Development of Multi-layer Films Containing Natural Antimicrobial Agents

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Declaration

"I, Chanun Rardniyom, declare that the PhD thesis entitled Development of Multi-layer Films Containing Natural Antimicrobial Agents is no more than 100,000 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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Abstract

In this research, multi-layer packaging films comprising an inner coating layer containing natural antimicrobial (AM) agents were developed. Volatile essential oils obtained from plant extracts including carvacrol, linalool and methylchavicol were used as the active AM agents in the coating layer. The amount of natural AM agents and the composition of the coating system required to provide the optimum AM effect in food media and on Cheddar cheese was explored. The stability of the AM coatings in various food simulants was also explored. In the film preparation, the AM coatings were embedded between low-density polyethylene (LDPE) layers to construct multi-layer AM films. Investigation of the retention and subsequent release of the AM agent from multi-layer films suggested that a coating layer consisting of ethylacrylate-methylmethacrylate performs satisfactorily as the carrier for the AM agent. Furthermore, LDPE is able to successfully control the release of the AM agent into food simulants. The controlling layer thickness, AM layer thickness and the solubility of AM agent are critical factors affecting the overall effectiveness of the multi-layer AM film. At subambient temperatures, the multi-layer AM films successfully inhibited the growth of *E. coli* in liquid food media and on Cheddar cheese.

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List of Abbreviations

A	Area of AM film
A. niger	*Aspergillus niger*
A. oryzae	*Aspergillus oryzae*
AIT	Allyl isothiocyanate
AM	Antimicrobial
AMP	Antimicrobial peptide
ANOVA	Analysis of variance
B. thermosphacta	*Brochothrix thermosphacta*
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C_F	Concentration of AM agent in food simulant
CMC	Carboxy methylcellulose
C_P	Concentration of the AM agent in controlling layer
D_0	Pre-exponential factor
D_F	Diffusion coefficient of the AM agent in food simulant
D_P	Diffusion coefficient of AM agent in controlling layer
E. coli	*Escherichia coli*
E_a	Activation energy of diffusion
EA-MMA	Ethylacrylate-methylmethacrylate
EDTA	ethylenediamine tetraacetic acid
EVOH	Ethylene vinyl alcohol
F. oxysporum	*Fusarium oxysporum*
FDA	Food and Drug Administration
FID	Flame ionization detection
FT-IR	Fourier Transform Infrared
GFSE	Grapefruit seed extract
GRAS	Generally Recognised As Safe
HMT	Hexamethylenetetramine
HPMC	Hydroxypropyl methylcellulose
K	Kinetic rate constant
k_0	Pre-exponential factor
$K_{F/P}$	Partition coefficient of AM agent between food simulant and controlling layer
$K_{P/F}$	Partition coefficient of AM agent between controlling layer and food simulant

$K_{P/S}$	Partition coefficient of AM agent between controlling layer and reservoir
$K_{S/P}$	Partition coefficient of AM agent between reservoir and controlling layer
Lb. leichmannii	*Lactobacillus leichmannii*
Lb. sakei	*Lactobacillus sakei*
Lb. plantarum	*Lactobacillus plantarum*
Lb. reuteri	*Lactobacillus reuteri*
LDPE	Low-density polyethylene
Leu. Mesenteroides	*Leuconostoc Mesenteroides*
l_F	Boundary layer in food simulant
Lis. innocua	*Listeria innocua*
Lis. monocytogenes	*Listeria monocytogenes*
LLDPE	Linear low density polyethylene
l_P	Thickness of controlling layer
M_∞	Total of mass of AM agent released from films at equilibrium
M. flavus	*Micrococcus flavus*
MAP	Modified atmosphere packaging
MC	Methylcellulose
MIC	minimum inhibitory concentration
M_t	Mass of AM agent released from films at time *t*
P. chrysogenum	*Penicillium chrysogenum*
PE	Polyethylene
PEG	Polyethylene glycol
PEMA	Poly(ethylene-co-methacrylic acid)
Pen. Spp.	*Penicillium spp.*
PE-OPA	Polyethylene-oriented polyamide
PET	Polyethylene terephthalate
PP	Polypropylene
PPBAC	Partially purified bacteriocin
PVC	Polyvinyl chloride
PVOH	Polyvinyl alcohol
R	Ideal gas constant (8.3143 J mol^{-1} K^{-1})
RCF	Regenerated cellulose films
S	Solubility coefficient of AM agent
S. aureus	*Staphylococcus aureus*

S. cerevisiae	*Saccharomyces cerevisiae*
S. enteritidis	*Salmonella enteritidis*
S. typhimurium	*Salmonella typhimurium*
SPI	Soy protein isolate
T	Temperature
t	Time
$t_{½}$	Half-life of release
TBHQ	Tertiary butylhydroquinone
t_s	Storage time
v	Release rate
v_0	Initial release rate
V_1	Volume of reservoir
V_2	Volume of food simulant
WPI	Whey protein isolate
WVTR	Water vapour transmission
ΔC	Concentration gradient

1 INTRODUCTION

This chapter introduces the background of food packaging and, in particular, antimicrobial (AM) packaging – a novel packaging technology that aims to prolong the shelf-life of food by inhibiting the growth of contaminating microorganisms. It also examines the advantages and various aspects of AM food packaging. In addition, it explores the aims and scope of this research.

1.1 Background

In food manufacturing, "packaging" is a discipline of a post-production process, where a product is enclosed in a container (or wrapping) for many purposes, including protection, transportation, distribution, storage, retailing and end-use (Robertson, 2006). In 1985, the Codex Alimentarius Commission referred to the roles of food packages, as follows: "Food is packaged to preserve its quality and freshness, add appeal to customers and to facilitate storage and distribution". Although "packing" means the enclosing procedure and "package" is the container such as a pouch, a bag, a box, a cup, a tray, a can, a tube, a bottle, *etc*., the statement used the verb "be packaged" in place of "be packed". In addition, it described four basic functions of food packaging *i.e.* containment, protection, convenience and communication (Robertson, 2006).

1.1.1 Conventional food packaging

Although it is not known when humans first made containers for storing food, various types of food containers are often found in historical sites such as the Fukui and Sempukuji caves in Nagasaki, Japan, where 10,000 year-old earthenware containers were found (Weeder, 1991). Most of these containers were prepared from fired clay lasting for thousand of years. Other forms of food packages or containers that were portable might have been made of organic, light-weight materials such as shells, animal organs, woven grasses, gourds, parts of trees including hollowed logs and leaves (Hook and Heimlich, 2007). These organic materials would easily decompose in the environment and would therefore leave little evidence of their use. Fabrics made of furs and felts woven from fibres, for example, were used to create clothing and wrapping materials such as bags (Hook and Heimlich, 2007). The weaving of

grasses and reeds was extended to provide baskets to store food and metal and pottery were available after ores and other useful compounds were discovered. Table 1.1 shows a series of historical events in the development of conventional packaging, which was described by Hook and Heimlich (2007) and Den Hartog (2007).

Table 1.1 Time-line of developments in conventional food packaging.

Year/Century	Development in packaging
7000 B.C.	Pottery invented
1500 B.C.	Glass industrialised in Egypt
200 B.C.	Paper made in China
1200	Tinplate made in Bohemia
1400s	Iron cans coated with tin made in Bavaria
16th	Cardboard made in China
1809	Tin cans made
1810	Tinplate patented
1841	Collapsible soft aluminium tubes invented
1870	Celluloid patented
1898	Polyethylene (PE) made accidently
1880s	Paraffin coating made fat and fluid impermeable paper and cartons
1900	Cellulose acetate derived from wood pulp
1910	Aluminium foils made
1924	Cellophane made in New York
1933	Styrene process refined in German
1935	Synthesis for PE developed in UK
1939	Low-density polyethylene (LDPE) industrialised in UK
1940s	Food packaging entered era of disposable packaging
1950s	Polypropylene (PP) invented
1950	Aluminium foil containers developed
1950	Foam boxes, cups and meat trays made from styrene
1950	Cellophane commercialised and used for packaging
1952	Cartons coated with PE replaced glass and cans for milk and fruit drinks
1958	Heat shrinkable films developed from styrene and synthetic rubber
1959	Aluminium cans made
1977	Polyethylene terephthalate (PET) used for beverages

Source: (Den Hartog, 2007; Hook and Heimlich, 2007)

1.1.2 Modern food packaging

There are typically three circumstances where a food package interacts with the environment (Robertson, 2006). Firstly, the package is in physical contact with its surroundings that may cause damage to the packaged food. Secondly, it is in contact with the ambient surroundings which can include gases, water, light, temperature, microorganisms and pests. Thirdly, it can affect potential purchasers with its appearance and the information on it. However, other than the interaction of food package with its environment, there is another category of food package that is designed to have the ability to interact with the packed constituents (Ahvenainen, 2003). Table 1.2 shows a series of historical events that lead the development of packaging entered this new era.

Table 1.2 Time-line of developments in active food packaging.

Year/Century	Development in active packaging
1810	Tinplate used for consuming residual O_2 in cans and reacting to protect iron base
1938	Fe, Zn, Mn used to remove O_2 from cans
1943	Fe used to absorb O_2 from vacuum packs / gas packs
1954	Regenerated cellulose films (RCF) impregnated with sorbic acid used for cheese
1955	Catalytic conversion of H_2 used to remove O_2 from tinplate cans and laminate pouches
1956	Enzyme impregnated in particular glucose oxide to remove O_2 from food packages patented
1958	Packets and sachets of chemicals for removing O_2 from food packages first published
1968	Sodium carbonate powder for removing O_2 from food packages patented
1970	Potassium permanganate used to absorb ethylene in LDPE bags
1973	Antimicrobial wrappers used to extend food shelf life
1976	Iron-based O_2 scavengers commercialised
1980s	Packaging made for microwave uses and modified atmosphere packaging (MAP) developed for ready-to-eat fresh vegetables and fruits
1986	First use of term “Smart packaging” and “Interactive packaging”
1987	First use of term “active packaging”
1995	Carbon dioxide generators commercialised
1995	Sulfur dioxide-releasing pad invented
1997	Ethanol-generating films or sachets patented

Source: (Ahvenainen, 2003; Robertson, 2006)

This novel stream of packaging technology is given many names such as “smart packaging”, “interactive packaging”, “active packaging”, “clever packaging” or “intelligent packaging” (Ahvenainen, 2003). The purpose of this food packaging is not only to ensure that the product will be transported safely, distributed and stored conveniently, displayed appealingly in the retail shops and used conveniently by the customers (Robertson, 2006) but also it can to a certain extent regulate the condition of the packaged food. This regulation leads to an advantageous performance of packaging such as releasing preferential additives or absorbing undesired substances, which in most cases extends the product’s shelf-life (Ahvenainen, 2003). For example, some are designed to release ethanol (Floros *et al.*, 1997; Smith *et al.*, 1987), carbon dioxide (Floros *et al.*, 1997; Vermeiren *et al.*, 1999), sulfur dioxide (Christie *et al.*, 1997; Thomas *et al.*, 1995), antioxidants (Hotchkiss, 1997) or AM agents (Vermeiren *et al.*, 2002) into the packages. Other active packages may be designed to absorb excessive moisture (Rooney, 1995), unwanted odours, flavours (Ahvenainen, 2003) or gases like oxygen (Smith *et al.*, 1986; Teumac, 1995), and ethylene (Rooney, 1995) from the package. In some advanced designs, active packaging may be able to improve the quality of the food. For instance, some packages can remove allergens from food such as the packages that remove lactose or cholesterol from milk and other dairy products (Ahvenainen, 2003).

1.1.3 Antimicrobial packaging

Antimicrobial packaging is a type of active packaging, where the package is designed to release active agents to inhibit the growth of microorganisms inside the package. This type of packaging is in contrast with that involving the addition of chemical preservatives directly into the food matrix, where an excess amount of these synthetic additives is believed to be of concern. For the consumer, it seems safer when active agents are indirectly integrated in the food package and released into the food product thereafter. Moreover, consumers tend to accept products to which naturally occurring substances have been added more than those containing synthetic agents (Han, 2005). This trend subsequently draws many researchers to integrate natural AM agents into food packaging materials, especially plant-derived AM agents (Nicholson, 1998; Suppakul, 2004).

Many studies on AM packaging have been conducted, particularly in the last 10 years (Suppakul, 2004). Typically the AM agents are incorporated in a polymer matrix or coated on a polymer film where the AM layer is in direct contact with the packaged food. The migration of the AM agent from this type of film is categorised as the "matrix model" (Richards, 1985). The AM film can also be manufactured as a multi-layer structure where an additional layer of polymer film is laminated on the active layer. The release of the AM agent from a multi-layer film is commonly known as a "reservoir model" (Richards, 1985). The release of AM agents from a composite or multi-layer film is inhibited by the additional layer which works as a controlling layer. Manufacturers can choose one material with a high capacity in which to store the AM agent in order to create the AM layer and use another material that has an optimum barrier property for the controlling layer (Brandsch *et al.*, 1999). Consequently, the advantage of the multi-layer film is that the direction of the release of active agents is somewhat controllable. Nevertheless, it is necessary to select the right materials and choose the right thicknesses of the film structure so that the produced film can release the AM agent at an adequate rate and over an appropriate period of time (Brandsch *et al.*, 1999).

In the pharmaceutical industry recently copolymers of polyacrylates were introduced for manufacturing drug capsules. Such polymers are claimed to have a good capacity for controlled drug-release and even have an ability to target the release in the human digestion system (Khan, 2001; Kshirsagar, 2000). In the packaging industry, polyacrylates are also used as adhesives for laminating extruded polyamide (Nylon™) with polyethylene (PE), a common laminated film used in vacuum packaging for food (Sidwell, 2007). Therefore, it is of interest to study the possibility of using polyacrylates as a potential controlled release carrier for natural AM agents in the structure of an AM packaging film.

The ability to prolong the shelf-life of food with synthetic or natural preservatives was a major development in the storage of many food products. The safety of synthetic preserving agents, however, has been challenged in regard to the potential side-effects of these on the human body (Lück and Jager, 1997). Recently, the use of AM agents derived from naturally occurring substances is back in fashion. These include compounds derived from animal, plant and microbial sources (Devidson and

Zivanovic, 2003). Antimicrobial extracts from spices and their essential oils have been said to have the greatest potential in food application. For example, allium from onion and garlic would be most suitable for incorporating into some types of Indian foods (Minakshi De, 1999). Linalool derived from sweet basil would be acceptable to be added into some Thai foods (Chaisawadi *et al.*, 2005). Thymol and carvacrol from oregano would be suitable for use in pizza (Bertelli *et al.*, 2003; Ultee and Smid, 2001).

1.2 Aim of this research

The aim of this research is to investigate multi-layered packaging films containing natural AM agents, with the following specific objectives:

1. Creating a new coating system that contains natural AM agents applied to an existing, commercially available packaging film;
2. Testing whether the produced films retain the incorporated AM substances at a level that is high enough to be active;
3. Testing whether and how the thickness of the film layers influence the release of the incorporated AM agents into selected food simulants;
4. Testing whether and to what extent the produced AM films demonstrate the inhibition of microorganism growth on a range of media.

1.3 Scope of this research

This research focused on exploring the development of multi-layer AM packaging film comprising a PE carrier film coated with a variety of coating materials such as cellulose derivatives and polyacrylates. These coating materials were tested for their ability to retain natural AM agents in the matrix. The AM agents that were incorporated in the selected coating system are linalool and methylchavicol – the principal constituents of sweet basil (*Ocimum basilicum*); and carvacrol – a major component of oregano (*Origanum vulgare*). Methylchavicol, linalool and carvacrol were used in this work because they are plant-derived AM agents that can inhibit a wide range of bacteria (Suppakul *et al.*, 2003a; Ultee *et al.*, 2002). The integrated AM substances were tested for their AM activity against *Escherichia coli* in solid agar media, liquid broth media and on commercial Cheddar cheese.

2 LITERATURE REVIEW

This chapter reviews current advancements in the use of naturally occurring AM agents in food packaging. Manufacturing methods of AM films, the properties of AM films and the controlled release of AM agents from these films are also reviewed.

2.1 Food spoilage by microorganisms

Excessive growth of microorganisms in foods can change the taste, odour, appearance and texture of foods and may even become health hazards. In many circumstances microorganisms are directly added into foods during production and processing in order to change the properties of these foods or even enhance their nutritional content. Certain yeasts, for example, are used to produce vitamin B in yoghurts. Most foods can be unsafe to consume when they are contaminated with foreign microorganisms. Unfortunately, consumers are unable to recognize foods as spoiled until the items start presenting an unsatisfactory taste, odour appearance or texture. In addition, their decision is often associated with their personal preference, ethnic origin and individual background (Garbutt, 1997).

2.1.1 Contamination by microorganisms

Contamination of foods by a small number of hazardous microorganisms is generally undetectable and people may consume these without recognition. The subsequent consumption of contaminated foods can cause food poisoning and other food-borne diseases (Garbutt, 1997).

Adequate hygiene is very important in food processing in order to prevent or minimize contamination by microorganisms. All raw materials handling, machinery and the surrounding environment must therefore be in sanitary condition. Manufacturing in completely germ-free conditions is very expensive and some food industries cannot afford this. It is therefore accepted that most manufactured foods contain microorganisms to some level. As a result, it is intended that these foods be in the market, purchased and consumed within a limited time while the number of microorganisms is still at an adequate level for consumption. In fact, raw materials

can carry natural microorganisms and during transport they may be contaminated with microorganisms from the surrounding environments such as air, water, soil and sewage. Moreover, they may be polluted by diseased plants and animals, including pests. During processing, they may be contaminated by humans, processing equipment and packaging materials (Garbutt, 1997). Paulsen and Smulders (2003) also reviewed four important causes of microbial contamination of meat, *i.e.* material which is the animal itself, man which is the worker in the industry, machine which includes all the equipment and method such as slaughter, chilling and cutting.

Microorganisms from a variety of sources can contaminate the surface of solid foods and penetrate into the bulk of the food. In liquid foods, the contaminating microorganisms can spread relatively easily through the foods whereas in solid foods the contamination generally remains on the surface of the food (Gill, 1979; Maxcy, 1981).

2.1.2 Domination of spoilage microorganisms

This section reviews factors that affect the growth of contaminating microorganisms in food that lead to spoilage. This knowledge should help manufacturers to target the appropriate microorganism and choose the right action to inhibit the growth of such microorganisms. This includes the selection of antimicrobial (AM) agents when they are needed.

When food is contaminated, the entire population of contaminating microorganisms will not grow uniformly and will spoil the food. There will be a competition amongst the contaminating microorganisms and finally the fastest genera will dominate (Sutherland, 2003). For example, fresh meat is likely contaminated by approx. 30 genera of bacteria in sub-classes of Gram-positive rods, Gram-positive cocci, Gram-negative rods and coccobacilli including 12 genera of moulds and 6 genera of yeasts. Only one or a few genera of microorganisms, however, appear to dominate and subsequently spoil the products. Species of dominant microorganisms are altered in different foods and at different storage conditions. For example, fresh meat can be spoiled at ambient temperature by psychrotrophic microorganisms. However, at

temperatures above 25°C it will be spoiled by mesophilic Enterobacteriaceae and Acinetobacter (Garbutt, 1997).

Table 2.1 Intrinsic and extrinsic factors causing food spoilage.

Intrinsic factors	• Nutrients in food • Antimicrobial residues in food • pH of food • Resistance to pH change • Oxidation-reduction potential • Resistance to redox change • Water activity of food • Physical barrier to microbial invasion
Extrinsic factors	• Storage temperature • Storage atmosphere • Storage humidity • Storage time

Source: Garbutt (1997)

Table 2.2 Examples of intrinsic and extrinsic factors on some foods.

Food types	**Intrinsic factors**	**Extrinsic factors**
Apple	Adequate nutrient content, a_w 0.98, pH 3.0, redox at surface positive, wax coated epidermis, organic acids present	Stored in air/CO_2 enhanced atmosphere at ambient or cool temperature, variable relative humidity
Apple juice in carton	Adequate nutrient content, a_w 0.98, pH 3.2, redox +400mV, organic acids present	Stored at ambient temperature
MAP packaged minced beef	Adequate nutrient content, a_w 0.98, pH 5.2, redox positive	Stored in $CO_2/N_2/O_2$ atmosphere at chill temperature, high relative humidity
Canned meat	Adequate nutrient content, a_w 0.98, pH 5.2, redox negative	Stored at ambient temperature
Pasteurised milk	Adequate nutrient content, a_w 0.98, pH 6.6, redox positive	Stored at chill temperature

Source: Garbutt (1997)

To determine the species of microorganisms that are responsible for the spoilage of a food, it is important to evaluate factors that influence their growth. These factors are categorised into two groups - intrinsic factors and extrinsic factors, as shown in Table 2.1. Some examples are given as shown in Table 2.2 (Garbutt, 1997).

2.1.3 Growth of microorganisms

The growth of individual microbial cells can be monitored by measuring the increase in their size and mass. However, measuring the dimensions and the dry weight of single cells on the micro-scale level requires very accurate instrumentation and is inherently difficult. Unless there is a particular reason for studying the growth of unicellular microorganisms, measuring the growth of their populations is more convenient. In addition, it is more practical to study the population growth in order to understand the activities of microorganisms in their environment and their effect on foods (Garbutt, 1997).

Growth of microbial populations can be studied in a liquid batch culture where the optimum conditions of nutrition, pH, redox, water activity, temperature, oxygen and humidity can be maintained. In general, the number of cells doubles in every cycle of cell division and the time taken for each doubling cycle is called the generation time or population doubling time. The population size at any period of time can be estimated from the initial number of cells and the generation time as shown in equation 2.1 (Garbutt, 1997):

$$N_t = 2^n \cdot N_o \tag{2.1}$$

where N_t is the population size at time t, N_o is the initial population size and n is the number of generations. The number of generations can be estimated by equation 2.2 (Garbutt, 1997):

$$n = \frac{t}{t_d} \tag{2.2}$$

where t is the time taken to generate the population size (N_t) from the initial size (N_0) and t_d is the time taken for the cell to double in size. The ideal relation between the population size and time is given by equation 2.3 (Garbutt, 1997):

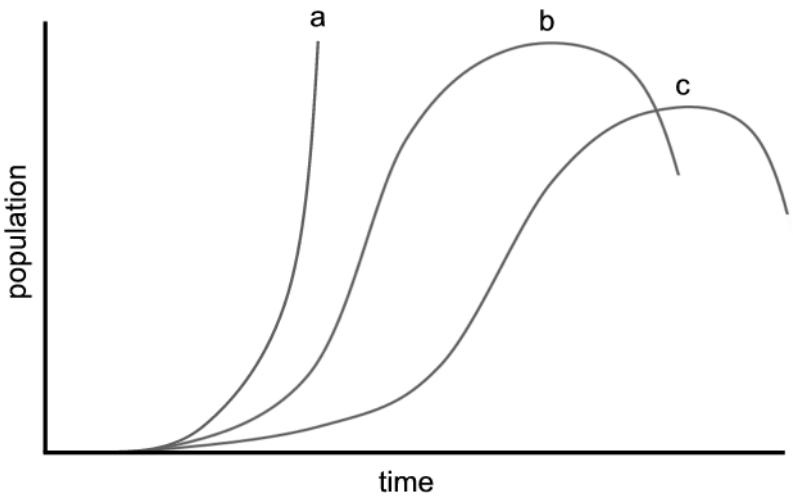
$$N_t = 2^{t/t_d} \cdot N_o \tag{2.3}$$

or

$$\log N_t = \frac{0.3010}{t_d} \cdot t + \log N_o \tag{2.4}$$

where the number of cells increase exponentially or logarithmically as a function of time as shown in Figure 2.1(a). When microorganisms are firstly inoculated in a food medium, they need some time to adjust to the new environment before they start growing, and start dividing. Furthermore, while the cells are growing, they consume nutrition in the media and consequently produce waste. The cell division will stop when the environmental conditions in the media are no longer suitable for them to grow and when the nutrients are depleted or when the level of waste is too high, the cells will die. As a result of these factors, the shape of the growth curve differs from the ideal one as shown in Figure 2.1(b). The growth of microorganisms is divided into four phases – lag phase, log phase, stationary phase and death phase. The ideal system (equation 2.3 or 2.4) can only apply to the log phase of population growth (Garbutt, 1997).

Moreover, microbial growth in real food cannot be predicted simply on the basis of an ideal, homogeneous system. In reality, food is generally heterogeneous with concentration gradients associated with the different components (Dens and Van Impe, 2003). Wilson *et al.* (2002) divided food into four micro-architectures which are: liquid, gel, surface and emulsions. These exhibit different characteristics of microbial growth as presented in Table 2.3.



Source: modified from Garbutt (1997)

Figure 2.1 Microbial growth: (a) ideal growth curve (b) actual growth curve and (c) actual growth curve with inhibition.

Table 2.3 Characteristics of microbial growth in different micro-structures of food.

Micro-structure	Microbial growth
Liquid	Grow planktonically*
Surface	Grow as colonies
Gel	Immobilised, limited to grow as colonies
Emulsion	Limited by availability of liquid phase

Source: Wilson *et al.* (2002)

* Individual cells spread freely in liquid

2.2 Antimicrobial food packaging

Antimicrobial (AM) food packaging is a packaging system that performs inhibitory properties against the growth of microorganisms in food. Antimicrobial packaging can minimise food spoilage caused by microorganisms contaminating the food surface (Gill, 1979; Maxcy, 1981). By releasing preservatives from the package (rather than mixing preservatives into the bulk of the food), a smaller amount of AM agent is needed to prevent the surface growth of microorganisms (Yalpani *et al.*, 1992).

This idea was first developed in the early 1980's when Ghosh *et al.* (1973) developed a cheese wrapping paper containing sorbates as antifungal agents using carboxy methyl cellulose (CMC) as a binder. In the present decade, AM packaging has become more interesting as shown by the increasing number of publications on this topic (Suppakul, 2004). Several types of packaging materials, in which various polymer matrices are mixed with different AM agents, have been described. This technology has resulted in advancements in food packaging and has opened up a new option for AM packaging systems. Such packaging systems do not only protect the food from post-contamination but can also inhibit the growth of pre-contaminating microorganisms (Appendini and Hotchkiss, 2002; Suppakul, 2004; Suppakul *et al.*, 2003a; Suppakul *et al.*, 2003b).

2.2.1 Potential antimicrobial agents used in packaging systems

There are several types of AM agents used in packaging systems. Several publications have reviewed previous studies and advances in AM food packaging using a wide range of AM agents. Brody *et al.* (2001) reviewed 33 AM packaging systems that were studied during 1973-1999. A further review by Ahvenainen (2003) assessed 61 research studies published between 1973-2002 and Han (2005) extended the review to 79 studies between 1973-2003. Some are synthetic chemicals, some are naturally occurring substances, and some are products of biotechnology. Suppakul (2004) and Han (2005) have summarised the antimicrobial agents that are potentially used in packaging materials as categorised in Table 2.4.

Table 2.4 Examples of AM agents used in packaging.

Classification	Antimicrobial agents
Acid Anhydrides	Benzoic anhydride, sorbic anhydride
Alcohol	Ethanol
Amine	Hexamethylenetetramine (HMT)
Ammonium Compound	Silicon quaternary ammonium salt
Antibiotics	Natamycin, neomycin sulfate, reuterin
Antimicrobial Peptides	Attacin, cecropin, defensin, magainin, 14-residue synthetic peptide (6K8L)
Antioxidants	Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), iron salts
Bacteriocins	Bavaricin, brevicin, carnocin, lacticin, mesenterocin, nisin, pediocin, sakacin, subtilin
Chelating agents	Citrate, conalbumin, ethylenediamine tetraacetic acid (EDTA), lactoferrin, polyphosphate, protoporphyrin IX, zinc protoporphyrin IX
Enzymes	Chitinase, ethanol oxidase, b-Glucanase, glucose oxidase, lactoperoxidase, lysozyme, myeloperoxidase
Fatty Acids	Lauric acid, palmitoleic acid
Fatty Acid Esters	Glycerol mono-laurate, monolaurin (Lauricidin®)
Fungicides	Benomyl, imazalil, sulfur dioxide
Inorganic Acids	Phosphoric acid
Metals	Copper, silver, zirconium
Oligosaccharides	Chitooligosaccharide
Organic Acids	Acetic acid, *p*-aminobenzoic acid, benzoic acid, citric acid, lactic acid, malic acid, propionic acid, sorbic acid, succinic acid, tartaric acid
Organic Acid Salts	Potassium sorbate, sodium benzoate, sodium citrate, sodium propionate
Parabens	Ethyl paraben, methyl paraben, propyl paraben
Phenols	Catechin, *p*-cresol, hydroquinones
Plant-Volatiles	Allyl isothiocyanate (AIT), carvacrol, cineole, cinnamaldehyde, citral, *p*-cymene, estragole (methylchavicol), eugenol, geraniol, hinokitiol (b-thujaplicin), linalool, pinene, terpineol, thymol
Plant/spice extracts	Grape seed extract, grapefruit seed extract, hop beta acid, Brassica erucic acid oil, rosemary oil, oregano oil, basil oil
Polysaccharides	Chitosan, konjac glucomannan
Probiotics	Lactic acid bacteria
Sanitising gas	Ozone, chlorine dioxide, carbon monoxide, carbon dioxide
Sanitisers	Cetyl pyridinium chloride, acidified NaCl, triclosan

Source: modified from Han (2005) and Suppakul (2004)

2.3 Active agents in AM packaging

This section reviews the AM activity of common active agents used in AM packaging systems. In particular, the conditions and limitations of using these active agents in developing packaging system are discussed.

2.3.1 Organic acids

Organic acids including their salts and esters are the most extensively studied AM agents for the feasibility to combine them with packaging films. The inhibitory action of organic acids is to acidify the environment of the microorganism in order to suppress its growth. However, this action is only effective against bacteria which normally cannot survive at a pH lower than 4. Mildly lowering the environmental pH is ineffective against most yeast and mould species because they can survive at a pH as low as 1.6. Unfortunately, producing such acidic conditions to inhibit yeast and mould in food is not practical. Another possible mechanism of the AM action of organic acids is the "weak acid theory". Most organic acids used as AM agents are weak lipophilic organic acids or weak fatty acids and can form equilibrium between the undissociated acids and their charged ions. While the pH of a food is low, the proportion of undissociated acids increases but in acidic aqueous foods, salts of organic acids such as sorbates and benzoates can react with water and produce acid forms. Only the undissociated acids can diffuse through lipid cell walls. Longer chain fatty acids, that are lipophilic, can pass the membrane better than short chain fatty acids. As a result, the shorter chain fatty acids need generally to be at a higher concentration to inhibit microorganism (Stratford and Eklund, 2003).

Inside the cell cytoplasm, where the pH is close to neutral, the acids dissociate into charged anions and protons. These charged ions accumulate in the cytoplasm and cause an inhibitory action. Moreover, organic acids with carboxylic groups such as ethylenediamine tetraacetic acid (EDTA) (4 groups), citric acid (3 groups), succinic acid (2 groups) and even lactic acid (1 group) can form complexes with metal cations and thereby form a precipitate. This chelation can remove the key nutritional cations from media or remove cations from the cell wall. All these disturbances can affect the metabolism of microorganisms and inhibit their growth (Stratford and Eklund, 2003).

Most esters of organic acids used as AM agents are phenol esters. The smaller esters are less lipophilic and cannot diffuse readily through the lipid cell membrane. The larger esters are more hydrophobic and insoluble in an aqueous environment thus appearing as oily micelles. The major phenol esters that are used as food preservatives are parabens – five alkyl esters of *p*-hydroxybenzoic acid. It is assumed that their hydroxyl group is the active site that interacts with enzymes. Therefore, phenol esters with more hydroxyl groups are likely to perform the inhibition better than phenol esters that have less hydroxy groups. As a result, they are suitable for non-acid foods. Unlike organic acids, their AM activity is less pH-dependent. Their disadvantages are their low solubility, bitter taste and high cost. Nevertheless, their AM activity requires very low concentration in comparison with organic acids (Stratford and Eklund, 2003). Table 2.5 shows a number of previous studies in food packaging system that incorporate with organic acids reviewed by Brody (2001), Ahvenainen (2003) and Han (2005).

Table 2.5 Examples of AM packaging using organic acids as active agents.

AM agents	Packaging	Foods	AM test
Acetic, propionic acid	Chitosan	Water	Migration test
Benzoic acid	Ionomer	Culture media	*Pen.* spp., *A. niger*
Benzoic, sorbic acid	PEMA	Culture media	*Pen.* spp., *A. niger*
Benzoic anhydride	PE	Tilapia fillets	Total bacteria
Calcium sorbate	CMC/paper	Bread	Mould
Potassium sorbate	LDPE	Cheese, culture media	*S. cerevisiae*
Potassium sorbate	LDPE	Cheese, culture media	*Pen.* spp., *Candida* spp., *Pichia* spp., *Trichosporon* spp.
Potassium sorbate	MC/chitosan	Culture media	
Potassium sorbate	MC/HPMC/fatty acid	Water	Migration test
Potassium sorbate	MC/Palmitic acid	Water	Migration test
Potassium sorbate	Starch/glycerol	Chicken breast	
Propionic acid	Chitosan	Water	Migration test
Propyl paraben	Styrene-acrylates	Culture media	*S. cerevisiae*
Propyl paraben	PE	Water, simulants	Migration test
Sodium benzoate	MC/chitosan	Culture media	
Sorbic anhydride	PE	Culture media	*S. cerevisiae*

Source: modified from Brody (2001), Ahvenainen (2003) and Han (2005)

2.3.2 Enzymes

A number of naturally occurring enzymes can be used as AM agents. Lysozyme is among the most common enzymes used as a food preservative against Gram-positive bacteria. This enzyme damages the cell membrane by catalysing the hydrolysis of the *ß*-1,4-glycosidic bonds between *N*-acetylmuramic acid C1 and the *N*-acetylglucosamine C4 of peptidoglycan layer that is present in Gram-positive bacteria with up to 50% by weight of the cell wall. The peptidoglycan layer is lighter in the Gram-negative bacteria cell wall and is protected by lipopolysaccharides, phospholipids and protein. Therefore, they are more resistant to lysozyme (Meyer, 2003).

Meyer (2003) summarised the mechanisms of some other enzymes that also show antimicrobial effects as shown in Table 2.6. Some examples of AM packaging systems that use enzymes as active agents are shown in Table 2.7.

Table 2.6 Mechanisms of some antimicrobial enzymes.

Mechanism	Enzyme type	Enzyme source	Effect
Catalytic cleavage of peptidoglycan or cell wall junctions	Lysozyme	Hen egg white	Bacteriolysis (Gram-positive microorganisms)
Channel formation and membrane destabilisation	Lysozyme as a cationic peptide	Hen egg white	Increased membrane permeability
Oxidative catalysis releasing or producing toxic or inactivating products	Lactoperoxidase Glucose oxidase	Cow's milk *A. niger*	Growth inhibition Growth inhibition
Oxidative catalysis removing essential substrates or nutrients	Glucosidase	*A. niger*	Growth inhibition
Enzyme catalysed degradation of extracellular polysaccharides	Hydrolases	Several different	Biofilm degradation

Source: Meyer (2003)

Table 2.7 Examples of AM packaging using enzymes as active agents.

AM agents	Packaging	Foods	AM test
Lysozyme	PVOH	Water	Migration test
Lysozyme, nisin, EDTA	Soy protein isolate (SPI), Zein	Culture media	*E. coli*, *Lb. plantarum*
Lysozyme, nisin, propyl paraben, EDTA	Whey protein isolate (WPI)	Culture media	*Lis. monocytogenes*, *S. typhimurium*, *E. coli* O157:H7, *B. thermosphacta*, *S. aureus*
Immobilised lysozyme	PVOH, nylon	Culture media	Lysozyme activity test
Glucose oxidase		Fish	

Source: modified from Brody (2001), Ahvenainen (2003) and Han (2005)

2.3.3 Antimicrobial peptide

Antimicrobial peptides (AMPs) are natural AM agents against a wide spectrum of pathogens. They are generally found in the host defence system of most living things' most exposed tissues such as skin, eyes and lungs of animals including lymph of insects. The active compounds are also produced in microorganisms (Rydlo *et al.*, 2006).

Microbial derivatives

Antimicrobial peptides derived from microbial source are typically made from bacteria. They are low molecular weight AMPs that can inhibit other bacteria and are also called "bacteriocins". Rydlo *et al.* (2006) summarised amino acid sequence of some bacteriocins as shown in Table 2.8. Some examples of AM packaging systems that use bacteriocins as active agents are shown in Table 2.9.

Table 2.8 Amino acid sequence of some microbial derived AMPs.

AMPs	Amino acid sequence *
Nisin	ITSISLCTPGC**K**TGALMGCNM**K**TATC**H**CSI**H**VS**K**
Pedioocin PA1	**K**YYGNGVTCG**KH**SCSVDWG**K**ATTCIINNGAMAWATGG**H**QGN**HK**C
Leucocin A	**K**YYGNGV**H**CT**K**SGCSVNWGEAFSAGV**H**RLANGGNGFW
Sakacin P	**K**YYGNGV**H**CG**Kh**SGCTVDWGTAIGNIGNNAAANWATGGNAGWN**K**
Bacteriocin 31	ATYYGNGLYCN**K**Q**K**CWVDWN**K**AS**R**EIG**K**IIVNGWVQ**H**GPWAP**R**
Enterocin A	TT**H**SG**K**YYGNGVYCT**K**N**K**CTVDWA**K**ATTCIAGMSIGGFLGGAIPGQC
Enterocin P	AT**R**SYGNGVYCNNS**K**CWVNWGEA**K**ENIAGIVISGWASGLAGMG**H**

* Positive charged residues are marked in bold and underlined

Source: modified from Rydlo *et al.* (2006)

Table 2.9 Examples of AM packaging using bacteriocins as active agents.

AM agents	Packaging	Foods	AM test
Nisin	PE	Beef	*B. thermosphacta*
Nisin	HPMC	Culture media	*Lis. monocytogenes, S. aureus*
Nisin	HPMC/stearic acid	Culture media	*Lis. monocytogenes, S. aureus*
Nisin	Corn zein	Shredded cheese	Total aerobes
Nisin	Corn zein, wheat gluten	Culture media	*Lb. plantarum*
Nisin	Ethylene-co-acrylic	Culture media	*Lb. leichmannii*
Nisin, lacticin	LDPE, polyamide	Culture media	*M. flavus, Lis. monocytogenes*
Nisin, lacticin	LDPE, polyamide	Oyster, beef	Total aerobes, coliform
Nisin, EDTA	PE, PE-PE oxide	Beef	*B. thermosphacta*
Nisin, citrate, EDTA	PVC, nylon, LLDPE	Chicken	*S. typhimurium*
Nisin, organic acids mixture	Acrylics, PVA-co-PE	Water	Migration test
Nisin, lauric acid	Zein	Simulants	Migration test
Nisin, lauric acid	Soy protein	Turkey bologna	*Lis. monocytogenes*
Nisin, pediocin	Cellulose casing	Turkey breast, ham, beef	*Lis. monocytogenes*
Pediocin	WPI	Culture media	*Lis. innocua*

Source: modified from Brody (2001), Ahvenainen (2003) and Han (2005)

In the 1950's, one of the first bacteriocins, named nisin, was identified from the product of *Lactococcus lactis* in raw milk (Ray, 1992). This followed the discovery of the inhibitory effect of *Streptococcus lactis* on *Lactobacillus bulgaricus* in 1928

(Rydlo *et al.*, 2006). Although there have been several other bacteriocins discovered since, it seems that nisin is still the only one that has been widely used as a food preservative.

This may be because nisin has the broadest AM spectrum, especially against many Gram-positive pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* and thermophilic spore-forming Gram-positive bacteria like *Bacillus stearothermophilus, Clostridium thermosaccharolyticum* and *Clostridium botulinum*. In some fermentation processes, nisin is effectively used to control lactic acid bacteria because it does not interfere with the growth of yeast (Ray, 1992). Moreover, nisin has very low toxicity and was accepted as a Generally Recognised As Safe (GRAS) substance by the US Food and Drug Administration (FDA) since 1988 (Abee and Delves-Broughton, 2003; Cooksey, 2000) and is the only bacteria-derived peptide approved by the FDA (Rydlo *et al.*, 2006).

Although it has been reported that nisin has no effect on Gram-negative bacteria, some studies have shown that the presence of chelating agents decreases the endurance of Gram-negative bacteria to nisin (Devidson and Zivanovic, 2003). For example, Stevens *et al.* (1991; 1992) reported that nisin incorporated with ethylenediamine tetraacetic acid (EDTA), citrate or phosphate can inhibit Gram-negative *Salmonella* serotypes and *Escherichia coli*. The study used chelating EDTA to react with magnesium and other divalent cations from the outer membrane of Gram-negative *Salmonella* sp., which leads to the damage of lipopolysaccharide in cell membrane and the increases of cell permeability. This assists nisin to permeate through the cell membrane of Gram-negative bacteria and cause cell lysis by interfering with the surface tension of the cytoplasmic membrane.

Natrajan and Sheldon (2000) studied the antibacterial efficiency of nisin against Gram-negative *Salmonella Typhimurium* on broiler skin. In their study, chelating EDTA was also used to destabilise the cell membrane of the bacteria. In addition, citric acid was added to the formulation to maximise the chelation and also act as an acidulant. Moreover, non-ionic emulsifying Tween^{TM} 80 was used to decrease the surface tension between nisin and bacteria cells. The results showed that nisin combined with EDTA and citric acid contributed to a better AM activity than nisin

associated with citric acid and Tween™ 80. However, the formulation of nisin with EDTA, citric and Tween™ 80 did not show a significantly better AM activity when compared with the activity of nisin combined with EDTA and citric acid.

Stevens *et al.* (1992) found that nisin activity is temperature-dependent. Nisin was found to posses a higher AM activity against *Salmonella* sp. at higher temperatures. When they varied the incubation temperature from 4 to 42°C, the highest AM efficacy was obtained in the range of 30-42°C.

Animal derivatives

Miltz *et al.* (2006) studied an AMP, called Dermaseptin S4, extracted from the skin of *Phyllomedusa* tree frogs. The AM agent was incorporated in a corn starch-based coating and applied on cucumber. They reported that Dermaseptin S4 had an inhibitory effect on moulds and aerobic bacteria. Rydlo *et al.* (2006) also summarised amino acid sequence of some animal derived AMPs as shown in Table 2.10.

Table 2.10 Amino acid sequence of some animal derived AMPs.

AMPs	Amino acid sequence *
Magainin	GIG**K**FL**H**SA**KK**FG**K**AFVGEIMNS
MSI-78	GIG**K**FL**KK**A**KK**FG**K**AFV**K**IL**KK**$_{CONH2}$
PR-39	**RRR**P**R**PPYLP**R**P**R**PPPFFPP**R**LPP**R**IPPGFPP**R**FPP**R**FP
Spheniscin	SFGLC**R**L**RR**GFCA**H**G**R**C**R**FPSIPIG**R**CS**R**FVQCC**RR**VW
Pleurocidin	GWGSFF**KK**AA**H**VG**KH**VG**K**AAL**H**TYL
Dermaseptin S4	ALWMTLL**KK**VL**K**AAA**K**ALNAVLVGANA
K4S4(1-14)	**A**LW**K**TLL**KK**VL**K**AA$_{CONH2}$
Cecropin P1	SWLS**K**TA**KK**LENSA**KKR**ISEGIAIAIQGGP**R**
Melittin	GIGAVL**K**VLTTGLPALISWI**KRKR**QQ
LL-37	LLGDFF**RK**S**K**E**K**IG**K**EF**KR**IVQ**R**I**K**DFL**R**NLVP**R**TES
Clavanin A	VFQFLG**K**II**HH**VGNFV**H**GFS**H**VF
Curvacin A	A**R**SYGNGVYCNN**KK**CNVN**R**GEATQSIIGGMISGWASGLAGM

* Positive charged residues are marked in bold and underlined

Source: modified from Rydlo *et al.* (2006)

2.3.4 Plant extracts

Some natural agents extracted from plants are claimed to have the ability to inhibit the growth of a wide range of microorganisms in food. For example, grapefruit seed extract was shown to have an efficacy against *Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* (Ha *et al.*, 2001). Natural agents have been studied with the intention of replacing conventional preservatives in a response to consumers objecting to having synthetic substances in their food (Nicholson, 1998). Table 2.11 lists examples of herbs and spices containing antimicrobial compounds.

Table 2.11 Examples of common herbs and spices containing AM compounds.

Achiote	Cornmint	Mace	Rosemary
Allspice	Cortuk	Mandarin	Rosewood
Almond	Cumin	Marigold	Saffron
Aloe Vera	Cymbopogon	Marjoram	Sage
Anethole	Dill	Martich gum tree	Sagebrush
Angelica	Elecampane	Melissa	Savoury
Anise	Eucalyptus	Mint (Apple)	Sassafras
Asafoetida	Evening primrose	Mugwort	Sideritis
Basil	Frankincense	Musky bugle	Senecio
Bay (Laurel)	Fennel	Mustard	(Chachacoma)
Bergamot	Fenugreek	Mountain tea	Spearmint
Birch	Gale	Neroly	Spike
Cajeput	Gardenia	Nutmeg	Star Anise
Calmus	Garlic	Onion	St John's Wort
Camomile	Geranium	Orange	Tangarine
Cananga	Ginger	Oregano	Tarragon
Caraway	Grapefruit	Ocicum	(Estragon)
Cardamom	Horseradish	Palmarosa	Tea tree
Carrot seed	Hassaku fruit peel	Paprika	Thuja
Cedarwood	Jasmine	Parsley	Thyme
Celery	Lavender	Patchouli	Tuberose
Chilli	Leek	Pennyroyal	Turmeric
Cinnamon	Lemon	Pepper	Valerian
Citronella	Lemongrass	Peppermint	Vanilla
Clove	Lime	Pettigrain	Verbena
Cocoa	Linden flower	Pimento	Wintergreen
Coffee	Liquorice	Ravensara	Wormwood
Coriander	Lovage	Rose	

Source: modified from Nychas and Skandamis (2003); Roller and Board (2003)

According to a discussion by Lück and Jager (1997), essential oils have no direct AM action to microorganisms but they likely have a great capacity to limit the growth factor such as oxygen and water activity in the applied area. Namely, they decrease

the partial pressure of oxygen and water that are necessary for microbial growth. Nevertheless, some studies reported the biological effects of essential oils on the cells. For example, Ultee *et al*. (2002; 1999) found that carvacrol can decrease intracellular ATP, reduce the pH gradient across the cytoplasmic membrane and fail the proton motive force of *Bacillus cereus*. These effects can finally cause death.

Suppakul *et al.* (2003a) studied by means of a solid diffusion test the AM activity of packaging films containing the essential oils extracted from sweet basil (*Ocimum basilicum* L.) which consist mainly of linalool and methylchavicol by means of a solid diffusion tests. The sweet basil extract was shown to have inhibitory effects against a wide range of Gram-positive and Gram-negative bacteria, food-borne pathogens and food spoilage bacteria, yeasts and moulds such as *Aeromonas hydrophila*, *Bacillus cereus*, *E. coli*, *Listeria monocytogenes*, *S. aureus*, *S. cerevisiae*, *Aspergillus sp.*, and *Penicillium sp.* (Singha and Gulati, 1990; Wan *et al.*, 1998).

The AM activity of a selection of volatile oils extracted from six natural plants was studied by Sánchez *et al.* (2005). Vapours of cinnamon, clove, basil, rosemary, thyme and oregano were tested for the inhibition against Gram-positive bacteria (*S. aureus, Bacillus cereus, Listeria monocytogenes, Enterococcus faecalis*), Gram-negative bacteria (*E. coli, Salmonella choleraesuis, Pseudomonas aeruginosa, Yersinia entericolitica*), yeast (*Candida albicans*) and moulds (*Penicillium islandicum, Aspergillus flavus*). Cinnamon and clove vapours showed the best inhibitory effects, followed by oregano and thyme whereas rosemary and basil had no activity. This investigation found that eugenol, which exists at high levels in both cinnamon and clove, is the key component responsible for the AM activity. On the other hand, estragol, a similar component of basil, is not responsible for significant inhibition. Some examples of AM packaging systems that use plant extracts as active agents are shown in Table 2.12.

Table 2.12 Examples of AM packaging using plant extracts as active agents.

AM agents	Packaging	Foods	AM test
Grape fruit seed extract	LDPE, nylon	Ground beef	Total aerobes, coliform bacteria
Grape fruit seed extract, Lysozyme, nisin	Na-alginate, K-carrageenan	Culture media	
Clove extract	LDPE	Culture media	*Lb. plantarum, F. oxysporum, E. coli, S. cerevisiae*
Herb extract, Ag-zirconium	LDPE	Lettuce, cucumber	*E. coli, S. aureus, Leu. mesenteroides, S. cerevisiae, A. niger, A. oryzae, P. chrysogenum*
Cinnamaldehyde, eugenol, organic acid	Chitosan	Bologna, ham	Enterobater, Lactic acid bacteria, *Lb. sakei, Serratia spp.*
Horseradish oil	Paper in pouch	Ground beef	*E. coli* O157:h7
Horseradish extract and *Lb. reuteri* (probiotics)	PE/EVOH/PET pouch	Ground beef	*E. coli* O157:h7
Allyl isothiocyanate	PE film/pad	Chicken, meats, smoked salmon	*E. coli, S. enteritidis, Lis. monocytogenes*
Green tea extract (catechins)	PVA/starch	Culture media	*E. coli*
Basil extract	LDPE	Culture media	*E. coli*

Source: modified from Brody (2001), Ahvenainen (2003) and Han (2005)

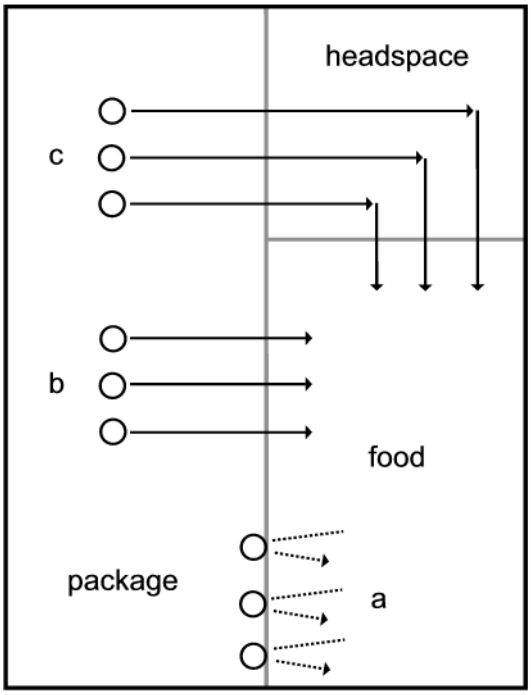
2.4 Antimicrobial packaging systems

The composition of food packaging systems is food, the package and the headspace in the package (Ahvenainen, 2003). In AM packaging systems, the AM package can function in alternative ways to inhibit the microbial growth in food.

2.4.1 Functioning modes of antimicrobial packaging

Antimicrobial packaging can be categorised into two types: one functions by a non-migratory mechanism and the other works by releasing the active agents into food. For the non-migratory type, the active substances are immobilized onto the package

surface and the inhibition happens when microorganisms contact with this active site. This packaging type needs the circulation of food in the package, which normally is practical with liquid food. For the migratory AM packaging, there are two sub-categories *i.e.* the packages that are incorporated with non-volatile active agents and the packages with volatile substances (Han, 2005). Figure 2.4 shows the schematic diagram of three functioning modes of AM packaging described above.



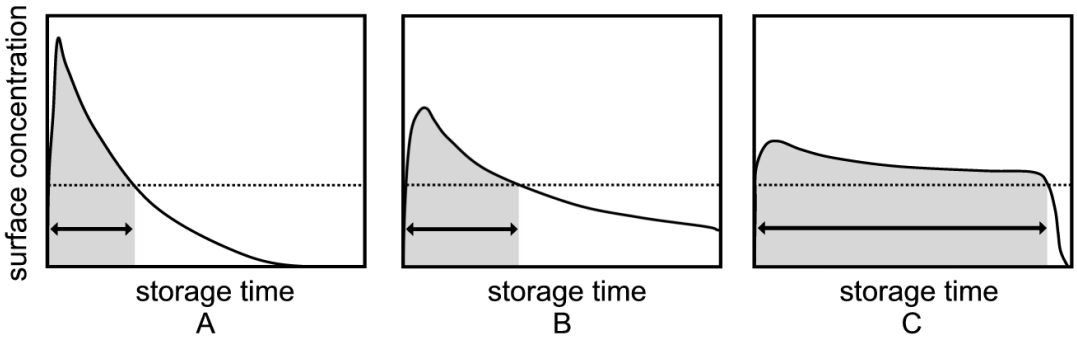
Source: modified from Ahvenainen (2003)

Figure 2.2 Functioning modes of AM packaging: (a) non-migration; (b) non-volatile migration; and (c) volatile migration.

Sánchez *et al.* (2005) produced and tested a multi-layer AM film made of polypropylene (PP), ethylene vinyl alcohol (EVOH) and polyethylene (PE) containing cinnamon extract. The PP/EVOH/PE film was observed to remotely inhibit the AM activity of fungi. In this case, the vapours of the AM agents released from the multi-layer films could create a protective antimicrobial atmosphere and direct contact was not necessary.

2.4.2 Controlled release

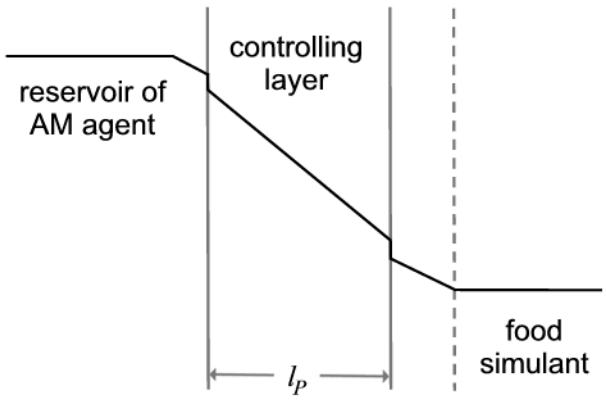
Generally, AM agents that are safely used in food products are also assumed to be safe when incorporated in packaging materials. Rather than adding a high dose of AM agents directly into food, the application of AM packaging offers potentially the advantage of a slow release of the AM agents onto the food surface and retains the required concentrations to inhibit microbial growth for longer periods (Ouattara *et al.*, 2000b). The continuous slow release should be advantageous for resealable packages for consumers as they may be opened and closed numerous times (Chung *et al.*, 2001). The release of AM agents from polymeric packaging materials is claimed to be systematically controllable (Sebti *et al.*, 2003). A number of mathematical models of AM food packaging systems were described by Brody *et al*. (2001). A multi-layer design is favoured because it is easier to control the diffusion of AM agents onto the food by selecting appropriate materials and thicknesses.



Source: modified from Han (2005)

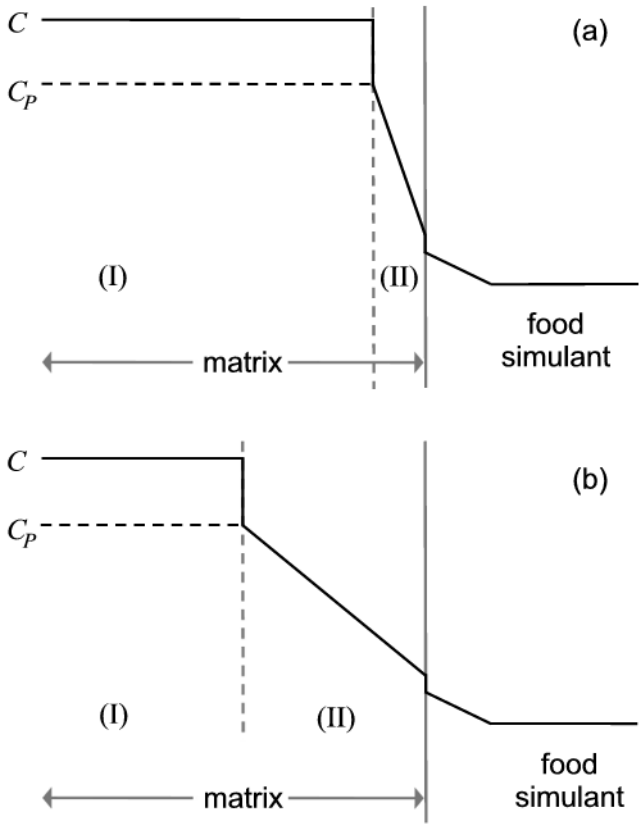
Figure 2.3 Release profiles of AM agents from different AM packaging films.

Figure 2.3 shows AM agent release profiles from three different AM packaging films. System A and B show the release from a monolithic film or so-called "matrix model". System A demonstrates a rapid AM agent release featuring a high solubility in food media while system B shows a slower release due to a lower solubility in food. The dashed line shows the minimum inhibitory concentration (MIC), at which food may be at risk of microbial deterioration when the concentration of the AM agent at the food surface becomes lower than this line. The concentration reduction in systems A and B is faster in comparison to the decrease in system C. The latter demonstrates the



Source: modified from Richards (1985)

Figure 2.4 Concentration profile during release from inner reservoir into the receptor phase.



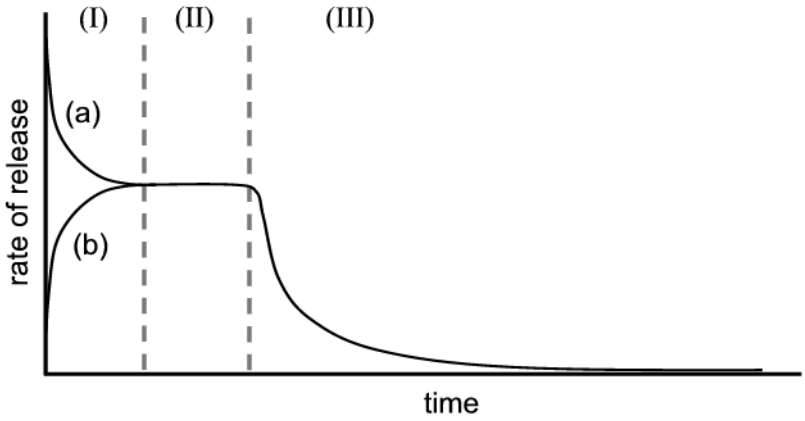
Source: modified from Richards (1985)

Figure 2.5 Concentration profile during release from a matrix containing AM agent into a receptor phase: (a) short-term, (b) long-term.

AM agent release from a multi-layer or so-called “reservoir model” indicating the release from a multi-layer film is to a certain extent manageable by the covering layer.

There are two approaches to the controlled release. One is the release from a reservoir and the other is the release from a matrix or monolithic material. Figure 2.4 and 2.5 demonstrate concentration profile of reservoir model and matrix model respectively.

The difference is, in reservoir model, the volume of AM layer does not change and the concentration of AM agent does not drop whereas, in matrix model, the volume of AM layer reduces and the concentration of AM agent depletes as releasing. The theoretical profile of release rate from a reservoir model is shown in Figure 2.6.



Source: modified from Richards (1985)

Figure 2.6 Overall profile of release rate versus time for a reservoir model.

Figure 2.6 shows three stages of release from a reservoir film. The first stage is the initial release that might start with an extremely high rate (a) or with a low rate (b) before the system reaches the steady state. The cause of the higher initial rate is that the active agent migrates and pre-exists at the surface, which is called “burst effect”, whereas the lower initial rate is obtained from the surface without pre-existing migrant, which is called “lag effect”. The second stage is the steady state, which the release rate is zero order. This stage can be the main advantage of the reservoir model if the polymer releases an active agent with constant rate for a prolonged time. The final part is the first order release where the release rate decreased exponentially with

respect to time. This is because the AM agent in the reservoir is lowering. At the end, the release rate approaches zero which is the equilibrium. The release of an AM agent when the system approaches the equilibrium corresponds to the first order rate as shown in Table 2.13 (Richards, 1985).

Table 2.13 Relation of migration parameters for release in a reservoir model.

Mass fraction	**Full form**	$\frac{M_t}{M_\infty} = 1 - \frac{1}{V_1 + V_2}\left[V_2 \exp\left(\frac{-AD_P K_{P/S}(V_1 + V_2)t}{l_P V_1 V_2}\right) + V_1\right]$
	Short form[a]	$\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$
Rate of release	**Full form**	$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S}(V_1 + V_2)t}{l_P V_1 V_2}\right)$
	Short form[a]	$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$

[a] $V_2 >> V_1$

V_1 is the volume of the reservoir

V_2 is the volume of the food simulant

M_t is the mass of AM agent released from films at time t

M_∞ is the total of mass of AM agent released from films at equilibrium

A is the area of AM film

D_P is the diffusion coefficient of AM agent in controlling layer

$K_{P/S}$ is the partition coefficient of AM agent between reservoir and controlling layer

l_P is the thickness of controlling layer

t is the time

The equations in Table 2.13 are derived from the following relationships (Richards, 1985):

$$\frac{dM_t}{dt} = \frac{AD_P K_{P/S} \Delta C}{l_P} \qquad (2.5)$$

where △C is the concentration gradient between the reservoir and the food simulant.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{- A D_P K_{P/S} (V_1 + V_2) t}{l_P V_1 V_2} \right) \tag{2.6}$$

If $V_2 >> V_1$, the equation will be shortened as the following expression.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{- A D_P K_{P/S} t}{l_P V_1} \right) \tag{2.7}$$

The relationship of the mass fraction of active agents retaining in the film with time can be described as below.

$$\frac{M_t}{M_\infty} = 1 - \frac{1}{V_1 + V_2} \left[V_2 \exp\left(\frac{- A D_P K_{P/S} (V_1 + V_2) t}{l_P V_1 V_2} \right) + V_1 \right] \tag{2.8}$$

When $V_2 >> V_1$, the equation is re-written as follows.

$$\frac{M_t}{M_\infty} = 1 - \exp\left(\frac{- A D_P K_{P/S} t}{l_P V_1} \right) \tag{2.9}$$

The kinetic rate constant (k) can be obtained from the following equation.

$$\frac{M_t}{M_\infty} = 1 - \exp(-kt) \tag{2.10}$$

The mass fraction of the retained migrant as a function of time can be expressed as follows.

$$1 - \frac{M_t}{M_\infty} = \exp(-kt) \tag{2.11}$$

From Equation 2.11, a plot of $\ln(1 - M_t/M_\infty)$ versus time will provide a straight line with a slope of $-k$, which the rate constant can be obtained.

Half-life ($t_{½}$) of release can be calculated from Equation 2.9, where M_t is $½M_\infty$.

$$t_{1/2} = \frac{0.693 \cdot l_P V_1}{AD_P K_{P/S}} \tag{2.12}$$

In a strong receptor or a well-agitating receptor, the release is dominated by the polymer at the contact surface.

$$\frac{dM_t}{dt} = \frac{AD_P C_P}{l_P} \tag{2.13}$$

or

$$\frac{dM_t}{dt} = \frac{AD_P K_{P/S} C_S}{l_P} \tag{2.14}$$

In a poor receptor or a stagnant layer, the boundary effect is taken into account; the release depends on the condition in the receptor.

$$\frac{dM_t}{dt} = \frac{AD_F C_F}{l_F} \tag{2.15}$$

or

$$\frac{dM_t}{dt} = \frac{AD_F K_{F/P} C_P}{l_F} \tag{2.16}$$

For a matrix model, equations describing the relationship between the mass fraction (M_t/M_∞) of low molecular weight substances migrating from a polymer film and time were described by Miltz (1987). For the short-term migration, where $(M_t/M_\infty) < 0.6$, the diffusion is given by:

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_P t}{\pi l_P^2}\right)^{1/2} \tag{2.17}$$

where D_P is the diffusion coefficient and l_P is the thickness of the film. A plot of (M_t/M_∞) versus $t^{1/2}$ should yield a straight line from which the diffusion coefficient can be calculated.

Half-life ($t_{1/2}$) of release can be calculated from Equation 2.17, where M_t is $M_\infty/2$.

$$t_{1/2} = \frac{0.049 \cdot l^2}{D_P} \tag{2.18}$$

For long-term migration, where (M_t/M_∞) > 0.6, the diffusion is given by:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \exp\left(\frac{-\pi^2 D_P t}{{l_P}^2}\right) \tag{2.19}$$

Activation energy of diffusion (E_a) can also be calculated from k or D_P as follow:

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \tag{2.20}$$

or

$$\ln k = \ln k_0 - \frac{E_a}{RT} \tag{2.21}$$

and

$$D_P = D_0 \exp\left(\frac{-E_a}{RT}\right) \tag{2.22}$$

or

$$\ln D_P = \ln D_0 - \frac{E_a}{RT} \tag{2.23}$$

where k_0 and D_0 are pre-exponential factor, R is the ideal gas constant 8.3143 J mol^{-1} K^{-1} and T is the absolute temperature.

Ouattara *et al.* (2000a) studied the diffusion of organic acids *i.e.* acetic acid and propionic acid from a chitosan film into pH 6.4 sodium phosphate buffer at 4, 10 and 24°C. For the first part of the release, the mass fraction of the released active agents (M_t/M_∞) was also subjected to the linearity test with respect to $t^{1/2}$, which is consistent with Equation 2.17 of the matrix model. The diffusion coefficient was calculated from the half-time equation (Equation 2.18). The second part of the release curve is an exponential curve, which was treated with Equation 2.19. The rate constant (k) was obtained from the kinetic equation (Equation 2.10). Finally, the activation energy (E_a) was calculated from Equation 2.20.

2.5 Manufacturing AM films

There are four main methods used to produce AM packaging films: (i) use of polymers that have AM properties, (ii) immobilisation of AM agents in polymers by chemical bonding, (iii) incorporation of the AM agents into the polymer matrix during processing without chemical bonding such as by absorption or impregnation and (iv) coating of AM agents onto a polymer surface (Appendini and Hotchkiss, 2002).

2.5.1 Antimicrobial polymers

There are a number of polymers that have an inhibitory effect against the growth of some particular microorganisms. Brody (2001), Ahvenainen (2003) and Han (2005) describes a number of AM packaging systems using the packaging material itself as an active substance as shown in Table 2.14.

Table 2.14 Examples of polymers that have AM properties.

Polymers	Treatments	Foods	AM test
Chitosan		Cheese	*Lis. monocytogenes, Lis. innocua*
Chitosan/paper		Strawberry	*E. coli*
Chitosan/LDPE	Herb extract	Culture media	*Lb. plantarium, E. coli, S. cerevisiae, F. oxysporum*
Chitosan acetate		Culture media	*E. coli, Vibrio vulnificus, S. typhimurium, S. enteritidis, Shigella sonnei*
Nylon	UV radiation, excimer laser	Culture media	*Pseudomonas fluorescens, Enterococcus faecalis, S. aureus*

Source: modified from Brody (2001), Ahvenainen (2003) and Han (2005)

2.5.2 Immobilisation

The production of AM films by the method of immobilisation involves binding AM enzymes to the polymer, in which produces non-migratory bioactive polymers. Unlike other types of AM packaging, the AM enzymes do not migrate into the food but remain active on the packaging surface. This advantage makes this type of packaging desirable for the modern market, which customer requirements are high and safety regulation are strict. Nevertheless, the non-migration systems have limited application to some food types which lack the circulation of the food in the package. The utility of this packaging is then restricted for liquid food only (Han, 2005).

2.5.3 Incorporation

Antimicrobial films produced by incorporation involve the integration of AM agents into the polymer matrix. The molecules of AM additives are physically bound in the structure of the polymer, and are released during the storage of the product. There are several types of incorporation mechanisms including absorption and impregnation.

Incorporation by absorption

Weng *et al.* (1999) studied the absorption of benzoic and sorbic acids into poly(ethylene-co-methacrylic acid) (PEMA). The PEMA films were prepared in a hydraulic heat press and pre-treated by two different treatments - soaking in HCl and in NaOH. The films were then soaked in an acetone solution containing the AM agents. The AM films that were pre-treated with NaOH had better inhibitory effects on *Penicillium* sp. and *Aspergillus niger* than those pre-treated by HCl. The release study showed a higher concentration of the AM agents released from NaOH-treated films whereas the release from the HCl-treated films was very low. Infrared (IR) spectra of AM films confirmed that there was a higher absorption of AM agents in the NaOH-treated films than in HCl-treated films. The higher polarity of NaOH was discussed as the explanation of its higher ability to absorb the AM organic acids.

During the absorption process, a swelling agent can be used to enhance the absorption of AM agent in the film. For example, Weng *et al.* (1999) used acetone as a swelling agent, which can be evaporated afterwards. A swollen polymer can uptake more AM

agents in the matrix. Following evaporation, there will be AM agents remained active in the polymer.

Incorporation by impregnation

Antimicrobial agents can be mixed with polymer pellets and melted together before the AM films are extruded from the polymer blend (Suppakul *et al.*, 2003a). However, it was reported that the retention of AM agents in extruded films is limited by the processing conditions. Most of the volatile AM agents are evaporated during the extrusion process and low extrusion operating temperature profiles are suggested (Han and Floros, 1997).

2.5.4 Coating

After Ghosh *et al.* (1973; 1977) first introduced the coating method by mixing calcium sorbate with CMC and coating on a paper wrap, several coating methods for producing AM polymeric films have been developed such as immersing, spraying and solvent casting.

Coating by immersion/soaking

Leung *et al.* (2003) studied hydrophobic nisin-coated films using an immersion method. Five commercial films were immersed in coating solutions having different concentrations of nisin for different immersion times, volume/area ratios, solution pH and coating temperatures. The AM activity against *Lactobacillus leichmannii* by the agar diffusion method was then used to evaluate the produced films. The films that had a higher hydrophobicity, as indicated by surface tension measurements, showed a higher AM activity. The efficiency of nisin-coated films depends therefore on the binding affinity of nisin to the polymer films. Increasing the concentration of nisin in the coating solution from 1.25 to 25 μg mL^{-1} increased linearly the inhibitory efficiency of the films. However, further increasing the concentration of nisin from 25 to 250 μg mL^{-1} did not enhance significantly the AM activity of the produced films due to surface saturation. In the soaking process, varying the contact time from 30 s to 2 h did not show any effect on the nisin binding, when the films were immersed in coating solution with a concentration lower than 6.25 μg mL^{-1}. However, contact time effectively promoted the binding of nisin to the films when the nisin concentration in the coating solution was higher (6.25-250 μg mL^{-1}), although this factor is still limited

by the surface saturation. It was also found that the higher volume/area ratio increases greatly the binding efficiency of nisin to the polymeric film, which promotes the AM activity of the produced films. However, the surface saturation still limits the binding when the concentration is higher that 25 μg mL^{-1}.

The previous study showed that the optimal stability of nisin was at pH 3, at which the solubility is also the best (Rollema *et al.*, 1995). Leung *et al.* (2003) also reported that the AM activity of films coated by a nisin solution at pH 3 is significantly higher than those that were coated at pH 2, 4, 5, 6 and 8. It was suggested that pH influences the charge density on both the nisin and the polymer surface, which influences greatly the binding efficiency. In addition, the study found that coating at a temperature of 2°C or 22°C did not affect nisin binding but coating at 80°C completely disabled the AM activity of nisin. It was proposed that coating at such a high temperature for 1 h changed the polymer surface and therefore decreased its ability to bind with nisin but not degrading this AM agent. It was reported previously that nisin is very resistant to heat and an AM activity is observed even after heating at 121°C for 15 min (Ray, 1992).

Coating by spraying

Natrajan and Sheldon (2000) investigated the antibacterial efficiency of nisin associated with EDTA, citric acid and Tween™ 80 when sprayed on three packaging films of a different hydrophobicity – polyvinyl chloride (PVC), linear low density polyethylene (LLDPE) and Nylon™. The coated films were dried under ambient temperature for 15 min before testing for inhibition against *Salmonella typhimurium* on broiler skin at a storage temperature of 4°C. It was reported that higher AM activity of nisin film was obtained when the film had a higher concentration of nisin incorporated and when it was in contact with the testing area for a longer period. The AM films coated with 300 and 175 μg mL^{-1} nisin can result in complete inhibition of microbial growth at 4°C after 72 and 96 h respectively.

In the spray coating studied by Natrajan and Sheldon (2000), the spraying solution was prepared from nisin diluted in distilled water, combined with chelating agents and pH adjusted with NaOH solution. Repulsion between the aqueous nisin solution and hydrophobic films was observed, especially with LLDPE. The coating solution

coalesced into droplets and lacked the ability to produce an even AM film although the coating solution had pH levels in the range of 3.5-3.8 which is close to the most effective range of nisin binding (pH 3). This incompatibility greatly affected the coating when the solution did not contain Tween^{TM} 80 (a surfactant) in the formulation. This repulsion also decreased the overall AM activity of the produced films.

In a similar study by Leung *et al.* (2003), nisin films were produced by soaking in a coating solution of nisin diluted in distilled water with pH adjustments using an HCl solution. In this case, the highest AM activity of nisin films was obtained when nisin was coated on the most hydrophobic films including LLDPE. This result is in contrast with the results reported by Natrajan and Sheldon (2000). Leung *et al.* (2003) claimed that nisin, an amphipathic molecule having ability to interact with both hydrophilic and hydrophobic substances, shows slightly higher hydrophobic characteristics. Therefore, nisin can adhere well to hydrophobic films and enables the production of hydrophobic films with AM properties. Leung *et al.* (2003) concluded that soaking time is a key factor and the immersion of the AM agent for a longer period to bind with the polymer surface would result in a higher retention of the AM agent on surface of the final film.

Mauriello *et al*. (2004) studied antilisterial coated-films produced by spray coating and compared this technique with immersion coating. A solution of partially purified bacteriocin 32Y (PPBAC) was applied onto polyethylene-oriented polyamide (PE-OPA) films. The coating solution for spraying comprised of 70% (v/v) isopropanol containing PPBAC 6400 AU mL L^{-1} whereas the coating solution for soaking was PPBAC with the same concentration diluted in phosphate buffer (50 mmol L^{-1}). In the coating process, the sprayed films were dried in warm air and the soaked films were dried with air at ambient temperature. Each of the films was tested for AM activity against *Listeria monocytogenes* V7 by the agar diffusion pour plate method. A clear zone was observed underneath and slightly around the spray coated film area while an irregular, more widely spread area of inhibition was observed around the soaked film. From these results it was not clear whether the soaked films had absorbed PPBAC into the polymer matrix and released it horizontally from the film edge or whether the films had absorbed and subsequently released unevenly PPBAC

from the surface. Further experiments were conducted to test the AM activity at different contact times. The inhibition zones produced by different treatments were similar and it was concluded that the contact time had no effect on the AM activity. In a similar study by Leung *et al.* (2003), the contact time had no influence on the AM activity of a film when the concentration of the bacteriocin in the coating solution was low.

In a further study, spray coated films were examined for the surface inhibition by a modified agar diffusion method in which *Listeria monocytogenes* V7 was inoculated on the agar surface by the spread plate method instead of seeding by the pour plate method. The coated films were placed on the agar and the AM effect was determined by the presence of an inhibition zone. In this case there was an inhibition zone underneath the film confirming the surface inhibition of the films. There was no clear zone around the film, however, suggesting that the AM agent is unable to diffuse from the spray coated films (Mauriello *et al.*, 2004).

Coating by solvent casting

This method can be applied to various food wraps (Grower *et al.*, 2004; Hoffman *et al.*, 2001; Leung *et al.*, 2003; Padgett *et al.*, 1998; Sebti *et al.*, 2002; Sebti *et al.*, 2003). Ouattara *et al.* (2000b) made a chitosan film incorporated with acetic acid and propionic acid by pouring the mixed solution in a plexiglass mould. The thickness of film was varied by changing the volume of the casting solution in the mould before drying at 80°C for 4-5 h. The AM activity and the release rate of acetic acid released from the produced film was studied aiming to develop a slow-release packaging for applying the acid onto processed meat. Hydrophobic compounds *i.e.* cinnamaldehyde and lauric acid were also added into the matrix of the hydrophilic chitosan to increase the tortuosity of the film. The polymer modification was expected to obstruct the diffusion of molecules through it, which could slow the release rate and extend the period of being active. As a result, only lauric acid decreased the release of acetic acid but the AM activity was not significantly changed. Cinnamaldehyde did not decrease the release but on the other hand increased the inhibitory effect due to its own AM activity.

Coma *et al.* (2001) casted an edible AM film made of melted HPMC incorporated with different hydrophobic fatty acids and derivatives *i.e.* stearic acid, oleic acid, methylpalmitate and methylstearate. Nisin as an AM agent was added into the film-forming solution prior to the casting process. The activity of the produced AM film was tested against *Listeria monocytogenes* and *Staphylococcus aureus*. As a result, the HPMC merged with stearic acid showed the weakest inhibitory effect of nisin. It was explained that the cationic nisin was bonded well with anionic stearic acid, which prevent the release of the active agent. However, the desorption of AM agent is not actually required in the edible film because the film is to protect itself as a part of food from the contaminated microorganisms (Han, 2005).

Limjaroen *et al.* (2003) studied an AM coating material produced by a casting method. Saran® F-310 resin (a copolymer of vinylidene chloride) was dissolved in methyl ethyl ketone at a concentration of 18% w/v and AM agents nisin, lactoferrin, sodium diacetate, sorbic acid or potassium sorbate were added at different concentrations. Lactic acid and 2 M NaOH were used to adjust the pH of the coating solution to 5.2. The coating material was applied to a glass plate, dried and removed from the plate then tested for AM activity by the agar diffusion method. All films except those containing lactoferrin or sodium diacetate showed positive inhibitory effects against *L. monocytogenes*. Furthermore, increasing the concentration of AM agent promoted the inhibitory effectiveness of the produced films. It was claimed that the coating material containing 3% w/v sorbic acid without pH adjustment demonstrated the best AM activity due to the greatest solubility and more homogeneous distribution of the AM agent in the polymer structure.

Buonocore *et al.* (2003) also produced an AM film made from polyvinylalcohol (PVOH) by casting method. The AM agents *i.e.* lysozyme, nisin and sodium benzoate were added while the polymer was dissolved in hot water, which was crosslinked by glyoxal with HCl as a catalyser. The solution was then cast on a plate and dried at room temperature for 48 h. A kinetic model of releasing the AM agents from the produced film into water was approached relating to water diffusion, macromolecular matrix relaxation and AM agent diffusion through the polymer.

Mauriello *et al.* (2004) successfully tested AM film production on an industrial scale by spreading a thin layer of bacteriocin solution onto a PE-OPA film with an offset roller that was immersed in the solution. The volatile solvent was immediately evaporated from the coating in a warm air tunnel. The film showed good inhibitory effectiveness by decreasing the viable cells of *Listeria monocytogenes* on hamburger meat stored at 4°C in the first 24 h.

Ha *et al*. (2001) compared the AM activity of two types of AM films that were multi-layered by a co-extrusion method and a solution-coating method. Grapefruit seed extract (GFSE) was used as an AM agent. The results showed that co-extruded AM films had a lower AM activity than the coated films. This is because the co-extrusion was done at 160-190°C while the GFSE is stable in the heat up till only 120°C. The films produced by solution coating used polyamide as a binder which did not require heat and had a higher AM retention.

In this research, the solvent casting is therefore chosen to develop a multi-layer AM film, for which a type of polymer can be used as binder with a high retention of AM agent while another polymer having slow-release property can be selected as a controlling layer.

2.6 Properties of AM films

Depending on the method of film production, AM additives can affect some physical properties of the AM film such as increasing the coefficient of friction as well as decreasing the water vapour and oxygen permeabilities (Dobias *et al.*, 2000). Conversely, Limjaroen *et al.* (2003) reported that water vapour and oxygen permeabilities of polyvinylidene copolymer (Saran® F-310) films increased after incorporating with potassium sorbates.

Addition of potassium sorbate in LDPE films was also studied (Han and Floros, 1997; Weng and Hotchkiss, 1993). It was found that the transparency of the films reduced with the increasing concentration of the AM agent, whereas the tensile strength of the AM films was similar to films without the AM agent. Han and Floros (1997) explained that the tensile strength of the AM films was not changed because the size

of potassium sorbate molecule was small and the tested concentration was low – 3% (w/w). The active agent could be captured in the void of the amorphous LDPE that had a large volume. It was suggested that a higher concentration of the small active molecule compared with the volume of polymer could affect the tensile strength of the films. However, Limjaroen *et al.* (2003) reported that 3% (w/w) potassium sorbate in Saran® F-310 decreased the tensile strength and also the toughness of the film.

Besides the AM agent, other additives could also affect some physical properties of the AM films. For instance, water vapour transmission (WVTR) of HPMC film incorporated with stearic acid as a hydrophobic compound was also decreased comparing with the non-additive HPMC film. On the contrary, adding higher hydrophobic methylstearate increased the WVTR. An analysis by electron microscopy confirmed a higher pore formation in the film, which was an explanation of the WVTR increase. The infrared spectra confirmed that there was no ester bond between the carboxylic group (-COOH) of the additive and the hydroxyl group (-OH) of HPMC (Coma *et al.*, 2001).

2.7 Future of AM packaging research

It is believed that the most interesting design of AM packaging for further development is the multi-layer type. This is because the multi-layer packaging consists of different materials which can be chosen to control the diffusion properties. The AM layer should retain a high amount of an AM agent while the controlling layer should restrict the diffusion of the active agent and release controlled level. This research also aims to impregnate a natural AM agent in polymers without applying heat where the solvent casting method is used. A short drying time at ambient temperature is another indicator of the achievement, which should maximise the AM retention. In this work, the release mechanism is expected to reach the steady state in a relative short time and proceeds with the first order declining rate until approaching the equilibrium where the equilibrium will be estimated from the asymptotic value.

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Polymers

The polymer films used in this research were a commercial *ca.* 50 µm thick low-density polyethylene (LDPE) and a *ca.* 50 µm thick nylon/LDPE obtained from Amyl Media Pty. Ltd. A *ca.* 10 µm thick LDPE film was purchased from a local retail outlet. The polymers used in the coating solutions were ethylacrylate-methylmethacrylate copolymer dispersion (EA-MMA) (Eudragit NE 40 D) supplied by Degussa Pty. Ltd., Australia; methylcellulose (MC) (18,804-2); hydroxypropyl methylcellulose (HPMC) (42,321-1); and polyethylene glycol (PEG) (20,236-3) were supplied by Aldrich Chemical Company Inc., USA (refer Appendix A).

3.1.2 AM agents and solvent

The AM additives used in this research were methylchavicol with a purity of 98% (AUSTL 21320) supplied by Aurora Pty. Ltd., Australia; linalool with a purity of 97% (L2602) and carvacrol with a purity of 98% (W224502) supplied by Sigma-Aldrich Pty. Ltd., Australia. The solvent used is ethanol (95SG) supplied by CSR Distilleries Ltd., Australia.

3.1.3 Media and microorganisms

The media used in the present studies were nutrient broth (AM 131), nutrient agar (AM 130) and plate count agar (AM 144) purchased from Amyl, Australia. The microorganism *Escherichia coli* (UNSW 080300) was obtained from the culture collection of the University of New South Wales, Australia.

3.1.4 Others

Masking tape 3M™ (2214) measuring *ca.* 140 µm thickness was purchased from a local stationary store. Cheddar cheese slices having pH 5.5 and 5.9 were purchased from a local retail outlet and stored at 4°C until use.

3.2 Activity of AM agents

3.2.1 Preparation of microorganism

The stock cultures of *E. coli* were kept in nutrient broth (Amyl, AM 131) containing 30% (v/v) glycerol at -80°C. The working culture was obtained by growing cells on nutrient agar overnight at 37°C. The *E. coli* cells were then sub-cultured into nutrient broth twice before use while they were in the early stationary growth phase. In order to harvest *E. coli* cells, nutrient broth that contained the twice-passaged *E. coli* was centrifuged (Sorvall®, Kendro Laboratory Products, U.S.A.) at 4000 × *g* for 10 min at 4°C. The supernatant was discarded and the precipitated cells were washed with a sterile 1% (w/v) peptone solution twice before being suspended in fresh nutrient broth and enumerated for the cell density. A certain density of *E. coli* was prepared by dilution with 1% (w/v) peptone solution.

3.2.2 Activity of natural AM agents

Potential AM agents for coating on packaging films were tested for their inhibitory effect against *E. coli* prior to preparing the films. Methylchavicol, linalool and carvacrol were selected for this experiment. One drop of each AM agent was placed on the surface of individual nutrient agars that were inoculated with 10^5 cfu mL^{-1} of *E. coli* by the pour-plate method. The plates were then aerobically incubated for 24 h at 37˚C. The AM activity of the tested agents was observed from the appearance of the inhibition zone. The experiment was performed in quadruplicate and the effect of the selected AM agents on the growth of *E. coli* was evaluated visually. The results were recorded photographically.

3.3 Film preparation and formulation

3.3.1 Coating procedure

Prepared AM coating solutions were poured on a *ca.* 50 μm thick LDPE film that was framed with two layers (*ca.* 280 μm thickness) of 3M™ masking tape around each edge. The solution was evenly spread using a glass rod and dried at ambient temperature for 1 h. The thickness of the dried coating was measured and films were prepared in duplicate.

3.3.2 Formulation of AM coating solution

A coating solution was made from methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), ethanol, distilled water and PEG with the percentage of each component shown in Table 3.1.

Table 3.1 Formulation of MC-HPMC coating solution

Component	Percent by weight
MC	2.6
HPMC	1.1
Ethanol	56.6
Water	37.7
PEG	1.9

MC and HPMC were dispersed into ethanol and heated with a heating magnetic stirrer. The heater was stopped when the temperature reached 60°C. With continuous agitation, PEG and distilled water was slowly added into the dispersion while the temperature was cooling down, which resulted in the formation of a uniformly clear gel solution. The solution was stored at 4°C until use.

The active AM agents selected from the activity testing (Section 3.2.2) were added to the prepared coating solution to obtain different concentrations ranging from 1-5% (w/w) and the films were prepared in accordance with Section 3.3.1. The maximum concentration of the AM agents obtainable in the dried coating was determined visually from the appearance of the dried coating surface. Segregation of AM agents from the dried coating indicated the maximum concentration had been exceeded.

3.3.3 Activity of AM agents released from coating materials

Coated films containing the optimal concentration of selected AM agents were prepared in accordance with the method described in Section 3.3.1 and 3.3.2 and cut in *ca.* 2 cm × 2 cm squares. The coated surface of dried AM films was placed on the surface of nutrient agars that had 10^5 cfu mL^{-1} of *E. coli* inoculated by the spread-plate method and were then aerobically incubated for 24 h at 37°C. The AM effect of the selected agents released from the coating onto the solid media was evaluated

visually by the appearance of inhibition zones and the results were recorded photographically. The best performing AM agent was selected for further experiments.

3.3.4 Stability of coating materials

The stability of two coating materials was compared. In addition to MC-HPMC coating solution, an alternative coating solution was prepared from EA-MMA copolymer dispersion (20% w/w) dissolved in ethanol (80% w/w). The solution was mixed with a magnetic stirrer for 5 min without heating until a homogenous clear gel was obtained. Films containing MC-HPMC (see Table 3.1) and EA-MMA coating solutions were prepared in accordance with the method described in Section 3.3.1 and 3.3.2 and cut in *ca.* 2 cm × 2 cm squares. Dried films were immersed in various food simulants *i.e.* water, 10% (v/v) ethanol, 50% (v/v) ethanol, 95% (v/v) ethanol and olive oil and stored at ambient temperature for 24 h on an automatic shaker with a rotating speed of 30 rpm. The stability of the coating materials was determined from the apparent solubility in the different food simulants.

3.3.5 Retention of AM agent in coating material

Coated glass plates containing the maximum concentration of selected AM agent in the selected coating solution were prepared in accordance with the method described in Section 3.3.1 and 3.3.2 with the exception of coating the AM solution on glass plates (*ca.* 2 cm x 7 cm) instead of LDPE carrier films. The coated glass plates were immersed in 100 mL of 95% (v/v) ethanol in a closed container at ambient temperature for 24 h and were shaken on an automatic shaker with a rotating speed of 30 rpm. The concentration of AM agent that had migrated from the fresh and dried coating material into the 95% (v/v) ethanol was analysed by gas chromatography.

3.3.6 Gas chromatography analysis

A gas chromatograph (Varian Star 3400-CX GC) equipped with a fused silica capacity column DB-5 (30 m length × 0.25 mm internal diameter, 0.25 μm film thickness, J & W Scientific, USA) was used. The following conditions were applied: injected volume, 1.0 μL; initial column temperature, 110°C, kept at this temperature for 15 min; injector temperature, 250°C, splitless; FID detector temperature 300°C; carrier gas, nitrogen. The AM agent contents of samples were calculated from

prepared standard curves. The data points were represented by the mean values and subjected to analysis of variance (ANOVA) and Tukey tests at the significance level of 0.05 using the statistical software Statistix™ 1.0 (Analytical Software Inc., USA).

3.3.7 Preparation of multi-layer AM films

Selected AM coating solutions containing the optimal concentration of selected AM agent were applied on *ca.* 50 μm thick LDPE or nylon/LDPE film that was framed with two layers (*ca.* 280 μm thickness) or four layers (*ca.* 560 μm thickness) of 3M™ masking tape around each edge. The solution was evenly spread using a glass rod and dried at ambient temperature for 1 h. Additional layers of LDPE film with different thickness ranging from *ca.* 10 to 50 μm were separately laminated on top of the AM coating layer. The selected polymer coating layers are self-adhesive therefore the respective LDPE films can be joined to the coating layer without any additional adhesive or bonding agent. The *ca.* 10 μm and *ca.* 50 μm laminated films were made of single LDPE films whereas the *ca.* 20 μm and *ca.* 30 μm films were composed of layers of *ca.* 10 μm LDPE films compressed with a pressure of 40 MPa at a temperature of 185°C for 2 min. The prepared AM films were folded and heat-sealed in order to prepare bags as shown in Figure 3.1.

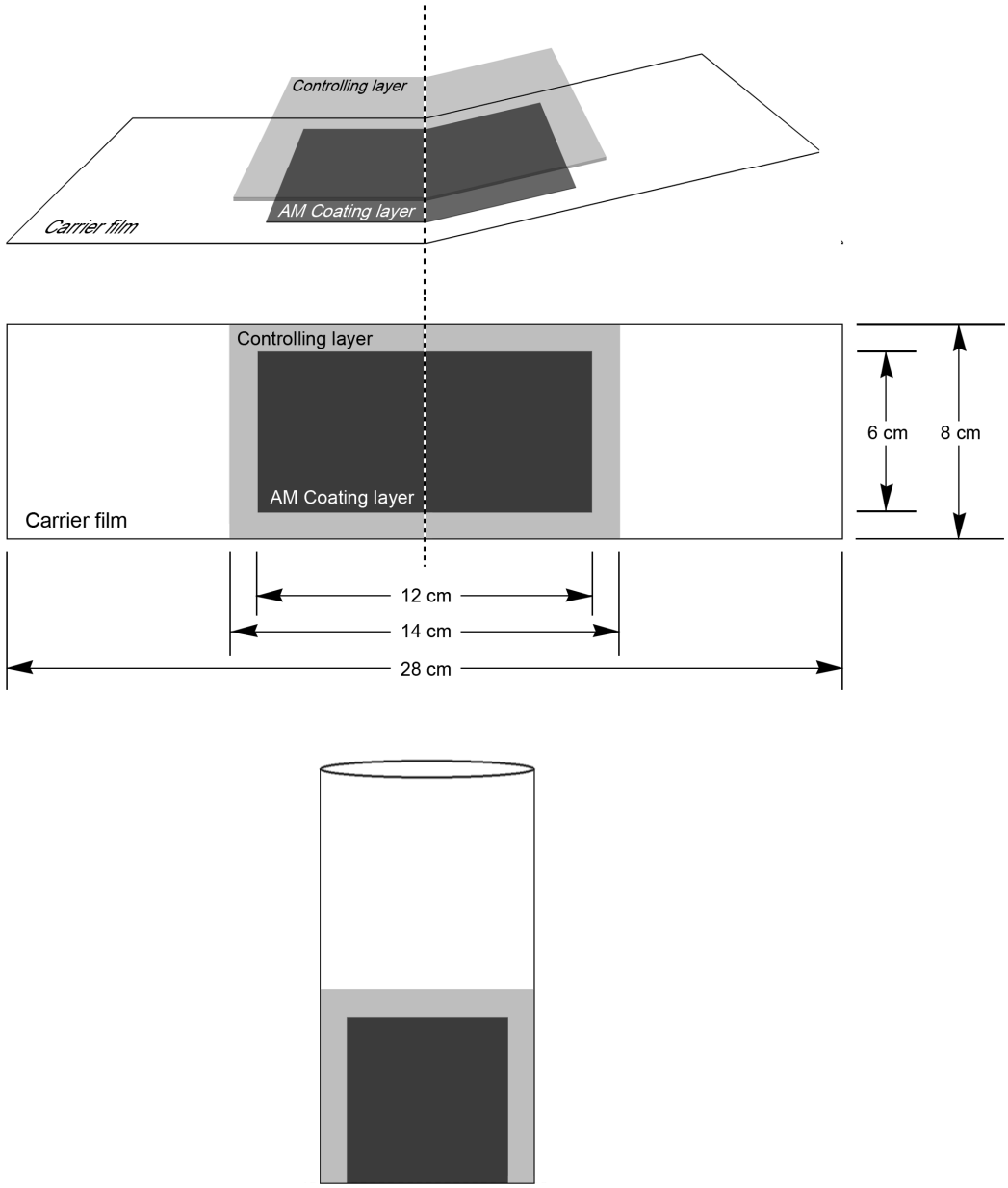


Figure 3.1 Schematic representation of the multi-layer film.

3.4 Release of AM agents

3.4.1 Effect of controlling layer thickness on release

Release of AM agent into food simulants

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of framing the *ca.* 50 μm thick nylon/LDPE carrier film with only four layers (*ca.* 560 μm thickness) of 3M™ masking tape. Samples were tested following the experimental treatment as shown in Table 3.2 to study the effect of temperature and thickness of the LDPE controlling layer on the release.

Table 3.2 Experimental treatment to study the effect of the controlling layer thickness on the release.

	Controlling layer thickness/ μm*			
Temperature / °C	**10**	**20**	**30**	**50**
4	✓	✓	✓	✓
20	✓	✓	✓	✓
37	✓	✓	✓	✓

* Approximated

The prepared AM bags were then filled with 50 mL of 95% (v/v) ethanol or water and were stored at 4, 20 or 37˚C. The mass (M_t) of the AM agent migrating from the multi-layer AM bags into 95% (v/v) ethanol was analysed by gas chromatography as described in Section 3.3.6 until it approached the asymptote.

The mean values of the data points were plotted as a function of time (t).The mass balance of the AM agent in different parts of the film was analysed. The concentration profile of the AM agent in the migration pathway was determined. The partition coefficients of the AM agent migrating between the various materials were also determined. The initial rate of release was obtained from the slope of the linear part of the release curve (M_t versus t), which was tested for linearity by calculating the regression parameter (R^2).

The total mass (M_∞) of release and permeability ($D_P K_{P/S}$) were obtained from the plot of the logarithm of the release rate ($\ln(dM_t/dt)$) versus time (t) using equation 2.6 and 2.7. The rate constant (k) of the release was determined from the plot of the logarithm of the retained mass fraction ($\ln(1-M_t/M_\infty)$) as a function of time (t) using equation 2.11 and 2.7. The activation energy (E_a) of the release was obtained from a plot of the logarithm of the rate constant ($\ln(k)$) versus the inverse of the absolute temperature (T^{-1}) using equation 2.21.

Release of AM agent to the atmosphere

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of framing the *ca.* 50 μm thick nylon/LDPE carrier film with only four layers (*ca.* 560 μm thickness) of 3M™ masking tape and laminating the coating layer with only *ca.* 50 μm thick LDPE film. The produced multi-layer AM films were then exposed to the air at 20°C for up to 20 d. The multi-layer AM films were analysed for the retained AM agent at regular intervals using a Fourier Transform Infrared (FT-IR) spectrophotometer (Bruker model Vector 22). The IR absorbance spectra were measured in the wave-number range of 4000 to 370 cm^{-1} (32 scans at a resolution of 4.0 cm^{-1}). A wave-number of the spectra that was representative of the AM agent was selected and its peak height was recorded. The concentration of the AM agent that was retained in the films was calculated as a proportion of the initial concentration. The release was represented as a plot of the mass fraction of retained AM agent ($\ln(1-M_t/M_\infty)$) versus time (t) to describe the diffusion process. With regard to the kinetic process, the logarithm of the retained mass fraction ($\ln(1-M_t/M_\infty)$) was plotted as a function of time (t), which was tested for linearity by calculating the regression parameter (R^2). Rate constant (k) of the release and permeability ($D_P K_{P/S}$) were obtained from the slope using equation 2.11 and 2.7.

3.4.2 Effect of AM layer thickness on the release

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of laminating the coating layer with only *ca.* 50 μm thick LDPE film. Samples were tested following the experimental treatment as shown in Table 3.3 to study effect of temperature and AM layer thickness on the release.

Table 3.3 Experimental treatment to study the effect of the AM layer thickness on the release of AM agent.

	Number of 3M™ framing tape / layers	
Temperature / °C	**2**	**4**
4	✓	✓
20	✓	✓
37	✓	✓

The prepared AM bags were then filled with 50 mL of the food simulant 95% (v/v) ethanol and were stored at 4, 20 or 37˚C. The mass (M_t) of AM agent migrating from the multi-layer AM bags into the food simulant was analysed by gas chromatography as described in Section 3.3.6 until it approached the asymptote.

The mean values of the data points were plotted as a function of time (t). The mass balance of the AM agent in different parts of the film was analysed. The concentration profile of the AM agent in the migration pathway was determined. The partition coefficients of the AM agent migrating between the various materials were also determined. The initial rate of the release was obtained from the slope of the linear part of the release curve (M_t versus t), which was tested for linearity by calculating the regression parameter (R^2).

The total mass (M_∞) of release and permeability ($D_P K_{P/S}$) were obtained from the plot of logarithm of the release rate ($\ln(dM_t/dt)$) versus time (t) using equation 2.6 and 2.7. Rate constant (k) of the release was determined from the plot of logarithm of the retained mass fraction ($\ln(1-M_t/M_\infty)$) as a function of time (t) using equation 2.11 and 2.7. The activation energy (E_a) of release was obtained from a plot of the logarithm of the rate constant ($\ln k$) versus the inverse of the absolute temperature (T^{-1}) using equation 2.21.

3.4.3 Effect of the solubility of the AM agent in the food simulant on the release

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of laminating the coating layer with only *ca.* 50 μm thick LDPE film. Samples were tested following the experimental treatment as shown in Table 3.4 to study effect of solubility of AM agent in food simulant on the release.

Table 3.4 Experimental treatment to study the effect of the solubility of the AM agent in the food simulant on release.

	Number of 3M™ framing tape / layers	
Food simulants	**2**	**4**
95 % (v/v) Ethanol	✓	✓
water	✓	✓

The prepared AM bags were then filled with 50 mL of 95% (v/v) ethanol or water and were stored at 20˚C. The mass (M_t) of the AM agent migrating from the multi-layer AM bags into the respective food simulants was analysed by gas chromatography as described in Section 3.3.6 until it approached the asymptote.

The mean values of the data points were plotted as a function of time (t). The mass balance of the AM agent in different parts of the film was analysed. The concentration profile of the AM agent in the migration pathway was determined. The partition coefficients of the AM agent migrating between the various materials were also determined. The initial rate of release was obtained from the slope of the linear part of the release curve (M_t versus t), which was tested for linearity by calculating the regression parameter (R^2).

The total mass (M_∞) of release and permeability ($D_P K_{P/S}$) were obtained from the plot of logarithm of the release rate ($\ln(dM_t/dt)$) versus time (t) using equation 2.6 and 2.7. Rate constant (k) of the release was determined from the plot of logarithm of the retained mass fraction ($\ln(1-M_t/M_\infty)$) as a function of time (t) using equation 2.11 and 2.7.

3.4.4 Data analysis

The mean values obtained from the experiments were reported and subjected to analysis of variance (ANOVA) with replication as well as the Tukey test at the significance level of 0.05 using statistical software Statistix™ 1.0 (Analytical Software Inc., USA).

3.5 Antimicrobial activity of multi-layer AM films

3.5.1 Activity in food media

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of using only *ca.* 50 μm thick nylon/LDPE carrier film, framing the carrier film with only four layers (*ca.* 560 μm thickness) of 3M™ masking tape and laminating the coating layer with only *ca.* 50 μm thick LDPE film. Multi-layer films without AM agent added in the coating layer were also prepared and used as the control. The bags were filled with 50 mL nutrient broth containing 10^3 cfu mL^{-1} of *E. coli* and were incubated at 8, 12, and 20°C. Samples of broth were taken for bacterial enumeration every 1 h at 20°C and every 5 d at 8 and 12°C and the time taken for *E. coli* to grow from the initial density of 10^3 cfu mL^{-1} to 10^6 cfu mL^{-1} was determined.

3.5.2 Activity on Cheddar cheese

The AM films were prepared in accordance with the method described in Section 3.3.7 with the exception of using only *ca.* 50 μm thick nylon/LDPE carrier film, framing the carrier film with only four layers (*ca.* 560 μm thickness) of 3M™ masking tape and laminating the coating layer with only *ca.* 50 μm thick LDPE film. For each experiment, a Cheddar cheese slice (*ca.* 4.5 cm × 4.5 cm × 0.2 cm) weighing *ca.* 5 g was sterilized by UV light for 1 h on each side in a laminar flow cabinet. The *E. coli* was inoculated on the top and bottom surfaces of the cheese slices and then distributed using a sterile glass rod to obtain *ca.* 10^4 cfu g^{-1}. The cheese samples were placed in the prepared bags and stored at 4°C for 1 day. The samples were then subjected to a temperature abuse by storing at 8 and 12°C for 20 and 10 d respectively. Samples of cheese were taken for bacterial enumeration every 60 h at 8°C and every 30 h at 12°C.

In order to enumerate *E. coli*, cheese samples were aseptically transferred to stomacher bags and each 5 g sample was dispersed in a 45 mL aliquot of 0.1 M sterile sodium phosphate buffer solution (pH 7.0 ± 0.1 at 25ºC). The samples were then blended in a laboratory blender (Seward Stomacher® 400, Seward Medical, UK) for 1 min. The blended samples were serially diluted in sterile peptone saline diluent solutions (pH 7.0 ± 0.1 at 25ºC). Samples of 0.1 mL of the diluted solutions were spread on plate count agar prior to aerobic incubation for 24 h at 37°C and the *E. coli* count was recorded.

4 RESULTS AND DISCUSSION

This chapter examines the *in vitro* AM activity of methylchavicol, linalool and carvacrol against the microorganism *E. coli*. The stability of films, containing these AM agents, in contact with a variety of food simulants and the production of multi-layer AM films are discussed. The retention of the AM agents in the prepared film samples and the subsequent release of agents from the films into a range of liquid food simulants and into the atmosphere were investigated. The application of the AM films on Cheddar cheese was then studied in order to assess the effectiveness of the multi-layer AM film.

4.1 Activity of natural AM agents

A number of AM agents that have been previously investigated (Suppakul, 2004), namely, methylchavicol and linalool, were tested for their AM activity. The AM activity of the natural agents against *E. coli* at the density level of 10^5 cfu mL^{-1} on nutrient agar is shown in Figures 4.1, 4.2 and 4.3 for methylchavicol, linalool and carvacrol respectively. In each case a clear zone is observed indicating significant microbial inhibition. It is evident that methylchavicol (Figure 4.1) provides the weakest activity against *E. coli* as indicated by the growth of some bacterial colonies. The growth of *E. coli* is entirely inhibited, however, on plates containing linalool and carvacrol (Figures 4.2 and 4.3 respectively). Therefore, both linalool and carvacrol were selected for further investigation.

The selection of the AM agents was based on the criterion that these agents inhibited effectively the growth of *E. coli*. Another important factor in the selection process was the ability of the AM agents to diffuse into various media. This includes the diffusion of the AM agent through the agar and evaporation to the atmosphere. Factors such as the solubility of the AM agents in the medium, the complexity of the lipid-aqueous phase in the medium and the vapour pressure of AM agents influence the diffusion process (Nychas and Skandamis, 2003). In addition, the evaluation of the AM effect is also influenced by the tested strains of *E. coli*. Although methylchavicol did not show a significant AM effect in the current experiment using faecal *E. coli* from the culture collection of University of New South Wales, earlier

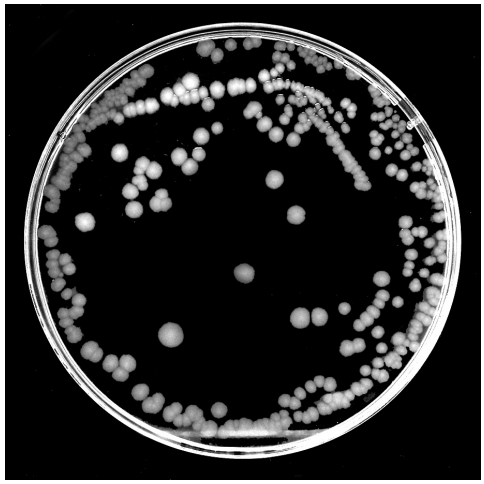


Figure 4.1 Photograph of inhibitory effect of methylchavicol against *E. coli.*

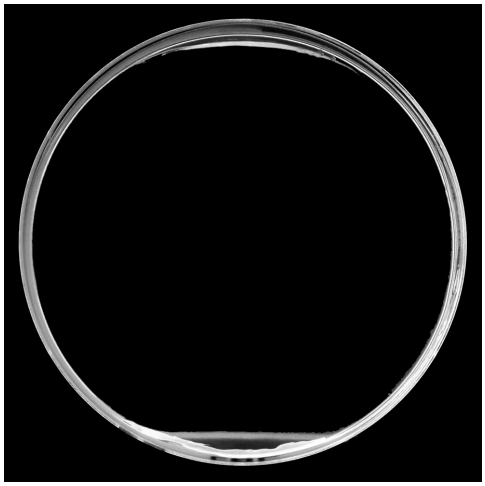


Figure 4.2 Photograph of inhibitory effect of linalool against *E. coli.*

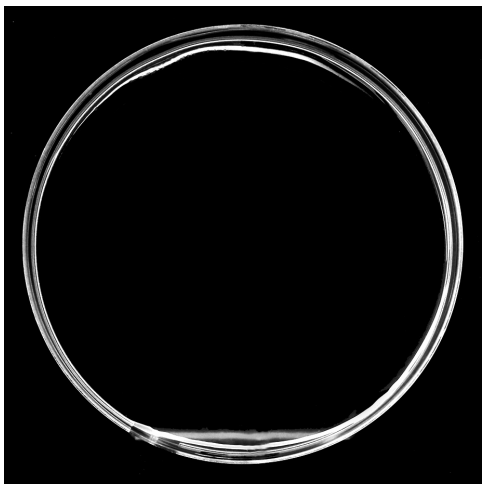


Figure 4.3 Photograph of inhibitory effect of carvacrol against *E. coli.*

research showed a distinct inhibitory effect against oral *E. coli* from the culture collection of Food Science Australia, Werribee (Suppakul, 2004; Suppakul *et al.*, 2003a).

4.2 Formulation of AM coating solution

The production of packaging films containing volatile additives must take into account the potential loss of the additives by minimizing or avoiding evaporation processes, including those involving heat. It is therefore advantageous to integrate such volatile (natural) AM agents in a polymer solution prior to coating on a packaging film. In order to achieve minimal loss of active volatile AM agents in the present work, methylcellulose and hydroxypropyl methylcellulose (MC-HPMC) was added as a coating solution (see Table 3.1).

Linalool or carvacrol were mixed in the coating solution at different concentrations in the range of 1-5% (w/w) and coated on LDPE films. The measured thickness of the dried AM coating was 20 μm approximately. The ability of the coating material to retain the AM agent in the matrix could be observed by the appearance of the coating surface. The solutions containing more than 4% (w/w) of the AM additives appeared to segregate on the surface when dry. An optimal concentration for both, linalool and carvacrol, to be incorporated in MC-HPMC solutions was therefore assumed to be 4% (w/w) and subsequent experiments were performed using this formulation as the maximum level.

Most natural AM agents extracted from plants, also termed essential oils, are hydrophobic substances. They are naturally immiscible in hydrophilic or aqueous solutions and organic solvents are needed for their dissolution. In this study, ethanol was used as a dispersing agent for methylcellulose, which is immiscible in water. Ethanol is also an effective solvent for AM agents so aqueous ethanol solutions with a 2:1 ratio of ethanol to water were used as suggested by Cooksey (2000). Suppakul (2004) also successfully prepared MC-HPMC coating solutions using the same formula. After the ethanol evaporates, the remaining system is a hydrophilic film containing a hydrophobic AM agent. Naturally, such a system has a limited uptake of AM agents. However, the concentration of the AM agents at the level of 4% (w/w) in the coating solution should be high enough for effective AM activity after drying.

Suppakul (2004) reported a positive result for the AM activity of a film containing linalool at the level of 1% (w/w), based on the dry weight of the film.

4.3 Activity of AM agents released from coating materials

The AM activity of the produced films containing a maximum concentration of linalool and carvacrol in the AM coating layer with a measured thickness of 20 μm, was examined by the agar disc diffusion test (see Section 3.3.3). A clear zone around the film indicates the inhibition caused by the AM agent that is released from the coated polymer. The AM activity of the control film (without an AM agent), the film containing linalool and the film containing carvacrol are shown in Figures 4.4, 4.5 and 4.6 respectively. No microbial growth inhibition was observed for the control film (Figure 4.4) as expected. For the sample containing linalool (Figure 4.5), a clear zone was observed indicating some microbial growth inhibition and suggesting that linalool has some inhibitory effect on 10^5 cfu mL^{-1} *E. coli*. The film containing carvacrol (Figure 4.6) resulted in a larger inhibition zone compared to the one containing linalool, and this zone spreaded symmetrically around the film sample. This confirms the higher AM activity of carvacrol compared with linalool when incorporated in MC-HPMC.

The inhibition zone surrounding the linalool films did not extend significantly beyond the edge of the film sample, which is similar to the result reported by Suppakul (2004). This result suggests that linalool has limited solubility in aqueous-based agar media and is therefore unable to diffuse through it effectively. The clear zone surrounding the carvacrol film is approximately double the size of the film sample. This is in recognition of the fact that the solubility of carvacrol is approximately 3 g in 100 mL water whereas the solubility of linalool is approximately 0.16 g in 100 mL water (see Appendices A.4 and A.6). Therefore, carvacrol was selected for further experiments.

Figure 4.4 Inhibitory effect of control film against *E. coli*.

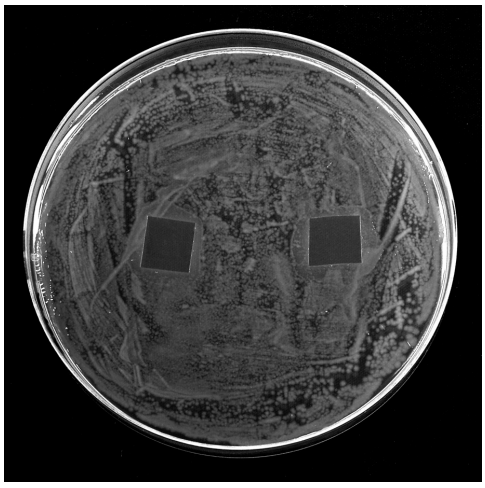


Figure 4.5 Inhibitory effect of linalool film against *E. coli*.

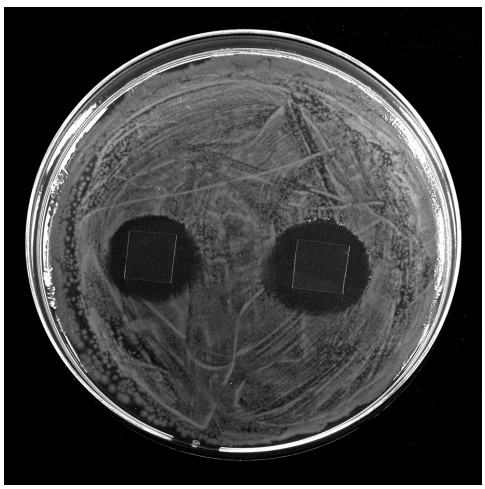


Figure 4.6 Inhibitory effect of carvacrol film against *E. coli*.

4.4 Stability of coating materials

A polymeric packaging material must remain stable while in contact with food. The MC-HPMC coating material is water-soluble and is therefore unsuitable for contact with high-moisture foods. An alternative coating material is EA-MMA, which is water insoluble and therefore more suitable for moist food contact applications. Similar to the MC-HPMC formulations, a maximum concentration of 4% (w/w) carvacrol can be incorporated into the EA-MMA casting solution formulation without surface separation being observed in the dry film.

The stability of AM film coatings made from MC-HPMC or EA-MMA containing the same concentration of carvacrol were compared by immersion in different food simulants. The thickness of both coating materials was 20 µm. Table 4.1 shows the relative stability of the MC-HPMC and EA-MMA formulations based on the apparent solubility in different food simulants.

Table 4.1 Stability of cellulose derivative and polyacrylate in food simulants.

Food simulants	MC-HPMC	EA-MMA
Water	Dissolved	Undissolved, turbid
10% (v/v) ethanol	Dissolved	Partly dissolved, turbid
50% (v/v) ethanol	Dissolved	Partly dissolved, turbid
95% (v/v) ethanol	Dissolved	Completely dissolved
Olive oil	Undissolved	Undissolved

The MC-HPMC formulation was completely dissolved in every food simulant containing water. Structurally, the MC-HPMC includes methyl, ethyl and hydroxyl groups that are highly reactive with water. The EA-MMA formulation remains undissolved in water due to the lower polarity of its structure. An undesirable characteristic of EA-MMA is that it temporarily turns white when absorbing water and becomes transparent again when the water is desorbed. The EA-MMA formulation dissolves in food simulants containing ethanol and there is an increasing solubility with increasing ethanol concentration. Furthermore, the food simulant containing 95% (v/v) ethanol completely dissolves the EA-MMA formulation. Both

the MC-HPMC and EA-MMA formulations were stable in olive oil and therefore could potentially be used in contact with oily food. For contact with aqueous foods, the EA-MMC formulation is more suitable.

A potentially stable food contact film formulation would comprise the AM agent/coating material covered with a protective moisture barrier material such as another LDPE film. The EA-MMA formulation is adhesive so it was anticipated that the LDPE could be directly laminated to produce a multi-layered film with an AM coating layer between two LDPE layers.

4.5 Retention of AM agent in coating material

During the coating process and the drying stage, carvacrol was unavoidably evaporated with the ethanol and moisture in the EA-MMA formulation. The retention of carvacrol in the AM layer was investigated by comparing its concentration in the AM films before and after drying. The results are shown in Table 4.2.

Table 4.2 Retention of carvacrol in EA-MMA coating formulation (see Appendix B.1 and B.2).

	AM coating layer thickness / μm	
	20	40
Concentration of carvacrol in dry coating / % (w/w)	29	30.6
Concentration of carvacrol in dry coating / g cm^{-3}	0.22	0.21
Retention of carvacrol / % (w/w)	95	96.2

The EA-MMA formulation containing initially 4% (w/w) carvacrol resulted in a dry AM layer that contained about 30% (w/w) or *ca.* 0.2 g cm^{-3} of the AM agent upon evaporation of the solvent. The results show that *ca*. 95% of carvacrol was retained in the dry AM layer. The drying process of the EA-MMA formulation took approximately one hour and the results show that the EA-MMA formulation has a high capacity for retaining carvacrol. This may also contribute to a long-term activity of the produced AM films, which enables them to remain active for a long period as illustrated schematically in Figure 2.5.

4.6 Release of AM agent from multi-layer films

4.6.1 Effect of controlling layer thickness

Release of AM agent into food simulants

Although carvacrol is adequately retained in the film, the performance of AM films in releasing the AM agent into food simulants required further investigation. An aqueous solution containing 95% (v/v) ethanol – a recommended fatty food simulant for polyolefin packaging materials by the Food and Drug Administration (FDA) in the US (McCort-Tipton and Pesselman, 2000), was used in the current work. In order to study the release of AM agents from multi-layered films in contact with food, an additional LDPE film was laminated over the EA-MMA layer to protect it from dissolution when in direct contact with food simulants and to control the release rate of the AM agent. In order to minimize the undesirable loss of AM agent to the atmosphere through the outer LDPE film layer (that is not in contact with the food), a relatively high barrier nylon backing film was used. The structure of the entire multi-layer AM film was nylon/LDPE/EA-MMA/LDPE (from outside to inside) as shown in Figure 4.7.

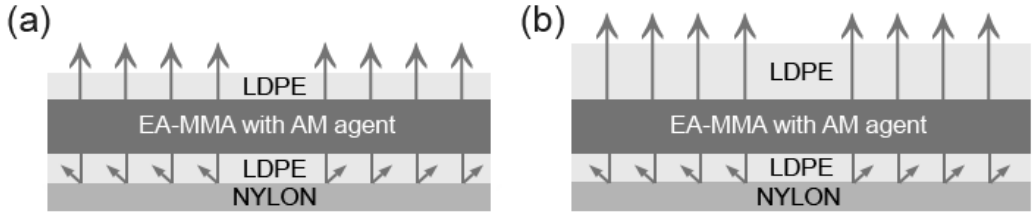


Figure 4.7 Nylon/LDPE/EA-MMA/LDPE multi-layer AM film.

Figure 4.8 shows the release of carvacrol into 95% aqueous ethanol at 37, 20 and 4°C from a 40 μm thick EA-MMA layer (or AM layer) through a LDPE controlling layer of (a) 10 μm or (b) 50 μm in thickness.

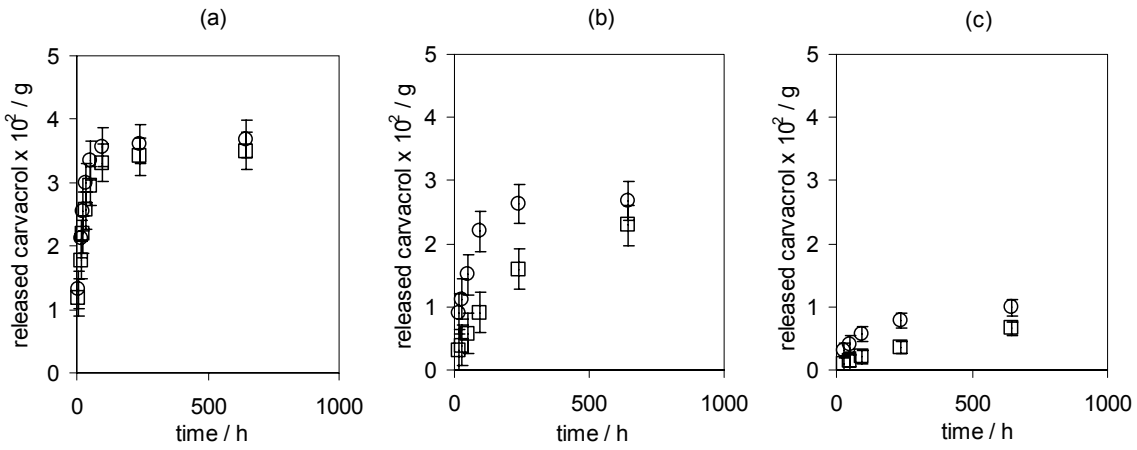


Figure 4.8 Amount of carvacrol released into 95% ethanol from 40 μm thick AM layer through (○) 10 μm or (□) 50 μm thick controlling LDPE layer at: (a) 37°C, (b) 20°C, (c) 4°C.

From the previous section that described the retention of carvacrol, the AM films contained up to *ca.* 0.2 g cm^{-3} carvacrol in the EA-AMM layers. Therefore, the total mass of carvacrol contained in the reservoir of the film samples (6 cm × 12 cm × 40 μm) was approximately 6.1 × 10^{-2} g. Figure 4.8(a) shows the release of carvacrol from the AM films approaching equilibrium when 3.7 × 10^{-2} g carvacrol was released into 50 mL of ethanol 95% (v/v) at 37°C. Figure 4.8(b) and 4.8(c) show that, at 20°C and 4°C respectively, the release did not reach equilibrium even after 1000 h.

Piringer (1999) reported that the partition coefficients ($K_{P/F}$) of many phenol compounds between LDPE (*P*) and ethanol (*F*) at 23°C are all less than unity. The partition coefficient of 2, 3, 5, 6-tetramethylphenol (an isomer of carvacrol ($C_{10}H_{14}O$) of the same molecular weight) between LDPE and 95% (v/v) ethanol is *ca.* 0.03. This means that the solubility of the phenol compound in the LDPE matrix is *ca.* 33 times lower than in 95% (v/v) ethanol. This also suggests that at equilibrium, the concentration of carvacrol in ethanol represents quite well the concentration in LDPE. The mass balance and the concentration of carvacrol present in the AM film of a 50 μm thick LDPE film as a controlling layer and in contact with a food simulant at 37°C are shown in Table 4.3.

Table 4.3 Mass balance and concentration of carvacrol released from nylon/LDPE/EA-MMA/LDPE film at initial and equilibrium state at 37°C (see Appendix B.3).

	Multi-layer system					
	40 μm AM layer		50 μm controlling layer		Ethanol	
Volume / cm^3	0.288		0.36		50	
	Amount of carvacrol					
	Initial	Equilibrium	Initial	Equilibrium	Initial	Equilibrium
Mass balance / g	6.1×10^{-2}	2.4×10^{-2}	0	2.6×10^{-4}	0	3.7×10^{-2}
Concentration / g cm^{-3}	2.1×10^{-1}	8.3×10^{-2}	0	7.4×10^{-4}	0	7.4×10^{-4}

Note: The initial mass and concentration presented in the table are the values considered directly after the films were produced, not at the commencement of the test.

While the concentration of carvacrol in the LDPE cover layer was assumed to be equal to the concentration in the food simulant (*ca.* 7.4×10^{-4} g mL^{-1}), the mass of the AM agent in the 50 μm thick LDPE layer (*ca.* 0.36 cm^3) was *ca.* 2.6×10^{-4} g. The mass balance shows that, at equilibrium, carvacrol still remained inside the film (reservoir, *ca.* 2.4×10^{-2} g). The concentration of the AM agent retained in the reservoir of the AM film (*ca.* 8.3×10^{-2} g cm^{-3}) was relatively high when compared with the released concentration (*ca.* 7.4×10^{-4} g mL^{-1}). This indicates that the LDPE controlling layer functioned as a selective barrier to restrict the migration of AM agent through it. In addition, the conservation of mass evident in Table 4.3 supports the notion that the nylon/LDPE backing film provides a relatively high barrier to the permeation of carvacrol. The partition coefficient of carvacrol migrating from the AM coating layer (reservoir) to LDPE ($K_{P/S}$) can be calculated from the ratio of the equilibrium concentrations of carvacrol in the AM layer and the controlling layer, which is approximately 8.9×10^{-3} (see Appendix B.4).

The concentration profile of carvacrol in the migration pathway from the AM layer to the receptor phase (or food simulant) is described in the multi-layer model as shown in Figure 4.9. The figure demonstrates the availability of AM agent that is contained inside the film and the capacity of the LDPE layer to control the migration. This

corresponds to the modelling of migration from multi-layer structures presented by Brandsch *et al.* (1999). The model was introduced as an example to demonstrate a migration study, in which at equilibrium, the concentration of migrant contained inside the AM layer is 100 times higher than the concentration in the controlling layer and also in the food simulant. Therefore, the partition coefficient between the AM layer (*S*) and the controlling layer (*P*) is $K_{S/P}$ = 100 (Brandsch *et al.*, 1999).

In the current study, the partition coefficient of carvacrol between the EA-MMA layer and the LDPE layer is approximately: $K_{S/P}$ = 112 (or $K_{P/S}$ = 8.9 × 10^{-3}) at 37°C, according to the model mentioned above.

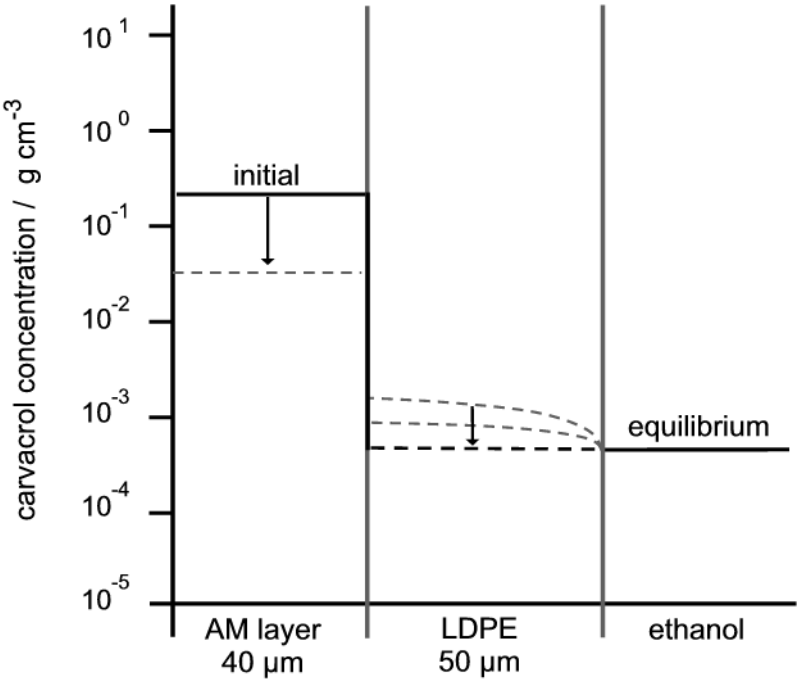


Figure 4.9 Concentration profiles of release from nylon/LDPE/EA-MMA/LDPE film at the initial and equilibrium states.

Without the controlling layer, the entire amount of AM agent would be released rapidly because of the direct contact with the food simulant. In a strong solvent such as ethanol in which the solubility of AM agent is much higher than in the AM layer, the concentration of carvacrol in the food simulant would rise rapidly whereas the

concentration in the AM film would drop quickly. Finally the concentration in both phases would be equal when the system reaches equilibrium.

For the release from a multi-layer films, the AM agent must migrate across two layers, namely the AM layer itself and the protecting layer. This would cause a lag time before the first release would start from the surface of the cover layer (Piringer, 2007). Nevertheless, multi-layer films can also release AM agents quickly provided that the AM agent already exists in the controlling layer. Figure 4.10 shows the early stage of the release, which proceeds according to a zero-order rate (Richards, 1985). Figure 4.10 shows the early stage of the release. The dashed lines suggest that all extrapolated Y-intercepts were above the origin, which refers to the "burst effect" during the unsteady state (see Section 2.4.2). The graphs proceed with the linear solid lines which demonstrate the steady state of release having a zero-order rate.

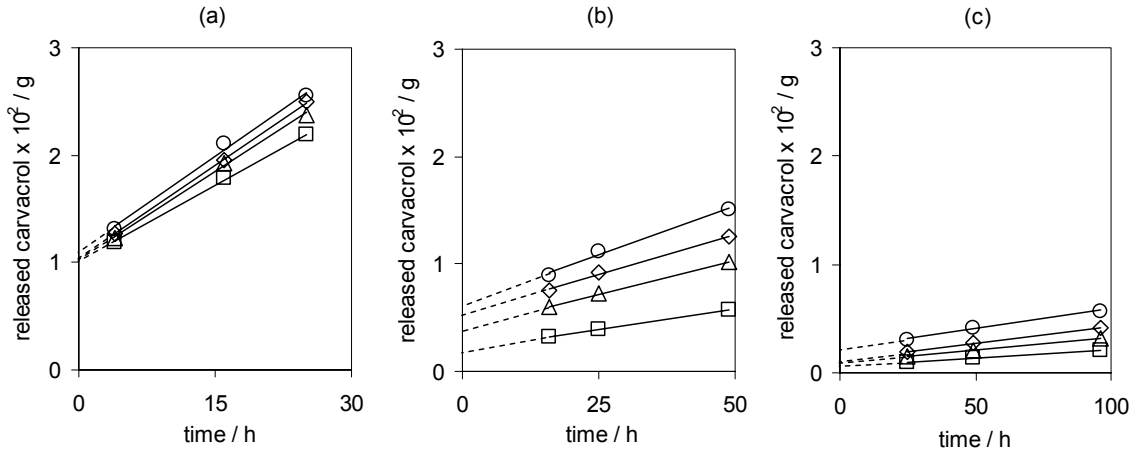


Figure 4.10 Zero-order initial rate of carvacrol released from 40 µm thick AM layer through: (○) 10 µm, (◊) 20µm, (Δ) 30 µm, (□) 50 µm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C.

The results clearly show an immediate release of AM agent into the food simulant, which suggests that during the film preparation, carvacrol was absorbed and present in the controlling layer prior the testing. The reason for it is because the AM layer was produced by a casting method, which used ethanol as a solvent. After casting, the films were left for only one hour before laminating with a protecting LDPE layer. The lamination was intentionally performed at a stage that the cast AM layer was not

completely dry in order to achieve adequate adhesion. The ethanol and carvacrol remaining in the undried AM layer was able to migrate into the matrix of the controlling layer thus allowing the films to release carvacrol in the early stage of the experiment. With the thin controlling layer, the absorption process could rapidly approach the saturation and the initial release then proceeded with the steady state. The zero-order rates from AM films with different controlling layer thicknesses ranging from 10 to 50 μm at three different temperatures are obtained from the slope in Figure 4.10. The values are given in Table 4.4.

Table 4.4 Initial rate of release of carvacrol from AM films with different controlling layer thicknesses at different temperatures.

	Test temperature		
	37°C	20°C	4°C
Controlling layer thickness / μm	Initial release rate × 10^4 / g h^{-1}		
10	5.95	1.81	0.37
20	5.82	1.47	0.30
30	5.50	1.27	0.23
50	4.77	0.77	0.15

Table 4.4 shows that the initial release rate decreased with decreasing temperature. Decreasing the thickness of the controlling layer resulted in a higher initial rate of release. These results are consistent with equation 2.7, in which the initial rate of release is inversely proportional to the thickness of the controlling layer.

If the initial rate $v_0 = dM_t/dt$ when $t = 0$, and the rate constant $k = AD_PK_{P/S}/l_PV_1$, then $v_0 = M_\infty k$. In a system, where A, $D_PK_{P/S}$ and V_1 are constant, the rate constant k is inversely proportional to the thickness l_p of the film ($k \propto 1/l_P$). Consequently, the initial rate (v_0) is also inversely proportional to the film thickness ($v_0 \propto 1/l_P$).

After the release started and carvacrol migrated from the reservoir, the concentration gradient between the two sides of the controlling layer decreases and therefore the

rate of release is reduced. Due to the higher initial rate of release, the thinner controlling layer had less carvacrol remaining in the reservoir than in the thicker controlling layer. With time, when the concentration gradient between the opposite sides of the thinner controlling layer became significantly low, the release rate became lower than the release through the thicker controlling layer.

When the system is agitated well or when the solubility of migrant in the food simulant is high, the partition coefficient of the AM agent migrating from the controlling layer to the food simulant ($K_{P/F}$) may be assumed to be less than or equal to unity (Piringer, 2007). The release rate of such system is dominated by the dimensions of the film*, namely* the film surface area (A), the thickness of the controlling layer (l_P), as well as by the concentration of the AM agent in the reservoir (C_S); the diffusion coefficient of the controlling layer (D_P) and the partition coefficient associated with the AM agent migrating between the reservoir and the controlling layer ($K_{P/S}$) as shown in equation 2.14 (Richards, 1985).

Following equation 2.7, the rate of release is exponentially related to the time of release. The plots of the release experiments conducted at 37°C, 20°C and 4°C are shown in Figure 4.11 from which the total mass of released AM agent and the permeability can be calculated (see Appendix B.5).

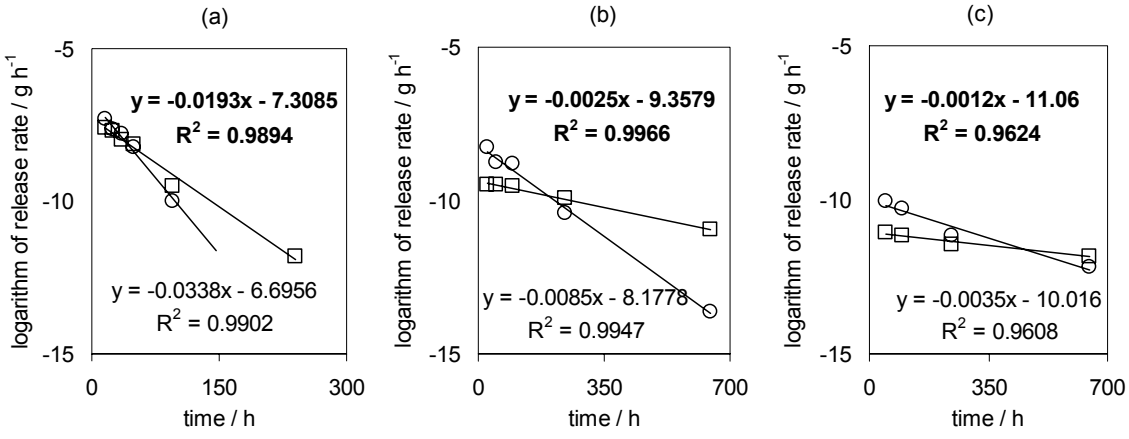


Figure 4.11 Logarithm of release rate of carvacrol migrating from 40 µm thick AM layer through: (○) 10 µm, (□) 50 µm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C versus time.

Figure 4.11 shows the logarithmic change in the release rate with time where the thinner controlling layer releases carvacrol at an initially high rate and decreases significantly with time. The figure is derived from Figure B.1 in Appendix B.5 and shows only migration from 40 µm thick AM layer through 10 and 50 µm thick controlling layer. According to equation 2.7, it can be seen that the release rate (dM/dt) is not only inversely proportional to the thickness of the controlling layer (l_P) but is also a function of $\exp(-1/l_P)$. The results demonstrated in Figure 4.11 show that during the early stages the release rate through the thicker controlling layer is slower than through the thinner layer. Consequently, the AM agent concentration in the reservoir behind the thicker controlling layer decreased slower while the AM agent concentration in the reservoir with the thinner controlling layer was undergoing fast exhaustion. At a point in time, both the concentrations of the AM agent behind the thinner controlling layer and the concentration gradient across this layer become lower than those of the thicker controlling layer. This causes the release rate across the thicker controlling layer to become higher than that through the thinner layer.

Table 4.5 shows estimates of the total mass of carvacrol that should be released at equilibrium calculated from equation 2.6 and 2.7. The results calculated from the short-form equation (equation 2.7) were similar to the results from the full-form equation (equation 2.6). This indicates that the testing condition met the assumption used to derive the short-form equation, a situation in which the volume of the food simulant must be much higher than the volume of the film *i.e.* $V_2 >> V_1$.

The results show that the total mass of carvacrol released at 4°C was much lower than that at 37°C and 20°C. Since the diffusion process is temperature and time dependent, the time of release at the lower temperature is longer for reaching the same equilibrium condition (Figge, 1996). At 4°C, the experimental data were collected during the very early stages of release which could lead to an underestimation. Considering the results at 37°C and 20°C, it was found that the total mass of the release is insignificantly dependent of the thickness of the controlling layer. Since the solubility of the AM agent in the food simulant (ethanol) is greater than the solubility in the controlling layer (PE), the partition coefficient ($K_{P/F}$) may be assumed to be less than or equal to unity (Piringer, 2007). The concentration of migrated AM agent in the food simulant is then equal to the concentration in the controlling layer at equilibrium

(see Figure 4.9). Therefore, the change of the controlling layer thickness has a negligible impact on the observed concentration of AM agent in the food simulant. Although the thicker the controlling layer, the more AM agent will remain in this layer at equilibrium for a constant AM agent concentration in the AM layer, the volume of food simulant is extremely greater than the volume of the film, the amount of AM agent remaining in the film is then relatively insignificant.

Table 4.5 Estimated total mass of released carvacrol from AM films with different controlling layer thicknesses at various temperatures (see Appendix B.5).

		Total released mass $(M_\infty) \times 10^2$ / g	
Temperature / °C	Controlling layer thickness / μm	*	**
37	10	3.68	3.66
	20	3.55	3.53
	30	3.71	3.69
	50	3.49	3.47
20	10	3.32	3.30
	20	3.09	3.07
	30	3.20	3.18
	50	3.47	3.45
4	10	1.28	1.28
	20	1.21	1.20
	30	1.25	1.24
	50	1.32	1.31

* Full-form equation (Equation 2.6)
** Short-form equation (Equation 2.7) that is valid when the release is not limited by the volume of food simulant (see Section 2.4.2)

The permeability is temperature dependent and is equal to the product of the diffusion coefficient and the solubility coefficient (D_PS) or the diffusion coefficient and the partition coefficient ($D_PK_{P/S}$) at the equilibrium state. It is also affected by the dimension of the film, the released amount, the release time and the concentration gradient of migrant between the two sides of the film (Piringer, 1999). Since the equilibrium concentration was estimated from M_∞ obtained from Table 4.5, "transmissibility" was then used in place of the term permeability. Table 4.6 shows

the transmissibility of carvacrol through the different thicknesses of the controlling layer as determined from the slope of the plots in Figure 4.11. A lack of correlation between the controlling layer thicknesses and the transmissibility values obtained at 4°C and 20°C is observed as the thickness increases from 30 to 50 μm. This is because 10-30 μm films had controlling layers that were comprised of multi-layers of 10 μm films each while the 50 μm sample was made of a single film. This evidence is observed at 4°C and 20°C, when the release was much slower than the release at 37°C.

Table 4.6 Estimated transmissibility of carvacrol released from AM films with different controlling layer thicknesses at different temperatures (see Appendix B.5).

		Transmissibility × 10^{11} / m^2 h^{-1}	
Temperature / °C	Controlling layer thickness / μm	*	**
37	10	1.34	1.35
	20	2.66	2.68
	30	3.23	3.25
	50	3.84	3.86
20	10	0.34	0.34
	20	0.58	0.58
	30	0.61	0.61
	50	0.50	0.50
4	10	0.14	0.14
	20	0.26	0.26
	30	0.26	0.26
	50	0.24	0.24

* Full-form equation (Equation 2.6)
** Short-form equation (Equation 2.7) that is valid when the release is not limited by the volume of food simulant (see Section 2.4.2)

The natural logarithm of the mass fractions of carvacrol retained in the AM films with a controlling layer of thickness ranging from 10 μm to 50 μm at three different temperatures during the initial state of release were plotted versus time as shown in Figure 4.12, which the release is demonstrated as the first-order kinetic rate.

The empirical rate constant (k) of the first-order release rate can be calculated from a plot of the mass fraction of carvacrol retained in the reservoir versus time (Figure 4.12) in accordance with equation 2.11. The rate constants for the release of carvacrol with different thicknesses of the controlling layer at the three temperatures are shown in Table 4.7. In this table, the rate constants obtained from different plots of ln(1-M_t/M_∞) versus time and dM/dt versus time, show direct correlation with temperature and inverse correlation with thickness of the controlling layer.

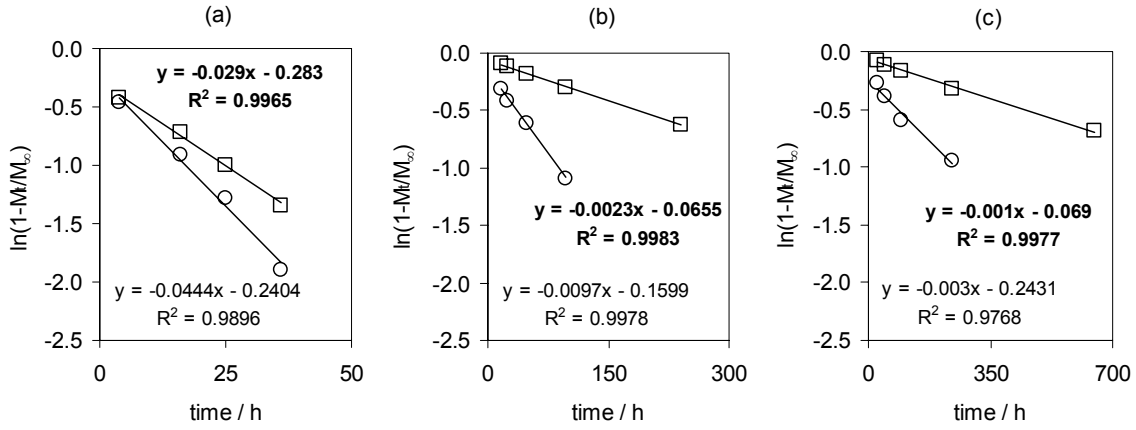


Figure 4.12 Natural logarithm of the mass fraction of carvacrol retained in the AM films of 40 μm in thickness and a controlling layer of: (○) 10 μm, (□) 50 μm thick at (a) 37°C, (b) 20°C, (c) 4°C.

From Table 4.7, the release rate is high at the highest test temperature and decreases with the decrease in temperature. Furthermore, at a given temperature, the rate constant of the release through the thinner controlling layer is higher than the release from the thicker layer during the initial state of release where the first-order kinetic is applied. With the same total mass of carvacrol to be released, the films with thinner controlling layers release the AM agent faster and approach equilibrium earlier. Namely, when the release started ($t = 0$), the rate constant k is proportional to $1/l_P$ and v_0 is proportional to $1/l_P$ because $v_0 = M_\infty k$. After the release started, the release rate is $v = v_0 \exp(-kt)$, or $v = M_\infty k \exp(-kt)$, therefore v is proportional to $1/l_P$ and also to $\exp(-1/l_P)$.

Table 4.7 Rate constants of release of carvacrol from AM films with different thicknesses of controlling layer at different temperatures (see Appendix B.6).

		Rate constant (k) × 10^2/ h^{-1}	
Temperature / °C	Controlling layer thickness / μm	*	**
37	10	4.44	3.38
	20	4.10	3.35
	30	3.31	2.71
	50	2.90	1.93
20	10	0.97	0.85
	20	0.57	0.72
	30	0.43	0.51
	50	0.23	0.25
4	10	0.30	0.35
	20	0.27	0.32
	30	0.21	0.22
	50	0.10	0.12

* Calculated from ln(1-M_t/M_∞) *vs* time

** Calculated from dM/dt *vs* time

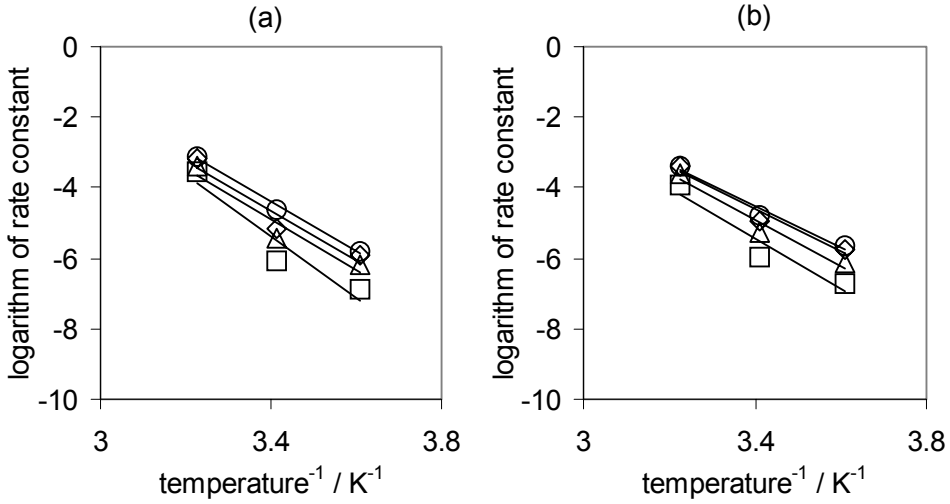


Figure 4.13 Logarithm of the rate constant obtained from: (a) mass fraction plot, (b) release rate plot; versus the reciprocal of the absolute temperature through a controlling layer of thickness: (○) 10 μm, (◊) 20μm, (Δ) 30 μm, (□) 50 μm.

The rate constants of the release rate at different temperatures conform to the Arrhenius equation 2.21. The plot of ln(k) versus the inverse of the absolute temperature is linear and has a slope of -Ea/R as shown in Figure 4.13. The results are summarized in Table 4.8, where the rate constants were obtained from different plots of ln(1-M_t/M_∞) versus time and from dM/dt versus time.

Table 4.8 Activation energy for release of carvacrol from AM films with different thicknesses of controlling layer (see Appendix B.7).

	Activation energy / J mol^{-1}	
Controlling layer thickness / μm	*	**
10	58.22	48.92
20	58.61	50.66
30	59.40	54.16
50	72.52	59.84

* Calculated from ln(1-M_t/M_∞) *vs* time

** Calculated from dM/dt *vs* time

The results show that the films with a thicker controlling layer have a higher energy barrier for the release process. Although the activation energy is the sensitivity of the rate constant to temperature and is independent of film thickness, in this experiment, the controlling layer was constructed from different numbers of 10 μm LDPE layer, which the interface can become resistant to the migration.

Release of AM agent to the atmosphere

When the nylon/LDPE/EA-MMA/LDPE multi-layer film is exposed to air at 20°C, the concentration of carvacrol retained in the film can be investigated by Fourier transform infrared (FT-IR) spectroscopy using a method that involves comparing data derived from sample spectra with standard curves. The relative loss of AM agent to the atmosphere can be estimated by assigning an IR peak to the AM agent and measuring the absorbance of the peak over time. Carvacrol has a hydroxyl group in its structure that is not present in any other components (see Appendix B.8). The IR absorbance band at the wave number of 3450 cm^{-1} corresponds to the hydroxyl group of carvacrol. The peak height of the absorbance measured at this wave number is proportional to the mass fraction of the AM agent retained in the films. The peak

height at t = 0 corresponds to the initial retained mass fraction. The peak height measured in the blank films was used as the reference, where $1-M_\infty/M_t = 0$ at $t = \infty$.

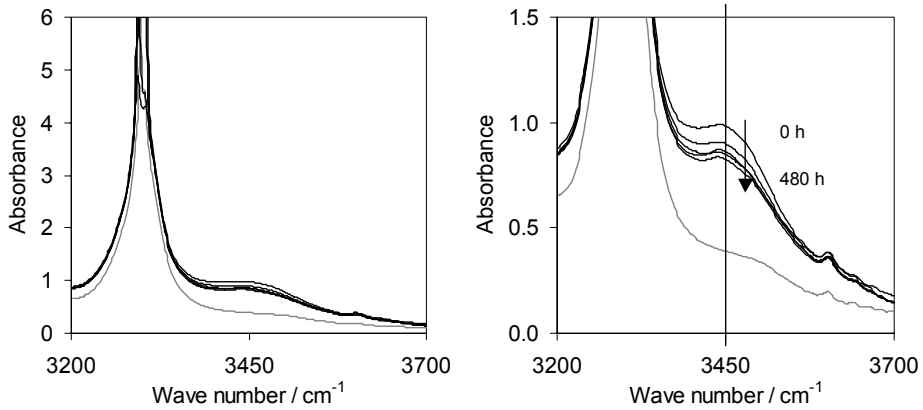


Figure 4.14 IR peak of carvacrol retained in the AM film with 50 µm thick controlling layer at 0, 120, 240, 360, 480 h.

Figure 4.14 shows that the peak height measured in the films with 50 µm thick controlling layer decreased with time between zero and 480 h whereas Figure 4.15 shows that the peak height measured in different controlling layer thicknesses after 480 h decreased with the decrease in thickness.

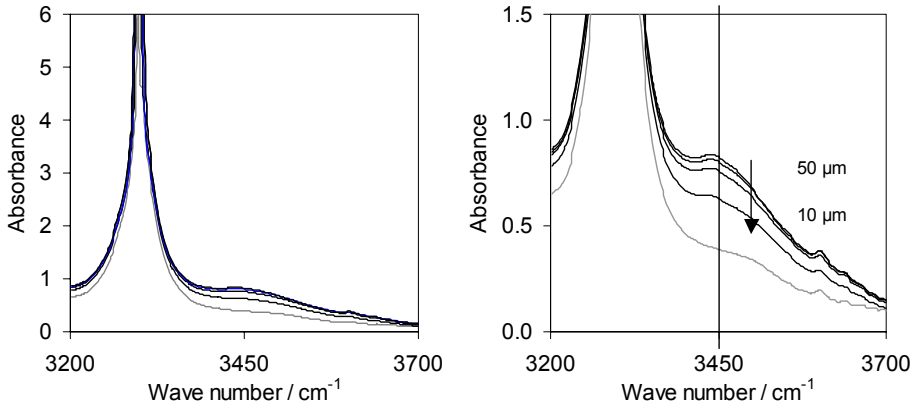


Figure 4.15 IR peak of carvacrol retained in the AM film with 10, 20, 30, 50 µm thick controlling layer after 480 h.

While the decrease in the absorbance peak related to carvacrol indicates the loss of the AM agent to the atmosphere, it also represents the capacity of the AM film to release

of the AM agent from the film. The release to the atmosphere is regarded as the release to an infinite sink for the volatilising AM compound as is assumed in Equation 2.11. The volume of a relatively large package headspace can also be considered as an infinite sink for the AM agent. On the other hand, considering a relatively small package headspace, the accumulation of volatilised AM compound may affect the partitioning of the AM compound between the AM film and the headspace and therefore may restrict the volatilisation. Figure 4.16 shows a plot of the mass fraction of AM agent that is retained in the film versus time. From this plot it is evident that a thicker controlling layer can effectively reduce the amount of released AM agent. Values of the rate constant (k) calculated from equation 2.11 are shown in Table 4.9.

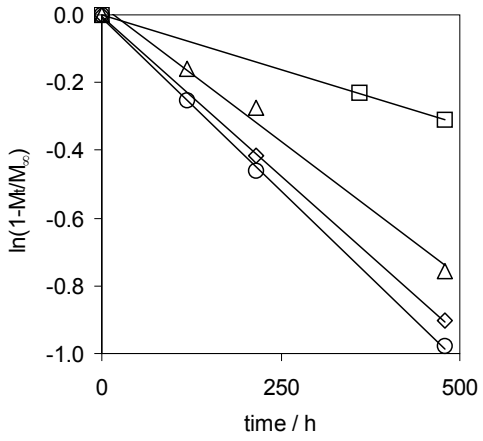


Figure 4.16 Logarithm of mass fraction of carvacrol retained in the AM films covered with a controlling layer of thickness: (○) 10 µm, (◊) 20µm, (Δ) 30 µm, (□) 50 µm when exposed to the air at 20°C for 20 days.

The results in Table 4.9 show that the films with thinner controlling layers release carvacrol into the atmosphere faster than the films with thicker controlling layers. It can be seen that the reduction of the release rate from the films with 10 to 30 µm thick controlling layers is much lower than the rate reduction from 30 to 50 µm. This is because the 50 µm layer was made of a single film whereas the other films had controlling layers that comprised of multi-layers of 10 µm films.

Table 4.9 Rate constants and transmissibility of carvacrol release to the atmosphere at 20°C from AM films with various thicknesses of the controlling layer (see Appendix B.9).

Thickness of controlling layer / μm	Rate constant (k) × 10^2 / h^{-1}	Transmissibility* × 10^{12} / m^2 h^{-1}
10	0.20	0.80
20	0.19	1.52
30	0.16	1.92
50	0.06	1.20

* Transmissibility was calculated from the short-form equation (Equation 2.7) because the volume of the air is infinite (see Section 2.4.2)

It is also evident that the transmissibility of the volatile AM agent in migrating through a single film is not consistent with that observed for the series of laminated films. However, it can be seen that the release through the 50 μm single layer has higher transmissibility than the 10 μm single layer.

4.6.2 Effect of AM layer thickness

It is known that the diffusion process is driven by the concentration gradient between two points (Chatwin, 1996). In this work, the concentration difference of the AM agent between the AM layer (reservoir) and the food simulant plays a key role. In the previous experiments the concentration gradient in conjunction with the thickness of the controlling layer was demonstrated to affect the diffusion rate. In this section the AM films were studied for the effect of variations in AM layer thickness, which is anticipated to affect the retention of the AM agent in the reservoir when the films were exposed to different food simulants.

In previous section (Section 4.6.1), the films comprised of a nylon barrier as a backing layer, which forced the release to occur primarily in one direction - into the food simulant. In this section, the backing layer was made of a LDPE film, through which carvacrol was allowed to migrate. The release then occurred from two sides of the films: one, the release to 95% (v/v) ethanol food simulant; the other, the release to the atmosphere. It was expected that while evaporation would take place on the outer side, the thickness of the AM layer would play a key role in supplying carvacrol for

the release to the other side. Accordingly, a higher capacity of AM agent retained in the reservoir would result in a higher AM agent release into the food simulant. The alternative structure of the resulting multi-layer film comprising LDPE/EA-MMA/LDPE is shown in Figure 4.17.

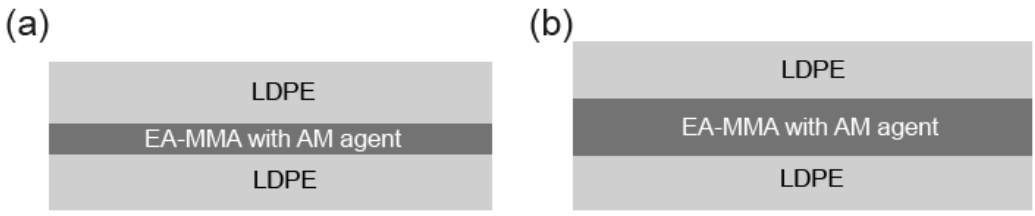


Figure 4.17 Schematic representation of the LDPE/EA-MMA/LDPE multi-layer AM film with: (a) 20 µm and (b) 40 µm thick EA-MMA layer.

The EA-MMA layer or AM layer containing up to *ca.* 0.2 g mL^{-1} carvacrol served as a reservoir of the AM agent while the outer LDPE layers function was to control the release of the AM agent. The total mass of carvacrol contained in the AM layers (6 cm × 12 cm) of 20 µm or 40 µm thicknesses was 3.3×10^{-2} g and 6.1×10^{-2} g respectively. When the AM film is brought in contact with a food simulant, the AM agent would commence releasing until the system reaches equilibrium. The equilibrium concentration can be approximated by the concentration of AM agent released into the food simulant when it remained constant or approached an asymptote.

Figure 4.18 shows the amount of AM agent released versus time from a 20 µm AM layer and a 40 µm AM layer through a 50 µm controlling layer into 50 mL ethanol (95% v/v) at 37°C, 20°C and 4°C. The results show that the 40 µm thick AM layer released more carvacrol and approached a higher asymptote than the 20 µm thick AM layer at the same temperatures.

It is anticipated that the release from the AM films with a nylon backing layer would result in a higher total mass of AM agent delivered to the food simulant. In a film without a backing layer, carvacrol can migrate in both directions: to the film surface in contact with the food simulant and to the opposite film surface exposed to the

atmosphere. A backing layer is therefore expected to be able to satisfactorily prevent unacceptable loss of AM agent to the atmosphere.

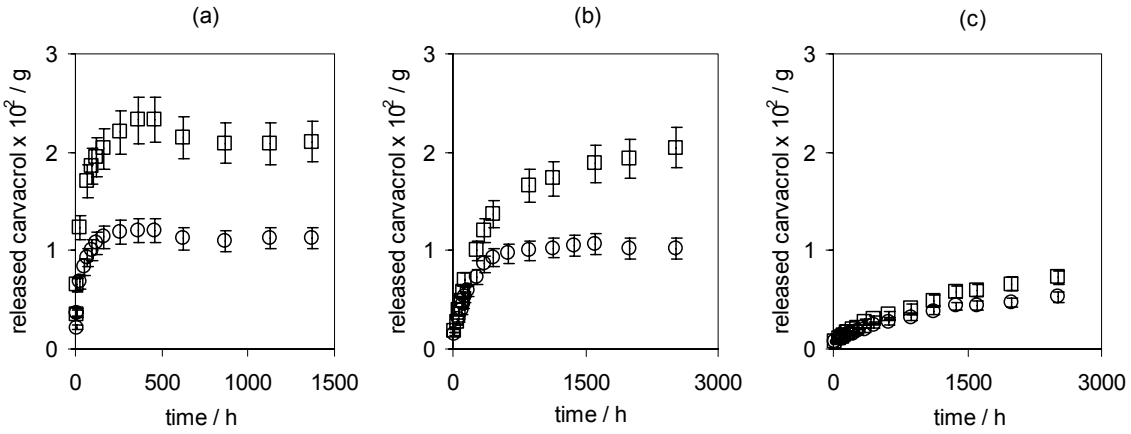


Figure 4.18 Amount of carvacrol released into 95% (v/v) ethanol from (○) 20 μm and (□) 40 μm thick AM layer through a 50 μm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C.

At the equilibrium state of the system at 20°C, the mass balance and the carvacrol concentration retained in the film as well as the concentration released from 20 and 40 μm thick AM layers into the food simulant were estimated (see Appendix B.10 and B.11) and shown in Tables 4.10 and 4.11 respectively. At equilibrium as demonstrated in Figure 4.19, the concentration of carvacrol retained in the film can be estimated from the partition coefficient of carvacrol migrating from the AM layer to LDPE ($K_{P/S}$ = 8.9 × 10^{-3}). This was obtained from the previous experiment where LDPE/nylon was used as the backing film.

Table 4.10 Mass balance and concentration of carvacrol released from LDPE/EA-MMA/LDPE film with 20 μm thick AM layers at initial and equilibrium states at 20°C (see Appendix B.10).

	Multi-layer system					
	20 μm AM layer		50 μm controlling layer		Ethanol	
Volume / cm^3	0.144		0.36		50	
	Amount of carvacrol					
	Initial	Equilibrium	Initial	Equilibrium	Initial	Equilibrium
Mass balance / g	3.3×10^{-2}	3.6×10^{-3}	0	7.9×10^{-5}	0	1.1×10^{-2}
Concentration / g cm^{-3}	2.3×10^{-1}	2.5×10^{-2}	0	2.2×10^{-4}	0	2.2×10^{-4}

Note: The initial mass and concentration presented in the table are the values considered directly after the films were produced, not at the commencement of the test.

Table 4.11 Mass balance and concentration of carvacrol released from LDPE/EA-MMA/LDPE film with 40 μm thick AM layers at initial and equilibrium states at 20°C (see Appendix B.11).

	Multi-layer system					
	40 μm AM layer		50 μm controlling layer		Ethanol	
Volume / cm^3	0.288		0.36		50	
	Amount of carvacrol					
	Initial	Equilibrium	Initial	Equilibrium	Initial	Equilibrium
Mass balance / g	6.1×10^{-2}	1.4×10^{-2}	0	1.5×10^{-4}	0	2.1×10^{-2}
Concentration / g cm^{-3}	2.1×10^{-1}	4.7×10^{-2}	0	4.2×10^{-4}	0	4.2×10^{-4}

Note: The initial mass and concentration presented in the table are the values considered directly after the films were produced, not at the commencement of the test.

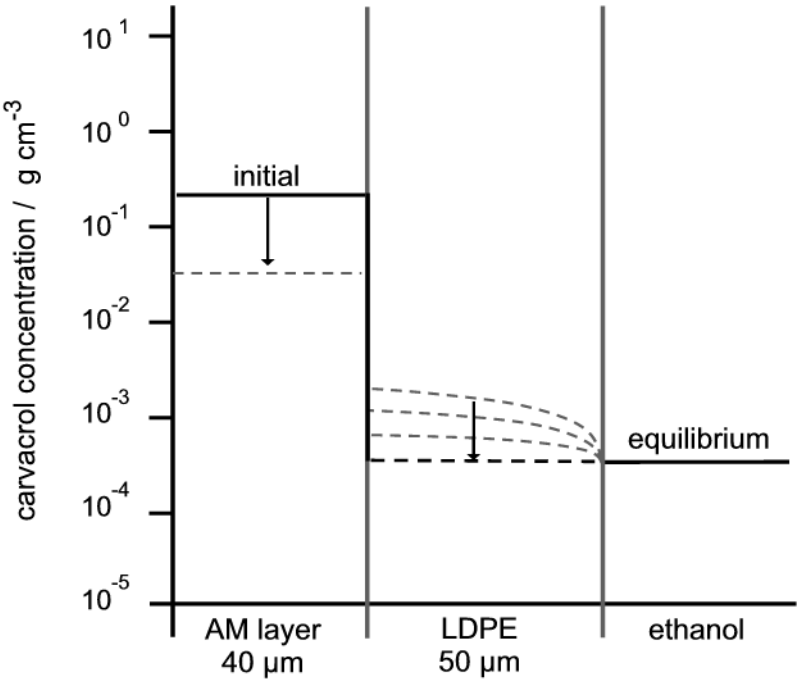


Figure 4.19 Concentration profile of release from LDPE/EA-MMA/LDPE film at the initial and equilibrium states.

The mass balance in Tables 4.10 and 4.11 shows that there is carvacrol missing from the systems. This corresponds to the result in the previous experiment, in which the release of carvacrol through the LDPE film into the atmosphere that was observed (see Section 4.6.1). In the absence of AM agent barrier film (nylon), the amount of carvacrol that can escape from the films with 20 µm and 40 µm thick AM layer through the LDPE film was 1.8×10^{-2} g and 2.6×10^{-2} g respectively (see Appendix B.10 and B.11). The percentage of loss was 54% and 43% by weight of the initial amount respectively. The films having a thicker AM layer could result in a higher AM agent retention and therefore a higher concentration of AM agent to be released into the food simulant. In an application where such loss is unavoidable, the equilibrium concentration of a system can be satisfied by a sufficient thickness of the reservoir. An increased volume of the reservoir can compensate for the AM agent escaping from the film. Nevertheless, changing the thickness of the AM layer may not be preferred as a method to further increase the equilibrium concentration of the AM agent. Increasing the concentration of AM agent in the reservoir might be a better option. However, since the capacity of the EA-MMA layer to retain carvacrol in its matrix is

limited (see Section 4.2), the selection of the polymer types in the film composite is another available option.

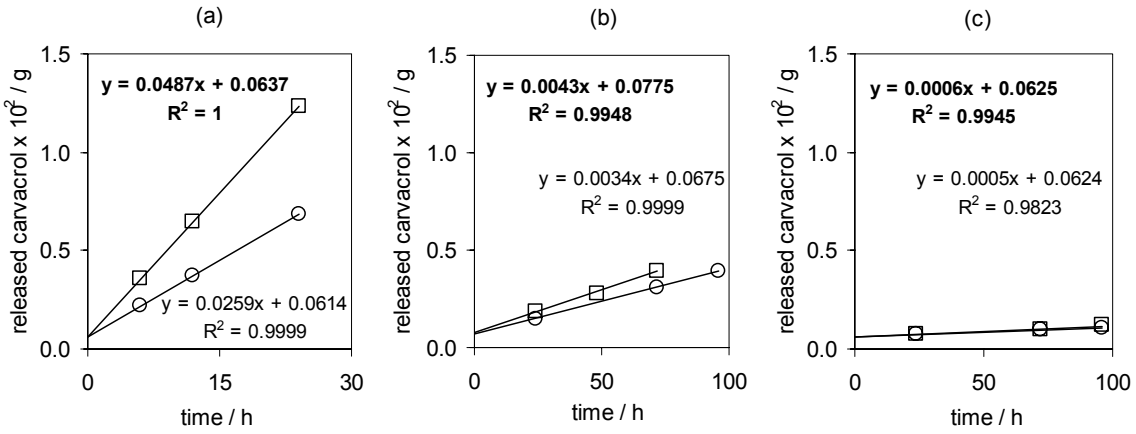


Figure 4.20 Initial rate of carvacrol released from (○) 20 µm and (□) 40 µm thick AM layer through 50 µm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C in the first stage.

The zero-order release rate was calculated from the plot of the released mass of carvacrol versus time when the release started as shown in Figure 4.20. The release of carvacrol from the produced films shows the immediate release, which is caused by the pre-existing AM agent in the controlling layer as discussed in Section 4.6.1. The initial release rates are shown in Table 4.12. While the concentration of carvacrol in the reservoir is initially high, the gradient is not significantly changed and the release rate therefore remains constant.

Table 4.12 Initial rate of release of carvacrol from AM films with different AM layer thicknesses at different temperatures.

Temperature / °C	AM layer thickness / µm	Initial release rate / g h^{-1}
37	20	0.26×10^{-4}
	40	0.49×10^{-4}
20	20	0.34×10^{-5}
	40	0.43×10^{-5}
4	20	0.5×10^{-6}
	40	0.6×10^{-6}

At the initial release point, the released amount of carvacrol from AM films with either a 20 μm or 40 μm thick AM layer was similar because they were constructed of EA-MMA with the same concentration of carvacrol. The migration of carvacrol across the controlling layer is driven by the concentration gradient between the reservoir and the food simulant.

If the initial rate is $v_0 = dM_t/dt$ at $t = 0$ and the rate constant is $k = AD_PK_{P/S}/l_PV_1$, then $v_0 = M_\infty k$. Since $k \alpha 1/V_1$, therefore $v_0 \alpha 1/V_1$. However, increasing V_1 will cause M_∞ to increase because the concentration of AM agent in the AM layer is constant. Therefore, from equation 2.6, the effect of $v_0 \alpha 1/V_1$ is negligible and the release rate is more affected by $v \alpha \exp(-1/V_1)$. If V_1 is high, v is also high. The plot of the release at 37°C (which is the highest abused release temperature) shows that the release of the two films proceeded at different rates. The release from the thicker AM layer was higher than from the thinner AM layer. While the AM agent was being removed from the reservoir layer, the rate of the release decreases exponentially (Richards, 1985). In the thicker reservoir, featuring a larger volume of AM agent, the concentration reduction is therefore slower. As time passes, the concentration of the AM agent in the thicker reservoir remains high and the release rate remains high also.

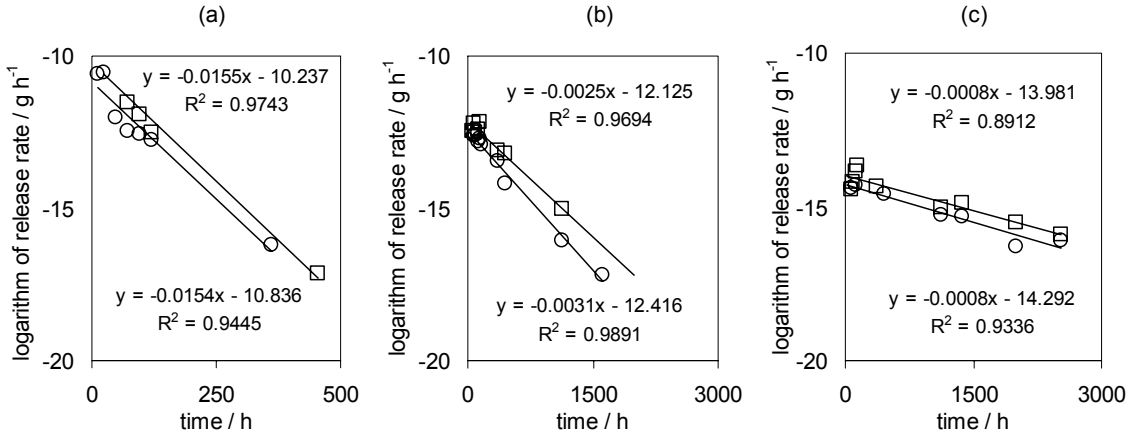


Figure 4.21 Logarithm of first-order rate of carvacrol released from (○) 20 μm and (□) 40 μm thick AM layer through 50 μm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C.

Figure 4.21 suggests that the release rate of carvacrol from the AM films decreases exponentially in accordance with equation 2.6 and 2.7. The plots clearly show the decrease of the release rate is in accordance with first-order kinetics at a similar rate. Furthermore, the time for achieving equilibrium was also longer at lower testing temperatures.

Table 4.13 Total mass release of carvacrol from AM films with different AM layer thicknesses at different temperatures (see Appendix B.12).

		Total released mass $(M_\infty) \times 10^2$ / g	
Temperature / °C	AM layer thickness / μm	*	**
37	20	1.28	1.28
	40	2.32	2.31
20	20	1.34	1.34
	40	2.18	2.17
4	20	0.78	0.78
	40	1.07	1.06

* Full-form equation (Equation 2.6)
** Short-form equation (Equation 2.7) that is valid for when the release is not limited by the volume of food simulant (see Section 2.4.2)

The plots of the logarithm of the release rate versus time (Figure 4.21) can be fitted to equation 2.6 and 2.7. The total mass of released carvacrol (M_∞) and the transmissibility at the test temperatures and different AM layer thicknesses are shown in Table 4.13 and 4.14 respectively. The results show that the total mass of released carvacrol from the 40 μm thick AM layer is higher than from the 20 μm thick AM layer. The transmissibility of carvacrol from the 40 μm thick AM layer is also higher than that from the 20 μm thick AM layer. Both parameters increased with increasing test temperature.

Table 4.14 Transmissibility of carvacrol through a 50 μm LDPE cover layer from AM films with different AM layer thicknesses at different temperatures (see Appendix B.12).

		Transmissibility × 10^{11} / m^2 h^{-1}	
Temperature / °C	AM layer thickness / μm	*	**
37	20	1.54	1.54
	40	3.08	3.10
20	20	0.31	0.31
	40	0.50	0.50
4	20	0.08	0.08
	40	0.16	0.16

* Full-form equation (Equation 2.6)
** Short-form equation (Equation 2.7) that is valid for when the release is not limited by the volume of food simulant (see Section 2.4.2)

Although the films prepared using the same concentration of carvacrol in the EA-MMA layer with a thickness of either 20 μm or 40 μm have similar initial AM release rates, the 40 μm thick EA-MMA contains more carvacrol that can be released into the food simulant. The concentration of the AM agent in the latter film therefore decreases more slowly. The release rate of carvacrol from the film with 40 μm AM layer was always higher than the rate from the 20 μm AM layer film. The results also show that the temperature affects greatly the transmissibility of carvacrol through the controlling layer. At 4°C, the carvacrol permeation was significantly less in compared to 20°C and 37°C and, therefore, the release data were only collectable in the very early stages of release. As a consequence, the total mass of released carvacrol calculated by using equations 2.6 and 2.7 are probably an underestimate, as discussed in Section 4.6.1.

According to the assumption used to derive the short-form equation, the solubility of AM agent in the food simulant and the volume of food simulant must be high enough to avoid the accumulation of AM agent at the releasing surface. This is the so-called "boundary effect" (Richards, 1985). When the volume of food simulant is much larger than the volume of the AM film, the short-form equation (equation 2.7) can be used instead of the full-form equation (equation 2.6). In Tables 4.14 and 4.15, the

calculated data obtained from the full-form and short-form equations provide similar results. Therefore, it is suggested that the ratio of the volume of the food simulant to the area of the tested films used in the present study 0.7 mL cm^{-2} is appropriate for the short form equation to be used. However, this ratio applied well to the experiment described in this section, which comprised 95% (v/v) ethanol – a strong solvent acting as a food simulant for fatty food and also suitable for hydrophobic AM agents from plant extracts. In a weak solvent, the distribution of the AM agent will be limited and the release will be dominated by the partition coefficient between the controlling layer and the food simulant (Figge, 1996).

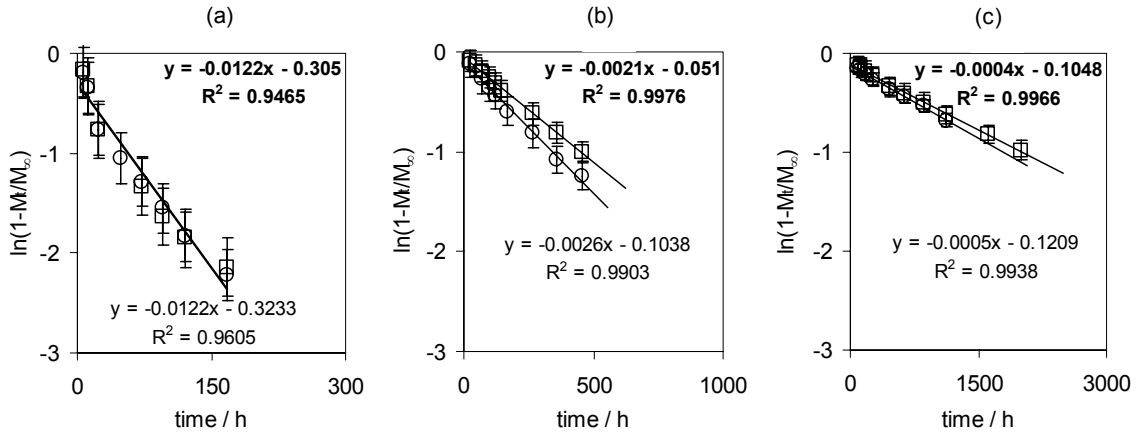


Figure 4.22 Logarithm of mass fraction of carvacrol retained in (○) 20 µm and (□) 40 µm thick AM layer at: (a) 37°C, (b) 20°C, (c) 4°C.

The mass fractions of carvacrol released from AM films with AM layer thicknesses of 20 µm and 40 µm at three different temperatures were plotted versus time as shown in Figure 4.21. In addition, plots of the mass fraction of carvacrol retained in the films versus time are also shown in Figure 4.22.

The empirical rate constant (k) can be obtained from a plot of the logarithm of the mass fraction of carvacrol retained in the reservoir versus time (Figure 4.22) in accordance with equation 2.11 (see Section 2.4.2). Rate constants for the release of carvacrol from an AM layer thickness of 20 µm and 40 µm are shown in Table 4.15. In the table the rate constants were obtained from different plots: $\ln(1-M_t/M_\infty)$ versus time and dM/dt versus time.

Table 4.15 Effect of temperature and AM layer thickness on the rate constant for the release of carvacrol from AM films (see Appendix B.13).

		Rate constant (k) × 10^2/ h^{-1}	
Temperature / °C	AM layer thickness / μm	*	**
37	20	1.22	1.54
	40	1.22	1.55
20	20	0.25	0.31
	40	0.21	0.25
4	20	0.05	0.08
	40	0.04	0.08

* Calculated from ln(1-M_t/M_∞) *vs* time

** Calculated from *dM/dt vs* time

The data show that, at a given temperature, the rate constants for the release of carvacrol from films with either a 20 μm thick AM layer or with a 40 μm thick AM layer are similar because these release constants were considered from the ratio of M_t/M_∞, in which the actual quantity of the release was ignored. The actual rates of release from the two films are not equal, as shown in Figure 4.20, as the release is from films that contain different amounts of AM agent and subsequently reach a different equilibrium concentration. For example, at 20°C, the release from AM films containing the 40 μm AM layer (carvacrol) in the range of $M_t/M_\infty = 0$ to $M_t/M_\infty = 1$ was 2.1×10^{-2} g but the release of carvacrol in the same range from the 20 μm AM layer was 1.3×10^{-2} g .

In regards to the previous discussion that the release rate was more affected by the term; "exp(-1/V_1)" than by the term "1/V_1", the results in Figure 4.18 show that the release rate of carvacrol from the films containing a 40 μm AM layer is always higher than the release rate from the films containing a 20 μm AM layer. Also, the rate constants were shown to conform to the Arrhenius equation 2.21. The plot of ln(k) versus 1/T is linear and has a slope of -Ea/R as shown in Figure 4.23.

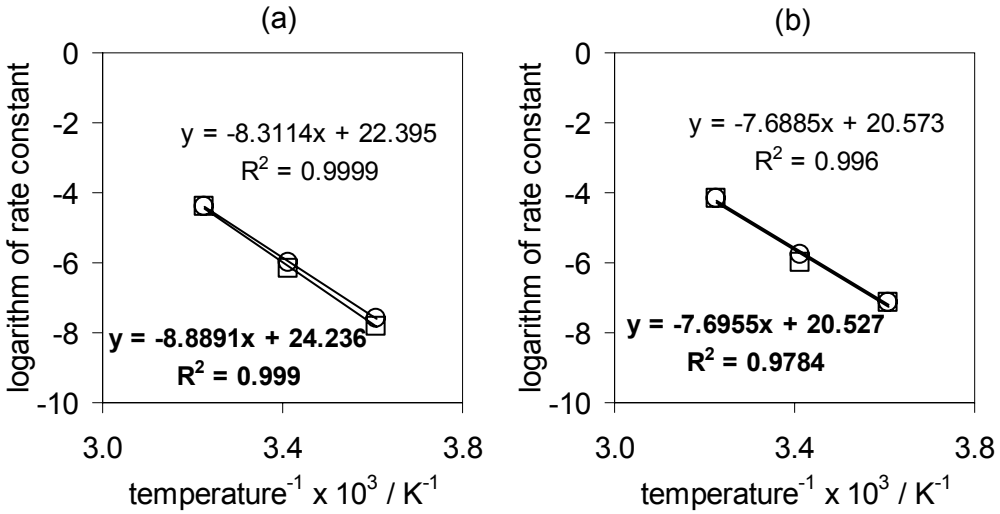


Figure 4.23 Logarithm of the rate constant obtained from: (a) mass fraction plot, (b) release rate plot; versus the reciprocal of the absolute temperature in the release of carvacrol from (○) 20 μm and (□) 40 μm thick AM layer.

Table 4.16 Activation energy of the release of carvacrol from the films with different AM layer thicknesses.

	Activation energy / J mol^{-1}	
AM layer thickness / μm	*	**
20	69.10	63.92
40	73.91	63.98

* Calculated from ln(1-M_t/M_∞) *vs* time

** Calculated from *dM/dt vs* time

The activation energy (E_a) for the release can be calculated from the slope of the plot of the logarithm of the rate constant versus the reciprocal of the absolute temperature (Figure 4.23). The rate constants can be obtained both from the plot of the logarithm of the mass fraction of carvacrol retained in the films versus time and from the plot of the release rate versus time. The results are shown in Table 4.16.

The similar activation energies for the release from the 20 and 40 μm AM layer suggests that the temperature dependence of the release does not depend on the

thickness of the AM layer. Although the composition of the film was of different thicknesses of the AM layer, the concentration of AM agent was the same. The driving force, which is the concentration gradient between the opposite sides of the controlling layer, was therefore equal when the release started. The activation energy of the films composed of 40 μm thick AM layer and 50 μm thick controlling layer obtained from this experiment and previous section (refer Table 4.8) are similar.

4.6.3 Effect of solubility of the AM agent

In the previous section, the films were tested for the effect of increasing the capacity of AM agent retained in the reservoir, which resulted in a higher equilibrium concentration of the agent released into 95% (v/v) ethanol – a simulant for fatty foods. In such a strong food simulant, the release profile of carvacrol – a hydrophobic AM agent, was achieved without the solubility limit. Nevertheless, it was interesting to demonstrate the effect of solubility when the films containing carvacrol were subject to the release test in an aqueous food simulant. To investigate the release in an aqueous food simulant, the films with either a 20 μm or a 40 μm thick AM layer were tested for their AM agent release into water at 20°C and the release curves are shown in Figures 4.24 and 4.25.

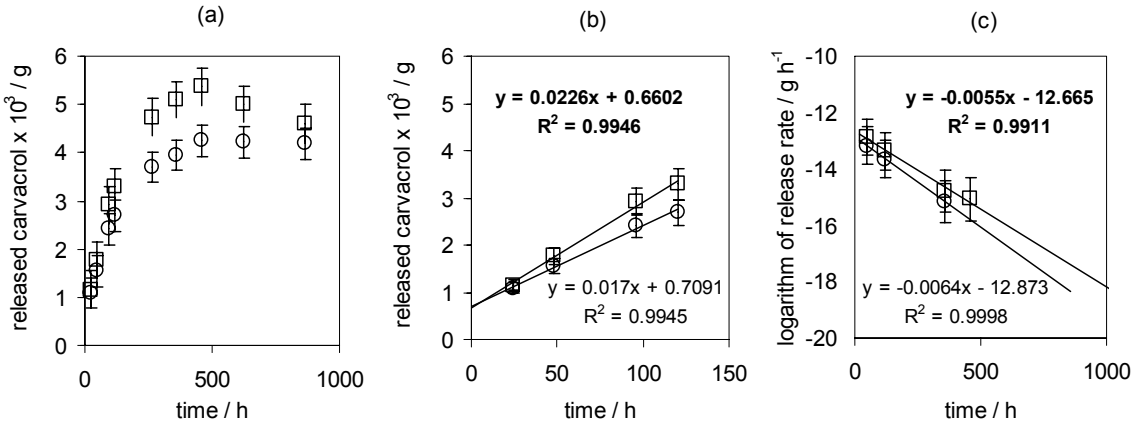


Figure 4.24 Release of carvacrol from (○) 20 μm and (□) 40 μm thick AM layer into water at 20°C: (a) mass of released carvacrol, (b) initial release assuming zero-order rate, (c) release rate assuming first-order kinetics.

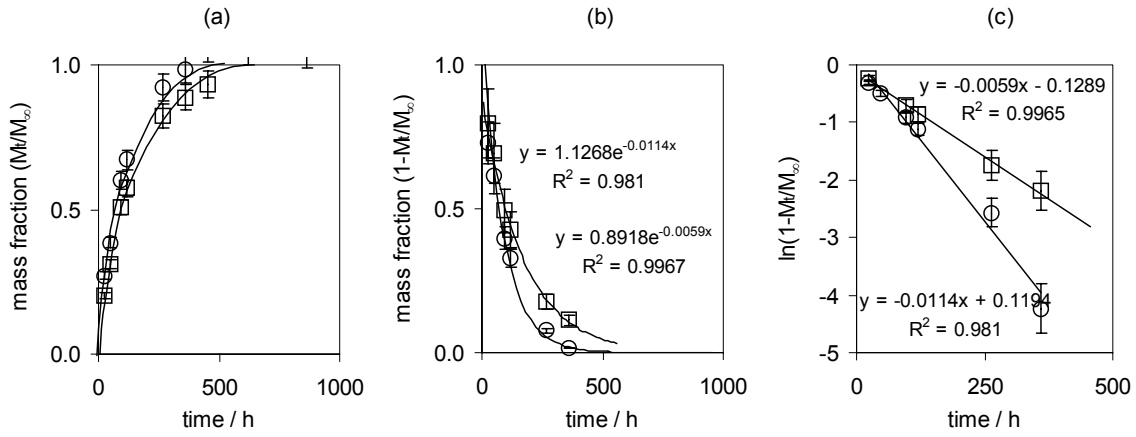


Figure 4.25 Release of carvacrol from (○) 20 µm and (□) 40 µm thick AM layer into water at 20°C: (a) mass fraction of released carvacrol, (b) mass fraction of carvacrol retained in the film, (c) logarithm of mass fraction of carvacrol retained in the film.

Table 4.17 Migration parameters of the release of carvacrol into water at 20°C from AM films with different AM layer thicknesses (see Appendix B.14).

	Thickness of AM layer	
Migration parameters	20 µm	40 µm
Initial rate / g h^{-1}	1.70×10^{-5}	2.26×10^{-5}
Total released mass (M_∞) / g		
From short form equation	4.01×10^{-3}	5.74×10^{-3}
From full form equation	4.02×10^{-3}	5.78×10^{-3}
Transmissibility / m^2 h^{-1}		
From short form equation	0.64×10^{-11}	1.10×10^{-11}
From full form equation	0.64×10^{-11}	1.09×10^{-11}
Rate constant (k) / h^{-1}		
From mass fraction plot	1.14×10^{-2}	0.59×10^{-2}
From release rate plot	0.64×10^{-2}	0.55×10^{-2}

The migration parameters for the release of carvacrol into water at 20°C are reported in Table 4.17. The data show that the release of carvacrol from the 20 µm and the 40 µm thick AM layers at 20°C are very similar. Although the 40 µm thick layer

contained twice the amount of carvacrol, the calculated total mass of carvacrol in the system with the 40 μm thick AM layer was only slightly higher than the system with the 20 μm thick AM layer. This may be the result of the limited solubility of the hydrophobic AM agent in water. This conclusion is supported by the release of carvacrol from the reservoir into ethanol, a hydrophobic food simulant used in the previous experiment.

Table 4.18 shows the mass balance and the concentration of carvacrol that was released into water and retained in the AM layer. At equilibrium, the mass of carvacrol retained in the films was estimated based on the percentage of evaporation obtained from Section 4.6.3. The concentration gradient of carvacrol between the reservoir and the controlling layer could also be estimated from the partition coefficient of carvacrol migration from the AM layer to LDPE ($K_{P/S}$), obtained in section 4.6.3. The partition coefficient of carvacrol during migration from the LDPE layer into water ($K_{P/F}$) was then calculated from the concentration gradient between the LDPE layer and water at equilibrium. The approximate values were in the range of 2.8 - 3.5.

Comparing this observation to the release of carvacrol into 95% (v/v) ethanol, the equilibrium concentration of carvacrol released into water was lower. The solubility of carvacrol in water is *ca.* 0.03 g mL^{-1} whereas the solubility in 95% (v/v) ethanol is more than 0.24 g mL^{-1} (see Table A.6 in Appendix A). In this experiment, the system reached the equilibrium concentration of only 0.8-1.2 $\times$ 10^{-4} g mL^{-1}, which suggests that the migration of carvacrol into water is depressed due to the solubility limit. According to equation 2.16, in applying such a relative poor solvent, the release is controlled by the film surface area (A), the thickness of the diffusion pathway into the food simulant, the so-called boundary layer (l_F), the diffusion coefficient of the AM agent in the food simulant (D_F), the partition coefficient of the migrant between the food simulant and the controlling layer ($K_{F/P}$), and the availability (or the solubility) of the AM agent in the controlling layer (C_P) as demonstrated in Figure 4.26 (Richards, 1985).

Table 4.18 Mass balance and concentration of carvacrol released from AM films with 20 μm thick AM layer into water at 20°C (see Appendix B.15).

	Multi-layer system					
	20 μm AM layer		50 μm controlling layer		Water	
Volume / cm^3	0.144		0.36		50	
	Amount of carvacrol					
	Initial	Equilibrium	Initial	Equilibrium	Initial	Equilibrium
Mass balance / g	3.3×10^{-2}	3.6×10^{-3} *	0	7.9×10^{-5} *	0	4.0×10^{-3}
Concentration / g cm^{-3}	2.3×10^{-1}	2.5×10^{-2} *	0	2.2×10^{-4} *	0	8.0×10^{-5}

* estimated from Section 4.6.3

Note: The initial mass and concentration presented in the table are the values considered directly after the films were produced, not at the commencement of the test.

Table 4.19 Mass balance and concentration of carvacrol released from AM films with 40 μm thick AM layer into water at 20°C (see Appendix B.16).

	Multi-layer system					
	40 μm AM layer		50 μm controlling layer		Water	
Volume / cm^3	0.288		0.36		50	
	Amount of carvacrol					
	Initial	Equilibrium	Initial	Equilibrium	Initial	Equilibrium
Mass balance / g	6.1×10^{-2}	1.4×10^{-2} *	0	1.5×10^{-4} *	0	5.8×10^{-3}
Concentration / g cm^{-3}	2.1×10^{-1}	4.7×10^{-2} *	0	4.2×10^{-4} *	0	1.2×10^{-4}

* estimated from Section 4.6.3

Note: The initial mass and concentration presented in the table are the values considered directly after the films were produced, not at the commencement of the test.

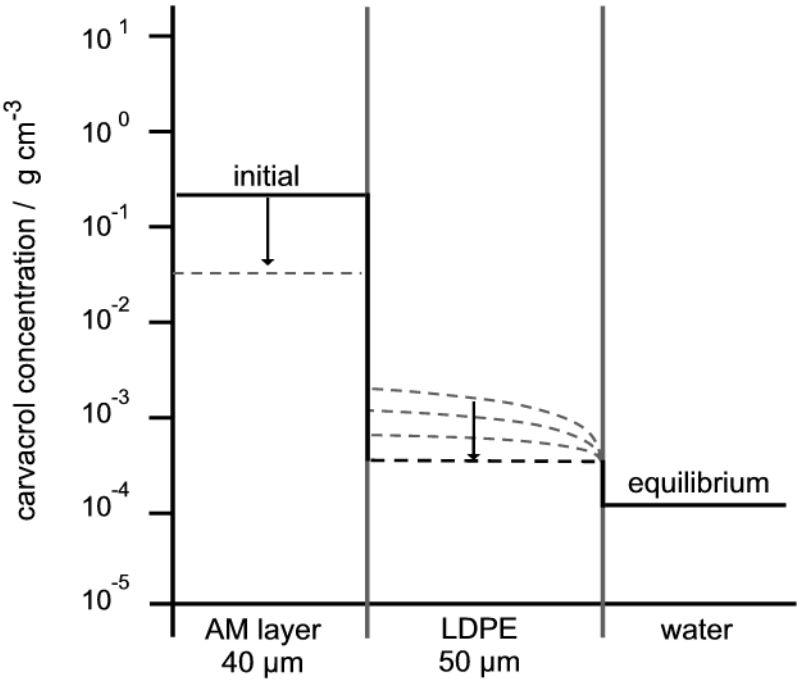


Figure 4.26 Concentration profile at the initial and equilibrium states for the release of carvacrol into water.

In some applications, the release may be affected by the solubility of the AM agent in the food system or food simulant. The solubility of hydrophilic AM agents in foods that contain a high proportion of fat, in the form of an oil-water emulsion, is limited. In that case, the AM agent can only be dissolved in the limited fraction of water (Garbutt, 1997). Similarly, the release of a hydrophobic AM agent into aqueous foods, with a limited concentration of fat, is limited, Therefore, carvacrol, a hydrophobic AM agent, was subjected to a release test into fatty food simulants in order to achieve the release profile throughout the experiment without reaching the solubility limit.

4.7 Antimicrobial activity of multi-layer AM films

As shown in Section 4.6, the release of AM agent from a multi-layer AM film can be controlled by varying the AM layer thickness and film composition. For an optimal inhibition, the released AM agent should meet the minimum inhibitory concentration (MIC) for the spoilage microorganisms (Han, 2003). However, in food products, a complete inhibition of the microorganisms is not necessary. A concentration lower than the MIC can still create a longer lag phase, lower growth rate or initial death before the growth (Devidson and Zivanovic, 2003). The ability of the AM film to inhibit the growth of *E. coli* is described in the following sections.

4.7.1 Antimicrobial activity of multi-layer AM films in food media

The effect of temperature on the growth of *E. coli* in a food medium (nutrient broth) is shown in Table 4.20 and in Figure 4.27. The results show that the growth of *E. coli* is slower at lower temperatures. Furthermore, the growth of *E. coli* at temperatures below 20°C is much slower when the medium is contained in the AM packaging material as compared to the growth that occurs in the non-AM packaging medium. The logarithm of the storage time (t_s) has a linear correlation with the inverse of the storage temperature, following the Arrhenius equation as shown in Figure 4.28.

Table 4.20 Time taken for *E. coli* to grow from 10^3 to 10^6 cfu mL^{-1} in nutrient broth with and without an AM packaging film.

	Storage temperature / °C		
	8	**12**	**20**
Film type	**Time taken / h**		
Non-AM film	576	108	15
AM film	2160	504	16

The multi-layer AM film inhibited the growth of *E. coli* at 8 and 12°C but the growth was similar at 20°C. Although the higher temperature can enhance the release of the AM agent from the film (refer to Section 4.6), increasing the temperature concurrently accelerates the microbial growth.

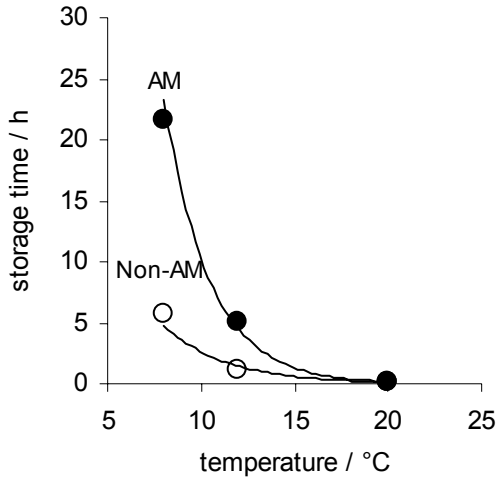


Figure 4.27 Plot of time for *E. coli* to grow from 10^3 to 10^6 cfu mL^{-1} in nutrient broth contained in (○) non-AM packaging and (●) AM packaging as a function of temperature; solid lines (—) show trends of *E. coli* growth.

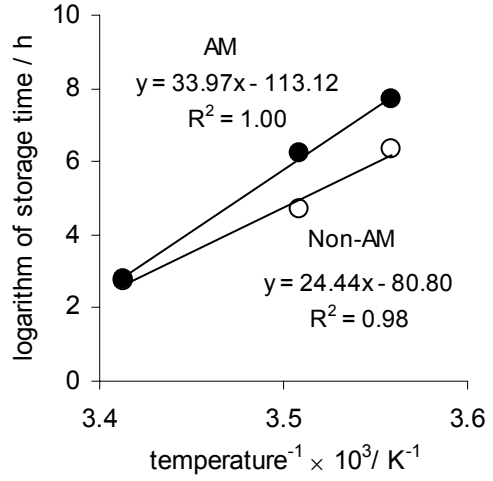


Figure 4.28 Plot of the logarithm of time required for *E. coli* to grow from 10^3 to 10^6 cfu mL^{-1} in nutrient broth contained in (○) non-AM packaging and (●) AM packaging versus the reciprocal of the absolute temperature.

4.7.2 Antimicrobial activity of multi-layer AM films in Cheddar cheese

Although the multi-layer AM films containing carvacrol show an effective inhibition of *E. coli* in a food medium, particularly at lower than ambient temperatures, the effect on real foods is to be investigated to confirm their AM activity. The AM activity of these films to inhibit the growth of *E. coli* in Cheddar cheese was investigated and described in the present section.

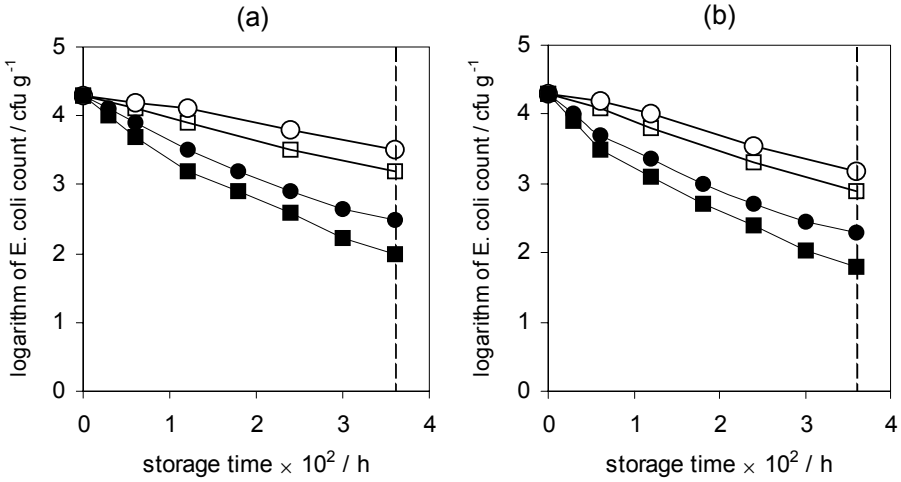


Figure 4.29 Plot of *E. coli* count on Cheddar cheese with (a) pH 5.9 and (b) pH 5.5, wrapped in non-AM packaging at (□) 8°C and (○) 12°C or wrapped in AM packaging at (■) 8°C and (●) 12°C.

Figures 4.29(a) and 4.29(b) show plots of the inhibition of *E. coli* on two types of Cheddar cheese with pH 5.5 and 5.9 respectively, wrapped in AM films containing a 40 µm thick AM layer and a 50 µm thick LDPE cover layer, and non-AM films having a 40 µm thick blank EA-MMA layer and a 50 µm thick LDPE layer. The samples with cell density of 4.3 $\log_{10}$ cfu g^{-1} were incubated at 8°C or 12°C and the results indicate that the number of viable *E. coli* in Cheddar cheese decreased at both storage temperatures. The effect of temperature on *E. coli* growth was studied by Wahi *et al.* (2006), in which the bacterium (4 $\log_{10}$ cfu g^{-1}) was tested for survival in cheese at 4, 8 and 28°C in a vacuum package. They found that *E. coli* could grow at 28°C but not at 4 and 8°C. In that research, the growth of *E. coli* in cheese samples at 12°C was also suppressed.

Table 4.21 Reduction of *E. coli* in Cheddar cheese packed in AM film and a non-AM film stored at different temperatures for 360 h.

	pH 5.9		**pH 5.5**	
Storage temperature / °C	**8**	**12**	**8**	**12**
Film type	**Reduction of *E. coli* / $\log_{10}$ cfu g^{-1}**			
Non-AM film	1.1	0.8	1.4	1.1
AM film	2.3	1.8	2.5	2.0

The Cheddar cheese samples obtained from a local retail outlet were mildly acidic with pH levels of 5.5 and 5.9, which also naturally suppress the growth of *E. coli*. A previous study reported that the minimum temperature for *E. coli* to grow in Cheddar cheese (pH 6.6) is 10°C under vacuum condition (Kasrazadeh and Genigeorgis, 1995). In that study it was found that addition of sodium benzoate or potassium sorbate to the cheese made from acidified milk with propionic acid could delay or prevent the growth of *E. coli*. These results correlate also to the reduction of the *E. coli* count observed in the current work that incubated acidic cheese (pH 5.5-5.9) at 8 and 12°C. The reductions of the *E. coli* population in Cheddar cheese stored in an AM packaging and non-AM packaging for 360 h are shown in Table 4.21.

A study was undertaken aiming to exempt the requirement of FDA's Food Code for refrigerated storage of processed cheese slices (Glass *et al.*, 2000). The Food Code currently considers pasteurised processed cheese slices as a potentially hazardous food, which requires refrigeration at 5°C or less to prevent rapid and progressive growth of foodborne pathogens. The researchers reported that eleven processed cheese slice formulations (pH 5.9 - 6.0) from seven manufacturers decreased the populations of *E. coli* by *ca.* 1.35 $\log_{10}$ cfu g^{-1}during 168 h storage at 27°C whereas the number of *E. coli* in this experiment was reduced by *ca.* 1.1 and 1.3 $\log_{10}$ cfu g^{-1} after 180 h storage at 8 and 12°C respectively. This difference in the results may stem from the fact that the two experiments were conducted using different initial cell densities of *E. coli*. Glass *et al.* studied the reduction of *E. coli* starting from 3 $\log_{10}$ cfu g^{-1} while the current study, started from 4.3 $\log_{10}$ cfu g^{-1} *E. coli*. However, both investigations found similarly, that the number of *E. coli* tended to remain constant after the course of population reduction.

The inhibitory effect of reuterin which is a bacteriocin produced from anaerobic fermentation of glycerol by *Lactobacillus reuteri* was studied by El-Ziney and Debevere (1998). It was reported that concentrations of the AM agent of 50, 100, and 150 units g^{-1} could decrease the population of *E. coli* by 2, 3, and 6 $\log_{10}$ cfu g^{-1} respectively in cottage cheese within a seven-day storage time. The results indicate that a higher concentration of AM agent results in a higher inhibitory effect on microbial growth. The results of the release experiments (Section 4.6) suggest that the release of AM agent from AM films is accelerated at increased temperature.

Therefore, in this experiment, the release rate of carvacrol at the high temperature was challenged by the growth rate of *E. coli*. While the higher temperature speeded up the release rate, the growth of the microorganism was also accelerated. Table 4.21 shows that the growth of *E. coli* in Cheddar cheese wrapped in an AM packaging is suppressed at both 8°C and 12°C. However, the population of *E. coli* reduced in the AM packaging faster than in non-AM packaging, which suggests that the AM films can inhibit the growth of *E. coli* in addition to the effect of the temperature and the acidity of product. This potentially prolongs the shelf life of Cheddar cheese and can be a packaging option to keep Cheddar cheese safer and longer during out-of-refrigeration storage.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In a multi-layer AM packaging system, an AM agent has to migrate across different phases, from the AM layer into the controlling layer, before it is released from the film system onto the food medium. The AM layer must be comprised of a material that has high capability to retain the AM agent in the matrix while the controlling layer is usually comprised of different materials in order to restrict the migration of an AM agent. In this research, a coating material comprised of EA-MMA is shown to perform satisfactorily as an AM layer. Furthermore, the results show that LDPE is capable of controlling the release of an AM agent into a food simulant. Without the LDPE controlling layer, direct contact with a food simulant at the surface of the AM layer results in a high rate of release of AM agent analogous to the release by a monolithic system. For a multi-layer AM packaging system, the retention of the AM agent behind the controlling or protecting layer can promote not only the slower release but also a longer period of AM activity.

5.1.1 Activity of natural AM agent and coating formulation

The AM agents carvacrol and linalool exhibited positive antimicrobial activity against *E. coli* at a density of 10^5 cfu mL^{-1} whereas methylchavicol showed a weaker activity in solid media. These AM agents were successfully incorporated in a MC-HPMC coating solution at a maximum level of 4% (w/w). A dried coating containing the maximum concentration of carvacrol showed a stronger AM activity against 10^5 cfu mL^{-1} *E. coli* when compared with the activity of the coating that contained linalool. In order to obtain higher stability in aqueous food media, the hydrophobic EA-MMA coating was selected to replace the hydrophilic MC-HPMC coating. Following the evaporation of the solvent from the EA-MMA coating solution during the drying process, *ca.* 95% (w/w) of the carvacrol was retained in the film product. The absolute concentration of carvacrol was *ca.* 30% (w/w) in the dry coating equating to *ca.* 0.2 g carvacrol per 1 cm^3 of dry coating. Multi-layer AM films comprised of nylon/LDPE/EA-MMA/LDPE incorporated with carvacrol were successfully produced. The release of AM agent was studied in order to investigate the effects of the thickness of controlling and AM layers as well as solubility. In this study, a

coating system that contains natural AM agents applied to an existing, commercially available packaging film was successfully formulated and the produced films can adequately retain the incorporated AM substances.

5.1.2 Effect of controlling layer thickness on AM agent release

The slow release of AM agent from the films occurred before the release reached equilibrium therefore the diffusion of AM agent is controlled by the thickness of the LDPE layer. In a food simulant such as ethanol that can effectively dissolve many active agents, equilibrium was achieved when the concentration of carvacrol in the LDPE controlling layer reached the solubility limit. Consequently, the diffusion of carvacrol through the controlling layer was restricted, which maintained the level of the AM agent in the EA-MMA layer. As expected, the release rate was found to be a function of temperature. After observing the release for 1000 h at 4°C, the multi-layer films continued releasing the AM agent without approaching equilibrium. At 20°C, however, the release was approaching equilibrium and at 37°C the system had reached equilibrium. The equilibrium concentration of carvacrol in ethanol obtained from the AM film with a 40 μm thick AM layer and a 50 μm thick controlling layer was 7.4×10^{-4} g cm^{-3} while there was 2.4×10^{-2} g carvacrol left in the AM layer.

The release of AM agent to the atmosphere was studied by FT-IR spectroscopy. The IR absorbance band at wavenumber 3450 cm^{-1} was successfully used to determine the retention of carvacrol in the AM film. The results of the IR study showed that the AM agent could migrate through the LDPE to the atmosphere, indicating the capability of the film to release the active agent to a packaging headspace. Furthermore, the diffusion rate was controlled by the thickness of the LDPE controlling layer. The effect of the thickness of the controlling layer on the release of the incorporated AM agents into selected food simulants was successfully investigated.

5.1.3 Effect of AM layer thickness on AM agent release

In an application where the AM agent is a volatile substance and there is a potential loss from evaporation, a higher amount of the agent must be incorporated in the AM layer to maintain the activity of the film. A thicker AM layer, however, could potentially retain a greater concentration of carvacrol in the reservoir. As expected, the release of carvacrol from the multi-layer AM film with a thicker AM layer

achieved a higher equilibrium concentration of AM agent in the AM film. Under the same release conditions at 20°C, a 40 μm thick AM layer retained 1.4×10^{-2} g carvacrol left and provided an equilibrium concentration of 4.2×10^{-4} g cm^{-3} in ethanol whereas a 20 μm thick AM layer retained 3.6×10^{-3} g carvacrol with an equilibrium concentration of 2.2×10^{-4} g cm^{-3}. The effect of AM layer thickness on the release of the incorporated AM agents into selected food simulants was successfully investigated.

5.1.4 Effect of solubility of AM agent on release

In a compatible food simulant that is a good receptor, the AM agent present in the controlling layer can be released almost entirely into the receptor phase. Therefore, the release of the agent will be dominated by the controlling layer. Conversely, in a poor receptor the release is restricted by the solubility limit of the AM agent in receptor phase. Under the same release conditions at 20°C, the AM film with a 40 μm thick AM layer yielded equilibrium concentrations of 4.2×10^{-4} g cm^{-3} in ethanol and 1.2×10^{-4} g cm^{-3} in water. In this case, ethanol is a more compatible food simulant for carvacrol than water.

5.1.5 Antimicrobial activity of multi-layer AM films

In food media, the AM films produced in this work demonstrated satisfactory inhibition of the growth of *E. coli* at 8°C and 12°C. However, since a higher temperature accelerates the microbial growth, the activity of the films was negligible at 20°C. On Cheddar cheese, the multi-layer AM films successfully inhibited the growth of *E. coli* with initial cell density of 4.3 $\log_{10}$ cfu g^{-1}. The films reduced the population to *ca.* 2.4 and 1.9 $\log_{10}$ cfu g^{-1} after storage at 8°C and 12°C respectively for 360 h.

5.2 Significance of the findings

This research examined the workability of multi-layer packaging materials containing natural AM agents – linalool, methylchavicol and carvacrol. The most suitable AM agent was carvacrol. The ability of multi-layer films to store a high concentration of AM agent in a coating beneath a barrier layer to sustain a long release was demonstrated. The AM layer studied primarily in this research was EA-MMA which contained a maximum of 0.2 g cm^{-3} or 30% (w/w) carvacrol. The subsequent release

of AM agent from the multi-layer system was successfully able to maintain the release of the active agent at a slow rate. More specifically, the initial rate of release was constant at the level of 10^{-6} - 10^{-4} g h^{-1} and decreased exponentially to the level of 10^{-12} - 10^{-10} g h^{-1} at the later part of the release. The effect of the film structure on the amount of AM agent released and the release rate was established. Whereas the thickness of the controlling layer controlled the rate of release, the level of equilibrium concentration was a function of the amount of the active agent that is retained in the AM layer or the thickness of the AM layer. This knowledge can enable the release of AM agent from a packaging system to be adequately controlled.

The competition between the rate of release of the AM agent and the rate of microbial growth is a critical factor with both the rate of release and rate of microbial growth temperature-dependent. In this research, after increasing the storage temperature from 8°C to 12°C, the AM film continued to suppress the increased microbial growth in Cheddar cheese. This suggests that the release of AM agent increased concurrently and maintained a higher concentration of AM agent. At 20°C, however, the release of the AM agent was not able to compete with the increased microbial growth in a simulated food medium.

5.3 Recommendations for further research

To increase the speed of the AM activity, it is recommended that the controlling layer could be thinner in order to speed up the initial release of AM agent from the film during the unsteady state. This would create an initially higher concentration of carvacrol and the initial release of the AM agent from the film should therefore be fast enough to suppress the multiplication of microorganisms. However, sufficient AM agent should be retained in the reservoir to continue releasing throughout the anticipated storage time in order to maintain the activity of the film.

When the release of the AM agent reaches the equilibrium, the concentration should remain constant unless the AM agent is used by microorganism or decomposed naturally. Further research could be conducted to study the release of an AM agent from a multi-layer film where it is being used or decomposed concurrently with the release. A study of the ability of multi-layer films to maintain equilibrium or create a new equilibrium could lead to the development of packaging systems that have a

longer duration of activity. Moreover, determining the maximum concentration of AM agent necessary for optimum AM activity in the reservoir should avoid over adding AM agent and therefore assist in optimising the film construction. This will also enable prediction of the active film shelf life for particular food packaging systems.

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Appendix A

Table A.1 Properties of methylcellulose.

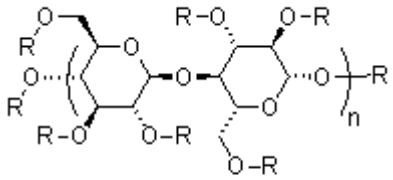
	Methylcellulose
Structure:	 R = CH_3 or H
Features:	Dissolves in water, undergoes reversible gelation upon heating, non-ionic, does not complex with ionic species and is surface active and enzyme resistant. Solutions are pseudoplastic.
Extent of Labelling:	~30 wt. % Methoxy
Molecular Weight:	Average M_n ~17,000
CAS Number:	9004-67-5
Surface Tension:	53-59 × 10^{-5} N cm^{-1} (at 25°C, 0.05 wt. %)
Viscosity:	25 mPa s (2 % in H_2O, at 20°C) (lit.)

Table A.2 Properties of hydroxy propyl methylcellulose.

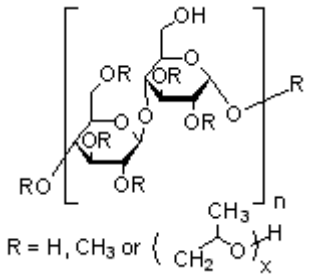
Hydroxypropyl methylcellulose	
Structure:	
Features:	Dissolves in water, undergoes reversible gelation upon heating, non-ionic, does not complex with ionic species and is surface active and enzyme resistant. Solutions are pseudoplastic.
Extent of Labelling:	1.8-2.0 mol methoxy per mol cellulose (D.S.) 29 wt. % methoxy 0.2-0.3 mol propylene oxide per mol cellulose (M.S.) 7 wt. % propylene oxide
Molecular Weight:	Average M_n ~11,500
CAS Number:	9004-65-3
Surface Tension:	43-55 × 10^{-5} N cm^{-1} (at 25°C, 0.05 wt. %)
Viscosity:	40-60 mPa s (2 % in H_2O, at 20°C) (lit.)
Transition Temperature:	Gel point 58-64°C

Table A.3 Properties of Eudragit NE 40 D.

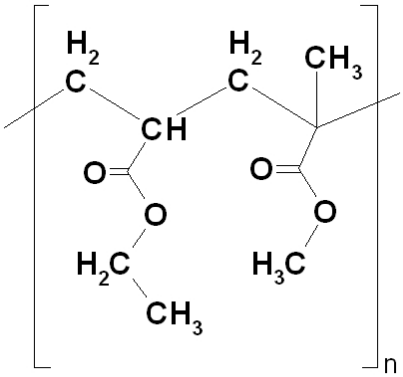
	Eudragit NE 40 D
Synonyms:	Polyacrylate dispersion 40 percent (Ph. Eur.) Ethyl acrylate methyl methacrylate copolymer dispersion (JPE)
Structure:	
Features:	Milky-white liquid of low viscosity with a faint characteristic odour. The aqueous dispersion is miscible with water in any proportion, the milky-white appearance being retained. When one part of Eudragit NE 40 D is mixed with 5 parts acetone, a clear to slightly cloudy, viscous solution is obtained. The same occurs when mixed with ethanol or isopropyl alcohol; initially the polymer is precipitated, but then dissolves again in the excess organic solvent. When mixed with 1 N sodium hydroxide in a ratio of 1:2, the dispersion does not dissolve. The milky-white appearance is retained. When the dispersion is poured onto a glass plate, a clear film forms upon evaporation of the water. The dispersion contains 2.0% Nonoxynol 100 as an emulsifier.
Molecular Weight:	Average M_n ~800,000
Dry substance:	38.5-41.5 %
Emulsifier:	2.0 % nonoxynal 100
Viscosity:	150 mPa s (at 20°C)
Relative Density:	1.055-1.064 (at 20°C)

Table A.4 Properties of linalool.

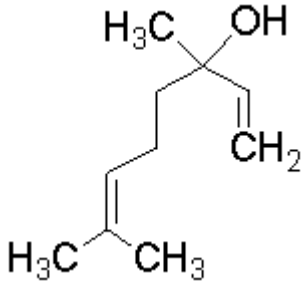
Linalool	
Synonyms:	(±)-3,7-Dimethyl-1,6-octadien-3-ol (±)-3,7-Dimethyl-3-hydroxy-1,6-octadiene
Structure:	
Molecular Formula:	$(CH_3)_2C=CHCH_2CH_2C(CH_3)(OH)CH=CH_2$
Molecular Weight:	154.25
CAS Number:	78-70-6
Beilstein Registry Number:	1721488
EG/EC Number:	2011344
Vapour Pressure:	0.17 mm Hg (at 25°C)
Boiling Point:	194-197°C (at 720 mm Hg) (lit.)
Melting Point:	<20°C (lit.)
Flash Point:	78.8°C
Density:	0.87 g mL^{-1} (at 25°C) (lit.)
Solubility in water:	0.16 g $(100\ mL)^{-1}$

Table A.5 Properties of methylchavicol.

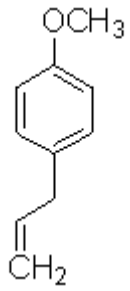
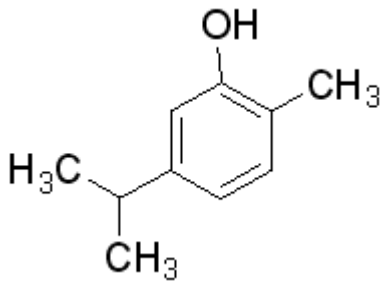
	Methylchavicol
Synonyms:	4-Allylanisole Chavicol methyl ether Estragole
Structure:	
Molecular Formula:	$H_2C{=}CHCH_2C_6H_4OCH_3$
Molecular Weight:	148.20
CAS Number:	140-67-0
Beilstein Registry Number:	1099454
EG/EC Number:	2054278
Boiling Point:	215-216°C (lit.)
Flash Point:	81.1°C
Density:	0.965 g mL^{-1} (at 25°C) (lit.)
Solubility in water:	<0.1 g $(100\ mL)^{-1}$

Table A.6 Properties of carvacrol.

	Carvacrol
Synonyms:	5-Isopropyl-2-methylphenol 2-Methyl-5-isopropylphenol Isothymol
Structure:	
Molecular Formula:	$(CH_3)_2CHC_6H_3(CH_3)OH$
Molecular Weight:	150.22
CAS Number:	499-75-2
Beilstein Registry Number:	1860514
EG/EC Number:	2078896
Boiling Point:	234-236°C (lit.)
Melting Point:	2°C (lit.)
Flash Point:	106°C
Density:	0.965 g mL^{-1} (at 25°C) (lit.)
Solubility in 60% ethanol	24 g $(100\ mL)^{-1}$
Solubility in water:	3 g $(100\ mL)^{-1}$

Appendix B

B.1 Calculation of retention of carvacrol in 20 µm dry EA-MMA coating formulation

(Table 4.2)

Before drying

Weight of coating	0.1673 g
Weight of carvacrol	0.0064 g
Concentration of carvacrol	(0.0064 x 100) / 0.1673 = 3.8% (w/w)

After drying

Weight of coating	0.0217 g
Weight of carvacrol	0.0061 g
Concentration of carvacrol	(0.0061 x 100) / 0.0217 = 29% (w/w)
Dimension of dry coating	
Width	2.00 cm
Length	7.00 cm
Thickness	0.0020 cm
Volume	0.028 cm^3
Concentration of carvacrol	0.0061 / 0.028 = 0.22 g cm^{-3}

Retention of carvacrol (0.0061 x 100) / 0.0064 = 95% (w/w)

B.2 Calculation of retention of carvacrol in 40 µm dry EA-MMA coating formulation

(Table 4.2)

Before drying

Weight of coating	0.3098 g
Weight of carvacrol	0.0119 g
Concentration of carvacrol	(0.0119 x 100) / 0.3098 = 3.84% (w/w)

After drying

Weight of coating	0.0389 g
Weight of carvacrol	0.0115 g
Concentration of carvacrol	(0.0115 x 100) / 0.0389 = 30.6% (w/w)
Dimension of dry coating	
Width	2.00 cm
Length	7.00 cm
Thickness	0.0040 cm
Volume	0.056 cm^3
Concentration of carvacrol	0.0115 / 0.056 = 0.21 g cm^{-3}

Retention of carvacrol (0.0115 x 100) / 0.0119 = 96.2% (w/w)

B.3 Calculation of mass balance and concentration of carvacrol released from nylon/LDPE/EA-MMA/LDPE film at initial and equilibrium state (Table 4.3)

Initial stage in AM layer

Dimension of AM layer	
Width	6.00 cm
Length	12.00 cm
Thickness	0.0040 cm
Volume	0.288 cm^3
Concentration of carvacrol	0.21 g cm^{-3}
Initial mass of carvacrol	0.21 x 0.288 = 0.061 g

Equilibrium stage in ethanol

Volume of ethanol	50 cm^3
Equilibrium concentration of carvacrol	0.00074 g cm^{-3}
Equilibrium mass of carvacrol	50 × 0.00074 = 0.037 g

Equilibrium stage in controlling layer

Dimension of controlling layer	
Width	6.00 cm
Length	12.00 cm
Thickness	0.0050 cm
Volume	0.36 cm^3
Equilibrium concentration of carvacrol	0.00074 g cm^{-3}
Equilibrium mass of carvacrol	0.36 × 0.00074 = 0.00026 g

Equilibrium stage in AM layer

Volume of AM layer	0.288 cm^3
Equilibrium mass of carvacrol	0.061 - 0.037 - 0.00026 = 0.024 g
Equilibrium concentration of carvacrol	0.024 ÷ 0.288 = 0.083 g cm^{-3}

B.4 Calculation of partition coefficient of carvacrol migrating from the AM coating layer (reservoir) to controlling LDPE layer ($K_{P/S}$)

(Table 4.3)

Equilibrium concentration of carvacrol

In controlling layer	0.00074 g cm^{-3}
In AM layer	0.083 g cm^{-3}
Partition coefficient; $K_{P/S}$	0.00074 ÷ 0.083 = 0.0089

B.5 Calculation of total mass and transmissibility of released carvacrol from AM films with different controlling layer thicknesses at various temperatures

(Table 4.5 and 4.6)

The full form of rate equation (Equation 2.6) is considered in an alternative form as follows.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S}(V_1+V_2)t}{l_P V_1 V_2}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}(V_1+V_2)}{l_P V_1 V_2}$$

$$M_\infty = \frac{a(V_1+V_2)}{bV_2}$$

$$D_P K_{P/S} = \frac{b l_P V_1 V_2}{A(V_1+V_2)}$$

For the short form (Equation 2.7), the same parameters can be calculated as below.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}}{l_P V_1}$$

$$M_\infty = \frac{a}{b}$$

$$D_P K_{P/S} = \frac{b l_P V_1}{A}$$

The values of term *a* and *b* can be obtained from the plots of dM_t/dt versus *t* as shown in Figure B.1 and Table B.1.

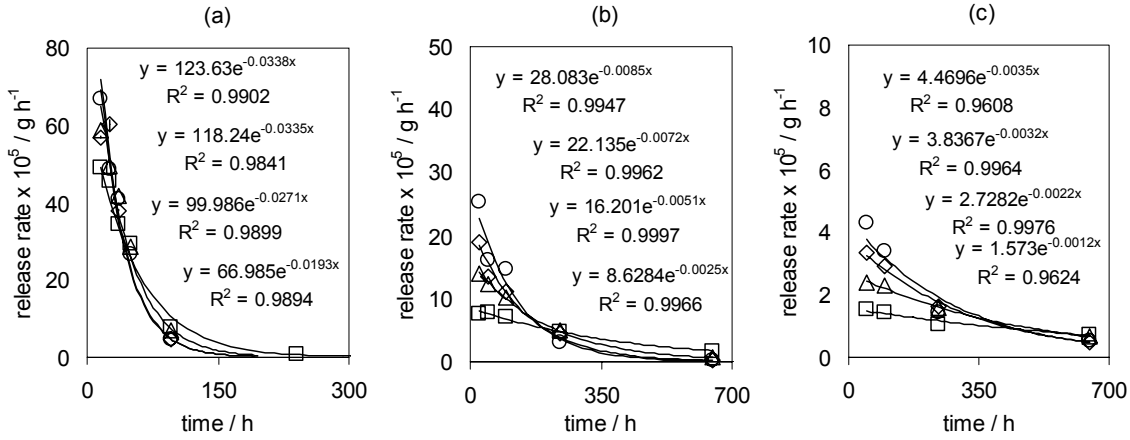


Figure B.1 Release rate of carvacrol migrating from 40 µm thick AM layer through: (○) 10 µm, (◇) 20 µm, (△) 30 µm, (□) 50 µm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C versus time.

Table B.1 Term *a* and *b* obtained from the release rate of carvacrol from AM films with different controlling layer thicknesses at various temperatures.

Temperature / °C	Controlling layer thickness / µm	Value	
		$a \times 10^5$	*b*
37	10	123.63	0.0338
	20	118.24	0.0335
	30	99.986	0.0271
	50	66.985	0.0193
20	10	28.083	0.0085
	20	22.135	0.0072
	30	16.201	0.0051
	50	8.6284	0.0025
4	10	4.4696	0.0035
	20	3.8367	0.0032
	30	2.7282	0.0022
	50	1.5730	0.0012

Dimension of AM layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0040 cm
Volume (V_1)	0.288 cm^3
Volume of ethanol (V_2)	50 cm^3

At 37°C, the AM film with 10 μm thick controlling layer has the dimension as follows.

Width	6.00 cm
Length	12.00 cm
Thickness (l_P)	0.0010 cm

From the plots, a = 0.0012363 and b = 0.0338.

For full form equation

$$M_\infty = \frac{a(V_1 + V_2)}{bV_2}$$

$$M_\infty = \frac{0.0012363(0.288 + 50)}{(0.0338)(50)} = 0.0368$$

and

$$D_P K_{P/S} = \frac{bl_P V_1 V_2}{A(V_1 + V_2)}$$

$$D_P K_{P/S} = \frac{(0.0338)(0.000010)(0.288)(50)}{(6 \times 12)(0.288 + 50)} = 1.34 \times 10^{-11}$$

For short form equation

$$M_\infty = \frac{a}{b}$$

$$M_\infty = \frac{0.0012363}{0.0338} = 0.0366$$

and

$$D_P K_{P/S} = \frac{bl_P V_1}{A}$$

$$D_P K_{P/S} = \frac{(0.0338)(0.000010)}{(6 \times 12)} = 1.35 \times 10^{-11}$$

B.6 Calculation of rate constants of release of carvacrol from AM films with different thicknesses of controlling layer at different temperatures (Table 4.7)

From Equation 2.11

$$1-\frac{M_t}{M_\infty}=\exp(-kt)$$

$$\ln(1-\frac{M_t}{M_\infty})=-kt$$

Rate constant, k, can be obtained from the slope of the plot of $\ln(1-M_t/M_\infty)$ versus time as shown in Figure B.2.

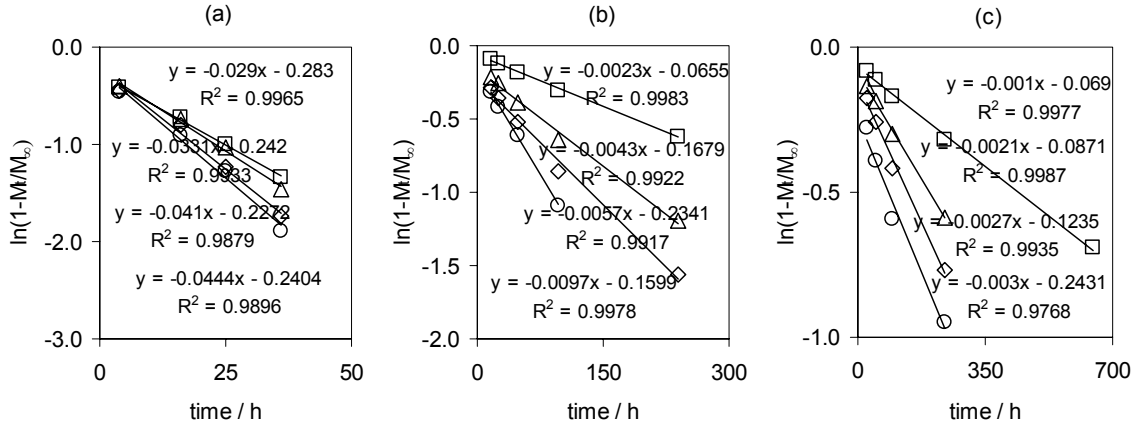


Figure B.2 Natural logarithm of the mass fraction of carvacrol retained in the AM films of 40 μm in thickness and a controlling layer of (○) 10 μm, (◇) 20 μm, (△) 30 μm, (□) 50 μm thick at (a) 37°C, (b) 20°C, (c) 4°C.

From Equation 2.7

$$\frac{dM_t}{dt}=\frac{M_\infty AD_P K_{P/S}}{l_P V_1}\exp\left(\frac{-AD_P K_{P/S}t}{l_P V_1}\right)$$

$$k=\frac{AD_P K_{P/S}}{l_P V_1}$$

$$\frac{dM_t}{dt}=M_\infty k\exp(-kt)$$

Term k is then equal to b in Table B.2, which is obtained from the plot of dM/dt versus time as shown in Figure B.1.

$$\frac{dM_t}{dt}=a\cdot\exp(-bt)$$

B.7 Calculation of activation energy for release of carvacrol from AM films with different thicknesses of controlling layer

(Table 4.8)

From equation 2.21

$$\ln k = \ln k_0 - \frac{E_a}{RT}$$

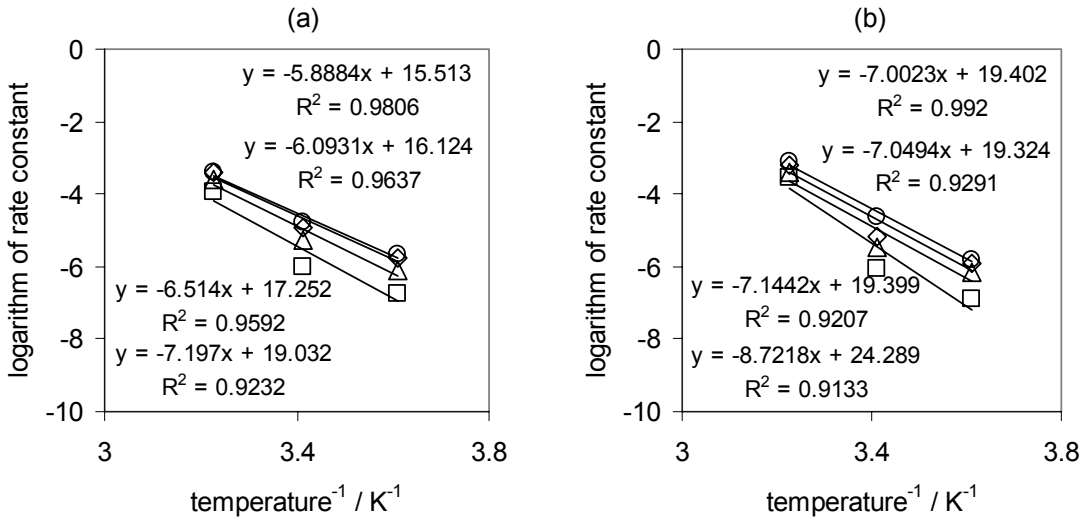


Figure B.3 Logarithm of the rate constant obtained from: (a) mass fraction plot, (b) release rate plot; versus the reciprocal of the absolute temperature through a controlling layer of thickness: (○) 10 μm, (◊) 20μm, (Δ) 30 μm, (□) 50 μm.

E_a is then obtained from -*slope/R*.

B.8 Spectrum of hydroxyl group in carvacrol structure

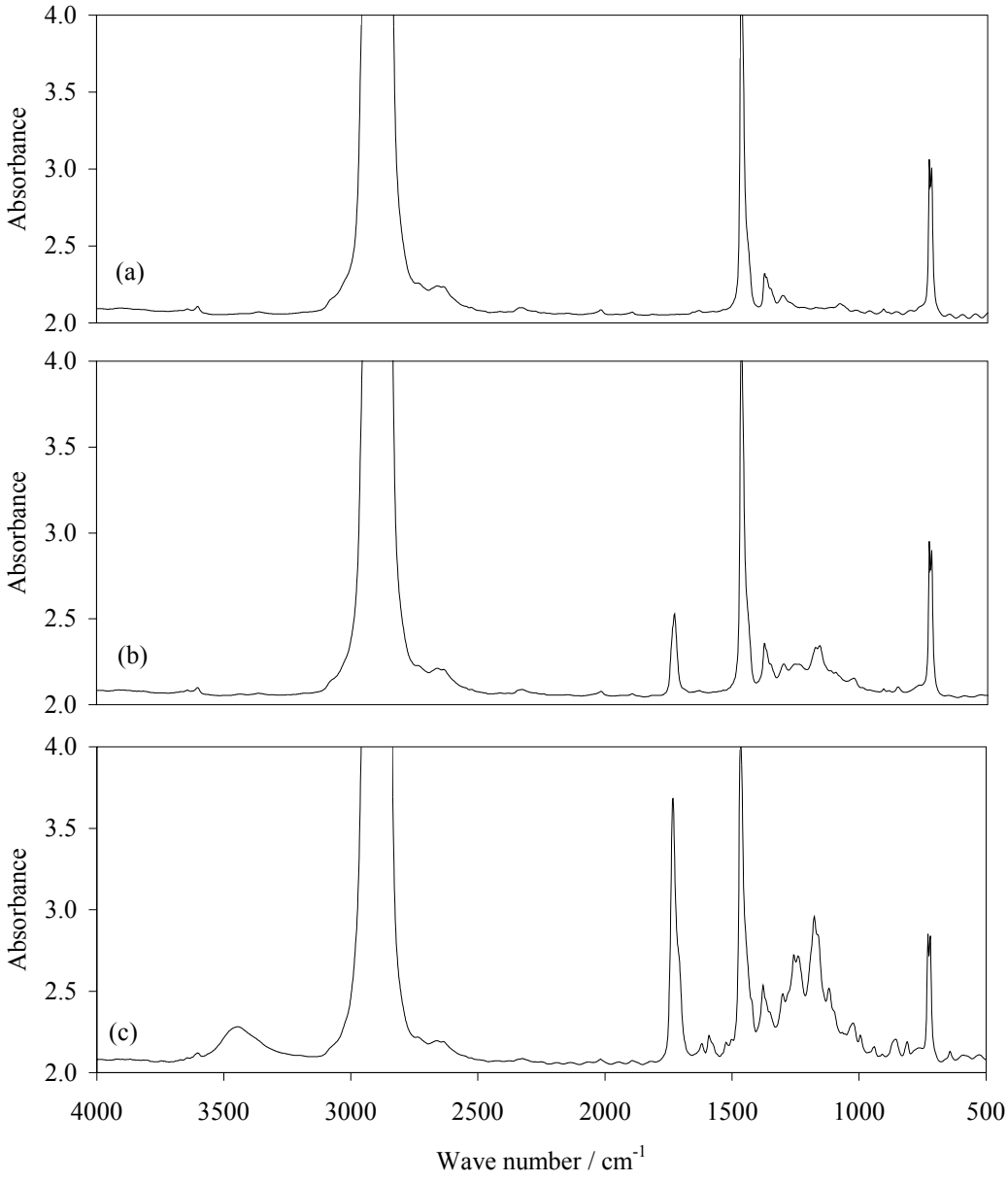


Figure B.4 The IR absorbance band at the wave number of 3450 cm^{-1} corresponds to the hydroxyl group of carvacrol where sample (a) is pure PE, sample (b) is PE coated with EA-MMA and sample (c) is PE coated with carvacrol-incorporated EA-MMA.

B.9 Calculation of rate constants and permeability of carvacrol release to the atmosphere at 20°C from AM films with various thicknesses of the controlling layer

(Table 4.9)

From Equation 2.11

$$1-\frac{M_t}{M_\infty}=\exp(-kt)$$

$$\ln(1-\frac{M_t}{M_\infty})=-kt$$

Rate constant, k, can be obtained from the slope of the plot of $\ln(1-M_t/M_\infty)$ versus time as shown in Figure B.5.

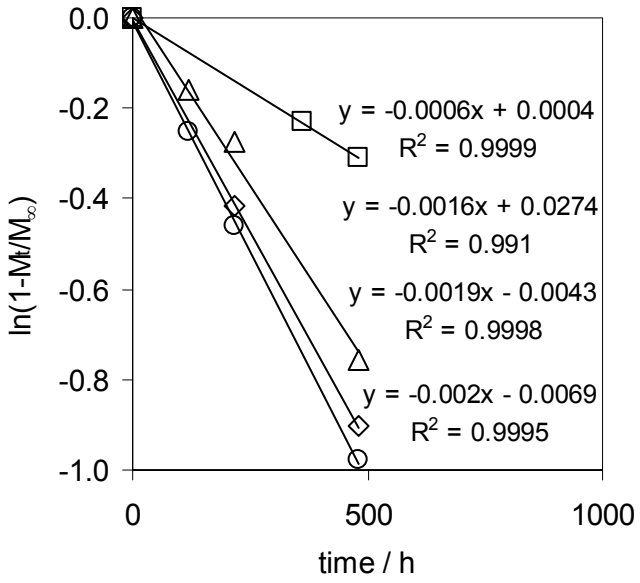


Figure B.5 Logarithm of mass fraction of carvacrol retained in the AM films covered with a controlling layer of thickness: (○) 10 μm, (◊) 20μm, (Δ) 30 μm, (□) 50 μm when exposed to the air at 20°C for 20 days.

From Equation 2.7

$$\frac{dM_t}{dt}=\frac{M_\infty AD_PK_{P/S}}{l_PV_1}\exp\left(\frac{-AD_PK_{P/S}t}{l_PV_1}\right)$$

$$k=\frac{AD_PK_{P/S}}{l_PV_1}$$

Permeability ($D_PK_{P/S}$) is then obtained from l_PV_1k/A.

B.10 Calculation of mass balance and concentration of carvacrol released from LDPE/EA-MMA/LDPE film with 20 µm thick AM layers at initial and equilibrium states

(Table 4.10)

Initial stage in AM layer

Dimension of AM layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0020 cm
Volume	0.144 cm^3
Concentration of carvacrol	0.23 g cm^{-3}
Initial mass of carvacrol	0.23 x 0.144 = 0.033 g

Equilibrium stage in ethanol

Volume of ethanol	50 cm^3
Equilibrium concentration of carvacrol	0.00022 g cm^{-3}
Equilibrium mass of carvacrol	50 × 0.00022 = 0.011 g

Equilibrium stage in controlling layer

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0050 cm
Volume	0.36 cm^3
Equilibrium concentration of carvacrol	0.00022 g cm^{-3}
Equilibrium mass of carvacrol	0.36 × 0.00022 = 0.000079 g

Equilibrium stage in AM layer

Volume of AM layer	0.144 cm^3
Partition coefficient ($K_{P/S}$)	0.0089
Equilibrium concentration of carvacrol	0.00022 ÷ 0.0089 = 0.025 g cm^{-3}
Equilibrium mass of carvacrol	0.025 x 0.144 = 0.0036 g

Mass of escaped carvacrol = initial mass in the AM layer - equilibrium mass in ethanol- equilibrium mass in controlling layer - equilibrium mass in AM layer

Evaporated carvacrol	0.033 – 0.011 – 0.000079 – 0.0036 = 0.018 g

B.11 Calculation of mass balance and concentration of carvacrol released from LDPE/EA-MMA/LDPE film with 40 µm thick AM layers at initial and equilibrium states

(Table 4.11)

Initial stage in AM layer

Dimension of AM layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0040 cm
Volume	0.288 cm^3

Concentration of carvacrol: 0.21 g cm^{-3}

Initial mass of carvacrol: 0.21 x 0.288 = 0.061 g

Equilibrium stage in ethanol

Volume of ethanol: 50 cm^3

Equilibrium concentration of carvacrol: 0.00042 g cm^{-3}

Equilibrium mass of carvacrol: 50 × 0.00042 = 0.021 g

Equilibrium stage in controlling layer

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0050 cm
Volume	0.36 cm^3

Equilibrium concentration of carvacrol: 0.00042 g cm^{-3}

Equilibrium mass of carvacrol: 0.36 × 0.00042 = 0.00015 g

Equilibrium stage in AM layer

Volume of AM layer: 0.288 cm^3

Partition coefficient ($K_{P/S}$): 0.0089

Equilibrium concentration of carvacrol: 0.00042 ÷ 0.0089 = 0.047 g cm^{-3}

Equilibrium mass of carvacrol: 0.047 x 0.288 = 0.014 g

Mass of escaped carvacrol = initial mass in the AM layer - equilibrium mass in ethanol- equilibrium mass in controlling layer - equilibrium mass in AM layer

Escaped carvacrol	0.061 – 0.021 – 0.00015 – 0.014 = 0.026 g

B.12 Calculation of total mass and transmissibility of released carvacrol from AM films with different AM layer thicknesses at various temperatures (Table 4.13 and 4.14)

The full form of rate equation (Equation 2.6) is considered in an alternative form as follows.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S}(V_1+V_2)t}{l_P V_1 V_2}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}(V_1+V_2)}{l_P V_1 V_2}$$

$$M_\infty = \frac{a(V_1+V_2)}{bV_2}$$

$$D_P K_{P/S} = \frac{b l_P V_1 V_2}{A(V_1+V_2)}$$

For the short form (Equation 2.7), the same parameters can be calculated as below.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}}{l_P V_1}$$

$$M_\infty = \frac{a}{b}$$

$$D_P K_{P/S} = \frac{b l_P V_1}{A}$$

The values of term *a* and *b* can be obtained from the plots of dM_t/dt versus *t* as shown in Figure B.6 and Table B.3.

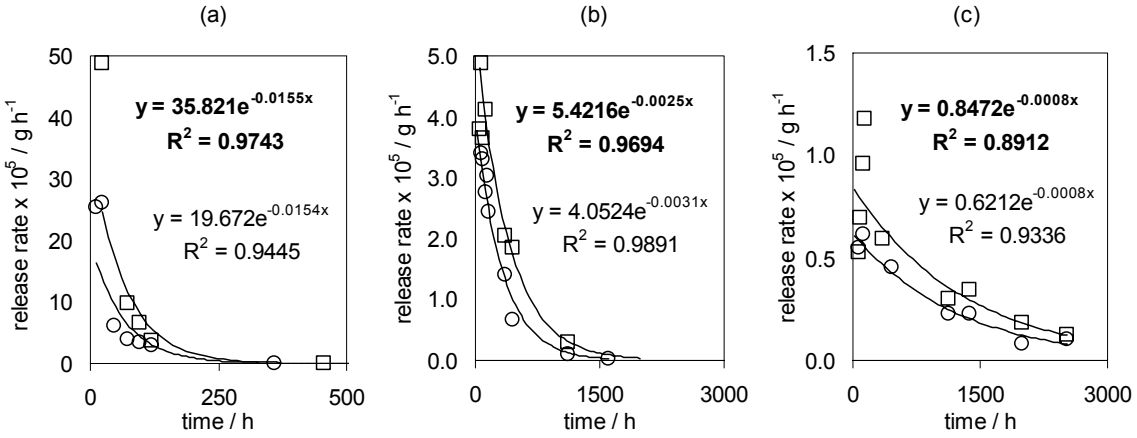


Figure B.6 Release rate of carvacrol migrating from (○) 20 μm, (□) 40 μm thick AM layer through 50 μm thick controlling layer at: (a) 37°C, (b) 20°C, (c) 4°C versus time.

Table B.2 Term *a* and *b* obtained from the release rate of carvacrol from AM films with different AM layer thicknesses at various temperatures.

Temperature / °C	AM layer thickness / μm	Value	
		$a \times 10^5$	*b*
37	20	19.672	0.0154
	40	35.821	0.0155
20	20	4.0524	0.0031
	40	5.4216	0.0025
4	20	0.6212	0.0008
	40	0.8472	0.0008

At 37°C, the AM film with 20 μm thick AM layer has the dimension as follows.

Width	6.00 cm
Length	12.00 cm
Thickness	0.0020 cm
Volume (V_1)	0.144 cm^3
Volume of ethanol (V_2)	0.36 cm^3

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness (l_P)	0.0050 cm

From the plots, a = 0.00019672 and b = 0.0154.

For full form equation

$$M_\infty = \frac{a(V_1 + V_2)}{bV_2}$$

$$M_\infty = \frac{0.00019672(0.144 + 50)}{(0.0154)(50)} = 0.0128$$

and

$$D_P K_{P/S} = \frac{bl_P V_1 V_2}{A(V_1 + V_2)}$$

$$D_P K_{P/S} = \frac{(0.0154)(0.000010)(0.144)(50)}{(6 \times 12)(0.144 + 50)} = 1.54 \times 10^{-11}$$

For short form equation

$$M_\infty = \frac{a}{b}$$

$$M_\infty = \frac{0.00019672}{0.0154} = 0.0128$$

and

$$D_P K_{P/S} = \frac{bl_P V_1}{A}$$

$$D_P K_{P/S} = \frac{(0.0154)(0.000050)(0.144)}{(6 \times 12)} = 1.54 \times 10^{-11}$$

B.13 Calculation of rate constant for the release of carvacrol from AM films affected by temperature and AM layer thickness

(Table 4.15)

From Equation 2.11

$$1-\frac{M_t}{M_\infty}=\exp(-kt)$$

$$\ln(1-\frac{M_t}{M_\infty})=-kt$$

Rate constant, k, can be obtained from the slope of the plot of $\ln(1-M_t/M_\infty)$ versus time as shown in Figure 4.22.

From Equation 2.7

$$\frac{dM_t}{dt}=\frac{M_\infty AD_P K_{P/S}}{l_P V_1}\exp\left(\frac{-AD_P K_{P/S}t}{l_P V_1}\right)$$

$$k=\frac{AD_P K_{P/S}}{l_P V_1}$$

Rate constant can also be calculated from the plot of dM_t/dt versus time as shown in Figure B.6.

B.14 Calculation of migration parameters of the release of carvacrol from AM films with different AM layer thickness into water at 20°C

(Table 4.17)

The full form of rate equation (Equation 2.6) is considered in an alternative form as follows.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S}(V_1+V_2)t}{l_P V_1 V_2}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}(V_1+V_2)}{l_P V_1 V_2}$$

$$M_\infty = \frac{a(V_1+V_2)}{bV_2}$$

$$D_P K_{P/S} = \frac{b l_P V_1 V_2}{A(V_1+V_2)}$$

For the short form (Equation 2.7), the same parameters can be calculated as below.

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$$

$$\frac{dM_t}{dt} = a \cdot \exp(-bt)$$

$$a = \frac{M_\infty A D_P K_{P/S}}{l_P V_1}$$

$$b = \frac{AD_P K_{P/S}}{l_P V_1}$$

$$M_\infty = \frac{a}{b}$$

$$D_P K_{P/S} = \frac{b l_P V_1}{A}$$

The values of term *a* and *b* can be obtained from the plots of dM_t/dt versus *t* as shown in Figure B.7 and Table B.4.

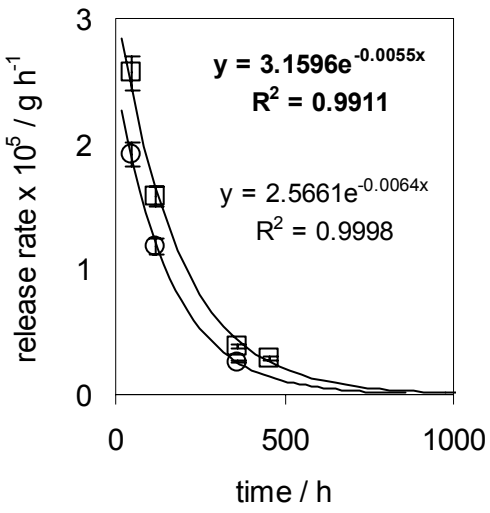


Figure B.7 Release rate of carvacrol migrating from (○) 20 μm, (□) 40 μm thick AM layer through 50 μm thick controlling layer into water at 20°C versus time.

Table B.3 Term *a* and *b* obtained from the release rate of carvacrol from AM films with different AM layer thicknesses into water at 20°C.

AM layer thickness / μm	Value	
	$a \times 10^5$	*b*
20	2.5661	0.0064
40	3.1596	0.0055

The AM film with 20 μm thick AM layer has the dimension as follows.

Width	6.00 cm
Length	12.00 cm
Thickness	0.0020 cm
Volume (V_1)	0.144 cm^3
Volume of ethanol (V_2)	0.36 cm^3

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness (l_P)	0.0050 cm

From the plots, a = 0.000025661 and b = 0.0064.

For full form equation

$$M_\infty = \frac{a(V_1 + V_2)}{bV_2}$$

$$M_\infty = \frac{0.000025661(0.144 + 50)}{(0.0064)(50)} = 0.00402$$

and

$$D_P K_{P/S} = \frac{bl_P V_1 V_2}{A(V_1 + V_2)}$$

$$D_P K_{P/S} = \frac{(0.0064)(0.000050)(0.144)(50)}{(6 \times 12)(0.144 + 50)} = 0.64 \times 10^{-11}$$

For short form equation

$$M_\infty = \frac{a}{b}$$

$$M_\infty = \frac{0.000025661}{(0.0064)} = 0.00401$$

and

$$D_P K_{P/S} = \frac{bl_P V_1}{A}$$

$$D_P K_{P/S} = \frac{(0.0064)(0.000050)(0.144)}{(6 \times 12)} = 0.64 \times 10^{-11}$$

From Equation 2.11

$$1 - \frac{M_t}{M_\infty} = \exp(-kt)$$

$$\ln(1 - \frac{M_t}{M_\infty}) = -kt$$

Rate constant, k, can be obtained from the slope of the plot of $\ln(1-M_t/M_\infty)$ versus time as shown in Figure 4.25(c).

From Equation 2.7

$$\frac{dM_t}{dt} = \frac{M_\infty A D_P K_{P/S}}{l_P V_1} \exp\left(\frac{-AD_P K_{P/S} t}{l_P V_1}\right)$$

$$k = \frac{AD_P K_{P/S}}{l_P V_1}$$

Rate constant can also be calculated from the plot of dM_t/dt versus t as shown in Figure B.7.

B.15 Calculation of mass balance and concentration of carvacrol released from AM films with 20 µm thick AM layer into water at 20°C

(Table 4.18)

Initial stage in AM layer

Dimension of AM layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0020 cm
Volume	0.144 cm^3

Concentration of carvacrol: 0.23 g cm^{-3}

Initial mass of carvacrol: 0.23 x 0.144 = 0.033 g

Equilibrium stage in water

Volume of ethanol: 50 cm^3

Equilibrium concentration of carvacrol: 0.00008 g cm^{-3}

Equilibrium mass of carvacrol: 50 × 0.00008 = 0.004 g

Equilibrium stage in controlling layer

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0050 cm
Volume	0.36 cm^3

Equilibrium concentration of carvacrol: 0.00022 g cm^{-3}

Equilibrium mass of carvacrol: 0.36 × 0.00022 = 0.000079 g

Equilibrium stage in AM layer

Volume of AM layer: 0.144 cm^3

Partition coefficient ($K_{P/S}$): 0.0089

Equilibrium concentration of carvacrol: 0.00022 ÷ 0.0089 = 0.025 g cm^{-3}

Equilibrium mass of carvacrol: 0.025 x 0.144 = 0.0036 g

Partition coefficient ($K_{P/F}$): 0.00022 ÷ 0.00008 = 2.8

B.16 Calculation of mass balance and concentration of carvacrol released from AM films with 40 µm thick AM layer into water at 20°C

(Table 4.19)

Initial stage in AM layer

Dimension of AM layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0040 cm
Volume	0.288 cm^3

Concentration of carvacrol 0.21 g cm^{-3}

Initial mass of carvacrol 0.21 x 0.288 = 0.061 g

Equilibrium stage in water

Volume of water 50 cm^3

Equilibrium concentration of carvacrol 0.00012 g cm^{-3}

Equilibrium mass of carvacrol 50 × 0.00012 = 0.0058 g

Equilibrium stage in controlling layer

Dimension of controlling layer

Width	6.00 cm
Length	12.00 cm
Thickness	0.0050 cm
Volume	0.36 cm^3

Equilibrium concentration of carvacrol 0.00042 g cm^{-3}

Equilibrium mass of carvacrol 0.36 × 0.00042 = 0.00015 g

Equilibrium stage in AM layer

Volume of AM layer 0.288 cm^3

Partition coefficient ($K_{P/S}$) 0.0089

Equilibrium concentration of carvacrol 0.00042 ÷ 0.0089 = 0.047 g cm^{-3}

Equilibrium mass of carvacrol 0.047 x 0.288 = 0.014 g

Partition coefficient ($K_{P/F}$) 0.00042 ÷ 0.00012 = 3.5

Appendix C

Table C.1 Time-line of developments in food packaging – glass.

Year/Century	Development in glass
7000 B.C.	Pottery made
1500 B.C.	Glass industrialised in Egypt
1200 B.C.	Glass cups and bowls moulded
300 B.C.	Blowpipe invented
0	Transparent glass made
10th	Glass making process commenced across Europe
17th	Split mould invented
1795	Food preserved in glass jars by hot-fill method to remove air
18th	Irregular shaped glass and raised decoration made
1858	Metal caps designed for glass jars
1889	Automatic rotary bottle making machine invented
1898	Crown caps invented for glass bottles
19th	Microorganism found to cause food spoilage
1903	Semiautomatic machine making jars and bottles invented

Source: (Den Hartog, 2007; Hook and Heimlich, 2007)

Table C.2 Time-line of developments in food packaging – paper.

Year/Century	Development in paper
200 B.C.	Paper made in China
1310	Paper made in UK
16th	Cardboard made in China
1690	Paper made in US
1817	Cardboard box made in UK
1844	Paper bags commercialised in UK
1850s	Corrugated paper made in UK
1852	Bag making machine made in US
1867	Paper made from wood pulp
1870	Glued paper sack invented
1880s	Paraffin coating made fat and fluid impermeable paper and cartons
19th	Shipping carton made of corrugated paperboard replaced wooden crates
1905	Printed paper bags made
1925	Large paper sack ends sewn
1952	Cartons coated with PE replaced glass and cans for milk and fruit drinks

Source: (Den Hartog, 2007; Hook and Heimlich, 2007)

Table C.3 Time-line of developments in food packaging – metal.

Year/Century	Development in metal
1200	Tin plate made in Bohemia
1400s	Iron cans coated with tin made in Bavaria
1600s	Plating process commenced in Europe, France and UK
1764	Snuff packed in metal canisters in London
1809	Tin cans made
1810	Tinplate patented
1810	Uses of metal containers to replace fragile glass patented
1811	Iron containers introduced for food packaging
1812	Meat packed in iron cans
1813	Meat packed in tin cans
19th (early)	Steel replaced iron in US
1819	Canning method arrived US
1821	Canned food commercialised in US
1825	Al powder first extracted from bauxite ore but expensive
1830s	Cookies and matches packed in tin cans
1841	Collapsible soft Al tubes invented
1852	Al making process developed
1856	Sweetened condensed milk packed in cans
1861-1865	American civil war made canned food significantly popular
1866	Keywind metal tear-strip developed
1866	Metal boxes first printed in US
1868	Enamel for can developed
1868	Hand-made cans replaced by machine-cut types
1875	Can opener invented
1888	Double seam closure and sealing compound developed
1890	Toothpaste packed in collapsible metal tubes
1904	Contamination by lead soldering solved
1910	Aluminium foils made
1920s	Nutritional value of canned food developed
1930s	Small portable canning machine invented and replaced glass jars and bottles
1942	Aluminium price declined 40 times
1950	Aluminium foil containers developed
1950	Pop top/tear tab lids made for cans
1959	Aluminium cans made
1960s	Food packed in collapsible metal tubes
1970s	Metals and plastics gained popularity

Source: (Den Hartog, 2007; Hook and Heimlich, 2007)

Table C.4 Time-line of developments in food packaging – plastic.

Year/Century	Development in plastic
1831	Styrene first distilled from balsam tree
1835	Vinyl chloride discovered
1870	Celluloid patented
1898	Polyethylene (PE) made accidently
1900	Cellulose acetate derived from wood pulp
1909	Cellulose acetate developed for photographic uses
1924	Cellophane made in New York
1933	Styrene process refined in German
1933	PE used for military and drug tablets
1935	Synthesis for PE developed in UK
1939	Low-density polyethylene (LDPE) industrialised in UK
1940s	Food packaging entered era of disposable packaging
1950s (early)	Polypropylene (PP) invented
1947	Vinyl chloride used for deodorant squeeze bottle
1950	Foam boxes, cups and meat trays made from styrene
1950	Cellophane commercialised and used for packaging
1958	Heat shrinkable films developed from styrene and synthetic rubber
1977	Polyethylene terephthalate (PET) used for beverages
1980s	Packaging made for microwave uses and modified atmosphere packaging (MAP) developed for ready-to-eat fresh vegetables and fruits
1980	Hot-fill food products (jam) packaged in PET

Source: (Den Hartog, 2007; Hook and Heimlich, 2007)