1-1-2014

Antimicrobial Activity Of Essential Oil Emulsions And Possible Synergistic Effect On Food Borne Pathogens

Varun Tahlan
Wayne State University,

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL EMULSIONS AND POSSIBLE SYNERGISTIC EFFECT ON FOOD BORNE PATHOGENS

by

VARUN TAHLAN

THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2014

MAJOR: NUTRITION AND FOOD SCIENCE

Approved by:

_____

Advisor Date

DEDICATION

This thesis is dedicated to my parents for their support and guidance.

ACKNOWLEDGEMENTS

First and foremost, I owe my deepest gratitude to my supervisor, Dr. Yifan Zhang, whose endless guidance and support from the very beginning has inspired me. Thanks to all the faculty and staff of the Department of Nutrition and Food Science for the opportunities and experiences. I would like to thank Dr. Heydari and Dr. Zhou for taking the time to be on my committee and give me their valuable input. Thanks to Dr. Kanika Bhargava for teaching me and for her support and all my fellow lab members for their support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ATP	Adenosine triphosphate
CAMHB	Cation-adjusted Mueller-Hinton broth
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide
EO	Essential oil
FIC	Fractional inhibitory concentration
GRAS	Generally recognized as safe
GTP	Guanosine triphosphate
LPS	Lipopolysaccharides
MIC	Minimum inhibitory concentration
NCCLS	National Committee of Clinical Laboratories Standards
PPM	Parts per million
TSA	Tryptic soy agar

CHAPTER 1

1. Introduction

Industrialization has led to major improvements in food quality and safety, yet food borne infections remain an important public health concern [1]. It was estimated in 2011 that 9.4 million people suffered from food borne illnesses in the United States [2]. Hence, developing new methods for eliminating food borne pathogens and improving existing techniques is essential. This is furthermore stressed by the shift in consumer trends towards organic, non-processed foods. These changes have occurred due to growing concerns over the use of a variety of synthetic additives to food products such as sorbate, benzoate, etc., which are not considered 'natural' [3]. Adding complexity to the problem is the emergence of antibiotic-resistant bacteria species in the food system [4]. These evolved bacteria demonstrate the necessity of improvement in food control techniques. Utilizing naturally occurring substances to control food borne bacteria is a logical approach that may provide consumers with many benefits. One such option is the use of essential oils as antibacterial additives in food.

In addition to enhancing flavor, herbs and spices have long been known for their antimicrobial use [5]. It is believed that the Romans used mustard to prevent the spoilage of fruit juice by fermenting bacteria [6]. Furthermore, reviews from the past demonstrate oil extracts from plant materials (flowers, herbs, spices, bark, seed, leaves, roots and fruit) known as 'Essential Oils" (EOs), volatile or ethereal oils to have antimicrobial properties [6, 7]. The term "essential oil" is believed to have been derived from *Quinta essential*, which was defined as the effective component of a drug by Paracelsus von Hohenheim in the 16th century [7]. They may be obtained via fermentation, enfleurage, or extraction, but the most common commercial

method employed is steam distillation [8, 9]. EOs and their constituents have been known to have other activities besides antibacterial properties, such as antimitotic[10], antiparasitic [11, 12] , insecticidal [13-15], and antiviral [16, 17] properties.

2. Historical and current use of essential oils

Herbs and spices have been known to have been used for their preservative, perfume and flavor properties since ancient times [5]. However, it was the Greek and Roman historians who first documented the use of EOs for medical treatment and aromatherapy [18]. By the 13th century, pharmacological effects of EOs were described in many pharmacopeias of the time, yet their use was not wide spread until the 16th century [5]. It is believed 1881 De La Croix was the first person to carry out antimicrobial analysis of EO vapors [19].

The most common use of EOs today is as flavoring agents in food, essences in perfumes and in pharmaceutical products for their functional properties [5, 20]. A variety of commercially available products exploit the antibacterial properties of EOs, like antiseptics and animal feed supplements [21, 22]. However, the potential of EOs in food safety has yet to be elucidated.

3. Composition of essential oils

Plants produce a variety of antimicrobial compounds, most of which are always present in the system while others are produced in response to injury or invasion [23]. However, the composition of the EOs produced differ depending on the season of harvest and geographical origin [24-26]. In addition, the composition of EOs extracted from different parts of the same plant may vary [27]. The most controllable factor by which EOs vary is the method of extraction. A difference in organoleptic profile indicates a difference in composition of oils due to solvent extraction as opposed to distillation of oils. It has been found that to maintain higher organoleptic

properties of EOs, extraction under low pressure with liquid carbon dioxide as a solvent is effective [28]. Herb EOs extracted using hexane have shown greater antimicrobial activity than similar steam distilled EOs [29]. However, this method is very expensive, so steam distillation is the most commonly used method for producing EOs on a commercial scale [18].

EOs can be made up of more than sixty individual components with the major components consisting up to about 85 % of the EOs total while minor components are present in trace amounts [30]. These molecules are low molecular weight organic compounds with diverse antimicrobial activities [31]. The active components can be classified according to their chemical structures: terpenes, phenylpropenes, terpenoids and “others” [31]. The major components of common EOs are presented in Table 1 and the structural formula of some of the components are presented in Figure 1.

The organic chemistry of each EO compound has a profound effect on its character. The structure of these individual components, such as different chemical groups, side chains and ring structures, affect their antimicrobial activity. EOs that have a higher composition of phenolic compounds such as carvacrol, thymol and euganol tend to show higher antimicrobial activity [32, 33]. It can be reasoned that their mechanism of antimicrobial action is similar to other phenolic compounds that contain a hydroxyl group.[34].In the case of non-phenolic compounds, the type of alkyl group present influences antimicrobial activity [35], though the position of the group does not seem to affect the level of antimicrobial activity [36].

3.1 Terpenes

Terpenes are hydrocarbons produced by a combination of several isoprene units and are synthesized in the cytoplasm of plant cells. Synthesis starts with an acetyl–CoA and proceeds

through the mevalonic acid pathway [31]. These compounds can be arranged into cyclic structures via the action of cyclases.. Monoterpenes (chemical formula: $C_{10}H_{16}$) and sesquiterpene (chemical formula: $C_{15}H_{24}$) are the main terpenes, but diterpenes and triterpenes also exist. Limonene, *p*- cymene, pinene and terpinene are some examples of common terpenes.

3.2 Terpinoids

Terpenes can undergo enzymatic biochemical modifications that add oxygen molecules and move or remove methyl groups, thereby forming terpenoids [37]. They can be divided into aldehydes, ketones, alcohols, phenols, epoides and esters. Common terpenoids are carvacrol, linalool, menthol, and thymol.

3.3 Phenylpropenes

Phenylpropenes are a subfamily of compounds under phenylpropanoinds that are synthesized in plants using phenylalanine. Few phenylpropenes have been studied in detail, but euganol, isoeuganol, cinnamaldehyde are some of EO phenylpropenes that have been elucidated [31].

Table 1. Composition of common essential oils

Common name of EO	Latin name of plant it is derived from.	Major Costituents of EO	Approximate %	Reference
Bay	*Laurus nobilis*	1,8-cineole α-terpinene Sarbinene	60% 13% 13%	[38]
Bergamot	*Citrus bergamia*	Limonene Linalool Linalyl acetate	59 % 9.5% 17%	[39]
Cinnamon	*Cinnamomum zeylanicum*	Trans – Cinnamaldehyde Euganol Linalool	65% 3% 4%	
Clove	*Syzygium aromaticum*	Euganol Eugenyl acetate	75 – 85% 8 – 15 %	
Lemongrass	*Cymbopogon flexuosus*	Geranial Myrcene 6-methylhept-5-en-2-one	46% 4% 3%	
Nutmeg	*Myristica fragrans*	Sabinene Euganol	50% 2%	[40]
Oregano	*Origanum vulgare*	Carvacrol α–pinene *p*–cymene Myrcene	Trace – 80% 3% 16% 2%	
Rosemary	*Rosmarinus officinalis*	α–pinene Camphor 1,8-cineole Bornyl acetate	2 – 25% 2 – 14% 3 – 89% 0 – 17%	
Sage	*Salvia officinalis*	α–pinene β–pinene α–tujone 1,8-cineole	4 – 5% 2 – 10% 20 – 42% 6 – 14%	
Thyme	*Thymus vulgaris*	Thymol Carvacrol *p*-cymene γ-terpinene	10 – 64% 2 – 11 % 10 – 56% 2 – 31%	

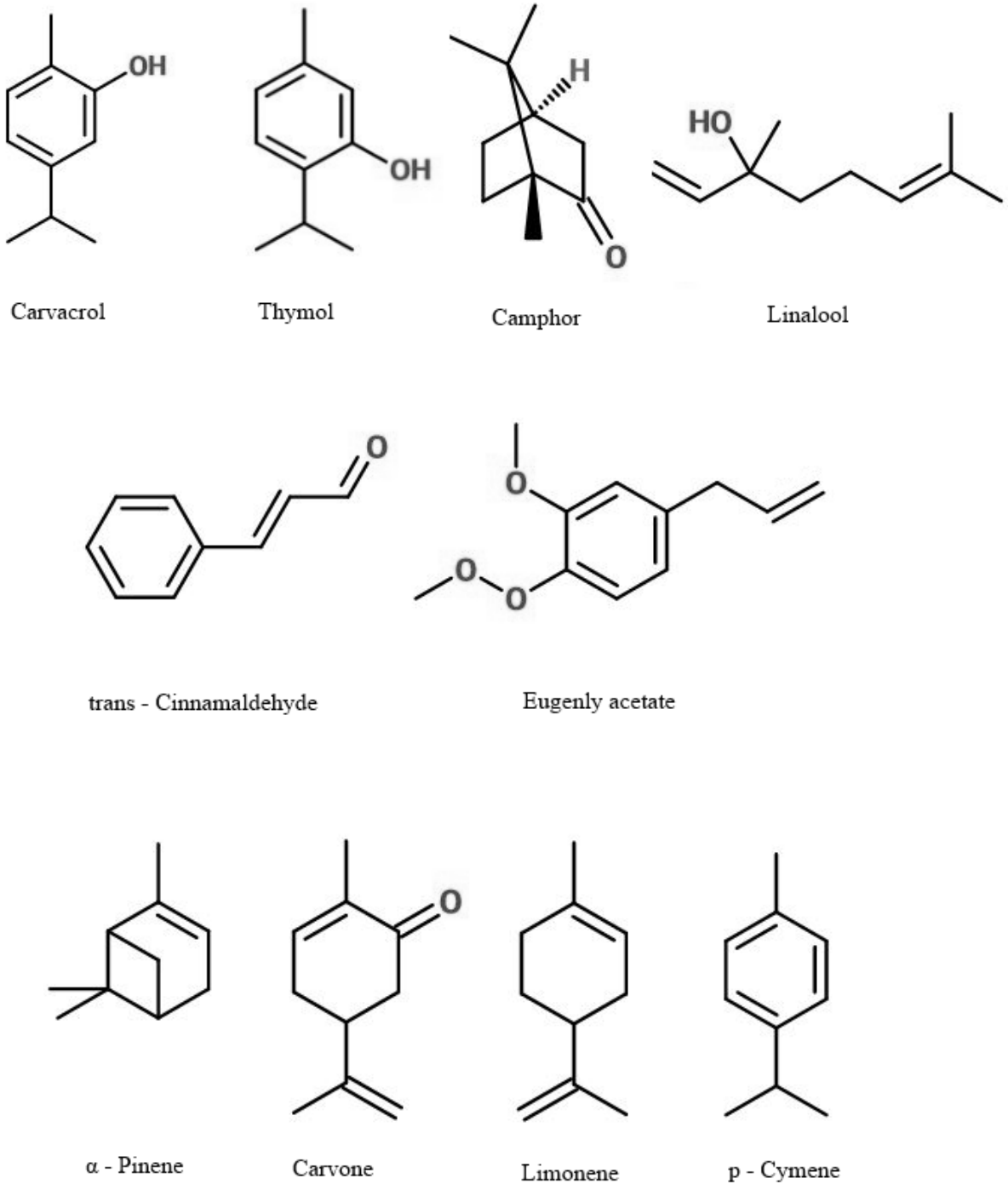

Figure 1. Chemical structures of selected essential oil components

4. Antimicrobial activity and mode of action

Although the food industry primarily uses EOs as flavorings, they also function as an interesting source of natural antimicrobials [31]. Utilization of these properties requires an understanding of their antimicrobial mode of action. The antimicrobial activity of EOs cannot be attributed to a single mechanism; it is likely that several sites in a cell act as targets [41]. It is difficult to predict the susceptibility of an organism to a certain EO, as it varies from strain to strain. However it is known that Gram-negative bacteria are generally less susceptible in comparison to Gram-positive species [42]. This occurs due to the presence of lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria, which acts as a barrier towards macromolecules and hydrophobic compounds. This provides Gram-negative bacteria with a higher tolerance towards the mostly hydrophobic antimicrobial components of EOs [43]. In Gram-positive bacteria, and to some extent in Gram-negative bacteria, this hydrophobic nature helps EOs to disturb the lipids of the bacterial cell membranes, thereby making them permeable [34, 44], and allowing the leakage of cellular material and ions [45, 46]. This does not necessarily mean cell death as some leakage from the cell is tolerated, but extensive loss or loss of essential components can lead to death [47].

4.1 Terpenes

Terpenes do not possess high antimicrobial activity, as evidenced by large scale experimentation with limonene, α-pinene, β-pinene and α-terpinene that show low or absent antimicrobial activity [35]. *p*-cymene, one of the major constituents of thyme, shows no antimicrobial activity at high concentrations [48] , but has the potential to promote the activity of compounds like carvacrol [36]. *p*-cymene has a high affinity for membranes and causes membrane expansion, but does not influence membrane permeability. It does cause a decrease in

the melting point and enthalpy of the membranes [49]. It has an insignificant effect on protein synthesis of the cell but its effect on membrane potential can affect cell motility in *E.coli* [50].

4.2 Terpenoids

The antimicrobial properties of terpenoinds are linked to the functional groups present. In phenolic terpenoids, it has been found that the presence of delocalized electrons and a hydroxyl group are essential for antimicrobial affect [31]. Carvacrol and thymol are able to disintegrate the outer membrane of Gram-negative cells, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. It is believed that carvacrol forms channels through the membrane by pushing apart the fatty acid chains in the phospholipids increasing membrane permeability [51].

4.3 Phenylpropenes

The antimicrobial activity of phenylpropenes depends on the number and type of substituents present on the aromatic ring [52]. The antimicrobial activity of phenylpropenes such as euganol occurs via non-specific membrane permeabilization. It has been demonstrated in various studies via the increased transport of ATP and potassium out of the cell [53, 54]. Euganol has also been shown to inhibit ATPase, histidine decarboxylase, protease and amylase activity [53, 55]. By inhibiting ATPase activity euganol essentially restricts energy production required for cellular repair. The hydroxyl group present in euganol is believed to affect the properties of proteins by binding to them. This supports euganols activity at sub lethal concentrations.

On the other hand the antimicrobial mode of action of cinnamaldehyde, a phenylpropene aldehyde, is not clear. There are three things that are believed to occur: at low concentrations it inhibits enzymes involved in cytokinesis; at sub lethal concentrations it acts as an ATPase

inhibitor; at lethal concentrations it agitates the cell membrane [56]. In another study, it was shown that cinnamaldehyde inhibits GTP dependent polymerization by binding to a protein required for cell division, FtsZ [57].

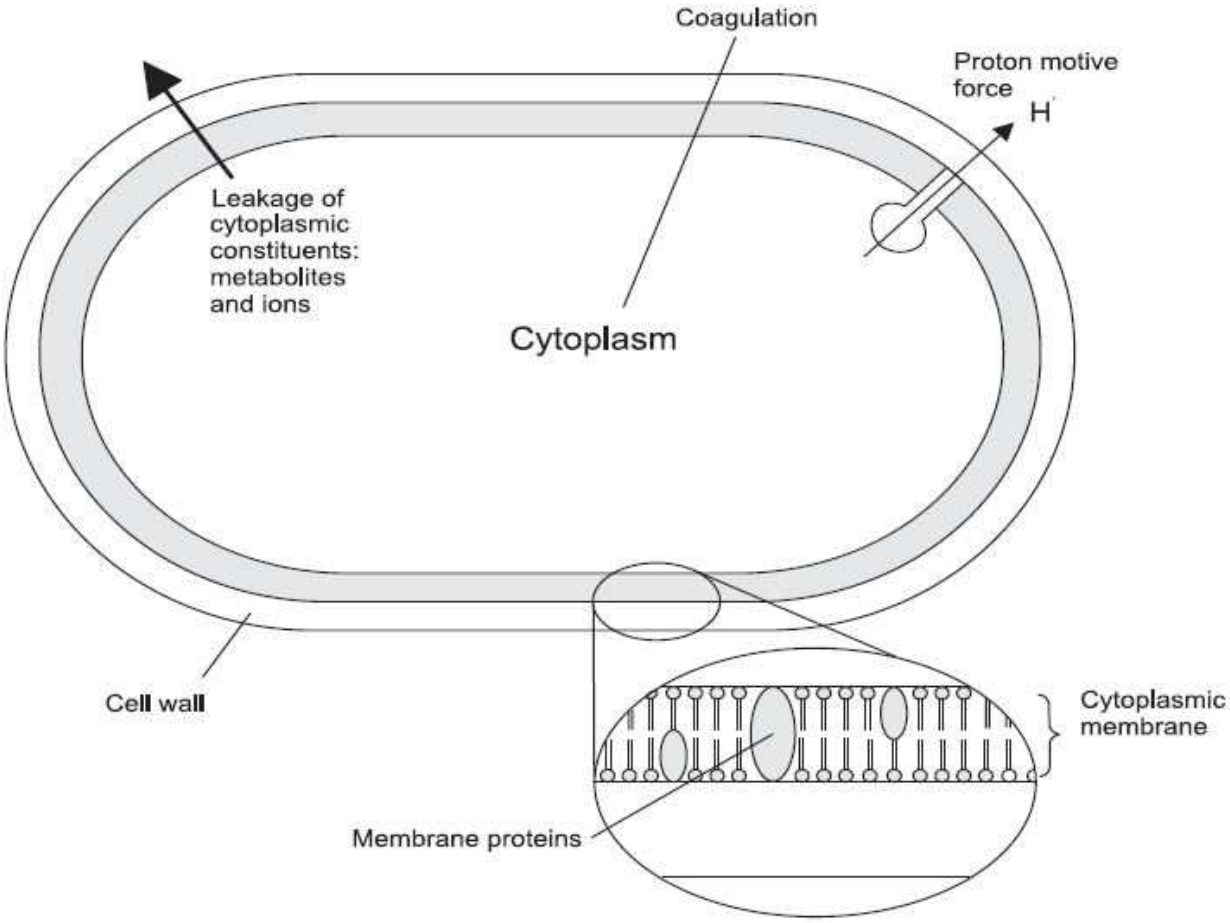

Figure 2. Possible mechanisms and sites of action for EO components in bacterial cell wall. Adapted from Burt et al [18].

Table 2. Overview of EOs and their mechanisms of action

Common name of EO	Model organism	Mechanism of action	Reference
Cinnamon	*E.coli* *L.monocytogens* *S.aureus* *S.enteritidis* *C.jejuni*	Inhibition of histidine decarboxylase; leakage and coagulation of cytoplasmic content; depolarization and membrane permeabilization	[49]
Clove	*C.jejuni* *E.coli* *L.monocytogens* *S.aureus* *S.enteritidis*	Inhibition of histidine decarboxylase	[31]
Lemon grass	*L. innocua* *L. monocytogens* *S. aureus*	Permeabilization of membrane	[44]
Oregano	*P. aeruginosa* *S. aureus*	Disspation of potassium gradient, depolarization of membrane, coagulation of cytoplasmic content.	[57, 58]
Rosemary	*E.coli* *B. subtilis* *S. aureus*	Increase in membrane rigidity, affect on lipid polymorphism	
Thyme	*E. coli* *L. innocua* *L. monocytogens* *S. aureus* *S. enteritidis*	Permeabilization of membrane, damage to cell envelope.	[59]

5. Application to food products

Essential oils and the compounds that make them are generally recognized as safe (GRAS) for human consumption, however there are many challenges involved in using them in the food industry. Most difficulties arise due to factors such as composition of the food product, interaction of the EO with the food or other extrinsic factors such as pH, packaging environment etc. [58]. A lower pH tends to show higher inhibitory effects on bacteria as it increases hydrophobicity, which enables it to easily dissolve in the lipids present in the cell membrane of the target bacteria [59]. In addition to pH, it is believed that low oxygen concentrations cause fewer oxidative changes to the EOs [18, 60]. The hydrophobic nature of EOs is a limiting factor in terms of application, but can be overcome by the use of stabilizing agents such as Tween-80, Tween-20 and lecithin.

The inherent antimicrobial ability of an oil can be related to the chemical configuration of the components, the concentrations in which they are present, and also the interactions between them [27, 35]. An antagonistic effect is observed when compounds are applied together. The effect of one or both compounds is reduced when applied together in comparison to when they are used alone. Additive properties are expressed when the combined effect is equal to the individual effect, while synergism is expressed when the sum of the combined effect is greater than the individual effect [61]. Hence by trying to develop suitable synergistic combinations of EOs, we can effectively apply them to food in lower concentrations than required individually, along with the use of a suitable stabilizing agent.

CHAPTER 2: MATERIALS AND METHODS

1. Essential oils

The essential oils used in this study were all culinary grade. Bay (*Laurus nobilis*), bergamot (*Citrus bergamia),* cinnamon (*Cinnamomum verum*), oregano (*Origanum vulgare*), clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon flexuosus),* nutmeg (*Myristica fragrans),* thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) were analyzed. These essential oils were selected based on their reported antimicrobial activity, sensory properties and the presence of different components in the EOs. The oils were obtained from Lorann oils and flavors (Lansing, Michigan).

2. Test strains and cultures

The cultures used in this study were *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 700927), *Salmonella* Typhimurium (ATCC 19585), *Listeria innocua* (ATCC 33090) and *Listeria monocytogens* (ATCC 19115). Working cultures were prepared by sub-culturing and maintaining on tryptic soy agar (TSA, BD Difco, Detroit, Michigan). Test inoculums were prepared by transferring 24-hour old cultures via a cotton swab to 5 ml of 0.85% saline. The saline suspension was adjusted to an optical density of 0.1 for each bacteria, which corresponds to 0.5 McFarland standard (1x 10^8 cfu/ml). Once standardized, 50µl of the saline suspension was transferred to 10 ml of cation-adjusted Mueller-Hinton II broth (CAMHB, BD Difco, Detroit, Michigan).

3. Essential oil emulsions

Due to the insolubility of EOs in water, oil-in-water emulsions were prepared using Tween-80 as the emulsifying agent. Oil and Tween-80 were mixed at a ratio of 1:0.5 in an aqueous phase, to give a final oil concentration of 20µl/ml. The mixture was then subjected to sonication for 5 minutes using an ultrasonicator (Fisher Scientific Sonic Dismembrator Model 300) to achieve potential nano particulate dispersion of the EOs.

4. Determination of minimum inhibitory concentration (MIC)

The MIC for the oil emulsions was determined by broth micro dilution method according to the National Committee of Clinical Laboratories Standards (NCCLS) guidelines [62]. The prepared oil emulsions were diluted two-fold in CAMHB to a concentration of 10,000 ppm. 100 µl of each emulsion was loaded into the first row of a 96-well plate and 50 µl of CAMHB was added to each subsequent row. The emulsions were serially diluted to obtain final concentrations of 5000, 2500, 1250, 625, 312, 156, 78 and 39 parts per million (ppm). To each well 50 µl of standardized inoculum was added, giving a bacterial concentration of 5×10^5 CFU/ml.

A positive control (containing inoculum but no essential oil) and negative control (containing essential oil but no inoculum) were included in each 96-well plate. Effect of Tween-80 alone on bacterial growth was examined and no effects were seen. Plates were incubated for 24 hours at 37ºC and observed after 24 hours. MIC was determined as the lowest concentration showing no visible signs of growth.

5. Determination of synergy between essential oil emulsions using checkerboard method

Synergy between oil emulsions was determined using the checkerboard method [63, 64]. Oil emulsion (A) was diluted along the x-axis, while oil emulsion (B) was diluted along the y-axis. The final volume in each well was 100 µl, comprised of 50 µl of emulsion dilution and 50 µl of bacteria standardized in CAMHB. Plates were incubated at 37ºC for 24 hours. The fractional inhibitory concentration (FIC) indices were calculated as $FIC_A + FIC_B$, where FIC_A and FIC_B are the respective MIC of oil emulsion A and B. Therefore FICs were calculated as:

$$FIC_A = \frac{MIC_A \text{ Combination}}{MIC_A \text{ Alone}} \qquad FIC_B = \frac{MIC_B \text{ Combination}}{MIC_B \text{ Alone}}$$

The combination was considered synergistic if the sum of the FICs was equal to or less than 0.5. If the values were between 0.5–1.0, 1.0–4.0 or higher than 4.0, they were considered to be additive, indifferent or antagonistic respectively.

6. Preparation and treatment of chicken sample

Chicken was obtained from Blimpie's Sandwich Shop (Detroit, MI) and transported on ice to be used for experimental purposes and processed as described by Kim et al [65]. The pieces were uniformly cut and weighed into 5 gram amounts. Each piece was sterilized in a 100 ppm sodium hypochlorite solution for 30 minutes and rinsed with deionized water. The samples were then inoculated with the bacteria standardized in CAMHB, and consequently treated with a twofold concentration of the individual *in vitro* MIC of the EOs that expressed synergism. The pieces were placed in 60 mm dishes and stored under refrigeration at 4ºC. Samples were prepared for day 0, day 1, day 3 and day 6 for each bacterial treatment.

5 ml of 0.1% peptone water was added to each sample and transferred to a stomacher bag. It was then mixed vigorously for 60 seconds at 230 rpm in a stomacher. 0.1 ml of the solution was taken and serially diluted from 10^{-1} to 10^{-5}. Each dilution was inoculated to TSA and incubated for 24 hours at 37ºC. The colonies formed were counted using a colony counter. The bacterial count was multiplied by the dilution factor and converted to Log CFU/gm.

7. Statistical analysis

Statistical analysis of the data was performed using SPSS 22.0 (IBM corp, Chicago, IL). Data represents the means of experiments performed in triplicate. The means were compared using ANOVA (Analysis of Variance). Furthermore, Tukey's test was applied with a significance level $p<0.05$.

CHAPTER 3: RESULTS

1. Determination of minimum inhibitory concentration

All EO emulsions were evaluated for their potential antimicrobial activity against *E. coli* (ATCC 25922), *E. coli* (ATCC 700927), *S.* Typhimurium (ATCC 19585), *L. innocua* (ATCC 33090) and *L. monocytogens* (ATCC 19115). Bergamot and nutmeg did not exhibit any antimicrobial activity against any of the test organisms within the selected test parameters and were therefore excluded from further analysis. Sage also showed no activity towards *S.* Typhimurium and was excluded. The MIC of the remaining EO emulsions against *E. coli* (ATCC 25922) and *E. coli* (ATCC 700927) can be seen in Figure 3. Cinnamon expressed the lowest MIC at 312.5 ppm for both organisms, followed by oregano at 625 ppm. Sage showed the highest MIC for both organisms at 5000 ppm while rosemary only expressed 5000 ppm for *E. coli* ATCC 700927.

Figure 4 shows the MIC for the EOs against *L. innocua* and *L. monocyatogens*. Cinnamon showed the lowest MIC for both organisms at 625 ppm, followed by lemongrass and sage at 1250 ppm for *L. monocytogens* and thyme at 1250 ppm for *L. innocua*. The highest MIC of 5000 ppm was seen in case of rosemary and clove for *L. innocua*. All remaining MICs were at 2500 ppm.

Figure 5 represents the MIC for all EOs excluding bergamot, nutmeg and sage against *S.typhimurium*. The lowest MIC was expressed by cinnamon at concentration of 625ppm, followed by thyme at 1250 ppm. Bay, oregano and lemongrass all exhibited a MIC of 2500 ppm. The highest MIC was seen in case of rosemary and clove at 5000 ppm.

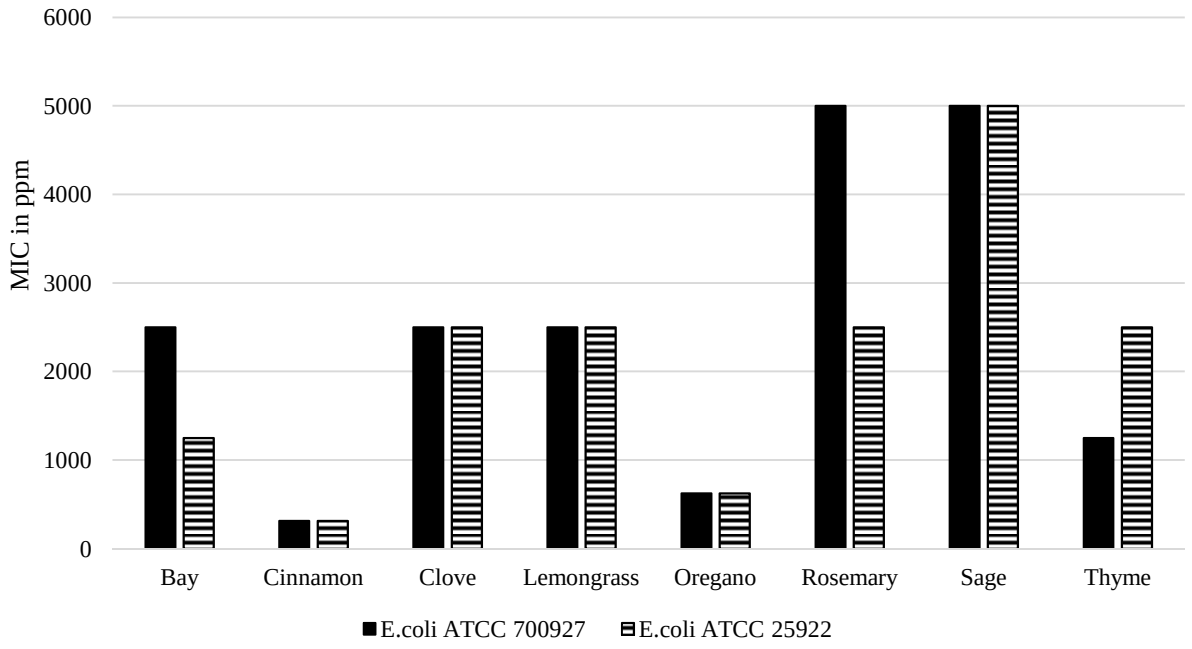

Figure 3. MIC of selected EOs against *E. coli* ATCC 25922 and *E. coli* ATCC 700927

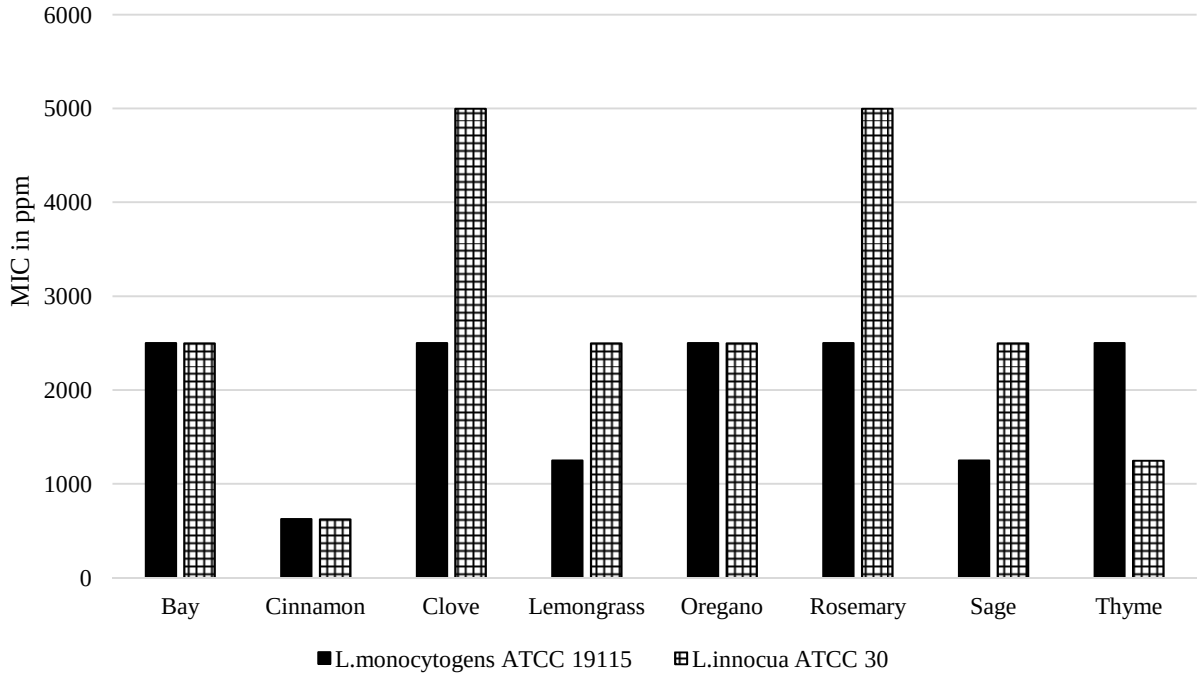

Figure 4. MIC of selected EOs against *L. innocua* ATCC 33090 and *L. monocytogens* ATCC 19115

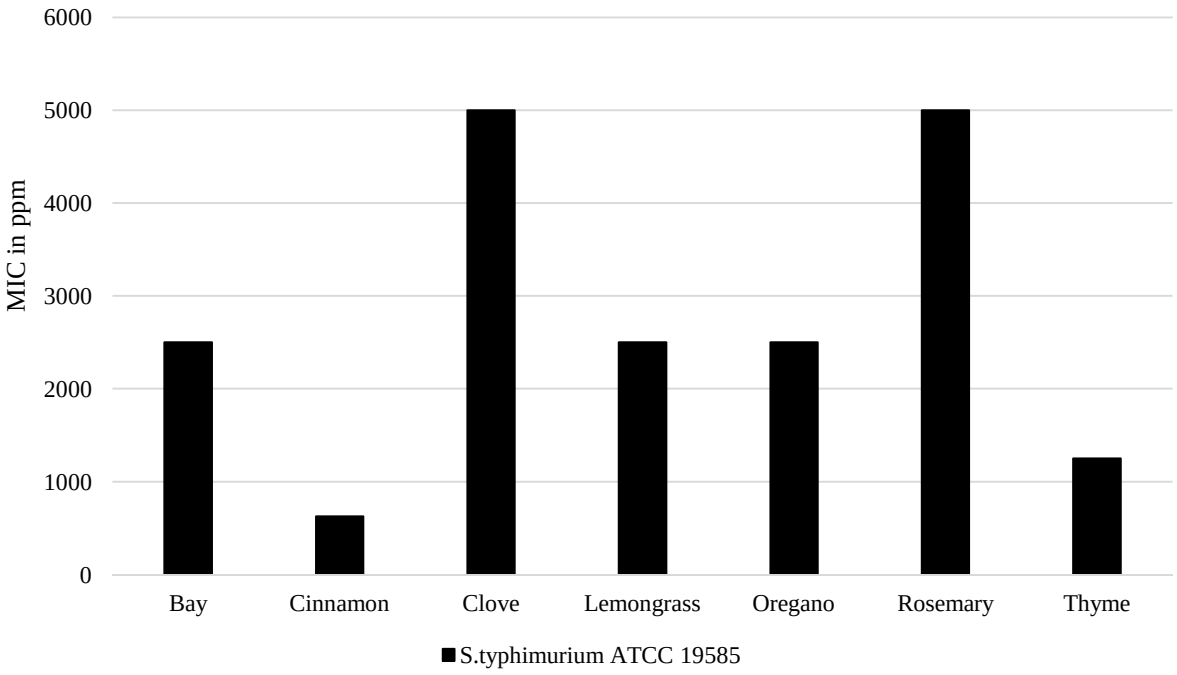

Figure 5. MIC of selected EOs against *S.* Typhimurium ATCC 19585

2. Determination of synergy between cinnamon and selected EOs

The quantitative effect of cinnamon in combination with other EO emulsions is expressed in terms of FIC indices. The FICs of the combinations are shown in Table 3. Synergy was observed only in case of cinnamon and oregano against *E. coli* ATCC 700927 and *L. innocua*, while cinnamon and clove showed synergism towards *L. innocua.* Cinnamon and oregano showed an additive affect towards *E. coli* ATCC 25922 and *L. monocytogens*. Another additive effect was seen in case of cinnamon and thyme towards *E. coli* ATCC 25922 and *S. typhimurium*. All other combinations of selected EOs with cinnamon were antagonistic towards all test organisms.

Table 3. FIC indices of cinnamon and selected EOs against test organisms

Foodborne Pathogen	Cinnamon & Oregano	Cinnamon & Thyme	Cinnamon & Clove	Cinnamon & Bay	Cinnamon & Lemon grass
E.coli ATCC 700927	0.46±0.3 (S)	1.05±0.07 (I)	1.2±0.17 (I)	1.27±0.02 (I)	1.58±0.04 (I)
E.coli ATCC 25922	0.8±0.005 (A)	0.57±0.03 (A)	1.3±0.005 (I)	1.06±0.11 (I)	1.24±0.12 (I)
S.typhimurium	1.03±0.05 (I)	0.91±0.015 (A)	1.06±0.11 (I)	1.24±0.07 (I)	1.03±0.05 (I)
L.innocua	0.44±0.02 (S)	1.06±0.11 (I)	0.5±0.01 (S)	1.10±0.18 (I)	1.14±0.12 (I)
L.monocytogens	0.65±0.005 (A)	1.06±0.11 (I)	1.14±0.15 (I)	1.20±0.10 (I)	1.10±0.17 (I)

Results are interpreted as synergy (S, FIC < 0.5), addition (A, 0.5 ≤ FIC ≤1), indifference (I, 1< FIC≤ 4) or antagonism (AN, FIC > 4).

3. Antimicrobial action of selected EOs and EO combinations to chicken

To determine the antimicrobial efficacy of the selected EOs and their combinations in a food model, chicken samples were treated and inoculated. During storage at 4 ºC all selected combinations and samples showed a significant ($p < 0.01$) reduction in bacterial load in comparison to control samples.

Figure 6 – figure 10 show the action of cinnamon alone on *E. coli* (ATCC 25922), *E. coli* (ATCC 700927), *L. monocytogens* (ATCC 19115), *L. innocua* (ATCC 33090) and *S.* Typhimurium (ATCC 19585). It reduced growth in comparison to control samples by 2.885, 3.39, 3.275, 4.29 and 3.06 Log respectively. It can be seen that it reduced cell load between day 1 and day 6 in case of *L. innocua*. Though all other samples on their own showed slight growth.

Figure 11 – figure 13 represent the action of thyme alone on *E.coli* ATCC 700927, *S.* Typhimurium and *L. innocua.* All samples were significantly lower in bacterial load in comparison to control samples, but showed slight growth between day 1 and day 6. It showed a reduction of *E. coli* (ATCC 700927), *L. innocua* (ATCC 33090) and *S.* Typhimurium (ATCC 19585) in comparison to control by 1.23, 1.065 and 1.38 respectively.

Figure 14 and 15 show the action of oregano individually on *E. coli* ATCC 700927 and *E. coli* ATCC 25922. Both samples showed similar activity as reported for thyme and showed a reduction of 3.21 for *E. coli* (ATCC 25922) and 3.535 for *E. coli* (ATCC 700927) between control and test samples.

All combinations of cinnamon and selected EOs exhibited similar trends of significant reduction in comparison to control, while showing mild growth between day 1 and day 6. Cinnamon and oregano in combination reduced *E. coli* (ATCC 25922), *E. coli* (ATCC 700927),

L. monocytogens (ATCC 19115) and *L. innocua* (ATCC 33090) by 2.215, 3.175, 2.2 and 2.085 Log. While cinnamon in combination with thyme showed a reduction of 1.76 Log for *E. coli* (ATCC 25922) and 2.71 Log for *S.* Thyphimurium in comparison to the control samples. Cinnamon and clove was only tested against *L. innocua* and reduced the bacterial load by 2.215 Log when compared to the control.

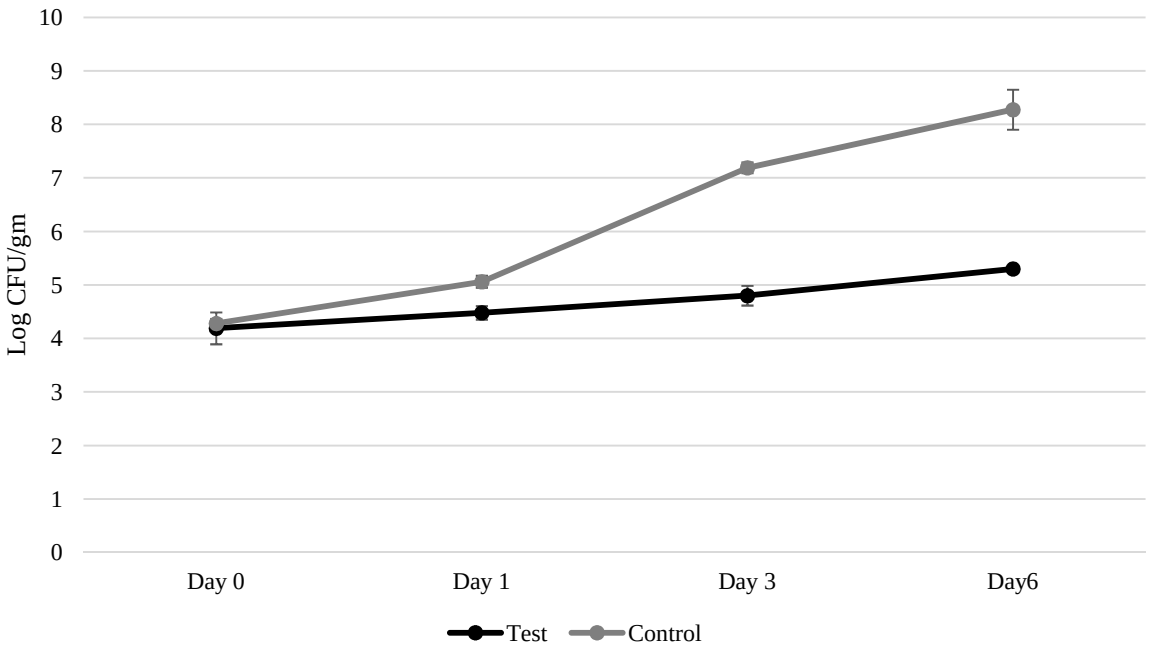

Figure 6. Antimicrobial activity of cinnamon at 625 ppm on *E. coli* ATCC 25922 growth on chicken in comparison to control at 4 ºC.

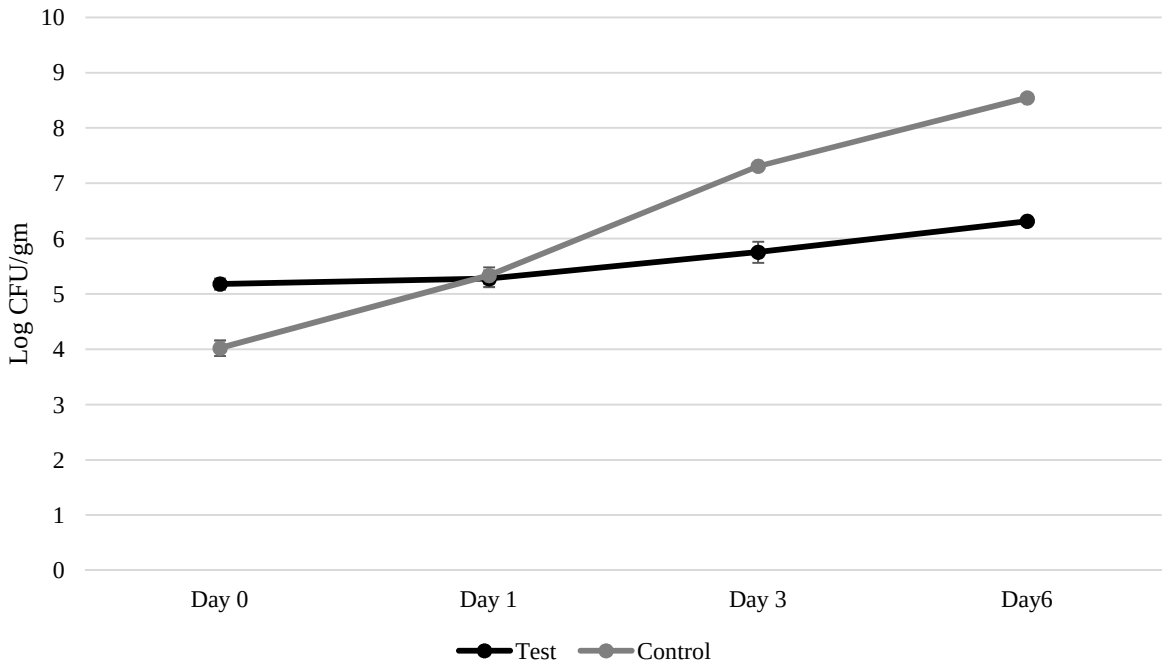

Figure 7. Antimicrobial activity of cinnamon at 625 ppm on *E. coli* ATCC 700927 growth on chicken in comparison to control at 4 ºC.

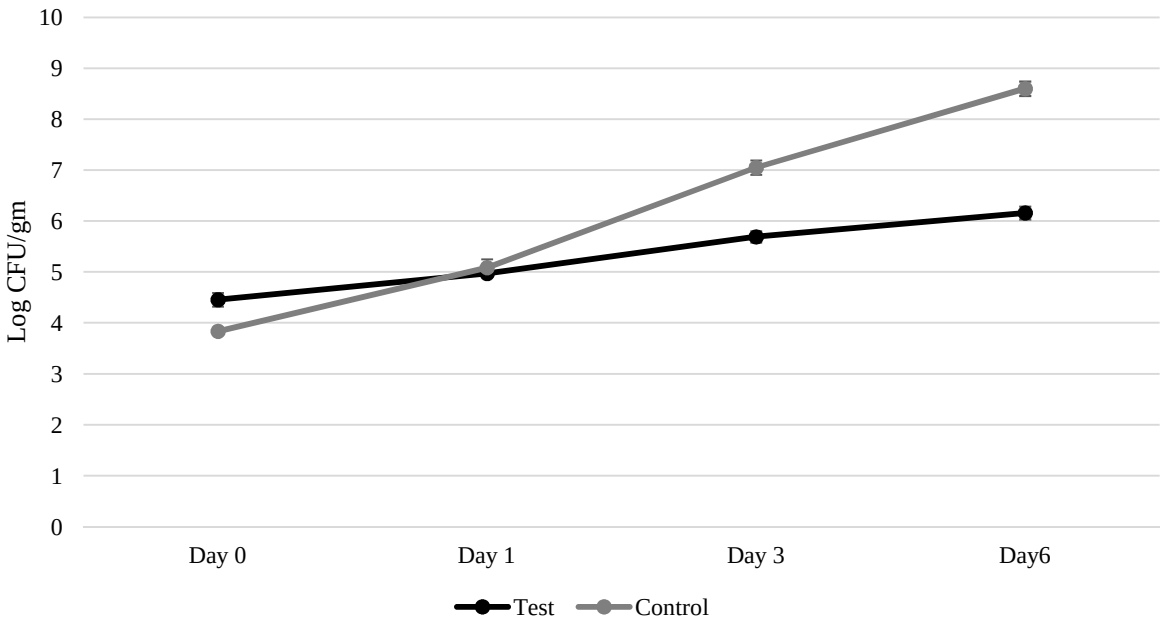

Figure 8. Antimicrobial activity of cinnamon at 1250 ppm on *S.* Typhimurium growth on chicken in comparison to control at 4 ºC.

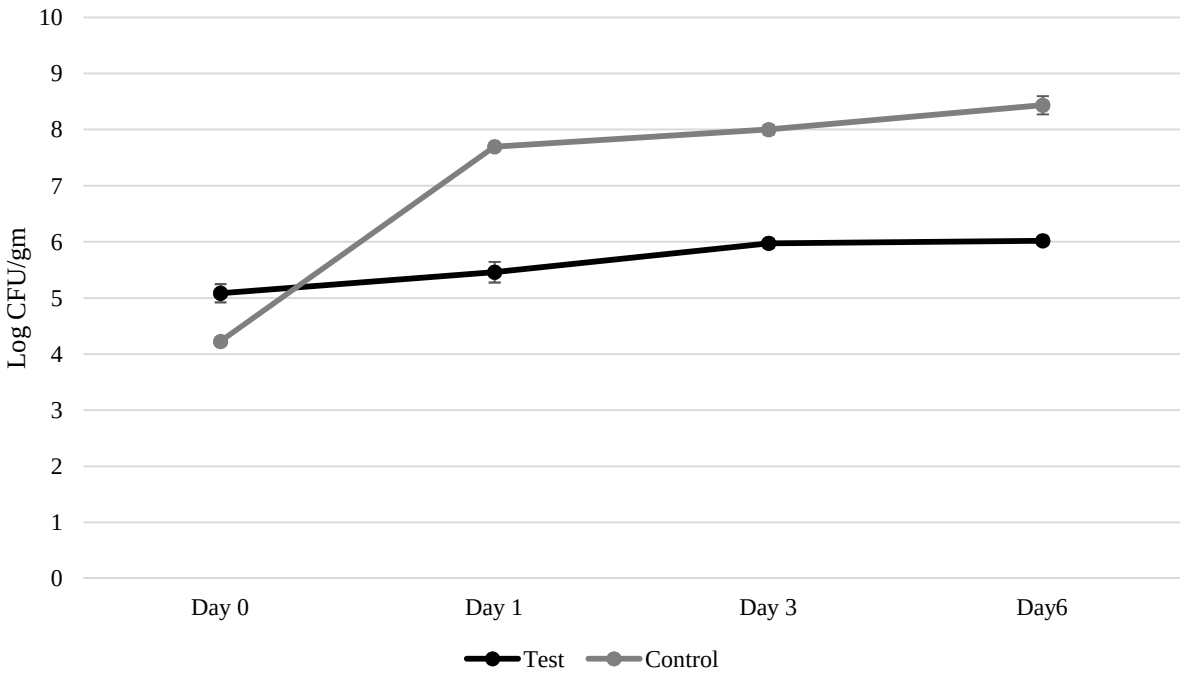

Figure 9. Antimicrobial activity of cinnamon at 1250 ppm on *L. monocytogens* growth on chicken in comparison to control at 4 ºC.

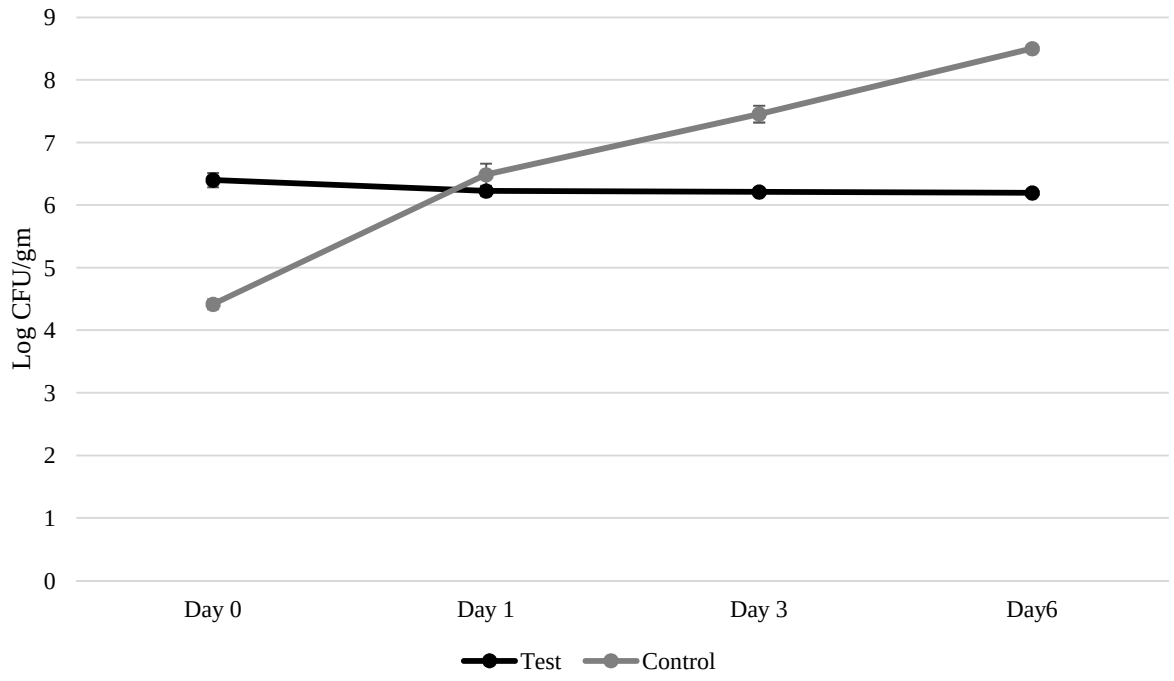

Figure 10. Antimicrobial activity of cinnamon at 1250 ppm on *L. innocua* growth on chicken in comparison to control at 4 ºC.

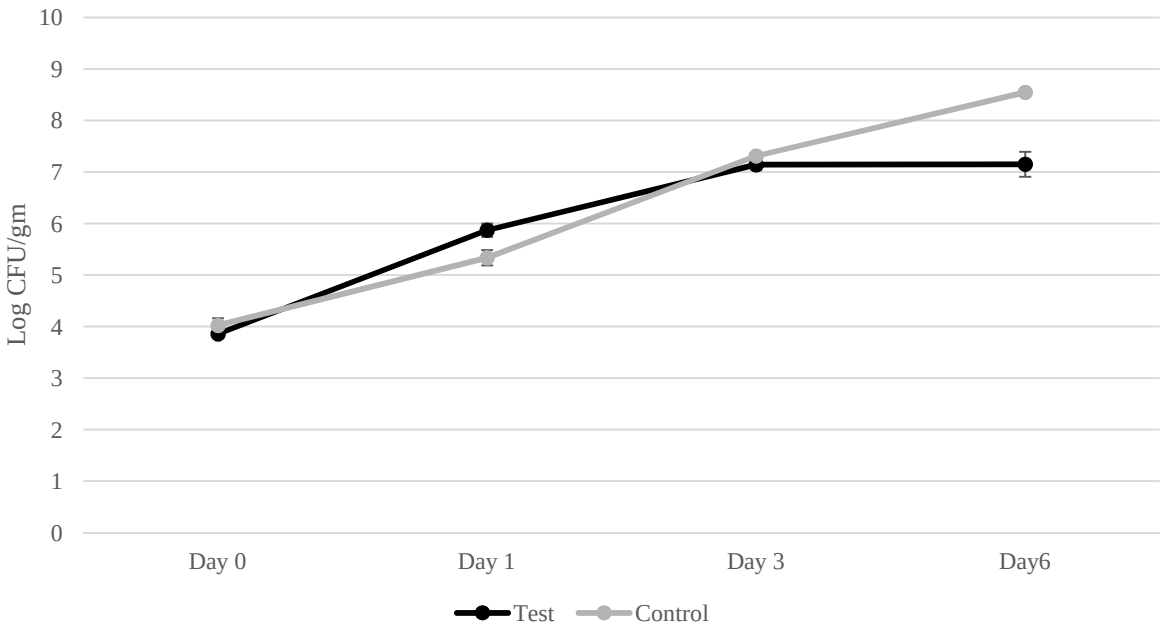

Figure 11. Antimicrobial activity of thyme at 2500 ppm on *E. coli* ATCC 700927 growth on chicken in comparison to control at 4 ºC.

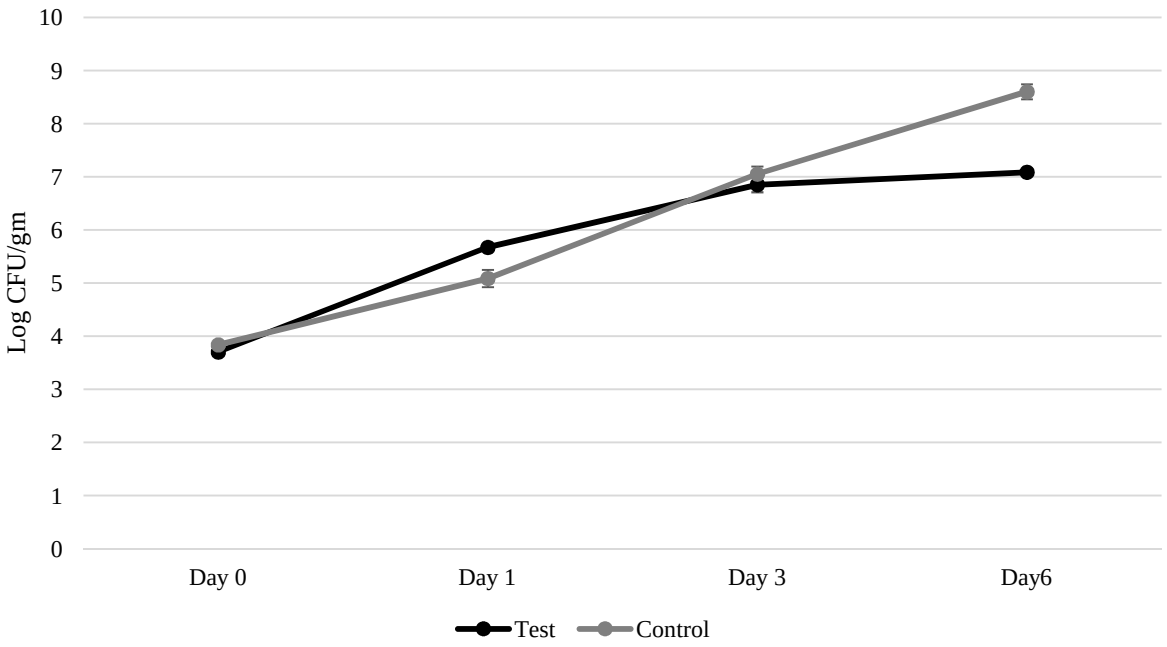

Figure 12. Antimicrobial activity of thyme at 2500 ppm on *S.typhimurium* growth on chicken in comparison to control during 4 ºC.

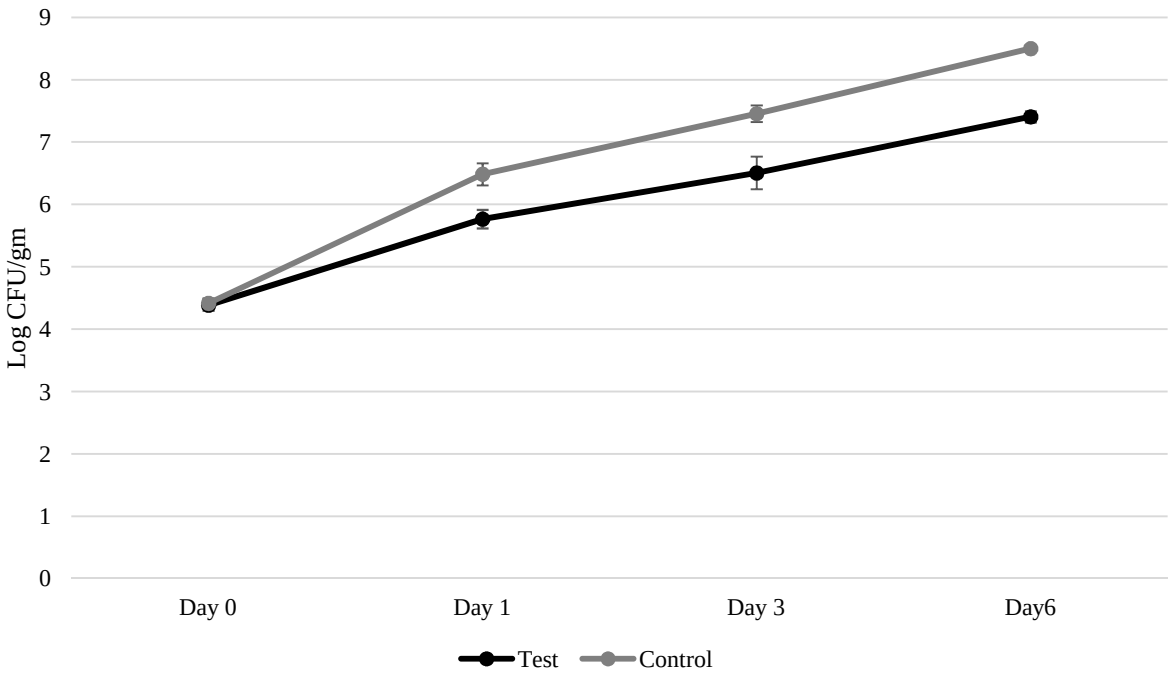

Figure 13. Antimicrobial activity of thyme at 2500 ppm on *L. innocua* growth on chicken in comparison to control during 4 ºC.

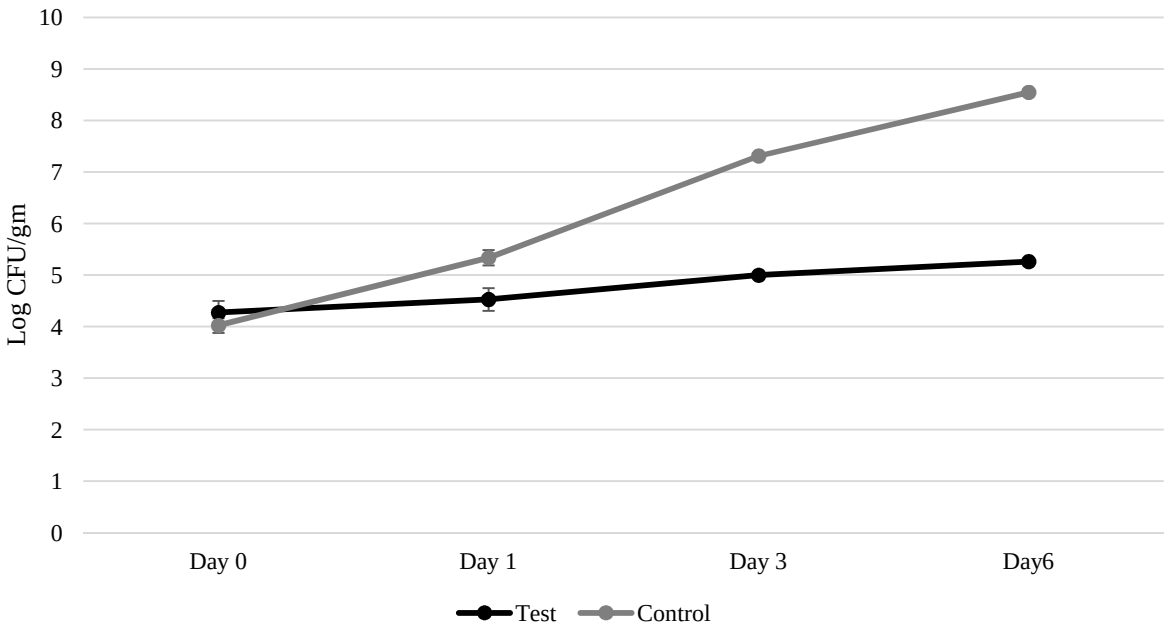

Figure 14. Antimicrobial activity of oregano at 1250 ppm on *E.coli* ATCC 700927 growth on chicken in comparison to control during 4 ºC.

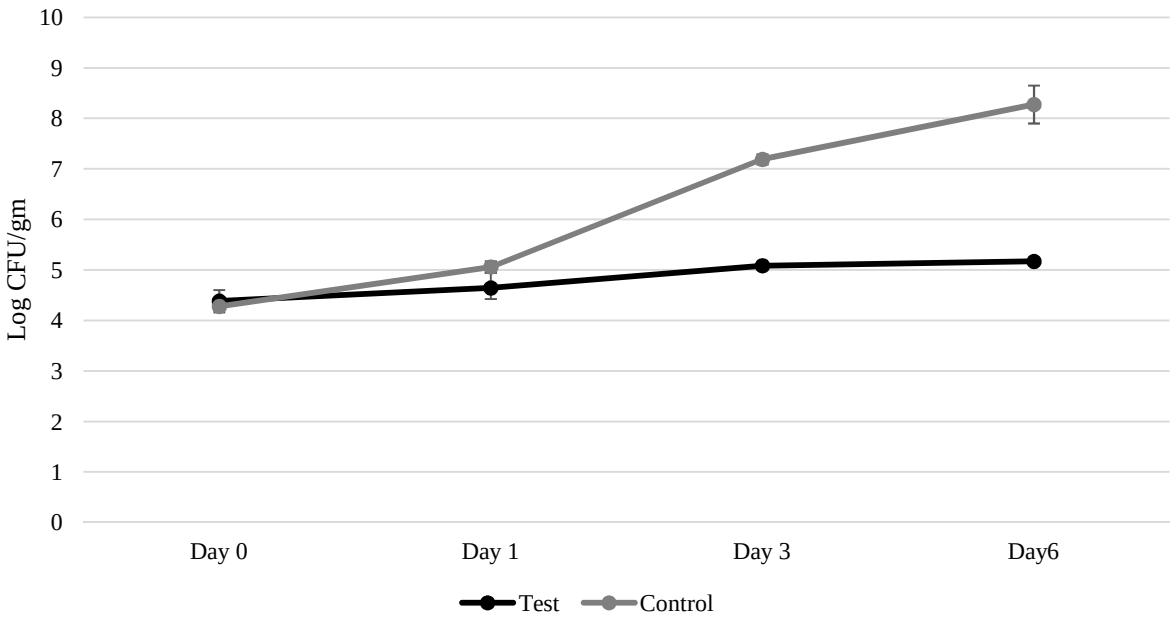

Figure 15. Antimicrobial activity of oregano at 1250 ppm on *E.coli* ATCC 25922 growth on chicken in comparison to control during 4 ºC.

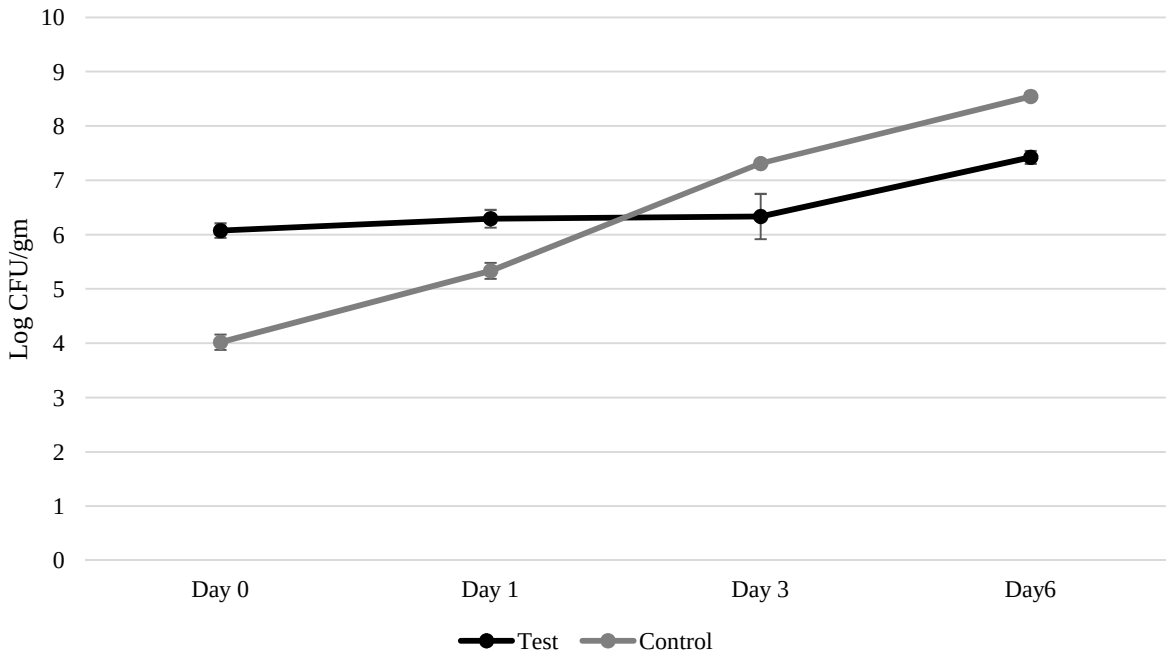

Figure 16. Antimicrobial activity of cinnamon at 625 ppm and oregano at 1250 ppm on *E.coli* ATCC 700927 growth on chicken in comparison to control during 4 ºC.

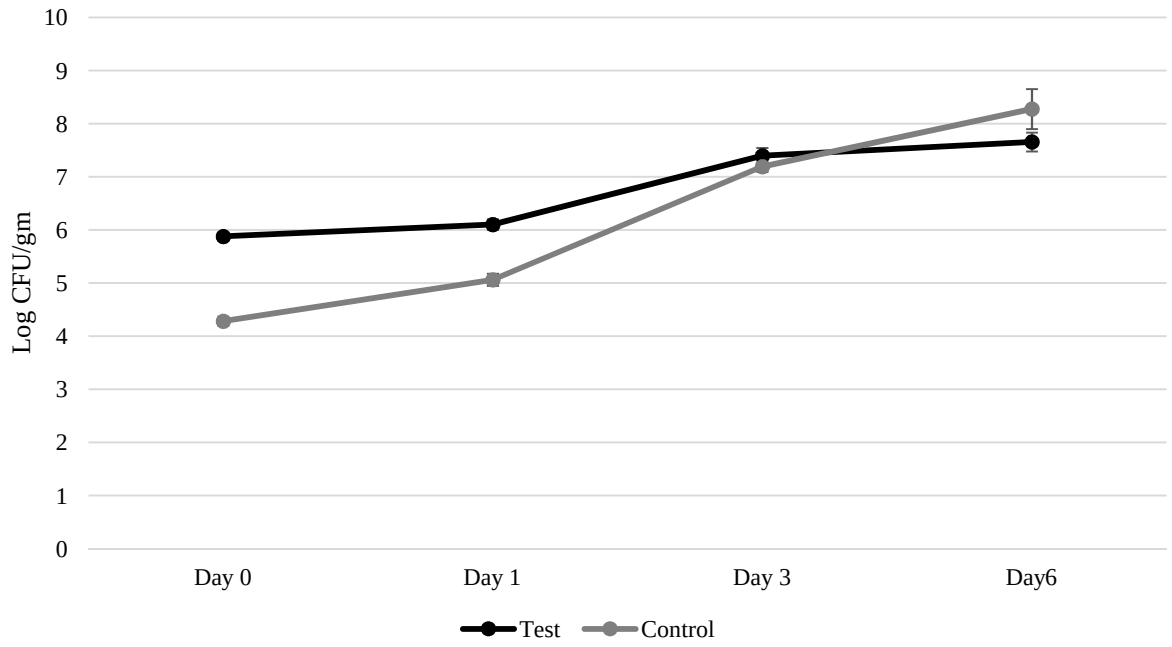

Figure 17. Antimicrobial activity of cinnamon at 625 ppm and oregano at 1250 ppm on *E.coli* ATCC 25922 growth on chicken in comparison to control during 4 ºC.

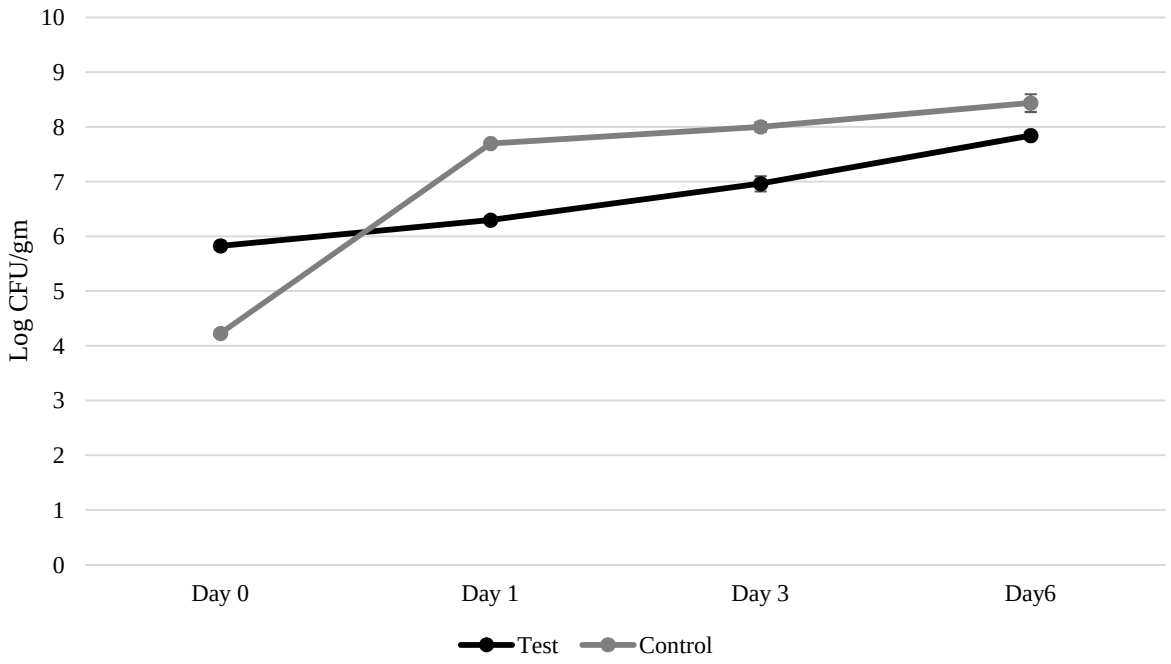

Figure 18. Antimicrobial activity of cinnamon at 1250 ppm and oregano at 2500 ppm on *L. monocytogens* growth on chicken in comparison to control during 4 ºC.

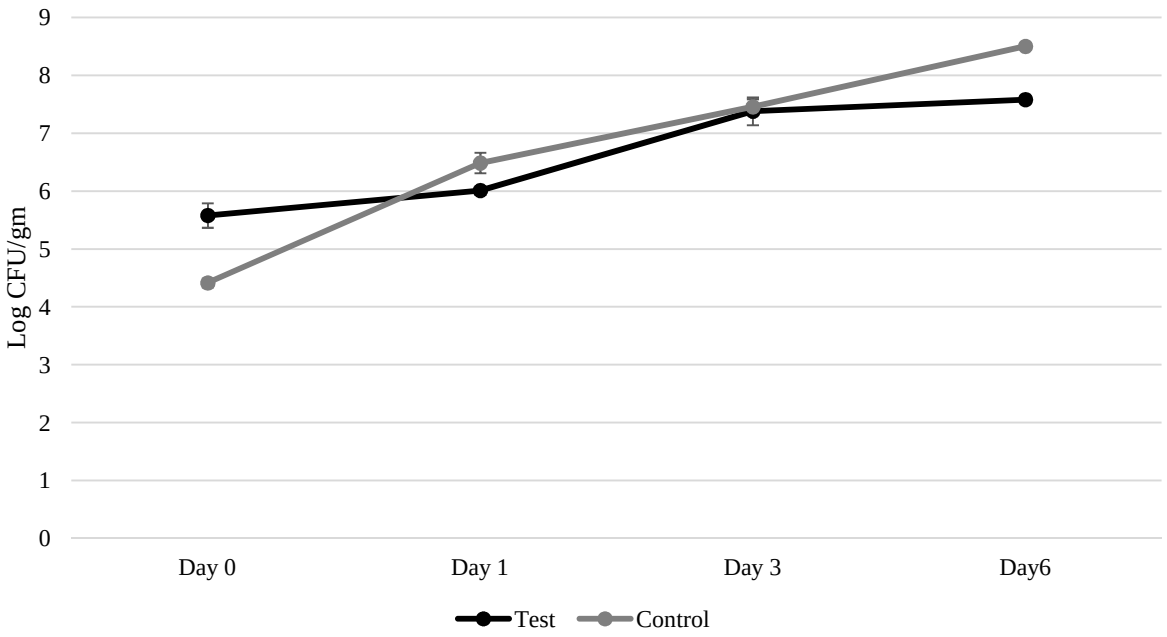

Figure 19. Antimicrobial activity of cinnamon at 1250 ppm and oregano at 1250 ppm on *L. innocua* growth on chicken in comparison to control during 4 ºC.

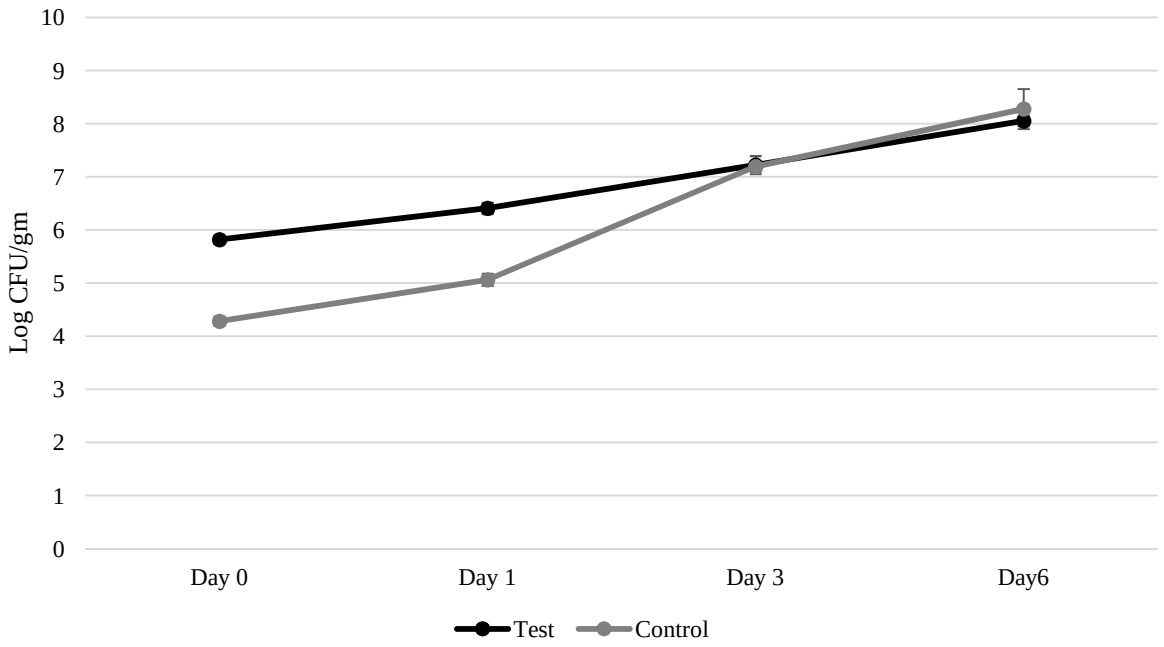

Figure 20. Antimicrobial activity of cinnamon at 1250 ppm and thyme at 1250 ppm on *E.coli* ATCC 25922 growth on chicken in comparison to control during 4 ºC.

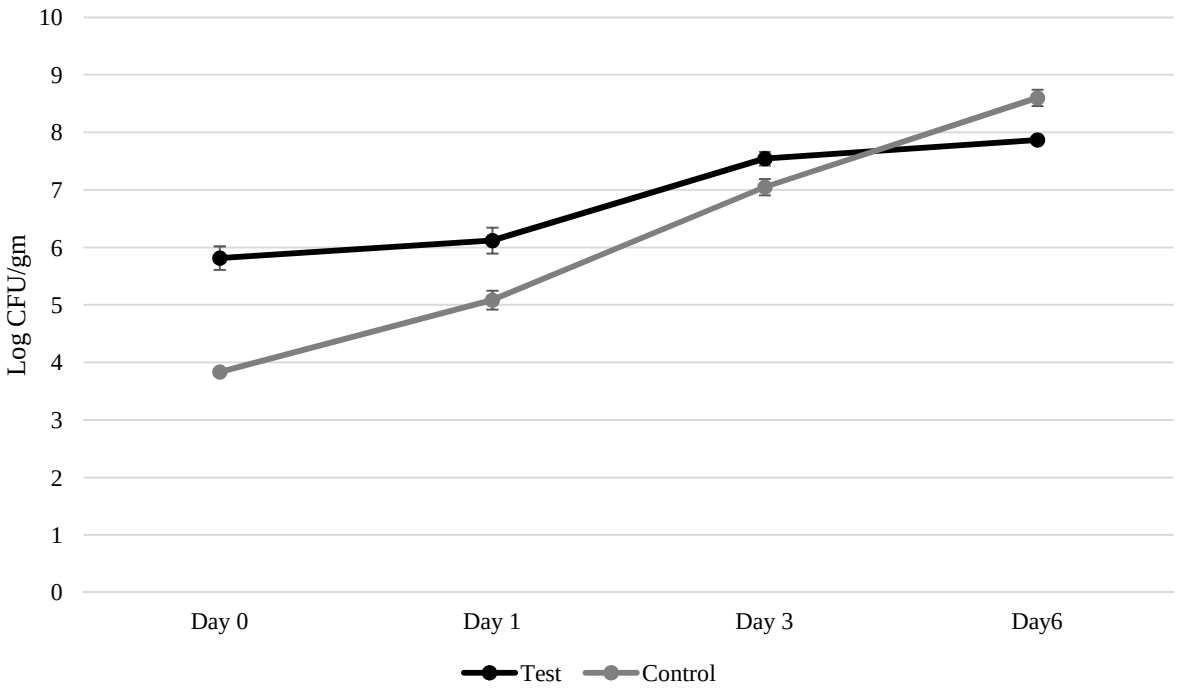

Figure 21. Antimicrobial activity of cinnamon at 1250 ppm and thyme at 2500 ppm on *S. typhimurium* growth on chicken in comparison to control during 4 ºC.

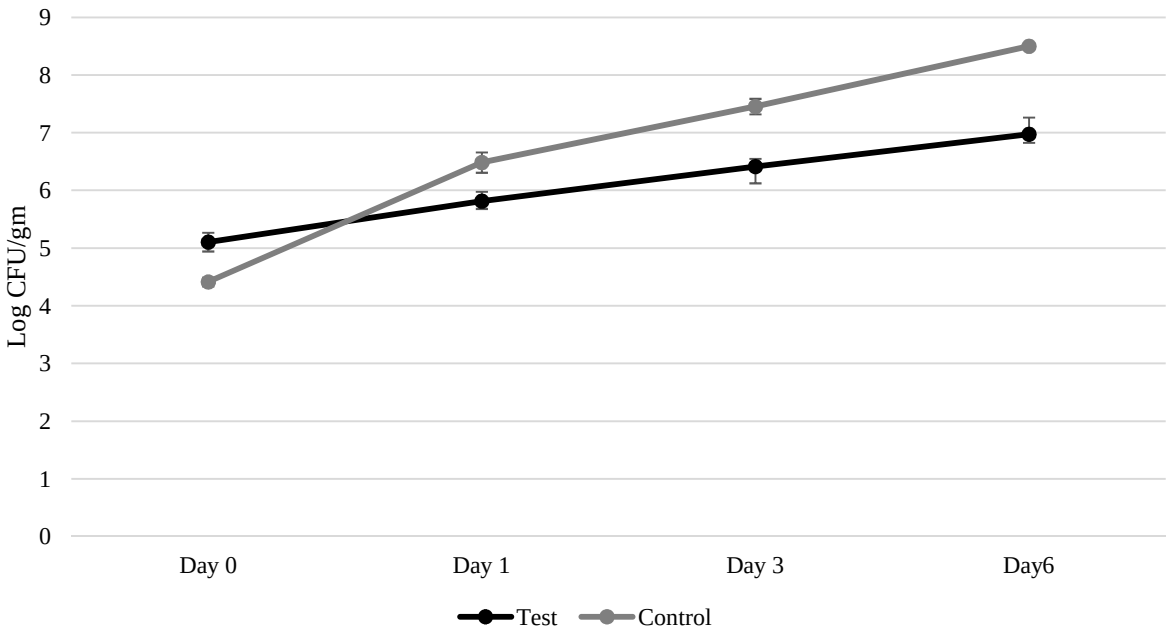

Figure 22. Antimicrobial activity of cinnamon at 1250 ppm and clove at 2500 ppm on *L. innocua* growth on chicken in comparison to control during 4 ºC.

CHAPTER 4: DISCUSSION

EOs have shown to be effective against a variety of foodborne microorganisms under *in vitro* conditions but have presented with application and concentration issues when used in actual food products. Since higher concentrations of EOs are required when used as additives to food, it is a challenge to develop optimized low concentrations to be used for product safety. The use of a surfactant like Tween-80 aids in the stabilization of the oil-in-water emulsion, thereby overcoming the inherent hydrophobic nature of the essential oils and extending its range of product application. Attempting to find synergistic combinations allows us to achieve the required low optimal concentration that minimizes the effect on the organoleptic properties and interactions with other food components.

In the initial study the selected EOs were screened for their potential antimicrobial activity against the selected foodborne bacteria. Preliminary findings showed that bergamot and nutmeg expressed no antimicrobial activity relative to the other oils towards any of the test organisms since possible inhibitory concentrations exceeded the selected parameter of 5000 ppm as the highest MIC. This can be attributed to the high composition of limonene and sabinene respectively for bergamot and nutmeg. Both limonene and sabinene are major constituents in the terpenes family, a group that lacks high inherent antimicrobial activity [48]. Even though nutmeg has phenylpropenes like euganol present, the amounts are insufficient to express antimicrobial activity at the concentrations of EO used in the study. The same can be seen in case of bergamot, where the presence of linalool and linalyl acetate, two terpenoids, is not sufficient to show antimicrobial activity. Other studies suggest they are required in concentrations ranging from 2000 µg–5000 µg to show effect [41, 48]. Bay expressed a consistent inhibitory effect on all test organisms, which is in agreement with findings from other studies [38]. This can be reasoned by

the presence of 1,8-cineole, a terpenoid [31]. The difference in activity between *E.coli* ATCC 700927 and *E.coli* ATCC 25922 may be attributed to differences in biological properties of the two organisms. The most effective inhibitor of all test organisms was cinnamon. It had the lowest MIC amongst all the oils with 312 ppm for both *E.coli* strains while 625 ppm for *S.typhimurium* and both species of *Listeria*. This activity can be explained by the high concentration of trans-cinnamaldehyde present in it [31], which results in loss of membrane integrity and decrease ATPase activity due to depolarization of the cell [53, 66]. The antimicrobial efficacy of cinnamon can also be attributed to euganol. Although euganol is not present in very high concentrations in cinnamon, it binds and affects the properties of proteins at sub lethal concentrations [55]. Rosemary predominantly expressed a high MIC of 5000 ppm towards *S.typhimurium*, *L.innocua* and *E.coli* ATCC 700927. This can be due to the presence of α–pinene and bronyl acetate in varying concentrations, as both terpenes have very low antimicrobial activity [35]. Rosemary's MIC was on the lower end of the spectrum at a concentration of 2500 ppm for *L.monocytogens* and *E.coli* ATCC 25922. This may be due to differences in structural and physiological properties [67]. Sage showed similar results, with MICs at 5000 ppm. The major components of sage are terpenes, which supports the low antimicrobial activity seen. The antimicrobial activity of clove and lemongrass was similar in a few cases: *S.typhimurium, E.coli* ATCC 700927, and *E.coli* ATCC 25922. While the primary constituent of clove is euganol and the primary constituent of lemongrass is gerinal, they both are effective in expressing antimicrobial effect on the cell [31]. The variation seen in case of the two *Listeria* species could be due to the presence of teichoic acid in the cell wall of *L. innocua*, which makes it less susceptible to hydrophobic compounds such as EOs [67]. Oregano was most effective on both *E.coli* species, and this activity can be explained by the presence of high

amounts of carvacrol as well as the presence of *p*–cymene has been reported to promote carvacrol activity [51]. Oregano had a slightly lower antimicrobial effect on the other test organisms. Thyme also showed a varying range of antimicrobial activity on the test organisms. Most of the oils did not follow the reported trend of increased activity towards Gram-positive species [6, 27, 33]. It is believed that individual components of EOs express different degrees of activity against Gram-positive and Gram-negative organisms and as it is known that the composition of EOs can vary by time and place of harvest or methods of extraction [18]. It is therefore possible that variation between batches is sufficient to present a range in variability of antimicrobial action on Gram-negative and Gram-positive bacteria. A direct comparison of these essential oil emulsions with previous reported activity against the selected organisms is difficult, due to the use of different solvents as ethanol and dimethyl sulfoxide (DMSO) [68, 69].

Combinations of cinnamon with other EOs were screened to determine if they were synergistic with each other. Cinnamon was selected as the primary EO as it exhibited the highest and most consistent antimicrobial activity. Only two combinations showed synergistic activity: cinnamon and oregano against *E.coli* ATCC 700927 and *L.innocua*, and cinnamon and clove against *L.innocua*. Additive effects were seen for combinations of cinnamon with oregano and thyme. It is believed that minor components present in the EOs are important to the activity of the EOs main components, and may even have a synergistic or potentiating impact [18, 68]. As most plant essential oils possess a similar make up of chemical constituents, their combinations are more likely to exhibit addition or indifference rather than synergism. Combinations with compounds containing different chemical structures might show better antimicrobial activity [68]. Since antimicrobial activity is not only influenced by chemical composition but also by

lipophilic properties, the potency of functional groups or aqueous solubility using a mixture of compounds can increase antimicrobial activity [35].

A crucial aspect of optimizing the application of EOs to food products is determining their antimicrobial efficacy with a food model. The findings of the food model study were promising. Three of the synergistic and four of the additive combinations were used to treat the chicken along with samples treated with their individual EOs. Clove was excluded from the study as it had an individual MIC above 2500 ppm. At first glance one can clearly see a difference in growth between the test samples and the control samples, even though all except one showed minimal growth in the presence of the EOs. Cinnamon was effective in reducing *L.innocua* by 0.205 Log CFU/gm between day 0 and day 6, which is very minute but taking into consideration the low concentration of cinnamon used is of note. All the EO treated samples showed a significant ($p<0.01$) lowering of bacterial count in comparison with control. When examined independently, they showed minor increase in growth. Even though there was minor growth, the EO had an effect as they were able to restrict it to a certain level. The variation between the *in vitro* activities of the oils seen in the food model study could be due to interaction of the EOs with components of the food such as fats and proteins. A high concentration of fats has been reported to have a negative influence on the activity of cinnamon and clove EOs [70], while high concentrations of proteins promote the antimicrobial activity of EOs when applied to food [71]. Therefore, the composition of the food product is also an influencing factor to the efficacy of EOs as antimicrobial agents in food.

CHAPTER 5: CONCLUSION

The control of foodborne pathogens is a major challenge confronting the food industry [72, 73]. Hot water treatments, steam and organic acids are commonly used in the decontamination process, but are not 100% effective since many pathogenic bacteria can survive and thrive. It has been suggested that the use of EOs in combination with other conventional treatments like preservatives or low temperature can be used as a synergistic alternative to existing methods [72]. This study focuses on the potential of using EOs in food products as a means to prevent further infections. Tween–80 is a crucial element to the EO composition, as it allows for the application of the EOs to products without their inherent hydrophobic nature affecting their potential activity.

The EO combinations revealed possible combinations that may be used. Cinnamon and oregano showed an additive effect against *E.coli* ATCC 700927, *E.coli* ATCC 25922, *L.innocua* and *L.monocytogens*. In addition, cinnamon with thyme and clove were selectively effective. The results of this study suggest that these combinations should be considered as potential alternatives for control of pathogens and to reduce microbial spoilage. A difference in activity between *in vitro* and actual food application was seen in the study, which is consistent with findings from previous studies [3, 6]. At twofold the concentration used in the *in vitro* study, the EOs were only able to reduce growth rate rather than completely inhibit growth over a 6-day period. However, there was significant reduction in the growth rate when compared to the control group. The results of this study support the argument that EOs have the potential to be used as antimicrobial control agents, and future studies should elucidate the potential of these synergistic EO combinations in various food models as an alternative to synthetic preservatives.

REFERENCES

1. WHO: **Food safety and foodborne illnesses. Fact Sheet No. 237.** 2006.
2. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM: **Foodborne illness acquired in the United States—major pathogens.** *Emerging infectious diseases* 2011, **17:**7.
3. Smid EJ, Gorris LG: **Natural antimicrobials for food preservation.** *Food science and technology* 1999**:**285-308.
4. Bhargava K, Wang X, Donabedian S, Zervos M, da Rocha L, Zhang Y: **Methicillin-resistant Staphylococcus aureus in retail meat, Detroit, Michigan, USA.** *Emerging infectious diseases* 2011, **17:**1135.
5. Bauer K, Garbe D, Surburg H: *Common fragrance and flavor materials.* Wiley. com; 2008.
6. Shelef L: **Antimicrobial effects of spices.** *Journal of Food Safety* 1984, **6:**29-44.
7. Guenther E, Althausen D: *The essential oils.* Van Nostrand New York; 1952.
8. Cassel E, Vargas R, Martinez N, Lorenzo D, Dellacassa E: **Steam distillation modeling for essential oil extraction process.** *Industrial crops and products* 2009, **29:**171-176.
9. Charles DJ, Simon JE: **Comparison of extraction methods for the rapid determination of essential oil content and composition of basil.** *Journal of the American Society for Horticultural Science* 1990, **115:**458-462.
10. Kalemba D, Kunicka A: **Antibacterial and antifungal properties of essential oils.** *Current Medicinal Chemistry* 2003, **10:**813-829.
11. Moon T, Wilkinson JM, Cavanagh HM: **Antiparasitic activity of two Lavandula essential oils against Giardia duodenalis, Trichomonas vaginalis and Hexamita inflata.** *Parasitology research* 2006, **99:**722-728.
12. Pessoa L, Morais S, Bevilaqua C, Luciano J: **Anthelmintic activity of essential oil of< i> Ocimum gratissimum</i> Linn. and eugenol against< i> Haemonchus contortus</i>.** *Veterinary Parasitology* 2002, **109:**59-63.
13. Regnault-Roger C, Hamraoui A, Holeman M, Theron E, Pinel R: **Insecticidal effect of essential oils from mediterranean plants uponAcanthoscelides Obtectus Say (Coleoptera, Bruchidae), a pest of kidney bean (Phaseolus vulgaris L.).** *Journal of chemical Ecology* 1993, **19:**1233-1244.
14. Prajapati V, Tripathi A, Aggarwal K, Khanuja S: **Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against< i> Anopheles stephensi</i>,< i> Aedes aegypti</i> and< i> Culex quinquefasciatus</i>.** *Bioresource Technology* 2005, **96:**1749-1757.
15. Isman MB: **Plant essential oils for pest and disease management.** *Crop protection* 2000, **19:**603-608.
16. Chao SC, Young DG, Oberg CJ: **Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses.** *Journal of Essential Oil Research* 2000, **12:**639-649.
17. Sökmen M, Serkedjieva J, Daferera D, Gulluce M, Polissiou M, Tepe B, Akpulat HA, Sahin F, Sokmen A: **In vitro antioxidant, antimicrobial, and antiviral activities of the**

essential oil and various extracts from herbal parts and callus cultures of Origanum acutidens. *Journal of agricultural and food chemistry* 2004, **52:**3309-3312.

18. Burt S: **Essential oils: their antibacterial properties and potential applications in foods—a review.** *International journal of food microbiology* 2004, **94:**223-253.
19. Boyle W: **Spices and essential oils as preservatives.** *Pharm J Spices (EBBD, 190317544)* 1955.
20. Weiss EA: *Essential oil crops.* Cab International; 1997.
21. Cox S, Mann C, Markham J, Bell H, Gustafson J, Warmington J, Wyllie S: **The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil).** *Journal of applied microbiology* 2000, **88:**170-175.
22. Windisch W, Schedle K, Plitzner C, Kroismayr A: **Use of phytogenic products as feed additives for swine and poultry.** *Journal of animal science* 2008, **86:**E140-E148.
23. Roller S: *Natural antimicrobials for the minimal processing of foods.* CRC Press; 2003.
24. Nannapaneni R, Chalova VI, Crandall PG, Ricke SC, Johnson MG, O'Bryan CA: ***Campylobacter* and *Arcobacter* species sensitivity to commercial orange oil fractions.** *International journal of food microbiology* 2009, **129:**43-49.
25. Paibon W, Yimnoi CA, Tembab N, Boonlue W, Jampachaisri K, Nuengchamnong N, Waranuch N, Ingkaninan K: **Comparison and evaluation of volatile oils from three different extraction methods for some Thai fragrant flowers.** *International journal of cosmetic science* 2011, **33:**150-156.
26. Demuner AJ, Almeida Barbosa LC, Gonçalves Magalhaes C, Da Silva CJ, Alvares Maltha CR, Lelis Pinheiro A: **Seasonal variation in the chemical composition and antimicrobial activity of volatile oils of three species of Leptospermum (Myrtaceae) grown in Brazil.** *Molecules* 2011, **16:**1181-1191.
27. Delaquis PJ, Stanich K, Girard B, Mazza G: **Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils.** *International journal of food microbiology* 2002, **74:**101-109.
28. Moyler DA: **Extraction of essential oils with carbon dioxide.** *Flavour and fragrance journal* 1993, **8:**235-247.
29. Packiyasothy E, Kyle S: **Antimicrobial properties of some herb essential oils.** *Food Australia* 2002, **54:**384-387.
30. Senatore F: **Influence of harvesting time on yield and composition of the essential oil of a thyme (Thymus pulegioides L.) growing wild in Campania (Southern Italy).** *Journal of agricultural and food chemistry* 1996, **44:**1327-1332.
31. Hyldgaard M, Mygind T, Meyer RL: **Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components.** *Front Microbiol* 2012, **3:**12.
32. Farag R, Daw Z, Hewedi F, El-Baroty G: **Antimicrobial activity of some Egyptian spice essential oils.** *Journal of Food Protection* 1989, **52:**665-667.
33. Juliano C, Mattana A, Usai M: **Composition and in vitro antimicrobial activity of the essential oil of Thymus herba-barona Loisel growing wild in Sardinia.** *Journal of Essential Oil Research* 2000, **12:**516-522.
34. Knobloch K, Weigand H, Weis N, Schwarm H, Vigenschow H: **Action of terpenoids on energy metabolism.** *Progress in essential oil research* 1986**:**429-445.
35. Dorman H, Deans S: **Antimicrobial agents from plants: antibacterial activity of plant volatile oils.** *Journal of applied microbiology* 2000, **88:**308-316.

36. Ultee A, Bennik M, Moezelaar R: **The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus.** *Applied and environmental microbiology* 2002, **68:**1561-1568.
37. Caballero B, Trugo L, Finglas P: *Encyclopedia of food sciences and nutrition: Volumes 1-10*. Elsevier Science BV; 2003.
38. Dadalioglu I, Evrendilek GA: **Chemical compositions and antibacterial effects of essential oils of Turkish oregano (Origanum minutiflorum), bay laurel (Laurus nobilis), Spanish lavender (Lavandula stoechas L.), and fennel (Foeniculum vulgare) on common foodborne pathogens.** *Journal of agricultural and food chemistry* 2004, **52:**8255-8260.
39. Nabiha B, Faten K, Hervé C, Moncef CM: **Chemical Composition of Bergamot (Citrus Bergamia Risso) Essential Oil Obtained by Hydrodistillation.** *Journal of Chemistry and Chemical Engineering* 2010, **4:**60-62.
40. Gopalakrishnan M: **Chemical composition of nutmeg and mace.** *Journal of Spices and Aromatic Crops* 1992, **1:**49-54.
41. Carson C, Riley T: **Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia.** *Journal of applied bacteriology* 1995, **78:**264-269.
42. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G: **Mechanisms of antibacterial action of three monoterpenes.** *Antimicrobial Agents and Chemotherapy* 2005, **49:**2474-2478.
43. Nikaido H: **Molecular basis of bacterial outer membrane permeability revisited.** *Microbiology and Molecular Biology Reviews* 2003, **67:**593-656.
44. Sikkema J, De Bont J, Poolman B: **Interactions of cyclic hydrocarbons with biological membranes.** *Journal of Biological Chemistry* 1994, **269:**8022-8028.
45. Carson CF, Mee BJ, Riley TV: **Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy.** *Antimicrobial agents and chemotherapy* 2002, **46:**1914-1920.
46. Helander IM, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LG, von Wright A: **Characterization of the action of selected essential oil components on Gram-negative bacteria.** *Journal of agricultural and food chemistry* 1998, **46:**3590-3595.
47. Denyer S, Hugo W: **Biocide-induced damage to the bacterial cyctoplasmic membrane.** *Society for Applied Bacteriology Technical Series* 1991, **27:**171-187.
48. Bagamboula C, Uyttendaele M, Debevere J: **Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and< i> p</i>-cymene towards< i> Shigella sonnei</i> and< i> S. flexneri</i>.** *Food microbiology* 2004, **21:**33-42.
49. Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D: **Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity.** *Journal of Agricultural and Food Chemistry* 2007, **55:**6300-6308.
50. Burt SA, van der Zee R, Koets AP, de Graaff AM, van Knapen F, Gaastra W, Haagsman HP, Veldhuizen EJ: **Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in Escherichia coli O157: H7.** *Applied and environmental microbiology* 2007, **73:**4484-4490.

51. Ultee A: *Bactericidal action of carvacrol towards the food pathogen Bacillus cereus: a case study of a novel approach to mild food preservation.* Wageningen Universiteit; 2000.
52. Pauli A, Kubeczka K-H: **Antimicrobial properties of volatile phenylpropanes.** *Natural product communications* 2010, **5:**1387-1394.
53. Gill A, Holley R: **Disruption of< i> Escherichia coli</i>,< i> Listeria monocytogenes</i> and< i> Lactobacillus sakei</i> cellular membranes by plant oil aromatics.** *International journal of food microbiology* 2006, **108:**1-9.
54. Hemaiswarya S, Doble M: **Synergistic interaction of eugenol with antibiotics against Gram negative bacteria.** *Phytomedicine* 2009, **16:**997-1005.
55. Wendakoon CN, Sakaguchi M: **Inhibition of amino acid decarboxylase activity of Enterobacter aerogenes by active components in spices.** *Journal of Food Protection®* 1995, **58:**280-283.
56. Kwon J, Yu C, Park H: **Bacteriocidal effects and inhibition of cell separation of cinnamic aldehyde on Bacillus cereus.** *Letters in applied microbiology* 2003, **37:**61-65.
57. Hemaiswarya S, Soudaminikkutty R, Narasumani ML, Doble M: **Phenylpropanoids inhibit protofilament formation of Escherichia coli cell division protein FtsZ.** *Journal of medical microbiology* 2011, **60:**1317-1325.
58. Skandamis P, Nychas GJ: **Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres.** *Journal of Applied Microbiology* 2001, **91:**1011-1022.
59. Juven B, Kanner J, Schved F, Weisslowicz H: **Factors that interact with the antibacterial action of thyme essential oil and its active constituents.** *Journal of Applied Microbiology* 1994, **76:**626-631.
60. Tassou C, Drosinos E, Nychas G: **Effects of essential oil from mint (Mentha piperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 and 10 C.** *Journal of Applied Microbiology* 1995, **78:**593-600.
61. Davidson P, Parish M: **Methods for testing the efficacy of food antimicrobials.** *Food technology (USA)* 1989.
62. Jorgensen JH: *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard: NCCLS document M7-A3.* NCCLS; 1993.
63. Moody J: **Synergism testing: broth microdilution checkerboard and broth macrodilution methods.** *Clinical microbiology procedures handbook Washington, DC: American Society for Microbiology* 1992, **5:**22.
64. Schelz Z, Molnar J, Hohmann J: **Antimicrobial and antiplasmid activities of essential oils.** *Fitoterapia* 2006, **77:**279-285.
65. Kim J-S, Kim Y: **The inhibitory effect of natural bioactives on the growth of pathogenic bacteria.** *Nutrition research and practice* 2007, **1:**273-278.
66. Bouhdid S, Abrini J, Zhiri A, Espuny M, Manresa A: **Investigation of functional and morphological changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Origanum compactum essential oil.** *Journal of applied microbiology* 2009, **106:**1558-1568.
67. Pine L, Malcolm G, Brooks J, Daneshvar M: **Physiological studies on the growth and utilization of sugars by Listeria species.** *Canadian journal of microbiology* 1989, **35:**245-254.

68. Gutierrez J, Barry-Ryan C, Bourke P: **The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients.** *International journal of food microbiology* 2008, **124:**91-97.
69. Palaniappan K, Holley RA: **Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria.** *International journal of food microbiology* 2010, **140:**164-168.
70. Cava R, Nowak E, Taboada A, Marin-Iniesta F: **Antimicrobial activity of clove and cinnamon essential oils against Listeria monocytogenes in pasteurized milk.** *Journal of Food Protection*® 2007, **70:**2757-2763.
71. Baranauskienė R, Venskutonis PR, Dewettinck K, Verhé R: **Properties of oregano (< i> Origanum vulgare</i> L.), citronella (< i> Cymbopogon nardus</i> G.) and marjoram (< i> Majorana hortensis</i> L.) flavors encapsulated into milk protein-based matrices.** *Food research international* 2006, **39:**413-425.
72. Singh A, Singh R, Bhunia A, Singh N: **Efficacy of plant essential oils as antimicrobial agents against< i> Listeria monocytogenes</i> in hotdogs.** *LWT-Food Science and Technology* 2003, **36:**787-794.
73. Mytle N, Anderson G, Doyle M, Smith M: **Antimicrobial activity of clove *Syzgium aromaticum* oil in inhibiting *Listeria monocytogenes* on chicken frankfurters.** *Food control* 2006, **17:**102-107.

ABSTRACT

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL EMULSIONS AND POSSIBLE SYNERGISTIC EFFECT ON FOOD BORNE PATHOGENS

by

VARUN TAHLAN

May 2014

Advisor: Dr. Yifan Zhang

Major: Nutrition and Food Science

Degree: Master of Science

The objective of this study was to evaluate the antimicrobial activity of essential oil emulsions against food borne pathogenic bacteria and determine potential applications. The oils used for this study were cinnamon, oregano, clove, thyme, rosemary, sage, bergamot, nutmeg, lemon grass and bay. Oil in water emulsions were prepared using Tween 80 as an emulsifying agent, with a stock oil concentration in the emulsions of 20,000 ppm. Essential oil emulsions were individually screened against *E. coli* (ATCC 25922), *E. coli* (ATCC 700927), *L. monocytogens* (ATCC 19115), *L. innocua* (ATCC 33090) and *S.* Typhimurium (ATCC 19585) using the broth micro dilution method. Cinnamon showed the highest antimicrobial efficacy against all test organisms, as determined by the minimum inhibitory concentration (MIC). Oregano had the second highest efficacy, while the other oils did not exhibit high antimicrobial activities. To determine synergistic effect of the emulsions, combinations were tested using checkerboard method. The only synergism observed was between cinnamon and oregano against *E. coli* (ATCC 700927) and *L. innocua* (ATCC 33090) and also between cinnamon and clove

towards *L. innocua* (ATCC 33090). All other combinations were additive or indifferent in nature to the test organisms. To determine antimicrobial activity of the essential oils on food, chicken pieces were inoculated with the bacteria standardized in CAMHB, and consequently treated with a twofold concentration of the individual *in vitro* MIC of the EOs that expressed synergism. The pieces were placed in 60 mm dishes and stored under refrigeration at 4ºC. Samples were prepared for day 0, day 1, day 3 and day 6 for each bacterial treatment.Cinnamon in comparison to control showed Log reduction of *E. coli* (ATCC 25922), *E. coli* (ATCC 700927), *L. monocytogens* (ATCC 19115), *L. innocua* (ATCC 33090) and *S.* Typhimurium (ATCC 19585) by 2.885, 3.39, 3.275, 4.29 and 3.06. While oregano reduced *E. coli* (ATCC 25922) and *E. coli* (ATCC 700927) by 3.21 and 3.53 Log. All bacterial species showed significant reduction ($p < 0.05$) in comparison to control samples. These results suggest that essential oil emulsions have the potential to be used as antimicrobial agents for enhancing food safety.

AUTOBIOGRAPHICAL STATEMENT

Varun Tahlan, MS

Wayne State University – Detroit, MI

- **Master of Science in Nutrition & Food Science** 2012- Present

M.S. Ramaiah College of Arts, Science and Commerce

Bangalore University - Bangalore, Karnataka, India

- **Master of Sciences in Microbiology** 2008-2010
- **Bachelor of Science in Biochemistry, Microbiology and Genetics** 2005-2008