

**Development of LDPE-based  
Antimicrobial Films for Food  
Packaging**

**Yogesh Mistry**

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**Packaging and Polymer Research Unit  
School of Molecular Sciences  
Faculty of Health, Engineering and Science  
Victoria University**

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## **Abstract**

The integration of antimicrobial (AM) agents into packaging materials is aimed at killing or inhibiting the spoilage and pathogenic microorganisms that may contaminate packaged food products. Over the years there has been an increased emphasis on naturally derived AM agents and polymer films containing AM agents derived from basil, for example, exhibit an AM effect against a wide spectrum of microorganisms. Due to the relatively high temperatures involved in manufacturing such films, however, there is a considerable evaporation loss of AM agent during the film blowing process. The present study aims at developing effective AM films and subsequently reducing the loss of active AM agents. The effect of polyethylene glycol (PEG) and ethylene vinyl acetate (EVA) in minimising the loss of active AM agent during the manufacturing of low-density polyethylene (LDPE) film is explored by measuring the release of AM agent into food simulants. The release of AM agents from the film is satisfactorily and consistently described by short-term and long-term migration equations and by first order kinetics. Furthermore, the polymer additives PEG and EVA play a role in controlling the release of the AM agents. The incorporation of AM agent does not adversely affect the mechanical or optical properties of the extruded LDPE/EVA film and the films retain *ca.* 75% of AM agents after extrusion.

## **Declaration**

I, Yogesh Mistry, declare that the Master by Research thesis entitled “Development of LDPE-based Antimicrobial Films for Food Packaging” is no more than 60,000 words in length, exclusive of tables, figures, appendices, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

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Signed

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Date

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## Abbreviations and Nomenclature

AM	Antimicrobial
EVA	Ethylene Vinyl Acetate
EVOH	Ethylene Vinyl Alcohol
FDA	Food and Drug Administration
GC	Gas Chromatography
GFSE	Grapefruit Seed Extract
LDPE	Low-Density Polyethylene
MAP	Modified Atmosphere Packaging
MIC	Minimum Inhibitory Concentration
PEG	Polyethylene Glycol
SF	Supercritical Fluid
$D$	diffusion coefficient
$k$	rate constant
$l$	film thickness
$m_{\infty}$	equilibrium amount of AM agent released from film
$m_p$	amount of AM agent in packaging material
$m_t$	amount of AM agent released from film
$t$	time
$v_t$	rate of release of AM agent

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# 1 Introduction

This chapter provides an insight into the role of food packaging materials and in particular, polymer films. The concept of active packaging is introduced with an emphasis on antimicrobial additives in polymer film formulations.

## 1.1 Background

Many food products can be subjected to contamination by undesirable microbes such as fungi, yeast and bacteria (Hotchkiss, 1997). In order to prevent or impede such contamination, novel packaging technologies are continually being developed to prolong the shelf-life and improve the safety or sensory properties of fresh foods (Ahvenainen, 2003). Food packaging therefore plays a significant role in the food supply chain and is an integral part of both food processes and the whole food supply chain.

Food packaging must perform several tasks as well as fulfilling many demands and requirements. Basic packaging requirements include good marketing properties, reasonable price, technical feasibility (e.g., suitability for automatic packaging machines, seal-ability, etc.), suitability for food contact, low environmental stress and suitability for recycling and refilling (Ahvenainen, 2003). A package must satisfy each of these requirements both effectively and economically. Furthermore, packaging has a more significant role in the preservation of food and in ensuring the safety of food in order to avoid wastage, food poisoning and to reduce allergies. To ensure a longer shelf-life, packaging must play an active role in processing, preservation and in retaining the quality of food products (Floras *et al.*, 1997).

In the past, packaging was expected to play a passive role as active roles were considered to result in damage to either the product or the package (Yam and Lee, 1995). The development of modified atmosphere packaging (MAP) over two decades ago was one of the first examples showing that some product/package interactions may have a positive effect (Farber, 1991; Parry, 1993). A more recent and advanced class of food packaging systems is known as “active packaging” (Rooney, 1995a).

## **1.2 Active Packaging**

Active packaging has been defined as a system in which the product, the package, and the environment interact in a positive way to extend shelf-life or to achieve some characteristics that cannot be obtained otherwise (Miltz *et al.*, 1995; Yam, *et al.*, 2005). The main aim of active packaging is to change the condition of packaged food in order to extend the shelf-life (Ahvenainen, 2003). This practice can improve food safety and sensory properties, while maintaining the quality of packaged food. Active packaging techniques for preservation and improving quality and safety of foods can be divided into three categories: (i) absorbing systems; (ii) releasing systems; and (iii) other speciality system for temperature, ultraviolet light and microwave control systems (Han, 2003). Active packaging materials that can release active compounds for enhancing the quality and safety of a wide range of foods during extended storage are particularly important. The release of active compounds plays an important role in determining the activity of the packaging material as well as in the inhibitory effect the packaging has on the spectrum of microorganisms.

### **1.3 Antimicrobial Food Packaging**

In most foods, the surface growth of microorganisms is the major cause of food spoilage (Maxcy, 1981). Microbial contamination in packaging materials is typically controlled by heat, steam or radiation treatment or by the addition of antimicrobial (AM) additives (Hotchkiss, 1997). Antimicrobial agents are often mixed directly into foods to control microbial growth and to extend shelf life (Weng and Hotchkiss, 1992). The vast majority of these AM agents, however, are synthetic materials that have the following disadvantages: (i) they are distributed in the bulk of the food at relatively large quantities and therefore may impart an off-flavour; and (ii) consumers are concerned about the possible side-effects of synthetic additives. To assist in solving these problems, AM packaging systems have started to evolve and these are primarily based on natural AM additives.

#### **1.3.1 Design Factors for Antimicrobial Packaging**

Antimicrobial agents have a specific inhibition activity against particular microorganisms. The selection of an AM agent therefore depends primarily on its activity against the target microorganisms (Ahvenainen, 2003). Many other factors, however, need to be considered when designing AM packaging systems. Specific gravity, water solubility, organoleptic properties, toxicity and resistance to microorganisms are important characteristic properties of the AM agent (Han, 2003). Other factors such as the method of incorporation into the packaging, permeation and evaporation, controlled release, and the physicochemical properties of the packaging materials should also be considered (Han, 1996). The characteristics of food products such as the composition and chemical nature (i.e.

pH, water activity) as well as manufacturing, storage and distribution conditions such as temperature are also important (Parry, 1993). Each of these factors should be carefully considered in accordance with the relevant regulations in order to design an effective AM package.

The design of an AM packaging system requires knowledge of controlled release technology and microbial growth kinetics (LaCoste *et al.*, 2005). When the migration rate of an AM agent is faster than the growth rate of the target microorganisms, the AM agent will be depleted before the expected storage period and the packaging system will lose its AM activity. This may result in the growth of microorganisms after the depletion of the AM agent. On the other hand, when the release rate is too slow to control the growth of the microorganisms, the microorganisms may grow before the AM agent is released. Therefore, the release rate of AM agents from the packaging material onto food should be specifically controlled to be similar to the growth rate of the target microorganisms (Han, 2000).

### **1.3.2 Release of Antimicrobial Agents**

Antimicrobial activity can be achieved by adding AM agents to a packaging system during manufacturing or by using AM polymeric materials (Hotchkiss 1997). There are three typical systems of AM agent activity: (i) absorption; (ii) immobilisation; and (iii) release systems. The absorption mode removes the essential factors of microbial growth from the food systems and inhibits the growth of microorganisms. The immobilisation system does not release AM agents but suppresses the growth of microorganisms at the contact surface. Immobilisation systems are considered less effective in the case of solid foods compared to liquid foods because there is

generally less contact between an AM package and a solid food product (Han, 2000).

The release system allows the migration of the AM agent (solute or gas) into the food or the headspace inside the package to inhibit the growth of microorganisms. Whereas a gaseous AM agent can penetrate through any space, a solute AM agent cannot migrate through the air space between the food and the packaging material. The release kinetics of a packaging system is studied by measuring the release rate of the AM agent into a food simulant or by measuring the effectiveness in inhibiting microbial growth and extending the shelf life of food. Control of the release rates and migration of the AM agent from the food packaging are very important (LaCoste *et al.*, 2005). Biochemical factors affecting the mass transfer characteristics of an AM substance include AM activity and the mechanism and kinetics of the selected substance to target microorganisms. Furthermore, the release kinetics should be designed to control the growth of the microbes and maintain the AM concentration above the critical inhibitory concentration (Han, 2000).

### **1.3.3 Migration of Antimicrobial Agents into Foods**

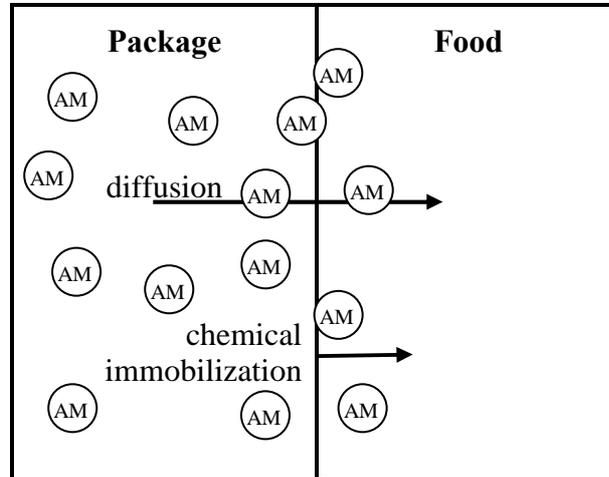
In food packaging, migration is used to describe the transfer of a substance from the packaging material into a food (Choudhry *et al.*, 1998). The migration of AM agents from the package into the food product is an essential in order to effectively inhibit the growth of microorganisms on the surface of food products. While the concentration of AM agent is maintained over the minimum inhibitory concentration (MIC) on the food surface, the system actively presents effective AM activity (Suppakul, 2004). It is technically difficult to measure the migration of a given

active agent into the food, however, because most foodstuffs are comprised of a complex mixture of substances such as water, carbohydrates, fats, lipids, proteins, vitamins, fibres and minerals. For this reason, migration studies are usually performed using food simulants (Dopico *et al.*, 2003). In current European food-packaging regulations (European Standard EN 1186-1, 1999), various food simulants that can be used for migration testing have been identified. These include: water (simulant A), 3% (v/v) acetic acid in water (simulant B); 15% (v/v) ethanol in water (simulant C); olive oil; sunflower oil; and synthetic fat simulant HB 307 (simulant D) where each simulant is representative of a particular type of food. Traditional liquid solvent/polymer extraction methods, which involve dissolution/precipitation, are used for studying the migration of additives from polymer (Zhou, 1998). In recent years, analysis with supercritical fluids (SFs) has emerged as an alternative analytical technique because SFs afford higher diffusivity and lower viscosity.

#### **1.4 Food Packaging Systems**

Most food packaging systems consist of packaging materials, food products and the headspace of the package (Ahvenainen, 2003). If the void volume of solid food products is considered as headspace, most food packaging systems represents either a package/food system or package/headspace/food system. A package/food system is a package in contact with a solid food product, or a low viscosity/liquid food without head space. Examples of food packaged in this type of system include wrapped cheese, deli products and aseptic meat packages. Diffusion between the packaging material and the food and partitioning at the interface are the main migration phenomena involved in this system (Han, 2003). An AM agent

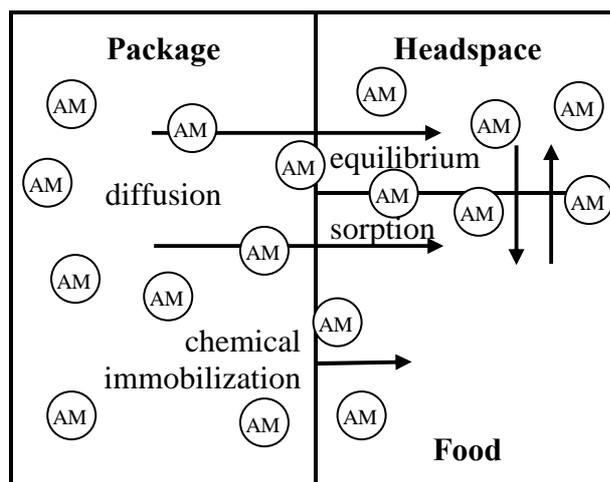
incorporated into the packaging material can migrate into the food through diffusion and partitioning as shown in Figure 1.1.



**Figure 1.1.** Diffusion of AM agent in a package/food system.

Examples of package/headspace/food systems include flexible packages, bottles, cans, cups and cartons. Evaporation or equilibrated distribution of a substance among the head space, packaging materials and food are to be considered as a part of main migration mechanism to estimate the interfacial distribution of the substance (Han, 2003). Compared to a non-volatile substance which can only migrate through the contact area between the package and the food, a volatile substance can migrate through the headspace and air gap between the package and the food as shown in Figure 1.2.

Other than diffusion and equilibrated sorption, some AM packaging systems use covalently immobilized antibiotics or fungicides. In this case, surface microbial growth is suppressed by immobilization of a non-food grade AM substance without diffusional mass transfer.



**Figure 1.2.** Diffusion of AM agent in a package/ headspace/food system.

### 1.5 Polymers and Active Packaging

Polymers such as low-density polyethylene (LDPE) constitute a majority of primary packages for foods and beverages and a great deal of research has been devoted to the development of active polymer packaging (Rooney, 1995b). Polymers can be activated by the addition of components such as antioxidants, oxygen scavengers, carbon dioxide absorbers/emitters, AM agents and ethanol emitters during their conversion into packaging materials. Most forms of active polymeric packaging involve an intimate interaction between the food and its package so that the layer in contact with the food is chosen to be active (Rooney, 1995b). Such polymer films can be used in laminates and in edible films that contain the active additives for wrapped food products (Han, 2000).

Antimicrobial agents in particular can be incorporated into a packaging system to form AM packages (Vartiainen *et al.*, 2003). The incorporation of these agents can be achieved by simple blending with the packaging materials, as well as

immobilisation or coating depending on the characteristics of packaging systems, AM agent and the food. Blended volatile AM agents can migrate from the packaging material into the food via diffusion, evaporation or slow release, while the immobilised agents remain bound to the polymer. A number of naturally derived AM agents have been shown to possess AM activity (Azaz *et al.*, 2005). Packaging materials containing natural AM agents have shown inhibitory effect against selected microorganisms such as *Staphylococcus aureus*, *Listeria innocua*, *Escherichia coli* and *Saccharomyces cerevisiae* (Suppakul, 2004). The manufacturing of films containing naturally derived AM agents, however, showed a considerable amount of loss due to evaporation occurring at extrusion conditions (Suppakul, 2004).

### **1.5.1 Polymer Processing**

In order to develop optimal AM packaging systems, the processability of the polymers, the AM constituents and any other additives should be understood. This will enable the production of AM products with sound morphological structures that will yield the required physical properties at an economical cost (Matthews, 1982). In the processing of polymeric materials it is necessary to achieve not only the required shape, but also suitable degree of homogeneity in composition and properties (Kim and Kwon, 1996). Vibratory blenders, tumble blenders, stirrer mixers, ribbon blenders, buss turbine mixers and roll mills are commonly used for dry blending polymers in powder or granule form with powdered additives such as pigments to produce master batches in powder or granule form for batch compounding (Matthews, 1982). Continuous compounding can be achieved cheaper and with better uniformity by using screw-extruders (Hess, 1978).

The function of an extruder in multi-component polymer processing is to transfer, soften, compact, compress and uniformly blend the components (Matthews, 1982). Compared with single-screw extruder, a twin-screw extruder can increase mixing by shearing in the regions between the surfaces of the screws (Cassagnau *et al.*, 2005). Mixing in extruders depends upon detailed material flow patterns, which largely occurs by laminar shearing in the molten polymer (Tadmor and Gogos, 1979). Dry blends of polymers having particle size of average diameter greater than 100  $\mu\text{m}$  may be feed satisfactorily with gravity (Todd, 1999). Blends made from smaller polymer particles or containing unabsorbed soft or liquid additives, however, will generally not feed smoothly from a gravity operated hopper. Positive forced feeding is achieved with such blends into the feed section of the screw by screw feeding devices (Matthews, 1982).

Matthews (1982) reviewed different screw designs and feeding mechanism for direct blending of liquids as well as solids additives with polymers. The compounding of LDPE with pigments can be aided by the addition of 0.2% to 0.5% (w/w) light oil or plasticizers with intensive non-fluxing mixers (Cudworth, 1976) of relatively low speed. Blending of polymers in powder form presents no special problems although the apparent melt viscosity and flow behaviour at compounding temperatures are significant in determining the degree and mode of dispersion (Han *et al.*, 1975). For processing complex blends where low viscosity additives are incorporated with a high viscosity molten polymer, the ratio of zero shear viscosity of additive to polymer must ideally be less than  $10^{-7}$ . Furthermore, the distribution and dispersion of the dispersed phase are decisive properties for blending of polymers (Cassagnau, *et al.*, 2005).

### 1.5.2 Blown Film Processing

The production of plastic film is primarily achieved by blown film extrusion (Middleman, 1977). In this process, the molten polymer enters a die, flows around a mandrel, and emerges through a ring-shaped opening in the form of a tube. Air is introduced into the tube causing it to expand and form a bubble while air-flow around the outside of the bubble cools and solidifies the melt. The air is contained in the bubble by the die at one end and by the nip rolls at the other end. An even pressure of air is maintained to ensure uniform thickness of the film bubble. The air-cooling is an integral part of a blown film line which affects the heat transfer from molten polymer film and the stability and bubble formation. Sidiropoulos and Vlachopoulos (2005) investigated the temperature distribution in machine direction and normal (thickness) direction the melt phase of a typical blown film bubble.

Both stretching the bubble radially, and pulling it away from the die biaxially orient the plastic, improving its strength and properties. After solidification, the film bubble moves into a set of pinch rolls where it is flattened and rolled onto a winder. Some extrusion lines have printing equipment and bag-making machines on-line. Simpler extrusion lines form the film or sheet and then perform the printing and bag making functions off-line. Bubble stability plays a very important role in evaluating the possibility of a polymer. A stable bubble is a requirement not only for continuous operation of the process but also for the production of an acceptable film. The key parameters affecting the film blowing process are bubble diameter and velocity. Ghaneh-Fard *et al.* (1996) studied in detail the criteria's for bubble stability during film blowing and demonstrated that the cooling of bubble is

controlled by amount of air inside the bubble, die diameter, height, melt temperatures and the velocity of the nip rollers.

Co-extrusion is a variation of the blown film process where the die is designed with multiple flow channels so that multiple layers may be formed. In food packaging, multiple layers are typically used in order to create barrier layers to protect the product from moisture, air, etc. The number of layers can range from two to as many as ten layers in more complex systems and the typical range of film thickness is 0.0001-0.050 inch. Common products formed by blown film extrusion or co-extrusion include garbage bags, can liners, agricultural films, grocery bags, and thin films for paper and tissue products (Pirkle and Braatz, 2003). Typical polymers used for blown film processing include polyethylenes, polypropylenes, ethylene vinyl acetate (EVA), and flexible polyvinyl chloride (Berins, 1991).

### **1.5.3 Additives in Polymers**

The extrusion processing of polymer products is often aided by the addition of additives such as polyethylene glycol (PEG). Polymer additives represent many classes of compounds, which possess a wide variety of chemical (i.e., phenols, amides, esters) and physical (i.e., volatility, solubility) properties (Berins, 1991). They are often incorporated into polyolefins and other polymeric materials for several reasons including: (i) to prevent of degradation by ultraviolet light, heat, and oxygen; (ii) to aid in the processing of the polymer; and (iii) to modify the physical properties of the polymer. Since the purity and amount of additive can affect polymer properties, it is very important to characterize and quantify additives in polymer products (Berins, 1991).

Traditionally, the incorporation of an AM agent into food can cause the consumption of the active compound by reaction with the food resulting in a loss of protection and an increased rate of food spoilage (Han, 2003). In active packaging AM systems, the AM agents can be incorporated directly into a polymer during processing. The loss of an AM agent during processing or manufacturing of packaging material, however, can result in the unnecessary and undesirable release into the food products. In order to control the retention within the polymer during manufacturing or any post packaging processing stages of the AM agent, polymer additives are often incorporated during processing.

#### **1.5.4 Antimicrobial Film Production**

Low-density polyethylene films are used in many food packaging applications and are primarily produced by blown film extrusion (Pirkle and Braatz, 2003). Due to the high temperatures required during the extrusion process, volatile natural AM agents can be subsequently lost which may cause a lack of AM activity (Suppakul, 2004). Since the release of active compounds is directed toward the food surface, however, relatively low quantities of active compounds are needed. Reducing the amount of active compounds in food packaging may also improve quality of flavour, since many additives can cause off-flavour (Han, 2003).

In order to develop effective AM films, a number of important factors need to be carefully considered. These include:

- The types and grades of polymers and additives for the production of the film.

- The method used to incorporate the AM agent into the polymer or master-batch.
- The optimal extrusion conditions to minimize the loss of AM agents.
- The extent to which an additive polymer could reduce the evaporation losses of volatile AM agents.
- The extent to which an additive polymer could control the release of the AM agent into the food product.
- The effect that any added polymer has on the ultimate properties of the film.

## 1.6 Aims

In view of the potential economical, environmental and general health benefits imparted by natural AM additives in food packaging films, the present study is aimed at the following:

- To prepare active LDPE films, using natural AM agents (linalool and thymol) with EVA and/or PEG as the AM binding agents, by compression moulding or blown film extrusion.
- To investigate the ability of the additive polymers EVA or PEG to retain the AM agents during film processing by determining the release of the AM agents from the films.
- To investigate the migration of AM agents into food simulants by conventional diffusion analysis.
- To investigate the migration of AM agents using an alternative kinetics analysis.

- To compare the release of AM agents from extruded film into different food simulants.
- To study the effect of AM agent on the properties of extruded films.

### **1.7 Scope of Work**

The natural AM agents linalool and thymol were selected for the studies and a standard commercial film grade LDPE was chosen as the packaging material. Additive polymers EVA and PEG were selected to improve solubilization by partially binding the AM agents in the polymer matrix. Samples were prepared using a compression moulding press and by blown film extrusion in order to study the release kinetics of the AM agent. The release rate of the AM agent into food simulants was also studied. The release of AM agents was also investigated using thermogravimetric analysis and some properties of the extruded films were measured.

## **2 Literature Review**

This chapter reviews the recent developments in active packaging and naturally derived AM additives in particular. A review of blending AM additives and AM film production is also presented.

### **2.1 Progress in Antimicrobial Packaging**

Active packaging is one of the innovative food packaging concepts and has been introduced in response to the continuous changes in consumer demands and market trends. This practice can improve food safety and sensory properties, while maintaining the quality of packaged food by changing the condition of packaged food to extend the shelf-life (Ahvenainen, 2003). Active packaging and AM packaging in particular, plays a very important role in the protection of food products (Robertson, 1993) and the cost saving potentials of active packaging systems have been demonstrated by Hotchkiss (1997).

Antimicrobial packaging systems are able to kill or inhibit spoilage and pathogenic microorganisms that can potentially contaminate food products (Hotchkiss, 1997). The inhibition of microbial activity is achieved by slow release of AM agents from the packaging system onto the food surface (Han, 2000). When a packaging system acquires AM activity, the packaging system limits or prevents microbial growth by extending the lag period and reducing the growth rate or decrease live counts of microorganisms. The goals of an AM system are safety assurance, quality maintenance and shelf-life extension (Ahvenainen, 2003). The development and application of AM films with an emphasis on active and AM packaging have been

recently reviewed (Suppakul *et al.*, 2003a; Suppakul *et al.*, 2003b). Table 2.1 summarizes recent advances in AM packaging development.

**Table 2.1** Summary of some recent AM packaging developments

AM Compounds	Trade Name(s)	Producer	Packaging Type
Silver zeolite	Aglon	Aglon Technologies	Paper, milk containers
	Novaron	Toagosei Co	Plastic
Triclosan	Microban	Microban	Deli-wrap, re-heatable containers
Allylthiocyanate	WasaOuro	Lintec Corp.	Labels, sheets
		Dry Company	Sachets
Chlorine dioxide	Microsphere	Bernard Tech Inc.	Bags, coatings, labels
Carbon dioxide	Freshpax	Multisorb Tech.	Sachets
	Verifrais	Sarl Codimer	Sachets
Ethanol vapour	Ethicap, Negamold,	Freund	Sachets
	Fretek		
	Oitech		Nippon Kayaku
Glucose oxidase	Bioka	Bioka Ltd	Sachets (Finland)

Source: Adapted from Han (2000).

The current approach to AM film development is to control the undesirable microorganisms present in the food products by incorporating AM agents directly into the polymer matrix (Labuza and Breene, 1989). Excellent processing properties of LDPE and application of transparent LDPE films in food packages and agricultural were studied by LaMantia *et al.* (1986). These studies suggest that it is highly desirable to have polymers with good rheological properties that will provide sound tubular film stability without the need to perform time-consuming and

expensive pilot runs. Fang *et al.* (2003) investigated the correlation between rheological properties and processability of polyethylene in film blowing and found that, the more elastic polymers were found to be more stable in film blowing. Also, the more stable polymer melts were found to be those possessing larger elongation properties.

Hong *et al.* (2000) incorporated naturally derived compounds such as propolis extract and clove extract into LDPE to form AM food packaging films. The compounds were directly blended through master batch processing and films were fabricated by blown film extrusion process. The direct incorporation of the additives into the LDPE film resulted in a uniform film matrix that was observed via Fourier transform infrared spectrometry. The incorporation of these natural AM agents did not significantly affect the mechanical or permeability properties of the films. Furthermore, the films incorporated with natural AM agents showed positive AM ability against *L. plantarum* and *F. oxysporum*.

An *et al.* (1998) incorporated a combination of AM agents including *Rheum palmatum* extract, *Coptis chinensis* extract, sorbic acid and silver substituted inorganic zirconium matrix in a 1% (w/w) concentration with LDPE. The incorporation of these natural and inorganic AM additives into LDPE did not adversely affect the mechanical tensile strength, heat shrinkage or wet-ability of the resulting film. The films, which were used for packing cucumber and curled lettuce, showed reduced growth of total aerobic bacteria compared with a control film without any AM additive. Furthermore, the presence of the AM agent into LDPE did not adversely affect the other quality attributes of the vegetables during storage.

Antimicrobial LDPE films can be also used in MAP applications. For example, Chung *et al.* (1998) incorporated 1% (w/w) AM agents of *Rheum palmatum* extract, *Coptis chinensis* extract and silver substituted inorganic zirconium into LDPE for the preservation of strawberries. The studies were conducted under a modified atmosphere produced by hermetic sealing to maintain the oxygen and carbon dioxide concentrations. The AM LDPE film successfully retarded the growth of total aerobic bacteria, lactic acid bacteria and yeast on fruits and resulted in significantly lower rate of decay. The hermetically sealed packages of AM LDPE films showed better retention of fruit firmness and did not impart any negative effect on the physical or chemical qualities of strawberries.

Lee *et al.* (1998) developed LDPE films of 30  $\mu\text{m}$  thickness, containing 1% (w/w) grapefruit seed extract by blown film extrusion processing at 150°C. An LDPE master batch containing 10% (w/w) of this extract was prepared in a twin-screw extruder prior to proportional mixing with LDPE pellets and fabrication into films. The resulting films showed inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. The films, when used for packing curled lettuce and soybean sprouts, also successfully inhibited the growth rate of lactic acid bacteria. Furthermore, the growth rate of aerobic bacteria and yeast were considerably reduced.

Grower *et al.* (2004) developed a nisin-containing solution for coating the surface of LDPE films in order to release nisin to inhibit the growth of *Listeria monocytogenes*. These AM coatings were effective against *L. monocytogenes* on solid micro-

biological media and on the surface of individually packed hotdogs. Coatings containing nisin (10,000, 7,500 and 2,500 IU mL<sup>-1</sup>) inhibited the growth of *L. monocytogenes* on modified oxford agar and tryptic soy agar. Films coated with solutions containing 156.3 IU mL<sup>-1</sup> of nisin, however, had no effect against *L. monocytogenes* grown on either agar.

Cahan *et al.* (2003) introduced 1% (w/w) of AM agent (Melcaptobenzothiazol, Polyacrylamid and starch potato) into EVA film. The AM agent was compounded with polyethylene wax prior to extrusion with EVA via a single-screw extruder equipped with a blown film die and film stretching unit. These films successfully reduced the target bacterial growth by *ca.* 50 to 70%.

Other than active food packaging applications, polymers can also be blended with insecticides to protect against rodent damage. “Rodrepel®” containing oleoresin derivatives extracted from green peppers are often used in the manufacturing of aromatic polymers (Joshi, 2006). They are compounded in low concentrations (*ca.* 200 ppm) into a master batch which is subsequently used in manufacturing underground pipes, cables, optical fibre and metal coatings. Low-density polyethylene cable insulations and coating can also be prepared from a master batch containing less than 5% (w/w) Thermirepel® to repel termites (Joshi, 2006).

Halek and Anita (1989) prepared an antifungal ionomeric film by compressing Surlyn® pellets (Dupont Laboratories, Delaware) with an ethylene copolymer containing 15% (w/w) methacrylic acid. The films were doped into an antifungal benomyl solution for six days to couple the fungicide to the ionomeric film and the

films successfully inhibited the growth of *Aspergillus flavus* and *Penicillium notatum*. Aitor *et al.* (2002) studied the tensile properties of polyethylene films (of 200 µm thickness) containing additives such as Irganox®1010, Irganox®1330 and Lowinox®CA22 prepared using a compression moulding press to study the effect of additives against environmental conditions.

Donghwan *et al.* (2003) evaluated the release kinetics of triclosan from a polymer coating on LDPE, as an AM layer for packaging materials, into 10% aqueous ethanol and n-heptane. Using pure water, no release of triclosan was observed. Studies on 10% ethanol showed that a 1.2% (w/w) triclosan was quickly released. Using n-heptane to simulate fatty foods 65% (w/w) of the triclosan was quickly released. Wang *et al.* (2005) investigated the antibacterial activity of LDPE films containing nanoparticles of TiO<sub>2</sub> prepared as a master batch by melt blending. Biodegradable polymers that demonstrated AM activity are currently being studied as edible coatings. Padgett *et al.* (1998) demonstrated the AM activity of lysozyme and nisin in soy protein isolate and corn zein films.

A summary of some typical applications of AM additives in LDPE packaging is presented in Table 2.2. This table illustrates a wide range of applications of AM packaging as well as a broad range of microbes that can be targeted by the AM agents.

## **2.2 Developments in Polymers and Packaging**

Many primary packages for foods and beverages are comprised of synthetic polymers and as such, polymers have become the medium for the incorporation of

**Table 2.2.** Some typical applications of AM LDPE food packaging

<b>AM Agents</b>	<b>Packaging Material</b>	<b>Testing Media</b>	<b>Test or Target Microorganisms</b>
Parabens	LDPE	Simulant	Migration test
Sorbates	LDPE	Culture media	<i>S. cerevisiae</i>
Sorbates	LDPE	Cheese	Yeast moulds
Nisin, lacticins	Polyamide/LDPE	Culture media	<i>M. flavus</i>
Nisin, lacticins, salt	Polyamide/LDPE	Culture media	<i>L. monocytogenes</i>
Imazalil	LDPE	Bell pepper	Moulds
Grape fruit seed extract	LDPE, nylon	Ground beef	Aerobes, coli-forms
Grape fruit seed extract	LDPE	Lettuce, cucumber	<i>E. coli</i> , <i>S. aureus</i>
Clove extract	LDPE	Culture media	<i>L. plantarum</i> , <i>E. coli</i> , <i>F. oxysporum</i> , <i>S. cerevisiae</i>
Herb extract, silver-Zirconium	LDPE	Strawberry	Fruit firmness
Hexamethylenetetramine	LDPE	Orange juice	Yeast, lactic acid bacteria
Silver zeolite, silver nitrate	LDPE	Culture media	<i>S. cerevisiae</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>Sal. Typhimurium</i> , <i>Vibrio parahaemolyticum</i>

active substances such as antioxidants, oxygen scavengers, flavour compounds and natural AM agents (Hotchkiss, 1997). In recent years, a great deal of research has been dedicated to polymeric food packaging materials that possess AM properties. Polymers containing AM agents from plant and herb extracts in combination with citric acid extracts were found to be very effective against a variety of different microorganisms including bacteria, viruses and fungi (Seabrook *et al.*, 1997). Polymer additives other than AM agents can include antioxidants, flavours to offset degradation on storage, insecticides to repel or kill insects, and fumigants in plastic films for packing of grains (Sherman and Manolis, 1998). The ability of such polymers to possess AM activity with food additives was studied by Halek and Anita (1989). The controlled slow release of these AM agents is essential to maintain the required concentration of the AM agent on the food surfaces to retard microbial growth (Han, 2000).

The incorporation of AM agents into polymers can affect the physical and mechanical integrity of packaging materials (Han, 2003). If an AM agent is compatible with a particular packaging material and does not interfere with the polymer structure, a substantial amount of the AM agent may be impregnated into the packaging material without any physicochemical integrity deterioration (Han, 1996). An excess amount of AM agent that is not capable of being blended with packaging material, however, can result in a detriment to the physical strength and mechanical integrity of the package (Cooksey, 2000). Polymer morphological studies are thus helpful in predicting the impact of the addition of an AM agent on the physical integrity of the packaging product.

### 2.3 Polymer Additives for AM Film Development

The production of successful AM films can be achieved by the use of additives that can serve as compatibilizers between AM agents and polymer materials. Polyethylene glycol, for example, is a biocompatible, non-toxic and non-immunogenic polymer used in biomaterials and biotechnology (Harris, 1992) that has been approved for internal consumption by the United States Food and Drug Administration. Several PEG gels have been studied for drug delivery and the controlled release of various therapeutic drugs (Kanjickal *et al.*, 2005). The terminal hydroxyl groups and ether groups are hydrophilic structures that are suitable for the controlled delivery of low molecular weight drugs. The use of PEG as a plasticiser can result in film products with improved tensile strength. Furthermore, PEG has the potential to form hydrogen bonds and to provide hydrophilic sites in otherwise hydrophobic polymer systems (Tillekeratne and Easteal, 2000).

Polyethylene glycol is well known for its low viscosity and lubrication properties (Liu *et al.*, 2005). The incorporation of small amounts of PEG can improve the melt rheology of polyolefins. Liu *et al.* (2005) incorporated small amounts (*ca.* 1-5 phr by weight) of PEG resin into mLLDPE to investigate the flow performance during extrusion. The rheological experiments showed that PEG/ mLLDPE blends exhibit lower apparent shear stress compared to that of pure mLLDPE. Furthermore, there was a synergistic improvement in the processability of mLLDPE. Liu *et al.* (2005) also studied the effect of PEG on the viscosity of mLLDPE compounded with inorganic fillers. A similar reduction in the viscosity of mLLDPE was observed with the incorporation of 1-5 phr and PEG also assisted in delaying the development of sharkskin fracture during the extrusion. More recently, Xie *et al.* (2006)

investigated the influence of PEG containing additives on the extrusion behaviour of ultrahigh molecular weight polyethylene and polypropylene blends. Blending 1% (w/w) PEG in polyethylene blends resulted in significant reduction of die pressure and melt viscosity and an increase in flow rate at a given die pressure.

Suyatma *et al.* (2005) studied the effect of PEG as a hydrophilic plasticizer on the mechanical, thermal and surface properties of chitosan used as potential AM films. The plasticisation efficiency of PEG in chitosan films was improved and films containing PEG showed better stability during storage, better elastic properties, high strain and lower tensile stress values than films that had no PEG.

The microstructure or the morphology of a polymer film can greatly influence the mobility of active compounds in the film. For an immiscible phase containing PEG and LDPE, the approach of smart blending (LaCoste *et al.*, 2005) may be applied to alter the blend morphology in order to provide the controlled release of linalool and thymol. The technique of smart blending may also provide a wide range of film permeability to suit different applications.

Other possible additive polymers that may contribute to blend compatibility between some polymers and AM agents are EVA and ethylene vinyl alcohol (EVOH). Moly *et al.* (2005) performed crystallisation studies on the blending of EVA with LLDPE and showed that blending EVA does not affect crystalline structure, but the crystallinity decreases with EVA content. Lee and Kim (1996) studied the morphology and oxygen barrier properties of LDPE/EVOH blends whereby the blends were prepared using a single screw extruder. The extruded blends were

compression moulded into thin films to study the morphology of the dispersed phase in immiscible polymer blends and demonstrated that the processing conditions influence the shape and dimension of the polymer structure. Polymer-based nanocomposites prepared by melt compounding have also been reported to exhibit markedly improved properties over neat polymers and micro-sized-particle-filled polymer composites (Walter *et al.*, 1999).

#### **2.4 Types and Uses of Antimicrobial Agents**

There are many different types of AM agents, natural or synthetic, that can be used in a variety of applications in the food, pharmaceutical and cosmetic industries. Several categories of AM agents have been tested for suitability in AM packaging applications including organic acids, fungicides, bacteriocins, proteins, enzymes, inorganic gases, and metal substituted zeolite (Ming *et al.*, 1997; Scannell *et al.*, 2000). Antimicrobial agents used in food packaging may be organic or inorganic materials or their salts (Cahan *et al.*, 2003).

Various chemicals like antioxidants, AM polymers, natural AM agents and gases, which have the potential to inhibit microorganisms, can be incorporated in packaging systems (Suppakul *et al.*, 2003b). Among the synthetic AM agents used are organic acids, fungicides, alcohols and antibiotics. Organic acids such as benzoic acids, parabenes, sorbic acid, propionic acid, acetic acid, lactic acid and their mixture possess a strong AM activity (Han, 2005). Table 2.3 lists some typical natural and synthetic AM agents that are used in food packaging.

**Table 2.3** Examples of typical AM agents used in food packaging

<b>Class of AM agents</b>	<b>Examples</b>
Organic acids	Propionic, benzoic, sorbic, acetic, lactic, malic, succinic, tartaric
Mineral acids	Phosphoric acid
Inorganics	Sulphites, sulfur dioxide
Parabens	Methyl, propylparaben
Antibiotics	Natamycin
Enzymes	Lactoperoxidase, lysozyme, lactoferrin
Metals	Silver, copper
Chelating agents	Ethylene diamine tetra acetate, purphosphate, citrates
Bacteriocins	Nisin, pediocins
Fungicides	Benomyl, imazalil
Essential oils	Eugenol, thymol, salicylaldehyde, cinnamic acid
Proteins	Conalbumin, cathepsin
Phenolic antioxidants	Butylatedhydroxyanisole, Butylatedhydroxytoluene 2-terbutylhydroquinone
Isothiocyanates	Allyl isothiocyanate, hypothiocyanate
Fatty acids and esters	Monolaurin
Others	Reuterin (3-hydroxypropionaldehyde), hydrogen peroxide, ozone, sulfur dioxide

Source: Adapted from Hotchkiss (1997).

Food-grade antioxidants can be incorporated into packaging materials creating an anaerobic atmosphere inside the package and eventually protect the food against aerobic spoilage. Various bacteriocins that are produced by beneficial microorganisms can also inhibit the growth of spoilage and pathogenic micro-

organisms (Ahvenainen, 2003). Fermentation products or by-products such as *nisin*, *lacticins*, *pediocin*; *diolococcin* and *propionic* can also impart AM activity (Daeschel, 1989). Some natural or synthetic polymers such as chitosan can possess AM activity (Hong *et al.*, 2000) while ultra-violet radiation can excite the structure of polymers such as nylon and stimulate AM activity (Paik *et al.*, 1998).

Gaseous AM agents can offer protection in the headspace of food packaging. Chlorine dioxide, allyl isothiocyanates, hinokithiol and ozone are examples of gaseous AM agents that have been successfully incorporated into packages (Gontard, 1997). Chemicals that produce chlorine dioxide when in contact with moisture can be incorporated into film during extrusion processing (Podhajny, 2004). MicroActive Corporation (Bernard Technologies) recently developed chlorine dioxide liberating films under the trade name Microsphere® (Podhajny, 2004).

## **2.5 Natural Antimicrobial Agents**

With the increase in consumer awareness for food safety and health standards, there is a general concern for use of chemical preservatives in food chain (Azaz *et al.*, 2005). In response to this, bio-preservatives and naturally derived AM additives are becoming more important as they represent a perceived lower risk to consumers (Nicholson, 1998). More extensive attempts are being made in the search for alternative AM compounds based on plant extracts (Hotchkiss, 1997). For example, the AM effect of essential oils and their active constituents against many food borne pathogenic bacteria including *Salmonella enterica*, *Campylobacter jejuni* (Friedman

*et al.*, 2002), *Staphylococcus aureus* and *Vibrio parahaemolyticus* (Juven *et al.*, 1994) have been studied.

The use of natural extracts is often preferred due to less complex regulation processes and consumer preference when compared to chemical AM agents (Baratta *et al.*, 1998). Plant extracts in particular such as grapefruit seed, cinnamon, horseradish and cloves have been added to packaging system to demonstrate effective AM activity against spoilage and pathogenic bacteria (Ha *et al.*, 2001; Lee *et al.* 1998; Hong *et al.*, 2000). The essential oils of various biologically active plant species have become popular in recent years (Ayse Dilek *et al.*, 2005). Some essential oils are known to possess AM activity in liquid as well as vapour media (Ayse Dilek *et al.*, 2005). Friedman *et al.* (2002) analysed a broad variety of naturally occurring and potentially food compatible plant-derived oils and oil compounds for AM activity. The extract showed promising AM activity against several species of bacterial food-borne pathogens including *C. jejuni*, *E. coli*, *L. monocytogenes* and *S. enterica*.

The use of natural AM compounds is not only important in the control of human and plant diseases of microbial origin but also in preservation and packaging food products (Baratta *et al.*, 1998). Fyfe *et al.* (1998) studied the inhibition of *L. monocytogenes* and *S. enteritis* by combinations of plant essential oils with either benzoic acid or methyl-paraben. This work highlighted the fact that essential oil of basil was a potent inhibitor of both the species. Koga *et al.* (1999) studied the bacteriocidal activity of basil and sage essential oil against a range of bacteria and their findings showed that gram-positive bacteria showed higher resistance to basil essential oil than gram-negative bacteria. In addition to AM activity, basil oil is

often used as a flavourant in tomato based products that have high acidity and that are prone to spoilage by acid-tolerant food microflora (Dziezak, 1989; Frierheller, 1991).

Deans and Ritchie (1987) screened the AM spectrum of 50 plants essential oils against twenty-five genera of bacteria and all bacteria showed a reasonably broad sensitivity to the oils tested. The AM and antifungal properties of essential oils of different species of *Ocimum* have been predominantly associated with the main constituent *linalool* (Sinha and Gulathi, 1990) and there have been synergistic effects attributed to these two components against *Rhizopus nigrans* (Reuveni *et al.*, 1984). Couladis *et al.* (2004) demonstrated the antifungal activity of thymol and also proved that it was a potent inhibitor of moulds thus confirming its potential for using in food preservation. Prasad *et al.* (1986) studied the AM activity of essential oils of *O. basilicum* which were rich in linalool against 11 gram positive and 7 gram negative bacteria. They discovered that these oils were more effective against the gram positive than the gram-negative bacteria. All gram positive bacteria including *Bacillus sacharolyticus*, *Bacillus stearothermophilus*, *B. subtilis*, *Bacillus thurengiensis*, *Micrococcus glutamicus* and *Sarcina lutea* were inhibited by each of these basil essential oils. Only the gram-negative strain *Salmonella weltevreden*, however, was suppressed by the oils. Lahariya and Rao (1979) studied the AM effectiveness of the essential oil of *O. basilicum* tested in vitro against 10 different microorganisms. They discovered that the essential oil was more active in inhibiting the growth of *Bacillus pumilus* and had less activity against the fungi.

Sweet basil (*Ocimum basilicum L.*) is a popular culinary herb that has been widely used as a food ingredient (Dziezak, 1989). Sweet basil has also been used for many

years as a food flavourant and as an ingredient in dental and oral health care products (Guenther, 1952). Additionally, basil essential oils have been reported to possess AM activity against a spectrum of gram-positive and -negative bacteria as well as important food borne pathogens (Fyfe *et al.*, 1998), moulds (Arora and Pandey, 1977) and yeasts (Conner and Beuchat, 1984). Coating of LDPE films or blending LDPE with basil extracts prior to extrusion are some of the techniques used for obtaining AM films (Han, 2000).

Suppakul *et al.* (2003b) recently published articles focussing on potentials of basil extracts in the field of AM food packaging. The investigation, evaluation, efficacy and feasibility effect of basil AM agents when incorporated into LDPE films against a wide section of microorganisms including *Staphylococcus aureus*, *Listeria innocua*, *Escherichia coli* and *Saccharomyces cerevisiae* were discussed. The resulting LDPE films proved to be promising as an active AM packaging material.

### **2.5.1 Properties and Uses of Thymol**

The natural product of the essential oil of *Thymus vulgaris*, thymol, is a phenolic monoterpene that has received considerable attention as a possible AM agent (Tepe *et al.*, 2004; Olasupo *et al.*, 2004) and as a possible food antioxidant (Youdim and Deanes, 2000; Shen *et al.*, 2005). Sefidkon *et al.* (2005) extracted 19.6% thymol by hydrodistillation from aerial parts of *Thymus eriocalyx jalas* growing in various locations in central Iran with the major component observed to be linalool (1.8% - 60.4%). Kalvandi *et al.* (2005) extracted 42.8% to 43.1% thymol from essential oils obtained from *Thymus eriocalyx* (Ronniger) species. Couladis *et al.* (2004) obtained 59% thymol from essential oils extracted from *Thymus striatus* collected from the

Orjen Mountains. Thymol was observed to be the major constituent in *T. kotschyanus* (19.6%), *T. carnosus* (36.6%), *T. pubescisus* (27.1%) and *T. serpullum* (18.7%) (Sefidkon *et al.*, 2005). The hydrodistillation of essential oils from the *Saturja* species in Turkey contained 17.5% - 43.5% thymol (Ayse Dilek *et al.*, 2005). These examples illustrate the diversity of locations and variation in extractable quantities of naturally occurring thymol.

As an AM agent, thymol possesses very high antifungal activity with very low MIC values (Thompson, 1989). Couladis *et al.* (2004) reported thymol to possess a significant antifungal activity, a low MIC and potent mould inhibitory properties. Ayse Dilek *et al.* (2005) reported that essential oils containing thymol possess strong antibacterial and antifungal action. Radulovic *et al.* (2006) showed that the essential oil of *Equisetum arvense L.* possesses a broad spectrum of strong AM activity attributed to the presence of thymol (12.9%) and linalool (2.77%).

### **2.5.2 Properties and Uses of Linalool**

Basil is a popular culinary herb and its essential oils have been used in wide applications in perfumery and oral products (Guenther, 1952). Basil oil has been shown to contain biologically active constituents that are fungistatic and have AM properties (Simon *et al.*, 1990). Several types of essential oils are extracted from basil oils and classified according to their geographic origin (Marotti *et al.*, 1996). The oils containing linalool are extracted traditionally by steam distillation from the leaves, stems and flowers of the plant. An alternative to the conventional steam distillation method is carbon dioxide extraction under supercritical extraction. Roberto and James (2006) detected 21 different volatile constituents of *O. basilicum*

and linalool was observed to be the major constituent. Lorenzo *et al.* (2003) extracted linalool by the hydrodistillation of essential oils obtained from the leaves of *H. angustifolium* and *H. scabrum*. Linalool was found to be the most abundant component (23.8%) of bark oil obtained by the hydrodistillation of wood oils from *Cinnamomum sintoc Blume* found in the forest of peninsular Malaysia by Jantan *et al.* (2005). Raina *et al.* (2001) performed gas chromatographic analysis of the hydrodistillation extract of fresh leaves of *Cinnamomum zeylanicum Blume* grown in Little Andaman and observed that linalool was a major constituent of the 47 constituents identified representing the 99.96% of the oil. Certain *Lippia alba* grown in Indian plains and their cultivated clones have been reported to yield 65% linalool from the extracted essential oils (Bahl *et al.*, 2000). Studies conducted by hydrodistillation on *Zanthoxylum alatum* seeds (Neetu *et al.*, 2001) reported high levels of linalool (70.6%) from seeds obtained in northern India. Singh *et al.* (2005) demonstrated by gas chromatographic analysis that coriander seed essential oils contain more than 52 components with the major component being linalool (75.3%). Furthermore, this study explored the potent antifungal activity of linalool suggesting it can be used as an alternative source of natural antioxidants.

The active volatile components of essential oils (e.g. linalool) are responsible for the AM activity of these essential oils (Bezic *et al.*, 2003). It has been shown that phenolic components of essential oils have the strongest AM activity followed by camphor compounds (Mario *et al.*, 1998). Linalool has been previously reported to have effective antibacterial (Onawunmi *et al.*, 1984) and antifungal (Reuveni *et al.*, 1984) properties that would be ideal for its use in AM film development.

## 2.6 The Properties of Antimicrobial Films

Antimicrobial agents that are blended with polymeric materials are most likely to be dispersed in the amorphous region of the polymeric structure. If an excessive amount of AM agent is mixed into the polymer, the amorphous region can be saturated and the additive can interfere with the polymer-polymer interactions in the crystalline regions (Han, 2003). The selection of polymer, polymer additive and AM agents is therefore important in developing an AM packaging system. Antimicrobial agents and additives that are blended with a polymer packaging material may affect the processability, physicochemical properties and optical properties of the resulting polymer product.

Transparent film materials are highly desired for food packaging applications for product visibility (Park *et al.*, 1998; Wang *et al.*, 2005). Han and Floras (1997) suggested an optimum transparency for LDPE films is *ca.* 15-20%. These workers also reported no significant difference in the tensile properties before and after the incorporation of potassium sorbate in LDPE films although the transparency of the films deteriorated as the sorbate concentration was increased. Weng and Hotchkiss (1993) reported no noticeable difference in clarity and strength of an LDPE film containing 0.5% and 1% (w/w) benzoic anhydride. Similar results were reported for naturally derived plant extracts such as 5% propolis, 5% clove (Hong *et al.*, 2000), 1% *R. palmatum* (An *et al.*, 1998) and 1% *C. chinensis* (Chung *et al.*, 1998). Although no physical integrity damage is observed after a low level of AM agent addition, optical properties can be changed with a loss in transparency or colour change of some packaging materials (Han and Floras, 1997). Studies conducted by Baldev *et al.* (2000) showed that the optical properties of polymer film were

adversely affected after incorporation of low molecular weight fillers. The percentage transmission of these films was reduced from 71% to 36% and the haze increased from 23% to 55%, with the increase in additive content from 0% to 50% (w/w).

Studies conducted by Park *et al.* (1998) on coated AM films containing polysaccharide and additives showed that the haze index was higher than that of the normal uncoated film. The haze was produced due to irregularities and the heterogeneity of the polymer. The transparency of the coated films appeared to decrease with the increasing molecular size of the plasticiser used for coating. Wang *et al.* (2005) investigated the transparency of antibacterial LDPE films containing titanium dioxide and observed that the transparency of the films decreases with the increase in dosage of the incompatible additive. Inclusion of another incompatible additive, silica gel, in LDPE blown films produced by Chuayjuljit *et al.* (2003) showed that the haze and gloss characteristics of the film were altered with the increase in the particle size and the amount of additive. Vartiainen *et al.* (2003) concluded that the transparency of AM films containing EDTA decreases rapidly with the increase in EDTA content. Recent studies conducted by Suppakul (2004) on LDPE-EVA blown film containing natural AM agents showed that there is a statistically significant decrease in transparency of AM films. The transparency of films containing linalool is less when compared to that of the LDPE-EVA control films without linalool.

Mechanical and sealing properties can also be adversely affected by the addition of AM agents. Dobias *et al.* (2000) observed that samples containing AM agents such

as benzoic anhydride, ethyl paraben or propyl paraben had poor tensile and sealing properties. Cooksey (2000) reported that LDPE films coated with nisin are difficult to heat seal.

## **2.7 The Future of AM Films for Packaging Applications**

Since most AM agents have different AM activities, a combination of different agents may improve the overall AM efficacy and the safety of packaged foods. Balancing AM products and their effectiveness depends on the AM additives, the substrate and the food products themselves. Another possibility for AM films may be incorporation of radiation emitting material into the films. Materials that emit long wavelength IR radiation have been developed in an attempt to control microorganisms without the risk associated with high-energy radiation (Rooney, 1995a). However, little evidence for the efficacy of this technology has been published in the scientific literature to date.

Although the current literature widely reports the properties and AM activity of a variety of agents in polymer films, the kinetics of migration of these agents are yet to be fully investigated. Studies of the loss of AM agents from extruded films, the effect of polymer additives on the retention of AM agents, and studies of the morphology of polymers blended with AM agents would also be beneficial to the current literature. Further studies of the controlled release of AM agents from polymer films would be of particular interest for the development of successful active packaging systems.

## 3 Experimental Work

### 3.1 Materials

#### 3.1.1 Polymers

The polymers that were used to prepare the films for the present study were low-density polyethylene (LDPE, XJF143/1700 Qenos, Australia), ethylene vinyl acetate copolymer (EVA, ELVAX® 3120, Dupont, Australia) and polyethylene glycol (PEG, A1683 Peg 4000 Ajax Finechem, Australia). The characteristic properties of the polymers are presented in Table 3.1 and additional details of the polymers are presented in Appendix A.

**Table 3.1** Characteristic properties of the polymers used to prepare the AM films

Polymer	MFI / dg min <sup>-1</sup>	T <sub>m</sub> / °C	Density / g cm <sup>-3</sup>	MW / Daltons <sup>1</sup>
LDPE	5.5	110	0.92	-
EVA	1.2	99	0.93	-
PEG1	-	55	1.2	4000
PEG2	-	60	1.2	200000
PEG3	-	60	1.2	500000

Note: 1. Molecular weights presented are average values.

#### 3.1.2 Antimicrobial Additives

The AM additives used in the experiments were: (i) linalool with a purity of 97% (L260-2, Aldrich Chemical Company, USA) and (ii) thymol with a purity of 98%

(AUSTL 21320, Aurora Pty Ltd, Australia). Additional details of the AM additives are presented in Appendix A.

### **3.1.3 Solvents**

The chemicals used in the experiments were isooctane (Unichrom 2516-2.5L GL, purchased from APS Chemicals, Australia) and ethanol (95 SG, CSR Distilleries, Australia).

## **3.2 Blend Preparation and Film Production**

### **3.2.1 Incorporation of AM Agent into the Polymer**

Film grade LDPE resin pellets and EVA resin pellets were ground to a powder in an industrial grinder (CTS Plastics Machinery Pty Ltd, Australia). Flakes of the PEG were crushed to powder using a glass rod. The AM agents were blended directly and mixed to ensure uniformity at room temperature.

### **3.2.2 Compression Moulded Film Production**

Films were prepared by compression moulding of the LDPE formulation to a thickness of *ca.* 2 mm using a compression moulding press (Laboratory press 15T, L0003, IDM Instrument Pty Ltd, Australia). A hard-chromed steel frame of 2 mm thickness was placed between the two platens of the press with the temperatures of the upper and lower platens set to 120°C. The polymer formulation was placed at the centre of the frame and was sandwiched between the two platens. As the polymer formulation melted, a compression force was gradually applied up to

130 kPa. The platens were then allowed to cool to 20°C by water circulation through a coil in the platens. The pressure was released and the films that were produced were folded and again heated in the press up to 3 times to facilitate uniform mixing. After the pressing operation, the films were immediately wrapped in aluminium foil to prevent loss of the AM agent. A hand held micrometer (Mitutoyo, Japan) was used for measuring the thickness.

### 3.2.3 Film Production to Study the Retention Ability of PEG

Polymer blends consisting of LDPE, EVA, AM agent and varying concentrations of PEG were compressed to films in accordance with the method described in Section 3.2.2. Details of the film formulations that were prepared are given in Table 3.2.

**Table 3.2** Film formulations used to study the retention ability of PEG

<b>Formulation</b>	<b>P0</b>	<b>P1</b>	<b>P2</b>	<b>P3</b>	<b>P4</b>
LDPE	88	87	86	86	86
Linalool	2	2	2	2	2
EVA	10	10	10	10	10
PEG1	-	1	2	-	-
PEG2	-	-	-	2	-
PEG3	-	-	-	-	2

Note: Values shown are % (w/w).

### 3.2.4 Film Production to Study the Retention Ability of EVA

Polymer blends consisting of LDPE, AM agent and varying concentrations of EVA were compression moulded into films in accordance with the method described in

Section 3.2.2. Details of the film formulations that were prepared are given in Table 3.3.

**Table 3.3** Film formulations used to study the retention ability of EVA

<b>Formulation</b>	<b>E0</b>	<b>E1</b>	<b>E2</b>
LDPE	98	88	48
Linalool	2	2	2
EVA	0	10	50

Note: Values shown are % (w/w).

### 3.2.5 Film Production to Study the Effect of AM Agent

Polymer blends consisting of LDPE, AM agent, EVA or PEG were compression moulded into films in accordance with the method given in Section 3.2.2. Details of the film formulations that were prepared are given in Table 3.4.

**Table 3.4** Film formulations used in AM agent release experiments

<b>Formulation</b>	<b>L0</b>	<b>L1</b>	<b>T0</b>	<b>T1</b>
LDPE	88	87	88	87
Linalool	2	2	-	-
Thymol	-	-	2	2
EVA	10	10	10	10
PEG1	-	1	-	1

Note: Values shown are % (w/w).

### 3.2.6 Production of Film by Extrusion

Films of *ca.* 50  $\mu\text{m}$  thickness were prepared from a pre-blended master batch of LDPE containing EVA and different concentrations of AM agents as shown in Table 3.5. A standard single-screw extruder was used with a diameter of 50 mm (Telford Smith, Australia) using an operating speed of 40 rpm. The temperature profile was maintained at 150°C from the first barrel zone to the die (high density 190 mm centre feed die with a die gap of 1.6 mm). The extruded film was immediately wrapped in aluminium foil to prevent loss of the AM agent by evaporation. The thickness of the film was measured using a micrometer (Mitutoyo, Japan) with an average of five readings taken at different points on the film sample.

**Table 3.5** Polymer formulations for blown film extrusion

<b>Formulation</b>	<b>F1</b>	<b>F2</b>	<b>F3</b>
LDPE	90	88	88
Linalool	-	2	-
Thymol	-	-	2
EVA	10	10	10

Note: Values shown are % (w/w).

## 3.3 Release Experiments

### 3.3.1 Quantification of AM Agents by Gas Chromatography

The concentration of AM agent in the prepared samples was determined by gas chromatography (GC). A sample of film was extracted using isooctane and an aliquot of the extract of a precisely known volume was sampled for GC analysis

using a Varian Star 3400-CX GC equipped with fused silica capillary column DB-5 (30 × 0.25 mm inner diameter, film thickness 0.25 μm, J. & W. Scientific, USA). The GC was operated using the following conditions: injection volume: 1.0 μl; initial column temperature: 80°C; heating rate: 5°C min<sup>-1</sup>; injector temperature: 250°C; split ratio 1:100; FID detector temperature: 300°C; and carrier gas: nitrogen. The concentration of AM agent was calculated from standard curves.

### **3.3.2 Antimicrobial Agent Release using Incubators**

Compression moulded film samples of approximately 5 × 5 cm were immersed in a sealed vessel of 100 mL of isooctane and were placed in an incubator shaker (Innova™ 4230, New Brunswick Scientific, U.S.A.) maintained at 25°C. The amount of AM agent released was monitored until equilibrium was attained. An aliquot of the solution was analysed by GC at different time intervals as described in Section 3.3.1. The release of AM agents from the extruded films into the food simulants was investigated by immersing *ca.* 0.5 g (4 pieces, 5 × 5 cm) of weighed film sample into 100 mL of isooctane, ethanol (95% and 15%) or distilled water in a sealed vessel as described in Section 3.3.1.

### **3.3.3 Antimicrobial Agent Quantification using Soxhlet Extraction**

In order to determine the amount of AM agent retained in the film after extrusion, the film samples were cut into small pieces and *ca.* 5 g of each film sample was extracted for 18 h by Soxhlet extraction using 150 mL of isooctane. An aliquot of the extract of a precisely known volume was sampled for GC analysis as described in Section 3.3.1.

### 3.4 Data Analysis

In order to determine the release of the AM agent from the polymer film formulations, two data analysis treatments were applied to the release data.

#### 3.4.1 Migration as a Diffusion Process

The release of the AM agent from the film into a food simulant is a diffusion process. Equations relating the mass fraction of molecules migrating from a polymer film with time have been derived by Miltz (1987).

For short-term migration ( $m_t/m_\infty$ ) < 0.6:

$$\frac{m_t}{m_\infty} = 4 \left( \frac{Dt}{\pi l^2} \right)^{\frac{1}{2}} \quad (1)$$

where  $m_t$  is the amount of AM agent released from the film,  $m_\infty$  is the equilibrium amount of AM agent released from the film,  $D$  is the diffusion coefficient and  $l$  is the thickness of the film. A plot of ( $m_t/m_\infty$ ) versus  $t^{1/2}$  should yield a straight line from which the diffusion coefficient can be obtained.

For long-term migration ( $m_t/m_\infty$ ) > 0.6:

$$\frac{m_t}{m_\infty} = 1 - \frac{8}{\pi^2} \exp\left( \frac{-\pi^2 Dt}{l^2} \right) \quad (2)$$

Rearranging equation (2) becomes:

$$\ln\left( 1 - \frac{m_t}{m_\infty} \right) = \ln\left( \frac{8}{\pi^2} \right) - \frac{\pi^2 Dt}{l^2} \quad (3)$$

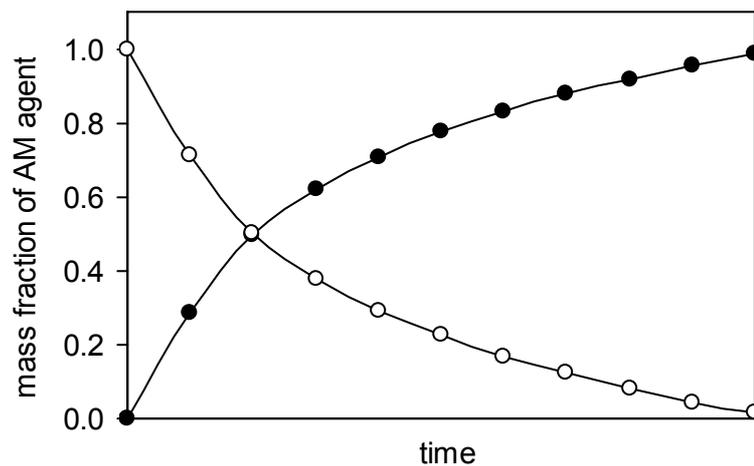
$$\ln\left(1 - \frac{m_t}{m_\infty}\right) = \ln\left(\frac{8}{\pi^2}\right) - kt \quad (4)$$

where  $k$  is the rate constant. From equation (4), a plot of  $\ln(1 - m_t/m_\infty)$  versus time should yield a straight line with slope,  $-k$ .

For the release experiments, the diffusion coefficients were calculated using equation (1) for short-term migration. The rate constants were calculated using equation (4) for long-term migration.

### 3.4.2 Migration as a Chemical Process

In addition to the diffusion analysis, the release of AM agent into the food simulant was further analysed for the fit to first-order kinetics. This analysis technique can provide an initial release rate as well as a rate constant. Figure 3.1 shows a plot of a typical first-order kinetics system.



**Figure 3.1.** Plot of mass of AM versus time for a first order kinetic system where: (○) mass of AM in packaging material and (●) mass of AM released.

A similar experimental plot of mass fraction of AM agent released versus time would confirm that first order kinetics adequately models the release process.

For a first-order system, the rate of loss of the active agent from the packaging material is given by:

$$\frac{dm_t}{dt} = -km_p \quad (5)$$

where  $m_t$  is the amount of AM agent released into the food simulant,  $m_p$  is the amount of AM agent in the packaging material, and  $k$  is the rate constant. At any point in time, the equilibrium concentration of the active agent,  $m_\infty$ , is given by:

$$m_\infty = m_t + m_p \quad (6)$$

Rearranging and substituting equation (6) in equation (5), the equation becomes:

$$\frac{dm_t}{dt} = -k(m_\infty - m_t) \quad (7)$$

Integrating equation (7) from time  $t = 0$  to  $t$ , and concentration  $m_p = m_\infty$  to  $m_p = m_\infty - m_t$ , the equation becomes:

$$\ln\left(1 - \frac{m_t}{m_\infty}\right) = -kt \quad (8)$$

From equation (8), a plot of  $\ln(1 - m_t/m_\infty)$  versus time should yield a straight line with slope,  $-k$ . Equation (9) is obtained by re-arranging equation (8):

$$m_t = m_\infty(1 - e^{-kt}) \quad (9)$$

The rate of release of the AM agent,  $v_t$ , at time  $t$  is obtained by taking the first time derivative of equation (9) thus:

$$\frac{dm_t}{dt} = m_{\infty}ke^{-kt} \quad (10)$$

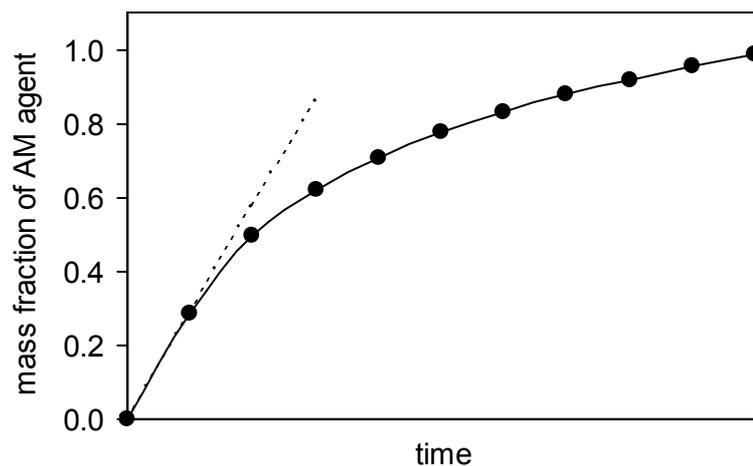
Whence:

$$v_t = m_{\infty}ke^{-kt} \quad (11)$$

At time,  $t = 0$ , the initial release rate,  $v_0$ , is given by:

$$v_0 = m_{\infty}k \quad (12)$$

Figure 3.2 shows a typical plot of mass fraction of AM released versus time demonstrating the initial rate of release. For the release experiments, the rate constants were calculated using equation (8) and the initial release rates of AM agent were calculated using equation (12).



**Figure 3.2.** Plot of mass fraction of AM released versus time demonstrating the initial rate of release of the AM agent (---).

### **3.5 Thermogravimetric Analysis**

A Perkin-Elmer Thermogravimetric Analyser (TGA 7) was used to obtain the mass loss of AM agents from the extruded films in nitrogen at elevated temperatures. The mass loss for films containing linalool and thymol were measured in the TGA apparatus by heating the samples from 35°C to *ca.* 200°C at a heating rate of 20°C min<sup>-1</sup>, using a nitrogen flow rate of 20 mL min<sup>-1</sup>.

### **3.6 Mechanical and Optical Properties of AM Films**

The effect of AM agents on the mechanical properties of the extruded films was investigated by measuring the tensile strength. The peak load of the film section (1 × 10) inch was determined using an Instron 4465 (USA) tensile tester in accordance with ASTM Method D 882-97. The percent haze of some of the films was measured using a Gardener hazemeter in accordance with ASTM Method D 1003-97. A total of five and four replicates were tested for the tensile and haze measurements respectively.

## 4 Results and Discussion

This chapter examines the processing of blends of LDPE with AM agents and additive polymers by a compression moulding technique and by extrusion film blowing. The release of the AM agents from the resulting films into food simulants is analysed. In addition, the use of TGA analysis was explored.

### 4.1 Effect of Compounding LDPE with PEG

Prior to preparation of the blends by blown film extrusion, the effect of compounding the additive polymer (PEG1) with film-grade LDPE was observed using compression moulding. Due to the differences in melting temperatures of the additive polymer (PEG, *ca.* 55°C) and LDPE (*ca.* 110°C), the PEG melted first and separated out to the periphery of the solid LDPE. As a compression force was applied, the LDPE gradually melted with the resultant film comprising two separate phases, with particles of PEG clearly visible on the boundary of the film.

With a decrease in PEG concentration, the phase separation was observed to decrease, with a subsequent increase in homogeneity and clarity. At a low concentration of PEG (*ca.* 1% (w/w)), a semi-transparent homogenous film was obtained. The immiscibility of PEG and LDPE may be due to the high difference in melt viscosity between PEG and LDPE (see Appendix A). A technique of melt-mixing the constituents and using finely ground PEG may improve miscibility and subsequent film clarity.

## **4.2 Blown Film Extrusion of LDPE/EVA/PEG Blends**

### **4.2.1 Effect of EVA and PEG on LDPE Extruded Films**

Blown film extrusion processing of LDPE/EVA blends containing as low as 1% (w/w) PEG caused choking in the feed section of the extrusion unit and the throat of the gravity feed hopper. The relatively low melting point of PEG and the subsequent softening of this component in the feeding zone of the extruder may account for the observed choking. The softening of the PEG caused agglomeration and adhesion of LDPE pellets to the single-screw and obstructed the progression of the polymer into the melting zone of the extruder and consequently no stable bubble was obtained during the process. Furthermore, bubble instability and holes were evident during extrusion that may be due to the incompatibility of the PEG/LDPE/EVA blend. The hydrophilic nature of PEG in contrast to the hydrophobic nature of LDPE may contribute to the blend incompatibility (Tillekeratne and Easteal, 2000). The inefficiency of mixing in the single-screw extruder and the particle size of PEG may also have interfered with the crystalline structure of the LDPE/EVA blend.

When a batch containing EVA and LDPE without PEG was extruded under identical conditions, a stable bubble was observed during extrusion and a film of uniform thickness was obtained. The bubble diameter was observed to be constant and uniform throughout the extrusion process while a constant screw speed was maintained. A stable film was produced with the incorporation of 2% (w/w) linalool or thymol and there was no significant difference observed in the bubble diameter and film thickness with the incorporation of either AM agent at this level.

Furthermore, no discoloration of the film was observed during extrusion, which may suggest that there was no thermal decomposition of the AM agents under the current extrusion conditions.

#### 4.2.2 Loss of AM Agent During Blown Film Extrusion

The amount of AM agents retained in the films produced by film blowing at an extrusion temperature of 150°C are summarised in Table 4.1. The loss of volatile AM agents due to evaporation under high extrusion temperatures may be due to the high volatility of natural AM agents (see Appendix A). Suppakul (2004) reported significant losses of AM agents by solvent blending with isooctane. The loss of AM agents, however, can be greatly reduced by direct blending with LDPE/EVA pellets prior to extrusion. Although the Soxhlet extraction process used to measure the loss of AM agent is relatively efficient, losses of solvent and volatile AM agents are inevitable. Efficiencies of less than 90% are typical for such extractions and more effective quantification of AM agent retentions could be explored.

**Table 4.1** Quantification of AM agent lost during blown film extrusion

<b>Formulation</b>	<b>AM Agent</b>	<b>% AM Agent Lost</b>
F2	2% Linalool	39
F3	2% Thymol	28

#### 4.2.3 Mechanical and Optical Properties of Extruded AM Films

The strength and ductility of plastic materials is often determined by measuring the tensile properties (Han and Floras, 1997). The effect of AM agents on tensile

properties of extruded film was studied by measuring the peak load of films containing AM agents and a control film produced under same extrusion conditions. Table 4.2 shows that the addition of natural AM agents into the LDPE film did not significantly influence the mechanical properties of the film. This may be due to the possibility that these natural AM agents are present in the amorphous regions of the polymer structure (Han, 2003). Considering the relatively large ratio of the amorphous to crystalline regions, the presence of a relatively small amount of AM does not affect the mechanical properties of the film to any observable extent. Haze is produced by irregularities on the surface of a film (Park *et al.*, 1998) and the results in Table 4.2 show that the film containing linalool shows a lower percentage haze than that of the control film and the film containing thymol. This may suggest that that the film containing linalool has a more regular, homogenous surface under the current extrusion conditions.

**Table 4.2** Tensile and optical properties of extrusion blown LDPE/EVA/AM films

Formulation	AM Agent	Peak Load / kN $\pm$ 0.001		% Haze
		MD <sup>1</sup>	TD <sup>1</sup>	
F1	-	7.5	6.6	16.8
F2	2% Linalool	7.4	6.2	8.2
F3	2% Thymol	7.2	6.1	15.1

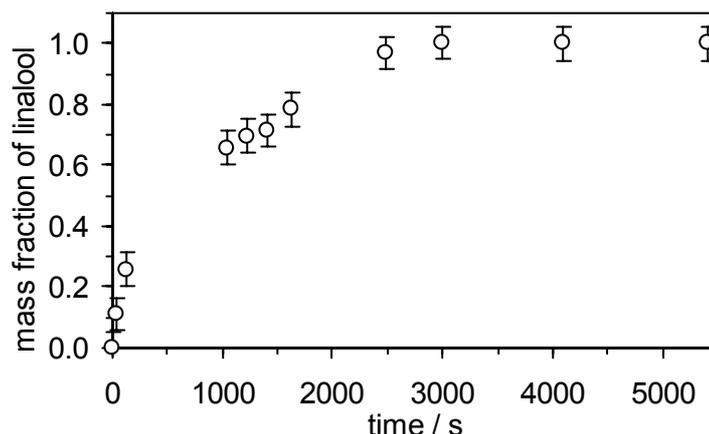
Note: 1. MD is the machine direction of the film, TD is the transverse direction of the film

### 4.3 Release of AM Agent from Films

The ability of the films produced by compression moulding and by blown film extrusion to retain the AM agents after processing was explored by various techniques.

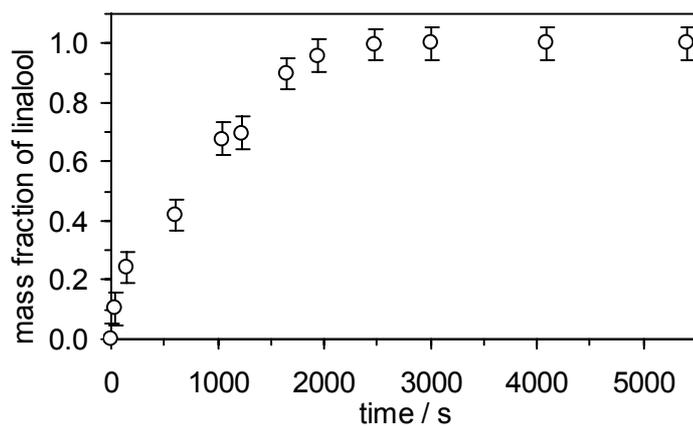
### 4.3.1 Effect of PEG on the Release of AM Agent

The effect of varying the amount and MW of PEG on the release of the AM agent linalool was explored. Plots of mass fraction of linalool released versus time for blends containing 1% and 2% (w/w) PEG1 are presented in Figure 4.1 and Figure 4.2 respectively. From these plots it is evident that the release of linalool with time is similar for blends containing 1% or 2% (w/w) PEG1. Furthermore, the time taken to release almost all of the linalool from the film is *ca.* 90 min in each case. Similar plots were obtained for formulations containing PEG2 and PEG3 with linalool (see Appendix B).

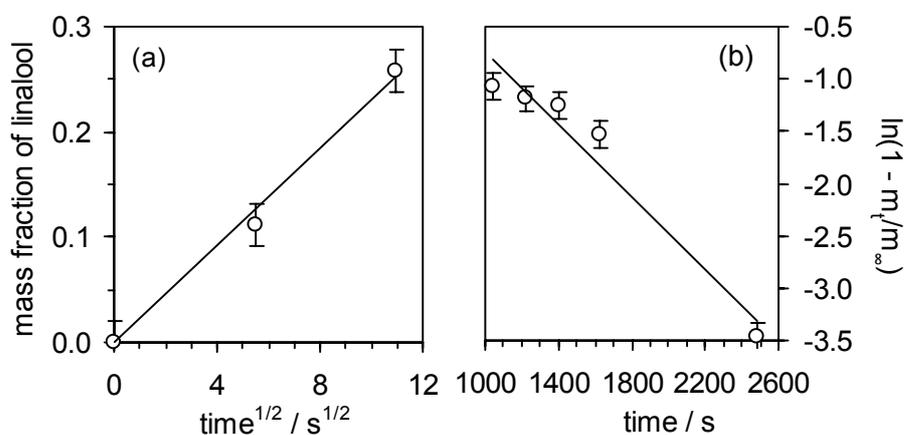


**Figure 4.1.** Plot of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from the compression moulded film containing 1% (w/w) PEG1 (P1).

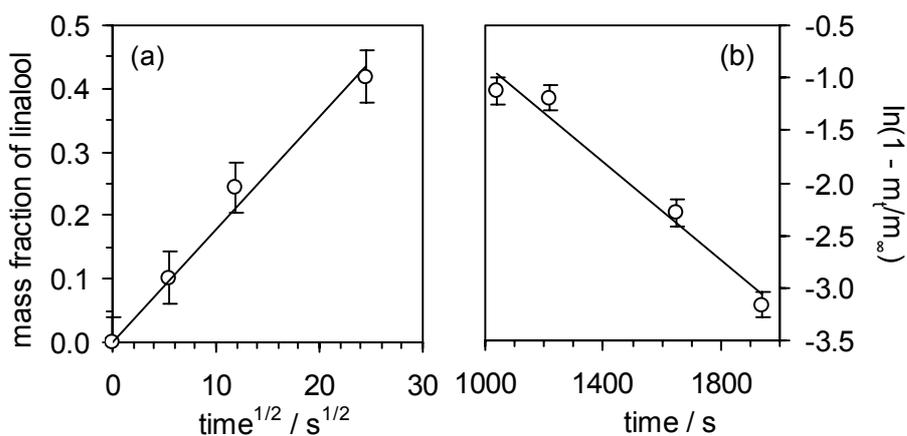
Plots of ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and of  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 1% and 2% (w/w) PEG1 are shown in Figure 4.3 and 4.4 respectively. Similar plots were obtained for formulations containing PEG2 and PEG3 with linalool (see Appendix B). The linearity of these plots confirms the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration.



**Figure 4.2.** Plot of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from the compression moulded film containing 2% (w/w) PEG1 (P2).

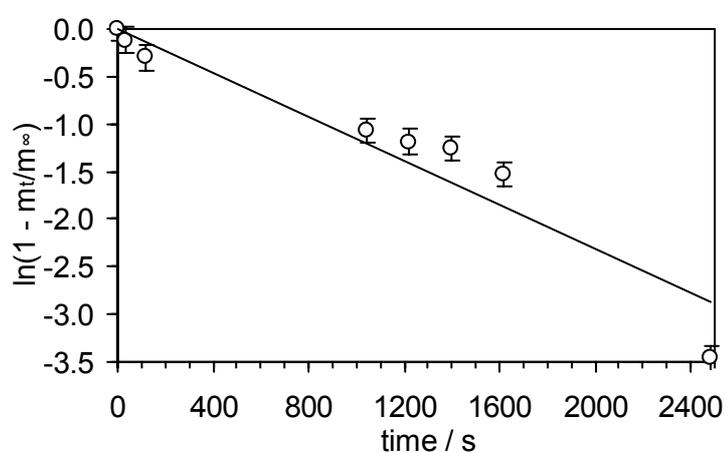


**Figure 4.3.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 1% (w/w) PEG1 (P1) in isooctane at 25°C.

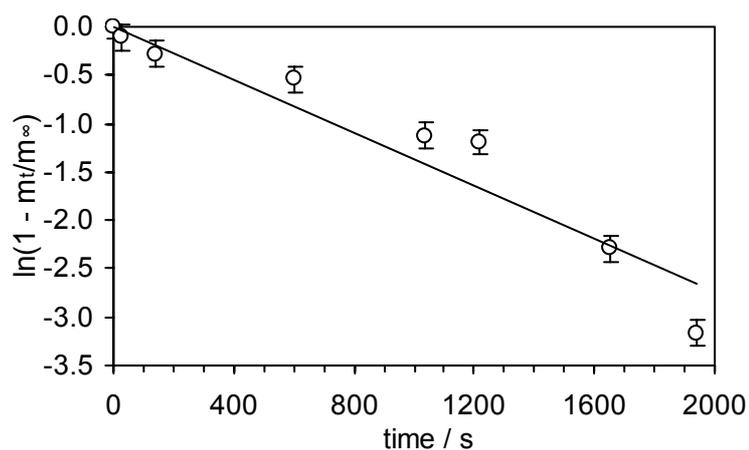


**Figure 4.4.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 2% (w/w) PEG1 (P2) in isooctane at 25°C.

Plots of  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 1% and 2% (w/w) PEG1 are presented in Figure 4.5 and Figure 4.6 respectively. The linearity of these plots suggests that these systems are consistent with first order kinetics (equation (8)). Similar plots were obtained for formulations containing PEG2 and PEG3 with linalool (see Appendix B).



**Figure 4.5.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from the compression moulded film containing 1% (w/w) PEG1 (P1).



**Figure 4.6.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from the compression moulded film containing 2% (w/w) PEG1 (P2).

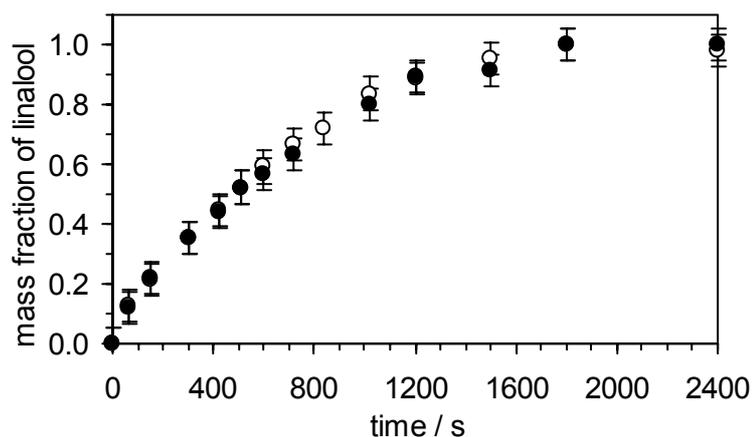
The diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses are given in Table 4.3. The blend comprising the highest MW PEG has the lowest diffusion coefficient. This suggests that the addition of 2% (w/w) of a high MW PEG may have a positive effect in retaining the AM volatile compounds containing hydroxyl groups such as linalool and thymol in the short-term.

**Table 4.3** Effect of PEG on the release of AM agent

Formulation	% (w/w) PEG	Diffusion Analysis		Kinetic Analysis	
		$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$v_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
P0	0	267	152	10.2	105
P1	1 (PEG1)	415	174	10.6	116
P2	2 (PEG1)	248	234	16.7	174
P3	2 (PEG2)	289	117	11.9	121
P4	2 (PEG3)	5.3	152	11.7	121

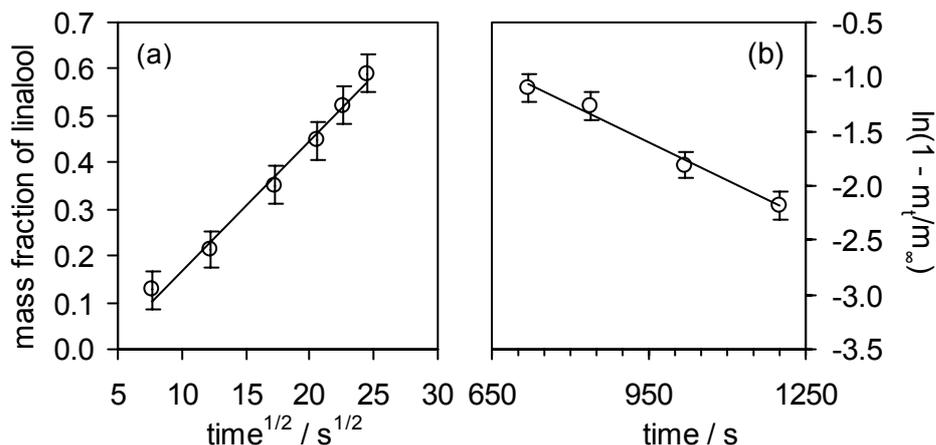
#### 4.3.2 Effect of AM Agent and PEG on the Release from Film

The effect of varying the AM agent and the addition of PEG on the release into a food simulant was studied using linalool and thymol. Figure 4.7 shows a plot of the mass fraction of linalool released versus time for blends containing 0% and 1% (w/w) PEG1. From this plot it is evident that the addition of 1% (w/w) PEG1 has little effect in slowing the release of linalool with time and that all of the linalool is released after *ca.* 40 min with or without the incorporation of the PEG1.

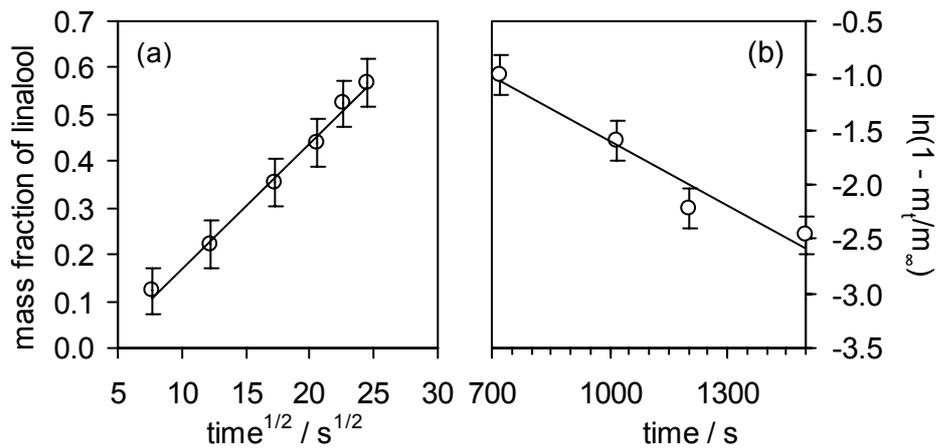


**Figure 4.7.** Plots of mass fraction ( $m_t/m_\infty$ ) of linalool released into isoctane at 25°C versus time from AM film prepared by compression moulding containing: (○) 0% (w/w) PEG1 (L0) and (●) 1% (w/w) PEG1 (L1).

Figure 4.8 and 4.9 show plots of ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 0% and 1% (w/w) PEG1 respectively. The linearity of these plots confirms the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration of linalool.

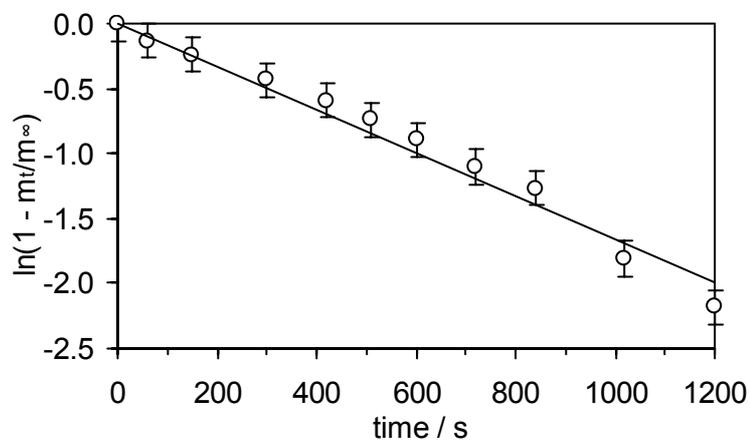


**Figure 4.8.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 0% (w/w) PEG1 (L0) into isoctane at 25°C.

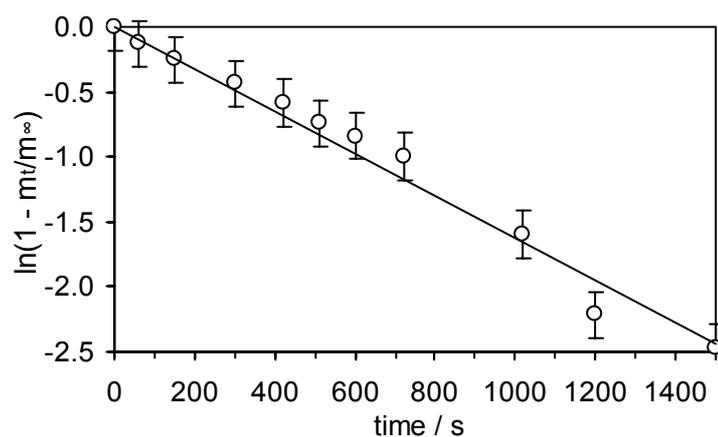


**Figure 4.9.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 1% (w/w) PEG1 (L1) into isooctane at 25°C.

Figure 4.10 and 4.11 show plots of  $\ln(1 - m_t/m_\infty)$  versus time for blends containing linalool with 0% and 1% (w/w) PEG respectively. The linearity of these plots suggests that the release of the AM agent follows first order kinetics systems.

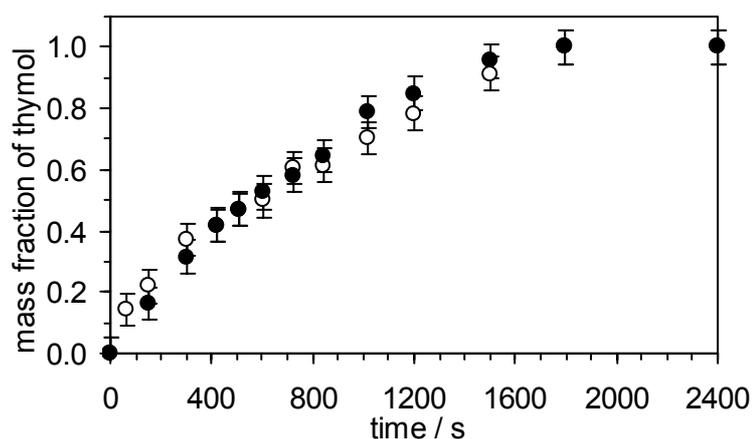


**Figure 4.10.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool from compression moulded film containing 0% (w/w) PEG1 (L0) into isooctane at 25°C.



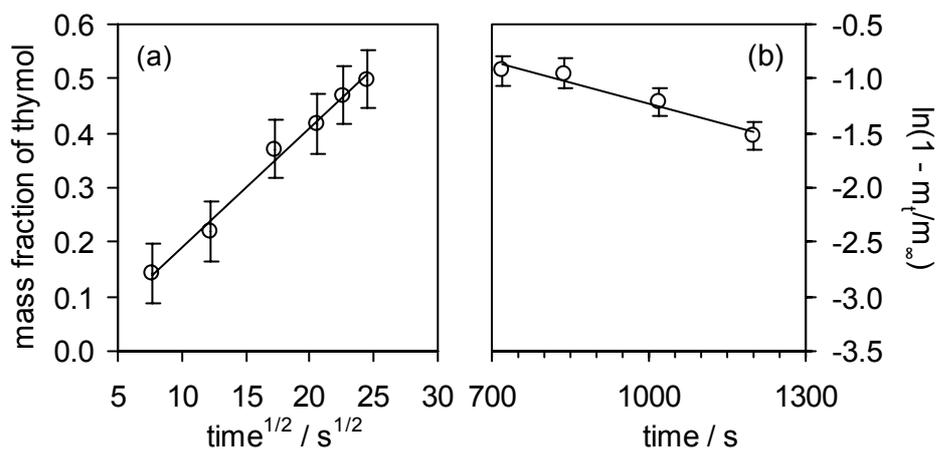
**Figure 4.11.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool from compression moulded film containing 1% (w/w) PEG1 (L1) into isooctane at 25°C.

Figure 4.12 shows a plot of the mass fraction of thymol released versus time for blends containing 0% and 1% (w/w) PEG1. From this plot it is evident that the addition of 1% (w/w) PEG1 is effective in slowing the initial release of thymol although all of the thymol is released after *ca.* 40 min with or without the incorporation of the PEG1.

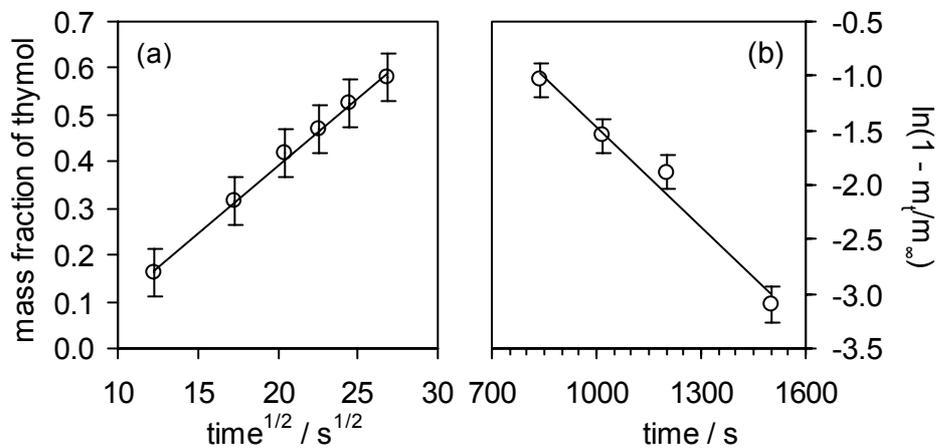


**Figure 4.12.** Plots of mass fraction ( $m_t/m_\infty$ ) of thymol released into isooctane at 25°C versus time from AM film prepared by compression moulding containing: (O) 0% (w/w) PEG1 (T0) and (●) 1% (w/w) PEG1 (T1).

Figure 4.13 and 4.14 show plots of  $(m_t/m_\infty)$  versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 0% and 1% (w/w) PEG1 respectively. The linearity of these plots confirms the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration of thymol.



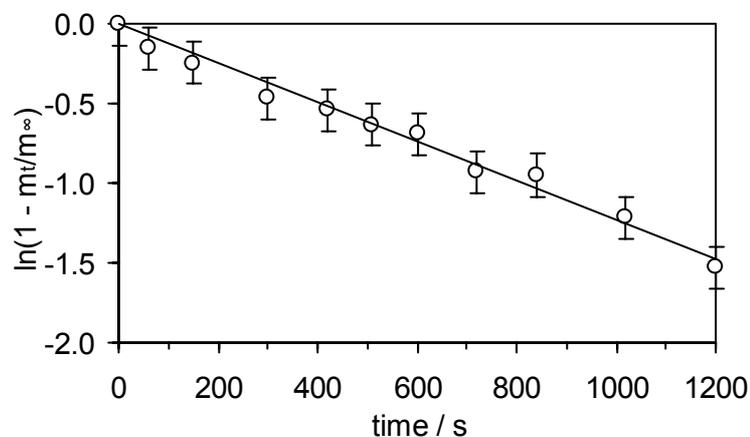
**Figure 4.13.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 0% (w/w) PEG1 (T0) into isooctane at 25°C.



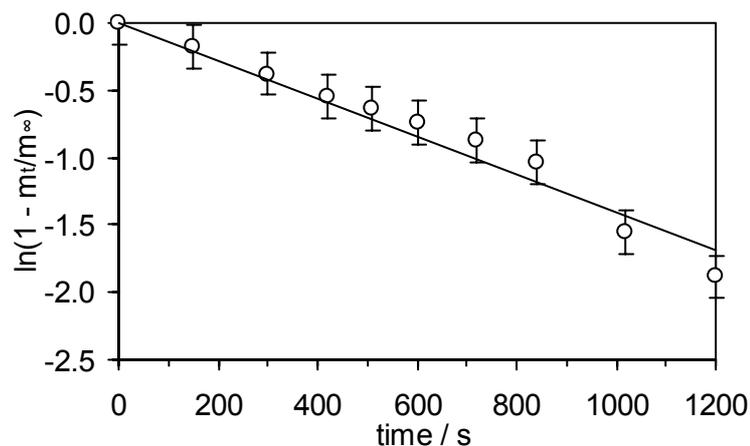
**Figure 4.14.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 1% (w/w) PEG1 (T1) into isooctane at 25°C.

Plots of  $\ln(1 - m_t/m_\infty)$  versus time for blends containing thymol with 0% and 1% (w/w) PEG respectively are shown in Figure 4.15 and 4.16 respectively. The

linearity of these plots suggests that the release of the AM agent follows first order kinetics systems.



**Figure 4.15.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol from compression moulded film containing 0% (w/w) PEG1 (T0) into isooctane at 25°C.



**Figure 4.16.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol from compression moulded film containing 1% (w/w) PEG1 (T1) into isooctane at 25°C.

The diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses are given in Table 4.4. The diffusion coefficients are lower for the blends containing thymol and the initial release rates of thymol are also

significantly lower. This suggests that thymol may be retained longer in the film initially which may be due to the presence of PEG.

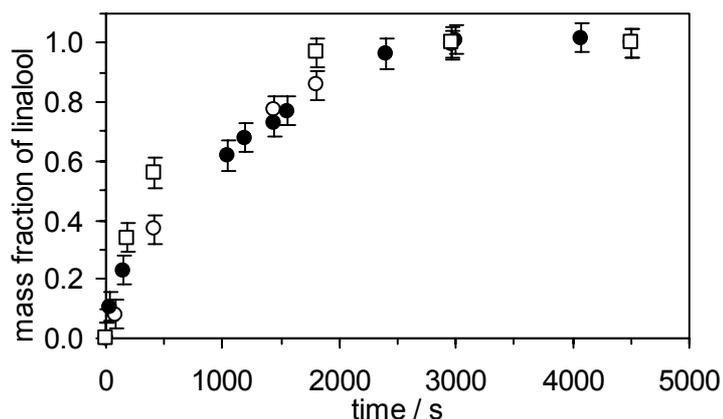
**Table 4.4** Effect of AM Agent and PEG on the release of AM agent

Formulation	% (w/w) PEG	Diffusion Analysis		Kinetic Analysis	
		$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$v_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
L0 (2% linalool)	0	384	235	22.3	166
L1 (2% linalool)	1	374	196	22.8	162
T0 (2% thymol)	0	326	131	14.0	123
T1 (2% thymol)	1	327	308	10.3	140

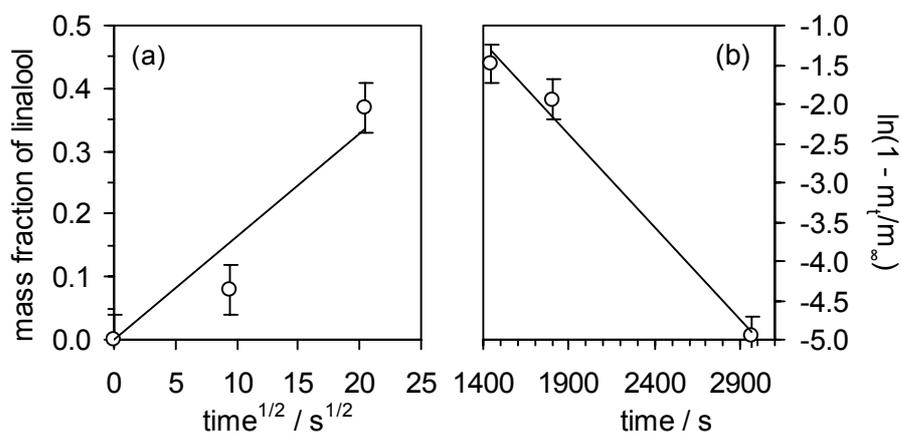
#### 4.3.3 Effect of EVA on the Release of AM Agent

The effect of a second polymer, EVA, was explored as a possible additive polymer to retain the AM agents in the LDPE film. A plot of mass fraction of linalool released versus time for blends containing 0%, 10% and 50% (w/w) EVA is presented in Figure 4.17 (and separately in Appendix B). From this plot it is evident that although the initial rate of release of linalool is higher for blends containing 50% (w/w) EVA, the time taken to release all of the linalool from the film is similar for each blend. Furthermore, the blend containing 10% (w/w) EVA retains slightly more linalool than any other blend at any point in time.

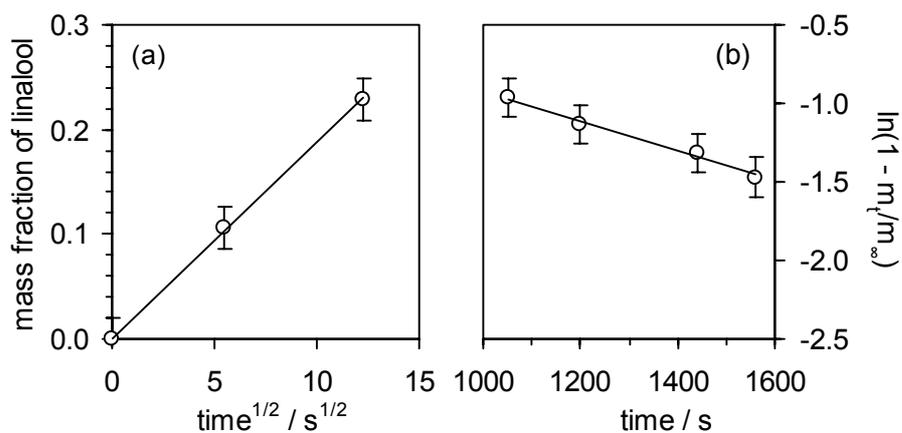
Figures 4.18 and 4.19 show plots of  $(m_t/m_\infty)$  versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 0% and 10% (w/w) EVA respectively. The linearity of these plots confirms the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration of linalool.



**Figure 4.17.** Plots of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from compression moulded film containing: (O) 0% (w/w) EVA (E0), (●) 10% (w/w) EVA (E1) and (□) 50% (w/w) EVA (E2).

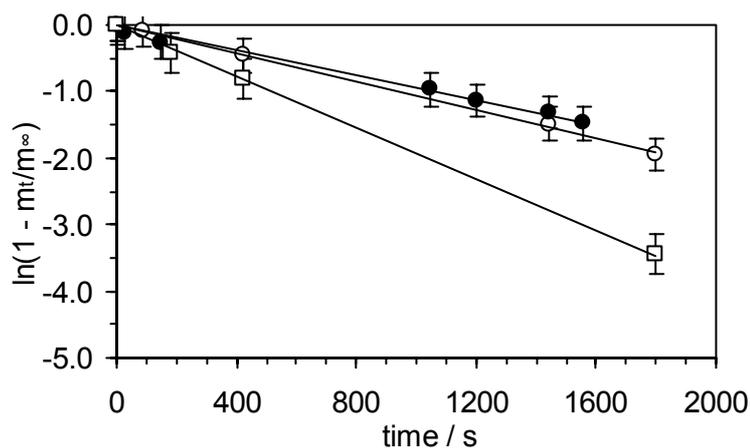


**Figure 4.18.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 0% (w/w) EVA (E0) into isooctane at 25°C.



**Figure 4.19.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 10% (w/w) EVA (E1) into isooctane at 25°C.

Figure 4.20 shows plots of  $\ln(1 - m_t/m_\infty)$  versus time for blends containing 0%, 10% and 50% (w/w) EVA. The linearity of these plots suggests that the release of the AM agent follows a first order kinetics system and confirms that the blend containing 50% (w/w) EVA releases the AM agent at a faster rate as suggested by the diffusion analysis.



**Figure 4.20.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool from compression moulded film containing: (○) 0% (w/w) EVA (E0), (●) 10% (w/w) EVA (E1) and (□) 50% (w/w) EVA (E2) into isooctane at 25°C.

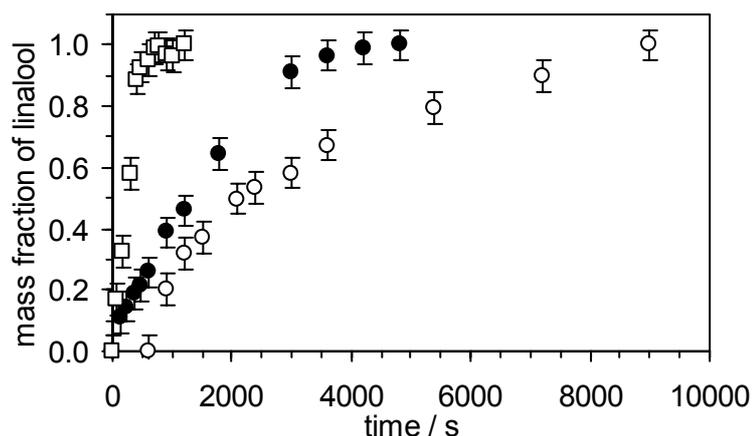
Table 4.5 presents the diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses. The diffusion coefficients are observed to increase with an increase in EVA content. Furthermore, the rate constants are lower for the blend containing 10% (w/w) EVA suggesting that EVA may be effective in retaining the AM agent linalool, possibly due to the presence of the hydroxyl group in the EVA structure. These results also suggest that the AM agent migrates from the film at a faster rate with a higher EVA content which may be due to the lower crystallinity of blends formed by compression moulding (Dalai and Wenxiu, 2002). The presence of the AM agent in the amorphous region of the blend may also contribute to the higher release rate.

**Table 4.5** Effect of EVA on the release of AM agent

Formulation	% (w/w) EVA	Diffusion Analysis		Kinetic Analysis	
		$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$v_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
E0	0	209	235	30.6	144
E1	10	278	91	8.8	93.4
E2	50	560	-	45.8	193

#### 4.3.4 Effect of Food Simulant on the Release of AM Agent

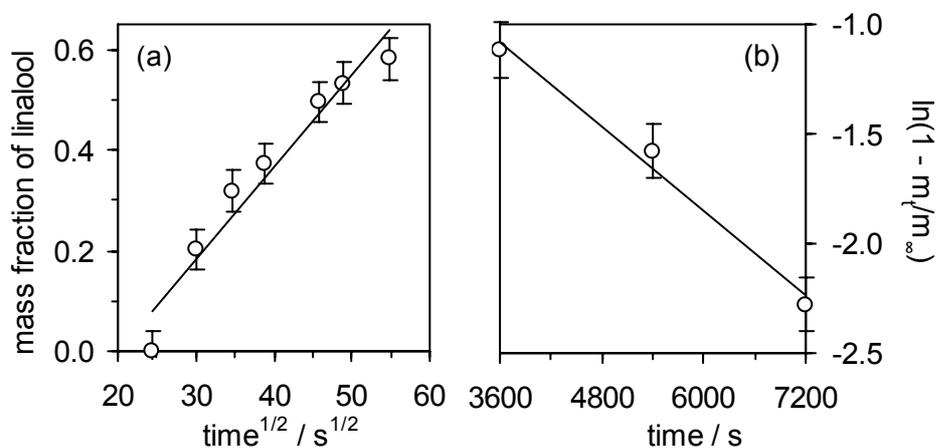
The release of the AM agent linalool or thymol into various food simulants including water, isooctane and ethanol was investigated. When using pure water as a food stimulant, no release of linalool or thymol was observed due to the insolubility of these AM agents in water. Plots of mass fraction of linalool released versus time into isooctane, 15% ethanol and 95% ethanol from extrusion blown film is presented in Figure 4.21. From this plot it is evident that the release of linalool into isooctane occurs faster than in any other solvent studied and that the slowest release is observed for 15% ethanol.



**Figure 4.21.** Plots of mass fraction ( $m_t/m_\infty$ ) of linalool released at 25°C versus time for F2 films into: (□) isooctane, (●) 95% ethanol, and (○) 15% ethanol.

The release of linalool into isooctane reaches equilibrium in *ca.* 20 minutes whereas the release into 15% ethanol reaches equilibrium in *ca.* 150 minutes with a lag time of *ca.* 15 minutes. The observed quick release into isooctane may be due to the swelling effect of this solvent on LDPE as reported by Helmroth *et al.* (2003). The high solubility of linalool in isooctane may also contribute to the quick release of the AM agent into this food simulant.

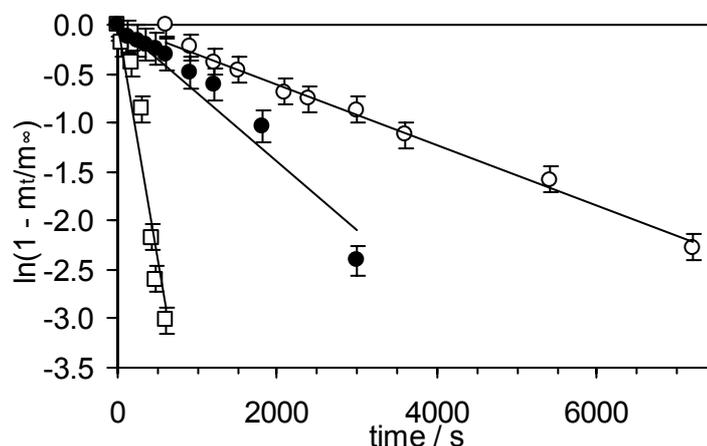
Figure 4.22 shows plots of  $(m_t/m_\infty)$  versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into 15% ethanol for extruded F2 film blends. The linearity of these plots confirms that the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration of linalool. Similar plots were obtained for the release of linalool into isooctane and 95% ethanol (see Appendix B).



**Figure 4.22.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into 15% ethanol at 25°C for the F2 film.

Figure 4.23 shows plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane, 15% ethanol and 95% ethanol from extrusion blown films. The linearity

of these plots suggests that the release of the AM agent follows a first order kinetics system and confirms that the release of linalool occurs fastest in isooctane and slowest in 15% ethanol.



**Figure 4.23.** Plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool from F2 film formulations into: ( $\square$ ) isooctane, ( $\bullet$ ) 95% ethanol and ( $\circ$ ) 15% ethanol at 25°C.

Table 4.6 presents the diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses. The kinetic values in Table 4.6 consistently decrease in the order: isooctane > 95% ethanol > 15% ethanol. The results suggest that amount of linalool released from the packaging material into liquids would decrease with the increasing affinity to the polymeric system. It can be assumed that its release into aqueous or acidic foods would be even lower because of the low solubility of linalool in these foods.

Plots of mass fraction of thymol released versus time into isooctane, 15% ethanol and 95% ethanol from extrusion blown film are presented in Figure 4.24. From this plot it is evident that the release of thymol into isooctane is similar to that of linalool

**Table 4.6** Effect of food simulant on the release of linalool from F2 films

Food Simulant	Diffusion Analysis		Kinetic Analysis	
	$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$v_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
Isooctane	41.4	450	37	483
95% Ethanol	6.7	142	4.1	70.0
15% Ethanol	4.5	32.2	0.7	29.9

in that the release occurs faster than in any other solvent studied. The slowest release of thymol, however, is observed using 95% ethanol then in 15% ethanol. The release of thymol into isooctane reaches equilibrium in *ca.* 30 minutes whereas the release into 15% ethanol and 95% ethanol reaches equilibrium in *ca.* 200 and 180 minutes respectively. The reason for this behaviour is not clear although one reason could be that thymol is less soluble in 95% than in 15% ethanol.

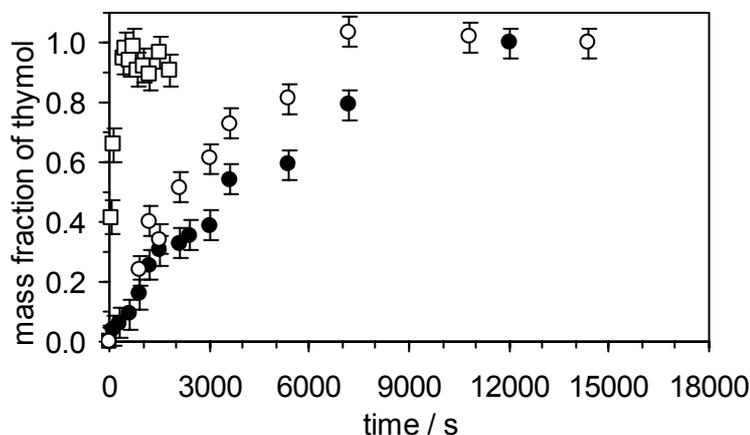
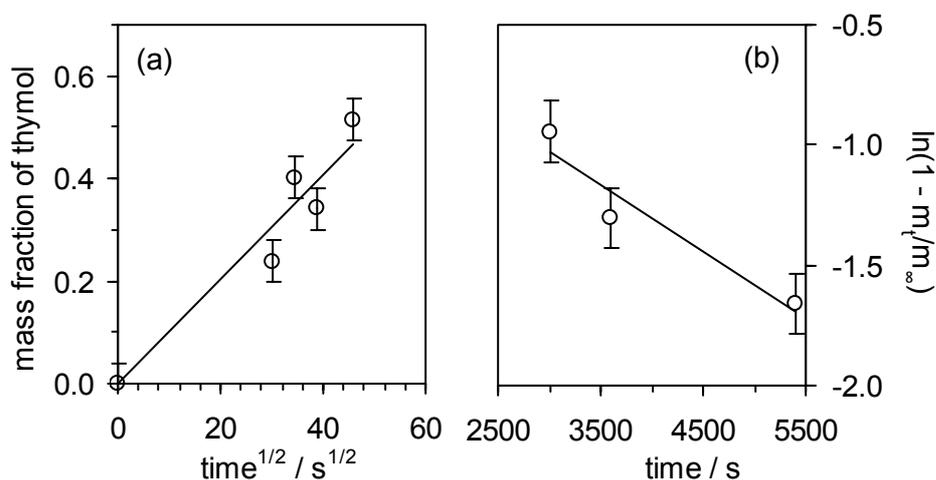
**Figure 4.24.** Plots of mass fraction ( $m_t/m_\infty$ ) of thymol released at 25°C versus time for F3 films into: (□) isooctane, (●) 95% ethanol, and (○) 15% ethanol.

Figure 4.25 shows plots of ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol into 15% ethanol for extruded F3 film blends. The linearity of

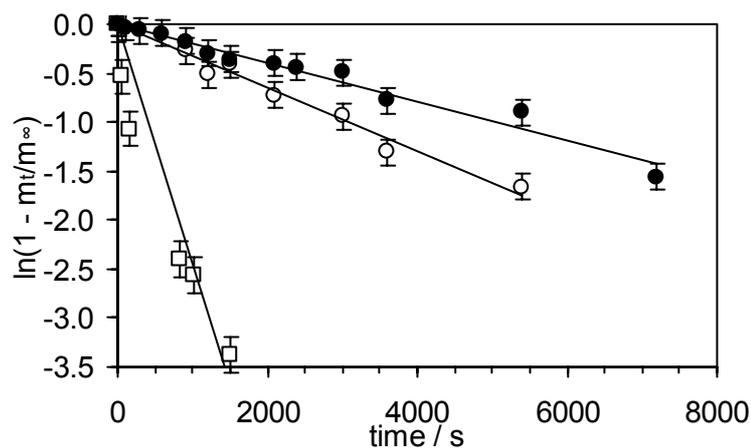
these plots confirms the data are adequately described by equation (1) for short-term migration and equation (4) for long-term migration of thymol. Similar plots were obtained for the release of thymol into 95% ethanol and isooctane (see Appendix B).



**Figure 4.25.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol into 15% ethanol at 25°C for the F3 films.

Figure 4.26 shows plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol into isooctane, 15% ethanol and 95% ethanol from extrusion blown films. The linearity of these plots suggests that the release of the AM agent follows a first order kinetics system and confirms that the release of thymol occurs fastest in isooctane and slowest in 95% ethanol.

Table 4.7 presents the diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses. The data consistently decreases in the order: isooctane > 15% ethanol > 95% ethanol.



**Figure 4.26.** Plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol from F3 film formulations into: (□) isooctane, (●) 95% ethanol and (○) 15% ethanol at 25°C.

**Table 4.7** Effect of food simulant on the release of thymol from F3 films

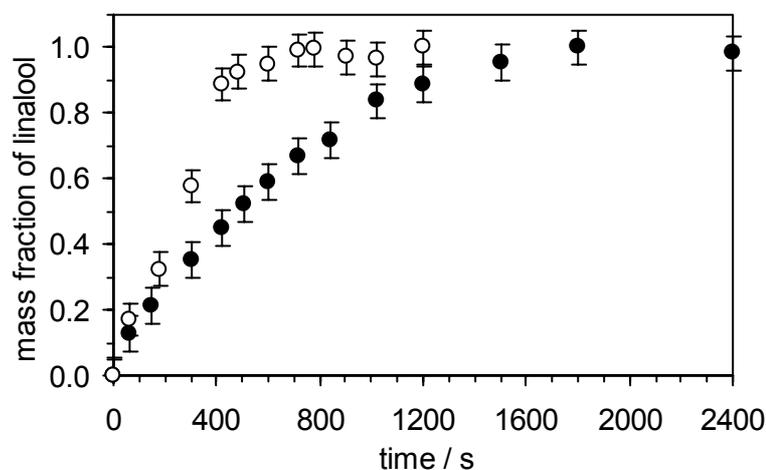
Food Simulant	Diffusion Analysis		Kinetic Analysis	
	$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$\nu_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
Isooctane	141	152	8	246
95% Ethanol	2.3	21.7	0.66	19.8
15% Ethanol	5.1	27.6	0.74	32.5

The differences in the migration of thymol and linalool may be due to the physical states of the AM agents which are crystalline and liquid at room temperature for thymol and linalool respectively. The results with the different food simulants suggest that the release of volatile natural AM agents is significantly affected by the contact medium and solubility of the AM agents. As a result of the low release rates into aqueous simulants, the diffusion of these AM agents into the aqueous food would be low, which may reduce the possibility of off-flavours in packed aqueous food products. Due to the relatively high vapour pressure of the AM agents,

however, a high release of these agents into the food package headspace would be expected. These results suggest that volatile AM agents such as linalool and thymol may be suitable for package/headspace/food systems as discussed by Han (2000).

#### 4.3.5 Effect of Film Fabrication on the Release of AM Agent

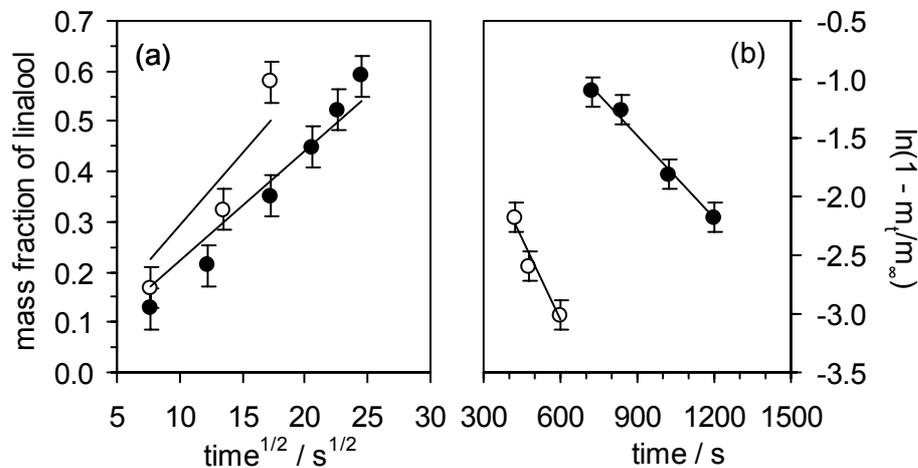
Figure 4.27 shows a plot of the mass fraction of linalool released versus time for film formulations produced by melt compression or melt extrusion. This plot suggests that melt compression is more effective in controlling the release of the AM agent. Furthermore, the film produced by melt compression releases entirely the equilibrium concentration of the AM agent in about twice the time compared to the film produced by melt extrusion.



**Figure 4.27.** Plots of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from film formulations produced by: (●) melt compression (L1) and (○) melt extrusion (F2).

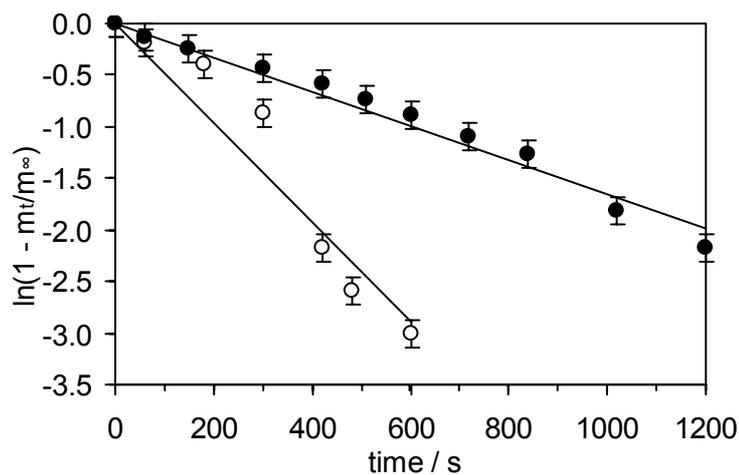
Figure 4.28 shows plots of ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool for film formulations produced by melt compression or melt

extrusion. These plots confirm that melt compression is more effective in controlling the release of the AM agent.



**Figure 4.28.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from film formulations produced by: (●) melt compression (L1) and (○) melt extrusion (F2).

Figure 4.29 shows plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool from film formulations produced by melt compression or melt extrusion. These plots also confirm that melt compression is more effective in controlling the release of linalool.



**Figure 4.29.** Plots of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from film formulations produced by: (●) melt compression (L1) and (○) melt extrusion (F2).

Table 4.8 shows the diffusion coefficients, rate constants and initial release rates for the diffusion and kinetic analyses. Although the release of linalool occurs faster from the extruded film, the diffusion coefficient is significantly lower than that of the melt compressed film. The differences in the film fabrication method (see Table 4.8), would suggest that the short-term release of the AM agent from samples produced by melt compression is considerably faster than that of samples produced by melt extrusion. This may be due to the more uniform distribution of AM agent in the polymer matrix (LaCoste *et al.*, 2005) imparted by melt-mixing during extrusion. Furthermore, in the thick, non-uniform films produced by melt compression, part of the AM agent additive could be concentrated on the film surface resulting in an apparent higher calculated diffusion coefficient. The diffusion coefficient determined for the melt extruded film is of a similar order of magnitude to values obtained in a previous study (Suppakul, 2004).

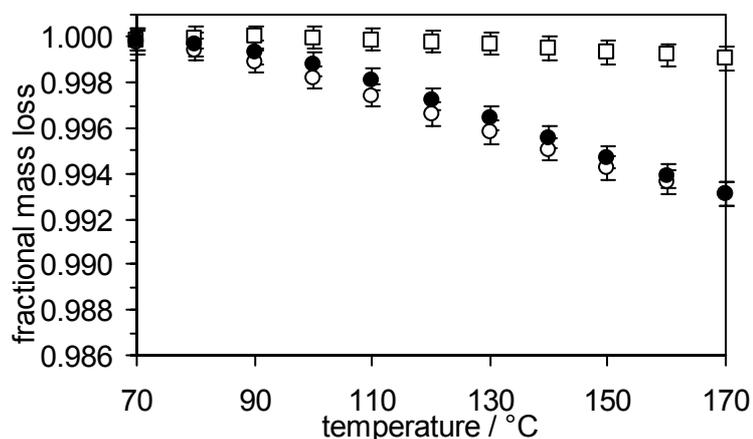
**Table 4.8** Effect of film fabrication on the release of linalool

Film Fabrication	Film Thickness	Diffusion Analysis		Kinetic Analysis	
		$D \times 10^{-14}$ / $\text{m}^2 \text{s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$	$v_0 \times 10^{-5}$ / $\text{g s}^{-1}$	$k \times 10^{-5}$ / $\text{s}^{-1}$
Melt compression	2 mm	384	235	22.3	166
Melt extrusion	50 $\mu\text{m}$	41.4	450	37	483

#### 4.3.6 TGA Analysis of AM Films

A possible alternative technique to conventional release kinetic analysis of AM agents using TGA was explored. Figure 4.30 shows plots of the fractional mass loss versus temperature for the control, linalool and thymol films. The fractional mass loss of linalool and thymol films followed a similar trend over the temperature range

which may indicate a loss of volatile AM agents from the molten polymer matrix in each case. Furthermore, the fractional mass losses increased significantly above 90°C which is consistent with the loss of the AM agent in the extrusion temperature range. The fractional mass loss of the linalool containing film was observed to be higher than that of the thymol containing film which is consistent with previous results that showed that linalool was released faster than thymol (see Table 4.4). This technique offers a potential alternative to the more conventional release experiments.



**Figure 4.30.** Plot of fractional mass loss of film versus temperature obtained by TGA analysis from extruded films: (□) control film (F1), (●) linalool film (F2) and (○) thymol film (F3).

## **5 Conclusions, Recommendations, Future work**

### **5.1 Conclusions**

The results of the present study highlight the promising potential and feasibility of incorporating natural AM agents such as linalool and thymol in conventional LDPE films to produce AM food packaging.

#### **5.1.1 Effect of Blending LDPE with PEG**

The incorporation of the additive polymer PEG into the LDPE/AM film blend played an important role in controlling the release rate of the AM agents: linalool and thymol. This was possibly due to the introduction of hydrophilic sites into the polymer matrix. For compression moulded AM films the initial rate of release of AM agent linalool decreased with an increase in PEG content. Furthermore, the highest molecular weight PEG significantly decreased the short-term release of linalool. The AM agent thymol was released at a comparatively slower rate than linalool and the release was further reduced by the addition of PEG.

#### **5.1.2 Effect of Blending LDPE with EVA**

The incorporation of the additive polymer EVA resulted in higher initial release rates of AM agents with high content (50% (w/w)) of EVA. The release rates were lower at the lower EVA content (10% (w/w)). The diffusion coefficient and initial rate of release of AM was lower in samples formed by extrusion mixing which may be due to the introduction of more uniform melt mixing that cannot be achieved during compression moulding.

### **5.1.3 Development of Extruded AM Films**

The natural AM agents were effectively incorporated into the polymer pellets by direct blending prior to blown film extrusion with significant retention of the AM agents. The amount of AM agent retained in the film was observed to be high when the agent was directly blended without using any solvent. The incorporation of linalool and thymol did not adversely affect the mechanical or optical properties of extruded LDPE-EVA films. Furthermore, the release rate of linalool was higher than that of thymol; that may be due to the higher volatility of linalool relative to thymol. A study using a TGA technique suggested that the loss of the volatile AM agents was high at the processing extrusion temperatures. Furthermore, the AM agent linalool was released faster than thymol from the polymer film.

### **5.1.4 Release of AM Agents from Film**

The release of the AM agents from film produced by compression moulding or by blown film extrusion can be adequately and consistently described by short-term and long-term migration equations. Furthermore, all of the release experiments revealed that the release of linalool and thymol from the polymer consistently obeyed first order kinetics. Indeed, satisfactory fits to first-order kinetics were obtained for all systems studied in this work and are confirmed by the linearity of the processed data. In addition to the diffusion coefficient and rate constant provided by the diffusion analysis, the kinetics analysis can provide an initial rate of release of AM agent. The release of the AM agents from extruded film was consistently high into isooctane and considerably slower into ethanol solutions.

## **5.2 Recommendations**

### **5.2.1 Blending Improvements**

The use of a single-screw extruder with a normal gravity feed hopper was ineffective in compounding PEG with LDPE. The problem of choking of the hopper at the throat caused by PEG may be overcome by using a screw feed hopper during extrusion. Application of twin-screw extruders may also be explored to improve the blend morphology. A detailed study on the compatibility of PEG with EVA/LDPE blends using morphological techniques is recommended for the production of stable films with good mechanical properties. Further morphological and rheological studies of PEG/EVA/LDPE blends with AM agents could be conducted to optimize the extrusion parameters. Investigation by X-ray diffraction, DSC evaluation and microscopy to measure the extent of crystallinity, flow properties and particle size distribution could be undertaken to develop such an optimum extrusion process.

### **5.2.2 Additive Quantification**

As the natural AM agents are volatile, losses of solvent and the AM agent are inevitable using a Soxhlet extraction method. Moreover, the loss of simulant and solvent were very difficult to control. Traditional liquid solvent/polymer extraction methods involving dissolution and precipitation are generally time consuming, uneconomical and the recoveries are significantly lower than 90%. An on-line supercritical fluid extraction/chromatography system could offer efficient extraction and separation of polymer additives.

### **5.2.3 Release Experiments**

The use of migration cells coupled with automatic sampling and analysis would help in the accurate determination of the release of linalool and thymol. The use of alternative food simulants such as hexane or acetic acid could be further explored for monitoring the release of linalool and thymol from AM films.

### **5.2.4 Development of TGA Methodology**

Foods packaged with AM films are often stored at different temperatures so the release kinetics are consequently different under different storage conditions. The use of a relatively unexplored technique of TGA to monitor the release of AM agents from film could be further investigated. Isothermal experiments in particular could be important in the development of optimal extrusion conditions of the AM film blends. Diffusion coefficients, temperature dependence of the diffusion coefficients, and Arrhenius activation energy of linalool and thymol could be investigated by monitoring the mass loss of the AM films by TGA analyser at different temperatures and isothermal conditions.

## **5.3 Scope for Future Work**

### **5.3.1 Barrier Properties**

Moisture, oxygen and carbon dioxide are very crucial for the preservation of foods and as such, the determination of the transmission rates of these gases and vapours through the AM/LDPE/EVA film blend could be investigated.

### **5.3.2 Multi-Layer Film Packaging**

The manufacture of multi-layer films containing natural AM agents can be made by co-extrusion or by extrusion lamination. In order to achieve the controlled release of the active compounds to the surface of the food and not into the atmosphere, the use of multi-layer film (control layer/active layer/barrier layer) as proposed by Han and Floras (1997) could be explored. An ideal structure would consist of an outer AM barrier layer, an AM containing matrix layer, and a release control layer. The outer layer should be a barrier layer to prevent loss of active agent from the polymer matrix layer. The release control layer may consist of a PEG/food grade resin blend to control the release of the AM agent. Release experiments with multi-layer films containing linalool and thymol may prove to be crucial in controlling the release into the food.

### **5.3.3 Antimicrobial Activity**

The AM activity of the packaging materials can be measured by microbiology experiments. Before food samples are packaged in the AM packaging material, the activity of LDPE films containing linalool and thymol films may be tested against variety of microorganisms. Determination of the minimum inhibition concentration of linalool and thymol would prove to be crucial in developing an AM film. The applicability of thymol and linalool films for the preservation of other types of food such as meat, poultry, seafood and high moisture bakery food products could be investigated. Microbial studies of LDPE films containing combinations of volatile linalool and thymol AM agents with other non-releasing traditional AM agents would also be of interest for the application of packaging different types of food.

#### **5.3.4 Modelling AM Release**

Mathematical modelling of the diffusion of the AM agent may be used to establish a release profile of natural AM agent from a packaging material into the food product. This may permit the estimation of accurate concentration pattern, provide diffusion profile of real food packaging systems and predict the period during which the AM concentration will be maintained above the critical inhibitory concentration in the packaged food (Han, 2003). Furthermore, the determination of the Sherwood number, which is the ratio of surface mass transfer coefficient to the diffusion coefficient, may be also used in modelling the mass transfer to predict the concentration of these active natural AM agents at any point in time.

## References

- Ahvenainen, R., Active and Intelligent Packaging. In: Novel Food Packaging Techniques, Woodhead Publishing Limited, Cambridge, Ahvenainen, R. (Ed), 2003.
- Aitor, P., Norman, S., Michele, E., and Eusebio, F. (2002). Additive interactions in stabilization of film grade high density polyethylene. *Journal of Vinyl and Additive Technology* **8**, 90-102.
- An, D., Hwang, Y.-I., Cho, S.-H., and Lee, D. (1998). Packaging of fresh curled lettuce and cucumber by using low density polyethylene films impregnated with antimicrobial agents. *Journal of Korean Society Food Science and Nutrition* **27**, 675-681.
- Arora, R., and Pandey, G.N. (1977). The application of essential oils and their isolates for blue mould decay control in Citus reticulata Blanco. *Journal of Food Science and Technology* **14**, 137-146.
- ASTM Method D 882 - 97, "Standard Test Method for Tensile Properties of Thin Plastic Sheeting", Plastics (I), 08.01, pp. 163-171, 1998.
- ASTM Method D 1003 - 97, "Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics", Plastics (I), 08.01, pp. 199-204, 1998.
- Azaz, A.D., Kurkcuoglu, M., Satil, F., Baser, H.C., and Tumen, G. (2005). In vitro antimicrobial activity and chemical composition of some Satureja essential oils. *Flavour and Fragrance Journal* **20**, 587-591.
- Bahl, J.R., Garg, S.N., Sinha, S.C., Bansal, R.P., Naqvi, A.A., and Kumar, S. (2000). Composition of linalool rich essential oil from Lippia alba grown in Indian plains. *Flavour and Fragrance Journal* **15**, 199-200.
- Baldev, R., Sankar, U., and Siddaramaiah, K. (2000). Low density polyethylene/starch blend films for food packaging applications. *Advances in Polymer Technology* **23**, 32-45.

- Baratta, T.M., Dorman, D.J.H., Denes, G.S., Figueiredo, C.A., Barroso, G.J., and Ruberto, G. (1998). Antimicrobials and antioxidant properties of some commercial oils. *Flavour and Fragrance Journal* **13**, 235-244.
- Berins, M., *Plastics Engineering Handbook of the Society of Plastics Industry*, 102-1991.
- Bezic, N., Skocibusic, M., Dunkic, V., and Radonic, A. (2003). Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. *Phytotherapy Research* **17**, 1037-1040.
- Cahan, R., Vadim, G., Binjamin, F., and Edward, B. (2003). Development of novel active packaging film preventing migration of antimicrobial component. *The College of Judea and Samaria, Israel* **4**, 89
- Cassagnau, P., Courmont, M., Melis, F., and Puaux, J.P. (2005). Study of mixing of liquid/polymer in twin screw extruder by residence time distribution. *Polymer Engineering and Science* **45**, 926-934.
- Choudhry, M.S., Lox, F., Beukens, A., and Decroly, P. (1998). Evaluation of migrational behaviour of plastic food-contact materials: a comparison of methods. *Packaging Technology and Science* **11**, 275-283.
- Chuayjuljit, S., Kunsawat, C., and Potiyaraj, P. (2003). Use of silica from rice husk ash as an antiblocking agent in low-density polyethylene film. *Journal of Applied Polymer Science* **88**, 848-852.
- Chung, S.K., Cho, S.H., and Lee, D.S. (1998). Modified atmosphere packaging of fresh strawberries by antimicrobial plastic films. *Korean Journal of Food Science and Technology* **30**, 1140-1145.
- Conner, D.E., and Beuchat, L.R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. *Journal of Food Science* **49**, 429-434.
- Cooksey, K., *Utilisation of Antimicrobial Packaging Films for Inhibition of Selected Microorganisms*. In: *Food Packaging Testing Methods and Applications*, American Chemical Society, South Carolina, Risch, S. (Ed), 2000.

- Couladis, M., Tzakou, O., Kujundzic, S., Sokovic, M., and Mimica-Dukic, N. (2004). Chemical analysis and antifungal activity of *Thymus striatus*. *Phytotherapy Research* **18**, 40-42.
- Cudworth, J., Farrel Bridge-Engineers to Rubber and Plastic Processing Industries, Farrel Bridge Ltd, Rochdale, 1976.
- Daeschel, M.A. (1989). Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technology* **43**, 164-167.
- Dalai, S., and Wenxiu, C. (2002). Radiation effects on LDPE/EVA blends. *Journal of Applied Polymer Science* **86**, 1296-1302.
- Deans, J.G., and Ritchie, G. (1987). Antimicrobial properties of plant essential oils. *International Journal of Food Microbiology* **5**, 165-180.
- Dobias, J., Chudackova, K., Voldrich, M., and Marek, M. (2000). Properties of polyethylene films with incorporated benzoic anhydride and/or ethyl and propyl esters of 4-hydroxybenzoic acid and their suitability for food packaging. *Food Additives and Contaminants* **17**, 1047-1053.
- Donghwan, C., Spyridon, E., and Yam, K.L. (2003). Evaluation of a polymer coating containing triclosan as the antimicrobial layer for packaging materials. *International Journal of Food Science and Technology* **38**, 165-169.
- Dopico, M.S., Lopez-Vilarino, J.M., and Gonzalez-Rodriguez, M.V. (2003). Determination of antioxidant migration levels from low density polyethylene film into food simulants. *Journal of Chromatography* **1018**, 53-62.
- Dziezak, J.D. (1989). Spices. *Food Technology* **43**, 102-115.
- Fang, Y., Carreau, P., and Lafleur, P. (2003). Rheological effects of polyethylenes in film blowing. *Polymer Engineering and Science* **43**, 1391-1406.
- Farber, J.M. (1991). Microbiological aspects of modified atmosphere packaging technology - a review. *Journal of Food Protection* **54**, 58-70.

- Floras, J.D., Dock, L.L., and Han, J.H. (1997). Active packaging technologies and applications. *Food Cosmetics and Drug Packaging* **20**, 10-17.
- Friedman, M., Henika, P.R., and Mandrell, R.E. (2002). Bacterial activity of plants essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. *Journal of Food Protection* **65**, 1545-1560.
- Frierheller, M.C., Modified Atmosphere Packaging of Miscellaneous Products. In: Modified Atmosphere Packaging of Food, Ellis Horwood limited, Chichester, UK, Ooraikul, B. and Stile, M.E. (Eds), 1991.
- Fyfe, L., Armstrong, F., and Stewart., J. (1998). Inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations. *International Journal of Antimicrobial Agents* **9**, 130-136.
- Ghaneh-Fard, A., Carreau, P.J., and Lafleur, P.G. (1996). Materials, interfaces, and electrochemical phenomena study of instabilities in film blowing. *Aiche Journal* **42**, 1388-1396.
- Gontard, N. Proceedings of Workshop sobre Biopolymers, Active Packaging, Pirassununga, FZEA. Brazil, April 22-24, 1997, pp. 23-27.
- Grower, J.L., Cooksey, D.K., and Getty, K.J.K. (2004). Development and characterization of an antimicrobial packaging film coating containing nisin for inhibition of *Listeria monocytogenes*. *Journal of Food Protection* **67**, 475-479.
- Guenther, E., The Essential Oils, Van Nostrand Co. Inc., New York, 1952.
- Ha, J.U., Kim, Y.M., and Lee, D.S. (2001). Multilayered antimicrobial polyethylene films applied to the packaging of ground beef. *Packaging Technology and Science* **14**, 55-62.
- Halek, W.G., and Anita, G. (1989). Fungal inhibition by a fungicide coupled to an ionomeric film. *Journal of Food Safety* **9**, 215-222.

- Han, C.D., Kim, Y.W., and Chen, S.J. (1975). Melt behaviour and blending of polymers. *Journal of Applied Polymer Science* **19**, 28-31.
- Han, J.H. (1996). Developing antimicrobial packaging materials: review. *The Journal of Korean Food Research Institute* **9**, 111-127.
- Han, J.H., and Floras, J.D. (1997). Casting antimicrobial packaging films and measuring their physical and antimicrobial activity. *Journal of Plastic Film and Sheeting* **13**, 287-298.
- Han, J.H., *Novel Food Packaging Techniques*. In: Antimicrobial Food Packaging. Woodhead Publishing Limited, Cambridge, Ahvenainen, R. (Ed), 2003.
- Han, J.H. (2000). Antimicrobial food packaging. *Food Technology* **54**, 56-65.
- Han, J.H., *Antimicrobial Packaging Systems*. In: Innovations in Food Packaging. Elsevier Academic Press, Amsterdam, The Netherlands, pp. 92-93, 2005.
- Harris, J.M., *Biotechnical and Biomedical Applications*. In: Polyethylene Glycol Chemistry, New York, 1992.
- Helmroth, I.E., Dekker, M., and Hankemeier, Th. (2003). Additive diffusion from LDPE slabs into contacting solvents as a function of solvent absorption. *Journal of Applied Polymer Science* **90**, 1609-1617.
- Hess, K.M. PRI International Conference on PVC Processing, Engineering Royal Holloway college, London, 1978, pp. 1-10.
- Hong, S., Park, J., and Him, D. (2000). Antimicrobial and physical properties of food packaging films incorporated with some natural compounds. *Food Science and Biotechnology* **9**, 38-42.
- Hotchkiss, J.H. (1997). Food packaging interactions influencing quality and safety. *Food Additives and Contaminants* **14**, 601-607.
- Jantan, I.b., Yalvema, M.F., Ayop, N., and Ahmad, S.A. (2005). Constituents of the essential oils of *Cinnamomum sintoc* Blume from mountains forests of peninsular Malaysia. *Flavour and Fragrance Journal* **20**, 601-604.

- Joshi, C. (2006). Nontoxic Animal, Rodent and Insect Repellants for Polymers, [www.rodrepel.com](http://www.rodrepel.com). [www.ctechcorporation.com](http://www.ctechcorporation.com).
- Juven, B.J., Kanner, J., Sched, F., and Weisslowicz., H. (1994). Factors that interact with the antibacterial action of thyme oil and its active constituents. *Journal of Applied Bacteriology* **76**, 626-631.
- Kalvandi, R., Sefidkon, F., Atri, M., and Mirza, M. (2005). Analysis of the essential oil of *Thymus eriocalyx* from Iran. *Flavour and Fragrance Journal* **19**, 341-343.
- Kanjickal, D., Lopina, S., Schmidt, S., and Donovan, D. (2005). Improving delivery of hydrophobic drugs from hydrogels through cyclodextrins. *Journal of Biomedical Materials* **74A**, 454-460.
- Kim, S.J., and Kwon, T.H. (1996). Enhancement of mixing performance of single screw extrusion processes via chaotic flows: Part 1. Basic concepts and experimental studies. *Advances in Polymer Technology* **15**, 41-54.
- Koga, T., Hirota, N., and Takumi, K. (1999). Bactericidal activities of essential oils of basil and sage against a range of bacteria and the effects of these essential oils on *Vibrio parahaemolyticus*. *Microbiology Reserve* **154**, 267-273.
- Labuza, T.P., and Breene, W.M. (1989). Applications of active packaging for improvements of shelf life and nutritional quality of fresh and extended shelf life foods. *Journal of Food Protection* **13**, 1-69.
- LaCoste, A., Schaich, K.M., Zumbrunnen, D., and Yam, K.L. (2005). Advancing controlled release packaging through smart blending. *Packaging Technology and Science* **18**, 77-87.
- Lahariya, A.K., and Rao, J.T. (1979). In Vitro antimicrobial studies of the essential oils of *Cyprus scariosus* and *Ocimum basilicum*. *Indian Drugs* **1916**, 150-152.
- LaMantia, F.P., Valenza, A., and Acierno, D. (1986). Influence of the structure of linear density polyethylene on the rheological and mechanical properties of blends with low density polyethylene. *European Polymer Journal* **22**, 647-652.

- Lee, D.S., Hwang, Y., and Cho, S.H. (1998). Developing antimicrobial packaging film for curled lettuce and soyabean sprouts. *Food Science and Biotechnology* **7**, 117-121.
- Lee, S.Y., and Kim, S.C. (1996). Morphology and oxygen barrier properties of LDPE/EVOH blends. *International Journal of Polymer Processing* **3**, 238-247.
- Liu, X., Xie, M., and Li, H. (2005). Effect of inorganic fillers in binary processing aids on the rheology of a metallocene linear low density polyethylene. *Journal of Applied Polymer Science* **96**, 1824-1829.
- Lorenzo, D., Loayza, I., and Dellacassa, E. (2003). Composition of the essential oils from leaves of two *Hedyosmum* spp. and bolovia. *Flavour and Fragrance Journal* **18**, 32-35.
- Mario, D., Alessandra, T., Antinio, F., and Camella, C. (1998). In vivo activity of *Salvia officinalis* oil against *Botrytis cinerea*. *Journal of Essential Oil* **10**, 157-160.
- Marotti, M., Piccaglia, R., and Giovanelli, E. (1996). Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. *Journal of Agricultural and Food Chemistry* **44**, 3926-3929.
- Matthews, G., *Polymer Mixing Technology*, Applied Science Publishers, New York, pp. 10-61, 1982.
- Maxcy, R.B. (1981). Surface microenvironment and penetration of bacteria into meat. *Journal of Food Protection* **44**, 550-552.
- Middleman, S., *Fundamental Studies of Polymer Processing*, McGraw-Hill, New York, 1977.
- Miltz, J., *Migration of Low Molecular Weight Species from Packaging Materials: Theoretical and Practical Considerations*. In: *Food Product-Package Compatibility*, Technomic Pub. Co., Gray, J.I., Harte, B.R., and Miltz, J. (Eds), pp. 1987.
- Miltz, J., Passy, N., and Mannhwim, C.H., *Trends and Applications of Active Packaging Systems*. In: *Food and Packaging Materials - Chemical Interaction*, The Royal

Society of Chemistry, Ackerman, P., Jägerstad, M., and Ohlsson, P. (Eds), pp. 201-210, 1995.

- Ming, X., Weber, G.H., Ayres, J.W., and Sandine, W.E. (1997). Bacteriocins applied to food packaging materials to inhibit *Listeria monocytogenes* on meats. *Journal of Food Science* **62**, 413-415.
- Moly, A.K., Radush, J.H., Androsch, R., Bhagawan, S.S., and Thomas, S. (2005). Nonisothermal crystallisation, melting behaviour and wide angle X-ray scattering investigations on linear low density polyethylene (LLDPE)/ethylene vinyl acetate (EVA) blends: effects of compatibilisation and dynamic crosslinking. *European Polymer Journal* **41**, 1410-1419.
- Neetu, J., Srivastava, S.K., Aggarwal, K.K., Ramesh, S., and Kumar, S. (2001). Essential oil composition of *Zanthoxylum alatum* seeds from northern India. *Flavour and Fragrance Journal* **16**, 408-410.
- Nicholson, M.D. (1998). The role of natural antimicrobials in food/packaging biopreservation. *Journal of Plastic Film and Sheeting* **14**, 234-241.
- Olasupo, N.A., Fitzgerald, D.J., Narbad, A., and Gasson, M.J. (2004). Inhibition of *Bacillus subtilis* and *Listeria innocua* by nisin in combination with some naturally occurring organic compounds. *Journal of Food Protection* **67**, 596-600.
- Onawunmi, G.O., Yisak, W.A., and Ogunlana, E.O. (1984). Antibacterial constituent in essential oil of *Cymbopogon citratus*. *Journal of Ethnopharmacol* **12**, 279-286.
- Padgett, T., Han, I.Y., and Dawson, P.L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection* **61**, 1330-1335.
- Paik, J.S., Dhanasekharan, M., and Kelly, M.J. (1998). Antimicrobial activity of UV-irradiated nylon film for packaging applications. *Packaging Technology and Science* **11**, 179-187.

- Park, J.S., Kim, J.H., Nho, Y.C., and Kwon, O.H. (1998). Antibacterial activities of acrylic acid-grafted polypropylene fabric and its metallic salt. *Journal of Applied Polymer Science* **69**, 2213-2220.
- Parry, R.T., Introduction in Principles and Applications of Modified Atmosphere Packaging of Food, Blackie Academic and Professional, Glasgow, pp. 1-18, 1993.
- Pirkle, C., and Braatz, R. (2003). Dynamic modeling of blow film extrusion. *Polymer Engineering and Science* **43**, 398-418.
- Podhajny, R.M. (2004). Antimicrobials on the fast track. *Material Columnist Science* **20**, 20.
- Prasad, G., Kumar, A., Singh, A.K., Bhattacharya, A.K., Singh, K., and Sharma, V.D. (1986). Antimicrobial activity of essential oils of some Ocimum species and clove oil. *Fitoterapia* **57**, 429-432.
- Radulovic, N., Stojanovic, G., and Palic, R. (2006). Composition and antimicrobial activity of Equisetum arvense L. essential oil. *Phytotherapy Research* **20**, 85-88.
- Raina, V.K., Srivastava, S.K., Aggarwal, K.K., Ramesh, S., and Kumar, S. (2001). Essential oil composition of Cinnamomum zeylanicum Blume leaves from Little Andaman, India. *Flavour and Fragrance Journal* **16**, 374-376.
- Reuveni, R., Fleischer, A., and Putievsk, E. (1984). Fungistatic activity of essential oils from ocimum basilicum. *Journal of Essential Oil* **110**, 20-22.
- Roberto, V., and James, S. (2006). Chemical characterization of basil (Ocimum spp.) based on volatile oils. *Flavour and Fragrance Journal* **21**, 214-221.
- Robertson, G.L., Food Packaging: Principles and Practice, Marcel Dekker, New York, 1993.
- Rooney, M.L., *Plastics in Active Packaging*. In: Materials and Development of Plastic Packaging, Rooney, M.L. (Ed), 1995a.
- Rooney, M.L., *Active Packaging in Polymer Films*. In: Active Food Packaging, Blackie Academic & Professional, London, 1995b.

- Scannell, A.G.M., Hill, C., Ross, R.P., Marx, S., Hartemeier, W., and Arendt, E.K. (2000). Development of bioactive food packaging materials using immobilised bacteriocins Lactin 3147 and Nisaplin. *International Journal of Food Microbiology* **60**, 241-249.
- Seabrook, Jr., Samuel, G., Willam, E., and Craver, I. Polymer Containing Antimicrobial Agents and Methods for Making and Using Same, Patent Issue 953908, 1997, Magellan Companies Inc., USA.
- Sefidkon, F., Kalvandi, R., Arti, M., and Barazandeh, M.M. (2005). Essential oil variability of *Thymus eriocalyx* (Ronniger) Jalas. *Flavour and Fragrance Journal* **20**, 521-524.
- Shen, A.-Y., Huang, M.-H., Liao, L.-F., and Wang, T. (2005). Thymol analogues with antioxidant and L-type calcium current inhibitory activity. *Drug Development Research* **64**, 195-202.
- Sherman, and Manolis, L. (1998). Biocides keep the bugs off your plastics. *Plastic Technology* **44**, 45
- Sidiropoulos, V., and Vlachopoulos, J. (2005). Temperature gradient in blown film bubbles. *Advances in Polymer Technology* **24**, 83-90.
- Simon, J.E., Quinn, J., and Murray, R.G., *Advances in New Crops Research*, Portland, Oregon, Simon, J.E. (Ed), pp. 484-489, 1990.
- Singh, G., Maurya, S., and Catalan, C. (2005). Studies on essential oils, Part 41. Chemical composition, antifungal, antioxidant and sprout suppressant activities of coriander (*Coriander sativum*) essential oil and its oleoresin. *Flavour and Fragrance Journal*, In press.
- Sinha, G.K., and Gulathi, B.C. (1990). Antimicrobial and antifungal study of some essential oils and some of their constituents. *Indian Perfumer* **34**, 126-129.
- Suppakul, P., Miltz, J., Sonneveld, K., and Bigger, S.W. (2003a). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science, Concise Reviews and Hypotheses* **68**, 408-420.

- Suppakul, P., Miltz, J., Sonneveld, K., and Bigger, S.W. (2003b). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry* **51**, 3197-3207.
- Suppakul, P. Study of Antimicrobial Films Containing Basil Extracts, PhD Thesis, Polymer Packaging Research Unit, Victoria University, Melbourne, Australia, 2004.
- Suyatma, N., Tighzert, L., and Copinet, A. (2005). Effects of hydrophilic plasticizers on mechanical, thermal and surface properties of Chitosan films. *Journal of Agricultural and Food Chemistry* **53**, 3950-3957.
- Tadmor, Z., and Gogos, C.G., Principles of Polymer Processing, Wiley Interscience, New York, pp. 89-92, 1979.
- Tepe, B., Daferera, D., Sokmen, M., Polissiou, M., and Sokmen, A. (2004). In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigi*. *Journal of Food Protection* **52**, 1132-1137.
- Thompson, D.P. (1989). Fungitoxic activity of essential oil components on food storage fungi. *Mycologia* **81**, 151-153.
- Tillekeratne, M., and Easteal, A. (2000). Modification of zein films by incorporation of polyethylene glycols. *Polymer International* **49**, 127-134.
- Todd, D. (1999). Improving the incorporation of fillers in plastics. A special report. *Advances in Polymer Technology* **19**, 54-64.
- Vartiainen, J., Skytta, E., and Ahvenainen-Rantala, R. (2003). Antimicrobial and barrier properties of LDPE films containing Imazalil and EDTA. *Journal of Plastic Film and Sheeting* **19**, 249-261.
- Walter, P., Mader, D., and Reichert, P. (1999). Novel polypropylene materials. *Journal of Macromolecular Science* **36**, 1613
- Wang, X., Wang, Z., and Wang, X. (2005). Preparation and characterization of linear low-density polyethylene/low-density polyethylene/TiO<sub>2</sub> membranes. *Journal of Applied Polymer Science* **98**, 216-221.

- Wang, Z., Li, G., Xie, g., and Zhang, Z. (2005). Dispersion behaviour of titanium dioxide nanoparticles in LLDPE and LDPE nanocomposites. *Macromolecular Chemistry and Physics* **206**, 258-262.
- Weng, Y.M., and Hotchkiss, J.H. (1992). Inhibition of surface moulds on cheese by polyethylene film containing the antimycotic imazalil. *Journal of Food Protection* **55**, 367-369.
- Weng, Y.M., and Hotchkiss, J.H. (1993). Anhydrides as antimycotic agents added to polyethylene films for food packaging. *Packaging Technology and Science* **6**, 123-128.
- Xie, M., Liu, X., and Li, H. (2006). Influence of polyethylene glycol containing additives on the extrusion of ultra high molecular weight polyethylene/polypropylene blend. *Journal of Applied Polymer Science* **100**, 1282-1288.
- Yam, K.L., and Lee, D.S., *Design of Modified Atmosphere Packaging for Fresh Produce*. In: Active Food Packaging, Blackie Academic & Professional, London, Rooney, M.L. (Ed), p. 55, 1995.
- Yam, K.L., Takhistov, P.T., and Miltz, J. (2005). Intelligent packaging: Concepts and applications. *Journal of Food Science, Concise Reviews and Hypotheses* **70**, R1-R10.
- Youdim, K.A., and Deanes, S.G. (2000). Effect of thyme oil and thymol dietary supplementation on the antioxidant status and fatty acid composition of the ageing rat brain. *Journal of Nutrition* **83**, 87-93.
- Zhou, L.Y. Quantitative Analysis of Additives in Low Density Polyethylene Using On-line Supercritical Fluid Extraction/Supercritical Fluid Chromatography, Masters Thesis, Faculty of the Virginia Polytechnic Institute and State University, 1998.

## Appendix A Material Properties

**Table A.1.** Typical properties of LDPE resin

<b>Polymer Resin: Qenos™ ALKATHENE® XLF197, Low-Density Polyethylene</b>			
<b>Product Characteristics</b>			
Processing method	Blown film extrusion grade		
Additives	No additives (could be incorporated to enhance cling performance)		
Features	Suitable for thin film guage (less than 20 microns)		
Uses	Packaging		
Manufacturer	Qenos Pty Ltd.		
<b>Physical Properties</b>			
Density	0.920 g cm <sup>-3</sup>	ASTM D1505	
Melt Index (190°C/2.16 kg)	5.5 dg min <sup>-1</sup>	ASTM D1238	
<b>Typical Film Properties</b> (15 µm thickness; blow ratio 3.2 to 1)			
Haze	3.5%	ASTM D1003	
Gloss	74 units	ASTM D2457	
Dart Impact	45 g	ASTM D1709	
Tear strength (N)	2.9 (MD)	0.8 (TD)	ASTM D1922
Tensile yield (MPa)	9 (MD)	10 (TD)	ASTM D882
Tensile strength (MPa)	22 (MD)	15 (TD)	ASTM D882
% strain at break	120% (MD)	670% (TD)	ASTM D882
Stiffness modulus (MPa)	150 (MD)	190 (TD)	ASTM D882
<b>Processing Information</b>			
FDA Status	Complies with Food and Drug Administration Regulation 21 CFR 177.1520(c) 2.1 and AS2070-1999 section 4.1.1(a). Not applicable for use in articles that contact food except for articles used for packaging or holding food during cooking.		

**Table A.2.** Typical properties of EVA resin

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<b>Polymer Resin: Dupont™ ELVAX® 3120, Ethylene Vinyl Acetate</b>		
<b>Product Characteristics</b>		
Processing method	Extrusion	
Composition	7.5 % by weight Vinyl Acetate	
Additives	Antiblock and slip	
Features	Copolymer	
Uses	Packaging	
Manufacturer	Dupont Packaging and Industrial Polymers	
<b>Physical Properties</b>		
Density	0.93 g cm <sup>-3</sup>	ASTM D792-ISO 1183
Melt Index (190°C/2.16 kg)	1.2 dg min <sup>-1</sup>	ASTM D1238-ISO 1133
Vicat Softening Point	84°C	ASTM D1525-ISO 306
Melting Point	99°C	ASTM D3417
Freezing Point	82°C	ASTM D3417
<b>Processing Information</b>		
FDA Status	Complies with Food and Drug Administration Regulation 21 CFR 177.1350(a)(1). This regulation describes polymers to be used in contact with food, subject to finish food-contact article meeting extractive limitations under the intended conditions of use as shown in paragraph (b)(1) of the regulation, for use in articles that contact food except for articles used for packaging or holding food during cooking.	
General processing information	Can be used in conventional extrusion equipment designed to process polyethylene resins. Applicable for blown film grades. Selection of desire temperature is subject to desire gauge, height of tower, cooling capacity, extruder hold-up time and machine variables.	

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**Table A.3.** Typical properties of PEG resins

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**Polymer Resin: A1683 PEG 4000, PEG 200000, PEG 500000**

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**Product Characteristics**

Molecular Weight	4000 / 200000 / 500000
Appearance	White waxy solid
Stability	Stable
Features	Soluble in most organic solvents
Uses	Binding agent and laboratory applications
Manufacturer	Ajax Finechem, Australia
Applications	Food and cosmetic applications

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**Physical Properties**

Melting Point	35°C – 60°C
Boiling Point	Not available
Vapour Pressure	Not available
Specific Gravity	1.2 @ 20°C
Flammability Limits	Not available
pH	4.0 – 7.5 (5% aqueous solution)

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**Table A.4.** Properties of AM agent linalool

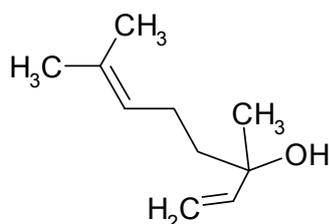
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**AM Agent: Linalool, Product Code L2602 Linalool  $\geq 97\%$**

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Synonyms: ( $\pm$ )-3,7-Dimethyl-1,6-octadien-3-ol  
( $\pm$ )-3,7-Dimethyl-3-hydroxy-1,6-octadiene  
( $\pm$ )-Linalool

Structure:



Molecular Formula:  $(\text{CH}_3)_2\text{C}=\text{CHCH}_2\text{CH}_2\text{C}(\text{CH}_3)(\text{OH})\text{CH}=\text{CH}_2$

Molecular Weight: 154.25

CAS Number: 78-70-6

Beilstein Registry Number: 1721488

EG/EC Number: 2011344

Vapour Pressure: 0.17 mm Hg (25 °C)

Boiling Point: 194-197 °C/720 mm Hg (lit.)

Flash Point: 174 °F

Density: 0.87 g mL<sup>-1</sup> at 25°C (lit.)

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**Table A.5.** Properties of AM agent thymol

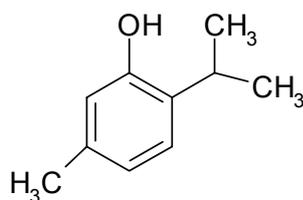
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**AM Agent: Thymol, Product Code T0501 Thymol  $\geq 99.5\%$**

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Synonyms: 2-Isopropyl-5-methylphenol  
5-Methyl-2-(1-methylethyl)phenol  
5-Methyl-2-isopropylphenol

Structure:



Molecular Formula: 2-[(CH<sub>3</sub>)<sub>2</sub>CH]C<sub>6</sub>H<sub>3</sub>-5-(CH<sub>3</sub>)OH

Molecular Weight: 150.22

CAS Number: 89-83-8

Beilstein Registry Number: 1907135

EG/EC Number: 2019448

Vapour Pressure: 1 mm Hg (64°C)

Boiling Point: 232°C (lit.)

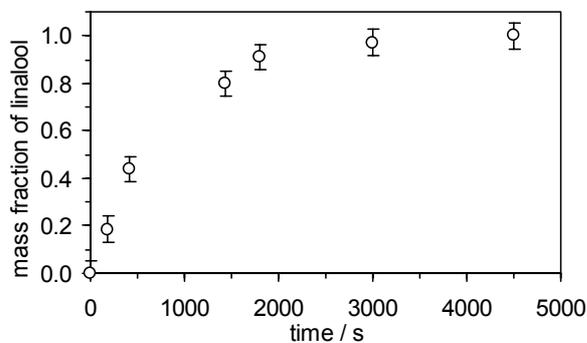
Melting Point: 48-51°C (lit.)

Density: 0.965 g mL<sup>-1</sup> at 25°C (lit.)

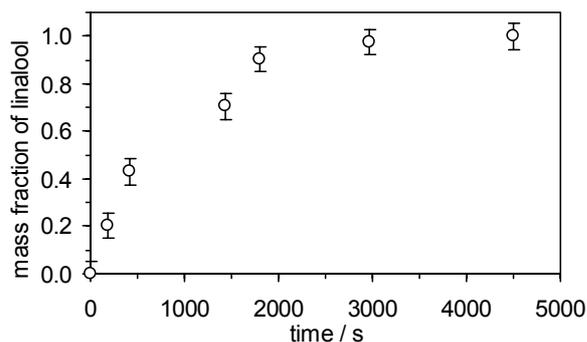
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## Appendix B Supplemental Figures

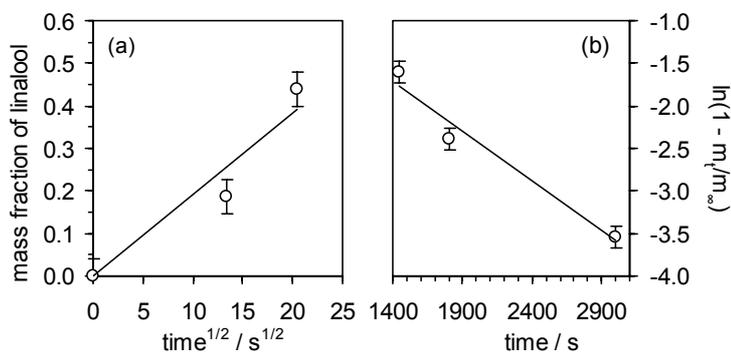
### Effect of PEG on the Release of AM Agent



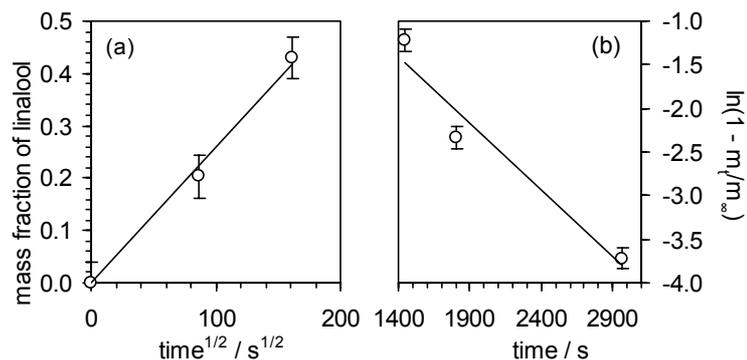
**Figure B.1.** Plot of mass fraction of linalool ( $m_t/m_\infty$ ) released into isooctane at 25°C versus time from the compression moulded film containing 2% (w/w) PEG2 (P3).



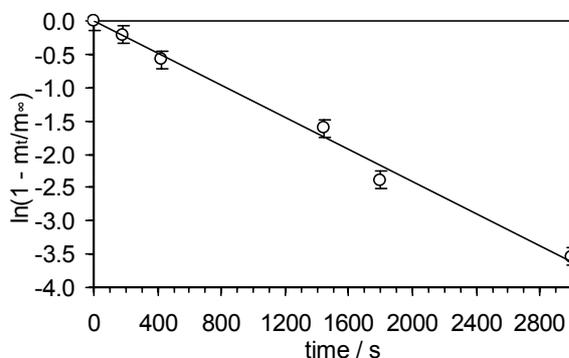
**Figure B.2.** Plot of mass fraction of linalool ( $m_t/m_\infty$ ) released into isooctane at 25°C versus time from the compression moulded film containing 2% (w/w) PEG3 (P4).



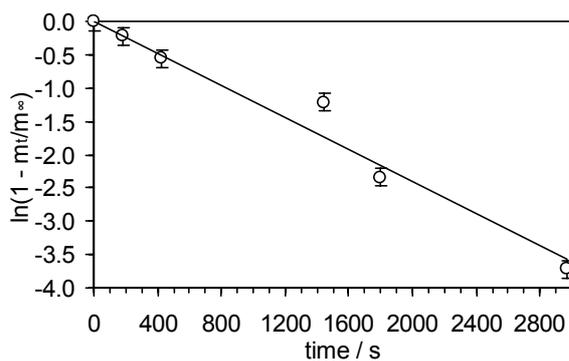
**Figure B.3.** Plots of: (a) ( $m_t/m_\infty$ ) versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 2% (w/w) PEG2 (P3) in isooctane at 25°C.



**Figure B.4.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for a blend containing 2% (w/w) PEG3 (P4) in isooctane at 25°C.

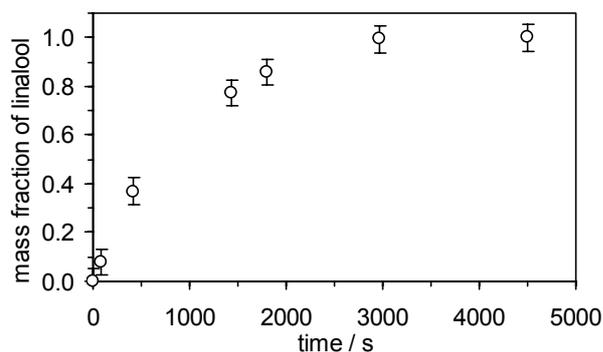


**Figure B.5.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from the compression moulded film containing 2% (w/w) PEG2 (P3).

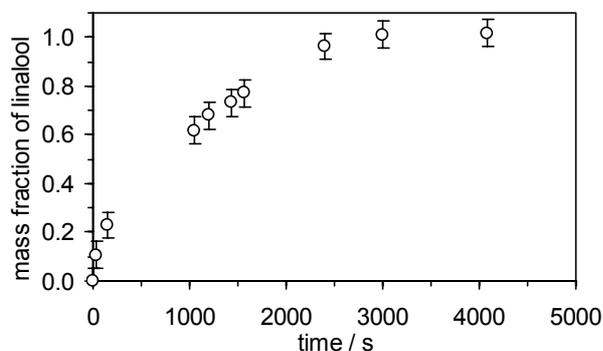


**Figure B.6.** Plot of  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C from the compression moulded film containing 2% (w/w) PEG3 (P4).

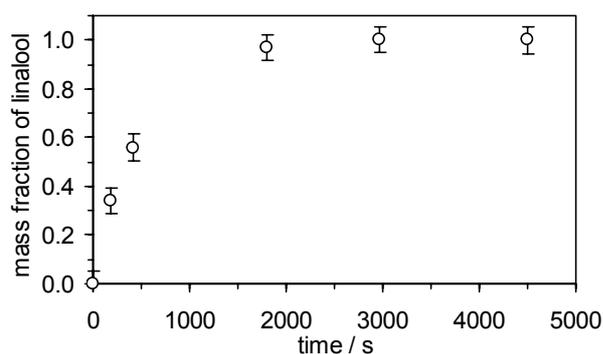
## Effect of EVA on the Release of AM Agent



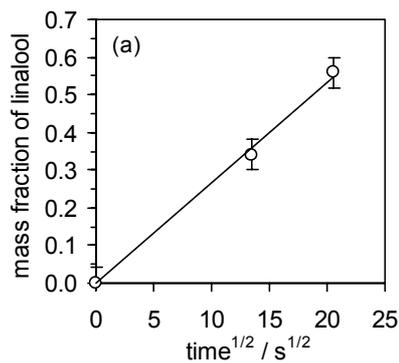
**Figure B.7.** Plot of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from compression moulded film containing 0% (w/w) EVA (E0).



**Figure B.8.** Plot of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from compression moulded film containing 10% (w/w) EVA (E1).

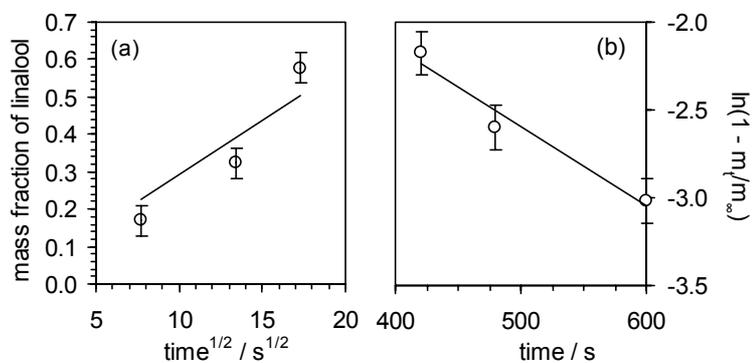


**Figure B.9.** Plot of mass fraction ( $m_t/m_\infty$ ) of linalool released into isooctane at 25°C versus time from compression moulded film containing 50% (w/w) EVA (E2).

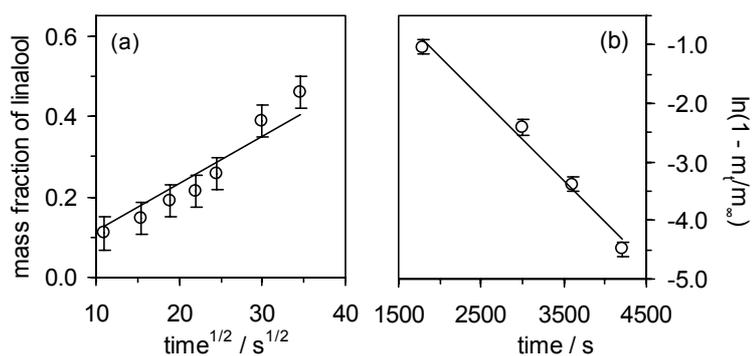


**Figure B.10.** Plot of  $(m_t/m_\infty)$  versus  $t^{1/2}$  versus time for a blend containing 50% (w/w) EVA (E2) into isooctane at 25°C.

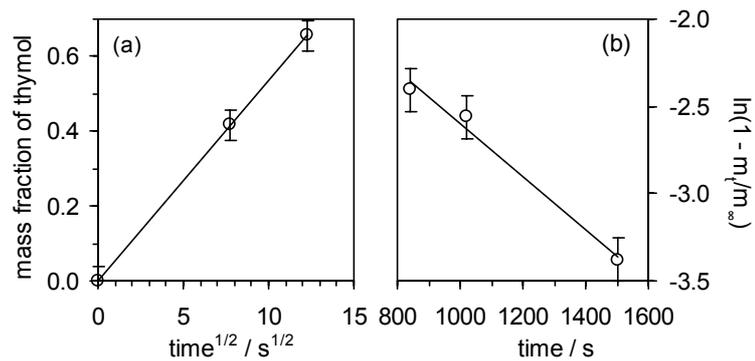
### Effect of Food Simulant on the Release of AM Agent



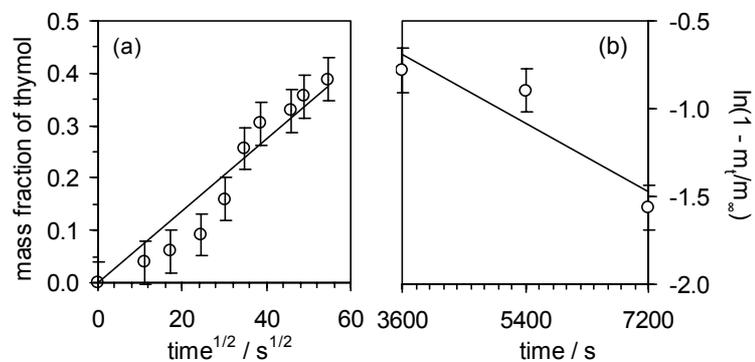
**Figure B.11.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into isooctane at 25°C for the F2 film.



**Figure B.12.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of linalool into 95% ethanol at 25°C for the F2 film.



**Figure B.13.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol into isooctane at 25°C for the F3 film.



**Figure B.14.** Plots of: (a)  $(m_t/m_\infty)$  versus  $t^{1/2}$  and (b)  $\ln(1 - m_t/m_\infty)$  versus time for the release of thymol into 95% ethanol at 25°C for the F3 film.