyles, 2007); additional classifications include Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), and Diffusely adherent *E. coli* (DAEC) (CDC, 2012b). *Escherichia coli* O157:H7 primarily belong to *E. coli* pathogroup EHEC (CDC, 2012b).

2. Pathogenicity

It is believed that the main source of O157 EHEC's virulence is their ability to produce two Shiga-toxins (also called verocytotoxin). The development of Shiga-toxins in EHEC are said to be encoded by a lamba-like bacteriophage genome inverted into the pO157 plasmid (Kaper et

al., 2004). These Shiga-toxins are classified as either Shiga toxin 1 (Stx1), or Shiga toxin 2 (Stx2). Shiga-toxin 2 is more likely to be found in O157:H7 then Stx1 (Mead and Griffin, 1998). Karmali (2004) reported Stx2 to have a superior cytotoxic effect on human glomerular endothelial cells than that of Stx1, thus increasing the virulence of the Stx2 strain in human hosts, including a higher risk of HUS. The endothelial cell is the primary target for Shiga-toxins, but renal tubular cells, meningeal cells, monocytes, and platelets are also subject to Shiga-toxin targeting as well (Karmali, 2004). The binding of Shiga-toxins to target cells induces coagulation and inflammation, which can be associated with both hemorrhagic colitis and HUS symptoms (Karmali, 2004). Shiga-toxin's mode of action is the feat of A/B subunit structures of the Shiga-toxin. Five identical B subunits of the toxin bind to the glycolipid globotriaosylceramide (Gb3), a glycolipid found in select eukaryote cell membranes. Once bound, the A subunit of the toxin enzymatically inactivates the 60S ribosomal subunit, inhibiting protein synthesis of the cell (Mead and Griffin, 1998; Endo et al., 1988); exposing the target cell to detrimental damage, which most likely will result in cell death. The death of colonic cells and lesions in small blood vessels from cell damage via the Shiga-toxin induced protein synthesis inhibition is the cause of the bloody diarrhea in hosts (Naylor et al., 2003).

Additional virulent factors of pathogenic EHEC's include the aptitude of the pathogen to survive in an enteric environment. These include the ability of the pathogen to adhere to the intestinal wall via a protein adhesive. The coding for this adhesive is a part of the Locus of Enterocyte Effacement (LEE), a pathogenicity island in the *E. coli* genome that encodes for epithelial attachment (Perna et al., 2001). In addition, *E. coli*'s high acid tolerance contributes to its virulence, therefore allowing pathogenic *E. coli* O157:H7 to surpass the hurdle effect of high acidic conditions of the stomach and intestinal tract (Naylor et al., 2003). Like other Gram-negative pathogenic bacteria, *E. coli* O157:H7 contain lipopolysaccharides (LPS) in their outer membranes. Lipid A, a substructure in the LPS, acts as a heat-stable endotoxin. The host cell

interaction with this structure upon release after cell lysis is known to cause fever, hemorrhagic shock, and diarrhea (Naylor et al., 2003).

3. Reservoirs in Leafy Green Production

While bovine species are considered the main reservoir of *E. coli* O157:H7, this pathogen can still cycle through the environment and food chain through assorted reservoirs including, water, manure, and soil; as well as vectors such as wildlife and insects (Franz and van Bruggen, 2008). In agricultural growing practices, it is believed that the main source of *E. coli* O157:H7 introduction come from reservoirs of untreated manure or manure amended soils, and irrigation water used to water and spray pesticide on crops (Franz and van Bruggen, 2008). Erickson et al. (2010) suggest that once pathogens are introduced in the fields, the soil could also become a pathogen reservoir. In a study by Islam et al. (2004), both *E. coli* and *Salmonella* spp. were found to be able to survive in the field for 177 and 231 days, respectively.

Conditions for *E. coli* O157:H7 in manure and manure-amended soils are unfavorable due to the lack of nutrients, compared to the rich supply found in the animal's gut (Franz and van Bruggen, 2008; Franz et al., 2008). However, pathogens like *E. coli* O157 and *S. enterica* have been known to survive for long periods in manure (Scott et al., 2006). In organically managed soils, manure is commonly used as a natural fertilizer. Organic soils naturally hold a higher microbial biomass and diversity than conventional soils (van Diepeningen et al., 2006). Based on the previous statement, pathogen contamination, like that from *E. coli* O157:H7, may cause a higher risk of contamination. However, studies have shown that because of the high microbial diversity in the organic soils, pathogens like *E. coli* O157:H7, which are out of their natural reservoir, are not able to survive due to competitive exclusion and inhibitory effects from bi-products of the other natural microorganisms in the soil. *Escherichia coli* O157:H7, nevertheless, can survive through environmental stress if the conditions and specific opportunity is given to them. As seen in a study by Franz et al. (2008), the survival of *E. coli* O157:H7 in manure-

amended soils was dependent on organic nitrogen (positive effect), and the richness of Eubacteria species in the soil (negative effect).

Irrigation water is a resource used by farmers to manage the health of their crops. However, water that comes in contact with pathogen contamination may become another pathogen reservoir. Solomon et al. (2003) found that lettuce, when sprayed with a cocktail of *E. coli* O157:H7, had surviving *E. coli* O157:H7 populations on the leafy surface 30 days following the initial spray. They further suspected that surface irrigation (i.e. topical spray irrigation) led to a detectable recovery of the pathogen from the leafy surface, whereas water irrigation from drip and sprinklers showed lower recovery.

4. Contamination in Leafy Green Production

Increased contamination from *E. coli* O157:H7 has been linked to intensive agriculture, i.e. the introduction of the pathogen from its original reservoir (cattle) to an unexposed area, such as a vegetable growing area (Lynch et al., 2009). Lynch et al. (2009) suggest that within the food production chain *E. coli* O157:H7 can enter at three specific points: in the field, during industrial processing, and during the preparation in the kitchen. Contamination in the field can derive from wild animals, irrigation water, water to apply fungicides and pesticides, soil, inadequately composted manure, and human handling (Beuchat, 2002; Delaquis et al., 2007). An investigation following an *E. coli* O157:H7 outbreak in spinach traced the infected leaves back to a growing region where feral swine existed in close proximity to the fields growing the spinach (Jay et al., 2008); providing evidence of possible contamination from an outside farm animal source.

Even after leafy greens are harvested from the fields and are sent to the post-harvest processing facilities, enteric pathogens, such as *E. coli* O157:H7, can survive on the leafy green under the given environment (temperature, water availability, available nutrients, and amount of natural micro flora still present on the leaf). Additionally, contamination may occur during post-harvest processing from contaminated water used for washing, sprays, (Solomon et al., 2003) and

shipping ice (Kim and Harrison, 2008). Penteado et al. (2004) considers that contamination can be amplified during this point due to the plunging of warm produce into a cold-water bath, which causes the internal airspaces to contract drawing water and contaminants in that water into the fruit, internalizing possible pathogens or spoilage microorganisms. Further post-harvest processing, which may include cutting, shredding, or storage of produce, may provide an ample opportunity for the pathogen to invade leafy green tissue (Delaquis et al., 2007).

The final stage, in the kitchen, contamination can arrive from hygiene of food handler/preparer. Once the contaminated leafy green has reached this stage the risk of infection is increased by of improper handling of the leafy green (i.e. failing to wash the leafy greens thoroughly before preparing them) (Franz and van Bruggen, 2008).

5. Survival in Post-Harvest Processing

Resistance to intrinsic and extrinsic factors such as, temperature, acidic conditions, moisture content, and available oxygen, has occurred in foodborne pathogens. Many of these factors are products of human microbial intervention during the growing and processing of food products. A variety of factors play a large role in the survival of pathogens in food products, including, storage temperature, package atmosphere, product type and bacterial strain (Francis and O'Beirne, 2001).

`Francis and O'Beirne (2001) found that *E. coli* O157:H7 and *Listeria monocytogenes* were able to survive at refrigerated temperatures (4 ^{o}C), with populations of *E. coli* O157:H7 decreasing slightly over time. In the same study, abused refrigerated temperatures (≥8 ^{o}C) were shown to provide *E. coli* O157:H7 the opportunity to increase 1-log CFU g^{-1} after 12 days in 8 ^{o}C storage. In addition, Li et al. (2001) noticed significant growth (3-log CFU g^{-1}) of *E. coli* O157:H7 on iceberg lettuce during storage at 15 ^{o}C, providing evidence that temperature control is essential in minimizing microbial growth. The use of low and high temperatures is a common use of microbial control in food products. In fresh leafy green production, the use of refrigeration

is necessary to control for microbial hazards on leafy green, particularly in damaged areas that may serve as harborage sties on the leaves. Produce growers utilize the method of composting as a control for microbial contamination when applying natural fertilizers, such as animal and green manure. The composting process can be divided up into four phases: the mesophilic phase, thermophillic phase, cool-down phase, and maturing phase. In each phase the microbial populations alter depending on the environmental conditions. Jiang et al. (2003) studied the effect of composting on coliform bacteria in static compost piles of dairy waste solids, and found that certain bacteria decline during various phases of the composting process. They mentioned most coliforms survived the mesophillic phase, but were reduced during the thermophillic phase, offering evidence that coliforms are not as resistant to the environmental factor of increased temperatures. Temperatures in composting usually range from 50 to 70°C for an allotted amount of time in order to kill the microbial pathogens, weed seeds, and fly larvae (Rynk, 1992). Compost temperatures begin increasing after 5 days, peaking at 7 to 10 days of composting (Rynk, 1992). Jiang et al. (2003) suggest that this slow heat up in the compost may enable the pathogen *E. coli* O157:H7 to become accustomed to the heat and become more sustainable in the compost environment, an influence that may be the cause of expression of heat shock genes, which will result in resistance.

Many food-grade sanitizers are used in processing to reduce microbial populations from both food products and equipment. The most widely used sanitizer in the fresh produce industry is chlorine; sometimes labeled as chlorine monoxide (Franz and van Bruggen, 2008). Aruscavage et al. (2006) found that the concentration of chlorine allowed to be used in food products (<200 ppm) is not very effective at reducing pathogens on lettuce surfaces, and that the effectiveness of sanitizers is dependent on the produce it is used on, as well as the contact time. If a sanitizer is found to be effective, Aruscavage et al. (2006) predicted that it might completely eliminate the competitive natural micro flora on the produce's surface. This may, in part, develop a resistant

pathogen to have little to no competition of nutrient source and will enumerate under the allowed conditions.

B. Leafy Greens

1. *Escherichia coli* O157:H7 Outbreaks in Leafy Greens

The rise in foodborne outbreaks in fresh produce can be associated with the rapid distribution, and consumption of raw products (Lynch et al., 2009). The consumption of produce has increased with a consumer trend of eating more healthy diets. However, improper washing techniques of produce in the consumer kitchens, and the lack of cooking the produce at high temperatures have increased the risk of ingestion of pathogenic organisms, like *E. coli* O157:H7 (Lynch et al., 2009). In a survey conducted between 1990 through 2004, produce was said to be the second leading cause of foodborne disease outbreaks (22% reported cases), as well as the leading cause of disease (38% of total foodborne disease cases) among five major food categories (produce, beef, poultry, seafood, and eggs) (Anonymous, 2006). Among produce outbreaks the most common occurrence is seen in pre-packaged salad, lettuce, juice, melon, and sprouts (Sivapalasingam et al., 2004). Pathogens *Salmonella spp*. and *E. coli* O157:H7, have been associated as the more dominant pathogens involved in produce outbreaks (Franz and van Bruggen, 2008).

In 2006, two multi-state *E. coli* O157:H7 outbreaks occurred from both spinach and iceberg lettuce. The first, involving baby spinach, was sourced to 3 bags of a single brand of spinach. The outbreak involved 26 states, with Utah and New Mexico as the source of an epidemiological study (Grant et al., 2008). Grant et al. (2008) concluded that washing spinach before consumption did not affect the odds of infection, which they believe to be from the pathogen being internalized in the spinach leaf, or strategically adhered to the spinach leaf surface, avoiding removal from washes. Jay et al. (2007) further researched the source of the outbreak and concluded that initial contamination of the spinach could have been sourced from

nearby wildlife or agricultural animal operations via contaminated water. Isolates were found in feral swine and cattle, of which, 14.9% and 33.8% were recovered, respectively, for each sampled animal. The second outbreak, involving iceberg lettuce, occurred a few months following the spinach outbreak. Sourced in the northeastern United States and involving a large restaurant chain, Taco Bell, this *E. coli* O157:H7 iceberg lettuce outbreak sickened 71 people across 5 states, of which 53 were hospitalized; 8 of these cases developed hemolytic uremic syndrome (HUS) (CDC, 2006). In 2012, a similar outbreak occurred where Shiga-toxin producing *E. coli* O157:H7 infections were linked to a bagged organic spinach blend. This multi-state outbreak had a total of 33 people reported as being infected with this particular Shiga-toxin producing O157 strain. Of those reported, 46% were hospitalized; two of those cases developed HUS, however, no deaths were reported (CDC, 2012a). Continuing investigation found the source might have come from one producer in Massachusetts, however, no further evidence indicates that this was the true source of the outbreak (CDC, 2012a).

2. Foodborne Pathogen Interaction with Leafy Green

Microbial colonization is selective to external factors, including the congested microenvironment and risk of predation, the continuous supply of nutrients and temperature fluctuations in the soil. These factors would usually favor the colonization of the rhizosphere of the leaf, instead of phyllosphere, because the phyllosphere is a nutrient-poor, arid environment subject to large temperature fluctuations and UV radiation (Yang et al., 2001). If colonization does occur on the phyllosphere microorganisms will not be uniformly distributed on leaf surfaces. Most are located at the base of trichomes, on the outer rim of stomates, or in cell grooves along veins, with each distributed in intricate communities resembling biofilms that are referred to as "aggregates" (Nibuerm and Lindow, 2004).

When human pathogens enter the plant phyllosphere and rhisosphere, they come in contact with established microbial communities (Delaquis et al., 2007). Native or introduced

microorganisms associated with entire plants are presented with highly altered physical and chemical environments in packaged leafy vegetables (Mercier and Lindow, 2000), with some of the organisms native in the soil microflora having antagonistic effects against pathogenic bacteria introduced into soil (Johannessen et al., 2005). Although nutritional conditions at the leaf surface are growth limiting, the release of cell sap from bruised, punctured, or cut tissues provides a supply of nutrients that can support extensive microbial growth (Mercier and Lindow, 2000; Delaquis et al., 2007). Initial attachment of phytopathogens to leaf surfaces is often at the stomata, broken trichomes, or cracks in the cuticle. Seo and Frank (1999) showed *E. coli* O157:H7 can attach at these sites as well, but mainly were found at the cut portions of the damaged leaf. In a study by Schuenzel and Harrison (2002), recovered bacterial strains *Pseudomona fluorescens*, *P. aerugilnosa*, and *Aeromonas hydrophila* acquired from fresh-cut produce were shown to demonstrate inhibitory activity toward *E. coli* O157:H7. When *E. coli* O157:H7 was introduced at lower cell densities (10^2 to 10^4 CFU/ml), the extent of colonization was comparatively low, although the pathogen was recovered from lettuce roots after a 10-day cultivation period (Warriner et al., 2003). Warriner et al. (2003) further explained that *E. coli* could become established in germinated seedlings, but is restricted to only the roots in mature spinach plants. Therefore, there is a low risk that the edible portion of the spinach leaves will harbor *E. coli* O157:H7 in the inner tissue.

Organisms, like *E. coli*, enter into a stationary phase when under environmental stress including starvation, UV-radiation, heat, salinity, and bi-products of organisms naturally occurring in the reservoir. When the cell enters this phase they undergo physiological changes that enable them to survive in these conditions (Abee and Wouters, 1999). The ability of enteric pathogens to multiply on the surface of leafy greens may be a critical factor in the epidemiology of zoonotic diseases linked to leafy greens (Brandl and Amundson, 2008).

Research has provided that enteric pathogens have the capabilities to grow and persist on agricultural plant surfaces even though they are not fit for the plant habitat as the plant's native

microflora (Brandl, 2006). *Escherichia coli* O157 can also reach the sub-stomatal cavity and the spongy mesophyll and survive in this environment (Xicohtencatl-Cortes et al., 2009). Bacteria can survive on plant surfaces in a heterogeneous distribution, preferring to reside at the base of the trichomes, in the cell grooves along the veins, and on the outer rim of the stomata (Moneir and Lindow, 2004).

Survival on plant surfaces is dependent on a variety of factors including nutrient availability, competition with indigenous micro flora, and relative humidity (Erickson et al., 2010). The survival rates of pathogens occurred greater on the interior of the plant, rather then the exterior (Johannessen et al., 2008), unless leaves become damaged in the field, which showed an increase in persistence of pathogen contamination (Baker-Reid et al., 2009).

Girón (2008) used high-resolution electron microscopy to study *E. coli* O157:H7 infected spinach leaves and found pre-harvest internalization of *E. coli* O157:H7 within the plant stomata, with visible flagella-like structures stemming from the stomata. The same study further found that a type III secretion system from *E. coli* O157:H7 appeared to activate stomata opening, which enabled stomata colonization for the pathogen. In a study by Brandl (2008b), *E. coli* O157:H7 load increased 4 to 11-fold on damaged lettuce leaves and 2-fold on intact ones. The study further clarified that the leaves affected by soft rot harbored 27 more bacteria than healthy ones.

Attachment to the plant surface is a necessary feat for a pathogen to help facilitate colonization on the leafy green surface. Once on the produce surface, it is very difficult to remove attached pathogens with standard produce washes (Beuchat and Scouten, 2002). Unlike non-pathogenic *E. coli*, the O157:H7 EHEC strain is able to adhere to plant surfaces, including tomato skin, alfalfa roots, and spinach leaves very efficiently (Jeter and Matthysse, 2005). Jeter and Matthysse (2005) believed that curli, a proteinaceous component involved in adhesion and cell aggregation, played a role in pathogen attachment to fruit and vegetable surfaces. However, when the gene for curli translation was deleted from *E. coli* O157:H7 there was still adequate attachment from *E. coli* O157. In addition, Xicohtencatl-Cortes et al. (2009) showed that flagella

could play a role in *E. coli* O157 leaf attachment by deleting of the fliC sequence, encoding flagellin. Their results revealed a reduced level of adhesion, suggesting that *E. coli* O157 attachment was repressed, but not fully inhibiting it's attachment significantly. These may be an indication that there is not one primary, but multiple factors and/or structures that mediate *E. coli* O157:H7 attachment on produce surfaces (Xicohtencatl-Cortes et al., 2009).

Studies have revealed that phylloepiphytic bacteria preferentially adhere to epidermal cell wall junctions, glandular and non-glandular trichomes, veins, stomata, and epidermal cell wall surfaces and in furrows between epidermal cells; therefore, *E. coli* O157:H7 could also selectively attach at these sites. When bacteria attach to sites with anatomical features such as veins and glandular trichomes, which are used to aid in protection of the plant from pathogen, their survival was shown to be enhanced (Monier and Lindow, 2004).

C. Essential Oils

1. Chemical Composition of Essential Oils

Essential oils are synthesized oily compounds formed from plant organs, which can include buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark. Each are stored within secretory and epidermic regions of the plant organism (Bakkali et al., 2008). Each essential oil contains very complex mixtures of isolated compounds, ranging from 20-60 components (Bakkali et al., 2008). The chemical constitution of these essential oils provides a specific effect in the antimicrobial activity, which can be correlated with the structure and functional groups of that compound (Gutierrez et al., 2008).

The most common compound found in plant-derived essential oils are terpenes and other terpenoids (isoprenoids) (Ait-Ouazzou et al., 2011) Terpenes are complex hydrocarbon compounds that contain multiple isoprene units, which may or may not be cyclic (Sikkema et al., 1995) Monoterpenes, a terpene with one isoprene subunit, is the most representative compound in essential oils, constituting 90% of the total compounds (Bakkali et al., 2008). Other assorted

aromatic compounds deriving from phenylpropone make up the remaining composition (Bakkali et al., 2008). Terpenes and phenylpropanic compounds are usually separated in plants, but may coexist. When these compounds do coexist together, one or the other is usually more dominant (Bakkali et al., 2008).

Phenolic compounds consist of a phenol ring with a hydroxyl group attached (Veldhuizen et al., 2006). Veldhuizen et al. (2006) explored the antimicrobial activity of carvacrol and thymol, both phenolic monoterpenes, each differing in location of the hydroxyl group on the benzene ring. They compared the effect of influence chemical structure on antimicrobial activity by displacing functional groups (e.g. isoprene, hydroxyl, etc.) from the original structure (benzene ring), and tested antimicrobial efficacy for each new change in structure. These changes formed compounds p-cymene, carvone, and other assorted compounds. The study's results found reduced antimicrobial activity when the ring substitutes were taken away, or moved to a different position on the phenolic ring. Veldhuizen et al. (2006) further hypothesized that the hydroxyl group, and the delocalized electrons on the benzene ring provided carvacrol its characteristic antimicrobial activity, offering evidence that the efficacy of antimicrobial activity can even be credited to the functional group placement, let along the lack of the functional group itself.

The chemical composition of essential oils can vary based on the geographical location of the plant, harvesting season, extraction method, and the region of the plant used to acquire the essential oil (McGimpsy et al., 1994; Friedman et al., 2002). As seen in cinnamon essential oil, the oils deriving from cinnamon bark contain 81% trans-cinnamaldehyde, with little traces of eugenol; whereas cinnamon leaf oil contains 70% eugenol, with little traces of cinnamaldehyde (Friedman et al., 2000). Also, oregano essential oil was found to contain compounds: carvacrol, thymol, ρ-cymene, and γ-terpene in concentrations ranging from trace amounts to the highest concentrations at 80, 64, 52, and 52%, respectively, in various oregano species (Sivropoulou et al., 1996).

2. Mechanisms of Antimicrobial Activity

a. Activity of Phenolic Compounds

Phenols are a class of compounds composed of an aromatic benzene ring, with a hydroxyl functional group bonded to the carbon ring (Dorman and Deans, 2000; Arfa et al., 2006). Burt and Reinders (2003) hypothesized that phenolic compounds attack the cell by sensitizing the membrane, which will induce the cell wall to saturate, increasing permeability. This phenomenon will then cause the integrity of the cytoplasmic membrane to collapse causing a sudden leakage of intracellular constituents (Joven et al.,1994). This antimicrobial activity is best defined as the phenolic compound changing the membrane functionality and protein-to-lipid ratios in the membrane (Sikkema et al., 1995), a process that is irreversible (Kisko and Roller, 2005). The high activity of phenols may be credited to alkyl substitution into the phenol nucleus, which is known to enhance their activity (Dorman and Deans, 2000).

Effects of phenolic compounds may be directed to non-specific inhibition of membrane bound enzymes caused by small hydrophobic molecules due to changes in protein conformation (Gill and Holley, 2006a). This activity can be supported from studies examining the antimicrobial effects of phenolic compounds carvacrol and thymol, the primary constituents in oregano and thyme essential oils. Both compounds are linked to the accumulation of the lipophilic character of the cell membrane causing leakage of protons and intermembrane compounds such as potassium (Xu et al., 2008), as well as other membrane-associated events such as ATP leakage (Gill and Holley, 2006b; Oussalah et al., 2006). As seen through a study on carvacrol; the activity can be pinned to the phenol's method of attacking the cell membrane, increasing the membrane permeability allowing a release of potassium ions and protons, thus taking away the essential components for the ATP synthesis, thereby decreasing intracellular ATP (Gill and Holley, 2006b; Oussalah et al., 2006). Carvacrol's hydrophobicity allows the compound to be accumulated the cell membrane. Once embedded the hydrogen bonding and proton release ability of carvacrol

may prompt reformation of the structure of the membrane, which can induce cell death (Arfa et al., 2006). Additionally, when carvacrol crosses through the bacterial cell membrane it interacts with the periplasmic enzymes. Then when introduced into the lipid-rich interior of the cytoplasmic membrane it can interact with the membranal proteins, affecting the cellular activities involved in proton motive force (Juven et al., 1994). Carvacrol, also, may act continuously, and diffuse back and forth through the cell membrane, while exchanging an acidic proton for another cation from the cytosolic side of the membrane, with a cation exchange on the exterior side (Veldhuizen et al., 2006). Juven et al. (1994) believed that the inhibition of growth of *Salmonella typhimurium* and *Staphylococcus aureus* by thyme essential oil can be associated by the hydrophobic and hydrogen bonding of the phenolic compounds to the membranal proteins, changing the membrane permeability characteristics. Similarly, Oussalah et al. (2006) found carvacrol and thymol to inhibit *E. coli* O157:H7 when introduced at 1 to 3 mM concentrations, which they believe to be caused from the disintegration of outer membrane, and release of the membrane associated materials out from the cell.

b. Action of Hydroxyl Functional Group

Dorman and Deans (2000) found that the interaction of the hydroxyl group in the phenolic structure could affect the antimicrobial activity of the compound. They did so by comparing the phenolic compounds, carvacrol to its methyl ether. Their findings further provided evidence that the position of the hydroxyl group exerted influence on the compound's effectiveness, as well as the difference in activity on Gram-negative and Gram-positive bacteria (Dorman and Deans, 2000).

Afra et al. (2006) hypothesized that the hydroxyl group on phenolic compounds, when in the presence of a system of delocalized electrons, reduced the gradient across the cytoplasmic membrane, resulting in a collapse of the proton motive force and depletion of the ATP pool. Additionally, the hydroxyl group can change the membrane physical and chemical properties that

affect the lipid ordering and stability of the membrane bilayer, causing a flux in proton passage across membranes (Afra et al., 2006).

Gill and Holley (2006a) noted the ability of the cyclic hydrocarbon's interactions with the cellular membrane might be limited to the solubility into the cellular membrane. As seen from phenolic compounds carvacrol and eugenol, the presence of the hydroxyl group may help increase the solubility of the essential oil constituent into the hydrophilic outer cell membrane (Sikkemma et al., 1995).

c. Activity of Aldehyde Compounds

Moyleyar and Narasimham (1986) proposed that an aldehyde group conjugated to a carbon-to-carbon double bond, similar to those found in benzene aromatic rings is highly electronegative. This interaction suggests that the activity to aldehyde containing compounds interacts with biological processes involving electron transfer, which in part, reacts with nitrogen containing compounds such as surface proteins and nucleic acids, providing an intercepting point of cell growth inhibition (Dorman and Deans, 2000). A study by Gill and Holley (2006) found that with the treatment of cinnamaldehyde, an aromatic aldehyde, against *E. coli* and *Listeria monocytogenes* resulted in a significant reduction in cellular ATP; thus providing support to the hypothesis that this compound's interaction with the cell interrupts protein cell function such as ATP synthesis.

d. Bactericidal and Bacteriostatic Effects on Bacterial Cells

Essential oils and their constituents vary in their effectiveness against a large array of organisms, in particular, bacteria (Bakkali et al., 2008). Bacteria are divided into two categories, Gram-negative and Gram-positive, based on their outer cell membrane. All Gram-negative bacteria contain two lipid bilayers, and possess a hydrophobic lipopolysaccharide (LPS) layer on the outer cell membrane, which can be resistant to many hydrophobic drugs (Nikaido, 1996).

Gram-positive bacteria contain a thick layer of peptidoglycan on the outer layer, with a single phospholipid inner bilayer. Due to the structural difference between both Gram-negative and –positive bacteria, essential oil treatments can vary in efficacy, with more restraint from Gram-negative bacteria (Ait-Ouazaou et al., 2011; Russell, 1991).

The addition of the LPS layer on Gram-negative bacteria can prevent accumulation of oils on the outer cell membrane, making them impermeable to entering the cellular envelope (Bezic et al., 2003). However, the hydrophobic constituents found in some essential oils are able to penetrate the cell wall through the porin proteins on the outer membrane (Helendar et al., 1998). Once they have entered into the cellular membrane, lipophilic essential oils are able to disrupt structures of the cellular membrane, including, polysaccharides, fatty acids, and phospholipids, proceeding to permeablize them (Bakkali et al., 2008). This occurrence of sub-lethal injuries may be enough to drive the cells to subsequent inactivation (Ait-Ouazzou et al., 2011). In a study by Gill and Holley (2006a), the interactions between cyclic hydrocarbons and *E. coli* liposomes resulted in a swelling of the liposomes, increased membrane fluidity, as well as the release of phospholipids and an efflux of protons. This effect can also be observed in Gill and Holley's (2006b) study, which found essential oil derived compounds eugenol and carvacrol to affect the motility of *E. coli* and *Listeria monocytogenes*. These planktonic cells require the use of flagella to mobilize, which is structurally integrated into the membrane, and are provided energy through the membrane proton gradient instead of an ATP intermediate (Silverman, 1980). The disruption of the proton gradient through the interaction of carvacrol and eugenol in the cell membrane can cause the impairment of the flagella, thereby inhibiting the cell to mobilize, which can have adverse effects on metabolic stability (Gill and Holley, 2006b).

Essential oil compounds were shown to affect the metabolic activity of Gram-negative bacteria *E. coli O157:H7* and *S. enterica* (Chorianopoulos et al., 2004; Oussalah et al., 2007). As previously discussed, the permeabilization of the membrane causes the loss of ions and membrane potential, which in part induces the collapse of the proton pump and depletion of the

ATP pool (Di Pasqua et al., 2006). The inhibition of ATPase including ATP dependent transport proteins involved in ATP generation and pH regulation would disrupt cell metabolic activity, thereby impairing cell survival (Gill and Holley, 2005; Gill and Holley, 2006; Shabala et al., 2002). Oussalah et al. (2006) suggests that the phenomenon associated with the release of ATP from the cells may be the cause of envelope damage from the antimicrobial treatment. This action is thought to be the stimulus caused from the lipophilic compounds in essential oils on the proton and/or ion trans-locating ATPase, limiting ATP hydrolysis in intact cells (Oussalah et al., 2006). Although, effective in cellular metabolic disruption, Gill and Holley (2006b) found that the concentrations of essential oils needed to inhibit ATP synthesis is within the same range needed to disrupt the cellular membrane, suggesting that this antimicrobial activity is a secondary, rather than primary, cause of cell death.

e. Synergistic and Antagonist Effects of Essential Oils and Their Constituents

Gutierrez et al. (2008) proposes that the minor components are critical to the antibacterial activity of essential oils by providing a synergistic influence with the primary constituents of the essential oil composition. This synergism may be more effective between different species of organisms based on their outer cell membrane (Gutierrez et al., 2008). The most effective combined preservation treatments are believed to include those that provide a hurdle effect. In multi-hurdle concepts, the combination of antimicrobials to achieve synergistic or additive antimicrobial efficacy are reasonable enough to counteract organoleptic or textural effects on food products, as well as continuing to reduce microbial activity (Ait-Ouazzou et al., 2011; Gutierrez et al., 2008).

Alternatively, the combination of some essential oils may act antagonistically in antimicrobial activity, and may be a component in the increase of antimicrobial activity. If the essential oil treatment is added consistently at a sub-lethal concentration the cells using a similar adaptation mechanism involving membrane structure and functions may possess the ability to

become resistant to essential oil treatments (Di Pasqua et al., 2006). In a study by Di Pasqua et al. (2006), the consistent addition of carvacrol at a sub-lethal level did not kill the desired bacteria (*Salmonella enterica* serovar Typhimurium, *E. coli* O157:H7), but resulted in a stress response, which caused unsaturation of the cellular membrane, changing membrane fluidity, thereby inhibiting the antimicrobial potential of the essential oil.

3. Organoleptic Interaction with Food

Essential oil interaction with food is somewhat similar to the interactions with the bacterial cells. Food contains slightly different cell structures depending on the type of food, so interactions can vary between items. When essential oils are introduced to the food matrix their efficacy as antimicrobials is reduced due to the high aqueous properties of food items, compared to the hydrophobic properties associated with essential oils (Burt et al., 2005; Gutierrez et al., 2008). Some studies involving essential oil efficacy determine the effects of essential oils on bacteria coinciding in microbial media, which may have different organoleptic properties then that of most food items, which may limit the true potential of the antimicrobial essential oils (Skandamis and Nychas, 2000).

A major concern of essential oil use on food items is the effect they have on the sensory properties of the foods. Essential oils are very aromatic, and contain various compounds that provide distinctive aromas varying from spicy to mild (cinnamon and oregano, respectively) (Friedman et al., 2002). Although, essential oils are the essence of spices used in adding flavor and appealing sensory properties to foods, they can also provide negative sensory properties to foods not appropriate to the particular aroma, e.g. fresh produce, dairy products, and meat and poultry. If the use of certain essential oils were applied to certain food products to negate negative sensory aspects, the quality of the food would be less affected (Gutierrez et al., 2008).

Because of the varied organoleptic and textural components in foods, i.e. fat, protein, carbohydrates, pH, etc. the use of essential oils on food products generally requires a higher

concentration in order to obtain effective results (Guitierrez et al., 2008). The sensory impact of essential oils on lettuce in a study by Gutierrez et al. (2008) exhibited an acidic flavor and strong aroma. However, in the same study the negative sensory effects were less adverse when essential oils were applied to carrots instead of lettuce leaves.

METHODOLOGY

A. Bacterial Culture Preparation.

A cocktail of three *Escherichia coli* O157:H7 strains (ATCC 43895, 43888 and 35150) were prepared for the inoculation. Strains originated from human feces associated with hemorrhagic colitis (ATCC 35150), a non-Shiga-toxin producing strain (ATCC 43888), and raw hamburger meat associated from a hemorrhagic colitis outbreak (ATCC 43895). Two of these strains (ATCC 35150 and 43895) were Shiga-toxin I and II producers. Each strain was maintained as a frozen stock culture at -80 ^{o}C. Preceding an experiment, each test strain was revived by taking a swab from the frozen culture, transferring it to tryptic soy broth (TSB; Bacto^{TM}, BD, Sparks, MD), and incubating at 37 ^{o}C for 18-24 h. The restored culture was then sub-cultured into a fresh 10 ml TSB and grown for 18-24 h at 37 ^{o}C. From this subculture, an overnight culture was prepared by adding 100 µl to 9 ml of TSB and incubating for 18-20 h at 37 ^{o}C to obtain a population of approximately 10^{8} $\log_{10}$ CFU ml^{-1}. This overnight culture of each *E. coli* O157:H7 strain was then used to prepare a cocktail by mixing equal parts of each strain. A dip inoculation was prepared from the cocktail using appropriate dilutions to obtain approximately 10^{6} $\log_{10}$ CFU ml^{-1} of bacterial population.

B. Preparation of Organic Leafy Greens

Organic baby and mature spinach, and romaine and iceberg lettuce were used as our organic leafy green samples in this study. Each leafy green was purchased from a local market in

the northern Oklahoma region. Romaine and iceberg lettuce were purchased as whole heads. Individual outer layer leaves were separated into individual leaves, disposing of the core portion. Baby and adult spinach leaves were acquired as already portioned individual leaves. The leaves were washed in sterilized distilled water to remove soil and other organic matter. Washed leaves were then exposed to ultraviolet (UV) radiation (254 nm) for 30 minutes; 15 minutes on each side of the leafy green, to remove any potential remaining natural background micro flora accumulated on the leafy green surface. A portion (5 g) of the un-inoculated leafy green sample was tested prior to treatment exposure to validate removal of background micro flora and that it was not interfering with the test results. Following the preparation of the dip inoculum, leafy greens were dip inoculated for 2 minutes, then allowed to dry in a biosafety hood for 30 minutes allowing *E. coli* O157:H7 to adhere to leafy green surface. Leafy green samples were set aside before the inoculation, and after time allowed for adherence for negative and positive controls, respectively.

C. Preparation of Antimicrobial Treatments

The antimicrobial treatments selected for this study were plant-derived essential oils: oregano, cinnamon, and lemongrass essential oils, and their primary constituents: carvacrol, cinnamaldehyde, and citral, respectively. In addition to the aforementioned treatments, sterile distilled water, and 3% hydrogen peroxide were used as controls. Both hydrogen peroxide and water are common washing solutions used in the organic produce industry (USDA, 2013), and were used to compare efficacy of compound treatments and industry standard washes.

The wash treatments of oregano, cinnamon, and lemongrass essential oils, and compounds, carvacrol, cinnamaldehyde, and citral were prepared in PBS (Phosphate Buffered Saline; sodium chloride, Fisher Scientific, NJ, USA; potassium chloride, sodium phosphate monobasic, and sodium phosphate dibasic, Sigma-Aldrich, MO, USA), at 0.1, 0.3, and 0.5%

concentrations. Treatment solutions were gently mixed and used immediately for leafy green washes.

Phosphate buffered saline (PBS) was also tested as a control due to its use in the wash solutions to help disperse essential oil and compound treatments. Enumerated results from PBS were compared against water's, the traditional medium for washing leafy greens in the industry, to determine if any differences in reduction of *E. coli* O157:H7 occurred between the two mediums. We found no significant difference ($P<0.05$) in *E. coli* O157:H7 reduction between water and PBS on all leafy greens tested in the study (Tables 1-8).

D. Microbial Analysis

The leafy greens were separated into 5 g samples and placed into a 24 oz Whirl-Pak™ bag (Nasco, Fort Atkison, WI, USA). Each sample was washed in the appropriate antimicrobial treatment solution (50 ml each) for 1 or 2 minutes, with gentle agitation. The liquid wash was then poured out, leaving only the treated leafy greens. Each Whirl-Pak™ bag was sealed lightly, and stored at either 4 or 8 ^{o}C for essential oil treated leaves, and only 4 ^{o}C for compound treated leaves. The survival of *E. coli* O157:H7 on leafy greens was determined by sampling on the initial day (day 0), the following day (day 1), and three days following the initial day (day 3). A 1 g sample of the treated leafy green was placed into a Whirl-Pak™ bag containing 9 ml of buffered peptone water (BPW, Oxord ltd., Basingstoke, Hampshire, England) and stomached (Stomacher 400 Circulator, Seward, Davie, Florida, USA) for 1 minute at 230 rpm. Appropriate dilutions were then plated on to Sorbitol MacConkey (SMAC, Remel, Thermo Fisher Scientific, Lenexa, Kansas, USA) agar. The SMAC plates were incubated at 37 ^{o}C for 18-24 hours. Surviving populations of *E. coli* O157:H7 were determined the following day. A level of detection was not recorded below 10^1 $\log_{10}$ CFU due to the method of acquiring microbiological samples of leaves.

In order to determine if the recovery of injured *E. coli* O157:H7 cells was affected by the selective growth medium (SMAC), treated samples were also plated on the non-selective

medium, Tryptic Soy Agar (TSA, Neogen, Lansing, Michigan, USA). No differences were observed (data not reported) between the *E. coli* O157:H7 cells recovered on both mediums.

E. Statistical Analysis

Surviving bacterial populations, obtained at CFU g^{-1}, were converted to log_{10} CFU g^{-1} and analyzed in SAS v9.3 (SAS, Cary, NC, USA) using Least Square Means (LSMEANS) to separate means. The PROC GLM procedure was used to compare means, with a significance level of $P<0.05$. Treatment interactions were analyzed using a factorial treatment design, which included the following treatments: Plant-derived essential oils (oregano, cinnamon, lemongrass) and compounds (carvacrol, cinnamaldehyde, citral), concentration of essential oils (0.1, 0.3, 0.5% vol/vol), storage-time (day 0, 1, 3), wash-time (1, 2 minutes), and storage temperature (4, 8°C), and leafy greens (baby spinach, adult spinach, romaine lettuce, iceberg lettuce). Each experiment was replicated three times.

RESULTS

In this study, multiple treatment factors were presented in a combined design where each treatment could be influenced by causative effects from other treatments. In order to determine treatment effects for both individual and multiple interactions we used a factorial experimental design to analyze the response of microbial growth between and among treatment factors. Due to the sparcity-of-effects principle, we did not further analyze high order interactions, based on the premise that the system is dominated by a main or low-order interaction. In this section, results will be divided into four parts: *Baby Spinach, Adult Spinach, Romaine Lettuce*, and *Iceberg Lettuce*.

A. Baby Spinach

Baby spinach leaves were dip inoculated with *Escherichia coli* O157:H7 at 6.0-log CFU ml^{-1}. Recovery of *E. coli* O157:H7, after time given for attachment, was recorded at ~5.5 log CFU g^{-1} (Tables 1.1-2 and 5.1-2).

1. *Essential Oil and Compound Treatments at Varied Concentrations*

Essential Oils. All essential oil and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on baby spinach following their wash (Tables 1.1-2). Essential oil treatments collectively showed a greater or equal reduction of *E. coli* O157:H7 than control treatments. Hydrogen peroxide (2.4-log), however, showed no difference in reduction when compared to 0.1% cinnamon (2.5-log) and lemongrass (2.4-log) essential oil

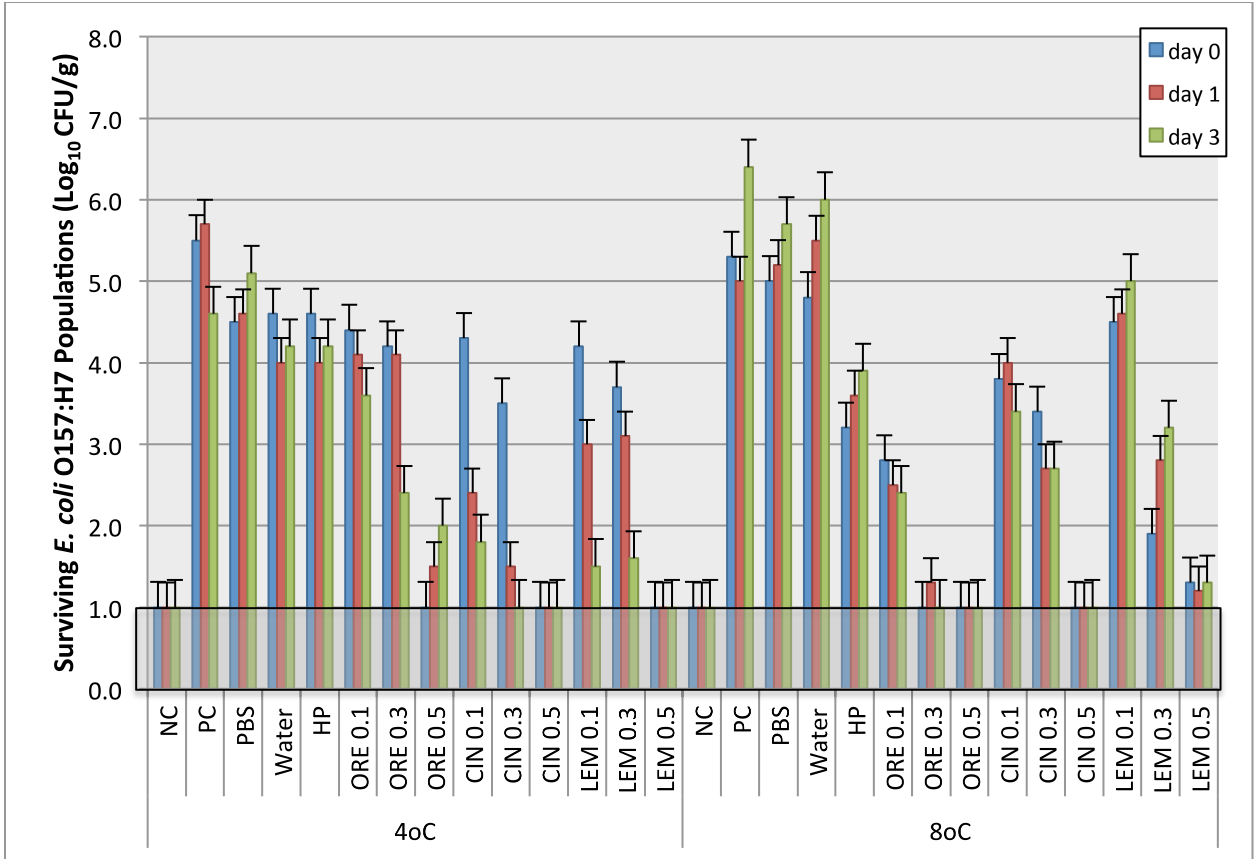
treatments, but was less effective than 0.1% oregano essential oil treatments (2.9-log_{10} CFU g^{-1}). Oregano essential oil was the overall most effective essential oil treatment, showing a greater reduction ($P<0.05$) than other essential oil treatments at both 0.1% (2.9-log) and 0.3% (3.9-log) concentration. Cinnamon and lemongrass essential oil treatments showed no difference in reduction of *E. coli* O157:H7 on baby spinach (Tables 1.1-2). All essential oil treatments at a 0.5% concentration reduced *E. coli* O157:H7 populations to undetectable levels (Figure 1-2). Each increasing concentration of essential oil showed a trend of increased reduction of *E. coli* O157:H7 populations (Figure 1-2).

Compounds. All compound and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on baby spinach following their wash (Tables 5.1-2). Compound treatments collectively showed a greater or equal reduction of *E. coli* O157:H7 than control treatments. However, 0.1% citral treatment (2.3-log) exhibited no significant difference ($P<0.05$) in reduction when compared to hydrogen peroxide (2.6-log). Both carvacrol and cinnamaldehyde treatments showed similar efficacy in reduction of *E. coli* O157:H7 on baby spinach (Table 5.1-2), with the lowest concentration reducing *E. coli* O157:H7 populations by ~5.3-log following the wash treatment. Both 0.3% and 0.5% carvacrol and cinnamaldehyde treatments reduced *E. coli* O157:H7 populations to undetectable levels (Figure 9). Citral was the less effective of the tested compounds (Table 5.1-2). However, at its lowest concentration (0.1%), citral treatments reduced *E. coli* O157:H7 by 2.3-log. Similar to essential oils, each increasing concentration of compound treatments showed a trend of increased reduction of *E. coli* O157:H7 populations (Figure 9).

2. *Treatment Exposure Time*

Essential Oils. Two-minute essential oil treatment exposures showed no significant difference ($P<0.05$) in reduction of *E. coli* O157:H7 populations on baby spinach, when compared to a 1-minute exposure (Tables 1.1-2). However, one treatment, 0.1% oregano essential

Figure 1. *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C



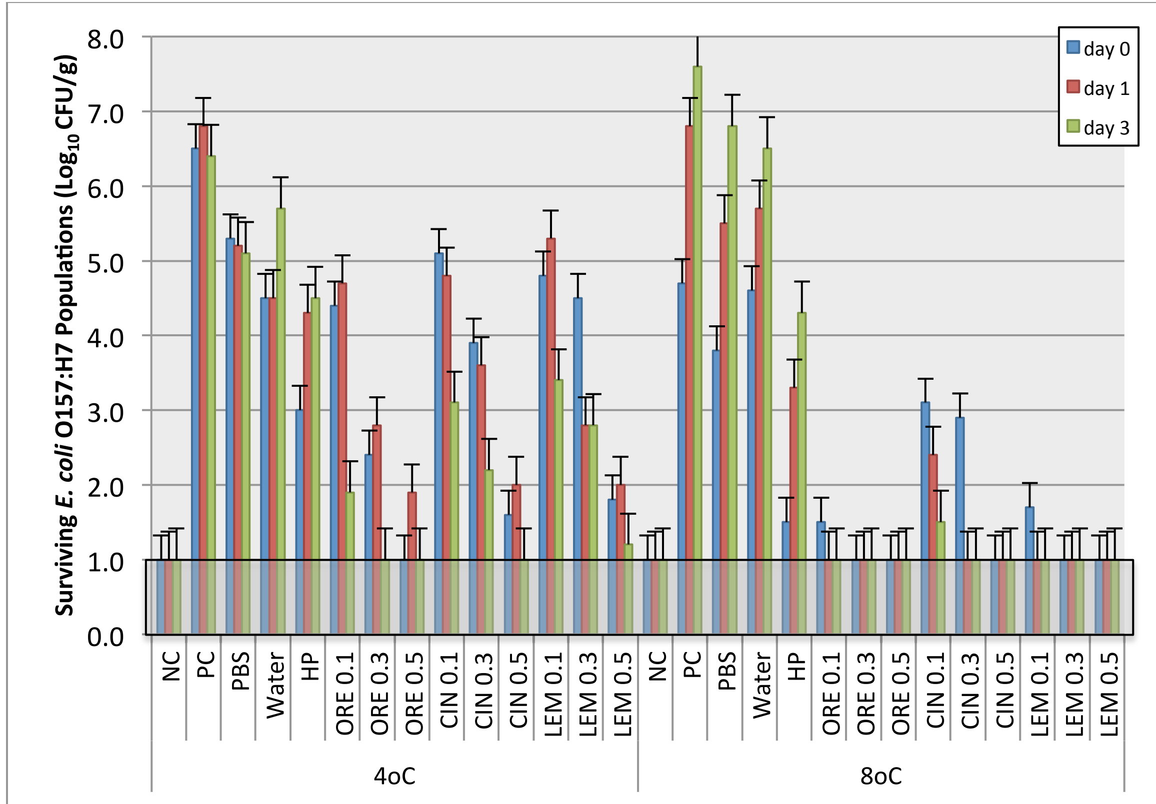
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Figure 2. *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 2-minute Essential Oil Treatment Held at 4 and 8 °C



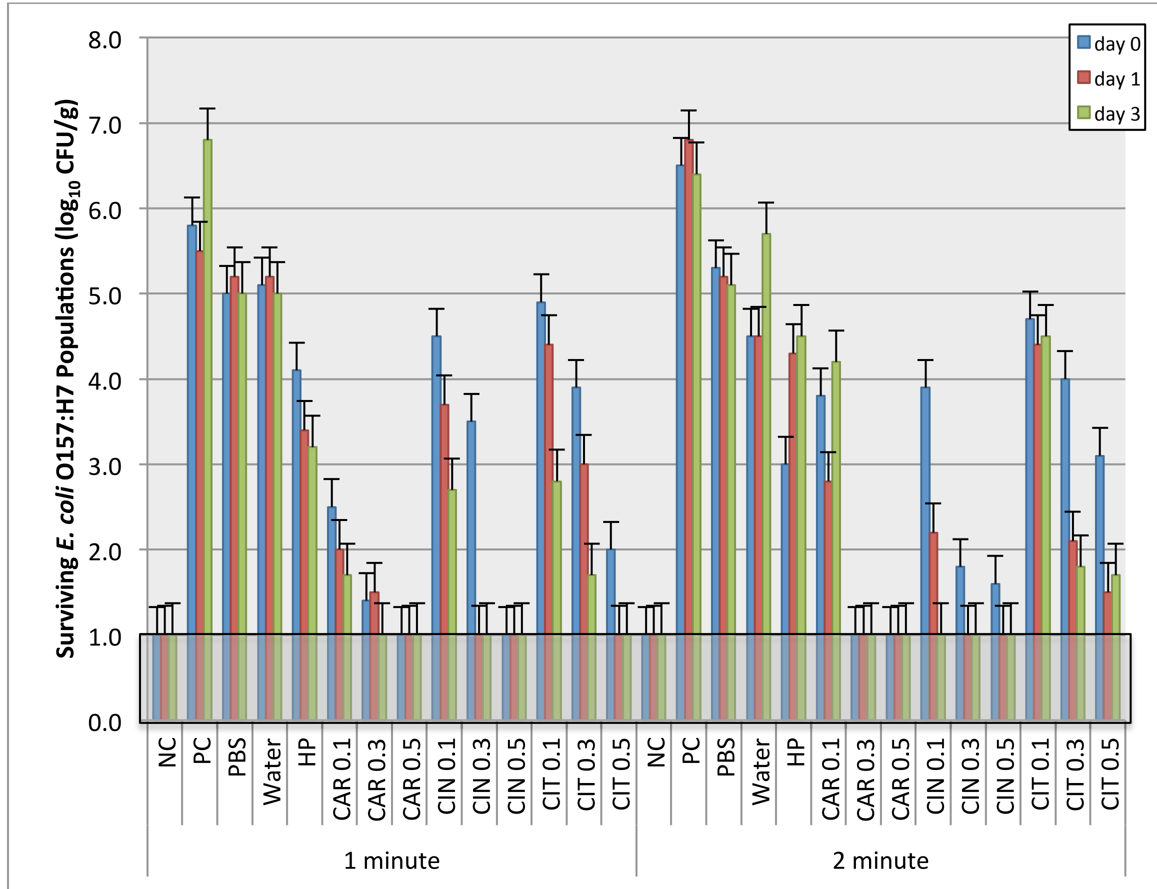
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Figure 9. *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 1 and 2-minute Plant-Derived Compound Treatment Held at 4 °C



[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[b]Values represent average mean of three repetitions.
[c]Bars represent standard error.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

oil, showed greater reduction at 2-minute exposure (3.9-log), than 1-minute (2.7-log). Hydrogen peroxide and water treatments showed no difference ($P<0.05$) in reduction between 1 and 2-minute exposure (Table 1.1-2).

Compounds. Carvacrol and cinnamaldehyde treatments at 0.1% concentration had greater reduction of *E. coli* O157:H7 with a 1-minute exposure (~4.0 and 2.4-log, respectively). Conversely, 0.1% and 0.5% citral treatments showed greater reduction at a 2-minute exposure (2.1 and 4.5-log, respectively). Higher concentrations of compounds (0.3 and 0.5%), with

exception of 0.5% citral, showed no difference between 1 and 2-minture exposures (Table 5.1-2). Hydrogen peroxide and water treatments showed no difference in reduction between 1 and 2-minute exposure (Table 5.1-2).

3. *Duration in Refrigerated Storage—4 °C*

Essential Oils. Essential oil treated baby spinach exhibited a continuous reduction trend against *E. coli* O157:H7 over the duration of 3 days in 4 °C storage (Figure 1-2). Oregano, cinnamon, and lemongrass essential oil treatments at 0.1% concentration significantly reduced ($P<0.05$) surviving *E. coli* O157:H7 populations an additional 1.9, 2.2, and 2.1-log, respectively, after 3 days in 4 °C storage. Similarly, 0.3% essential oil treatments additionally reduced surviving *E. coli* O157:H7 by 3.0, 3.2, and 2.7-log for oregano, cinnamon, and lemongrass, respectively, after 3 days in 4 °C storage. Essential oil treatments at 0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to undetectable levels by day 3 in 4 °C storage (Table 1.1-2). Oregano and lemongrass essential oils at 0.1% showed no change in reduction between day 0 and day 1 (0.3 and 0.8-log, respectively), but continued reduction between day 1 and day 3 (1.8 and 1.8-log, respectively) in 4 °C storage. Conversely, 0.1 and 0.3% cinnamon essential oil continued reduction ($P<0.05$) throughout the 3 days in 4 °C storage. Hydrogen peroxide and water treatments showed no significant change ($P<0.05$) in remaining *E. coli* O157:H7 populations through the 3 days in 4 °C storage (Tables 1.1-2).

Compounds. Compound treatments generally showed continuous reduction of *E. coli* O157:H7 on baby spinach (Tables 5.1-2), with exception of 2-minute exposed 0.1% citral, which showed no change in surviving *E. coli* O157:H7 after treatment on day 0 (Table 5.2). Cinnamaldehyde treatments at 0.1% concentration significantly reduced surviving *E. coli* O157:H7 populations an additional 2.4-log after 3 days in 4 °C storage. Similarly, 0.3% compound treatments additionally reduced surviving *E. coli* O157:H7 by 1.7 and 2.2-log for cinnamaldehyde and citral, respectively, after 3 days in 4 °C storage. Compound treatments at

0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to <1.7-log CFU g^{-1} (most at undetectable levels) by day 3 in 4 °C storage (Table 5.1-2). Treatments of 0.1% and 0.3% citral showed no change in surviving *E. coli* O157:H7 populations after day 1 in 4 °C storage, but exhibited a significant reduction (P<0.05) between day 1 and day 3 in storage (Table 5.1-2). Cinnamaldehyde treatments at 0.1% and 0.3% concentration showed continuous reduction after day 1 (2.4 and 1.7-log, respectively) in 4 °C storage, with 0.1% cinnamaldehyde treatments showing a 1.1-log reduction from day 1 to day 3 as well. Hydrogen peroxide and water treatments showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations through the 3 days in 4 °C storage (Tables 5.1-2).

4. *Duration in Refrigerated Storage—8 °C (Only for Essential Oil Treatments*)

Essential Oils. During the 3 days in 8 °C storage, oregano essential oil treatments showed no change in surviving *E. coli* O157:H7 after initial treatment on day 0 (Figure 1-2). Cinnamon essential oil treatments for 0.1 and 0.3% concentrations reduced surviving *E. coli* O157:H7 populations from day 0, by 2.3 and 1.3-log, respectively, after three days in 8 °C storage. Lemongrass essential oil treatments showed no change in surviving *E. coli* O157:H7 after initial treatment on day 0 (Figure 1-2). However, 1-minute exposed 0.3% lemongrass essential oil treatment had increased *E. coli* O157:H7 populations (2.3-log) over the three days in 8 °C storage. Essential oil treatments at 0.3 and 0.5% concentrations reduced *E. coli* O157:H7 to undetectable levels by day 1 in storage, showing no significant change (P<0.05) in *E. coli* O157:H7 populations throughout the 3 days in 8 °C storage (Table 1.1-2). Hydrogen peroxide and water treatments showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations through the 3 days in 8 °C storage (Tables 1.1-2).

5. *Comparison of Refrigeration Storage Temperatures—4 and 8 °C (Only for Essential Oil Treatments)*

Essential Oils. Storage temperatures, 4 and 8 ^oC exhibited no significant difference ($P<0.05$) among all 0.5% essential oil treatments (Table 1.1-2). This is believed to be evident due to the reduction of *E. coli* O157:H7 from baby spinach to <1.3-log_{10} CFU g^{-1} (most at undetectable levels) surviving *E. coli* O157:H7 on day 0. Oregano essential oil at 0.1% concentration had a greater reduction of *E. coli* O157:H7 at 4 ^oC (1.7-log), than at 8 ^oC (0.5-log), through the 3 days in storage. Similarly, 0.3% oregano essential oil treatments had a greater *E. coli* O157:H7 reduction at 4 ^oC (1.6-log), than 8 ^oC (<0.1- log). However, 0.3% oregano essential oil treatments held in 8 ^oC storage showed reduction of *E. coli* O157:H7 to much lower levels (<0.5- log_{10} CFU g^{-1}) than 4 ^oC on day 0 (Figure 1-2); preventing further analysis of continuing treatment effects under 8 ^oC storage. Cinnamon essential oil at 0.1 and 0.5% concentration showed no difference in reduction of remaining *E. coli* O157:H7 between 3 days at 4 and 8 ^oC storage (Figure 1-2). Cinnamon essential oil at 0.3%, however, exhibited a greater reduction throughout the 3 days in storage for 4 ^oC storage (2.1-log), than 8 ^oC (1.3-log). Similar to cinnamon essential oil treatments, 0.3% lemongrass essential oil treatments showed a continuous reduction during the 3 days in 4 ^oC storage (1.9-log), but had no significant variation ($P<0.05$) in remaining *E. coli* O157:H7 while in 8 ^oC storage (Table 1.1-2). Treatments of 0.1 and 0.5% lemongrass essential oil treatments showed no significant difference ($P<0.05$) in remaining *E. coli* O157:H7 between the 3 days in 4 and 8 ^oC storage (Tables 1.1-2). Hydrogen peroxide and water treatments behaved similarly at both 4 and 8 ^oC, showing either a significant increase ($P<0.05$) or no change in remaining *E. coli* O157:H7 over the duration in storage (Table 1.1-2).

B. Mature Spinach

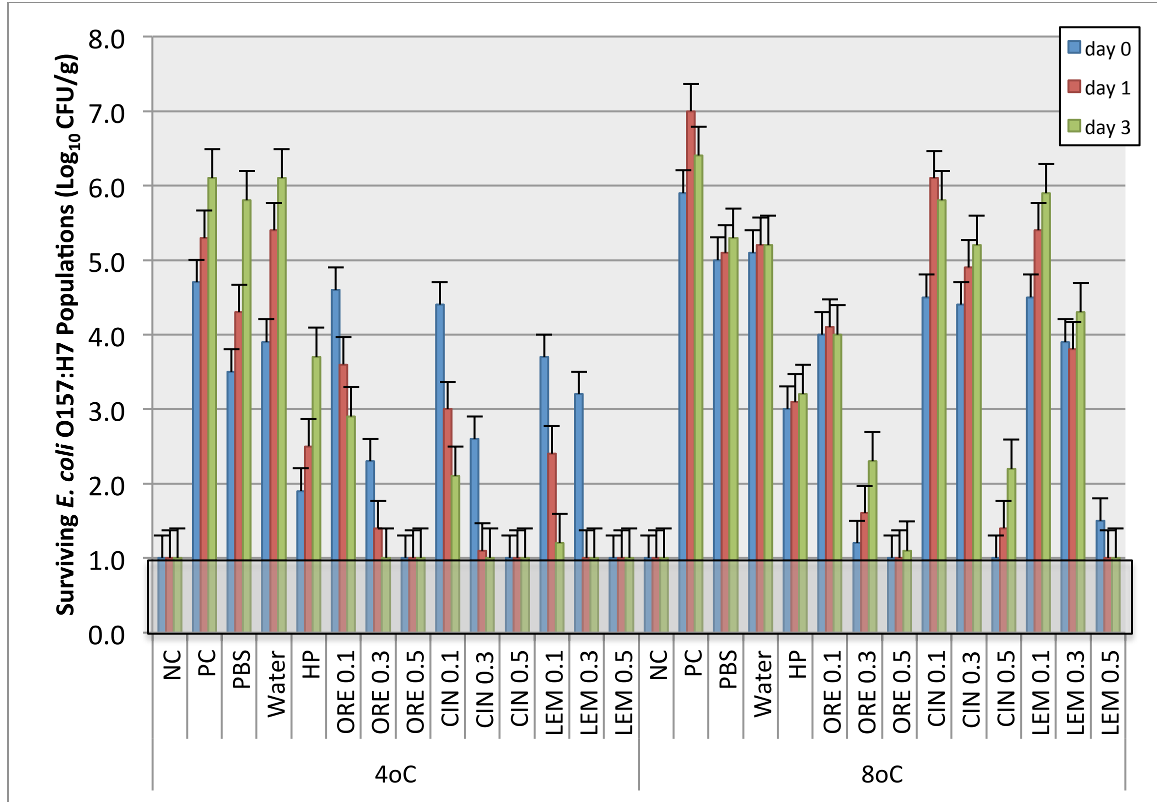
Mature spinach leaves were dip inoculated with *E. coli* O157:H7 at 6.0-log CFU ml^{-1}. Recovery of *E. coli* O157:H7, after time given for attachment, was recorded at ~5.0-log CFU g^{-1} (Tables 2.1-2 and 6.1-2).

1. *Essential Oil and Compound Treatments at Varied Concentrations*

Essential Oils. All essential oil and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on mature spinach following their wash (Tables 2.1-2). Hydrogen peroxide effectively reduced *E. coli* O157:H7 by 2.7-log, showing greater efficacy than water (~1.0- log). When compared to essential oil treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in reduced *E. coli* O157:H7 with both 0.1% oregano and lemongrass essential oil treatment (2.6 and 2.2-log, respectively). However, hydrogen peroxide effectively reduced a significantly greater ($P<0.05$) amount of *E. coli* O157:H7 than 0.1% cinnamon essential oils (1.8-log). Oregano essential oil had a more efficient reduction at 0.3% concentration (4.5- log) than both 0.3% cinnamon and lemongrass essential oils (3.3 and 3.4- log, respectively). All essential oil treatments at 0.5% concentration exhibited reduction of *E. coli* O157:H7 to non-detectable levels (Figure 3-4). Each increasing concentration of essential oil treatment showed a trend of greater reduction of *E. coli* O157:H7 populations (Figure 3-4).

Compounds. All compound and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on mature spinach following their wash (Tables 6.1-2). Hydrogen peroxide effectively reduced surviving *E. coli* O157:H7 by 2.6-log, as well as showing better efficacy than water. When compared to compound treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in reduced *E. coli* O157:H7 with both 0.1% cinnamaldehyde and citral treatments (2.7 and 2.2-log, respectively), but was not as effective as 0.1% carvacrol treatments (4.2- log). Carvacrol 0.3% concentration effectively reduced *E. coli* O157:H7 to undetectable levels (Tables 6.1-2). At 0.3% concentration, cinnamaldehyde and citral reduced *E. coli* O157:H7 by 3.9 and 3.8-log, respectively. All treatments at 0.5% concentration exhibited $<0.5\text{-}\log_{10}$ CFU g^{-1} (mostly undetectable growth) surviving *E. coli* O157:H7. Similar to essential oils, each increasing concentration of compound treatments showed a trend of greater reduction of *E. coli* O157:H7 populations on mature spinach (Figure 10).

Figure 3. *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C



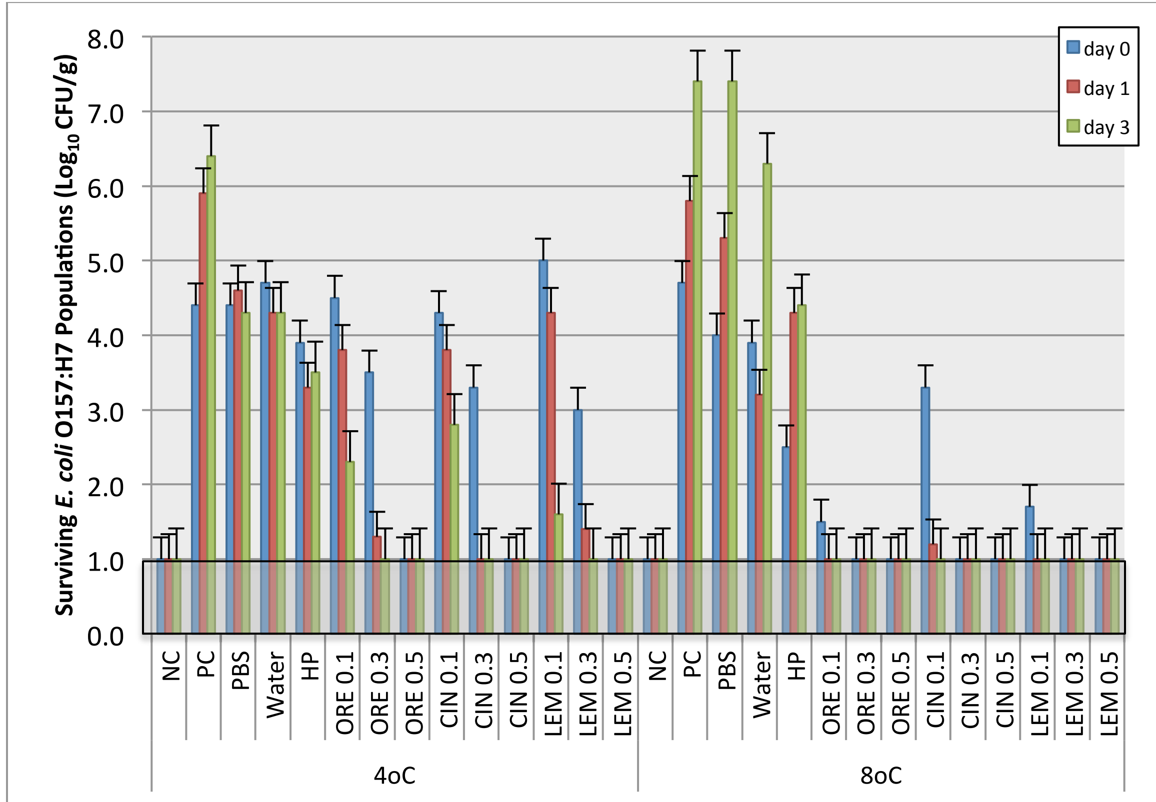
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Figure 4. *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 2-minute Essential Oil Treatment Held at 4 and 8 ^{o}C



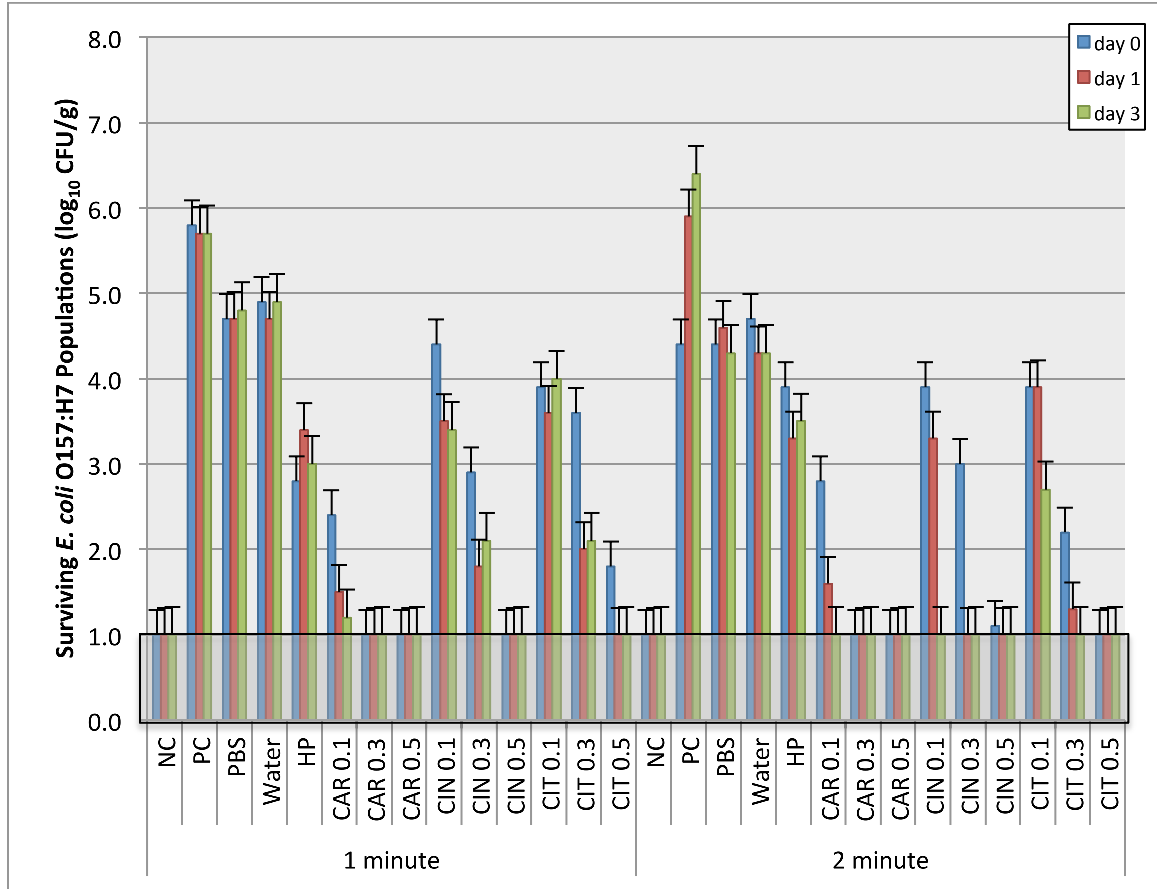
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Figure 10. *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 1 and 2-minute Plant-Derived Compound Treatment Held at 4 °C



[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[b]Values represent average mean of three repetitions.
[c]Bars represent standard error.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

2. *Treatment Exposure Time*

Essential Oils. Two-minute treatment exposures showed no significant ($P<0.05$) difference in reduction of *E. coli* O157:H7 populations on mature spinach, when compared to 1-minute treatment exposure (Table 2.1-2). Water control treatments, however, had a greater reduction of *E. coli* O157:H7 after a 2-minute treatment exposure (1.2-log), than 1-minute (0.8-log).

Compounds. There was no significant difference ($P<0.05$) between 1 and 2-minute treatment exposures on carvacrol treated mature spinach (Table 6.1-2). Both 0.1 and 0.3% cinnamaldehyde treatments had greater reduction of *E. coli* O157:H7 after 2-minute treatment exposure (2.3 and 3.8-log, respectively), compared to 1-minute (1.9 and 3.4-log, respectively). Similarly, 0.3% citral treatments exhibited greater reduction at 2-minute exposure (4.6-log), than 1-minute (3.2-log). Hydrogen peroxide and water treatments had no significant difference ($P<0.05$) in reduction of *E. coli* O157:H7 between 1 and 2-minute treatment exposures (Tables 6.1-2).

3. *Duration in Refrigerated Storage—4 ^{o}C*

Essential Oils. Essential oil treated mature spinach exhibited a continuous reduction trend against *E. coli* O157:H7 over the duration of 3 days in 4 ^{o}C storage (Figures 3-4). Oregano, cinnamon, and lemongrass essential oil treatments at 0.1% concentration significantly reduced ($P<0.05$) surviving *E. coli* O157:H7 populations by an additional 2.0, 1.4, and 3.0-log, respectively, after 3 days in 4 ^{o}C storage. Similarly, 0.3% essential oil treatments additionally reduced 1.9, 1.9, and 2.1-log of surviving *E. coli* O157:H7 for oregano, cinnamon, and lemongrass, respectively, after 3 days in 4 ^{o}C storage. Essential oil treatments at 0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to undetectable levels by day 3 in 4 ^{o}C storage (Table 2.1-2). Oregano essential oil at 0.1 and 0.3% concentration showed continued reduction between day 0 and day 1 (1.2 and 1.6-log, for 0.1 and 0.3% concentration, respectively), but had no significant difference ($P<0.05$) in remaining *E. coli* O157:H7 populations in 4 ^{o}C storage (Tables 2.1-2). Cinnamon and lemongrass essential oils at a 0.1% concentration exhibited continuing reduction of *E. coli* O157:H7 from day 0 to day 1 (1.1 and 1.5-log, respectively), as well as day 1 to day 3 (1.0 and 1.4-log, respectively). Both cinnamon and lemongrass at 0.3% concentration only displayed continuing reduction of *E. coli* O157:H7 from day 0 to day 1 (1.4 and 1.9-log, respectively). Hydrogen peroxide and water treatments

showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations during the 3 days in 4 ^{o}C storage at a 1-minute exposure (Tables 1.1). However, with a 2-minute exposure, both hydrogen peroxide and water treatments increased 1.4 and 2.9-log, respectively, over the duration of 3 days in 4 ^{o}C storage.

Compounds. Compound treatments generally showed continuous reduction of *E. coli* O157:H7 on mature spinach (Figures 10), with the exception of 1-minute exposure of 0.1% citral, which showed no change in surviving *E. coli* O157:H7 after treatment on day 0 (Table 6.1). Carvacrol and cinnamaldehyde treatments at 0.1% concentration significantly reduced (P<0.05) surviving *E. coli* O157:H7 populations an additional 1.3 and 1.5-log, respectively, after 3 days in 4 ^{o}C storage. Similarly, 0.3% compound treatments additionally reduced surviving *E. coli* O157:H7 by 1.4 and 1.4-log for cinnamaldehyde, and citral, respectively, after 3 days in 4 ^{o}C storage. Compound treatments at 0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to undetectable levels by day 3 in 4 ^{o}C storage (Table 5.1-2). Treatments of 0.1% cinnamaldehyde showed no change in surviving *E. coli* O157:H7 from day 0 to day 1, but did show a reduction of 1.3-log from day 1 to day 3 in 4 ^{o}C storage. Carvacrol at 0.3% concentration reduced *E. coli* O157:H7 populations to undetectable levels after initial treatment on day 0, and exhibited no significant change (P<0.05) over the duration in 4 ^{o}C storage. Cinnamaldehyde and citral at 0.3% concentration had a continuing reduction of surviving *E. coli* O157:H7 by 1.9 and 1.3-log, respectively, from day 0 to day 1; no significant (P<0.05) change in remaining *E. coli* O157:H7 occurred from day 1 to day 3 in 4 ^{o}C storage for 0.3% cinnamaldehyde treatments (Table 6.1-2). Hydrogen peroxide and water treatments showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations through the 3 days in 4 ^{o}C storage (Tables 6.1-2).

4. *Duration in Refrigerated Storage—8 ^{o}C (Only for Essential Oil Treatments*)

Essential Oils. During the duration of 3 days in 8 ^{o}C storage, oregano essential oil treatments showed no change in surviving *E. coli* O157:H7 after initial treatment on day 0

(Tables 2.1-2). Cinnamon essential oil treatments at 0.1% concentration reduced surviving *E. coli* O157:H7 by 1.9-log after three days in 8 ^{o}C storage. Concentrations of 0.3 and 0.5% cinnamaldehyde showed no change in remaining *E. coli* O157:H7 over the course of 3 days in 8 ^{o}C (Tables 2.1-2). Similarly, lemongrass essential oil at each concentration showed no change in remaining *E. coli* O157:H7 through the duration of 3 days in 8 ^{o}C (Tables 2.1-2). Control treatment, hydrogen peroxide, exhibited no change in remaining *E. coli* O157:H7 through the duration of 3 days in 8 ^{o}C storage (Tables 2.1-2). However, water treatments showed significant growth ($P<0.05$) of *E. coli* O157:H7 from day 0 to day 1 (1.1-log), but remained unchanged from day 1 to day 3 (Tables 2.1-2).

5. *Comparison of Refrigeration Storage Temperatures—4 and 8 ^{o}C (Only for Essential Oil Treatments)*

Essential Oils. Storage temperatures, 4 and 8 ^{o}C, exhibited no difference ($P<0.05$) among all 0.5% essential oil treatments (Table 2.1-2). This is believed to be evident due to the reduction of *E. coli* O157:H7 from mature spinach to undetectable levels of *E. coli* O157:H7 on day 0 (Figure 3-4). Oregano essential oil at 0.1% concentration showed no difference in reduction between storage temperatures from day 0 to day 1, but had a continuing reduction of *E. coli* O157:H7 at 4 ^{o}C (1.1-log), with no change in remaining *E. coli* O157:H7 at 8 ^{o}C (Tables 2.1-2). No significant difference ($P<0.05$) was found in remaining *E. coli* O157:H7 for both 0.3 and 0.5% oregano essential oil treatments at 4 and 8 ^{o}C (Tables 2.1-2). In comparison with 8 ^{o}C storage (<0.1 and 1.5-log, respectively), cinnamon essential oil at 0.1 and 0.3% concentrations showed a greater reduction of *E. coli* O157:H7 under the duration in 4 ^{o}C storage (1.9 and 1.9-log, respectively). Similarly, lemongrass essential oil at 0.1 and 0.3% concentration showed a greater reduction of *E. coli* O157:H7 through the 3 days in 4 ^{o}C (3.0 and 2.1-log, respectively), than 8 ^{o}C storage (Table 2.1-2). Hydrogen peroxide and water treatments behaved similarly at

both 4 and 8 ^{o}C, showing no change in remaining *E. coli* O157:H7 over the duration in storage (Table 2.1-2).

C. Romaine Lettuce

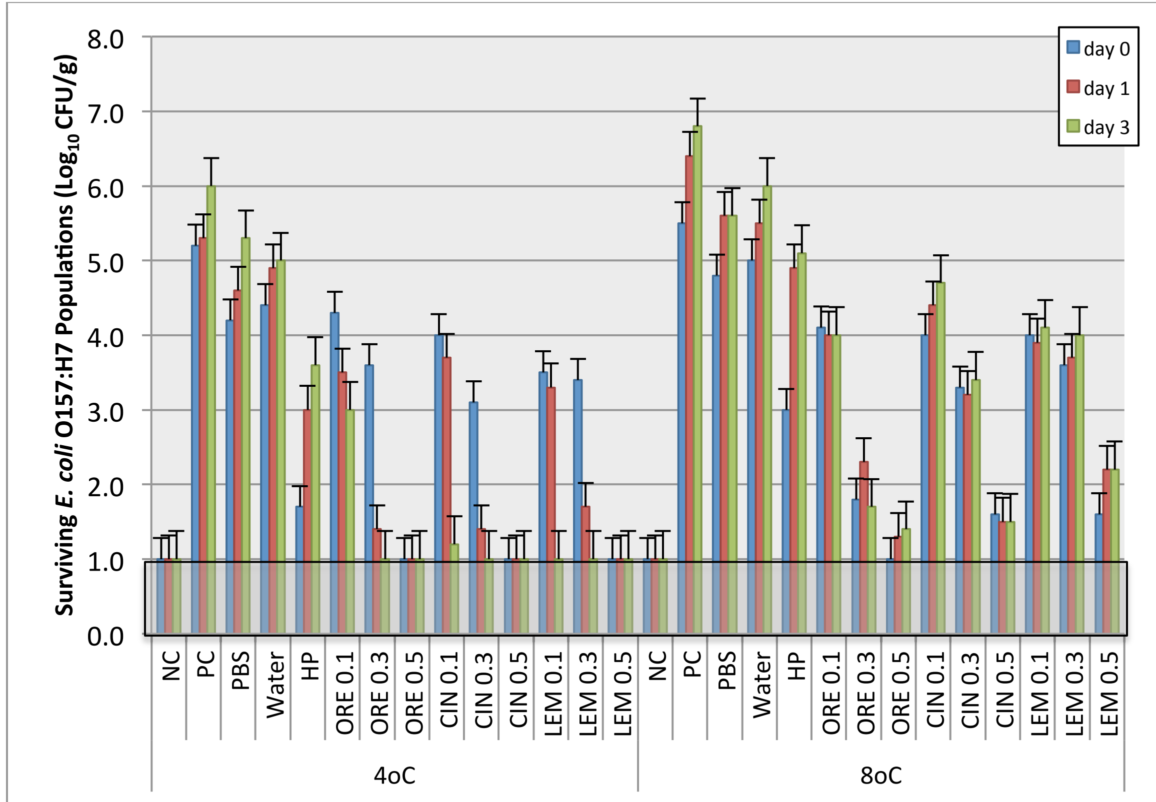
Romaine lettuce leaves were dip inoculated with *E. coli* O157:H7 at 6.0-log CFU ml^{-1}. Recovery of *E. coli* O157:H7, after time given for attachment, was recorded at ~5.5-log CFU g^{-1} (Tables 3.1-2 and 7.1-2).

1. *Essential Oil and Compound Treatments at Varied Concentrations*

Essential Oils. All essential oil and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on romaine lettuce following their wash (Tables 3.1-2). Hydrogen peroxide effectively reduced *E. coli* O157:H7 by ~3.0-log, as well as showing greater reduction efficacy than water (1.1-log). When compared to essential oil treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in reduced *E. coli* O157:H7 for 0.1% oregano, cinnamon, and lemongrass essential oil treatments (3.0, 2.7, and 2.9-log, respectively). Oregano essential oil had significantly reduced ($P<0.05$) more *E. coli* O157:H7 at 0.3% concentration (4.7-log) than both 0.3% cinnamon and lemongrass essential oils (3.9 and 3.9-log, respectively). All essential oil treatments at 0.5% concentration exhibited reduction of *E. coli* O157:H7 to <1.6 log CFU g^{-1} (with most treatments showing undetectable growth). Each increasing concentration of essential oil treatments showed a trend of increased reduction of *E. coli* O157:H7 populations (Figure 5-6).

Compounds. All compound and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on romaine lettuce following their wash (Tables 7.1-2). Hydrogen peroxide effectively reduced *E. coli* O157:H7 by 3.1-log, as well as showing greater reduction than water (1.6-log). When compared to compound treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in *E. coli* O157:H7 reduction when compared to 0.1% cinnamaldehyde

Figure 5. *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C



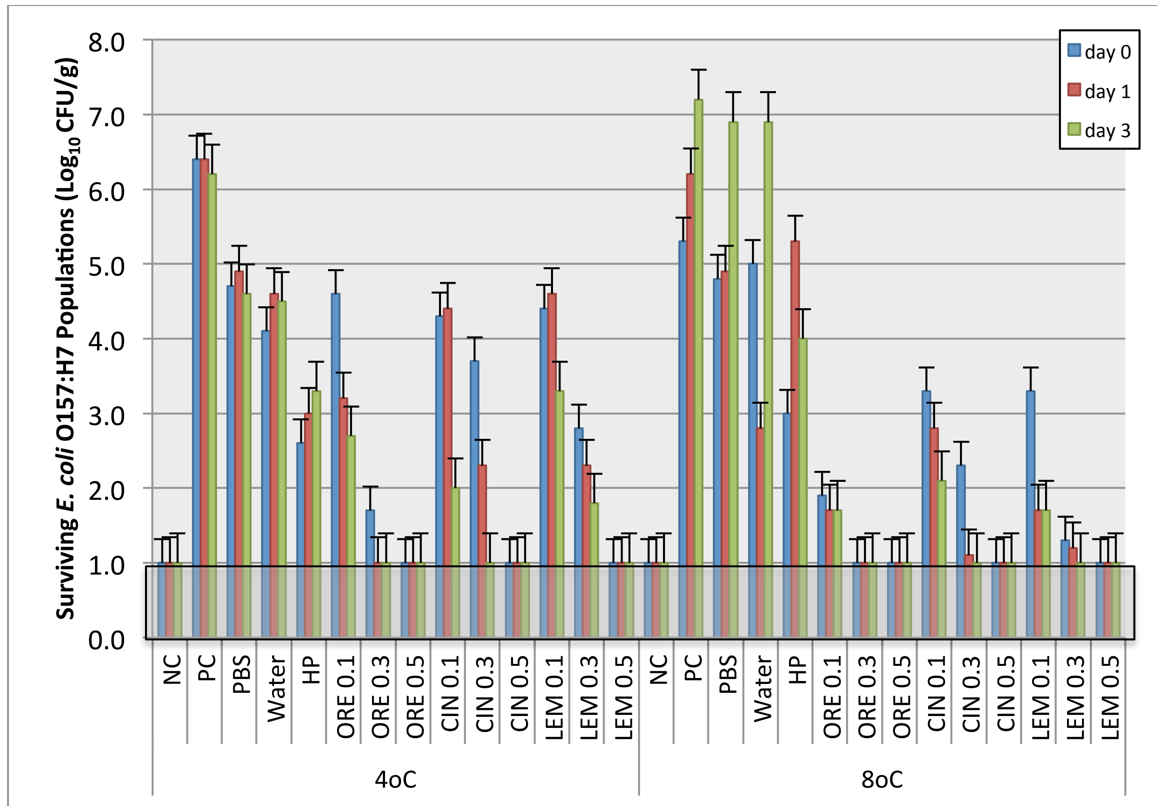
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

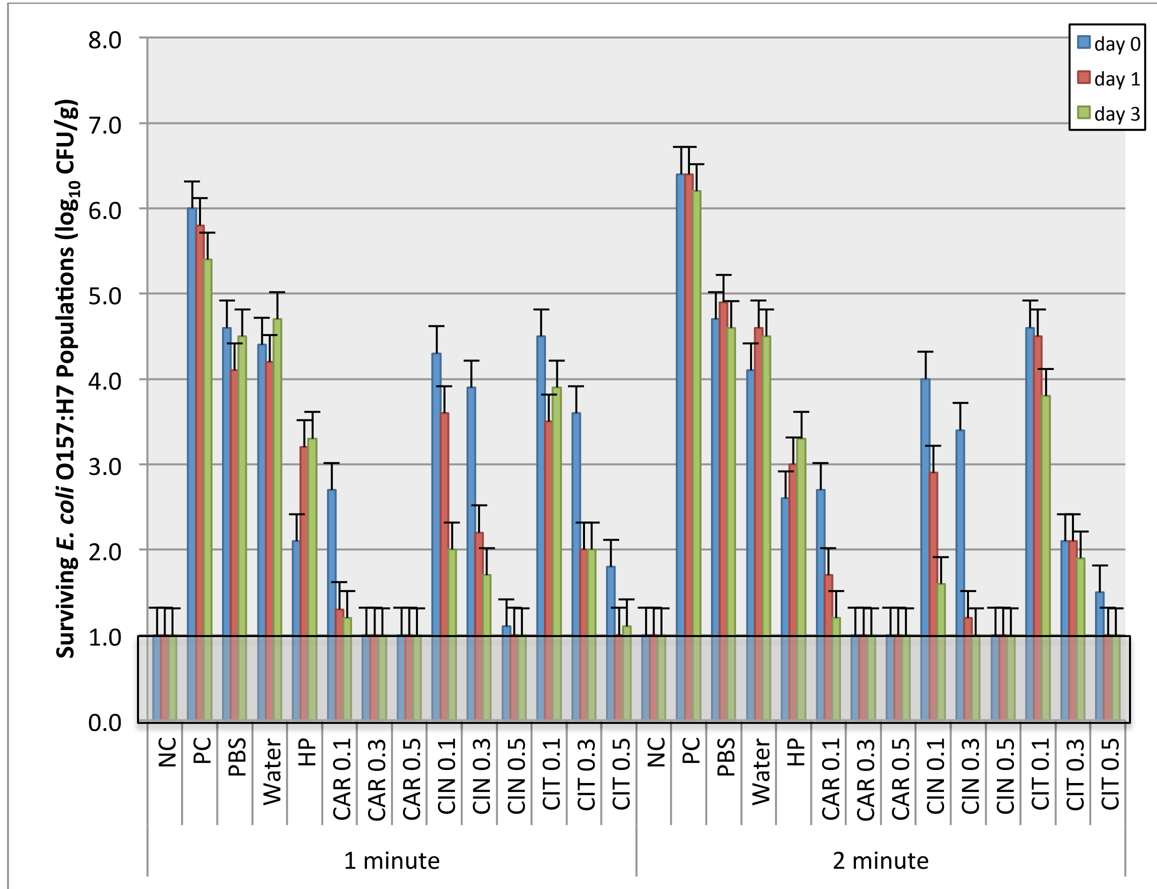
Figure 6. *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 2-minute Essential Oil Treatment Held at 4 and 8 °C



[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.
[b]Values represent average mean of three replications.
[c]Bars represent standard error.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

treatment (3.0-log), but was not as effective as 0.1% carvacrol treatments (4.2-log). However, hydrogen peroxide did significantly reduce ($P<0.05$) more *E. coli* O157:H7 than 0.1% citral treatments (1.9-log). Carvacrol was shown to be the most effective compound treatment, with both 0.1 and 0.3% concentrations significantly reducing more *E. coli* O157:H7 than 0.1 and 0.3% cinnamaldehyde (3.0 and 3.8-log, respectively) and citral (1.9 and 3.7-log, respectively) treatments. All treatments at 0.5% concentration exhibited less than 1.8-log_{10} CFU g^{-1} (with most treatments showing no detectable growth) surviving *E. coli* O157:H7 populations.

Figure 11. *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 1 and 2-minute Plant-Derived Compound Treatment Held at 4 °C



[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[b]Values represent average mean of three repetitions.
[c]Bars represent standard error.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Similar to essential oils, each increasing concentration of compound treatments showed a trend of increased reduction against *E. coli* O157:H7 populations (Figure 11).

2. *Treatment Exposure Time*

Essential Oils. Two-minute treatment exposures showed no significant difference (P<0.05) in reduction of *E. coli* O157:H7 populations on romaine lettuce, when compared to 1-minute treatment exposure for essential oil and control treatments (Table 3.1-2). Lemongrass

essential oil at 0.3% concentration, however, did exhibit a greater reduction of *E. coli* O157:H7 from 2-minute treatment exposure (4.6-log), than a 1-minute exposure (2.9-log).

Compounds. Two-minute treatment exposures showed no significant difference (P<0.05) in reduction of *E. coli* O157:H7 populations on romaine lettuce, when compared to 1-minute treatment exposure for compound and control treatments (Table 7.1-2). Citral at 0.3% concentration, however, exhibited a greater reduction of *E. coli* O157:H7 from a 2-minute treatment exposure (4.3-log), than a 1-minute exposure (2.4-log).

3. *Duration in Refrigerated Storage—4 ^{o}C*

Essential Oils. Essential oil treated romaine lettuce exhibited a continuous reduction trend or showed no change against *E. coli* O157:H7 over the duration of 3 days in 4 ^{o}C storage (Figure 5-6). Oregano and Cinnamon essential oils at 0.1% concentration showed no change in remaining *E. coli* O157:H7 populations (Tables 3.1-2). However, 0.1% lemongrass essential oil treatments significantly reduced (P<0.05) surviving *E. coli* O157:H7 populations an additional 1.8-log after 3 days in 4 ^{o}C storage. Similarly, 0.3% cinnamon essential oil treatments reduced surviving *E. coli* O157:H7 by 2.4-log after 3 days in 4 ^{o}C storage. Both 0.3% oregano and lemongrass treatments showed no change in remaining *E. coli* O157:H7 populations after 3 days in 4 ^{o}C storage. All essential oil treatments at 0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to undetectable levels by day 3 in 4 ^{o}C storage (Table 3.1-2). Hydrogen peroxide exhibited a significant growth (P<0.05) in *E. coli* O157:H7 populations from day 0 to day 1 (0.9-log), but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 3.1-2). Water treatments showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations through the 3 days in 4 ^{o}C storage (Tables 3.1-2).

Compounds. Compound treatments generally showed a continuous reduction of *E. coli* O157:H7 on romaine lettuce (Figure 11), with exception of 2-minute exposure of 0.1% citral, which showed no significant change (P<0.05) in surviving *E. coli* O157:H7 after the initial

treatment on day 0 (Table 7.2). Carvacrol and cinnamaldehyde treatments at 0.1% concentration significantly reduced ($P<0.05$) surviving *E. coli* O157:H7 populations an additional 1.5 and 2.4-log, respectively, after 3 days in 4 ^{o}C storage. Similarly, 0.3% compound treatments additionally reduced surviving *E. coli* O157:H7 by 2.3 and 0.9-log for cinnamaldehyde and citral, respectively, after 3 days in 4 ^{o}C storage. Carvacrol at 0.3% concentration reduced *E. coli* O157:H7 to undetectable levels, deterring further analysis of effects under 3 days of 4 ^{o}C storage. Similarly, compound treatments at 0.5% concentration further reduced surviving *E. coli* O157:H7 to undetectable levels by day 3 in 4 ^{o}C storage (Table 7.1-2). Carvacrol at 0.1% concentration reduced surviving *E. coli* O157:H7 by 1.2-log from day 0 to day 1 in 4 ^{o}C storage, but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 in storage (Table 7.1-2). Cinnamaldehyde at 0.1% concentration exhibited a significant reduction ($P<0.05$) of *E. coli* O157:H7 from day 0 to day 1 (0.9-log), and day 1 to day 3 (1.4-log), in 4 ^{o}C storage. Hydrogen peroxide exhibited a significant reduction ($P<0.05$) in *E. coli* O157:H7 populations from day 0 to day 1 (0.8-log), but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 7.1-2). Water treatments showed no significant change ($P<0.05$) in remaining *E. coli* O157:H7 populations through the 3 days in 4 ^{o}C storage (Tables 7.1-2).

4. *Duration in Refrigerated Storage—8 ^{o}C (Only for Essential Oil Treatments*)

Essential Oils. During the duration of 3 days in 8 ^{o}C storage, oregano essential oil treatments showed no change in surviving *E. coli* O157:H7 after initial treatment on day 0 (Tables 3.1-2). Cinnamon essential oil at 0.1 and 0.5% concentration showed no change in remaining *E. coli* O157:H7 through the duration of 3 days in 8 ^{o}C (Tables 3.1-2). Lemongrass and cinnamon essential oils at a 0.1% concentration had no change in surviving *E. coli* O157:H7 populations over 3 days in 8 ^{o}C storage. Both 0.3 and 0.5% lemongrass treatments showed no change in the surviving *E. coli* O157:H7 population while under 8 ^{o}C storage (Tables 3.1-2). Hydrogen peroxide exhibited a significant growth ($P<0.05$) in *E. coli* O157:H7 from day 0 to day

1 (2.1-log), but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 7.1-2). Water treatments showed no significant change ($P<0.05$) in remaining *E. coli* O157:H7 populations through the 3 days in 8 ^{o}C storage (Tables 7.1-2).

5. *Comparison of Refrigeration Storage Temperatures—4 and 8 ^{o}C (Only for Essential Oil Treatments)*

Essential Oils. Storage temperatures, 4 and 8 ^{o}C, exhibited no difference among all 0.5% essential oil treatments (Table 3.1-2). This is believed to be evident due to the reduction of *E. coli* O157:H7 from romaine lettuce to undetectable levels of *E. coli* O157:H7 on day 0. Oregano essential oil at 0.1 and 0.3% concentration showed no difference in levels of surviving *E. coli* O157:H7 over the 3 days in 4 and 8 ^{o}C storage (Table 3.1-2). Similarly, cinnamon essential oil at 0.1 and 0.3% concentration showed no difference in surviving *E. coli* O157:H7 over the 3 days in 4 and 8 ^{o}C storage (Table 3.1-2). Lemongrass at 0.1% concentration displayed higher surviving *E. coli* O157:H7 populations by day 3 in 8 ^{o}C storage (<1.8-log_{10} CFU g^{-1}), than 4 ^{o}C (2.9-log_{10} CFU g^{-1}). On day 3, there was, however, no difference in remaining *E. coli* O157:H7 for 4 and 8 ^{o}C (Table 3.1-2). Lemongrass essential oil at 0.3% concentration showed no difference in surviving *E. coli* O157:H7 over the 3 days in 4 and 8 ^{o}C storage (Table 3.1-2). Hydrogen peroxide treatments behaved similarly at both 4 and 8 ^{o}C, showing no change in remaining *E. coli* O157:H7 over the duration in storage (Table 3.1-2). Water treatments had a larger surviving population when held at 8 ^{o}C (6.5-log_{10} CFU g^{-1}), than at 4 ^{o}C (4.8-log_{10} CFU g^{-1}).

D. Iceberg Lettuce

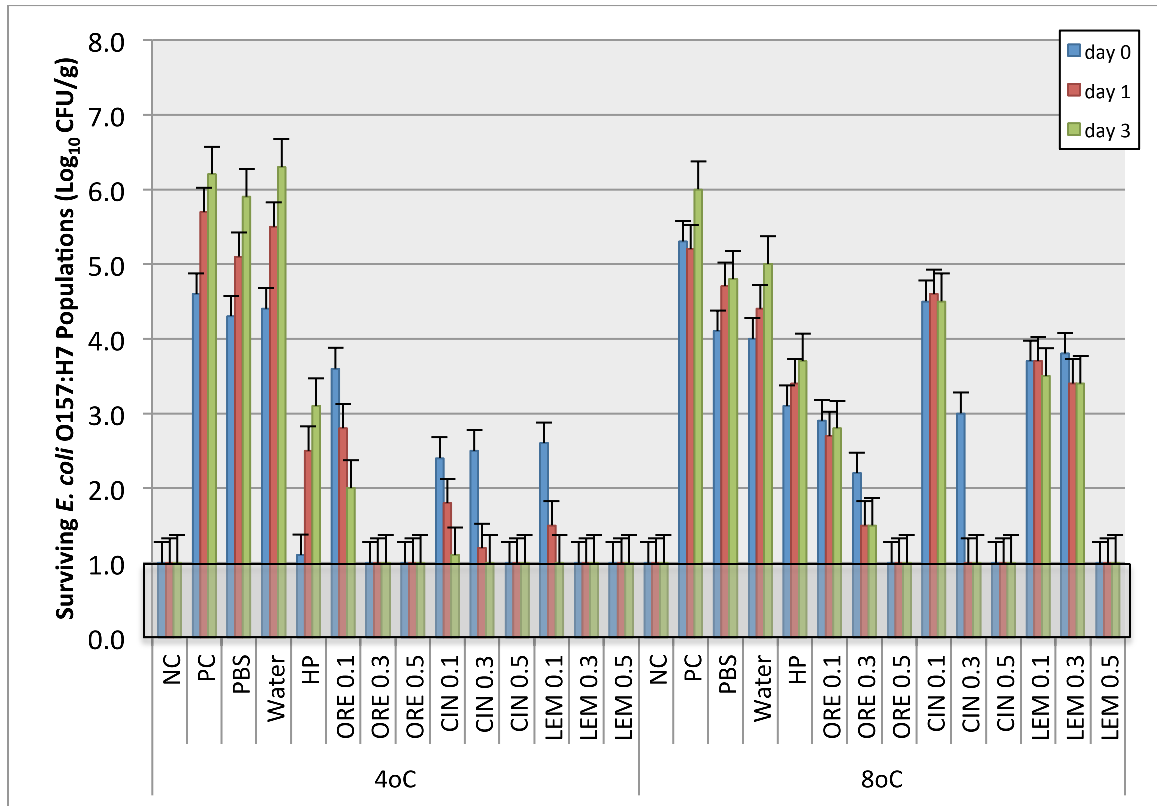
Iceberg lettuce leaves were dip inoculated with *E. coli* O157:H7 at 6.0-log CFU ml^{-1}. Recovery of *E. coli* O157:H7, after time given for attachment, was recorded at ~5.0-log CFU g^{-1} (Tables 4.1-2 and 8.1-2).

1. *Essential Oil and Compound Treatments at Varied Concentrations*

Essential Oils. All essential oil and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on iceberg lettuce following their wash (Tables 4.1-2). Hydrogen peroxide effectively reduced *E. coli* O157:H7 by 2.7-log, as well as showing greater efficacy in reduction than water (0.5-log). When compared to essential oil treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in reduced *E. coli* O157:H7 for 0.1% oregano, cinnamon, and lemongrass essential oil treatments (2.8, 2.6, and 2.7-log, respectively). Oregano essential oil had a more efficient reduction at 0.3% concentration (4.2-log) than both 0.3% cinnamon and lemongrass essential oils (3.8 and 3.8-log, respectively). All essential oil treatments at 0.5% concentration exhibited reduction of *E. coli* O157:H7 to undetectable growth (Tables 4.1-2). Each increasing concentration of essential oil treatment showed a trend of increased reduction against *E. coli* O157:H7 populations (Figure 7-8).

Compounds. All compound and control treatments significantly reduced ($P<0.05$) *E. coli* O157:H7 on iceberg lettuce following their wash (Tables 8.1-2). Hydrogen peroxide effectively reduced surviving *E. coli* O157:H7 by 3.2-log, as well as showing greater efficacy in reduction than water (1.6-log). When compared to compound treatments, hydrogen peroxide showed no significant difference ($P<0.05$) in reduced *E. coli* O157:H7 when compared to 0.1% carvacrol, cinnamaldehyde, and citral treatments (4.3, 3.4, and 2.8-log, respectively). Carvacrol (4.4-log) and cinnamaldehyde (4.0-log) at 0.3% concentration showed no difference in reduction of *E. coli* O157:H7. They both however had a more significant reduction of *E. coli* O157:H7 than 0.3% citral (2.0-log). All treatments at 0.5% concentration exhibited undetectable growth of *E. coli* O157:H7 (Tables 8.1-2). Similar to essential oils, each increasing concentration of compound treatments showed a trend of increased reduction against *E. coli* O157:H7 populations (Figure 12).

Figure 7. *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 1-minute Essential Oil Treatment Held at 4 and 8 °C



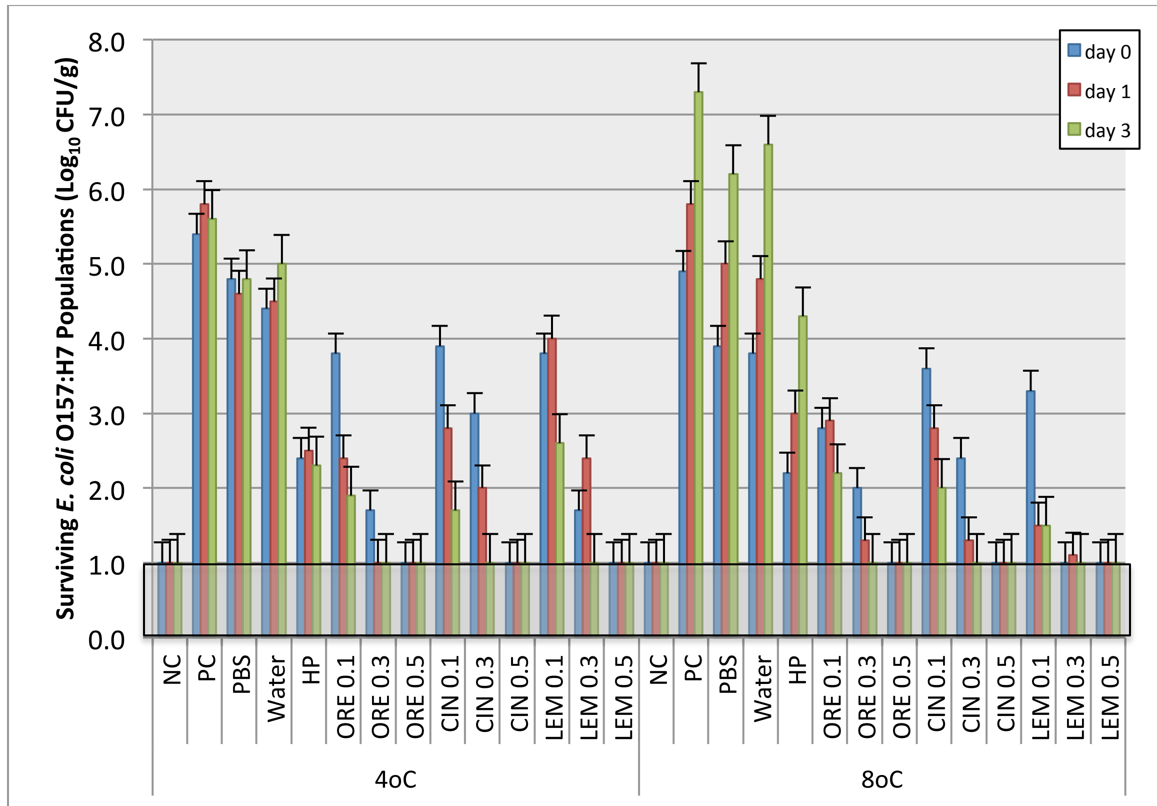
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Figure 8. *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 2-minute Essential Oil Treatment Held at 4 and 8 ^{o}C



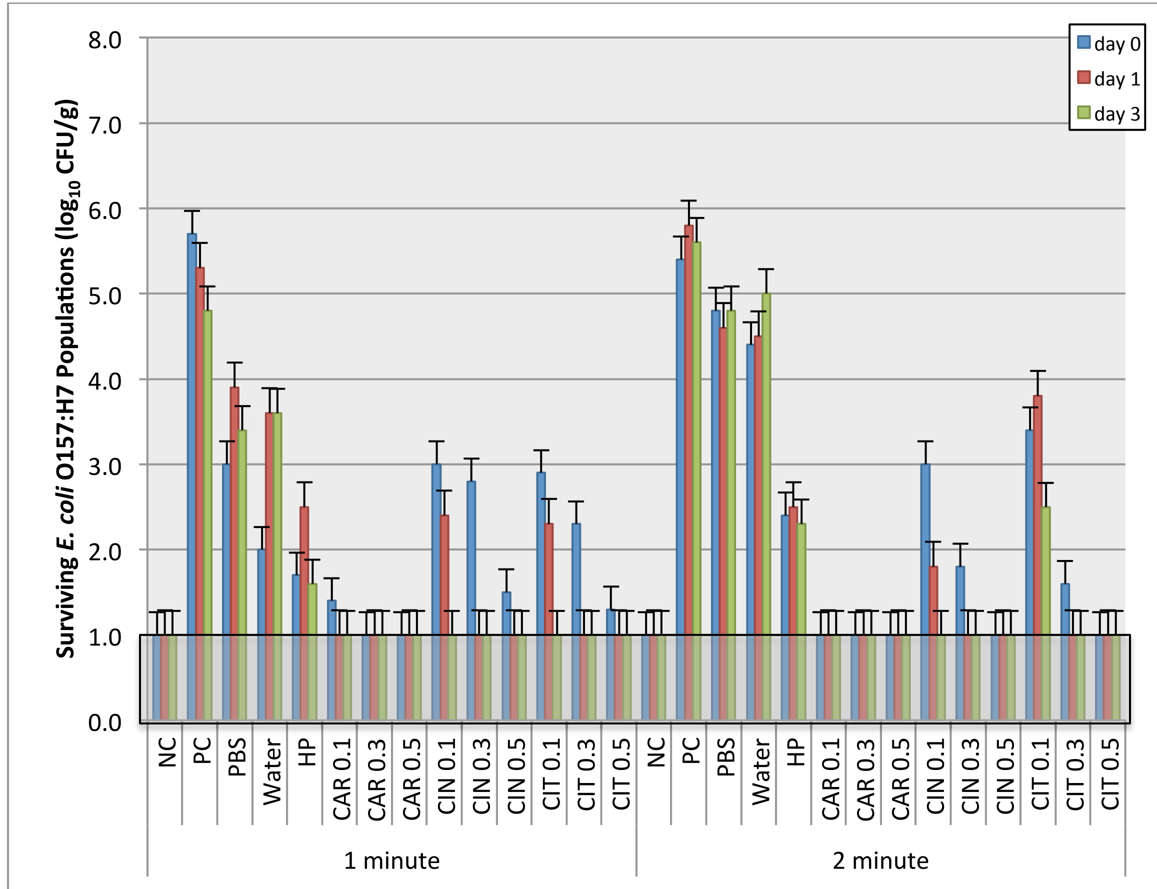
[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil.

[b]Values represent average mean of three replications.

[c]Bars represent standard error.

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Figure 12. *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 1 and 2-minute Plant-Derived Compound Treatment Held at 4 ^{o}C



[a]PC: Positive Control; NC: Negative Control; PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[b]Values represent average mean of three repetitions.
[c]Bars represent standard error.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

2. *Treatment Exposure Time*

Essential Oils. Two-minute treatment exposures showed no significant ($P<0.05$) difference in reduction of *E. coli* O157:H7 populations on iceberg lettuce, when compared to 1-minute treatment exposure for essential oil and control treatments (Table 4.1-2). Lemongrass essential oil at 0.3% concentration, however, did exhibit a greater reduction of *E. coli* O157:H7 from 2-minute treatment exposure (4.4-log), than a 1-minute exposure (3.3-log).

Compounds. Two-minute treatment exposures showed no significant ($P<0.05$) difference in reduction of *E. coli* O157:H7 populations on iceberg lettuce, when compared to 1-minute treatment exposure for carvacrol, cinnamaldehyde, and control treatments (Table 8.1-2). Citral at 0.3% concentration had greater reduction of *E. coli* O157:H7 from 2-minute treatment exposure (4.4 log), than 1-minute exposure (3.4-log).

3. *Duration in Refrigerated Storage—4 ^{o}C*

Essential Oils. Essential oil treated iceberg lettuce exhibited a continuous reduction trend against *E. coli* O157:H7 over the duration of 3 days in 4 ^{o}C storage (Figure 7-8). Oregano, cinnamon, and lemongrass essential oil treatments at 0.1% concentration significantly reduced surviving *E. coli* O157:H7 populations an additional 1.8, 2.3, and 1.4-log, respectively, after 3 days in 4 ^{o}C storage. Similarly, 0.3% cinnamon essential oil treatment reduced surviving *E. coli* O157:H7 on iceberg by 1.8-log after 3 days in 4 ^{o}C storage. Essential oil treatments at 0.3 and 0.5% concentration further reduced surviving *E. coli* O157:H7 from day 0 to undetectable levels by day 3 in 4 ^{o}C storage (Table 4.1-2). Oregano essential oils at 0.1% concentration showed significant reduction ($P<0.05$) between day 0 and day 1 (1.1-log), but showed no difference in remaining *E. coli* O157:H7 populations from day 1 and day 3 in 4 ^{o}C storage. Cinnamon essential oil at 0.1 and 0.3% concentration showed continued reduction from day 0 to day 1 (0.9 and 1.2-log, respectively), but showed no difference in remaining *E. coli* O157:H7 populations from day 1 and day 3 in 4 ^{o}C storage (Tables 4.1-2). Lemongrass essential oil at 0.1% concentration showed no change in remaining *E. coli* O157:H7 populations from day 0 to day 1, but did exhibit a significant reduction ($P<0.05$) from day 1 to day 3 (1.0-log) in 4 ^{o}C Storage. Hydrogen peroxide exhibited a significant growth ($P<0.05$) in *E. coli* O157:H7 from day 0 to day 1 (0.8-log), but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 8.1-2). Water treatments, when washed for two minutes, showed continuous growth in *E. coli* O157:H7

populations from day 0 to day 1 (1.1-log), as well as day 1 to day 3 (0.8-log) while in 4 ^{o}C storage (Table 4.1).

Compounds. Compound treatments generally showed continuous reduction of *E. coli* O157:H7 on iceberg lettuce (Figure 12). With exception to 2-minute treatment of 0.1% citral (Table 8.2), all compound treatments at each concentration reduced *E. coli* O157:H7 to undetectable levels (Table 8.1-2). Carvacrol treatments at each concentration effectively reduced *E. coli* O157:H7 populations to undetectable levels, deterring any further analysis of effects under 3 days in 4 ^{o}C storage (Table 8.1-2). Cinnamaldehyde and citral treatments at 0.1% concentration significantly reduced (P<0.05) surviving *E. coli* O157:H7 populations an additional 2.0 and 0.9-log, respectively, after 3 days in 4 ^{o}C storage. Similarly, 0.3% compound treatments additionally reduced surviving *E. coli* O157:H7 by 1.3 and 1.3-log for cinnamaldehyde and citral, respectively, after 3 days in 4 ^{o}C storage. Cinnamaldehyde at 0.1% concentration showed significant reduction (P<0.05) of remaining *E. coli* O157:H7 from day 0 to day 1 (1.3-log), but showed no difference in remaining *E. coli* O157:H7 populations from day 1 and day 3 in 4 ^{o}C storage (Table 4.1-2). Citral at 0.1% concentration showed no change in surviving *E. coli* O157:H7 between day 0 to day 1; it did however significantly reduce (P<0.05) *E. coli* O157:H7 from day 1 to day 3 (1.3-log) in 4 ^{o}C storage. Similarly, 0.3% cinnamaldehyde and citral significantly reduced *E. coli* O157:H7 from day 0 to day 1 (1.3 and 1.3-log, respectively) in 4 ^{o}C storage, but had no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 8.1-2). Hydrogen peroxide and water treatments showed no significant change (P<0.05) in remaining *E. coli* O157:H7 populations through the 3 days in 4 ^{o}C storage (Tables 8.1-2).

4. *Duration in Refrigerated Storage—8 ^{o}C (Only for Essential Oil Treatments*)

Essential Oils. During the duration of 3 days in 8 ^{o}C storage, 0.1 and 0.5% oregano essential oil treatments showed no change in surviving *E. coli* O157:H7 after initial treatment on day 0 (Tables 4.1-2). Oregano essential oil at 0.3% concentration reduced 0.9-log *E. coli*

O157:H7 after 3 days in 8 ^{o}C storage. Cinnamon essential oil at 0.3% concentration reduced 1.6-log *E. coli* O157:H7 after 3 days in 8 ^{o}C storage. Cinnamon essential oil at 0.5% concentration showed no change in remaining *E. coli* O157:H7 through the duration of 3 days in 8 ^{o}C storage (Tables 4.1-2). Lemongrass essential oil at 0.1% concentration showed a reduction *E. coli* O157:H7 by 0.9-log over 3 days in 8 ^{o}C storage. Both 0.3 and 0.5% lemongrass essential oil treatments showed no change in remaining *E. coli* O157:H7 through the duration of 3 days in 8 ^{o}C storage (Tables 4.1-2). Cinnamon essential oil at 0.1% and 0.3% concentration significantly reduced ($P<0.05$) surviving *E. coli* O157:H7 populations from day 0 to day 1, but showed no change in remaining populations from day 1 to day 3 in 8 ^{o}C storage. Similarly, lemongrass essential oil at a 0.1% concentration significantly reduced ($P<0.05$) surviving *E. coli* O157:H7 populations from day 0 to day 1, but showed no change in remaining populations from day 1 to day 3 in 8 ^{o}C storage (Tables 4.1-2). Hydrogen peroxide exhibited a significant growth ($P<0.05$) in *E. coli* O157:H7 populations from day 0 to day 1 (1.4-log), but showed no change in remaining *E. coli* O157:H7 populations from day 1 to day 3 (Tables 4.1-2). Water treatments showed no significant change ($P<0.05$) in remaining *E. coli* O157:H7 populations from day 0 to day 1, but had a significant growth ($P<0.05$) from day 1 to day 3 (~1.0-log) through the 3 days in 8 ^{o}C storage (Tables 4.1-2).

5. *Comparison of Refrigeration Storage Temperatures—4 and 8 ^{o}C (Only for Essential Oil Treatments)*

Essential Oils. Storage temperatures, 4 and 8 ^{o}C, exhibited no difference among all 0.5% essential oil treatments (Table 4.1-2). This is believed to be evident due to the reduction of *E. coli* O157:H7 from iceberg lettuce to undetectable levels of *E. coli* O157:H7 on day 0. Oregano essential oil at 0.1% concentration showed no difference in surviving *E. coli* O157:H7 from day 0 to day 1 at both 4 and 8 ^{o}C, but had lower surviving *E. coli* O157:H7 from day 1 to day 3 when stored at 4 ^{o}C (Table 4.1-2). At a 0.3% concentration, oregano essential oil showed no difference

in surviving *E. coli* O157:H7 over the 3 days in 4 and 8 ^{o}C storage (Table 4.1-2). Cinnamon essential oil treatments at 0.1% concentration had less surviving *E. coli* O157:H7 from day 1 to day 3 in 4 ^{o}C (1.4-$\log_{10}$ CFU g^{-1}), in comparison to that in 8 ^{o}C storage (3.4-$\log_{10}$ CFU g^{-1}). Cinnamon essential oil at a 0.3% concentration had no difference in surviving *E. coli* O157:H7 populations over the 3 days in 4 and 8 ^{o}C storage (Table 4.1-2). Lemongrass essential oil at a 0.1% concentration had no difference in remaining *E. coli* O157:H7 populations from day 0 to day 1 in both 4 and 8 ^{o}C storage, but from day 1 to day 3, remaining *E. coli* O157:H7 populations were lower when stored at 4 ^{o}C (1.8-$\log_{10}$ CFU g^{-1}), than 8 ^{o}C storage (2.5-$\log_{10}$ CFU g^{-1}). Hydrogen peroxide treatments showed similar remaining *E. coli* O157:H7 populations for both 4 and 8 ^{o}C from day 1, but had significantly more ($P<0.05$) *E. coli* O157:H7 in 8 ^{o}C storage (4.1-$\log_{10}$ CFU g^{-1}), than 4 ^{o}C (2.7-$\log_{10}$ CFU g^{-1}), on day 3.

DISCUSSION

A. Efficacy of Essential Oils and Compounds at Varied Concentrations

In this study, it was determined that all essential oil and compound treatments were able to reduce *Escherichia coli* O157:H7 populations significantly ($P<0.05$) even at the lowest concentrated treatment (0.1% v/v) after their initial application (Day 0). Multiple studies have demonstrated essential oil antimicrobial efficacy (Friedman, Henika, and Mandrell, 2002; Gutierrez, Rodriguez, Barry-Ryan, and Bourke, 2008), as well as antimicrobial efficacy from individual essential oil derived compounds (Friedman, Henika, and Mandrell, 2002; Burt, Vlielander, Haagsman, and Veldhuizen, 2005).

Studies have shown that essential oils, when tested in vitro, are effective against specific pathogens, but may require a higher concentration in foods to acquire the same lethality due to influence from varied components in the total food makeup (i.e. fat, protein, carbohydrates, available nutrients) (Smid and Gorris, 1999). In addition, foodborne pathogens vary in their retention on or in the food product based on their attachment and survival abilities (Tian, Bae, and Lee, 2013; Giaouris et al., 2013). There have been varied studies examining the antimicrobial efficacy of plant-derived essential oils against foodborne pathogens *Salmonella enterica* and *Salmonella enterica* serovar Newport on organic leafy greens (Todd et al., 2013; Moore-Neibel et al., 2013), as well as the use of cinnamaldehyde to reduce *E. coli* O157:H7 and *Salmonella enterica* on spinach leaves (Yossa et al. 2012). In the study by Todd et al. (2013), they found varied reduction of *Salmonella enterica* serovar Newport between leafy greens, iceberg lettuce

and romaine lettuce. This may suggest that that essential oil treatments may vary based on the attachment of the pathogen to the leafy green surface. Similarly, in the current study, variation among leafy greens was not significant ($P<0.05$), with the exception of iceberg lettuce, which had significantly ($P<0.05$) lower surviving *E. coli* O157:H7 populations.

Both oregano essential oil and carvacrol were found to be the most effective treatments in the two experiments of this study. Burt and Reinders (2003) found oregano essential oil to be bactericidal (no viable cells detected; $>10^4$ log reduction) within one minute of its application at a concentration of 625 μ l^{-1}, and at 5 minutes at concentrations of both 312 μl L^{-1} and 156 μl L^{-1}. In our study we dispersed essential oils in PBS at 0.1% v/v, 0.3% v/v, and 0.5% v/v; 100 μ l^{-1}, 300 μ l^{-1}, and 500 μ l^{-1}, respectively. Evidence of no detectable growth of *E. coli* O157:H7 at 0.5% concentrations coincides with Burt and Reinders' (2003) findings, providing evidence that the *E. coli* O157:H7 cells were eradicated shortly after being exposed to the treatments. Conversely, in the current study oregano oil at 0.1% concentration showed significant ($P<0.05$) reduction (0.1-2.1 log CFU g^{-1}) after the initial treatment (Day 0), but was not significantly ($P<0.05$) more effective than the hydrogen peroxide control treatment. In the same study, Burt and Reinders (2003) found oregano essential oil concentrations of 78 μ l^{-1} to reduce *E. coli* O157:H7 2-logs within 5 minutes, but noticed that essential oils showed no continuation in reduction in the following 15 minutes of examining the treated culture. This phenomenon may be dependent on the essential oils specific activity, as well as the microbial capacity of the sample. Sokmen et al. (2004) analyzed *Oreganum* plant species and tested their antimicrobial, antiviral, and antioxidant activities. Their study found oregano essential oil, which is composed of 72% carvacrol, to have the greatest effectiveness on *Bacillus macarens*, *Salmonella enteritidis*, and *Escherichia coli* out of 35 assorted bacterial species. In addition, evidence of oregano essential oil's notable antimicrobial activity against *E. coli* O157:H7 is also present in additional studies (Gutierrez-Larrainzar et al., 2002), as well as from carvacrol (Friedman et al., 2004).

Cinnamon essential oil can vary in its chemical composition dependent on where the essential oil is acquired. Friedman et al. (2000) reported cinnamon essential oil to be diverse, with the oils deriving from cinnamon bark to contain 81% trans-cinnamaldehyde, with little traces of eugenol; whereas cinnamon leaf oil contains 70% eugenol, contains little traces of cinnamaldehyde. In our study, we focused and utilized cinnamon essential oil derived from cinnamon bark, clarifying our use of cinnamaldehyde as an essential oil derived compound treatment. In the current study cinnamon essential oil at 0.1% (100 ppm) and 0.3% (300 ppm) exhibited reduction of 0.1-2.2 log CFU g^{-1} and <0.1-2.0 log CFU g^{-1} *E. coli* O157:H7, respectively, after initial application (Day 0). Both had no significant difference ($P<0.05$) between the control treatments (<0.1-2.3 log CFU g^{-1}) (water and PBS) after the initial application (Day 0). Similar results from Yossa et al. (2012) exhibited significant ($P<0.05$) reduction of *E. coli* O157:H7 (3.23 log CFU g^{-1}) from spinach leaves when cinnamaldehyde was sustained in an emulsification solution, Tween 20. In the same study, cinnamaldehyde, individually, was only significantly ($P<0.05$) effective at reducing *E. coli* O157:H7 at a larger concentration of 1000 ppm, compared to the smaller 800 ppm. Variation of significant reduction at much lower concentrations in the current study may be the cause of the treatment application method, or other related factor.

Lemongrass essential oil and isolated citral are found to be effective antifungal agents (Tzortzakis and Economakis, 2007). The use of them in this study was to determine their efficacy with the problematic foodborne pathogen, *E. coli* O157:H7 on leafy greens. In our study, both lemongrass and citral showed the least activity as an antimicrobial treatment against *E. coli* O157:H7 compared to the other treatments. However, at high concentrations (0.5%) both lemongrass and citral reduced *E. coli* O157:H7 populations to <1.0 log CFU g^{-1} or undetectable levels after the initial application (Day 0). Somolinos et al. (2009) studied citral's inactivation of *E. coli*. Their research suggested evidence that when citral is at neutral pH its efficacy was a function of inoculum size, treatment compound concentration, and storage and time; otherwise

citral was said to be more active at an alkaline pH. Somolinos et al. (2009) further found that citral treated to an inoculum (*E. coli* O157:H7) at 10^7 to 10^9 log_{10} CFU g^{-1} the antimicrobial efficacy of citral would decline, especially at smaller concentrations of 100 and 200 μl l^{-1}. Evidence of this phenomenon may also be apparent for all essential oil and essential oil derived compounds based on their hydrophobic nature. Lemongrass essential oil was found to inhibit enteric pathogen, *Salmonella enterica*, on organic leafy greens (Moore-Neibel et al., 2011).

B. Effects of Treatment Exposure Time

Treatment exposure times were examined in the current study to determine if longer exposure to the plant-based essential oils and compounds provide higher reductions of *E. coli* O157:H7 on the leafy greens. With a few arbitrary occurrences, there was no trend toward increased reduction seen from longer treatment exposure during the leaf wash. In a study conducted by Todd et al. (2013) similar methods comparing cinnamon essential oil wash treatments against *Salmonella* Newport tested for differences in 1 and 2-minute treatment exposures. Their results found a higher rate of reduction from 2-minute treatment exposure. This may be the action of a more effective antimicrobial activity against that particular organism. However, in a similar study, Moore-Neibel et al. (2013) examined antimicrobial effects of oregano essential oil against *Salmonella enterica*, showing differing results where no significant difference was seen between 1- and 2-minute exposure times, with exception to 0.3% oregano essential oil on romaine and iceberg lettuce.

C. Effects of Refrigerated Temperatures and Storage Duration

In accordance to a FDA guidance regulation, leafy greens that have been cut, chopped, or torn must be kept at refrigerated temperatures, 41 ^{o}F (5 ^{o}C) or less, during post-harvest processing (FDA, 2010). Refrigerated temperatures are set to inhibit growth of any spoilage or pathogenic bacteria, keeping them from causing any damage to both, quality and safety of the product.

Francis and O'Beirne (2001) found that *E. coli* O157:H7 growth was halted at temperatures below 4 ^{o}C, however temperatures at 6-8 ^{o}C still allow for minimum growth (Delaquis, Bach & Dinu, 2007). This poses a problem for leafy green handlers when environmental conditions exceed the safe parameters for controlling microbial growth. In the current study, effects of refrigerated storage were studied over a period of 3 days to examine *E. coli* O157:H7 survival on treated leafy greens. Results showed either a decrease or no significant change (P<0.05) in surviving *E. coli* O157:H7 populations after each day in 4 ^{o}C refrigerated storage. This may indicate that the applied essential oil and/or compound treatments were still active, or had caused enough damage to the bacterial cell for it to not be able to maintain functioning under low temperature conditions.

Francis and O'Beirne (2001) stated that refrigerated temperatures (4 ^{o}C) keep *E. coli* O157:H7 activity limited, but the cell is kept viable, and undamaged. They further provide that if temperatures reach 5 ^{o}C *E. coli* O157:H7 cells will begin to regain a slow, but measurable metabolic activity. In the essential oil portion of the current study, two storage temperatures were compared to examine the effects of essential oil antimicrobial and control treatments when held in refrigerated temperatures (4 ^{o}C), or at an abused temperature (8 ^{o}C). As seen in Tables 1-4, when stored at temperatures of 8 ^{o}C, *E. coli* O157:H7 increased in growth on water and hydrogen peroxide treated leafy greens at 1.0-2.8 $\log_{10}$ CFU g^{-1} and 0.6-2.8 $\log_{10}$ CFU g^{-1}, respectively. In contrast to the observed growth in the controls, essential oil treatments continued to reduce or maintained a consistent microbial population with no significant growth or reduction (P<0.05) between each day. When comparing temperatures 4 and 8 ^{o}C for essential oil treated leafy greens, both temperatures had similar remaining *E. coli* O157:H7 populations, with no significant difference (P<0.05). Similar studies tested the effects of temperature on oregano (Moore-Neibel et al., 2013) and lemongrass (Moore-Neibel et al., 2011) essential oils against *Salmonella enterica* on organic leafy greens. Both studies found converse results, in which oregano essential oil had better reduction at 8 ^{o}C, and lemongrass having greater reduction at 4 ^{o}C. Skandamis and

Nychas (2000) studied the effects of essential oils against *E. coli* O157:H7 while under refrigerated temperatures. Their results indicate that the volatile essential oils are limited from their full potential in antimicrobial activity while under colder storage, protecting the intended bacteria from any further destruction.

D. Control Treatments

Control treatments were used as standards to test the plant-derived essential oil and primary compound's efficacy against *E. coli* O157:H7 on leafy green surfaces. Overall, essential oils and compound treatments at high concentrations (0.3%, 0.5%) showed a more significant ($P<0.05$) reduction than the control treatments. Some essential oil and compound treatments at 0.1% showed no difference in reduction when compared to hydrogen peroxide treatments. However, unlike the essential oil and compound treatments in this study, hydrogen peroxide did not show a trend of continuing reduction over the time in refrigerated storage for both 4 and 8 ^{o}C. It is hypothesized that hydrogen peroxide may only have short term effects on the bacteria, in comparison to a continuous effect found in the essential oils and compound treatments. This may be the cause of *E. coli* O157:H7's adaptability towards the hydrogen peroxide antimicrobial exposure.

E. Conclusion

The findings from the current study have provided evidence that essential oils from oregano, cinnamon and lemongrass, as well as their primary constituents, carvacrol, cinnamaldehyde, and citral, respectively, can provide significant antimicrobial effects against *E. coli* O157:H7 when applied to leafy green surfaces via an agitated wash similar to those found in produce post-harvest handling. It was shown that increasing concentrations of essential oils and compounds correlates with an increase in antimicrobial efficacy. Treatment exposure times (1 and 2 minutes) were determined to show no significant difference in reduction. A storage holding

temperature of 4 ^{o}C showed significant reduction of *E. coli* O157:H7 over a duration of 3 days for essential oil and compound treatments. When under 8 ^{o}C storage, essential oil treated leafy greens displayed evidence of either unchanged or less reduction of *E. coli* O157:H7 over the duration of 3 days, when compared to 4 ^{o}C storage. Some treatments at 0.1% concentration, as well as some control treatments, exhibited an increase in *E. coli* populations over duration of 3 days in storage. While in low-temperature storage, plant-derived essential oil and compound treated leafy greens showed signs of continuing reduction of *E. coli* O157:H7 populations, with higher concentrations of 0.3% and 0.5% resulting in no detectable growth by day 3. Thus, plant-derived essential oil and compound washes for leafy greens are an effective, natural antimicrobial treatment for organic leafy greens. Additional assessments in both sensory, processing functionality, and cost analysis are needed to determine whether essential oil treatment is probable for leafy green post-harvest processing.

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APPENDICES

Table 1.1. *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	5.5 ± 0.5 a	5.7 ± 0.2 a	4.6 ± 0.2 a,b
PBS	-	4.5 ± 0.3 a,b	4.6 ± 0.4 a,b	5.1 ± 0.4 a
HP	-	4.6 ± 0.1 a,b	4.0 ± 0.4 b	4.2 ± 0.9 b
Water	-	4.6 ± 0.4 a,b	4.2 ± 0.8 b	3.8 ± 1.0 b
ORE	0.1	4.4 ± 0.7 b	4.1 ± 0.4 b	3.6 ± 2.3 b
ORE	0.3	4.2 ± 0.4 b	4.1 ± 1.4 b	2.4 ± 2.4 c
ORE	0.5	<1.0 ± 0.0 d	1.5 ± 0.0 c,d	2.0 ± 0.3 c,d
CIN	0.1	4.3 ± 0.7 b	2.4 ± 1.3 d	1.8 ± 1.4 c,d
CIN	0.3	3.5 ± 0.3 b	1.5 ± 0.9 b,c	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	4.2 ± 0.7 b	3.0 ± 1.0 b,c	1.5 ± 0.9 c,d
LEM	0.3	3.7 ± 1.0 b	3.1 ± 0.7 b,c	1.6 ± 1.0 c,d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	1.4 ± 0.3 d
8 ^{o}C				
Control	-	5.3 ± 0.4 a	5.0 ± 0.8 a	6.4 ± 0.8 a
PBS	-	5.0 ± 0.3 a	5.2 ± 0.6 a	5.7 ± 0.7 a
HP	-	3.2 ± 0.8 b	3.6 ± 0.8 b	3.9 ± 0.8 b
Water	-	4.8 ± 0.3 a	5.5 ± 0.6 a	6.0 ± 0.5 a
ORE	0.1	2.8 ± 0.3 b	2.5 ± 0.9 b	2.4 ± 0.2 b
ORE	0.3	<1.0 ± 0.0 c	1.3 ± 0.5 c	1.2 ± 0.5 c
ORE	0.5	<1.0 ± 0.0 c	1.1 ± 0.2 c	<1.0 ± 0.0 c
CIN	0.1	3.8 ± 0.4 b	4.0 ± 1.3 b	3.4 ± 1.5 b
CIN	0.3	3.4 ± 0.6 b	2.7 ± 0.3 b	2.7 ± 0.7 b
CIN	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.1	4.5 ± 0.2 a,b	4.6 ± 0.4 a	5.0 ± 1.0 a
LEM	0.3	1.9 ± 0.8 c	2.8 ± 0.1 b	3.2 ± 0.1 b
LEM	0.5	1.3 ± 0.6 c	1.2 ± 0.3 c	1.3 ± 0.6 c

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil

[b]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 1.2. *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 2-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	6.5 ± 0.1 a	6.8 ± 0.2 a	6.4 ± 0.6 a
PBS	-	5.3 ± 0.1 b	5.2 ± 0.1 b	5.1 ± 0.1 b
HP	-	3.0 ± 1.9 c	4.3 ± 0.9 b	4.5 ± 0.4 b
Water	-	4.5 ± 0.4 b	4.5 ± 0.5 b	5.7 ± 0.7 a,b
ORE	0.1	4.4 ± 1.3 b	4.7 ± 1.4 b	1.9 ± 1.6 c,d
ORE	0.3	2.4 ± 1.2 c	2.8 ± 2.0 c	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	1.9 ± 0.0 c,d	<1.0 ± 0.0 d
CIN	0.1	5.1 ± 0.3 b	4.8 ± 0.4 b	3.1 ± 1.9 c
CIN	0.3	3.9 ± 0.6 b,c	3.6 ± 0.7 c	2.2 ± 2.1 c,d
CIN	0.5	1.6 ± 1.0 d	2.0 ± 1.0 c,d	<1.0 ± 0.0 d
LEM	0.1	4.8 ± 0.2 b	5.3 ± 0.3 b	3.4 ± 2.1 c
LEM	0.3	4.5 ± 0.9 b	2.8 ± 1.6 c	2.8 ± 1.8 c
LEM	0.5	1.8 ± 1.4 c,d	2.0 ± 1.7 c,d	1.2 ± 0.4 d
8 ^{o}C				
Control	-	4.7 ± 0.8 a	6.8 ± 0.2 b	7.6 ± 0.1 b
PBS	-	3.8 ± 0.5 a,d	5.5 ± 0.2 a	6.8 ± 1.0 b
HP	-	1.5 ± 0.3 c	3.3 ± 0.3 d	4.3 ± 0.4 a,d
Water	-	4.6 ± 0.1 a	5.7 ± 0.1 a,d	6.5 ± 0.7 b
ORE	0.1	1.5 ± 0.8 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
ORE	0.3	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
ORE	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
CIN	0.1	3.1 ± 0.1 d	2.4 ± 0.5 d	1.5 ± 0.8 c
CIN	0.3	2.9 ± 1.1 d	<1.0 ± 0.0 c	<1.0 ± 0.0 c
CIN	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.1	1.7 ± 0.6 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.3	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 2.1. *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	4.7 ± 0.6 a	5.3 ± 0.5 a,b	6.1 ± 0.2 b
PBS	-	3.5 ± 0.8 c	4.3 ± 0.3 a,c	5.8 ± 0.8 b
HP	-	1.9 ± 1.5 d	2.5 ± 1.1 c,d	3.7 ± 0.6 a,c
Water	-	3.9 ± 1.2 a,c	5.4 ± 0.3 a,b	6.1 ± 0.2 b
ORE	0.1	4.6 ± 0.2 a	3.6 ± 0.1 a,c	2.9 ± 0.7 c,d
ORE	0.3	2.3 ± 1.2 c,d	1.4 ± 0.6 d	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.1	4.4 ± 0.6 a,c	3.0 ± 0.7 c,d	2.1 ± 0.2 d
CIN	0.3	2.6 ± 0.2 c,d	1.1 ± 0.2 d	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	3.7 ± 0.1 a,c	2.4 ± 0.2 c,d	1.2 ± 0.2 d
LEM	0.3	3.2 ± 0.0 c	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
8 ^{o}C				
Control	-	5.9 ± 0.2 a	7.0 ± 0.5 a	6.4 ± 0.2 a
PBS	-	5.0 ± 0.0 a	5.1 ± 0.2 a	5.3 ± 0.1 a
HP	-	3.0 ± 0.4 b	3.1 ± 0.2 b	3.2 ± 0.5 b
Water	-	5.1 ± 0.3 a	5.2 ± 0.1 a	5.2 ± 0.1 a
ORE	0.1	4.0 ± 0.1 a,b	4.1 ± 0.0 a,b	4.0 ± 0.0 a,b
ORE	0.3	1.2 ± 0.2 c	1.6 ± 0.8 c	2.3 ± 0.2 b,c
ORE	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	1.1 ± 0.0 c
CIN	0.1	4.5 ± 0.0 a	6.1 ± 0.4 a	5.8 ± 0.1 a
CIN	0.3	4.4 ± 0.3 a	4.9 ± 0.1 a	5.2 ± 0.4 a
CIN	0.5	<1.0 ± 0.0 c	1.4 ± 0.6 c	2.2 ± 0.2 b,c
LEM	0.1	4.5 ± 0.2 a	5.4 ± 0.2 a	5.9 ± 0.3 a
LEM	0.3	3.9 ± 0.2 a,b	3.8 ± 0.2 a,b	4.3 ± 0.2 a
LEM	0.5	1.5 ± 0.3 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 2.2. *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 2-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	4.4 ± 0.3 a	5.9 ± 0.7 b	6.4 ± 0.2 b
PBS	-	4.4 ± 0.7 a	4.6 ± 1.0 a	4.3 ± 0.9 a
HP	-	3.9 ± 1.0 a,b	3.3 ± 1.1 b	3.5 ± 0.7 a,b
Water	-	4.7 ± 1.0 a	4.3 ± 0.8 a	4.3 ± 0.9 a
ORE	0.1	4.5 ± 1.2 a	3.8 ± 1.4 a,b	2.3 ± 1.2 c
ORE	0.3	3.5 ± 0.5 b	1.3 ± 0.6 c,d	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.1	4.3 ± 0.8 a	3.8 ± 1.1 a,b	2.8 ± 1.8 b,c
CIN	0.3	3.3 ± 1.4 b,c	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	5.0 ± 0.4 a	4.3 ± 0.4 a	1.6 ± 1.0 b,c
LEM	0.3	3.0 ± 1.8 b,c	1.4 ± 0.7 c,d	<1.0 ± 0.0 d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
8 ^{o}C				
Control	-	4.7 ± 0.9 a	5.8 ± 0.3 b	7.4 ± 0.4 c
PBS	-	4.0 ± 0.0 a,d	5.3 ± 0.5 a,b	7.4 ± 0.4 c
HP	-	2.5 ± 1.5 d	4.3 ± 0.7 a	4.4 ± 0.6 a
Water	-	3.9 ± 0.0 a,d	3.2 ± 0.3 d	6.3 ± 0.5 b
ORE	0.1	1.5 ± 0.8 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
ORE	0.3	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
ORE	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
CIN	0.1	3.3 ± 1.3 d	1.2 ± 0.2 e	<1.0 ± 0.0 e
CIN	0.3	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
CIN	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.1	1.7 ± 0.4 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.3	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 3.1. *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 1-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	5.2 ± 0.5 a	5.3 ± 0.6 a	6.0 ± 0.5 a
PBS	-	4.2 ± 0.1 a	4.6 ± 0.1 a	5.3 ± 0.7 a
HP	-	1.7 ± 1.2 b	3.0 ± 0.4 c	3.6 ± 0.4 c
Water	-	4.4 ± 0.2 a	4.9 ± 0.9 a	5.0 ± 1.2 a
ORE	0.1	4.3 ± 0.2 a	3.5 ± 0.4 c	3.0 ± 0.7 c
ORE	0.3	3.6 ± 0.5 c	1.4 ± 0.4 b	<1.0 ± 0.0 b
ORE	0.5	<1.0 ± 0.0 b	<1.0 ± 0.0 b	<1.0 ± 0.0 b
CIN	0.1	4.0 ± 0.3 c	3.7 ± 0.8 c	1.2 ± 0.2 b
CIN	0.3	3.1 ± 0.5 c	1.4 ± 0.7 b	<1.0 ± 0.0 b
CIN	0.5	<1.0 ± 0.0 b	<1.0 ± 0.0 b	<1.0 ± 0.0 b
LEM	0.1	3.5 ± 1.3 c	3.3 ± 0.2 c	<1.0 ± 0.0 b
LEM	0.3	3.4 ± 0.5 c	1.7 ± 0.7 b	<1.0 ± 0.0 b
LEM	0.5	<1.0 ± 0.0 b	<1.0 ± 0.0 b	<1.0 ± 0.0 b
8 ^{o}C				
Control	-	5.5 ± 0.6 a	6.4 ± 1.3 a,b	6.8 ± 1.4 b
PBS	-	4.8 ± 0.6 a	5.6 ± 1.0 a	5.6 ± 0.8 a
HP	-	3.0 ± 0.2 c	4.9 ± 0.5 a	5.1 ± 0.5 a
Water	-	5.0 ± 0.7 a	5.5 ± 0.5 a	6.0 ± 1.1 a,b
ORE	0.1	4.1 ± 0.8 a	4.0 ± 1.1 a,c	4.0 ± 1.0 a,c
ORE	0.3	1.8 ± 0.9 d	2.3 ± 0.8 c,d	1.7 ± 0.7 d
ORE	0.5	<1.0 ± 0.0 d	1.3 ± 0.4 d	1.4 ± 0.5 d
CIN	0.1	4.0 ± 0.4 a,c	4.4 ± 0.8 a	4.7 ± 1.1 a
CIN	0.3	3.3 ± 1.0 c	3.2 ± 1.3 c	3.4 ± 1.5 c
CIN	0.5	1.6 ± 0.7 d	1.5 ± 0.5 d	1.5 ± 0.6 d
LEM	0.1	4.0 ± 0.7 a,c	3.9 ± 0.6 a,c	4.1 ± 0.8 a,c
LEM	0.3	3.6 ± 0.5 c	3.7 ± 0.6 c	4.0 ± 0.8 a,c
LEM	0.5	1.6 ± 0.7 d	2.2 ± 0.7 c,d	2.2 ± 0.8 c,d

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Table 3.2. *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 2-minute Essential Oil Treatment Held at 4 and 8 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
4 ^{o}C				
Control	-	6.4 ± 0.9 a	6.4 ± 1.1 a	6.2 ± 1.3 a
PBS	-	4.7 ± 1.1 b	4.9 ± 0.4 b	4.6 ± 1.0 b
HP	-	2.6 ± 1.6 c	3.0 ± 1.8 c	3.3 ± 0.7 c
Water	-	4.1 ± 1.0 b	4.6 ± 1.1 b	4.5 ± 0.9 b
ORE	0.1	4.6 ± 0.5 b	3.2 ± 2.2 c	2.7 ± 1.0 c
ORE	0.3	1.7 ± 0.6 c,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.1	4.3 ± 0.8 b	4.4 ± 0.9 b	2.0 ± 0.9 c,d
CIN	0.3	3.7 ± 0.6 b,c	2.3 ± 1.2 c	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	4.4 ± 0.4 b	4.6 ± 0.3 b	3.3 ± 2.0 b,c
LEM	0.3	2.8 ± 1.5 c	2.3 ± 1.4 c	1.8 ± 0.5 c,d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
8 ^{o}C				
Control	-	5.3 ± 0.1 a	6.2 ± 0.1 a,b	7.2 ± 0.1 b
PBS	-	4.8 ± 0.1 a	4.9 ± 0.2 a	6.9 ± 0.2 b
HP	-	3.0 ± 0.0 c	5.3 ± 0.7 a	4.0 ± 0.0 c
Water	-	5.0 ± 0.1 a	2.8 ± 0.8 c	6.9 ± 0.0 b
ORE	0.1	1.9 ± 0.1 c,d	1.7 ± 0.0 d	1.7 ± 0.0 d
ORE	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.1	3.3 ± 0.7 c	2.8 ± 0.3 c	2.1 ± 0.2 c
CIN	0.3	2.3 ± 0.1 c	1.1 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	3.3 ± 0.1 c	1.7 ± 0.0 d	1.7 ± 0.0 d
LEM	0.3	1.3 ± 0.0 d	1.2 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 4.1. *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 1-minute Essential Oil Treatment Held at 4 and 8 °C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
4 °C				
Control	-	4.6 ± 0.9 a	5.7 ± 0.3 a,b	6.2 ± 0.3 b
PBS	-	4.3 ± 0.2 a	5.1 ± 0.6 a,b	5.9 ± 0.3 b
HP	-	1.1 ± 0.2 c	2.5 ± 0.1 d	3.1 ± 0.1 d
Water	-	4.4 ± 0.2 a	5.5 ± 0.6 a,b	6.3 ± 0.3 b
ORE	0.1	3.6 ± 0.1 a,d	2.8 ± 0.2 d	2.0 ± 0.2 c,d
ORE	0.3	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
ORE	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
CIN	0.1	2.4 ± 0.3 d	1.8 ± 0.7 c,d	<1.1 ± 0.2 c
CIN	0.3	2.5 ± 0.2 d	1.2 ± 0.4 c	<1.0 ± 0.0 c
CIN	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.1	2.6 ± 0.2 d	1.5 ± 0.5 c	<1.0 ± 0.0 c
LEM	0.3	<1.0 ± 0.0 c	1.0 ± 0.0 c	<1.0 ± 0.0 c
LEM	0.5	<1.0 ± 0.0 c	1.0 ± 0.0 c	<1.0 ± 0.0 c
8 °C				
Control	-	5.3 ± 1.0 a	5.2 ± 0.8 a	6.0 ± 0.7 a
PBS	-	4.1 ± 0.7 b	4.7 ± 1.8 a,b	4.8 ± 1.7 a,b
HP	-	3.1 ± 1.2 b	3.4 ± 1.6 b,c	3.7 ± 1.5 b,c
Water	-	4.0 ± 0.8 b	4.4 ± 1.3 a,b	5.0 ± 1.2 a,b
ORE	0.1	2.9 ± 0.5 c,e	2.7 ± 0.4 c,e	2.8 ± 0.2 e
ORE	0.3	2.2 ± 0.8 c	1.5 ± 0.5 d,e	1.5 ± 0.5 d,e
ORE	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 c	<1.0 ± 0.0 e
CIN	0.1	4.5 ± 0.7 a,b	4.6 ± 0.5 a,b	4.5 ± 0.6 a,b
CIN	0.3	3.0 ± 0.4 c,d	<1.0 ± 0.0 e	<1.0 ± 0.0 e
CIN	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.1	3.7 ± 0.3 b,c	3.7 ± 0.3 b,c	3.5 ± 0.5 b,c
LEM	0.3	3.8 ± 0.3 b,c	3.4 ± 1.4 b,c	3.4 ± 1.4 b,c
LEM	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Table 4.2. *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 2-minute Essential Oil Treatment Held at 4 and 8 °C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
4 °C				
Control	-	5.4 ± 0.8 a	5.8 ± 1.5 a	5.6 ± 1.4 a
PBS	-	4.8 ± 0.4 a,c	4.6 ± 1.0 a,c	4.8 ± 0.4 a,c
HP	-	2.4 ± 1.3 b	2.5 ± 1.3 b	2.3 ± 1.1 b
Water	-	4.4 ± 0.3 a,c	4.5 ± 1.0 a,c	5.0 ± 1.0 a
ORE	0.1	3.8 ± 1.1 c	2.4 ± 1.3 b	1.9 ± 0.9 b,d
ORE	0.3	1.7 ± 1.2 b,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
ORE	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
CIN	0.1	3.9 ± 1.0 c	2.8 ± 1.8 b	1.7 ± 1.2 b,d
CIN	0.3	3.0 ± 1.7 b,c	2.0 ± 0.8 b	<1.0 ± 0.0 d
CIN	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
LEM	0.1	3.8 ± 0.5 c	4.0 ± 0.9 c	2.6 ± 1.4 b
LEM	0.3	1.7 ± 1.2 b,d	2.4 ± 1.3 b	<1.0 ± 0.0 d
LEM	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
8 °C				
Control	-	4.9 ± 0.3 a	5.8 ± 0.3 a	7.3 ± 0.6 b
PBS	-	3.9 ± 0.8 c	5.0 ± 0.5 a	6.2 ± 0.4 a,b
HP	-	2.2 ± 1.3 d	3.0 ± 1.0 c,d	4.3 ± 0.2 a,c
Water	-	3.8 ± 0.6 c	4.8 ± 0.4 a	6.6 ± 0.0 b
ORE	0.1	2.8 ± 0.4 d	2.9 ± 2.0 c,d	2.2 ± 1.9 d
ORE	0.3	2.0 ± 0.1 d,e	1.3 ± 0.7 e	<1.0 ± 0.0 e
ORE	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
CIN	0.1	3.6 ± 0.0 c	2.8 ± 0.8 c	2.0 ± 0.2 d,e
CIN	0.3	2.4 ± 0.3 d	1.3 ± 0.5 e	<1.0 ± 0.0 e
CIN	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.1	3.3 ± 0.8 c	1.5 ± 0.0 d,e	1.5 ± 0.0 d,e
LEM	0.3	<1.0 ± 0.0 e	1.1 ± 0.0 e	<1.0 ± 0.0 e
LEM	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e

[a]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; ORE: Oregano Essential Oil; CIN: Cinnamon Essential Oil; LEM: Lemongrass Essential Oil
[b]Values represent average mean of three replications. Standard deviation is presented following mean value.
[c]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Table 5.1 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 1-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	5.8 ± 0.6 a	5.5 ± 0.1 a	6.8 ± 0.7 a
PBS	-	5.0 ± 0.4 b	5.2 ± 0.4 b	5.0 ± 0.5 b
HP	-	4.1 ± 0.5 c	3.4 ± 0.7 c	3.2 ± 0.5 c,d
Water	-	5.1 ± 0.2 b	5.2 ± 3.0 b	5.1 ± 0.2 b
Carvacrol	0.1	2.5 ± 1.7 d	2.0 ± 1.7 d	1.7 ± 1.7 d,e
Carvacrol	0.3	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
Carvacrol	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
Cinnamaldehyde	0.1	4.5 ± 0.2 c	3.7 ± 0.5 c	2.7 ± 1.5 c,d
Cinnamaldehyde	0.3	3.5 ± 0.4 c	<1.0 ± 0.0 e	<1.0 ± 0.0 e
Cinnamaldehyde	0.5	<1.0 ± 0.0 e	<1.0 ± 0.0 e	<1.0 ± 0.0 e
Citral	0.1	4.9 ± 0.6 c	4.4 ± 0.5 c	2.8 ± 1.6 c,d
Citral	0.3	3.9 ± 0.7 c	3.0 ± 0.3 c,d	1.7 ± 1.1 d,e
Citral	0.5	2.0 ± 1.4 d,e	<1.0 ± 0.0 e	<1.0 ± 0.0 e

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Table 5.2 *Escherichia coli* O157:H7 Population on Organic Baby Spinach after 2-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	6.5 ± 0.1 a	6.8 ± 0.2 a	6.4 ± 0.6 a
PBS	-	5.3 ± 0.1 b	5.2 ± 0.1 b	5.1 ± 0.1 b
HP	-	3.0 ± 1.9 c	4.3 ± 0.9 b	4.5 ± 0.4 b
Water	-	4.5 ± 0.4 b	4.5 ± 0.5 b	5.7 ± 0.7 a,b
Carvacrol	0.1	3.8 ± 0.7 b,c	2.8 ± 1.6 c	4.2 ± 1.0 b
Carvacrol	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Carvacrol	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.1	3.9 ± 0.7 b	2.2 ± 1.0 c	<1.0 ± 0.0 d
Cinnamaldehyde	0.3	1.8 ± 1.4 c,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.5	1.6 ± 1.0 c,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Citral	0.1	4.7 ± 0.8 b	4.4 ± 0.5 b	4.5 ± 0.7 b
Citral	0.3	4.0 ± 0.6 b	2.1 ± 2.0 c	1.8 ± 1.4 c,d
Citral	0.5	3.1 ± 1.9 b,c	1.5 ± 0.9 c,d	1.7 ± 1.2 c,d

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

Table 6.1 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 1-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	5.8 ± 0.3 a	5.7 ± 0.2 a	5.7 ± 0.2 a
PBS	-	4.7 ± 0.8 b	4.7 ± 0.2 b	4.8 ± 0.4 b
HP	-	2.8 ± 0.6 c	3.4 ± 0.4 c	3.0 ± 0.1 c
Water	-	4.9 ± 0.7 b	4.7 ± 0.7 b	4.9 ± 0.5 b
Carvacrol	0.1	2.4 ± 0.6 c	1.5 ± 0.5 c,d	1.2 ± 0.3 d
Carvacrol	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Carvacrol	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.1	3.4 ± 0.4 b,c	3.5 ± 1.1 c	3.4 ± 0.8 b,c
Cinnamaldehyde	0.3	2.9 ± 1.6 c	1.8 ± 1.4 c,d	2.1 ± 1.0 c,d
Cinnamaldehyde	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Citral	0.1	3.9 ± 1.0 c	3.6 ± 1.3 c	4.0 ± 1.0 c
Citral	0.3	3.6 ± 2.3 c	2.0 ± 1.7 c,d	2.1 ± 1.0 c,d
Citral	0.5	1.8 ± 1.3 c,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 6.2 *Escherichia coli* O157:H7 Population on Organic Mature Spinach after 2-minute Plant-Derived Compound Treatment Held at 4 °C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	4.4 ± 0.3 a	5.9 ± 0.7 b	6.4 ± 0.2 b
PBS	-	4.4 ± 0.7 a	4.6 ± 1.0 a	4.3 ± 0.9 a
HP	-	3.9 ± 1.0 a	3.3 ± 1.1 a	3.5 ± 0.7 a
Water	-	4.7 ± 1.0 a	4.3 ± 0.8 a	4.3 ± 0.9 a
Carvacrol	0.1	2.8 ± 1.6 c	1.6 ± 1.0 d	<1.0 ± 0.0 d
Carvacrol	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Carvacrol	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.1	3.9 ± 0.5 a	3.3 ± 0.8 a,e	<1.0 ± 0.0 d
Cinnamaldehyde	0.3	3.0 ± 0.7 c	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.5	1.1 ± 0.1 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Citral	0.1	3.9 ± 1.3 a	3.9 ± 0.3 a	2.7 ± 1.5 c
Citral	0.3	2.2 ± 1.5 c,d	1.3 ± 0.5 d	<1.0 ± 0.0 d
Citral	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 7.1 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 1-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

		Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
Treatments	Conc. (%)	Day 0	Day 1	Day 3
Control	-	6.0 ± 0.6 a	5.8 ± 1.1 a	5.4 ± 0.5 a
PBS	-	4.6 ± 0.7 b	4.1 ± 0.6 b	4.5 ± 0.7 b
HP	-	2.1 ± 2.0 c,d	3.2 ± 0.2 c	3.3 ± 0.6 c
Water	-	4.4 ± 0.8 b	4.2 ± 0.5 b	4.7 ± 0.9 b
Carvacrol	0.1	2.7 ± 0.4 c	1.3 ± 0.7 d	1.2 ± 0.3 d
Carvacrol	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Carvacrol	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.1	4.3 ± 0.5 b	3.6 ± 0.6 b,c	2.0 ± 1.7 c,d
Cinnamaldehyde	0.3	3.9 ± 0.3 b,c	2.2 ± 1.5 c	1.7 ± 1.3 c,d
Cinnamaldehyde	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Citral	0.1	4.5 ± 0.7 b	3.5 ± 0.7 b,c	3.9 ± 1.6 b,c
Citral	0.3	3.6 ± 0.7 b,c	2.0 ± 1.7 c,d	2.0 ± 0.9 c,d
Citral	0.5	1.8 ± 0.7 d	<1.0 ± 0.0 d	1.1 ± 0.1 d

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 7.2 *Escherichia coli* O157:H7 Population on Organic Romaine Lettuce after 2-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	6.4 ± 0.9 a	6.4 ± 1.1 a	6.2 ± 1.3 a
PBS	-	4.7 ± 1.1 b	4.9 ± 0.4 b	4.6 ± 1.0 b
HP	-	2.6 ± 1.6 c	3.0 ± 1.8 c	3.3 ± 0.7 b,c
Water	-	4.1 ± 1.0 b	4.6 ± 1.8 b	4.5 ± 0.9 b
Carvacrol	0.1	2.7 ± 1.5 c	1.7 ± 1.2 c,d	1.2 ± 0.4 d
Carvacrol	0.3	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Carvacrol	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.1	4.0 ± 0.4 b,c	2.9 ± 1.6 c	<1.6 ± 1.1 d
Cinnamaldehyde	0.3	3.4 ± 0.7 b,c	1.2 ± 0.3 d	<1.0 ± 0.0 d
Cinnamaldehyde	0.5	<1.0 ± 0.0 d	<1.0 ± 0.0 d	<1.0 ± 0.0 d
Citral	0.1	4.6 ± 0.7 b	4.5 ± 0.6 b	3.8 ± 1.1 b,c
Citral	0.3	2.1 ± 1.0 c	2.1 ± 1.6 c	1.9 ± 1.5 c
Citral	0.5	1.5 ± 0.8 c,d	<1.0 ± 0.0 d	<1.0 ± 0.0 d

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral

[2]Values represent average mean of three replications. Standard deviation is presented following mean value.

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

[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 8.1 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 1-minute Plant-Derived Compound Treatment Held at 4 ^{o}C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population (log_{10} CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	5.7 ± 0.9 a	5.3 ± 0.4 a	4.8 ± 0.3 a
PBS	-	3.0 ± 1.7 b	3.9 ± 0.5 b	3.4 ± 0.4 b
HP	-	1.7 ± 0.6 c	2.5 ± 0.2 b,c	1.6 ± 0.5 c
Water	-	2.0 ± 1.7 b,c	3.6 ± 0.4 b	3.6 ± 0.0 b
Carvacrol	0.1	1.4 ± 0.4 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Carvacrol	0.3	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Carvacrol	0.5	<1.0 ± 0.0 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Cinnamaldehyde	0.1	3.0 ± 1.7 b	2.4 ± 0.1 b,c	<1.0 ± 0.0 c
Cinnamaldehyde	0.3	2.8 ± 0.1 b	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Cinnamaldehyde	0.5	1.5 ± 0.6 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Citral	0.1	2.9 ± 1.6 b	2.3 ± 0.6 b,c	<1.0 ± 0.0 c
Citral	0.3	2.3 ± 0.4 b,c	<1.0 ± 0.0 c	<1.0 ± 0.0 c
Citral	0.5	1.3 ± 0.5 c	<1.0 ± 0.0 c	<1.0 ± 0.0 c

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[2]Values represent average mean of three replications. Standard deviation is presented following mean value.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 log_{10} CFU

Table 8.2 *Escherichia coli* O157:H7 Population on Organic Iceberg Lettuce after 2-minute Plant-Derived Compound Treatment Held at 4 °C

Treatments	Conc. (%)	Surviving *E. coli* O157:H7 Population ($\log_{10}$ CFU g^{-1})		
		Day 0	Day 1	Day 3
Control	-	4.4 ± 0.8 a	4.8 ± 1.5 a	4.6 ± 1.4 a
PBS	-	3.8 ± 0.4 a,b	3.6 ± 1.0 b	3.8 ± 0.4 a,b
HP	-	1.4 ± 1.3 c	1.5 ± 1.3 b	1.3 ± 1.1 b
Water	-	3.4 ± 0.3 a,b	3.5 ± 1.0 a,b	4.0 ± 1.0 a,b
Carvacrol	0.1	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Carvacrol	0.3	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Carvacrol	0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Cinnamaldehyde	0.1	2.0 ± 0.4 b	0.8 ± 0.8 c	0.0 ± 0.0 c
Cinnamaldehyde	0.3	0.8 ± 0.8 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Cinnamaldehyde	0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Citral	0.1	2.4 ± 1.1 b	2.8 ± 0.6 a,b	1.5 ± 1.4 b
Citral	0.3	0.6 ± 0.7 c	0.0 ± 0.0 c	0.0 ± 0.0 c
Citral	0.5	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c

[1]PBS: Phosphate Buffered Saline; HP: Hydrogen Peroxide; CAR: Carvacrol; CIN: Cinnamaldehyde; CIT: Citral
[2]Values represent average mean of three replications. Standard deviation is presented following mean value.
[3]Mean values with letters a, b, c, etc. provide evidence of significant difference ($P<0.05$), with different letters representing statistical significance, and same letters representing no statistical significance across both rows and columns.
[d]Level of detection for values did not proceed below 10^1 $\log_{10}$ CFU

VITA

Jordan James Denton

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Master of Science

Thesis: ANTIMICROBIAL EFFICACY OF ESSENTIAL OILS AND THEIR PRIMARY CONSTITUENTS AGAINST *ESCHERCHIA COLI* O157:H7 ON ORGANIC LEAFY GREENS

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