1   **Essential Oils and Their Principal Constituents as Antimicrobial Agents for Synthetic**

2   **Packaging Films**

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4   Kuorwel K. Kuorwel[1], Marlene J. Cran[2], Kees Sonneveld[3],

5   Joseph Miltz[4] and Stephen W. Bigger[1*]

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7   1. School of Engineering and Science, 2. Institute for Sustainability and Innovation

8   Victoria University, PO Box 14428, Melbourne, 8001, Australia.

9   3. KS PackExpert & Associates, PO Box 399, Mansfield, 3724, Australia

10   4. Department of Biotechnology and Food Engineering, Technion-Israel Institute of

11   Technology, Haifa, 3200, Israel

12

13

14   ***Corresponding Author**

15   Prof. Stephen W. Bigger

16   School of Engineering and Science, Victoria University,

17   PO Box 14428, Melbourne, 8001, Australia

18   Tel: +61 3 9919 2959

19   Fax: +61 3 9919 2005

20   E-mail: stephen.bigger@vu.edu.au

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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.

2

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).

92   Although AM agents such as essential oils (EOs) and/or their principal components may

93   exhibit AM activity against various microorganisms when incorporated into packaging

94   materials, the organoleptic properties of the packaged food products are one of the important

95   factors that must also be taken into consideration. According to Davison and Zivanovic

96   (2003), the concentration of AM agents required to demonstrate AM activity against various

97   microorganisms on food products might be higher than the concentration applied for

98   flavouring purposes. As a result, this might cause food tainting and/or adverse sensorial

99   effects to food products (Smith-Palmer and others 2001; Bagamboula and others 2004). The

100   adverse sensorial effects of AM agents to food products can be overcome by masking the

101   odor of AM agents with other approved aroma compound as suggested by Gutiérrez and

102   others (2009). An understanding of the relationship between minimum inhibitory

103   concentration and acceptable organoleptic properties of AM Agents such as EOs and/or their

104   constituents is also important (Lambert and others 2001). In some cases, the replacement of

105   EOs with one or a number of their principal constituents may provide equal AM effectiveness

106   but with milder flavouring attributes (Lambert and others 2001; Smith-Palmer and others

107   2001).

108

109   In a recent review, the current authors presented an evaluation of the AM activity of

110   biodegradable polysaccharide and protein based films containing natural agents (Kuorwel and

111   others 2011b). These films showed the potential for wide-range of applications in food

112   packaging where undesirable microbial growth is a concern. Moreover, these films degrade

113   readily in the environment but the acquisition of this attribute may harm the processability

114   and mechanical stability of the film. Thus, in spite of the increasing concern in recent years

115   about the use of synthetic polymers due to their poor biodegradability, these materials have

116   several advantages including low cost, good processability and sound mechanical and

117 physical properties. Therefore, development of AM packaging materials manufactured from

118 synthetic polymers such as low-density polyethylene (LDPE), high-density polyethylene

119 (HDPE), polystyrene (PS), polyethylene terephthalate (PET) and polypropylene (PP) is still

120 important in offering commercial benefits for packaging food products. In the current review,

121 a detailed summary of synthetic films utilising common synthetic and natural AM agents is

122 presented with an emphasis on the principal components of basil, oregano, and thyme

123 essential oils (EOs) namely, linalool, carvacrol and thymol respectively. This is followed by a

124 list of other natural AM agents that have the potential for controlling microbial growth on

125 foods.

126

127 **2   Antimicrobial Packaging Systems**

128 Studies have shown that AM packaging systems can increase the shelf life of packaged foods

129 by extending the lag phase and reducing the growth rate of spoilage microorganisms (Han

130 2000; Cooksey 2001; Appendini and Hotchkiss 2002; Rydlo and others 2006; Coma 2007; de

131 Oliveira and others 2007; Gutierrez and others 2008; Rardniyom and others 2008; Rupika

132 and others 2008). In the past, preservatives were directly added into food products to protect

133 them from microbial contamination. This process of direct addition of preservatives into

134 foods may result in levels of additives in excess to those required for an efficient AM effect.

135 New AM packaging systems have attracted much attention in the food industry with the aim

136 of replacing the conventional food preservation systems (Weng and Hotchkiss 1993; An and

137 others 1998; Quintavalla and Vicini 2002; Bagamboula and others 2004; Devlieghere and

138 others 2004b; Miltz and others 2006).

139

140 An AM packaging system can be produced by: directly incorporating AM agents into

141 packaging films; coating of the packaging films with AM agents; developing packaging

142 materials from polymers that have inherent AM properties (Vermeiren and others 2002;

143 Suppakul and others 2003b). Typically, AM packaging systems can be regarded as migrating

144 or non-migrating with the distinction depending on the specific AM agent used and on its

145 interactions with the packaging and food matrix (Cooksey 2000). In a migrating system, the

146 AM agent is released from the packaging film into the package headspace and onto the food

147 surface and such systems are most useful when direct contact between the packaging film and

148 food product is not required for efficient AM activity (Weng and Hotchkiss 1993; Cooksey

149 2000). The non-migrating systems involve packaging materials in which the AM agent is

150 immobilised within the material (Brody and others 2001) and these systems can be applied

151 where direct contact between the food and the material can be achieved or is required for

152 effective AM activity (Vermeiren and others 2002; Suppakul and others 2003b). In either AM

153 packaging system, both synthetic and natural AM agents can be incorporated into or coated

154 onto the packaging material. The mode of action of AM agents incorporated in a packaging

155 material is influenced by the controlled and slow release of the agent onto the food surfaces.

156 This is required in order to maintain an adequate concentration of the agent on the food and

157 effectively inhibit microbial growth throughout the product shelf life (Cooksey 2005; Salleh

158 and others 2007; Cran and others 2010; Tunç and Duman 2011). Han (2005) suggested that

159 the mass transfer rate of an AM agent should not be faster than the growth rate of the target

160 microorganism, otherwise the AM agent might be diluted on the surface of the packaged food

161 product, thus limiting the AM activity.

162

163 During extrusion or compression moulding of AM films, the temperature and mechanical

164 energy input, such as shearing forces, must be carefully considered (Han 2003). High-

165 processing temperatures, for example, may result in considerable losses of volatile AM

166 agents (Han and Floros 1997; Han 2000; Rupika and others 2005; Suppakul and others

167 2011b). Moreover, Cooksey (2005) suggested that an AM agent might partly or completely

168 lose its AM activity if incorporated into a film under harsh processing conditions. Therefore,

169 to minimise the loss of AM agent during processing, temperatures that are as low as possible

170 should be applied (Han and Floros 1998; Suppakul and others 2011b). The storage

171 temperature may also influence the activity of AM agents that are incorporated into

172 packaging films (Vojdani and Torres 1989; Han 2005). The concentration of AM agents

173 retained in the film may decrease during long-term storage. However, the amount of AM

174 agent retained in the film after a long storage period may be sufficient to demonstrate AM

175 activity as shown by Suppakul and others (2011b). Du and others (2008) reported AM

176 activity against *E. coli* (using an agar disc diffusion method) of carvacrol incorporated into

177 films for edible apples that were stored for 7 weeks.

178

179 **2.1   Synthetic AM Agents**

180 In the past few decades, various synthetic AM agents have been investigated and developed

181 into food packaging materials (Weng and Hotchkiss 1992; Weng and Hotchkiss 1993). Many

182 of these agents including various organic acids and salts have been approved by regulatory

183 agencies and have since been used for the preservation of food products (Davidson and

184 Taylor 2007). Synthetic AM agents that have demonstrated inhibitory activity against

185 different microorganisms include sodium benzoates and propionates, potassium sorbates,

186 sulfites, chlorides, nitrites, triclosan, fungicides (e.g. benomyl, imazalil) and various metal

187 ions including silver zeolites, quaternary ammonium salts and copper ions (Chen and others

188 1996; Devlieghere and others 2000a; Han 2000; Ouattara and others 2000; Hoffman and

189 others 2001; Cooksey 2005). Other AM agents such as acetic acid from vinegar and benzoic

190 acid from cranberries are found in nature, but are classified as synthetic AM agents when

191 produced synthetically (Davidson and Taylor 2007).

192 Many synthetic AM compounds have been evaluated in synthetic polymeric materials by

193 various researchers. Table 1 summarises these synthetic AM agents incorporated into or

194 coated onto packaging materials as potential candidates for food packaging. Although Table

195 1 contains a large amount of information on the activity of AM agents successfully

196 incorporated into various synthetic polymers, comparison between the different AM agents

197 and/or AM films is difficult due to variations in strains of microorganisms and different

198 experimental conditions or equipment used by the various researchers. In order to compare

199 the results of various experiments involving AM agents, there is a need for a standardisation

200 of the test methods as suggested by Suppakul and others (2003a).

201

202 Numerous studies have concentrated on incorporating common food preservatives such as

203 organic acids, their salts and anhydrides into packaging films (see Table 1). Studies on

204 benzoic or sorbic acid incorporated into packaging materials have evaluated their action

205 against various microorganisms in laboratory media such as agar plates and/or in actual food

206 products. The packaging films incorporated with these organic acids or anhydrides have

207 demonstrated inhibitory effects against various spoilage and pathogenic microorganisms.

208 Weng and others (1999) showed that benzoic acid or sorbic acid incorporated into

209 poly(ethylene-co-methacrylic acid) (PEMA) film inhibited the growth of *A. niger* and

210 *Penicillium* sp. on solid media. Weng and Chen (1997) investigated the AM activity of

211 benzoic acid or benzoyl chloride incorporated into ionomer films. The AM activity of these

212 films was demonstrated by their ability to inhibit the growth of *Penicillium* sp. and *A. niger*.

213 In an earlier study, Weng and Hotchkiss (1993) incorporated benzoic acid or benzoic

214 anhydride into LDPE films which significantly suppressed the growth of *Rhizopus stolonifer*,

215 *Penicillium* sp. and *A. toxacarius* on potato dextrose agar and on the surface of Cheddar

216 cheese. Matche and others (2006) examined the AM activity of benzoyl chloride incorporated

217 into modified ethylene acrylic acid films against *Penicillum* sp. and *A. niger* sp. on solid

218 media for 15 days with the film demonstrating inhibition against both species. Silveira and

219 others (2007) incorporated sorbic acid into LDPE films with the aim of preserving fresh

220 pastry dough. It was found that 3% (w/w) sorbic acid incorporated into a 70 μm film reduced

221 2 and 1.5 log cycles of mesophilic and psychrotrophic bacteria respectively on the pastry

222 dough after 40 days of storage at 8°C compared to the control film. Limjaroen and others

223 (2005) coated sorbic acid onto polyvinylidene chloride copolymer films to control the growth

224 of *L. monocytogenes* on beef bologna and Cheddar cheese. It was found that sorbic acid

225 coated on the films inhibited microbial growth on cheese by log 0.6 $CFUg^{-1}$ after 28 days of

226 storage at 4°C. They further reported that the population of *L. monocytogenes* on beef

227 bologna was reduced by log 0.6 and 1.4 $CFUg^{-1}$ for films containing 1.5% and 3.0% (w/v)

228 respectively compared to the control film.

229

230 Other researchers have studied the AM activity of salts against several microorganisms as

231 shown in Table 1. Han and Flores (1997) developed LDPE films containing potassium

232 sorbate and found that these films successfully reduced the growth of *S. cerevisiae in vitro*

233 experiments. Vartiainen and others (2003b) demonstrated that potassium sorbate, sodium

234 benzoate and sodium nitrate incorporated into LDPE, poly(maleic acid-co-olefine), PET or

235 PS films inhibited the growth of *B. cereus* on culture media. Limjaroen and others (2003)

236 coated potassium sorbate onto polyvinylidene chloride copolymer films and reported that the

237 films inhibited the growth of *L. monocytogenes* on solid media.

238

239 In addition to the antibacterial activity, the antifungal activity of synthetic AM agents

240 incorporated in polymeric materials has been investigated (Halek and Anita 1989; López-

241 Malo and others 2007). Vartiainen and others (2003a) examined the inhibitory effects of

10

242 imazalil incorporated into LDPE against the growth of *A. niger* by the agar diffusion assay

243 with films containing 0.05-0.25% (w/w) imazalil demonstrating significant inhibitory

244 activity. Weng and Hotchkiss (1992) incorporated imazalil into LDPE film and evaluated the

245 antimycotic activity of this agent on the growth of *A. toxacarius* and *Penicillium* sp. on potato

246 dextrose agar (PDA) and Cheddar cheese. They reported that 2 g kg^{-1} of imazalil suppressed

247 the growth of *A. toxacarius* on PDA whereas a film containing 1 g kg^{-1} of imazalil reduced

248 the growth of *Penicillium* sp. The latter film inhibited the growth of both mould species on

249 the surface of Cheddar cheese. López-Malo and others (2002; 2005) examined the antifungal

250 activity of potassium sorbate, sodium benzoate and sodium bisulfite against the growth of

251 *Aspergillus flavus* inoculated on laboratory media with each of the agents imparting an

252 inhibitory effect. López-Malo and others (2007) investigated the antifungal activity of sodium

253 benzoate and cinnamon extract, separately or in combination, against the growth of *A. flavus*

254 on potato dextrose agar or a checkerboard array respectively. They found that both AM

255 agents demonstrated antifungal activity on *A. flavus*, with cinnamon extract being more

256 effective than sodium benzoate. They claimed that mixtures of cinnamon extract and sodium

257 benzoate showed promising antifungal activity.

258

259 Triclosan and hexamethylenetetramine are common synthetic AM agents that have been

260 evaluated in packaging systems with some commercial developments. Vermeiren and others

261 (2002) investigated the AM activity of triclosan incorporated into LDPE film and reported

262 that concentrations of 0.5 and 1.0% (w/w) demonstrated AM activity against *L.*

263 *monocytogenes, Sal. enteritidis, Staph. aureus, E. coli* O157:H7 and *Brocothrix*

264 *thermosphacta* in an agar diffusion assay. Cutter (1999) reported that triclosan was effective

265 against bacteria on the surface of beef. Recently, Camilloto and others (2010) studied the

266 activity of triclosan incorporated into LDPE against *Staph. aureus*, *E. coli*, *L. innocua* and *P.*

267 *aeruginosa* in the agar disc diffusion test and found that the AM film inhibited the growth of

268 *Staph. aureus* and *E. coli*. Chung and others (2003a) investigated the AM activity of triclosan

269 coated onto styrene-acrylate copolymer against *Enterococcus faecalis* on solid and in liquid

270 media and showed effective inhibition of the bacteria. Ji and Zhang (2009) reported that

271 triclosan incorporated into PVC film inhibited the growth of *Staph. aureus* and *E. coli* using

272 the plate-counting technique. Devlieghere and others (2000b) studied the AM activity of

273 hexamethylenetetramine impregnated into an LDPE film and found it to be effective against

274 spoilage microorganisms on cooked ham.

275

276 **2.2   Natural Antimicrobial Agents**

277 In recent years, natural AM agents have attracted much attention in the food and packaging

278 industries as a replacement for synthetic ones for food preservation. According to Davidson

279 and Zivanovic (Davidson and Zivanovic 2003), natural AM agents are classified by their

280 sources: AM agents derived from plant EOs (e.g. basil, thyme, oregano, cinnamon, clove and

281 rosemary); animal sources (e.g. lysozyme, lactoferrin); microbial sources (nisin, natamycin);

282 and naturally occurring polymers (chitosan). The EOs extracted from plant sources consist of

283 various mixtures including terpenoids, esters, aldehydes, ketones, acids and alcohols

284 (Dorman and Deans 2000). These plant EOs are volatile and generally possess relatively

285 strong odours (Bakkali and others 2008).

286

287 Extracts derived from various herbs and EOs contain a range of natural compounds such as

288 thymol, linalool and carvacrol which have a broad AM spectrum against different pathogenic

289 and spoilage microorganisms including Gram-negative species such as *E. coli, Yersinia*

290 *enterocolitica, P. aeruginosa* and *Sal. choleraesuis* (López and others 2007a; Suppakul and

291 others 2011b), Gram-positive bacteria such as *L. monocytogenes, Staph. aureus, B.* cereus

292 (Friedman and others 2002; López and others 2007b; Gutiérrez and others 2009), yeasts such

293 as *S. cerevisiae, Candida albicans, Debaryomyces hansenii* (Rupika and others 2006;

294 Suppakul and others 2008; Kuorwel and others 2011a) and moulds such as *Alternaria*

295 *alternate, A. niger, Botrytis cinerae, A. flavus, penicllium roqueforti* (López-Malo and others

296 2007; Rodríguez-Lafuente and others 2010). These additives are considered to be safe and

297 have the "Generally Recognised As Safe" (GRAS) status as designated by the American

298 Food and Drug Administration (Zaika 1988; Han 2005; Matan and others 2006).

299 Antimicrobial agents derived from plant sources are produced as secondary metabolites and

300 are associated generally with the volatile EO fractions. The mode of action of AM agents

301 and/or AM activity of plant EOs is related to their chemical structure namely, the presence of

302 hydrophilic functional groups such as the hydroxyl groups of phenolic components and/or

303 lipophilicity of the components in the EOs which depends on their concentration (Farag and

304 others 1989; Davidson and Naidu 2000; Dorman and Deans 2000; Friedman and others 2002;

305 Bagamboula and others 2004). Essential oils and their principal constituents inhibit

306 microorganisms *via* a range of mechanisms such as: disruption of the cyctoplasmic

307 membrane (Knobloch and others 1989; Sikkema and others 1995; Helander and others 1998);

308 leakage of intracellular constituents such as metabolites and ions (Sikkema and others 1995;

309 Lambert and others 2001); coagulation of cell content (Gustafson and others 1998; Pauli

310 2001); inhibition of protein synthesis (Helander and others 1998), enzymes associated with

311 cell wall synthesis (Conner and Beuchat 1984), DNA/RNA synthesis (Ultee and others 1999;

312 Tassou and others 2000), general/metabolite pathways (Ultee and others 2002); and/or the

313 destruction of the osmotic integrity of the cell membrane (Ultee and Smid 2001). The AM

314 activity of different EOs is very difficult to compare given the variation of EOs compositions

315 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

341 Table 2 summarises the AM activity of a range of common natural agents that have been

342 incorporated into or coated onto synthetic packaging films. Table 2 also lists other studies

343 that have evaluated the inhibitory effects of natural AM agents *in vitro* or directly on food

344 products without incorporating them into packaging films.

345

346 **2.2.1   Antimicrobial Activity of Basil Essential Oils**

347 Basil EOs contain primarily linalool and methylchavicol as the active volatile components

348 which are responsible for their AM activity (Simon and others 1990; Fyfe and others 1998;

349 Wan and others 1998; Bezic and others 2003; Suppakul 2004). Many studies have evaluated

350 the AM activity of basil EOs against various microorganisms both *in vitro* and on a range of

351 food products as shown in Table 2. Prasad and others (1986) investigated the AM activity of

352 the EOs of *O. basilicum* against various Gram-positive and Gram-negative bacteria with the

353 oils shown to be more effective against Gram-positive bacteria including *Bacillus*

354 *sacharolyticus, B. stearothermophilus, B. subtilis, B. thurengiensis, Micrococcus glutamicus*

355 and *Sarcina lutea* than the Gram-negative ones. Lachowicz and others (1998) evaluated the

356 AM effects of EOs of sweet basil against acid-tolerant food microflora. They reported greater

357 inhibitory effects of the tested EOs against the Gram-positive bacteria *Bacillus* sp., *Staph.*

358 *aureus* sp., *Micrococcus* sp., *Sarcina* sp., *Lactobacillus* sp. than against the Gram-negative

359 bacteria *E. coli, Salmonella* sp., *Enterobacter* sp. and *Pseudomonas* sp. In contrast to these

360 studies, Koga and others (1999) found that the Gram-positive bacteria were more resistant to

361 basil EOs than the Gram-negative ones.

362

363 Various researchers have reported also the inhibitory effect of basil EOs against fungi. Rai

364 and others (1999) evaluated the antifungal activity of the EOs of ten plant species (including

365 *O. basilicum*) and reported that the EOs of basil were active against all *Fusarium* species

15

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.

390 The AM activity of linalool against several microorganisms has also been reported in studies

391 conducted *in vitro* only. Kim and others (1995) investigated the AM activity of some EO

392 components including linalool against five food-borne pathogens (*E. coli, E. coli* O157:H7,

393 *Sal. typhimurium, L. monocytogenes and V. vulnificus*) and found a dose-related increase in

394 the zone of inhibition against all tested strains except for *L. monocytogenes*. Mazzanti and

395 others (1998) reported that linalool completely inhibited the growth of all yeasts (seven

396 strains of *C. albicans, C. krusei* and *C. tropicalis*), *Staph. aureus* and *E. coli* using the agar

397 disc diffusion method. Dorman and Deans (2000) investigated the antibacterial activity of 21

398 plant volatile oil components including linalool against 25 bacterial strains using the agar

399 well diffusion method. It was reported in this study that linalool was an effective AM agent

400 against a broad spectrum of 23 out of the 25 bacterial strains investigated.

401

402 **2.2.3   Antimicrobial Activity of Oregano and Thyme Essential Oils**

403 Oregano and thyme are popular culinary herbs with their EOs containing terpenoid

404 compounds, mainly the monoterpenoid phenols of thymol (5-methyl-2-[1-methylethyl]

405 phenol) and carvacrol (5-isopropyl-2-methyl phenol). These EOs have been claimed to

406 demonstrate potential health benefits, antioxidant activity and AM properties (Nychas 1995;

407 Baratta and others 1998; Youdim and Deans 2000; Olasupo and others 2004; Tepe and others

408 2004; Davidson and Taylor 2007). The AM activity of thyme and oregano EOs is primarily

409 attributed to their major components thymol and carvacrol respectively (Farag and others

410 1989; Cosentino and others 1999; Davidson and Naidu 2000; Dorman and Deans 2000;

411 Lambert and others 2001; Bagamboula and others 2004; Davidson and Taylor 2007).

412

413 The AM activity of oregano and thyme EOs against various microorganisms has been

414 investigated on media and on a range of food products as summarised in Table 2. For

17

415 example, Lin and others (2004) evaluated the AM activity of phenolic compounds derived

416 from oregano against *L. monocytogenes* on solid media, on beef and on fish products and

417 reported that the extracts exhibited AM activity against *L. monocytogenes* in the agar

418 diffusion assays. Friedman (2004) studied the antibacterial activity of ten different EOs

419 including that of oregano against *E. coli* and *Sal. enterica* in apple juice. They reported that

420 the selected EOs exhibited greater AM activity against *Sal. enterica* than *E. coli*.

421

422 The AM activity of oregano and thyme EOs was investigated in liquid culture and solid

423 media against various microorganisms. Becerril and others (2007) investigated the AM

424 activity of oregano EOs incorporated into a patented plastic packaging material against *E.*

425 *coli* and *Staph. aureus*, using a "kill time" assay. The authors claimed that oregano EOs

426 exhibited significant AM activity with a kill time of approximately 90 min for *E. coli* and 104

427 min for *Staph. aureus*. Marques and others (2008) studied the AM activity of three natural

428 AM agents: oregano, garlic and chitosan against the growth of *Sal. enterica* in liquid culture

429 at 10 and 20°C. They reported that all of the natural agents inhibited significantly the

430 microbial growth at both temperatures with oregano demonstrating the highest inhibitory

431 effects followed by garlic then chitosan. Rodriguez-Lafuente and others (2010) investigated

432 the AM activity of oregano and cinnamon EOs incorporated into packaging-paper against

433 *Alternaria alternata* using an *in vitro* antifungal assay. The authors reported that oregano and

434 cinnamon EOs inhibited the growth of *A. alternata* on solid media.

435

436 Nielsen and Rios (2000) reported that oregano EOs exhibit an inhibitory activity against

437 microorganisms commonly associated with bread spoilage. Tepe and others (2004) examined

438 the AM activity of *Thymus eigii* EOs and its main constituents carvacrol, thymol and *p*-

439 cymene against *B. catarrhalis*, *C. perfringens*, *B. cereus*, *Staph. aureus*, *S. pneumoniae*, *M.*

440 *smegmatis* and *P. aeruginosa in vitro* and found that these EOs demonstrate AM activity

441 against the tested microorganisms. More recently, Gutierrez and others (2008) evaluated the

442 synergistic effect of the EOs of thyme, oregano, lemon balm, marjoram, rosemary and sage

443 against *B. cereus*, *E. coli*, *L. monocytogenes* and *P. aeruginosa* using the spot test on agar

444 media. They reported a significant AM activity of oregano in combination with basil, thyme

445 or marjoram against *B. cereus*, *E. coli* and *P. aeruginosa*. Similarly, Gutierrez and others

446 (2009) determined the AM activity of the EOs of thyme, oregano, lemon balm and marjoram

447 against *Enterobacter* sp., *Listeria* sp., *Lactobacillus* sp. and *Pseudomonas* sp. using foods

448 based on lettuce, meat and milk. Their findings demonstrated that minimum inhibitory

449 concentrations were significantly lower in lettuce and beef media than in tryptic soy broth

450 and that oregano and thyme produced the most active EOs. Lopez and others (2007b)

451 reported AM activity of oregano, cinnamon and clove EOs incorporated into PP or PE/EVOH

452 against various Gram-negative bacteria (*E. coli, Y. enterocolitica, P. aeruginosa* and *Sal.*

453 *choleraesuis*), Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and *E.*

454 *faecalis*), yeasts (*C. albicans, D. hansenii, Z. rouxii)* and moulds (*B. cinerae, A. flavus, E.*

455 *repens, p. roqueforti, P. islandicum, P. commune, P. nalgiovensis*). Similarly, Lopez and

456 others (2007a) reported AM activity of cinnamon, oregano and thyme EOs against various

457 Gram-negative bacteria (*E. coli, Y. enterocolitica, P. aeruginosa* and *Sal. choleraesuis*),

458 Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and *E. faecalis*), yeasts

459 (*C. albicans)* and moulds (*A. flavus, P. islandicum*). The main constituents: cinnamaldehyde,

460 carvacrol and thymol also demonstrated inhibitory effect against *L. monocytogenes, Sal.*

461 *choleraesuis, A. flavus* and *C. albicans* using a modified vapour diffusion test.

462

463 Sagdiç and Özcan (2003) investigated the AM activity of various EOs including oregano and

464 thyme EOs against different microorganisms including *Bacillus amyloliquefaciens, B. cereus,*

465   *Enterobacter aerogenes, E. coli, Sal. enteritidis, Staph. aureus* and *Yersinia enterocolitica.*

466   They reported that oregano was particularly effective against all bacteria during incubation.

467   Oussalah and others (2006) studied the inhibitory effects of sixty different EOs including

468   oregano and thyme against *Pseudomonas putida*. The results of their study showed that many

469   EOs possess *in vitro* antibacterial activity against *P. putida* with oregano and thyme EOs

470   demonstrating the highest AM activity. Viuda-Martos and others (2008) evaluated the

471   effectiveness of the EOs of oregano, sage, clove, thyme, rosemary and cumin on the growth

472   of various microorganisms including *Lactobacillus curvatus*, *Lactobacillus sakei, Staph.*

473   *carnosus* and *Staph. xylosus*, *Enterobacter gergoviae* and *Enterobacter amnigenus*. They

474   found that each of the EOs demonstrated inhibitory activity against all bacteria tested with

475   oregano showing the highest AM activity and reported that the effects of thyme, sage and

476   rosemary were concentration dependent. Baydar and others (2004) studied the antibacterial

477   activity of EOs of thyme, oregano and savoury against various pathogenic bacteria including

478   *B. cereus, E. coli* and *L. monocytogenes.* They reported positive AM activity against the

479   tested bacteria and suggested that the inhibition may be attributed to the action of the

480   components carvacrol, γ-terpinene and *p*-cymene (a constituent of cumin or thyme EOs). The

481   results of these studies demonstrate that oregano and thyme EOs have the potential to be used

482   as AM agents in the food industry for better preservation of quality, enhancement of safety

483   and extension of shelf life. Nevertheless, additional information is required on the benefits of

484   these EOs before considering them as potential candidates for manufacturing of AM films

485   with commercial applications.

486

487   **2.2.4   Antimicrobial Efficacy of Carvacrol and Thymol**

488   Carvacrol and thymol are the major components of oregano and thyme EOs. They have

489   received substantial attention as useful natural AM agents due to their natural origin and

490 GRAS status, as well as them exhibiting a broad AM spectrum against different

491 microorganisms, and possessing heat stability when incorporated into packaging materials

492 (Deans and Ritchie 1987; Zaika 1988; Ultee and others 1998; Lorenzo and others 2003;

493 Couladis and others 2004; Azaz and others 2005; Han 2005; Matan and others 2006). Table 2

494 shows that carvacrol and/or thymol can be applied in food products to control microbial

495 contamination by various microorganisms including bacteria, yeasts and moulds.

496

497 Bagamboula and others (2004) determined the AM effect of carvacrol or thymol against

498 *Shigella* sp. (*S. sonnei* and *S. flexneri*) on lettuce. They observed a decrease in *Shigella* sp.

499 after washing the lettuce with 0.5% and 1% (v/v) thymol or carvacrol and found that at 1%

500 (v/v) of each agent, the population decreased to an undetectable level. They also reported

501 significant inhibition of *Shigella* sp. using the agar diffusion method. The AM activity of

502 carvacrol has also been reported by Ultee and others (2000) when studying the preservation

503 of rice against *B. cereus*. Roller and Seedhar (2002) investigated the effectiveness of

504 carvacrol against the natural flora of freshly cut melons and kiwifruit. They found that

505 carvacrol reduced significantly the viable count of natural flora on kiwifruit dipped in a

506 solution of the agent, but it was less effective on honeydew melons. Kiskó and Roller (2005)

507 explored the AM effectiveness of carvacrol against *E. coli* inoculated into unpasteurised

508 apple juice. They found that carvacrol reduced the bacteria to an undetectable level within the

509 first two days of storage. Ultee and Smid (Ultee and Smid 2001) found carvacrol to be

510 effective against *B. cereus* toxin production in soups to an undetectable level. Chiasson and

511 others (2004) reported effective AM activity of carvacrol and thymol against *E. coli* and *Sal.*

512 *typhimurium* in minced meat products. Seaberg and others (2003) reported inhibitory effects

513 of carvacrol against *L. monocytogenes* in ready-to-eat beef slices. Recently, Rardniyom

514 (2008) coated carvacrol onto LDPE and nylon films and reported that the AM film inhibited

21

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*.

540

541 Falcone and others (2005) reported that thymol inhibited significantly the growth of *S.*

542 *cerevisiae* and *B. cereus* in liquid media. They reported that the growth kinetics of *B. cereus*

543 in liquid media is a function of thymol concentration. Ultee and others (1998) investigated

544 the AM activity of carvacrol against *B. cereus* using a liquid culture media and reported that

545 the activity depends on the concentration, exposure time, temperature and pH. Periago and

546 others (2004) studied the AM activity of carvacrol and cymene against the growth of two

547 strains of *L. monocytogenes* and found that carvacrol and cymene reduced microbial growth

548 during the lag and exponential phases. They found that the combination of carvacrol and

549 cymene resulted in a larger decrease in viable counts of *L. monocytogenes* compared with the

550 separate application of these agents. Burt and others (2005) conducted a comparative study of

551 the AM activity of oregano and thyme EO components (carvacrol, thymol, *p*-cymene and γ-

552 terpinene) against the growth of *E. coli* O157:H7 and also found synergistic effects of these

553 components using the checkerboard assay. They reported that carvacrol and thymol

554 demonstrated individual and additive antibacterial activity against *E. coli* O157:H7, but no

555 observable AM activity by *p*-cymene and γ-terpinene was found. Although the vast majority

556 of studies involving essential oils or their extracts suggest a positive and broad spectrum of

557 AM activity, an important aspect that needs more attention is how to minimise the loss of

558 these volatile agents during processing, particularly at high temperatures. Gutiérrez and

559 others (2009) reported AM activity of carvacrol, thymol and cinnamadehyde incorporated

560 into PP against various Gram-negative (*E. coli, Yersinia enterocolitica, P. aeruginosa* and

561 *Sal. choleraesuis*), Gram-positive bacteria (*L. monocytogenes, Staph. aureus, B. cereus* and

562 *Enterococcus faecalis*), yeasts (*Candida Albicans, Debaryomyces hansenii,*

563 *Zygosaccharomyces rouxii)* and moulds (*Botrytis cinerae, A. flavus, Eurotium, repens,*

564 *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

into multi-layered PE films against *M. flavus, E. coli, Staph. aureus* and *B. subtilis* on ground beef. The coated films demonstrated an AM activity against all the microorganisms studied. Lee and others (1998) developed an LDPE packaging film incorporated with GFSE and reported that the film containing narigin, ascorbic acid, hesperidin and various organic acids was shown to possess a wide spectrum of AM activity. However, although the LDPE films containing GFSE had an inhibitory effect on the growth of *E. coli* and *Staph. aureus* on solid media, they were unable to inhibit the growth of *Leuconostoc mesenteroides*, *S. cerevisiae*, *Aspergillus oryzaei*, *A. niger* and *Penicillium chrysogenum.* Rodriguez and others (2008) investigated the AM activity of an active packaging film incorporated with cinnamon EOs against *Rhizopus stolonifer* both *in vitro* and on sliced bread. They reported that cinnamon EOs inhibited the growth of *R. stolonifer* on solid media and on sliced bread.

The AM films incorporated with EOs and/or their principal constituents have the potential for packaging of many food products such as bakery (Suhr and Nielsen 2003; Rodríguez and others 2008; Rokchoy and others 2009; Mehyar and others 2011), dairy (Suppakul and others 2008; Kuorwel and others 2011a), meat, chicken and fish (Suppakul and others 2003a; Kerry and others 2006; Wu and others 2010), and fresh produce (Rodríguez-Lafuente and others 2010). There are already some commercial applications for AM packaging systems such as wasabi extract (or Japanese horseradish) used for Japanese rice lunch boxes (Koichiro 1993), Piatech manufactured by Rhone-Poulene (USA) and Daikoku Kasei Co (Japan) (Brody and others 2001).

3   Conclusions

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

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

659

List of Abbreviations and Nomenclature

660		
661	AM	Antimicrobial
662	AP	Active Packaging
663	CFU	Colony Forming Units
664	EOs	Essential Oils
665	EVOH	Ethylene Vinyl Alcohol
666	GRAS	Generally Recognized As Safe
667	GFSE	Grapefruit Seed Extract
668	HDPE	High-Density Polyethylene
669	LB	Lactic-acid Bacteria
670	LDPE	Low-Density Polyethylene
671	LLDPE	Linear Low-Density Polyethylene
672	MAP	Modified Atmosphere Packaging
673	OEO	Oregano Essential Oil
674	PDA	Potato Dextrose Agar
675	PE	Polyethylene
676	PEG	Polyethylene Glycol
677	PEMA	Poly(Ethylene-*co*-Methacrylic Acid)
678	PET	Polyethylene Terephthalate
679	PP	Polypropylene
680	PS	Polystyrene
681	PVC	Polyvinyl Chloride
682	PVDC	Poly(Vinylidene Chloride) or Poly(Vinyl Dichloride)
683	TEO	Thyme Essential Oil

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