ANTIMICROBIAL PROPERTIES AND CHARACTERIZATION OF PLASTIC PACKAGING FILM INCORPORATED WITH GARLIC OIL

By

SUNG SUET YEN

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ABSTRACT

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Sung Suet Yen

The recent food-borne microorganisms outbreaks in some countries have gained the public awareness on food safety. It is important to develop more innovative approaches to inhibit microbial growth on foods while maintaining food quality. Antimicrobial (AM) packaging is one of the novel developments that involves the incorporation of AM agent into polymer film. Owing to the demand of natural AM agent from plant extract due to safety concern, this research is focus on the development of AM plastic packaging by using garlic oil extracts as AM agent. Blown film extrusion method was employed to produce film samples added with garlic oil in 0, 2, 4, 6 and 8% w/w. Challenge test showed that this film was able to reduce the number of *L. monocytogenes* on ready-to-eat beef loaves after 3, 6, 9 and 15 days of storage at 4^{o}C. Nevertheless, the effectiveness on *E. coli* and *B. thermosphacta* were not significant. Meanwhile, high amount of garlic oil only slightly reduced the films' mechanical strength. Flow mark on the films surface as observed by scanning electron micrograph (SEM) might be related to such reduction. On the other hands, differential scanning calorimeter (DSC) thermogram showed that the film crystallinity increased significantly in the addition of garlic oil. For water vapor barrier properties, film with higher amount of garlic oil

proved to have weaker barrier. There was no significant difference in the thermal stability for all samples when tested with thermogravimetry analyser (TGA). Infrared spectroscopy analysis showed garlic oil does not change the polymer structure. While for melt flow testing, the melt flow rate increased linearly in relation to the amount of garlic oil. Overall, this research exhibited that plastic film contained garlic oil has good potential to be used as food packaging especially to inhibit *L. monocytogenes*, without ruins the film physical properties.

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APPROVAL SHEET

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Approved by:

_____

(Ir. Dr. LEE TIN SIN) Date:……..
Associate Professor/ Supervisor
Department of Chemical Engineering
Faculty of Engineering and Science
Universiti Tunku Abdul Rahman

_____

(Ir. Dr. TEE TIAM TING) Date:……..
Professor/ Co-supervisor
Department of Chemical Engineering
Faculty of Engineering and Science
Universiti Tunku Abdul Rahman

_____

(Dr. ABDUL RAZAK RAHMAT) Date:……..
Associate Professor/ Co-supervisor
Department of Polymer Engineering
Faculty of Chemical engineering
Universiti Teknologi Malaysia

FACULTY OF ENGINEERING SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

SUBMISSION OF FINAL YEAR PROJECT /DISSERTATION/THESIS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATIONS

m	average mass
χ	degree of crystallinity
Ɛ	elongation at break
T_i	initial decomposition temperature
ΔH_m	latent heat of fusion
m_{nom}	nominal load
T_p	peak decomposition temperature
W	polymer weight fraction
T	temperature
ΔH^o_m	theoretical latent heat of fusion for 100% crystalline polyethylene
t	time-interval
% v/v	volume of solute per volume of total mixture
% w/v	weight of solute per volume of total mixture
% w/w or % weight	weight of solute per weight of total mixture

$-NH_2$	amino group
C=O	carbonyl
CO_2	carbon dioxide
Na_2EDTA	disodium ethylenediaminetetraacetic acid
$Na_2EDTA{\cdot}2H_2O$	ethylenediaminetetraacetic acid disodium salt
Au	gold
O_2	oxygen
Pd	palladium
NaCl	sodium chloride
$Na_2S_2O_5$	sodium metabisulfite
(-SH)-	sulfhydryl group
H_2SO_4	sulfuric acid
TiO_2	titanium dioxide
ZnO	zinc oxide

A. Oris	*Actinomyces oris*
A. flavus	*Aspergillus flavus*
A. fumigatus	*Aspergillus fumigatus*
A. niger	*Aspergillus niger*
B. cereus	*Bacillus cereus*
B. subtilis	*Bacillus subtilis*
B. thermosphacta	*Brochothrix thermosphacta*
C. utilis	*Calathea utilis*
C. albicans	*Candida albicans*
C. botulinum	*Clostridium botulinum*
C. fasciculate	*Crithidia fasciculate*
E. histolytica	*Entamoeba histolytica*
E. faecium	*Enterococcus faecium*
E. coli	*Escherichia coli*
G. Lamblia	*Giardia Lamblia*
H. alvei	*Hafnia alvei*
H. pylori	*Helicobacter pylori*
L. plantarum	*Lactobacillus plantarum*
L. lactis	*Lactococcus lactis*
L. colosoma	*Leptomonas colosoma*
L. major	*Leishmania major*
L. innocua	*Listeria innocua*
L. monocytogenes	*Listeria monocytogenes*
M. flavus	*Micococcus flavus*
P. italicum	*Penicillium italicum*
P. nigricans	*Penicilium nigricans*
P. membranifaciens	*Pichia membranifaciens*
P. gingivalis	*Porphyromonas gingivalis*
P. aeruginosa	*Pseudomonas aeruginosa*
R. mucilaginosa	*Rhodotorula mucilaginosa*
S. cerevisiae	*Saccharomyces cerevisiae*
S. enterica	*Salmonella enterica*
S. enteritidis	*Salmonella enteritidis*
S. typhimurium	*Salmonella typhimurium*

S. liquefaciens	*Serratia liquefaciens*
S. putrefacens	*Shewanella putrefacens*
S. dysenteriae	*Shigella dysenteriae*
S. flexneri	*Shigella flexneri*
S. sonnei	*Shigella sonnei*
S. aureus	*Staphylococcus aureus*
S. faecalis	*Streptococcus faecalis*
S. mutans	*Streptococcus mutans*
S. sobrinus	*Streptococcus sobrinus*
Z. aouxii	*Zygosaccharomyces aouxii*
Z. bisporus	*Zygosaccharomyces bisporus*

AAPEF	alginate-apple puree edible film
AIT	allyl isothiocyanate
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
AM	antimicrobial agent
CPAE	chickpea albumin extract
CFU	colony forming unit
DNA	deoxyribonucleic acid
DAD	diallyl disulfide
DAS	diallyl sulfide
DAT	diallyl trisulphide
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
DSA	differential scanning calorimetry
EB	electron beam
EVA	ethylene vinyl acetate
EVA/LDPE	ethylene vinyl acetate blended low density polyethylene
EVOH	ethylene vinyl alcohol
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drugs Administration
FTIR	fourier transform infrared spectroscopy
GA	glutaraldehyde

GRAS	generally recognized as safe
GFSE	grape fruit seed extract
HDPE	high density polyethylene
HIV	human immunodeficiency virus
HPMC	hydroxypropyl methylcellulose
LA	lactic acid
LAB	lactic acid bacteria
LLDPE	linear low density polyethylene
LPS	lipopolysaccharide
LDPE	low density polyethylene
MD	machine direction
MPa	mega Pascal
MFR	melt flow rate
MAP	modified atmosphere packaging
OPP	oriented polypropylene
ppm	part per million
PA	polyamide
PCL	polycaprolactone
PE	polyethylene
PEG	polyethylene glycol
PET	polyethylene terephthalate
PLA	polylactic acid
PP	polypropylene
PS	polystyrene
PU	polyurethane
PVA	polyvinyl acetate
PVC	poly(vinyl chloride)
PVdC	poly(vinylidene chloride)
KS	potassium sorbate
RTE	ready-to-eat
RH	relative humidity
rpm	revolution per minute
RNA	ribonucleic acid
SB	sodium benzoate

SD	sodium diacetate
SL	sodium lactate
TS	tensile strength
TGA	thermogravimetric analysis
TPS	thermoplastic starches
TVC	total viable count
TSA	tryptone soya agar
TSB	tryptone soya broth
UV	ultraviolet
UTM	universal testing machine
WVP	water vapor permeability
WVTR	water vapor transmission rate
WPI	whey protein isolate

CHAPTER 1

INTRODUCTION

1.1 Background

It is well known that the primary functions of packaging are to isolate foods from external environment and protects the foods against deterioration by the actions of microorganisms, moisture, gases, dusts, odors as well as mechanical forces (Cooksey, 2010). The exposures of these degradation agents tend to reduce the shelf-life of foods as well as affecting consumers' health when foods are contaminated by microorganisms related to food-borne diseases. Contamination could occur anywhere when food is being exposed to open environment such as during slaughtering, post processing, distribution, shipping, and storage or retail display stages. Thus, a good packaging should act as a barrier system to reduce passage of surrounding contaminants into foods. Meanwhile, the packaging must be inert, non-toxic, impermeable to microorganisms and strong enough to withstand possible amount of mechanical forces from easily rupture. In addition to the functions of extending shelf-life and maintaining food quality, packaging also important for marketing and advertising, standardizing, provides useful information to consumers while making products more usable and convenience (Cooksey, 2010). In short speaking, packaging functions for containment, protection, convenience, and communication.

Commonly, microbial contamination is the main reason for food spoilage. Traditional methods of preserving foods include fermentation, drying, adding antimicrobial agents (organic acids, plants, and salts), thermal processing, freezing, refrigeration, modified atmosphere and irradiation have been employed long times ago. All these methods possess their own limitation especially when attempting to apply on fresh meats (Quintavalla and Vivini, 2002). Inasmuch, varieties of packaging systems have been developed as alternatives to preserve foods for different attributes and applications. For example, overwrap packaging are designed for short term refrigeration storage, whereas modified atmosphere packaging (MAP) or vacuum packaging are utilized for long term storage.

In addition to the public demand for foods with extended shelf-life, the safety of foods has become the major consideration especially after the World Trade Centre tragedy happened in year 2001. Public started to concern on foods and water supplies as a form of bioterrorism (Nestle, 2003). Besides, food-borne pathogenic microorganisms' outbreaks issue has been frequently occurring without effective solution. Hence, the current trend of food packaging system are concerning about developing more innovative approaches to inhibit pathogenic microbial activities in foods. Plenty of products such as active packaging and intelligent packaging have been developed to meet crucial safety requirements. For instance, antimicrobial (AM) packaging which is under the family of active packaging is made from packaging system containing AM agents. The usage of AM packaging has more advantages compared to direct adding of AM agents onto foods because

AM agents added on food surfaces by sprays or drips are not effective enough to inhibit microorganisms. This is due to rapid diffusion of AM agent into foods and denaturization of the active substances by food constituents which reduce the reactivity of AM agent. Whereas, AM packaging offers slow and continuous transferring of AM agent from packaging to food surfaces which enable AM agent to maintain at high concentration over a long period (Quintavalla and Vicini, 2002).

1.2 Problem Statements

The purposes of AM agents in packaging are to provide safety assurance, shelf-life extension and quality maintenance on food. AM packaging are able to inhibit spoilage and suppress food-borne illness microbial that potentially contaminates food products (Hotchkiss, 1997). Generally, AM packaging is designed to address one property or requirement of the foods. For example, for the purpose of extend food shelf-life, the AM agents chosen must be able to inhibit the food native spoilage microorganisms. In the past decades, large numbers of AM food packaging products were developed from novel plastic materials and AM agents. Most of the products are proven able to control the growth of microbial and prolong food shelf-life effectively. However, there are only a few commercialised products found in the market. This may be due to several reasons such as strict safety and hygiene regulations, limited consumer acceptance on product effectiveness and high cost. Table 1.1 lists numerous commercial products of AM food packaging.

Table 1.1: Commercial Products of AM Food Packaging

Active Compound	Matrix	Application	Trade Name
Silver substituted Zeolite	LLDPE, PE, rubber	Film, Wrap, milk containers, paperboard cartons	AgIon®, Zeomic™, Cleanaid™, Novaron®
Chlorine dioxide	Polyolefin	Film, sachet	MicroGarde™, Microsphere™
Ethanol	Silicon dioxide	Sachet	Ethicap™
Sulfur dioxide	Laminated plastic sheet with $Na_2S_2O_5$	Sheet or pad for postharvest storage of grape fruits	Uvasy™
Triclosan	Polymer, rubber	Food container	Microban®
Wasabi extract	Encapsulation in cyclodextrin	Coated PET film, tablet	Wasapower™

The most popular commercialised products available in the market are those packaging that use volatile gas-form AM agents such as chlorine dioxide, ethanol and sulfur dioxide. These AM agents are often enclosed separately in sachets/pads that are attached to the internal part of the package. The AM agents will release in vapor form to the headspace of packaging to contact with food products (Appendini and Hotchkiss, 2002). Commercial product includes Microgarde™ as marketed by BarrierSafe Solutions International Inc., USA exists in stickers or sheets form containing sodium chlorite and acid precursors. When humidity or moisture in the air is high, it enters the Microgarde™ sheet and initiates the solid-state dry reaction subsequently producing chlorine dioxide that diffuses throughout the package to inhibit microbial contamination and control odor. Microgarde™ is found effective to inhibit aerobic total viable count on iceberg lettuce. Another commercial product called Ethicap™ from Japan, developed by Freund Corp., is ethanol vapor emitter. Ethicap™ is a paper wafer contains ethanol-silicon dioxide in acetic acid that sandwiched between layers of plastic films that permeable to ethanol. The ethanol vapor released into packaging headspace is able to retard mold growth. This technology is often applied for bakery and dried fish products.

Besides, silver compound is also employed as the AM agent in food packaging film. Silver is generally recognized by public as hygiene-improving and antitoxic material. In fact, silver substituted Zeolite products have been widely accepted by public when applied in plastic films for food packaging. Examples of commercialized products included Zeomic™, AgIon®, Novaron® and Cleanaid™. Sodium ions of Zeolites are substituted by silver ions to inhibit

a wide range of microorganisms such as mold and bacteria by disrupting the microbial enzymes activities (Appendini and Hotchkiss, 2002). For example, Zeomic™, product of Sinanen Zeomic Co. is able to control the growth of gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungi (*Aspergillus niger* and *Penicilium nigricans*). Zeomic™ has been widely applied in chopping board, food packaging films, glove and lunch box.

On the other hand, sanitizer and fungicide are non-food grade AM agents that allowed to be added into food packaging material. These AM agents are usually immobilized through covalently bonded to polymer materials for limiting the migration of AM agents into food products. Example of commercialized product that incorporates sanitizer to food packaging system is Microban® which is developed by Microban International Ltd in USA. Microban® utilizes triclosan as AM agent and this triclosan-incorporated food packaging was approved by European Union who regulates that migration of triclosan to foods should not exceed 5mg/kg (Quintavalla and Vicini, 2002). There were a wide range of microorganisms claimed sensitive to this packaging. Cutter (1999) investigated on the effectiveness of Microban® product with 1500ppm triclosan in plastic against population of meat surface related microorganisms. By using plate overlay assay method, it was found that this product inhibited the growth of *Salmonella typhimurium* (ATCC 14028), *S. aureus* (ATCC 12598), *Brochothrix thermosphacta* (ATCC 11509), *Bacillus subtilis* (ATCC 605), *E. coli* (ATCC 25922), *Shigella flexneri* (ATCC 12022) and several strains of *E. coli* O157:H7. However, when tested on refrigerated,

vacuum-packaged meat surfaces, that product did not effectively reduce the number of bacteria strains.

Among the plant extract AM agents, only wasabi extract AM products have been commercialized. The antimicrobial packaging used wasabi extract called Wasapower™ is well known developed by Sekisui Plastics Co. in Japan. Volatile allyl isothiocyanate (AIT) is the main AM component in wasabi extract that inhibits bacteria such as *E. coli* and *S. aureus* as well as fungi, *A. niger* and *Penicillium italicum*.

The commercial products show that the AM agents used are dominantly artificial where excessive amount of these agents can endanger public health. Consumers nowadays tend to accept products with natural substances more than those containing artificially-produced agents. Thus, it is necessary to replace chemically synthesized AM agents with natural alternative in order to ensure food safety. For this reason, essential oils became a good candidate for antimicrobial agents. The main focus of this study is to investigate the AM activity and characterization of polyethylene film incorporated with different weight percent of garlic oil as natural AM agent.

1.3 Objectives

The aim of this research is to investigate plastic packaging film containing natural AM agent from plant extract – garlic oil, with the following objectives:

1. To determine the incorporation method of garlic oil into low density polyethylene (LDPE) film with ethylene vinyl acetate (EVA) copolymer as the AM binding agent by using blown film extrusion technique.
2. To investigate the AM effectiveness of garlic oil-incorporated LDPE film against beef related bacteria in-vitro.
3. To study the AM effectiveness of garlic oil-incorporated LDPE film on ready-to-eat beef loaves.
4. To study the influence of garlic oil on package characteristics such as mechanical properties, physical properties, thermal stability and rheological behavior.

1.4 Scope of Study

This research focused on exploring the development of AM packaging comprising LDPE/EVA as packaging material and garlic oil as the natural AM additive. This packaging was produced by conventional method known as blown film extrusion technique. For AM effectiveness, agar disk diffusion test and challenge test were carrying out and focused on beef related bacteria namely *B. thermosphacta*, *L. monocytogenes* and *E. coli*. Film characterization

analyses were also carried out to determine the plastic packaging behaviour as affected by the incorporation of garlic oil in terms of tensile and tear properties, thermal stability, water vapor barrier properties, rheological behaviour, polymer morphology and microstructure.

CHAPTER 2

LITERATURE REVIEW

2.1 Antimicrobial Food Packaging

2.1.1 Antimicrobial Packaging System and Design

Packaging is a system consists of packaging material, foods and in-package atmosphere, for the purpose of food protection. The form of packaging system present is depends on the incorporation method of AM agent into packaging material. Whereby, the incorporation method is relying on the properties of AM agent (volatile or non-volatile). Therefore, selection of AM agent and incorporation method is major factor for the AM effectiveness of the packaging system.

Non-volatile AM agents are generally incorporate into packaging directly during film manufacturing process (Figure 2.1a and 2.1f). AM agent selected should have appropriate diffusivity in packaging material and solubility on food surfaces to ensure mass transfer kinetics of AM agent is over the kinetics of microbial growth (Han, 2005). The non-volatile agents are unable to transfer to foods through in-package atmosphere, thus, the packaging are requires to have intact contact with foods. The foods should have smooth surface without any air gaps, pores or holes which could restrict the migration

of AM agent. The benefit of this packaging system is the simple incorporation process which does not change the current existing manufacturing process.

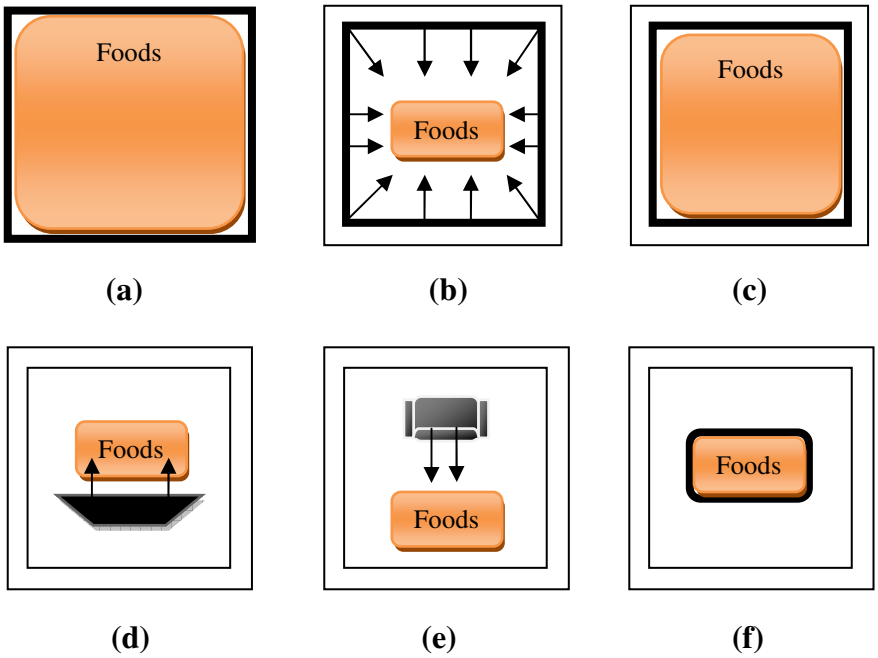


Figure 2.1: AM Food Packaging Systems. (a) Used of AM Packaging Material, (b) AM Coating on Conventional Packaging Materials, (c) Immobilization of AM Agents in Polymeric Materials, (d) The Use of AM Trays, (e) The Use of Sachet Containing Volatile AM Agents, (f) AM Edible Coating on Foods (Han, 2005).

Volatile AM agents are generally sensitive to heat and more suitable to be coated on packaging material or place inside sachet and tray (Figure 2.1b, 2.1d and 2.1e). Due to the ability of AM agent to diffuse to foods through in-package gas, it is not necessary for foods to contact with packaging material. The microbial inhibition effectiveness is dependent on volatility of AM agents, chemical interaction between the agents with packaging material and composition of foods. AM volatility can control by adding retention agent into

packaging system such as EVA and polyethylene glycol (PEG) (Suppakul, 2004; Cran et. al, 2010). The advantage of this packaging system is that it is effective for foods with high porosity, irregular shape and in shredded form.

Another AM packaging system uses covalently immobilized AM agents (Figure 2.1c). In this case, AM agents are attach with packaging material to prevent it from migrate to foods especially when non-food grade agent such as fungicides and antibiotics are used. Since the agent is immobilized, AM activities only take place on food surfaces that contact with packaging. This packaging system having advantages in marketing and regulation due to the inability of AM agent from migrate to foods, which can ensure food safety. However, it is very limited on the selection of AM agents and food application (Han, 2005).

2.1.2 Antimicrobial Packaging Material

AM packaging are divided into two major groups called biodegradable packaging and non-biodegradable packaging. Most synthetic polymers are non-biodegradable and being superior candidates for usage as food packaging material with the advantages of low cost, low density, inert, excellent barrier properties, good mechanical strength, high transparency, ability to be heat-sealed and easy to be printed on. The properties of common plastic materials used for food packaging are summarised in Table 2.1. The most widely used plastics in packaging included low density polyethylene (LDPE), linear low density polyethylene (LLDPE), high density polyethylene (HDPE),

polypropylene (PP), ethylene vinyl acetate (EVA), polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC). However, these petrochemical products possess negative impact to the environment. It causes landfill depletion, environmental pollution and high energy consumption of manufacturing process. The diffusion of additives from polymers into food products could also endanger human health. Therefore, there has been a shift towards increasing usage of biodegradable materials in recent years.

Table 2.1: Properties of Major Packaging Materials Used for Meat and Poultry (Ščetar et al., 2010)

Packaging Material (0.025mm thickness)	Water Vapor Transmission rate, $g/m^2/24h$	O_2 Transmission Rate, $cm^3/m^2/24h$	Tensile Strength, g/mL	Tear Strength, g/mL	Impact Strength, J/m	Light Transmission, %
Poly(vinyl chloride) PVC	1.5 – 5	8 – 25	9 – 45	400 - 700	180 – 290	90
Poly(vinylidene chloride) PVdC	0.5 – 1	2 – 4	55 -110	10 – 19	-	90
Polypropylene (PP)	5 – 12	2000 – 4500	35.8	340	43	80
High density polyethylene (HDPE)	7 – 10	1600 – 2000	38.2	200 – 350	373	-
Low density polyethylene (LDPE)	10 – 20	6500 – 8500	11.6	100 – 200	375	65
Linear low density polyethylene (LLDPE)	15.5 – 18.5	200	7 – 135	150 – 900	200	-
Ethylene vinyl acetate copolymer, EVA	40 – 60	12500	14 – 21	40 – 200	45	55 – 75
Ethylene vinyl alcohol, EVOH	1000	0.5	8 – 12	400 – 600	-	90
Polyamide, PA	300 – 400	50 – 75	81	15 – 30	50 -60	88
Poly(ethylene terephthalate), PET	15 – 20	100 – 150	159	20 – 100	100	88
Polystyrene, PS	70 – 150	4500 – 6000	45.1	2 – 15	59	92

Generally, biodegradable AM films were produced by natural polymer possesses inherently AM reactivity or through addition of AM agents into natural polymer. Examples of renewable biopolymers are polysaccharides, proteins, fibres, gums, lipids and their complexes, obtained from animal and plant origin. Most of the biodegradable-based packaging researches are focused on the blending of thermoplastic starches (TPS) with biodegradable polyesters such as polycaprolactone (PCL), polylactic acid (PLA), polyhydroxybutyrate-co-hydroxyvalerate, polybutylene succinate-adipate, poly(butylenes adipate-co-terephtalate), and poly(hydroxyl ester ether). Among the biodegradable ingredients, PLA is the most widely used polymer. PLA is a biocompatible thermoplastic which is generated by fermentation from renewable resources. It can also be synthesized by ring opening polymerization of lactide in the presence of a catalyst condensation or polymerization of lactic acid (Sin et al., 2013). PLA-based films could perform better than other AM films because of several advantages, such as competitive price, regulation matter and eco-friendliness. On the other hand, a natural polymer called chitosan possesses inherent AM activities. It is one of the ideal degradable films but costly to produce due to hassle process yields limited amount. The popularity of chitosan in AM development is mainly due to its inherent AM properties and biodegradability. It can be used as AM agent by its own or blending with other natural polymers as the structural support as well as cost reduction. Meanwhile, the poly(vinyl alcohol) (PVA) is a special synthetic polymer with biodegradable characteristic. Generally, it is blended with different synthetic and natural polymers because it is innocuous, non-carcinogenic, good biocompatible properties, hydrophilic, water-soluble and

chemically stable. Tripathi et al. (2009) suggested that chitosan-PVA blends possess advantages in the biological characteristics due to biological compatibility in respective chitosan and PVA blends.

Besides the biodegradable materials that discussed previously, edible films made from edible biopolymer (e.g.: proteins, lipids, polysaccharides) and food-grade additives (e.g.: plasticizers, AM agents, colorant, flavours, emulsifiers) are also the good candidate for fabrication of AM films. By incorporation of AM agents and antioxidants into films, food products can be protected from microbial growth, moisture migration and nutrient oxidation. The application of edible films is mostly on nuts, candies and fruits. Examples of edible films are soy film, alginate-based film and whey protein film.

2.1.3 Antimicrobial Agents-Matrixes Incorporation Methods

There are plenty of researches on AM packaging; however up to date the development is still not effective enough to inhibit the growth of spoilage microorganisms and extend food shelf-life. Many of the AM agents used are highly sensitive to film production process condition. Under high processing pressure and temperature, deformation or evaporation of volatile AM agents happened (Jari et al., 2003). Nevertheless, out of the plastic production technology, compression molding and blown film extrusion are commonly employed for heat-stable AM agents that required mild production conditions. In order to reduce the loss of AM agents when process under elevated conditions, it is crucial to employ alternative approaches for rapid processing.

By using conventional processing method which involves high temperature, researchers found that the loss of AM agents occurred tremendously. Ha et al. (2001) used high temperature profile 160-190°C to extrude AM LLDPE-based film resulted in high loss of grape fruit seed extract (GFSE) which showed no AM activity. Marino et al. (2012) reported 25-44 % weight of thymol and carvacrol remained in PP film when subjected to temperature 190°C for 18 minutes of hot press process. As a result, antibacterial activities towards *S. aureus* only effective when high initial concentration (8% w/w) of AM agent were used. They also found that the AM agent only retained 3.5% w/w after hot press process. AM LLDPE-based film developed by Suppakul et al. (2002) experienced even greater loss of AM agent at about 96.7% weight after blown film extrusion process. This was due to higher melting temperature of LLDPE compared with LDPE.

Alternative method to incorporate volatile AM agents had been studied in order to prevent unnecessary loss during film processing. Some researchers produced masterbatch of EVA/AM compound at relatively lower temperature prior to undergo film production process (Suppakul, 2004; Mistry, 2006; Suppakul Sonneveld et al., 2007; Cran et al., 2010; Herath, 2010). They first mixed EVA with AM agents to obtain homogeneous compound, and then mixed the compound with LDPE powder at room temperature before undergo blown film process. They successfully proved that EVA has effectively retained higher amount of volatile AM agent attributed to the polarity affinity of hydroxyl group in EVA towards AM agents which consisted some degree of polarity (Suppakul, 2004; Mistry, 2006; Herath, 2010). By incorporating

EVA into LDPE, EVA/LDPE film produced by blown film extrusion at 150^{o}C retained carvacrol up to 66 % weight, thymol 80% weight (Herath, 2010), linalool 60% weight (Suppakul, 2004), and methylchavicol 35% weight (Suppakul, 2004). Cran et al. (2010) studied on the release kinetics of AM agents from EVA/LDPE film suggested that 10% weight of EVA in LDPE is the most optimum concentration to achieve minimum release rate of AM agents. At higher concentration, the bulky side chain of EVA could reduce crystallinity of LDPE matrix, subsequently causing adverse accelerates the release of AM agent during application. Besides, Mistry (2006) and Herath (2010) have studied on the addition of polyethylene glycol (PEG) and PEG/EVA respectively into LDPE on the retention of AM agents. The function of PEG is to assist binding of AM agents to polymer and enhance retention of AM agents. In the absence of EVA, PEG was insufficient to retain AM agents due to poor interaction of PEG with LDPE matrix. EVA with bulky side chain created spaces for PEG to occupy between LDPE chains and hence enhancing PEG-LDPE interaction. Subsequently, a more uniform dispersion of PEG in LDPE matrix could be formed and retained up to 8% weight more of thymol and cavacrol respectively when compared to EVA merely.

Heat-sensitive AM agents such as volatile compounds are preferable to be produced by non-heating methods such as solvent compounding, electrospinning and surface coating. Among these methods, coating was the most popular approach to apply AM agents onto polymer surface due to simplicity of the process. In order to enhance the attachment of AM onto

plastic matrix, plastic films usually undergo surface modification process by corona treatment or UV radiation prior application of AM agents. Normally, the whole process was done in room temperature. Examples of AM-coated films studied included chitosan/essential oil-coated PP film (Torlak and Nizamlioğlu, 2011), chitosan/bacteriocin-coated plastic film (Ye et al., 2008a, 2008b), bacteriocin-coated LDPE film (Iseppi et al., 2008), cinnamaldehyde, garlic oil and rosemary oil-coated PP/LDPE film (Gamage et al., 2009), oregano essential oil and citral-coated PP/EVOH film (Muriel-Galet et al., 2012), chitosan-coated plastic film (Ye et al., 2008a, 2008b), thyme and oregano-coated LDPE (Solano and Gante, 2010) and etc. Interestingly, AM film produced by elevated temperature process shows better inhibition against microbial compared to coating method as reported by Solano and Gante (2010). They found that AM film produced by extrusion method is more effective towards *E. coli*, *S. typhimurium* and *L. monocytogenes* compared to ionising-coated AM film with the identical amount of AM agent incorporated. The results suggest that blown film extrusion method enhance the incorporation of active compounds on the polymer. However, there were a limited number of studies done in comparing the both methods; thus, the comparison of effectiveness is still required further exploration.

In addition, an alternative method to prevent loss of AM activities is production via cast film. It is generally produced at relatively much lower temperature without subject to mechanical shear forces. However, this method is usually limited for natural polymer which having lower melting temperature. Thus, the film is usually biodegradable and edible depends on the

ingredients. Examples of natural polymer can be prepared using for this method is starch, chitosan, alginate, WPI, PLA, PVA and etc. Researchers usually employed several types of treatments in final stage of the processes to improve the mechanical properties of the cast film. For instance, chitosan and potato starch film preparation involve the microwave treatment (Tripathi et al., 2008) and electron beam (EB) irradiation. Another example of film produced by casting technique is chitosan/PVA film. The film was kept in desiccators to dry up at room temperature for 24 h, and then cross-linked using 0.01% weight glutaraldehyde (GA) and 0.5% weight sulfuric acid (H_2SO_4) as a catalyst for 1 h. The final product was obtained after washing with distilled water.

2.1.4 Antimicrobial Effectiveness of Antimicrobial Packaging

Numerous studies found that AM packaging can effectively inhibit targeted bacteria when appropriate amount of AM agents incorporated into polymer film. The effectiveness of AM packaging are greater compared to direct addition of preservative agents into food due to two important reasons. Firstly, the attachment of AM agents with polymer film enables slow release of AM agents function over longer period. Secondly, AM activities of preservative agents may experience inactivation (such as neutralization, hydrolysis, dilution and etc.) by food matrixes and components when added directly into the food (Appendini and Hotchkiss, 2002; Muriel-Galet et al., 2012). Besides, direct addition of preservative agents into food can reduce the food quality as resulted by changing the organoleptic and textural qualities of

the food. Thus, AM packaging are playing important roles to inhibit the growth of targeted bacteria on foods while improving foods safety and prolong shelf-life without scarifying the foods quality.

The functionality of AM agents in foods can be examined from the number of microorganism growth as described by spoilage index in Table 2.2 (Fung et al., 1980). Most of the time, food is considered spoil when exceed a total microbial count of 10^7 CFU/g. When total count reaches 10^8 CFU/g and above, unpleasant odor started to build up. Most researchers studied on AM packaging referred bacteria count of 10^7 CFU/ml or g or cm^2 as a standard for shelf-life indication.

Table 2.2: Fung Scale for Liquid, Solid, and General Surfaces (Fung et al., 1980)

Total counts for spoilage consideration	Ranges (CFU/ml or g or cm^2)
Low count (no concern)	10^{0-2}
Intermediate count (slight concern)	10^{3-4}
High count (serious concern)	10^{5-6}
Index of spoilage	10^7
Odor development	10^8
Slime development	10^9
Unacceptable, too high	10^{10}

2.1.4.1. Antimicrobial Packaging with Essential Oils and Plant Extracts

The AM activities of essential oils and plant extracts are well known for a long time and numerous of researches outcomes have been published on the AM activities of plant essential oils against food-borne pathogens. Since essential oils are rich in volatile terpenoids and phenolic particles, they are highly potential to inhibit a wide spectrum of microorganisms. Generally, the active components of plant essential oils inhibit microorganisms through disrupting of the cytoplasmic membrane, disturbance the electron flow, proton motive force, active transport and inhibition of protein synthesis. Examples of plant extracts and essential oils that most widely incorporated into food packaging are linalool, thymol, carvacrol, clove oil, cinnamaldehyde and basil essential oils.

There are plenty of reports found related to the AM effectiveness of plant extract components in food packaging. Ha et al. (2001) reported when comparing film without AM agent, linear low density polyethylene (LLDPE) co-extruded film with 0.5% and 1% w/w grape fruit seed extract (GFSE) has extended the time of beef stored under 3°C resulted total aerobic bacteria count of 10^7 CFU/g from 13 to 14 days. When the identical amount of GFSE coated on LLDPE film was tested, the shelf-life of beef has extended from 9 to 14 days. Meanwhile, there was no significant different between result of 0.5% and 1% GFSE in LLDPE film can be observed. GFSE can effectively inhibit the growth of pathogenic bacteria. AM activities of GFSE incorporated with soy protein edible film showed a positive result towards *L. monocytogenes*

population by reduction of 1 log CFU/ml after 1 hour incubation at 25 °C. However, *E. coli* and *S. Typhimurium* showed only 0.1 and 0.2 log CFU/ml reduction respectively (Sivarooban et al., 2008).

Muriel-Galet et al. (2012) studied on polypropylene (PP) film coated with oregano essential oil and citral. By comparing with the control specimen (PP film without AM agent), oregano essential oil-coated PP film with 6.7% carvacrol active ingredient has successfully reduced the number of *E. coli*, *Salmonella enterica* and *L. monocytogenes* in salad for 1.4 log, 0.5 log and 0.36 log CFU/g respectively after storage under temperature 4°C, packaging environment of 12% CO_2 and 4% O_2 for two days. Meanwhile, 5% of citral coated PP film was able to reduce the number of *E. coli* and *S. enterica* by 0.36 log and 0.41 log CFU/g respectively. However, the AM effectiveness of citral coated PP film is lesser compared to oregano. In addition, the citral coated-PP film was not effective towards *L. monocytogenes*. Carvacrol and citral were reported to be more effective towards gram-negative bacteria due to the high affinity. Especially carvacrol can affect the cell wall lipids of gram-negative bacteria. However, this was inconsistent with some researchers who claimed there were no differences between the AM effectiveness on the gram-positive and gram-negative bacteria (Quattara et al., 1997).

Other essential oils-coated film that have been studied extensively included allyl isothiocyanate (AIT) (Nadarajah et al., 2005), rosemary (Han et al., 2007), garlic oil (Pranoto et al., 2005; Gamage et al., 2009) and cinnamaldehyde (Han et al., 2007). By incorporating 1.2% w/w of AIT into

oriented polypropylene/polyethylene (OPP/PE) film, the microbial count on sprouts stored at 10°C (Gamage et al., 2009) has reduced significantly. Nadarajah et al. (2005) also reported AIT was effective against *E. coli* on ground meat patties with a reduction of 3 log CFU/g within 10 days of storage. Garlic oil-incorporated film exhibits effectiveness only when high concentration of garlic oil was used. Gamage et al. (2009) found that plastic film with 1.2% w/w garlic oil inhibited the growth of microbial on sprout, whereas no significant differences when 0.6, 0.8 and 1.0% w/w of garlic oil were incorporated. However, garlic oil was reported to effectively reduce the number of gram-negative and gram-positive bacteria when used in small amount in edible film, where 0.2% w/w garlic oil-incorporated alginate film inhibited the growth of *S. aureus* and *Bacillus cereus* (Pranoto et al., 2005).

Essential oils were also incorporated into protein-based film to evaluate its AM performance. Seydim and Sarikus (2006) have prepared whey protein isolate (WPI) films containing 1.0-4.0% w/v ratios of rosemary, oregano and garlic essential oils to test the AM effective against *S. aureus* (ATCC 43300), *E. coli* O157:H7 (ATCC 35218), *L. monocytogenes* (NCTC 2167), *Lactobacillus plantarum* (DSM 20174) and *Salmonella enteritidis* (ATCC 13076). The researchers found that when 3% w/v of garlic essential oil is added in WPI films, a significant inhibition was observed against all tested strains. Compared to rosemary essential oil, oregano and garlic essential oil respectively exhibited larger inhibition zones on *S. enteritidis*, *L. monocytogenes*, *S. aureus*, *E. coli* and *L. plantarum*. In addition, WPI films added with 1.5% w/w of oregano oil prolonged the shelf-life of beef by a

factor of 2 while minimizing changes in beef color. Emiroğlu et al. (2010) studied the AM activities of soy edible films added with thyme and oregano oils on fresh ground beef patties showed that there were lack of significant inhibition on *S. aureus* when tested with meat even though it showed a significant inhibitory effect when tested in-vitro. *P. aeruginosa* exhibited better result where reduction of colonies observed when the film incorporated with thyme or oregano plus thyme essential oils. On the other hand, Oussalah et al. (2004) studied the AM effects of milk protein-based film on *Pseudomonas* spp. and *E. coli.* They reported that the usage of films with essential oils significantly reduced the amount of microorganism in meat during 7 days of storage at 4°C. Among the essential oils, oregano oil was the most effective combination that works against the growth of the respective bacteria. Oregano oil:pimento oil in 1:1 (w/w) ratio was more effective against the growth of *E. coli* than a pimento oil-coated film, whereas the AM effectiveness against the growth of *Pseudomonas* spp. was identical for both films.

2.1.4.2. Antimicrobial Packaging with Enzyme

Lysozyme, obtained from hen egg white is an enzyme classified as natural AM agent and commonly incorporated into packaging materials. Similar with other AM agents, lysozyme is less effective towards gram-negative bacteria due to the existing of protective lipopolysaccharide (LPS) layer on the bacteria cell wall. Gram-positive bacteria are highly susceptible to lysozyme, because their membrane is made up of 90% peptidoglycan where

hydrolyzation of β-1-4 glycosidic linkage between the *N*-acetyl muramic acid and *N*-acetyl glucosamine take place. In order to further improve the AM effectiveness of enzyme, other AM agents such as detergents and chelators were usually added.

In early year, Ellison and Giehl (1991) proposed to use lactoferrin to improve the activity of lysozyme towards gram-negative bacteria. Lactoferrin is a natural component of milk with large cationic patches that disrupting the outer membrane permeability resulting the release of LPS. It has been used in AM spray for beef carcasses decade ago. Recently, Barbiroli et al. (2012) developed lysozyme/lactoferrin-containing paper and assessed on *E. coli* (DSMZ 50902) and *Listeria innocua* (DSMZ 20649). When tested on gram-positive bacteria, *L. innocua*, the presence of both the AM agents induce a significant increment of the lag phase duration which prolonged from 1.86 to 6.5 h, whereas lysozyme alone only prolongs the lag phase from 1.86 to 5.81 h. For gram-negative bacteria *E. coli*, lysozyme alone only slightly increases the lag phase from 1.08 to 1.79 h whereas, lactoferrin-containing paper showed lag phase increment from 1.8 to 3.25 h.

Mecitoğlu et al. (2006) proposed the combination usage of lysozyme and disodium ethylenediaminetetraacetic acid (Na_2EDTA) as the AM agent. EDTA is a chelating agent that capable to destabilize the LPS layer of gram-negative bacteria. Zein films incorporated with lysozyme showed AM effect on *B. subtilis* (ATCC 6633) and *L. plantarum* (DSMZ 1954). By the addition of Na_2EDTA, the films also became effective on gram-negative

bacteria, *E. coli* (ATCC 53868). Similarly, in the studied of Gemili et al. (2009), agar disk diffusion test conducted for *E. coli* showed significant formation of inhibition zone when using cellulose acetate film containing both AM agents. By using lysozyme and Na_2EDTA alone, no zone formation can be observed. Unalan et al. (2011) studied on zein films containing lysozyme (700μg cm^{-1}) and Na_2EDTA (300μg cm^{-1}) found that through agar disk diffusion test, the inhibition zone produced by using zein films containing both lysozyme and Na_2EDTA exhibited 1.5 and 2.4 fold greater zones on gram-positive bacteria, *L. monocytogenes,* than using films containing Na_2EDTA or lysozyme alone. The zein films added with both lysozyme and Na_2EDTA and Na_2EDTA alone were effective towards *E. coli* O157:H7 and *S. typhimurium*. Through the challenge test, compared to those of control beef patties, the films containing both AM agents decreased the total viable counts (TVC) and total coliform counts on beef patties after 5 days of storage. In another study, Gucbilmez et al. (2007) utilized functional protein extracts- chickpea albumin extract (CPAE) to control lysozyme distribution and release rate while improving antioxidant activity in zein film. In the result, zein film incorporated with 277μg/cm^2 $Na_2EDTA{\cdot}2H_2O$ and 1400U/cm^2 lysozyme tested on *E. coli* (ATCC 53868) showed uneven distribution of these AM agents in zein films. For the addition of 530μg/cm^2 CPAE into the mentioned films, the AM effectiveness did not change significant but the distribution of lysozyme were improved.

2.1.4.3. Antimicrobial Packaging with Chitosan

Chitosan are highly attractive to researchers due to some interesting properties such as it is natural polymer, nontoxic, film-forming ability and biodegradable (Cooksey, 2010; Torlak and Nizamlioğlu, 2011). Besides, chitosan has inherent AM properties and able to inhibit the growth of wide range of food pathogens. However, owing to the poor solubility of chitosan at high pH value, its application is only effective in acid medium. In the study of Liu et al. (2004), chitosan solutions reduced the numbers of *E. coli* and *S. aureus* by ~ 1 log in 5 minutes. Almost all *E. coli* were killed in 120 min, but the quantities of *S. aureus* were not affected.

The only weakness of chitosan film is the poor mechanical properties compared to synthetic polymer. Thus, chitosan can be coated on plastic film to compromise mechanical properties and enhance AM activities. Torlak and Nizamlioğlu (2011) studied AM effectiveness of 2% w/v chitosan coated on PP films towards Kashar cheese slice inoculated with bacteria. The cheese slices were stored in vacuum packaging at 4°C for 14 days. PP films without AM agent were used as control. After 14 days, control cheese exhibited 5.11, 4.92 and 4.88 log CFU/g of *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 respectively. Chitosan-coated PP films found reduction count of *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 at 0.7, 0.61 and 0.49 log CFU/g, respectively compared to PP films without AM agent. Similarly, Duan et al. (2007) also reported significant reduction of *L. monocytogenes* in mozzarella cheese and camembert cheese stored at 10°C and 4°C respectively.

Packaging coated with 2% and 2.5% v/v chitosan under vacuum condition has reduced the population of total aerobic count in pork from 6 log (control) to 3.75 and 3.61 log CFU/g respectively within 14 days of storage in refrigerator (Yingyuad et al., 2006). On the other hand, chitosan-coated film prepared using high pressure treatment on turkey breast was studied by Joerger et al. (2009). They reported a lower count of *L. monocytogenes* by 3 log after one week of storage compared to plastic film without chitosan and high pressure treatment. Most researchers found that chitosan are highly effective towards gram-negative bacteria. This is because the positively charged chitosan molecules can interact better with negatively charged teichoic acid backbone in cell wall of gram-negative bacteria.

In order to further enhance the AM properties of chitosan, other AM agents were also added into chitosan-coated films. These synergistic combinations can inhibit wider range of microorganisms due to the ability of chitosan to function as carrier for other additives. Zivanovic et al. (2005) studied on bologna wrapped with chitosan that stored at 10°C for 5 days. They reported chitosan films reduced the number of *L. monocytogenes* by 2 log, whereas addition of 1% oregano oil into chitosan film reduced *L. monocytogenes* by 3.6 log. By incorporating 1% oregano and clove essential oil respectively into chitosan incorporated-PP film, the inhibition effects against bacteria were significant, especially when added of oregano essential oil. The reduction of *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 count compared with control film (without AM agents) was 1.31, 1.4 and 1.18 log CFU/g respectively in vacuum sealed cheese stored at 4°C after two weeks of

storage (Torlak and Nizamlioğlu 2011). Ye et al. (2008a, 2008b) enhanced the efficacy of chitosan-coated film against *L. monocytogenes* by adding nisin, sodium diacetate (SD), sodium lactate (SL), potassium sorbate (KS) and sodium benzoate (SB) into chitosan. Such combination of AM agents were generally used as food preservatives and recognized as GRAS. They found that chitosan-coated plastic films alone with 0.0025 and 0.0055g/cm^2 of chitosan were insufficient to control the growth of *L. monocytogenes* on ham steaks stored at 20°C although the counts were consistently lower about 0.7 log CFU/cm^2 than those films without chitosan. Nevertheless, such films did not extend the shelf-life of ham steaks. Furthermore, the coated films contained 0.01 g/cm^2 of SL and 0.003 g/cm^2 of PS respectively exhibited the most satisfied results. The reduction of *L. monocytogenes* was 1.9 log CFU/cm^2 more pronounced when compared to chitosan-coated film after 10 days of storage under similar condition. At storage temperature of 4°C, SL/chitosan-coated film showed even greater inhibition effect with a reduction of more than 5 log CFU/cm^2 compared to chitosan-coated film.

Chitosan has been widely studied as AM and edible packaging application due to its biodegradability characteristic. The recent development of edible chitosan films included adding of AM agent such as essential oils and nisin, or blending with other materials such as chitosan-glucomannan-nisin blends and chitosan-starch blends to produce highly effective AM films (Du et al., 2008). In-vitro AM activity of edible chitosan-starch film with *Thymus kotschyanus* essential oil as studied by Mehdizadeh et al. (2012) found that *Thymus kotschyanus* essential oil showed different inhibition levels on *E.*

coli, *S. aureus, L. monocytogenes*, and *S. typhimurium*. The films without essential oils were not effective against *S. typhimurium* where clear zone of inhibition was not observed. *L. monocytogenes* was the most sensitive bacteria inhibited against the films added with *kotschyanus* essential oil, followed by *E. coli*, *S. aureus* and *S. typhimurium*. As the concentration of *kotschyanus* essential oil increased, the inhibition zone of *E. coli* expanded significantly. This indicates that the *kotschyanus* essential oil has selective reactivity over the range of gram-positive and gram-negative bacteria.

2.1.4.4. Antimicrobial Packaging with Bacteriocin

Other than natural AM agents extracted from plants, animals and essential oils, AM agents generated by bacteria called bacteriocin were gradually gaining popularity attribute to their ability to withstand elevated temperatures and acidic environment. Bacteriocin is metabolic by-product (antimicrobial peptide) produced by bacteria defence system from almost all types of bacteria. This naturally occurred activity allowed bacteria of one strain to inhibit the growth of other strains in adjacent. Bacteriocin produced by lactic acid bacteria (LAB) has highly acceptance from public, because LAB has been the important bacteria for food fermentation for centuries. Example of traditional fermented foods that consume for centuries included cheese, wine, kimchi, miso, soysauce, bean paste, and etc. Nisin, produced by *Lactococcus lactis* bacteria, commonly present in milk is designated by Food and Drug Administration (FDA) as “Generally Recognized as Safe” (GRAS) AM agent and employed commercially for food preservation. It is the first

isolated bacteriocin and specially used to prevent the outgrowth of *Clostridium botulinum* spores in cheese.

Bacteriocins such as enterocins A and B, Sakacin, Enterocin 416K1, pediocin AcH have been proven able to control the proliferation of *L. monocytogenes* in artificially contaminated foods (Iseppi et al., 2008). Siragusa et al. (1999) reported that bacteriocin-coated PE film fabricated by blown film extrusion process examined for vacuum packaging of meat inoculated with *B. thermosphacta* has significantly inhibited the growth of this bacterial. An et al. (2000) also reported that bacteriocin-coated PE film inhibits against *Micococcus flavus* and *L. monocytogenes*. Kim et al. (2002) studied on bacteriocin-coated LDPE film found that the usage of nisin and lacticin NK24-coated films extended the shelf-life of oysters and beef stored at 3°C from 10 to 12 days and from 9 to 13 days, respectively. Meanwhile, the shelf-life of oyster and beef extended from 5 to 12 days and 5 to 9 days respectively when stored at 10°C.

Besides, pediocin-plastic film was also reported to exhibit inhibition effects over several pathogenic and gram-positive bacteria on foods. Particularly, their effectiveness is most prominent towards *L. monocytogenes*. In the research of Goff et al. (1996), a preparation containing pediocin was added to sterilize raw chicken inoculated. The results showed a reduction from approximately 3.0 log CFU/g of *L. monocytogenes* to below detectable levels after 28 days of storage at 5°C, whereas the control treatment of the counting reached 1.9 log CFU/g. Santiago-Silva et al. (2009) developed cellulose film

containing pediocin found that the film with 50% pediocin promoted an inhibition towards *L. innocua* counting on sliced ham, whereas films with 25% pediocin and control unable to suppress the microorganism growth during storage at 12°C. At the end of storage period (15 days), the inhibitory was 2 log cycles reduced for the sliced ham in contact with film of 50% pediocin, while less than 1 log cycle for the film with 25% pediocin in relation to the control. Ming et al. (1997) reported total inhibitory effects towards *L. monocytogenes* when used cellulosic casings sprayed with pediocin on the meats (chicken, beef and ham) for 12 storage-weeks at 4°C. Iseppi et al. (2008) studied on electrocin-coated LDPE reported reduction of *L. monocytogenes* count on contaminated frankfurters that stored at temperature of 4°C. During the first day, there was significant drop at more than 1 log observed. Whereby, such level of decrement was maintained until the 14^{th} day. Massani et al. (2012) have produced multilayer-plastic films incorporated with lactocin and tested on *L. planturum*. The films were composed of an outer layer of PP, an inner layer of PA–PE structure, a barrier layer of EVOH co-polymer, and a LLDPE food contact layer. The researchers reported that the films' AM activity was influenced by time and temperature. Through agar disk diffusion test on *L. planturum*, it was found that after 7 days of storage at 30, 10 and 5°C, the inhibition zones achieved 32, 66 and 100% respectively. After 14 days of storage, no AM activity was detected or those packages stored at 30 and 10°C.

2.1.4.5. Antimicrobial Packaging with Inorganic Nanoparticles

The utilization of inorganic nanoparticles as AM agents has recently gaining popularity which attributed to the good stability of these materials to withstand harsh process conditions such as elevated temperatures in plastic fabrication process. The most extensively studied inorganic nanoparticles for AM purposes were titanium dioxide (TiO_2) and zinc oxide (ZnO). Titanium dioxide (TiO_2) is non-toxic and approved by FDA for used in foods, drugs and food contact materials. It is a photocatalyst widely used to inactivate wide range of microorganisms. TiO_2 generated hydroxyl radicals and reactive oxygen species via light reaction which can inactivate microorganisms by oxidizing the polyunsaturated phospholipids component of the cell membrane. Chawengkijwanich and Hayata (2008) developed TiO_2-coated OPP films to investigate the AM effects on *E. coli* in-vitro and in actual case. The results showed that when two 20W black-light illumination was applied, the film coated with TiO_2 powder having greater AM effects with 3 log CFU/ml reduction of *E. coli* compared to uncoated film with only 1 log CFU/ml reduction after 180 min of illumination. In the actual test, a more significant result obtained where under irradiation of ultraviolet A light, *E. coli* counts on cut lettuce stored in a TiO_2-coated film bag decreased from 6.4 on day 0 to 4.9 log CFU/g on day 1, while the uncoated film only exhibited slightly decreased from 6.4 to 6.1 log CFU/g after 1 day of storage. TiO_2-incorporated PE film developed by Xing et al. (2012) using blown film extruder exhibited that, by antibacterial drop-test method, the blank film did not possess any antibacterial activities where the untreated TiO_2 exhibited inhibition ratios of 50.4% for *E.*

coli and 58.0% for *S. aureus*. Whereas, the antibacterial activities of TiO_2-incorporated PE film treated with ultraviolet light for 1 hour improved significantly, which were 89.3% for *E. coli* and 95.2% for *S. aureus*. Recently, Bodagh et al. (2013) developed TiO_2-LDPE film by using blown film extruder and tested on *Pseudomonas spp*. and *Rhodotorula mucilaginosa*. Through the modified in-vitro test method developed by Chawengkijwanich and Hayata (2008), the number of *Pseudomonas spp*. decreased significantly by 4 log CFU/ml and 1.35 log CFU/ml after 3 hours of ultraviolet A illumination on TiO_2 film and blank film respectively. Meanwhile, the number of *R. mucilaginosa* decreased by 2 log CFU/ml and 0.64 log CFU/ml on TiO_2 film and blank film respectively. In an in-vivo test done on fresh pears packaged in TiO_2 nanocomposite film and stored under lighting of fluorescent light at 5°C for 17 days, the level of mesophilic bacteria reduced from 3.14 to less than 2 log CFU/g whereby the bacteria count increased from 3.19 to 4.02 log CFU/g for pears packaged in blank films. On the other hand, it was observed a reduction of yeast count from 2.45 to less than 2 log CFU/g obtained by fruits packaged in TiO_2 films, whereas for blank film, the yeast count increased from 2.1 to 3.37 log CFU/g. Such results proven that the nanoparticles-incorporated plastic films prepared by extrusion are effective for fruit packaging applications.

The applications of ZnO nanoparticles coating systems have recently attracted a great deal of attention due to its AM activity towards both the gram-positive and gram-negative bacteria. In the study of Li et al. (2010), ZnO-coated PVC films exhibited significant AM effects on *E. coli* and *S.*

aureus compared with blank film. When tested on films that been exposed to ultraviolet light for 3 hours with the present of identical amount of ZnO nanoparticles, the inhibition improved by 29.8% for *E. coli* and 26.0% for *S. aureus*. This result indicated that ZnO breakdown products were the key AM particles that inhibit the growth of bacteria. Tankhiwale and Bajpai (2012) also developed PE film that coated with ZnO. The ZnO-coated PE film without treatment with light could also retard the growth of *E. coli* by showing a clear zone of inhibition around the film during agar disk diffusion test, whereas a clouded bacteria was observed in the blank film. The inhibition activities were further confirmed by the kinetics of bacterial growth which showed that the growth rate of bacterial was suppressed appreciable in the solution containing ZnO-coated film.

2.1.5 Effects of Antimicrobial Agents on Mechanical and Barrier Properties

The incorporation of AM agents into polymer can adversely affect the physical properties, mechanical integrity and thermal stability of packaging when the AM agents used are not compatible with the polymer. Whereas, AM agents that are compatible with packaging materials can impregnate well into spaces between polymer chains. In other words, it does not influence the film properties when reasonable amount is added (Han, 1996). Therefore, the study of polymer chemistry and structure are important in predicting the influence of particular AM agents on the packaging. Subsequently, the selection of AM agents, packaging polymers and incorporation methods can be more effective.

Some researchers have thoroughly investigated the packaging mechanical properties when substantial amount of AM agents incorporated. The mechanical properties of film as represented by tensile strength (TS) and elongation at break (Ɛ) are the most important properties to be analyzed and benchmarking. It measures film strength and stretchability prior to breakage when extension force applied. The incorporation of additives into molten polymers other than crosslinking agents generally detriment the packaging tensile strength due to the additives is usually small components that tend to occupy between polymer chains and cause slippage when external forces being applied. This phenomenon is called plasticizing effect which can be proven by comparing AM agent incorporation methods between both coating method and extrusion method. Solano and Gante (2010) compared both methods by adding 4% w/w oregano essential oil and thyme essential oil into LDPE film. Through the coating method, polymer chains were radiated and applied with layers of essential oils. When comparison was done on blank film, coated film has no significant changes in mechanical properties. Whereas, the essential oil incorporated-LDPE film produced by using single screw extruder was slightly inferior in tensile strength as compared to film without essential oil that produced by the similar method.

Generally, TS did not reduce significantly when small amount of AM additives applied. Mistry (2006) reported that the incorporation of 2% w/w linalool and thymol respectively into LDPE did not significantly affect the mechanical properties of the film. This was due to the presence of small amount of AM does not interrupt the crystalline phase and intermolecular

interaction of polymer chains. Similar result was also found when incorporate 1% w/w oregano essential oil and thyme essential oil respectively into LDPE film (Solano and Gante, 2010). Besides, the AM particle size is also an important factor that affects the mechanical characteristics of the polymer films. Most of the time, particles in nano size are unlikely to interrupt the polymer chains morphology. In contrast, some specialty mineral types' AM agents may enhance the film stiffness. Xing et al. (2012) studied on PE film incorporated with 2% w/w TiO_2 nanoparticles produced by single-screw extruder found that both the TS and Ɛ were higher when compared to film without additive. The similar result obtained by Li et al. (2009) when carried out study on polyurethane (PU) films incorporated with 2% w/w ZnO nanoparticles. This can be explained with the so-called exfoliation effect helps to strengthen the interactions of polymer chains and nanoparticles.

Biodegradable films generally possess weak mechanical strength while the addition of AM agents would further weaken the films. According to Kechichian et al. (2010), when cinnamon and clove powders were incorporated into films made by cassava starch, both the TS and Ɛ decreased dramatically. Kiam wood extract-incorporated hydroxypropyl methylcellulose (HPMC) film was having weaker TS compared to blank film due to the incompatibility characteristics of kiam wood extract and HPMC. The incomplete dispersal of kiam wood extract produced heterogeneous film structure thus weakened the film properties (Maria et al., 2007).

Another important property that should take into account is water vapor transmission. Water vapor transmission represents the ease of moisture to penetrate and transfer through a material (Li et al. 2006). It is important for a packaging to have good water vapor barrier properties not only to prevent excessive water loss from foods, but also resists moisture from atmosphere to migrate into foods. Water can accelerate microorganisms' growth and reduce shelf-life of foods. Water vapor transmission of AM packaging is very much dependent on the hydrophilic-hydrophobic ratio of AM-matrix material. Hydrophilic material tends to increase packaging water vapor transmission. Suppakul et al. (2006) studied on basil extract-EVA/LDPE film manufactured by extrusion method. They found that a reduction of water vapor transmission from 13.7 (blank EVA/LDPE) to 10.5 and 5.2 $g/m^2/day$ respectively for linalool-incorporated LDPE film and methylchavical-incorporated LDPE film. Methylchavical reduces water vapor transmission greater than Linalool due to higher hydrophobicity characteristic. Jari et al. (2003) reported the addition of 5% EDTA with hydrophilic characteristic in LDPE film has increased water vapor transmission from 5.85 to 6.6 $mg/dm^2/day$. The water vapor transmission values are in relation to the concentration of EDTA added.

For biodegradable film, water vapor transmission was dependent on AM agents' hydrophilicity as well. Kechichian et al. (2010) obtained the lower value of water vapor transmission for cassava starch films added with cinnamon and clove powders attributed to the hydrophobic properties of these ingredients. Maria et al. (2007) studied on alginate-apple puree edible film (AAPEF) reported the incorporation of essential oil and oil compounds such

as lemongrass, cinnamon oil, citral, oregano oil, carvacol and cinnamaldehyde did not significantly affect the water vapor permeability of the edible film. Similarly, addition of oregano essential oil into WPI film developed by Zinoviadou et al. (2009) did not change the water vapor permeability of film though 1.5% w/w of oil was added. These may be due to essential oils are mostly terpene-like compound which is water resistant. According to Pranoto et al. (2005), garlic oil with hydrophobic characteristic did not significantly affect the water vapor permeability on chitosan film. However, the addition of potassium sorbate (KS) into chitosan-tapioca films increased WVP of the film. Even though chitosan possesses higher hydrophobicity compared to tapioca starch, the addition of potassium sorbate (KS) with hydrophilic hydrogen bond has increased the hydrophilic:hydrophobic ratio of the film (Maria et al., 2009). Hydrophilic additives like kiam wood extract increased the water vapor permeability when the content of kiam wood extracts was higher (Jutaporn et al., 2011). Nevertheless, addition of lactic acid (LA) into whey protein isolated (WPI) film did not change the water vapor permeability (WVP) (Oscar et al., 2012).

Other than the characteristic of AM agent, the method of incorporating AM also affected the changes of water vapor transmission. AM coated film possesses lower water vapor transmission since coating could prevent part of moisture from passing through packaging. Xing et al. (2012) reported water vapor transmission value of TiO_2-incorporated PE film produced by extrusion method increased from 18.1 (blank PE) to 20.1 g/m^2/day. It was probably due to the TiO_2 nanoparticle caused irregularity of the crystallinity structure of PE

thin film, subsequently promoting the moisture passing through the PE film. In contrast, addition of ZnO nanoparticles by coating method decreased water vapor transmission from 128 to 85 g/m^2/day.

2.2 Natural Antimicrobial Agent – Garlic

Numerous studies showed that allicin are natural agent for antibacterial and antifungal activities (Cavallito et al., 1944; Shadkchan et al., 2004; Davis, 2005). Allicin, chemically known as allyl 2-propenylthiosulfinate is found only in crushed garlic and it is suggested that allicin is the product of enzymatic reaction between alliin (allicin precursor) and allinase (enzyme) (Stoll and Seebeck, 1951; Rabinkov et al., 1994). The enzymatic reaction of garlic allinase is shown in Figure 2.2. Cross section of garlic studied by Koch and Lawson (1996) shows that alliin and allinase are located in two separated compartment. When these compartments destroyed by pathogens action or animal bite, allinase transform alliin to allicin which able to inactivate or kill the microbial immediately. The transformation is extremely rapid within seconds as this is the natural defence mechanism of garlic.

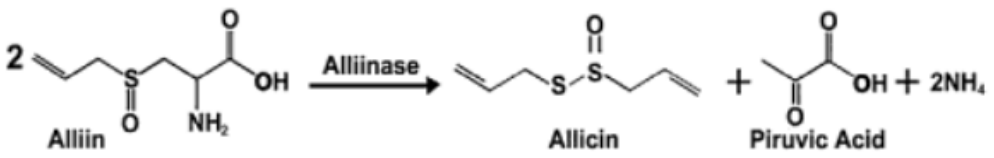


Figure 2.2: Enzymatic Reaction of Garlic Allinase (Maria et al., 2010)

Allicin is highly volatile and unstable. Upon extracted from fresh garlic by steam distillation process, allicin is stable up to 23^{o}C with half-life of

10 days. It tends to breakdown and transform to a more stable compound known as alkyl sulfides consists of ajoene, diallyl sulfide (DAS), diallyl disulfide (DAD) and diallyl trisulfide (DAT) (Shi et al., 2005). At temperature of 4°C, Hitoyuki (2008) estimated that allicin having half-life of 346 days. Although excessive amount of allicin would loss when expose to high temperature, the AM properties are retained. Alkyl sulfides have been reported able to kill a wide range of microbial and the AM strength is comparable with allicin (Banerjee et al., 2003; Corzo-Martinez et al., 2007). Figure 2.3 illustrates the chemical structures of DAS, DAD, DAT and ajoene.

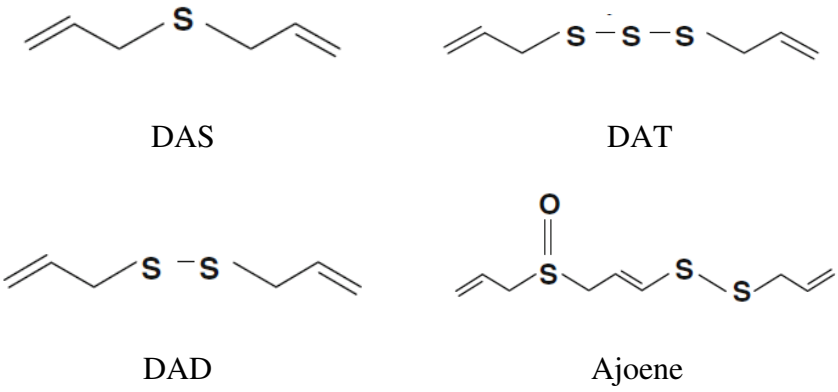


Figure 2.3: Chemical Structures of Allicin Metabolic Products

2.2.1 Antimicrobial Effects of Garlic

The AM mechanism of garlic is still not clearly defined. Most researchers assumed the reaction between thiosulfinates of allicin with thiol groups of microbial is the main mechanism (Ankri and Mirelman, 1999) in where a low concentration of allicin is sufficient to block the microbe's virulence activities in host tissues. A slightly higher concentration would

affect other enzymes in microorganisms such as thioredoxin reductases. In another study, low concentration of allicin partially inhibited protein and DNA synthesis in *S. typhimurium*, whereby, RNA synthesis was rapidly inhibited. This suggests that RNA is the first target of allicin (Feldberg et al., 1988). Sagdic and Tornuk (2012) suggest garlic compounds may alter the permeability of microbial cell walls and interchange the intracellular materials with extracellular materials which could retard bacteria growing process.

The antibacterial activities of allicin and its breakdown products have been well documented. Cavallito et al. (1944) were the first to report that allicin in garlic is the major agent to inhibit the growth of various bacterial strains. There are a wide spectrum of gram-negative and gram-positive bacteria that highly sensitive to garlic, such as, strains of *Salmonella*, *Staphylococcus*, *Klebsiella*, *Bacillus*, *Proteus*, *Clostridium*, *Escherichia*, *Micrococcus* and *Mycobacterium* (Ankri and Mirelman, 1999; Sagdic and Tornuk, 2012). Besides, garlic also effective against disease-causes bacteria such as *Helicobacter pylori*, which contribute to gastric ulcers and stomach cancer (Cellini et al., 1996). A common psychotropic pathogenic bacterium called *L. monocytogenes* found in foods that could cause meningitis disease had been widely investigated in-vitro and reported sensitive to garlic (Sagun et al., 2006; Kim et al., 2007). Allicin and its alkyl sulfides products are potential to control oral disease by suppress the growth of *Streptococcus sobrinus*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Actinomyces oris* (Bakri and Douglas, 2005). Moreover, some bacteria resistant to multidrug antibiotics such as *E. coli*, *enterococcus*, *Shigella dysenteriae*,

Shigella sonnei and *Shigella flexneri* are all sensitive to allicin. However, there are some bacteria strains that resistant to allicin such as *Streptococcus β hemolyticus*, *Enterococcus faecium* and *Pseudomonas aeruginosa*. The reason is assumed to be the hydrophilic membrane layer of those bacteria prevents the penetration of allicin (Ankri and Mirelman, 1999).

Garlic had been reported for its strong antifungal effect in-vitro and in-vivo studies. This effect are mainly attributed to allicin breakdown products – DAS, DAD, DAT and ajoene rather than allicin alone as reported by Tansey and Appleton (1975) and Corzo-Martinez et al. (2007). Yamada and Azuma (1977) evaluate the in-vitro antifungal activity of allicin against *Candida*, *Cryptococcus*, *Epidermophyton*, *Microsporum* and *Trichophyton* found that all these fungus were inhibited in the presence of allicin in concentration as low as 1.57µg/mL to 6.25µg/mL. Besides, *Aspergillus flavus*, *Aspergillus fumigatus*, *A. niger*, *Zygosaccharomyces bisporus* and *Zygosaccharomyces aouxii* are found sensitive to garlic (Kim and Kyung, 2003; Shams-Ghahfarokhi et al., 2006). Interestingly, heated garlic retains its antifungal activities towards *Candida albicans*, *Calathea utilis*, *Pichia membranifaciens*, *Saccharomyces cerevisiae*, *Z. bisporus* and *Z. ouxii*. The effect even increase when garlic exposed to 121^{o}C for 45 minutes was used for the test. This suggested that the breakdown products of allicin are the main antifungal agents in garlic (Kim and Kyung, 2003).

There are only few reports regarding antiparasitic properties of garlic. The earliest record was in ancient China, where people treat intestinal disease

by consuming alcoholic extract of crush garlic. In recent years, some studies show that little amount of allicin (30µg/mL) able to inhibit *Entamoeba histolytica* (human intestinal parasite), *Giardia Lamblia*, *Leptomonas colosoma, Leishmania major*, and *Crithidia fasciculata* (Mirelman et al., 1987) whereby a more recent study claimed that only 5µg/mL of allicin is able to inhibit 90% of *E. histolytica* (Ankri et al., 1997).

The antiviral properties of garlic studied by Weber et al. (1992) reported ajoene and allicin are effective against human cytomegalovirus, influenza B, vaccinia virus, vesicular stomatitis virus, herpes simplex virus type 1 & 2, parainfluenza virus type 3 and human rhinovirus type 2, whereby, DAD and DAT have no effect. Ajoene seems to have greater antiviral capability compared to allicin in most studies. Ajoene and DAD are active against human immunodeficiency virus (HIV) infected cells as well (Tatarintsev et al., 1992).

2.2.2 Garlic Oil Incorporated Film and Effective Microorganisms

There are only a few published papers related to garlic oil used as AM agent in food packaging system. Most of the studies are focus on edible film, and as per known, no research is study on garlic oil incorporated-plastic film produce by blown film extrusion technique. The reason may be due to allicin is very unstable and easily decompose under high processing temperature and pressure. However, the antimicrobial activities may not loss since allicin tend to transform to DAS, DAD, DAT and ajoene which is stable under high

temperature (Tansey and Appleton, 1975; Corzo-Martinez et al., 2007). Blown film extrusion technique is the conventional method used commercially for mass production; therefore, the feasibility of this technique should be study.

Edible AM film incorporated with garlic oil had obtained positive results against wide range of bacteria strains. Garlic essential oil incorporated into whey protein (WIP) based film was studied by Seydim and Sarikus (2006). WIP film is a milk protein based film that claimed to have good mechanical strength, and excellent in oxygen, aroma and lipids barriers compared to other edible films (Gennadios et al., 1994; Miller and Krochta, 1997). Seydim and Sarikus (2006) add 1-4% w/v of garlic oil, which obtained by steam distillation process for 3 hours, into WIP film. The result shows incorporation of 1 and 2% garlic oil in WIP film is not effective enough towards all strains of food pathogenic bacteria included *L. monocytogenes*, *E. coli* O157:H7, *S. aureus*, *S. enteritidis* and *L. plantarum*. The film only shows inhibition area when tested by using 3 and 4% garlic oil. The effectiveness is highest against *S. aureus* followed by *L. monocytogenes*.

Pranoto et al. (2005) studied on garlic oil-incorporated alginate film with 0.1-0.4 %v/v of garlic oil against meat product related bacteria such as *E. coli*, *S. typhimurium*, *S. aureus* and *B. cereus*. They found that a clear inhibition zone formed when 0.3 and 0.4% garlic oil incorporated alginate film tested with *S. aureus* and *B. cereus*. However, only weak inhibition against *E. coli* and *S. typhimurium* found even when 0.4% garlic oil used.

Chitosan film incorporated with different ratio of garlic oil is most studies among the AM edible film. Chitosan itself having wide range of antimicrobial activities possess amino group (-NH_2) that tends to bond with allyl and disulfide group in allicin. The formation of chitosan-allicin complex increase the AM activities compare to when use of allicin and chitosan alone. Chitosan:allicin in the ratio of 1:1 is effective towards *E. coli* and *S. aureus* whereby *Streptococcus faecalis* and *S. typhimurium* are not inhibited (Pirak et al., 2012). This finding is in contrast with others which claimed that *E. coli* is not significantly sensitive to garlic oil-incorporated film. This may be due to chitosan having greater ability to inhibit *E. coli* compare to allicin.

Gamage et al. (2009) produced garlic oil-plastic film by coated a layer of garlic oil onto corona treated OPP/PE film and laminated with another layer of LLDPE film. The film with 1.2% v/v garlic oil significantly suppressed the total microbial count on sprouts of broccoli, radish and alfalfa.

2.2.3 Garlic Oil in Local Market

Garlic is well known as a natural healthy food that has been consumed to enhance body immune system and to treat various diseases. Due to the benefits, garlic is widely manufactured into garlic oil tablets and capsules. Garlic oil is as effective as the fresh garlic and convenient to consume everyday. Around the world, China is the major country that produces garlic, with about 77% of the total amount, followed by India with about 4%, South Korea with 2%, Egypt and Russia with 1.6% and United States with 1.4%.

Locally, since Malaysia is not suitable for garlic cultivation, garlic is mainly imported from China, whereby, garlic oil is available in capsule or tablet form. The oil is mainly produced in China, Egypt, France, Bulgaria, Germany and Japan.

Although Malaysia is neither garlic nor garlic oil production country, however, garlic oil can be produced easily from fresh garlic. There are two ways to produce garlic oil – steam distillation and maceration. By using steam distillation method, steam is passed through crushed garlic and the resultant oil is captured by condensation process. Steam distillation produces concentrated oil rich of AM active compounds. Maceration is carried out by chopping garlic cloves and soaking them in vegetable oil for more than 24 hours. The AM compounds which are oil soluble will be extracted out through the process. Due to the readily available, well known medical benefits and AM effectiveness, garlic is selected as AM agent for this research and garlic oil is function as a medium to bring in the garlic active compounds to the plastic film.

2.3 Safety and Shelf-Life of Raw Beef

Evolution of consumer requirements related to life-style changes have led to increase demand for fresh meat. They seek food of better quality in terms of safety, sensory, functionality and nutritional properties (Nychas et al., 2008). At the same time, extended shelf-life of food with no or minimum added of synthetic preservative are most preferred.

2.3.1 Spoilage Related Bacteria

Shelf-life is defined by The Institute of Food Technologies in the USA as 'the period between manufacture and retail purchase of a food product, during which time the product is in a state of satisfactory quality in terms of nutritional value, taste, texture and appearance' (Singh and Singh, 2005). Meat spoilage can be indicated from the number of microorganism growth on meat surfaces as described by Fung Scale in Table 2.2 (Fung et al., 1980). The number and species of microorganism growth depends greatly on the conditions under where they were stored (Fung, 2009). Generally, beef considered spoil when reach a total microbial count of 10^7-10^8CFU/g. When total count reaches 10^8 CFU/g and above, strange odor started to build.

Microbial groups living on meat are just like a small ecosystem where different species will compete with each other and only those microorganism best adapted to the overall environment will outgrow the others and become high in numbers. Thus, the shelf-life and spoilage pattern of meat are highly dependent on storage environment. Bacteria species that frequently reported to cause meat spoilage are *Pseudomonas spp*., *B. thermospacta*, *Enterobacteriaceae* and lactic acid bacteria (Borch et al., 1996; Russo et al., 2006; Nychas et al., 2008; Doulgeraki et al., 2012).

During food spoilage process, at initial state, Pseudomonas spp. dominated on aerobically stored meat (Gill and Gill, 2010). They first utilize glucose for enumeration (Gill and Newton, 1979) and only consume amino

acid when glucose level is too low to support the growth. The breakdown products of amino acids such as ammonia and sulfides give slime odor to beef (Borch, 1996; Nychas et al., 1998).

B. thermospacta, another competitive bacterium after *Pseudomonas spp.*, had frequently reported as important spoilage bacteria on meat and beef products (Borch et al., 1996; Nychas et al., 2008; Gill and Gill, 2010; Doulgeraki et al., 2012). The ability of *B. thermospacta* to compete with other meat spoilage microbial groups (*Psuedomonas spp.*, *Enterobacteriaceae* and lactic acid bacteria) has been proven by Russo et al. (2006). *B. thermospacta* is facultative anaerobe that able to grow under the presence and absence of oxygen. Glucose was the main nutrient required for the growth of *B. thermospacta* in meat. Under aerobic condition, *B. thermospacta* produce acetic, diacetyl, isovaleric acids and 3-mthylbutanol as a result of metabolisms (Dainty et al., 1985) which give rise to cheesy and slime odor. Under anaerobic condition, *B. thermospacta* become occasionally the dominant microbial. The metabolism products are mainly lactic acid and ethanol which give milder odor compare with the products generated under aerobic condition.

Enterobacteriaceae, a facultative anaerobe bacteria, is more competitive under anaerobic condition although they also reported to exist on chilled meat stored aerobically (Nychas et al., 1998). Prolonged storage in a vacuum pack favored their growth. The generation of amines and sulfides produced slime odor.

Lactic acid bacteria are aerotolerant anaerobe bacteria that predominated under low oxygen availability. They have been reported as major spoiling microorganisms of meat under vacuum condition (Dainty et al., 1983; Borch et al., 1996). Whereby, under aerobic condition, they tend to grow slowly at chilled temperature but generally out-compete by *Pseudomonas spp*. Therefore, lactic acid bacteria rarely considered as spoilage bacteria under aerobic condition (Jos, 1996). Lactic acid and acetic acid, the metabolic products generated by lactic acid bacteria, give rise to sourly odor.

2.3.2 Factors of Spoilage

There are many factors or combined factors that caused meat spoilage, included implicit factors and extrinsic factors. Implicit factors are related to microbial action and meat indigenous enzyme activities (Nychas et al., 2008).

During slaughter process, microorganisms from the animal's intestinal tract, slaughterer's hand, cut tools, air and environment contacted with meat surfaces would probably causes contamination and lead to meat spoilage (Koutsoumanis and Sofos, 2004). The shelf-life and spoilage pattern of beef are dependent on the types of microorganisms presented initially and their subsequent growth (Borch et al., 1996). Beef provided a good medium rich of glucose, glucogen, lactate, amino acids and etc., which are required for the growth of bacteria groups such as *B. thermosphacta*, *Carnobacterium spp.*, *Enterobacteriaceae*, *Lactobacillus spp*., *Psuedomonas spp*. and *Shewanella putrefacens* (Gill, 1986; Borch et al., 1996). Indeed, compare to microbial

action, indigenous enzymatic activities contributed to meat spoilage are negligible. The post mortem glycolysis caused by indigenous enzymes stop when pH of meat reaches a value of 5.4-5.5 (Tsigarida and Nychas, 2001).

For extrinsic factors, it included the environmental condition of meat such as temperature, surrounding gaseous and pH during the process of slaughter, storage, distribution and retail. The expected shelf-life under refrigerated storage with various environmental conditions is shown in Table 2.3. Among the mentioned factors, temperature is the most important factor that influences the spoilage of meat (Koutsoumanis and Taoukis, 2005). Inappropriate temperatures or fluctuation of temperature always lead to meat spoilage in a relatively shorter time. In the presence of oxygen, muscle tissues tend to discolor 2-5 times faster at 10^{o}C compared to at 0^{o}C (Hood 1980). Lipid oxidation that gives rise to rancid odors and flavors in meats also accelerated when temperature increase. At temperature higher than -1.5^{o}C, the optimum temperature of chilled meat, microbial activities increased abruptly with further increment of temperature (Gill et al., 1988). As a result, a significant effect on sensory quality and enzymatic browning and shriveling could happen at higher storage temperature (Willocx, 1995). In some countries, the minimum requirement temperature for fresh meats be stored, transported and displayed is below 4^{o}C (Pastors, 2005). However, this is hardly to achieve due to product temperature may rise during processing, packing and transportation. Torstveit and Magnussen (1998) reported that the mean temperature for meat retail display is above 4^{o}C. Thus, it is not

surprising that microorganisms could grow rapidly and lead to spoilage during retail display.

Environmental gaseous level is also an important factor related to food spoilage. In the presence of oxygen, a composed of aerobic bacteria and facultative anaerobic bacteria such as *Pseudomonas spp*., *B. thermosphata*, *S. putrefaciens* and *Enterobacter spp*. tend to grow and dominated on beef surfaces even at temperature as low as -1.5^{o}C (Gill et al., 1988). In the reduced oxygen availability, facultative anaerobic bacteria and anaerobic bacteria such as *B. thermosphata* and lactic acid bacteria will be more preferable to grow, whereby, *Psuedomonas spp.* which are strictly aerobic, cannot grow under anaerobic conditions (Gill and Gill, 2010).

Meat pH will affect the selection and growth of bacteria. Meat with higher pH tends to spoil more rapidly than normal pH due to amino acids is more susceptible to bacteria at higher pH level (Borch et al., 1996). Bacteria reported to grow well at high pH vacuum condition are *B. thermosphacta*, *Enterobacter spp.*, *S. putrefaciens*, *Hafnia alvei*, *Serratia liquefaciens* and *Lactobacillus spp*. (Gill and Newton, 1979; Patterson and Gibbs, 1997).

Table 2.3: Expected Life Span under Refrigerated Storage, Growth Ability of Bacterial on Meat and Meat Products (Borch et al., 1996)

Product	Storage	Expected Life Span	Growth[a]			
			Pseudomonas spp.	*Enterobacteriaceae*	Lactic acid bacteria	*B. thermosphacta*
Meat, normal pH	Air	Days	+ + +	+ + +	+ +	+ +/+ + +
	High O_2 – MA	Days	+ + +	+ +/+ + +	+ +/+ + +	+ + +
	Vacuum	Weeks-months	+	+/+ +	+ + +	+ +/+ + +
	100% CO_2	Months	+	+/+ +	+ + +	+
Meat, high pH	Vacuum	Days	+	+ +/+ + +	+ + +	+ +/+ + +
	100% CO_2	Weeks-months	+	+/+ +	+ + +	+
Meat products	Air	Days	+/+ +	+	+ +	+ + +
	Vacuum	Weeks	+	+	+ + +	+ +/+ + +
	100% CO_2	Weeks	+	+	+ + +	+

a + + +, dominant part of the microflora; + +, intermediate part of the microflora; +, minor part of the microflora.

2.3.3 Beef Related Pathogenic Bacteria – *L. monocytogenes* and *E. coli*

L. monocytogenes is a gram-positive, facultative anaerobe food-borne illness bacterial that commonly found in meat product. Consuming foods contaminated by this bacterial would lead to fatal diseases such as meningitis and pneumonia which often occurs in the new born, pregnant women and elder folk. *L. monocytogenes* had been involved in food-borne illness outbreak at 1998 and 1999 which causes 21 deaths among 100 reported cases and at year 2002, 10 deaths occurred in United States related to consumption of contaminated meat (Ye et al., 2008a, 2008b). Reported by the government of United State at year 2000, the annual economic loss associated with *L. monocytogenes* was $2.3 billion.

L. monocytogenes can be found in many organisms that live in different environment such as silage, sewage, soil, fresh water, sea water, and even drains in food production unit. The high capacity of *L. monocytogenes* to tolerate with the wide range of environmental conditions is the main factor of the spread of this bacterium. Although the optimum growth temperature for *L. monocytogenes* is 30-35^{o}C, it can still grow at temperature as low as 0^{o}C and as high as 42^{o}C. In the presence of high salt concentration at about 16% w/v NaCl, *L. monocytogenes* can survive for up to one year and can grow in 10% w/v NaCl (Aldsworth et al., 2009).

Numerous studies show that beef is closely related to the growth of *L. monocytogenes*. The number detected is often higher than other pathogenic

bacteria such as *Salmonella* and *E. coli*. In Ireland, a survey conducted in the early 1990s reported detection of *L. monocytogenes* in 16% of 50 minced beef in retail (Sheridan et al., 1997). In Seattle, Washington, 18 cases out of 512 of retail beef samples were detected contaminate by *L. monocytogenes* (Samadpour et al., 2006). Season could be the major contribution to the growth of *L. monocytogenes*. In Alberta, Canada, a larger number of *L. monocytogenes* (52%) was detected in beef samples during late spring and summer in year 2001 (Bohaychuck et al., 2006).

E. coli O157:H7 is a gram-negative, facultative anaerobe food-borne illness bacterial that particularly problematic for the beef industry. Consuming foods contaminated by *E. coli* O157:H7 could cause hemorrhagic colitis disease which resultant in bloody diarrhea, severe cramping and occasional vomiting that last for 2 to 9 days (Feng, 2000). In more serious case, it could cause fatal as reported by Mead et al. (1999), 52 people out of total 1843 are death. It is estimated that *E. coli* O157:H7 contamination has caused economic loss of up to $2.7 billion from year 1993 to 2003.

The capability of *E. coli* to tolerate with the changes of environmental conditions is not as good as *L. monocytogenes*. It can only grow at temperature as low as 7^{o}C and as high as 46^{o}C. The optimum temperature for the growth is in the range of 35-40^{o}C. However, it can survive well in foods at refrigeration temperature in the range of 3-7^{o}C, with a reduction of 0.5-1.5 log bacterial within 5 weeks of storage. Pathogenic *E. coli* is able to grow in saline broth with 6% of NaCl (ICMSF, 1996).

Numerous studies on raw beef microorganisms sampled from retailers, caterers and processors have found the exits of *E. coli* O157. In Switzerland, 2.4% of total 211 minced beef investigated are found positive to *E. coli* O157 (Fantelli and Stephan, 2001). In United States, national survey reported 0.54% of samples from processors and 0.75% of samples from retailer contain *E. coli* O157 (Naugle et al., 2005). The growth of *E. coli* in beef is varied with season. In Ireland, January to August is the most active season with average 3.6% of beef found to contain *E. coli*, whereby September to December only 0.28% found (Cagney et al., 2004).

CHAPTER 3

METHODOLOGY

3.1 Materials

Raw material for film production, low density polyethylene (LDPE), was purchased from Titan Petchem (M) Sdn. Bhd., Malaysia. Ethylene vinyl acetate copolymer (EVA) with the grade of UE629 (10% vinyl acetate content) was supplied by USI Corporation, Taiwan. EVA was added to LDPE as the compatibilizer of deodorized garlic oil and LDPE matrix. On the other hand, the culture medium, namely Tryptone Soya (Agar, CM0131B and broth, CM0129B) and saline peptone powder (CM0733B) were purchased from Oxoid Microbiology, United Kingdom. The AM agent, i.e. deodorized garlic oil was obtained from Xiamen Forever Green Source Biochem Tech. Co., Ltd., China.

3.2 Garlic Oil-Polymer Incorporation method

By referring to the literature review, garlic oil can be incorporated into plastic film by either direct adding method or coating method. By using coating method, allicin can be retained due to low temperature required for the process. By using direct adding method, the main active AM agent exist is the breakdown products of allicin known as alkyl sulfides. Both methods can be

used to produce AM films since both having active AM agents. In coating method, in order to enhance the attachment of AM agent onto plastic matrix, plastic films need to undergo surface modification process by corona treatment or UV radiation before coating. This method is time consuming, costly and less suitable for fast-paced production environment; therefore, direct adding method is used in this study

3.3 Antimicrobial Film Preparation

The AM plastic films were prepared from pre-mixed 90% low density polyethylene (LDPE) pellet and 10% ethylene vinyl acetate copolymer (EVA) powder. EVA copolymer pellet was initially ground into powder by using ball mill grinder, then blended with garlic oil (0, 2, 4, 6, 8% w/w respectively) thoroughly in drum tumbler and continued with addition of LDPE pellet. The masterbatch was manufactured into films by blown film extrusion machine (Tai King Machinery, Taiwan). The temperature in the extruder was set to 170^{o}C in all heating zones. After extrusion, the films were immediately wrapped with aluminium foil to prevent evaporation of AM agent. Summary of film formulation is listed in Table 3.1.

Table 3.1: Summary of Film Formulations

Weight of LDPE (kg)	Weight of EVA (kg)	Weight of Garlic Oil (kg)	Weight Percent of Garlic Oil (% w/w)
3.6	0.4	0.00	0 (Control)
3.6	0.4	0.08	2
3.6	0.4	0.16	4
3.6	0.4	0.24	6
3.6	0.4	0.32	8

3.4 Bacterial Cultures

Bacterial cultures used in this study were typical beef contaminants included gram-negative bacteria, *E. coli* (ATCC 10536) and gram-positive bacteria, *L. monocytogenes* (ATCC 13932) and *B. thermosphacta* (ATCC 11509). *L. monocytogenes* and *E. coli* are pathogenic bacteria and *B. thermosphacta* is beef spoilage bacteria. They were purchased from American Type Culture Collection (ATCC), US. The bacteria cultures were maintained according to the steps describe in manual obtained from supplier. For ceasing bacteria activities, the bacteria strains were stored at -80^{o}C in Tryptone Soy Broth (TSB) that contained 20% glycerol. For experiment purpose, the bacteria were regularly subculture on Tryptone Soya Agar (TSA) and stored at 4^{o}C. In the preparation for antibacterial test, one colony of bacteria was transferred from TSA into 50 ml Tryptone Soya Broth (TSB) and incubated in incubator shaker at 37^{o}C (*E. coli* and *L. monocytogenes*) or 25^{o}C (*B.*

thermosphacta), 200 rpm for 18 hours. Serial dilution was conducted to obtain required concentration of bacteria.

3.5 Agar Disk Diffusion

The AM activities were carried out by agar disk diffusion test. The plastic films were cut into 26 mm diameter discs and placed on TSA plates with approximately 10^6 CFU/ml of tested bacteria. The plates were incubated for 24 hours at 37^{o}C for *L. monocytogenes* and *E. coli*, and 25^{o}C for *B. thermosphacta*. After 24 hours, the growth of bacteria surrounding and underneath the film was observed and the area of inhibitory/retraction zone was measured. The test was done in triplicate.

3.6 Challenge Test

The AM activity of the films was tested on ready-to-eat (RTE) beef loaves. Once purchased from local retailer, the meats were steam to cooked and cut into loaf shape that weighted 5g per piece. In order to sterilize the beef loaves, every single side of the meat surfaces was exposed to UV light for 15 minutes in prior to test. The meat were then randomly divided into three sets for different bacteria inoculations, and each set divided into five lots for different packaging formulation, i.e., garlic oil in 0, 2, 4, 6 and 8% w/w respectively. To inoculate bacteria on beef, 0.1 ml of each bacteria strain (*E. coli, L. monocytogenes and B. thermosphacta*) with concentration of 10^6-10^7 cfu/ml was transfer onto top and bottom surfaces of meat and spread evenly to

obtain bacteria concentration of approximately 10^5 cfu/g. The samples were leave for 5 minutes to allow the inoculums to soak in and attach to the meats before wrapped with plastic films containing 0, 2, 4, 6 and 8% w/w of garlic oil respectively. The meats were make sure to be tightly contacted with the films and the three open sides of films were sealed and stored immediately at 4^oC for 15 days to mimic the normal retail display temperature (Torstveit and Magnussen, 1998). The counts of bacteria were determined by using serial dilution method immediately after inoculation and periodically after 3, 6, 9 and 15 days of inoculation. To determine the number of bacteria grown on the sampling days, two packages for each formulation were open. The bacteria on beef are extracted by added 50 ml saline peptone water into each sample and homogenized with laboratory blender (Bagmixer) for 2 minutes. 0.1 ml homogenate was pipette into centrifuge tube and serially diluted with 0.9 ml saline peptone water. 0.1 ml of each diluted homogenate were then transferred onto TSA plate and incubated at 37^oC (*E. coli and L. monocytogenes*) or 25^oC (*B. thermosphacta*) in incubator chamber. After 24 hours, the number of colonies formed was calculated and expressed as cfu/g. The procedure is illustrated in Figure 3.1.

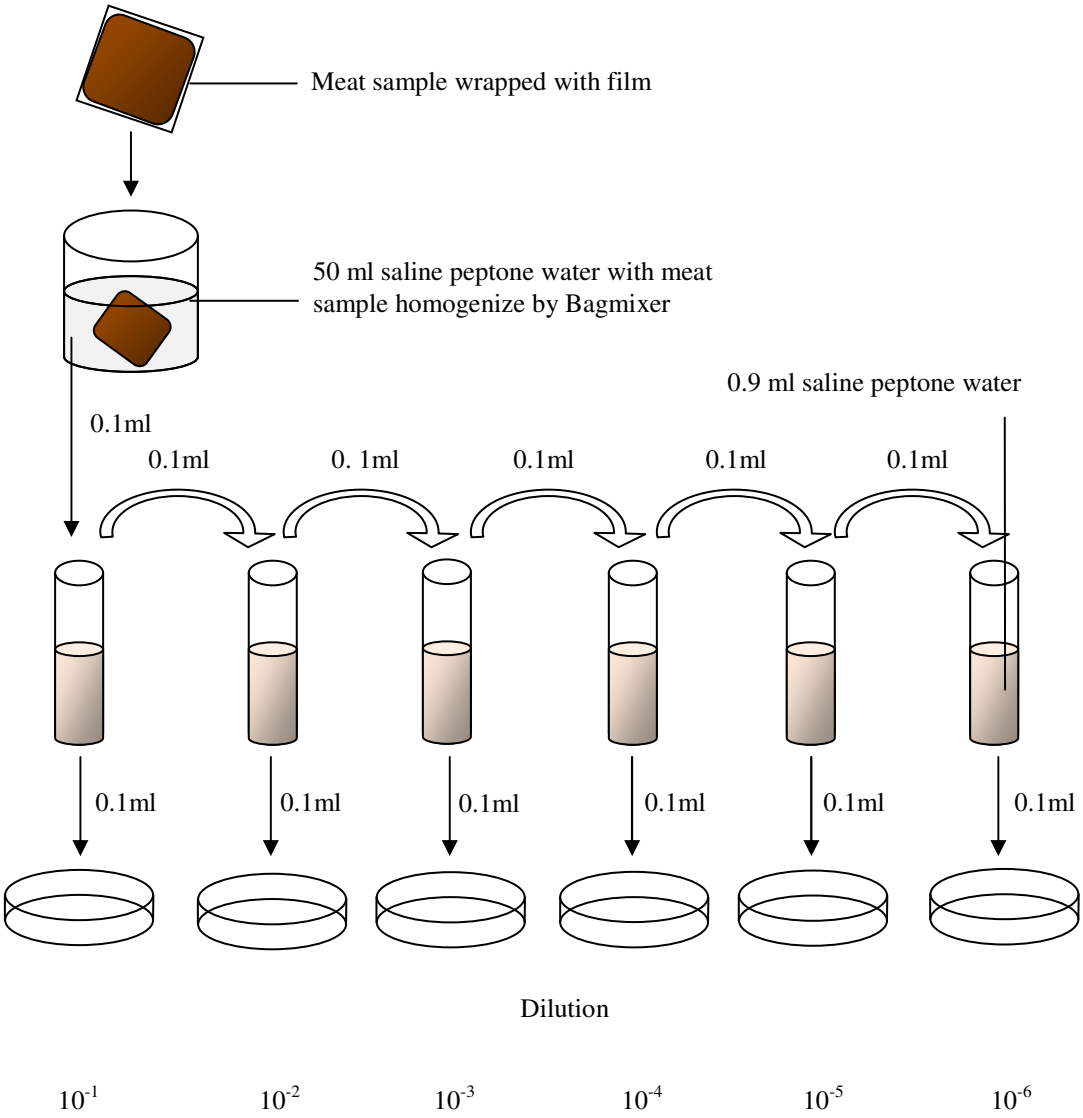


Figure 3.1: Serial Dilution on Meat Sample Illustration

3.7 Tensile Test

Tensile properties of the plastic films were determined according to ASTM D882–10 (ASTM, 2010b) by using Universal Testing Machine Instron 5567. Prior to test, the film was conditioned to room temperature for 48 hours. Each film with different formulation was cut in machine direction (MD) for five sheets with dimension of 100 mm length and 12.7 mm width. The

specimen was placed in grips of machine with initial grip distance of 50 mm and the rate of grip separation used was 500 mm/min.

3.8 Tear Propagation Force

Tear propagation force of plastic films was determined according to ASTM D1938 – 08 (ASTM, 2008a) by using Universal Testing Machine (UTM) Instron 5567. Prior to test, the films were conditioned to room temperature for 48 hours. Each of the film samples were cut to five trouser sheets in the machine direction (MD) according to the dimension specified in ASTM. The thickness of specimens were measured at three points and averaged. The specimen was then placed in grips of machine with initial grip distance of 50 mm and the rate of grip separation used was 250 mm/min. The average tear propagation force was calculated by averaging the load over 25.4 mm interval.

3.9 Differential Scanning Calorimetry (DSC)

The melting behavior of plastic films was determined by using Mettler Toledo DSC823E DSC according to ASTM D3418-08 (ASTM, 2008b). Samples were cut into approximately 5 mg and sealed by aluminum pan. Another empty pan was used as reference. The DSC machine was set to a constant heating and cooling rate of 10^{o}C/min under nitrogen atmosphere to prevent thermal degradation during the test. The temperature range used was

50 to 250^{o}C. The degree of crystallinity from the normalized DSC curves was calculated according to equation (1)

$$\chi\,(\%) = 100 \times (\Delta H_m / W \Delta H^{o}_{m}) \qquad (1)$$

where ΔH_m (J/g) is the latent heat of fusion, W is the polymer weight fraction in the sample, and ΔH^{o}_{m} is the theoretical latent heat of fusion for 100% crystalline polyethylene, 293J/g (Suppakul et al., 2006). The tests were repeated for three times in order ensure the result accuracy.

3.10 Water Vapor Barrier Properties

The water barrier properties of the film samples were determined in term of water vapor transmission rate (WVTR) at 23 °C and 60 % relative humidity (RH) according to the ASTM E96/E96M-10 (ASTM, 2010c). The circular test cup was filled with silica gel (desiccant) activated at 200^{o}C (0 %RH) and then sealed by the test films. It was covered with lid having 60 mm of opening (film tested surface area) which allowed vapor to pass through. The cups were placed inside a control chamber which was maintained at 60 %RH and 23°C. The test was carried out in triplicate for each film formulation. Weight gain of the test cups from time to time was recorded periodically until the weight gain become constant. Through weighing, the transfer of water vapor from surrounding air via the film into the desiccant can be calculated and expressed as WVTR in g/m^2/day.

3.11 Films Microstructure

The surface and the cross-section of the films were tested in Hitachi VP-SEM S-3400N to get the microstructure graphics. Films were cut into small pieces in order to be mounted on specimen stubs with double-sided adhesive tape. SC7620 Mini Sputter Coater was used to coat the samples with a layer of gold (Au) and palladium (Pd). Then, the image was observed under Scanning Electron Microscope (SEM) using an accelerating voltage of 15 kV with working distance of 5.7 mm. For cross-section observation, the samples were previously elongated and fractured during tensile test.

3.12 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) test is conducted by using Mettler Toledo TGA/SDTA851e. Samples of 5mg to 6mg were cut from the plastic films. The sample is heated from 25^{o}C to 600^{o}C at heating rate of 20^{o}C/min under nitrogen gas flow. Through TGA, the weight change of sample as a function of temperature is calculated. The decomposition points of polymer material were determined from the graph.

3.13 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) analysis is carried by using Perkin Elmer Spectrum RX1 analyzer. In this analysis, the type of chemical bonds (functional groups) and molecular structures of the polymer

matrix and garlic components can be identified. For the sample preparation, small pieces of plastic films with different amount of garlic oil were cut. The wavelength of the analyzer was set in the range of 400 to 4000cm^{-1}. The sample is placed on a plate in the analyzer and FTIR spectra were recorded.

3.14 Melt Flow Rate (MFR)

Melt flow rate (MFR) is a measurement scale of how easily the molten plastic can flow and represents a typical index for quality control. In this experiment, melt flow testing was carried out by using Dynisco D4002HV machine according to ASTM D1238-10 Testing Standard (ASTM, 2010a) under the condition of 190/2.16 (190 °C with 2.16 kg of load) which the value of MFR was determined in g/10minutes. The temperature of barrel is set up to 190 °C and a portion of the sample was charged within 60 seconds so that the piston was in proper position. When desired temperature reached, the load was applied and the melted sample was allowed to flow and cut in every 60 seconds. The extrudate specimen was collected and weighed to nearest 1 mg once they were cooled. The weight of extrudate was then converted to obtain the flow rate in grams per 10 seconds as calculated by the Equation 2.

$$MFR\ (T, m_{nom}) = \frac{600m}{t} \quad (2)$$

where

T = test temperature, °C

m_{nom} = nominal load, kg

m = average mass of the cut-offs, g

t = cut-off time-interval, s

600 = factor converting grams per second into grams per 10 min (600 seconds)

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Agar Disk Diffusion

The AM effect of garlic oil-incorporated LDPE/EVA films was studied by agar disk diffusion test. The bacteria selected for the test were pathogenic and spoilage bacteria related to beef products. Table 4.1 and 4.2 shows the antibacterial activities of films against gram-positive bacteria: *L. monocytogenes, B. thermosphacta*; and gram-negative bacteria: *E. coli*. For all bacteria tested, it has neither inhibition nor retraction zone formed for control film, i.e. film without garlic oil.

Among the tested bacteria, *L. monocytogenes* is the most susceptible bacteria with obvious retraction zone of 10.22±0.87 and 12.51±0.60 cm^2 when tested by film with 6% and 8% w/w garlic oil respectively (Table 4.1). When tested with 2% w/w and 4% w/w AM-films, the inhibition zones were not obvious. Instead, a clear retraction area formed underneath the films was observed (Table 4.2). Meanwhile, *B. thermosphacta* and *E. coli* showed retraction zone of 9.63±0.18 and 9.02±1.76 cm^2 respectively when tested with 8% w/w garlic oil-film. The concentrations of garlic oil from 2 to 6% w/w did not significantly expand the area of the retraction zone. This result shows that gram-negative bacteria (*E. coli*) are more resistant to garlic oil compare to

gram-positive bacteria (*L. monocytogenes* and *B. thermosphacta*). The additional layer of outer membrane built by lipopolysaccharide and protein that presented on gram-negative bacteria may affect the permeability of garlic oil inhibitory compounds (Perry et al., 2009; Ramos et al., 2012). Thus, the amount of garlic oil needed to inhibit gram-negative bacteria is higher than gram-positive bacteria (Pranoto et al., 2005; Seydim and Sarikus, 2006; Gamage et al., 2009).

The dosage of garlic oil to show significant retraction zone in this study is much higher when compared to other researches; this is expected since the films are produced by conventional blown film extrusion machine under high temperature (170^{o}C) and pressure. Most of the plant extracts-AM agents are highly sensitive to film production process condition. Under high processing pressure and temperature, deformation or evaporation of AM agent can be happened (Jari et al., 2003). According to Kamel and Saleh (2000), the active AM component of garlic, known as allicin, is highly unstable and tended to degrade to yield mainly ajoenes and dially trisulfide (DAT) when subjected to elevated temperature. Thus, it is believed that most of the allicin evaporates during the processing period. Nevertheless, the remaining component ajoene, which is a more stable lipid-soluble allylsulfides is believed to devastate a wide range of microbial (Banerjee et al., 2003; Corzo-Martinez et al., 2007). In allicin, the thiosulfinate structure (R-S(=O)-S-R´) is thought to be responsible for the antibacterial activity by inhibiting sulfhydryl group (-SH)-containing enzymes in microorganisms. Ajoene also contains S=O group that believed almost as active as allicin. Whereas, when the film

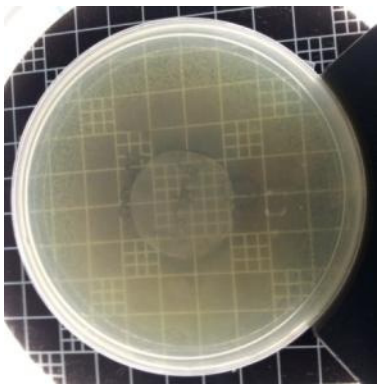
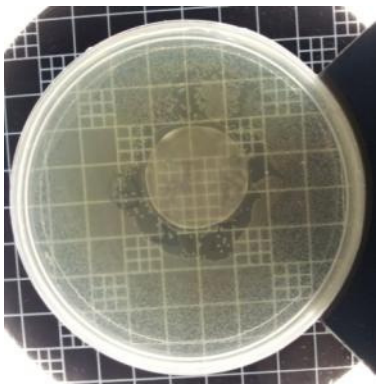
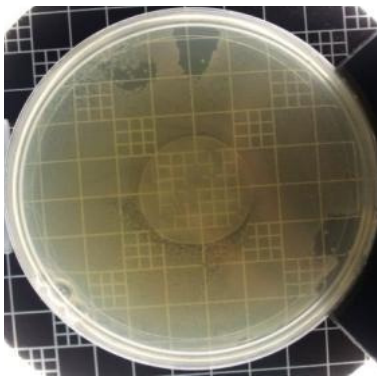
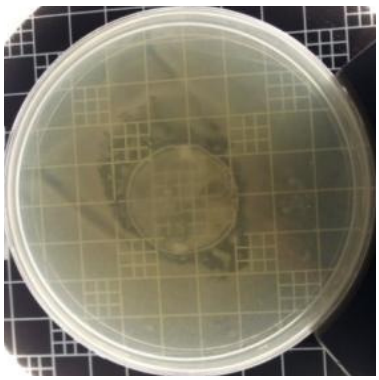
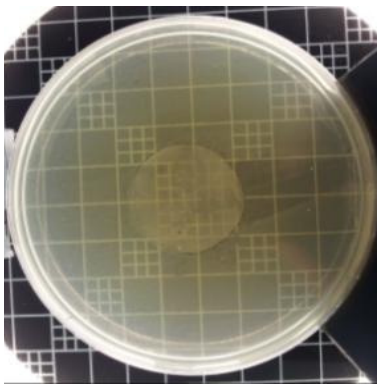
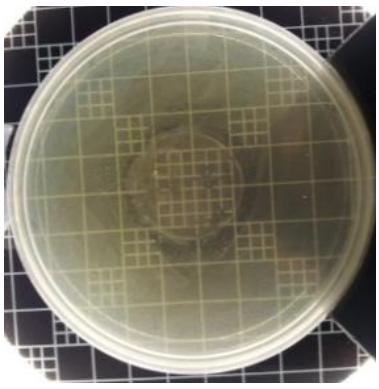
processing temperature is insignificant like the solution casting approach in preparation of AM film, lower amount of AM agent could exhibit a clear inhibition zone. For example, edible whey protein isolated (WPI) AM film produced under maximum temperature of 90 ± 2°C significantly inhibits bacteria growth, including *L. monocytogenes* and *E. coli* O157:H7, when 3-4% wt/vol garlic oil is incorporated (Seydim and Sarikus, 2006).

Table 4.1: Area of Retraction Zones* Produced by Different Percentage of Garlic oil-containing LDPE/EVA Films Observed by Agar Disk Diffusion Method

Garlic Oil (% w/w)	Retraction Zone (cm^2)		
	L. monocytogenes	*B. thermosphacta*	*E. coli*
0	0.00	0.00	0.00
2	5.31 ± 0.00	7.08 ± 0.92	5.31 ± 0.00
4	5.31 ± 0.00	7.56 ± 0.07	5.31 ± 0.00
6	10.22 ± 0.87	8.07 ± 0.58	7.55 ± 1.31
8	12.51 ± 0.60	9.63 ± 0.18	9.02 ± 1.76

*retraction zone: a visible decrease in microorganism density on the petri dish

Table 4.2: Antimicrobial Effect of LDPE/EVA with 0 and 8% w/w Garlic Oil against (a) *L. monocytogenes* (b) *B. thermosphacta* and (c) *E. coli*

	0% w/w Garlic Oil	8% w/w Garlic Oil
(a)		
(b)		
(c)		

4.2 Challenge Test

4.2.1 Challenge Test of Films against *L. monocytogenes*

Figure 4.1 shows the growth behavior of *L. monocytogenes* on artificially contaminated RTE beef that packaged in films with different amount of garlic oil. Regardless on the amount of garlic oil added into plastic packaging, the beef samples having similar bacteria growth curve where the number of *L. monocytogenes* increased rapidly within the 15 days of storage at 4^{o}C. The survival of *L. monocytogenes* in meat products is mainly depends on the temperature and pH value during storage. According to Aldsworth et al. (2009), although the optimum growth temperature for *L. monocytogenes* is 30-35^{o}C, it is capable to grow at temperature range of 0-42^{o}C. Barker and Park (2001) also suggested the optimum pH for *L. monocytogenes* growth is 7-8 but they still can grow in the range of 5-10. In our study, the pH value of beef samples was 6.6±0.1 which expected to support the growth of *L. monocytogenes*. Results indicated that RTE beef products that stored at refrigerator having high potential for causing health problems when the products were contaminated with *L. monocytogenes.*

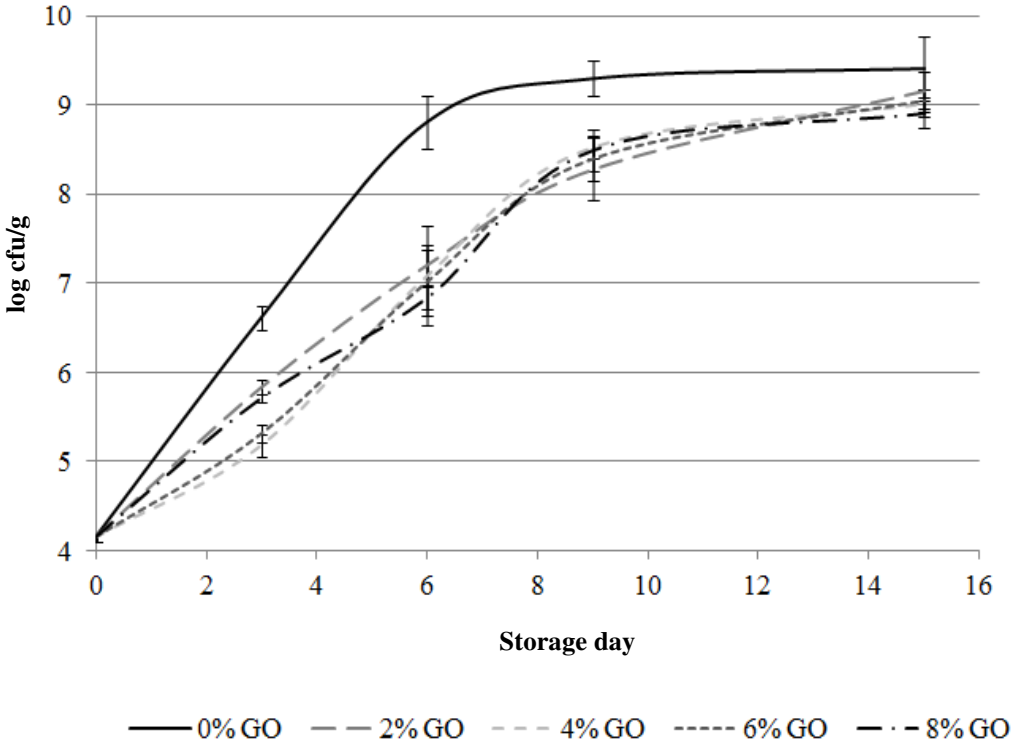


Figure 4.1: Effect of Garlic oil-incorporated Plastic Films on the Growth of *L. monocytogenes* on RTE Beef Loaves Stored at 4°C

Throughout the storage period, *L. monocytogenes* grow most rapidly on beef samples packaged in control film. The counts of *L. monocytogenes* reached 7 log cfu/g within 4 days of storage and reached the initial stage of stationary phase on day 7. Whereby, the incorporation of garlic oil into plastic films effectively reduced the bacteria growth rate. As in the case of film with 8% w/w garlic oil, the counts of *L. monocytogenes* in this treatment were 1.98 log cfu/g lower than those in control film on the 6th day of storage and only reach the initial stage of stationary phase on day 10. According to Quintavalla and Vicini (2002), the effectiveness of AM packaging was attributed to the entrapment of AM agents within polymer chains that offer slow and continuous migration of the agents from packaging material to food surfaces.

Besides, Herath (2010) studied on the release kinetics of AM agents into food simulants also suggested that the intermolecular interaction between AM agents and polymer was one of the factors to maintain the AM agents in high concentration over a long period. On the other hand, the AM effectiveness of film with 2% w/w garlic oil was comparable with film containing higher amount of garlic oil. The further additions of garlic oil only slightly increase the AM effectiveness of packaging at the end of storage day, thus, 2% w/w garlic oil is good enough to suppress *L. monocytogenes* in this particular study. Du et al. (2009) reported 0.5% w/w garlic oil in tomato cast film can effectively suppressed the growth of *L. monocytogenes*. Kim et al. (2007) also reported that garlic oil is highly effective on different strains of *L. monocytogenes*.

4.2.2 Challenge Test of Films against *E. coli*

The antimicrobial test of films against *E. coli* presented in Figure 4.2 shows that the bacteria count in all beef samples only increased slightly throughout the 15 days storage at 4^{o}C. As in the case of control film, *E. coli* count only increased 0.342 log after 15 days of storage. This is reasonable since temperatures lower than 7^{o}C does not support the growth of *E. coli* and the optimum temperature for the growth is in the range of 35-40^{o}C. However, *E. coli* can still survive well in foods at refrigeration temperature in the range of 3-7^{o}C within 5 weeks of storage period (ICMSF, 1996).

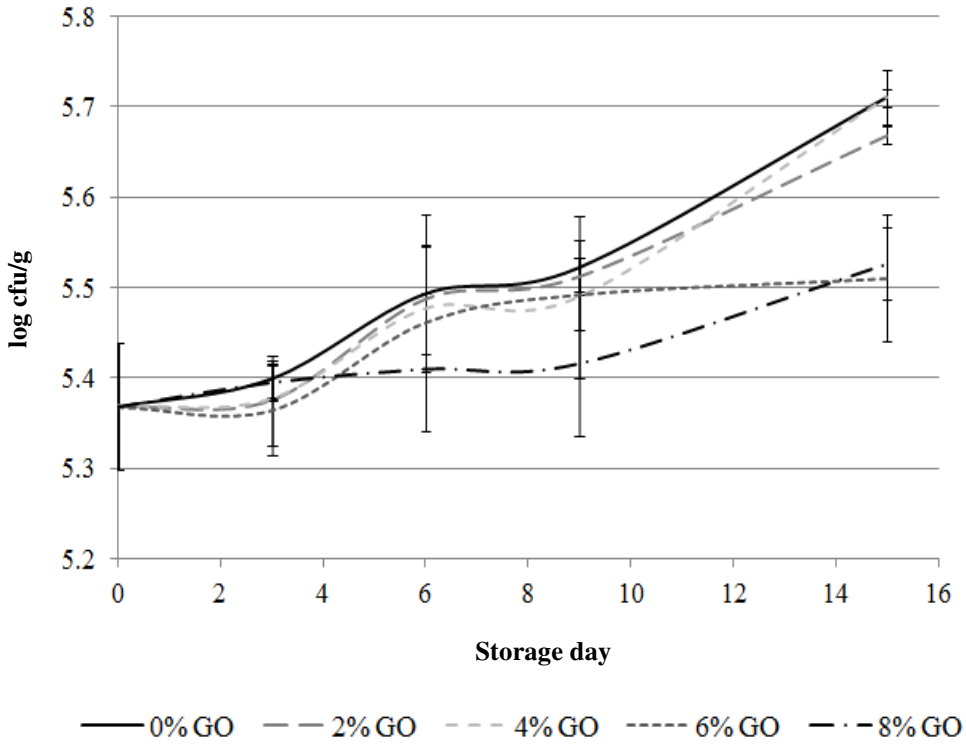


Figure 4.2: Effect of Garlic oil-incorporated Plastic Films on the Growth of *E. coli* on RTE Beef Loaves Stored at 4°C

Result shows that the counts of *E. coli* on beef packaged in garlic oil-containing films were consistently lower than those in the control film sample throughout the storage period. However, the difference in counts between control film and AM films were low at ≤0.2log cfu/g for all data points. Thus, it is concluded that garlic oil-containing films in this case having minimal AM effects on *E. coli*. Du et al. (2009) developed AM films also found that tomato films formulated with garlic oil at 0.5-3% w/w were not effective against *E. coli* (gram-negative bacteria). They were however effective against *L. monocytogenes* (gram-positive bacteria) at all concentration in agar disk diffusion test. This can be due to two reasons, firstly, the high resistance of lipopolysaccharide layer on gram-negative bacteria towards garlic active

compounds (Sivarooban et al., 2008), secondly, the lack of allicin in the AM films. There are many reports claimed that allicin in garlic is an effective bactericidal against *E. coli* (Ankri and Mirelman, 1999; Curtis et al., 2004). However, allicin become unstable when exposed to high temperature and tends to decompose to sulfide compounds. The half-life of allicin is about a year at 4^{o}C, and 32 days at 15^{o}C, but only 1 day at 37^{o}C (Fujisawa et al., 2008). Thus, it is expected no allicin exist in garlic oil that been exposed to elevated temperature such as in this study, garlic oil has been exposed to 170^{o}C during blown film extrusion process. Although the breakdown products of allicin, sulfide compounds, having AM effects against wide range of bacteria (Yin and Cheng, 2003), the sensitivity of *E. coli* to these compounds is relatively lower compared to allicin (Sagdic and Tornuk, 2012). Thus, higher amount of garlic oil is needed to show inhibitory effects as reported by Seydim and Sarikus (2006) who found that whey protein isolated (WPI) film inhibit the growth of microbial on sprout only when high amount of garlic oil is incorporated.

4.2.3 Challenge Test of Films against *B. thermosphacta*

B. thermosphacta is a competitive bacterium after *Pseudomonas spp.* that had frequently reported as important spoilage bacteria on beef products (Borch et al., 1996; Nychas et al., 2008; Doulgeraki et al., 2012). It is a facultative anaerobe that able to grow under the presence and absence of oxygen. Under aerobic condition, *B. thermosphacta* produce acetic, diacetyl, isovaleric acids and 3-mthylbutanol as a result of metabolisms (Dainty et al.,

1985) which give rise to cheesy and slime odor. *B. thermosphacta* was detected in the aerobic flora of chilled meat (Nychas et al., 2008); therefore, it is not surprising that this bacterium could grow rapidly during retail display process.

The growth of *B. thermosphacta* on beef loaves packaged in films with different amount of garlic oil is shown in Figure 4.3. Under storage temperature of 4^{o}C, the counts of *B. thermosphacta* increase rapidly from 5.41 log cfu/g on day 0 to 9.01, 8.90, 8.92, 8.79 and 8.86 log cfu/g for films with 0, 2, 4, 6 and 8% w/w garlic oil in respective on day 6 of storage. Similar with *L. monocytogenes*, *B. thermosphacta* is gram-positive bacteria. However, it is more resistance to AM agent compared to *L. monocytogenes*. As observed from the result curve, although film with garlic oil having lower counts of *B. thermosphacta*, it is consider not effective since the differences in counts between control film and AM films were low at <0.3log cfu/g for all data points.

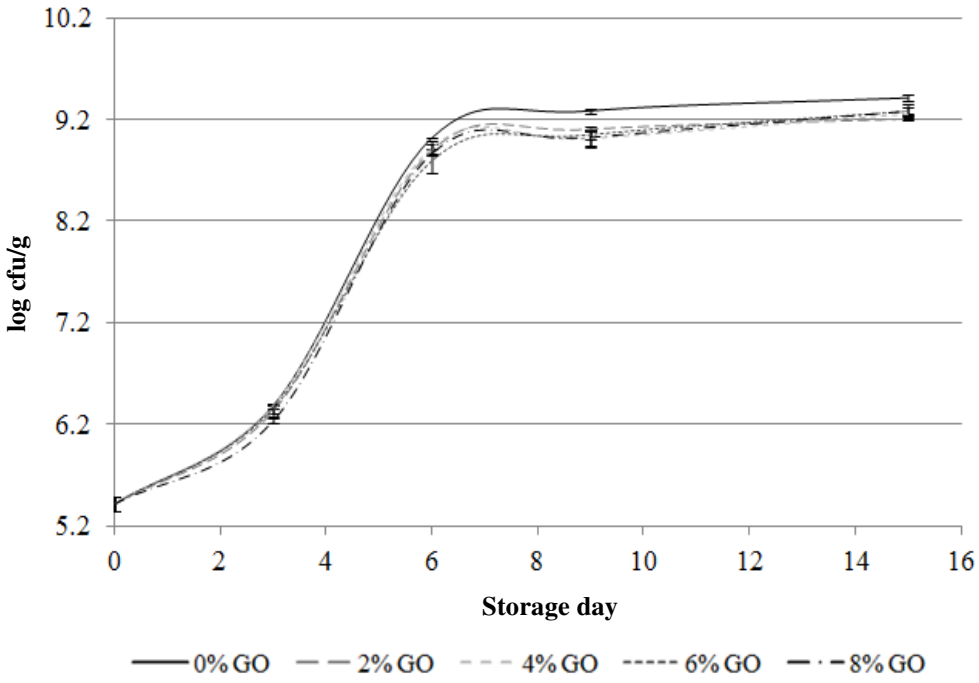


Figure 4.3: Effect of Garlic oil-incorporated Plastic Films on the Growth of *B. thermosphacta* on RTE Beef Loaves Stored at 4°C

4.3 Tensile Test

AM additives could change the film properties and adversely affect the packaging performance. Figure 4.4 and Figure 4.5 show the effect of different concentrations of garlic oil on the tensile strength (TS) and elongation at break (Ɛ) of LDPE/EVA film respectively. Comparing to the control film, TS of films decreased gradually with the higher amount of garlic oil incorporated. When incorporated with garlic oil in 2, 4, 6 and 8%, TS reduced gradually to 15.40, 14.88, 13.73 and 13.52 MPa respectively as compared to control film with 16.96 MPa TS. This is because additives other than cross-linking agents could interrupt the film integrity and resultant in lower TS (Cagri et al., 2001). Nevertheless, polyethylene matrix can tolerate with small quantity of garlic oil

added. This indicates that small amount of garlic oil having good compatibility with the polymer matrix. The AM agents may be first impregnated at the less dense area of polymeric structure – the amorphous region, without significant interference with polymer-polymer interaction. In contrast, when high level of AM agent is incorporated, the space within amorphous region is filled and the AM agent will start to fill the crystalline region and interfere with the polymer-polymer interactions (Han, 2005). Thus, the crystalline structure became weaker at higher amount of garlic oil. Besides, high additives level would lead to agglomeration within polymer chains and contributed to stress concentration area which further reduce the TS. According to Bastarrachea et al. (2001), who reviewed engineering properties of AM films, reported that even small quantities of AM can change the tensile properties when they interact with the packaging material's matrix. Similar results were obtained by Pranoto et al. (2005), Gamage et al. (2009), Solano and de Rojas Gante (2010), Nostro et al. (2012), who tested on AM-incorporated natural polymer and synthetic polymer.

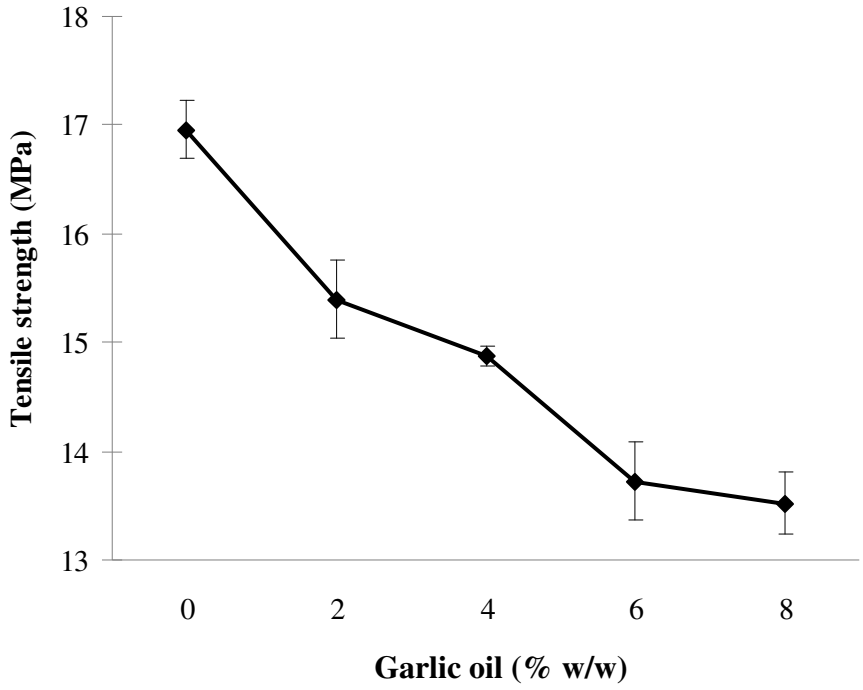


Figure 4.4: Tensile Strength of LDPE/EVA Film Incorporated with Different Amount of Garlic Oil

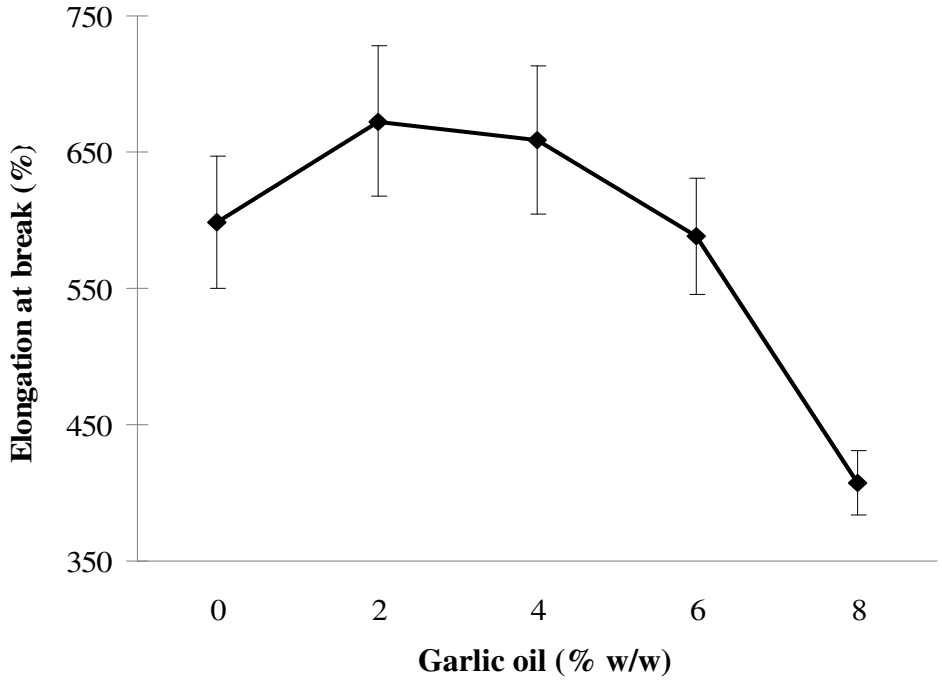


Figure 4.5: Elongation at Break of LDPE/EVA Film Incorporated with Different Amount of Garlic Oil

On the other hands, addition of garlic oil at 2% and 4% w/w increased Ɛ from 598.23% of control film to 672.5% and 658.95% respectively. Garlic oil with low molecular weight particles may be first positioned in the space provided by amorphous phase of polymer structure (Han, 2005) and further acting as plasticizing agent. These particles tend to reduce the intermolecular forces of polymer chains. When applied with tensile force, polymer chains slipped and pass through each other, thus improving the flexibility and stretch ability of the film (Nostro et al., 2012). However, Ɛ drop slightly in 6% w/w garlic oil-film whereby a significant drop was observed in 8% garlic oil-film with Ɛ value of 407.21%. This show that high amount of garlic oil greatly weakened the film structure by occupying the AM agents within the spaces between polymer chains. This further reduces the intermolecular forces of polymer chains and caused the chains unable to hold each other when slippage occurs during external drawing. Thus, the films became less extendable. Similar trend was obtained by Pranoto et al. (2005). Although increasing amount of garlic oil has reduced both TS and Ɛ value of plastic film, it still fall within common value of food packaging material that used in commercial. In this study, garlic oil-incorporated LDPE/EVA film has TS value of 13.52-15.40 MPa and Ɛ value of 407.21-672.50% which is within the value of the most common used packaging material – LDPE with TS value of 7-25 MPa and Ɛ value of 300-900% (Bastarrachea et al., 2001). This indicates that the mechanical properties of the AM films are still acceptable for its application.

4.4 Tear Propagation Force

Plastic packaging is required to have good resistance to tear propagation in order to prevent package damage during processing, transportation and application. ASTM D1938 (trouser tear test) is a widely used test for highly ductile materials such as plastic films (Chang et al., 2002). It covers the force required to propagate a tear at a specially created single tear region. When the stresses reached a certain level, tear propagate from the single tear region. Tear properties of plastic films are mainly affected by the structure and alignment of the crystalline and amorphous phases as reported by Seyed et al. (2009). The higher is the orientation of crystalline and amorphous phases, the lower is the tear resistance along machine direction. Besides, films with higher crystallinity tend to have greater tear resistance because the crystalline regions act as reinforcement region (Nikolaos, 1977). Figure 4.6 summarizes the force required to propagate the initial tear of LDPE/EVA films (along machine direction) with different level of garlic oil. It shows that the tear force did not significantly affected by garlic oil. For film with 2% garlic oil, tear force increase slightly from 1.537N of control film to 1.594N. This is due to the higher crystallinity of the film. When garlic oil level further increase, tear force decrease to 1.51N, 1.458N and 1.347N respectively for film with 4, 6 and 8% of garlic oil. Oil possessing lubricating effects facilitated the movement of polymeric chains and makes the chains easier to align during blown film extrusion process. Although these films having higher crystallinity than control film (Figure 4.8), the strength of crystalline region was probably overwrite by the highly oriented polymer phase.

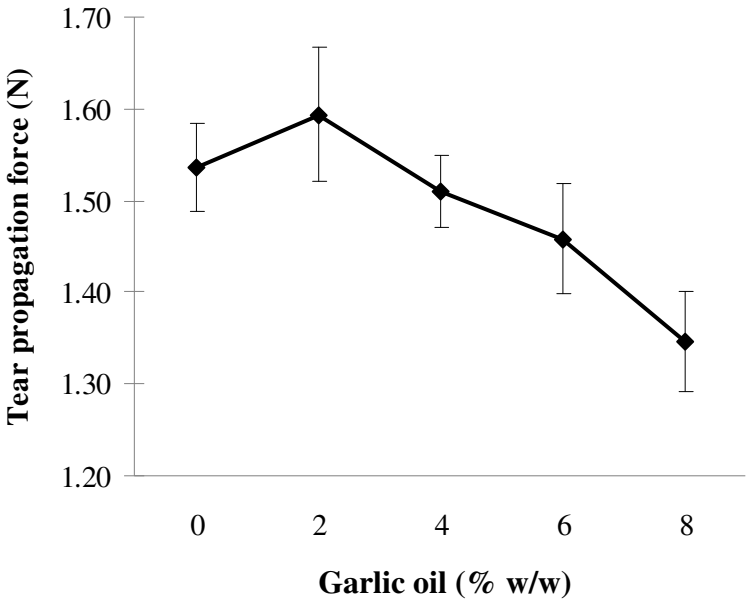


Figure 4.6: Tear Propagation Force of LDPE/EVA Films with Different Amount of garlic Oil Added

4.5 Differential Scanning Calorimetry (DSC)

The effect of adding different garlic oil concentrations on thermal properties of LDPE/EVA films was studied by using differential scanning calorimetry (DSC). Figure 4.7 shows the DSC thermogram of the five formulations obtained during the second heating process. The result of first heating which reflects thermal history has been eliminated in order to obtained result with better accuracy. Regardless of the amount of garlic oil added, there are only one endothermic peak obtained during the heating process with melting temperature remains practically unchanged at around 92.7-93.7^{o}C (Table 4.3). Although high amount of garlic oil is added, it does not affect the melting temperature of the film because most of them have been evaporated during film manufacturing process. The loss of garlic oil and its compounds is

explained by the TGA result in section 4.8. Besides, the amount of active compounds exist in the films is not significant to affect the melting temperature as well. Thus, the films thermal stability is unaffected by the incorporation of garlic oil. This is supported by many researchers who evaluated the thermal properties of AM food packaging film. Many of them found no significant changes in both the glass transition temperature and melting temperature, as highlighted by Bastarrachae *et al.* (2011) who review on AM films for food packaging. Ramos *et al.* (2012) found adding of 8%w/w carvacrol did not change the melting temperature of PP film as well. Similarly, Herath (2010) who studied on thymol and carcacrol added LDPE/EVA films found that addition of 5% w/w AM agent into polymer films did not change the films melting temperature. Besides, Sappakul *et al.* (2006) also found the addition of basil extracts in LDPE/EVA films did not change the melting temperature significantly.

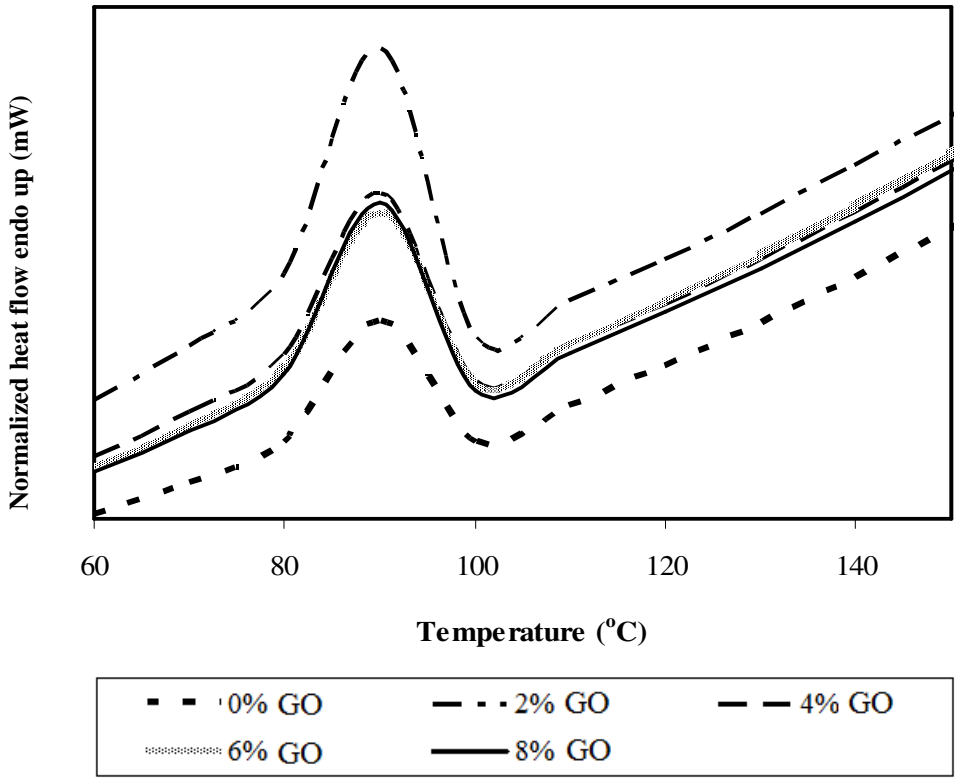


Figure 4.7: DSC Thermograms of LDPE/EVA Films with Different Percentage of Garlic Oil Incorporated

Table 4.3: Enthalpy of Melting and Melting Temperature of LDPE/EVA Films with Garlic Oil Incorporated

Garlic Oil (% w/w)	Enthalpy of Melting (J/g)	Melting Temperature (°C)
0	39.0	92.7
2	63.3	93.3
4	55.8	93.7
6	54.3	93.7
8	49.4	92.8

Besides, all the films having melting endothermic transition at the region 86-96^{o}C with different area (Figure 4.6). Melting enthalpy represented the magnitude of intermolecular bonding among polymer chains and with other additives (Tiam-Ting et al., 2013). The greater the enthalpy, the greater the amount of thermal energy required to transform into kinetic energy in order to weaken the bonding and thus free the polymer molecules from ordered crystalline structure. DSC thermogram showed that adding of garlic oil in 2% and 4% greatly increases the melting enthalpy from 39.0 J/g of control film to 63.3 J/g and 55.8 J/g respectively (Table 4.3). Melting enthalpy further reduces to 49.4 J/g when the amount of garlic oil added increase to 8%. This shows that garlic oil has induced pronounces effect on formation of secondary bonding especially when small amount of garlic oil is added. On the other hand, the crystallinity of the films is further studied and summarized in Figure 4.8. The crystallinity was calculated based on melting enthalpy ratio of the blended films to 100% crystalline LDPE. It was found that addition of garlic oil has greatly increased the films crystallinity from 13.3% of control film to 21.6%, 19%, 18.5% and 16.9% respectively for films with 2%, 4%, 6% and 8% garlic oil. Crystalline structure formed in polymer when the molecular chains fold orderly into regular repeating pattern. Importantly, this is highly depends on how easily the chains can move and fold. In this case, it is believe that addition of garlic oil would initially promote reorganization of polymer chains followed by vaporization due to heating process. Subsequently, the remaining polymer chains after the vaporization of garlic oil would rearrange in more compact structure. This can be well observed in the shrink/rougher

surfaces of microstructure images as shown in Section 4.7. Thus, this can be correlated with the crystallinity of the films improved.

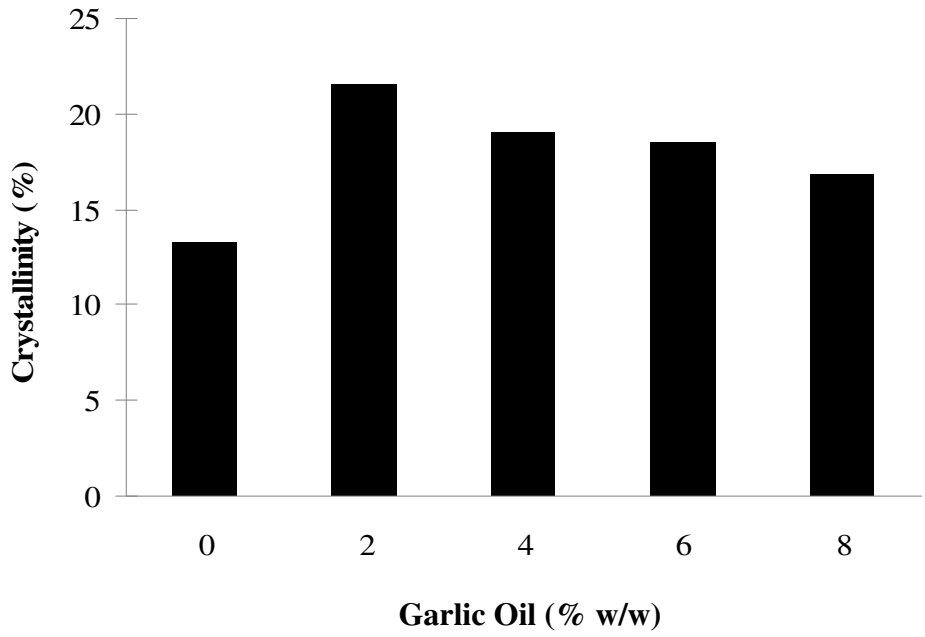


Figure 4.8: Crystallinity of LDPE/EVA Films with Different Amount of Garlic Oil Added

4.6 Water Vapor Barrier Properties

Water vapor transmission rate (WVTR) represents the ease of moisture to penetrate and pass through a material (Li et al., 2006). It is important for a packaging to have good water vapor barrier properties not only to prevent excessive water loss from foods, but also resists moisture from atmosphere to migrate into foods since water can accelerate microorganisms' growth and reduce shelf-life of foods. Figure 4.9 shows the WVTR of plastic film with different percentage of garlic oil incorporated. The addition of garlic oil on the film increased WVTR from 0.85 g/m^2/day to 0.92, 1.27, 1.34 and 1.56

g/m^2/day in respect to film with 2, 4, 6 and 8% w/w of garlic oil. Although the hydrophobic compound of garlic oil tends to inhibit the moisture contact, there was more weight gain for film with higher garlic oil percentage. This is probably caused by the evaporation of volatile compound of garlic oil - sulfur components from the film and left behind the porous matrix. The porous matrix of the film allowed the vapor transmission and contributed to the weight gain. Besides, these results can be verified by the study from Pranoto et al. (2005) where the weight gained was greater with the higher amount of garlic oil added into alginate films. The author suggested that it might be due to the structure of alginate film was changed by addition of garlic oil which resulted in intermolecular extension and furthermore, loosening the compactness of the structure. Hence, it encouraged the water vapor to pass through the film.

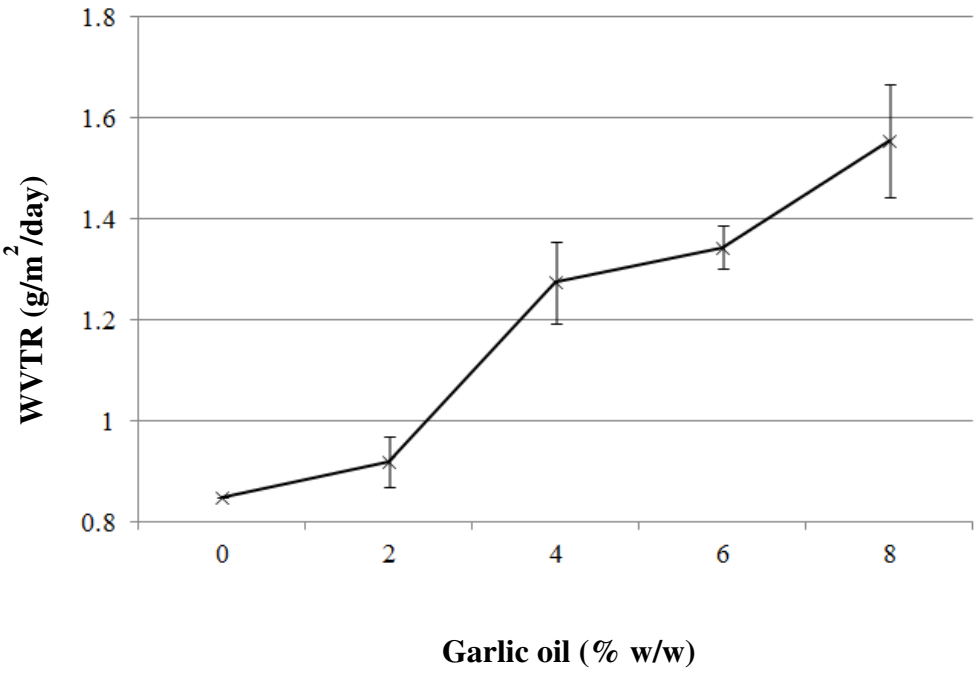
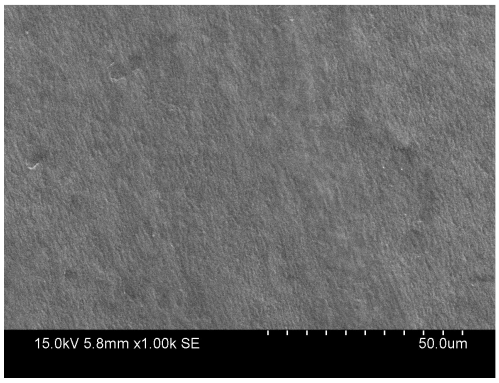


Figure 4.9: Effect of Different Amount of Garlic Oil on Water Vapor Transmission Rate (WVTR) of Film Samples

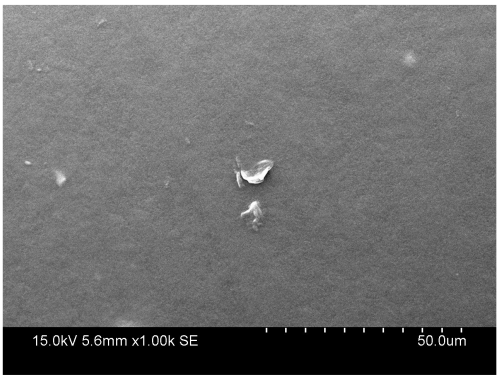
4.7 Films Microstructure

It is important to examine the microstructure changes happened in the plastic film after the addition of AM agent by the means of scanning electron microscope (SEM). SEM micrographs of LDPE/EVA film added with garlic oil ranging from 0% w/w to 8% w/w before and after tensile test were obtained by scanning the surface of the films and the fracture part of film after tensile test. Initially by observing the images as shown in Figure 4.10, there was no obvious different of appearance for both control film and film added with 2% w/w of garlic oil. The films were smooth and having homogeneous surface without pores, indicating that ordered matrix was formed in both films. Whereas, films incorporated with 4-8% w/w garlic oil has relatively rougher surfaces. The rough surfaces were observed throughout the films and most pronounce in film with 8% w/w garlic oil. The existence of irregularity of the surface was possibly due to the flow of the essential oil during film production process. This flow mark may reduce film integrity and cause reduction of films' mechanical strength. Besides, evaporation of volatile components in garlic oil during blown film process created the empty micro-holes (Sanchez-Gonzalez et al., 2011) and these might be associated with the reduction of tensile strength and barrier properties. Further examining the fracture part of the films as shown in Figure 4.11 found that the cross-sections of control film showed more regular breakage and smoother surface as highlighted in Figure 4.11a. Whereas, when the garlic oil content increased, the cross-section of the fractured film showed rougher surfaces. This was clearly highlighted in Figure 4.11b, 4.11c, 4.11d and 4.11e. This can be caused by the addition of oil

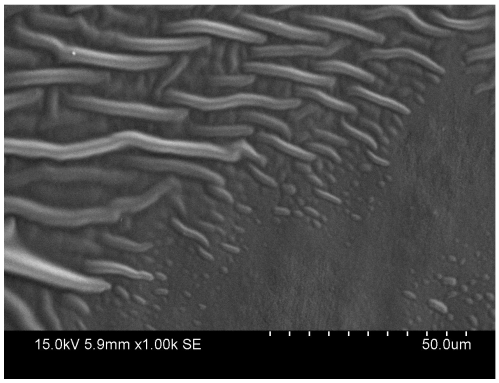
induced greater flexibility of polymer chains which enhanced higher ductility of the films.



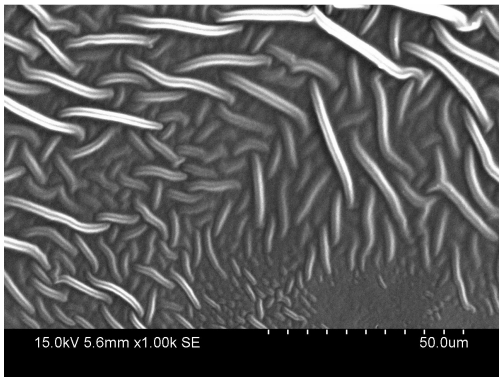
(a)



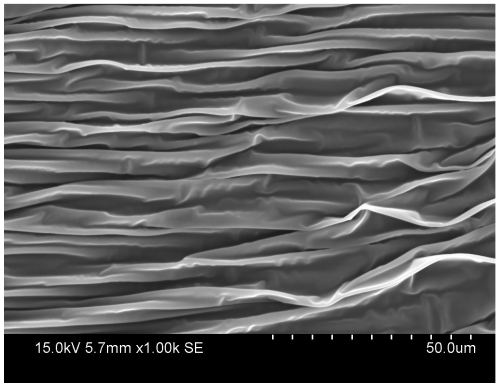
(b)



(c)

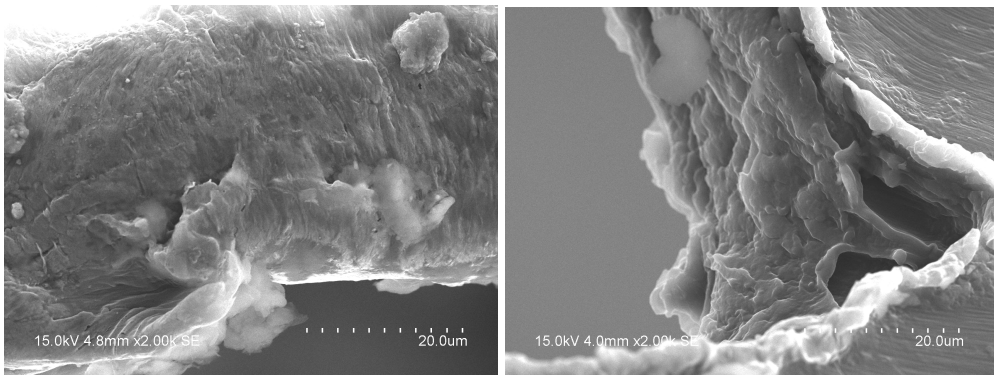


(d)

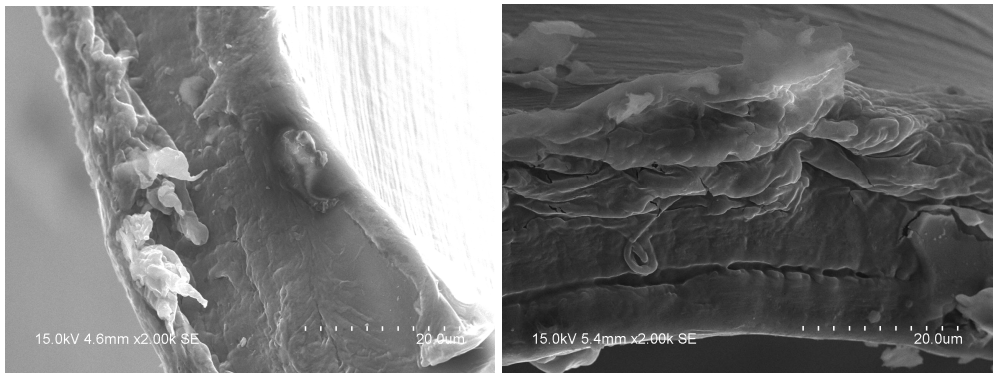


(e)

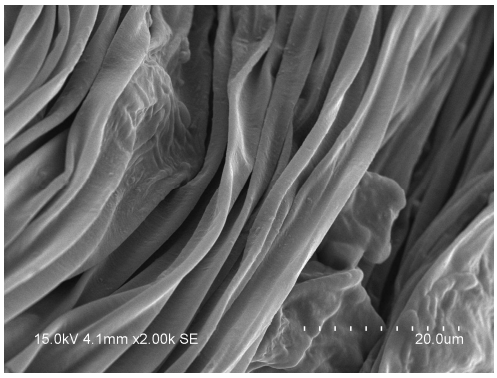
Figure 4.10: Micrographs (1000×) of Antimicrobial Films Incorporated with Garlic Oil at (a) 0% w/w, (b) 2% w/w, (c) 4% w/w, (d) 6% w/w and (e) 8% w/w



(a) **(b)**



(c) **(d)**



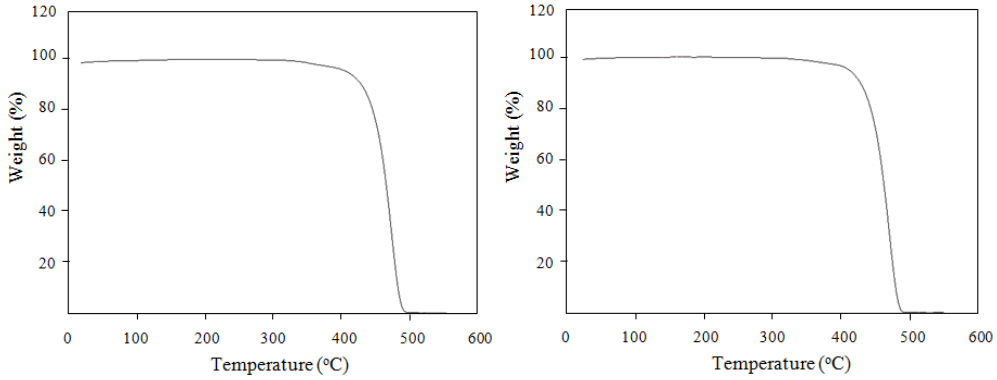
(e)

Figure 4.11: Micrographs (2000x) of Tensile Fracture Surface of Antimicrobial Films Incorporated with Garlic Oil at (a) 0% w/w, (b) 2% w/w, (c) 4% w/w, (d) 6% w/w and (e) 8% w/w

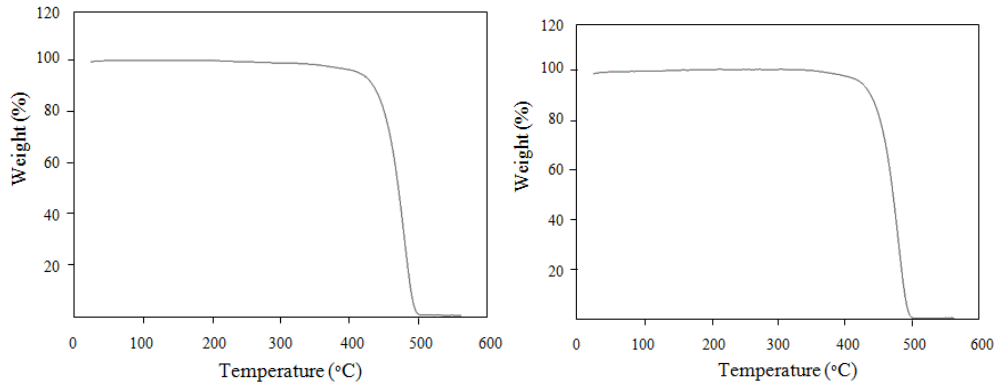
4.8 Thermogravimetric Analysis (TGA)

The study on thermal stability of plastic films with different percentage of garlic oil was carried out through TGA under nitrogen atmosphere. Figure 4.12 shows the TGA weight loss curves as the function of temperature obtained for LDPE/EVA films containing 0, 2, 4, 6 and 8% w/w of garlic oil. For all the formulations, only single peak of weight loss correspoding to the polymer blends are observed. No noticeable step of mass loss corresponding to garlic oil detected. This is expected that high amount of garlic oil and its compound have been lost during the blown film extrusion process which exposed the additives to elevated temperature. Similar finding is obtained by Suppakul et al. (2006) who incorporated 1% w/w linalool and methylchavicol into LDPE-based film. After processed into films at 160^{o}C, the remaining amount of the additives in the extruded films was only 0.34% w/w, in where, this very low concentration is not detectable by TGA. Although the amount of linalool and methylchavicol is low, it is good enough to show positive AM activity against *E. coli*. The manufacturing temperature used in this study was 170^{o}C, whereby garlic oil boiling point is around 155^{o}C. Therefore, it is believe that the loss of the oil is great. Besides, allicin is unstable and volatile so it is expect that some amount of allicin has been evaporated and some brokekdown to alkyl sulfides. The alkyl sulfides – DAD, DAT and ajoene are more stable, therefore it is believe to be retained and embedded inside the polymer chains. However, the amount may be too little to be detected by TGA. Although the amount is not significant, but it is effective enough to reduce the growth rate of *L. monocytogene* on beef as shown in challenge test

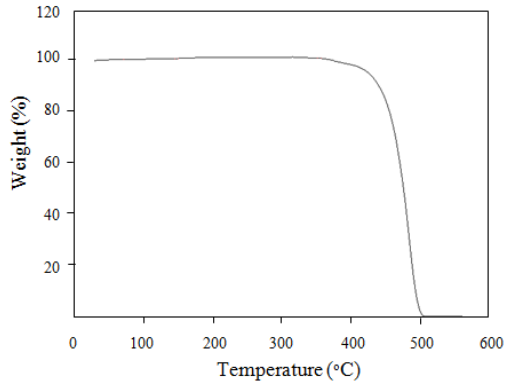
that discussed in section 4.2. The results of AM test prove the existence of garlic active agent in the films and this is further confirmed by FTIR result that discussed in section 4.9.



(a) **(b)**



(c) **(d)**



(e)

Figure 4.12: TGA Weight Lost (%) Curves of LDPE/EVA Film with (a) 0% w/w Garlic Oil, (b) 2% w/w Garlic Oil, (c) 4% w/w Garlic Oil, (d) 6% w/w Garlic Oil, and (e) 8% w/w Garlic Oil

Table 4.4 summarizes the initial decomposition temperature (T_i) and peak decomposition temperature (T_p) obtained from TGA data. As it can be seen, no significant distinction was observed for T_i and T_p values in all the samples. LDPE/EVA matrix started to decompose at around 318^{o}C with peak decomposition temperature at approximately 463^{o}C. Results indicated that the incorporation of garlic oil does not affect the thermal stability of the plastic packaging. Similar result was obtained by Suppakul et al. (2006) who incorporated linalool and methylchavicol into LDPE-based film. The authors found that linalool only slightly reduce the T_p of LDPE from 466 ^{o}C to 461 ^{o}C.

Table 4.4: Decomposition Temperature of LDPE/EVA Film Incorporated with Different Amount of Garlic Oil

Garlic oil (% w/w)	T_i (^{o}C)	T_p (^{o}C)
0	318	464
2	317	463
4	318	463
6	320	462
8	319	462

4.9 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is widely used to study the functional group and structural changes of polymeric material at molecular level through a detail spectral analysis (Ahmad et al., 2012). In this study, FTIR is performed to identify the interaction between polymer matrixes with garlic oil. Figure 4.13 represents

the spectra of LDPE/EVA films incorporated with different amount of garlic oil ranging from 0 to 8 % w/w. In overall, there have no significant differences between the spectra. The spectra with garlic oil incorporated are similar to the control film. All characteristic peaks are identical such as at the bands of 2800–3100 cm^{-1}, 1740 cm^{-1}, 1464 cm^{-1}, 1375 cm^{-1}, 1241 cm^{-1}, 1021 cm^{-1}, 729 cm^{-1} and 720 cm^{-1}. These bands can be related to absorbance of ethylene groups as 2920 cm^{-1}, 2850 cm^{-1}, 1470 cm^{-1}, 1375 cm^{-1} and 720 cm^{-1}, and vinyl acetate groups as 1740 cm^{-1}, 1240 cm^{-1}, 1020 cm^{-1} (Jamroz, 2003). These showed the absence of major structural change in the polymer matrixes. Similar results were reported by Pranoto et al. (2005) for chitosan film incorporated with *Allium sativum.*

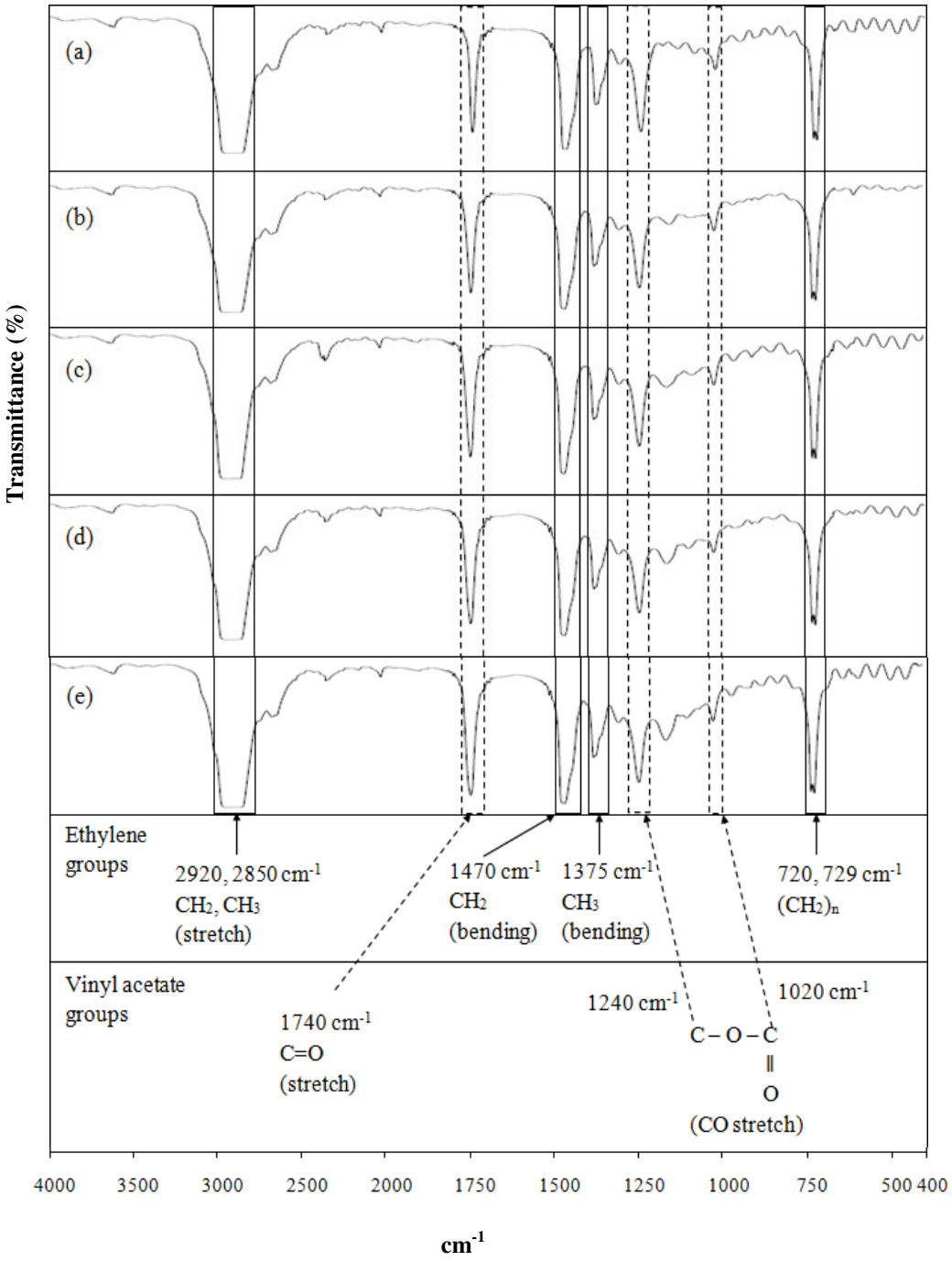


Figure 4.13: FTIR Spectra of (a) Control Film, (b) Film with 2% w/w Garlic Oil, (c) Film with 4% w/w Garlic Oil, (d) Film with 6% w/w Garlic Oil, (e) Film with 8% w/w Garlic Oil, (——) Ethylene Groups, and (----) Vinyl Acetate Groups.

Nevertheless, there are some minor distortions of bands at the absorbance range of 1030-1200 cm^{-1}. A very weak peak can be observed at 1050 cm^{-1} for films added with garlic oil (Figure 4.14). This peak represented S=O bond (Ilić et al., 2012) that existed in garlic oil active component, namely ajoene. Ajoene is thought to be responsible for the antibacterial activity by inhibiting sulfhydryl group (-SH)-containing enzymes in microorganisms. Since only small quantity of garlic oil had been added into plastic film, the amount of ajoene existed is very much lower than the polymer chains, and thus the peak represented S=O bond is relatively much weaker. Besides, the absorbance at 1131 cm^{-1} and 1177 cm^{-1} in control film are integrated into one at 1153 cm^{-1} in film with 2% w/w garlic oil and 1161 cm^{-1} in films with 4-8% w/w garlic oil. As the amount of garlic oil increase, this peak becomes sharper in significant. Peak at 1177 cm^{-1} in control film is probably stand for carbonyl, C=O stretching of ketone structure which formed by main chain scission of EVA copolymer (Jin et al., 2010) during the film forming process. In the addition of garlic oil, this peak was overwrite by peak at 1153 cm^{-1} (film with 2% w/w garlic oil) and 1161cm^{-1} (film with 4-8% w/w garlic oil) which is most likely due to the vibration of C–OH groups of amino acids (Wong et al., 1991) from garlic oil. Other important vibration represented the existence of garlic oil active components such as S–C bond (700-800 cm^{-1}) is hardly to be detected since it is probably obscured by the vibration of ethylene units.

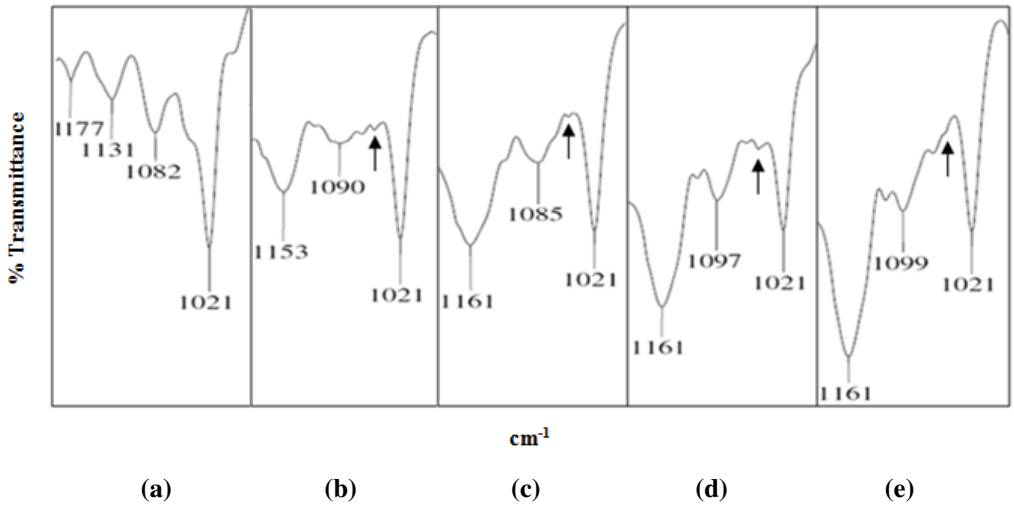


Figure 4.14: A Section of FTIR Spectra of (a) Control Film, (b) Film with 2% w/w Garlic Oil, (c) Film with 4% w/w Garlic Oil, (d) Film with 6% w/w Garlic Oil, (e) Film with 8% w/w Garlic Oil. Arrow Represented S=O Group at 1050 cm^{-1}

4.10 Melt Flow Rate (MFR)

Melt flow rate (MFR) is always important for the plastic processing industry because different melt rate is used for different process such as injection molding and film extrusion. According to Vasile and Pascu (2005), the processing technique basically decides the selection of melt flow index. The weight of extrudates increased (Table 4.5) as the garlic oil content of film increased which explained that the higher flow rate was achieved. According to Rosato and Rosato (2003), flow rate is indirectly proportional to the viscosity. As the flow rate increases, the viscosity decreases. Hence, it can deduce that the film with more garlic oil has lower viscosity compare to the film with lesser amount of garlic oil. Figure 4.15 shows the average MFR with

standard deviation for different film samples. Obviously, the MFR values were proportional to the amount of garlic oil added which was ranging from 3.01g/10min to 4.93 g/10min.

Table 4.5: Average Melt Flow Rate (MFR) of Film Samples

Incorporation of garlic oil (% w/w)	Average weight of extrudates (g)	Average MFR ± standard deviation (g/ 10 min)
Control	0.30	3.01 ± 0.05
2%	0.36	3.63 ± 0.17
4%	0.40	3.96 ± 0.11
6%	0.43	4.32 ± 0.06
8%	0.49	4.93 ± 0.18

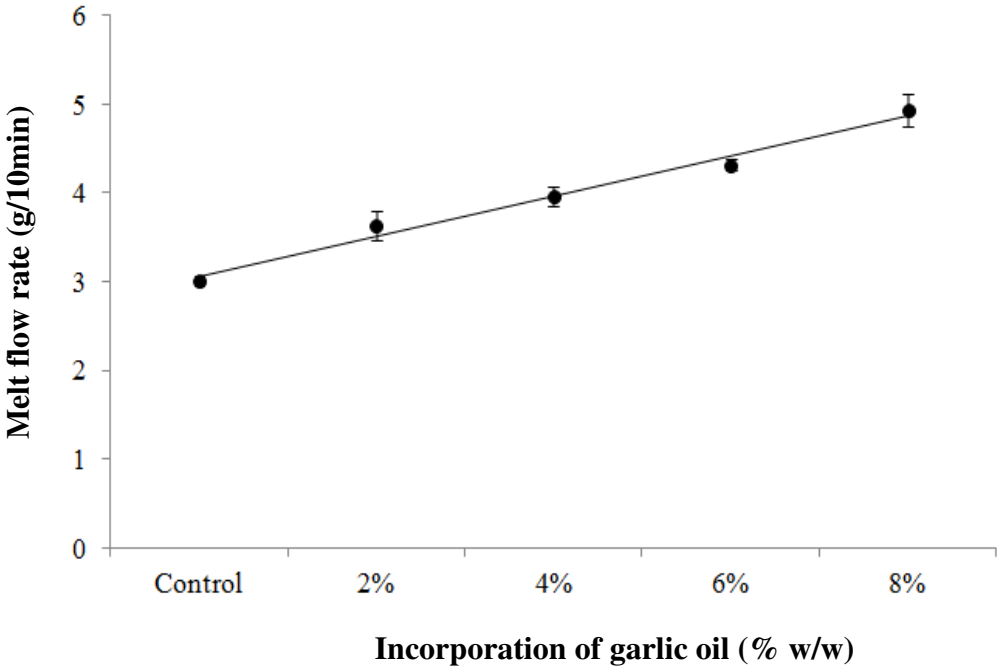


Figure 4.15: Average Melt Flow Rate (MFR) of Films Added with Different Amount of Garlic Oil (% w/w)

This result was probably induced by the incorporation of garlic oil itself where this oil extract has provided lubricating effect to ease the melt flow. The incorporation of garlic oil acted as lubricant to enhance the flow performance by reducing the overall viscosity of the molten fluid in the bore. Thus, the lubricating effect was demonstrated in reducing the sliding friction between the molten flow and the die. The weight of the extrudates was observed to have increment from control film to film added with 8% w/w of oil as shown in Table 4.5. Besides, less bubbles and lower roughness were observed in the extrudates. MFR is an important parameter not only in polymer product processing, but also in polymer masterbatch processing as shown in this research because the MFR tells how easily a masterbatch can be distributed during a compounding operation or in film extruders. In overall, the higher the amount of garlic oil added, the greater is the flow rate and hence the higher is the MFR.

CHAPTER 5

CONCLUSION

There are varieties of AM packaging available in the market. The AM agents used are dominantly artificial where excessive amount of these agents can endanger public health. Consumers nowadays are preferred to choose products with natural substances rather than those containing artificial additives. For this reason, garlic oil with active AM compounds is used in this study. LDPE films with 10% w/w EVA as the AM compounds compatibilizer were successfully manufactured using blown film extrusion method. The films were then tested to determine the AM effectiveness and plastic packaging behaviour as affected by the incorporation of garlic oil in terms of tensile and tear properties, thermal stability, water vapor barrier properties, rheological behaviour, polymer morphology and microstructure.

The inhibition of bacteria growth on solid media is studied by agar disk diffusion test. The retraction zone underneath and nearby the film shows that LDPE/EVA film with 8% w/w garlic oil can significantly reduce the concentration of tested bacteria with inhibition strength of *L. monocytogenes* > *B. thermosphacta* > *E. coli*. This result suggests the need of direct contact between the food surface and the AM film for effective bacteria inhibition. In the challenge test, plastic film incorporated with different amount of garlic oil had sufficiently reduced the growth rate of *L. monocytogenes* on RTE beef

after 3, 6, 9 and 15 days of storage at 4^{o}C. However, the effect is minimal on both *E. coli* and *B. thermosphacta*. This result shows *L. monocytogenes* on RTE beef is sensitive to small amount of garlic oil. Agar disk diffusion test is good for preliminary study to estimate the AM effectiveness on different bacteria type whereby real food is required to get a more representative result.

Addition of substances into polymer could adversely affect the packaging performance. In this study, the incorporation of garlic oil did not tremendously reduce the film integrity in terms of tensile strength, elongation at break and force for tear propagation (tear resistance). This indicates garlic oil has good compatibility with LDPE/EVA matrix and successfully acts as a medium to bring in the active compounds. Whereby, the films crystallinity increased significantly especially when small amount of garlic oil incorporated as calculated based on the DSC analysis. This suggests garlic oil has also performed as plasticizer that smoothen the flow of polymer chains and allowed them to arrange better to form the crystalline structure. Theoretically, higher crystallinity should improve the films mechanical strength, however, the films tensile strength did not increase with the increasing of crystallinity. This can be caused by sitting of AM compounds in crystalline region which weaken the structure. It is also suggested that the films have been weakening by the flow mark of garlic oil as can be observed under SEM.

For barrier properties, the incorporation of garlic oil had slightly reduced the film water vapor barrier properties. This is probably caused by the formation of pores when the active compounds are evaporated during film

manufacturing process or storage. The porous matrix encouraged the passing through of water vapor. According to TGA result, there have no significant changes on the films thermal stability as affected by garlic oil. This is because high amount of garlic oil and its compounds have been lost during the blown film extrusion process which exposed the additives to elevated temperature. The remaining amount is not significant to affect the thermal stability of the films however it is effective enough to reduce the growth rate of *L. monocytogene* on beef as shown in challenge test. The existence of the active compounds is supported by FTIR result as well. MFR result shows garlic oil acted as lubricant which enhances the flow performance of polymer material during manufacturing process. Overall, this research indicated that the plastic film contained garlic oil has good potential to be used as food packaging especially to inhibit *L. monocytogenes*.

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APPENDICES

APPENDIX A:

TABLE A1: Area of Retraction Zones (cm^2) Produced by Different Percentage of Garlic oil-containing LDPE/EVA Films Observed by Agar Disk Diffusion Method

Garlic Oil (% w/w)	Sample	*L. monocytogenes*	*B. thermosphacta*	*E. coli*
0	1	0.00	0.00	0.00
	2	0.00	0.00	0.00
	3	0.00	0.00	0.00
2	1	5.31	6.06	5.31
	2	5.31	7.28	5.31
	3	5.31	7.89	5.31
4	1	5.31	7.60	5.31
	2	5.31	7.48	5.31
	3	5.31	7.60	5.31
6	1	9.48	8.04	8.85
	2	11.18	8.66	7.56
	3	10.01	7.50	6.23
8	1	11.90	9.79	10.78
	2	13.10	9.43	9.02
	3	12.52	9.68	7.26

TABLE A2: Effect of Garlic oil-incorporated Plastic Films on the Number (log CFU/g) of *L. monocytogenes* on RTE Beef Loaves Stored at 4^oC

	Garlic Oil (% w/w)				
Day	0% GO	2% GO	4% GO	6% GO	8% GO
0	4.146±0.05	4.146±0.05	4.146±0.05	4.146±0.05	4.146±0.05
3	6.613±0.13	5.830±0.08	5.180±0.12	5.307±0.10	5.710±0.05
6	8.810±0.30	7.204±0.23	7.086±0.56	7.013±0.37	6.835±0.13
9	9.297±0.20	8.277±0.35	8.530±0.13	8.391±0.25	8.495±0.23
15	9.409±0.36	9.160±0.21	9.020±0.15	9.045±0.13	8.910±0.17

TABLE A3: Effect of Garlic oil-incorporated Plastic Films on the Number (log CFU/g) of *E. coli* on RTE Beef Loaves Stored at 4°C

Day	Garlic Oil (% w/w)				
	0% GO	2% GO	4% GO	6% GO	8% GO
0	5.368±0.07	5.368±0.07	5.368±0.07	5.368±0.07	5.368±0.07
3	5.399±0.02	5.375±0.05	5.376±0.00	5.364±0.05	5.395±0.02
6	5.493±0.00	5.487±0.06	5.476±0.07	5.461±0.12	5.410±0.00
9	5.522±0.03	5.512±0.02	5.489±0.09	5.492±0.04	5.416±0.08
15	5.710±0.03	5.668±0.01	5.710±0.01	5.510±0.07	5.526±0.04

TABLE A4: Effect of Garlic oil-incorporated Plastic Films on the Number (log CFU/g) of *B. thermosphacta* on RTE Beef Loaves Stored at 4°C

Day	Garlic Oil (% w/w)				
	0% GO	2% GO	4% GO	6% GO	8% GO
0	5.413±0.15	5.413±0.15	5.413±0.15	5.413±0.15	5.413±0.15
3	6.379±0.48	6.327±0.33	6.384±0.23	6.352±0.16	6.230±0.47
6	9.012±0.11	8.900±0.26	8.920±0.45	8.790±0.09	8.860±0.15
9	9.280±0.18	9.108±0.33	9.012±0.23	9.047±0.16	9.022±0.10
15	9.408±0.32	9.210±0.45	9.252±0.15	9.276±0.06	9.280±0.30

TABLE A5: Tensile Strength of LDPE/EVA Film Incorporated with Different Amount of Garlic Oil

Garlic Oil (% w/w)	Tensile Strength (N/mm^2)
0	16.961±0.27
2	15.395±0.36
4	14.880±0.09
6	13.725±0.36
8	13.519±0.28

TABLE A6: Elongation at Break of LDPE/EVA Film Incorporated with Different Amount of Garlic Oil

Garlic Oil (% w/w)	Tensile Strength (N/mm^2)
0	598.233±48.39
2	672.500±55.42
4	658.947±54.72
6	588.213±42.99
8	407.213±23.83

TABLE A7: Heat Flow Endo Up (mW) of LDPE/EVA Films with Different Percentage of Garlic Oil Incorporated

Temperature (°C)	Heat Flow Endo Up (mW)				
	0% GO	2% GO	4% GO	6% GO	8% GO
30	19.9994	19.9991	19.9979	19.9994	19.9991
40	21.2432	23.1405	22.0790	21.8028	21.7082
50	21.6050	23.8034	22.6210	22.3795	22.2768
60	22.1048	24.7922	23.4733	23.2195	23.1118
70	22.8200	26.0447	24.5109	24.1982	24.0704
80	23.8064	27.7559	25.8415	25.5075	25.4024
90	26.6282	33.0114	29.6201	29.1783	29.4275
100	23.8058	26.2308	25.2229	25.1938	24.9861
110	24.6444	27.1322	26.1117	26.1105	25.8783
120	25.5688	28.1081	27.0493	27.1005	26.8413
130	26.5738	29.1589	28.0863	28.1764	27.875
140	27.6613	30.2916	29.1837	29.3429	28.9766
150	28.8054	31.4576	30.3637	30.5544	30.1482
160	30.0288	32.7459	31.6242	31.8487	31.4010
170	31.3220	34.0974	32.9545	33.2029	32.7286
180	32.6899	35.5252	34.3727	34.6397	34.1432
190	34.1330	37.0312	35.8589	36.1531	35.6428
200	35.7431	38.6146	37.4346	37.7422	37.2245
210	37.3538	40.2950	39.1036	39.4166	38.8977
220	39.0563	42.0685	40.8468	41.1629	40.6392
230	40.9570	43.9132	42.6634	42.9944	42.4426
240	43.2853	45.8273	44.5602	45.1216	44.2597
250	46.0422	47.8043	46.5444	46.9358	46.2349

TABLE A8: Enthalpy of Melting (ΔH_m) and % Cyrstallinity of LDPE/EVA Films with Different Percentage of Garlic Oil Incorporated

Garlic Oil (% w/w)	ΔH_m	Crystallinity (%)
0	38.9652	13.299
2	63.2622	21.591
4	55.7847	19.039
6	54.2929	18.530
8	49.3782	16.853

TABLE A9: Tear Propagation Force (N) of LDPE/EVA Films with Different Amount of Garlic Oil Added

Garlic Oil (% w/w)	Tearing Force (N)
0	1.537±0.048
2	1.594±0.073
4	1.510±0.039
6	1.458±0.060
8	1.347±0.054

TABLE A10: Effect of Different Amount of Garlic Oil on Water Vapor Transmission Rate (WVTR) of Film Samples

Garlic Oil (% w/w)	WVTR ($g/m^2/day$)			
	1st	2nd	3rd	Average ± Standard Deviation
0	0.0354	0.0354	0.0354	0.0354 ± 0.0000
2	0.0354	0.0354	0.0442	0.0383 ± 0.0051
4	0.0619	0.0531	0.0442	0.0531 ± 0.0088
6	0.0531	0.0619	0.0531	0.0560 ± 0.0051
8	0.0619	0.0796	0.0531	0.0648 ± 0.0135