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INHIBITORY EFFECTS OF ESSENTIAL SPICE OILS AND CONSTITUENTS AGAINST SELECTED FOODBORNE PATHOGENS

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# INHIBITORY EFFECTS OF ESSENTIAL SPICE OILS AND CONSTITUENTS AGAINST SELECTED FOODBORNE PATHOGENS

By

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## ABSTRACT

## INHIBITORY EFFECTS OF ESSENTIAL SPICE OILS AND CONSTITUENTS AGAINST SELECTED FOODBORNE PATHOGENS

**By**

**Jamie Sue Merritt**

Five essential spice oils and a major constituent from each oil were tested for antibacterial activity against *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Bacillus cereus* and *Staphylococcus aureus* using a paper disk assay. With this methodology, 2.5 and 5.0% oregano, thyme, cinnamon, bay and clove oils and their constituents, carvacrol, thymol, cinnamic aldehyde, eugenol and caryophyllene, respectively, were tested for inhibitory activity against the foodborne pathogens. Caryophyllene was ineffective against *Salmonella typhimurium*, *Yersinia enterocolitica* and *Staphylococcus aureus* and was dropped from the remaining studies. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for each of the remaining oils and constituents was determined using a broth dilution method. Thyme oil exhibited the strongest antibacterial activity of all compounds tested with a MIC and MBC of 250 ppm and was selected to continue into the ground meat studies. Oregano and bay oils were effective (MIC and MBC = 250 ppm) against three of the five organisms. Oregano oil was also selected to continue into the ground meat studies due to its effectiveness against *Escherichia coli* 0157:H7 and *Salmonella typhimurium* (250 ppm) and *Yersinia enterocolitica* (500 ppm) which were the organisms chosen for the remaining studies. *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* were added to irradiated

ground beef, turkey and pork, respectively, then the inhibitory effects of the oregano and thyme oils were tested. At 4°C, high levels of oregano and thyme oils were required for bactericidal activity against the organisms, with 5000 ppm needed for *Salmonella typhimurium*, 10,000 ppm for *Yersinia enterocolitica* and 20,000 ppm for *Escherichia coli* 0157:H7. Apparently meat components (protein, fat, etc.) in addition to the cold temperature, diminished the effectiveness of the oils. In temperature abuse conditions, oregano and thyme oils prevented growth and lowered bacterial counts, but high (8000 ppm) levels were required. Thermal inactivation studies were conducted on inoculated ground meats with added oregano and thyme oil (500 ppm). *Escherichia coli* 0157:H7 was inactivated at 58°, 63° and 68°C, *Salmonella typhimurium* at 53°, 58° and 63°C and *Yersinia enterocolitica* at 52°, 57° and 62°C. D values for samples with the oils were lower for all organisms at all temperatures when compared to controls (no oil). The addition of oregano and thyme oils increased z values for *Escherichia coli* 0157:H7 and *Yersinia enterocolitica*. *Salmonella typhimurium* had slightly lower z values when oils treatments were compared to a control. The oils were more effective at higher temperatures (versus 4°C) which was illustrated by the lower levels required for bactericidal activity. When oregano oil (8000 ppm) was added to pepperoni inoculated with *Escherichia coli* 0157:H7 and processed with a mild heat treatment (53°C), bacterial counts were lower before and after fermentation even though the pH only reached ~5.2 (versus 4.8 – 4.9 for controls). Complete elimination of the organism was achieved after the heat treatment (controls still viable).

This dissertation is dedicated to my parents, Gene and Betty Cherry, my brother Chris Cherry, and my late husband, John Merritt, without whom I would not have completed my Ph.D program. Also, I would like to dedicate this work to all the people in my life that make life enjoyable.

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# INTRODUCTION

For many years, illnesses caused from the consumption of food contaminated with pathogenic bacteria and/or toxins produced by these bacteria has been a major concern to public health officials. These pathogenic microorganisms are associated with millions of foodborne illnesses each year, with associated costs and productivity losses estimated in the billions of dollars (Jay, 1997). Controlling bacterial pathogens could reduce foodborne illness outbreaks and help insure a safe, wholesome food supply.

Antimicrobial agents, including food preservatives and organic acids, have been used to inhibit foodborne bacteria and extend the shelf life of processed foods. Researchers have also found that antimicrobial agents exist in edible medicinal plants, herbs and spices (Kim et al., 1995 a,b). Essential oils of many spices have been shown to posses antimicrobial activity including thyme, cinnamon, bay, clove, oregano, sage and mace (Deans and Ritchie, 1987; Morozumi, 1978; Bullerman et al., 1977; Shelef, 1983). Constituents of essential spice oils including thymol, carvacrol and eugenol also have been shown to have antimicrobial activity (Kim et al., 1995 a,b). The use of essential spice oils and constituents as antimicrobial agents would not only assist in providing a safe food supply, but would also satisfy the consumer demand for natural additives in foods.

The purpose of this research was to determine how effective selected essential spice oils and constituents from these oils were against several foodborne pathogens. The first objective was to screen oregano, thyme, clove, bay and cinnamon oils and a corresponding constituent from each oil including carvacrol, thymol, caryophyllene, eugenol and cinnamic aldehyde, respectively, for antibacterial activity against *Escherichia coli* 0157:H7, *Salmonella typhimurium, Yersinia enterocolitica, Bacillus cereus* and *Staphylococcus aureus.* To determine which components were most effective, the paper disk assay and broth dilution methods were used. By determining the minimum inhibitory and bactericidal concentrations, the two most effective compounds (oregano and thyme oils) were selected to continue into the ground meat studies. The second objective was to determine how effective oregano and thyme oils were against *Escherchia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in aseptic ground beef, turkey and pork, respectively. The ground meat studies included refrigeration, thermal inactivation and temperature abuse trials. A study was also conducted with a commercial rapid test kit to determine if the oils would interfere with the immunologically based kit. The third objective included the preparation of a dry, fermented sausage (pepperoni) inoculated with *Escherichia coli* 0157:H7 and processed with a mild heat treatment. Oregano oil treatments were compared to a control treatment to determine how effective the oil was at controlling the organism.

# CHAPTER 1

## 1.0 REVIEW OF LITERATURE

### 1.1 INTRODUCTION: FOOD POISONING PERSPECTIVES

#### 1.1.1 DEFINING THE NATURE AND SCALE OF FOOD POISONING

A wide variety of diseases are caused by the ingestion of foods contaminated with pathogenic microorganisms or their toxic products. Foodborne diseases are defined as any illness that results from the ingestion of food; however, this definition is broad and leaves room for further interpretation. Food poisoning has become a topic for both public and scientific debate. Consumers are very concerned with the safety and quality of foods they purchase for consumption. They are inundated with information and misinformation on a wide variety and seemingly endless list of food safety problems including chemical, physical and microbiological hazards. Public health officials and scientists are faced with increasing incidences of food poisoning and food related diseases each year. The rising numbers of food poisoning cases are due partially to actual increased incidences as well as increased reporting of incidences to public health facilities. Microorganisms that were once thought incapable of growing and multiplying in certain foods have been discovered to withstand modern processing and storage techniques leading to increased food poisoning cases (Cliver, 1990).

Defining food poisoning, foodborne diseases and food-related diseases is difficult due to the misuse and misunderstanding of the terminology. Classical food poisoning is considered the rapid onset of gastrointestinal symptoms after

the ingestion of contaminated foods. The transmission agents for disease can be intrinsic, such as allergens and toxic compounds, that are naturally found in the food or extrinsic, such as microorganisms and toxic compounds (i.e. pesticides and heavy metals) that are contaminants in food. When microorganisms are cause for food poisoning, food can be an active transmission medium in which growth occurs or a passive transmission medium in which no growth occurs, but toxins are present. The symptoms of classical food poisoning include gastrointestinal disturbances (diarrhea and vomiting); however, when serious and life threatening symptoms occur from the ingestion of pathogenic microorganisms or their toxins the resulting illnesses are termed food-related or foodborne diseases. They can be defined as immunological rather than gastrointestinal in nature and are caused directly by foodborne pathogens or indirectly by their toxins. Some foodborne or food-related diseases include Grave's disease, hemolytic uremic syndrome (HUS), septic and aseptic arthritis, Guillian-Barre Syndrome and myocarditis (Eley, 1992).

Food poisoning and foodborne or food-related diseases caused by microorganisms are classified as infections or intoxications. Infections are a result of viable, usually multiplying pathogens at the site of inflammation (i.e. intestinal mucosa). The organisms have been consumed with a food product. The dose required for onset of disease depends on the type of organism, age and health of the host and environmental conditions (Taylor, 1990). Intoxications are caused by the ingestion of a hazardous dose of toxic chemical that is not a result of allergies or metabolic disorders. The toxic agents are non-viable in

contrast to infections where in the organisms are viable. Pathogenic microorganisms actively multiply and produce toxin in the food which is then consumed. Once the toxin is consumed intoxication may occur. The dose required for and intoxication depends on the host and circumstances of exposure. Many of these toxins are heat labile and can be deactivated by proper food processing techniques; Others are heat stable and pose serious threat to food safety (Cliver, 1990).

Food poisoning and foodborne diseases are global problems. The poorer, less industrialized nations (Third World Nations) have a very high mortality rate attributed to diarrhea resulting from foodborne diseases. This mortality rate is especially high in infants (Varnam, 1991; Eley, 1992). Richer, more industrialized nations, such as the United States, have fewer incidences of foodborne diseases; however, the scale of the problem is still significant. Difficulty arises when trying to assess the scale of the problem due to underreporting. It is estimated that 10 to 100 times (or higher) more incidences occur than are reported (Varnam, 1991). The economic impact that foodborne diseases and food poisoning have is staggering. The incidence rate of food poisoning is increasing in the United States and globally. Control measures are needed to help reduce or eliminate pathogens from the food and water supply.

### 1.1.2 MICROBIAL FOOD POISONING: CAUSES AND EFFECTS

Bacteria are the most important vectors in classical food poisoning where in enteric symptoms such as diarrhea and vomiting occur. These foodborne bacteria are considered pathogens which are defined as microorganisms that

have the ability to cause disease, with the pathogenicity of each organism varying among and between different species (Sonnenwirth and Swartz, 1980). From the production source to the consumer, many avenues exist for these pathogenic bacteria to contaminate the food and water supply. Mishandling at farms, dairies, and aquatic and land environments where food is produced can cause contamination (Wang et al., 1997; Hancock et al., 1997). Processing facilities, food service establishments, homes and transportation services are also very important sources of contamination if mishandling and poor sanitation occur (Bryan et al., 1997). If conditions are favorable, these disease-producing bacteria can multiply and/or produce toxin in the mishandled food, which can lead to a food poisoning outbreak. The effects of food poisoning and food-related diseases can range from discomfort to death. Generally, classic food poisoning leads to tremendous discomfort due to the acute gastrointestinal symptoms associated with it. Death can occur, especially in infants, elderly and immune-compromised patients, if the symptoms are not treated carefully by medical professionals (Eley, 1992).

## 1.2 ORGANISMS AND SYMPTOMS ASSOCIATED WITH BACTERIAL FOOD POISONING AND FOOD RELATED DISEASES

### 1.2.1 INTRODUCTION

Many pathogenic microorganisms can cause food poisoning and food-related disease. Table 1.2.1A summarizes several of the common pathogenic bacteria found in contaminated food.

Table 1.2.1A: Common Foodborne Pathogens Associated with Food Poisoning Outbreaks and Food-Related Diseases

| Organism | Disease |
|---|---|
| *Salmonella* spp. | Classic Food Intoxication<br>Joint Disease<br>Vascular Disease<br>Respiratory Disease<br>Renal Disease<br>Skin Disease |
| *Shigella* spp. | Classic Food Infection<br>Joint Disease<br>Renal Disease |
| *Campylobacter* spp. | Classic Food Infection<br>Joint Disease<br>Respiratory Disease<br>Renal Disease<br>Skin Disease<br>Joint Disease<br>Neural Disorders |
| *Escherichia coli* spp. | Classic Food Infection<br>Joint Disease<br>Renal Disease<br>Neural Disorders |
| *Yersinia enterocolitica* | Classic Food Infection<br>Heart Disease<br>Skin and Soft Tissue Disease<br>Joint Disease<br>Autoimmune Disorders<br>Neural Disorders |
| *Staphylococcus aureus* | Classic Food Intoxication<br>Joint Disease |
| *Bacillus cereus* | Classic Food Intoxication |
| *Clostridium botulinum* | Classic Food Intoxication |
| *Clostridium perfringens* | Classic Food Infection |
| *Listeria monocytogenes* | Classic Food Infection<br>Neural Disorders<br>Fetal Tissue Disease |

(Varnam, 1991;Jay, 1997:Cliver,1990)

*Escherichia coli* 0157:H7, *Yersinia enterocolitica, Salmonella typhimurium, Staphylococcus aureus* and *Bacillus cereus* will be discussed in depth as these organisms were used in the experimental design.

## 1.3 MORPHOLOGICAL, BIOCHEMICAL AND VIRULOGICAL ASPECTS OF ORGANISMS ASSOCIATED WITH BACTERIAL FOOD POISONING AND FOOD RELATED DISEASES

### 1.3.1 *Escherichia coli* 0157:H7

The genus *Escherichia* is in the *Enterobacteriaceae* family and contains six species including *Escherichia coli*. These organisms are gram negative, facultatively anaerobic, non-sporeforming bacilli. The pathogenic strains are categorized into four groups (Jay, 1997;Eley, 1992;Cliver,1990; Varnam, 1991). These groups are enterotoxigenic (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC). *Escherichia coli* 0157:H7 is included in the EHEC group which has different epidemiological characteristics, pathogenicity and O:H serovars than the other three groups (Jay, 1997; Eley, 1992; Doyle and Cliver,1990; Varnam, 1991).

Isolates of *E. coli* 0157:H7 have three distinct properties that may be used in the isolation and differentiation of the serovar. Failure to ferment sorbitol is a characteristic of *E. coli* 0157:H7, which is rare among other species of *E. coli*. Isolates of *E. coli* 0157:H7 also fail to produce β-glucuronidase, which is another useful method to differentiate from other *E. coli* species on selective media.

Another less known feature of *E. coli* 0157:H7 is the sensitivity to bromothymol blue at high incubation temperatures. This feature allows for a simple confirmation test (Jay, 1997;Eley, 1992;Cliver,1990; Varnam, 1991).

Genetic control, virulence and adherence factors have been studied extensively since the first cases of *E.coli* 0157:H7 poisoning occurred in 1975 (California) and 1982 (Michigan and Oregon). Genetic control of the virulence in the organism is provided by a 60 megadalton plasmid, which encodes for the production of fimbrial adhesions. Large quantities of shiga-like toxin (SLT) are produced and are mediated by phages. The mechanism by which the phage controls production of the SLT is not known (Jay, 1997;Eley, 1992;Cliver,1990; Varnam, 1991).

*E. coli* 0157:H7 is associated with food-related diseases rather than classic food poisoning. Generally, *E. coli* infections require $10^5 - 10^7$ colony forming units (CFU) per gram or milliliter to cause illness (Eley, 1992). However, levels as low as 50 CFU's of the verotoxic *E.coli* 0157:H7 have been shown to cause illness (Tilden et al., 1996). Although initial contamination levels may not be very high in some food products, this organism has the ability to multiply to infectious levels (Eley, 1992).

*E. coli* 0157:H7 can induce serious illnesses including hemorrhagic colitis and hemolytic uremic syndrome. Hemorrhagic colitis results from edema, corrosion and hemorrhage of the colon's mucosal lining. The incubation period seems to vary greatly in outbreaks where three to fourteen days (average 4 to 8 days) have been noted (Carter et al., 1987). Symptoms include sudden

abdominal pain followed by watery diarrhea, nausea, vomiting and abdominal distention (Varnam, 1991). Once the illness progresses 1 to 2 days, bloody diarrhea occurs. Death is usually rare unless the disease is allowed to progress, especially in children, elderly and immuno-compromised people. Fever usually does not develop; however, if it develops in the late stages of the disease prognosis can be poor (Jay, 1997; Doyle and Cliver,1990; Eley, 1992; Varnam, 1991).

Hemolytic uremic syndrome (HUS) usually begins with diarrhea and progresses to microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure (sometimes requiring renal dialysis). Less commonly, thrombocytopenic purpura occurs as an extension of HUS and includes fever and neurological symptoms. Long term kidney damage can result and death years after the illness has occurred (Varnam, 1991; Eley, 1992; Carter et al., 1987).

*E. coli* 0157:H7 was first isolated from an infected woman in California in 1975; however, concern did not arise until outbreaks in Michigan and Oregon (1982) occurred. Since then several outbreaks have been documented and many different foods were found to be the transmission medium (Varnam, 1991).

Until 1982, only one case of *E. coli* 0157:H7 had been isolated. The outbreaks in Michigan and Oregon first led to the recognition that this organism caused hemolytic colitis. At least 47 people were affected as a result of consuming undercooked beef patties from a fast food chain. Each year since 1982, outbreaks have occurred throughout the United States. Some outbreaks

were very small (2 to 3 cases), while others were quite large (400 to 500 cases) with several deaths (Dorn, 1993; Molenda, 1994; Jay, 1997; Keene et al., 1997).

Most outbreaks have been associated with the ingestion of undercooked meat and unpasteurized milk (Dorn, 1993; Wang et al., 1997). It appears that dairy cattle have a higher prevalence of *E. coli* 0157:H7 than the general cattle population (Wells et al., 1991; Rice et al., 1997). Since the early 1980's, several vectors of transmission have appeared including potatoes, apple cider, fruits and vegetables, water, processed meat, yogurt and mayonnaise (Dorn, 1993; Besser et al., 1993; Tomicha et al., 1997; Calicioglu et al., 1997; Zhao et al., 1994; Hara-Kudo et al., 1997; Wang and Doyle, 1998). Many of these foods (pre and post harvest) were at some time in contact with manure from cattle (Dorn, 1993). Person to person transmission has also been documented (Dorn, 1993). Many discovered and undiscovered transmission vectors are possible making control of the organism difficult.

More recently, processed meats and sausages have been found to harbor the organism resulting in illness. The United States Department of Agriculture's Food Safety and Inspection Service recommends a 5 $\log_{10}$ reduction of Escherichia coli 0157:H7 in fermented dry and semi-dry sausages (Reed, 1995); however, traditional batter conditioning, fermentation and drying steps are only sufficient to decrease the level of the organism 1 to 2 $\log_{10}$ CFU's. Heat treatments and low pH help achieve the 5 $\log_{10}$ decrease recommended (Faith et al.,1998a,b; Hinkens et al., 1996; Riordan et al., 1998; Ellajosyula et al., 1998). Danger still exists if the initial load of organisms is extremely high. Another

control method, in addition to the heat processing and low pH, would be beneficial to help assure safe and wholesome meat products.

### 1.3.2 *Salmonella typhimurium*

The genus *Salmonella* is a member of the *Enterobacteriaceae* family and consists of gram negative , facultatively anaerobic, oxidase negative, nonsporeforming bacilli. Approximately 2300 serovars exist, of which 50 to 150 have been implicated in disease outbreaks (Clarke and Gyles, 1993). These organisms are catalase positive and have both respiratory and fermentative metabolism of carbohydrates (Niven,1987; Varnam, 1991, Eley, 1992).

These organisms are motile; however, it is unclear how motility affects pathogenicity (Bauer and Hormansdorfer, 1996). *Salmonella typhimurium* are ubiquitous and can affect man and a range of other warm and cold blooded animal hosts (Liu et al., 1993). *Salmonella typhimurium* may be present in the intestinal tracts of poultry and other larger meat animals without any signs of infection. Healthy animals may also carry these organisms in their feces, lymph nodes, spleen, kidneys, liver and gall bladder. Shipping and slaughter stress can increase the frequency of the organism in feces and organ tissue. The carrier rate of asymptomatic animals at the time of slaughter may be as high as 20% (Niven, 1987).

The genetic control of virulence has not been completely elucidated. It appears that both plasmid-born and chromosomal genes are involved. *Salmonella typhimurium* carries a 50 to 60 megadalton plasmid which is called the virulence plasmid (Varnam, 1991). This plasmid controls the ability of the

organism to spread beyond the initial site of infection. Chromosomal genes are also important in determining the ability to survive and multiply in cells of the epithelial system (Varnam, 1991). Motility appears to be an important factor in virulence. Motility helps the organism to maintain close contact between the bacteria and the HeLa cells in the stages before adherence. Chemotaxis plays a role in virulence as well. HeLa cells release an attractant, which increases the collision frequency and aids in the attachment of *Salmonella typhimurium* to the HeLa cells themselves. *Salmonella typhimurium* adheres to the epithelial cells of the ileum via fimbrae attachment. The cells can then penetrate (via receptor mediated endocytosis and between adjacent cells) and enter deeper tissues such as the *lamina propria*. The bacteria enter individually into a vacuole which then coalesce with other vacuoles. Next, intracellular multiplication of the bacteria in the membrane bound vacuole occurs. The cells are extruded from the *lamina propria* and elicit an inflammatory response. This inflammatory response, in addition to endotoxin, enterotoxin and cytotoxin production, leads to the symptoms of acute gastroenteritis (Varnam, 1991; Bauer and Hormansdorfer, 1996).

*Salmonella typhimurium* is one of the leading serovars associated with Salmonellosis (Eley, 1992). Symptoms of Salmonellosis begin after a 5 to 72 hour incubation (average 12 to 36 hours) and include diarrhea, abdominal pain, chills, fever, vomiting, dehydration and headache. Symptoms generally last 1 to 4 days. The severity of these symptoms depends on the host's immunocompetence (Jay, 1997; Varnam, 1991; Eley, 1992; Doyle and Cliver,

1990). The dose level for illness has been cited as low as 10 organisms per gram (Varnam, 1991), but dose levels required for illness usually ranges from $10^8$ to $10^9$ CFU's (Jay, 1997). This level is misleading because several disease outbreaks have been caused by food which contained only $10^4$ to $10^5$ CFU/gram of food material (Eley, 1992). The level of organisms depends on the type of food and strain present (Doyle and Cliver,1990). Typically, the disease is not fatal, but like most diseases it affects the young, old and immunocompromised individuals more severely (Eley, 1992). Antibiotics are normally not recommended for gastrointestinal symptoms to prevent antibiotic resistant strains from evolving. Antibiotics are usually only used if septicemia develops (Doyle and Cliver, 1990).

Four major factors contribute to outbreaks of Salmonellosis. These include temperature abuse, inadequate cooking, contaminated raw ingredients and cross contamination (Doyle and Cliver, 1990). *Salmonella typhimurium* is found in a range of different foods. Some common foods associated with Salmonellosis include meat and meat products, poultry and poultry products (including eggs), salads, chocolate, pasta and an large number of other miscellaneous foods (Varnam, 1991). Each year the number of Salmonellosis cases increases. Control measures are needed to slow or decrease this increasing incidence rate (Jay, 1997).

### 1.3.3 *Yersinia enterocolitica*

The genus *Yersinia* is a member of the *Enterobacteriaceae* family by virtue of its common antigens and its biochemical and core DNA similarities. However, it has several distinctive qualities including the size and morphology of the colonies (Varnam, 1991; Stern and Pierson, 1979, Brenner, 1981). *Yersinia enterocolitica* is a gram negative, nonsporeforming , facultatively anaerobic, coccibacilli. Depending on the growth medium and incubation temperature, pleomorphisms have been known to occur leading to mixtures of cocci and rods (Varnam, 1991). Stern and Pierson (1979) reported that the organism was a rod. Vegetative cells in a young culture are ovoid, but later develop into a rod. Varnam (1991) stated that *Yersinia* cells are coccoid and small, while Sonnenwirth and Swartz (1980) reported the cells as relatively large coccibacilli. Morphology and size seem to depend on the temperature, growth medium and age of culture. *Yersinia enterocolitica* are psychrotrophic in nature which presents a unique problem in the food industry as related to food safety. They can multiply at temperatures ranging from -2° to 45°C with an optimum between 25° C and 37°C. Many of the organisms characteristics are temperature dependent. Generally, they are motile at temperatures below 30°C and non-motile at 37°C. Incubation at 37°C results in a loss of the virulence plasmid, thus the loss of plasmid mediated properties (Varnam, 1991; Lee,1977).

Many biochemical properties are also temperature dependent. For example, indole is differentially produced; the methyl red is positive and the Voges-Proskauer test is negative at 37°C. These reactions are generally

reversed at 22°C to 30°C. Typically 30°C is the transition point for many reactions. Reactions are generally positive at temperatures below 30°C and negative above 30°C (Varnam, 1991; Eley, 1992).

The virulence of *Yersinia enterocolitica* differs between humans and animals. In many cases, serovars that are pathogenic to animal reservoirs are not to the human reservoirs. The swine species are the predominant carriers of the human pathogenic *Yersinia enterocolitica* (Hurvell et al., 1977; Wauters, 1977; Pedersen, 1977;Funk et al., 1998; Andersen et al., 1991). Many other non-human sources also carry the organism, including cows, dogs, cats, horses, deer, rabbits and fleas (Wormser and Keusch, 1981). As with most members of the *Enterobacteriaceae* family, *Yersinia enterocolitica* has both chromosomal and plasmid genes that are involved in the control of virulence (Varnam, 1991). Chromosomes appear to be the major factor mediating cell invasion. Plasmids are involved with adherence, but not cell invasion. *Yersinia entercolitica* contains a 40 to 48 Megadalton plasmid designated pVYe. The virulence plasmid is involved in a number of temperature regulated phenotypes including the expression of certain outer membrane proteins, agglutination, serum resistance, macrophage cytotoxicity, V antigen production and low calcium response. The low calcium response is manifested as a growth restriction when temperatures are raised from 30°C to 37°C in the absence of calcium. Outer membrane proteins are produced in the absence of calcium until all growth ceases (Wauters, 1981). It has been suggested that this organism primes itself for virulence outside the host's body at lower temperatures. Once inside the host's

body an initial burst of activity is all that is required for an infection. Down regulation of the genes may be required for adherence and cell invasion. The adhesion of *Yersinia enterocolitica* to epithelial cells of the ileum is regulated by outer membrane proteins that recognize several receptors. Receptor mediated endocytosis is the mechanism involved in internalization which is identical to the Salmonella species. Endocytosis by HeLa cells is also stimulated by non-viable cells. *Yersinia enterocolitica* does not multiply intracellularly in HeLa cells after release from the endocytotic vacuole. Subsequently, invasion into the epithelial cells occurs and the organism progresses to Peyer's patches. *Yersinia enterocolitica* multiplies in the follicles and spreads to the *lamina propria* . Multiplication is extracellular with the exception of a small percentage of organisms multiplying in phagocytes. *Yersinia enterocolitica* survives in the hosts tissue with the aid of a V antigen. Protection from phagocytosis is considered one of the major roles of the plasmid encoded outer membrane proteins. These proteins have the ability to obstruct the antibacterial activity of phagocytes. The role of enterotoxins in the pathogenicity of *Yersinia enterocolitica* is questionable because both virulent and non-virulent strains produce a toxin. Toxin production ceases above 30°C and cannot be demonstrated in-vivo. High levels of lipolytic activity may be an additional virulence factor. By acting on the host's lipid layers, the organism may be able to penetrate and infect adjacent tissues easier (Larsen, 1977; Lee, 1977; Sonnenwirth and Swartz, 1980). Many of the temperature regulated, plasmid mediated, calcium dependent virulence factors are unknown or unclear. It is

evident that more research is required to fully understand how *Yersinia enterocolitica* acts as a dangerous foodborne pathogen.

The pathogenicity of *Yersinia enterocolitica*, like other pathogens, depends on strain, ingested dose, and the hosts genetic factors, age and health. In most humans, the result of an infection is gastroenteritis or classic food poisoning. The pathogenic serovars which are predominantly found in human disease cases are 0:8, 0:9, 0:5,27 and 0:3, with 0:8 most frequently found in cases from North America (Jay, 1997; Varnam, 1991). The average infectious dose level is $10^8$ - $10^9$ CFU's; however, $10^4$ - $10^5$ CFU's may be reported to be infectious also (Stern and Pierson, 1979). The symptoms of an infection include abdominal pain and diarrhea, which can vary from loose stools to ulcerative lesions. A fever and vomiting usually result. The abdominal pain is frequently in the lower right abdomen and many times has been diagnosed as appendicitis. In some cases unnecessary surgical interventions have occurred from misdiagnosis. The incubation period is about 36 to 48 hours, and the duration of the illness is approximately 1 to 3 days. In adults, however, complications can occur weeks after the initial infection affecting skin and connective tissue. Erythema nodosum is caused by *Yersinia enterocolitica* and results in painful red swellings, which are most common in older women. Connective tissue disorders include painful joints and various forms of arthritis. In a small percentage of cases complications can progress to long term syndromes including rheumatoid arthritis, cornea and pharynx inflammation, gland enlargement, arterial inflammation and damage, inflammatory dermatitis, hardening and shrinking of

connective tissue and autoimmune thyroid disease (Grave's disease). Only serovar 0:3 appears to be associated with the autoimmune disorders. Mortality is rare unless septicemia occurs which affects children, elderly and previously immunocompromised most severely. The fatality rate may exceed 50% if septicemia sets in (Varnam, 1991; Doyle and Cliver, 1990; Eley, 1992; Larsen, 1977; Pearson, 1977).

Yersinial infections are on the rise just like most foodborne bacterial infections. The first reported outbreak was in Japan in 1972 where 188 school children and one teacher were diagnosed. The source of transmission was never found (Cliver,1990).

*Yersinia enterocolitica* has been isolated from swine, beef, lambs, chicken, brown trout, oysters, shrimp, crab and water. The organism is generally associated with the animal kingdom. Although this organism is not as ubiquitous as many foodborne pathogens, it does offer unique problems to the industry if contamination occurs. The organism can grow readily at refrigeration temperatures so the use of cold temperatures to prevent growth is not successful. However, Lee et al., (1981) reported that freezing may have adverse effects on the survival of the organism over a period of time (~28 days).

### 1.3.4 *Bacillus cereus*

For many years *Bacillus cereus* has been known as a ubiquitous organism found in air, soil and water, but it was only considered as background contamination. More recently, it has received attention as an actual pathogen

and has been recognized as a serious threat to food safety (Hassan and Nabbut, 1996).

The genus *Bacillus* is a member of the *Bacillaceae* family and consists of gram positive, aerobic, catalase positive bacilli. A distinctive feature of the genus is the formation of heat resistant endospores. *Bacillus* species are classified depending on the shape of the spore and the swelling of the mother sporangium by the endospore. *Bacillus cereus* is in group 1 in which the spores are ellipsoidal and do not distend the sporangium. The vegetative cells of *Bacillus cereus* are motile by petrichous flagella and are very large. The cells often appear in chains. *Bacillus cereus* has a requirement for amino acids as growth factors, but vitamins are not essential for growth. It produces a variety of toxins, proteases, amylases, antibiotics and hemolysins which invariably add to its ubiquitous nature. Spores are formed during environmental duress such as heating and drying. The spores have a higher heat resistance than vegetative cells, thus allowing it to survive inadequate heat processes. Spore germination can occur from 8°C to 30°C and has an absolute requirement for glycine or a neutral L-amino acid and purine ribosides. L-alanine is the most effective amino acid for stimulating germination (Varnam, 1991; Eley, 1992; Cliver, 1990; Jay, 1997; Davis et al., 1980).

The genus *Bacillus* comprises of a large number of organisms, some of which are important in heat-treated foods and food poisoning. *Bacillus cereus* is the most important species associated with food poisoning.

*Bacillus cereus* produces a number of toxins including, two of which are responsible for producing human disease. A diarrheal toxin and an emetic toxin are produced by the organism. Many other toxins are produced, but they do not play a role in food poisoning (Varnam, 1991). The diarrheal toxin is a protein (MW~50,000) which is fairly heat labile (inactivation at 56°C for 5 min). It is also sensitive to trypsin and pronase and unstable at refrigeration temperatures. The emetic toxin is a small protein (MW~5,000) which is heat stable to 126°C for 90 minutes and is not sensitive to trypsin or pepsin. The emetic toxin is also stable at refrigeration temperatures for approximately two months. The productions of both toxins are associated with the growth of the organism. Diarrheal toxin is produced during exponential growth, while emetic toxin production appears to be during sporulation. Little is known about the genetic control of virulence (Varnam, 1991; Christiansson et al., 1989; Griffiths, 1990; Johnson et al., 1983).

The most common type of food poisoning caused by *Bacillus cereus* is associated with the diarrheal toxin. Incubation time ranges from 8 to 16 hours. Disease characteristics include abdominal pain, cramps and profuse watery diarrhea. Fever and vomiting are rarely seen. The emetic form of food poisoning has a much faster onset, which is usually within 1 to 6 hours after the ingestion of a contaminated food. Symptoms include a rapid onset of nausea, vomiting and malaise. With both syndromes recovery is usually within 24 hours. Treatment is not required because the diseases are self-limiting (Cliver, 1990; Varnam, 1991; Eley, 1992; Jay, 1997).

*Bacillus cereus* was thought to be a pathogen in the early twentieth century, but it was not until the 1950's that it was proved to be a pathogen by a Norwegian scientist. The first well-documented outbreak of diarrheal poisoning was in 1969. Like other forms of bacterial food poisoning, occurrences are increasing (Cliver, 1990).

Meat and meat products have been known to carry *Bacillus cereus* including stews, pies, soups and roasts (diarrheal poisoning). The endospore can survive the cooking processes, but significant numbers are usually not seen unless temperature abuse occurs post-heating (Juneja et al., 1997). Milk and milk products are recognized to have been contaminated with *Bacillus cereus* for years, but with the development of pasteurization the incidence has dropped to almost zero. The organism has been found in yogurt and cheese, but findings are rare. Cereal products, mainly rice, have been well-documented sources of *Bacillus cereus* emetic poisoning. Chinese restaurants have the highest rate of emetic food poisoning. This is due to traditional cooking practices. The Chinese boil large quantities of rice and allow it to stand at room temperature before frying. This allows for spore germination and toxin production (Varnam, 1991). Other less common cereal foods include pasta and wheat flour. Bread can also, to a limited extent, harbor the organism (Johnson, 1984). The incidence rate of *Bacillus cereus* in bread is increasing, mainly because antimicrobial agents are no longer used in the product formulation. Many dried foods, such as eggs, potatoes, legumes, sauces and mixes have been known to harbor the organism (Jaquette and Beuchat, 1998). Cross contamination is another vector for *Bacillus*

*cereus* (Pfeifer and Kessler, 1995). Although *Bacillus cereus* infections are a very small percentage of the total food poisoning cases, control measures are needed to help reduce the incidence rate (Jay, 1997).

### 1.3.5 *Staphylococcus aureus*

The Staphylococcal genus contains at least 23 species, most important being *Staphylococcus aureus*. This organism is of major concern to the meat and poultry industries (Miller et al., 1997).

*Staphylococcus aureus* is gram positive, facultatively anaerobic, non-sporeforming cocci. It is coagulase positive which differs from other *Staphylococcal* species. The organism belongs to the *Micrococcaceae* family, which has both oxidative and fermentative metabolism of glucose. *Staphylococcus aureus* also produces a golden carotenoid pigment.

The symptoms of *Staphylococcus aureus* food poisoning are entirely due to pre-formed enterotoxins and under most conditions no growth of the organism occurs in the host's intestine. Eight enterotoxins have been identified in food poisoning cases. These enterotoxins are similar in composition and biological activity, but they are identified as separate proteins because they are immunologically distinct (antigenicity differences). The eight serologically distinct enterotoxins are identified as A, B, C1, C2, C3, D, E and H. Other enterotoxins exist and have not yet been identified (Su and Wong, 1995). The identified toxins are heat stable, water soluble, single chain globular proteins with molecular weights between 26,900 and 35,000 Daltons. The toxins are resistant to digestive enzymes such as trypsin (pancreas) and pepsin (stomach) allowing

them to pass through the digestive tract to the site of action. Generally, contamination levels of approximately $10^6$ CFU's are sufficient to produce enough toxin for disease (Pereira et al., 1996). It is estimated that between 1 microgram and 100 nanograms of toxin will cause sickness.

The site of action in the intestinal tract is not known. Experiments in primates to determine the emetic action of the enterotoxins has determined that the mode of action by the toxins is not like other food poisoning organisms or toxins. The enterotoxins do not act directly on intestinal cells like other toxins. Instead, they act on receptors in the intestine with the stimulus reaching the vomit center in the brain through the vagus nerve. Enterotoxin A is the most commonly observed cause of *Staphylococcus aureus* poisoning. The diarrheal actions of the toxins are different from other diarrheal toxins. They do not cause fluid accumulation when injected into experimental ileal loops in rabbits. However, research has been unable to determine what action actually stimulates diarrhea (Varnam, 1991; Jay, 1997; Eley, 1992; Cliver, 1990). Based on work cited, it is evident that more research is needed to elucidate the action of the toxins produced by *Staphylococcus aureus*.

The symptoms of *Staphylococcus aureus* food poisoning usually appear within 2 to 4 hours after consumption of a contaminated food. Incubation periods as short as 30 minutes and as long as 8 hours have been reported. Commonly, reported symptoms include nausea, retching, vomiting and less frequently diarrhea. Body temperature can drop in some cases and in others low-grade fevers develop. The illness is usually self-limiting and lasts about 24

hours. Treatment is not recommended unless dehydration occurs. Death is rare except among the young, old and immunocompromised (Cliver, 1990; Eley, 1992; Varnam, 1991; Jay, 1997).

*Staphylococcus aureus* is the second leading cause of food poisoning in the United States (Eley, 1992). Little progress was made in identifying the organism before 1914 when Dr. Barber isolated the organism from milk obtained from the Philippines. Many cases were reported and described before this, but the actual causative factor was not described until 1914. The toxins were not discovered until 1930. Outbreaks are generally small; however, large outbreaks (>1000) have been documented (Cliver, 1990).

*Staphylococcus aureus* is almost always transmitted to food from a human source (i.e. food handlers, cross contamination). Human to human transmission is also of importance since it is estimated that 25 to 50% of the population may be carriers of the organism (Eley, 1992). *Staphylococcus aureus* has been found on the skin, nose, throat, hair and sometimes stools of humans. Domestic and food animals are also carriers of the organism. Foods commonly associated with *Staphylococcus aureus* poisoning include meat and poultry products. Typically the organism is not able to grow on meat, but cross contamination, poor hygiene and temperature abuse occur and outbreaks from meat products are common (Niven, 1987). Many other foods including dairy products, vegetables, fish and fish products, eggs and egg products, fermented foods and bakery products have been implicated in *Staphylococcal* poisoning; however, the

actual source of contamination almost always is the food handler (Juneja et al., 1997).

Each year the incidence rate increases for all of the described organisms and other foodborne pathogens as well. Control and prevention measures are needed to help provide a safer food supply.

## 1.4 PREVENTION OF FOODBORNE DISEASE

### 1.4.1 GOVERNMENT INTERVENTION

From the producer to the consumer, food safety is a major concern. Impressive gains have been made in supplying a healthy and safe food supply. An absolute risk free food supply is not attainable due in part to human contamination and the ability of microorganisms to grow and adapt to a variety of environments. Production and marketing of a safe food supply requires the interaction of several agencies.

A safe food supply begins with the food industry. Proper sanitary procedures by producers, processors and distributors is required to help reduce the potential of foodborne disease outbreaks. Government regulatory agencies work closely with the food industry in attempt to provide safe food products. Inspection services have been established for many years. Currently, food safety advocates are appealing for a safer food supply. Stringent new laws and inspection systems are being established. Trained microbiologists are working closely with quality assurance groups and federal inspectors on the implementation of the new laws and regulations (Jay, 1997). The Food and Drug Administration (FDA) has jurisdiction over all foods traveling interstate, except

meat and poultry. The FDA's regulatory approach is based on Good Manufacturing Practice (GMP) codes. In addition to food processing and distribution, the FDA regulates eating establishments on common carriers, federal property and interstate highways. The United States Department of Agriculture (USDA) has jurisdiction over meat and poultry in interstate commerce and in about half the states' intrastate commerce. The USDA's authority is derived from the federal Meat and Poultry Inspection Acts. Inspection activity is from the Food Safety and Inspection Service (FSIS). State agencies also exist to work in cooperation with federal agencies to aid in providing the safest food supply possible (Jay,1997). State and local health agencies are also involved in food safety issues. These agencies handle issues regarding food poisoning, food related diseases, hazardous diseases and environmental health, which includes food service establishment inspections (Ingham County Health Department, 1998). Regardless of the services provided by the government, foodborne disease outbreaks continue to rise.

The Hazard Analysis and Critical Control Point (HACCP) concept was developed in 1971 by H.E. Bauman and other scientists at the Pillsbury Company in collaboration with the National Aeronautics and Space Administration (NASA) and the U.S. Army Research Laboratories. It was first applied to low-acid canned foods. HACCP is a system that leads to the production of microbiologically safe foods by controlling the organisms at the point of production and preparation. It focuses on raw materials as well as end products (Bauman, 1990). More recently, HACCP systems have been

mandated in meat and poultry programs as a systematic approach to food safety. In 1992 , the National Advisory Committee on Microbiological Criteria for Foods developed and adopted the HACCP program for the meat and poultry industry. In 1995, the initial proposal for a pathogen reduction/HACCP program was made. This program was termed the Mega-Reg. The Mega-Reg is the FSIS's regulatory overhaul of all federally and state inspected facilities. The final rule was made on July 25, 1996 and required the implementation of HACCP-based systems for all inspected facilities. Additionally, the development and implementation of sanitation standard operating procedures (SSOPs) and pathogen testing programs for *E.coli* (slaughter facilities) and *Salmonella* performance standards (slaughter and raw ground meat producing operations) were required. The SSOP and pathogen (*E.coli*) testing requirements became mandatory on January 27, 1997. The compliance deadlines for HACCP programs and *Salmonella* performance standards were staggered over three years ranging from January 25, 1998 for facilities with over 500 employees to January 25, 2000 for facilities with fewer than 10 employees (Gombas, 1998).

Other government agencies are involved to a greater or lesser extent in the production of a safe food supply including the Center for Disease Control, Environmental Protection Agency, National Marine and Fisheries Service and state run Departments of Health and Agriculture (Cliver,1990). All of the regulatory agencies attempt to work together to provide a safe and wholesome food supply. However, as mentioned before, foodborne disease outbreaks are increasing. Alternative methods of control in conjunction with the newly devised

pathogen reduction/HACCP plans are essential to decrease or possibly eliminate the threat of meatborne disease outbreaks.

## 1.5 SCIENTIFIC AND APPLIED APPROACHES TO PATHOGEN CONTROL AND PREVENTION OF FOODBORNE DISEASE IN MEAT AND MEAT PRODUCTS

Pathogen control research is increasing with the enhanced concern from consumers regarding food safety. Meat and meat products are especially rich environments for both pathogens and spoilage organisms, which makes control difficult.

The initial selection of an antimicrobial agent depends on the microbial spectrum of the chemical. Rarely does an antimicrobial inhibit or kill all organisms; therefore, it is important to select the chemical that best fits the need of the food product (Branen, 1993). Many antimicrobial compounds are being used in the meat industry including sorbic acid and sorbates, organic acids, nitrite, bacteriocins, parabens and phenolic compounds. Consumer acceptance trends, however, are pushing for natural and safe products such as essential spice oils and compounds within these oils. In order for the meat and food industry to remain competitive, it must meet the demands of the consumers (Conner,1993). Recent trends also include a desire for high quality foods that are minimally processed (free from chemical additives, less salt, less heat). This has major microbiological implications. By altering traditional practices such as salting and preservative addition and lower heating, shelf life and safety of the foods may be adversely affected. The loss of preservation and safety must be

compensated, and it is here that natural antimicrobials may have an important function.

A wide range of effective natural antimicrobial agents exist. Examples include animal derived antimicrobial agents such as lysozyme, lactoperoxidase, lactoferrin and serum transferrin and plant derived antimicrobials such as essential spice oils, low molecular weight compounds of herbs and spices, phenolics from herbs and spices and phytoalexins. Natural antimicrobial compounds are also derived from microorganisms including bacteriocins such as nisin and pediosin. An increasing number of these natural systems are being utilized or studied in food products. The future potential is great for these compounds, especially plant derived antimicrobial agents. More research is necessary to fully understand how they are effective and what levels are needed for effectiveness (Jay, 1997; Davis et al., 1980).

Currently, very little is known about the mechanisms involved in the biocidal activity of essential spice oils and their constituents. Some attempts have been made to determine these mechanisms. For example, Deans and Ritchie (1987) determined when an essential spice oil exhibited biocidal activity, it appeared to be equally effective against gram positive and gram negative types of bacteria. This would tend to suggest that the inhibitory mode of action is common to all bacteria, possibly acting in the cytoplasm rather than the cell wall where chemical and physical makeup differs between bacterial types (Hay and Waterman, 1993).

The control of microbial organisms by plant essential oils can vary due to the fluctuating biological activity of the oils. This is not surprising since the chemical makeup of the oils ranges from relatively inert hydrocarbons to highly reactive phenols. Additionally, the chemical composition of an oil originating from a single plant species can vary based on chemotype, geographic origin, climate fluctuations and stage of growth or ontogeny at harvest. All of the factors listed have an impact on the antimicrobial activity of essential oils (Rhyu, 1979; Deans and Ritchie, 1987;Guenther, 1950).

Attempts have been made to identify which functional groups or spatial arrangements are responsible for the biocidal action of the oils. Villar and co-workers (1986) and Kabara (1984) found that the cis-configuration around the double bonds of the oils related to greater antimicrobial activity than the trans-configuration. They also noted that the most active functional group was the hydroxy group in both aliphatic alcohols and phenols. Chain length in aliphatic compounds also was important in antibacterial activity. Even though a great deal is known about functional group activity of essential spice oils and their constituents, the actual mechanisms of action is unclear (Knobloch et al., 1986).

The essential oils of oregano, thyme, bay, clove and cinnamon will be discussed in detail as they were the basis for the research to be described later. Additionally, a major constituent of each oil, carvacrol, thymol, eugenol, caryophyllene and cinnamic aldehyde, respectively, will be reviewed also. The following discussion will focus on how these compounds act as antimicrobials

against pathogenic microorganisms with emphasis on *Escherichia coli, Salmonella, Yersinia, Bacillus* and *Staphylococcus* species.

## 1.6 HISTORY, CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL SPICE OILS AND CONSTITUENTS

### 1.6.1 OREGANO OIL

The term *Origanum* is used to describe a variety of plants in the *Origanum (Corido)* and *Thymus* species, which are native to the Mediterranean area. *Origanum* is a member of the *Labiatae* family (Salzer and Furia, 1977). Important phenolic species within the *Origanum* group include Greek oregano (*Origanum vulgare Linne ssp viride (Boiss.) Hayak*), Turkish oregano (*Origanum onites Linne*), Spanish oregano (*Coridothymus capitatus Linne Hoffman* and *Link*), and Mexican oregano (*Lippia graveolens* HBK) (Putievsky et al., 1985). Spanish oregano is the most common variety used in the manufacture of essential oregano oil with other varieties used to a lesser extent. Botanically, the *Origanum* species are closely related. Therefore, chemical compositions are also similar (Salzer and Furia, 1977). The essential oil has been analyzed extensively by gas chromatography and contains between 20 and 50 components. Predominant compounds include carvacrol and thymol with a higher ratio of carvacrol to thymol. Thyme oil contains a higher ratio of thymol to carvacrol. It is difficult to distinguish between oregano and thyme oil organoleptically, but the general rule is thyme oil has a hard medicinal quality while oregano is more herbal-spicy (Salzer and Furia, 1977).

The Food Chemical Codex (1963) describes *Origanum* oil (Spanish) as follows: "The volatile oil obtained by steam distillation from the flowering herb, *Thymus capitatus* and various species of *Origanum*. It is a yellowish red to dark

brownish red liquid, having a pungent spicy odor suggestive of thyme oil. It is soluble in most fixed oils, and in propylene glycol. It is soluble, with turbidity, in mineral oil, but is insoluble in glycerine."

Oregano oil is used as a flavoring agent, but it does possess other useful characteristics to the food industry, such as antimycotic, antiaflatoxigenic and antioxidant capabilities (Hitokoto et al., 1980; Shelef, 1983). The antimicrobial activity of oregano oil could be of importance due to greater government restrictions and consumer demands regarding the use of "unnatural" antimicrobial agents (Davidson and Parish, 1989).

The antimicrobial activity of oregano oil has been studied to a small degree. The antifungal, antimycotic and antiaflatoxigenic activity of oregano oil has been studied extensively; however, the following discussion will only cover antimicrobial activity since bacteria differ greatly from molds and fungus.

Beuchat (1976) studied the effects of several spices, including oregano, on *Vibrio parahemolyticus*. Dried, ground oregano was added to agar at concentrations up to 1.0%. The oregano was highly inhibitory to the organism as noted by lack of colony growth. Inhibition was observed at levels as low as 0.1% and complete bactericidal activity was noted at 0.5%. Beuchat also screened the essential oil of oregano by the broth dilution method with final concentrations of 10 and 100μg of oil per milliliter of broth. The oregano oil was bactericidal at 100μg/ml. Ismaiel and Pierson (1990a, b, c) studied the effects of oregano and other spice oils on Clostridium botulinum in three separate trials. The first two studies demonstrated the inhibition of germination, outgrowth and vegetative

growth of four different serotypes of *Clostridium botulinum* (33A, 40B, 1623E and 67B). Spice oils were added (10, 50, 100, 150 and 200 ppm) to medium then inoculated with spores. The tubes were held in an anaerobic environment for three days at 35°C. The number of colonies were counted. Oregano oil was considered a very active inhibitor at 200ppm. The effect of oregano oil (10, 50,100, 150 and 200 ppm) on spore germination was studied using the microculture method (oil smeared on glass slide then covered with spores dried on cover slip then placed in growth agar). The oregano oil was highly inhibitory at 150 and 200 ppm, while at 10 ppm little inhibition occurred. To observe the effects of oregano oil on outgrowth and vegetative growth, germinated spores were placed in tubes with media and oil (same levels mentioned) and incubated for a week. Growth of germinated spores was monitored by measuring absorbance (600 nm). Vegetative cells (in late logarithmic phase) were placed in media and incubated (7 days) with the spice oil. Oregano oil had no effect on outgrowth of *Clostridium botulinum* spores. Vegetative cell growth was prevented with 150 and 200 ppm oil, while 10 ppm inhibited growth slightly. Ismaiel and Pierson's third study (1990c) observed the effects of oregano oil (100 and 200 ppm) on *Clostridium botulinum* toxin production in broth and ground pork. Oregano oil was effective at inhibiting growth in broth. The inhibitory effect was dramatically reduced, however, when added to inoculated ground pork. In ground pork, oregano oil was only effective at a higher level (400 ppm) in combination with 50 ppm sodium nitrite. Huhtanen (1980) also studied the antimicrobial activity of oregano oil on *Clostridium botulinum*. The organism was

grown in media with varying degrees of oils. Turbidity measurements were made to determine if any growth occurred. Oregano oil was somewhat inhibitory (not bactericidal) at 500 ppm.

Paster and co-workers (1990) studied the inhibitory action of oregano and thyme oils on foodborne bacteria. They used the pour plate method with oils incorporated into the agar up to 350 ppm. Several pathogens were tested including *Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa, Campylobacter jejuni* and *Clostridium sporogenes*. Under aerobic conditions, oregano oil (250 ppm) slightly inhibited *Salmonella typhimurium* and *Staphylococcus aureus*. The inhibitory effect was greatly enhanced when organisms were incubated at low oxygen tensions. *Pseudomonas aeruginosa* was unaffected, while *Clostridium sporogenes* and *Campylobacter jejuni* were highly inhibited at low oxygen tensions. They speculated that low oxygen tension results in less oxidative changes in the oils. Antimicrobial mixtures with oregano oil have also been researched. Tassou and co-workers (1996) inoculated (*Staphylococcus aureus* and *Salmonella enteriditis*) fish and mixed it with a mixture of olive oil, oregano oil and lemon juice. They measured the inhibitory effects of the mixture on the pathogens as well as resident microflora. A modified atmosphere (40% $CO_2$, 30% $0_2$, 30% $N_2$) was used. The mixture had a bacteriostatic effect on the pathogens and resident microflora; though, it was difficult to say which component or components were the actual antimicrobial agent. *Staphylococcus aureus* was inhibited to a greater extent in the modified atmosphere versus aerobic conditions. In an informational article (Anonymous,

1997) oregano and other spices were described as powerful antibiotics. The article stated that: "garlic, onion, allspice and oregano killed all the bacteria they were tested against including *Salmonella* and *Staphylococcus*". This statement was made by Paul Sherman who is an evolutionary biologist at Cornell University. He is studying traditional meat recipes from 31 countries. He stated that: "Their case was supported by the fact that the hotter the climate, the more danger of food poisoning; hence, the more spices are used. Therefore, spices have more uses than just seasoning—POWERFUL ANTIBIOTICS". Based on the research published, it is evident that oregano oil could be a powerful antibiotic in food products.

### 1.6.2 THYME OIL

Thyme oil extract is obtained from the leaves and flowering tops of the plant *Thymus vulgaris Linne*. As mentioned earlier, this plant is closely related, botanically and chemically, to the *Origanum* species. The essential oil content usually comprises about 10% of the extract, while *Origanum* species tend to be higher ranging from 20 – 25%. As with oregano oil, thyme oil has two components, including thymol and carvacrol with thymol present in larger quantities (Salzer and Furia, 1977). The Food Chemical Codex (1963) describes thyme oil as: "The volatile oil obtained by distillation from the flowering plant *Thymus vulgaris Linne* or *Thymus zygis Linne* and its variety *gracilis Boissier* (Family *Labiatae).* It is a colorless, yellow or red liquid, with a characteristic

pleasant odor, and a pungent, persistent taste. It is affected by light. The specifications state that not less than 40% by volume must be phenols".

Like oregano, extensive research has been conducted on the antimycotic, antiaflatoxigenic and antifungal properties of thyme oil. Little has been published on the antimicrobial aspects of the oil. The same antimicrobial research conducted on oregano oil was conducted on thyme oil. Beuchat (1976) observed the effects of thyme oil on *Vibrio parahemolyticus*. He determined that dried thyme was highly toxic to the organism at a concentration of 0.5%. The essential oil of thyme was bactericidal at 100 ppm. Ismaiel and Pierson (1990 a, b, c) studied the effects of thyme oil on *Clostridium botulinum* spore germination, outgrowth and vegetative cell growth. Thyme oil actively inhibited the organisms growth at 200 ppm, similar to oregano oil. At 150 and 200 ppm, thyme oil prevented germination of spores. Thyme oil completely inhibited spore outgrowth and vegetative growth at 200 ppm, while oregano oil was also bactericidal at 150 ppm. Inhibition of *Clostridium botulinum* by thyme oil was studied by Huhtanen (1980). Thyme oil was inhibitory, but not bactericidal, at 500 ppm. Paster and co-workers (1990) determined that 350 ppm thyme oil (versus 250 ppm for oregano oil) inhibited *Staphylococcus aureus* and *Salmonella typhimurium*. The oil had no effect on *Pseudomas aeruginosa* up to 500 ppm. Thyme oil was very effective against *Campylobacter jejuni*. The inhibitory effect was enhanced for thyme oil when incubated in low oxygen tensions.

Deans and Ritchie (1987) conducted a screening experiment to determine the antibacterial properties of selected essential plant oils. Twenty-five organisms were tested including generic *E. coli, Staphylococcus aureus* and *Yersinia enterocolitica*. Fifty essential oils were used in the screening process including bay, cinnamon, clove and thyme. The technique used to determine the inhibitory abilities of the oils was the well diffusion method. The method involved punching a hole in agar that created wells into which a specific (10µl) quantity of oil could be placed. This technique insured that the radial diffusion from each well was easily measured which allowed the researcher to assess actual inhibition intensity. Thyme oil was highly inhibitory to twenty-three of the organisms including *E. coli, Staphylococcus aureus* and *Yersinia enterocolitica*. Another screening experiment was led by Aktug and Karapinar (1986). Various concentrations of thyme oil were tested against *Salmonella typhimurium* and *Staphylococcus aureus*. Oil levels of 250, 500, 1000, 2000, 4000, 5000, 6000, 8000 and 10,000 ppm were added to growth media and autoclaved. The organisms were then plated and growth observed for seven days. Thyme oil inhibited *Staphylococcus aureus* at 500 ppm while *Salmonella typhimurium* displayed little sensitivity to the oil. Thyme and oregano oils both exhibited antimicrobial activity against several foodborne pathogens and have potential in food products as natural antibiotics.

### 1.6.3 BAY OIL

Bay oil is also referred to as Myrcia oil. The Food Chemical Codex (1963) describes bay oil as: "The volatile oil distilled from the leaves of *Pimenta racemosa (Miller) J.W. Moore* (Family *Myrtaceae*). It occurs as a yellow to brownish yellow liquid with a pleasant aromatic odor, and a pungent, spicy taste. It is soluble in alcohol and in glacial acetic acid". Bay oil should not have less than 50% or more than 65% by volume of phenols. The bay or bay rum tree occurs wild or semi-wild and is cultivated on several West Indian Islands including Dominica and Puerto Rico. The leaves of the tree are harvested and distilled for the bay oil. Eugenol, which is an aromatic phenol, is a major constituent of bay oil (Guenther, 1950).

Two studies were located on the antimicrobial effects of bay oil and bay extract. Huhtanen (1980) determined that bay leaves in ethanol (125 ppm) inhibited *Clostridium botulinum.* Deans and Ritchie (1987) screened selected plant essential oils against many foodborne pathogens including *E. coli, Staphylococcus aureus* and *Yersinia enterocolitica.* Bay oil (1:5 oil/ethanol) was found to be inhibitory against all the organisms.

### 1.6.4 CLOVE OIL

Another popular spice, clove oil, also has antimicrobial activity. The Food Chemical Codex (1963) defines clove oil as: "The volatile oil obtained by steam distillation from the dried flower buds of *Eugenia caryophyllus (Sprengel) Bullock et Harrison* (Family *Myrtaceae*). It is a colorless or pale yellow liquid having the

characteristic clove odor and taste. It darkens and thickens upon aging or exposure to air".

Since ancient times, the preserving properties of essential spice oils have been known. The Egyptians used oils such as cloves, cinnamon and cassia to enhance the mummification process. Early Romans also left literature describing the use of spice oils for medicinal properties (Bullerman et al., 1977).

Clove extract is generally about 80% essential oil. The oil is comprised of eugenol, eugenyl acetate and caryophyllene. Very little antimicrobial research has been published. Huhtanen (1980) described the inhibition of *Clostridium botulinum* by clove and other oils previously described. When compared to the other oils, clove oil was a less potent inhibitor of the organism. Conversely, Ismaiel and Pierson (1990a, b, c) determined that clove oil was a very potent inhibitor of spore germination, outgrowth and vegetative cell growth. Deans and Ritchie (1987) found that clove oil (1:5 oil/ethanol) inhibited *E. coli, Staphylococcus aureus* and *Yersinia enterocolitica.* Despite the conflicting reports on clove oil, it could still be considered a usable antimicrobial agent. Further research is necessary to determine the actual potential of clove oil.

### 1.6.5 CINNAMON OIL

Cinnamon (leaf) oil is extracted from the variety *Cinnamomum cassia Linne Blume.* The Food Chemical Codex (1963) describes cinnamon oil as: "The volatile oil obtained by steam distillation from the leaves and twigs of Cinnamomum cassia Nees Blume (Family:*Lauraceae*). It is a yellowish or

brownish liquid having the characteristic odor and taste of cassia cinnamon. Upon aging or exposure to air, it darkens and thickens. It is soluble in glacial acetic acid and in alcohol". The Food and Drug Administration describes the oil similarly, but they also state that the oil must contain no less than 80% , by volume, of cinnamic aldehyde (Guenther, 1950). The *Cinnamomum cassia* species is also termed Chinese cinnamon versus Ceylon or Padang cinnamon. Chinese cinnamon is an oleoresin comprised of about 66% essential oil, 10% fatty oil and small levels of coumarin and alkaloids (Salzer and Furia, 1977). The antimicrobial properties have been studied to a very small extent.

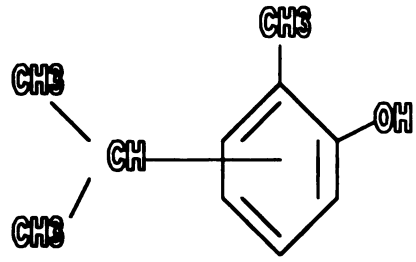
Ismaiel and Pierson (1990 a, b, c) tested cinnamon oil against *Clostridium botulinum*. Like oregano and thyme oils, the effects of cinnamon oil on spore germination, outgrowth and vegetative cell growth were studied. Cinnamon oil at 100 ppm prevented spore germination, but had no effect on outgrowth. Vegetative growth was diminished, but not as effectively as clove oil. Deans and Ritchie (1987) found that cinnamon oil (1:5 oil/ethanol) inhibited *E.coli, Staphylococcus aureus* and *Yersinia enterocolitica*. More research is necessary to elucidate the antibacterial activity of cinnamon oil.

Essential spice oils are composed of many different chemical compounds. Some which have antibacterial properties. These antibacterial compounds include many low molecular weight substances among which phenolic compounds predominate. The aldehydes, alcohols and esters are also potential antibacterials (Salzer and Furia, 1977;Gould, 1996). Carvacrol, thymol, eugenol, caryophyllene and cinnamic aldehyde are constituents of oregano, thyme, bay,

clove and cinnamon oils respectively. Each of these compounds are known to have antibacterial properties to some extent and will be discussed further.

### 1.6.6 CARVACROL

The Food Chemical Codex (1963) describes carvacrol as: "A colorless to pale yellow liquid consisting mainly of a mixture of isomeric carvacrols (isopropyl-o-cresols), and having a pungent, spicy odor resembling that of thymol. It is freely soluble in alcohol and in ether, but is insoluble in water". Carvacrol is a major component of both oregano and thyme oil. The structure of carvacrol is as follows:



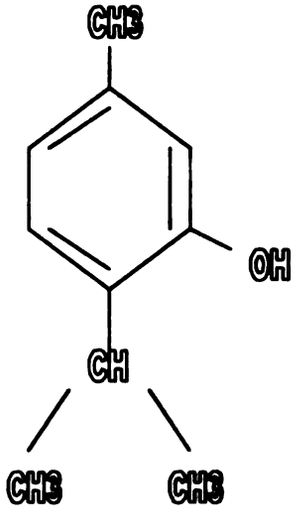

(Food Chemical Codex, 1963)

Kim and co-workers (1995a) studied the antibacterial effects of several spice oil constituents including carvacrol. The first study tested the activity of carvacrol against *Salmonella typhimurim* in culture medium and fish cubes. Carvacrol at 5 and 10% was highly inhibitory to the organism. The minimum inhibitory concentration of carvacrol was 250 ppm. Minimum inhibitory concentration is the lowest concentration at which no growth occurred in media. Fish cubes were inoculated with the organism and dipped into 0.5, 1.5 and 3% carvacrol. The fish cubes were stored at 4°C in whirlpack bags and sampled on

day 1, 2 and 4. Carvacrol, at 0.5%, decreased the organism 1 $\log_{10}$ CFU/g over all the days compared to a control. Carvacrol, at 1.5%, reduced the organism 3 to 6 $\log_{10}$ CFU/g over the testing period. Complete inhibition was observed with 3.0% carvacrol. Kim and co-workers (1995b) screened several essential oil components against five foodborne pathogens. The organisms included *E. coli*, *E. coli* 0157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Vibrio vulnificus*. Carvacrol exhibited strong antibacterial activity against all organisms at levels from 250 to 500 ppm. Based on work published, carvacrol is an effective inhibitor of major foodborne bacteria and has potential as a natural antibiotic in foods.

### 1.6.7 THYMOL

Thymol (5-methyl-2-isopropylphenol) is a low molecular weight, phenol with known antibacterial activity. It is a major component of thyme and oregano oils (Gould, 1996; Salzer and Furia, 1977; Putievsky et al., 1985; Putievsky, 1985). The structure of thymol is as follows:




(Hay and Waterman, 1993)

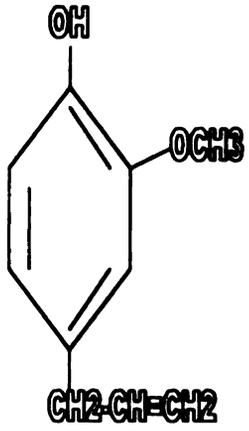
Thymol has been used for years as a therapeutic agent in the treatment of feline fungal infections (Windholz, 1976). The antimicrobial effect of thymol is poorly understood; though, it has been shown that the hydroxy group of both aliphatic alcohols and phenols was the most active biocidal functional group of the compound (Putievsky, 1985).

Research has been conducted on the antifungal and antimycotic abilities of thymol, but no antibacterial research was located. Due to the lack of research in this area, opportunities exist to determine if thymol is a potential antibacterial agent in foods.

### 1.6.8 EUGENOL

Eugenol is a phenolic compound primarily found in bay, clove and cinnamon oils. Eugenol can be as high as 50 to 65% in bay oil and 95% in clove oil. Cinnamon oil ranges from 4 to 10% eugenol (Guenther, 1950). Eugenol is also known as 4-allyl-2-methoxyphenol, eugenic acid and 4-allylguaiacol. The Food Chemical Codex (1963) defines eugenol as: "The main constituent of carnation, cinnamon leaf and clove oils. It is obtained from clove oil and other sources (Bay oil). It is a colorless to pale yellow liquid having a strongly aromatic odor of clove, and a pungent, spicy taste. It darkens and thickens upon exposure to air. It is slightly soluble in water and is miscible with alcohol, with chloroform, with ether and with fixed oils".

The structure of eugenol is as follows:



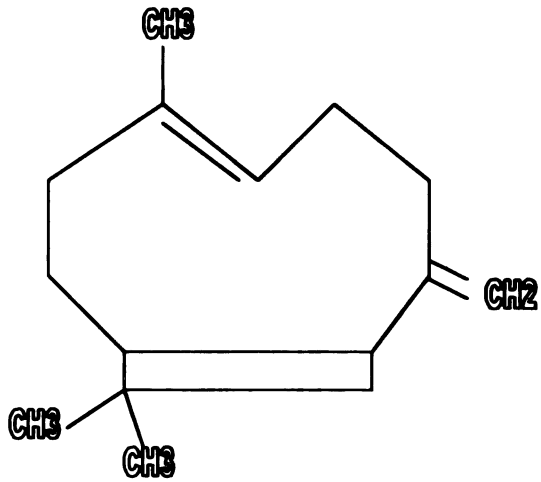

(Food Chemical Codex, 1963)

Research involving the inhibition of foodborne pathogens by eugenol is minimal. A study by Kim and co-workers (1995b) observed the effects of essential oil components against several foodborne pathogens. Eugenol (5, 10, 15 and 20%) was included in the study as a possible biocidal agent against *E. coli* 0157:H7, *Salmonella typhimurium* and other pathogens. Eugenol exhibited a dose related response and was effective against the pathogens. More research is warranted to determine if eugenol is a possible antibacterial against other common foodborne pathogens.

### 1.6.9 CARYOPHYLLENE

Caryophyllene is a constituent of many essential spice oils, especially clove oil. The Food Chemical Codex (1963) defines caryophyllene as: "A mixture of sesquiterpenes differing slightly in structure and occurring in many essential

oils, especially clove oil. It is a colorless to slightly yellow oily liquid having a light clove-like odor. It is soluble in alcohol and in ether, but insoluble in water". The structure of caryophyllene is as follows:



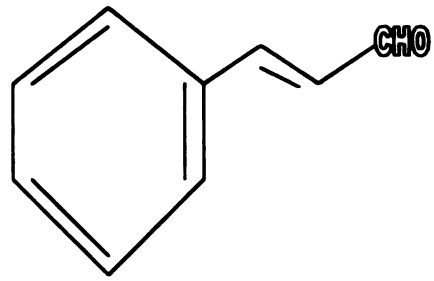

(Food Chemical Codex, 1963)

No research publications were located on the antibacterial, antifungal or antimycotic ability of the compound. However, caryophyllene is a sesquiterpene which may reduce its antibacterial capacity since these types of compounds are less volatile than monoterpenes (Hay and Waterman, 1993).

### 1.6.10 CINNAMIC ALDEHYDE

Cinnamic aldehyde is a major component of cinnamon oil. Cinnamic aldehyde comprises about 65 to 75% of the volatile portion of the oil. Depending on the growth conditions, upwards of 90% cinnamic aldehyde is possible in the oil (Morozumi, 1978; Bullerman et al., 1977; Salzer and Furia, 1977). Cinnamic

aldehyde is defined by the Food Chemical Codex as: "The main constituent of oils of cassia, cinnamon bark and root. It is usually prepared synthetically. It is a yellow, strongly refractive liquid, having an odor resembling that of cinnamon oil, and a burning aromatic taste. It is affected by light. One gram dissolves in about 700 ml of water. It is miscible with alcohol, with chloroform, with ether and with fixed or volatile oils". The structure of cinnamic aldehyde is as follows:




(Food Chemical Codex, 1963)

Published research on the antibacterial effects of the compound were unavailable. The antifungal and antimycotic properties have been studied somewhat. Interestingly, Morozumi (1978) studied 50 essential oils and components against fungal species and found that cinnamic aldehyde exhibited the highest antifungal ability. Antibacterial research may prove that cinnamic aldehyde is an effective agent against foodborne pathogens also.

### 1.6.11 CONCLUSION

Realistically, extensive research is needed to determine all the possible essential oils and constituents that are effective against foodborne pathogens. Additional research should address the efficacy and functionality in food systems, the toxicology and safety in food formulations, the interactions with food components and preservation system and the mechanisms in which the essential oils and constituents act against the microorganisms. However, consumers are demanding a safe food supply, preferably provided by natural methods. The research to be presented henceforth will describe how selected essential oils and constituents of the oils affect several pathogens commonly found in meat and meat products.

# CHAPTER 2

## 2.0 DETERMINATION OF ANTIBACTERIAL POTENTIAL OF SELECTED ESSENTIAL PLANT OILS AND CONSTITUENTS AGAINST SEVERAL FOODBORNE PATHOGENS

### 2.1. ABSTRACT

Antibacterial activity of five essential plant oils and a major constituent from each (0, 2.5 and 5.0% in Tween 20/ethanol) were tested against five foodborne pathogens including *Escherichia coli* 0157:H7, *Salmonella typhimurium, Yersinia enterocolitica, Staphylococcus aureus* and *Bacillus cereus* using the paper disk method. Treatments were run in duplicate. Any degree of inhibition (clear zone around disk) was considered a positive or antibacterial response, while no clearing around the disk was considered a negative response. Caryophyllene was negative against *Yersinia enterocolitica, Staphylococcus aureus* and *Salmonella typhimurium* at both test levels. Cinnamon oil at 2.5% was ineffective (negative) against *Staphylococcus aureus*; however, at 5.0% a zone of inhibition was noted. All other test combinations were positive. Therefore, all test compounds, with exception to caryophyllene, could serve as potential antibacterial agents to inhibit pathogen growth in food.

### 2.2 INTRODUCTION

The antifungal and antimycotic activity of essential spice oils and constituents within these oils are well documented (Bullerman et al., 1977; Buchanan and Shepard, 1981; Paster et al., 1990; Salmeron et al., 1990;

Paster et al., 1995; Azzouz and Bullerman, 1982). Successful antibacterial research has been conducted on essential spice oils and constituents. Compounds with known antibacterial activity include clove, oregano, thyme, cinnamon and bay oils. Carvacrol, eugenol and thymol are phenolic constituents of essential spice oils that have documented antibacterial properties (Deans and Ritchie, 1987; Shelef, 1983; Kim et al., 1995 a,b; Morozumi, 1978; Aktug and Karapinar, 1986; Hall and Maurer, 1986; Beuchat, 1976). All of the studies use a range of pathogens which makes comparison and evaluation of the compounds difficult. A comprehensive study is needed to determine how these compounds affect a range of common pathogenic microorganisms and what levels are required for inhibition. The objective of this study was to screen five common foodborne pathogens against five essential plant oils and one major constituent from each oil to test for antibacterial potential. Compounds with weak inhibitory activity would be dropped from further studies which were more labor intensive than the paper disk assay.

## 2.3 MATERIALS AND METHODS

### 2.3:1 TEST COMPOUNDS

Oregano, clove, thyme, bay and cinnamon oils were obtained from Kalsec, Inc. (Kalamazoo, MI). Carvacrol, eugenol and cinnamic aldehyde were purchased from Aldrich Chemical (Milwaukee, WI). Caryophyllene and thymol were purchased from Sigma Chemical Company (St. Louis, MO). All test compounds were used without further purification. Solutions with the desired

level of test compound (0, 2.5 or 5.0%) were prepared fresh before each use by dissolving in 10 ml of sterile distilled, deionized water containing 0.2g filter sterilized ethanol and 0.2g Tween 20 (Aldrich Chemicals). Control treatments were 10 ml of sterile distilled, deionized water with 0.2g sterile ethanol and 0.2g Tween 20 and no test compound.

### 2.3:2 TEST MICROORGANISMS

*Escherichia coli* 0157:H7 (ATCC 43894), *Salmonella typhimurium* (ATCC 29630), *Yersinia enterocolitica* (Microbiology Culture Lab,Michigan State University ), *Bacillus cereus* (14579) and *Staphylococcus aureus* (25923) stock cultures were maintained tryptic soy broth (Difco: Detroit, MI) except for *Yersinia enterocolitica* which was maintained in brain-heart infusion broth (Difco:Detroit, MI). The cultures were transferred into fresh broth and incubated 22 to 24 hr at 35°C before use . Organisms were centrifuged (Sorvall Superspeed RC2, Norwalk, CT) at 4,340 x g for 20 minutes. The pelleted sediments were resuspended in sterile 0.1% buffered peptone water (Becton Dickinson Microbiology Systems, Cockeysville, MD). Inoculations were enumerated by serial dilution in sterile 0.1% buffered peptone water and plated on Petrifilm™ Coliform or *E. coli* Count Plates (3M, St. Paul, MN) for *E. coli* 0157:H7 and *Salmonella typhimurium*. *Bacillus cereus* and *Staphylococcus aureus* were plated on tryptic soy agar (Difco: Detroit, MI). *Yersinia enterocolitica* were enumerated on *Yersinia* Selective Agar with *Yersinia* antimicrobic supplement CN (Difco: Detroit, MI).

### 2.3:3 ANTIBACTERIAL ASSAY USING A PAPER DISK METHOD

The methodology used was adapted with select changes from Kim and co-workers (1995b). Five essential oils and five constituents were screened against five pathogenic bacteria using a zone of inhibition assay on tryptic soy agar or *Yersinia* selective agar with *Yersinia* antimicrobic supplement CN. A 100µl aliquot of each organism was spread evenly on appropriate agar plates using a sterile glass rod spreader. The plates were left at room temperature for 15 min to allow agar surface to dry. Five sterilized filter paper disks (Whatman No. 1 filter paper, 0.6cm diameter) were spaced evenly on each plate. For example, a plate would have one control and two duplicate treatments for a total of five disks. A 10µl aliquot of each test solution was added to the disks in duplicate. Plates were incubated at 35°C for 24 hours. A positive result was any clear zone of inhibition around a disk (small or large), while a negative result did not have a clear zone around a disk.

### 2.3:4 RESULTS AND DISCUSSION

Table 2.3:4A contains the results of the antibacterial screening. All compounds except caryophyllene and cinnamon oil were completely effective at both 2.5 and 5.0%. Cinnamon oil was ineffective against *Staphylococcus aureus* at 2.5%, but effective at 5.0%. Caryophyllene was ineffective against *Yersinia enterocolitica, Staphylococcus aureus* and *Salmonella typhimurium* at both concentrations. Kim and co-workers (1995a,b) found similar results with carvacrol and eugenol against E.coli 0157:H7 and *Salmonella typhimurium*. Although they used higher concentrations, their results indicated that both

compounds were effective antibacterials against the two organisms. In this study, all compounds, except caryophyllene, were effective antibacterial agents against the test pathogens and were further screened in the following study to determine the minimum inhibitory and bactericidal concentrations. Caryophyllene was dropped from the trial due to its lack of antibacterial potential and its unacceptability for the remainder of the experiments.

**Table 2.3:4A Results of Antibacterial Screening Using Paper Disk Method[1]**

| | ORGANISMS[2] | | | | |
|---|---|---|---|---|---|
| | EC | ST | YE | SA | BC |
| **Oregano** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Thyme** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Bay** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Clove** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Cinnamon** | | | | | |
| 2.5% | + | + | + | - | + |
| 5.0% | + | + | + | + | + |
| **Carvacrol** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Thymol** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Eugenol** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |
| **Caryophyllene** | | | | | |
| 2.5% | + | - | - | - | + |
| 5.0% | + | - | - | - | + |
| **Cinnamic Al.** | | | | | |
| 2.5% | + | + | + | + | + |
| 5.0% | + | + | + | + | + |

[1] (+) = positive for inhibition zone (-) = no inhibition zone
Results are from duplicate samples

[2]EC=*E. coli* 0157:H7, ST=*S. typhimurium*, YE=*Y. enterocolitica*, SA=*S. aureus*, BC=*B. cereus*

# CHAPTER 3

## 3.0 DETERMINATION OF THE MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR SELECTED ESSENTIAL SPICE OILS AND CONSTITUENTS AGAINST SEVERAL FOODBORNE PATHOGENS

## 3.1 ABSTRACT

Antibacterial activity of five essential spice oils and four constituents of these oils against *E. coli* 0157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Bacillus cereus* was determined by the broth dilution method. The compounds (250, 500, and 1000 ppm) were tested (in duplicate) in liquid medium for 36 hours to determine the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC). Additionally, thymol was tested at 2000 ppm due to lack of bacteristatic or bactericidal activity at lower levels. Thyme oil exhibited the strongest bactericidal activity of all compounds tested. Thyme oil had MIC and MBC values of 250 ppm for all organisms. Oregano oil had MIC and MBC values of 250 ppm for *E. coli* 0157:H7, *Salmonella typhimurium* and *Staphylococcus aureus*; however, *Yersinia enterocolitica* and *Bacillus cereus* required 500 ppm. Bay oil was effective against *Staphylococcus aureus, Yersinia enterocolitica* and *Bacillus cereus* with MIC and MBC values of 250 ppm; conversely, bay oil was the least effective against *E. coli* 0157:H7 and *Salmonella typhimurium* (MIC and MBC = 1000 ppm). *Staphylococcus aureus* and *Bacillus cereus* required 1000 ppm eugenol for complete inhibition. Since bay oil was effective against *Staphylococcus aureus* and *Bacillus cereus* and eugenol was not, this would suggest that eugenol is not the major antibacterial agent in bay oil. Thymol was

the least effective compound against all organisms, requiring 2,000 ppm for complete inhibition. Several of the tested compounds could be considered potential antibacterial agents; however, thyme and oregano oils were the most effective against the organisms to be tested in the ground meat studies.

## 3.2 INTRODUCTION

Based on the screening results from the paper disk assay, all essential spice oils and constituents, except caryophyllene, were screened again in liquid medium to determine the minimum inhibitory and bactericidal concentrations (MIC and MBC). The minimum inhibitory concentration is the lowest concentration of test compound at which no growth of an organism occurs. The minimum bactericidal concentration is the lowest concentration of test compound that kills the organism (Kim et al., 1995a,b). In most cases, the MIC and MBC are the same *in vitro*; however, in food systems these values tend to vary and increase due to components (proteins, fat, water) in the food (Kim et al., 1995a). The objective of this study was to determine the MIC and MBC of selected essential spice oils and constituents using a broth dilution method. The two most effective compounds will be carried into the remaining experiments.

## 3.3 MATERIALS AND METHODS

### 3.3:1 TEST COMPOUNDS

Methods for determination of the MIC and MBC of oregano, thyme, bay, clove and cinnamon oil and carvacrol, thymol, eugenol and cinnamic aldehyde were adapted from Kim and co-workers (1995a,b) with select changes. Test compounds (250, 500, 1000 ppm) were added to 19.6 ml of appropriate broth

with 2.0% Tween 20. Thymol was also tested at 2000 ppm (1000 ppm ineffective) with 4.0% Tween 20 added for solubility purposes. The protocol included test compounds at 100 and 1500 ppm to be screened; however, at 1500 ppm the compounds were poorly soluble and 100 ppm was ineffective against all organisms. Consequently, these test levels were dropped from the experimental design. Controls were flasks containing 2.0% or 4.0% Tween 20 with no test compounds.

### 3.3:2 TEST MICROORGANISMS

*E.coli* 0157:H7, *Salmonella typhimurium, Yersinia enterocolitica, Staphylococcus aureus* and *Bacillus cereus* were transferred from stock cultures to fresh tryptic soy broth or brain-heart infusion broth and incubated at 35°C for 22 to 24 hr before use. Before use, the cultures were centrifuged at 4,340 x g for 20 min. Broth was poured from the cultures and the sedimented pellets were then resuspended in equivalent sterile 0.1% buffered peptone water. Previous trials indicated that the inoculum levels reached $10^8$ colony forming units (CFU)/ ml; thus, inoculum were diluted with 0.1% sterile buffered peptone water to the desired level of $10^6$ CFU/ml. Inoculum were diluted to allow for growth during the study if the oils were ineffective.

### 3.3:3 DETERMINATION OF MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS

The broth dilution method was used to determine the MIC of test compounds. In duplicate 50 ml Erlenmeyer flasks, 19.6 ml of sterile tryptic soy broth (all organisms except *Yersinia enterocolitica*) or sterile brain-heart infusion

broth (*Yersinia enterocolitica*) were added. A 200 µl aliquot of bacterial suspension ($10^6$ CFU/ml) was added to each appropriate flask. Tween 20 (2.0%) was added followed by the test compounds at 250, 500 and 1000 ppm. Control treatments were the broth, organism and Tween 20 in a flask with no test compound added. Flasks were incubated at 35°C in a shaking water bath (65 rev/min) for 36 hours. A 1.0 ml sample was taken at 0, 6, 12, 24 and 36 hours. Duplicate flasks were treated with each compound at each concentration. *E. coli* 0157:H7 and *Salmonella typhimurium* were serially diluted with sterile 1.0% buffered peptone water and plated on Petrifilm™ Coliform plates. *Bacillus cereus* and *Staphylococcus aureus* were also serially diluted, but were plated on Petrifilm™ Aerobic Plates. *Yersinia enterocolitica* was serially diluted and plated on *Yersinia* selective agar with *Yersinia* antimicrobic supplement. Two plates per dilution were used for the determination of the most probable number of organisms at that dilution. The lowest concentration at which no growth occurred in either flask was the MIC. A 1 ml aliquot was taken from the flasks showing no growth and added to 10 ml of fresh sterile tryptic soy broth or brain-heart infusion broth. The sample was then incubated at 35°C for 24 hr. If no growth (no turbidity) occurred in the transferred sample, that level was considered the MBC. If growth occurred, the next level of test compound was transferred and checked for growth for determination of MBC. This procedure continued until the MBC was determined. Duplicate flasks were treated for each compound at each concentration.

## 3.4 STATISTICAL ANALYSIS

The MIC and MBC values for each essential spice oil or constituent against each pathogen were the same in this experiment; hence, values may only be labeled as MBC. Results were categorized with a logistic regression model using SAS (Version 6.12, SAS Institute, Inc.,Cary, NC). If organisms survived a treatment they were designated as 1. If the organism did not survive they were designated as 0. Using the logistic regression procedure, results were separated by organism, compound level and time. At the end of 36 hr, the two compounds that exhibited the lowest MBC's (greatest number of 0's) for all pathogens were selected to continue into the ground meat studies against *E.coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica*. Treatments were replicated twice. Additionally, two platings per serial dilution within a treatment were used.

## 3.5 RESULTS AND DISCUSSION

Figures 3.5.A through 3.5.J display the effects of each essential spice oil and constituent (controls, 250 and 500 ppm) against the five test pathogens. In almost all cases, test compounds at 1000 ppm were past the MBC so only 250 and 500 ppm were displayed. For example, *Salmonella typhimurium* and *E.coli* 0157:H7 required 1000 ppm bay oil to reach the MBC and *Staphylococcus aureus* and *Bacillus cereus* required 1000 ppm eugenol. All others required 500 ppm or less to reach the MBC. All results can be located in Appendix A. Thyme oil was the most effective antibacterial compound with MIC and MBC values of

250 ppm against all organisms. Oregano and bay oils were both effective against three of the five organisms at 250 ppm. Bay oil (250 ppm) was lethal to *Bacillus cereus, Yersinia enterocolitica* and *Staphylococcus aureus.* However, oregano oil (in addition to thyme oil) was chosen for the ground meat studies because it was highly effective against *E. coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* which were used in those studies. Oregano oil appeared to have a MBC value of 250 ppm for *Staphylococcus aureus,* but the organism recovered when transferred to fresh broth after 36 hours (See figure 3.5.G). Thus, the MBC of oregano oil for *Staphylococcus aureus* was 500 ppm.

In all cases, essential spice oils were more effective than the constituents, except cinnamic aldehyde which was more lethal (MBC = 250 ppm) than cinnamon oil (MBC = 500 ppm) against *Bacillus cereus.* Additionally, thymol was a very poor inhibitor/antibacterial agent (MBC =2000 ppm). Thyme and oregano oils (thymol is a major constituent of both) were very effective suggesting that the essential spice oils are composed of compounds (in addition to constituent tested) that work individually or together to form an effective antibacterial substance.

Differences exist when comparing this research to previously published work. For example, Kim and co-workers (1995a,b) MBC values for carvacrol against *E.coli* 0157:H7 (500 ppm) and *Salmonella typhimurium* (250 ppm) were contrary to our work. We noted MBC's of 250 ppm for *E.coli* 0157:H7 and 500 ppm for *Salmonella typhimurium.* Our MBC values for eugenol against the same two organisms were half that of Kim and co-workers (1995b). We recorded MBC's of

500 ppm for *E.coli* 0157:H7 and 250 ppm for *Salmonella typhimurium* (versus 1000 and 500 ppm respectively for Kim et al.1995b). The varying results between studies could be due to different enumeration methods or isolates used. Aktug and Karapinar (1986) determined that 5000 ppm ground thyme was required to completely inhibit growth of *Salmonella typhimurium*. This is much higher than the 250 ppm noted in this experiment. However, the researchers used ground thyme in media where as we used essential thyme oil. Essential oils are more concentrated than the dried spice; hence, much lower levels are needed for bactericidal action. Although differences do exist, they are relatively small and could be due to experimental conditions or other uncontrollable error.

In conclusion, thyme oil was the most effective antibacterial agent with a MBC of 250 ppm for all organisms, while thymol was the least effective requiring 2000 ppm for bactericidal activity against the test organisms. Oregano oil was also an effective antibacterial agent requiring levels of 250 to 500 ppm for bactericidal activity against the test microorganisms. The constituents were not as effective as the essential oil from which they were selected, suggesting that other components in the oils act as antibacterial agents.

Due to the potent antibacterial activity exhibited, thyme and oregano oils were chosen to continue into the ground meat studies. Most of the compounds tested (especially thyme oil) were effective antibacterial compounds (MBC values ranging from 250 to 1000 ppm) and show promise as food safety control measures. Future work may include further screening of different pathogenic microorganisms with the test compounds or combinations of test compounds.

Also, elucidation of the specific antibacterial components contained within the essential spice oils would be beneficial in controlling the organoleptic changes that may occur from the addition of intact oils. The use of these antibacterial agents in food products should be studied extensively.

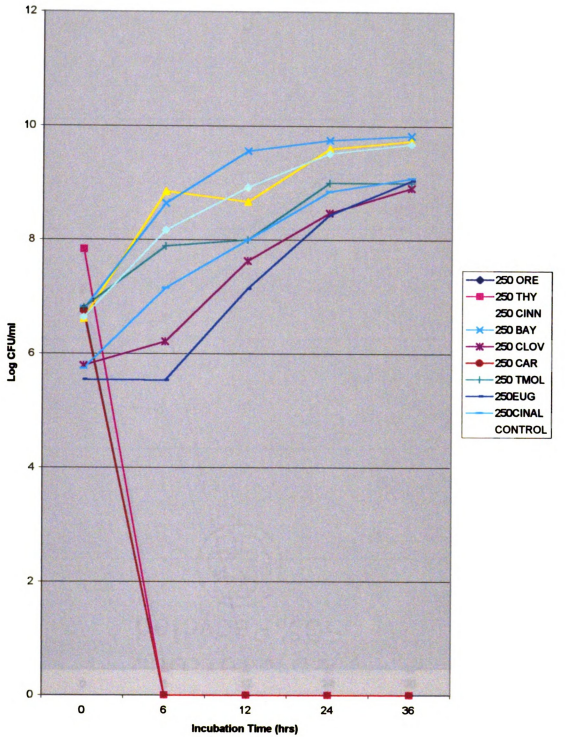



**Figure 3.5.A: Effects of essential oils and constituents (250 ppm) against *Escherichia coli* 0157:H7[1]**

[1] **Measures in duplicate**

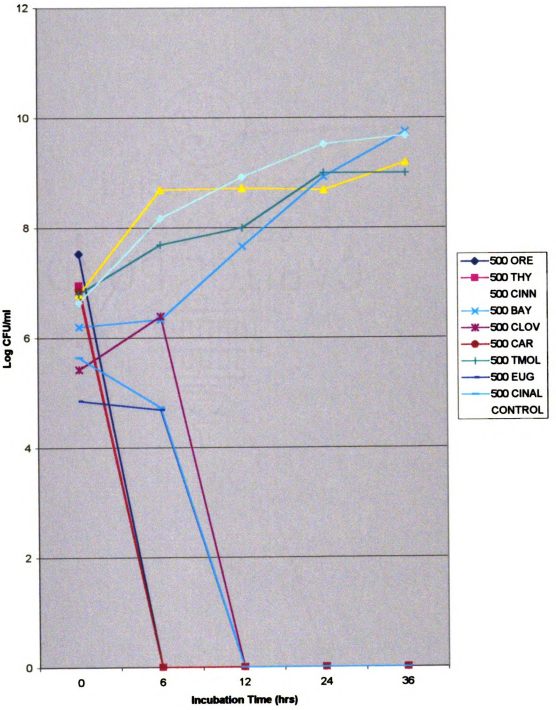



**Figure 3.5.B: Effects of essential oils and constituents (500 ppm) against *Escherichia coli* 0157:H7**

[1] Measures in duplicate

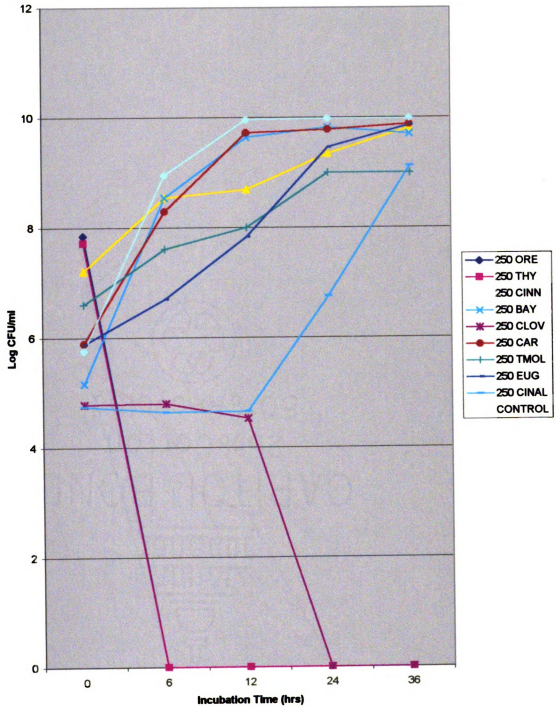



**Figure 3.5.C: Effects of essential oils and constituents (250 ppm) against *Salmonella typhimurium***

[1] Measures in duplicate

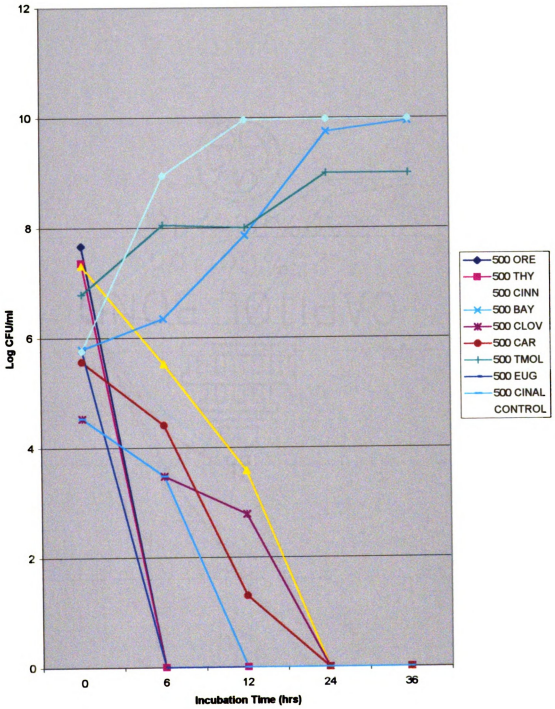



**Figure 3.5.D: Effects of essential oils and constituents (500 ppm) against *Salmonella typhimurium***

[1] Measures in duplicate

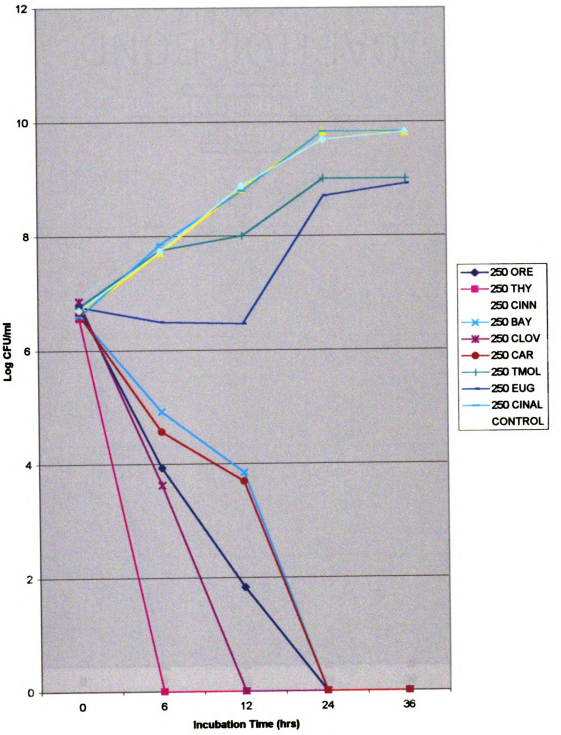



**Figure 3.5.E: Effects of essential oils and constituents (250 ppm) against *Yersinia enterocolitica***

[1] **Measures in duplicate**

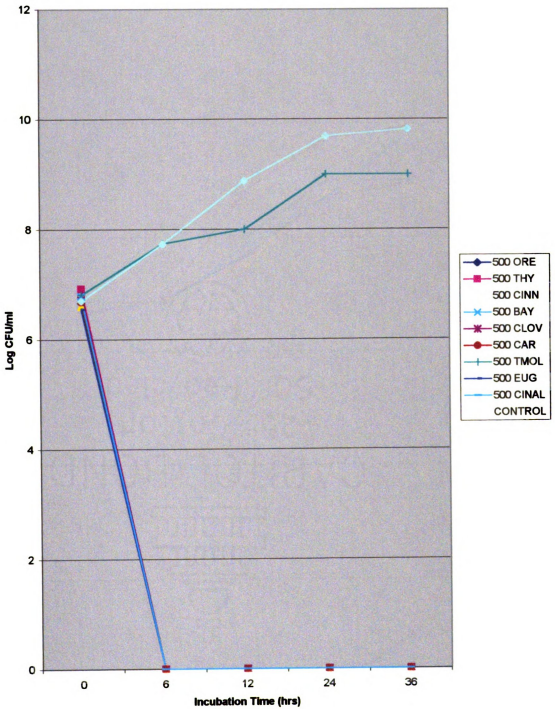



**Figure 3.5.F: Effects of essential oils and constituents (500 ppm) against *Yersinia enterocolitica***

[1] **Measures in duplicate**

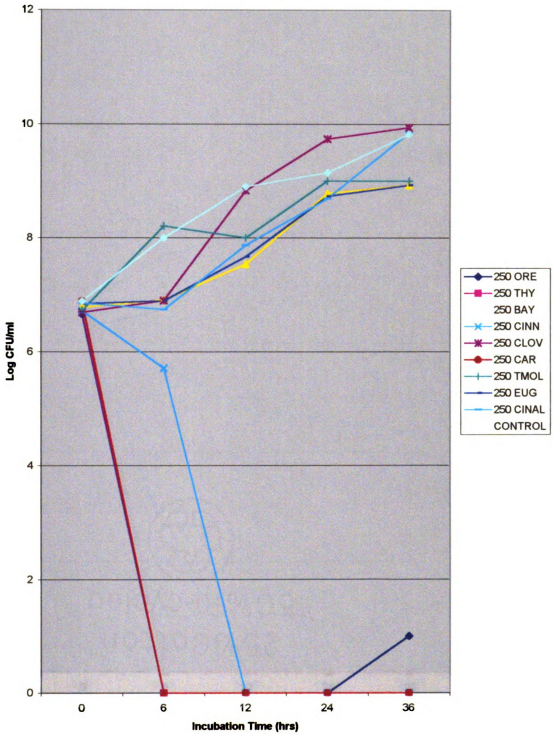



**Figure 3.5.G: Effects of essential spice oils and constituents (250 ppm) against *Staphylococcus aureus***

[1] **Measures in duplicate**

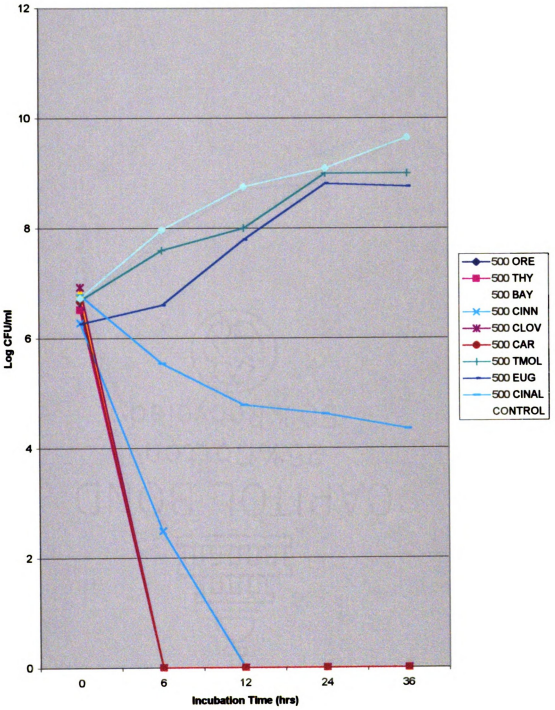



Figure 3.5.H: Effects of essential oils and constituents (500 ppm) against *Staphylococcus aureus*

[1] Measures in duplicate

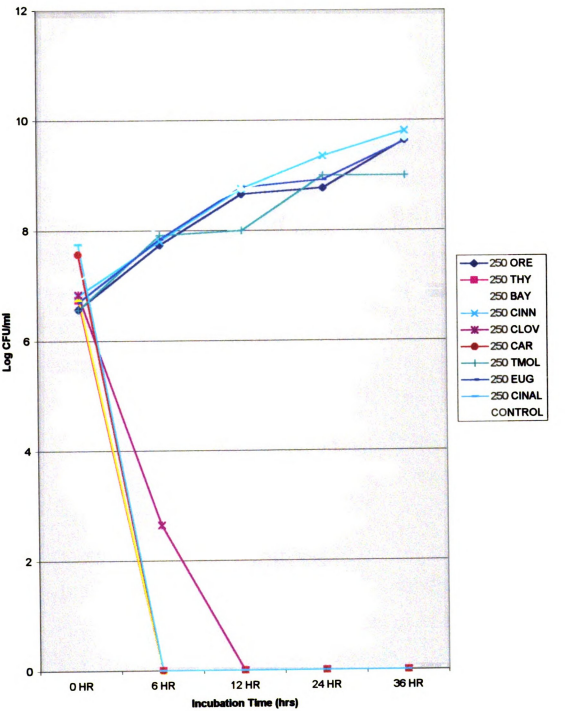



**Figure 3.5.I: Effects of essential oils and constituents (250 ppm) against *Bacillus cereus***

[1] Measures in duplicate

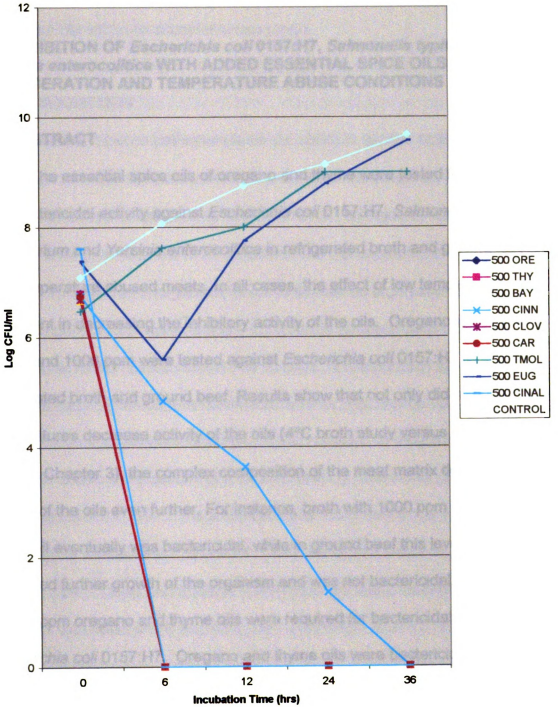



**Figure 3.5.J: Effects of essential oils and constituents (500 ppm) against *Bacillus cereus***

[1] Measures in duplicate

# CHAPTER 4

## 4.0 INHIBITION OF *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* WITH ADDED ESSENTIAL SPICE OILS IN REFRIGERATION AND TEMPERATURE ABUSE CONDITIONS

## 4.1 ABSTRACT

The essential spice oils of oregano and thyme were tested for inhibitory and bactericidal activity against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in refrigerated broth and ground meats and temperature abused meats. In all cases, the effect of low temperature was significant in decreasing the inhibitory activity of the oils. Oregano and thyme oils at 500 and 1000 ppm were tested against *Escherichia coli* 0157:H7 in refrigerated broth and ground beef. Results show that not only did refrigeration temperatures decrease activity of the oils (4°C broth study versus MIC and MBC study in Chapter 3), the complex composition of the meat matrix decreased the activity of the oils even further. For instance, broth with 1000 ppm oregano or thyme oil eventually was bactericidal, while in ground beef this level only prevented further growth of the organism and was not bactericidal. Additionally, 20,000 ppm oregano and thyme oils were required for bactericidal effect against *Escherichia coli* 0157:H7. Oregano and thyme oils were bactericidal to *Salmonella typhimurium* at 5000 ppm, while *Yersinia enterocolitica* required 10,000 ppm. In temperature abused meats, relatively high levels (5000 ppm for *Salmonella typhimurium* and 8000 ppm for *Escherichia coli* 0157:H7, *Yersinia enterocolitica)* of the oils were required to prevent growth or decrease bacterial

counts. At refrigeration temperatures, the bactericidal levels required in ground meats are not within an acceptable use range.

## 4.2 INTRODUCTION

Many foodborne pathogens have the ability to survive and/or grow at refrigeration (4 - 6°C) temperatures and below. *Yersinia enterocolitica* is considered a psychrophile, which is defined as an organism that can grow over a range of subzero to 20°C. *Salmonella typhimurium* is categorized as a psychrotrophic microorganism, which is defined as organisms that can grow at temperatures between 0 and 7°C (optimum between 30 to 40°C) and produce visible colonies within 7 to 10 days. At refrigeration temperatures, *Salmonella typhimurium* is considered a minor psychrotroph due to the lengthy time to grow at low temperatures; however, at incubation temperatures the organism is a serious threat to food safety (Jay, 1997). *Escherichia coli* 0157:H7 is a mesophile, which is defined as those organisms that grow well between 20 and 45°C with the optima between 30 and 40°C (Jay, 1997). Doyle and Schoeni (1984) reported that *Escherichia coli* 0157:H7 grows well between 30 and 42°C with an optimum of 37°C. The organism grows poorly between 42 and 45°C and does not grow at all at 4, 10 and 45.5°C. Even though this organism cannot grow at low temperatures, it does have the ability to survive for long periods at these temperatures (Zhao et al., 1993). Because all three of the pathogens can grow and/or survive at

refrigeration temperatures and lower, control measures are needed to prevent serious foodborne illnesses.

We have established that oregano and thyme oils (250 ppm) are effective inhibitors of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in broth at incubation temperatures (35°C). The antimicrobial effects of many spices have been studied against numerous foodborne pathogens, but the experimental temperatures are 35 to 37°C. Kim and co-workers (1995a) studied the effects of spice oils constituents (carvacrol, citral and geraniol) against *Salmonella typhimurium* in culture medium and on fish cubes. In growth media, the organism was completely inhibited with 250 ppm carvacrol and 500 ppm citral and geraniol at 35°C. Very high levels of the compounds were required to inhibit or kill the organism on fish cubes at 4°C over four days. Carvacrol at 5000 ppm was required for a 3-log reduction, while 30,000 ppm was required for complete inhibition. Citral and geraniol, at 30,000 ppm, did not inhibit the organism at refrigeration temperature (4°C). Based on research published, it is apparent that essential spice oils and constituents are not nearly as effective at refrigeration temperatures. Also, much higher levels of these compounds are needed for bactericidal action in broth and food products at lower temperatures. The objective of this study was to determine the effects of oregano and thyme oils against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* under refrigeration temperatures and temperature abuse conditions.

## 4.3 MATERIALS AND METHODS

### 4.3.1 TEST COMPOUNDS

For the broth study using *Escherichia coli* 0157:H7, oregano and thyme oils were added at 500, 1000 and 2000 ppm with 2% added Tween 20 to disperse the oils. In the refrigerated ground meat studies, oregano and thyme oils (Kalsec Inc., Kalamazoo, MI) were diluted (1:10) with 70% sterile ethanol to insure uniform mixing within the meats. In the first refrigeration trial, oils were tested at 500 and 1000 ppm; however, these levels were inhibitory, not bactericidal so a separate study was conducted with 5000, 10,000 and 20,000 ppm oil. Temperature abuse trials were conducted with oregano and thyme oils at 5000 ppm for *Salmonella typhimurium* and 8000 ppm for *Escherichia coli* 0157:H7 and *Yersinia enterocolitica*. The controls for the broth study was inoculated broth with 2% Tween 20, while the controls for the ground meat studies were inoculated meats with an equivalent amount of 70% sterile ethanol as the highest test oil. For all ground meat studies, oil/ethanol preparations were added dropwise to inoculated, ground meat at the desired level then mixed by hand with sterile latex gloves for 3 min.

### 4.3.2 TEST MICROORGANISMS

For broth and ground meat studies, *Escherichia coli* 0157:H7 (204P), *Salmonella typhimurium* (ATCC 29630) and *Yersinia enterocolitica* (Michigan State University isolate) were transferred from stock cultures to 10 ml sterile tryptic soy broth (*Escherichia coli* 0157:H7 or *Salmonella typhimurium*) or brain-

heart infusion broth (*Yersinia enterocolitica*) and incubated at 35° for 20 to 22 hr. After incubation, the cultures were centrifuged (Sorvall Superspeed RC2-B, Ivan Sorvall Inc., Norwalk, CT) at 4,340 x g for 20 min at 4°C. Broth was poured from the cultures and the sedimented pellets were then resuspended to their original volume with sterile 0.1% buffered peptone water. For the broth study and the refrigerated ground meat studies, the desired starting level for each organism was approximately $10^6$ colony forming units (CFU)/ g or ml. The desired starting level for each organism in the temperature abuse trials was approximately $10^3$ CFU/g. A lower level of organisms was used to allow for growth (if any) during the trial.

### 4.3.3 ASEPTIC GROUND MEAT PREPARATION

Research protocol developed for the ground meat studies required sterile raw material. This was achieved by the following methods. Vacuum packaged beef top round (*semimembranosus*-Excel, Plainview, TX) and center cut pork loin (*Longissimus dorsi*-IBP, Dakota City, NE) and modified atmosphere packaged turkey (*Pectoralis major and minor*-Jerome Foods, Barrow, WI) were purchased from a local grocery. Each product was washed quickly in soapy water then rinsed to kill surface bacteria prior to grinding. All excess fat and connective tissue was removed. The meats were ground (6.35mm then 3.18mm plates) in a clean Hobart Grinder Chopper (Model 84181D, Hobart Mfg. Co., Troy, OH)). Each meat was then separated into Stomacher bags (Sizes: 80 and 400, Seward Medical, London, UK) then vacuum packaged and sealed (Settings: 5.4 Vacuum

and 4.2 Seal: MultiVac, Model A300/16, Kansas City, MO). Several Stomacher bags were then placed in a Koch vacuum package bag ( Size: 10x12" and 12x18",Kansas City, MO) then vacuum packaged and sealed. This step was continued until all Stomacher bags were in vacuum package bags. All meats were then frozen (-30°C) until shipment to the irradiation facility. Frozen meat was shipped in insulated containers overnight to the Iowa State University Linear Accelerator Facility (Ames, IA). Dose rate was 106.2 Kilogray/min. Absorbed dose ranged from 5.38 to 12.58 Kilogray, which was within the required dosage for sterilization. Sterile product was then returned via overnight shipping and kept frozen until use. The ground meat and pepperoni studies used the sterile meats. The meats were tested for sterility before each trial with general aerobic plate count methods and were found to be sterile throughout the entire study. Moisture, fat and protein contents of the meat were determined by AOAC (1990) methods 950.46B, 991.36 and 981.10, respectively. Results can be found in Table 4.3.3A.

### 4.3.4 REFRIGERATED BROTH STUDY

A preliminary trial to determine the effects of refrigeration on essential spice oil inhibitory activity was conducted with *Escherichia coli* 0157:H7. Oregano and thyme oils were tested at 500, 1000 and 2000 ppm with 2% Tween 20 added to disperse the oils. Sterile (50 ml) tryptic soy broth was inoculated with 0.5 ml of prepared culture (See section 4.2.2). Initial levels of organisms (day 0) were enumerated then all treatments were refrigerated (4°C) for 30 days. Inhibitory activity of the oils were tested on days 1, 2, 5, 7, 9, 16, 23 and 30. The

**TABLE 4.3.3A: RESULTS OF PROXIMATE ANALYSIS OF RAW GROUND BEEF, TURKEY AND PORK SAMPLES**

| SAMPLE | MOISTURE (%) | FAT (%) | PROTEIN (%) | pH |
|---|---|---|---|---|
| Beef 1 | 80.1 | 6.6 | 20.7 | 6.2 |
| Beef 2 | 79.0 | 6.0 | 19.9 | 6.3 |
| Beef 3 | 80.0 | 6.5 | 19.6 | 6.3 |
| Mean[1] | 79.7±0.6 | 6.4±0.3 | 20.1±0.5 | 6.3±0.05 |
| Turkey 1 | 80.8 | 1.4 | 22.2 | 6.3 |
| Turkey 2 | 81.1 | 1.2 | 22.5 | 6.3 |
| Turkey 3 | 80.6 | 1.3 | 22.4 | 6.3 |
| Mean[1] | 80.9±0.2 | 1.3±0.1 | 22.4±0.1 | 6.3±0.03 |
| Pork 1 | 79.3 | 3.6 | 22.1 | 6.2 |
| Pork 2 | 79.9 | 3.8 | 21.1 | 6.3 |
| Pork 3 | 79.0 | 3.5 | 22.2 | 6.3 |
| Mean[1] | 79.4±0.4 | 3.6±0.1 | 21.8±0.6 | 6.3±0.04 |

[1]Values expressed as mean±SD

organisms were enumerated by taking a 1.1 ml sample and serially diluted in sterile 0.1% buffered peptone water then plating twice on Petrifilm™ coliform plates. Plates were incubated for 24 hr at 35° then counted. The control treatment was inoculated broth with 2% Tween 20 added. Each treatment was run in duplicate.

### 4.3.5 REFRIGERATED GROUND MEAT STUDY

Ground beef, turkey and pork were inoculated to a target level of approximately $10^6$ CFU/g (5.5 ml of prepared inoculum in 550g meat) with the *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* respectively. Ethanol (70%) diluted oregano and thyme oils (1:10) were added to the meat at 500 and 1000 ppm and mixed for 3 min. Samples were then placed in sterile Stomacher bags (Size 400: Seward Medical, London, UK). Anaerobic treatments were vacuum packaged (setting 5.5) and sealed (setting 4.2) with a MultiVac (Model A300/16, Kansas City, MO), while aerobic samples were transferred to sterile petridishes. Initial inoculum counts were made for day 0 then treatments were refrigerated (4°) for 20 days. The organisms were counted on days 4, 8, 12, 16 and 20. A 1.1 g sample was taken from each treatment and serially diluted with 0.1% sterile buffered peptone water. Organisms were plated as described in the previous section. All treatments were run in duplicate.

### 4.3.6 TEMPERATURE ABUSE STUDY

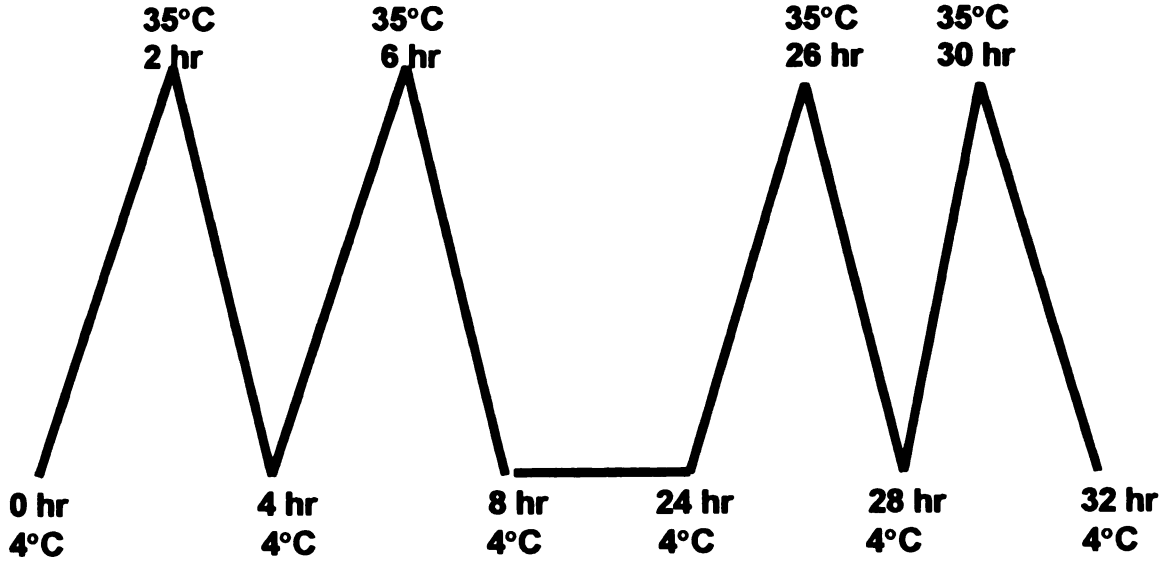
Ground beef, turkey and pork were inoculated to an initial level of approximately $10^6$ CFU/g with *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica*, respectively. Oregano and thyme oils were added at

8000 ppm as described in section 4.2.1. Preliminary studies determined that 5000 ppm was not sufficient to inhibit the organisms, while 10, 000 ppm killed the organisms within 2 hrs; therefore, 8000 ppm was selected as the lowest effective level for use. Controls were inoculated meat with the equivalent amount of 70% ethanol as in the test treatments. A 2.2 g sample of each treatment was extruded using a sterile modified syringe into a sufficient number of thermal death time tubes (10 x 75mm). Tubes were sealed using a gas-oxygen flame (MAPP gas, Bernz-O-Matic, Medina, NY). Tubes were placed in a wire rack then immersed in a circulating water bath (Polystat Model 1268-52, Cole-Parmer Instrument Co., Chicago, IL) set at 4°C. The bath was programmed to linearly increase in temperature to 35°C over 2 hr then decrease linearly back to 4°C over another 2 hr. This cycle was repeated, then the temperature was held at 4°C for 16 hr. After the 16 hr hold time the cycle was run twice again (see Table 4.3.6A). Tubes were drawn at 0,4,8, 24, 28 and 32 hr (when the water temperature returned to or was at 4°C) and organisms were enumerated within 1 hr as described in 4.2.4. All treatments were done in duplicate.

### 4.3.7 STATISTICAL ANALYSIS

Statistical analysis was conducted using SAS ( Version 6.12, SAS Institute, Cary, NC). For all ground meat studies, the mixed procedure to analyze least square means was used for each meat model with all possible interactions analyzed. All treatments in each study were replicated twice.

**TABLE 4.3.6A: TIME / TEMPERATURE MODEL FOR THE TEMPERATURE ABUSE TRIALS**




Samplings for enumeration were at 0, 4, 8, 24, 28 and 32 hr.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 PRELIMINARY BROTH STUDY WITH Escherichia coli 0157:H7 IN GROUND BEEF WITH ADDED OREGANO AND THYME OILS

A preliminary study of the inhibitory effects of oregano and thyme oils at 4°C was conducted in broth against *Escherichia coli* 0157:H7. The objective of the study was to determine if low levels of oils were inhibitory at refrigeration temperature (4°C) when compared to incubation temperature (35°C). Also, when compared to refrigerated ground meat studies, the broth study would help determine if a decrease in oil inhibitory activity was due to temperature or the food product or both. Only *Escherichia coli* 0157:H7 was chosen for the broth study due to its seemingly higher resistance to the oils. Results are displayed in Figure 4.4.1A. The control group decreased approximately 2 $\log_{10}$ CFU/ml values over a 30 day period. Oregano and thyme oils (500 ppm) started to decrease below the control counts from day 16 on, but the organism was never completely eliminated in the trial period. At 1000 ppm, oregano and thyme oils were inhibitory starting at day 7 with total elimination of the organism at day 23. At 2000 ppm, oregano and thyme oils were inhibitory at day 2 with total elimination on day 7 for thyme oil and day 23 for oregano oil. When compared to incubation temperature, inoculated broth at 4°C required 8 times (250 vs 2000 ppm) more oregano and thyme oil for bactericidal action (see chapter 3). Based on these results, the initial refrigerated ground meat studies were designed using 500 and 1000 ppm oregano and thyme oils to determine how they affected the three test organisms in a food system.

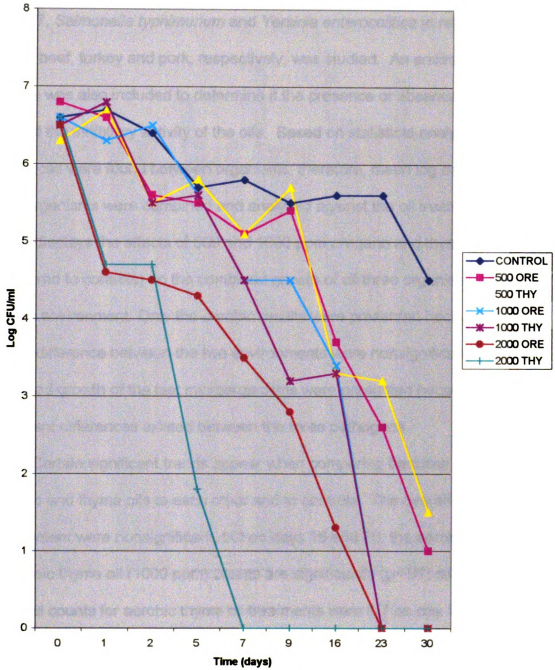



Figure 4.4.1A: Effects of oregano and thyme oils in refrigerated broth containing *Escherichia coli* 0157:H7[1]

[1] Measures in duplicate

### 4.4.2 INHIBITORY ACTIVITY OF OREGANO AND THYME OILS IN AEROBIC AND ANAEROBIC REFRIGERATED GROUND MEATS

The inhibitory action of oregano and thyme oils against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in refrigerated ground beef, turkey and pork, respectively, was studied. An environmental variable was also included to determine if the presence or absence of oxygen affected the inhibitory activity of the oils. Based on statistical analysis, no differences were found between organisms; therefore, mean log counts of all three organisms were combined and analyzed against the oil treatments. Figure 4.4.2A displays the effects of 500 and 1000 ppm oregano and thyme oils (compared to controls) on the combined growth of all three organisms in an aerobic environment. Only the aerobic results were presented because the overall difference between the two environments were nonsignificant. The combined growth of the test microorganisms were presented because no significant differences existed between the three pathogens.

Certain significant trends appear when comparing bacterial counts of oregano and thyme oils to each other and to controls. The overall effects of environment were nonsignificant, but on days 16 and 20, the aerobic and anaerobic thyme oil (1000 ppm) counts are significantly ($p<.01$) different. The bacterial counts for aerobic thyme oil treatments were 6.7 on day 16 and 6.5 on day 20 where as bacterial counts for anaerobic thyme oil treatments were 5.7 and 5.8 for days 16 and 20, respectively. It is possible that toward the end of the experiment the oxygen was totally depleted and the effect of environment became significant rather than the oils themselves. Other differences between

Figur

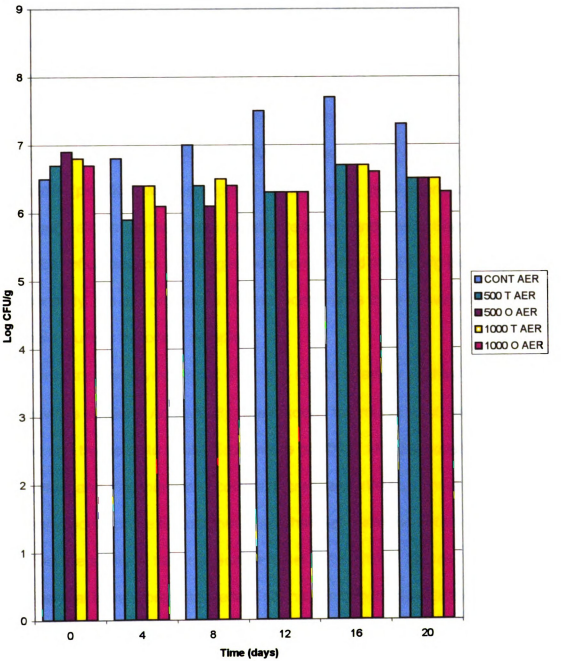



**Figure 4.4.2A:** Effects of oregano and thyme oils on the combined growth of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in an aerobic environment at 4°C[1]

[1] Measures in duplicate

oil treatment counts and control counts were not affected by environment (aerobic and anaerobic counts were similar). On day 0, no differences between any treatment counts or control counts existed. Bacterial log counts for control treatments were significantly higher ($p<.01$) than 1000 ppm oregano and 500 ppm thyme oil treatment counts on day 4. On days 8, 12, 16 and 20, all oregano and thyme oil treatments (500 and 1000 ppm) had lowered log counts significantly ($p<.0001$) when compared to the control. It is important to note that even though all the oil treatments counts are significantly lower than the control, the number of organisms still ranged between $10^6$ and $10^7$ CFU/g (see Appendix B). Essentially, the oils prevented the microorganisms from growing where as they could multiply in control samples.

Oregano and thyme oils at 4°C were not as effective as they are at 35°C (see previous section). Additionally, when added to ground meat, the ability of oregano and thyme oil to inhibit *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* decreased further as noted in this study. Apparently, some of the factors that affect the heat resistance of an organism also affect the ability of the organism to resist antimicrobial agents. For instance, protein and fat have a protective effect on the microorganisms allowing them to resist harmful agents (Jay, 1997). Also, meat components (proteins) may react with the bactericidal constituents of the oils lowering their effectiveness or increasing the concentrations required to kill the organisms. Phenols, which are major components of many spice oils, have a high affinity for proteins (Davis et al., 1980). Additionally, *Escherichia coli* 0157:H7 decreased approximately 2

$\log_{10}$ in refrigerated broth controls over 30 days, while in ground beef controls the microorganism increased approximately 1 $\log_{10}$ CFU/g. The difference may be due to the growth medium.

Again, it is important to note that even though oregano and thyme oils prevented the three test organisms from growing at 4°C, they did not completely eliminate the pathogens. A separate study was developed to determine at what levels the oils were lethal to the test microorganisms in refrigerated ground meat.

### 4.4.3 DETERMINATION OF BACTERICIDAL LEVELS OF OREGANO AND THYME OILS IN REFRIGERATED GROUND MEATS

Because ground meats have a very short shelf life and essential spice oils are very pungent, the bactericidal level of oregano and thyme oils in refrigerated meat must be as low as possible and function in a short period of time (<7 days). Preliminary studies determined that >4000 ppm oregano and thyme oils were required to completely inhibit the organisms over a 7 day trial period. Consequently, levels of 5000, 10,000 and 20,000 ppm were chosen for this study. These levels were tested against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in refrigerated ground meats at 0, 1, 2, 5 and 7 days. *Salmonella typhimurium* was the least resistant to the two oils even at day 0 where all oil treatments had significantly ($p<.0001$) lower counts than the controls (see Figure 4.4.3B). When determining D values (Chapter 5), *Salmonella typhimurium* exhibited the same lower resistance to the oils when compared to the other two test microorganisms. Both 5000 and 10,000 ppm thyme oil treatments were approximately 2 log counts lower when compared to control, while 10,000 ppm oregano and both 20,000 ppm oil treatments lowered

counts 4 to 6 logs. *Salmonella typhimurium* and *Yersinia enterocolitica* were eliminated by day 1 with 20,000 ppm oil treatments, while *Escherichia coli* 0157:H7 required seven days for total elimination. By day 2, 10,000 ppm of either oil had eliminated the *Salmonella typhimurium*. By day 5 the microorganisms were eliminated by all oil levels. The microbes had grown significantly ($p<.05$) by day 7 in the control group.

*Yersinia enterocolitica* was also susceptible ($p<.05$) to all levels of oils at day 0 when compared to controls (see Figure 4.4.3C). Additionally at day 0, the counts in 5000 ppm oregano and thyme oil treatments were significantly higher ($p<.0001$) than in the 10,000 and 20,000 ppm oil treatments, and counts in 10,000 ppm treatments were higher ($p<.05$) than in the 20,000 ppm treatments indicating a dose related response. Unlike *Salmonella typhimurium* and *Yersinia enterocolitica, Escherichia coli* 0157:H7 was not completely eliminated by 20,000 ppm of either oil by day 1, but required seven days. However, 5000 ppm oil did not eliminate the organism in the 7 day trial period and the 10,000 ppm oil was not bactericidal until day 7. The control treatments grew significantly ($p<.05$) over the 7 days.

*Escherichia coli* 0157:H7 was the most resistant to the oregano and thyme oils. Like the other test microorganisms, *Escherichia coli* 0157:H7 was significantly ($p<.05$) inhibited by all oil treatments when compared to controls on day 0 (see Figure 4.4.3D). Additionally on day 0, *Escherichia coli* 0157:H7 also exhibited a dose related response to the oils ($p<.05$) similar to *Yersinia enterocolitica*. *Escherichia coli* 0157:H7 was the most resistant to the oils as

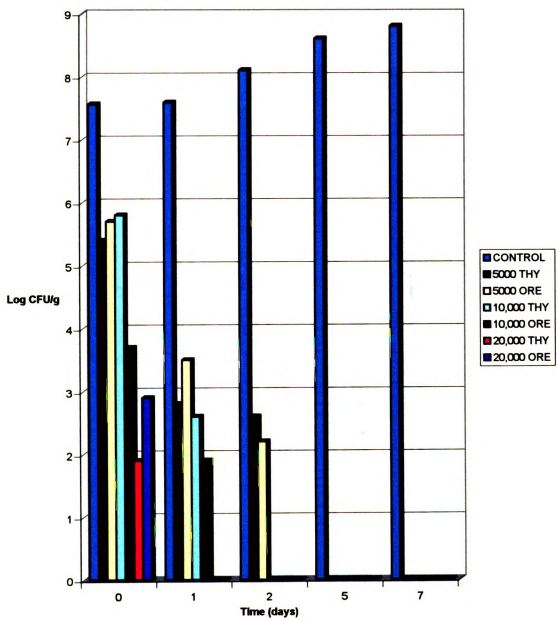



Figure 4.4.3B: Effects of high levels of thyme and oregano oils on *Salmonella typhimurium* at 4°C[1]

[1] Measures in duplicate

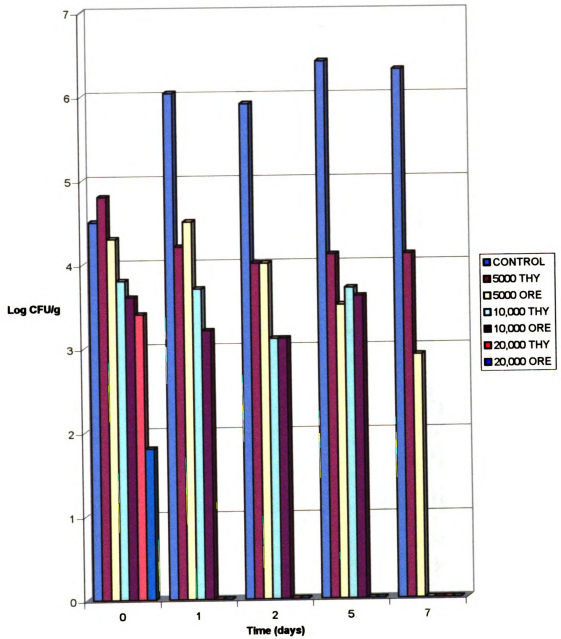



Figure 4.4.3C: Effects of high levels of thyme and oregano oils on *Yersinia enterocolitica* at 4°C[1]

[1] Measures in duplicate

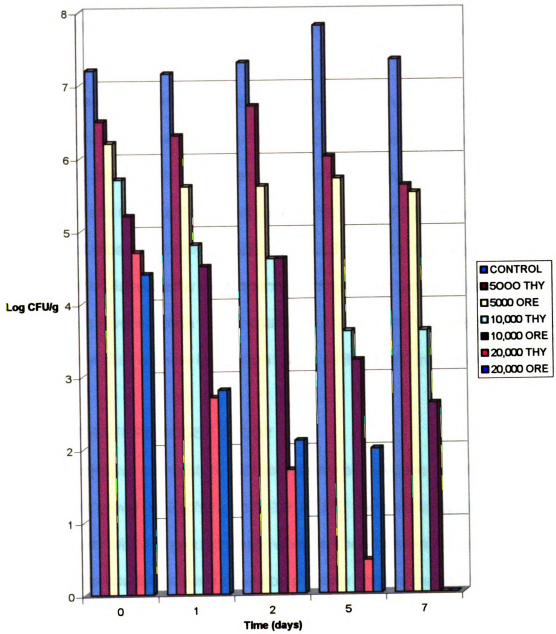



**Figure 4.4.3D: Effects of high levels of thyme and oregano oils on *Escherichia coli* 0157:H7 at 4°C[1]**

[1] Measures in duplicate

noted by the level and time required to eliminate the organism. For bactericidal action, 20,000 ppm oregano and thyme oils were required by day 7. The lower levels of oils were inhibitory; however, these lower levels did not eliminate the organism. The inoculum grew significantly ($p<.05$) over the test period in controls.

The bactericidal levels of oregano and thyme oils were 5000, 10,000 and 20,000 ppm for *Salmonella typhimurium*, *Yersinia enterocolitica* and *Escherichia coli* 0157:H7, respectively. These levels are lower than the 30,000 ppm required to kill *Salmonella typhimurium* in fish cubes (Kim et al., 1995a). However, these levels are not organoleptically acceptable in food products. Consequently, use of essential spice oils as bactericidal agents at refrigeration temperatures is not practical. To prevent growth of pathogens already present in a food product, the essential spice oils (at lower levels) are useful as illustrated in section 4.4.2.

### 4.4.4 TEMPERATURE ABUSE STUDY

The objective of this study was to determine if oregano and thyme oils would prevent growth of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in ground meats when temperatures mimicked abuse conditions. The conditions were based on possible abuses that might occur during distribution or in consumer homes. For example, when consumers buy a meat product it has potential to increase in temperature when transported from the purchase location to the home and then the product is re-chilled. Other potential warm-up and cool down periods could occur in consumer's homes and

commercial establishments. A potential existed for essential spice oils to prevent or inhibit pathogen growth. Preliminary results indicated that >7000 ppm oregano and thyme oil was required for inhibition of *Yersinia enterocolitica* and *Escherichia coli* 0157:H7 and >4000 ppm was required for *Salmonella typhimurium*; therefore, test levels were set at 8000 ppm and 5000 ppm. Again, *Salmonella typhimurium* displayed the least resistance to the oils by requiring a lower level for inhibition. Figures 4.4.4E, 4.4.4F and 4.4.3G display the effects of oregano and thyme oils on the test microorganisms in temperature abuse situations (see Table 4.3.6A for depiction of experimental design).

*Escherichia coli* 0157:H7 grew significantly ($p<.0001$) over the 32 hr test period, while counts with thyme and oregano oil treatments (8000 ppm) remained the same after 32 hr (Figure 4.4.3E). Both *Yersinia enterocolitica* and *Salmonella typhimurium* grew significantly ($p<.0001$) over the test period. However, both spice oil treatments lowered ($p<.01$) bacterial counts over the test period (see Figures 4.4.4F and 4.4.4G).

In conclusion, it is apparent that all three pathogens have the ability to grow and multiply in temperature abuse conditions. Oregano and thyme oils prevented further growth and lowered bacterial counts; however, similar to the refrigeration studies, high levels of the oils were required. Essential spice oils have more potential as bactericidal additives in heat-treated meats when compared to refrigerated or temperature abused meats. They also have potential in processed meat products, such as sausages, where spices are already

present and high levels of essential spice oils may not be distasteful (See Chapter 7).

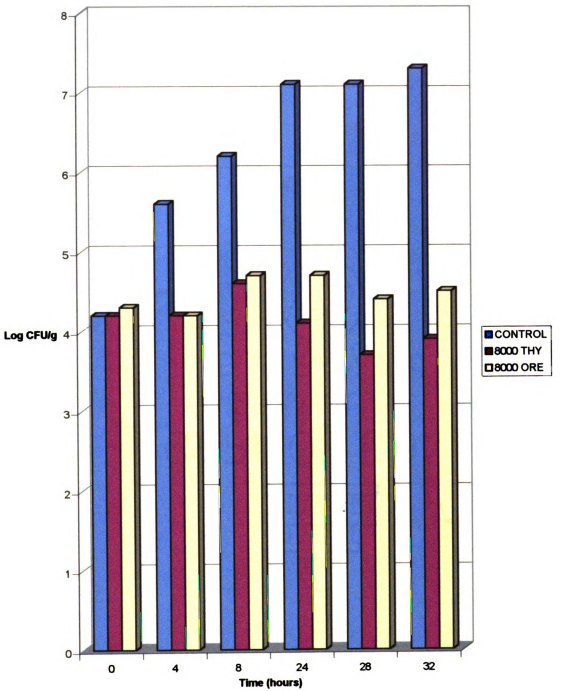



Figure 4.4.4E: Effects of thyme and oregano oils on *Escherichia coli* 0157:H7 In temperature abused ground beef[1]

[1] Measures in duplicate

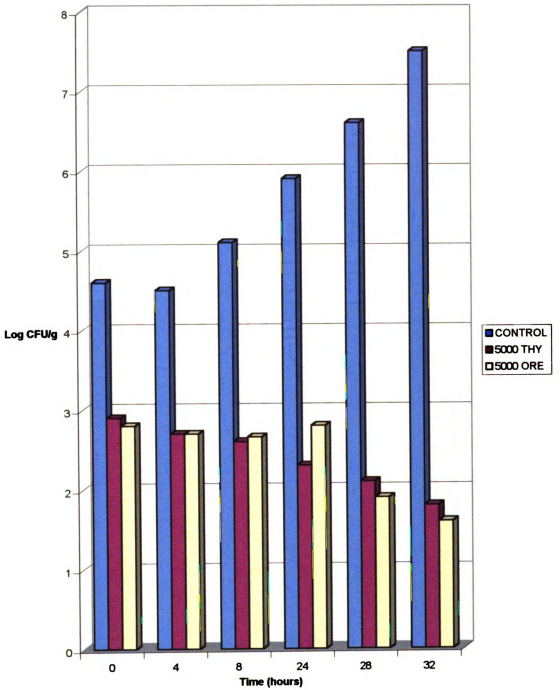



Figure 4.4.4F: Effects of thyme and oregano oils on *Salmonella typhimurium* In temperature abused ground turkey[1]

[1] Measures in duplicate

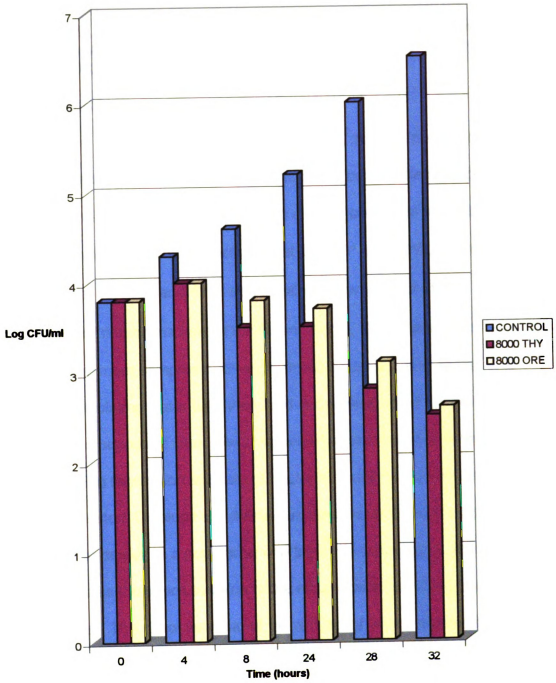



Figure 4.4.4G: Effects of thyme and oregano oils on *Yersinia enterocolitica* In temperature abused ground pork[1]

[1] Measures in duplicate

# CHAPTER 5

## 5.0 THERMAL INACTIVATION OF *Escherichia coli* 0157:H7, *Salmonella typhimurium* AND *Yersinia enterocolitica* AS AFFECTED BY THYME AND OREGANO OILS.

### 5.1 ABSTRACT

Thermal inactivation of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* in sterile, ground meat (beef, turkey and pork, respectively) with added thyme or oregano oil (500 ppm) was studied. *Escherichia coli* 0157:H7 was inactivated at 58°, 63° and 68°C, *Salmonella typhimurium* at 53°, 58° and 63°C and *Yersinia enterocolitica* at 52°, 57° and 62°C. Thermal death time tubes (10x75mm, sterile, disposable glass) were filled with inoculated meat samples (with and without spice oil), heat sealed and placed in a circulating water bath then held at the designated temperatures for predetermined periods of time. The D values (decimal reduction time) were calculated using regression analysis of bacterial counts versus time at each temperature tested. D values are defined as the time required at a specific temperature to decrease a population one log cycle (reduce population 90%) and are calculated as the absolute value of the reciprocal of the slope from the survivor curves. The absolute value of the reciprocal of the thermal death time (TDT) phantom curve slope was defined as the z value. TDT phantom curves were determined by plotting the log of D values versus temperature. The D values for samples with oregano and thyme oils were lower for all organisms at all temperatures when compared to controls (no oils). Thyme and oregano oils, when compared to controls, raised z values slightly for *Escherichia coli* 0157:H7

(5.64 and 5.58 vs 5.47°C) and to a larger extent for *Yersinia enterocolitica* (8.28 and 7.74 versus 6.91°C). The combination of lower D values and higher z values suggest that the oils help to increase the organism's heat sensitivity. *Salmonella typhimurium* with added spice oils had lower D values; however, the z values for oregano and thyme oil were slightly lower than the control (4.41 and 4.24°C versus 4.66°C). Although not to the extent of the other two organisms, the spice oils were still successful in increasing the heat sensitivity of *Salmonella typhimurium*.

## 5.2 INTRODUCTION

Heat resistance in microorganisms varies tremendously. Several criteria are involved in the heat resistance and heat destruction of bacterial cells, including external environmental and internal physiological factors. External factors include water availability, fat content, salt, carbohydrate and protein concentration, presence of inhibitory compounds and surrounding temperature. Factors associated with the organisms specifically include number and age of organisms present, growth temperature and spore formation (Hansen and Riemann, 1963; Ng et al., 1969; Goepfert et al., 1970; Baird-Parker et al., 1970; Jay, 1997).

Thermal destruction of microorganisms is important in food safety and preservation. Thermal death time (TDT) is the time required to kill a specified number of organisms at a certain temperature, while decimal reduction time (D value) is the time required to destroy 90% of the organisms. Various methods are available to determine TDT or D value. The most common procedure is to

place a known number of cell or spores in sealed containers in order to get the desired number of survivors over a time period at a certain temperature. The organisms are heated to the specified time at the chosen temperature, then placed in an ice bath. The samples are then placed on suitable growth medium and enumerated after incubation. Death is defined as the inability of the organisms to form colonies (Jay, 1997).

Decimal reduction time (D value) is the time required to reduce a population by 90% at a specified temperature. This value is equal to the number of minutes required for the survivor curve to traverse one log cycle at a specific temperature. Mathematically, it is equal to the reciprocal (absolute value) of the slope of the survivor curve (log survivors versus time at a specific temperature) and is a measure of the death rate (Jay, 1997; National Canners Association, 1968). The z value refers to the degrees (temperature) required for the TDT curve to traverse one log cycle. Mathematically, this value is equal to the reciprocal (absolute value) of the slope of the TDT curve, which plots log D values against the corresponding temperatures used to determine and calculate the D values. D values reflect the resistance of an organism to a specific temperature, while z values provide information on the relative resistance of an organism to different destructive temperatures (it allows for the calculation of equivalent thermal processes at different temperatures.

Based on previous essential spice oil research and the results from this work, we know that oregano and thyme oils are effective inhibitors against several foodborne pathogens. The two oils, however, appear to be more effective

at higher temperatures. The objective of this study was to determine how effective oregano and thyme oils (via D and z value comparison) were against *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* at three higher (cooking) temperatures when compared to controls. The D and z values were determined using the oils at the lowest effective level.

## 5.3 MATERIALS AND METHODS

### 5.3:1 TEST COMPOUNDS

Oregano and thyme oils (Kalsec, Inc., Kalamazoo, MI) were diluted (1:10) with 70% sterile ethanol to insure that they uniformly mixed within the meats. Both oils were very pungent, especially when heated, therefore; the oils were tested prior to the study to determine the lowest effective use level. Preliminary trials indicated that oregano and thyme oils were ineffective at 250 ppm (no difference in D or z values compared to controls). Levels of 1000 ppm were extremely effective due to the immediate death of all organisms within seconds of immersion into the heated water circulator. At 500 ppm, both oils were effective and provided a destruction rate or survivor curve that could be analyzed. Controls (no oils) were inoculated meat samples with the equivalent amount of 70% sterile ethanol as the test oils. Preliminary studies showed that the ethanol containing controls were no different than controls with inoculum only; therefore, only the ethanol controls were used in the study. Oil/ethanol preparations were added dropwise to inoculated, ground meat at the desired level then mixed by hand with sterile latex gloves for 3 min.

### 5.3:2 TEST MICROORGANISMS

Methodology for the thermal inactivation trials was adapted from Orta-Ramirez and co-workers (1997) and Veeramuthu and co-workers (1998) with select alterations. Cultures of *Escherichia coli* 0157:H7 (204P obtained from L. Beuchat, Univ. of GA), *Salmonella typhimurium* (ATCC 29630) and *Yersinia enterocolitica* (Michigan State University isolate) were transferred from stock cultures to 10 ml fresh tryptic soy broth (*Escherichia coli* 0157:H7and *Salmonella typhimurium*) or 10 ml brain-heart infusion broth (*Yersinia enterocolitica)* and incubated at 35°C for 22 to 24 hr. After incubation, the cultures were centrifuged (Sorvall Superspeed RC2-B, Ivan Sorvall Inc., Norwalk, CT) at 4,340 x g for 20 minutes at 4°C. Broth was poured from the cultures and the sedimented pellets were then resuspended to their original volume with sterile 0.1% buffered peptone water. Cultures of *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica* were added dropwise to irradiated ground beef, turkey and pork samples, respectively, at approximately $10^6$ colony forming units (CFU)/g. The inoculated meats were mixed thoroughly by hand, using sterile latex gloves, for 3 min.

Within 6 hr of each thermal treatment, test samples were homogenized for 90 sec. in sterile 0.1% buffered peptone water. Organism enumeration was completed by serial dilution with sterile 0.1% buffered peptone water and plating in duplicate on Petrifilm™ Aerobic Count Plates (3M, St. Paul, MN) or Yersinia

Selective Agar with Yersinia antimicrobic supplement (Difco: Detroit, MI). Plates were incubated at 35°C for 48 hr.

**5.3:3 THERMAL INACTIVATION STUDIES**

Bacterial inactivation experiments were conducted using irradiated ground beef, turkey and pork inoculated and prepared as described in sections 5.3:1 and 5.3:2. Two grams of meat were extruded using a sterile modified syringe into thermal death time tubes (10 x 75mm). Tubes were sealed using a gas-oxygen flame (MAPP gas, Bernz-O-Matic, Medina, NY)). Tubes were placed in a wire rack then immersed in a circulating water bath (Polystat Model 1268-52, Cole-Parmer Instrument Co., Chicago, IL for beef and turkey samples and Polyscience Model 9510, Niles, IL for pork samples) and held at the temperature/time relationships depicted in Table 5.3:A. Original protocol designated the thermal inactivation temperatures for *Yersinia enterocolitica* to be the same as *Salmonella typhimurium* (53, 58 and 63°C); however, each test temperature for thermal inactivation of *Yersinia enterocolitica* was one degree lower (52, 57 and 62°) due to differences in circulatory water baths used. For all temperatures, the bath was set 0.5° above the target temperature and monitored using a thermocouple inserted into the center of a non-test control sample containing 2 g of meat. The thermocouple was attached to a Solomat MPM 200 Modumeter (Solomat Partners LP, Stamford, CT). For the 52, 53 and 58°C trial (see Table 5.3:A), zero time was when the internal temperature reached the target temperature. For 57, 62, 63 and 68°C trials (See Table 5.3:A), zero time was when the internal temperature reached 10° below the target temperature. This

**TABLE 5.3.A. TIME AND TEMPERATURE SCHEDULES FOR THERMAL INACTIVATION OF *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica***

***E. coli* 0157:H7**

**58°C CONTROL:** 0, 4, 8, 10, 15, 20, 30, 40 and 50 min
**58°C THYME OIL:** 0, 4, 8, 10, 15, 20, 25, 30 and 40 min
**58°C OREGANO OIL:** 0, 4, 8, 10, 15, 20, 25, 30 and 40 min

**63°C CONTROL:** 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 min
**63°C THYME OIL:** 0, 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2 and 2.5 min
**63°C OREGANO OIL:** 0, 0.25, 0.5, 0.75, 1, 1.5, 1.75, 2 and 2.5 min

**68°C CONTROL:** 0, 0.17, 0.33, 0.5, 0.58, 0.67 and 0.83 min
**68°C THYME OIL:** 0, 0.08, 0.17, 0.25, 0.42, 0.58 and 0.67 min
**68°C OREGANO OIL:** 0, 0.08, 0.17, 0.25, 0.42, 0.58 and 0.67 min

***S. typhimurium***

**53°C CONTROL:** 0, 15, 30, 45, 60, 90, 120, 150 and 180 min
**53°C THYME OIL:** 0, 15, 30, 45, 60, 90, 120, 150 and 180 min
**53°C OREGANO OIL:** 0, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min

**58°C CONTROL:** 0, 0.5, 1, 2, 3, 4, 5, 8, 9, 10, 11 and 12 min
**58°C THYME OIL:** 0, 0.5, 1, 2, 3, 4, 5, 8, 9 and 10 min
**58°C OREGANO OIL:** 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 7 min

**63°C CONTROL:** 0, 0.25, 0.5, 0.75, 1, 1.25 and 1.5 min
**63°C THYME OIL:** 0, 0.08, 0.16, 0.25, 0.33, 0.42, 0.5, 0.58 and 0.67 min
**63°C OREGANO OIL:** 0, 0.08, 0.16, 0.25, 0.33, 0.42, 0.5, 0.58 and 0.67 min

***Y. enterocolitica***

**52°C CONTROL:** 0, 5, 10, 20, 30, 40, 50 and 60 min
**52°C THYME OIL:** 0, 5, 10, 15, 20, 25, 30, 35 and 40 min
**52°C OREGANO OIL:** 0, 5, 10, 15, 20, 25, 30, 35 and 40 min

**57°C CONTROL:** 0, 0.5, 1, 1.5, 2, 4, 6, 7, 8 and 10 min
**57°C THYME OIL:** 0, 0.5, 1, 1.5, 2, 3, 3.5, 4, 5 and 6 min
**57°C OREGANO OIL:** 0, 0.5, 1, 1.5, 2, 3, 4, 5, and 6 min

**62°C CONTROL:** 0, 0.33, 0.67, 0.83, 1, 1.33, 1.67 and 2 min
**62°C THYME OIL:** 0, 0.17, 0.33, 0.5, 0.67, 1, 1.5, 2 and 2.5 min
**62°C OREGANO OIL:** 0, 0.17, 0.33, 0.5, 0.67, 1, 1.5, 2 and 2.5 min

was done to detect any reduction in bacterial counts prior to reaching the target temperature. Heating times and temperatures were selected to achieve at least a 4-log reduction in bacterial counts. Tubes were removed from the bath at specified times (based on preliminary work) and placed immediately in an ice bath for at least 20 min. Samples that were not plated directly from the ice bath were held at 4°C up to 6 hr then plated. Tubes were broken open carefully and the samples placed in 10 ml sterile 0.1% buffered peptone water then homogenized for 90 sec. Bacteria were plated as described in section 5.3:2.

### 5.3:4 STATISTICAL ANALYSIS: CALCULATION OF D AND z VALUES

Bacterial plate counts were converted to logarithmic units. Plate counts below 10 were entered as zeros on the log scale. D values were calculated as recommended in the *Laboratory Manual for Food Canners and Processors* (National Canners Association,1968); however, the survivor curves were analyzed using the regression analysis function of Lotus 1-2-3 (97 edition, Lotus Development Corp., Cambridge, MA). The D values were the absolute value of the reciprocal of the slope. The z values were calculated using Lotus 1-2-3 also. They were plotted as log D values versus temperature. The z value was the absolute value of the reciprocal of the slope of the thermal death time curve. Three replications of each treatment were heated at each temperature.

## 5.4 RESULTS AND DISCUSSION

### 5.4:1 INACTIVATION OF *Escherichia coli* 0157:H7

Figures 5.4A through 5.4C display the survivor curves for *Escherichia coli* 0157:H7 in ground beef. The z values are portrayed in Figure 5.4J. Table 5.4

summarizes the thermal inactivation results for all organisms, treatments and times. Other research was not located on the effect of oregano and thyme oils (or any essential oils or constituents) on thermal inactivation; however, Orta-Ramirez and co-workers (1997) did determine D and z values for *Escherichia coli* 0157:H7 (ATCC 43894) at 58, 63 and 68°C without additives. They had D values of 6.44, 0.43 and 0.12 min at 58, 63 and 68°C respectively, while this study had D values of 8, 0.414 and 0.118 min at the same temperatures respectively. They calculated a z value of 5.60°C, which was slightly higher than z value (5.47°C) in this study. Doyle and Schoeni (1984) and Line and co-workers (1991) also studied D and z values of this organism in ground beef at very similar temperatures, with D values of 4.5 and 4.1 min at 57.2°C and 0.4 and 0.3 min at 62.8°C, respectively. They had z values of 4.1 and 4.61°C, respectively. In most cases, differences between all of the studies were very small and could possibly be attributed to experimental error (heat fluctuations, stuffing and plating variations, recovery methods, etc) or other uncontrollable parameters such as fat, protein and moisture variations. Veeramuthu and co-workers (1998) studied thermal inactivation of *Escherichia coli* 0157:H7 in ground turkey meat; however, the test temperatures were different so no D value comparisons will be made. However, z values can be compared and were found to be very similar (5.7 or 6.0 depending on plating medium used).

At the test temperatures in this study, thyme and oregano oils were effective in lowering the D value (increasing heat sensitivity) for *Escherichia coli* 0157:H7. D values for thyme oil were 5.66, 0.391 and 0.095 min at 58, 63 and

68°C, respectively, while oregano oil had D values of 6.04, 0.391 and 0.097 at the same temperatures. Differences between z values for the two spice oils compared to the control were small, but the spice oils did increase the z value, which means the organisms were more heat sensitive. Based on the results of this experiment, we conclude that oregano and thyme oils are effective compounds in reducing the heat resistance of *Escherichia coli* 0157:H7 at higher temperatures.

### 5.4:2 INACTIVATION OF *SALMONELLA TYPHIMURIUM*

Figures 5.4D through 5.4F display the survivor curves for *Salmonella typhimurium* in ground turkey. Figure 5.4K displays the z values. Table 5.4 and 5.5 exhibits thermal inactivation results for all treatments, temperatures and organisms. *Salmonella typhimurium* had lower D values and higher z values than *Escherichia coli* 0157:H7 at 58 and 63°C (with or without spice oils), suggesting that the organism is more sensitive to heat. Oregano and thyme oils also decreased D values and had similar z values when compared to the control suggesting that the compounds are effective at increasing the heat sensitivity of the organism similar to *Escherichia coli* 0157:H7. Studies determining the heat resistance of *Salmonella typhimurium* have been made using eggs as a food medium. Doyle and Cliver (1990) reported a D value (in eggs) for *Salmonella typhimurium* of 0.27 min at 60°C. This value is slightly lower than the values we reported in ground turkey, which were 1.67 min for 58°C and 0.217 min at 63°C. The differences could be due to the food medium in which the thermal inactivation studies were conducted. Many thermal inactivation studies

conducted on *Salmonella* species are done on *Salmonella senftenberg*, which is the most heat resistant of all the *Salmonella* serovars. It has been reported that *Salmonella senftenberg* is about 10 to 30 times more heat resistant than *Salmonella typhimurium* in moist heat conditions. However, it has also been noted that latter organism is more resistant to dry heat (Doyle and Cliver, 1990; Jay, 1997). Our results, when compared specifically to Orta-Ramirez et al., (1997), indicated that *Salmonella typhimurium* was about 2 times less heat resistant than *Salmonella senftenberg* at 53°C and about 9 times less heat resistant at 58 and 63°C using the same research methodology. The differences in heat resistance between the two organisms in both studies is lower than the value (30x) reported by Jay (1997), possibly due to moist versus dry heat conditions. Based on the results provided, we concluded that oregano and thyme oils assist in lowering the heat resistance of *Salmonella typhimurium* at higher temperatures. Additionally, *Escherichia coli* 0157:H7 is more heat resistant than *Salmonella typhimurium* (with or without spice oils) at temperatures between 58 and 63°C.

### 5.4:3 THERMAL INACTIVATION OF *Yersinia enterocolitica*

Figures 5.4G through 5.4I exhibit the survivor curves for *Yersinia enterocolitica* at 52, 57 and 62°C. The z values can be found in Figure 5.4L and Table 5.5. Table 5.4 provides results D value analysis for all treatments, organisms and temperatures. At 52 and 57°C, *Yersinia enterocolitica* exhibited the greatest decrease in heat resistance due to oregano and thyme oils when compared to the control and other organisms. However, part of this difference

could be due to the psychrophilic nature of the *Yersinia* species and the inability to withstand high temperatures (Jay, 1997). Little difference existed between control counts versus spice oil counts at 62°C. Francis et al. (1980) reported a D value of 0.3 min at 62.8°C very similar to our control D value at 62°C of 0.33 min. As with the other organisms, *Yersinia enterocolitica* is less heat resistant when oregano and thyme oils were present.

In conclusion, the addition of oregano and thyme oils were effective in lowering the D values for all organisms at all temperatures when compared to controls with no oils. This means that at the same temperature, ground beef with oregano or thyme oil added would require less time to decrease (90%) organism counts when compared to ground beef with no additives. The addition of the oils increased z values for *Escherichia coli* 0157:H7 and *Yersinia enterocolitica*, while slightly decreasing z values for *Salmonella typhimurium.* When compared to refrigeration or temperature abuse conditions, the oils were more effective inhibitors at higher temperatures. This would suggest that temperature played an important role in the biocidal activity of the essential plant oils. In the future, the use of essential oils in cooked meats (and other foods) could aid in decreasing the incidence of food poisoning cases by decreasing or eliminating the number of organisms present in the food.

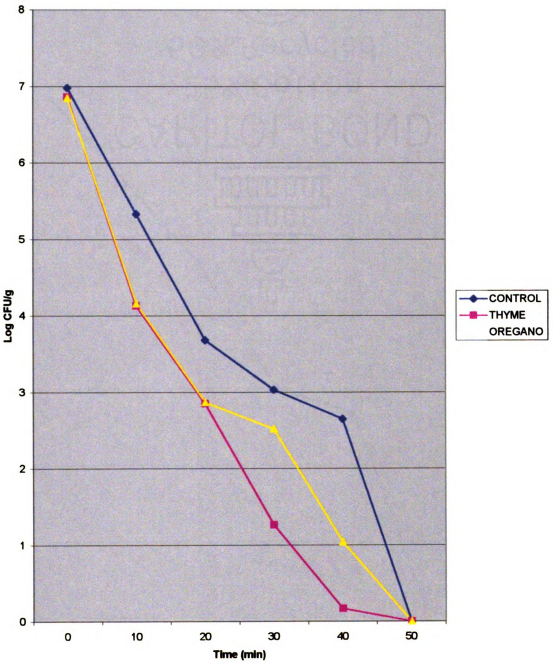



Figure 5.4.A: Survivor curves for *Escherichia coli* 0157:H7 at 58°C[1]

[1] Measures in triplicate

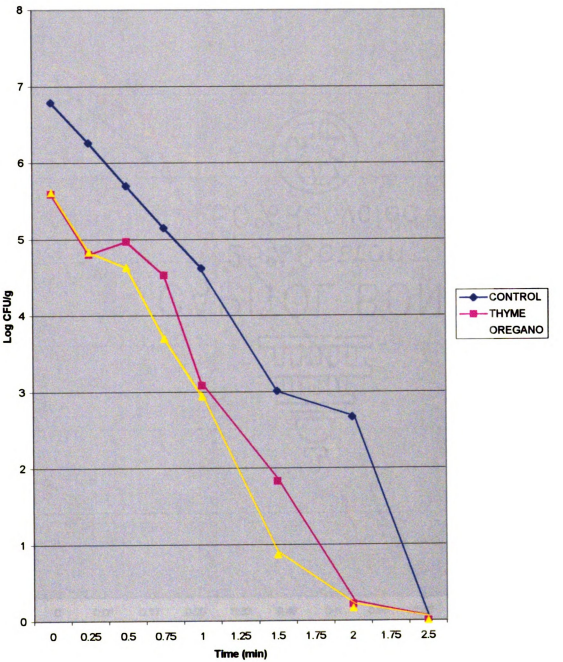



Figure 5.4.B: Survivor curves for *Escherichia coli* 0157:H7 at 63°C[1]

[1] Measures in triplicate

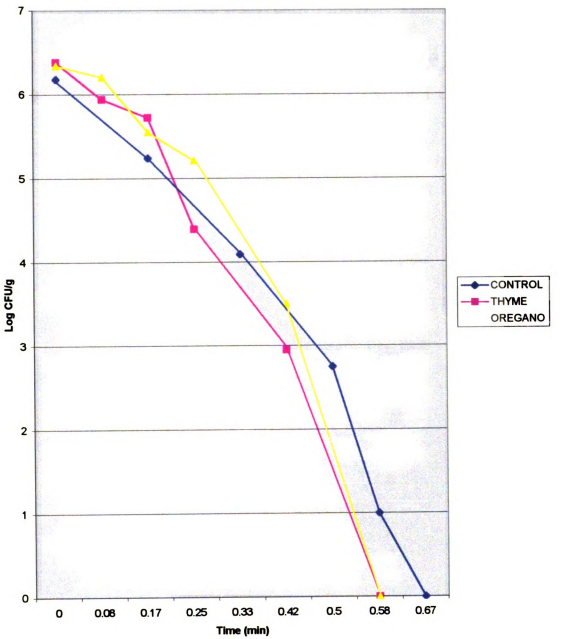



Figure 5.4.C: Survivor curves for *Escherichia coli* 0157:H7 at 68°C[1]

[1] Measures in triplicate

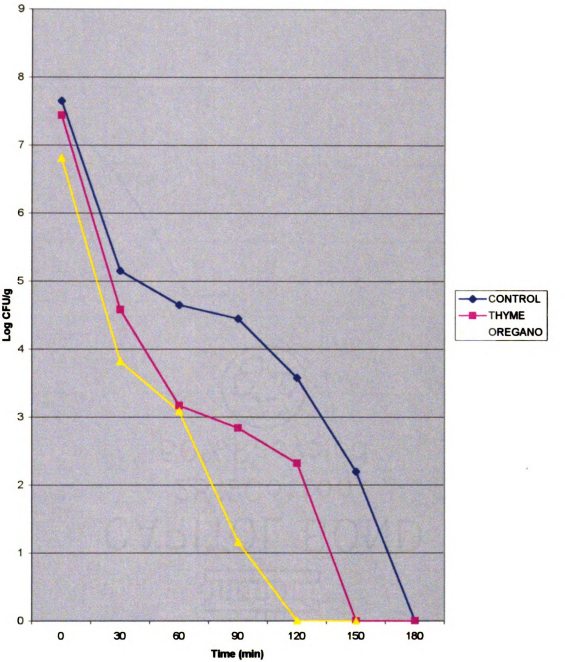



Figure 5.4.D: Survivor curves for *Salmonella typhimurium* at 53°C[1]

[1] Measures in triplicate

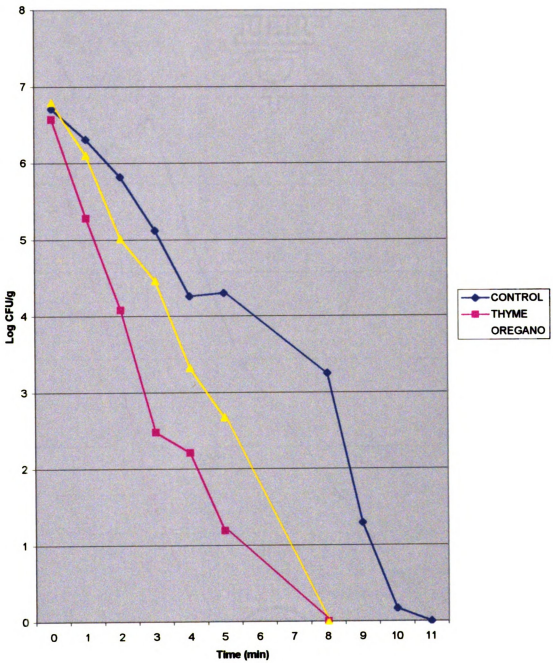



Figure 5.4.E: Survivor curves for *Salmonella typhimurium* at 58°C[1]

[1] Measures in triplicate

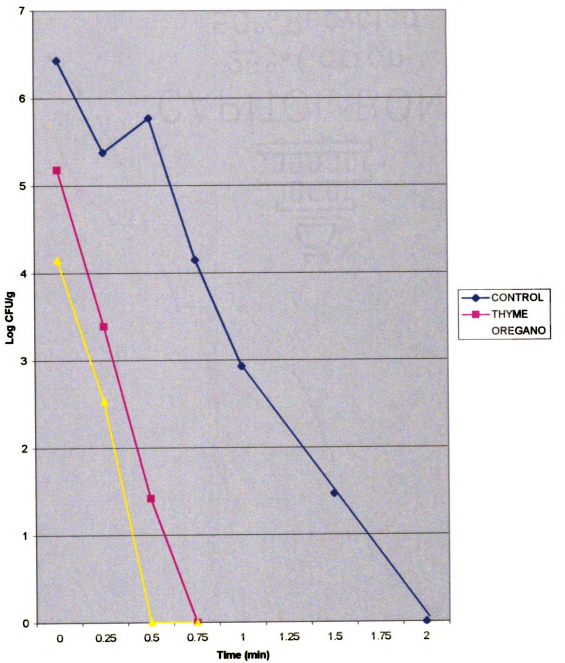



Figure 5.4.F: Survivor curves for *Salmonella typhimurium* at 63°C[1]

[1] Measures in triplicate

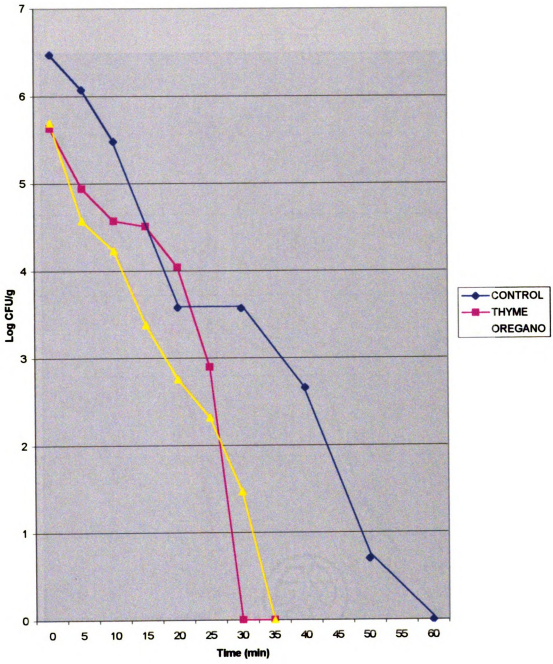



Figure 5.4.G: Survivor curves for *Yersinia enterocolitica* at 52°C[1]

[1] Measures in triplicate

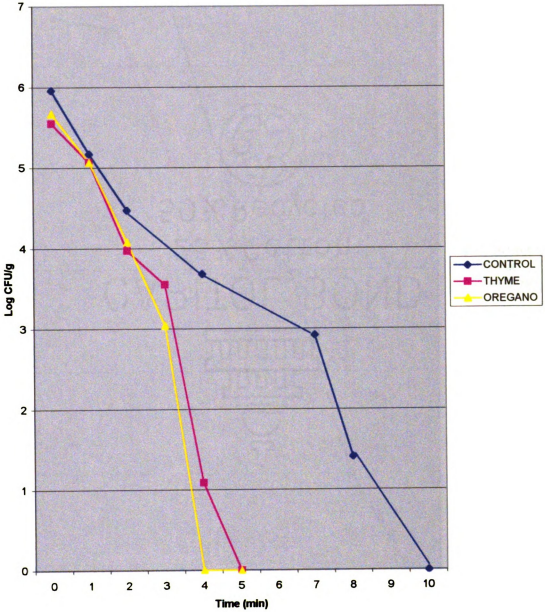



Figure 5.4.H: Survivor curves for *Yersinia enterocolitica* at 57°C[1]

[1] Measures in triplicate

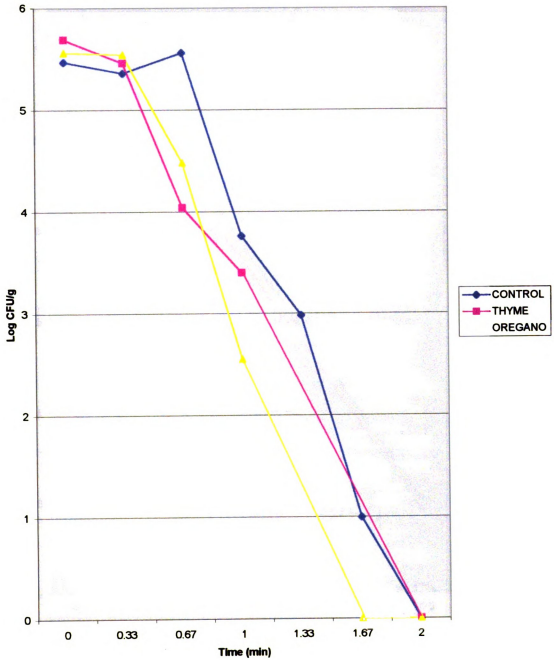



Figure 5.4.I: Survivor curves for *Yersinia enterocolitica* at 62°C[1]

[1] Measures in triplicate

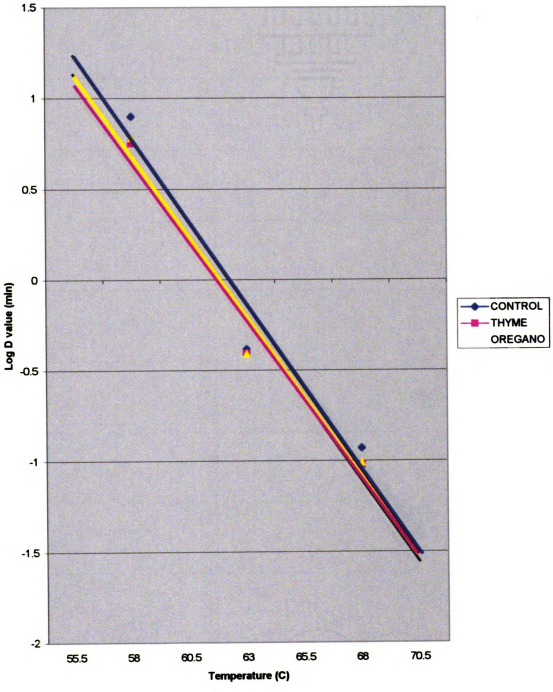



Figure 5.4.J: Thermal death curves for *Escherichia coli* 0157:H7

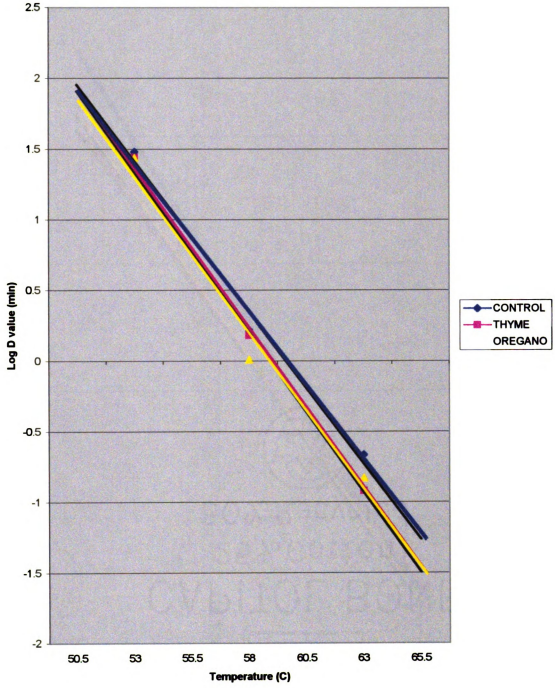



Figure 5.4.K: Thermal death curves for *Salmonella typhimurium*

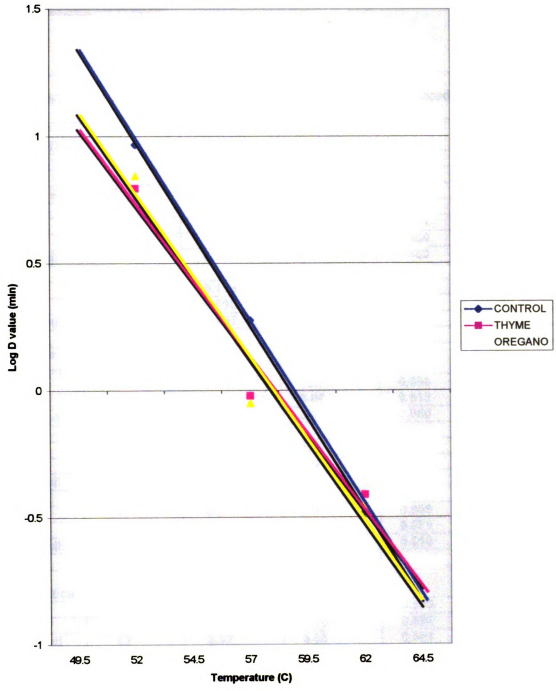



Figure 5.4.L: Thermal death curves for *Yersinia enterocolitica*

**TABLE 5.4: SUMMARY OF D VALUES FOR *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica***

| TREATMENT | T ( °C ) | D Value(min) | Constant | R Squared | X coefficient |
|---|---|---|---|---|---|
| *Escherichi coli* 0157:H7 | | | | | |
| Control | 58 | 8.00 | 6.70 | 0.952 | -0.124 |
| Thyme oil | 58 | 5.66 | 6.19 | 0.944 | -0.176 |
| Oregano oil | 58 | 6.04 | 6.26 | 0.958 | -0.166 |
| | | | | | |
| Control | 63 | 0.414 | 6.88 | 0.974 | -2.41 |
| Thyme oil | 63 | 0.391 | 5.77 | 0.954 | -2.55 |
| Oregano oil | 63 | 0.391 | 5.49 | 0.950 | -2.56 |
| | | | | | |
| Control | 68 | 0.118 | 6.43 | 0.956 | -8.48 |
| Thyme oil | 68 | 0.095 | 6.88 | 0.973 | -10.49 |
| Oregano oil | 68 | 0.097 | 7.10 | 0.942 | -10.29 |
| | | | | | |
| *Salmonella typhimurium* | | | | | |
| Control | 53 | 30.09 | 13.92 | 0.998 | -0.033 |
| Thyme oil | 53 | 27.23 | 6.09 | 0.913 | -0.037 |
| Oregano oil | 53 | 27.49 | 5.23 | 0.860 | -0.036 |
| | | | | | |
| Control | 58 | 1.67 | 6.90 | 0.969 | -0.597 |
| Thyme oil | 58 | 1.52 | 5.53 | 0.903 | -0.654 |
| Oregano oil | 58 | 1.02 | 7.03 | 0.975 | -0.982 |
| | | | | | |
| Control | 63 | 0.218 | 6.51 | 0.986 | -4.58 |
| Thyme oil | 63 | 0.125 | 5.18 | 0.979 | -7.99 |
| Oregano oil | 63 | 0.146 | 4.23 | 0.910 | -6.82 |
| | | | | | |
| Yersinia enterocolitica | | | | | |
| Control | 52 | 9.26 | 6.47 | 0.973 | -0.108 |
| Thyme oil | 52 | 6.23 | 6.16 | 0.886 | -0.160 |
| Oregano oil | 52 | 6.97 | 5.58 | 0.981 | -0.143 |
| | | | | | |
| Control | 57 | 1.89 | 5.87 | 0.951 | -0.530 |
| Thyme oil | 57 | 0.95 | 5.97 | 0.942 | -1.05 |
| Oregano oil | 57 | 0.889 | 5.97 | 0.925 | -1.12 |
| | | | | | |
| Control | 62 | 0.33 | 6.59 | 0.881 | -3.02 |
| Thyme oil | 62 | 0.388 | 5.92 | 0.972 | -2.58 |
| Oregano oil | 62 | 0.355 | 5.89 | 0.899 | -2.82 |

**Table 5.5: SUMMARY OF z VALUES AND REGRESSION ANALYSIS FOR *Escherichia coli* 0157:H7, *Salmonella typhimurium* and *Yersinia enterocolitica***

| | Z value (°C) | Constant | R squared | X coefficient |
|---|---|---|---|---|
| ***Escherichia coli* 0157:H7** | | | | |
| Control | 5.47 | 11.38 | 0.948 | -0.183 |
| Thyme oil | 5.64 | 10.93 | 0.969 | -0.177 |
| Oregano oil | 5.58 | 11.08 | 0.965 | -0.178 |
| | | | | |
| ***Salmonella typhimurium*** | | | | |
| Control | 4.66 | 12.76 | 0.989 | -0.214 |
| Thyme oil | 4.24 | 13.92 | 0.988 | -0.236 |
| Oregano oil | 4.41 | 13.37 | 0.977 | -0.227 |
| | | | | |
| ***Yersinia enterocolitica*** | | | | |
| Control | 6.91 | 8.51 | 0.999 | -0.145 |
| Thyme oil | 8.29 | 6.98 | 0.959 | -0.121 |
| Oregano oil | 7.74 | 7.48 | 0.953 | -0.129 |

# CHAPTER 6

## 6.0 EFFECTS OF OREGANO AND THYME OILS ON A COMMERCIAL IMMUNOLOGICAL RAPID TEST KIT


### 6.0 ABSTRACT

Use of rapid screening methodology by the food industry in steadily increasing. Immunological based screening assays are very popular due their speed, specificity and sensitivity. Oregano and thyme oils were added (500, 1000, 5000, 10,000 and 20,000 ppm) to ground beef inoculated with *Escherichia coli* 0157:H7. Samples were then analyzed with the Reveal® *Escherichia coli* 0157:H7 microbial screening test. Each level of essential oil was analyzed in triplicate. No interference or interaction occurred from the oils even at the highest test level. The objective of the study was to determine if any interference or interaction (possible false negatives in the rapid test kit) resulted from the addition of essential spice oils to ground meat.


### 6.1 INTRODUCTION

Immunoassays are rapidly becoming a popular application in the food industry. Detection and quantification of microorganisms, hormones, pesticides, antibiotics, mycotoxins and foreign proteins in food are some common applications of immunoassays. The specificity and sensitivity of an antibody for an antigen allows for the detection and quantification of a component without the lengthy separation and extraction steps required in conventional analysis methods (Smith, 1995; AOAC, 1990). The basis for all immunoassays is an interaction between an antibody (protein produced by immune cells of the body in

response to an antigen) and a corresponding antigen (substance with which an antibody will bind specifically), and the detection of the interaction using enzymes or radiolabels (Hefle, 1995).

The assay principles of the Reveal® *Escherichia coli* 0157:H7 screening test are cited from the instruction handbook (Neogen Corp., 1996). This system utilizes an 8 hr enrichment medium (contents trademarked and not published) that provides the organism with nutrients and other factors needed for survival and rapid growth. After 8 hr, a portion of the enrichment culture is placed into a round sample port of the Reveal detection device. The test portion diffuses through a nitrocellulose pad to a specific reaction zone containing colloidal gold-labeled *E.coli* 0157:H7-specific antibodies. The gold-labeled antibodies attach to the *E. coli* 0157:H7 forming a complex. These complexes migrate through the nitrocellulose pad to a reagent zone, which contains a second line of *E.coli* 0157:H7-specific antibodies. The complexes are immobilized by the second group of antibodies and a visible line in the test window appears as a result of the aggregation of gold. The remainder of the sample continues to migrate to a second binding reagent zone, which contains an antibody specific for a different gold-labeled analyte present in the nitrocellulose pad. This results in a second line being formed in the window of the kit. This line will form regardless if any organism is present or not to ensure that the test is working properly. Post-enrichment growth of *E.coli* 0157:H7 will yield a positive reaction with as few as $10^4$ to $10^5$ CFU/ml.

Possible interference and variation from food matrices or additives in the food can affect immunoassay results. For example, high levels of proteins can cause nonspecific interactions. Large molecules may cause steric hindrance of the antibody-antigen binding. Fatty acids have been reported to denature antibodies, and phenolic substances can interact nonspecifically with proteins and have potential to cause problems in the assay systems (Hefle, 1995). Since essential spice oils are composed of high levels of phenols and fatty acids, they may interfere with immunoassay results. Thus, the objective of the study was to determine if any interference from oregano and thyme oil occurred with the selected test kit.

## 6.2 MATERIALS AND METHODS

### 6.2.1 TEST MICROORGANISMS

*Escherichia coli* 0157:H7 (strain 204P) was transferred from a stock culture to 10 ml of fresh, sterile tryptic soy broth (Difco: Detroit, MI) 22 to 24 hr before use. On the day of the experiment, the culture was sedimented by centrifugation at 4340 x g for 20 min at 4°C (Sorvall Superspeed RC2-B, Ivan Sorvall Inc, Norwalk, CT) and resuspended to its original volume using sterile 0.1% buffered peptone water (Becton Dickinson Microbiology Systems, Cockeysville, MD). Inoculum was added to ground beef to achieve levels of $10^7$ to $10^8$ CFU/g.

### 6.3.2 TEST COMPOUNDS

Oregano and thyme oils (Kalsec Inc., Kalamazoo, MI) were used at

500, 1000, 5000, 10,000 and 20,000 ppm. The oils were diluted (1:10) in sterile 70% ethanol to insure complete dispersion in the ground meat.

### 6.3.3 CULTIVATION AND SAMPLE PREPARATION

Oregano and thyme oils (500, 1000, 5000, 10,000 and 20,000 ppm) in 70% ethanol (1:10 – oil/ethanol) were added to 50 ml of sterile ground beef. Controls were ground beef with no inoculum, ground beef with inoculum only and ground beef with 70% ethanol equivalent to level used in 20,000 ppm samples. Samples were mixed with a sterile mixing rod for 3 min. Oils and controls were tested in duplicate. Samples with 20,000 ppm oil were tested immediately after the 8 hr incubation then held at 4°C for 24 hr and retested. The 24 hr samples were tested to determine if low levels ($10^1$ to $10^2$ CFU/g) of the injured organism could be recovered sufficiently for positive identification. To measure initial inoculum levels in each sample, a 1.1g sample from each test group was serially diluted with 0.1% sterile buffered peptone water and plated in duplicate on Petrifim™ coliform plates (3M). Plates were incubated for 24 hr at 35°C then read. *Escherichia coli* 0157:H7 was enumerated after the eight-hour incubation in enrichment media. A 1.1 ml sample of the media was serially diluted, plated and incubated identical to the initial counts.

Methodology for the 8 hr Reveal® test system was followed directly from the instruction manual (Neogen Corp., 1996). Media preparation included the transfer of one Reveal 8 hr medium bottle to a Stomacher 400 bag (Tekmar, Inc., Cincinnati, OH). Sterilized water (225 ml @ 43°C) was added to the bag. The

bag was closed with a rubberband then shaken vigorously for 1 min. Each bag was held in an incubator at 43°C until used (within one hour). A 25 g sample of inoculated ground beef with different test levels of oregano and thyme oils was added to each bag then homogenized for 2 min. using a Stomacher lab blender (Model 400, Tekmar Company, Cincinatti, OH). The Stomacher bags were closed loosely and incubated for 8 hr at 43°C. After 8 hr the bags were removed from the incubator and shaken gently. Using a sterile pipette, 5 ml of sample was transferred to a 16 x 150mm sterile tube and placed in a boiling water bath for 10 min. The tubes were allowed to cool in cool water for 5 min.

**6.3.4 REVEAL DEVICE TEST PROCEDURE**

The procedure for the device was followed directly from the instruction booklet. The test devices were allowed to come to room temperature (~25°C) prior to use. Each device was labeled appropriately and placed on a flat surface. A 120 µl sample was taken from each cooled tube and placed in the port of the device. The test portion is wicked through a nitrocellulose pad to a specimen reaction zone containing colloidal gold labeled *Escherichia coli* 0157:H7-specific antibodies. The gold labeled antibodies attach to the organism forming a complex. These complexes migrate through the pad to a reagent zone, which contains a second line of *E. coli* 0157:H7-specific antibodies. The *E. coli* 0157:H7-antibody complexes are immobilized by the second group of antibodies producing a visible line in the test window (T zone) as a result of the aggregation of gold. The remainder of the sample continues to migrate to a second binding reagent zone, which contains an antibody specific for a different gold labeled

analyte present in the nitrocellulose pad. This results in the formation of a line in the control window. Regardless of whether or not the sample contains any *E.coli* 0157:H7, a line will form in the window (C zone), to ensure that the test is working properly. The lines in the C and T zones were read and interpreted 20 minutes after the addition of the sample to the device. Results were interpreted as follows: Lines in C and T zones were considered a positive result for *E. coli* 0157:H7, while a line in C, but no line in T was considered a negative result. If no line appeared in C, regardless if there was a line in T, the result was considered invalid. All materials were autoclaved (121°C for 30 min.) when study was complete.

## 6.4 RESULTS AND DISCUSSION

Table 6.4A summarizes the results of the Reveal devices. It is evident that essential spice oils, specifically oregano and thyme oil, do not interfere with the immuno-based assay (specifically Reveal®) even at very high levels of use. Other commercial immunoassays are based on the same principles as the Reveal® test kit, therefore, one could assume that using essential spice oils in food or meat will not interfere with any immuno-based microbiological testing.

**Table 6.4.A: Results of the Reveal® *Escherichia coli* 0157:H7 Microbial Screening Test when Tested Against Ground Beef with Various Levels of Oregano and Thyme Oils**

| SAMPLE | INITIAL CFU/g[1] | 8 hr CFU/g[1] | 24 hr CFU/g[2] | RESULT[3] |
|---|---|---|---|---|
| Control (No ethanol/oil) | 7.44 | 9.51 | – | Positive |
| Control (70% ethanol/no oil)[4] | 7.64 | 9.21 | – | Positive |
| Control (No inoculum) | 0 | 0 | – | Negative |
| 500 ppm oregano | 8.21 | 9.89 | – | Positive |
| 500 ppm thyme | 7.98 | 9.59 | – | Positive |
| 1000 ppm oregano | 7.57 | 9.46 | – | Positive |
| 1000 ppm thyme | 7.78 | 9.71 | – | Positive |
| 5000 ppm oregano | 7.88 | 9.53 | – | Positive |
| 5000 ppm thyme | 7.62 | 9.71 | – | Positive |
| 10,000 ppm oregano | 7.10 | 9.42 | – | Positive |
| 10,000 ppm thyme | 6.95 | 8.67 | – | Positive |
| 20,000 ppm oregano | 3.89 | 5.41 | – | Positive |
| 20,000 ppm thyme | 4.00 | 5.81 | – | Positive |
| 20,000 ppm oregano (24h) | 4.05 | – | 5.35 | Positive |
| 20, 000 ppm thyme (24h) | 3.54 | – | 5.40 | Positive |

[1]Average of two replications
[2] Only measured on 20,000 ppm samples held 24 hrs
[3]Results of Reveal™ devices were identical for duplicate replications
[4]70% Ethanol added at level equivalent to 20,000 ppm samples

# CHAPTER 7

## 7.0 SURVIVAL OF *Escherichia coli* 0157:H7 DURING THE MANUFACTURE OF PEPPERONI WITH ADDED OREGANO OIL

### 7.1 ABSTRACT

This study investigated the survival of *Escherichia coli* 0157:H7 during the manufacture of pepperoni with added oregano oil (8000 ppm) in combination with a mild heat treatment (128°F/53°C). Because this organism has a low infectious dose (<50 organisms), total elimination of the organism would insure a safe product. The U.S. Food Safety and Inspection Service (FSIS) has recommended a 5 $\log_{10}$ decrease of the organism during the processing of dry fermented sausages. If the contamination load is very high (>$10^6$), a 5 $\log_{10}$ decrease would still leave a possible infectious dose and pose a safety threat. A mild heat treatment had been shown to achieve the recommended decrease of the organism; however, it is not completely eliminated. With the addition of 8000 ppm oregano oil, pepperoni was completely devoid of *Escherichia coli* 0157:H7 after the heat treatment whereas control treatments contained approximately $10^4$ CFU/g. The pH after fermentation only dropped to ≅5.2, which is higher than the recommended pH of ≤ 5.0. Oregano oil is a protective measure against *Escherichia coli* 0157:H7 if processing conditions are not ideal. The addition of oregano oil to dry fermented sausages in combination with a mild heat treatment would insure a product that is absent of *Escherichia coli* 0157:H7.

## 7.2 INTRODUCTION

In December 1994 an outbreak of *Escherichia coli* 0157:H7 poisoning was linked to salami, which was the first reported incident in a dry fermented sausage (Nickelson, 1996). In 1995, the Center for Disease Control and Prevention (CDCP) reported that the organism was found on pre-sliced product from delicatessen counters in Washington. In early 1995, another incident of *Escherichia coli* 0157:H7 was linked to semi-dry (uncooked) fermented sausage in southern Australia (Center for Disease Control and Prevention, 1995). Studies have shown that other pathogenic species, such as *Salmonella* spp. and *Listeria* spp., could survive in fermented meat products (Glass and Doyle, 1989; Glass et al., 1992; Calicioglu et al., 1997; Tomicka et al., 1997). *Escherichia coli* 0157:H7 is more acid tolerant than other vegetative foodborne pathogens and has a low infectious dose. It is not surprising that this organism poses a safety threat in dry fermented sausages (Riordan et al., 1998; Tomicka et al. 1997). After the plant implicated in the 1994 outbreak was found to be following industry developed good manufacturing practices and federal requirements, the U.S. Food Safety and Inspection Service (FSIS) recommended that ready to eat fermented meat products obtain a 5 $\log_{10}$ CFU/g reduction of the organism.

Traditional pepperoni formulations and processes are not sufficient to achieve a 5 $\log_{10}$ reduction of *Escherichia coli* 0157:H7, rather the traditional process achieves a reduction of slightly over a 1 $\log_{10}$ decrease (Riordan et al., 1998; Hinkens et al., 1996). Traditional formulations and processes do, however, decrease the number of organisms to levels recommended by the FSIS if they

are allowed to stay in storage for 90 days (Faith et al., 1998a,b). Hinkens and co-workers (1996) outlined low (128°F/60 min) and high (145°F/instantaneous) temperature heating steps that helped achieve the required reduction of the organism. The objective of this study was to determine if oregano oil (8000 ppm) in combination with a low heat treatment (128°F/53°C) would increase the reduction rate and/or completely eliminate *Escherichia coli* 0157:H7 in a traditionally formulated pepperoni.

## 7.3 MATERIALS AND METHODS

### 7.3.1 OREGANO OIL PREPARATION

A preliminary study using 5000, 10,000 and 15,000 ppm of oregano oil was conducted to determine the amount of oil to use in the actual trial. At 5000 ppm, the sausages were no different than control treatments, while at 10,000 ppm the inhibitory action was too high nearly eliminating the organism before fermentation. Therefore, 8000 ppm was chosen for the actual study. Oregano oil was prepared by diluting (1:10) with sterile 70% ethanol. The oil was diluted to insure uniform mixing in the pepperoni batter. The oil/ethanol combination was added to the batter and mixed by hand with sterile latex gloves for 3 min. Controls were inoculated batter with the equivalent amount of 70% ethanol as in the oil treatment and inoculated batter with no additives (traditional formula).

### 7.3.2 PREPARATION OF BACTERIAL INOCULUM

Approximately 20 hr before use, *Escherichia coli* 0157:H7 (204P) was transferred from stock cultures to fresh tryptic soy broth and incubated at 35°C. On the day of the trial, inoculum was centrifuged (Sorvall SuperSpeed RC2-B, Ivan Sorvall Inc., Norwalk, CT) at 4,340 x g for 20 min. The pelleted inoculum was drained of media then resuspended in equivalent sterile 0.1% buffered peptone water. The desired inoculum level in the pepperoni treatments was $10^6$ to $10^7$ CFU/g. This level was chosen to insure that a 5 $\log_{10}$ decrease in bacterial counts could be detected.

For the P*ediococcal* starter culture, a mixture of thawed *Pediococcus* spp. were added to the batters at 0.028%. The desired level of lactic organisms in each batter was $10^8$ colony forming units (CFU)/g.

### 7.3.3 PEPPERONI PREPARATION

Basic pepperoni batters were prepared according to the following formulation (Riordan et al., 1998; Hinkens et al., 1996; Faith et al., 1998a,b):

| | |
|---|---|
| Lean beef | 36.8% |
| Lean pork | 36.8% |
| Fat (pork) | 26.3% |
| Salt | 2.5% |
| Dextrose | 0.625% |
| Spice | 0.12% |
| Starter culture | 0.028% |
| Modern cure | 0.25% |
| Erythorbate | 0.055% |

The ground beef and pork were irradiated according to the methodology in Chapter 4. Fat analysis had already been conducted on the ground meats and the results were used to calculate the exact quantity of each meat and fat pork trim needed to produce a pepperoni with 30:70 fat:lean. Fat pork trim was obtained from a local butcher shop then rendered and sterilized (121°C for 40 min). The sterilized fat was stored at -20°C until needed. The pork adipose tissue was considered to be 100% fat since it had been rendered. Lean meats were ground previous to irradiation and were stored at -20°C until needed. Both lean meats and fat tissue were thawed 24 hr before the processing day. Three treatments were designed including a control with no additives, a control with equivalent amounts of 70% ethanol as the oil treatment and the oregano oil treatment. All treatments were run in duplicate as separate batches. The target weight of each batch was 700 g. All processes were conducted as aseptically as possible to avoid any external contamination. On the processing day, fat pork trim was ground with a sterilized Hobart grinder (Model 84181D, Hobart Mfg. Co., Troy, OH) using a 3/16" (0.476 cm) plate. Meat and fat ingredients were weighed and placed in a sterile mixing bowl. *Escherichia coli* 0157:H7 was added drop wise to the ground meats and fat. A non-inhibitory, food grade, green dye (FD &C green, McCormick and Co. Inc., Hunt Valley, MD) was added (1 ml) to the mixture as an aid to monitor for uniform mixing. Mixing was done by hand for 2 min with sterile latex gloves. The remaining ingredients were added and mixing continued for 2 min. The pepperoni spice was provided by Kalsec Inc. (Kalamazoo, MI). For the ethanol controls and the oregano oil treatments,

another 2 min mixing step was added to incorporate the extra ingredient. After each batch was mixed, it was ground in a Hobart grinder using a 3/16" plate. The ground batter was stuffed into a pre-soaked (10 min) fibrous cellulose dry sausage casing (#1 1/2) manufactured by the Viskase Corporation (Chicago, IL). The sausages were then tied and weighed. A small portion of each batter was set aside for microbiological and chemical analysis. The sausages were placed inside a designed humidity chamber and transferred to an oven (Isotemp Oven, Model 655G, Fischer Scientific) for fermentation. The fermentation step was conducted at 35°C with a relative humidity ranging between 85 and 95%. After 18 hr (pH goal <5.0), samples were taken for pH and microbiological analysis by cutting the tie off one end of each sausage. Each sausage was weighed and placed in the humidity chamber then transferred back to the oven, which was then set at 53°C. This study was designed with a heat treatment (versus traditional processing without heat treatment) because essential spice oils are more effective at higher temperatures (See Chapters 3 and 4). Methodology was adapted from Hinkens and co–workers (1996) who compared *Escherichia coli* 0157:H7 levels in heat treated pepperoni to traditionally processed pepperoni with no heat treatment. After 8 hr, the oven temperature was increased to 55°C. Once the sausages reached an internal temperature of 53°C (approximately 12 hr), they were held for an additional hour. After the heat process, the sausages were placed in a steam of cold water until the internal temperature reached approximately 27°C. Sausages were then weighed, sampled, re-weighed and wrapped in brown butcher paper for drying. The drying step was at room

temperature (22.8°C) and was carried out until the sausages lost approximately 30% of their weight (16 days) or reached a moisture/protein ratio (M:Pr) of approximately 1.9:1 (Romans et al., 1994). Samples were then taken for microbiological and pH analysis.

### 7.3.4 MICROBIOLOGICAL ANALYSIS OF PEPPERONI

Pepperoni was tested for *Escherichia coli* 0157:H7 prior to stuffing and after fermentation, cooking and drying. A 1.1 g sample was serially diluted with sterile 0.1% buffered peptone water and plated on two Petrifilm™ coliform plates (3M, St. Paul, MN). From the lowest dilution tube, a 1 ml aliquot was placed in 10 ml sterile tryptic soy broth and incubated at 35°C for 24 hr. If the Petrifilms™ lacked the presence of *Escherichia coli* 0157:H7, the tryptic soy broth was checked for growth. This was conducted to positively identify complete inhibition of the organism. Sausages were also tested for viable *Pediococci* (starter culture) before and after fermentation by spread plating on Rogosa agar (Becton-Dickinson Microbiology Systems, Cockeysville,MD) and incubating 48 hr at 35°C.

### 7.3.5 CHEMICAL ANALYSIS OF PEPPERONI

The pH was determined by mixing a 5 g sample with 45 ml distilled water in a Waring blender for 30 sec. The pH was measured before and after fermentation, after heating and after drying. All sausages were sent to AAT

Laboratories, Inc. (Grand Rapids, MI) for proximate analysis (moisture, fat and protein) and titratable acid determination.

## 7.4 RESULTS AND DISCUSSION

### 7.4.1 RESULTS OF MICROBIOLOGICAL ANALYSIS

Microbiological analysis determined that the initial levels of *Escherichia coli* and *Pediococcus* spp. were $10^6 - 10^7$ CFU/g. This was the target level for *Escherichia coli* 0157:H7, but the *Pediococcus* spp. levels were lower than anticipated for all treatments (See Table 7.4.1A). Because all treatments including controls had low starter culture counts, the oregano oil was not the cause. The premature depletion of substrate (dextrose) may have been a factor in the low starter culture counts after fermentation. Interestingly, the oregano oil treatment had lower initial (pre-fermentation) counts for *Escherichia coli* 0157:H7 than the controls. Apparently the oregano oil was inhibitory to the organism during processing. After fermentation, *Escherichia coli* 0157:H7 levels were the same in both control treatments, while the oregano oil treatment had lower counts. The results of our controls are similar to other studies (Riordan et al., 1998; Faith et al., 1998 and Hinkens et al., 1996) where fermentation had no effect on *Escherichia coli* 0157:H7 levels when formulated with traditional ingredients (no antibacterial additives). After the heat treatment (53°C), both control treatments had *Escherichia coli* 0157:H7 present ($10^4$ CFU/g). The level of *Escherichia coli* 0157:H7 remaining was much higher than the level determined by Hinkens and co-workers (1996). These researchers achieved a 5

$log_{10}$ reduction with the heat treatment (See Table 7.4.1A). Different processing techniques and equipment could have negatively affected our results. Also, the test organism (204P) used in this study had known heat resistance (Ahmed et al., 1995) which possibly could have attributed to the higher log counts. Sausages with oregano oil were completely devoid of *Escherichia coli* 0157:H7 (no recovery in tryptic soy broth) after the heat treatment. Oregano oil was more efficient and effective at inhibiting *Escherichia coli* 0157:H7 in heat treated pepperoni, than in control sausages without oregano oil. It would be useful to determine how oregano oil affects the level of *Escherichia coli* 0157:H7 without any heat treatment (traditional process).

The level of *Pediococcus* spp. remained steady throughout the study with only a slight increase after fermentation. The initial levels were below what the study was designed for which possibly affected the processing pH.

### 7.4.2 CHEMICAL ANALYSIS OF PEPPERONI

The pH was measured before and after fermentation, after heat processing and after drying. The initial pH of each sausage was within the normal range when compared to other research. Table 7.4.2A displays the pH of each sausage and the sausages in Hinkens et al. (1996). The pH of all sausage treatments including controls were higher than the target pH of <5.0. This may be due to the low number of *Pediococci* spp. in each batter. The higher levels of *Escherichia coli* 0157:H7 in our control treatments, when compared to Hinkens et al. (1996), may have been due in part to a higher pH after fermentation. Consistent with other pepperoni research, our pH values remained steady

throughout the heat processing and drying stages. Moisture, fat and protein results are displayed in Table 7.4.2A.

In conclusion, the addition of oregano oil to a dry, fermented sausage insures that *Escherichia coli* 0157:H7 is completely eliminated from the product even when processing results are not ideal (i.e. pH to high and lactic acid content to low). Future work could include a study of unheated sausage with oregano oil (or other essential spice oils) to determine if a heat treatment is required for the elimination of the organism, which is the traditional processing practice. A taste panel would be beneficial to determine the organoleptic changes in the product with added oregano oil. The use of essential spice oils as antibacterial agents in processed meat products has future potential.

**TABLE 7.4.1A: *Escherichia coli* 0157:H7 COUNTS DURING PEPPERONI PROCESSING**

| | *Escherichia coli* 0157:H7 | |
|---|---|---|
| **Sampling Time** | **Log CFU/g[1]** | **Log CFU/g[2]** |
| **Pre-fermentation** | | |
| Control | 7.20 | 7.79 |
| Ethanol control | 7.28 | — |
| Oregano oil | 6.89 | — |
| **Post-fermentation** | | |
| Control | 7.73 | 6.89 |
| Ethanol control | 7.05 | — |
| Oregano oil | 5.54 | — |
| **Post-heat processing** | | |
| Control | 4.69 | <2.00 |
| Ethanol control | 4.69 | — |
| Oregano oil | 0.00 | — |
| **Post-drying** | | |
| Control | 4.44 | <2.00 |
| Ethanol control | 4.56 | — |
| Oregano oil | 0.00 | — |

[1]Log CFU/g based on average of two plate/repetition x two repetitions on Petrifilm™

[2]Results cited from Hinkens et al. (1996). Counts made from MRS agar

**Table 7.4.2A. CHEMICAL COMPOSITION OF PEPPERONI DURING PROCESSING[1]**

| Sampling Time | pH | pH[2] | (%) MOISTURE | (%) FAT | (%) PROTEIN |
|---|---|---|---|---|---|
| **Pre-fermentation** | | | | | |
| Control | 6.18 | --- | | | |
| Ethanol Control | 6.21 | --- | | | |
| Oregano Oil | 6.18 | --- | | | |
| **Post-fermentation** | | | | | |
| Control | 5.19 | 4.72 | | | |
| Ethanol Control | 5.21 | --- | | | |
| Oregano Oil | 5.21 | --- | | | |
| **Post-heat processing** | | | | | |
| Control | 5.19 | 4.78 | | | |
| Ethanol Control | 5.21 | --- | | | |
| Oregano Oil | 5.20 | --- | | | |
| **Post-drying** | | | | | |
| Control | 5.18 | 4.79 | 32.33 | 44.21 | 17.18 |
| Ethanol Control | 5.20 | --- | 29.45 | 45.95 | 19.58 |
| Oregano Oil | 5.17 | --- | 28.78 | 47.25 | 18.77 |

[1]Moisture, fat and protein analysis only conducted after drying period
[2]Results cited from Hinkens et al. (1996)

# SUMMARY AND CONCLUSIONS

Conclusions for each study can be found at the end of each chapter.

# APPENDICES

# APPENDIX A

## APPENDIX A

**Table A.1: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR *Escherichia coli* 0157:H7**

VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| | 250 ORE | 250 THY | 250 CINN | 250 BAY | 250 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.78 | 7.83 | 6.61 | 6.78 | 5.79 |
| 6 HR | 0 | 0 | 8.85 | 8.64 | 6.21 |
| 12 HR | 0 | 0 | 8.67 | 9.56 | 7.63 |
| 24 HR | 0 | 0 | 9.6 | 9.75 | 8.47 |
| 36 HR | 0 | 0 | 9.74 | 9.83 | 8.91 |

| | 250 CAR | 250 TMOL | 250EUG | 250CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.75 | 6.81 | 5.54 | 5.74 | 6.64 |
| 6 HR | 0 | 7.89 | 5.53 | 7.15 | 8.17 |
| 12 HR | 0 | 8 | 7.14 | 8 | 8.92 |
| 24 HR | 0 | 9 | 8.44 | 8.85 | 9.52 |
| 36 HR | 0 | 9 | 9.04 | 9.08 | 9.68 |

| | 500 ORE | 500 THY | 500 CINN | 500 BAY | 500 CLOV |
|---|---|---|---|---|---|
| 0 HR | 7.53 | 6.95 | 6.79 | 6.2 | 5.42 |
| 6 HR | 0 | 0 | 8.69 | 6.33 | 6.39 |
| 12 HR | 0 | 0 | 8.72 | 7.66 | 0 |
| 24 HR | 0 | 0 | 8.69 | 8.93 | 0 |
| 36 HR | 0 | 0 | 9.19 | 9.75 | 0 |

| | 500 CAR | 500 TMOL | 500 EUG | 500 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.87 | 6.8 | 4.85 | 5.64 | 6.64 |
| 6 HR | 0 | 7.69 | 4.69 | 4.73 | 8.17 |
| 12 HR | 0 | 8 | 0 | 0 | 8.92 |
| 24 HR | 0 | 9 | 0 | 0 | 9.52 |
| 36 HR | 0 | 9 | 0 | 0 | 9.68 |

ORE=OREGANO
THY=THYME
CINN=CINNAMON
BAY=BAY
CLOV=CLOVE
CAR=CARVACROL
TMOL=THYMOL
EUG=EUGENOL
CINAL=CINNAMON ALDEHYDE
CONTROL=CONTROL

## APPENDIX A

**Table A.2: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR *Salmonella typhimurium***

VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| | 250 ORE | 250 THY | 250 CINN | 250 BAY | 250 CLOV |
|---|---|---|---|---|---|
| 0 HR | 7.85 | 7.72 | 7.2 | 5.16 | 4.78 |
| 6 HR | 0 | 0 | 8.54 | 8.54 | 4.8 |
| 12 HR | 0 | 0 | 8.69 | 9.64 | 4.53 |
| 24 HR | 0 | 0 | 9.34 | 9.82 | 0 |
| 36 HR | 0 | 0 | 9.806 | 9.7 | 0 |

| | 250 CAR | 250 TMOL | 250 EUG | 250 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 5.89 | 6.6 | 5.88 | 4.74 | 5.76 |
| 6 HR | 8.29 | 7.61 | 6.7 | 4.64 | 8.95 |
| 12 HR | 9.72 | 8 | 7.83 | 4.66 | 9.96 |
| 24 HR | 9.78 | 9 | 9.46 | 6.75 | 9.98 |
| 36 HR | 9.88 | 9 | 9.86 | 9.12 | 9.99 |

| | 500 ORE | 500 THY | 500 CINN | 500 BAY | 500 CLOV |
|---|---|---|---|---|---|
| 0 HR | 7.67 | 7.36 | 7.31 | 5.79 | 4.53 |
| 6 HR | 0 | 0 | 5.52 | 6.35 | 3.48 |
| 12 HR | 0 | 0 | 3.58 | 7.86 | 2.79 |
| 24 HR | 0 | 0 | 0 | 9.75 | 0 |
| 36 HR | 0 | 0 | 0 | 9.95 | 0 |

| | 500 CAR | 500 TMOL | 500 EUG | 500 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 5.56 | 6.79 | 5.77 | 4.53 | 5.76 |
| 6 HR | 4.41 | 8.05 | 0 | 3.48 | 8.95 |
| 12 HR | 1.3 | 8 | 0 | 0 | 9.96 |
| 24 HR | 0 | 9 | 0 | 0 | 9.98 |
| 36 HR | 0 | 9 | 0 | 0 | 9.99 |

ORE=OREGANO CAR=CARVACROL
THY=THYME TMOL=THYMOL
CINN=CINNAMON EUG=EUGENOL
BAY=BAY CINAL=CINNAMON ALDEHYDE
CLOV=CLOVE CONTROL=CONTROL

## APPENDIX A

**Table A.3: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR *Yersinia enterocolitica***

VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| | 250 ORE | 250 THY | 250 CINN | 250 BAY | 250 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.77 | 6.56 | 6.64 | 6.61 | 6.86 |
| 6 HR | 3.93 | 0 | 7.71 | 4.92 | 3.63 |
| 12 HR | 1.83 | 0 | 8.82 | 3.85 | 0 |
| 24 HR | 0 | 0 | 9.83 | 0 | 0 |
| 36 HR | 0 | 0 | 9.81 | 0 | 0 |

| | 250 CAR | 250 TMOL | 250 EUG | 250 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.64 | 6.76 | 6.76 | 6.59 | 6.7 |
| 6 HR | 4.57 | 7.75 | 4.51 | 7.86 | 7.73 |
| 12 HR | 3.69 | 8 | 6.46 | 8.79 | 8.88 |
| 24 HR | 0 | 9 | 8.7 | 9.83 | 9.69 |
| 36 HR | 0 | 9 | 8.91 | 9.83 | 9.82 |

| | 500 ORE | 500 THY | 500 CINN | 500 BAY | 500 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.67 | 6.92 | 6.6 | 6.79 | 6.72 |
| 6 HR | 0 | 0 | 0 | 0 | 0 |
| 12 HR | 0 | 0 | 0 | 0 | 0 |
| 24 HR | 0 | 0 | 0 | 0 | 0 |
| 36 HR | 0 | 0 | 0 | 0 | 0 |

| | 500 CAR | 500 TMOL | 500 EUG | 500 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.68 | 6.81 | 6.55 | 6.74 | 6.7 |
| 6 HR | 0 | 7.74 | 0 | 0 | 7.73 |
| 12 HR | 0 | 8 | 0 | 0 | 8.88 |
| 24 HR | 0 | 9 | 0 | 0 | 9.69 |
| 36 HR | 0 | 9 | 0 | 0 | 9.82 |

ORE=OREGANO
THY=THYME
CINN=CINNAMON
BAY=BAY
CLOV=CLOVE
CAR=CARVACROL
TMOL=THYMOL
EUG=EUGENOL
CINAL=CINNAMON ALDEHYDE
CONTROL=CONTROL

## APPENDIX A

**Table A.4: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR *Staphylococcus aureus***

VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| | 250 ORE | 250 THY | 250 BAY | 250 CINN | 250 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.66 | 6.71 | 6.78 | 6.72 | 6.69 |
| 6 HR | 0 | 0 | 6.91 | 5.71 | 6.9 |
| 12 HR | 0 | 0 | 7.54 | 0 | 8.83 |
| 24 HR | 0 | 0 | 8.78 | 0 | 9.74 |
| 36 HR | 1 | 0 | 8.93 | 0 | 9.94 |

| | 250 CAR | 250 TMOL | 250 EUG | 250 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.89 | 6.73 | 6.84 | 6.85 | 6.89 |
| 6 HR | 0 | 8.21 | 6.89 | 6.74 | 8 |
| 12 HR | 0 | 8 | 7.67 | 7.87 | 8.9 |
| 24 HR | 0 | 9 | 8.73 | 8.69 | 9.15 |
| 36 HR | 0 | 9 | 8.93 | 9.85 | 9.82 |

| | 500 ORE | 500 THY | 500 BAY | 500 CINN | 500 CLOV |
|---|---|---|---|---|---|
| O HR | 6.54 | 6.52 | 6.82 | 6.28 | 6.93 |
| 6 HR | 0 | 0 | 0 | 2.49 | 0 |
| 12 HR | 0 | 0 | 0 | 0 | 0 |
| 24 HR | 0 | 0 | 0 | 0 | 0 |
| 36 HR | 0 | 0 | 0 | 0 | 0 |

| | 500 CAR | 500 TMOL | 500 EUG | 500 CINAL | CONTROL |
|---|---|---|---|---|---|
| O HR | 6.67 | 6.69 | 6.26 | 6.79 | 6.73 |
| 6 HR | 0 | 7.6 | 6.6 | 5.54 | 7.97 |
| 12 HR | 0 | 8 | 7.79 | 4.78 | 8.75 |
| 24 HR | 0 | 9 | 8.81 | 4.62 | 9.09 |
| 36 HR | 0 | 9 | 8.76 | 4.35 | 9.65 |

ORE=OREGANO CAR=CARVACROL
THY=THYME TMOL=THYMOL
CINN=CINNAMON EUG=EUGENOL
BAY=BAY CINAL=CINNAMON ALDEHYDE
CLOV=CLOVE CONTROL=CONTROL

## APPENDIX A

**Table A.5: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS FOR *Bacillus cereus***

VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| | 250 ORE | 250 THY | 250 BAY | 250 CINN | 250 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.57 | 6.74 | 6.79 | 6.82 | 6.84 |
| 6 HR | 7.74 | 0 | 0 | 7.81 | 2.65 |
| 12 HR | 8.66 | 0 | 0 | 8.76 | 0 |
| 24 HR | 8.77 | 0 | 0 | 9.35 | 0 |
| 36 HR | 9.64 | 0 | 0 | 9.81 | 0 |

| | 250 CAR | 250 TMOL | 250 EUG | 250 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 7.57 | 6.56 | 6.7 | 7.75 | 7.09 |
| 6 HR | 0 | 7.91 | 7.84 | 0 | 8.07 |
| 12 HR | 0 | 8 | 8.78 | 0 | 8.75 |
| 24 HR | 0 | 9 | 8.92 | 0 | 9.14 |
| 36 HR | 0 | 9 | 9.62 | 0 | 9.68 |

| | 500 ORE | 500 THY | 500 BAY | 500 CINN | 500 CLOV |
|---|---|---|---|---|---|
| 0 HR | 6.8 | 6.79 | 6.67 | 6.75 | 6.69 |
| 6 HR | 0 | 0 | 0 | 4.84 | 0 |
| 12 HR | 0 | 0 | 0 | 3.64 | 0 |
| 24 HR | 0 | 0 | 0 | 1.35 | 0 |
| 36 HR | 0 | 0 | 0 | 0 | 0 |

| | 500 CAR | 500 TMOL | 500 EUG | 500 CINAL | CONTROL |
|---|---|---|---|---|---|
| 0 HR | 6.74 | 6.47 | 7.38 | 7.61 | 7.09 |
| 6 HR | 0 | 7.62 | 5.59 | 0 | 8.07 |
| 12 HR | 0 | 8 | 7.75 | 0 | 8.75 |
| 24 HR | 0 | 9 | 8.79 | 0 | 9.14 |
| 36 HR | 0 | 9 | 9.57 | 0 | 9.68 |

| | |
|---|---|
| ORE=OREGANO | CAR=CARVACROL |
| THY=THYME | TMOL=THYMOL |
| CINN=CINNAMON | EUG=EUGENOL |
| BAY=BAY | CINAL=CINNAMON ALDEHYDE |
| CLOV=CLOVE | CONTROL=CONTROL |

# APPENDIX B

## APPENDIX B

**Table B.1: BROTH REFRIGERATION STUDY WITH *Escherichia coli* 0157:H7**
VALUES EXPRESSED AS $LOG_{10}$ CFU/ml

| TIME<br>DAY 0 | CONTROL | 500-OR | 1000-OR | 2000-OR | 500-TH | 1000-TH | 2000-TH |
|---|---|---|---|---|---|---|---|
| PLATE A | 6.46 | 6.51 | 6.69 | 6.61 | 6.23 | 6.51 | 6.62 |
| PLATE B | 6.77 | 6.78 | 6.69 | 6.48 | 6.39 | 6.49 | 6.6 |
| DAY 1 | | | | | | | |
| PLATE A | 6.78 | 6.58 | 6.32 | 6.49 | 6.78 | 6.68 | 6.56 |
| PLATE B | 6.76 | 6.67 | 6.39 | 6.48 | 6.76 | 6.84 | 6.46 |
| DAY 2 | | | | | | | |
| PLATE A | 6.48 | 5.59 | 6.45 | 4.59 | 5.56 | 5.54 | 4.79 |
| PLATE B | 6.45 | 5.61 | 6.54 | 4.62 | 5.48 | 5.46 | 4.75 |
| DAY 5 | | | | | | | |
| PLATE A | 5.79 | 5.56 | 5.62 | 4.34 | 5.81 | 5.6 | 1.75 |
| PLATE B | 5.78 | 5.46 | 5.62 | 4.28 | 5.77 | 5.62 | 1.73 |
| DAY 7 | | | | | | | |
| PLATE A | 5.89 | 5.18 | 4.56 | 3.3 | 5.11 | 4.51 | 0 |
| PLATE B | 5.94 | 5.04 | 4.58 | 3.46 | 5.04 | 4.45 | 0 |
| DAY 9 | | | | | | | |
| PLATE A | 5.54 | 5.56 | 4.46 | 2.73 | 5.69 | 3.23 | 0 |
| PLATE B | 5.76 | 5.3 | 4.68 | 2.78 | 5.79 | 3.17 | 0 |
| DAY16 | | | | | | | |
| PLATE A | 5.69 | 3.46 | 3.4 | 1.3 | 3.29 | 3.23 | 0 |
| PLATE B | 5.56 | 3.4 | 3.46 | 1.3 | 3.18 | 3.39 | 0 |
| DAY 23 | | | | | | | |
| PLATE A | 5.69 | 2.6 | 0 | 0 | 3.19 | 3 | 0 |
| PLATE B | 5.89 | 2.56 | 0 | 0 | 3.28 | 3.09 | 0 |
| DAY30 | | | | | | | |
| PLATE A | 4.46 | 0 | 0 | 0 | 1.3 | 3.69 | 0 |
| PLATE B | 4.56 | 0 | 0 | 0 | 1.69 | 3.81 | 0 |

OR=OREGANO OIL TH=THYME OIL

## APPENDIX B

**Table B.2: REFRIGERATION STUDY WITH *Escherichia coli* 0157:H7 *Salmonella typhimurium* AND *Yersinia enterocolitica* IN AEROBIC AND ANAEROBIC CONDITIONS WITH OREGANO AND THYME OILS ADDED**

GBEC=GROUND BEEF *Escherichia coli* 0157:H7
GPYE=GROUND PORK *Yersinia enterocolitica*
GTST=GROUND TURKEY *Salmonella typhimurium*

VALUES EXPRESSED AS $LOG_{10}$ CFU/g WITH TWO PLATES/TREATMENT

AEROBIC TRIAL START

| | REPITITION 1 | | | REPITITION 2 | | |
|---|---|---|---|---|---|---|
| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
| CONTROL | 6.46 | 6.48 | 6.91 | 6.27 | 6.04 | 6.99 |
| | 6.57 | 6.34 | 6.98 | 6.69 | 6.11 | 6.85 |
| 500-O | 6.93 | 5.61 | 6.72 | 6.96 | 5.9 | 6.89 |
| | 6.85 | 5.92 | 6.89 | 6.95 | 6.08 | 6.91 |
| 1000-O | 6.62 | 6 | 6.56 | 6.72 | 5.68 | 6.99 |
| | 6.69 | 6.11 | 6.68 | 6.89 | 5.64 | 6.94 |
| 500-T | 6.78 | 6.11 | 6.59 | 6.69 | 5.66 | 6.77 |
| | 6.54 | 6.56 | 6.69 | 6.9 | 5.77 | 6.89 |
| 1000-T | 6.89 | 5.49 | 6.79 | 6.83 | 5.43 | 6.71 |
| | 6.62 | 6.08 | 6.76 | 6.89 | 5.97 | 6.66 |

AEROBIC TRIAL DAY 4

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 6.93 | 6 | 7 | 6.69 | 6.78 | 7.04 |
| | 6.88 | 6.47 | 7.04 | 6.71 | 6.47 | 7.11 |
| 500-O | 6.53 | 5.69 | 6.54 | 6.25 | 5.3 | 6.7 |
| | 6.57 | 5.53 | 6.2 | 6.38 | 5.47 | 6.82 |
| 1000-O | 5.69 | 4.84 | 6.63 | 6 | 5.65 | 6.04 |
| | 6.46 | 4.94 | 6.56 | 6.11 | 5.72 | 5.3 |
| 500-T | 5.6 | 5.69 | 6.66 | 5.69 | 5.48 | 6.69 |
| | 6.23 | 5.95 | 6.74 | 6.32 | 5.84 | 6.62 |
| 1000-T | 6.95 | 5.3 | 6.88 | 6.43 | 5.3 | 6.77 |
| | 6.18 | 5.82 | 6.43 | 6.2 | 5.9 | 6.47 |

## APPENDIX B

### REFRIGERATION STUDY CONTINUED

AEROBIC TRIAL DAY 8

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.6 | 8.39 | 7.11 | 6.48 | 7.59 | 7.04 |
| | 7.48 | 8.2 | 7.23 | 6.48 | 7.83 | 7.04 |
| 500-O | 6.23 | 7.89 | 6.95 | 5.6 | 7.94 | 6.52 |
| | 6.39 | 7.96 | 6.86 | 6.04 | 7.96 | 6.73 |
| 1000-O | 6.45 | 7.62 | 6.77 | 6.41 | 7.6 | 6.63 |
| | 6.38 | 7.59 | 6.6 | 6.38 | 7.51 | 6.41 |
| 500-T | 6.51 | 7.85 | 6.66 | 6.38 | 7.98 | 6.46 |
| | 6.48 | 7.91 | 6.59 | 6.38 | 7.96 | 6.76 |
| 1000-T | 6.67 | 7.76 | 5.08 | 6.49 | 6.85 | 6.23 |
| | 6.39 | 7.6 | 5.16 | 6.52 | 6.69 | 6.6 |

AEROBIC TRIAL DAY 12

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.48 | 8.81 | 7.78 | 7.85 | 8.48 | 7.84 |
| | 7 | 8.69 | 7.54 | 7.69 | 8.69 | 7.69 |
| 500-O | 6.15 | 7.62 | 6.65 | 6.2 | 7.6 | 6.46 |
| | 6.45 | 7.56 | 6.79 | 6.3 | 7.77 | 6.56 |
| 1000-O | 6.41 | 7.77 | 5.78 | 6.3 | 7.56 | 6.04 |
| | 6.28 | 7.6 | 5.78 | 6.2 | 7.69 | 5.95 |
| 500-T | 6.11 | 7.95 | 6.78 | 6.38 | 7.69 | 6.59 |
| | 6.04 | 7.96 | 6.69 | 6.41 | 7.91 | 6.65 |
| 1000-T | 6.41 | 7.6 | 6.46 | 6.26 | 7.79 | 6.6 |
| | 6.46 | 7.93 | 6.53 | 6.23 | 7.99 | 6.17 |

## APPENDIX B

### REFRIGERATION STUDY CONTINUED

AEROBIC TRIAL DAY 16

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.56 | 8.92 | 7.46 | 7.69 | 8.79 | 7.77 |
| | 7.69 | 8.79 | 7.91 | 7.91 | 8.89 | 7.95 |
| 500-O | 6.62 | 7.75 | 6.6 | 6.63 | 7.6 | 6.58 |
| | 6.77 | 7.83 | 6.51 | 6.69 | 7.49 | 6.69 |
| 1000-O | 6.48 | 7.6 | 6.76 | 6.79 | 7.84 | 6.9 |
| | 6.6 | 7.46 | 6.59 | 6.46 | 7.65 | 6.81 |
| 500-T | 6.46 | 7.49 | 6.93 | 6.91 | 7.91 | 6.66 |
| | 6.69 | 7.76 | 6.86 | 6.78 | 8.69 | 6.49 |
| 1000-T | 6.75 | 7.46 | 6.84 | 6.77 | 7.62 | 6.95 |
| | 6.61 | 7.66 | 6.97 | 6.6 | 7.84 | 7.85 |

AEROBIC TRIAL DAY 20

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.51 | 8.62 | 7.79 | 7 | 8.51 | 7.6 |
| | 7.84 | 8.78 | 7.85 | | | |
| 500-O | 6.11 | 7.6 | 6.69 | 6.81 | 7.69 | 6.59 |
| | 6.11 | 7.46 | 6.48 | 6.94 | 7.48 | 6.74 |
| 1000-0 | 6 | 7.92 | 6.41 | 6.62 | 7.46 | 6.36 |
| | 6.11 | 7.62 | 6.59 | 6.49 | 7.85 | 6.69 |
| 500-T | 6.28 | 7.71 | 6.6 | 6.69 | 7.78 | 6.69 |
| | 6.3 | 7.78 | 6.6 | 6.56 | 7.59 | 6.6 |
| 1000-Y | 6.9 | 7.6 | 6.72 | 6.46 | 7.66 | 6.94 |
| | 6.04 | 7.48 | 6.56 | 6.48 | 7.69 | 6.65 |

## APPENDIX B

### ANAEROBIC REFRIGERATION STUDY

ANAEROBIC TRIAL START

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 6.5 | 5.78 | 6.54 | 6.6 | 6.2 | 6.77 |
| | 6.61 | 5.9 | 6.65 | 6.46 | 6.27 | 6.44 |
| 500-O | 6 | 6.14 | 6.49 | 6.3 | 6.27 | 6.49 |
| | 6.47 | 6.14 | 6.44 | 6.17 | 6.17 | 6.6 |
| 1000-O | 6.59 | 6.36 | 6.69 | 6.27 | 6.07 | 6.65 |
| | 6.32 | 6.26 | 6.51 | 6.47 | 6.34 | 6.49 |
| 500-T | 6.39 | 6.08 | 6.27 | 6.71 | 5.69 | 6.46 |
| | 6.48 | 6.17 | 6.46 | 6.6 | 5.84 | 6.48 |
| 1000-T | 6.59 | 6.11 | 6.55 | 6.14 | 6.91 | 6.27 |
| | 6.3 | 6.15 | 6.62 | 6.25 | 6.98 | 6.49 |

ANAEROBIC TRIAL DAY 4

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 6.6 | 7.07 | 6 | 6.83 | 7.11 | 6.49 |
| | 6.78 | 7.16 | 6.04 | 6.72 | 7.23 | 6.59 |
| 500-0 | 6.21 | 6.11 | 6.56 | 6.16 | 6.23 | 6.81 |
| | 6.32 | 6.15 | 6.65 | 6.18 | 6.08 | 6.69 |
| 1000-0 | 6.26 | 6.04 | 6.69 | 6.51 | 6.08 | 6.62 |
| | 6.17 | 6.08 | 6.54 | 6.6 | 6.14 | 6.26 |
| 500-T | 6.42 | 6.17 | 6.2 | 6.29 | 6.23 | 6.34 |
| | 6.27 | 6.2 | 6.39 | 6.17 | 6.07 | 6.59 |
| 1000-T | 6.48 | 6.19 | 6.46 | 6.24 | 6.05 | 6.62 |
| | 6.42 | 6.08 | 6.59 | 6.16 | 6.13 | 6.77 |

## APPENDIX B

### ANAEROBIC REFRIGERATION STUDY CONTINUED

ANAEROBIC TRIAL DAY 8

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 6.6 | 8.66 | 7.06 | 6.7 | 8.56 | 7.11 |
| | 7 | 8.76 | 7.32 | 6.9 | 8.61 | 7.17 |
| 500-0 | 6.49 | 6.94 | 6.76 | 6.46 | 6.72 | 6.28 |
| | 6.69 | 6.98 | 6.61 | 6.43 | 6.69 | 6.56 |
| 1000-O | 6.56 | 7.25 | 6.91 | 6.4 | 6.99 | 6.57 |
| | 6.49 | 7.19 | 6.18 | 6.28 | 7.08 | 6.46 |
| 500-T | 6.93 | 6.94 | 6.69 | 6.46 | 6.68 | 6 |
| | 6.94 | 6.78 | 6.79 | 6.39 | 6.83 | 6.43 |
| 1000-T | 6.59 | 6.15 | 5.78 | 6.56 | 5.9 | 6.25 |
| | 6.72 | 6.46 | 6.65 | 6.69 | 6.15 | 6.43 |

ANAEROBIC TRIAL DAY 12

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.28 | 8.11 | 7.23 | 7.11 | 8 | 7.36 |
| | 7.18 | 7.69 | 7 | 7.15 | 8.2 | 7.38 |
| 500-O | 6.16 | 6.22 | 6.96 | 6.65 | 6.63 | 6.81 |
| | 6.29 | 6.28 | 6.9 | 6.77 | 6.48 | 6.91 |
| 1000-O | 6.56 | 6.41 | 6.26 | 6.35 | 6.79 | 6.61 |
| | 6.69 | 6.51 | 6.59 | 6.24 | 6.38 | 6.6 |
| 500-T | 6.89 | 6.25 | 6.76 | 6.24 | 6.04 | 6.2 |
| | 6.98 | 6.35 | 6.84 | 6.28 | 6.78 | 6.3 |
| 1000-T | 6.69 | 6.11 | 6.72 | 6.04 | 6.67 | 6.28 |
| | 6.95 | 6.28 | 6.72 | 6.04 | 6.2 | 6.23 |

## APPENDIX B

### ANAEROBIC REFRIGERATION STUDY CONTINUED

ANAEROBIC TRIAL DAY 16

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.04 | 7.28 | 7.26 | 7 | 7.78 | 7 |
| | 7 | 7.41 | 7.23 | 7 | 7.69 | 7.28 |
| 500-O | 6.34 | 7.11 | 6.46 | 6.56 | 7.08 | 6.62 |
| | 6.66 | 7.18 | 6.28 | 6.61 | 7.15 | 6.48 |
| 1000-O | 6.46 | 7.04 | 6.36 | 5.6 | 7.28 | 6.48 |
| | 6.49 | 7.21 | 6.34 | 6.56 | 7.26 | 6.38 |
| 500-T | 6.88 | 6.85 | 6.72 | 6.77 | 6.72 | 6.95 |
| | 6.72 | 6.79 | 6.83 | 5.86 | 6.56 | 6.9 |
| 1000-T | 5.61 | 6.23 | 6.69 | 5.48 | 5.85 | 6.11 |
| | 5.84 | 6.39 | 6.69 | 5.85 | 6.28 | 6.53 |

ANAEROBIC TRIAL DAY 20

| | GBEC 1 | GPYE 1 | GTST 1 | GBEC 2 | GPYE 2 | GTST 2 |
|---|---|---|---|---|---|---|
| CONTROL | 7.62 | 7.71 | 7.78 | 7.69 | 7.78 | 7.71 |
| | 7.48 | 7.46 | 7.61 | 7.28 | 7.95 | 7.56 |
| 500-O | 6.59 | 7.62 | 6.56 | 6.77 | 7.63 | 6.66 |
| | 6.78 | 7.45 | 6.6 | 6.6 | 7.79 | 6.86 |
| 1000-0 | 6.77 | 7.41 | 6.76 | 6.56 | 7.71 | 6.39 |
| | 6.62 | 7.6 | 6.41 | 6.69 | 7.59 | 6.46 |
| 500-T | 6.79 | 6.76 | 6.85 | 6.85 | 6.81 | 6.77 |
| | 6.59 | 6.93 | 6.69 | 6.62 | 6.69 | 6.89 |
| 1000-T | 5.72 | 6.97 | 6.63 | 5.91 | 6.49 | 6.46 |
| | 5.85 | 6.71 | 6.65 | 5.87 | 6.66 | 6.68 |

## APPENDIX B

**Table B.3: BACTERICIDAL LEVEL OF OREGANO AND THYME OILS IN REFRIGERATED GROUND BEEF WITH *Escherichia coli* 0157:H7**

VALUES EXPRESSED AS $LOG_{10}$ CFU/g

| | PLATE | START | DAY 0 | DAY 1 | DAY 2 | DAY 5 | DAY 7 |
|---|---|---|---|---|---|---|---|
| 5K OR R1 | A | 7.81 | 6.28 | 5.33 | 5.75 | 5.9 | 5.46 |
| | B | 7.69 | 6.23 | 5.37 | 5.52 | 5.95 | 5.41 |
| 5K OR R2 | A | 7.81 | 5.95 | 5.9 | 5.59 | 5.49 | 5.48 |
| | B | 7.69 | 6.8 | 5.95 | 5.72 | 5.56 | 5.53 |
| 5K TH R1 | A | 7.81 | 6.61 | 6.74 | 6.48 | 6.08 | 6.14 |
| | B | 7.69 | 6.77 | 6.28 | 6.69 | 6 | 6.03 |
| 5K TH R2 | A | 7.81 | 6.38 | 6.08 | 6.6 | 5.9 | 5.86 |
| | B | 7.69 | 6.18 | 6.28 | 6.91 | 5.85 | 5.87 |
| 10K OR R | A | 7.81 | 5.04 | 4.41 | 4.56 | 4.48 | 3.85 |
| | B | 7.69 | 5.04 | 4.39 | 4.52 | 4.46 | 3.48 |
| 10K OR R | A | 7.81 | 5.46 | 4.3 | 4.83 | 2 | 1.9 |
| | B | 7.69 | 5.4 | 4.79 | 4.6 | 2 | 1 |
| 10K TH R1 | A | 7.81 | 5.73 | 4.6 | 4.59 | 3.56 | 3.13 |
| | B | 7.69 | 5.77 | 4.6 | 4.32 | 3.57 | 3.17 |
| 10K TH R | A | 7.81 | 5.79 | 4.95 | 4.69 | 3.56 | 3.98 |
| | B | 7.69 | 5.79 | 4.85 | 4.78 | 3.57 | 4.11 |
| 20K OR R | A | 7.69 | 4.48 | 2.56 | 2.21 | 1.48 | 0 |
| | B | 7.9 | 4.3 | 2.62 | 2.17 | 1 | 0 |
| 20 K OR R | A | 7.69 | 4.3 | 2.88 | 2.13 | 2.69 | 0 |
| | B | 7.9 | 4.48 | 2.94 | 2.08 | 2.75 | 0 |
| 20 K TH R | A | 7.69 | 4.9 | 2.99 | 1.69 | 0 | 0 |
| | B | 7.9 | 4.85 | 2.95 | 1 | 0 | 0 |
| 20K TH R | A | 7.69 | 4.3 | 2.77 | 1.78 | 1.78 | 0 |
| | B | 7.9 | 4.6 | 2.83 | 1.6 | 0 | 0 |
| C R1 ETO | A | 7.81 | 7.52 | 7.26 | 7.81 | 7.76 | 7.38 |
| | B | 7.69 | 7.45 | 7.04 | 6.69 | 7.83 | 7.28 |
| C R2 ETO | A | 7.81 | 7.08 | 6.3 | 6.59 | 7.32 | 6.08 |
| | B | 7.69 | 7 | 6.48 | 6.3 | 7.18 | 6.32 |

5K=5000 10K=10,000 20K=20,000
C =CONTROL OR=OREGANO TH=THYME
R=REPITITION ETO = ETHANOL

## APPENDIX B

**Table B.4: BACTERICIDAL LEVEL OF OREGANO AND THYME OIL IN REFRIGERATED GROUND TURKEY WITH *Salmonella typhimurium***

VALUES EXPRESSED AS $LOG_{10}$ CFU/g

| | PLATE | START | DAY 0 | DAY 1 | DAY 2 | DAY 5 | DAY 7 |
|---|---|---|---|---|---|---|---|
| 5K OR R1 | A | 7.84 | 5.53 | 3.69 | 2.6 | 0 | 0 |
| | B | 7.69 | 5.64 | 3.3 | 2.46 | 0 | 0 |
| 5K OR R2 | A | 7.84 | 5.79 | 3.34 | 1.78 | 0 | 0 |
| | B | 7.69 | 5.89 | 3.78 | 1.95 | 0 | 0 |
| 5K TH R1 | A | 7.84 | 5.53 | 2.78 | 2.84 | 0 | 0 |
| | B | 7.69 | 5.76 | 3.69 | 2.62 | 0 | 0 |
| 5K TH R2 | A | 7.84 | 5.18 | 2.18 | 2.41 | 0 | 0 |
| | B | 7.69 | 5.18 | 2.41 | 2.58 | 0 | 0 |
| 10K OR R | A | 7.84 | 3.46 | 1.85 | 0 | 0 | 0 |
| | B | 7.69 | 3.3 | 2.04 | 0 | 0 | 0 |
| 10K OR R | A | 7.84 | 3.99 | 2.08 | 0 | 0 | 0 |
| | B | 7.69 | 3.93 | 1.78 | 0 | 0 | 0 |
| 10K TH R1 | A | 7.84 | 5.69 | 2.2 | 0 | 0 | 0 |
| | B | 7.69 | 6.04 | 2.39 | 0 | 0 | 0 |
| 10K TH R | A | 7.84 | 5.81 | 2.91 | 0 | 0 | 0 |
| | B | 7.69 | 5.88 | 2.84 | 0 | 0 | 0 |
| 20K OR R | A | 7.77 | 2.95 | 0 | 0 | 0 | 0 |
| | B | 7.66 | 2.99 | 0 | 0 | 0 | 0 |
| 20 K OR R | A | 7.77 | 2.77 | 0 | 0 | 0 | 0 |
| | B | 7.66 | 2.81 | 0 | 0 | 0 | 0 |
| 20 K TH R | A | 7.77 | 1.48 | 0 | 0 | 0 | 0 |
| | B | 7.66 | 1.3 | 0 | 0 | 0 | 0 |
| 20K TH R | A | 7.77 | 2.65 | 0 | 0 | 0 | 0 |
| | B | 7.66 | 2.48 | 0 | 0 | 0 | 0 |
| C R1 ETO | A | 7.84 | 7.5 | 7.59 | 8.16 | 8.91 | 8.81 |
| | B | 7.69 | 7.62 | 7.6 | 7.96 | 8.26 | 8.62 |
| C R2 ETO | A | 7.84 | 7.16 | 7 | 7.62 | 8.46 | 8.75 |
| | B | 7.69 | 7.11 | 7.29 | 7.5 | 8.59 | 8.47 |

5K=5000 10K=10,000 20K=20,000
C=CONTROL OR=OREGANO TH=THYME ETO = ETHANOL
R=REPITITION

## APPENDIX B

**Table B.5: BACTERICIDAL LEVEL OF OREGANO AND THYME OILS IN REFRIGERATED GROUND PORK WITH *Yersinia enterocolitica***

VALUES EXPRESSED AS $LOG_{10}$ CFU/g

| | PLATE | START | DAY 0 | DAY 1 | DAY 2 | DAY 5 | DAY 7 |
|---|---|---|---|---|---|---|---|
| 5K OR R1 | A | 5.69 | 4 | 4.28 | 4 | 3.39 | 2.99 |
| | B | 5.45 | 4 | 4.34 | 4.04 | 3.51 | 2.94 |
| 5K OR R2 | A | 5.69 | 4.85 | 4.66 | 4.15 | 3.51 | 2.95 |
| | B | 5.45 | 4.48 | 4.91 | 3.85 | 3.46 | 2.89 |
| 5K TH R1 | A | 5.69 | 4.6 | 4.74 | 3.9 | 4.32 | 4.04 |
| | B | 5.45 | 4.85 | 4.51 | 3.9 | 4.26 | 4.11 |
| 5K TH R2 | A | 5.69 | 4.87 | 3.6 | 3.95 | 4.13 | 4.23 |
| | B | 5.45 | 4.84 | 3.86 | 4.41 | 3.86 | 4.2 |
| 10K OR R | A | 5.69 | 3 | 3.08 | 3.56 | 3.71 | 0 |
| | B | 5.45 | 3.3 | 2.9 | 3.69 | 3.86 | 0 |
| 10K OR R | A | 5.69 | 4.08 | 3.56 | 2.6 | 3.51 | 0 |
| | B | 5.45 | 4.14 | 3.4 | 2.48 | 3.43 | 0 |
| 10K TH R1 | A | 5.69 | 3.85 | 4.09 | 2.3 | 3.87 | 0 |
| | B | 5.45 | 3.95 | 3.83 | 2.3 | 3.76 | 0 |
| 10K TH R2 | A | 5.69 | 3.78 | 3.83 | 3.84 | 3.49 | 0 |
| | B | 5.45 | 3.48 | 3.11 | 3.87 | 3.71 | 0 |
| 20K OR R | A | 6.39 | 1 | 0 | 0 | 0 | 0 |
| | B | 6.86 | 1 | 0 | 0 | 0 | 0 |
| 20 K OR R | A | 6.39 | 2.69 | 0 | 0 | 0 | 0 |
| | B | 6.86 | 2.6 | 0 | 0 | 0 | 0 |
| 20 K TH R | A | 6.39 | 3.34 | 0 | 0 | 0 | 0 |
| | B | 6.86 | 3.54 | 0 | 0 | 0 | 0 |
| 20K TH R2 | A | 6.39 | 3.69 | 0 | 0 | 0 | 0 |
| | B | 6.86 | 3 | 0 | 0 | 0 | 0 |
| C R1 ETO | A | 5.69 | 5.3 | 6.21 | 5.84 | 6.37 | 6.19 |
| | B | 5.45 | 5.6 | 5.86 | 5.9 | 6.35 | 6.38 |
| C R2 ETO | A | 5.69 | 5.34 | 5.85 | 5.11 | 6.32 | 6.28 |
| | B | 5.45 | 5.67 | 6.23 | 5.04 | 6.34 | 6.11 |

5K=5000 10K=10,000 20K=20,000
C=CONTROL OR=OREGANO TH=THYME ETO=ETHANOL
R=REPITITION

## APPENDIX B

**Table B.6: TEMPERATURE ABUSE STUDY**

BEEF WITH *Escherichia coli* 0157:H7

| TIME | PLATE | C REP 1 | C REP 2 | 8K OR R1 | 8K OR R2 | 8K TH R1 | 8K TH R2 |
|---|---|---|---|---|---|---|---|
| 0 hr raw | A | 4.23 | 4.29 | 4.24 | 4.22 | 4.09 | 4.28 |
| | B | 4.13 | 4.17 | 4.42 | 4.29 | 4.23 | 4.35 |
| 4 hr | A | 5.81 | 5.62 | 4.11 | 4.27 | 4.04 | 4.17 |
| | B | 5.59 | 5.57 | 4.19 | 4.29 | 4.19 | 4.28 |
| 8 hr | A | 6.12 | 6.27 | 4.78 | 4.49 | 4.26 | 4.59 |
| | B | 6.17 | 6.23 | 4.75 | 4.69 | 4.72 | 4.65 |
| 24 hr | A | 7.06 | 7.14 | 4.71 | 4.62 | 4 | 4 |
| | B | 7.08 | 7.08 | 4.87 | 4.57 | 4.15 | 4.28 |
| 28 hr | A | 7.13 | 7.16 | 4.34 | 4.59 | 3.3 | 4.23 |
| | B | 7.08 | 7.1 | 4.38 | 4.41 | 3.68 | 3.85 |
| 32 hr | A | 7.16 | 7.37 | 4.51 | 4.57 | 4.04 | 4.08 |
| | B | 7.2 | 7.41 | 4.38 | 4.66 | 3.9 | 3.9 |

C=CONTROL
REP=REPITITION
8K=8000
OR=OREGANO
TH=THYME

Values are expressed as $\log_{10}$ CFU/g

## APPENDIX B

**Table B.7: TEMPERATURE ABUSE STUDY**

TURKEY WITH *Salmonella typhimurium*

| TIME | PLATE | C REP 1 | C REP 2 | 5K OR R1 | 5K OR R2 | 5K TH R1 | 5K TH R2 |
|---|---|---|---|---|---|---|---|
| 0 hr raw | A | 4.81 | 4.46 | 2.48 | 3.11 | 2.78 | 3 |
| | B | 4.69 | 4.54 | 2.78 | 3 | 3.04 | 2.78 |
| 4 hr | A | 4.46 | 4.66 | 2.85 | 3.28 | 2.9 | 3 |
| | B | 4.57 | 4.3 | 2.78 | 2 | 2.3 | 2.6 |
| 8 hr | A | 5.28 | 5.39 | 2.48 | 2.48 | 2.48 | 2.78 |
| | B | 4.6 | 5.18 | 2.9 | 2.78 | 2.95 | 2.3 |
| 24 hr | A | 6.3 | 6 | 2.95 | 2.48 | 2.28 | 2.51 |
| | B | 5.04 | 6.18 | 3.11 | 2.69 | 2.3 | 2 |
| 28 hr | A | 6.41 | 6.28 | 2.04 | 2.04 | 2.56 | 2.46 |
| | B | 6.3 | 7.3 | 1.78 | 2 | 1 | 2.3 |
| 32 hr | A | 7.6 | 7 | 1.48 | 1.69 | 1.78 | 1.78 |
| | B | 7.48 | 7.95 | 1.48 | 1 | 1.95 | 1.6 |

C=CONTROL
REP=REPITITION
5K=5000
OR=OREGANO
TH=THYME

Values expressed as $\log_{10}$ CFU/g

## APPENDIX B

**Table B.8: TEMPERATURE ABUSE STUDY**

PORK WITH *Yersinia enterocolitica*

| TIME | PLATE | C REP 1 | C REP 1 | 8K OR R1 | 8K OR R2 | 8K TH R1 | 8K TH R2 |
|---|---|---|---|---|---|---|---|
| 0 hr raw | A | 4.06 | 3.81 | 3.8 | 3.69 | 3.3 | 3.9 |
| | B | 4.04 | 3.58 | 3.95 | 3.69 | 3.78 | 4.18 |
| 4 hr | A | 4.79 | 3.95 | 3.95 | 3.69 | 3.9 | 4.48 |
| | B | 4.69 | 3.85 | 4 | 4.04 | 3.51 | 4.3 |
| 8hr | A | 4.36 | 4.78 | 3.78 | 3.95 | 3.3 | 3.6 |
| | B | 4.28 | 4.95 | 3.6 | 3.9 | 3 | 3.9 |
| 24 hr | A | 5.79 | 4.9 | 4.3 | 3.9 | 3.28 | 3.81 |
| | B | 5.9 | 4.3 | 3.51 | 3.18 | 3.41 | 3.6 |
| 28hr | A | 6.28 | 5.32 | 2.78 | 3.08 | 2.3 | 2.78 |
| | B | 6.39 | 5.81 | 3.28 | 3.2 | 3 | 2.9 |
| 32 hr | A | 6.41 | 6.3 | 2.78 | 2.6 | 2.69 | 2.04 |
| | B | 6.48 | 6.9 | 2 | 2.9 | 2.85 | 2.3 |

C=CONTROL
REP=REPITITION
8K=8000
OR=OREGANO
TH=THYME

Values expressed as $\log_{10}$ CFU/g

# APPENDIX C

## APPENDIX C

**Table C.1: THERMAL INACTIVATION OF *Escherichia coli* 0157:H7 IN GROUND BEEF AT 58, 63 AND 68C VALUES ARE LOG10 CFU/g**

**THERMAL DEATH TIME 58C BEEF**

| | | CONTROL | CONTROL | CONTROL |
|---|---|---|---|---|
| TIME | PLATE | REP 1 | REP 2 | REP 3 |
| raw | A | 6.93 | 6.93 | 6.93 |
| raw | B | 7.08 | 7.08 | 7.08 |
| 0s | A | 7.15 | 6.92 | 6.85 |
| 0s | B | 7.13 | 6.89 | 6.94 |
| 4m | A | 6.18 | 6.62 | 6.77 |
| 4m | B | 6.22 | 6.48 | 6.56 |
| 8m | A | 5.41 | 5.89 | 5.94 |
| 8m | B | 5.32 | 5.78 | 5.98 |
| 10m | A | 5.16 | 5.41 | 5.59 |
| 10m | B | 5.26 | 5.3 | 5.23 |
| 15m | A | 4.28 | 3.9 | 4.81 |
| 15m | B | 4.16 | 3.85 | 4.89 |
| 20m | A | 4.17 | 2.83 | 3.94 |
| 20m | B | 4.22 | 2.98 | 3.96 |
| 30m | A | 3.1 | 2.81 | 3.07 |
| 30m | B | 3.16 | 2.96 | 3.11 |
| 40m | A | 3.56 | 1.95 | 2.59 |
| 40m | B | 3.65 | 1.78 | 2.41 |
| 50m | A | 0 | 0 | 0 |
| 50m | B | 0 | 0 | 0 |
| | | THYME | THYME | THYME |
| TIME | PLATE | REP 1 | REP 2 | REP 3 |
| raw | A | 7.08 | 6.93 | 7.08 |
| raw | B | 6.93 | 6.99 | 6.99 |
| 0s | A | 6.99 | 6.75 | 6.86 |
| 0s | B | 7 | 6.76 | 6.85 |
| 4m | A | 5.13 | 6.04 | 5.6 |
| 4m | B | 5.15 | 5.9 | 5.85 |
| 8m | A | 4.17 | 4.4 | 4.24 |
| 8m | B | 4 | 4.27 | 4.35 |
| 10m | A | 4.19 | 4.32 | 3.95 |
| 10m | B | 4.24 | 4.34 | 3.78 |
| 15m | A | 4.41 | 3.59 | 3.6 |
| 15m | B | 4.24 | 3.52 | 3.41 |
| 20m | A | 3.22 | 2.6 | 3.04 |
| 20m | B | 3.18 | 2.3 | 2.78 |
| 25m | A | 1.48 | 1 | 1.47 |
| 25m | B | 1.3 | 1 | 1.3 |
| 30m | A | 0 | 1 | 0 |
| 30m | B | 0 | 0 | 0 |
| 40m | A | 0 | 0 | 0 |
| 40m | B | 0 | 0 | 0 |

## APPENDIX C

**THERMAL DEATH TIME 63C BEEF**

| TIME | PLATE | CONTROL REP 1 | CONTROL REP 2 | CONTROL REP 3 |
|---|---|---|---|---|
| raw | A | 7.41 | 7.41 | 7.41 |
| raw | B | 7.46 | 7.46 | 7.46 |
| 0s | A | 6.69 | 7.07 | 6.79 |
| 0s | B | 6.51 | 6.99 | 6.69 |
| 0.25m | A | 5.9 | 6.79 | 6.28 |
| 0.25m | B | 5.6 | 6.6 | 6.39 |
| 0.5m | A | 5.79 | 6 | 5.86 |
| 0.5m | B | 5.69 | 5.28 | 5.6 |
| 0.75m | A | 5.05 | 5.2 | 5.26 |
| 0.75m | B | 5.18 | 5.15 | 5.03 |
| 1m | A | 4.65 | 4.56 | 4.86 |
| 1m | B | 4.28 | 4.69 | 4.69 |
| 1.5m | A | 3.11 | 3.27 | 2.08 |
| 1.5m | B | 3.22 | 3.2 | 3.19 |
| 2m | A | 3.14 | 1.95 | 2.39 |
| 2m | B | 2.99 | 2.81 | 2.72 |
| 2.5m | A | 0 | 0 | 0 |
| 2.5m | B | 0 | 0 | 0 |
| 3m | A | 0 | 0 | 0 |
| 3m | B | 0 | 0 | 0 |
| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
| raw | A | 7.13 | 7.16 | 6.75 |
| raw | B | 6.71 | 7.13 | 6.75 |
| 0s | A | 6.28 | 5.17 | 5.3 |
| 0s | B | 6.2 | 5.34 | 5.28 |
| 0.25m | A | 5.46 | 4.34 | 4.41 |
| 0.25m | B | 5.79 | 4.37 | 4.43 |
| 0.5m | A | 5.28 | 4.84 | 4.66 |
| 0.5m | B | 5.45 | 4.74 | 4.86 |
| 0.75m | A | 4.32 | 4.66 | 4.77 |
| 0.75m | B | 4.46 | 4.59 | 4.41 |
| 1m | A | 3.56 | 2.92 | 2.79 |
| 1m | B | 3.48 | 2.9 | 2.88 |
| 1.5m | A | 2.77 | 1 | 1.6 |
| 1.5m | B | 2.58 | 1.3 | 1.78 |
| 1.45m | A | 2.18 | 0 | 0 |
| 1.45m | B | 2 | 0 | 0 |
| 2m | A | 1.3 | 0 | 0 |
| 2m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 68C BEEF

| TIME | PLATE | CONTROL REP 1 | CONTROL REP 2 | CONTROL REP 3 |
|---|---|---|---|---|
| raw | A | 7.06 | 7.02 | 713 |
| raw | B | 7.06 | 7.02 | 7.05 |
| 0s | A | 6.43 | 6.03 | 6.08 |
| 0s | B | 6.41 | 6.08 | 6.12 |
| 10s | A | 5.41 | 5.23 | 5.08 |
| 10s | B | 5.35 | 5.26 | 5.13 |
| 20s | A | 4.1 | 3.48 | 4.11 |
| 20s | B | 4.21 | 3.85 | 4.18 |
| 30s | A | 2.28 | 2.3 | 3 |
| 30s | B | 2.34 | 2.69 | 2.85 |
| 35s | A | 1.48 | 1 | 1 |
| 35s | B | 1 | 1 | 0 |
| 40s | A | 0 | 0 | 0 |
| 40s | B | 0 | 0 | 0 |
| 50s | A | 0 | 0 | 0 |
| 50s | B | 0 | 0 | 0 |

| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
|---|---|---|---|---|
| raw | A | 7.06 | 7.02 | 7.08 |
| raw | B | 7.02 | 7.08 | 7.15 |
| 0s | A | 6.25 | 6.46 | 6.48 |
| 0s | B | 6.17 | 6.58 | 6.34 |
| 5s | A | 5.81 | 6.51 | 5.85 |
| 5s | B | 5.72 | 5.78 | 6.04 |
| 10s | A | 5.34 | 5.86 | 5.95 |
| 10s | B | 5.41 | 5.78 | 5.98 |
| 15s | A | 4.23 | 4.39 | 4.36 |
| 15s | B | 4.26 | 4.62 | 4.48 |
| 25s | A | 3.11 | 2 | 3.08 |
| 25s | B | 3.06 | 2.48 | 2.9 |
| 35s | A | 1 | 0 | 0 |
| 35s | B | 0 | 0 | 0 |
| 40s | A | 0 | 0 | 0 |
| 40s | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 58C BEEF

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 6.99 | 7.25 | 6.99 |
| raw | B | 6.93 | 7.17 | 7.17 |
| 0s | A | 6.76 | 6.88 | 6.91 |
| 0s | B | 6.86 | 6.77 | 6.93 |
| 4m | A | 6.06 | 6.08 | 5.85 |
| 4m | B | 6.26 | 5.95 | 5.78 |
| 8m | A | 4.28 | 4.69 | 4.69 |
| 8m | B | 4.22 | 5.05 | 4.83 |
| 10m | A | 4.29 | 4.08 | 4.18 |
| 10m | B | 4.18 | 4.2 | 4.04 |
| 15m | A | 3.27 | 3.3 | 3.04 |
| 15m | B | 3.1 | 3.2 | 2.6 |
| 20m | A | 3.56 | 2.3 | 2.6 |
| 20m | B | 3.69 | 2.3 | 2.78 |
| 25m | A | 2.46 | 2.51 | 2.62 |
| 25m | B | 2.66 | 2.75 | 2.15 |
| 30m | A | 0 | 1.69 | 1.6 |
| 30m | B | 0 | 1.95 | 1 |
| 40m | A | 0 | 0 | 0 |
| 40m | B | 0 | 0 | 0 |

## APPENDIX C

**THERMAL DEATH TIME 63C BEEF**

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 7.13 | 6.71 | 7.13 |
| raw | B | 7.16 | 6.75 | 6.75 |
| 0s | A | 6.41 | 5.14 | 5.24 |
| 0s | B | 6.56 | 5.16 | 5.2 |
| 0.25m | A | 5.84 | 4.32 | 4.28 |
| 0.25m | B | 6.01 | 4.25 | 4.25 |
| 0.5m | A | 5.83 | 4.01 | 4.09 |
| 0.5m | B | 5.66 | 4.08 | 4.14 |
| 0.75m | A | 4.72 | 3.25 | 3.26 |
| 0.75m | B | 4.6 | 3.18 | 3.19 |
| 1m | A | 3.74 | 2.48 | 2.52 |
| 1m | B | 3.83 | 2.41 | 2.68 |
| 1.5m | A | 2.58 | 0 | 0 |
| 1.5m | B | 2.62 | 0 | 0 |
| 1.75m | A | 1.3 | 0 | 0 |
| 1.75m | B | 1.6 | 0 | 0 |
| 2m | A | 1 | 0 | 0 |
| 2m | B | 0 | 0 | 0 |
| 2.5m | A | 0 | 0 | 0 |
| 2.5m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 68C BEEF

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 7.06 | 7.02 | 7.08 |
| raw | B | 7.06 | 7.02 | 7.15 |
| 0s | A | 6.33 | 6.28 | 6.36 |
| 0s | B | 6.38 | 6.2 | 6.49 |
| 5s | A | 6.06 | 5.85 | 6.56 |
| 5s | B | 6.04 | 6.26 | 6.62 |
| 10s | A | 5.42 | 5.36 | 5.75 |
| 10s | B | 5.29 | 5.63 | 5.86 |
| 15s | A | 5.39 | 5.05 | 5.16 |
| 15s | B | 5.46 | 5.11 | 5.1 |
| 25s | A | 4.56 | 3.57 | 2.85 |
| 25s | B | 4.69 | 3.69 | 2.69 |
| 35s | A | 1 | 0 | 0 |
| 35s | B | 1.3 | 0 | 0 |
| 40s | A | 0 | 0 | 0 |
| 40s | B | 0 | 0 | 0 |

## APPENDIX C

**Table C.2: THERMAL INACTIVATION OF *Salmonella typhimurium* IN GROUND TURKEY AT 53, 58 AND 63C VALUES ARE LOG10 CFU/g**

**THERMAL DEATH TIME 53C TURKEY**

| | | CONTROL | CONTROL | CONTROL |
|---|---|---|---|---|
| TIME | PLATE | REP 1 | REP 2 | REP 3 |
| RAW | A | 7.33 | 7.33 | 7.33 |
| RAW | B | 7.3 | 7.3 | 7.3 |
| 0m | A | 7.28 | 7.84 | 7.96 |
| 0m | B | 7.18 | 7.76 | 7.9 |
| 15m | A | 6.15 | 5.84 | 6.04 |
| 15m | B | 6.11 | 5.88 | 5.96 |
| 30m | A | 5.46 | 5.07 | 5.03 |
| 30m | B | 5.41 | 4.84 | 5.05 |
| 45m | A | 4.04 | 4.59 | 4.76 |
| 45m | B | 4.08 | 4.41 | 4.78 |
| 60m | A | 4.24 | 4.81 | 4.88 |
| 60m | B | 4.27 | 4.77 | 4.95 |
| 90m | A | 4.11 | 4.84 | 4.59 |
| 90m | B | 4.15 | 4.69 | 4.3 |
| 120m | A | 3.5 | 3.79 | 3.89 |
| 120m | B | 3.38 | 3.26 | 3.69 |
| 150m | A | 2.17 | 2 | 2.6 |
| 150m | B | 2.12 | 2.48 | 1.83 |
| 180m | A | 0 | 0 | 0 |
| 180m | B | 0 | 0 | 0 |
| | | THYME | THYME | THYME |
| TIME | PLATE | REP 1 | REP 2 | REP 3 |
| RAW | A | 7 | 7 | 7 |
| RAW | B | 7.28 | 7.28 | 7.28 |
| 0m | A | 7.26 | 7.51 | 7.72 |
| 0m | B | 7.23 | 7.66 | 7.28 |
| 15m | A | 5.5 | 5.29 | 5.23 |
| 15m | B | 5.72 | 5.18 | 5.16 |
| 30m | A | 4.41 | 4.94 | 4.28 |
| 30m | B | 4.58 | 4.88 | 4.41 |
| 45m | A | 3.3 | 4.08 | 3.83 |
| 45m | B | 3.69 | 3.96 | 3.69 |
| 60m | A | 3.43 | 3.11 | 3.03 |
| 60m | B | 3.28 | 3.13 | 3.06 |
| 90m | A | 3.33 | 2.11 | 2.94 |
| 90m | B | 3.28 | 2.39 | 3.03 |
| 120m | A | 2.28 | 2.41 | 2.23 |
| 120m | B | 2.36 | 2.48 | 2.2 |
| 150m | A | 0 | 0 | 0 |
| 150m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 58C TURKEY

| TIME | PLATE | CONT 1 | CONT 2 | CONT 3 |
|---|---|---|---|---|
| raw | A | 6.89 | 6.94 | 6.96 |
| raw | B | 6.78 | 6.81 | 6.9 |
| 0s | A | 6.33 | 6.77 | 6.93 |
| 0s | B | 6.4 | 6.81 | 6.99 |
| 0.5m | A | 6.28 | 6.24 | 6.84 |
| 0.5m | B | 6.23 | 6.28 | 6.86 |
| 1m | A | 6.06 | 6.13 | 6.74 |
| 1m | B | 6.11 | 6.17 | 6.69 |
| 2m | A | 6.22 | 5.18 | 6.08 |
| 2m | B | 6.15 | 5.23 | 6.11 |
| 3m | A | 5.03 | 5.27 | 5.03 |
| 3m | B | 5.07 | 5.25 | 5.08 |
| 4m | A | 4.3 | 4.4 | 4.09 |
| 4m | B | 4.25 | 4.38 | 4.14 |
| 5m | A | 4.83 | 4.08 | 4.08 |
| 5m | B | 4.7 | 4.04 | 4.08 |
| 8m | A | 3.28 | 3.08 | 3.15 |
| 8m | B | 3.39 | 3.17 | 3.45 |
| 9m | A | 1.6 | 1 | 1 |
| 9m | B | 1 | 1.69 | 1.48 |
| 10m | A | 1 | 0 | 0 |
| 10m | B | 0 | 0 | 0 |

| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
|---|---|---|---|---|
| raw | A | 6.94 | 7.91 | 6.94 |
| raw | B | 6.78 | 6.84 | 6.83 |
| 0s | A | 6.26 | 6.76 | 6.65 |
| 0s | B | 6.3 | 6.72 | 6.77 |
| 0.5m | A | 5.08 | 5.91 | 5.96 |
| 0.5m | B | 5.04 | 5.98 | 5.93 |
| 1m | A | 5.39 | 5.18 | 5.11 |
| 1m | B | 5.56 | 5.08 | 5.36 |
| 2m | A | 4.06 | 4.26 | 4.08 |
| 2m | B | 3.99 | 4.04 | 4.06 |
| 3m | A | 2.6 | 2.3 | 2.48 |
| 3m | B | 2.76 | 2 | 2.78 |
| 4m | A | | 2.2 | 2.28 |
| 4m | B | 2.11 | 2.04 | 2.34 |
| 5m | A | 1 | 1.78 | 0 |
| 5m | B | 1.48 | 1.6 | 1.3 |
| 8m | A | 0 | 0 | 0 |
| 8m | B | 0 | 0 | 0 |

## APPENDIX C

**THERMAL DEATH TIME 63C TURKEY**

| TIME | PLATE | CONTROL REP 1 | CONTROL REP 2 | CONTROL REP 3 |
|---|---|---|---|---|
| raw | A | 7.23 | 7.23 | 7.23 |
| raw | B | 7.24 | 7.24 | 7.24 |
| 0s | A | 6.33 | 6.44 | 6.5 |
| 0s | B | 6.38 | 6.46 | 6.47 |
| 0.25m | A | 5.45 | 5.39 | 5.27 |
| 0.25m | B | 5.49 | 5.34 | 5.31 |
| 0.5m | A | 5.98 | 5.69 | 5.79 |
| 0.5m | B | 5.81 | 5.59 | 5.76 |
| 0.75m | A | 4.08 | 4.2 | 4.08 |
| 0.75m | B | 4.11 | 4.2 | 4.23 |
| 1m | A | 2.79 | 3.07 | 2.81 |
| 1m | B | 3.01 | 2.98 | 2.91 |
| 1.5m | A | 1 | 1.95 | 1 |
| 1.5m | B | 1.48 | 1.78 | 1.6 |
| 2m | A | 1 | 0 | 1.3 |
| 2m | B | 0 | 0 | 1 |
| 2.5m | A | 0 | 0 | 0 |
| 2.5m | B | 0 | 0 | 0 |
| 3m | A | 0 | 0 | 0 |
| 3m | B | 0 | 0 | 0 |

| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
|---|---|---|---|---|
| raw | A | 6.77 | 6.99 | 6.88 |
| raw | B | 6.6 | 6.94 | 6.76 |
| 0s | A | 5.15 | 5.15 | 5.08 |
| 0s | B | 5.26 | 5.18 | 5.23 |
| 5s | A | 4.81 | 4.72 | 4.46 |
| 5s | B | 4.75 | 4.46 | 4.56 |
| 10s | A | 3.62 | 3.28 | 3.69 |
| 10s | B | 3.78 | 3.4 | 3.61 |
| 15s | A | 3.48 | 3.06 | 3.84 |
| 15s | B | 3.3 | 2.99 | 3.72 |
| 20s | A | 2.69 | 2.59 | 2.46 |
| 20s | B | 2.75 | 2.62 | 2.56 |
| 25s | A | 2 | 2.28 | 2.2 |
| 25s | B | 2.17 | 1.3 | 2.04 |
| 30s | A | 1.48 | 1.69 | 1.78 |
| 30s | B | 1.69 | 0 | 1.9 |
| 35s | A | 0 | 0 | 0 |
| 35s | B | 0 | 0 | 0 |
| 40s | A | 0 | 0 | 0 |
| 40s | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 53C TURKEY

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| RAW | A | 7.34 | 7.34 | 7.34 |
| RAW | B | 7.3 | 7.3 | 7.3 |
| 0m | A | 6.87 | 6.83 | 6.72 |
| 0m | B | 6.88 | 6.89 | 6.65 |
| 15m | A | 4.3 | 5.38 | 5.04 |
| 15m | B | 4.48 | 5.36 | 5.11 |
| 30m | A | 3.69 | 4.11 | 3.48 |
| 30m | B | 3.69 | 4.28 | 3.69 |
| 45m | A | 3 | 2.69 | 2.85 |
| 45m | B | 2.78 | 3.15 | 3 |
| 60m | A | 3.06 | 2.84 | 3.47 |
| 60m | B | 2.66 | 3.48 | 3.03 |
| 75m | A | 2.2 | 2.08 | 1.78 |
| 75m | B | 2 | 1.78 | 1.6 |
| 90m | A | 1.3 | 1.78 | 1.3 |
| 90m | B | 1.6 | 0 | 0 |
| 105m | A | 1.69 | 1 | 1.3 |
| 105m | B | 1.6 | 1 | 1 |
| 120m | A | 0 | 0 | 0 |
| 120m | B | 0 | 0 | 0 |
| 150m | A | 0 | 0 | 0 |
| 150m | B | 0 | 0 | 0 |
| 180m | A | 0 | 0 | 0 |
| 180m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 58C TURKEY

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 6.81 | 6.69 | 6.98 |
| raw | B | 6.77 | 6.48 | 6.9 |
| 0s | A | 6.89 | 6.76 | 6.81 |
| 0s | B | 6.81 | 6.69 | 6.78 |
| 0.5m | A | 6.27 | 6.11 | 6.08 |
| 0.5m | B | 6.21 | 6.11 | 6.04 |
| 1m | A | 6.09 | 6.08 | 6.16 |
| 1m | B | 6.08 | 6.1 | 6.11 |
| 1.5m | A | 5.51 | 6.07 | 5.69 |
| 1.5m | B | 5.6 | 5.91 | 5.76 |
| 2m | A | 5.46 | 4.95 | 4.9 |
| 2m | B | 4.93 | 4.98 | 4.88 |
| 2.5m | A | 4.6 | 4.78 | 4.59 |
| 2.5m | B | 4.72 | 4.75 | 4.79 |
| 3m | A | 4.79 | 4.08 | 4.59 |
| 3m | B | 4.69 | 3.95 | 4.65 |
| 4m | A | 3.41 | 3.23 | 3.32 |
| 4m | B | 3.34 | 3.25 | 3.37 |
| 5m | A | 2.6 | 2.98 | 2.56 |
| 5m | B | 2.51 | 2.9 | 2.48 |
| 6m | A | 1 | 1.78 | 0 |
| 6m | B | 0 | 0 | 0 |
| 7m | A | 0 | 0 | 0 |
| 7m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 63C TURKEY

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 6.77 | 6.99 | 6.88 |
| raw | B | 6.6 | 6.94 | 6.76 |
| 0s | A | 4.01 | 4.29 | 4.12 |
| 0s | B | 4.05 | 4.32 | 4.13 |
| 5s | A | 3.08 | 3.12 | 3.08 |
| 5s | B | 3.1 | 3.15 | 3.13 |
| 10s | A | 3.8 | 3.5 | 3.75 |
| 10s | B | 3.86 | 3.6 | 3.79 |
| 15s | A | 2.4 | 2.64 | 2.28 |
| 15s | B | 2.56 | 2.83 | 2.45 |
| 20s | A | 2.81 | 2.46 | 2.28 |
| 20s | B | 2.78 | 2.56 | 2.46 |
| 25s | A | 1 | 1.95 | 1.6 |
| 25s | B | 1.69 | 1.78 | 1.69 |
| 30s | A | 0 | 0 | 0 |
| 30s | B | 0 | 0 | 0 |
| 35s | A | 0 | 0 | 0 |
| 35s | B | 0 | 0 | 0 |
| 40s | A | 0 | 0 | 0 |
| 40s | B | 0 | 0 | 0 |

## APPENDIX C

**Table C.3: THERMAL INACTIVATION OF *Yersinia enterocolitica* IN GROUND PORK AT 52, 57 AND 62C VALUES ARE LOG10 CFU/g**

THERMAL DEATH TIME 52C PORK

| TIME | PLATE | CONT 1 | CONT 2 | CONT 3 |
|---|---|---|---|---|
| raw | A | 6.54 | 6.32 | 6.18 |
| raw | B | 6.32 | 6.54 | 6.08 |
| 0s | A | 6.51 | 6.39 | 6.59 |
| 0s | B | 6.45 | 6.23 | 6.65 |
| 5m | A | 6.46 | 6.62 | 5.85 |
| 5m | B | 6.39 | 5.78 | 5.3 |
| 10m | A | 5.71 | 4.69 | 5.88 |
| 10m | B | 5.81 | 5.06 | 5.76 |
| 20m | A | 3.48 | 3.28 | 4.08 |
| 20m | B | 3.3 | 3.41 | 3.94 |
| 30m | A | 3.6 | 3.38 | 3.96 |
| 30m | B | 3.3 | 3.3 | 3.93 |
| 40m | A | 3.08 | 2.08 | 2.81 |
| 40m | B | 3.13 | 2.59 | 2.3 |
| 50m | A | 1 | 1 | 0 |
| 50m | B | 2.2 | 0 | 0 |
| 60m | A | 0 | 0 | 0 |
| 60m | B | 0 | 0 | 0 |
| TIME | PLATE | THYME 1 | THYME 2 | THYME 3 |
| raw | A | 5.68 | 5.68 | 5.68 |
| raw | B | 5.97 | 5.97 | 5.97 |
| 0s | A | 5.77 | 5.89 | 5.32 |
| 0s | B | 5.39 | 5.67 | 5.78 |
| 5m | A | 4.79 | 5.1 | 4.59 |
| 5m | B | 5.04 | 5.19 | 4.94 |
| 10m | A | 4.91 | 4.83 | 4.59 |
| 10m | B | 4.75 | 4.04 | 4.3 |
| 15m | A | 4.56 | 4.28 | 4.75 |
| 15m | B | 4.62 | 4.38 | 4.48 |
| 20m | A | 4.05 | 4.1 | 4 |
| 20m | B | 3.99 | 4.16 | 3.96 |
| 25m | A | 2.69 | 3.48 | 2.85 |
| 25m | B | 2.85 | 2.6 | 2.95 |
| 30m | A | 0 | 0 | 0 |
| 30m | B | 0 | 0 | 0 |
| 35m | A | 0 | 0 | 0 |
| 35m | B | 0 | 0 | 0 |
| 40m | A | 0 | 0 | 0 |
| 40m | B | 0 | 0 | 0 |

## APPENDIX C

**THERMAL DEATH TIME 57C PORK**

| TIME | PLATE | CONTROL REP 1 | CONTROL REP 2 | CONTROL REP 3 |
|---|---|---|---|---|
| raw | A | 5 | 5 | 5 |
| raw | B | 6 | 6 | 6 |
| 0s | A | 6.46 | 5.85 | 5.85 |
| 0s | B | 6.49 | 5.85 | 5.3 |
| 30s | A | 5.89 | 5.95 | 5.72 |
| 30s | B | 5.94 | 5.98 | 5.56 |
| 1m | A | 5 | 5.29 | 5.32 |
| 1m | B | 5 | 5.19 | 5.22 |
| 1.5m | A | 5.81 | 4.22 | 5.03 |
| 1.5m | B | 4.09 | 4.32 | 5.14 |
| 2m | A | 4.62 | 3.79 | 4.94 |
| 2m | B | 4.47 | 4.14 | 4.85 |
| 4m | A | 3 | 3.84 | 4.08 |
| 4m | B | 3.08 | 3.9 | 4.17 |
| 6m | A | 3.25 | 3.22 | 3.31 |
| 6m | B | 3.69 | 3.04 | 3.27 |
| 8m | A | 2.42 | 1 | 1.45 |
| 8m | B | 2.39 | 0 | 1.2 |
| 10m | A | 0 | 0 | 0 |
| 10m | B | 0 | 0 | 0 |

| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
|---|---|---|---|---|
| raw | A | 5.59 | 5.59 | 5.59 |
| raw | B | 5.89 | 5.89 | 5.89 |
| 0s | A | 5.46 | 5.62 | 5.88 |
| 0s | B | 5.49 | 5.26 | 5.59 |
| 30s | A | 5.21 | 5.33 | 5.15 |
| 30s | B | 5.17 | 5.27 | 5.27 |
| 1m | A | 5.66 | 5.51 | 4.85 |
| 1m | B | 4.6 | 5.3 | 4.48 |
| 1.5m | A | 4.41 | 4.95 | 4.77 |
| 1.5m | B | 4.72 | 4.66 | 4.43 |
| 2m | A | 3.79 | 4.24 | 4.08 |
| 2m | B | 3.69 | 4.19 | 3.88 |
| 3m | A | 3.69 | 3.79 | 3.46 |
| 3m | B | 3.9 | 2.9 | 3.56 |
| 3.5m | A | 2.78 | 2.69 | 3.28 |
| 3.5m | B | 3.18 | 2.85 | 3 |
| 4m | A | 0 | 1.9 | 1.6 |
| 4m | B | 1.78 | 0 | 1.3 |
| 6m | A | 0 | 0 | 0 |
| 6m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 62C PORK

| TIME | PLATE | CONTROL REP 1 | CONTROL REP 2 | CONTROL REP 3 |
|---|---|---|---|---|
| 0s | A | 5.6 | 5.53 | 5.62 |
| 0s | B | 5.84 | 5.26 | 5 |
| 20s | A | 5.28 | 5.32 | 5.7 |
| 20s | B | 5.06 | 5.38 | 5.42 |
| 40s | A | 5.11 | 5.69 | 5.48 |
| 40s | B | 5.45 | 5.84 | 5.78 |
| 50s | A | 4.78 | 5.07 | 5.21 |
| 50s | B | 4.59 | 5.2 | 4.99 |
| 1m | A | 3.56 | 3.84 | 3.59 |
| 1m | B | 4.04 | 3.75 | 3.75 |
| 1.33m | A | 2.79 | 3.04 | 2.98 |
| 1.33m | B | 3.08 | 3.07 | 2.93 |
| 1.67m | A | 0 | 1 | 1.6 |
| 1.67m | B | 0 | 1.48 | 1.9 |
| 2m | A | 0 | 0 | 0 |
| 2m | B | 0 | 0 | 0 |
| TIME | PLATE | THYME REP 1 | THYME REP 2 | THYME REP 3 |
| 0s | A | 5.46 | 5.69 | 5.77 |
| 0s | B | 5.66 | 5.62 | 5.91 |
| 10s | A | 5.72 | 5.41 | 5.98 |
| 10s | B | 5.26 | 5.76 | 5.85 |
| 20s | A | 5.6 | 5.1 | 5.66 |
| 20s | B | 5.9 | 4.93 | 5.58 |
| 30s | A | 4.2 | 4.62 | 4.94 |
| 30s | B | 4.28 | 4.49 | 4.88 |
| 40s | A | 4.3 | 4.17 | 3.69 |
| 40s | B | 4.68 | 3.93 | 3.49 |
| 60s | A | 3.79 | 3.76 | 2.27 |
| 60s | B | 3.68 | 2.99 | 3.91 |
| 1.5m | A | 1.9 | 2.48 | 1.6 |
| 1.5m | B | 2.46 | 2.3 | 2.46 |
| 2.5m | A | 0 | 0 | 0 |
| 2.5m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 52C PORK

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 5.83 | 5.83 | 5.83 |
| raw | B | 5.66 | 5.66 | 5.66 |
| 0s | A | 5.66 | 5.91 | 5.56 |
| 0s | B | 5.45 | 5.88 | 5.68 |
| 5m | A | 4.6 | 4 | 5.28 |
| 5m | B | 4.69 | 4.08 | 4.77 |
| 10m | A | 4.19 | 4.08 | 4.38 |
| 10m | B | 4.24 | 4.18 | 4.29 |
| 15m | A | 3.11 | 3.69 | 3.83 |
| 15m | B | 3 | 3.3 | 3.39 |
| 20m | A | 2.78 | 3 | 2.95 |
| 20m | B | 2.6 | 2.9 | 2.3 |
| 25m | A | 2.81 | 2.28 | 2.68 |
| 25m | B | 2.62 | 1.9 | 1.68 |
| 30m | A | 1.69 | 1.85 | 1.9 |
| 30m | B | 1.69 | 0 | 1.69 |
| 35m | A | 0 | 0 | 0 |
| 35m | B | 0 | 0 | 0 |
| 40m | A | 0 | 0 | 0 |
| 40m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 57C PORK

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 5.99 | 5.99 | 5.99 |
| raw | B | 5.9 | 5.9 | 5.9 |
| 0s | A | 5.79 | 5.96 | 5.48 |
| 0s | B | 5.66 | 5.54 | 5.59 |
| 30s | A | 5.2 | 5.09 | 5.42 |
| 30s | B | 5.25 | 5.19 | 5.31 |
| 1m | A | 4.81 | 5.19 | 5.16 |
| 1m | B | 5.04 | 5.09 | 5.1 |
| 1.5m | A | 4.62 | 4.93 | 4.96 |
| 1.5m | B | 4.45 | 4.72 | 4.66 |
| 2m | A | 4.1 | 4 | 4.11 |
| 2m | B | 4.15 | 3.98 | 4.16 |
| 3m | A | 3.46 | 2.69 | 2.78 |
| 3m | B | 3.62 | 2.78 | 2.9 |
| 4m | A | 0 | 0 | 0 |
| 4m | B | 0 | 0 | 0 |
| 5m | A | 0 | 0 | 0 |
| 5m | B | 0 | 0 | 0 |
| 6m | A | 0 | 0 | 0 |
| 6m | B | 0 | 0 | 0 |

## APPENDIX C

### THERMAL DEATH TIME 62C PORK

| TIME | PLATE | OREGAN REP 1 | OREGAN REP 2 | OREGAN REP 3 |
|---|---|---|---|---|
| raw | A | 5.59 | 5.59 | 5.59 |
| raw | B | 5.65 | 5.65 | 5.65 |
| 0s | A | 5.69 | 5.71 | 5.26 |
| 0s | B | 5.79 | 5.41 | 5.48 |
| 10s | A | 5.77 | 5.79 | 5.65 |
| 10s | B | 5.66 | 5.72 | 5.95 |
| 20s | A | 5.28 | 5.66 | 5.79 |
| 20s | B | 5.62 | 5.28 | 5.6 |
| 30s | A | 4.98 | 4.94 | 4.28 |
| 30s | B | 5.09 | 4.81 | 4.39 |
| 40s | A | 4.79 | 4.05 | 4.62 |
| 40s | B | 4.71 | 3.91 | 4.79 |
| 1m | A | 2.15 | 2.66 | 3.09 |
| 1m | B | 1.94 | 2.48 | 2.99 |
| 1.5m | A | 0 | 0 | 0 |
| 1.5m | B | 0 | 0 | 0 |
| 2m | A | 0 | 0 | 0 |
| 2m | B | 0 | 0 | 0 |

# APPENDIX D

## APPENDIX D

**Table D.1: SURVIVAL OF *Escherichia coli* 0157:H7 IN PEPPERONI**

| SAMPLING TIME | LOG CFU/g PLATE 1 | LOG CFU/g PLATE 2 |
|---|---|---|
| PRE-FERMENTATION | | |
| CONTROL REP 1 | 7.15 | 7.25 |
| CONTROL REP 2 | 7.24 | 7.2 |
| ETHANOL CONTROL REP 1 | 7.22 | 7.34 |
| ETHANOL CONTROL REP 2 | 7.28 | 7.28 |
| OREGANO OIL REP 1 | 6.81 | 6.96 |
| OREGANO OIL REP 2 | 6.89 | 6.88 |
| POST-FERMENTATION | | |
| CONTROL REP 1 | 7.04 | 7.08 |
| CONTROL REP 2 | 7.08 | 7.08 |
| ETHANOL CONTROL REP 1 | 6.3 | 7.08 |
| ETHANOL CONTROL REP 2 | 7.11 | 7.26 |
| OREGANO OIL REP 1 | 5.81 | 5.69 |
| OREGANO OIL REP 2 | 4.78 | 5.25 |
| POST-HEAT PROCESSING | | |
| CONTROL REP 1 | 4.3 | 4.78 |
| CONTROL REP 2 | 4 | 4 |
| ETHANOL CONTROL REP 1 | 4.81 | 4.69 |
| ETHANOL CONTROL REP 2 | 4.49 | 4.69 |
| OREGANO OIL REP 1 | 0 | 0 |
| OREGANO OIL REP 2 | 0 | 0 |
| POST-DRYING | | |
| CONTROL REP 1 | 4.51 | 4.09 |
| CONTROL REP 2 | 4.26 | 4.91 |
| ETHANOL CONTROL REP 1 | 4.69 | 4.5 |
| ETHANOL CONTROL REP 2 | 4.21 | 4.82 |
| OREGANO OIL REP 1 | 0 | 0 |
| OREGANO OIL REP 2 | 0 | 0 |

## APPENDIX D

**Table D.2: *Pediococcus* spp. COUNTS DURING MANUFACTURE OF PEPPERONI**

| PRE-FERMENTATION | LOG CFU/g (ROGOSA AGAR) | |
|---|---|---|
| | PLATE 1 | PLATE 2 |
| CONTROL REP 1 | 5.25 | 5.67 |
| CONTROL REP 2 | 5.45 | 5.55 |
| ETHANOL CONTROL REP 1 | 6.12 | 6.09 |
| ETHANOL CONTROL REP 2 | 5.23 | 5.48 |
| OREGANO OIL REP 1 | 6.07 | 5.09 |
| OREGANO OIL REP 2 | 5.42 | 6.26 |
| | | |
| POST-FERMENTATION | | |
| CONTROL REP 1 | 7.45 | 6.59 |
| CONTROL REP 2 | 7.05 | 6.89 |
| ETHANOL CONTROL REP 1 | 7.69 | 7.95 |
| ETHANOL CONTROL REP 2 | 6.58 | 7.12 |
| OREGANO OIL REP 1 | 6.87 | 6.78 |
| OREGANO OIL REP 2 | 6.68 | 7.58 |

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