# Essential Oils and Their Principal Constituents as Antimicrobial Agents for Synthetic Packaging Films

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**Abstract**

Spices and herbal plant species have been recognized to possess a broad spectrum of active constituents that exhibit antimicrobial (AM) activity. These active compounds are produced as secondary metabolites associated with the volatile essential oil (EO) fraction of these plants. A wide range of AM agents derived from EOs have the potential to be used in AM packaging systems which is one of the promising forms of active packaging systems aimed at protecting food products from microbial contamination. Many studies have evaluated the AM activity of synthetic AM and/or natural AM agents incorporated into packaging materials and have demonstrated effective AM activity by controlling the growth of microorganisms. This review examines the more common synthetic and natural AM agents incorporated into or coated onto synthetic packaging films for AM packaging applications. The focus is on the widely studied herb varieties including basil, oregano and thyme and their essential oils.

**Keywords**: Essential oils, natural AM agents, active packaging, antimicrobial packaging, microbial contamination

# 1 Introduction

Food products can be subjected to microbial contamination that is mainly caused by bacteria, yeasts and fungi. Many of these microorganisms can cause undesirable reactions that deteriorate the flavour, odour, colour, sensory, and textual properties of foods (Appendini and Hotchkiss 1997; Vermeiren and others 1999; Weng and others 1999; Appendini and Hotchkiss 2002; Vermeiren and others 2002; Devlieghere and others 2004a; Han 2005; Rupika and others 2005; Davidson and Taylor 2007; Gutierrez and others 2008). Microbial growth in food products is a major concern because some microorganisms can potentially cause food-borne illness (Padgett and others 1998; Natrajan and Sheldon 2000; Cha and Chinnan 2004; Davidson and others 2005; de Oliveira and others 2007). In packaged foods, the growth and survival of common spoilage and pathogenic microorganisms such as *Listeria monocytogenes*, *Escherichia coli* O157, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter*, *Clostridium perfringens, Aspergillus niger* and *Saccharomyce cerevisiae* are affected by a variety of intrinsic factors such as pH, water activity, and the presence of oxygen or by extrinsic factors associated with storage conditions including temperature, time and relative humidity (Singh and others 2003; López-Malo and others 2005; Rydlo and others 2006). Many food products including various types of cheeses, meats, poultry and baked products are highly susceptible to microbial spoilage (Weng and Hotchkiss 1993; Suppakul 2004; Limjaroen and others 2005; Schelz and others 2006; Silveira and others 2007).

To prevent the growth of spoilage and pathogenic microorganisms on foods, various traditional preservation techniques such as heat treatment, salting, acidification and drying are used in the food industry (Quintavalla and Vicini 2002; Ozdemir and Floros 2004; Davidson and Taylor 2007; Farkas 2007). In recent years, a rise in consumer demand for safe, fresh and minimally-processed foods, has led to the development of new preservation

techniques. Active packaging (AP) technologies, for example, can provide safe food products with longer shelf lives (Rooney 1995; Lau and Wong 2000; Vermeiren and others 2002; Fitzgerald and others 2003; Ozdemir and Floros 2004; Gutierrez and others 2008). In the food industry, spoilage of food products, including spoilage that is caused by microorganisms is a major concern. The AP technologies designed primarily to protect food products from deterioration and from the growth of microorganisms can involve the use of synthetic or natural antimicrobial (AM) agents (Juneja and Sofos 2005). To diminish food spoilage by microorganisms, different AM agents (primarily synthetic) are commonly incorporated directly into the food. This method has many disadvantages: (i) consumers prefer foods with no or minimal synthetic additives because of concerns about side-effects; (ii) since food spoilage occurs primarily on the surface, incorporation of relatively large quantities of the agents in the bulk of the food is not justified; (iii) some of the synthetic agents possess a distinct flavour that may rendered the food flavour, and (iv) synthetic additives have to be declared on the package. Therefore, packaging materials that incorporate in them the AM agent as an additional protective barrier are emerging as the preferred preservation method. Several authors have reported AP technologies that involve the use of films produced from synthetic polymers (Miltz and others 1995; Rooney 1995; Smith and others 1995). These materials can act as carriers for active agents, including AM compounds, in order to maintain high concentrations of the agent on or near the food surface to control or prevent the growth of spoilage and pathogenic microorganisms (Krochta and De Mulder-Johnston 1997; Joerger 2007; Raybaudi-Massilia and others 2009; Rojas-Graü and others 2009; Suppakul and others 2011a). Thus a packaging film impregnated or coated with an AM agent could potentially extend the shelf life and improve the microbial safety of food products (Appendini and Hotchkiss 2002; Suppakul and others 2003b; Burt 2004; Kuorwel and others 2011b).

Although AM agents such as essential oils (EOs) and/or their principal components may exhibit AM activity against various microorganisms when incorporated into packaging materials, the organoleptic properties of the packaged food products are one of the important factors that must also be taken into consideration. According to Davison and Zivanovic (2003), the concentration of AM agents required to demonstrate AM activity against various microorganisms on food products might be higher than the concentration applied for flavouring purposes. As a result, this might cause food tainting and/or adverse sensorial effects to food products (Smith-Palmer and others 2001; Bagamboula and others 2004). The adverse sensorial effects of AM agents to food products can be overcome by masking the odor of AM agents with other approved aroma compound as suggested by Gutiérrez and others (2009). An understanding of the relationship between minimum inhibitory concentration and acceptable organoleptic properties of AM Agents such as EOs and/or their constituents is also important (Lambert and others 2001). In some cases, the replacement of EOs with one or a number of their principal constituents may provide equal AM effectiveness but with milder flavouring attributes (Lambert and others 2001; Smith-Palmer and others 2001).

In a recent review, the current authors presented an evaluation of the AM activity of biodegradable polysaccharide and protein based films containing natural agents (Kuorwel and others 2011b). These films showed the potential for wide-range of applications in food packaging where undesirable microbial growth is a concern. Moreover, these films degrade readily in the environment but the acquisition of this attribute may harm the processability and mechanical stability of the film. Thus, in spite of the increasing concern in recent years about the use of synthetic polymers due to their poor biodegradability, these materials have several advantages including low cost, good processability and sound mechanical and

physical properties. Therefore, development of AM packaging materials manufactured from synthetic polymers such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polyethylene terephthalate (PET) and polypropylene (PP) is still important in offering commercial benefits for packaging food products. In the current review, a detailed summary of synthetic films utilising common synthetic and natural AM agents is presented with an emphasis on the principal components of basil, oregano, and thyme essential oils (EOs) namely, linalool, carvacrol and thymol respectively. This is followed by a list of other natural AM agents that have the potential for controlling microbial growth on foods.

## 2 Antimicrobial Packaging Systems

Studies have shown that AM packaging systems can increase the shelf life of packaged foods by extending the lag phase and reducing the growth rate of spoilage microorganisms (Han 2000; Cooksey 2001; Appendini and Hotchkiss 2002; Rydlo and others 2006; Coma 2007; de Oliveira and others 2007; Gutierrez and others 2008; Rardniyom and others 2008; Rupika and others 2008). In the past, preservatives were directly added into food products to protect them from microbial contamination. This process of direct addition of preservatives into foods may result in levels of additives in excess to those required for an efficient AM effect. New AM packaging systems have attracted much attention in the food industry with the aim of replacing the conventional food preservation systems (Weng and Hotchkiss 1993; An and others 1998; Quintavalla and Vicini 2002; Bagamboula and others 2004; Devlieghere and others 2004b; Miltz and others 2006).

An AM packaging system can be produced by: directly incorporating AM agents into packaging films; coating of the packaging films with AM agents; developing packaging

materials from polymers that have inherent AM properties (Vermeiren and others 2002; Suppakul and others 2003b). Typically, AM packaging systems can be regarded as migrating or non-migrating with the distinction depending on the specific AM agent used and on its interactions with the packaging and food matrix (Cooksey 2000). In a migrating system, the AM agent is released from the packaging film into the package headspace and onto the food surface and such systems are most useful when direct contact between the packaging film and food product is not required for efficient AM activity (Weng and Hotchkiss 1993; Cooksey 2000). The non-migrating systems involve packaging materials in which the AM agent is immobilised within the material (Brody and others 2001) and these systems can be applied where direct contact between the food and the material can be achieved or is required for effective AM activity (Vermeiren and others 2002; Suppakul and others 2003b). In either AM packaging system, both synthetic and natural AM agents can be incorporated into or coated onto the packaging material. The mode of action of AM agents incorporated in a packaging material is influenced by the controlled and slow release of the agent onto the food surfaces. This is required in order to maintain an adequate concentration of the agent on the food and effectively inhibit microbial growth throughout the product shelf life (Cooksey 2005; Salleh and others 2007; Cran and others 2010; Tunç and Duman 2011). Han (2005) suggested that the mass transfer rate of an AM agent should not be faster than the growth rate of the target microorganism, otherwise the AM agent might be diluted on the surface of the packaged food product, thus limiting the AM activity.

During extrusion or compression moulding of AM films, the temperature and mechanical energy input, such as shearing forces, must be carefully considered (Han 2003). High-processing temperatures, for example, may result in considerable losses of volatile AM agents (Han and Floros 1997; Han 2000; Rupika and others 2005; Suppakul and others

2011b). Moreover, Cooksey (2005) suggested that an AM agent might partly or completely lose its AM activity if incorporated into a film under harsh processing conditions. Therefore, to minimise the loss of AM agent during processing, temperatures that are as low as possible should be applied (Han and Floros 1998; Suppakul and others 2011b). The storage temperature may also influence the activity of AM agents that are incorporated into packaging films (Vojdani and Torres 1989; Han 2005). The concentration of AM agents retained in the film may decrease during long-term storage. However, the amount of AM agent retained in the film after a long storage period may be sufficient to demonstrate AM activity as shown by Suppakul and others (2011b). Du and others (2008) reported AM activity against *E. coli* (using an agar disc diffusion method) of carvacrol incorporated into films for edible apples that were stored for 7 weeks.

## 2.1 Synthetic AM Agents

In the past few decades, various synthetic AM agents have been investigated and developed into food packaging materials (Weng and Hotchkiss 1992; Weng and Hotchkiss 1993). Many of these agents including various organic acids and salts have been approved by regulatory agencies and have since been used for the preservation of food products (Davidson and Taylor 2007). Synthetic AM agents that have demonstrated inhibitory activity against different microorganisms include sodium benzoates and propionates, potassium sorbates, sulfites, chlorides, nitrites, triclosan, fungicides (e.g. benomyl, imazalil) and various metal ions including silver zeolites, quaternary ammonium salts and copper ions (Chen and others 1996; Devlieghere and others 2000a; Han 2000; Ouattara and others 2000; Hoffman and others 2001; Cooksey 2005). Other AM agents such as acetic acid from vinegar and benzoic acid from cranberries are found in nature, but are classified as synthetic AM agents when produced synthetically (Davidson and Taylor 2007).

Many synthetic AM compounds have been evaluated in synthetic polymeric materials by various researchers. Table 1 summarises these synthetic AM agents incorporated into or coated onto packaging materials as potential candidates for food packaging. Although Table 1 contains a large amount of information on the activity of AM agents successfully incorporated into various synthetic polymers, comparison between the different AM agents and/or AM films is difficult due to variations in strains of microorganisms and different experimental conditions or equipment used by the various researchers. In order to compare the results of various experiments involving AM agents, there is a need for a standardisation of the test methods as suggested by Suppakul and others (2003a).

Numerous studies have concentrated on incorporating common food preservatives such as organic acids, their salts and anhydrides into packaging films (see Table 1). Studies on benzoic or sorbic acid incorporated into packaging materials have evaluated their action against various microorganisms in laboratory media such as agar plates and/or in actual food products. The packaging films incorporated with these organic acids or anhydrides have demonstrated inhibitory effects against various spoilage and pathogenic microorganisms. Weng and others (1999) showed that benzoic acid or sorbic acid incorporated into poly(ethylene-co-methacrylic acid) (PEMA) film inhibited the growth of *A. niger* and *Penicillium* sp. on solid media. Weng and Chen (1997) investigated the AM activity of benzoic acid or benzoyl chloride incorporated into ionomer films. The AM activity of these films was demonstrated by their ability to inhibit the growth of *Penicillium* sp. and *A. niger*. In an earlier study, Weng and Hotchkiss (1993) incorporated benzoic acid or benzoic anhydride into LDPE films which significantly suppressed the growth of *Rhizopus stolonifer*, *Penicillium* sp. and *A. toxacarius* on potato dextrose agar and on the surface of Cheddar cheese. Matche and others (2006) examined the AM activity of benzoyl chloride incorporated

into modified ethylene acrylic acid films against *Penicillum* sp. and *A. niger* sp. on solid media for 15 days with the film demonstrating inhibition against both species. Silveira and others (2007) incorporated sorbic acid into LDPE films with the aim of preserving fresh pastry dough. It was found that 3% (w/w) sorbic acid incorporated into a 70 μm film reduced 2 and 1.5 log cycles of mesophilic and psychrotrophic bacteria respectively on the pastry dough after 40 days of storage at 8°C compared to the control film. Limjaroen and others (2005) coated sorbic acid onto polyvinylidene chloride copolymer films to control the growth of *L. monocytogenes* on beef bologna and Cheddar cheese. It was found that sorbic acid coated on the films inhibited microbial growth on cheese by log 0.6 $CFUg^{-1}$ after 28 days of storage at 4°C. They further reported that the population of *L. monocytogenes* on beef bologna was reduced by log 0.6 and 1.4 $CFUg^{-1}$ for films containing 1.5% and 3.0% (w/v) respectively compared to the control film.

Other researchers have studied the AM activity of salts against several microorganisms as shown in Table 1. Han and Flores (1997) developed LDPE films containing potassium sorbate and found that these films successfully reduced the growth of *S. cerevisiae in vitro* experiments. Vartiainen and others (2003b) demonstrated that potassium sorbate, sodium benzoate and sodium nitrate incorporated into LDPE, poly(maleic acid-co-olefine), PET or PS films inhibited the growth of *B. cereus* on culture media. Limjaroen and others (2003) coated potassium sorbate onto polyvinylidene chloride copolymer films and reported that the films inhibited the growth of *L. monocytogenes* on solid media.

In addition to the antibacterial activity, the antifungal activity of synthetic AM agents incorporated in polymeric materials has been investigated (Halek and Anita 1989; López-Malo and others 2007). Vartiainen and others (2003a) examined the inhibitory effects of

imazalil incorporated into LDPE against the growth of *A. niger* by the agar diffusion assay with films containing 0.05-0.25% (w/w) imazalil demonstrating significant inhibitory activity. Weng and Hotchkiss (1992) incorporated imazalil into LDPE film and evaluated the antimycotic activity of this agent on the growth of *A. toxacarius* and *Penicillium* sp. on potato dextrose agar (PDA) and Cheddar cheese. They reported that 2 g $kg^{-1}$ of imazalil suppressed the growth of *A. toxacarius* on PDA whereas a film containing 1 g $kg^{-1}$ of imazalil reduced the growth of *Penicillium* sp. The latter film inhibited the growth of both mould species on the surface of Cheddar cheese. López-Malo and others (2002; 2005) examined the antifungal activity of potassium sorbate, sodium benzoate and sodium bisulfite against the growth of *Aspergillus flavus* inoculated on laboratory media with each of the agents imparting an inhibitory effect. López-Malo and others (2007) investigated the antifungal activity of sodium benzoate and cinnamon extract, separately or in combination, against the growth of *A. flavus* on potato dextrose agar or a checkerboard array respectively. They found that both AM agents demonstrated antifungal activity on *A. flavus*, with cinnamon extract being more effective than sodium benzoate. They claimed that mixtures of cinnamon extract and sodium benzoate showed promising antifungal activity.

Triclosan and hexamethylenetetramine are common synthetic AM agents that have been evaluated in packaging systems with some commercial developments. Vermeiren and others (2002) investigated the AM activity of triclosan incorporated into LDPE film and reported that concentrations of 0.5 and 1.0% (w/w) demonstrated AM activity against *L. monocytogenes, Sal. enteritidis, Staph. aureus, E. coli* O157:H7 and *Brocothrix thermosphacta* in an agar diffusion assay. Cutter (1999) reported that triclosan was effective against bacteria on the surface of beef. Recently, Camilloto and others (2010) studied the activity of triclosan incorporated into LDPE against *Staph. aureus*, *E. coli*, *L. innocua* and *P.*

*aeruginosa* in the agar disc diffusion test and found that the AM film inhibited the growth of *Staph. aureus* and *E. coli*. Chung and others (2003a) investigated the AM activity of triclosan coated onto styrene-acrylate copolymer against *Enterococcus faecalis* on solid and in liquid media and showed effective inhibition of the bacteria. Ji and Zhang (2009) reported that triclosan incorporated into PVC film inhibited the growth of *Staph. aureus* and *E. coli* using the plate-counting technique. Devlieghere and others (2000b) studied the AM activity of hexamethylenetetramine impregnated into an LDPE film and found it to be effective against spoilage microorganisms on cooked ham.

## 2.2 Natural Antimicrobial Agents

In recent years, natural AM agents have attracted much attention in the food and packaging industries as a replacement for synthetic ones for food preservation. According to Davidson and Zivanovic (Davidson and Zivanovic 2003), natural AM agents are classified by their sources: AM agents derived from plant EOs (e.g. basil, thyme, oregano, cinnamon, clove and rosemary); animal sources (e.g. lysozyme, lactoferrin); microbial sources (nisin, natamycin); and naturally occurring polymers (chitosan). The EOs extracted from plant sources consist of various mixtures including terpenoids, esters, aldehydes, ketones, acids and alcohols (Dorman and Deans 2000). These plant EOs are volatile and generally possess relatively strong odours (Bakkali and others 2008).

Extracts derived from various herbs and EOs contain a range of natural compounds such as thymol, linalool and carvacrol which have a broad AM spectrum against different pathogenic and spoilage microorganisms including Gram-negative species such as *E. coli, Yersinia enterocolitica, P. aeruginosa* and *Sal. choleraesuis* (López and others 2007a; Suppakul and others 2011b), Gram-positive bacteria such as *L. monocytogenes, Staph. aureus, B.* cereus

(Friedman and others 2002; López and others 2007b; Gutiérrez and others 2009), yeasts such as *S. cerevisiae, Candida albicans, Debaryomyces hansenii* (Rupika and others 2006; Suppakul and others 2008; Kuorwel and others 2011a) and moulds such as *Alternaria alternate, A. niger, Botrytis cinerae, A. flavus, penicllium roqueforti* (López-Malo and others 2007; Rodríguez-Lafuente and others 2010). These additives are considered to be safe and have the "Generally Recognised As Safe" (GRAS) status as designated by the American Food and Drug Administration (Zaika 1988; Han 2005; Matan and others 2006). Antimicrobial agents derived from plant sources are produced as secondary metabolites and are associated generally with the volatile EO fractions. The mode of action of AM agents and/or AM activity of plant EOs is related to their chemical structure namely, the presence of hydrophilic functional groups such as the hydroxyl groups of phenolic components and/or lipophilicity of the components in the EOs which depends on their concentration (Farag and others 1989; Davidson and Naidu 2000; Dorman and Deans 2000; Friedman and others 2002; Bagamboula and others 2004). Essential oils and their principal constituents inhibit microorganisms *via* a range of mechanisms such as: disruption of the cyctoplasmic membrane (Knobloch and others 1989; Sikkema and others 1995; Helander and others 1998); leakage of intracellular constituents such as metabolites and ions (Sikkema and others 1995; Lambert and others 2001); coagulation of cell content (Gustafson and others 1998; Pauli 2001); inhibition of protein synthesis (Helander and others 1998), enzymes associated with cell wall synthesis (Conner and Beuchat 1984), DNA/RNA synthesis (Ultee and others 1999; Tassou and others 2000), general/metabolite pathways (Ultee and others 2002); and/or the destruction of the osmotic integrity of the cell membrane (Ultee and Smid 2001). The AM activity of different EOs is very difficult to compare given the variation of EOs compositions amongst the plant species, differences in the geographic origin of the plants, harvesting

316 season, extraction methods and the part of plant that is used (Zaika 1988; Elgayyar and others
317 2001).
318 There are a number of test methods used to determine the AM activity of various EOs and
319 their principal constituents. These include diffusion methods (agar diffusion), dilution
320 methods (broth and agar dilution) and microatmosphere methods (Davidson and Zivanovic
321 2003; Guynot and others 2003; Nedorostova and others 2011; Tunç and Duman 2011). These
322 test methods provide preliminary information on the possible effectiveness of the tested
323 active constituents. The agar diffusion method has been widely used in the past, but the
324 results obtained from this technique are qualitative. Although the agar diffusion method can
325 indicate the AM activity of EOs and/or their principal components on solid media, the high
326 hydrophobicity of EOs is always a major problem (Davidson and Zivanovic 2003). As a
327 result, the agar disc diffusion assays do not generally demonstrate a clear zone of inhibition at
328 very low concentrations; however these do exhibit a clear inhibition zone at high
329 concentrations of hydrophobic, lipophilic AM agents (Friedman and others 2002; Sanla-Ead
330 and others 2011). Conversely, microatmosphere methods, which allow the determination of
331 the AM activity of EOs and/or their constituents in the vapour phase, can be used with
332 lipophilic AM films at low concentrations of AM agents (López and others 2007a; Fisher and
333 others 2009; Goñi and others 2009; Kloucek and others 2011). Recently, Sanla-Ead and
334 others (2011) investigated the AM activity of cinnamaldehyde and eugenol incorporated into
335 cellulose-based packaging films against Gram-negative bacteria (*E. coli*, *Sal. enteritidis*),
336 Gram-positive bacteria (*L. monocytogenes*, *Staph. aureus*) and yeasts (*C. albicans*, *C.*
337 *cerevisiae)* using the vapour diffusion assay. The authors reported that cinnamaldehyde and
338 eugenol incorporated into cellulose-based packaging films demonstrated positive inhibitory
339 effects against the tested microorganisms.
340

Table 2 summarises the AM activity of a range of common natural agents that have been incorporated into or coated onto synthetic packaging films. Table 2 also lists other studies that have evaluated the inhibitory effects of natural AM agents *in vitro* or directly on food products without incorporating them into packaging films.

### 2.2.1 Antimicrobial Activity of Basil Essential Oils

Basil EOs contain primarily linalool and methylchavicol as the active volatile components which are responsible for their AM activity (Simon and others 1990; Fyfe and others 1998; Wan and others 1998; Bezic and others 2003; Suppakul 2004). Many studies have evaluated the AM activity of basil EOs against various microorganisms both *in vitro* and on a range of food products as shown in Table 2. Prasad and others (1986) investigated the AM activity of the EOs of *O. basilicum* against various Gram-positive and Gram-negative bacteria with the oils shown to be more effective against Gram-positive bacteria including *Bacillus sacharolyticus, B. stearothermophilus, B. subtilis, B. thurengiensis, Micrococcus glutamicus* and *Sarcina lutea* than the Gram-negative ones. Lachowicz and others (1998) evaluated the AM effects of EOs of sweet basil against acid-tolerant food microflora. They reported greater inhibitory effects of the tested EOs against the Gram-positive bacteria *Bacillus* sp., *Staph. aureus* sp., *Micrococcus* sp., *Sarcina* sp., *Lactobacillus* sp. than against the Gram-negative bacteria *E. coli, Salmonella* sp., *Enterobacter* sp. and *Pseudomonas* sp. In contrast to these studies, Koga and others (1999) found that the Gram-positive bacteria were more resistant to basil EOs than the Gram-negative ones.

Various researchers have reported also the inhibitory effect of basil EOs against fungi. Rai and others (1999) evaluated the antifungal activity of the EOs of ten plant species (including *O. basilicum*) and reported that the EOs of basil were active against all *Fusarium* species

including *F. acuminatum, F. solani, F. pallidoroseum* and *F. chlamydosporum.* Conner and Beuchat (1984) reported positive AM activity of basil EOs against *Kloeckera apiculata* on solid media. Basilico and Basilico (1999) investigated the inhibitory effects of some EOs, including that of basil (*O. basilicum*), against the growth of *A. ochraceus* and subsequent ochratoxin A production. They reported that at a level of 1000 ppm, only basil EO decreased the fungal growth and the production of ochratoxin A for up to 7 days after which mould growth occurred.

### 2.2.2 Antimicrobial Activity of Linalool

Linalool has been reported to possess both fungistatic and antibacterial properties against a wide spectrum of microorganisms such as *Staph. aureus, L. innocua, E. coli, A. niger* and *S. cerevisiae* (Lachowicz and others 1998; Friedman and others 2002; Suppakul and others 2003a). As shown in Table 2, numerous studies have evaluated the AM activity of linalool incorporated into packaging films. For example, Suppakul and others (2006; 2008) reported that linalool incorporated into LDPE film exhibited inhibitory activity against the growth of *Staph. aureus, L. innocua, E. coli* and *S. cerevisiae* on culture media and on the surface of Cheddar cheese. Rardniyom (2008) investigated the AM activity of linalool coated onto LDPE and nylon films against the growth of *E. coli* and reported effective inhibitory activity in liquid culture and on Cheddar cheese. Rupika and others (2006) reported that linalool incorporated into LDPE films demonstrated significant inhibitory activity against the growth of *L. innocua* and *E. coli* both *in vitro* and on the surface of Cheddar cheese. Suppakul and others (2011b) reported that linalool and/or methylchavicol incorporated into LDPE films demonstrated inhibitory activity against the growth of *E. coli* on agar disc media.

The AM activity of linalool against several microorganisms has also been reported in studies conducted *in vitro* only. Kim and others (1995) investigated the AM activity of some EO components including linalool against five food-borne pathogens (*E. coli, E. coli* O157:H7, *Sal. typhimurium, L. monocytogenes and V. vulnificus*) and found a dose-related increase in the zone of inhibition against all tested strains except for *L. monocytogenes*. Mazzanti and others (1998) reported that linalool completely inhibited the growth of all yeasts (seven strains of *C. albicans, C. krusei* and *C. tropicalis*), *Staph. aureus* and *E. coli* using the agar disc diffusion method. Dorman and Deans (2000) investigated the antibacterial activity of 21 plant volatile oil components including linalool against 25 bacterial strains using the agar well diffusion method. It was reported in this study that linalool was an effective AM agent against a broad spectrum of 23 out of the 25 bacterial strains investigated.

### 2.2.3 Antimicrobial Activity of Oregano and Thyme Essential Oils

Oregano and thyme are popular culinary herbs with their EOs containing terpenoid compounds, mainly the monoterpenoid phenols of thymol (5-methyl-2-[1-methylethyl] phenol) and carvacrol (5-isopropyl-2-methyl phenol). These EOs have been claimed to demonstrate potential health benefits, antioxidant activity and AM properties (Nychas 1995; Baratta and others 1998; Youdim and Deans 2000; Olasupo and others 2004; Tepe and others 2004; Davidson and Taylor 2007). The AM activity of thyme and oregano EOs is primarily attributed to their major components thymol and carvacrol respectively (Farag and others 1989; Cosentino and others 1999; Davidson and Naidu 2000; Dorman and Deans 2000; Lambert and others 2001; Bagamboula and others 2004; Davidson and Taylor 2007).

The AM activity of oregano and thyme EOs against various microorganisms has been investigated on media and on a range of food products as summarised in Table 2. For

example, Lin and others (2004) evaluated the AM activity of phenolic compounds derived from oregano against *L. monocytogenes* on solid media, on beef and on fish products and reported that the extracts exhibited AM activity against *L. monocytogenes* in the agar diffusion assays. Friedman (2004) studied the antibacterial activity of ten different EOs including that of oregano against *E. coli* and *Sal. enterica* in apple juice. They reported that the selected EOs exhibited greater AM activity against *Sal. enterica* than *E. coli*.

The AM activity of oregano and thyme EOs was investigated in liquid culture and solid media against various microorganisms. Becerril and others (2007) investigated the AM activity of oregano EOs incorporated into a patented plastic packaging material against *E. coli* and *Staph. aureus*, using a "kill time" assay. The authors claimed that oregano EOs exhibited significant AM activity with a kill time of approximately 90 min for *E. coli* and 104 min for *Staph. aureus*. Marques and others (2008) studied the AM activity of three natural AM agents: oregano, garlic and chitosan against the growth of *Sal. enterica* in liquid culture at 10 and 20°C. They reported that all of the natural agents inhibited significantly the microbial growth at both temperatures with oregano demonstrating the highest inhibitory effects followed by garlic then chitosan. Rodriguez-Lafuente and others (2010) investigated the AM activity of oregano and cinnamon EOs incorporated into packaging-paper against *Alternaria alternata* using an *in vitro* antifungal assay. The authors reported that oregano and cinnamon EOs inhibited the growth of *A. alternata* on solid media.

Nielsen and Rios (2000) reported that oregano EOs exhibit an inhibitory activity against microorganisms commonly associated with bread spoilage. Tepe and others (2004) examined the AM activity of *Thymus eigii* EOs and its main constituents carvacrol, thymol and *p*-cymene against *B. catarrhalis*, *C. perfringens*, *B. cereus*, *Staph. aureus*, *S. pneumoniae*, *M.*

*smegmatis* and *P. aeruginosa in vitro* and found that these EOs demonstrate AM activity against the tested microorganisms. More recently, Gutierrez and others (2008) evaluated the synergistic effect of the EOs of thyme, oregano, lemon balm, marjoram, rosemary and sage against *B. cereus, E. coli, L. monocytogenes* and *P. aeruginosa* using the spot test on agar media. They reported a significant AM activity of oregano in combination with basil, thyme or marjoram against *B. cereus, E. coli* and *P. aeruginosa*. Similarly, Gutierrez and others (2009) determined the AM activity of the EOs of thyme, oregano, lemon balm and marjoram against *Enterobacter* sp., *Listeria* sp., *Lactobacillus* sp. and *Pseudomonas* sp. using foods based on lettuce, meat and milk. Their findings demonstrated that minimum inhibitory concentrations were significantly lower in lettuce and beef media than in tryptic soy broth and that oregano and thyme produced the most active EOs. Lopez and others (2007b) reported AM activity of oregano, cinnamon and clove EOs incorporated into PP or PE/EVOH against various Gram-negative bacteria (*E. coli, Y. enterocolitica, P. aeruginosa* and *Sal. choleraesuis*), Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and *E. faecalis*), yeasts (*C. albicans, D. hansenii, Z. rouxii)* and moulds (*B. cinerae, A. flavus, E. repens, p. roqueforti, P. islandicum, P. commune, P. nalgiovensis*). Similarly, Lopez and others (2007a) reported AM activity of cinnamon, oregano and thyme EOs against various Gram-negative bacteria (*E. coli, Y. enterocolitica, P. aeruginosa* and *Sal. choleraesuis*), Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and *E. faecalis*), yeasts (*C. albicans)* and moulds (*A. flavus, P. islandicum*). The main constituents: cinnamaldehyde, carvacrol and thymol also demonstrated inhibitory effect against *L. monocytogenes, Sal. choleraesuis, A. flavus* and *C. albicans* using a modified vapour diffusion test.

Sagdiç and Özcan (2003) investigated the AM activity of various EOs including oregano and thyme EOs against different microorganisms including *Bacillus amyloliquefaciens, B. cereus,*

*Enterobacter aerogenes, E. coli, Sal. enteritidis, Staph. aureus* and *Yersinia enterocolitica*. They reported that oregano was particularly effective against all bacteria during incubation. Oussalah and others (2006) studied the inhibitory effects of sixty different EOs including oregano and thyme against *Pseudomonas putida*. The results of their study showed that many EOs possess *in vitro* antibacterial activity against *P. putida* with oregano and thyme EOs demonstrating the highest AM activity. Viuda-Martos and others (2008) evaluated the effectiveness of the EOs of oregano, sage, clove, thyme, rosemary and cumin on the growth of various microorganisms including *Lactobacillus curvatus, Lactobacillus sakei, Staph. carnosus* and *Staph. xylosus*, *Enterobacter gergoviae* and *Enterobacter amnigenus*. They found that each of the EOs demonstrated inhibitory activity against all bacteria tested with oregano showing the highest AM activity and reported that the effects of thyme, sage and rosemary were concentration dependent. Baydar and others (2004) studied the antibacterial activity of EOs of thyme, oregano and savoury against various pathogenic bacteria including *B. cereus, E. coli* and *L. monocytogenes*. They reported positive AM activity against the tested bacteria and suggested that the inhibition may be attributed to the action of the components carvacrol, γ-terpinene and *p*-cymene (a constituent of cumin or thyme EOs). The results of these studies demonstrate that oregano and thyme EOs have the potential to be used as AM agents in the food industry for better preservation of quality, enhancement of safety and extension of shelf life. Nevertheless, additional information is required on the benefits of these EOs before considering them as potential candidates for manufacturing of AM films with commercial applications.

### 2.2.4 Antimicrobial Efficacy of Carvacrol and Thymol

Carvacrol and thymol are the major components of oregano and thyme EOs. They have received substantial attention as useful natural AM agents due to their natural origin and

GRAS status, as well as them exhibiting a broad AM spectrum against different microorganisms, and possessing heat stability when incorporated into packaging materials (Deans and Ritchie 1987; Zaika 1988; Ultee and others 1998; Lorenzo and others 2003; Couladis and others 2004; Azaz and others 2005; Han 2005; Matan and others 2006). Table 2 shows that carvacrol and/or thymol can be applied in food products to control microbial contamination by various microorganisms including bacteria, yeasts and moulds.

Bagamboula and others (2004) determined the AM effect of carvacrol or thymol against *Shigella* sp. (*S. sonnei* and *S. flexneri*) on lettuce. They observed a decrease in *Shigella* sp. after washing the lettuce with 0.5% and 1% (v/v) thymol or carvacrol and found that at 1% (v/v) of each agent, the population decreased to an undetectable level. They also reported significant inhibition of *Shigella* sp. using the agar diffusion method. The AM activity of carvacrol has also been reported by Ultee and others (2000) when studying the preservation of rice against *B. cereus*. Roller and Seedhar (2002) investigated the effectiveness of carvacrol against the natural flora of freshly cut melons and kiwifruit. They found that carvacrol reduced significantly the viable count of natural flora on kiwifruit dipped in a solution of the agent, but it was less effective on honeydew melons. Kiskó and Roller (2005) explored the AM effectiveness of carvacrol against *E. coli* inoculated into unpasteurised apple juice. They found that carvacrol reduced the bacteria to an undetectable level within the first two days of storage. Ultee and Smid (Ultee and Smid 2001) found carvacrol to be effective against *B. cereus* toxin production in soups to an undetectable level. Chiasson and others (2004) reported effective AM activity of carvacrol and thymol against *E. coli* and *Sal. typhimurium* in minced meat products. Seaberg and others (2003) reported inhibitory effects of carvacrol against *L. monocytogenes* in ready-to-eat beef slices. Recently, Rardniyom (2008) coated carvacrol onto LDPE and nylon films and reported that the AM film inhibited

515 the growth of *E. coli* on Cheddar cheese by log 2.3 and 1.8 CFU $g^{-1}$ on samples stored at 8
516 and 12°C respectively for 15 days.
517 In addition to studies on real foods, several studies have reported the inhibitory effect of
518 carvacrol and thymol both on solid and liquid media as shown in Table 2. On solid media,
519 using the agar diffusion test, López-Malo and others (2005) found that carvacrol and thymol
520 had a significant inhibitory effect against *A. flavus*. Singh and others (2006) investigated the
521 AM activity of thymol against various microorganisms using the agar well diffusion method
522 and showed that thymol inhibited completely the growth of *B. cereus* and *P. aeruginosa*.
523 Tepe and others (2004), reported the positive AM activity of carvacrol and thymol against *B.*
524 *catarrhalis, C. perfringens, B. cereus, Staph. aureus, S. pneumoniae, M. smegmatis* and *P.*
525 *aeruginosa in vitro*. Sivropoulou and others (1996) reported significant AM activity of
526 carvacrol and thymol against *Staph. aureus*. Dorman and Deans (2000) reported effective
527 AM activity of thymol and carvacrol against selected microorganisms including *B. cereus,*
528 *Staph. aureus, L. monocytogenes, E. coli, A. niger* and *S. cerevisiae* using the agar well
529 diffusion method. Olasupo and others (2003b) reported that carvacrol and thymol
530 demonstrated the highest AM activity against *E. coli* and *Sal. typhimurium* using liquid
531 culture compared to other agents including eugenol, nisin, cinnamic acid and diacetyl
532 compounds. Rupika and others (2005) found that carvacrol and/or thymol impregnated into
533 LDPE films had a significant inhibitory activity against *E. coli, Staph. aureus, L. innocua, P.*
534 *aeruginosa, A. niger* and *S. cerevisiae* using the agar disc diffusion assay. Han and others
535 (2005) investigated the effectiveness of carvacrol and thymol coated onto LDPE film against
536 *L. innocua* and *E. coli* in solid and liquid media and observed an inhibitory effect using the
537 agar diffusion method. In the liquid culture test, carvacrol and thymol incorporated into the
538 film reduced significantly the specific growth rate and the final cell concentration of *L.*
539 *innocua*.

Falcone and others (2005) reported that thymol inhibited significantly the growth of *S. cerevisiae* and *B. cereus* in liquid media. They reported that the growth kinetics of *B. cereus* in liquid media is a function of thymol concentration. Ultee and others (1998) investigated the AM activity of carvacrol against *B. cereus* using a liquid culture media and reported that the activity depends on the concentration, exposure time, temperature and pH. Periago and others (2004) studied the AM activity of carvacrol and cymene against the growth of two strains of *L. monocytogenes* and found that carvacrol and cymene reduced microbial growth during the lag and exponential phases. They found that the combination of carvacrol and cymene resulted in a larger decrease in viable counts of *L. monocytogenes* compared with the separate application of these agents. Burt and others (2005) conducted a comparative study of the AM activity of oregano and thyme EO components (carvacrol, thymol, *p*-cymene and γ-terpinene) against the growth of *E. coli* O157:H7 and also found synergistic effects of these components using the checkerboard assay. They reported that carvacrol and thymol demonstrated individual and additive antibacterial activity against *E. coli* O157:H7, but no observable AM activity by *p*-cymene and γ-terpinene was found. Although the vast majority of studies involving essential oils or their extracts suggest a positive and broad spectrum of AM activity, an important aspect that needs more attention is how to minimise the loss of these volatile agents during processing, particularly at high temperatures. Gutiérrez and others (2009) reported AM activity of carvacrol, thymol and cinnamadehyde incorporated into PP against various Gram-negative (*E. coli, Yersinia enterocolitica, P. aeruginosa* and *Sal. choleraesuis*), Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and *Enterococcus faecalis*), yeasts (*Candida Albicans, Debaryomyces hansenii, Zygosaccharomyces rouxii)* and moulds (*Botrytis cinerae, A. flavus, Eurotium, repens, penicllium roqueforti, P. islandicum, P. commune, P. nalgiovensis*). Recently, Persico and

565 others (2009) claimed that carvacrol incorporated into a LDPE film demonstrated an AM
566 activity against *B. thermosphacta*, *L. innocua* and *Carnobacterium. sp* on agar medium.
567
568
569 **2.2.5 Other Natural Antimicrobial Agents**
570 Numerous studies have evaluated the inhibitory effects of other natural AM agents including
571 bacteriocins, plant extracts such as grapefruit seed extract (GFSE), enzymes and spices (see
572 Table 2). Bacteriocins such as nisin are ribosomally synthesised peptides produced by lactic
573 acid bacteria and possess bactericidal properties against a range of microorganisms (Siragusa
574 and others 1999). They were widely studied for their AM activity in packaging films.
575 Grower and others (2004) developed an AM film by coating nisin onto an LDPE film and
576 reported that these coatings were effective against *L. monocytogenes* on solid microbiological
577 media and on the surface of individually packed hotdogs. Natrajan and Sheldon (2000)
578 reported significant AM activity of nisin coated on three different packaging films: polyvinyl
579 chloride (PVC), linear low-density polyethylene (LLDPE) and nylon against *Sal.*
580 *typhimurium* on broiler drumstick skin stored at 4°C. The AM activity of the nisin film was
581 found to be at higher nisin concentrations and when the film was in direct contact with the
582 tested products for a longer period. Kim and others (2002) coated nisin onto LDPE film in
583 order to control naturally-occurring bacteria on packaged fresh oysters and ground beef
584 stored at 3 and 10°C. They claimed that nisin coated onto the film reduced microbial growth
585 at both temperatures in contrast to a non-coated LDPE film. The inhibitory effects of AM-
586 coated films on the growth of coliform bacteria were more evident at 10°C than at 3°C, while
587 the effect on the total aerobic bacteria count was consistently apparent at both temperatures.
588 Siragusa and others (1999) evaluated the AM effectiveness of nisin incorporated into LDPE
589 films against the growth of *B. thermosphacta* inoculated on the surface of a beef carcass.

590 They reported that the films reduced significantly the population of *B. thermosphacta* at the
591 end of a storage period at 4 and 12°C. Scannell and others (2000) investigated the AM
592 activity of nisin immobilised onto polyethylene (PE) and/or nylon films and found that the
593 films reduced the levels of *L. innocua* and *Staph. aureus* in sliced cheese and ham. Mauriello
594 and others (2004) investigated the anti-listerial effect of bacteriocin produced by
595 *Lactobacillus curvatus* 32Y incorporated PE and oriented nylon films. The films were coated
596 with the bacteriocin using three different methods: soaking, spraying and coating and all the
597 films inhibited the growth of *L. monocytogenes* on both solid media and pork steaks.
598 Mauriello and others (2005) coated nisin onto LDPE films in order to control the growth of
599 *Micrococcus luteus* in tryptone soya broth and in raw, pasteurised and UHT milk. The nisin
600 coated onto LDPE films was shown to have an inhibitory effect against the growth of the
601 bacteria in the broth and also reduced microbial counts in the milk products. Cooksey (2001)
602 coated nisin onto LDPE films and evaluated their inhibitory effect against *L. monocytogenes*
603 on packaged hotdogs and reported that coatings containing 2500 IU $mL^{-1}$ or greater of nisin
604 applied to the films effectively inhibited microbial growth on the hotdogs stored under
605 refrigeration for 60 days. Cutter and others (2001) investigated the AM activity of nisin
606 incorporated into PE food packaging films and reported a significant AM effect of the films
607 against *B. thermosphacta*.
608
609 The AM activity of plant extracts was investigated by several researchers and invariably
610 demonstrated an inhibitory effect against various microorganisms. For example, Hong and
611 others (2000) investigated the AM effectiveness of 5% (w/w) propolis extract or clove extract
612 incorporated into LDPE films against *E. coli*, *L. plantarum*, *S. cerevisiae* and *Fusarium*
613 *oxysporum*. All extracts demonstrated an inhibitory activity on the growth of *L. plantarum*
614 and *F. oxysporum*. Ha and others (2001) investigated the AM activity of GFSE incorporated

615 into multi-layered PE films against *M. flavus, E. coli, Staph. aureus* and *B. subtilis* on ground
616 beef. The coated films demonstrated an AM activity against all the microorganisms studied.
617 Lee and others (1998) developed an LDPE packaging film incorporated with GFSE and
618 reported that the film containing narigin, ascorbic acid, hesperidin and various organic acids
619 was shown to possess a wide spectrum of AM activity. However, although the LDPE films
620 containing GFSE had an inhibitory effect on the growth of *E. coli* and *Staph. aureus* on solid
621 media, they were unable to inhibit the growth of *Leuconostoc mesenteroides, S. cerevisiae,*
622 *Aspergillus oryzaei*, *A. niger* and *Penicillium chrysogenum.* Rodriguez and others (2008)
623 investigated the AM activity of an active packaging film incorporated with cinnamon EOs
624 against *Rhizopus stolonifer* both *in vitro* and on sliced bread. They reported that cinnamon
625 EOs inhibited the growth of *R. stolonifer* on solid media and on sliced bread.
626
627 The AM films incorporated with EOs and/or their principal constituents have the potential for
628 packaging of many food products such as bakery (Suhr and Nielsen 2003; Rodríguez and
629 others 2008; Rokchoy and others 2009; Mehyar and others 2011), dairy (Suppakul and others
630 2008; Kuorwel and others 2011a), meat, chicken and fish (Suppakul and others 2003a; Kerry
631 and others 2006; Wu and others 2010), and fresh produce (Rodríguez-Lafuente and others
632 2010). There are already some commercial applications for AM packaging systems such as
633 wasabi extract (or Japanese horseradish) used for Japanese rice lunch boxes (Koichiro 1993),
634 Piatech manufactured by Rhone-Poulene (USA) and Daikoku Kasei Co (Japan) (Brody and
635 others 2001).
636

637 ## 3 Conclusions

638 In recent years, an increasing interest has emerged in the development of various forms of
639 active packaging systems intended to protect food products from microbial contamination.

Many synthetic and natural AM agents incorporated into or coated onto synthetic polymer-based packaging materials have demonstrated significant AM activity against various microorganisms. Although incorporating synthetic AM agents directly into foods can effectively inhibit the growth and survival of various microorganisms, consumers today demand minimally processed, preservative-free food products with a longer shelf life. Thus, natural AM agents such as basil, thyme and oregano EOs with their main components linalool, thymol and carvacrol respectively are well suited to be utilised as preservatives in foods and as potential alternatives for synthetic food additives. Packaging materials containing AM agents demonstrate a potential for applications in AM packaging systems that could reduce the risk of food-borne illness associated with microbial contamination in food products. Although the commercial applications of these systems is not yet widespread, it is anticipated that AM packages containing natural AM agents would be one of the new developments in food packaging (cheeses, processed meats, fish, bakery, fruits and vegetables and more) in the near future.

654 **Table 1: Antimicrobial activity of common synthetic AM agents**

655

| AM Agent | Amount added | Packaging Material | Test Type/Media | Target Microorganism(s) | Findings | References |
|---|---|---|---|---|---|---|
| Benzoic acid | 0.5 mol/L | PEMA | PDA | *A. niger* and *Penicillium* sp. | Inhibited microbial growth | Weng and others (1999) |
| Benzoic acid | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Failed to inhibit mould growth | Weng and Hotchkiss (1993) |
| Benzoic anhydride | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Demonstrated antimycotic activity on media and cheese | Weng and Hotchkiss (1993) |
| EDTA | 5% w/w | LDPE | Agar diffusion | *B. subtilis*, *A. niger* and *E. coli* | Inhibited *B. subtilise* and *A. Niger* but not *E. coli* | Vartiainen and others (2003a) |
| Imazalil | 1000-2000 mg/kg | LDPE | Agar media; Cheddar | *A. toxacarius*, *Penicillium* sp. | All concentrations delayed microbial growth on media | Weng and Hotchkiss (1992) |

| AM Agent | Amount added | Packaging Material | Test Type/Media | Target Microorganism(s) | Findings | References |
|---|---|---|---|---|---|---|
| | | | cheese | | and cheese | |
| Imazalil | 0.05-0.25% w/w | LDPE | Agar diffusion | *B. subtilis*, *A. niger* and *E. coli* | Inhibited *B. subtilise* and *A. Niger* but not *E. coli* | Vartiainen and others (2003a) |
| Potassium sorbate | 2-3% w/v | PVC | Agar media | *L. monocytogenes* | Films inhibited microbial growth | Limjaroen and others (2003) |
| Propionic acid | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Failed to inhibit mould growth | Weng and Hotchkiss (1993) |
| Propionic anhydride | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Failed to inhibit mould | Weng and Hotchkiss (1993) |
| Sodium propionate | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Failed to inhibit mould | Weng and Hotchkiss (1993) |

| **AM Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Sodium diacetate | 0.5-3% w/v | PVC | Agar media | *L. monocytogenes* | No AM activity observed | Limjaroen and others (2003) |
| Sorbic acid | 1.5-3% w/v | PVC | Agar media | *L. monocytogenes* | Inhibited microbial growth | Limjaroen and others (2003) |
| Sorbic acid | 0.5-2% w/w | LDPE | Agar media; Cheddar cheese | *R. stolonifer*, *Penicillium* sp. and *A. toxacarius* | Failed to inhibit mould | Weng and Hotchkiss (1993) |
| Sorbic acid | 0.5 mol $L^{-1}$ | PEMA | PDA | *A. niger* and *Penicillium* sp. | Inhibited microbial growth | Weng and others (1999) |
| Triclosan | 500-1000 mg $kg^{-1}$ | LDPE | Agar diffusion; Chicken breasts | *L. monocytogenes, Sal. enteritidis, Staph. aureus, E. coli* O157:H7, *B. thermosphacta, B. cereus, L. sake, L. brevis, P.* | Inhibited *L. monocytogenes, Sal. enteritidis, Staph. aureus, E. coli* O157:H7 with slight inhibition of *B.* | Vermeiren and others (2002) |

| AM Agent | Amount added | Packaging Material | Test Type/Media | Target Microorganism(s) | Findings | References |
|---|---|---|---|---|---|---|
| | | | | *Roqueforti, A. niger* and *C. albicans* | *thermosphacta,* but no activity against, *B. cereus, L. sake, L. brevis, P. Roqueforti, A. niger* and *C. Albicans* | |
| Triclosan | 5% w/w | PVC | Plate count | *Staph. aureus, E. coli* | *Staph. aureus, E. coli* | Ji and Zhang (2009) |

656

657 **Table 2: Antimicrobial activity of natural AM agents**

658

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Carvacrol | 1.33-2.65% w/w | LDPE | Liquid culture | *E. coli* | Reduced microbial growth | Rupika and others (2008) |
| Carvacrol | 1-4% w/w | PP | Agar medium | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p. roqueforti, P. commune* | Carvacrol demonstrated AM activity against all the tested microorganisms | Gutiérrez and others (2009) |
| Carvacrol | 1-4% w/w 1-21.8µL/L | PP, PE/EVOH | Agar medium or vapour diffusion | *L. monocytogenes, Sal. choleraesuis, A. flavus* and *C. albicans* | carvacrol inhibited the growth of all the tested microorganisms | Lopez and others (2007b; 2007a) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | method | | | |
| Carvacrol | 0.2-2% w/w | LDPE | Agar media | *E. coli, Staph. aureus, L. innocua, P. aeruginosa, A. niger* and *S. cerevisiae* | Inhibited *E. coli, Staph. aureus, A. niger* and *S. cerevisiae* but not *L. innocua* or *P. aeruginosa* | Rupika and others (2005) |
| Carvacrol | 10% w/w | LDPE | Agar media | *B. thermosphacta, L. innocua* and *Carnobacterium. sp* | Carvacrol demonstrates *B. thermosphacta, L. innocua* and *Carnobacterium. sp* | Persico and others (2009) |
| Carvacrol | 4% w/w | LDPE and nylon film | liquid food media; Cheddar cheese | *E. coli* | Multilayer films inhibited microbial growth | Rardniyom and others (2008) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Cinnamaldehyde | 1-4% w/w | PP | Agar medium | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p. roqueforti, P. commune* | cinnamaldehyde demonstrated AM activity against all the tested microorganisms | Gutiérrez and others (2009) |
| Cinnamaldehyde | 1-4% w/w 0.4-21.8µL/L | PP, PE/EVOH | Agar medium or vapour diffusion method | *L. monocytogenes, Sal. choleraesuis, A. flavus* and *C. albicans* | Cinnamaldehyde inhibited the growth of all the tested microorganisms | Lopez and others (2007b; 2007a) |
| Clove extract | 20% w/w | LDPE | Liquid culture | *E. coli*, *L. plantarum*, *S. cerevisiae* and *F. oxysporum* | Effective against *L. plantarum* and *F. oxysporum* but not against | Hong and others (2000) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | | | *E. coli* and *S. cerevisiae* | |
| GFSE | 0.1 or 1% w/w | LDPE | Agar media; Curled lettuce; Soybean sprouts | *E. coli*, *Staph. aureus, L. mesenteroides, S. cerevisiae, A. oryzaei, A. niger, P. chrysogenum* | Inhibited *E. coli* and *Staph. aureus* but not *S. cerevisiae, A. oryzaei, A. niger,* or *P. chrysogenum*. | Lee and others (1998) |
| GFSE | 0.5% or 1% w/v | Multi-layered PE (coated) | Ground beef, Agar media | *M. flavus*, *P. aeruginosa, E. coli, Staph. aureus* and *B. subtilise, S. cerevisiae, A. Niger, P. chysogenum, L. mesenteroides* | AM activity against *M. flavus*, *E. coli, Staph. aureus* and *B. subtilise* | Ha and others (2001) |
| Lactoferrin | 0.5-2.5% w/v | PVC | Agar media | *L. monocytogenes* | No AM activity | Limjaroen and others (2003) |
| Lacticin NK24 | 20 $gL^{-1}$ | LDPE | Fresh oysters; | Coliform, total aerobic | Inhibited microbial growth | Kim and others (2002) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | ground beef | bacteria | | |
| Linalool | 0.037% w/w | LDPE | Agar media | *E. coli* | Linalool incorporated into LDPE film inhibit the growth of *E. Coli* after 1 year of storage | Suppakul and others (2011b) |
| Linalool | 0.338% w/w | LDPE | Agar media; Cheddar cheese | *E. coli, L. innocua, S. cerevisiae* | Inhibitory activity against *E. Coli* but not *L. innocua* or *S. cerevisiae* on agar media; reduced *E. coli* and *L. innocua* on cheese | Suppakul (2004; 2006; 2008) |
| Linalool | 4% w/w | LDPE and nylon film | Liquid culture; Cheddar cheese | *E. coli* | Multilayer films inhibited microbial growth | Rardniyom and others (2008) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Linalool | 0.54-1.19% w/w | LDPE | Agar and liquid media; Cheddar cheese | *E. coli, L. innocua* | Inhibited microbial growth | Rupika and others (2006) |
| | | | | | | |
| Methylchavicol | 0.028% w/w | LDPE | Agar media | *E. coli* | Methylchavicol inhibitory activity against the growth of *E. Coli* after 1 year of storage | Suppakul and others (2011b) |
| Methylchavicol | 0.345% w/w | LDPE | Agar media; Cheddar cheese | *E. coli, L. innocua, S. cerevisiae* | Inhibitory activity against *E. coli* but not against *L. innocua* or *S. cerevisiae* on agar media | Suppakul (2004; 2006; 2008) |
| Nisin | 0.05 or | LDPE | Beef carcass | *B. thermosphacta* | Inhibited microbial growth | Siragusa and others |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | 0.1% w/v | | | | | (1999) |
| Nisin | 2-2.5% w/v | PVC | Agar media | *L. monocytogenes* | Inhibited microbial growth | Limjaroen and others (2003) |
| Nisin | 157 mg/mL | LDPE | Agar media | *L. monocytogenes* | Inhibited microbial growth | Grower and others (2004) |
| Nisin | 100 µg/mL | LLDPE, PVC, nylon | Broiler skin | *Sal. typhimurium* | Significantly reduced microbial population | Natrajan and Sheldon (2000) |
| Nisin | 20 g/L | LDPE | Fresh oysters; ground beef | coliform, total aerobic bacteria | Suppressed coliform and bacterial growth | Kim and others (2002) |
| Nisin | 0.03 or 0.6 g/mL | PE or polyamide | Sliced cheese; ham | *L. innocua* and *Staph. aureus* | Reduced microbial growth in cheese | Scannell and others (2000) |
| Propolis | 20% w/w | LDPE | Liquid culture | *E. coli*, *L. plantarum*, *S. cerevisiae* and *F. oxysporum* | Inhibited *L. plantarum* and *F. oxysporum* but not *E. coli* or *S. cerevisiae* | Hong and others (2000) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Thymol | 0.85-3.15% w/w | LDPE | Liquid culture | *E. coli* | Inhibited microbial growth | Rupika and others (2008) |
| Thymol | 1-4% w/w | PP | Agar medium | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p. roqueforti, P. commune* | Thymol demonstrated AM activity against all the tested microorganisms | (Gutiérrez and others 2009) |
| Thymol | 0.23-1.6% w/w | LDPE | Agar media | *E. coli, Staph. aureus, L. innocua, P. aeruginosa, A. niger* and *S. cerevisiae* | Inhibited *E. coli, Staph. aureus, A. niger* and *S. cerevisiae* but not *L. innocua,* and *P. aeruginosa* | Rupika and others (2005) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Thymol | 1-4% w/w 1-21.8µL/L | PP, PE/EVOH | Agar medium or vapour diffusion method | *L. monocytogenes, Sal. choleraesuis, A. flavus* and *C. albicans* | Thymol inhibited the growth of all the tested microorganisms | Lopez and others (2007b; 2007a) |
| Basil EOs | | | | *E. coli, Staph. aureus, seven strains of Candida* | Inhibited microbial growth | Mazzanti and others (1998) |
| Basil EOs | | | Solid media | 25 strains of bacteria | Inhibited microbial growth | Dorman and Deans (2000) |
| Basil EOs | | | Solid media | *Staph. aureus* | Inhibited microbial growth | Baratta and others (1998) |
| Basil EOs | | | Solid media Food | *Bacillus* sp., *Staph. aureus* sp., *micrococcus* sp., *Sarcina* sp., *Lactobacillus* sp., *E. coli, Salmonella,* | Inhibitory effects against Gram-positives (*Bacillus* sp., *Staph. aureus* sp., *micrococcus* sp., *Sarcina* | Lachowicz and others (1998) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | | sp., *Enterobacter* sp., *Pseudomonas* sp. | sp. and *Lactobacillus* sp. Reduced effects against Gram-negatives (*E. coli, Salmonella* sp., *Enterobacter* sp. and *Pseudomonas* sp. | |
| Basil EOs | | | | *Fusarium acuminatum, F. solani, F. pallidoroseum* and *F. chlamydosporum* | EOs effective against all *Fusarium* species | Rai and others (1999) |
| Cinnamon EOs | 1-4% w/w 13.1-131µL/L | PP, PE/EVOH | Agar medium or vapour diffusion method | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A.* | Cinnamon EOs in PP or PE/EVOH demonstrated AM activity against all the tested microorganisms | Lopez and others (2007b; 2007a) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | | *flavus, E. repens, p. roqueforti, P. commune* | | |
| Cinnamon EOs | 3-6% (w/w) | Paraffin-based paper | *In vitro,* Sliced bread | *A. alternata* | Cinnamon EOs inhibited the growth of *A. alternata* on solid media | Rodriquez-Lafuente and others (2010) |
| Cinnamon EOs | 1-6% w/w | Paraffin-paper | *In vitro,* Sliced bread | *R. stolonifer* | Cinnamon EOs in paraffin film inhibited the growth of *R. stolonifer* | Rodríguez and others (2008) |
| Clove | 1-4% w/w | PP, PE/EVOH | Agar medium | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p.* | Clove EOs demonstrated AM activity against all the tested microorganisms | Lopez and others (2007b; 2007a) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | | | | *roqueforti, P. commune* | | |
| Oregano EOs | 0.8% (v/w) | surface dipping, $O_2$ permeable films | beef meat fillets | *L. monocytogenes,* autochthonous flora | Reduced growth by 2-3 $log_{10}$ | Tsigarida and others (2000) |
| Oregano EOs | 3-6% (w/w) | Paraffin-based paper | *In vitro,* Cherry tomato | *A. alternata* | Oregano EOs inhibited the growth of *A. alternata* on solid media | Rodriquez-Lafuente and others (2010) |
| Oregano EOs | | dressing, MAP | fresh fish fillets | *Staph. aureus, Sal. enteritidis,* Residential flora | Bacterostatic and bactericidal effects | Tassou and others (1996) |
| Oregano EOs | 800 ppm | surface spreading | thin-sliced beef | *L. monocytogenes* | Significant inhibition | Seaberg and others (2003) |
| Oregano EOs | 0.05-1% | PE bags | minced beef | spoilage microbiota | Reduction in microbial | Skandamis and |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | (v/w) | | | | loads | Nychas (2001) |
| Oregano EOs | | surface application | raw fish fillets | *Photobacterium phosphoreum* | No significant growth reduction | Mejlholm and Dalgaard (2002) |
| Oregano EOs | 1-4% w/w 13.1-175µL/L | PP, PE/EVOH | Agar medium or Vapour diffusion method | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p. roqueforti, P. commune* | Oregano EOs in PP or PE/EVOH demonstrated AM activity against all the tested microorganisms | Lopez and others (2007b; 2007a) |
| Oregano EOs | 50 µL | Suspensions of oils in apple juice | Apple juices | *E. coli, Sal. enterica* | Selected oils were bactericidal | Friedman and others (2004) |
| Oregano EOs | 0.1-10% | dissolved in | Liquid | *Sal. enterica* | OEO showed strongest | Marques and others |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| | (v/v) | brain heart infusion broth | culture | | AM activity | (2008) |
| Oregano EOs | | | *In vitro* | *B. cereus, E. coli L. monocytogenes* | Inhibited microbial growth | Baydar and others (2004) |
| Thyme EOs | 50 μL | vapour contact | sponge cake analogues | *Eurotium* sp., *Aspergillus* sp., *Pencillium* sp. | Significant reduction in microbial growth | Guynot and others (2003) |
| Thyme EOs | 135 or 270 μL/L | vapour contact | rye bread | *Pencillium* sp., *E. repens, A. flavus* | Significant reduction in microbial growth | Suhr and Nielsen (2003) |
| Thyme EOs | 0.1-1% (v/v) | cheese-EO-mixture | soft cheese | *L. monocytogenes* *Sal. enteritidis* | Significant inhibition in low-fat cheese; no inhibition in full-fat cheese | Smith-Palmer and others (2001) |
| Thyme EOs | 1:5 dilution | surface application | cooked poultry | *A. hydrophila, L. monocytogenes* | Inhibited growth of *A. Hydrophila* | Hao and others (1998) |

| **Antimicrobial Agent** | **Amount added** | **Packaging Material** | **Test Type/Media** | **Target Microorganism(s)** | **Findings** | **References** |
|---|---|---|---|---|---|---|
| Thyme EOs | 1-4% w/w 26.2-175µL/L | PP, PE/EVOH | Agar medium, Vapour diffusion method | *E. coli, Y. enterocolitica, P. aeruginosa, Staph. aureus, B. cereus* and *E. faecalis, C. albicans, D. hansenii, Z. Rouxii, A. flavus, E. repens, p. roqueforti, P. commune* | thyme EOs demonstrated AM activity against all the tested microorganisms | Lopez and others (2007b; 2007a) |
| Thyme EOs | | surface application | raw fish fillets | *Photobacterium phosphoreum* | No significant growth reduction | Mejlholm and Dalgaard (2002) |

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# List of Abbreviations and Nomenclature

| | |
|---|---|
| AM | Antimicrobial |
| AP | Active Packaging |
| CFU | Colony Forming Units |
| EOs | Essential Oils |
| EVOH | Ethylene Vinyl Alcohol |
| GRAS | Generally Recognized As Safe |
| GFSE | Grapefruit Seed Extract |
| HDPE | High-Density Polyethylene |
| LB | Lactic-acid Bacteria |
| LDPE | Low-Density Polyethylene |
| LLDPE | Linear Low-Density Polyethylene |
| MAP | Modified Atmosphere Packaging |
| OEO | Oregano Essential Oil |
| PDA | Potato Dextrose Agar |
| PE | Polyethylene |
| PEG | Polyethylene Glycol |
| PEMA | Poly(Ethylene-*co*-Methacrylic Acid) |
| PET | Polyethylene Terephthalate |
| PP | Polypropylene |
| PS | Polystyrene |
| PVC | Polyvinyl Chloride |
| PVDC | Poly(Vinylidene Chloride) or Poly(Vinyl Dichloride) |
| TEO | Thyme Essential Oil |

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