12-2008

"Intervention Strategies to Enhance the Safety of Ready-to-eat Meat Products by Plant Essential Oils

Chayapa techathuvanan
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Chayapa techathuvanan entitled ""Intervention Strategies to Enhance the Safety of Ready-to-eat Meat Products by Plant Essential Oils." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

David A. Golden, Major Professor

We have read this thesis and recommend its acceptance:

Qixin Zhong, P. Michael Davidson

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Chayapa Techathuvanan entitled "Intervention Strategies to Enhance the Safety of Ready-to-eat Meat Products by Plant Essential Oils." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

David A. Golden
Major Professor

We have read this thesis
and recommend its acceptance:

Qixin Zhong

P. Michael Davidson

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and
Dean of the Graduate School

(Original signatures are on file with official student records.)

INTERVENTION STRATEGIES TO ENHANCE THE SAFETY OF READY-TO-EAT MEAT PRODUCTS BY PLANT ESSENTIAL OILS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Chayapa Techathuvanan

December 2008

ACKNOWLEDGEMENTS

I would like to sincerely thank my major professor, Dr. David A. Golden, for giving me the opportunity to complete my M.S. degree, and for his technical insights, expertise, and guidance throughout this thesis work. I would like to acknowledge Dr. Qixin Zhong and Dr. P. Michael Davidson not only for serving on my M.S. committee members but also for imparting their knowledge, their humanity and guidance during my study. I am also grateful to Dr. William C. Morris and Dr. Faith Johnson Critzer for their support, encouragement, and valuable suggestions.

Moreover, I would like to take this opportunity to express my appreciation to all of the faculty, staff, and students in the Department of Food Science and Technology for creating an academic and friendly work environment.

Most importantly, I am extremely thankful to my parents, Mr. Suravit and Mrs. Phunthip Techathuvanan, who instilled in my dedication, quality, and perseverance, for their endless support and encouragement. Also, special thankfulness is given to Mr. Prachya Mruetusatorn for his love and care. I really appreciate everything they all have sacrificed to get me where I am today.

ABSTRACT

Components of plant essential oil (PEO) extracts are known to have antimicrobial properties. However, their antimicrobial efficacy in food systems is low due to their hydrophobic nature and association with other food components. Incorporation of PEO components into an appropriate carrier may offer a potential solution to improve their activity in food systems. This study was conducted to determine the effect of PEO components (thymol, eugenol, linalool, carvacrol, and cinnamaldehyde) incorporated into zein coating on inactivation of *Listeria monocytogenes* on a ready-to-eat meat model, frankfurters (hot dogs). Hot dogs were inoculated with 7 log CFU/sample and dipped into prepared PEO-zein coatings. Samples were vacuum packaged and stored at 10ºC for 9 days or 4ºC for 4 weeks. Survival of *L. monocytogenes* was investigated by direct plating onto modified Oxford agar. Enrichment using UVM broth and Fraser broth was conducted when the pathogen was not detected by direct plating. Generally, results show that the PEO loaded coatings are effective against *L. monocytogenes* at 10 and 4ºC. When compared to the coating control (zein coating without PEO) at 10ºC, coatings loaded with 10% cinnamaldehyde and 1% carvacrol showed the greatest inhibitory effect and suppressed growth of *L. monocytogenes* by 2.4 and 2.1 log CFU/sample, respectively, after 9 days of storage. At 4ºC, the coating loaded with carvacrol was most effective at suppressing growth of *L. monocytogenes* (1.54-log reduction). No or little dose-response association between PEO concentration and antimicrobial activity was observed in the study. While further research is still required, this study indicates that incorporation of PEOs in corn zein to be used as an edible coating has a high potential to enhance the safety in ready-to-eat meat.

TABLE OF CONTENTS

Chapter **Page**

LIST OF FIGURES

Figure **Page**

LIST OF TABLE

Table **Page**

1. INTRODUCTION

Despite being generally safe products, ready-to-eat (RTE) meat products are subject to be contaminated with the bacterial pathogen *Listeria monocytogenes*. Surface contamination typically occurs post-processing but before packaging. *L. monocytogenes* cells, that are undetectable by direct plating procedures, in vacuum-packaged RTE meats could potentially increase to infective doses during a long refrigerated storage period, since the organism is capable of growth under refrigeration conditions. Several methods have been proposed or utilized with the objective of either reducing the chances for survival of *L. monocytogenes* on RTE meats in the package and/or preventing growth or inactivating the pathogen during storage of RTE meats, including in-package thermal pasteurization, high hydrostatic pressure treatment, etc. Currently, generally recognized as safe (GRAS) antimicrobials, such as sodium lactate and sodium diacetate, and/or antimicrobial food additives, such as nisin, propionic acid, and calcium sulfate are commonly used in the RTE meat industry in order to control growth of *L. monocytogenes*. While effective in some instances, many of the intervention procedures used have drawbacks such as practicality, adverse effects on sensory quality, or high cost.

The most common natural compounds investigated for their antimicrobial efficacy include bacteriocins, such as nisin, natamycin, and pediocin, enzyme such as lysozyme, proteins such as lactoferrin, the polysaccharide chitosan, and essential oils and their constituents. Plant essential oils (PEOs) have been repeatedly shown to have antimicrobial activity. Many studies have determined that the antimicrobial effect of PEOs is mostly from their phenolic components. While those components are effective

antimicrobials, they are also generally amphiphilic, or partially hydrophobic and partially hydrophilic. They interact with or are solubilized by hydrophobic components of foods such as lipids or hydrophobic portions of proteins. This makes them unavailable to react with microorganisms that are either in the water phase or at interfaces, or often does not allow the concentrations necessary to produce significant antimicrobial activity. These challenges may be solved by incorporating antimicrobials such as PEOs in an appropriate carrier enabling a sustained release. Incorporation of PEO components into zein coatings could possibly reduce losses of active components and establish possibilities for prolonged antimicrobial action and improved safety of RTE meat products.

The objectives of this research were to evaluate the antimicrobial effects of PEOs incorporated in zein edible coatings against food and clinical isolates of *L. monocytogenes* on a model RTE meat (hot dogs), and to determine whether storage temperature has an effect in controlling the growth of *L. monocytogenes* on hot dogs coated by zein loaded with PEO coatings.

2. LITERATURE REVIEW

2.1 *Listeria monocytogenes*

2.1.1 Characteristics and Sources of Contamination

L. monocytogenes is a Gram-positive, non-spore forming bacterium. Cells are pleomorphic ovoid to rod-shaped with round ends. Fresh isolates which are in the smooth, pathogenic form appear as short diphtheroid-like rods gauging 1.0-2.0 μm x 0.5 μm when observed through a microscope. However, after 3-5 days of incubation, long rods measuring 6-20 μm are generally found in rough strains (Ryser and Marth, 1991). Possessing peritrichous flagella, *L. monocytogenes* expresses typical tumbling motility at 20-25ºC.

L. monocytogenes is ubiquitous, widely distributed in the environment. Although *L. monocytogenes* is found in a relatively low number in most natural habits, it has been isolated from a variety of sources, including soil, vegetation, fecal material, sewage, and water, where conditions may facilitate higher numbers,.

L. monocytogenes is a facultative anaerobe. It produces acid with no gas from glucose. The optimum temperatures for its growth are between 30- 37^{o}C; nevertheless, it has ability to grow over a temperature range of 0^{o}C and 42^{o}C (Ralovich, 1992). Thus, it is classified as a mesophilic, psychrotrophic bacterium. The organism can grow at pH between 4.5 and 9.6 (Seeliger and Jones, 1986), although the optimal pH is around neutral (pH 7.0); optimal water activity is $\geq$ 0.92 with sodium chloride (NaCl) as the solute (Miller, 1992). In meats, this organism can grow well at pH 6.0 or above and poorly or not at all below pH 5.0 (Glass and Doyle, 1989). While *L. monocytogenes* can

grow well at higher water activities, it is unique in its ability to grow at water activities as low as 0.90. It is tolerant to salt and nitrite (McClure and others, 1997). It is capable of growth at up to 10% NaCl, survives at 20-30% NaCl, and is relatively resistant to CO_2 treatment and several food processing or preservation treatments such as freezing and drying (Harris, 2002; Linton, 1992). *L. monocytogenes* can survive under unfavorable environmental conditions better than most other non-spore forming foodborne pathogens. Therefore, it is a contaminant in a variety of food products and represents a serious problem to the food industry.

2.1.2 Ready-to-eat Meat and Pathogen Relationship

In the United States, *L. monocytogenes* is estimated to account for almost 3,000 foodborne illness cases annually (USDA, Economic Research Service, 2007) at an approximate cost of more than $1 million per case (Cagri and others, 2004). Exposure to this organism by a pregnant woman may lead to stillbirth or miscarriage due to *in utero* exposure of the fetus. In non-pregnant adults, this organism can cause gastroenteritis and the more serious infectious disease, listeriosis, with the second highest mortality rate (20 to 25%) among foodborne infectious diseases (Swaminathan, 2001; CFSAN, 2003).

Despite being generally safe products, ready-to-eat (RTE) meat products are subject to contamination with *L. monocytogenes* on their surfaces after processing. Since *L. monocytogenes* is typically present in the environment, animals may be colonized by consuming contaminated feed or water (Husu and others, 1990). The organism may enter the food plant in the intestines of infected animals, and some strains may survive in biofilms and persist in the processing environment causing contamination of food

products (Giovannacci and others, 1999). *L. monocytogenes* is sensitive to heat and can easily be inactivated by heat treatment. Thus, prevention of recontamination after cooking, but before packaging, is a critical step that needs to be controlled. Due to the ability of *L. monocytogenes* to survive and grow at refrigeration temperatures and tolerate high salt concentrations and the fact that RTE products are designed to be consumed without further cooking or heating, these products can pose high risk to public health (Figure 1).

The cumulative 10-year *L. monocytogenes* prevalences in seven different tested RTE meat and poultry products between 1990 and 1999 listed jerky, 0.52%; cooked, uncured poultry products, 2.12%; large-diameter cooked sausages, 1.31%; small-diameter cooked sausages, 3.56%; cooked beef, roast beef, and cooked corned beef, 3.09%; salads, spreads, and pates, 3.03%; and sliced ham and luncheon meat, 5.16%. The cumulative three-year *L. monocytogenes* prevalence for dry and semidry fermented sausages was 3.25% (Levine and others 2001). As the United States has continued the zero tolerance policy for *L. monocytogenes* since 1985, product recalls due to potential contamination with this organism have been continuously issued. More than 350,000 pounds of RTE meat products, including hot dogs, luncheon meats, cold cuts, fermented and dry sausages, and other deli-style meat and poultry products, were recalled for possible *Listeria* contamination in 2005 (USDA-FSIS, 2005). Recently, approximately 7,000 pounds of RTE turkey products were recalled due to contamination with *L. monocytogenes* (USDA-FSIS, 2007), while the largest product recall, 27.4 million pounds of fresh and frozen RTE poultry products, was issued in 2002 because of potential *L. monocytogenes* contamination (Teratanavat and Hooker, 2004).

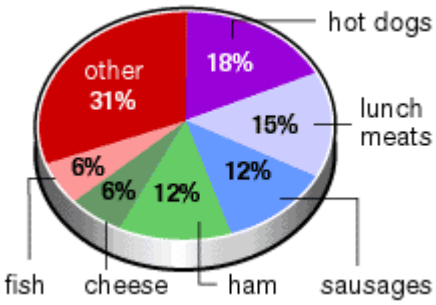


Figure 1. Foods involved in *Listeria* recalls between 1996 and 1999 (Thongson, 2005).

Data collected from published and unpublished outbreak-associated listeriosis cases between 1978 through 2000 emphasize that contaminated meat and dairy products were responsible for more than 90% of cases, and more than 60% of cases were associated with RTE meats (Thongson, 2005). In 1999, the Centers for Disease Control and Prevention (CDC, 1999) reported that 101 cases and 21 deaths caused by a multistate outbreak during 1998 and 1999 were associated to *L. monocytogenes* contamination in frankfurters and deli meats. A multistate outbreak in 2000 was linked to sliced deli turkey meat and resulted in 29 cases, four deaths, and three miscarriages or stillbirths (CDC, 2000). More recently, there were 46 confirmed cases, seven deaths, and three stillbirths or miscarriages associated with a multistate outbreak of *L. monocytogenes* contaminated in the sliceable deli turkey meat (CDC, 2002).

2.2 Plant Essential Oils

Plant essential oils (PEOs), also called volatile or ethereal oils (Guenther, 1948), are aromatic compounds obtained from various parts of plant materials via a variety of processes. Steam distillation is the most commonly used for essential oil production;

expression, fermentation, enfleurage or extraction can also be employed to obtain essential oils (Van de Braak and Leijten, 1999). PEOs can comprise more than 60 individual components (Senatore, 1996; Russo and others, 1998). Some of the main components of PEOs are alcohols, aldehydes, esters, ethers, ketones, phenols, and terpenes (Ouattara, 1997). Primarily, PEOs had been prepared and used in pharmaceutical and medical areas (Guenther, 1948; Bauer and others, 2001; Carson and Riley, 1993). Tea tree oil was used for medical purposes by native Australians at the end of the 18th century (Carson and Riley, 1993). However, during the 19th and 20th centuries, use of PEOs in medicine gradually became secondary to their use for flavor and aroma compounds (Guenther, 1948). Because of their pleasant fragrance, many PEOs have been used in cosmetic products. They have also been used for flavoring foods and beverages for hundreds of years.

2.2.1 Antimicrobial Activities of PEOs

It has long been known that some PEOs have antimicrobial properties (Guenther, 1948; Boyle, 1955; Daferera and others, 2003; Ela and others, 1996; Elgayyar and others, 2001; Reddy others, 1998; Tassou and others, 2000; Shelef, 1983; Nychas, 1995). In addition to antibacterial properties (Deans and Ritchie, 1987; Carson and others, 1995; Mourey and Canillac, 2002), PEOs or their components have shown evidence of antiviral (Bishop, 1995), antimycotic (Azzouz and Bullerman, 1982; Akgül and Kivanç, 1988; Jayashree and Subramanyam, 1999; Mari and others, 2003), antitoxigenic (Akgül and others, 1991; Ultee and Smid, 2001; Juglal and others, 2002), antiparasitic (Pandey and others, 2000; Pessoa and others, 2002), and insecticidal (Konstantopoulou and others,

1992; Karpouhtsis and others, 1998) properties. Many of the studies focused on the *in vitro* antimicrobial activity; however, since the interest of 'green' food consumerism desiring fewer synthetic food additives increases (Burt, 2004), the antimicrobial activity of these compounds on application in food products has been of more interest.

Although the PEOs and their components exhibit a good antimicrobial activity in *in vitro* assays, it has generally been shown that a higher concentration of PEO or its component is required to achieve the equivalent effect in food systems (Shelef, 1983; Smid and Gorris, 1999). This is due to interactions between the phenolic compounds and the food matrix (Nychas and Tassou, 2000). Various PEOs derived from plants used as herbs, spices or infusions in foods have been studied for their inhibitory properties against important foodborne pathogens and food spoilage microorganisms. Oregano, thyme, clove, basil, and coriander oils have shown inhibitory activity against *Escherichia coli, Salmonella* Thyphimurium*, Staphylococcus aureus, L. monocytogenes, Yersinia enterocolitica, Pseudomonas aeruginosa, Aspergillus niger, Geotrichum candidum* and *Rhodothorula* (Conner and Buchat 1984; Ela and others, 1996; Elgayyar and others, 2001; Prudent and others, 1995; Hammer and others, 1999; Burt and Reinders, 2003 Farag and others, 1989; Smith-Palmer and others, 1998; Cosentino and others, 1999). However, some minor components of PEOs such as sage, certain species of thyme, and oregano, have been suggested to play a significant role in antibacterial activity, possibly by producing a synergistic effect between other components (Marino and others, 2001; Lattaoui and Tantaoui- Elaraki, 1994; Paster and others, 1995; Marino and others, 1999). The phenolics and terpenoids appear to be the major active compounds that are chiefly responsible for the antimicrobial properties of PEOs (Cosentino and others, 1999;

Davidson and Naidu, 2000). PEOs containing a high percentage of eugenol, thymol, carvacrol, cinnamaldehyde, and linalool (Figure 2) effectively limit growth of a variety of microorganisms, including *L. monocytogenes, B.acillus cereus,* and *S. aureus* (Lis-Balchin and Deans, 1997; Lis-Balchin and others, 1998). Minimum inhibitory concentrations (MICs) of thymol and carvacrol against *L. monocytogenes* were determined to be in the range of 0.375-5 µl/ml (Cosentino and others, 1999).

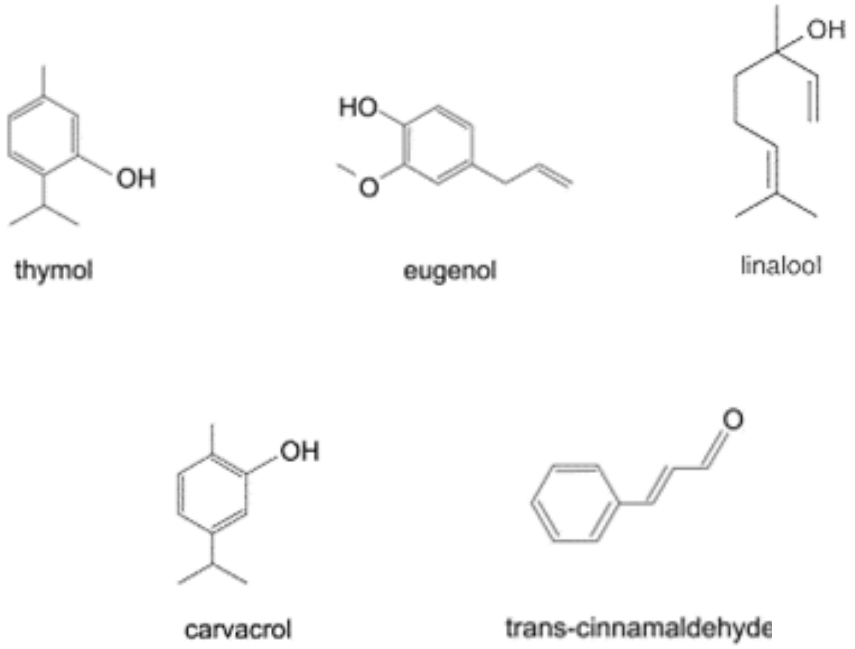


Figure 2. Structural formulae of essential oil components.

(A) Thymol

Dorman and Deans (2000) tested seven individual PEO components against 25 bacterial strains; thymol was shown to have the widest antimicrobial spectrum followed by carvacrol. Thymol at 0.5% concentration was observed to reduce shigellae in lettuce, and at 1.0%, shigellae could not be detected by direct plating (Bagamboula and others, 2004). Thymol was also found to have a higher bactericidal effect on *B. cereus* at 30ºC with increasing concentrations in the range of 0.2-1.0 mmol/l. A synergistic effect was observed to be more inhibitory against microorganisms when thymol and cymene were combined (Delgado and others, 2004). Ettayebi and others (2000) demonstrated a synergism with 0.02% thymol and 40 IU/ml nisin against *L. monocytogenens*. In *B. subtilis*, a similar synergistic effect was also achieved by using a combination of 0.03% thymol and 75 IU/ml nisin.

(B) Carvacrol

Carvacrol was shown to have an antibacterial effect towards *Salmonella* in refrigerated pieces of fish (Hulin and others, 1998). Ultee and others (2000) monitored an obvious reduction of the toxin production by *B. cereus* in brain heart infusion broth with carvacrol. However, approximately 50-fold higher concentrations of carvacrol were required to achieve this same effect in soup. Karatzas and others (2001) investigated the combined antimicrobial action of carvacrol and high hydrostatic pressure (HHP) against *L. monocytogenes* Scott A in semi-skimmed milk at 1, 8, and 20ºC. Carvacrol and HHP acted synergistically and the antimicrobial effects of the combined treatment were greater at lower temperatures. The application of 1.0% carvacrol decreased the number of *Shigella* sp. in lettuce to be below the detection limit (Bagamboula and others, 2004).

(C) Eugenol

Eugenol (a major clove oil component) had a significantly inhibited effect against *L. monocytogenes* in sliced roast beef (Hao and others, 1998a). Hao and others (1998b) also reported a 1-2 log decrease of *L. monocytogenes* on cooked chicken breast treated with 20% eugenol at 5°C during seven days storage. Similarly, clove oil at concentrations of 0.5% and 1% restricted growth of *L. monocytogenes* with 1-3 log reduction in meat and cheese samples at 30 and 7°C (Vrinda Menon and Garg, 2001).

(D) Linalool

Linalool was shown to have an antibacterial activity towards *Shigella flexneri, Shigella sonnei,* and *E. coli* (Bagamboula and others, 2004). Kim and others (1995) reported that linalool exerted a potent inhibitory effect against *E. coli*, *E. coli* O157:H7, *S.* Typhimurium, *Vibrio vulnificus*, and *L. monocytogenes* on agar plates. Moreover, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this PEO constituent were determined. Growth of all the tested bacterial strains except *L. monocytogenes* was completely inhibited by 1000 μg/ml of linalool. Although linalool failed to kill *L. monocytogenes* at 1000 μg/ml, an inhibitory effect was observed.

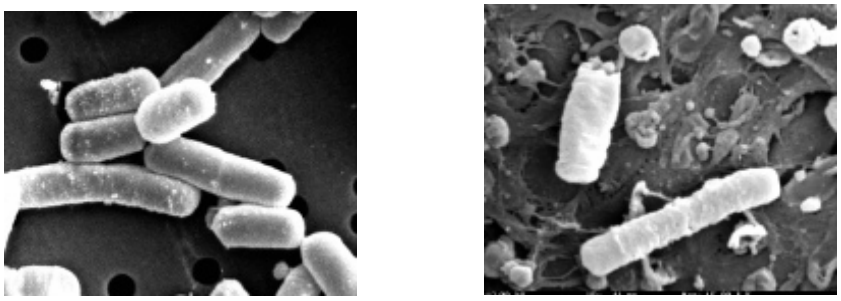
(E) Cinnamaldehyde

Gill and Holley (2004) reported bactericidal activity of cinnamaldehyde against *L. monocytogenes*. Within one hour at 20ºC, *L. monocytogenes* numbers were decreased by greater than 1-log with 30 mM of cinnamaldehyde. The mechanism of action of cinnamaldehyde was investigated, and potentially found to be due to its ability to inhibit the membrane bound ATPase activity of *L. monocytogenes* resulting in bacterial growth limitation (Gill and Holley, 2006).

2.2.2 Mechanisms of Antimicrobial Activity

Although the antimicrobial effects of PEOs are well recognized, the understanding of the mechanisms of activity of these substances is inadequate. Various mechanisms of their inhibitory effects against microorganisms have been continuously explored, including intracellular pH gradient, intracellular ATP, proton motive force, leakage of specific ion, alteration in nucleic acids and amino acids, and structural and functional damage of plasma membrane. Phenolic compounds in PEOs are known to be responsible for the antimicrobial activity against various types of organisms. Such compounds have been suggested to inhibit DNA, RNA, protein, lipid, and polysaccharide synthesis, inhibition of the respiratory chain and electron transfer, and inhibition of substrate oxidation. Uncoupling of oxidative phosphorylation, inhibition of active transport, loss of pool metabolites might also occur (Nes and Eklund, 1983; Denyer, 1990; Nychas, 1995).

The proposed mechanism of antimicrobial activity of phenolic compounds of PEOs is to disrupt the phospholipid cell membrane of bacteria because of their hydrophobic properties. Subsequently, the destabilizing of the membrane, loss of integrity, increase in permeability and leakage of cytoplasm are also possible (Kim and others, 1995; Sikkema and others, 1994, 1995; Weber and de Bont, 1996). Moreover, the interaction of phenolic compositions with enzymes located on the cell wall is also found to be another mechanism of PEOs for inhibiting growth of microorganisms (Farag and others, 1989; Wendakoon and Sakaguchi, 1995; Kreydiyyeh and others, 2000).



Non-treated cells Cinnamaldehyde-treated cells

Figure 3. Scanning electron micrographs of non-treated and cinnamaldehyde-treated *L. monocytogenes* (Shan and others, 2007).

Damage in membrane integrity could further affect pH homeostasis and equilibrium of ions of cells. Proton ion gradients are involved in the proton motive force, the pH gradient (ΔpH) and the electrical potential (Δ ψ) that are employed to generate ATP using ATPase at the membrane (Sikkema and others, 1995; Davidson, 1997). The interference of ion gradients, including H^+, could possibly alter ATP generation, and the ΔpH is also relevant to intra- and extracellular enzyme functions. At bactericidal concentrations, cinnamaldehyde, eugenol, and carbonyl cyanide *m*-chlorophenylhydrazone were found to prevent an increase in cellular ATP upon addition of glucose in non-energized *L. monocytogenes* (Gill and Holley, 2004). Similar results were observed by Helander and others (1998) in *E. coli* exposed to thymol or carvacrol. The possible mechanisms of inhibition of energy generation are inhibition of glucose uptake or utilization of glucose and effects on membrane permeability (Evans and Martin 2000; Gill and Holley, 2004). Cells that lose the ability to generate energy are unable to

reproduce or alter their metabolism to adapt to antimicrobial challenge (Gill and Holley, 2004).

Observation of morphological changes of bacterial cells treated with PEO components, such as cinnamaldehyde (Figure 3), was also demonstrated (Shan and others, 2007).

2.3 Biodegradable Packaging

Packaging is one of the key methods used to protect foods from outside contamination during distribution, transmission, and storage, and to maintain the quality of food products for storage, transportation, and end-use. As a consequence of rapid developments in food technology and the food industry, developments in the field of food packaging technology has been oriented towards processing food products more conveniently, more effectively, at less cost, and with higher quality and safety levels (Han, 2005). Among the many novel inventions, biodegradable coatings and films are notable. As concern for the environment increases, the importance of using biodegradable materials as an alternative to petrochemical-based plastic polymers has increased due to their functionality and environmental-friendly attributes. In addition to their inherent biodegradability and edibility, edible coating and film materials may simplify the total package structure due to their good protective functions (Krochta and De Mulder-Johnston, 1997; Debeaufort and others, 1998).

2.3.1 Edible Coatings and Films

Edible coatings and films are produced from edible biopolymers and food-grade additives (Han, 2005) should be stable before, during and after application (Risch, 2000),

and should have good sensory qualities and good barrier and mechanical properties. Film-forming biopolymers can be proteins, polysaccharides or lipids (Gennadios and others, 1997). Additives, including plasticizers and other active agents, are incorporated with biopolymers in order to improve the physical properties and/or functionality of the final films, depending on the food product. One of the promising applications of active food packaging is the use of edible coatings and films as carriers of active compounds, such as antioxidants, preservatives, antimicrobials, colors, and flavors (Cuq and others, 1995; Day, 1998; Han, 2000, 2001). Edible coatings are also found to provide a barrier against visible and/or UV light, which can alter food characteristics via oxidation reactions (Risch, 2000). Performing as a competent barrier against moisture, oxygen, light, and aroma, an edible coating/film may decrease packaging requirements and the use of multilayer synthetic polymers.

2.3.2 Zein

(A) Characteristics of Zein

Zein is a class of proteins found in corn endosperm. It is non-toxic and white to yellow in color with no odor or taste (Laurila and Bohlen, 2007). Since zein contains a low content of polar amino acids and a high content of non-polar amino acids, prolamines, it is insoluble in water but soluble in aqueous aliphatic alcohol solutions. The prolamines are a group of globular proteins found in grasses, cereal crops such as corn (zein), wheat (gliadin), and oats (avenin). Prolamines contain a high amount of the amino acids proline and glutamine (Figure 4) with only small portions of the amino acids arginine, lysine and histidine (Laurila and Bohlen, 2007).

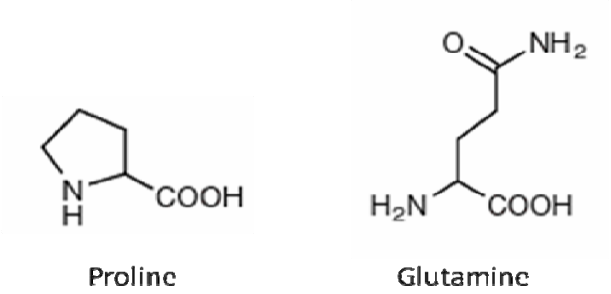


Figure 4. Structures of the amino acids proline and glutamine.

Zein is commercially produced as a by-product of the corn wet-milling industry. A centrifugal separation process of starch from the endosperm slurry leaves corn gluten meal, a protein-rich mass, from which zein is extracted with aqueous alcohol (Padua and Wang, 2002). Based on solubility distinction, zein comprises three proteins fractions, α-zein, β-zein, and γ-zein. The α-zein fraction is a chief component, accounting for 75-85% of total zein (Esen, 1987).

(B) Zein in Coatings and Films

Film-forming properties of zein have been documented for years and are the basis for most commercial utilization of zein (Padua and others, 2000; Andres, 1984). Films may be created by casting, drawing or extrusion techniques (Han, 1999; Lai and Padua, 1997; Reiners and others, 1973). The produced coatings and films have been found to be brittle, thus plasticizers such as glycerin, fatty acids and acetylated monoglycerides (Reiners and others, 1973) are required in order to improve their flexibility. Zein-based films show great potential for uses in edible coatings and biobased packaging (Padua and others, 2000).

Since zein has GRAS (generally recognized as safe) status (Anonymous, 1995), it has been employed as edible coatings and films in medical, pharmaceutical and food industries. Protective coatings prepared from zein are commercially available for use on confectionery products, shelled nuts, and pharmaceutical tablets (Gennadios and others, 1994). Park and others (1994) reported the effect of zein film on delaying ripening and color change in tomatoes during storage, depending on the thickness of film. The barrier properties of this edible material were additionally recognized (Torres and others., 1985a and b; Kester and Fennema 1986; Guilbert 1988; Vojdani and Torres 1989a and b, 1990). Zein coating alone showed a barrier effect against *L. monocytogenes* in cooked sweet corn. After eight days storage at 10°C, the number of *L. monocytogenes* on coated sweet corn was 10-fold lower than non-coated sweet corn (Carlin and others, 2001). Using zein film as a carrier of active agents can also create an efficient antimicrobial edible food packaging. Several studies have been conducted to investigate the inhibitory effects of zein-based coatings and films incorporated with active compounds. Lysozyme, nisin, EDTA, lauric acid, and/or calcium propionate incorporated in zein films demonstrated antimicrobial activity against *Lactobacillus plantarum, E. coli, L. monocytogenes* and *Salmonella* Enteritidis (Padgett and others, 1998; Hoffman and others, 2001; Janes and others, 2002). Janes and others (2002) incorporated nisin in edible zein film coating on chicken and reported a reduction of *L. monocytogenes* counts up to 3.2 log after 24 days at 4°C.

To develop an edible coating or film by incorporating an antimicrobial compound, there are some factors to be considered: (1) The safety of biopolymer and additive has to be approved since they directly contact the food surface; (2) the activity of the

incorporated antimicrobial substance may be affected by storage conditions (e.g., temperature, storing period), and characteristics of food (e.g., pH, water activity); (3) characteristics of the antimicrobial agent, including the solubility, molecular weight, polarity, and ionic strength, can affect the diffusion rate of such active compound from the coating/film into food; (4) characteristics of the biopolymer and film-forming process have an influence on adhesion between the coating and food surface; (5) physical properties of edible coating or film should match the packaging requirements depending on the applications (Min and Krochta, 2007). Because zein is GRAS and has the ability to act as a carrier of small molecules, a wide range of active agents have been applied into zein films and coatings to improve the functionality of edible food packaging.

Incorporation of essential oils into the zein films and coatings may enhance their antimicrobial properties by creating an appropriate environment inside the food package and could possibly reduce losses of active components due to neutralization or diffusion into the bulk of food products, maintain adequate concentrations of preservatives, establish possibilities for prolonged antimicrobial action and improve the safety of food products. Many times, an antimicrobial process may be very effective *in vitro* and have much less efficacy when applied to food products. Using the model system to investigate the effects of certain PEO constituents with a certain application is required in order to expand the knowledge on usage of such naturally occurring additives and food coatings in industrial practices.

3. MATERIALS AND METHODS

3.1 Bacteria Strains and Culture Maintenance

Five strains of *L. monocytogenes* from the University of Tennessee Food Microbiology collection were used in this study: Scott A (clinical isolate), V7 (raw milk isolate), ATCC19115 (human isolate), 310 (goat cheese isolate associated with spontaneous abortion), and 108 (hard salami isolate). Test strains cultured into trypticase soy broth (TSB; Difco Becton Dickinson Microbiology Systems, Sparks, MD) containing 0.6 % (w/v) yeast extract (Difco Becton Dickinson Microbiology Systems, Sparks, MD) at 35°C for 24 h. Cultures were maintained on trypticase soy agar (TSA) slants at 4°C until needed.

3.2 Preparation of Bacterial Suspension

Pure cultures (5 strains) of *L. monocytogenes* were taken from TSA slants and grown in 10 ml of TSB for 24 h at 35°C. Cultures were transferred a minimum of two times at 24 h intervals before use. The five test strains were combined to yield a mixed culture of equal proportions of each test strain immediately prior to use as an inoculum. The mixed culture was diluted in phosphate buffered saline (PBS; pH 7.2; Difco Becton Dickinson Microbiology Systems, Sparks, MD) to achieve a population of approximately 7 log CFU/ml and the diluted culture suspension was used to inoculate hot dogs.

3.3 Preparation and Inoculation of Hot Dog Samples

Commercially produced hot dogs made with mixed chicken and pork (approximately 45 g, 12 cm in length, and 2 cm in diameter per hot dog) were obtained from a local grocery store. Hot dogs were cut in half using a clean knife and tray. Each

piece of hot dog was surface inoculated with 10 spots of 10 µl inoculum biosafety level 2 (BSL-2) safety cabinet. Inoculated hot dogs were covered and held at 4°C overnight to allow the inoculums to dry.

3.4 Preparation of Zein-PEO Coating Solutions

Coating formulations tested on hot dog samples were prepared as stock solutions. Six grams of purified zein (Acros Organics, NJ) were dissolved in 58.5 ml of 85% (v/v) aqueous ethanol prepared in a sterile container at room temperature from 99.5% ethanol (Acros Organics, NJ); 1.5 ml of sterile propylene glycol (Acros Organics, NJ) was gradually added into the solutions. Each PEO component, including thymol (99% purity, Acros Organics, NJ), eugenol (99% purity, Acros Organics, NJ), carvacrol (Fluka Chemie, Sigma-Aldrich, Buchs, Switzerland), linalool (97% purity, Acros Organics, NJ), or *trans*-cinnamaldehyde (99% purity, Acros Organics, NJ), was then mixed thoroughly in the coating solutions at the desired concentration (0.5, 1.0, 1.5, 2.0, 5.0, and 10.0% w/v and v/v for thymol and other PEOs, respectively). The density of PEO components was assumed to be equivalent to the density of pure water. Percentage of PEO components in coating solutions are shown as molar concentrations in Table 1. Homogeneous zein-PEO coating solutions were formed by stirring using sterile magnetic stirrer at room temperature.

PEO (Molecular Weight)	Molarity of PEO in Coating Solution for:					
	0.5%	**1.0%**	**1.5%**	**2.0%**	**5.0%**	**10.0%**
Thymol (150.22)	0.0333	0.6657	0.9985	0.1331	0.3328	0.6657
Eugenol (164.20)	0.0304	0.0609	0.09135	0.1218	0.3045	0.6090
Carvacrol (150.22)	0.0333	0.6657	0.9985	0.1331	0.3328	0.6657
Linalool (154.25)	0.0324	0.06483	0.09724	0.1297	0.3241	0.6483
Cinnamaldehyde (132.16)	0.0378	0.07567	0.1135	0.1513	0.3783	0.7567

Table 1. Molarity of PEO components in coating solutions.

3.5 Coating Process and Storage Conditions

Dried, inoculated hot dogs were carefully dipped into the coating stock solutions for 5 sec to allow the solutions to coat the hot dog surface completely. Excess solutions were allowed to freely drip off the hot dogs surface. The samples were air-dried at ambient conditions inside a BSL-2 safety cabinet for 10 min prior to vacuum packaging with barrier film (Cryovac; Simpsonville, SC) using a Multivac vacuum packaging machine. Individually vacuum sealed hot dogs were stored at 10°C for 9 days and 4°C for up to 28 days, and analyzed at 3-day and 7-day intervals, respectively.

Figure 5. Vacuum packaged, inoculated hot dog with zein-PEO coating.

3.6 Enumeration of Bacteria

A modification of the USDA protocol (USDA protocol, 1998) for *Listeria* was utilized. Hot dogs were transferred into filter-stomacher bags and the package in which hot dogs have been vacuum packaged were rinsed with 100 ml of UVM *Listeria* enrichment broth (Difco Becton Dickinson Microbiology Systems, Sparks, MD), which then was added to stomacher bag. Hot dogs were pummeled in a stomacher blender at medium speed for two minutes. Serial dilutions were made using PBS and spiral plated onto duplicate modified Oxford agar (MOX; Difco Becton Dickinson Microbiology Systems, Sparks, MD) plates followed by incubation at 30°C for 48 h. Typical black colonies on MOX agar were counted and considered as *L. monocytogenes* without further confirmation. The UVM broth/meat packages were incubated for 22 ± 2 h at 30°C, mixed, and 0.1 ml was transferred to 9 ml of Fraser broth (Difco Becton Dickinson Microbiology

Systems, Sparks, MD). Inoculated Fraser broth was incubated for 27 ± 1 h at 35°C and streaked onto MOX agar. The presence of typical black colonies on MOX were considered as a positive test for *L. monocytogenes*. Since the results of the direct plating portion of each analysis time were available before enrichment procedures were complete, enrichment procedures for a given analysis time were not completed if direct plating already indicated a positive finding of *L. monocytogenes*.

Throughout this study, aseptic technique was used at all times. All contaminated materials were contained and subsequently sterilized in an autoclave at121°C for 30 min.

3.7 Statistical Analysis

The inhibitory effects of zein film coatings with PEOs against growth of *L. monocytogenes* on the surface of hot dogs at refrigerator temperatures were statistically analyzed using the mixed models procedure (PROC MIXED) of SAS® 8.2 (SAS Institute Inc.; Cary, NC). Analysis of variance was used to determine statistical differences in survival of pathogens on different coating formulations. Statistical significance was set at $P < 0.05$. All experiments were replicated three times with two samples collected for each treatment per replication.

4. RESULTS AND DISCUSSION

4.1 Overview

The antilisterial activity of individual PEO constituent or antimicrobial packaging loaded PEO component in *in vitro* study has been reported by several earlier works (Guenther, 1948; Boyle, 1955; Daferera and others, 2003; Ela and others, 1996; Elgayyar and others, 2001; Reddy others, 1998; Tassou and others, 2000; Shelef, 1983; Nychas, 1995). The present study revealed the action in controlling the growth of *L. monocytogenes* of zein film coating loaded PEO components in the RTE meat product model, hot dog. Although the pathogen was neither eliminated nor completely inhibited, selected PEO components restricted proliferation of *L. monocytogenes* on hot dogs. The effect was more pronounced with a higher concentration of PEOs as compared to a lower at both 10 and 4ºC. Survival of *L. monocytogenes* in hot dog model coated with zein loaded with various concentrations of PEO components stored at 10ºC and 4ºC is shown in Figures 5-14. Typically, all the coating formulations exhibited an inhibitory effect against *L. monocytogenes* immediately after coating. For the following incubation days, the trends of bactericidal effect, bacteriostatic effect, and bacterial recovery were observed depending on the type and concentration of PEO components in the coating, and storage temperature. Extended storage period of 28 days was conducted in the 4ºC study since the low temperature itself is capable in limiting the growth of *Listeria.* Moreover, a possible synergistic antilisterial effect of zein coating loaded PEO components and lower storage temperature was also expected.

Based upon the dilution and plating scheme used for this study, the limit of detection for *L. monocytogenes* was 3.3 log CFU/hotdog. Therefore, if direct plating yielded no detection, but enrichment was positive, 3.2 log CFU/sample (as opposed to using zero) was used in the data set to make the statistical analysis possible.

4.2 Growth of *L. monocytogenes* populations in Control

For 10 and 4ºC studies, the initial inoculums level on hot dogs was 6.54 and 6.09 log CFU/sample (e.g., per hot dog), respectively. After dipping, *L. monocytogenes* populations on hot dogs treated with water control and coating control (without PEO components incorporated) were 5.79 and 5.15 log CFU/sample for 10ºC, and 5.54 and 5.01 log CFU/sample for 4ºC, respectively. In the water control for both temperatures, of the reduction in bacterial numbers by 0.55-0.75 log CFU likely represents the proportion of cells washed off the hot dog surface by the dipping process. The *L. monocytogenes* populations on hot dogs immediately after dipping in zein coating solution alone were ca. 0.53-0.64 log CFU lower than those dipped in sterile deionized water. Since little or no effect on *L. monocytogenes* growth suppression of zein with propylene glycol film coating was reported (Janes and others, 2002), this reduction in microbial populations was likely caused by the inhibitory effect of ethanol used in the coating formula. CDC (2008) revealed that 60-90% v/v of ethanol is effective against typical pathogens that cause gastroenteritis. Since the concentration of ethanol in all coating formulations in this study was about 82.88% v/v, the inhibitory effect observed is most likely due to the antimicrobial consequence of ethanol in the coating solutions.

During nine days of storage of water control treated samples at 10ºC, *L. monocytogenes* populations increased by 0.85 log CFU/sample. The final bacterial count was observed to be 6.64 log CFU/sample. *L. monocytogenes* on hot dogs coated with the coating control increased by 0.5 log CFU, from 5.15 to 5.65 log CFU/sample, after storage at 10ºC for nine days. When the hot dogs coated with water control were stored at 4ºC, *L. monocytogenes* exhibited slower growth compared to those stored at 10ºC ($P<0.05$). Throughout the study at 4ºC, the *L. monocytogenes* populations in hot dogs coated by water control and coating control did not change ($P<0.05$). These results indicated that *L. monocytogenes* was able to survive, but unable to grow significantly at 4ºC ($P<0.05$). While *L. monocytogenes* is capable of growth at 4°C, the lack of growth on control hot dogs in this study is likely due to the antimicrobial components inherently present in commercial hot dogs. Hot dogs used in this study contained potassium lactate, sodium lactate, sodium diacetate, sodium erythorbate, sodium phosphate, and sodium nitrite. Use of preservative-free hot dogs in a separate study could confirm this theory.

4.3 Inhibition of Pathogen on Hot Dogs by Zein-PEOs Coating Films at 10ºC

In practice, the temperature of many domestic refrigerators is approximately 8ºC and abuse temperatures of up to 15ºC are not uncommon (Alzamora and others, 2000). The temperature 10ºC was chosen to use in this study as it is considered as "mild temperature abuse" at which the generation times of psychrotrophic bacteria are much shorter than those of mesophilic bacteria (Firstenberg-Eden and Tricarico, 1983).

All coating formulations (Figures 6-10) containing selected PEO components used in this study (thymol, eugenol, linalool, carvacrol, and cinnamaldehyde) are

effective in inhibiting the growth of *L. monocytogenes* on tested hot dogs except the coatings loaded 0.5% and 1.0% eugenol (Figure 7). As expected, the antilisterial effect was stronger at the higher concentrations of PEOs.

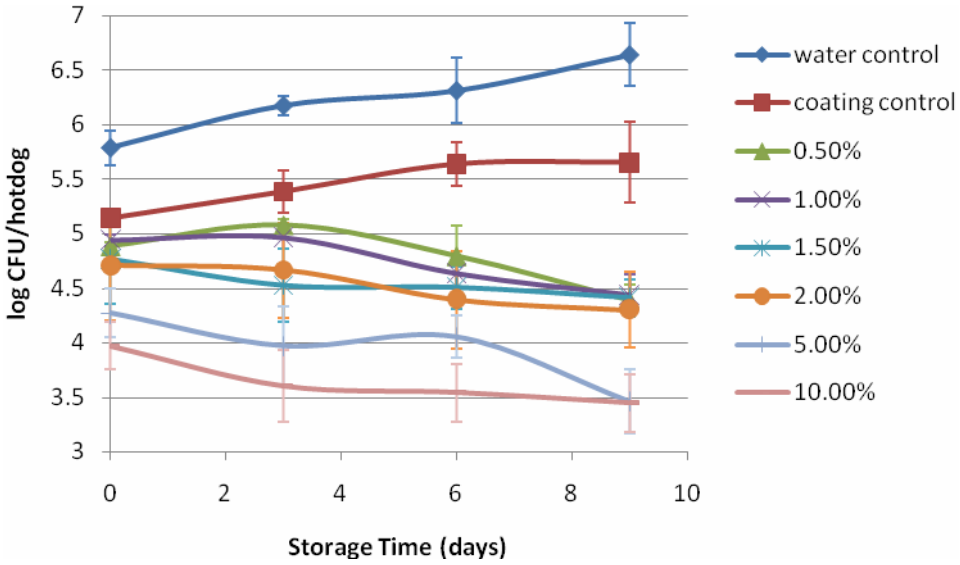


Figure 6. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% thymol at 10ºC.

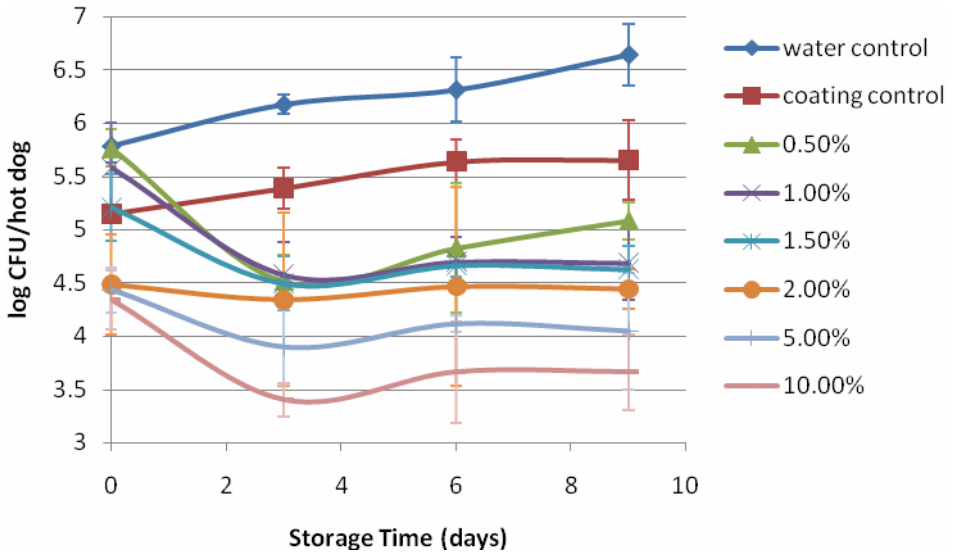


Figure 7. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% eugenol at 10ºC.

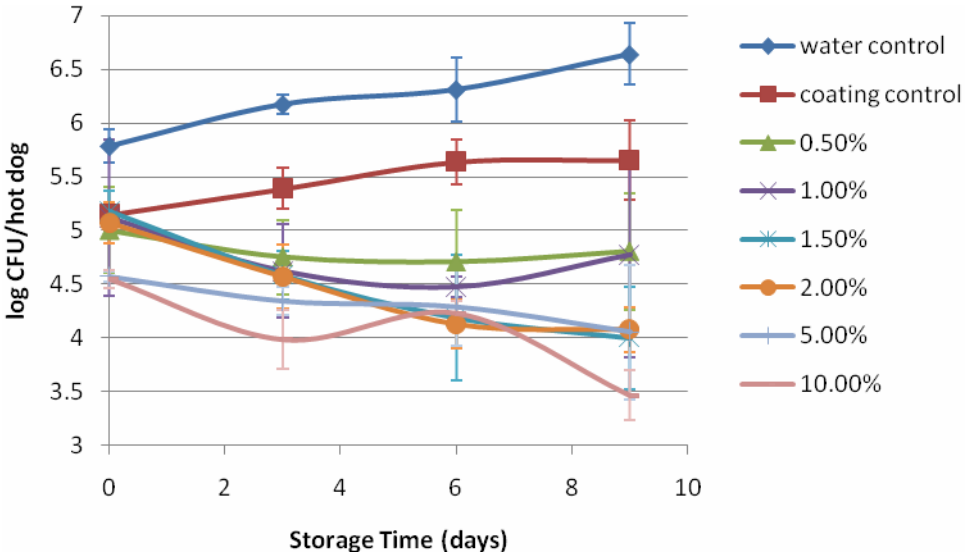


Figure 8. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% linalool at 10ºC.

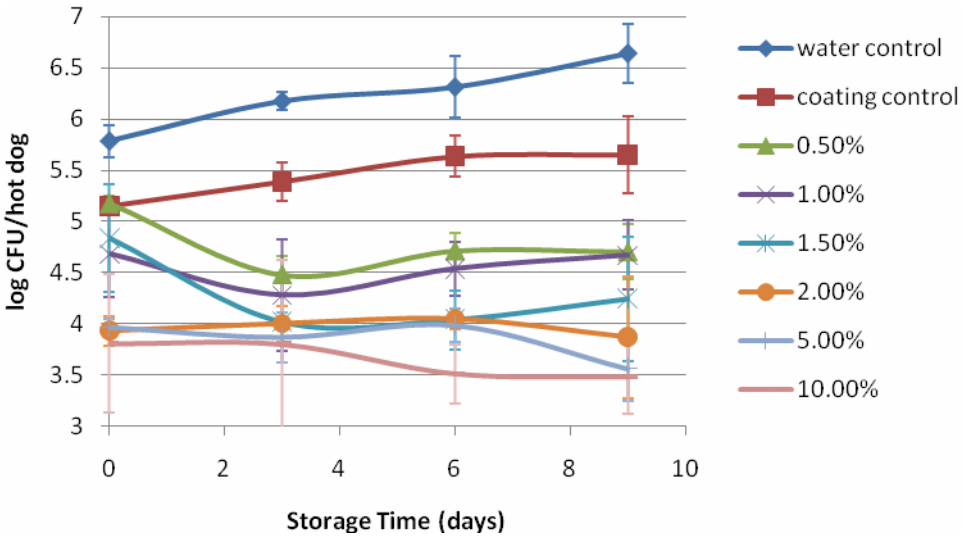


Figure 9. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% carvacrol at 10ºC.

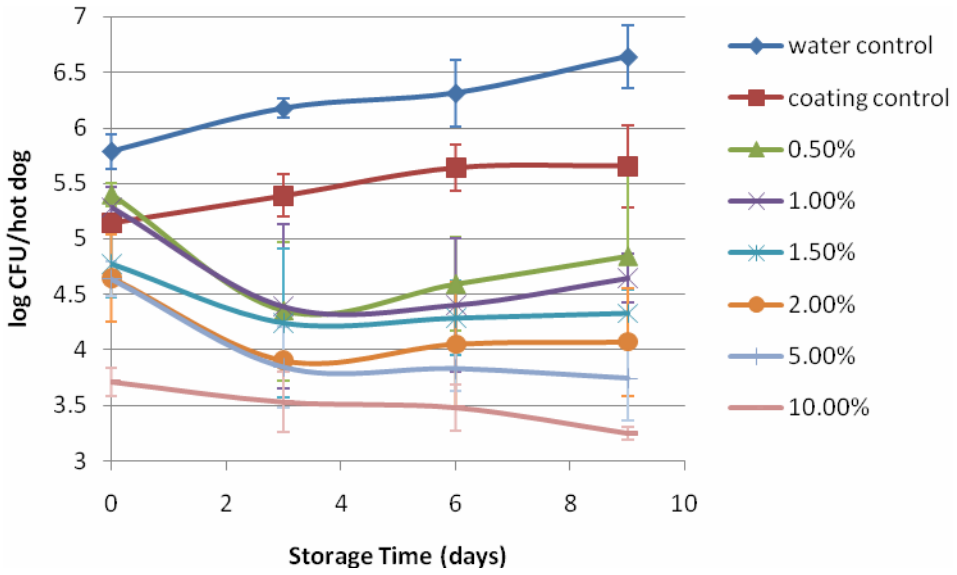


Figure 10. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% cinnamaldehyde at 10ºC.

Figure 5 shows the antibacterial effect of thymol at concentrations of 0.5-2.0%, and greater inhibition was shown with 5.0 and 10.0%. Immediately after dipping, the initial decline in viable counts of *L. monocytogenes* by 0.2-1.2 log CFU compared with the zein coating with no thymol added was observed. After nine days, all concentrations of thymol significantly suppressed the bacterial populations by up to 0.5 log CFU additional to the populations on the day 0 of each treatment. The coatings loaded with thymol in the range of 0.5-5.0% seemingly exhibited the bactericidal action as the pathogen populations kept decreasing.

Although 0.5 and 1.0% of eugenol incorporated in zein coatings limited the growth of *L. monocytogenes*, the effects were not significant ($P<0.05$) as compared with the coating with no PEO component loaded. As shown in Figure 7, eugenol at 0.5-1.5% did not exhibit an antilisterial activity instantaneously after the hot dogs were treated. After three days incubation, a 0.5-1.0 log CFU decrease in bacterial number was observed, depending on the initial number. Subsequently, slight growth of *L. monocytogenes* was observed with the 0.5% eugenol coating, and the number of bacteria was stable with other higher eugenol concentrations from day 3 through 9. The coatings loaded with eugenol in the range of concentration of 1.0-10.0% inhibited growth of *L. monocytogenes* without statistical significance ($P<0.05$.

After six days storage, 1.5, 2.0, 5.0, and 10.0% linalool coatings showed significant bactericidal effects, as compared to coating control, against *L. monocytogenes* by reducing bacterial counts by up to 1.52 log CFU/sample ($P<0.05$) as shown in Figure 8. The final listerial populations in hot dogs coated with such coatings were 4.00, 4.08, 4.05, and 3.46 log CFU/hotdog, respectively. While the coatings containing 1.5, 2.0, 5.0,

and 10% linalool reduced the pathogen numbers throughout the study, it was observed that the listerial populations increased by up to 0.3 log CFU during 6 through 9 days storage on hot dogs coated with zein loaded 0.5 and 1.0% linalool.

A similar listerial growth pattern as found in zein coatings loaded with 0.5 and 1.0% linalool from day 6 to 9 was also observed with zein coatings loaded 0.5, 1.0, and 1.5% carvacrol during day 3 to 9 (Figure 9). The trend of bacterial recovery is shown as the number of *L. monocytogenes* increased. After nine days incubation, the coatings loaded 5.0 and 10.0% carvacrol showed about a 2.1 log CFU reduction in bacterial counts.

It is worth noting that the greatest suppression of *L. monocytogenes* growth among every treatment in the study was determined with the coating loaded 10% cinnamaldehyde at day 9, when *L. monocytogenes* populations decreased by 2.4 log CFU compared to the coating control. As can be seen in Figure 10, like some other PEO components, slight bacterial growth of 0.5 and 0.3 log CFU from day 3 to 9 was observed with the zein coatings with 0.5, and 1.0% cinnamaldehyde, respectively. A bacteriostatic effect was clearly shown when using 1.5, 2.0, and 5.0% cinnamaldehyde, although without significant differences ($P<0.05$), and a slight inhibitory effect was seen when the higher concentration was used. The coating loaded 10% cinnamaldehyde reduced the populations of *L. monocytogenes* continuously throughout the experiment; hence, the higher concentrations are suggested to be tested for their bactericidal activities.

Our results demonstrated that coatings loaded with either cinnamaldehyde or carvacrol expressed the best antimicrobial action against *L. monocytogenes* among all tested coatings, following by thymol. The coating loaded with eugenol or linalool exhibited about the same activity in controlling the growth of *L. monocytogenes*, which is

less than the effect of cinnamaldehyde, carvacrol, and thymol. Voda and others (2004) investigated the anti-fungal properties of 22 PEO components. They theorized that a higher hydrophobic compound has a stronger antimicrobial activity. Among phenolic compounds, including thymol, carvcarol and eugenol, our results showed that zein coatings loaded with carvacrol, which is the most hydrophobic compound among the tested PEO components, showed the strongest antilisterial action against selected 5-strain mixture of *L. monocytogenes* on hot dog surfaces, while eugenol, which is less hydrophobic than carvacrol, expressed weaker antimicrobial effects in our experiment. Another hypothesis derived by Voda and others (2004) is that compounds with two or three oxygen-containing groups exhibit a weaker antimicrobial activity than compounds with only one oxygen-containing group in the aromatic ring. Concurrent with their hypothesis, eugenol loaded in zein coating was found to have only weak activity in limiting the growth of *L. monocytogenes* in this present study.

Typically, the active compound incorporated in the biopolymer diffuses through the polymer matrix out to the environment until a thermodynamic equilibrium between the two phases is achieved. Del Nobile and others (2008) determined the release kinetics of thymol loaded in zein film coating in water solution buffered at pH 7.0. Their results showed that the amount of released thymol increased until equilibrium between thymol in film and outer water solution was reached. This phenomenon may indicate that the higher concentration of PEO incorporated into the film matrix would diffuse out to the surface of hot dog more than the lower concentration in order to maintain the equilibrium between active coating film and food product. Therefore, *L. monocytogenes* on the hot dog coated with high percentage of PEO component in coating was more likely to be

inactivated than the coating with low percentage of PEO component. Due to the large difference of PEO concentration in coating and on hot dog surface at the beginning of the storage, resulting in high diffusion rate of PEO, the substantial decline in *Listeria* populations was observed. Also, a considerably higher concentration of each PEO component was required to achieve a bactericidal effect than to obtain an inhibitory effect during the experiment.

4.4 Inhibition of Pathogen on Hot Dogs by Zein-PEOs Coating Films at 4ºC

At 4ºC, the significant decline in *L. monocytogenes* growth was found instantly after tested bacteria on hot dogs exposed to each treatment of coatings, followed by a steady decrease in bacterial numbers (Figure 11-15). This reflects that each concentration of the PEO components in the coatings have a bactericidal effect against tested pathogen at the beginning of exposure. The stable number of survivors during longer incubation revealed that these bioactive coatings in combination with the refrigerated temperature (and the presence of other preservatives) provide a bacteriostatic effect at this storage temperature.

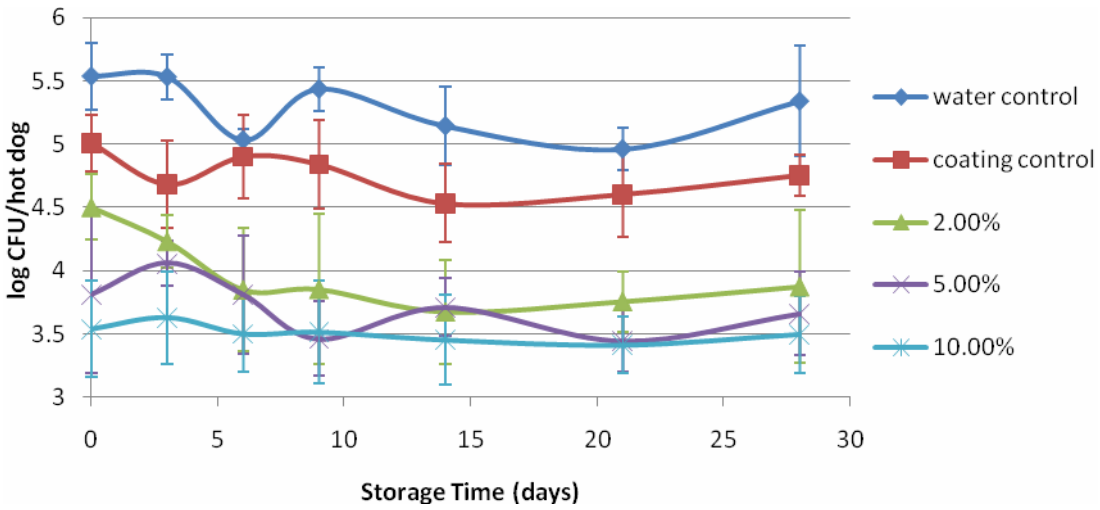


Figure 11. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% thymol at 4ºC.

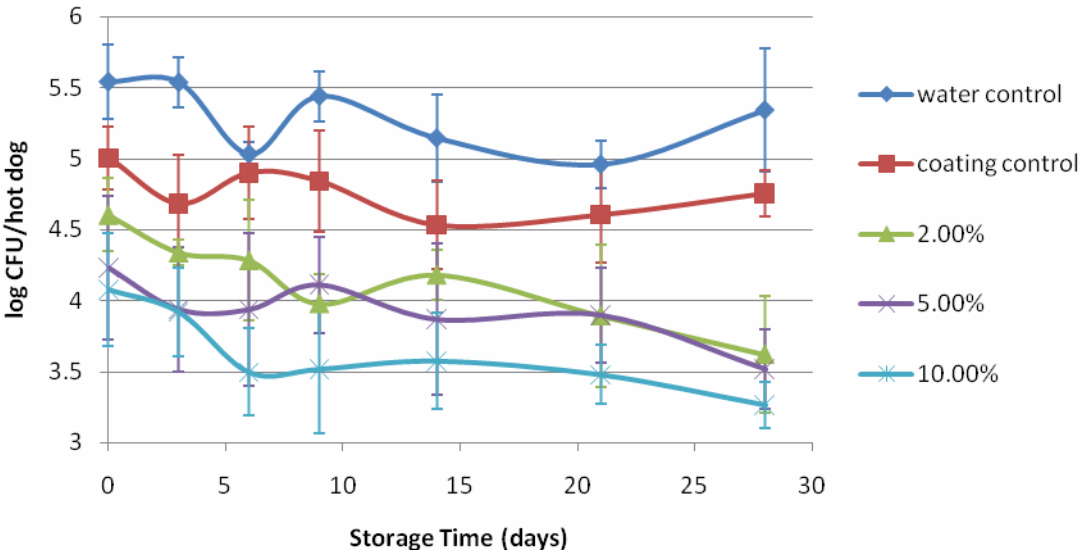


Figure 12. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% eugenol at 4ºC.

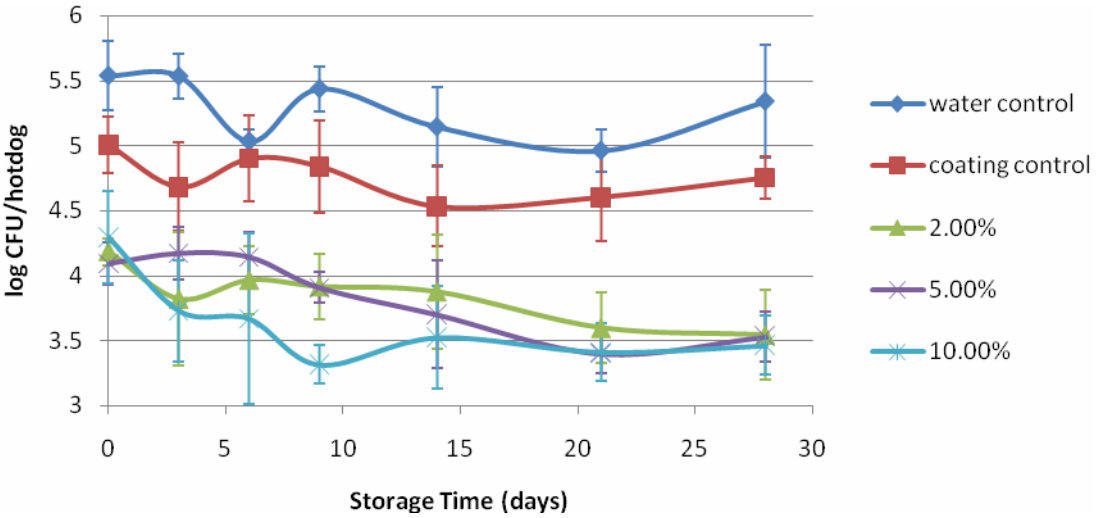


Figure 13. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% linalool at 4ºC.

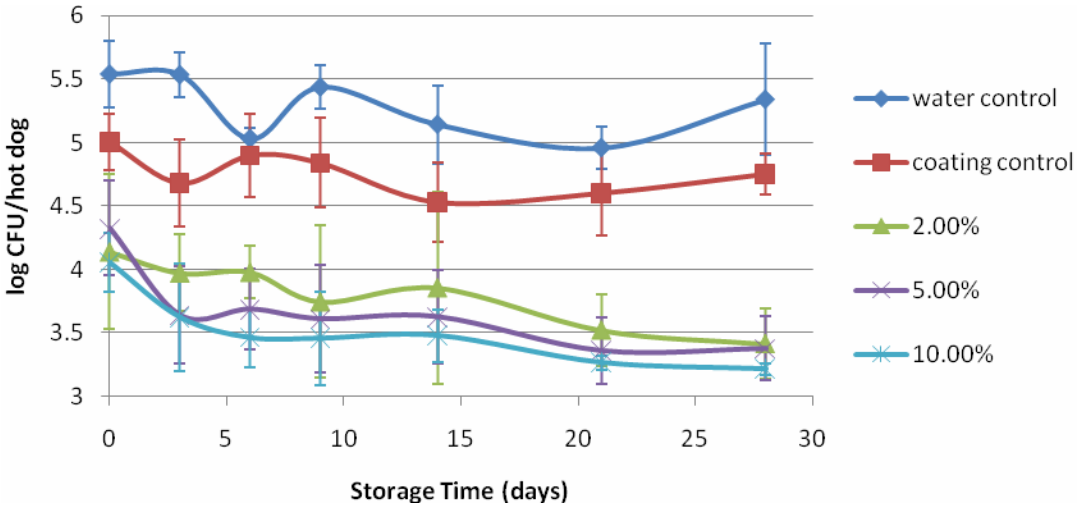


Figure 14. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% carvacrol at 4ºC.

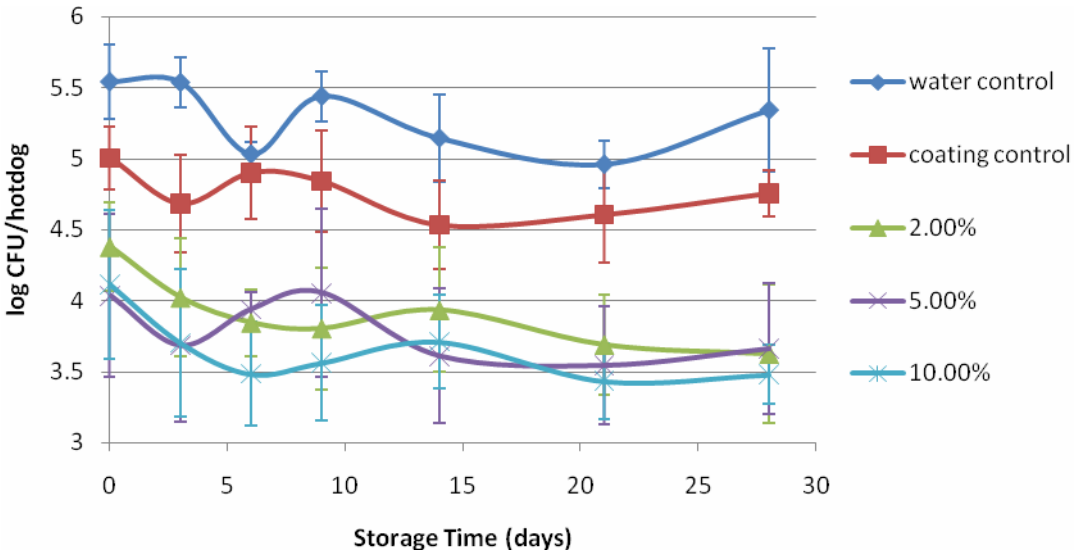


Figure 15. Survival of *L. monocytogenes* on hot dogs coated with zein loaded with 0.5-10.0% cinnamaldehyde at 4ºC.

Figure 11 shows that the coatings loaded with 5.0 and 10.0% thymol expressed a bactericidal effect against *L. monocytogenes* immediately after dipping. The *L. monocytogenes* population instantaneously decreased by 1.19 and 1.46 log CFU, as compared to coating control, and were observed to be 3.81 and 3.54 log CFU/sample once treated with 5.0 and 10.0% thymol coatings, respectively. Hot dogs coated with 2.0% thymol coating tended to reduce the pathogen population, although the effect was insignificant ($P<0.05$). Throughout 28 days storage, bacterial counts on hot dogs coated with every tested concentration were unchanged ($P<0.05$). Similar antimicrobial effects and bacterial growth patterns as observed in hot dogs coated with each concentration of thymol coatings were also found in each concentration of eugenol (Figure 12).

Linalool, carvacrol, and cinnamaldehyde coatings also showed bactericidal actions at day 0 (Figure 13-15). Subsequently, a bacteriostatic effect was observed during the storage period. Although the tested concentrations of PEOs considerably demonstrated differences in the survival populations as compared to coating control, by comparing within each PEO, insignificant different antibacterial effects among individual concentration (2.0, 5.0, and 10.0%) were identified throughout 28 days storage ($P<0.05$).

Based on the results of all tested antimicrobial coatings, even though almost identical curve patterns were observed, the present study showed that carvacrol coating seemed to have the best potential in inhibiting the growth of *L. monocytogenes*. The high hydrophobicity of this compound could be an explanation of the activity as mentioned in the 10ºC results.

4.5 Effect of Storage Temperature

Our results revealed that *L. monocytogenes* can survive but grow at 4ºC on hot dogs used in this study. As can be seen from the results, listerial growth was inhibited more when samples were stored at 4ºC as compared to those stored at 10ºC. At the same 2.0, 5.0, and 10.0% PEO concentrations incorporated in zein coatings for both incubation temperatures, the antimicrobial effect examined at 10ºC was observed to be dependent on the concentration of PEO components, where higher concentration showed the greater effect. On the other hand, the antimicrobial activity was evidently independent of the PEO concentration at 4ºC. It is well recognized that lower temperatures can cause sublethal damage to *L. monocytogenes* cells treated with nisin (Abee and others, 1994; De Martinis and others, 1997; Dykes, 1999; Dykes and Withers, 1999; Ter Steeg, 1999).

Dykes (1999) reported that long-term chill-storage of *L. monocytogenes* at 4°C for four weeks in nutrient-deprived buffers caused visible shrinkage of the cytoplasm and slight cell wall damage when examined by transmission electron microscopy. Solomakos and others (2007) found that minced beef treated with 0.6% of thyme essential oil, 500 or 1000 IU/g of nisin, and their combination at 4ºC resulted in lower decline in *L. monocytogenes* populations than at 10ºC. While generally *L. monocytogenes* was more sensitive to antimicrobial treatment at 4ºC, its survival was not appreciably poorer than the treatment at 10ºC in our study. Synergistic antimicrobial activity of tested bioactive coatings and low storage temperature (4ºC) still remains unclear, and study in this aspect should be further determined, preferably using preservative-free hot dogs.

4.6 Inactivation Mechanisms of PEOs

The PEO components possess an antimicrobial activity against both Gram-positive and Gram-negative bacteria, yet the inactivation mechanisms remain obscure. Several studies were conducted to investigate the mode of action of PEOs. The mechanisms of antimicrobial activity of essential oils have been proposed, including (a) the interference of active compounds of essential oils with the phospholipids bilayer of the cell membrane causing increased permeability and loss of cellular constituents (Lambert and others, 2001; Kim and others, 1995; Sikkema and others, 1994, 1995; Weber and de Bont, 1996); (b) dissipation of the proton motive force, the pH gradient, and the electrical potential ($\Delta\psi$) of cell (Sikkema and others, 1995; Davidson, 1997); (c) interruption of various enzyme systems, including those involved in the production of cellular energy and synthesis of structural components (Farag and others, 1989;

Wendakoon and Sakaguchi, 1995; Kreydiyyeh and others, 2000); and (d) damaging of genetic material (Kim and others, 1995).

The proposed mechanism of antimicrobial activity of phenolic compounds of PEOs is in their attack on the phospholipid cell membrane due to their hydrophobic nature, which causes destabilization of the membrane, loss of integrity, increase in permeability and leakage of cytoplasm (Kim and others, 1995; Sikkema and others, 1994, 1995; Weber and de Bont, 1996), or in their interaction with enzymes located on the cell wall (Farag and others, 1989; Wendakoon and Sakaguchi, 1995; Kreydiyyeh and others, 2000). Thus, the bacterial cell wall plays a major role on the resistance of bacteria against the antimicrobial effect of PEOs. In Gram-positive bacteria, including *L. monocytogenes*, the differences of each strain in cell wall compositions and the thickness of peptidoglycan layer are expected to be associated with different protection. Likewise, the resistance of Gram-negative bacteria to the PEOs may lie in the protective role of their cell wall lipopolysaccharides.

4.7 Ingredient Parameters

The sodium and potassium salts of short-chain organic acids such as citric, acetic, lactic, or their combinations have shown antimicrobial properties (Buchanan et al., 1993; Schlyter et al., 1993; Shelef and Addala, 1994; Shelef et al., 1997; Stekelenburg and Kant-Muermans, 2001; Luchansky, 2005). Sodium, potassium, and calcium lactates are approved for use as flavoring agents, shelf-life extenders, and/or antimicrobials. RTE meat manufacturers commonly use these GRAS ingredients in their meat products as additional hurdles to control or prevent proliferation of *L. monocytogenes* or other

foodborne pathogens (Mbandi and Shelef, 2002). Our selected RTE meat model was a hot dog that contains potassium lactate, sodium lactate, sodium diacetate, sodium erythorbate, sodium phosphate, and sodium nitrite as its ingredients. Therefore, it is important to note that these sodium and potassium salts in our food model may affect the antimicrobial properties of tested antimicrobial coatings either synergistically or antagonistically. Thus, further investigation on an antilisterial effect of zein coating loaded with PEO components without and in combination with these individual salts is recommended.

5. SUMMARY

The antimicrobial efficacy of PEO loaded zein coatings was tested against *L. monocytogenes*. Results showed that the tested antimicrobial coatings are effective against the investigated pathogenic microorganism, although only little in dose-response association between antilisterial effect and PEO concentration in the coating was found. Cinnamaldehyde- and carvacrol-zein coatings exhibited the strongest antimicrobial activity when comparing among tested PEO-zein coatings. However, the sodium and potassium salts in RTE meat model may play a role in inhibitory effect of zein-PEO coatings observed either synergistically or antagonistically. Thus, further research is needed in this area. Variation in microbial inactivation may be partially attributed to operational technique of the coating step, which can vary the number of *L. monocytogenes* washed off.

With the recurring recalls of ready-to- eat meat and poultry products due to contamination with *L. monocytogenes,* there is an obvious need to develop additional methods to prevent the economic loss and possible deaths and illness from foodborne listeriosis infections. Zein coating loaded with PEOs seems to be a promising method to control the growth of *L. monocytogenes* on the surface of RTE meats and prolong the shelf-life of the products by overcoming the major limitation that currently prevents application of PEO components to foods. By combining this bioactive coating use with other antimicrobial treatments, the ability to obtain safe and wholesome RTE products may be improved.

List of References

Abee, T., F.M. Rombouts, J. Hugenholtz, G. Guihard, and L. Letellier. 1994. Mode of action of Nisin Z against *Listeria monocytogenes* Scott A grown at high and low temperatures. Appl Environ Microbiol. 60: 1962-1968.

Akgül, A., and M. Kivanç. 1988. Inhibitory effects of selected Turkish spices and oregano components on some foodborne fungi. Intl J Food Microbiol. 6: 263– 268.

Akgü l, A., M. Kivanç, and S. Sert. 1991. Effect of carvacrol on growth and toxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. Sciences des Aliments. 11: 361–370.

Andres, C. 1984. Natural Edible Coating has Excellent Moisture and Grease Barrier Properties. Food Proc. 45(13): 48-49.

Anonymous. 1995. Substances added directly to human food affirmed as generally recognized as safe (GRAS). Code of Federal Regulations no. 184.1984. 21:511-512. Washington, D.C.: Office of the Federal Register, U.S. Government Printing Office.

Azzouz, M.A., and L.B. Bullerman. 1982. Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. J Food Prot. 45(14): 1298–1301.

Bagamboula, C.F., M. Uyttendaele, and J. Debevere. 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and *p*-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol. 21: 33-42.

Bauer, K., D. Garbe, and H. Surburg. 2001. Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Wiley-VCH, Weinheim, Germany. pp. 293.

Bishop, C.D., 1995. Antiviral activity of the essential oil of *Melaleuca alternifolia* (Maiden and Betche) Cheel (tea tree) against tobacco mosaic virus. J Ess Oil Res. 7: 641–644.

Boyle, W. 1955. Spices and essential oils as preservatives. Am Perfum Essent Oil Rev. 66: 25– 28.

Burt, S.A. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Intl J Food Microbiol. 94: 223– 253.

Burt, S.A., and R.D. Reinders. 2003. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett Appl Microbiol. 36 (3): 162– 167.

Buchanan, R.L., M.H. Golden, and R.C. Whiting. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. J Food Prot. 56: 474– 478.

Cagri, A., Z. Ustunol, and E.T. Ryser. 2004. Antimicrobial edible films and coatings. J Food Prot. 67: 833-848.

Carlin, F., N. Gontard, M. Reich, and C. Nguyen-The. 2001. Utilization of zein coating and sorbic acid to reduce *Listeria monocytogenes* growth on cooked sweet corn. J Food Sci. 66(9): 1385-1389

Carson, C.F., and T.V. Riley. 1993. Antimicrobial activity of the essential oil of *Melaleuca alternifolia*. Lett Appl Microbiol. 16: 49– 55.

Carson, C.F., and T.V. Riley 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol. 78: 264–269.

Center for Food Safety and Applied Nutrition. 2003. Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Washington, D.C.: Center for Food Safety and Applied Nutrition, Food and Drug Administration, U.S. Dept. of Health and Human Services and Food Safety and Inspection Service, U.S. Dept. of Agriculture.

CDC. 2008. OPRP – General Information on Hand Hygiene. Available from: http://www.cdc.gov/nceh/vsp/cruiselines/hand_hygiene_general.htm.
Accessed: June 21, 2008.

Conner, D.E., and L.R. Beuchat. 1984. Effect of essential oils from plants on growth of spoilage yeasts. J Food Sci. 49:429-434.

Cosentino, S., C.I.G. Tuberoso, B. Pisano, M. Satta, V. Mascia, E. Arzedi, and F. Palmas. 1999. *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett Appl Microbiol. 29: 130– 135.

Daferera, D.J., B.N.Ziogas, and M.G. Polissiou. 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium sp.* and *Clavibacter michiganensis* subsp. *michigansis*. Crop Prot. 22: 39-44.

Davidson, P.M. 1997. Chemical preservatives and natural antimicrobial compounds. In *Food Microbiology Fundamentals and Frontiers*, (Doyle, M.P., L.R. Beuchat, and T.J. Montville, ed.). ASM Press, New York, NY. pp. 520-556.

Davidson, P.M., and A.S. Naidu. 2000. Phyto-phenol. In *Natural Food Antimicrobial Systems* (Naidu, A.S., ed.). CRC Press, Boca Raton, FL. pp. 265–294.

Deans, S.G., and G. Ritchie. 1987. Antibacterial properties of plant essential oils. Intl J Food Microbiol. 5: 165– 180.

Debeaufort, F., J.A. Quezada-Gallo, and A. Voilley. 1998. Edible films and coatings: tomorrow's packaging: a review. Crit Rev Food Sci Nutr. 38(4): 299-313.

Delgado, B., P.S. Fernández, A. Palop, and P.M. Periago. 2004. Effect of thymol and cymene on *Bacillus cereus* vegetative cells evaluated through the use of frequency distributions. Food Microbiol. 21: 327-334.

Del Nobile, M.A., A. Conte, A.L. Incoronato, and O. Panza. 2008. Antimicrobial efficacy and release kinetics of thymol from zein films. J Food Eng. 89: 57-63.

Denyer, S.P. 1990. Mechanisms of action of biocides. Intl Biodeterior. 26: 89-100.

De Martinis, E.C.P., A.D. Crandall, A.S. Mazzotta, and T.J. Montville. 1997. Influence of pH, salt, and temperature on nisin resistance in *Listeria monocytogenes*. J Food Prot. 60: 420-421.

Dorman, H.J.D., and S.G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 88: 308–316.

Dykes, G.A., and K.M. Withers. 1999. Sub-lethal damage of *Listeria monocytogenes* after long-term chilled storage at 4°C. Lett Appl Microbiol. 28:45-48.

Dykes, G.A. 1999. Physical and metabolic causes of sub-lethal damage in *Listeria monocytogenes* after long-term chilled storage at 4°C. J Appl Microbiol. 87:915-922

Economic Research Service. 2007. Foodborne illness cost calculator. Available from: http://www.ers.usda.gov/data/foodborneillness/. Accessed: May 16, 2008.

Ela, M.A., N.S. El-Shaer, and N.B. Ghanem. 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. Pharmazie. 51:993-995.

Elgayyar, M., F.A. Draughon, D.A. Golden, and J.R. Mount. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot. 64(7): 1019-1024.

Esen, A. 1987. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zer mays*). J Cereal Sci. 5: 117-128.

Ettayebi, K., J. E. Yamani, and B. Rossi-Hassani. 2000. Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. FEMS Microbiol Lett. 183: 191-195.

Evans, J.D. and S.A. Martin. 2000. Effects of thymol on ruminal microorganisms. Current Microbiol. 41: 336–340.

Farag, R.S., Z.Y. Daw, F.M. Hewedi, and G.S.A El-Baroty. 1989. Antimicrobial activity of some Egyptian spice essential oils. J Food Prot. 52(9): 665– 667.

Firstenberg-Eden, R., and M.K. Tricarico. 1983. Impedimetric determinaton of total, mesophilic and psychrotrophic zounts in raw milk. J Food Sci. 48(6): 1750–1754.

Gennadios, A., T.H. Mchugh, C.L. Weller, and J.M. Krochta. 1994. Edible coatings and films based on proteins. In *Edible Coatings and Films to Improve Food Quality* (Krochta, J.M., E.A. Baldwin, and M. Nisperos-Carriedo, ed.). Technomic Publishing Company, Lancaster, PA. pp. 201–277.

Gill, A. O., and R.A. Holley. 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei.* Appl Environ Microbiol. 70(10): 5750-5755.

Gill, A. O., and R.A. Holley. 2006. Inhibition of membrane bound ATP*ases* of *Escherichia coli* and *Listeria monocytogenes* by plant oil aromatics. Intl J Food Microbiol. 111: 170–174.

Giovannacci, I., C. Ragimbeau, S. Queguiner, G. Salvat, J.L. Vendeuvre, V. Carlier, and G. Ermel. 1999. *Listeria monocytogenes* in pork slaughtering and cutting plants. Use of RAPD, PFGE and PCR-REA for tracing and molecular epidemiology. Int J Food Microbiol. 53:127–40.

Glass, K.A., and M.P. Doyl. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl Environ Microbiol. 55:1565–9.

Guenther, E., 1948. The Essential Oils. D. Van Nostrand, New York.

Guilbert, S. 1988. Use of superficial edible layer to protect intermediate moisture foods: Application to the protection of tropical fruit dehydrated by osmosis. In *Food Preservation by moisture control* (Seow, C.C., ed.). Elvesier Applied Science Publishers, London, UK. pp. 119-219.

Husu, J.R., J.T. Beery, E. Nurmi, M.P. Doyle. 1990. Fate of *Listeria monocytogenes* in orally dosed chicks. Intl J Food Microbiol. 11: 259–69.

Ha, T.T. 1999. Extrusion processing of zein-based biodegradable plastics. Ph.D. Thesis. University of Illinois, Urbana- Champaign, IL. pp. 166.

Hammer, K.A., C.F. Carson, and T.V. Riley. 1999. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 86: 985– 990.

Hao, Y.Y., R.E. Brackett, and M.P. Doyle. 1998. Inhibition of *Listeria monocytogenes* and *Aeromonas hydrophila* by plant extracts in refrigerated cooked beef. J Food Prot. 61(3): 307–312.

Harris, L.J. 2002. *Listeria monocytogenes*. In *Foodborne diseases*, second ed. (Cliver, D.O., and H. Reimann, ed.). Academic Press, San Diego, CA.

Helander, I.M., H. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E.J. Smid, L.G.M. Gorris, and A. von Wright. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agric Food Chem. 46: 3590–3595.

Hoffman, K.L., I.Y. Han, and P.L. Dawson. 2001. Antimicrobial effects of corn zein films impregnated with nisin, lauric acid, and EDTA. J Food Prot. 64(6): 885–889.

Janes, M.E., S. Kooshesh, and M.G. Johnson. 2002. Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein film coatings containing nisin and/or calcium propionate. J Food Sci. 67: 2754–2757.

Jayashree, T., and C. Subramanyam. 1999. Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation. Lett Appl Microbiol. 28: 179– 183.

Juglal, S., R. Govinden, and B. Odhav. 2002. Spice oils for the control of co-occurring mycotoxin-producing fungi. J Food Prot. 65(4): 683–687.

Karatzas, A.K., E.P.W. Kets, E.J. Smid, and M.H.J. Bennik. 2001. The combined action of carvacrol and high hydrostatic pressure on *Listeria monocytogenes* Scott A. J Appl Microbiol. 90: 463-469.

Karpouhtsis, I., E. Pardali, E. Feggou, S. Kokkini, Z.G. Scouras, and P. Mavragani-Tsipidou. 1998. Insecticidal and genotoxic activities of oregano essential oils. J Agric Food Chem. 46: 1111– 1115.

Kester, J.J., O.R. Fennema. 1986. Edible films and coatings--A Review. Food Technol. 40(12): 47-59.

Kim, J., M.R. Marshall, and C. Wei. 1995. Antimicrobial activity of some essential oils components against five foodborne pathogens. J Agric Food Chem. 43:2839-2845.

Kim, J.M., M.R. Marshall, J.A. Cornell, J.F. Preston III, and C.I. Wei. 1995. Antibacterial activity of carvacrol, citral, and geraniol against *Samonella typhimurium* in culture medium and on fish cubes. J Food Sci. 60(6): 1364-1368.

Konstantopoulou, I., L. Vassilopoulou, P. Mavragani-Tsipidou, and Z.G. Scouras, 1992. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *Drosophila auraria*. Experientia. 48(6): 616– 619.

Kreydiyyeh, S.I., J. Usta, and R. Copti. 2000. Effect of cinnamon, clove and some of their constituents on the Na+-K+-ATPase activity and alanine absorption in the rat jejunum. Food Chem Toxicol. 38: 755–762.

Krochta, J. M. and C.D. Mulder-Jphnston. 1997. Edible and biodegradable polymer films. Food Technol. 51: 61-74.

Lai, H-M., and G.W. Padua. 1997. Properties and microstructure of plasticized zein films. Cereal Chem. 74: 771-775.

Lambert, R.J.W., P.N. Skandamis, P.J. Coote, and J.E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 91: 453-462.

Lattaoui, N., and A. Tantaoui-Elaraki. 1994. Individual and combined antibacterial activity of the main components of three thyme essential oils. Rivista Italiana EPPOS. 13: 13–19.

Laurila, K., and M. Bohlen. 2007. Biopolymers as protection during transport of construction materials. M.S. Thesis. Hogskolaniboras, Ingenjorshogskolan Institution, Sweden. pp. 73.

Levine P, B. Rose, S. Green, G. Ransom, and W. Hill. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. J Food Prot 64(8): 1188–1193.

Linton, R.H., J.B. Webster, M.D. Pierson, J.R. Bishop, and C.R. Hackney. 1992. The effect of sublethal heat shock and growth atmosphere on the heat resistance of *Listeria monocytogenes* Scott A. J Food Prot. 55: 284-287.

Lis-Balchin, M., and S.G. Deans. 1997. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J Appl Microbiol. 82: 759-762.

Lis-Balchin, M., G. Buchbauer, K. Ribisch, and M.-T. Wenger. 1998. Comparative antibacterial effects of novel Pelargonium essential oils and solvent extracts. Lett Appl Microbiol. 27: 135–141.

Luchansky, J.B., J.E. Call, B. Hristova, L. Rumery, L. Yoder, and A. Oser. 2005. Viability of *Listeria monocytogenes* on commercially-prepared hams surface treated with acidic calcium sulfate and lauric arginate and stored at 4°C. Meat Sci. 71: 92-99.

Mari, M., P. Bertolini, and G.C. Pratella. 2003. Non-conventional methods for the control of post-harvest pear diseases. J Appl Microbiol. 94: 761–766.

Marino, M., C. Bersani, and G. Comi. 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. J Food Prot. 62(9): 1017– 1023.

Marino, M., C. Bersani, and G. Comi. 2001. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiacea* and *Compositae*. Intl J Food Microbiol. 67: 187–195.

Mbandi, E., and L.A. Shelef. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Intl J Food Microbiol. 76: 191– 198.

McClure, P.J., A.L. Beaumont, J.P. Sutherland, and T.A. Roberts. 1997. Predictive modeling of growth of *Listeria monocytogenes*. The effects on growth of NaCl, pH, storage temperature and $NaNO_2$. Intl J Food Microbiol. 34: 221–32.

Miller, A. 1992. Combined water activity and solute effects on the growth and survival of *Listeria monocytogenes* Scott A. J. Food Prot. 55: 414-418.

Min, S., and J.M. Krochta. 2007. Edible coatings containing bioactive antimicrobial agents. In *Packaging for nonthermal processing of food* (Han, J.H., ed), Backwell Publishing Professional, Ames, IA. pp. 29-51.

Mourey, A., and N. Canillac. 2002. Anti-*Listeria monocytogenes* activity of essential oils components of conifers. Food Control. 13: 289–292.

Nes, I.F., and T. Eklund. 1983. The effect of parabens on DNA, RNA and protein synthesis in Escherichia coli and Bacillus subtilis. J Appl Bacteriol. 54: 237–242.

Nychas, G.J.E. 1995. Natural antimicrobials from plants. In *New Methods of Food Preser*V*ation.* (Gould, G.W., ed.). Blackie Academic & Professional, London. pp 58-89.

Nychas, G.E., and C.C. Tassou. 2000. Traditional preservatives—oils and spices. In *Encylopedia of Food Microbiology* (Robinson, R.K., C.A. Batt, and P.D. Patel, ed.). Academic Press, London, UK. pp. 1717–1722.

Ouattara, B, R.E. Simard, R.A. Holey, G.J.P. Piette, and A. Begin. 1997. Antimicrobial activity of selected fatty acids and essential oils against six meat spoilage organisms. Intl J Food Microbiol. 37:155-162.

Padgett, T., I.Y. Han, and P.L. Dawson. 1998. Incorporation of foodgrade antimicrobial compounds into biodegradable packaging films. J Food Prot. 61: 1330–1335.

Padua, G.W., and Q. Wang. 2002. In *Protein-Based Films and Coatings* (A., Gennadios, ed.). CRC Press, Boca Raton, FL. pp. 43-67.

Padua, G.W., A. Rakoronirainy, and Q. Wang. 2000. Zein-based biodegradable packaging for frozen foods. Proceedings of the Food Biopack Conference, 27 – 29 August 2000, Copenhagen, Denmark, pp. 84 – 88.

Pandey, R., A. Kalra, S. Tandon, N. Mehrotra, H.N. Singh, and S. Kumar. 2000. Essential oil compounds as potent source of nematicidal compounds. J Phytopathol. 148 (7–8): 501–502.

Park, H.J., M.S. Chinnan, and R.L. Shewfelt. 1994. Edible corn-zein film coatings to extend storage life of tomatoes. J Food Proc Preserv. 18: 317–331.

Paster, N., M. Menasherov, U. Ravid, and B. Juven. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J Food Prot. 58(1): 81– 85.

Pessoa, L.M., S.M. Morais, C.M.L. Bevilaqua, and J.H.S. Luciano. 2002. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. Vet Parasit. 109 (1– 2): 59–63.

Prudent, D., F. Perineau, J.M. Bessiere, G.M., Michel, and J.C. Baccou. 1995. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.)—evaluation of its bacterioatatic and fungistatic properties. J Ess Oil Res. 7: 165– 173.

Ralovich, B. 1992. Data for the properties of *Listeria* strains—A Review. Acta Microbiol Hung. 39:105–32.

Reddy, M.V.B., P. Angers, A. Gosselin, and J. Arul. 1998. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonorifer* in strawberry fruits. Phytochem. 47(8): 1515-1520.

Reiners, R.A., J.S. Wall, and G.E. Inglett. 1973. Corn proteins: Potential for their industrial use. In *Industrial Uses of Cereals* (Pomeranz, Y., ed.), St. Paul, MN: American Association of Cereal Chemists. pp. 285-298.

Russo, M., G.C. Galletti, P. Bocchini, and A. Carnacini. 1998. Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): A preliminary evaluation of their use in chemotaxonomy by cluster analysis: 1. Inflorescences. J Agric Food Chem. 46: 3741– 3746.

Ryser, E.T. and E.H. Marth. 1991. *Listeria*, Listeriosis and Food Safety. , Marcel Dekker, New York.

Schlyter, J.H., K.A. Glass, J. Leoffelholz, A.L. Degnan, and J.B. Luchansky. 1993. The effect of diacetate with nitrite, lactate or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Intl J Food Microbiol. 19: 271– 281.

Seelinger HPR, Jones D. 1986. Genus Listeria. In *Bergey's manual of systemic bacteriology* (Sneath, P.H.A., N.S. Mair, G. H.J. Sharpe ME, ed.). Williams and Wilkins, Baltimore, MD. pp. 1235–1245.

Senatore, F., 1996. Influence of harvesting time on yield and composition of the essential oil of a thyme (*Thymus pulegioides* L.) growing wild in Campania (Southern Italy). J Agric Food Chem. 44: 1327– 1332.

Shan, B., Y.Z. Cai, J.D. Brooks, and H. Corke. 2007. Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (*Cinnamomum burmannii*): Activity against Foodborne Pathogenic Bacteria. J Agric Food Chem. 55: 5484-5490.

Shelef, L.A., 1983. Antimicrobial effects of spices. J Food Safety. 6: 29– 44.

Shelef, L.A., and L. Addala. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. J Food Saf. 14: 103– 115.

Shelef, L.A., S. Mohammed, W. Tan, and M.L. Webber, 1997. Rapid optical measurements of microbial contamination in raw ground beef and effects of citrate and lactate. J Food Prot. 60: 673– 676.

Sikkema, J., J.A.M. de Bont, and B. Poolman.1994. Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem. 269: 8022–8028.

Sikkema, J., J.A.M. de Bont, and B. Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev. 59: 201-222.

Smid, E.J., and L.G.M. Gorris. 1999. Natural antimicrobials for food preservation. In: Handbook of Food Preservation (Rahman, M.S., ed.). Marcel Dekker, New York, pp. 285–308.

Smith-Palmer, A., J. Stewart, and L. Fyfe. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett Food Microbiol. 26: 118–122.

Stekelenburg, F.K., and M.L.T. Kant-Muermans. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of Lactobacillus curvatus and Listeria monocytogenes. Intl J Food Microbiol. 66: 197–203.

Swaminathan, B. 2001. *Listeria monocytogenes*, p. 383-410. In *Food microbiology: fundamentals and frontiers*, 2nd ed. (M. P. Doyle, L. R. Beuchat, and T. Montville, ed.). ASM Press, Washington, D.C.

Tassou, C., K. Koutsoumanis, and G.J.E. Nychas. 2000. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Res Intl. 33: 273-280.

Teratanavat, R., and N.H. Hooker. Understanding the characteristics of US meat and poultry recalls: 1994–2002. Food Control. 15: 359–367.

Ter Steeg, P.F., J.C. Hellemons, and A.E. Kok. 1999. Synergistic actions of nisin, sublethal ultrahigh pressure, and reduced temperature on bacteria and yeast. Appl Environ Microbiol. 65:4148-4154.

Thongson, C. 2005. Antimicrobial activity of Thai spices against foodborne pathogens. Ph.D. Dissertations. Kasetsart University, Bangkok, Thailand.

Torres, J.A., M. Motoki, and M. Karel. 1985a. Microbial stabilization of intermediate moisture food surfaces. I Control of surface preservative concentration. J Food Proc Preserv. 9:75–92.

Torres, J.A., J.O. Bouzas, M. Karel. 1985b. Microbial stabilization of intermediate moisture food surfaces. II. Control of surface pH. J Food Process Pres 9:93- 106.

Ultee, A., R.A. Slump, G. Steging, and J. Smid. 2000. Antibacterial activity of carvacrol towards *Bacillus cereus* on rice. J Food Prot. 63(5): 620–624.

Ultee, A., and E.J. Smid. 2001. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. Intl J Food Microbiol. 64: 373–378.

USDA-FSIS. 2005. Michigan firm recalls ready-to-eat meat products for possible *Listeria* contamination. Available from: http://www.fsis.usda.gov/PDF/Recall_025_2005_Release.pdf. Accessed: May 16, 2008.

USDA-FSIS. 2007. California firm recalls ready-to-eat turkey products for possible *Listeria* contamination. Available from: http://www.fsis.usda.gov/News_&_Events/Recall_021_2007_Release/index.asp. Accessed: May 16, 2008.

USDA protocol. 1998. Chapter 8. Isolation and identification of *Listeria monocytogenes* from meat, poultry and egg products. Microbiology Laboratory Guidebook., 3rd Edition.

Van de Braak, S.A.A.J., and G.C.J.J. Leijten. 1999. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, pp. 116.

Voda, K., B. Boh, and M. Vrtačnik. 2004. A quantitative structure–antifungal activity relationship study of oxygenated aromatic essential oil compounds using data structuring and PLS regression analysis. J Mol Model. 10: 76-84.

Vojdani, F., and J. A. Torres. 1989a. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose multi-layer films. J Food Process Pres. 13: 417-430.

Vojdani, F., and J. A. Torres. 1989b. Potassium sorbate permeability of polysaccharide films: chitosan, methylcellulose and hydroxypropyl methylcellulose. J Food Process Eng. 12:33-48.

Vojdani, F., and J. A. Torres. 1990. Potassium sorbate permeability of methylcellulose and hydroxypropyl methylcellulose coatings: Effect of fatty acids. J Food Sci 55:841-846.

Vrinda Menon, K., and S.R. Garg. 2001. Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. Food Microbiol. 18: 647– 650.

Weber, F.J., and J.A.M. de Bont. 1996. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. Biochimica et Biophysica Acta 1286: 225–245.

Wendakoon, C.N., and M. Sakaguchi. 1995. Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J Food Prot. 58:280-283.

VITA

Chayapa Techathuvanan was born in Bangkok, Thailand on June 8, 1984. She received her B.S. in Food Science and Technology from Kasetsart University, Bangkok, Thailand in 2006. In August 2006, she began the M.S. program in Food Science and Technology focusing in Food Microbiology at the University of Tennessee at Knoxville, Tennessee and graduated from this program in December 2008. She has continued her education at the University of Tennessee pursuing her doctorate in Food Microbiology.