Rochester Institute of Technology

RIT Digital Institutional Repository

Theses

5-18-2012

Evaluation of natural plant powders with potential use in antimicrobial packaging applications

Yujie Cheng

Follow this and additional works at: https://repository.rit.edu/theses

Recommended Citation

Cheng, Yujie, "Evaluation of natural plant powders with potential use in antimicrobial packaging applications" (2012). Thesis. Rochester Institute of Technology. Accessed from

This Thesis is brought to you for free and open access by the RIT Libraries. For more information, please contact repository@rit.edu.

R·I·T

Evaluation of Natural Plant Powders with Potential Use in Antimicrobial Packaging Applications

By

Yujie Cheng

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Masters of Science

Department of Materials Science and Engineering
College of Science
Rochester Institute of Technology
Rochester, NY
May 18, 2012

Committee Approval:

K. S. V. Santhanam Date
Director of Materials Science and Engineering
Thesis Advisor

Changfeng Ge Date
Thesis Advisor

Jeffrey Lodge Date
Committee Membe

Abstract

This study investigates the antimicrobial effects of vanillin, turmeric and curcumin in packaging coating application. Regent alcohol and DMSO were introduced as two solutions for the three antimicrobial agents. The coating solutions involving these three antimicrobial agents are: vanillin/ Reagent alcohol (10, 5, 2.5 and 1.25% (w/w)), vanillin/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)), turmeric/ Reagent alcohol (10, 5, 2.5 and 1.25% (w/w)), turmeric/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)), curcumin/ Reagent alcohol (10, 5, 2.5 and 1.25 (w/w)) and curcumin/DMSO (10, 5, 2.5, 1.25, 0.625 and 0.3125% (w/w)). The antimicrobial activity effects of the aforementioned coating solutions were investigated for five types of common pathogens and food spoilage bacteria: *Staphylococcus aureus* and *Listeria monocytogenes* representing gram-positive bacteria; *Shigella sonnei*, *Salmonella typhimurium* as well as *E.coli O157:H7* representing gram- negative bacteria. Significant antimicrobial effects for gram-positive bacteria were observed for curcumin and turmeric in their Reagent alcohol and DMSO solutions. It is also noticed that *E.coli O157:H7* was more sensitive over vanillin/ Reagent alcohol solution, however, *Listeria monocytogenes* was more sensitive over vanillin/ DMSO solutions. The factors that influence the solution's antimicrobial activity were studied. Turmeric is chosen as the antimicrobial agent to be incorporated into polymers. In order to study the antimicrobial effect as packaging application, two types of the polymer based turmeric products were evaluated. They are LDPE based turmeric pellets produced by a single screw extruder, and turmeric coated BOPP films.

Acknowledgements

I would like to express my sincere gratitude to all people that helped me during my project. I would like to thank my advisor, Professor K. S. V. Santhanam and Associate Professor Changfeng Ge for their support, guidance and encouragement. I warmly thank to Associate Professor Jeffrey Lodge, for his assistance with microbiological techniques and many times answered questions related to this study.

I am grateful to Mr. Kai Lei for his assistance with the development of the LDPE based turmeric pellets and turmeric coated BOPP films. I thank all the laboratory staff, especially Leslie Gregg for all her technical support and friendship.

I express my deepest thanks to my parents, uncle, brother and sister for the support and encouragement for which I am very grateful.

Publications Arising from this Work

Journal Papers

Yujie Cheng, Changfeng Ge, Jeffrey Lodge & K.S.V.Santhanam. (2011). Evaluation Study of Vanillin, Curcumin and Turmeric with Potential Use in Antimicrobial Packaging Applications. *Journal of Applied Packaging Research,* **5(4)**, 215-226.

Table of Contents

Abstract i

Acknowledgements ii

Publications Arising from this Work iii

1 Introduction 1

1.1. Background 1

1.2. Active packaging 2

1.3. Antimicrobial packaging 4

1.4. Antimicrobial agents 5

1.5. Antimicrobial packaging system 7

1.6. Aims 9

1.7. Scope of work 10

2 Literature review 11

2.1. Progress in antimicrobial packaging 11

2.2. Types and uses of antimicrobial agents 13

2.3. Natural plant products as antimicrobial agents 15

2.3.1. Antimicrobial activity in plant essential oils 16

2.3.2. Antimicrobial activity in plant extracts from roots and seeds 17

2.3.3. Properties and uses of vanillin 18

2.3.4. Properties and uses of turmeric 20

2.3.5. Properties and uses of curcumin 22

2.4. Development in polymers and packaging 23

3 Materials and methods 26

3.1. Antimicrobial agent in solutions 26

3.1.1. Materials and methods 26

3.1.2. Antimicrobial testing 28

3.1.3. Data analysis 28

3.2. Antimicrobial agent in polymers 29

3.2.1. Materials and methods 29
3.2.2. Antimicrobial testing 30
3.2.3. Data analysis 31
4 Results and discussion 32
4.1. Antimicrobial agent in solutions 32
4.1.1. Antimicrobial Effects of Vanillin, Turmeric and Curcumin in Reagent alcohol Solution 33
4.1.2. Antimicrobial Effects of Vanillin, Turmeric and Curcumin in DMSO Solution 40
4.1.3. Comparison of the antimicrobial activity of agents in Reagent alcohol and DMSO 46
4.1.4. The influence of concentration on antimicrobial activity over each bacterial 51
4.1.5. The Minimum Inhibitory Concentration (MIC) 52
4.1.6. Combination of powder and solvent 53
4.2. Polymers containing antimicrobial agents 53
4.2.1. Turmeric containing LDPE discs 53
4.2.2. Turmeric coated BOPP films 54
5 Conclusions and Future work 59
5.1. Conclusions 59
5.1.1. Effect of natural powders 59
5.1.2. Effect of solvents 60
5.1.3. Combination of powder and solvent 62
5.1.4. Effect of turmeric incorporated polymer 62
5.2. Future work 63
5.2.1. Solvent properties 63
5.2.2. Antimicrobial susceptibility of bacteria by survival analysis 63
5.2.3. Polymer incorporated antimicrobial agents 63
Reference 65
Appendix A Material Properties 70

1 Introduction

In this chapter a brief review of the developments in food packaging and new food packaging technologies are presented with a special emphasis on antimicrobial packaging. The concept of active packaging is introduced with an emphasis on antimicrobial additives from natural plants extract. Additionally, current studies on natural plants extract with antimicrobial activity and the future development in antimicrobial packaging are also discussed.

1.1. Background

Food quality and safety are major concerns in the food industry. Many food contaminations are due to undesirable microbes such as bacteria and yeast. During distribution and transportation, the quality of food products can deteriorate biologically, chemically and physically (Han, 2005). One main role of food packaging materials is to control the mass and flavor lost during transportation as well as to extend the shelf life until consumption. Therefore, the food industry wants packaging materials that contribute to extending the shelf life and maintaining the quality and the safety of the food products.

Year after year, packaging technology has broader applications and has become better. Food packaging has evolved from a simple preservation method to a more complex one, and its functionality has changed from single to multiple. In the past, the main trend in

food packaging was to focus on lightweight, source reduction and energy saving in the 1970s. People were more concerned about the effectiveness and cost of packaging materials initially. However, from the 1980s to the present, most developments in the area of food technology have become more complicated, which not only relates to cost and functionality but also environmental issues and food safety.

Nowadays, many novel packaging technologies have arisen, which is leading to higher standards of regulation, hygiene, health and safety. The most remarkable new functional packaging systems include modified atmosphere packaging (MAP), active packaging and edible films/coating (Han, 2005). Among those technologies, active packaging, which has the same basic function as traditional packaging systems, has changed the condition of packaged food products and is designed to improve safety and/or enhance sensory properties in food and beverages, while protecting our environment.

1.2. Active packaging

Active packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product (P.Suppakul *et al*., 2003). The term “active packaging” was first applied by Labuza in 1987 (Rooney, 2005) and was introduced as recently as two decades ago. Labuza and Breene in 1988 defined active packaging as technologies, which involve interactions between the food, the packaging material, and the internal gaseous atmosphere.

Active packaging is designed to perform a role other than to provide an inert barrier between the product and the outside environment, using the possible interactions between food and package in a positive way to improve product quality and acceptability (Preeti *et al*., 2011). Various kinds of active substances can now be incorporated into the packaging material to improve its functionality and give it new or extra functions including oxygen scavenging, antimicrobial activity, moisture scavenging, ethylene scavenging, ethanol emitting, etc. (Preeti *et al*., 2011). The decision to consider active packaging is usually based on factors including economic advantage, process engineering limitations, time-dependent processes, secondary effects and environmental impacts (Rooney, 2005). Some of the driving forces in active packaging have been directed toward enhancing a limiting characteristic of packaging materials, however, others have brought a novel function to such technology to allow protection and/or preservation of the food (Boca Raton, 2007).

Food packaging technology has been developed over the past few decades to satisfy consumer demands relating to more natural forms of preservation, and methods to control packaging and storage for assurance and food safety. Active packaging is certainly one of the most impressive innovations in this particular field. Such importance is related to the area of fresh and extended shelf life of food products as originally described by Labuza and Breene in 1989. Some new definitions on active packaging materials were also presented by the European Union in 2004.They defined active packaging material as a kind of material, which is designed to deliberately incorporate components that will release or absorb substances into or from the packaged food or the environment

surrounding the food (Regulation, 2004). Nowadays, the commercial viability of active packaging is not only derived from an increased distributional life of the produce but also results from the logistical benefits including reduced wastage, cheaper packaging overall and easier handling (Boca Raton, 2007).

1.3. Antimicrobial packaging

Antimicrobial packaging is a form of active packaging technology, which is based on antimicrobial agents that are immobilized with the polymeric structure or incorporated in plastic resins, before film casting (Kim *et al.*, 2008). Similar to but more specific when compared with active packaging, antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself (Appendini & Hotchkiss, 2002). The driving force behind this development is the sporadic outbreak of contamination found in our daily food products (Nicholson, 1998).

Antimicrobial packaging technology can be classified into two types: (1) those that contain an antimicrobial agent that are released slowly from the packaged materials to the food products surface and (2) those that are effective against surface growth of microorganisms and do not migrate into the food products. The target for an antimicrobial packaging can also be focused in two fields: (1) pathogen inhibition to provide enhanced food safety and (2) shelf life extension as well as to protect the loss of flavor and odor in products (Nicholson, 1998).

This novel technology is widely used in the food industry, especially when it is incorporated into a polymer. In most, but not all, solid or semisolid food products, microbial growth occurs mainly at the surface (Brody *et al*., 2001). Thus elimination of microbial growth on the food product surface is considered to be one of the effective ways of improving the storage stability of packaged foods and their shelf life (Lee *et al.*, 2008). However, during the past few years, most of the antimicrobial agents in commercial food packaging were artificially synthetized. Due to negative effects from artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural and in particular, plant extracts, including their essential oils and essences (Smith-Palmer *et al.*, 1998). For this reason, naturally derived antimicrobial agents are becoming more important since they possess a perceived lower risk to the consumer. Recently, people have given more emphasis on the research of such next generation food packaging materials derived from natural plants with antimicrobial activity.

1.4. Antimicrobial agents

An antimicrobial agent is a chemical preservative that can be incorporated into a packaging material to add antimicrobial activity to it (Han, 2000). Various antimicrobial agents could be incorporated into conventional food packaging systems and materials to create new antimicrobial packaging systems. They could generally be divided into three major groups: chemical agents, natural spice, and probiotics (Han, 2005).

The most commonly used chemical antimicrobial agents are the various organic acids. Organic acids, including benzoic acid, lactic acid and mixture of organic acids, are widely used as chemical antimicrobial agents because their antibacterial efficiency is well understood and cost effective. Other chemical antimicrobial agents like acid salts, alcohol and fatty acids also have been used historically. When antimicrobial agents are incorporated into a polymer, the material limits or prevents microbial growth (Han, 2000). Most of the commonly used polymers such as LDPE could be incorporated with antimicrobial agents in food packaging. Potassium sorbate incorporated with LDPE has been applied in cheese packaging (Han, 1996); Benzoic acid anhydride incorporated with PE has been used in fish fillet packaging (Huang *et al.,* 1997) (Aaron L. Brody *et al.,* 2001). This early work has been followed by a much more divergent field of research into antimicrobial packaging in general for applications in food packaging technology.

Natural antimicrobial agents, which are naturally derived or occurring in nature, have been isolated from plants and animal sources. Considering the consumers' demand for chemical preservative-free food products, food manufacturers are now using naturally derived antimicrobial agents to replace the traditional chemical ones. There are many advantages to using natural plants as antimicrobial agents: firstly, it is cheap especially for use in underdeveloped nations with little access to expensive western medicines; secondly, natural spice without any chemical synthetic products should be safer and have fewer side effects. Thus, it is possible and effective to coat these natural plant powders on packaging materials in order to extend the shelf life of products. For example, some compounds reported as natural antimicrobial agents from plants include cinnamon, clove,

thyme, vanillin, and grapefruit seed extract. Although these natural plants agents were not applied in the food industry as early as synthetized agents were, they were already known as the main source of new leads for antimicrobial remedies and pharmaceutical development by ancient civilizations (Abdallah, 2011). In commercial packaging systems, naturally derived antimicrobial agents incorporated with polymer or paperboard could reduce the safety risk, which originates from chemical antimicrobial agents, effectively and significantly.

1.5. Antimicrobial packaging system

Most food packaging systems consist of the packaging materials, the food, and the headspace in the package. Suppose the void volume of solid products is assumed as headspace, most food packaging systems can be represented as either a package-food system or a package-headspace-food system. Patterns of migration and action in antimicrobial food packaging systems are illustrated in Figure1.5.1 (a) and (b). Diffusion between the packaging materials and products at the interface is the main migration phenomena in the food packaging system. The migration also can be achieved by gas phase diffusion from the packaging layer to product surface.

There are five types of antimicrobial packaging including:

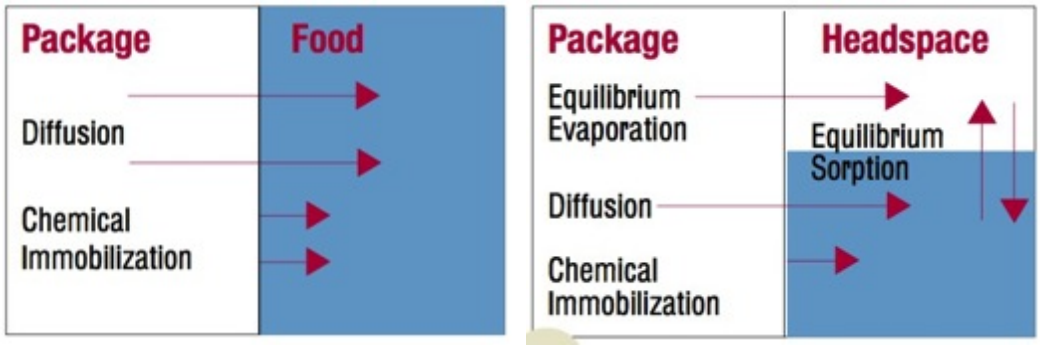
(1) Addition of pads containing volatile antimicrobial agents into packages;

(2) Incorporation of volatile and non-volatile antimicrobial agents directly into polymers;

(3) Coating or adsorbing antimicrobials onto polymer surfaces;

(4) Immobilization of antimicrobials to polymers by ion or covalent linkages;

(5) Use of polymers that are inherently antimicrobial.



(a) Direct contact (b) Direct and indirect contacts

Figure 1.5.1 Food packaging systems and migration phenomena (Han, 2000)

The mechanism of antimicrobial migration is critical for food packaging systems. Figure 1.5.2 shows the mass diffusion mechanism of an antimicrobial agent with different applications. Antimicrobial agents are incorporated into the packaging materials initially, then migrate into the food through diffusion and partitioning.

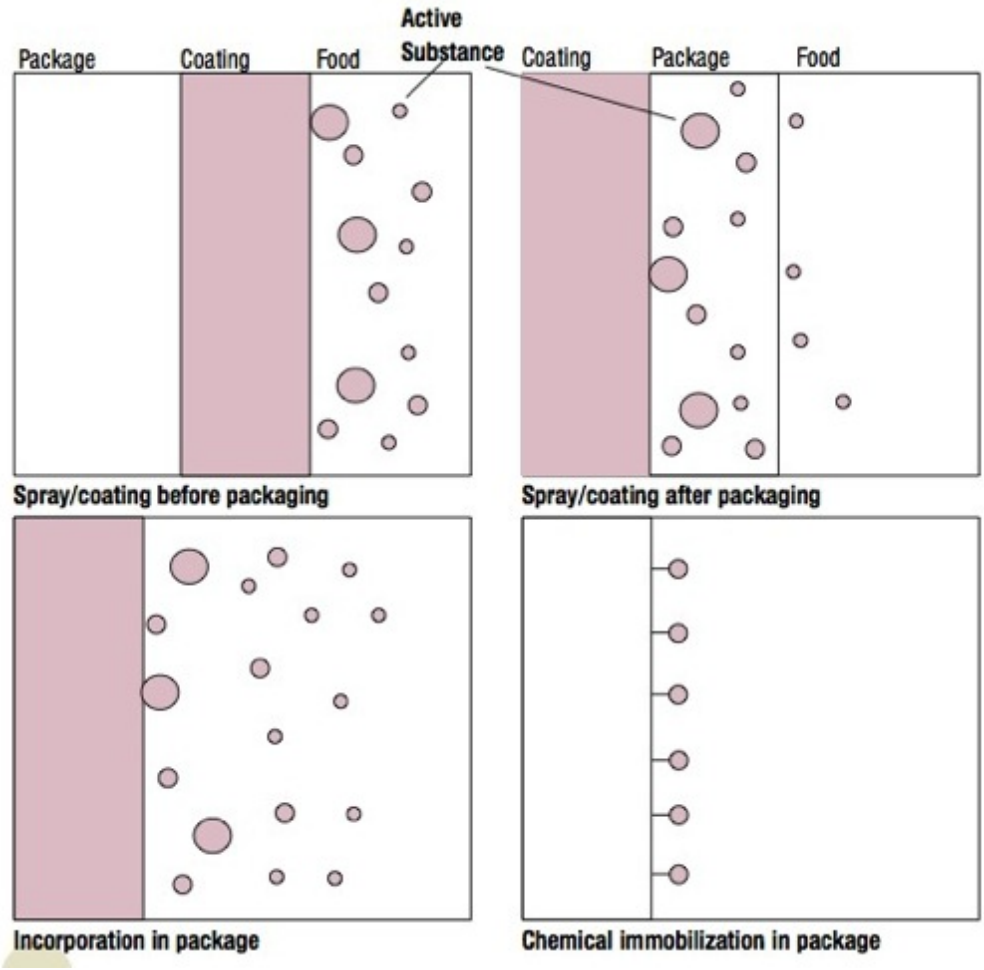


Figure 1.5.2 Migration of active particles (Han, 2000)

1.6. Aims

In view of the development history of active packaging and antimicrobial packaging, as well as environmental and general health benefits imparted by natural antimicrobial agents in food packaging system, the present study is aimed at the following:

- Evaluate the antimicrobial effects of three natural plant powders (vanillin, turmeric and curcumin) solutions with two kinds of solvent: Reagent alcohol and Dimethyl sulfoxide (DMSO).
- The inhibitory effects of all solutions were investigated on Mueller Hinton agar plates against five types of common bacteria: *E.coli O157:H7, Staphylococcus aureus, Shigella sonnei, Salmonella typhimurium,* and *Listeria monocytogenes*.
- Compare the results and find minimum inhibitory concentration (MIC) for each solution.
- Apply natural plant powders on LDPE resin and BOPP films to produce low cost and no toxins antimicrobial packaging materials.

1.7. Scope of work

The natural plant powders: Vanillin, Turmeric and Curcumin were chosen for the antimicrobial activity study. Samples were prepared by half fold dilution method using Reagent alcohol and DMSO respectively. The minimum inhibitory concentration (MIC) for each solution on different bacteria was studied. The relationship between concentration and antimicrobial effectiveness was analyzed and some assumptions were proposed. A comparison of antimicrobial activity among each solution was also illustrated. Turmeric powder was selected to incorporate into LDPE resin and coating on BOPP films, which were followed by antimicrobial activity studies.

2 Literature review

This chapter reviews the recent progress in antimicrobial packaging and provides a detailed discussion on the antimicrobial studies of antimicrobial agents derived from natural plants. The sources of natural spice, their antimicrobial activity and possible future applications in food packaging as well as other benefits are reviewed. A review of blending antimicrobial agents to LDPE resin as well as BOPP films with antimicrobial agents coating is also presented.

2.1. Progress in antimicrobial packaging

Packaging plays a significant role in the food supply chain, and it has to satisfy many demands and requirements. The basic functions of traditional packaging include protection, containment, convenience and communication. However, in recent years, consumer demands for minimally processed and naturally preserved food, increased regulatory requirements, and concern for products quality and safety. Previously, traditional packaging materials were considered as “passive” one, which means their function to protect the product against contamination is mainly based on an inert barrier (Lee *et al.,* 2008).

The first use of the term “active packaging ” was proposed by Labuza (1987), who defined active packaging as a kind of technology which perform some desired function other than an inert barrier to the external environment (Rooney, 1995). In recent years,

packaging technology has shifted from passive to active due to the development of novel packaging methods such as active packaging.

Antimicrobial packaging is a form of active packaging. Antimicrobial food packaging materials have to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety (Han, 2000). Such a system can be constructed by using antimicrobial materials and/or by incorporating antimicrobial agents into the packaging system. The development and application of antimicrobial packaging has been recently reviewed (Appendini & Hotchkiss, 2002).

The most common and successful commercial application of antimicrobial packaging in the food industry should be sachets that are enclosed loose or attached to the interior of a package (Appendini & Hotchkiss, 2002). Carbon dioxide emitters as sachets, which include ascorbic acid and sodium hydrogen carbonate, can inhibit the growth of gram-negative bacteria and moulds on vegetables, fruits as well as fish, meat and poultry (Ahvenainen, 2003). On the other hand, incorporation of antimicrobial agents directly in to polymers has been commercially used in drug delivery, food packaging and biomedical devices. For example, packaging films like antimicrobial preservative releasers including organic acids as well as spice and herb extracts can prevent the growth of spoilage and bacteria on meat, fish, bread, cheese, fruit and vegetables (Ahvenainen, 2003).

2.2. Types and uses of antimicrobial agents

There are many different types of antimicrobial agents, which can be used in a wide variety of applications in the food, pharmaceutical and cosmetic industries. Several types of antimicrobial agents have been tested for antimicrobial packaging applications including organic acids, fungicides, bacteriocins, proteins, enzymes, inorganic gases, and metals. Table 2.1 lists some typical antimicrobial agents used in food packaging (Han, 2000; Appendini & Hotchkiss, 2002).

Table 2.1 Examples of some typical antimicrobial agents used in food packaging

Class of antimicrobial agents	Examples
Organic acids	Potassium sorbate, benzoic acid, acetic acid, propionic acid
Inorganic gas	Carbon dioxide, sulphur dioxide, chlorine dioxide
Metals	Silver, copper
Bacteriocins	Nisin, pediocins, lacticin
Spices	Cinnamic, caffeic
Essential oils	Grapefruit seed extract, clove, thyme
Inherently bioactive polymers	Chitosan
Alcohol	Ethanol

A summary of some typical applications of antimicrobial packaging systems is presented in Table 2.2. Some common antimicrobial agents for food products are such preservatives as organic acid, inorganic gas, metal, bacteriocins and alcohol. Organic acids incorporated with chitosan, such as acetic acid and propionic acid have been studied as antimicrobial chemicals for the packaging of water (Ouattara *et al.,* 1999). Carbon dioxide, which is a kind of inorganic gas, has been studied as a preservative for the packaging of fruit (Sacharow, 1988). Other materials including silver (Ishitani, 1995), nisin (Daeschel, 1992) and ethanol (Smith *et al.,* 1987), have been tested as antimicrobial agents in food packaging.

Table 2.2 Examples of typical applications of antimicrobial packaging

Antimicrobial Agent	Packaging material	Food
Organic Acids (Acetic acid, propionic acid)	Chitosan	Water
Inorganic gas (Carbon dioxide)	Sodium metabisulfite	Fruit
Metal (Silver)	Various polyolefins	Culture media
Bacteriocin (Nisin)	Silicon coating	Culture media
Alcohol (ethanol)	Silicon oxide sachet	Bakery

2.3. Natural plant products as antimicrobial agents

Nowadays, the most commonly used commercial preservatives in the food industry are chemical preservatives. Although the chemical preservatives, synthetic and semi-synthetic, have been widely accepted in the modern era, the undesirable side effects cannot be neglected. With the increasing demand for food safety and health standards, consumers have become more concerned about the presence of chemical residues in the food products (Charu *et al*., 2008). Due to the negative impact from chemical preservatives, attention has been shifted to natural plant extracts, including their powders and essential oils (Smith-Palmer *et al*., 1998).

Natural plants, which have antimicrobial agents, have been widely used in ethno-medicine around the world dating back to a thousand years ago. In the first century A.D., Dioscorides wrote De Materia Medica, which offers descriptions of approximately 30 healing plants. However, since the development of antibiotics in the 1950s, the production of antimicrobial agents derived from plants has been rarely needed (Marjorie, 1999). Currently, the use of natural plants with antimicrobial activity have come back into our lives: essential oils such as Cinnamomum zeylanicum, Thymus vulgaris and Origanum vulgaris were studied as antimicrobial solutions in paper packaging (Rodr ıguez *et al*., 2007). Antimicrobial compounds from plants can be divided into several major groups, which include Phenolics and Polyphenols, Terpenoids

and Essential Oils, Alkaloids, Lectins and Polypeptides, Mixtures and Other Compounds (Marjorie, 1999). Our study focuses on plant essential oils and extracts.

2.3.1. Antimicrobial activity in plant essential oils

Essential oils offer a promising alternative to the synthetic preservatives used in food products. H.J.D. Dorman and S.G. Deans (2000) evaluated antimicrobial activity of six plant volatile oils against twenty-five common microorganisms. They conclude that the volatile oils showed considerable inhibitory effects against all tested bacteria. Smith-Palmer *et al*. (1998) studied the antimicrobial properties of twenty-one plant essential oils against five common food-borne pathogens. This work highlighted that Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria. Besides the previous study on antimicrobial activity of different plants essential oils, some research focused on certain essential oils was evaluated. Charu Gupta *et al.* (2008) screened ten herbal oils for antimicrobial activity using an agar well diffusion technique against ten common bacteria. They discovered that only cinnamon oil and clove oil showed a broad range of antimicrobial activity. The MIC is 1.25%(v/v) for cinnamon oil against *Listeria monocytogenes* and *E. coli*, and 2.5%(v/v) for clove oil against *Staphylococcus epidermidis* respectively. A. Rodr´ıguez *et al.* (2007) had assessed the antimicrobial activity of clove, cinnamon and oregano essential oils, and applied these essential oils on paraffin-based “active coating” for paper packaging materials. It has been shown that no inhibition of tested Gram-positive bacteria (*Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 29213)) was observed, and only the active

coating with cinnamon essential oil showed inhibitory activity against Gram-negative bacteria (*E.coli* (ATCC 29252), *Salmonella typhimurium* (CECT 4000*)*). It was also found that the percentage of essential oils added to the coating materials have a significant impact on their antimicrobial activities. Sudsuda Vanit *et al.* (2010) reported that pure clove oil possesses better antimicrobial inhibition on tested bacteria (*E.coli* (DMST 4212), *Staphylococcus aureus* (DMST 8840), *Bacillus cereus* (DMST5040)) than clove oil in starch matrixes. Natural plant essential oils showed promising antimicrobial activity against several species of bacterial food-borne pathogens including *E.coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* (Burt, 2004). Furthermore, Sara Burt (2004) highlighted some Gram-positive organisms are generally more sensitive to essential oils than Gram-negative organisms, which agrees with the previous study by Smith-Palmer et al. (1998).

2.3.2. Antimicrobial activity in plant extracts from roots and seeds

Plant extracts from roots and seeds are also reported to have wide-ranging bioactivity as antimicrobial agents. Daljit S. Arora and Jasleen Kaur (1999) compared the sensitivity of some human pathogenic bacteria and yeasts to various spice extracts. They discovered that only garlic and clove were found to possess antimicrobial activity, and garlic extracts inhibited *Staphylococcus epidermidis* (MTCC 435) and *Salmonella typhimurium* (MTCC 531) significantly. Prashant Tiwari *et al*. (2011) analyzed the antibacterial sensitivity of various solvent extracts of Carica papaya root by well diffusion method and observed that Carica papaya root extracts possess a broad spectrum of activity against a panel of

bacteria diseases. Recently, antibacterial and antifungal activity of root and root callus extracts of Trianthema decandra L. in different solvents were tested against both Gram-positive and Gram-negative bacteria (Radfar *et al*., 2012). In this work, significant antimicrobial activity was observed in root callus extract of chloroform, ethyl acetate and ethanol with a MIC of 3.12 to 12.5 µg/ml. It is also reported that antibacterial activity was more in root callus extract than root extract.

The antimicrobial effect from plant seed extracts, especially grape seed extract was demonstrated in recent studies (T. Sivarooban *et al*., 2008; Bledar Bisha *et al*., 2010). Antilisterial effect of grape seed extracts was evaluated using multiple tools (Bledar Bisha *et al*., 2010), and the results suggested that grape seed extract has significant antimicrobial effect against *Listeria* spp. at relatively low concentrations. T. Sivarooban *et al*. (2008) studied the antimicrobial properties of soy protein isolate (SPI) films containing grape seed extract (1% w/w), nisin (10,000IU/g), ethylenediaminetetraacetic acid (EDTA 0.16% w/w), and their combinations. The grape seed extract, nisin and EDTA incorporated with SPI film is effective to inhibit the growth of *Listeria monocytogennes*, *E. coli O157:H7* and *Salmonella typhimurium*, which has potential application in ready-to-eat food products.

2.3.3. Properties and uses of vanillin

The natural product of the extracts of vanilla, vanillin is a phenolic aldehyde that has received considerable attention as a possible antimicrobial agent (Suwarat *et al*., 2009; Suwarat *et al.*, 2010; Cheng *et al*., 2011; Jurmkwan *et al.*, 2008). The antimicrobial

property of vanillin is due to its phenolic compound in chemical structure, and the chemical structure of vanillin is shown in Figure 2.3.3.

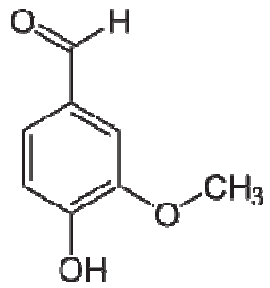


Figure 2.3.3

Vanillin has been selected as an antimicrobial agent for coating paperboard intended for packaging bakery products. Suwarat Rakchoy *et al.* (2009) evaluated the inhibitory effects of vanillin solutions and investigated MIC of the solution against selected bacteria including *E.coli* (DMST 4212), *Staphylococcus aureus* (DMST 8840) and *Bacillus cereus* (DMST 5040) by agar well diffusion method. Three types of vanillin coating solutions were prepared and evaluated, including vanillin/DMSO (10,5,2.5,1.25%(w/w)), vanillin/ ethyl alcohol (10,5,2.5,1.25%(w/w)) and vanillin/chitosan (10,5,2.5,1.25%(w/w)). Furthermore they assessed the shelf life of vanillin coated paperboard with bakery products, and the results showed that the shelf life was extended compared with the uncoated paperboard (Suwarat Rakchoy *et al.*, 2010). Vanillin coated solution significantly inhibit all tested bacteria, and the antimicrobial effect depends on the combination of substances in the solution and the solution concentration.

In order to achieve an economical method for producing antimicrobial packaging, Jurmkwan *et al.* (2008) incorporated vanillin directly into chitonsan-methyl cellulose based films with polyethylene glycol 400 (PEG) as a plasticizer. Furthermore, they analyzed the impact of different vanillin concentration on mechanical properties, water vapor and oxygen permeability, opacity and thermal properties of the coating films. The results indicated that both vanillin and PEG concentration affected all the tested properties of chitosan-methyl cellulose based film.

2.3.4. Properties and uses of turmeric

Turmeric, a bright yellow-orange substance, obtained from the root of the plant Curcuma longa, has long been used as a traditional spice in both China and India. Turmeric is mostly used in flavored milk drinks, cultured milk and desserts to obtain lemon and banana colors in dairy products. In previous research, turmeric has been shown to have anti-HIV activity (Marjorie, 1999), wound-healing (Ishita *et al.*, 2004) as well as antimicrobial activity (Seher *et al*., 2006). Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%) (Ishita *et al.*, 2004). The main components of turmeric are: curcumin (60%), desmethoxycurcumin, monodemethoxycurcumin, bisdemethoxycurcumin, dihydrocurcumin and cyclocurcumin (Priyanka *et al.,* 2010). The Structure of the main curcuminoids of turmeric is shown in Figure 2.3.4 (Ishita *et al.*, 2004).

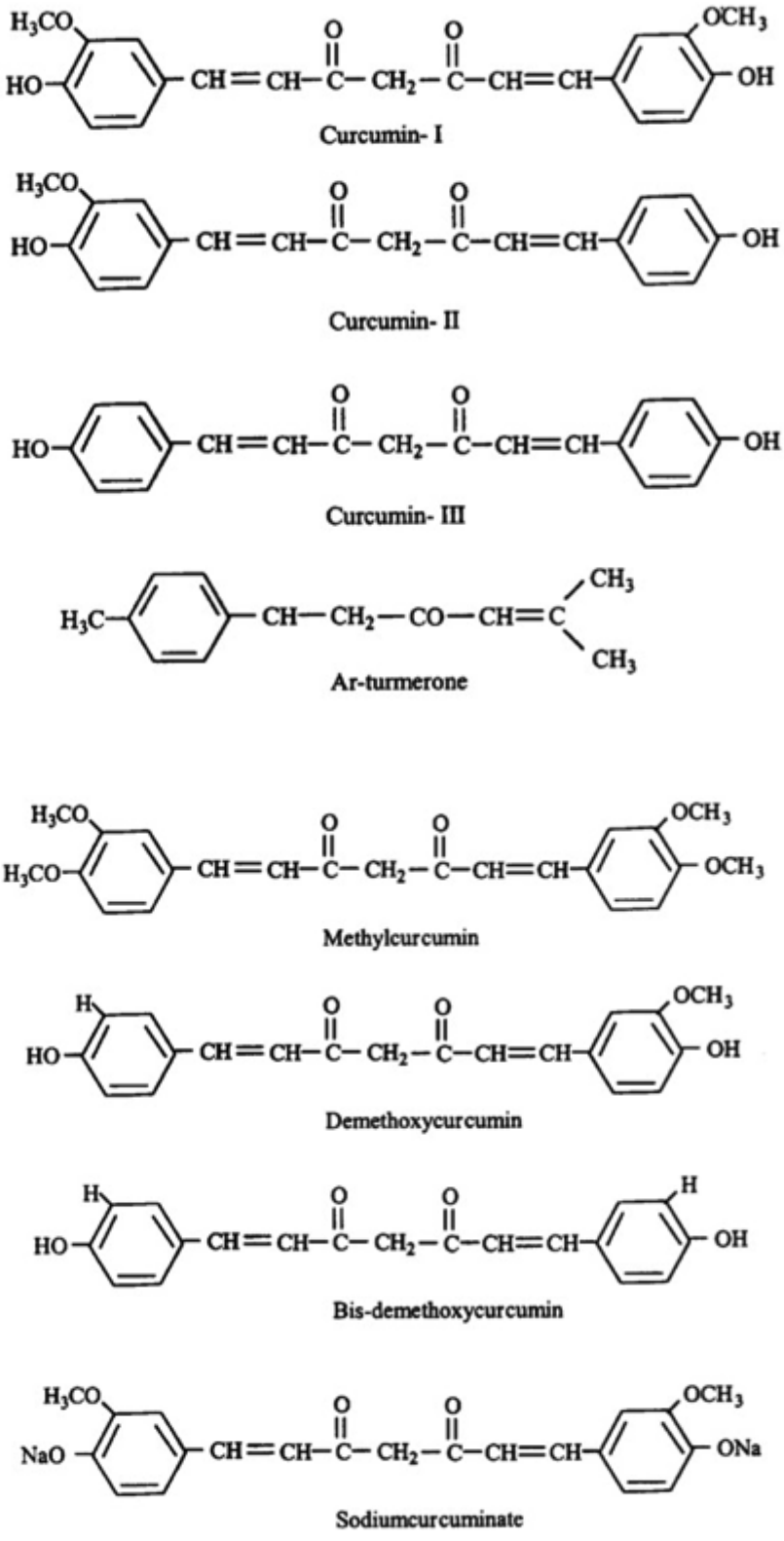

Curcumin- I
Curcumin- II
Curcumin- III
Ar-turmerone
Methylcurcumin
Demethoxycurcumin
Bis-demethoxycurcumin
Sodiumcurcuminate

Figure 2.3.4

P. S. Negi *et al.* (1999) extracted turmeric oil from the mother liquor using hexane at 60 °C, and then studied the antimicrobial activity by pour plate method. The results of the study show that turmeric oil possessed antibacterial activity against tested bacteria including *Staphylococcus aureus* and *E.coli*. Another antimicrobial study conducted by Seher *et al.* (2006) on methanolic extract of turmeric powder showed that turmeric powder was most effective on *Staphylococcus aurues* (COWAN 1) and had a slight effect on *Listeria monocytogennes* (SCOOT A). However, it was not effective on *E. Coli* (ATCC 25921).

2.3.5. Properties and uses of curcumin

Curcumin is the most biologically active constitute of turmeric. The characteristic yellow color of turmeric was isolated in the 19th century and was named curcumin (Araújo & LL Leon, 2001). Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, antitumor activities (Ishita *et al.*, 2004; Araújo & LL Leon, 2001; Preetha *et al*., 2008; Marjorie, 1999) and anti-cancer activities (Reason Wilken *et al*., 2011). The chemical structure of curcumin (Figure 2.3.5.) was determined by Roughley and Whiting in 1973 (Araújo & LL Leon, 2001).

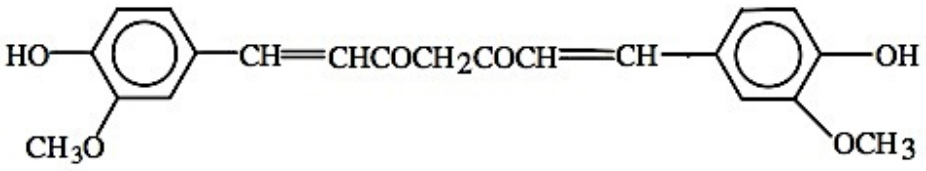


Figure 2.3.5

Bhavani Shankar and Sreenivasa Murthy (1979) evaluated the activity of turmeric fractions on the growth of some bacteria in vitro. In this work, both curcumin and the oil fraction were tested against several bacteria like *Staphylococcus* and *Lactobacillus*. However, curcumin only inhibited *Staphylococcus aureus* in the range of 2.5mg/ml to 50mg/ml (CAC Araújo and LL Leon, 2001).

Antimicrobial activity of indium curcumin against *S. aureus* (ATCC 25923) and *S.epidermidis* (ATCC 14990) as well as minimum inhibitory concentration (MIC) has been reported (Tajbakhsh *et al.*, 2008). It was reported that curcumin inhibits the growth of varieties of microbes such as viruses, bacteria and some pathogenic fungi (Chai, *et al.*, 2005). Other studies on indium curcumin and microcapsule curcumin evaluated the antimicrobial effects for particular bacteria (Tajbakhsh *et al*., 2008; Wang *et al*., 2009).

2.4. Development in polymers and packaging

As antimicrobial agents are incorporated into a polymer, the material inhibits microbial growth. Such applications could be effectively used for foods products, not only in the form of films but also as containers (Han, 2000).

The selection of an antimicrobial agent is often limited by the incompatibility of that agent with packaging material or by its heat instability during the extrusion process (Weng *et al*., 1993; Han & Floros, 1997). In order to minimize this restriction, Han (2000) suggested using master batches of the antimicrobial agent (1% potassium sorbate) in

LDPE resin to produce antimicrobial packaging materials that inhibited the growth of yeast on agar plates. Such a master batch was prepared, by mixing the LDPE resin and potassium sorbate powder, and extruded and pelletized (Pérez-Pérez *et al*., 2006). Andrea Carolina Valderrama Solano and Cecilia de Rojas Gante (2011) developed LDPE films, which were incorporated with known concentrations (1%(w/w) and 4%(w/w)) of natural essential oils of oregano and thyme directly by extrusion. The resulting films showed inhibitory activity against *Escherichia coli O157:H7, Salmonella typhimurium*, and *Listeria monocytogenes*. Panuwat Suppakul *et al.* (2006) studied the properties of LDPE based films containing either linalool or methylchavicol as antimicrobial additives. The results showed that, this incorporation of either linalool or methylchavicol by a single screw extruder into LDPE based film change some characteristic of the packaging material slightly. Abel Guarda *et al.* (2011) reported that BOPP films, with a coating of microcapsules containing 10% of carvacrol and 10% of thymol as natural AM agents, exhibited antimicrobial activity against *E.coli O157:H7* and *Staphylococcus aureus* (ATCC 25923) in agar plate test.

Figure 2.4 (Lee *et al*., 2008) shows a single screw extruder that consists of an electrically heated metal barrel, a hopper for feeding the resin usually in pellet form, a motor for rotating a screw, and a die where the polymer melts exits. The heat from the barrel is responsible for softening the surface of the resin pellets making them stick to each other, while the rotating screw is responsible for melting the resin pellets and forcing the polymer melt through the die (Lee *et al*., 2008).

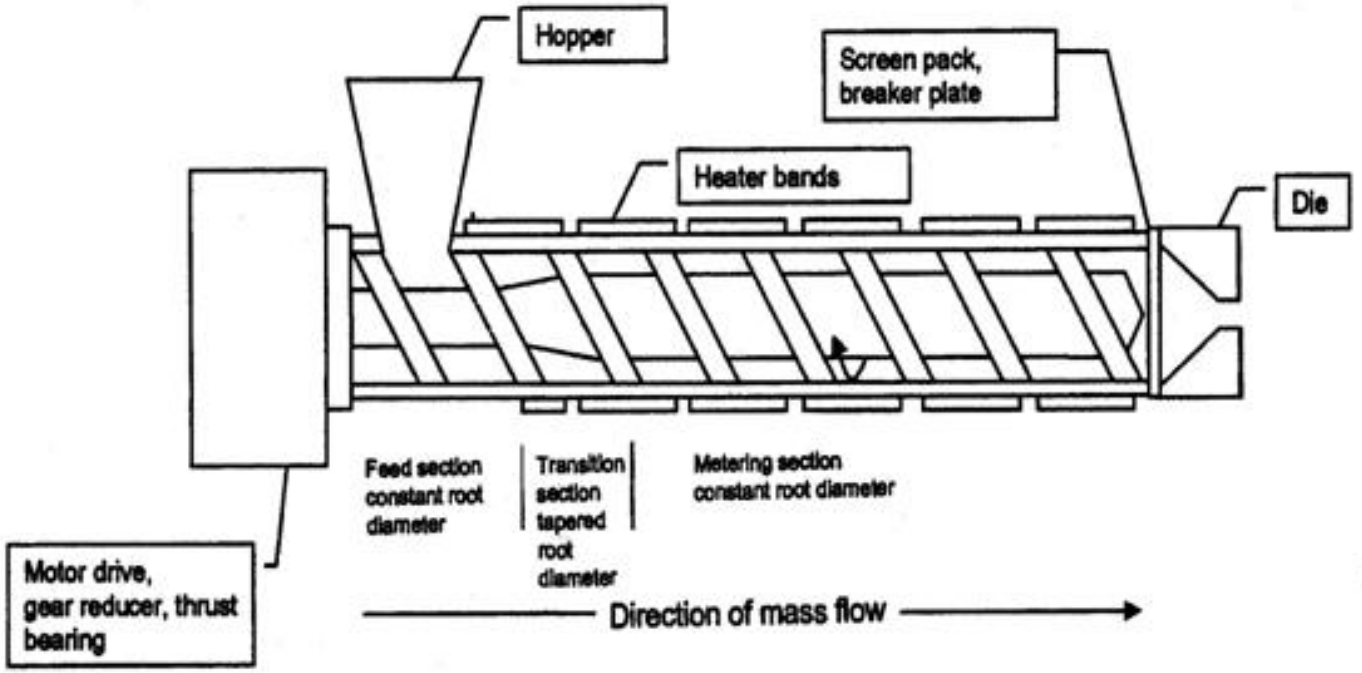

Hopper
Screen pack,
breaker plate
Heater bands
Die
Feed section
constant root
diameter
Transition
section
tapered
root
diameter
Metering section
constant root diameter
Motor drive,
gear reducer, thrust
bearing
Direction of mass flow

Figure 2.4

3 Materials and methods

3.1. Antimicrobial agent in solutions

3.1.1. Materials and methods

1) Antimicrobial agents

Turmeric powders were purchased from nuts.com. Vanillin (94752, Fluka) and Curcumin (C1386, Sigma) were obtained from Sigma-Aldrich Co. LLC. U.S.

Typical properties of the agents are presented in Appendix A.

2) Solvents

The solvents used in the experiment were: (i) Reagent alcohol (R8382, purchased from Sigma-Aldrich Co. LLC. U.S.). (ii) Dimethyl sulfoxide (DMSO, 276855 anhydrous, ≥99.9%, purchased from Sigma-Aldrich Co. LLC. U.S.).

3) Preparation of solution

In this experiment, three types of solutions were prepared for Reagent alcohol and DMSO respectively, using a series of half-fold dilutions. There were four concentrations for each Reagent alcohol solution and six concentrations for each DMSO solution. Details of solution's composition and concentration are given in Table 3.1.1

Table 3.1.1 Solution's composition and concentration prepared in antimicrobial test

Agents	Reagent alcohol as solvent	DMSO as solvent
Vanillin	10	10
Curcumin	5	5
Turmeric	2.5	2.5
	1.25	1.25
		0.625
		0.3125

Note: values shown are %(w/w)

4) Microorganisms

Five types of common pathogenic and food spoilage bacteria were selected. They are (a) gram-positive bacteria: *Staphylococcus aureus* and *Listeria monocytogenes*; (b) gram-negative bacteria: *Shigella sonnei, Salmonella typhimurium, E. coli O157:H7*. The Department of Biotechnology and Molecular Bioscience, Rochester institute of technology, NY, U.S, provided all bacterial strains.

5) Media and culture plates

The antimicrobial activities of vanillin, turmeric and curcumin solutions were analyzed on Mueller-Hinton II agar (VWR) by disc diffusion method.

3.1.2. Antimicrobial testing

The antimicrobial activities of vanillin, turmeric and curcumin solutions were determined by agar plate diffusion method. Each type of bacteria was streaked onto Mueller-Hinton agar plates. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates uniformly. Using blank antibiotic assay discs of 6mm in diameter impregnated with 40µl of vanillin, turmeric and curcumin solutions and was placed on the surface of the agar respectively. The solutions diffused out from the antibiotic assay disc into the agar. Then, the plates containing the testing samples were incubated for 24 hours at 35.5°C. Control samples were performed triplicate on each bacteria with 40µl solvent. Vanillin, turmeric and curcumin solutions were performed triplicate for different concentrations. In order to obtain comparable results, all prepared solutions were applied under the same conditions and the same incubation environment.

3.1.3. Data analysis

The antimicrobial activities of all solutions were detected as clear zones around the antibiotic assay discs, and the diameters of the clear zones measured by a ruler in millimeters (mm). If the solution is effective against bacteria at a certain concentration, no colonies will grow wherever the concentration in the agar is greater than or equal to that effective concentration. This region is called the “zone of inhibition.” Thus, the size of the zone of inhibition is a measure of the compound’s effectiveness. The larger the

clear zone around the antibiotic assay disc, the more effective the solution is. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of solutions that completely inhibit the growth of each bacterial strain being tested (Wang *et al*., 2009).

3.2. Antimicrobial agent in polymers

3.2.1. Materials and methods

1) Polymers

The polymers that were used to prepare the master batch and coating films for present study were low-density polyethylene resin (LDPE, NA 214-000, Lyondell Company) and biaxially oriented polypropylene (BOPP) film (Bicor™ 100 SLP, ExxonMobil).

Typical properties of the polymers are presented in Appendix A.

2) Antimicrobial agents

Turmeric powders were purchased from nuts.com.

Typical properties of the agents are presented in Appendix A.

3) Adhesive and preparation of coating solution

Adhesive and coating solutions only used in BOPP films. Adhesive was prepared by using Ethyl Acetate, 555 (Dow Chemical Company) and 536B (Dow Chemical Company) mixture in 33.46g, 38.46g and 5g respectively.

4) Production of pellets by single screw extruder

The turmeric powders were blended with polymer resin LDPE into a master batch in concentration of 2.5% (w/w). 600 grams of resin was added to the mixer with 7.5 grams of turmeric powders. The blended material was fed into a single screw extruder, with the working temperature at 305°F. Material was first cooling immediately by water after exited the die, then ground in a knife mill to produce pellets (5mm×3mm×2mm). The pellets were collected separately in ten containers every three munities. In order to obtain comparable results, all pellets used in antimicrobial testing was melted and reshaped into discs of approximately 6mm in diameter and 1mm in thickness.

5) Blend preparation and film coating

The turmeric powders were blended with prepared adhesive (Ethyl Acetate, 555,536B) made a mixture concentration of 5%(w/w). 3.85g turmeric powders were added to the prepared adhesive, forming a total of 80.77g coating solution.

BOPP film (22mm×40mm) coated by draw down machine (EZ Coater EC-100, Fairfield. Ohio, U.S.A.), with 3.5ml of the coating solution covering the entire film and was dried at 120°C-130°C in oven for 24 hours. Then the film was cut into test samples (13mm×13mm).

3.2.2. Antimicrobial testing

The antimicrobial activities of turmeric containing LDPE discs and turmeric coated BOPP films were determined by agar plate diffusion method. Each type of bacteria was streaked onto Mueller-Hinton agar plates. Sterilized cotton swabs were dipped in the

bacterial culture in nutrient broth and then swabbed on the agar plates uniformly. Turmeric containing LDPE samples were chosen randomly from each container made up totally ten samples on each agar plate. The active agents in the discs diffused out from the tested samples into the agar. Then, the plates containing the testing samples were incubated in an appropriate incubation chamber for 48 hours and 72 hours at 36°C. Control samples for turmeric containing LDPE discs were pure LDPE resin discs, and performed triplicate in this study. Testing was only done once in this study.
Turmeric coated BOPP films sample were randomly chose from all cut samples. The active agents in films diffused out from the tested samples into the agar. Then, the plates containing the testing samples were incubated in an appropriate incubation chamber for 72 hours at 36°C. Control samples for turmeric coated BOPP films were pure BOPP films. Testing was only done once in this study.

3.2.3. Data analysis

The antimicrobial activities of all tested samples were detected as clear zones around the antibiotic assay discs, and the diameters of the clear zones measured by a ruler in millimeters (mm).

4 Results and discussion

This chapter examines the antimicrobial activity of natural plant powders in solutions with different solvents and concentration. Antimicrobial effectiveness of the blends of LDPE resin and natural plants agent, as well as BOPP film with natural plants agent coating are also demonstrated.

4.1. Antimicrobial agent in solutions

In this study, turmeric and curcumin powders could not compeletely be solubilized in reagent alcohol. The insoluble particles in the solution might possibly prevent the migration of solution that contained antimicrobial agents, this results in a smaller inhibit zone with higher solution concentration (Cheng *et al.*, 2011). DMSO was used as an alternative solvent in the experiment. DMSO was a solvent was used in many previous studies: Antimicrobial properties of vanillin/DMSO solution were evaluated and compared with vanillin/chitosan solution and vanillin /ethyl alcohol solution (Rakchoy *et al*., 2009); DMSO made up solution to ensure a better solubilization of curcumin and indium curcumin (Tajbakhsh *et al*., 2008).

In this study, DMSO is chosen as solvent of natural plant powders in antimicrobial activity testing, and the results were compared with our previous work that used reagent alcohol as the solvent.

4.1.1. Antimicrobial Effects of Vanillin, Turmeric and Curcumin in Reagent alcohol Solution

The average diameter and standard deviation (STDEV) of clear zone of inhibition on control samples, vanillin/reagent alcohol solution, turmeric/reagent alcohol solution and curcumin/reagent alcohol solution are illustrated in Table 4.1.1. A comparison of antimicrobial activity of different solution over particular microorganisms is shown in Figure 4.1.1 (a)-(e). Furthermore, clear zone diameters for three types of solution over five microorganisms are also illustrated in Figure 4.1.2 (a)-(e).

If indistinct clear zone of inhibition was observed in the test, the diameter is marked as "-". Besides, standard deviation is "-" illustrated that there were no distinct clear zone of inhibition observed in three replicates.

According to the results in Table 4.1.1, vanillin/reagent alcohol solution is more effective over *E.coli O157:H7* with a minimum inhibitory concentration (MIC) at 2.5%(w/w). The results agree with previous studies on the effect of vanillin/alcohol solution in inhibiting *E. coli* (Rakchoy *et al*., 2009). In addition, vanillin/reagent alcohol solution has significant effectiveness on *Shigella sonnei* and *Listeria monocytogenes* with the MIC at 2.5%(w/w). Whereas the same solution has slight less antimicrobial activity on *Staphylococcus aureus* and *Salmonella typhimurium*. The results contradict previous study on the effect of vanillin/alcohol solution in inhibiting *E. coli* (DMST 4212) by Rakchoy *et al*. (2009), who reported that vanillin/alcohol was more effective over

Staphylococcus aureus (DMST 8840).

Turmeric/reagent alcohol solution has a weak antimicrobial activity on *Staphylococcus aureus* and *Listeria monocytogenes* with the MIC at 10%(w/w). This finding is in the agreement with Chaudhary et al. (2010) who reported the turmeric alcohol extract has antibacterial activity against Gram-positive bacteria. However, turmeric/reagent alcohol solution has no significant antimicrobial effect on *E.coli O157:H7, Shigella sonnei* and *Salmonella typhimurium*. While the antimicrobial activity of turmeric/reagent alcohol has some antimicrobial effective on Gram-positive bacteria, it appears no significant antimicrobial effect on Gram-negative bacteria.

As the most biologically active constitute of turmeric, curcumin has significant antimicrobial activity over *Staphylococcus aureus* and Listeria monocytogenes. According to the results, the MIC of curcumin/reagent alcohol solution was valued at 2.5%(w/w) for *Staphylococcus aureus*, but was only 1.25%(w/w) for *Listeria monocytogenes*. However, based on the results, curcumin has moderate effect on *Shigella sonnei* with a MIC at 1.25%(w/w), and no significant effect on *E.coli O157:H7* and *Salmonella typhimurium*. In summary, the results indicated that the selected Gram-positive bacteria had higher sensitivity than the selected Gram-negative bacteria. Both the results from turmeric/Reagent alcohol and curcumin/Reagent alcohol are agree with Smith-Palmer *et al*. (1998) who reported that Gram-positive bacteria were more sensitive to inhibition by plant agents than the Gram-negative bacteria. Bhawana *et al*. (2011) reported that such antimicrobial effect with selectivity could be due to differences in their

cell membrane constituents and structure.

In general, increasing its concentration should intensify the antimicrobial activity of certain powder/reagent alcohol solution. From the results, vanillin/reagent alcohol solution had increasing antimicrobial effect over all five bacterial strains when the concentration increased. Vanillin/Reagent alcohol solution showed a good agreement with this concentration and effectiveness relation. However, the data from turmeric and curcumin demonstrated that a decreasing concentration of solution results in an increased antimicrobial activity. This could be caused by poor solubility of turmeric or curcumin in reagent alcohol. The insoluble particle in turmeric or curcumin reagent alcohol solution might possibly prevent the migration of solution that contained antimicrobial agents. Therefore, when the solution was diluted in lower concentration, less insoluble particles in the solution could improve the migration of solution.

Bacterial	Control sample		Concentration (%w/w)	Vanillin		Turmeric		Curcumin	
	Average diameter (mm)	STDEV		Average diameter (mm)	STDEV	Average diameter (mm)	STDEV	Average diameter (mm)	STDEV
Listeria monocytogenes	10.00	1.00	10	17.00	1.00	15.67	0.58	11.00	4.00
			5	13.00	0	7.67	0.58	22.67	1.53
			2.5	11.67	1.53	10.33	0.58	23.00	1.00
			1.25	12.00	1.00	11.67	2.08	23.33	1.15
Staphylococcus aureus	9.00	1.00	10	12.33	2.89	14.33	1.53	14.33	1.53
			5	10.00	0	8.33	1.53	17.33	1.53
			2.5	9.67	0.58	9.00	0	25.00	1.73
			1.25	10.00	0	8.67	1.53	9.67	1.15
Shigella sonnei	10.33	0.58	10	18.33	3.79	11.33	3.79	8.33	2.89
			5	16.00	2.00	9.33	5.77	14.67	4.16
			2.5	12.67	1.15	8.00	1.73	17.67	3.51
			1.25	10.67	0.58	9.33	2.31	16.67	3.79
Salmonella typhimurium	17.67	3.79	10	21.67	1.53	7.33	2.08	12.00	1.00
			5	18.33	1.53	11.33	4.93	10.67	1.15
			2.5	15.33	0.58	11.00	2.65	12.00	1.73
			1.25	14.00	0	10.00	0	12.00	3.46
E.coli O157:H7	12.33	2.89	10	22.00	0	6.50	0.71	-	-
			5	15.00	1.00	-	-	8.67	1.53
			2.5	12.33	0.58	7.67	2.08	10.33	2.08
			1.25	11.67	1.53	11.33	3.79	13.00	2.00

Table 4.1.1

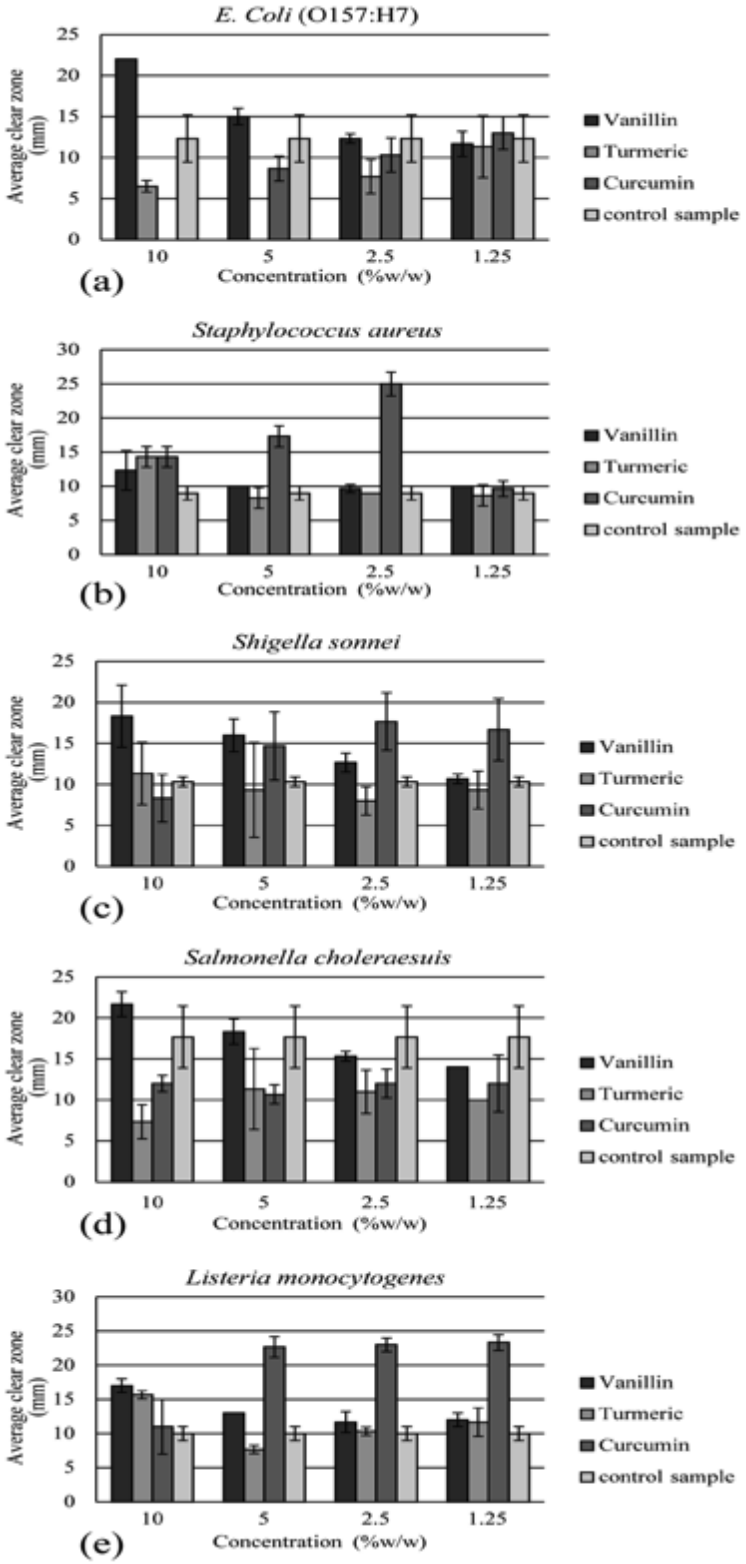


Figure 4.1.1 Antimicrobial activities and MICs of control sample and vanillin, Turmeric, Curcumin in Reagent alcohol solutions. (a) *E. Coli O157:H7*, (b) *Staphylococcus aureus*, (c) *Shigella sonnei*, (d) *Salmonella typhimurium*, (e) *Listeria monocytogenes*.

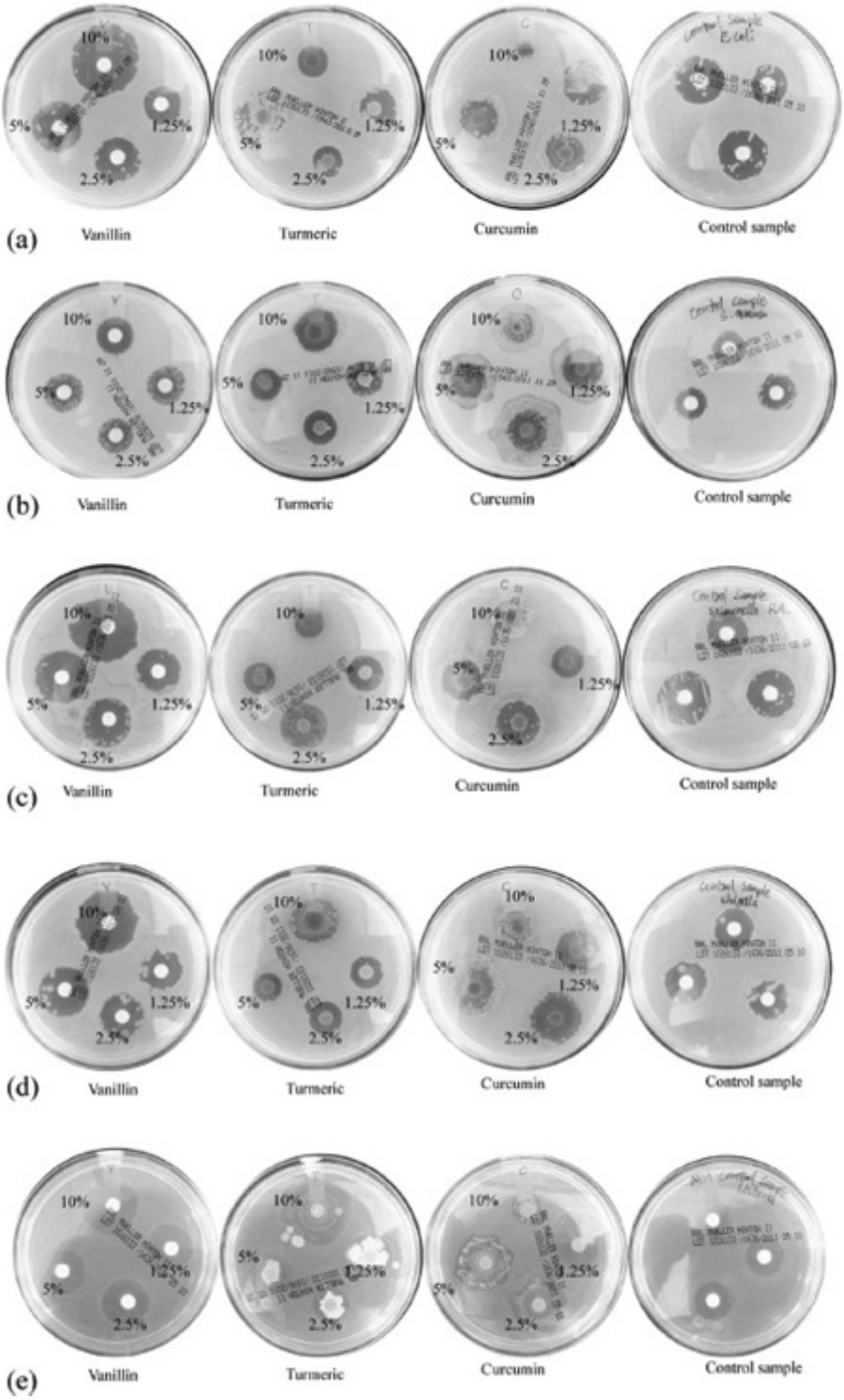


Figure 4.1.2 The illustration above shows zones of inhibition around antibiotic assay discs saturated with 40 µl of vanillin, turmeric and curcumin solutions respectively. (a) *E. Coli O157:H7*, (b) *Staphylococcus aureus*, (c) *Shigella sonnei*, (d) *Salmonella typhimurium*, (e) *Listeria monocytogenes*.

Bacteria	Control sample		Concentration (% w/w)	Vanillin		Turmeric		Curcumin	
	Average diameter (mm)	STDEV		Average diameter (mm)	STDEV	Average diameter (mm)	STDEV	Average diameter (mm)	STDEV
Listeria monocytogenes	0	0	10	29.67	0.58	4.33	1.53	4	0
			5	25	1	8.67	1.15	6.67	0.58
			2.5	19	0	7.67	0.58	6	0
			1.25	10.33	0.58	8	0	6.67	0.58
			0.625	-	-	-	-	7	0
			0.3125	-	-	-	-	7.67	0.58
Staphylococcus aureus	0	0	10	8.67	0.58	5.67	1.53	4	1
			5	5.33	0.58	7	1	6.33	0.58
			2.5	-	-	8.33	0.58	6.33	0.58
			1.25	-	-	8	0	8.68	0.58
			0.625	-	-	-	-	7.33	0.58
			0.3125	-	-	-	-	8	1
Shigella sonnei	0	0	10	6	0	4	1	4	1
			5	3.67	0.58	6.67	0.58	5.67	0.58
			2.5	-	-	6.33	0.58	6	0
			1.25	-	-	6	0	6.33	0.58
			0.625	-	-	5.67	0.58	7.33	2.31
			0.3125	-	-	5.33	0.58	7	0
Salmonella typhimurium	2.33	0.58	10	5.33	1.53	2.67	0.58	3.33	0.58
			5	4	0	5.33	1.15	5.67	0.58
			2.5	5	0	5.66	0.58	6	0
			1.25	5	0	7	0	8	1
			0.625	5.33	0.58	7.33	0.58	7	0
			0.3125	5	0	5.33	0.58	7	0
E.coli O157:H7	2.00	1.73	10	4.33	0.58	7	1	4	0
			5	4	0	7.67	0.58	6.33	0.58
			2.5	-	-	6.33	0.58	6.67	0.58
			1.25	-	-	7	0	7.33	0.58
			0.625	-	-	6	0	6.33	0.58
			0.3125	-	-	4	0	6.33	0.58

Table 4.1.2

4.1.2. Antimicrobial Effects of Vanillin, Turmeric and Curcumin in DMSO Solution

The average diameter and standard deviation of clear zone of inhibition on control sample, vanillin/DMSO solution, turmeric/DMSO solution and curcumin/ DMSO solution are illustrated in Table 4.1.2. The STDEV in Table 4.1.2 is much smaller when compared with Table 4.1.1, which means the results from Table 4.1.2 are more reliable. Moreover, as observed in the study, all tested powders were dissolved uniformly in DMSO. Thus, DMSO is considered as a better solvent for selected agents than Reagent alcohol. A comparison of antimicrobial activity of each solution over particular bacteria is represented in Figure 4.1.3 (a)-(e). Furthermore, clear zone diameter for three types of solution over selected bacteria is also illustrated in Figure 4.1.4 (a)-(e).

In Table 4.1.2, if there was no distinct clear zone of inhibition observed in the test, the diameter marked as "-". Standard deviation marked as "-" illustrated that there were no distinct clear zone of inhibition observed in three replicates.

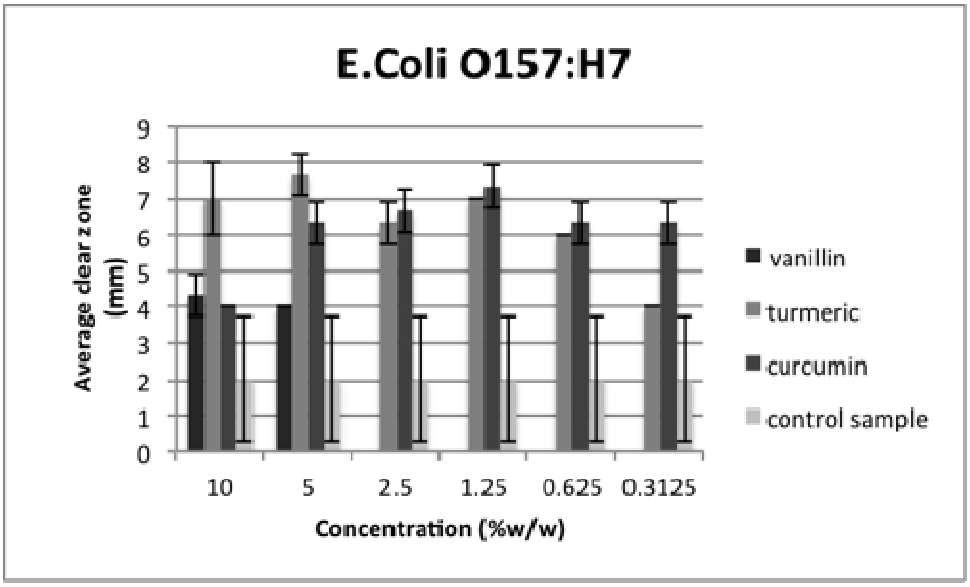
Different bacteria species showed variable sensitivity. Of the different agents tested for their antibacterial effect, vanillin/DMSO solution was the most susceptible to inhibited the growth of *Listeria monocytogenes* with the MIC at 1.25%(w/w). The result agrees with earlier observation (Yujie Cheng *et al*., 2011), but showed a lower MIC than previous study which given the MIC at 2.5%(w/w) in vanillin/Reagent alcohol soution. Furthermore, vanillin/DMSO solution inhibited the growth of *Staphylococcus aureus* and

Shigella sonnei to a moderate extent with the MIC at 5%(w/w). In addition, the same solution has slight less antimicrobial activity on *Salmonella typhimurium* and *E.coli O157:H7*, with the MIC at 0.3125%(w/w) and 5%(w/w) respectively. The results contradicts previous study on the antimicrobial effect of vanillin/ DMSO solution in inhibiting *E.coli O157:H7* by Rakchoy *et al*. (2009) who reported that vanillin/DMSO solution was more effective over *E.coli O157:H7* with the MIC at 2.5%(w/w). However, the results are in good agreement with pervious research from Rakchoy *et al.* (2009) who reported vanillin/DMSO solution was more effective over *Staphylococcus aureus*.

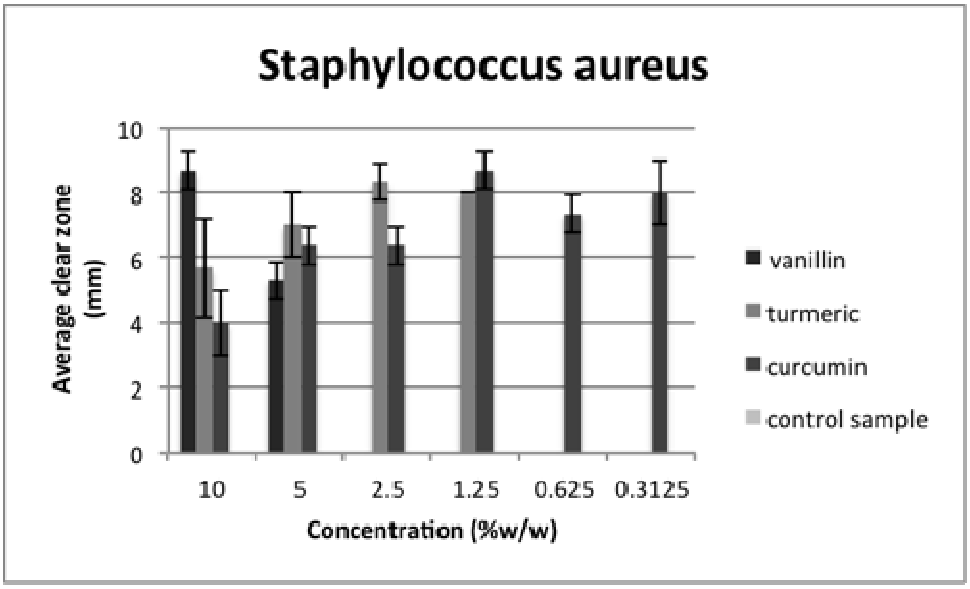
According to the results, turmeric/DMSO solution has a significant antimicrobial activity, with the MIC at 1.25%(w/w), towards Gram-positive bacteria: *Listeria monocytogenes* and *Staphylococcus aureus*. The results showed that turmeric in DMSO solvent possessed more antimicrobial activity than in Reagent alcohol. On the other hand, Gram-negative bacteria including *Shigella sonnei*, *Salmonella typhimurium* and *E.coli O157:H7* were sensitive to turmeric/DMSO solution and showed moderate sensitivity. The MIC of turmeric/DMSO solution is 0.3125%(w/w) on all tested Gram-negative bacteria.

Curcumin/DMSO solution possessed antibacterial activity against all tested bacteria. Amongst those tested bacteria, *Listeria monocytogenes, Staphylococcus aureus* and *Shigella sonnei* had a higher sensitivity on curcumin/DMSO solution than *Salmonella typhimurium* and *E.coli O157:H7*. Moreover, MIC was observed at 0.1325%(w/w) for all tested bacteria, which means such solution still possessed antimicrobial effect on all

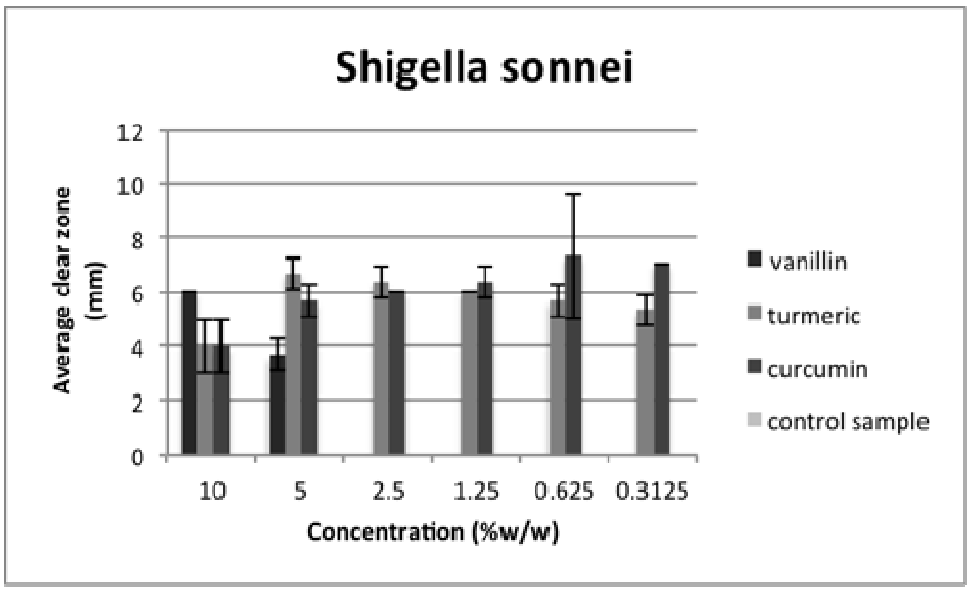
tested bacteria even though the concentration of solution decreased to the minimum in the experiment.



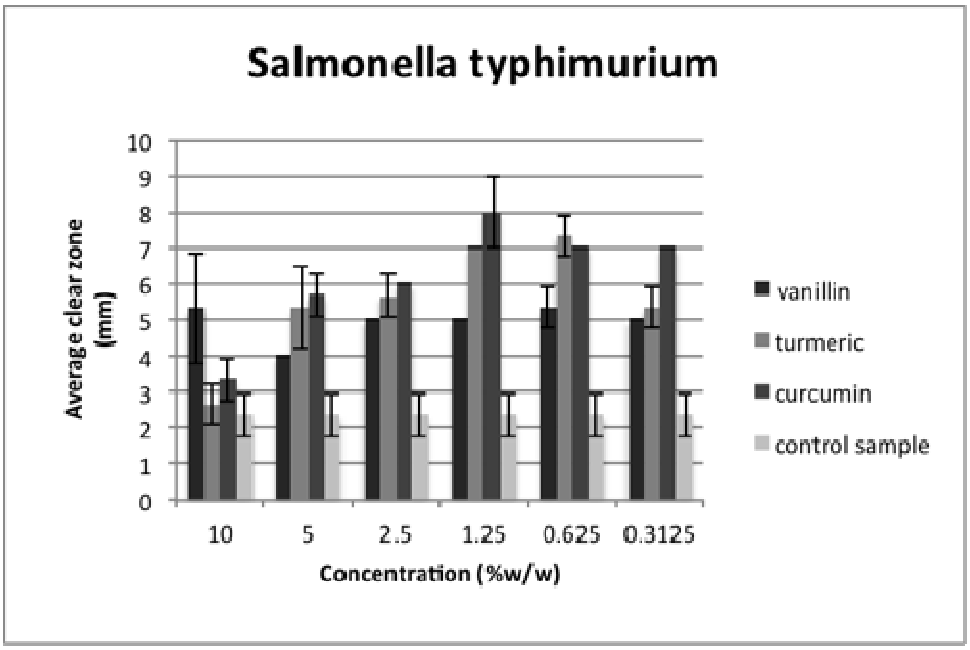
(a)



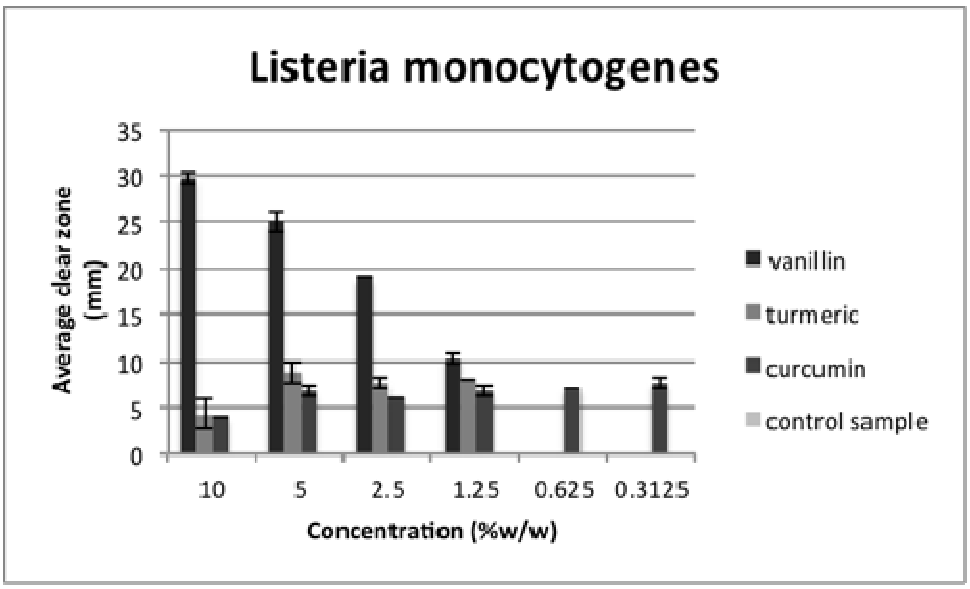
(b)


Shigella sonnei
Average clear zone (mm)
12
10
8
6
4
2
0
10
5
2.5
1.25
0.625
0.3125
Concentration (%w/w)
vanillin
turmeric
curcumin
control sample

(c)


Salmonella typhimurium
Average clear zone (mm)
10
9
8
7
6
5
4
3
2
1
0
10
5
2.5
1.25
0.625
0.3125
Concentration (%w/w)
vanillin
turmeric
curcumin
control sample

(d)



(e)

Figure 4.1.3 Antimicrobial activities and MICs of control sample and vanillin, turmeric, curcumin in DMSO solutions. (a) *E. Coli O157:H7*, (b) *Staphylococcus aureus*, (c) *Shigella sonnei*, (d) *Salmonella typhimurium*, (e) *Listeria monocytogenes*.

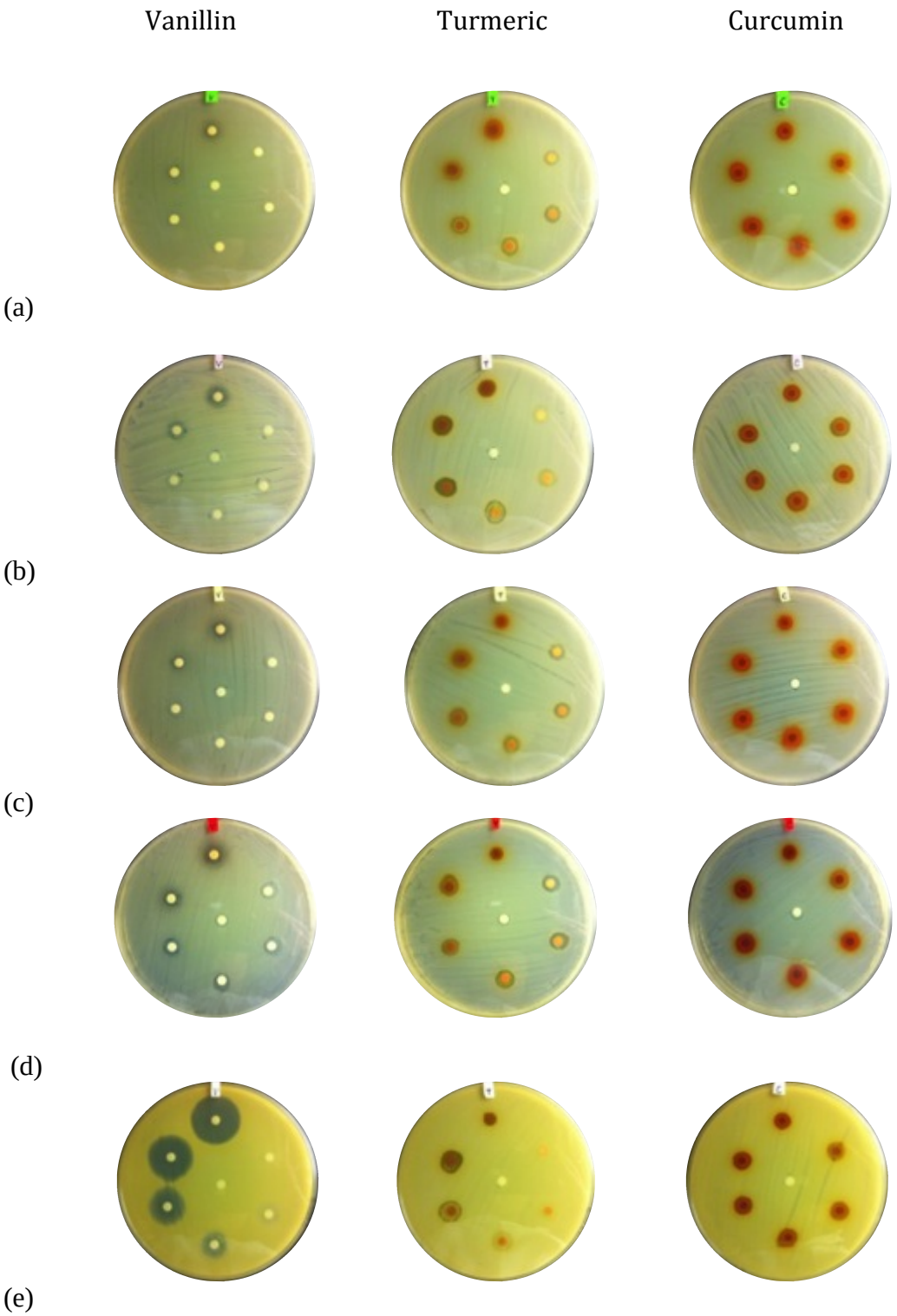


Figure 4.1.3 The illustration above shows zones of inhibition around antibiotic assay discs saturated with 40 µl of vanillin, turmeric and curcumin solutions respectively. (a) *E. Coli O157:H7*, (b) *Staphylococcus aureus*, (c) *Shigella sonnei*, (d) *Salmonella typhimurium*, (e) *Listeria monocytogenes*.

4.1.3. Comparison of the antimicrobial activity of agents in Reagent alcohol and DMSO

The measured clear zone of inhibition diameter after subtracts control sample effects for each tested Reagent alcohol solution is illustrated in Table 4.1.3. The negative diameter indicated control samples showed greater antibacterial effect than tested solution. Based on the previous study (Cheng *et al*., 2011), there are two possible reasons for such abnormal status. First, the poor solubility of powders in Reagent alcohol prevents effective particle diffusing in the medium. Second, the large Standard Deviation in the test affected the final results.

After subtract antibacterial effect from Reagent alcohol, curcumin/Reagent alcohol solution inhibited the growth of bacteria with *Listeria monocytogenes* being the most susceptible and *Salmonella typhimurium* the least. For turmeric/Reagent alcohol solution, even not as effective as curcumin/Reagent alcohol solution, still had a slight effect on *Listeria monocytogenes*. Vanillin/Reagent alcohol solution showed moderate antimicrobial activity on all tested bacteria.

Bacteria	Concentration (%w/w)	Reagent alcohol Average diameter (mm)		
		Vanillin	Turmeric	Curcumin
Listeria monocytogenes	10	7	5.67	1
	5	3	-2.33	12.67
	2.5	1.67	0.33	13
	1.25	2	1.67	13.33
Staphylococcus aureus	10	3.33	5.33	5.33
	5	1	-0.67	8.33
	2.5	0.67	0	16
	1.25	1	-0.33	0.67
Shigella sonnei	10	8	1	-2
	5	5.67	-1	4.34
	2.5	2.34	-2.33	7.34
	1.25	0.34	-1	6.34
Salmonella typhimurium	10	4	-10.34	-5.67
	5	0.66	-6.34	-7
	2.5	-2.34	-6.67	-5.67
	1.25	-3.67	-7.67	-5.67
E.coli O157:H7	10	9.67	-5.83	-
	5	2.67	-	-3.66
	2.5	0	-4.66	-2
	1.25	-0.66	-1	0.67

Table 4.1.3

Clear zone of inhibition diameter after subtract control sample effects for each Reagent alcohol solution

The measured clear zone of inhibition diameter after subtracted control sample effects for each tested DMSO solution are illustrated in Table 4.1.4. Compared with vanillin/DMSO solution, turmeric/ DMSO solution and curcumin/DMSO solution showed a greater inhibitory effect at lower concentrations on all tested bacteria. However, when the solution's concentration was above 1.25%(w/w), vanillin/DMSO solution had a stronger inhibition effect on *Listeria monocytogenes* than turmeric/ DMSO solution and curcumin/DMSO solution did.

DMSO, which used as cell protector for bacteria storage in the laboratory, nearly has no antimicrobial effect against all tested bacteria. Comparing Table 4.1.1 and Table 4.1.2, all tested powder solutions in Reagent alcohol showed larger diameter of inhibition zone than in DMSO. It seems that natural powder agents in Reagent alcohol possessed greater antimicrobial activity than in DMSO. However, the control samples with pure DMSO did not inhibit *Listeria monocytogenes*, *Staphylococcus aureus* and *Shigella sonnei*, and only showed slightly effect on *Salmonella typhimurium* and *E.coli O157:H7* (Table 4.1.2). On the other hand, there was more than 10mm inhibition diameter effect from all tested control sample with pure Reagent alcohol (Table 4.1.1). Thus, vanillin as well as turmeric and curcumin in their DMSO solutions reflected their actual antimicrobial effect against selected bacteria.

DMSO can solubilize the powders better than Reagent alcohol. In general, since DMSO has good solubility for selected powder, increasing the certain agent solution's concentration can intensify its antibacterial effect. During the preparation of solution, all

selected powders were uniformly dissolved in DMSO. However, only vanillin powder could dissolve uniformly in both Reagent alcohol and DMSO. Turmeric/Reagent alcohol solution and curcumin/Reagent alcohol solution existed insoluble particles in our previous study (Cheng *et al*., 2011). Based on the results, vanillin/DMSO solution showed increasing antimicrobial activity over selected bacteria strains as the concentration increased. However, data from turmeric/DMSO solution and curcumin/DMSO solution demonstrated a decreasing concentration of solution resulted in a first increased followed by decreased antimicrobial activity on certain concentration. We supposed that insoluble particles in the certain solution at higher concentration prevent the active agents diffusing in the agar plate.

Bacteria	Concentration (%w/w)	DMSO Average diameter (mm)		
		Vanillin	Turmeric	Curcumin
Listeria monocytogenes	10	29.67	4.33	4
	5	25	8.67	6.67
	2.5	19	7.67	6
	1.25	10.33	8	6.67
	0.625	-	-	7
	0.3125	-	-	7.67
Staphylococcus aureus	10	8.67	5.67	4
	5	5.33	7	6.33
	2.5	-	8.33	6.33
	1.25	-	8	8.68
	0.625	-	-	7.33
	0.3125	-	-	8
Shigella sonnei	10	6	4	4
	5	3.67	6.67	5.67
	2.5	-	6.33	6
	1.25	-	6	6.33
	0.625	-	5.67	7.33
	0.3125	-	5.33	7
Salmonella typhimurium	10	3	0.34	1
	5	1.67	3	3.34
	2.5	2.67	3.33	3.67
	1.25	2.67	4.67	5.67
	0.625	3	5	4.67
	0.3125	2.67	3	4.67
E.coli O157:H7	10	2.33	5	2
	5	2	5.67	4.33
	2.5	-	4.33	4.67
	1.25	-	5	5.33
	0.625	-	4	4.33
	0.3125	-	2	4.33

Table 4.1.4

4.1.4. The influence of concentration on antimicrobial activity over each bacterial

In summary, the results indicated that the selected bacteria's variable sensitivity depends on different solutions. If we subtracted the additional effect from control sample, the largest inhibition zone diameters for the selected bacteria should be showed in Table 4.1.5.

Bacteria	Solution	Concentration (%w/w)	Inhibition zone diameters (mm)
Listeria monocytogenes	Vanillin/DMSO	10	29.67
Staphylococcus aureus	Vanillin/DMSO	10	8.67
Shigella sonnei	Vanillin/Reagent alcohol	10	8.00
Salmonella typhimurium	Curcumin/DMSO	1.25	5.67
E.coli O157:H7	Vanillin/Reagent alcohol	10	9.67

Table 4.1.5

Selected bacteria can be effectively suppressed by using the particular solution with the largest inhibition zone diameter. However, agent's cost as well as antimicrobial activity should take into account when it comes to commercial use and industrial application. In general, optimum option must have lower cost with effective function for particular application. Thus, the Minimum Inhibitory Concentration (MIC) is an important index in this study.

4.1.5. The Minimum Inhibitory Concentration (MIC)

The MIC of selected agents solution on five bacteria is illustrated in Table 4.1.6. Although vanillin solution in both Reagent alcohol and DMSO solutions showed greater antimicrobial activity on selected bacteria, turmeric and curcumin were both have lower MIC, which indicates turmeric and curcumin, especially in their DMSO solutions, can inhibit selected bacteria when the concentration goes down. All the tested agents showed lower MIC in their DMSO solutions when compared with Reagent alcohol, which reconfirm our conclusion that DMSO has a better ability to solubilize the selected agents than Reagent alcohol. Turmeric/DMSO solution has MIC of 1.25%(w/w) on both *Listeria monocytogenes* and *Staphylococcus aureus,* and 0.3125%(w/w) on *Shigella sonnei*, *Salmonella typhimurium* and *E.coli O157:H7.* On the other hand, curcumin/DMSO solution showed 0.3125%(w/w) on all tested bacteria.

Bacteria	Vanillin (%w/w)		Turmeric (%w/w)		Curcumin (%w/w)	
	Reagent alcohol	DMSO	Reagent alcohol	DMSO	Reagent alcohol	DMSO
Listeria monocytogenes	2.5	1.25	10	1.25	1.25	0.3125
Staphylococcus aureus	10	5	10	1.25	2.5	0.3125
Shigella sonnei	2.5	5	-	0.3125	1.25	0.3125
Salmonella typhimurium	10	0.3125	-	0.3125	-	0.3125
E.coli O157:H7	2.5	5	-	0.3125	-	0.3125

Table 4.1.6 The MIC of selected agents in Reagent alcohol and DMSO solutions

4.1.6. Combination of powder and solvent

As the antimicrobial activity against certain bacteria is related to the type of bacteria as well as the type of powder's solution and its concentration, choose the combination of powder and solvent is crucial (Table 4.1.7).

Type of bacteria	Bacteria	Combination
Gram positive	Listeria monocytogenes	Vanillin/DMSO
	Staphylococcus aureus	Turmeric/DMSO; Curcumin/DMSO; Curcumin/Reagent alcohol
Gram negative	Shigella sonnei	Vanilli/Reagent alcohol; Turmeric/DMSO; Curcumin/DMSO
	Salmonella typhimurium	Turmeric/DMSO;Curcumin/DMSO
	E.coli O157:H7	Vanillin/Reagent alcohol

Table 4.1.7

4.2. Polymers containing antimicrobial agents

4.2.1. Turmeric containing LDPE discs

In this study, we chose turmeric as incorporated antimicrobial agent for the following reasons: (1) In all the three powders, turmeric powder has the acceptable melting point that can be used in single screw extruder. (2) It has antimicrobial activity. (3) Turmeric powder is the lowest cost among three powders.

After 48 hours (Figure 4.2.1), and 72 hours (Figure 4.2.2) in the incubation chamber at 36°C, there is no inhibition zone observed on the agar plate. Figure 4.2.3 is the control samples.

4.2.2. Turmeric coated BOPP films

In order to have comparable results, we chose turmeric as antimicrobial coating agent on BOPP films. However, there is no inhibition zone observed on the agar plates after 72 hours in the incubation chamber at 36°C (Figure 4.2.4).

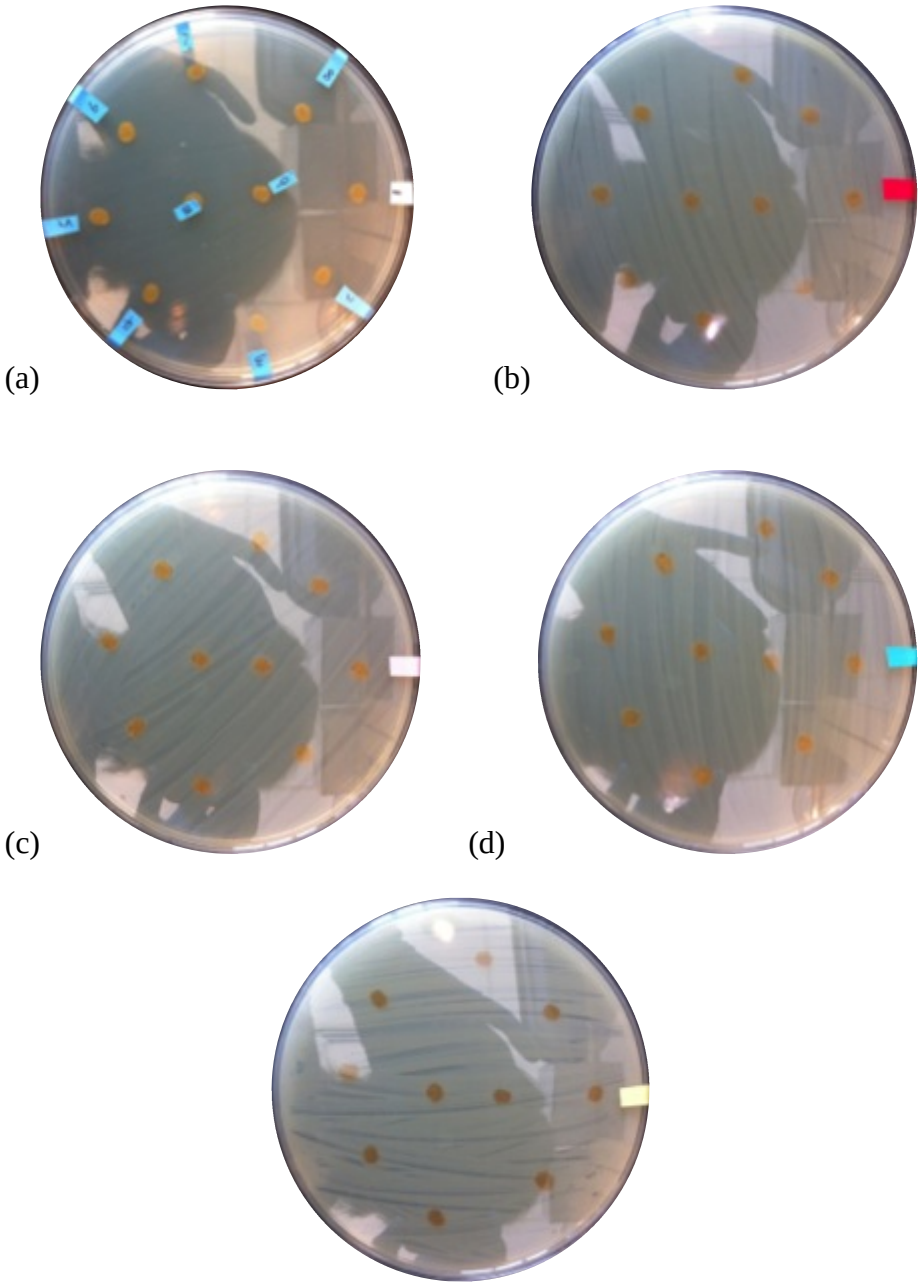


Figure 4.2.1. Turmeric containing LDPE resin after 48 hours: (a) *Listeria monocytogenes*, (b) *E. Coli O157:H7*, (c) *Salmonella typhimurium*, (d) *Shigella sonnei*, (e) *Staphylococcus aureus*.

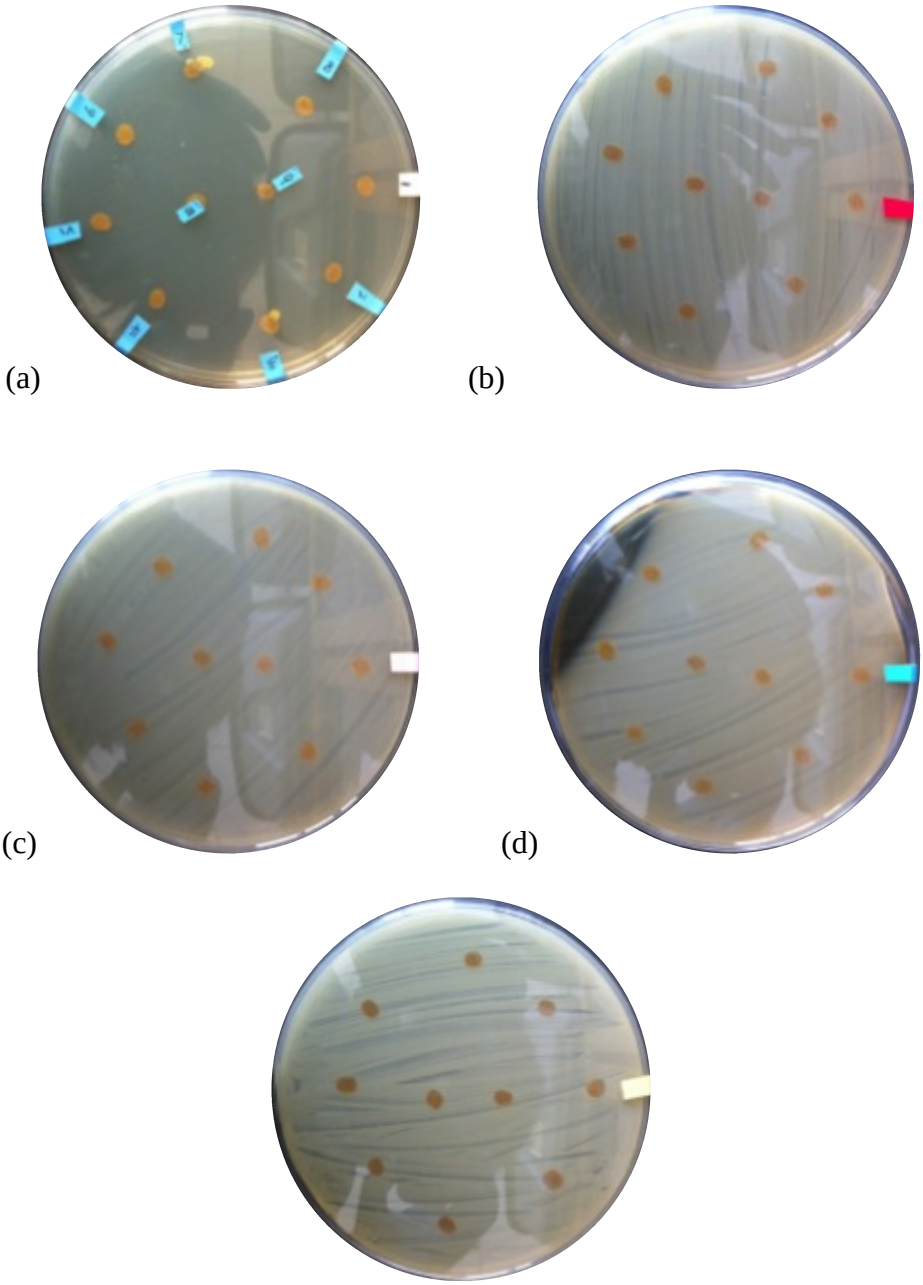


Figure 4.2.2 Turmeric containing LDPE resin after: (a) *Listeria monocytogenes*, (b) *E. Coli O157:H7*, (c) *Salmonella typhimurium*, (d) *Shigella sonnei*, (e) *Staphylococcus aureus*.

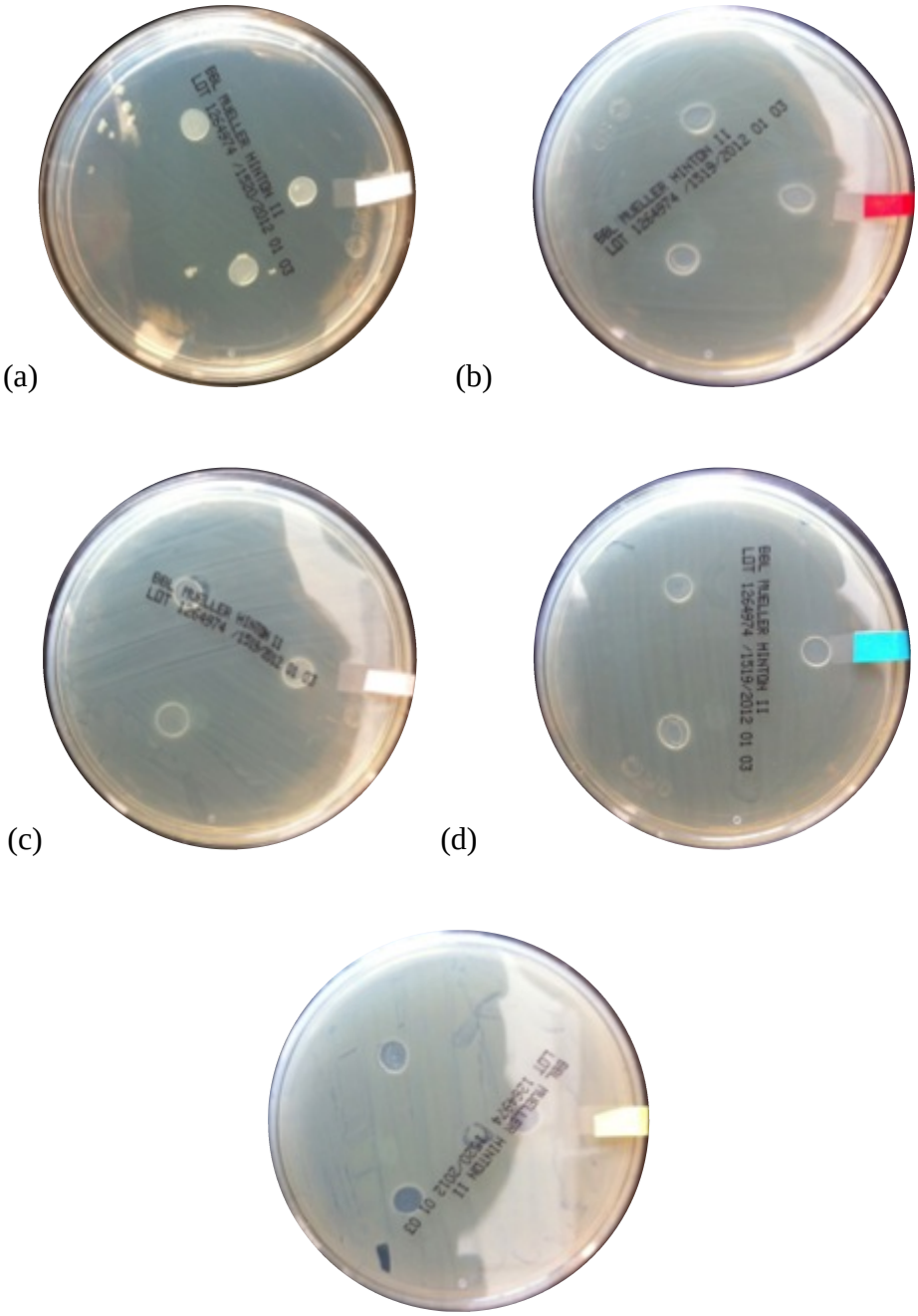


Figure 4.2.3 LDPE resin control samples: (a) *Listeria monocytogenes*, (b) *E. Coli O157:H7*, (c) *Salmonella typhimurium*, (d) *Shigella sonnei*, (e) *Staphylococcus aureus*.

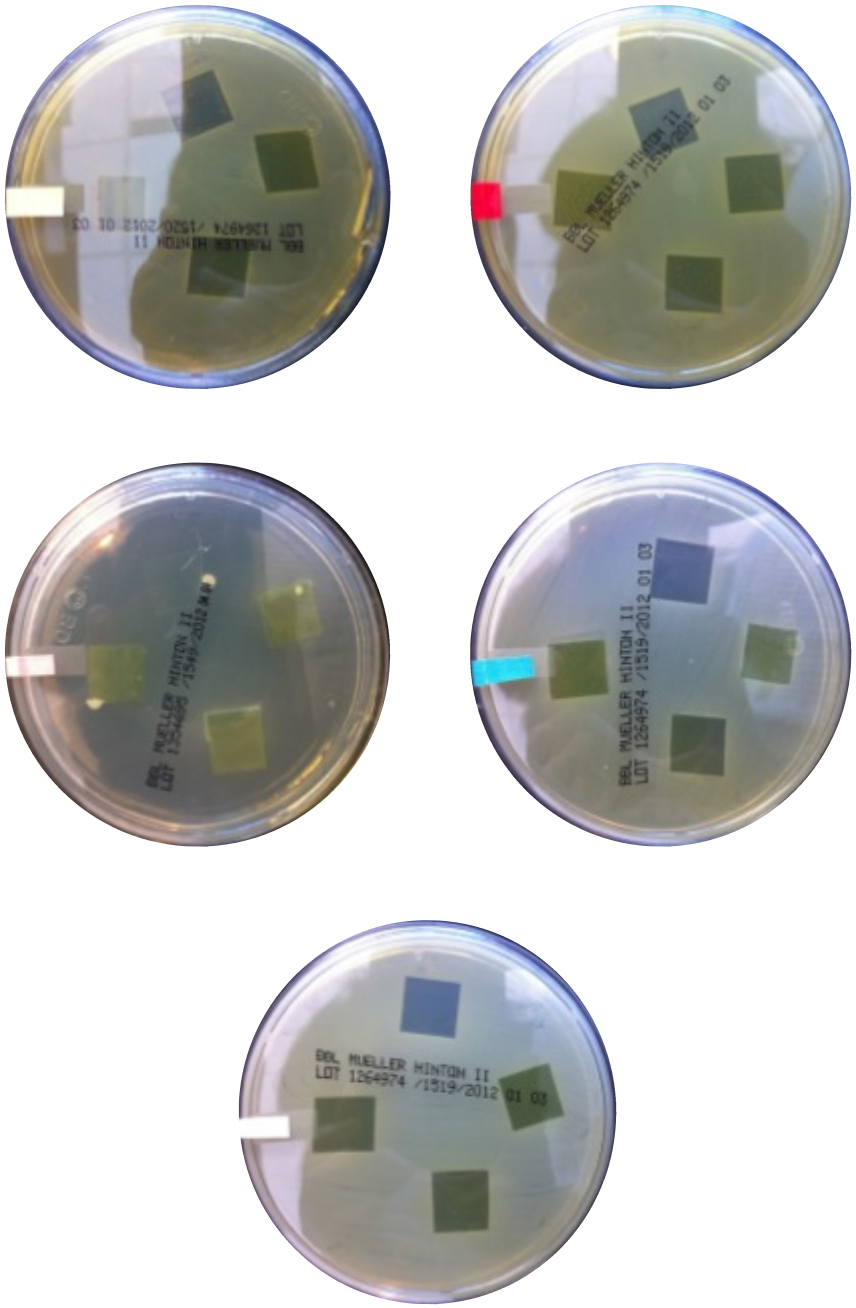


Figure 4.2.4 Turmeric coated BOPP films after 72 hours: (a) *Shigella sonnei*, (b) *E. Coli O157:H7*, (c) *Listeria monocytogenes*, (d) *Salmonella typhimurium*, (e) *Staphylococcus aureus*.

5 Conclusions and Future work

5.1. Conclusions

The results of the present study compares the antimicrobial activity of natural plant powders such as vanillin, curcumin and turmeric in different solvents, and highlight the promising potential of incorporating turmeric in industry using LDPE resin and commercial BOPP films to produce antimicrobial food packaging materials.

5.1.1. Effect of natural powders

According to this study, natural powders including vanillin, curcumin and turmeric are inhibitory to all tested bacteria to varying degrees. Vanillin/Reagent alcohol solution possesses the most significant antimicrobial activity on *E.coli O157:H7* with MIC at 2.5%(w/w), while it shows moderate effectiveness on *Shigella sonnei* and *Listeria monocytogenes* and slight activity on *Staphylococcus aureus* and *Salmonella typhimurium.* However, vanillin/DMSO solution inhibits the growth of bacteria with *Listeria monocytogenes* being the most susceptible with MIC at 1.25%(w/w). The same solution possesses a moderate to lower effect on *Staphylococcus aureus, Shigella sonnei, Salmonella typhimurium* and *E.coli O157:H7.* Based on the results, vanillin/Reagent alcohol solution is more effective against *E.coli O157:H7* (Gram negative bacteria). However, vanillin/DMSO solution is more effective against *Listeria monocytogenes*

(Gram positive bacteria). This may be due to the properties as well as chemical and physical structure of the solvents, powders and bacteria. The combination of those factors in the solution may possess a significant impact on the antimicrobial activity.

On the other hand, curcumin and turmeric powders in both Reagent alcohol and DMSO solution possessed similar antimicrobial effects. Gram-positive bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* are generally more sensitive to curcumin and turmeric than Gram-negative bacteria such as *Shigella sonnei, Salmonella typhimurium* and *E.coli O157:H7*, which agrees with previous studies by Smith-Palmer et al. (1998) (Burt, 2004). However, since DMSO solubilizing curcumin and turmeric better than Reagent alcohol, the results from DMSO are more reliable than Reagent alcohol.

5.1.2. Effect of solvents

Comparing the antimicrobial results from Reagent alcohol and DMSO, which are used as powder solvents, it seems that all natural plant powders in Reagent alcohol give a larger diameter of inhibition zone than in DMSO. However, after subtracting the side effect from the solvent, powders in DMSO give a better antimicrobial effect than Reagent alcohol.

This is due to the antimicrobial effect from the solvent and how the solvent solubilizes the powders. Pure Reagent alcohol, as the control sample in our study, has antimicrobial activity against all tested bacteria. The fact that vanillin/Reagent alcohol solution

possessed significant antimicrobial activity against Gram-negative bacteria: *E.coli O157:H7*, could explain by the possibility that Reagent alcohol in vanillin/Reagent alcohol solution is more toxicity on Gram-negative bacteria than Gram-positive bacteria. Turmeric/Reagent alcohol solution and curcumin/ Reagent alcohol solution contained visible insoluble particles. The insoluble particles, which prevent the migration of solution that contained antimicrobial agents, were considered to be the critical impact factor for the large STDEV and decreasing antimicrobial activity with increasing solution's concentration in this study.

DMSO, which is used as a cell protector for bacteria storage in the laboratory, has nearly no antimicrobial effect against all tested bacteria. Thus, compared with Reagent alcohol, the results in DMSO may reflect the actual effect from selected powders. DMSO has a better solubility than Reagent alcohol, especially when it is used with curcumin and turmeric powders. However, for curcumin and turmeric powders, a decreasing concentration of the solution results in an increased antimicrobial activity. This indicates DMSO, is even a better solvent than Reagent alcohol, but the DMSO still has limitation on solubilizing curcumin and turmeric powders.

In conclusion, antimicrobial activity of natural plant powders is complicated, and influenced by many factors such as the type of solvent, spice and bacteria used. Solvent may influence the effectiveness of selected powders in two aspects. First, solvents may possess antimicrobial activity, which causes an additional effect on tested bacteria. Second, if the solubility of a solvent is poor for some agents, those agents may migrate

slowly through the solvent to the agar and weaken the antimicrobial activity from agents. Thus, the selection of a solvent to dissolve an antimicrobial agent is important for this study.

5.1.3. Combination of powder and solvent

The antimicrobial activity against certain bacteria is related to the type of bacteria as well as the type of the powder's solution and its concentration. Therefore choosing the proper combination of powder and solvent is very important.

5.1.4. Effect of turmeric incorporated polymer

Based on the results from this study, there are no antimicrobial effectiveness of turmeric incorporated LDPE resin and BOPP films. This result might be due to several factors including the chemical and physical structure, temperature during processing and moisture of the environment. The configuration of the polymers' matrix, like the low presence of a porous medium, can influence the diffusion phenomenon of turmeric powders. On the other hand, the antimicrobial activity of turmeric powder may be influenced by high temperature during the single screw extruder processing. Moreover, insufficient moisture in the environment for such products prevent the migration of turmeric powders out of polymer matrix.

5.2. Future work

5.2.1. Solvent properties

The antimicrobial activity of tested powder is influenced by solvent properties, especially the solubility and antimicrobial activity from solvents. Chemical and physical properties for solvents should be investigated. Moreover, other natural spices such as cloves, oregano and cinnamon should be tested in both Reagent alcohol and DMSO solutions in order to justify the antimicrobial effect from solvents.

5.2.2. Antimicrobial susceptibility of bacteria by survival analysis

The antimicrobial susceptibility over time of bacteria for certain agents could be demonstrated by survival curves. Changes in antimicrobial susceptibility were monitored by a survival analysis approach that tests changes in bacterial growth inhibition for a given antimicrobial agent across the entire range of tested concentrations (Álvaro Hidalgo *et al*., 2011). The survival analysis can confirm the antimicrobial effectiveness of such agents against certain bacteria.

5.2.3. Polymer incorporated antimicrobial agents

Chemical and physical factors are related to the diffusion of antimicrobial agents through the packaging material. This diffusion may be influenced by hydrogen bond, ionic bond,

hydrophobic interactions, electrostatic interactions, and so on (Bastarraches *et al.*, 2011). Future studies on packaging materials with high a presence of a porous medium may be conducted to understand the feasibility of polymer incorporated antimicrobial material. In some other studies, it is not always possible to incorporate the studied antimicrobial agents directly in the films' matrix (Bastarraches *et al.*, 2011). If this is the case, other natural antimicrobial spices with a small particle size, good solubility and applicable property on industry processing, should be considered.

Reference

Aaron L. Brody, Eugene R. Strupinsky and Lauri R. Kline. (2001). *Active packaging for food applications.* Lancaster, PA : Technomic Publishing Co., Inc.

Abel Guarda, Javiera F. Rubilar, Joseph Miltz & Maria Jose Galotto. (2011). The antimicrobial activity of microencapsulated thymol and carvacrol. *International Journal of Food Microbiology*,146(2), 144–150.

Álvaro Hidalgo, Ana Carvajal, Birte Vester, Ma□rit Pringle, Germán Naharro & Pedro Rubio. (2011). Trends towards Lower Antimicrobial Susceptibility and Characterization of Acquired Resistance among Clinical Isolates of Brachyspira hyodysenteriae in Spain. *Antimicrobial Agents and Chemotheraoy*, 55(7), 3330-3337.

Andrea Carolina Valderrama Solano & Cecilia de Rojas Gante. (2011). Two Different Processes to Obtain Antimicrobial Packaging Containing Natural Oils. *Food Bioprocess Technology.*

Appendini, P. & Hotchkiss, J.H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, 3(2), 113-26.

A. Rodr´ıguez, R. Batlle & C. Ner´ın. (2007). The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. *Progress in Organic Coatings*, 60, 33–38.

A. Smith-Palmer, J. Stewart & L. Fyfe. (1998). Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*, 26, 118-122.

Bhavani Shankar, T. N. & Sreenivasa Murthy, V. (1979). Effect of turmeric (Curcuma *longa)* fractions on the growth of some intestinal and pathogenic bacteria *in vitro. Indian J. Exp. Biol*, 17, 1363– 1366.

Bhawana, Rupesh Kumar Basniwal, Harpreet Singh Buttar, V. K. Jain, & Nidhi Jain. (2011).Curcumin Nanoparticles: Preparation, Characterization, and Antimicrobial Study. *Journal of Agricultural and Food Chemistry*, 59(5), 2056–2061.

Bledar Bisha, Natalia Weinsetel, Byron F. Brehm-Stecher & Aubrey Mendonca. (2010). Antilisterial Effects of Gravinol-S Grape Seed Extract at Low Levels in Aqueous Media and Its Potential Application as a Produce Wash. *Journal of Food Protection*, 73(2), 266-273.

CAC Araújo & LL Leon. (2001). Biological Activities of Curcuma longa L. *Mem Inst Oswaldo Cruz, Rio de Janeiro*, 96(5), 723-728.

Chai, H., S. Yan, P. Lin, A. B. Lumsden, Q. Yao & C. Chen. (2005). Curcumin blocks HIV protease inhibitor ritonavir-induced vascular dysfunction in porcine coronary arteries. *J. Am. Coll. Surg*, 200, 820-830.

Charu Gupta, Amar P. Garg, Ramesh C. Uniyal & Archana Kumari. (2008). Antimicrobial activity of some herbal oils against common food-borne pathogens. *African Journal of Microbiology Research*, 2, 258-261.

Daeschel, M.A., McGuire, J., & Al-Makhlafi, H. (1992). Antimicrobial activity of nisin absorbed to hydrophilic and hydrophobic silicon surfaces. *J. Food Protect*, 55, 731-735.

Daljit S. Arora & Jasleen Kaur. (1999). Antimicrobial activity of spices. *International Journal of Antimicrobial Agents*, 12, 257–262.

Dong Sun Lee, Kit L.Yam, & Luciano Piergiovanni. (2008). *Food packaging science and technology*. Boca Raton : CRC Press.

Emad M. Abdallah. (2011). Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science*, 1 (6), 16-20.

Han JH. (2000). Antimicrobial food packaging. *Food Technology*, 54(3), 56-65.

Han JH & Floros JD. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *J Plastic Film Sheeting*. 3(4), 287-298.

Han Jung H. (Ed.). (2005). *Innovations in food packaging*. San Diego, Calif.: Elsevier Academic; Oxford: Elsevier Science.

H.J.D. Dorman & S.G. Deans. (2000). Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. *Journal of Applied Microbiology*, 88,308-316.

Ishitani, T. (1995). Active packaging for food quality preservation in Japan. In "Food and Food Packaging Materials— Chemical Interactions," ed. P. Ackermann, M. Jagerstad, & T. Ohlsson, pp. 177-188. Royal Soc. of Chemistry, Cambridge, England. Cited in Hotchkiss (1997).

Ishita Chattopadhyay, Kaushik Biswas, Uday Bandyopadhyay & Ranajit K. Banerjee. (2004). Turmeric and curcumin: Biological actions and medicinal applications. *Current Science*, 87(1), 44-52.

Jurmkwan Sangsuwan, Nithiya Rattanapanone & Pornchai Rachtanapun. (2008). Effects of vanillin and plasticizer on properties of chitosan-methyl cellulose based film. *Journal of Applied Polymer Science*, 109(6), 3540-3545.

Kim, Y.T., Kim, K., Han, J.H. & Kimmel, R.H. (2008). Antimicrobial packaging for foods. In Joseph Kerry & Paul Butler (Eds.), *Smart Packaging Technologies* (pp.111-123). Chichester, England; Hoboken, NJ: John Wiley.

Luis Bastarrachea, Sumeet Dhawan & Shyam S. Sablani. (2011). Engineering Properties of Polymeric-Based Antimicrobial Films for Food Packaging. *Food Eng Rev*, 3,79–93.

Marjorie Murphy Cowan. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews,* 12(4), 564-582.

Mohammadreza Radfar, M. S. Sudarshana, H. U. Kavitha, S. Satish & M. H. Niranjan. (2012).Evaluation of antibacterial and antifungal activity of root and root callus extracts of Trianthema decandra L. *African Journal of Biotechnology*, 11(2), 510-515.

Myron D. Nicholson. (1998). The role of natural antimicrobials in food/packaging biopreservation. *Journal of Plastic Film and Sheeting,* 14, 234-241.

Ouattara, B., Simard, S., Piette, G.J.P., Holley, R.A., & Begin, A. (1999). Diffusion of acetic and propionic acids from chitosan films immerged in water. Presented at Ann. Mtg. of Inst. of Food Technologists, Chicago, Ill., July 24-28, 1995.

Panuwat Suppakul, Joseph Miltz, Kees Sonneveld & Stephen W. Bigger. (2006). Characterization of Antimicrobial Films Containing Basil Extracts. *Packag. Technol. Sci.,* 19, 259–268.

Pérez-Pérez, C., Regalado-González, C., Rodríguez-Rodríguez, C. A., Barbosa-Rodríguez, J. R. & Villaseñor-Ortega, F. (2006). Incorporation of antimicrobial agents in food packaging films and coatings. In Guevara-González, R. G. & Torres-Pacheco, I. (Eds.), *Advances in agricultural and food biotechnology* (pp. 193-216). Research Signpost.

Prashant Tiwari, Kuldeep Kumar ,Rajnikant Panik ,Alok Pandey ,Ashish Pandey & Pratap Kumar Sahu. (2011). Antimicrobial activity evaluation of the root of Carica papaya Linn. *International Journal of PharmTech Research,* 3(3), 1641-1648.

Preetha Anand, Sherin G. Thomas, Ajaikumar B. Kunnumakkara, Chitra Sundaram, Kuzhuvelil B. Harikumar, Bokyung Sung, Sheeja T. Tharakan, Krishna Misra, Indira K. Priyadarsini, Kallikat N. Rajasekharan & Bharat B. Aggarwal. (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochemical Pharmacology,* 76, 1590–1611.

Priyanka Chaudhary, Pramod Kumar Sharma, Vipin Kumar Garg & Jonish Varshney. (2010). A review on pharmacological activities of turmeric. *Pharmacologyonline,* 3, 193-199.

Preeti Singh, Ali Abas Wani & Sven Saengerlaub. (2011). Active packaging of food products: recent trends. *Nutrition and Food Science,* 41(4), 249-260.

P. Suppakul, J. Milta, K. Sonneveld & S.W. Bigger. (2003). *Journal of Food Science,* 68(2), 408-420.

P. S. Negi, G. K. Jayaprakasha, L. Jagan Mohan Rao, & K. K. Sakariah. (1999). Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *J.Agric. Food Chem.,* 47, 4297-4300.

Raija Ahvenainen (Ed.). (2003). *Novel Food Packaging Techniques* (pp.6-19). Boca Raton: CRC Press; Cambridge, England: Woodhead Pub. Ltd.

Regulation (EC) No 1935/2004 Of The European Parliament and of The Council of 27 October 2004; on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC

Reason Wilken, Mysore S Veena, Marilene B Wang & Eri S Srivatsan. (2011). Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Molecular Cancer,* 10:12.

Rooney M.L. (Ed.). (1995). *Active food packaging* (pp.1-33). London: Blackie Academic & Professional.

Rooney Michael L. (2005). Introduction to active food packaging technologies. In Jung H. Han (Ed.), *Innovations in Food Packaging* (pp.63-79). San Diego, Calif.: Elsevier Academic; Oxford: Elsevier Science.

Sacharow, S. (1988). Freshness enhancers: The control in controlled atmosphere packaging. *Preserved Foods,* 157(5), 121-122.

Saeed Tajbakhsh, Khosro Mohammadi, Iman Deilami, Keivan Zandi, Moradali Fouladvand, ElissaRamedani & GolandamAsayesh. (2008). Antibacterial activity of indium curcumin and indium diacetylcurcumin. *African Journal of Biotechnology,* 7(21), 3832-3835.

Sara Burt. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology,* 94, 223–253.

Seher Gur,Dilek Turgut-Balik & Nazmi Gur. (2006). Antimicrobial activities and some fatty acids of Turmeric, Ginger Root and Linseed Used in the Treatment of infectious diseases. *World Journal of Agricultural Sciences,* 2(4), 493-442.

Smith, J.P., Ooraikul, B., Koersen, W.J., van de Voort, F.R., Jackson, E.D., & Lawrence, R.A. (1987). Shelf life extension of a bakery product using ethanol vapor. *Food Microbiol,* 4, 329-337.

Sudsuda Vanit, Panuwat Suppakul & Tunyarut Jinkarn. (2010). Antimicrobial effects of coating solution containing clove oil and hydrophobic starch for coating paperboard. *Asian Journal of Food & Agro-Industry,* 3(2), 204-212.

Suwarat Rakchoy, Panuwat Suppakul & Tunyarut Jinkarn. (2009). Antimicrobial effects of vanillin coated solution for coating paperboard intended for packaging bakery products. *Asian Journal of Food and Agro-Industry,* 2(4), 138-147.

Suwarat Rakchoy, Panuwat Suppakul & Tunyarut Jinkarn. (2010). Antimicrobial paperboard packaging for bakery products. Proceeding of the 17th IAPRI world conference on packaging, pp. 780-784.

T. Sivarooban, N.S. Hettiarachchy & M.G. Johnson. (2008). Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Research International,* 41,781–785.

Weng YM & Hotchkiss JH. (1993). Anhydrides as antimycotic agents added to polyethylene films for food packaging. *Packag. Technol. Sci.,* 6(3), 123-128.

Wilson, Charles L. (Ed.). (2007). *Intelligent and active packaging for fruits and vegetables*. Boca Raton : Taylor & Francis.

Yujie Cheng, Changfeng Ge, Jeffrey Lodge & K.S.V.Santhanam. (2011). Evaluation Study of Vanillin, Curcumin and Turmeric with Potential Use in Antimicrobial Packaging Applications. *Journal of Applied Packaging Research,* 5(4), 215-226.

Yu Wang, Zhaoxin Lu, Hao Wu & Fengxia Lv. (2009). Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens. *International Journal of Food Microbiology,* 136, 71–74.

Appendix A Material Properties

Table A.1. Typical properties of LDPE resin

Polymer resin: Petrothene® NA 214-000, Low Density Polyethylene		
Physical Properties		
Density	0.918 g/cc	ASTM D1505
Melt index	10 g/10min	ASTM D1238
Vicat Softening Point	86°C	ASTM D1525
Melting point	284°F	
Freezing point	122°F	
Applications	PETROTHENE NA 214-000 is a low density polyethylene resin specifically designed for high speed, light weight, extrusion coating applications. This resin can be drawn to a coating weight of three pounds per rem at line speed exceeding 1,500 fpm with minimum neck-in and no edge weave. NA 214-000 can be used for applications including sugar pouches, industrial and multi-wall bags, treated and primed films and laminations.	
Processing Techniques	A melt temperature of 590°-620°F is recommended for use in processing NA 214-000 in light-weight coating applications. Electrostatic, flame or chemical priming should be used to ensure good adhesion. The maximum melt temperature that is recommended for NA 214-000 is 625°F.	

Table A.2. Typical properties of BOPP films

Polymer film: Bicor™ 100 SLP, Biaxially Oriented Polypropylene	
Product Characteristics	
Product Name	POLYPROPYLENE FILM
Product Description	Polymer article
Intended Use	Labeling material, Packaging material
Supplier	EXXONMOBIL CHEMICAL COMPANY
General Information	
Physical State	Solid
Form	Film
Color	Colorless
Odor	None to mild
Physical Properties	
Melting point	116°C (240°F) - 171°C (340°F)
Relative Density (at 20 °C)	0.9
Freezing Point	N/D

Table A.3. Typical properties of Vanillin

Antimicrobial agent: Vanillin, product number 94752	
Synonyms	4-Hydroxy-3-methoxybenzaldehyde Vanillinum
Formula	$C_8H_8O_3$
Molecular Weight	152.15 g/mol
CAS Number	121-33-5
EC Number	204-465-2
Vapour pressure	1 hPa (1 mmHg) at 107 °C (225 °F)
Melting point	Melting point/range: 81 - 83 °C (178 - 181 °F) - lit.
Boiling point	170 °C (338 °F) at 20 hPa (15 mmHg) - lit.
Flash point	No data available
Density	No data available

Table A.4. Properties of turmeric

Antimicrobial agent: Turmeric, product number 330	
CAS Number	No data available
EC Number	No data available
Vapour pressure	No data available
Melting point	175 °C (347 °F)
Boiling point	No data available
Flash point	No data available
Density	No data available

Table A.5. Properties of curcumin

Antimicrobial agent: Curcumin, product number C1386	
Synonyms	Natural Yellow 3 (E,E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione Diferulylmethane Diferuloylmethane
Formula	$C_{21}H_{20}O_6$
Molecular Weight	368.38 g/mol
CAS Number	458-37-7
EC Number	207-280-5
Vapour pressure	No data available
Melting point	Melting point/range: 175 °C (347 °F)
Boiling point	No data available
Flash point	No data available
Density	No data available