

Effect of Alternative Household Sanitizing Formulations Including: Tea Tree Oil, Borax, and
Vinegar, to Inactivate Foodborne Pathogens on Food Contact Surfaces

Ashley Elizabeth Zekert

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Committee Members:

Robert C. Williams, Chair

Joseph D. Eifert

Renee R. Boyer

Susan S. Sumner

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Blacksburg, Virginia

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ABSTRACT

Current trends indicate that American consumers are increasingly selecting products that they believe to be environmentally friendly or “natural.” In the kitchen, this trend has been expressed through greater desire for using alternative or “green” sanitizers instead of bleach or other common chemical sanitizers. The purpose of this work was to evaluate the effectiveness of one suggested alternative, tea tree oil, as a food contact surface sanitizer. Three foodborne bacterial pathogens (*Listeria monocytogenes* N3-031 serotype 1/2a, *Escherichia coli* O157:H7 strain E009, and *Salmonella* Typhimurium ATCC 14028) were applied separately onto three different food contact surfaces (high density polyethylene, glass, and Formica® laminate). Tea tree oil (TTO), borax, and vinegar (5% acetic acid) were applied individually as well as in combination for a total of seven treatment solutions. In addition, household bleach (6.15% sodium hypochlorite), sterile reverse osmosis (RO) water, and no applied treatment were used as controls. Treatments were tested using an adaptation of the Environmental Protection Agency DIS/TSS-10 test method, whereby each contaminated surface was treated with 100 µl of test solution and held for 1 min followed by submersion in neutralizing buffer and microbiological plating. Samples (0.1 ml) were plated onto TSA and incubated at 35°C for 48 h prior to colony counting. Bleach reduced microbial populations significantly with greater than 5-log reduction reported for all surfaces (Formica® laminate, glass, and HDPE), against *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium*. TTO produced reductions between four and five logs for *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* and was not statistically different from the vinegar treatment ($P>0.05$). All combination recipes,

including the borax treatment, failed to produce reductions in microbial populations at levels considered to be appropriate for food contact surface sanitizers. Surface type did not play a significant role in the effectiveness of the treatment ($P>0.05$). Although TTO and vinegar did reduce pathogen populations on surfaces, reductions were not sufficient enough to be considered an equally effective alternative to household bleach.

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INTRODUCTION AND JUSTIFICATION

In today's society, consumers are striving to be more environmentally friendly by purchasing products that claim to be "green" or "natural." According to the Environmental Protection Agency (EPA) (1991), a product's environmental element is one of the most important factors in their purchasing decision. Recyclability, use of recycled content, and reduction of toxic content are some of the important green attributes consumers are looking for in a product (Chen 2001).

According to *Conscious Consumer Report*, 22% of eco-conscious consumers always use environmentally friendly cleaning products, and 55% of eco-conscious consumers sometimes use environmentally friendly cleaning products (Miller and Washington 2009). In terms of food contact surface sanitation, environmental concern has led consumers to replace conventional antimicrobials with environmental friendly or "green" alternative products (Rutala, Barbee et al. 2000). Consumers have also turned to "home mixtures" as safe alternatives for traditional cleaning products (Parnes 1997). More specifically, consumers are looking for alternatives to bleach because they want to reduce chlorine waste in the environment. This trend has led to many formulation recommendations by environmentalist groups (Bauer and Beronio 1995; Parnes 1997).

Many of these consumers have turned to homemade sanitizing formulations found via public websites for sanitizing food contact surfaces. These recipes contain ingredients such as borax, vinegar and essential oils such as tea tree oil (*Melaleuca alternifolia*). The use and claims of sanitizers is highly regulated in the food processing and restaurant industries. Products that claim to be effective antimicrobials on inanimate objects must be tested for efficacy (Lawrence and Bennet 2001). Research has shown that borax and vinegar alone are not effective

alternatives to bleach (Bauer and Beronio 1995). Tea tree oil, however, has been shown to be an effective skin antiseptic but no research has been shown to prove its effectiveness as a food contact sanitizing treatment (Raman, Weir et al. 1995).

Research has not shown the efficacy of alternative antimicrobials, such as borax, vinegar, and tea tree oil in combination with one another as recommended by public websites. Using suggested recipes found online, the effectiveness of the sanitizing treatments against *Escherichia coli* O157:H7, *Salmonella enterica* serotype Typhimurium, and *Listeria monocytogenes* strains was analyzed in the current study. Treatments were tested on Formica®, high density polyethylene (HDPE), and glass surfaces; all of which are contact surfaces commonly found in the kitchen. The intent of this work is to provide consumers with science-based information regarding the efficacy of recommended alternative sanitizing recipes.

OBJECTIVES

- To determine the efficacy of tea tree oil as a surface sanitizer to inactivate foodborne pathogens (*Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes*).
- To determine the efficacy of alternative household sanitizing formulations containing tea tree oil, borax and or vinegar to inactivate foodborne pathogens (*Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes*).
- To determine the influence of surface type on the efficacy of the alternative household sanitizers tested to inactivate foodborne pathogens (*Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes*).

LITERATURE REVIEW

Importance of Cross Contamination to Foodborne Illness

According to the Center for Disease Control (CDC), approximately 76 million foodborne illnesses occur each year in the United States. Of those 76 million, there are 325,000 hospitalizations and 5,000 deaths (CDC 2005). Several factors must be in place for foodborne illness to occur. An organism must contaminate the food, the organisms must grow or survive within the food, and the food must be ingested (Marriott and Gravani 2006).

Contamination of food commonly occurs by cross contamination (Scott 2000). Cross contamination is the transfer of microorganisms to foods from other foods, cutting boards, utensils, food handlers hands, wet cloths, etc (CDC 2005). Often wet cloths are used in the kitchen to “clean” a contaminated surface; however, Scott et al (1982) found that 48% of dish cloths and 52% of cleaning cloths were contaminated with over 100 organisms/20 cm² (Scott and Bloomfield 1990). Tebutt (1986) found 74% of cloths intended for food contact surfaces were contaminated with at least one of the following organisms: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, or *Clostridium perfringens*, with over half having colony counts greater than 10⁵ CFU/ cm² (Abrhami, Tall et al. 1994). It is ironic that the use of contaminated cloths to “clean” surfaces may in fact contaminate the surface even more. A contaminated surface increases the opportunity for the potential transfer of microorganisms to food. In a similar study, Scott and Bloomfield et al (1990) found that 10 to 24% of home food contact surfaces were contaminated with more than 200 organisms/20 cm².

Cross contamination accounts for 6% of foodborne illnesses, some of which occur from a low infectious dose (Bloomfield and Scott 1997). Furthermore, the risk of microorganism

transfer usually increases with the increase of microbial load (Moore, Blair et al. 2007). During slaughter, meat and poultry carcasses can be contaminated by contact with intestinal material (CDC 2005). Risk for cross contamination also increases when food debris remain on food contact surfaces (Moore, Blair et al. 2007). Debris can act as a food source for microorganisms allowing bacterial adhesion and growth (Bredholt, Maukonen et al. 1999; Moore, Blair et al. 2007).

Appropriate preventative measures may eliminate or reduce the risk of cross contamination. A recent study reported that 30-71% of consumers surveyed admitted to using the same cutting board or surface for preparation of raw meats and ready-to-eat foods (Moore, Blair et al. 2007). Using separate cutting boards or plates for raw food and ready-to-eat products can be an effective preventative measure (CDC 2005). Survey results suggest that 60% of consumers wash the surface of preparation area of raw and ready-to-eat foods (Moore, Blair et al. 2007). Washing food contact surfaces with something other than water, for example disinfectants or antimicrobials, may eliminate microorganisms that are present. Implementing proper food handler hygiene, i.e. washing hands, is a major key to preventing cross contamination. When preparing foods, it is important to appropriately wash hands and all surfaces in order to reduce potential pathogens from contaminating foods (Mosteller and Bishop 1993).

Food Contact Surfaces

A food contact surface is any surface that may come in contact with food. This includes but is not limited to processing equipment, utensils, food preparation areas, and food storage areas. Stainless steel, rubber, plastic, fiberglass, concrete, mesh belts, soft metals, and wood are

common surfaces found in the processing environment (Schmidt and Rodrick 2003). The domestic environment has similar surfaces to the processing environment. Some of the more common surfaces include laminate and granite countertops; plastic, wood, or glass cutting boards; stainless steel sinks and appliances. Food contact surfaces sometimes harbor foodborne pathogens (Bloomfield and Scott 1997). As a result, these surfaces may pose a constant risk of transferring contaminants. Therefore, all such surfaces should have antimicrobial treatment prior to use (Bloomfield and Scott 1997).

Bacterial Adherence

Bacterial contamination of the domestic kitchen is common. Scott et al. (2000) surveyed 200 homes and found that 90% of the domestic kitchens were contaminated with *Pseudomonas* (Kagan, Aiello et al. 2002). A subsequent study found that 69% of domestic kitchens were contaminated with *Enterobacteriaceae* (Kagan, Aiello et al. 2002). Some of the most common contaminated kitchen surfaces include kitchen sinks, draining boards, cleaning cloths and mops and dish cloths (Kagan, Aiello et al. 2002). Failure to remove bacteria from a food contact surface can greatly increase the risk of foodborne illness by cross contamination. When bacterial colonies dry, a significant reduction of recoverable organisms results (Scott and Bloomfield 1990). However, bacteria on dry surfaces have the ability to survive for an extended period of time due to starvation stress response (Yousef and Juneja 2003). Starvation stress is defined as “the survival of bacteria in environments with low or no available nutrients” (Dickson and Frank 1993). In low nutrient environments, the stringent response is activated (Yousef and Juneja 2003). This stringent response mechanism allows bacteria to regulate their metabolism by

induction of stationary phase genes. These genes ultimately stimulate biosynthetic pathways (Yousef and Juneja 2003).

Bacteria have the capability to attach to any surface including but not limited to glass, stainless steel, polypropylene, rubber, wood (Abrhami, Tall et al. 1994; Bower, McGuire et al. 1996). As a result, in the domestic environment, foodborne illness caused by cross contamination is a major issue (DeVere and Purchase 2007). Once attached to the surface, given acceptable conditions, bacteria have the ability to multiply (Scott and Bloomfield 1990). The colonization of cells results in extracellular polysaccharide production, often leading to a microbial ecosystem formation called a biofilm. It is metabolically favorable for microorganisms to form biofilms because nutrients are more concentrated at an interface (Bower, McGuire et al. 1996). Bacterial cells can be shed from the biofilms potentially contaminating foods (Sofos 2009). Contamination of foods can occur at high enough levels to cause illness. Potential for contamination increases in foods that are stored at abusive temperatures (Abrhami, Tall et al. 1994). Many factors influence an organism's ability to adhere to a surface. Factors that affect adherence include surface characteristics, organism characteristics, contact time, and nutrient availability (Bower, McGuire et al. 1996; Kusumaningrum, Riboldi et al. 2003; Cliver 2006; Whitehead and Verran 2006).

Surface roughness and hydrophobicity can significantly affect the attachment of an organism. Generally, the rougher the surface, there is greater risk for biofilm formation (Whitehead and Verran 2006). Whitehead et al. (2006) defines roughness as “the irregularities in the surface texture which are inherent in the production process but excluding waviness and errors of form” (Whitehead and Verran 2006). The roughness of a surface increases the surface area available for colonization. Organisms adhere to hydrophobic surfaces, such as plastics,

better than hydrophilic surfaces such as stainless steel (Lee, Lopes et al. 2007). This is due to the hydrophobic surface features present on the cell (Doyle 2000).

Characteristics of an organism can also aid in the attachment of bacteria to a surface. Some bacteria use structures such as flagella, fimbriae, pili, or other extracellular polymeric substances (Bower, McGuire et al. 1996; Cliver and Riemann 2002). These structures help anchor the organism to the surface (Bower, McGuire et al. 1996). The greatest microbial retention occurs when surface characteristics are similar to the organism's surface (smooth versus rough) (Whitehead and Verran 2006).

Microorganisms can attach within hours or it may take days depending on surface characteristics (Cliver and Riemann 2002). Depending on the organism, nutrient availability or organic soil may play an important role in the adhesion rate. When surfaces are dry, minimal bacterial growth and survival can be attributed to reduced bacterial attachment and lack of available nutrients (Kusumaningrum, Riboldi et al. 2003). Surface soiling then may preserve viability (Scott and Bloomfield 1990). Scott et al. (1990) found that on soiled surfaces Gram positive and some Gram negative bacteria were recoverable in significant numbers for 4 hours and up to 24 to 48 hours in some cases. Significant numbers of bacteria can be transferred to hands, dish cloths, food, etc. from contact with contaminated surface (Scott and Bloomfield 1990). For some organisms, like *Listeria spp.*, research has shown that low nutrient environments may enhance adherence and biofilm formation (Gustafson, Liew et al. 1998; Cliver and Riemann 2002).

Increasing biofilm age is associated with increasing bacterial resistance. Established biofilms are difficult to remove by cleaning and sanitizing (Mosteller and Bishop 1993). Since the surface hinders the access of disinfectant to the cell, cells that are attached to a surface are

more resistant to disinfection than planktonic cells which are vulnerable from all sides (Bower, McGuire et al. 1996). To prevent bacterial attachment, it is important to effectively clean and sanitize the surface before biofilm buildup (Mosteller and Bishop 1993). Despite the fact that drying can significantly reduce the numbers of bacteria, it is not reliable as the sole sanitizing method (Abrhami, Tall et al. 1994).

Organisms of Focus

***Salmonella enterica* serotype Typhimurium:** *Salmonella spp.* have been recognized for their ability to cause illness for more than 100 years (CDC 2008). Concern regarding *Salmonella spp.* has grown over the years due to evolution of the organism and its increasing ability to survive in unfavorable environments. There are more than 2400 serotypes of *Salmonella* (Schmidt and Rodrick 2003). Fifty-nine percent of all serotypes are *Salmonella enterica* subspecies I (Brenner, Villar et al. 2000). Of these subspecies, *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium are the most common causes of foodborne illness (Schmidt and Rodrick 2003). Responsible for 15% of foodborne illnesses in the United States, *Salmonella* Typhimurium has the ability to infect both humans and animals (Cliver 2006).

Salmonella is a Gram negative, non-spore forming, facultative anaerobe that is a member of the *Enterobacteriaceae* family (Cliver 2006). *Salmonella spp.* have the capability to grow in a wide range of conditions. They can grow in temperatures between 5.2 - 46.2°C but prefer optimal temperatures of 35 - 43°C (Schmidt and Rodrick 2003). *Salmonella* can grow at pH between 3.8 and 9.5; however, they prefer a more neutral pH (6.5 - 7.5) (Schmidt and Rodrick 2003; Cliver 2006). *Salmonella* requires environments with high water activities (>0.93) for growth, but can survive for extended periods under dry conditions (Schmidt and Rodrick 2003;

Cliver 2006). Exposure to environments of reduced water availability can induce heat shock proteins increasing the heat tolerance of the organism (Cliver 2006). At low sodium chloride concentrations (4%), the organism still has the ability to grow (Schmidt and Rodrick 2003). The hardiness *Salmonella spp.* enables it to survive and persist in many extreme environments.

Salmonella is considered to be practically ubiquitous in the environment, but is most commonly found in the intestinal tract of animals and humans. As a result, foods of animal origin are the most likely to be contaminated (Schmidt and Rodrick 2003). Undercooked eggs, raw meats and poultry, unpasteurized dairy products, shrimp, custards, sauces, and cream dishes are among the foods that are most susceptible to *Salmonella* contamination (Roberts 2001). However, any food can become contaminated, including fruits and vegetables, often from fecal contamination (CDC 2008). *Salmonella* can be transferred to any food via cross contamination from a contaminated food handler or any contaminated surface (CDC 2008).

Ingestion of contaminated food products may result in Salmonellosis. The average infective dose is estimated to be 10^4 cells but varies depending on the environment, virulence, and the individual consuming the contaminated food (Cliver 2006). Symptoms often develop within 12 to 72 hours and include diarrhea, nausea, fever, and abdominal cramps (CDC 2008). Symptoms often last 4-7 days and are typically self-limiting (Cliver 2006; CDC 2008). Determination of the illness severity is dependent on the susceptibility of the infected individual. The elderly, children, and the immunocompromised are the most susceptible to Salmonellosis (Roberts 2001). *Salmonella spp.* are responsible for 40,000 reported cases per year in the U.S. and 400 of these will result in death (CDC 2008). Often mild cases are not reported, so it is estimated that the number of cases is as much as 30 times greater (CDC 2008).

***Escherichia coli* O157:H7:** *Escherichia coli* is a Gram negative, non-spore forming, small rod. *E. coli* is facultative anaerobe whose optimal growth temperature is approximately 37°C (Montville and Matthews 2005). It is acid tolerant with the ability to survive at pH as low as 4 - 4.5 (Montville and Matthews 2005). Induction of *Escherichia coli*'s acid tolerance can allow survival for greater than 28 days at refrigeration temperatures. Acid tolerance can also induce other stress responses such heat, radiation, and antimicrobial tolerance (Montville and Matthews 2005).

Escherichia coli organisms are very diverse; and are classified based on virulence, pathogenicity, clinical symptoms, and serotypes. *E. coli* is categorized as follows: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Diffusely Adhering (DAEC), Enteroaggregative (EAEC), and Enterohemorrhagic (EHEC) (Montville and Matthews 2005). Some *E. coli*, such as O157:H7, are harmful and have the capability of inducing disease such as Hemolytic Uremic Syndrome (HUS) and Hemorrhagic Colitis (HC) (Jay 2000; Montville and Matthews 2005; CDC 2009). The average infectious dose of pathogenic *E. coli* is estimated to be 10^6 - 10^8 cells (Feng and Weagant 2008). However, the EHEC serotypes have been found to have an infectious dose as low as 10 - 100 cells (Feng and Weagant 2008). *Escherichia coli* O157:H7 is the primary cause of EHEC associated disease and is the most publicized *E. coli* (Montville and Matthews 2005). The symptoms of *E. coli* infection are variable depending on the category of *E. coli* (CDC 2009). Usually an incubation period of 3 to 4 days after exposure can be expected (CDC 2009). Symptoms include mild abdominal pain with or without worsening bloody diarrhea (CDC 2009). In a small number of cases, HUS development is seen approximately 7 days after initiation of symptoms (CDC 2009). HUS and HC are caused by toxin (stx) producing strains of *E. coli* such as O157:H7 (Jay 2000). The toxin produced

destroys the intestinal lining of the infected individual (FDA 2009). Approximately 2% to 7% of infections caused by *E. coli* O157:H7 will get HUS (Jay 2000). Elderly, children, and the immunocompromised are most susceptible for development of HUS and HC (Montville and Matthews 2005).

Escherichia coli is commonly found in the environment and is usually an indicator of fecal contamination (CDC 2009). Most strains are harmless and are found naturally in the gut and functions to suppress harmful bacteria (FDA 2009). Consumption of raw contaminated foods, cross contamination, consumption of contaminated water, and inadequate personal hygiene are the most common means of *E. coli* contamination (CDC 2009). Foods most commonly associated with *E. coli* include ground beef, roast beef, raw milk, produce, and unpasteurized apple cider (Montville and Matthews 2005). *E. coli* O157:H7 is believed to be responsible for the majority of cross contamination illnesses (Montville and Matthews 2005).

***Listeria monocytogenes*:** *Listeria monocytogenes* was first described by Murray et al. (1926), who initially named it *Bacterium monocytogenes* after its monocytosis characteristics observed in infected guinea pigs and rabbits (Farber and Peterkin 1991; Cossart 2007). In 1940, the organism adopted its current name, *Listeria monocytogenes*. However, it was not until 1983 that the first foodborne outbreak was reported (Cossart 2007). As a result, interest in the organism increased significantly during the 1980s (Farber and Peterkin 1991). *L. monocytogenes* is one of six species of *Listeria* (Cossart 2007). Other species include *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*; however, *L. monocytogenes* is the only species that is of significant public health concern (Schmidt and Rodrick 2003; Cossart 2007). Thirteen serovars comprise *L. monocytogenes*. Serovars 1/2a, 1/2b, 4b make up 95% of human isolates. Serovar

4b accounting for 33 - 50% of sporadic human cases and is responsible for most of the large outbreaks (Schmidt and Rodrick 2003).

Listeria monocytogenes is a Gram positive, non-sporeforming, facultative anaerobic rod (Farber and Peterkin 1991; Ryser and Marth 2007). Cells can be found in single units or small chains (Ryser and Marth 2007). *Listeria monocytogenes* can grow at temperatures as low as -0.4°C and at temperatures as high as 45°C with an optimal temperature range of 30 - 37°C (Schmidt and Rodrick 2003). As a stress response, *L. monocytogenes*, activates cold shock proteins, which enable it to survive at refrigeration temperatures (Puttamreddy, Carruthers et al. 2008). Growth can occur in a pH as low as 4.39 and as high as 9.4 with an optimal pH of 7.0. *Listeria monocytogenes* prefers growth in an environment with a water activity of ≥ 0.92 (Schmidt and Rodrick 2003). It can grow in environments of 10-12% sodium chloride and survive at higher salt concentrations (Montville and Matthews 2005). At room temperature (20-25°C), flagellin production and assembly occurs resulting in tumbling motility. At optimal growth temperature (37°C), flagellin production is significantly reduced (Ryser and Marth 2007). Under aerobic conditions, all strains utilize glucose to form lactate, acetate, and acetoin; however, in an anaerobic environment acetoin is not produced and hexose and pentose are used (Farber and Peterkin 1991; Ryser and Marth 2007).

Listeriosis is the infection that results as a consequence of ingesting foods contaminated with *L. monocytogenes*. Other routes of infection include: direct contact with the environment; contact with naturally infected animals; or cross infection between newborn infants (McLauchlin, Mitchell et al. 2004). Each year, there are 2,500 reported cases in the U.S., 500 of which result in death (CDC 2008). However, it is thought that cases of Listeriosis are under diagnosed (McLauchlin, Mitchell et al. 2004). The infective dose varies with virulence factors,

the food matrix, and the susceptibility of the victim, (McLauchlin, Mitchell et al. 2004; FDA 2009). The elderly, pregnant women, infants and children, and the immunocompromised are the most susceptible to Listeriosis (CDC 2008). Symptoms include flu-like symptoms that can result in septicemia, meningitis, encephalitis, and intrauterine/cervical infection often resulting in abortion in pregnant women (FDA 2009). The incubation period is variable between 1-91 days (Cliver and Riemann 2002).

Listeria monocytogenes is commonly found in the environment residing in soil or water and is often carried by asymptomatic animals (CDC 2008). Eleven to fifty-two percent of animals are carriers of the organism, with 45% of pigs and 24% of cattle harboring *L. monocytogenes* (Schmidt and Rodrick 2003). Foods most commonly associated with *L. monocytogenes* contamination include: unpasteurized milk, ready-to-eat (RTE) foods, and raw vegetables that have been fertilized with contaminated manure (Roberts 2001; Ryser and Marth 2007). In the processing environment, the hardiness of *L. monocytogenes* often allows for survival in floor drains, stagnant water, floors, and in food residue on processing equipment (Schmidt and Rodrick 2003). Since *L. monocytogenes* can grow at refrigeration temperature, post-processing contamination of RTE food products is the main concern of contamination. Consequently, the United States Department of Agriculture (USDA) strongly enforces a zero tolerance policy of *L. monocytogenes* in processed RTE products prohibiting sales of any contaminated foods (Cliver and Riemann 2002).

Alternative Sanitizers

Sanitizer

A sanitizer is a substance that reduces microbial contaminants to a level that is considered safe (Marriott and Gravani 2006). Often sanitizers are confused with disinfectants. Disinfectants destroy all vegetative cells that are present on inanimate objects. Sanitizers are either categorized as food contact or non-food contact (Lawrence and Bennet 2001). By definition, food contact sanitizers must reduce the microbial contamination 99.999% (5-log) in 30 seconds at 25°C (Lawrence and Bennet 2001). Food contact surface sanitizers are those sanitizers used in the processing industry on equipment and utensils as well in the restaurant industry (Lawrence and Bennet 2001). In the domestic environment, the use of sanitizers would most closely mimic the use of sanitizers in the restaurant environment.

Domestic sanitizers are most commonly used to reduce the risk of cross contamination of microorganisms that may lead to food spoilage. They also serve the purpose of preventing the spread of pathogens that could lead to illness. In the food-processing industry and in restaurants, sanitizers are used in conjunction with a Hazard Analysis Critical Control Points (HACCP) program (Lawrence and Bennet 2001). There is no HACCP plan in the domestic environment; however, sanitizers still serve the same functional goals. These functions include the ability to reduce outbreaks of pathogenic microorganisms, minimize the spread of nonpathogenic microorganism that are of public health significance, prevent development of undesired odors, deter the development of microbial end products, control of microorganism in reservoirs of infection of living animals, and increase shelf-life (Lawrence and Bennet 2001). Barker et al. (2003) found that wiping a counter in a model kitchen with a cloth resulted in the reduction of

microbial contamination risk by 38.6%. The risk reduction was not enough to consider the surface hygienic (Barker, Naeeni et al. 2003). This shows that the use of an antimicrobial agent is pertinent in reduction of microbial load.

There are several factors that affect the efficacy of a sanitizer (Marriott and Gravani 2006). Some of these factors include the exposure time, temperature, concentration, pH, presence of organic matter, water hardness, microbial population, and bacterial attachment (McDonnell and Russell 1999). In order to get the optimal results from the selected sanitizer it is important to use under the appropriate conditions. In addition to the optimal conditions, the ideal sanitizer must be economical, have easy application, shelf stability, limited odor, low toxicity, cleaning capability, penetrative power, residual activity, produce a 99.999% (5-log) reduction within 30 seconds, be active before and after dilution with hard water, not be affected by the presence of proteins or organic deposits, be noncorrosive, have a broad spectrum of activity against Gram positive and Gram negative microorganisms, be fast-acting, compatible with other chemicals, and not persist within the environment (Lawrence and Bennet 2001). DeVere et al. (2007) found that the use of antimicrobial products was most effective at reducing bacteria when products were used as instructed.

White Vinegar (5% Acetic acid)

The antimicrobial activity of distilled white vinegar (5% acetic acid) is primarily due to acetic acid. Acetic acid (CH_3COOH) is an organic acid that is commonly used in the food industry. Acetic acid is used in acidification in order to preserve foods, and as a surface spray or rinse on meat carcasses, and produce rinses to help reduce microbial populations (Parish, Beuchat et al. 2003).

Most foodborne bacterial pathogens cannot survive for long periods of time or grow at pH that is less than 4.5 (Parish, Beuchat et al. 2003). As a result, vinegar has shown to have bacteriostatic (inhibition of bacterial growth) and bactericidal (reduction of viable cells) effect on microorganism (Entani, Asai et al. 1998). Vinegar destroys microorganism by penetrating and disrupting the cell's membrane (Parish, Beuchat et al. 2003; Yousef and Juneja 2003; Marriott and Gravani 2006). The acid molecule then dissociates and acidifies the cell interior by accumulation of H⁺ ions (McDonnell and Russell 1999; Parish, Beuchat et al. 2003; Yousef and Juneja 2003; Marriott and Gravani 2006). Consequently, the homeostasis dissipation of the proton motive force is affected (Yousef and Juneja 2003).

The antimicrobial effect of vinegar is dependent on the concentration, the type of microorganism, the strain, and the contact time (Entani, Asai et al. 1998; Parish, Beuchat et al. 2003). Some microorganisms are more acid tolerant than others. Entani et al. (1998) found that the relationship between the log₁₀ of contact time for inactivation with the concentration of acetic acid is linear. Inoculum size did not have an effect on the effectiveness of the antimicrobial action and cells in log phase were more sensitive to vinegar compared to those in stationary phase (Entani, Asai et al. 1998).

Borax

Borax is a common product in the household described by borax manufacturer, 20 Mule Team®, as a laundry booster, multi-purpose cleaner, and home deodorizer. Other common names for borax (Na₂B₄O₇) include sodium borate, sodium baborate, sodium pyroborate, and sodium tetraborate (Windholz, Budavari et al. 1976). Borax has a mildly alkaline pH of 9.5

(Windholz, Budavari et al. 1976). It is a good scouring compound with bleaching action and biostatic activity (Ladaniya 2008).

There is limited research on the use and effectiveness of borax as an antimicrobial. Prior to the mid 1960s, borax was used to prevent harvest related infection; however, that practice was discontinued due to increasing concern of boron residue (Ladaniya 2008). Recently, borax has had growing interest because it is less toxic than some chemical pesticides (Ladaniya 2008). Menetrez et al. (2007) discovered that when a surface was inoculated with 10^5 CFU of *Stachybotrys chartarium*, the addition of borax reduced growth to minimal levels (Menetrez, Foarde et al. 2007).

Tea Tree Oil

Plant essential oils have been used for thousands of years (Gustafson, Liew et al. 1998). The antimicrobial characteristics of plant oils and extracts have been the source of many applications including food preservation, pharmaceuticals, alternative medicine, and natural therapies (Gustafson, Liew et al. 1998). Tea tree oil is derived from the Australian plant *Melaleuca alternifolia* (Carson and Riley 1995). Tea tree oil is slightly soluble in water, miscible with nonpolar solvents, and has a relative density of 0.885 to 0.906 (Carson and Riley 2001).

Tea tree oil has gained popularity recently due to its safe, natural, and antiseptic properties (56). It is composed of terpene hydrocarbons, mostly monoterpenes, sesquiterpenes, and their associated alcohols (Carson and Riley 1995). Terpenes are hydrocarbons polymers of isoprene (C_5H_8) (Carson and Riley 1995). The composition of tea tree oil is regulated by the international standard for tea tree oil (ISO 4730 Oil of *Melaleuca*-terpinen-4-ol type) (Carson,

Hammer et al. 2006). The standard specifies the limits for 14 of the 100 components of tea tree oil (Carson, Hammer et al. 2006). Tea tree oil is comprised of mostly terpinen-4-ol (>30%). Terpinen-4-ol (C₁₀H₁₈O) is a monocyclic terpene alcohol that is responsible for the broad-spectrum antimicrobial activity of tea tree oil (Cox, Mann et al. 1998).

Studies have shown that the antimicrobial activity of monoterpenes is a result of the damage it exerts on the cell membrane (Cox, Mann et al. 2000). Gustafson et al. (1998) examined *E. coli* under electron microscopy after exposure to tea tree oil and observed loss of cellular electron dense material and coagulation of cytoplasmic constituents (Gustafson, Liew et al. 1998). Some cell walls remained intact indicating that autolysis is a secondary event that occurred after cell death (Gustafson, Liew et al. 1998). Tea tree oil also induces K⁺ leakage and inhibits glucose-dependent respiration (Cox, Mann et al. 1998). Normally, entry and exit of compounds is highly regulated and the free passage of ions, such as, H⁺, K⁺, Na⁺, and Ca²⁺ is inhibited by the cytoplasmic membranes (Cox, Mann et al. 1998). Membrane permeability is necessary in the regulation of cellular functions necessary for maintaining energy status including membrane-coupled energy-transducing processes, solute transport, and regulation of metabolism, and turgor pressure control (Cox, Mann et al. 1998).

Although terpinen-4-ol is the main component of tea tree oil, plant antimicrobial compounds often include two or more active components that may react antagonistically or synergistically (Friedman, Henika et al. 2002). Carson et al. (2002) observed the individual components of tea tree oil (terpinen-4-ol, α -terpineol, and 1,8-cineole) on *Staphylococcus aureus* and discovered that none induced autolysis but all caused leakage of 260-nm-light-absorbing material (Carson, Mee et al. 2002). Tea tree oil kills a wide variety of microorganisms including Gram positive and Gram negative bacteria and yeasts (Carson, Hammer et al. 2006). Overall, its

antimicrobial action has been described to be similar to that of membrane-active disinfectants (Cox, Mann et al. 1998). Examples of membrane active disinfectants include: chlorhexidine, quaternary ammonia compounds, alcohols, and phenols (Samaranayake 2006). These disinfectants damage cell membrane causing leakage of cell constituents (Samaranayake 2006).

Tea tree oil is generally safe for topical use (Carson, Hammer et al. 2006). In some instances, tea tree oil can cause irritant and allergic reactions (Carson, Hammer et al. 2006). Evidence from studies involving animals and humans indicate that tea tree oil can be toxic if ingested (Carson, Hammer et al. 2006). The 50% lethal dose for tea tree oil in a rat model was found to be 1.9 to 2.6 ml/kg (Carson, Hammer et al. 2006). Although oral ingestion of tea tree oil has been reported in adults and children, no deaths have been recorded (Carson, Hammer et al. 2006).

Household Bleach (6.15% Sodium Hypochlorite)

Bleach is a very common substance used in the domestic environment and is commonly used for hard-surface disinfection (McDonnell and Russell 1999). Clorox® claims their household bleach has the ability to kill bacteria such as: *Staphylococcus aureus*, *Salmonella choleraesuis*, *Pseudomonas aeruginosis*, and *Streptococcus pyogenes*, *Escherichia coli* O157:H7, *Shigella dysenteriae*, and Methicillin Resistant *Staphylococcus aureus* (MRSA). Along with bacteria, Clorox® claims its bleach solution has the ability to kill a number of fungi and viruses. Chlorine compounds are desirable because they have a wide spectrum of use, are fast acting, and inexpensive (Lawrence and Bennet 2001).

Household bleach is an aqueous solution consisting of 6.15% sodium hypochlorite (NaOCl), the active ingredient, and less than 1% sodium hydroxide (Clorox 2005). In water,

sodium hypochlorite ionizes yielding sodium (Na^+) and the hypochlorite ion (OCl^-) which is in equilibrium with hypochlorous acid (HOCl) (McDonnell and Russell 1999; Estrela, Estrela et al. 2002; Marriott and Gravani 2006). The sodium hypochlorite reaction is described below:



Household bleach solution has a pH of 11.4 (Clorox 2005). At pH around 7.2, 68.5% of the solution is undissociated, whereas, at pH near 10.6, 0.1% of the solution is undissociated (Lawrence and Bennet 2001). The pH affects the concentration of the antimicrobial agents in the solution (Lawrence and Bennet 2001). In the hypochlorite solution, it is the undissociated hypochlorous acid (HOCl) that is the active species (Lawrence and Bennet 2001). However, Estrela et al. (2002) found that the minimum inhibitory concentration (MIC) of 5% household bleach solution to inhibit growth from all microorganisms was 0.5%. This indicates that bleach is a very potent antimicrobial.

The antimicrobial activity of bleach can be attributed to hypochlorous acid (Estrela, Estrela et al. 2002). Hypochlorous acid acts as a solvent when it comes in contact with an organism (Estrela, Estrela et al. 2002). Hypochlorous acid releases the chlorine which combines with protein amino groups resulting in the formation of chloramines (Estrela, Estrela et al. 2002). Chloramines interfere with cellular metabolism by amino acid degradation and hydrolysis (Estrela, Estrela et al. 2002). Overall, the antimicrobial effects of bleach are primarily due to HOCl 's ability to aggregate essential bacterial proteins (Winter, Ilbert et al. 2008). The alkaline nature of household surfaces also affects the antimicrobial activity of bleach. The high pH (~11.4) of sodium hypochlorite is due to the presence of hydroxyl ions (Estrela, Estrela et al.

2002). The pH interferes with the integrity of the cytoplasmic membrane, consequently, irreversibly alters cellular metabolism and degradation of phospholipids (Estrela, Estrela et al. 2002).

Sugars and starches have shown to have no effect on germicidal activity but the presence of tyrosine, tryptophan, cystine, egg albumin, peptone, body fluids, tissues, microbes, and vegetable matter have all shown to bind chlorine (Lawrence and Bennet 2001). Rudolph and Levine (1941) found that increasing the concentration of sodium hypochlorite four-fold resulted in a 50% reduction in killing time (Lawrence and Bennet 2001). The temperature in which sodium hypochlorite is used has a dramatic effect on the antimicrobial effectiveness. A 60-65% reduction in killing time is associated with every 10°C increase in temperature (Lawrence and Bennet 2001).

Sodium hypochlorite has been proven as an effective antimicrobial agent. Borneff et al. (1988) showed that a hypochlorite cleanser was more effective on contaminated food contact surfaces at reducing bacterial transmission compared to conventional cleansers (Cozad and Jones 2003).

Interest in chlorine containing compounds has increased over the years due to its potential adverse environmental and human health effects. The EPA reports that chlorine can cause environmental harm at low levels and is especially harmful to soil organisms in the water and soil (Meredith 1995). Sodium hypochlorite can cause corrosion of mucous membranes, esophageal, or gastric perforation, and laryngeal edema upon ingestion (Winter, Ilbert et al. 2008).

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CHAPTER II

METHODS AND MATERIALS

Culture and Culture Maintenance

The following organisms were obtained from the -70°C storage freezer at the Department of Food Science and Technology, Virginia Polytechnic Institute and State University: *Listeria monocytogenes* N3-031 serotype 1/2a, isolated from turkey franks, and obtained from Dr. Boor and Dr. Wiedmann of Cornell University (Fugett, Fortes et al. 2006); *Escherichia coli* O157:H7 strain E009 (beef isolate), provided by Dr. M. Doyle (University of Georgia, Griffin), and *Salmonella* Typhimurium ATCC 14028 (chicken isolate). Cultures were each revived individually in Tryptic Soy Broth (TSB, Difco, Franklin Lakes, NJ) at 35±2 ° C for 24 hours, maintained on Tryptic Soy Agar (TSA, Difco, Franklin Lakes, NJ) slants, and stored at 4°C.

Preparation of Inocula

Cultures of each bacterium were grown separately in TSB at 35±2°C. Two successive 24 hour transfers from the stock slant to 10 ml TSB were made before use in test media. Following the last transfer, the culture was centrifuged at 10000 x g for 5 minutes. The spent medium was decanted and the cell pellet was resuspended in 10 ml 0.1% buffered peptone water (BPW) (Difco, Franklin Lakes, NJ).

Test Method

An adaptation of the EPA Disinfectant Technical Science Section-10 (DIS/TSS-10) test method was used on 2.54 x 2.54 cm (1 in. x 1 in.) squares of laminate (Formica® Corporation

2009, Cincinnati, OH), and high density polyethylene (HDPE), or 2.5 cm x 2.5 cm glass (Fisherbrand®, Pittsburgh, PA) (EPA 2009).

Preparation of Carriers

Laminate, HDPE, and glass slide carriers were decontaminated by soaking surfaces in 70% ethanol for one hour. Surfaces were air dried in biological safety cabinet, level 2 (Nuair™, Plymouth, MN).

Application of Culture on Carrier

Separate prepared surface carriers were inoculated with 0.1 ml of *L. monocytogenes*, *E. coli* O157:H7, or *S. Typhimurium* suspended in 0.1% buffered peptone water (BPW). Resulting in approximately 1.0×10^8 CFU deposited onto the surface. Cultures were spread with a sterile, disposable, hockey stick spreader (Fisherbrand®, Pittsburgh, PA). Carriers were dried for approximately 40 minutes in the laminar flow hood or until cultures were visibly dry (EPA 2009).

Carrier Viable Bacterial Count

Following drying, recovery of bacterial number's "zero time" was performed to determine the viable bacterial count on surface carriers. Inoculated carriers were placed in 10 ml Neutralizing Buffer (NB; Difco, Franklin Lakes, NJ) and vortexed for 30 seconds to remove surviving organisms. One ml was transferred to BPW water and serially diluted with BPW and spread plated in duplicate on TSA. Plates were incubated for $35 \pm 2^\circ\text{C}$ for 48 hours.

Sanitizer Preparation

The following sanitizers were used: borax (20 Mule Team™, Henkel Corporation, Düsseldorf, Germany), distilled white vinegar (5% acetic acid; Heinz®, Pittsburgh, PA), tea tree oil (Thursday Plantation, Ballina NSW, Australia), alone as well as in combination to create a total of 7 treatments. In addition, treatments of bleach (Clorox®, Oakland, CA) and sterile reverse osmosis water were used for a means of comparisons to the alternative treatment solutions. The control was designated as an inoculated surface that received no sanitizing treatment. Alternative sanitizer formulations were prepared as directed by formulation directions recommended on New Wrinkles (<http://www.newwrinkles.com>) (Walton 2008). This site was chosen because it had many different recommendations that were unique. There are many other sites with other alternative sanitizing formulations.

Individual Ingredient Treatment

Tea tree oil and vinegar were used directly from the bottle. 66.0 g (1/4 cup) of borax was combined with 1.89 L (1/2 gallon) of hot tap water as instructed by formulation directions before use (Hunter and Halpin 2005). Temperature was not measured because it was not specified in formulation instructions. Water used in formulations was not sterile because the instructions did not indicate to do so.

Borax and Tea Tree Oil

The borax and tea tree oil combination was prepared by mixing 0.25 ml tea tree oil (5 drops), 132.0 g (1/2 cup) of borax powder, and 3.79 L (1 gallon) of water in a sterile container (Walton 2008).

Acetic acid and Tea Tree Oil

The acetic acid and tea tree oil combination was prepared by mixing 0.25 ml (5 drops) tea tree oil, 29.6 ml (1/8 cup) white acetic acid, and 236.6 ml (1 cup) hot water (Walton 2008).

Borax and Acetic acid

The borax and acetic acid combination was prepared by mixing 11 g borax (2 teaspoons), 59.2 ml (4 tablespoons) acetic acid, and 709.8 ml (3 cups) hot water (Walton 2008).

Borax, Tea Tree Oil, and Acetic acid

The borax, tea tree oil, and acetic acid combination was prepared by mixing 0.15 ml (3 drops) tea tree oil, 5.50 g (1 teaspoon) borax, 14.8 ml (3 teaspoon) white acetic acid, and 473.18 ml (2 cups) water (Walton 2008).

Control

Household bleach, approximately 6% sodium hypochlorite (Nalco) was used as a positive control. Sterilized reverse osmosis (RO) water (Eli Millipore™, Billerica, MA) was used a negative control.

Sanitizer Application

Sanitizer treatments were applied separately to each surface two times for each of three replications. One hundred µl of prepared sanitizer test solution was applied to the bacterial dried on the surface of each carrier. Sanitizers were allowed to remain in contact for 60 seconds.

Treated carriers were then placed in 20 ml of the NB and vortexed for 30 seconds to neutralize residual sanitizer and remove surviving organisms. One ml was transferred to BPW, serially diluted with BPW, and spread plated in duplicate on TSA. Plates were incubated for $35\pm 2^{\circ}\text{C}$ for 48 hours. A typical colony was selected from the incubated TSA and streaked onto selective media for colony confirmation. *E. coli* was streaked onto Sorbitol MacConkey Agar (Difco, Franklin Lakes, NJ). *S. Typhimurium* was streaked onto XLT-4 Agar (Difco, Franklin Lakes, NJ). *L. monocytogenes* was streaked onto Modified Oxford Agar (Difco, Franklin Lakes, NJ).

After vortexing, the remaining solution in the NB was enriched to allow bacteria that were not enumerated to grow. Twenty ml of TSB was added to the tube containing the NB and surfaces and incubated at $35\pm 2^{\circ}\text{C}$ for 24 hours. The enrichment broth was evaluated for turbidity to determine if bacterial presence still existed on the surface carrier. Positive TSB tubes associated with plates with limited growth were streaked onto selective media to confirm pathogen presence. Negative enrichment broth results were streaked onto appropriate selective medium to confirm pathogen absence. Carriers that had a considerable amount of growth from plates and have positive TSB tubes were not streaked onto selective medium but were considered to have positive pathogen presence.

Data Analysis

To assess the effectiveness of each treatment against each organism on each surface, each sampling procedure was replicated three times with duplicate analysis within each replicate (n=6). The mean cell recovery of duplicate plate counts was determined for each observation. Cell recovery counts were transformed to \log_{10} for statistical analysis and subtracted from the initial bacterial concentration on surface which also was transformed to \log_{10} . Initial bacterial

concentrations were determined by performing standard plate counts of the inoculums prior to application of inoculum to surface. The efficacy of each sanitizer was determined by calculating the log reduction of bacterial concentration after sanitizer application (Lindsay and von Holy 1999). Log reduction was calculated by the following equation:

$$\log K = \log N - \log n$$

where N is the initial bacterial count of the inoculum, n is the bacterial count of surface after sanitizer treatment, and K is the bacterial reduction value (Lindsay and von Holy 1999). A sanitizer was considered to be effective if bacterial reduction was greater than or equal to 5 logs ($K \geq 5$). Food contact sanitizers must reduce the microbial contamination 99.999% (5-log) to be considered effective (Marriott and Gravani 2006).

The data was analyzed using the General Linear Model (GLM) procedure of SAS (Version 9.03, Statistical Analysis Systems Institute, Inc. 2007). The factorial design with repeated measures were utilized within a randomized complete block design to test the effects of different sanitation formulations and different food contact surfaces and their interaction effects on the log value reductions of *S. Typhimurium*, *L. monocytogenes*, and *E. coli*. If the interactions between treatments were not significant ($P > 0.05$), the main effects (formula treatments and surfaces) were separated by Duncan's Test (SAS 2007) using the interaction as the error term.

RESULTS

E. coli O157:H7

Addition of tea tree oil for one minute contact time had a significant effect on the reduction of *E. coli* O157:H7 on HDPE, Formica® laminate, and glass (Table 2). Only bleach, was more effective at reducing the microbial population. Tea tree oil was no any more or less effective than the vinegar treatments. Tea tree oil was significantly more effective than borax, water, all of the combination treatments, as well as, the surface receiving no treatment. Combining treatments did not have a synergistic effect on the microbial reduction.

L. monocytogenes

Tea tree oil produced a significant reduction against *L. monocytogenes* (Table 3). The bleach treatment was the only treatment that was significantly more effective at reducing the bacterial population on the surface.. The vinegar treatment was not statistically more or less effective than tea tree oil.. The use of borax and combining treatments did not significantly affect the reduction of the microbial population. Borax, tea tree oil and vinegar; tea tree oil and borax; vinegar and borax; water; as well as the surface receiving no treatment were not statistically different. However, the surface receiving the tea tree oil, vinegar, and borax mixture was statistically the least effective treatment.

S. Typhimurium

Application of tea tree oil to surfaces contaminated with *S. Typhimurium* resulted in reductions in the 4-log range for all the surfaces (Table 4). The bleach treatments were more effective at the reduction of *S. Typhimurium* population. Vinegar was no more or less effective

than tea tree oil. The combination formulations were not effective nor were they statistically different from one another.

Surfaces

There appears to be no noticeable trends among the effectiveness of the treatment and the surface type (HDPE, glass, Formica® laminate).

DISCUSSION

Nine treatments (tea tree oil; vinegar; borax; tea tree oil and vinegar; tea tree oil and borax; tea tree oil, vinegar, and borax; vinegar and borax; household bleach; and sterile RO water) were applied to food contact surfaces contaminated with foodborne pathogens (*L. monocytogenes*, *E. coli* O157:H7, *S. Typhimurium*) previously associated with foodborne contamination. Treatments were left in contact with contaminated surfaces for one min at room temperature. Results suggest that household bleach (sodium hypochlorite) was the most effective sanitizer tested at pathogen reduction. Tea tree oil and vinegar treatments had the same effect on reduction of *L. monocytogenes* and *E. coli* O157:H7. However, tea tree oil was slightly less effective than vinegar at reducing *S. Typhimurium*.

A greater than 5 log₁₀ reduction is necessary for a sanitizing treatment to be considered effective (Marriott and Gravani 2006). The results show that tea tree oil and vinegar occasionally produced a greater than 5 CFU/cm² log₁₀ reduction against *S. Typhimurium*, *L. monocytogenes* and *E. coli* O157:H7. However, it cannot be concluded that a 5 log reduction was actually achieved for any treatment other than household bleach because sterile RO water produced log₁₀ reduction levels of approximately 3-log₁₀ against *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* respectively. This may be attributed to the detection or recovery limits of the testing. Moreover, results do confirm that tea tree oil and vinegar do cause some microbial reduction on food contact surfaces. The results support and expand studies indicating tea tree oil has antimicrobial effects (Carson and Riley 1995; Raman, Weir et al. 1995; Carson and Riley 2001; McMahon, Ann et al. 2007). The tea tree oil used had a pH of 3.34 (Table 5). Thus one possible mode of antimicrobial effect could be damage to bacterial organisms due to acidification of the micro-environment. This would be in addition to the

previously report mode of action. Cox et al. (1998) reported that tea tree oil induces K^+ leakage and inhibits glucose-dependent respiration. Since tea tree oil cannot consistently reduce pathogen levels to ranges considered safe, the risk of cross contamination is still present (Scott and Bloomfield 1990).

Since prior studies have concentrated on the antiseptic effects of tea tree oil; the majority of organisms studied have been associated with skin (Carson and Riley 1995; Raman, Weir et al. 1995; Cox, Mann et al. 1998; Carson, Mee et al. 2002). The results of the present study indicate that tea tree oil has similar effect on the reduction of foodborne pathogens. Although this study did not test the antibacterial mode of action, it is likely that the antimicrobial ability of tea tree oil can be attributed to potassium leakage and inhibition of glucose dependent respiration (Cox, Mann et al. 1998).

Vinegar (5% Acetic Acid) treatment resulted in 4-5 \log_{10} CFU/cm² of *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium*. Entani et al. (1998) found the effectiveness of acetic acid (vinegar) was dependent on the growth phase of the organisms. When organisms were in the stationary growth phase, treatment with Vinegar did not produce results differing from those seen with tea tree oil against *E. coli* O157:H7 and *L. monocytogenes*. Like tea tree oil, a greater than 5 \log_{10} reduction CFU/cm² was achieved. Once again, it cannot be concluded that this reduction of pathogens is adequate to prevent disease. Yang et al. (2009) found that a greater than 5 \log_{10} CFU/ml of *E. coli* O157:H7, *L. monocytogenes*, and *S. Typhimurium* resulted from treatment with vinegar in a suspension test. The differences between the present study and that of Yang et al. (2009) could be due to differences in the techniques used to test the sanitizer treatments (suspension vs. surface treated). In solution weak organic acids exist in equilibrium with their undissociated and dissociated forms (Brul and Coote 1999). When the pH and pKa are

equal, the undissociated and dissociated forms are equal. The surface of a cell is negatively charged; therefore, in order for a weak organic acid to cross the membrane to get to the cytoplasm the acid must be in its undissociated form (Brul and Coote 1999). Once in the cytoplasm, there is an accumulation of H⁺ ions, which acidifies the interior of the cell (Brul and Coote 1999; Yousef and Juneja 2003). Consequently, the pH balance is altered, throwing off the homeostasis balance of the proton motive force (Yousef and Juneja 2003).

Other than bleach, tea tree oil, and vinegar alone, all combination recipes including those containing borax did not produce significant reductions in microbial populations adequate to be considered an appropriate food contact surface sanitizer. This supports the findings of Bauer et al. (1995) that borax is not an effective alternative germicidal treatment. Expanding upon Bauer et al.'s findings, when alternative treatments were combined with one another, a synergistic effect was not created. Results seen with borax alone was equivalent to those seen when it was combined with other agents. Borax is a mineral that consists of sodium, boron, oxygen, and water. According to 20 Mule Team™, borax manufacturer claims the uses of borax are as follows: laundry boosting, stain removal, hard water treatment, cleaning and deodorizing, grease and grime removal, and odor elimination. 20 Mule Team™ does not make claims that their product is appropriate sanitizing agent. Therefore, consumers, using borax for sanitizing purposes are not utilizing it for its intended uses. The results of the study are consistent with a study performed by Olsen et al. (1994) which found borax had soil removal properties, however, was not an effective antimicrobial agent.

The treatment containing all three compounds (TTO, vinegar, and borax) produced the smallest log₁₀ reduction for each of the three tested organisms. This treatment could be less effective at removing organisms from the surface. The pH of this solution was 8.72 (Table 5).

An organism can maintain a normal cytoplasmic pH in environments with pHs 5.5-9.0 (Padan, Bibi et al. 2005). The addition of borax to vinegar and tea tree oil raised the pH significantly (Table 5). This may have caused more of the tea tree oil or vinegar to be in its dissociated (charged) form. The effectiveness of the agents was minimized because they were not able to penetrate the cell membrane in order to acidify the cytoplasm (Brul and Coote 1999). The formulation of vinegar and tea tree oil had the addition of water to the formulation mixture which also raised the pH, consequently, limiting the antimicrobial effectiveness.

Household bleach was the only treatment considered to be effective against the tested foodborne pathogens. With a greater than 5 CFU/cm² log₁₀ reduction reported for all organisms, it can be concluded that household bleach is an appropriate sanitizer. This supports the research of Borneff al. (1988) that sodium hypochlorite solution was more effective at bacterial reductions on surfaces when compared to conventional cleansers (Cozad and Jones 2003).

Overall, tea tree oil does have antimicrobial characteristics; however, the reduction capabilities are not as effective as household bleach. The results of this study further confirm the necessity of using the appropriate kitchen sanitizing product for the specified food contact surface and pathogen. Using products in a manner for which they are intended will help ensure microbial populations will be reduced to levels considered safe, and thus minimize the likelihood of foodborne illness. Conversely using agents with unproven antibacterial actions may lead to a false sense of safety.

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CHAPTER II TABLES

Table 1

The alternative sanitizer formulations as recommended by New Wrinkles.

Treatment	Converted Metric Formulation	Household Measurement Formulation
Tea Tree Oil	Used Directly from container	Used Directly from container
Vinegar	Used Directly from container	Used Directly from container
Borax	<ul style="list-style-type: none"> • 66.0 g borax • 1.89 L water 	<ul style="list-style-type: none"> • ¼ c borax • ½ gal water
Tea Tree Oil, Vinegar, Borax	<ul style="list-style-type: none"> • 0.15 ml TTO • 5.50g borax • 14.8 ml vinegar • 473.2 ml water 	<ul style="list-style-type: none"> • 3 drops TTO • 1 tsp borax • 3 tsp vinegar • 2 c water
Tea Tree Oil and Vinegar	<ul style="list-style-type: none"> • 0.25 ml TTO • 29.6 ml vinegar • 709.8 ml “hot” water 	<ul style="list-style-type: none"> • 5 drops TTO • 1/8 c vinegar • 1 c “hot” water
Tea Tree Oil and Borax	<ul style="list-style-type: none"> • 0.25 ml TTO • 132.0 g borax • 3.79 L water 	<ul style="list-style-type: none"> • 5 drops TTO • ½ c borax • 1 gal
Sterile RO water	Used Directly from container	Used Directly from container
Bleach	Used Directly from container	Used Directly from container
Control (No Treatment)	No Treatment	No Treatment

Table 2

The mean log₁₀ reduction of E. coli O157:H7 when treated with alternative sanitizer formulations on varying food contact surfaces.

Formulation Ingredients	Surface Type		
	HDPE	Glass	Formica®
Bleach	7.28 ± 0.42 ^{aA}	7.34 ± 0.17 ^{aA}	7.02 ± 0.07 ^{aA}
Tea Tree	5.77 ± 2.12 ^{bA}	4.99 ± 1.75 ^{bcA}	5.71 ± 1.11 ^{bA}
Vinegar	4.74 ± 2.10 ^{bcA}	5.66 ± 1.77 ^{bA}	5.36 ± 1.20 ^{bA}
Borax	3.32 ± 1.39 ^{cdeA}	4.36 ± 1.79 ^{cdA}	3.08 ± 0.42 ^{cdA}
Tea Tree Oil and Vinegar	3.64 ± 0.96 ^{cdeAB}	3.91 ± 1.07 ^{cdeA}	3.05 ± 1.23 ^{cdB}
Tea Tree Oil and Borax	3.62 ± 0.67 ^{cdeA}	3.28 ± 0.73 ^{eA}	3.62 ± 0.73 ^{cA}
Tea Tree Oil, Vinegar, and Borax	2.45 ± 0.45 ^{eA}	3.07 ± 0.86 ^{deA}	2.62 ± 0.80 ^{cdA}
Vinegar and Borax	2.58 ± 0.56 ^{deA}	2.65 ± 0.82 ^{deA}	2.01 ± 0.74 ^{dA}
Sterile RO Water	4.03 ± 2.21 ^{cdA}	3.44 ± 0.75 ^{deA}	3.54 ± 1.00 ^{cA}
Control (No Treatment)	2.75 ± 0.16 ^{deA}	3.84 ± 1.81 ^{cdeA}	3.26 ± 1.0 ^{cA}

^a Mean value, n = 6.

^b Values in the same column designated with the same lower case letter are not significantly different from one another (P ≤ 0.05).

^c Values in the same row designated with the same upper case letter are not significantly different from one another (P ≤ 0.05).

Table 3

The mean log₁₀ reduction of L. monocytogenes when treated with alternative sanitizer formulations on various food contact surfaces.

Formulation Ingredients	Surface Type		
	HDPE	Glass	Formica®
Bleach	5.80 ± 0.33 ^{aB}	6.80 ± 0.52 ^{aA}	6.01 ± 0.77 ^{aB}
Tea Tree	5.72 ± 0.36 ^{bA}	4.61 ± 1.50 ^{bA}	4.64 ± 1.43 ^{aA}
Vinegar	4.74 ± 1.97 ^{aB}	4.17 ± 1.21 ^{bcB}	5.93 ± 0.83 ^{abA}
Borax	3.72 ± 1.26 ^{dA}	4.01 ± 1.18 ^{bcA}	3.12 ± 0.79 ^{bcA}
Tea Tree Oil and Vinegar	3.24 ± 0.97 ^{bcA}	3.30 ± 0.53 ^{cdA}	4.26 ± 1.46 ^{cdA}
Tea Tree Oil and Borax	3.61 ± 0.91 ^{cdA}	4.28 ± 2.05 ^{bcA}	3.25 ± 0.43 ^{bcA}
Tea Tree Oil, Vinegar, and Borax	2.12 ± 0.75 ^{dA}	2.78 ± 0.55 ^{dA}	2.64 ± 0.79 ^{dA}
Vinegar and Borax	3.58 ± 1.38 ^{dA}	3.00 ± 0.78 ^{dA}	3.05 ± 0.99 ^{bcA}
Sterile RO Water	3.73 ± 1.90 ^{cdA}	3.31 ± 0.83 ^{cdA}	3.40 ± 1.32 ^{bcA}
Control (No Treatment)	3.01 ± 0.85 ^{cdA}	3.59 ± 1.07 ^{cdA}	3.38 ± 1.70 ^{cdA}

^a Mean value, n = 6.

^b Values in the same column designated with the same lower case letter are not significantly different from one another (P ≤ 0.05).

^c Values in the same row designated with the same upper case letter are not significantly different from one another (P ≤ 0.05).

Table 4

The mean log₁₀ reduction of S. Typhimurium when treated with alternative sanitizer formulations on various food contact surfaces.

Formulation Ingredients	Surface Type		
	HDPE	Glass	Formica®
Bleach	7.27 ± 0.47 ^{aA}	6.51 ± 0.83 ^{aA}	7.02 ± 0.71 ^{aA}
Tea Tree	4.83 ± 1.74 ^{bA}	4.43 ± 0.37 ^{bA}	4.10 ± 1.44 ^{cA}
Vinegar	6.17 ± 1.35 ^{abA}	4.33 ± 1.21 ^{bB}	5.37 ± 0.89 ^{bA}
Borax	2.39 ± 0.53 ^{cdA}	3.38 ± 2.19 ^{bcA}	3.12 ± 0.91 ^{cdA}
Tea Tree Oil and Vinegar	3.25 ± 0.78 ^{cA}	3.00 ± 0.29 ^{cA}	2.81 ± 0.51 ^{deA}
Tea Tree Oil and Borax	2.41 ± 0.61 ^{cdB}	2.33 ± 0.40 ^{cB}	3.30 ± 0.53 ^{cdA}
Tea Tree Oil, Vinegar, and Borax	1.67 ± 0.55 ^{dA}	2.16 ± 0.61 ^{cA}	2.16 ± 0.67 ^{eA}
Vinegar and Borax	2.01 ± 0.77 ^{cdB}	2.07 ± 0.47 ^{deA}	2.66 ± 0.63 ^{cB}
Sterile RO Water	3.13 ± 2.43 ^{cdA}	2.64 ± 1.38 ^{cA}	2.29 ± 0.43 ^{deA}
Control (No Treatment)	2.33 ± 0.83 ^{cdA}	2.50 ± 0.71 ^{cA}	2.75 ± 1.06 ^{deA}

^a Mean value, n = 6.

^b Values in the same column designated with the same lower case letter are not significantly different from one another (P ≤ 0.05).

^c Values in the same row designated with the same upper case letter are not significantly different from one another (P ≤ 0.05).

Table 5

The pH of the Sanitizing Treatment applied to Contaminated Food Contact Surfaces.

Treatment	pH
Bleach	11.34
Tea Tree Oil	3.34
Vinegar	2.63
Borax	9.19
Tea Tree Oil, Vinegar, Borax	8.72
Tea Tree Oil and Vinegar	2.83
Vinegar and Borax	7.80
Tea Tree Oil and Borax	9.19
Sterile RO Water	6.62