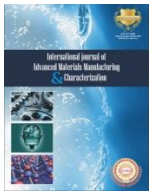

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Formulation and Characterization of Materials Containing Natural Antimicrobial Agents for Food Packaging Applications

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A B S T R A C T

An overview of some of the traditional as well as novel polymeric systems that are used for food packaging applications is presented where the system contains natural anti microbial agents. The background to the need for developing new active packaging systems that meet consumer preference in particular relation to the nature of the additives they release into the foodstuff as well as to their effect on the environment after disposal is discussed. Some novel systems that attempt to address these needs and operate within the constraints that are imposed by regulatory authorities, consumer expectations and environmental considerations are presented along with some of the main techniques that are commonly used to characterize such systems.

Introduction

Food packaging developed from the need to preserve food products for extended periods, to ensure freshness and safety, and to enable transportation over short or long distances. Products that can be recognized as modern food packaging date back to the early 19th century and include items made from paper and cardboard and metal cans [1]. Advances in packaging increased rapidly in the 20th century and many of these innovations coincided with the commercial development of commodity polymers such as polystyrene (PS), polyvinyl chloride (PVC) and polyethylene. Today there are many different types of materials that are used for food packaging and polymeric materials comprise a significant proportion of the packaging market.

The range of synthetic polymers currently used in food packaging include low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), polypropylene (PP), PS, polyethylene terephthalate (PET), copolymers of ethylene vinyl alcohol (EVOH), Surlyn™ ionomers, PVC, and ethylene vinyl acetate (EVA). Indeed, many of these polymers are highly adaptable and can be processed into films, bottles, trays, caps, lids, and containers of all shapes and sizes [2, 3]. These materials offer advantages of low cost, low density, have the ability to be thermo-formed and thermo-sealed, and some are printable. Unfortunately, these materials are inert and do not readily degrade in the environment or in landfill and this is particularly problematic for single-use, disposable packaging. The long-term environmental sustainability of these polymers is now being seriously questioned and interest is shifting to the development of biopolymers and biocomposites as alternatives to the more conventional commodity packaging polymers [4, 5].

There is a wide range of materials that can be produced from renewable sources and these include starch-based polymers and aliphatic polyesters such as poly(lactic acid) (PLA) [6, 7]. These materials are inherently biodegradable either under composting conditions or in landfill and many of them are suited to packaging applications. It is also possible to blend some of these materials with other synthetic and natural polymers to enhance the biodegradability of packaging products and starch-filled LDPE is one example [8]. In addition to these blends, there are additives that enhance the biodegradability of synthetic polymers such as

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expanded PS which is typically not recyclable and therefore must be disposed of to landfill [9].

Over the past few decades, consumer and commercial demand for high-quality food products has driven innovations in packaging and resulted in a new class of packaging materials. Active packaging (AP) technologies are based on a relatively new concept where the food, packaging and environment interact to further enhance food safety and storage [10, 11]. Active packaging systems are designed to change the condition of the packaged food in order to extend the shelf life or to improve safety or sensory properties whilst maintaining the quality of food products [2, 12]. There are several categories of AP systems including those that interact with the internal gaseous environment and/or directly with the product, to produce a beneficial outcome. Examples of AP systems include oxygen scavengers, moisture scavengers, carbon dioxide scavengers or emitters, humidity absorbers or controllers, ethylene scavengers, and aroma emitters or absorbers [2, 13].

One important class of AP involves the use of antimicrobial (AM) agents that are either incorporated directly into the packaging material or in a coating layer to prevent or minimize the growth of spoilage or pathogenic microorganisms [13-16]. Direct incorporation of AM agents can be achieved during normal processing such as film extrusion but this can often result in unacceptable losses of AM agents, particularly the more volatile agents [17]. Coating systems can minimize or prevent these losses and can also extend the application of post-processed packaging films by imparting AM activity [16]. Coating systems can be formulated from naturally derived or modified materials such as methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) or copolymer dispersions that are commonly used for targeted drug release such as ethyl acrylate-methyl methacrylate (EA-MMA) [16, 18].

Antimicrobial systems are designed to reduce or eliminate the need for adding preservatives directly into food products. It is known that microbial growth occurs primarily on the surface of food products so direct contact with films containing AM agents or release of AM agents into a packaging headspace can offer some protection and potentially extend the shelf-life of food products [19]. There are a wide range of AM agents that can be used in packaging including synthetic chemical compounds and naturally derived agents such as essential oils (EOs) and their extracts. Naturally derived materials are preferred by many consumers as these are often perceived as a safer alternative to synthetically derived chemical agents [20]. An AM system can be generally categorized as either a migratory or immobilized system. A migratory AM packaging system involves a slow or controlled release of a relatively volatile AM substance onto the food surface potentially resulting in a delay in the microbial growth [21]. Antimicrobial films that contain volatile EOs typically belong to the migratory system category. In an immobilized or non-migratory system, the AM agent must be in direct contact with the food to impart AM activity.

The development of new AM packaging products is a multi-step process that commences with formulating the AM materials. One of the first steps is to select an appropriate AM agent or agents. Clearly, the target microorganisms that may spoil or otherwise contaminate the packaged food product must be considered in making this choice. The AM agent(s) must be effective against these microorganisms and there are several techniques used to characterize the efficacy including *in vitro* tests and perhaps more importantly, tests on real food products [22]. Table 1 shows some common natural AM agents derived from EOs of thyme and basil and the microorganisms against which they are effective [23-33]. It is evident from this table that many of these naturally derived AM agents demonstrate effectiveness against a wide spectrum of microorganisms and thymol, in particular, shows a significantly high level of AM activity.

Table 1. Relative AM effectiveness of thyme, oregano, thymol and carvacrol.

Organism	Type of AM agent	Degree of inhibition[a]	Reference
Gram-negative bacteria	EOs	thyme, oregano > clove, bay	[23]
		thyme, oregano > basil, rosemary	[23]
		oregano >> coriander > basil > anise	[24]
	active constituents	thymol, carvacrol > *p*-cymene	[25]
		thymol, carvacrol > linalool	[25]
		thymol, carvacrol > eugenol	[26]
		thymol, carvacrol > eugenol, geraniol, citral	[27]
Gram-positive bacteria	EOs	thyme > oregano > clove, black pepper	[28]
		oregano, thyme > basil	[29]
		oregano, thyme > mint, angelica	[30]
	active constituents	thymol, carvacrol > *p*-cymene, linalool	[25]
		thymol, carvacrol > cinnamaldehyde, eugenol > linalool, allyisothiosyanate	[31]
Yeast and moulds	EOs	thyme > clove, rosemary, sage, bay	[32]
		thyme ≈ mustard ≈ lemon grass	[32]
	active constituents	thymol, carvacrol > eugenol	[33]
		thymol, carvacrol > *p*-cymene, linalool	[25]

[a]Comparisons based on inhibition results from the same study

The other considerations in the development of effective AM packaging include a thorough characterization of the AM food packaging materials themselves and one of the most important considerations is the physicomechanical properties. Clearly, it is very important to ensure that the addition of an AM agent does not adversely affect these properties [34] and in the case of

hydrophilic materials such as some starch-based films, it is also important that the environmental service-life conditions are taken into account so that factors such as high relative humidity or water activity do not adversely affect film properties [35]. Another important consideration is the regulatory status of the AM agent as it is only possible to use substances that are "generally recognized as safe" (i.e. those that have "GRAS" status) since AM agents that are incorporated into food packaging are considered in the USA to be food additives [36]. Moreover, under the European Union framework directive, substances classified as food additives or flavours can be released by active materials and must therefore not exceed the specific migration limits [37].

Another very important consideration in the characterization of such materials is therefore the migration or release of AM substances from the packaging matrix [38]. There are a number of diffusion models that can be applied to migrating AM systems [39] and the choice of model will depend on factors such as the distribution of AM agent in the packaging matrix, whether the diffusion is from one or both sides of the package, and the volume of solvent into which the migration occurs. It is also possible in some cases to modify the selection of diffusion boundaries to improve the quality of experimental data analysis and obtain constants and coefficients that describe the diffusion with a higher degree of certainty [40]. It is therefore clear that a comprehensive strategic approach is needed to develop effective, mechanically sound, and safe AM films.

This paper presents an overview of many of the approaches that can be applied to formulate and characterize novel AM materials used in the packaging of certain foodstuffs.

Materials and Methods

Materials

Polymers, filler and coating agents

The synthetic polymers used in the present study were LLDPE (Dowlex 2045 E, Dow Chemical, Australia), LDPE (XJF143/1700 Qenos, Australia), EVA (ELVAX® 3120, Dupont, Australia), and PEG (A1683 PEG 4000 Ajax Finechem, Australia). Thermoplastic starch (TPS), a chemically modified high amylose corn starch (Gelose 939) was supplied by Penford Ltd., Australia. A thermoplastic starch film blended with an aliphatic polyester (APTPS) was supplied by BioGrade®, Australia. Poly(lactic acid) (PLA, 7001D IngeoTM) was obtained from NatureWorks LLC, USA and kenaf fibre (bast) was purchased from Ecofibre Industries, Australia. The polymers used in the coating solutions were: (i) ethyl acrylate methyl methacrylate (EA-MMA) co-polymer dispersion supplied by Degussa Pty. Ltd., Australia and (ii) methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) purchased from Sigma-Aldrich, Australia.

AM agents

The AM agents were methylchavicol with a purity of 98% (AUSTL 21320) purchased from Aurora Pty. Ltd., Australia; linalool with a purity of 97% (L2602), carvacrol with a purity of 98% (W224502) and thymol with a purity of 99% (W306606) were purchased from Sigma-Aldrich, Australia.

Solvents and other chemicals

Other chemicals include ethanol (95SG) supplied by CSR Distilleries Ltd., Australia, iso-octane (2,2,4–Trimethylpentane, OmniSolv®, TX 1389-1) supplied by EMD™ Chemicals Inc., USA, and glycerol purchased from AnalaR, Australia. Sodium hydroxide and acetic acid were purchased from Merck Chemicals, Australia. The encapsulation material β-cyclodextrin (β-CD) with purity ≥ 97% and water content 1.5% w/w was purchased from Sigma-Aldrich, Australia.

Microbial growth media and *E. coli* culture

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

Activity of AM agents

Preparation of microorganism

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

Activity of natural AM agents

One drop of each AM agent, methylchavicol and linalool, was placed on the surface of individual nutrient agars that were inoculated with 105 CFU mL-1 of *E. coli* by the pour-plate method. The plates, prepared in quadruplicate, were then aerobically incubated for 24 h at 37°C. The AM activity of the tested agents was observed from the appearance of the inhibition zone.

AM Material Preparation

Films prepared from LLDPE and AM agent

Linear low-density polyethylene films of 45 - 50 μm thickness containing linalool or methylchavicol were prepared from LLDPE pellets. Additive-free LLDPE pellets were ground and the powder was doped with linalool or methylchavicol dissolved in isooctane. A pre-blended master batch powder containing approximately 15% w/w linalool or methylchavicol was mixed with additive-free LLDPE pellets and manufactured into films by the extrusion film blowing process using a single screw extruder with a diameter of 50 mm (Telford Smith, Australia). The films contained approximately 1.0% w/w linalool or methylchavicol. The temperature profile in the extruder was 195, 205, 210, 200, 210°C from the first barrel zone to the die [41].

Films prepared from LDPE, EVA and AM agent

Low-density polyethylene/EVA films with and without thymol or carvacrol were prepared from LDPE pellets. Master batches containing AM agents at 2% and 4% w/w and EVA were compounded with LDPE in a twin-screw extruder. The extrudate was immediately cooled in a water bath, dried and pelletized. Each compounded blend was blown into film of 40 - 50 μm in thickness in a single screw extruder using an operating speed of 30 rpm. The temperature profile in the extruder was maintained at approximately 150°C (all zones) [42].

Preparation of AM coating

The coating solution was prepared from EA-MMA copolymer dispersion (20% w/w) dissolved in ethanol (80% w/w). The solution was mixed with a magnetic stirrer for 5 min without heating until a homogenous clear gel was obtained and the AM agent carvacrol was added to obtain a final coating concentration of *ca*. 30% w/w. Prepared AM coating solutions were poured on a *ca.* 50 µm thick LDPE film that was framed with two layers (*ca.* 280 µm thickness) of 3M™ masking tape around each edge. The solution was evenly spread using a glass rod and dried at ambient temperature for 1 h. The thickness of the dried coating was measured using a micrometer and films were prepared in duplicate. The thickness of the coating layer was increased by adding additional layers of tape to the frame [18].

AM agent quantification by gas chromatography

The actual concentration of AM agent in the prepared samples was determined by gas chromatography (GC). The procedure was as follows: 5 g of film was extracted for 18 h by Soxhlet extraction using 150 mL of isooctane. An aliquot of the extract of a precisely known volume was sampled for GC analysis. A Varian Star 3400-CX GC equipped with a fused silica capillary column DB-5 (30 m × 0.25 mm i.d., film thickness 0.25 µm, J & W Scientific, USA) was used. The following conditions were applied: injected volume, 1.0 µL; initial column temperature, 80°C; heating rate, 5°C min^{-1} up to 180°C, kept at this temperature for an additional 5 min; injector temperature, 250°C; split ratio, 1:100; FID detector temperature, 300°C; carrier gas, nitrogen. The AM agent content in each of the samples was calculated from prepared standard curves.

Encapsulation of AM additive

Preparation of β-CD/thymol complex

The β-CD complex preparation was achieved as follows: 3.835 g of β-CD was dissolved in 203 mL of water and heated to 55°C. Whilst the mixture was being vigorously stirred on a hot plate a 1 mL aliquot of ethanol was added followed by the addition of 0.5 g of thymol. The solution was covered and maintained at 55°C for 4 h with sufficient stirring after which the solution was cooled to room temperature and stirring was continued for 2 d at that temperature. The mixture of β-CD/thymol was then cooled to 4°C and maintained at this temperature for 24 h. The precipitated β-CD/thymol complex was recovered by vacuum filtration. The collected filtrate was allowed to dry in air for 5-9 d. A mass recovery of *ca*.71.3% was obtained from the reaction [43].

Preparation of LDPE films with thymol and β-CD/thymol complex

Low-density polyethylene films containing thymol or the β-CD/thymol complex were prepared using a laboratory hot press (IDM Instruments Pty. Ltd., Australia, Model No. L0003). For samples containing LDPE and thymol, the thymol was dissolved in ethanol and combined with LDPE pellets to ensure an even distribution of thymol before the ethanol was evaporated at room temperature. For samples containing the β-CD/thymol complex, a dry mixture of LDPE with the complex was formed. In both cases, the target concentration of thymol was *ca*. 10% w/w. To prepare the films, a sample of *ca.* 2 g of the resultant solid mixture was placed between Mylar™ films that were positioned between a set of aluminum plates. Film samples were created by pressing the solid mixture in the laboratory press that was preheated to 120°C. The temperature of the upper and lower platens of the press was maintained at 120°C for 2 min under a pressure of 30 kPa. The plates were quench-cooled with water to 80°C, then removed from the press and the samples removed from between the aluminum plates. The films were peeled off gently from the Mylar™ after the film had cooled completely [43].

Quantification of thymol

The films samples were cut into small (*ca*. 1 g) pieces and each sample was extracted for 90 min under reflux extraction using 50.00 mL of ethanol. An aliquot of the extract was directly analyzed using GC in accordance with the method given above.**Preparation and Testing of Starch-Based Films**

Film preparation

The preparation of the starch-based film was achieved by heat pressing under compression. Master batches were prepared by gradually adding the starch-based material to a plasticiser made of a mixture of water and glycerol. The final composition of the formulation was 65% w/w starch-based material, 10% w/w water, and 25% w/w glycerol. A sample weighing *ca.* 15 g of the resultant mixture was placed between Mylar™ films that were then positioned between a set of aluminium plates and pressed in the laboratory press. The temperature of the upper and lower platens of the press was maintained at 125°C for 5 min under a pressure of 20 kPa. The plates were then quench-cooled, removed from the press and the films were peeled off the Mylar™ film after cooling was complete [44].

Coating preparation

The coating solution for APTPS films was prepared using MC and HPMC materials. The MC and HPMC were added slowly to absolute ethanol and heated, with stirring, on a magnetic hotplate. The heating was discontinued when the temperature reached 65°C. With continuous agitation, a mixture of PEG and distilled water, as plasticiser, was added slowly to the MC/HPMC dispersion whilst the dispersion cooled. This resulted in the formation of a uniformly clear coating solution or gel. The AM agent carvacrol was then added to the coating solution to form the final coating material with the AM agent at a target level of 3% w/w. The coating medium was applied to the starch-based material using a hand drawn glass roller and the film was then dried under ambient conditions (21°C, relative humidity, RH, 38%) for 24 h. To control the thickness of the coating, the starch-based material was taped onto a 30 x 30 cm glass plate and the edges were framed using 3M™ masking tape.

Water uptake of TPS films

The water uptake of TPS films was measured by conditioning film samples at different levels of RH. The film samples were contained in desiccators and exposed for 7 days over saturated solutions (in distilled water) of P_2O_5, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, NaCl, KCl, K_2SO_4 and pure distilled water. These solutions provided relative humidities of: 0, 11, 23, 33, 43, 53, 66, 75, 85, 97 and 100% respectively at 20 ± 1°C. The water content of the equilibrated film samples was determined gravimetrically by firstly weighing the exposed samples and then drying them at 105°C in a laboratory oven for 24 h before re-weighing.

Mechanical properties of starch-based films

The physico-mechanical properties of the TPS and APTPS starch-based systems were investigated in accordance with ASTM Method D 882-97. Films were cut into strips of 20 × 100

mm. The measurements were made using an Instron 4465 (USA) tensile tester with an R 2797 (500 N) peak load cell and crosshead speed of 50 mm min-1.

Preparation of PLA/kenaf fibre composites

Preparation of kenaf fibres

Kenaf fibres were soaked in 5% w/v sodium hydroxide solution for 2 h at room temperature. The fibers were filtered and washed with distilled water before being neutralized by adding a few drops of acetic acid. The fibres were then filtered, washed and rinsed with distilled water to remove excess acid and the latter confirmed using a pH meter. Finally, the fibres were dried overnight in an air oven at 105°C. Prior to mixing, the PLA resin and kenaf fibres were dried in an oven at 60°C overnight before blending with 5% and 10% w/w thymol.

Processing PLA/kenaf and PLA/kenaf/thymol composites

The melt-blending process was used to prepare the composites using an internal mixer (Haake PolyLab OS, Germany) at a fixed screw speed of 50 rpm. The processing temperature and time were set at 155°C and 8 min respectively and a total mass of 50 g was required to fill the mixing chamber. The mixing torque curves together with stock temperature (actual temperature of the blend) were simultaneously monitored during the mixing process using Polysoft OS software.

AM Migration

The release of thymol from the extruded LDPE/EVA/AM agent films into 95% v/v ethanol/water or carvacrol from the MC-HPMC coatings on APTPS films into isooctane at 25°C was assessed in accordance with the double-sided, total immersion migration test (i.e. both sides of films immersed into the liquid stimulant). Film samples weighing *ca.* 0.5 g (4 pieces, 5 × 5 cm) were immersed into 100 mL of food simulant in a tightly sealed vessel that was mildly agitated (50 rpm) in an incubator shaker (InnovaTM 4230, New Brunswick Scientific, USA.). The migration test for each film in each simulant was performed at three temperatures: 10, 15 and 20°C. The amount of AM agent released was monitored until equilibrium was attained. An aliquot of the solution was analysed by GC analysis at different time intervals. The AM release in food simulants was quantified by the GC conditions as described above.

Results and Discussion

Preparation and Characterization of Antimicrobial Films and Composites

The steps involved in the preparation and characterization of AM films and composites include the characterization of the efficacy of the AM agent against the target microorganism, material formulation and processing, as well as the consideration of techniques to reduce loss of natural AM agents during film processing.

Effectiveness of AM Agents

Prior to the incorporation of AM agents into packaging films, it is important to establish the efficacy of the selected AM agents against previously identified target microorganisms. This is usually achieved *in vitro* by preparing a culture of the bacteria on a growth plate, exposing the culture to the AM agent, incubating the plate, and finally performing a plate count. Figure 1 shows the AM activity of the natural agents' methylchavicol and linalool against *E. coli* at the density level of 105 CFU mL-1 on nutrient agar. It is evident that methylchavicol (Figure 1(a)) provides the weaker activity against *E. coli* as indicated by the growth of some bacterial colonies. The growth of *E. coli* is entirely inhibited, however, on plates containing linalool (Figure 1(b)) suggesting linalool is a suitable AM agent for *E. coli*. Similar tests can be performed following the incorporation of the AM agent into a film or coating.

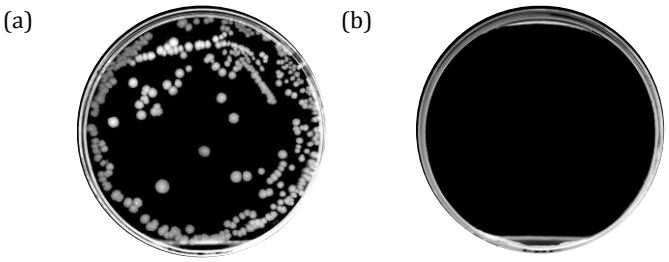

Figure 1. Photographs showing the inhibitory effect of: (a) methylchavicol and (b) linalool against E. coli.

Processing Antimicrobial Films

There are a number of techniques that can be used to prepare AM films from polymers such as LDPE or LLDPE but the most common technique is film blowing. The process of film blowing and indeed any extrusion or molding technique for thermoplastics involves the use of elevated temperatures. These temperatures can result in a loss of AM agent, particularly volatile EOs and their extracts, so it is important to minimize these losses by lowering extrusion temperatures if possible, or by using additives that help to retain the AM agent. The post-processing concentration of the AM agent in the film is a very important parameter as it relates directly to the AM activity. For samples of LLDPE AM films prepared with linalool or methylchavicrol by extrusion at *ca.* 200°C, losses of up to 95% of AM agent were observed [41]. Understandably, this would be deemed an unacceptable loss if these films are to be commercially manufactured.

Reducing Loss of Natural AM Agents during Processing

There have been a number of studies in the literature that have identified the problem of unacceptably high losses of naturally-derived AM agents from polymeric materials during extrusion and/or film blowing processes [17, 45]. Various attempts to rectify this problem have been reported.

Additives to Enhance AM Retention

Polymeric additives such as PEG and/or EVA can be used to enhance the retention of volatile AM agents during thermal processing [41]. To investigate the ability of EVA to enhance the retention of thymol or carvacrol during processing, blends of LDPE, EVA and AM agent were prepared by extrusion at *ca.*

150°C. Table 2 shows the film formulation details and the post-processing concentrations and retention of AM agent in the films.

Table 2. Post-processing retention of AM agents in extruded films

AM Agent	Film Formulation[#]		Post Processing	AM Agent Loss
	AM Agent	LDPE	Concentration	
	% w/w	% w/w	% w/w	%
Thymol	2	88	0.9	55
	4	86	3.2	20
Carvacrol	2	88	1.3	33
	4	86	2.7	35

[#]Balance of formulation is 10% EVA.

The data in Table 2 indicate that the retention of the AM agents under investigation is considerably higher than that of linalool in LLDPE without EVA where the loss of linalool has been reported to be as high as 70% in some cases [41]. Moreover, a relatively high retention of thymol and carvacrol (Table 2) suggests that these agents can withstand a temperature of 150°C during extrusion. This might be attributed to the higher boiling points of thymol (*ca.* 232°C) and carvacrol (*ca.* 236°C) compared with that of linalool (*ca.* 196°C).

Antimicrobial Film Coatings

For some materials, it may not be possible to directly incorporate AM agents during processing and in these cases, techniques such as surface coating can enable the delivery of AM activity. Coating can be achieved by several methods including immersion or soaking, spraying, or solvent casting with the latter particularly useful for preparing AM films for food packaging [46, 47]. Coating by solvent casting is achieved by dissolving an AM agent in a solvent or solvent mixture, coating the mixture onto the film substrate to a desired thickness, then evaporating the solvent. This process is achieved in the absence of excessive heat although it may be necessary to apply mild heating to dissolve some of the components. Nevertheless, coating by solvent casting results in significantly lower volatile AM agent losses in most cases. Table 3 shows the retention of carvacrol in coating formulations prepared at different thicknesses using an EA-MMA coating system [18]. The results show that a significantly high proportion of carvacrol is retained in the coating and that there is little difference in the retention when the thickness is doubled.

Table 3. Retention of carvacrol in EA-MMA coating formulations

AM coating layer thickness	Concentration of carvacrol in dry coating		Retention of carvacrol
µm	% w/w	g mL-1	%
20	29	0.22	95.1
40	30.6	0.21	96.2

Encapsulation of AM agents using inclusion compounds

Another technique currently under investigation to minimize significant losses of AM agents during extrusion of films is the use of inclusion compounds such as β-cyclodextrin (β-CD) to encapsulate the AM agents. The β-CD molecule is a cyclic oligosaccharide consisting of seven glucose units and the molecule is shaped like a hollow truncated cone as shown in Figure 2. Complexation of AM agents in the β-CD matrix can be achieved by a relatively facile method that enables more than 90% of the AM agent thymol to be complexed and subsequently extracted into an ethanol solution [43]. This retention of AM agent is similar to that observed in coating systems and therefore presents a viable option for commercial film production providing the encapsulation process is optimized, the overall process is cost effective and, most importantly, the AM agent is sufficiently mobile and available to be effective within the formulation.

(a) (b)

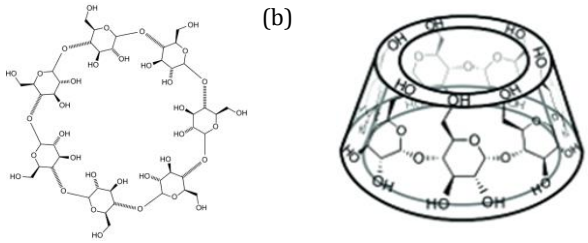

Figure 2. Schematic representations of: (a) β-CD molecular structure and (b) possible tropoidal structure.

Figure 3 shows that the AM agent thymol when encapsulated within the β-CD complex is significantly retained within the LDPE film over the experimental time period whereas films that were processed with the free thymol lose most of this agent in less than 100 h. Although the current evidence suggests that the formation and incorporation of these complexes can be successfully achieved, further work needs to be conducted to assess the efficacy of films produced with AM/β-CD complexes. In particular, the β-CD complex should eventually release the AM agent in order to impart AM activity to the formulation and the presence of the complex should not adversely affect the physicomechanical properties of the film. These aspects are currently under investigation.

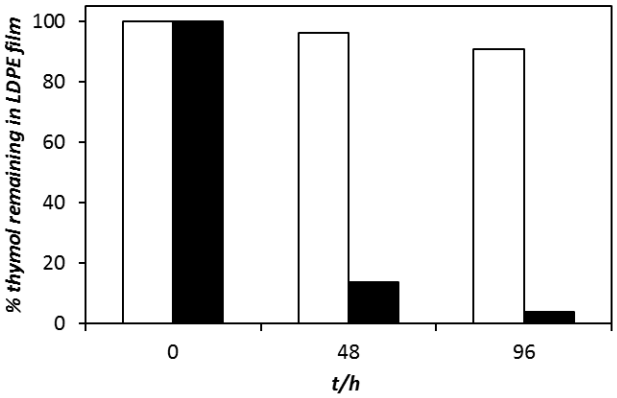

Figure 3. Percentage of thymol remaining in LDPE film: (■) without β-CD complex and (□) with β-CD complex.

AM Packaging from Biopolymers and Biocomposites

There is an increasing need to find environmentally friendly yet cost effective alternatives to conventional, synthetically derived plastics. Recent advances in the development of biopolymers derived from renewable resources have given rise to new possibilities in the field of packaging materials.

Starch-Based Materials

Starch-based materials are inherently hygroscopic and lose significant mechanical integrity in the presence of water or high humidity [35]. Figure 4 shows the effect of the RH on the percentage elongation at break and percentage water absorbed for the case of TPS films.

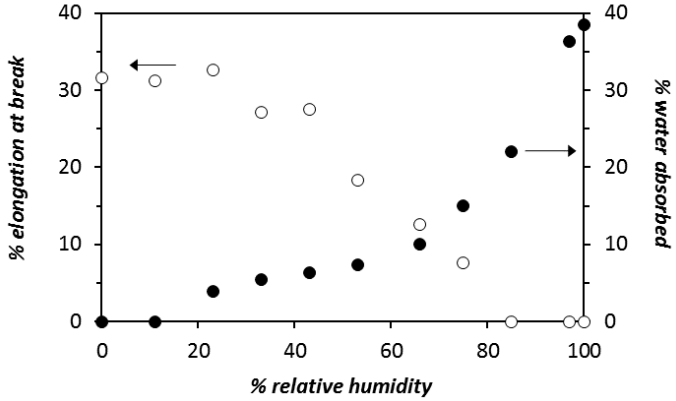

Figure 4. Percentage elongation at break and percentage water absorbed for heat pressed TPS films as a function of percent RH.

The percent elongation starts to decline significantly above 25% RH which corresponds to a moisture uptake of only *ca.* 5%. Moreover, a 50% decrease in the percent elongation is observed at an RH level of *ca.* 53% which corresponds to a moisutre uptake of less than 8%. This confirms the high sensitivity of this material to the presence of moisture and can limit the application of this and similar materials only to the packaging of foods with relatively low water activity.

Poly(lactic acid)/Fibre Biocomposites

Fortunately, there are many other biopolymers that have similar molecular structures and physicomechanical characteristics to the more conventional synthetic polymers. For example, PLA is in the same class of polymers as poly(ethylene terephthalate) (PET); it has similar properties, can be processed using conventional equipment, and is biodegradable but it is, unfortunately, relatively expensive to produce [48]. Cost savings can be made, however, by introducing inexpensive fillers that can often impart higher mechanical strength to the biocomposite materials that are formed [49]. As expected, the addition of fillers can adversely affect the processability of biocomposites during extrusion so processing aids often are added to improve the rheological properties. Figure 5 shows examples of the rheological profiles of PLA/kenaf fibre biocomposites in the presence of the AM agent thymol. In this case, the addition of the AM agent has the added benefit of imparting a lubricating effect during processing resulting in a significant reduction in the processing torque.

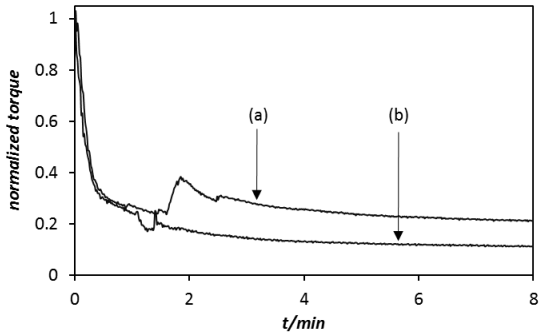

Figure 5. Normalized torque for PLA biocomposites containing: (a) 10% w/w kenaf fibre and (b) 10% w/w kenaf fiber and 10% w/w thymol.

The peak in the melt torque (see Figure 5, curve (a)) observed at *ca.* 2 min corresponds to the point in time when the kenaf fibres are introduced into the mixer. This peak is absent in the case where AM-doped kenaf fibres are introduced (see Figure 5, curve (b)) and may be attributed to the lubricating effect of the AM agent. The relatively lower equilibrium torque that is observed after *ca.* 6 min of the formulation containing the thymol compared with the formulation without thymol is also indicative of the lubricating effect of the thymol.

Characterizing the Rate of AM Release

Various equations have been derived in the literature for the time dependence of the mass of additive that migrates from a polymer film into the simulant. The period over which migration occurs has been divided into "short" and "long" terms [39, 50].

The short-term migration of an additive is defined as the time for which $m_t/m_\infty < 0.6$ and is described by the following equation:

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

where m_t and m_∞ are the amounts of additive released from the film up to time t and equilibrium ($t = \infty$) respectively, D is the diffusion coefficient and l is the thickness of the film. A plot of m_t/m_∞ versus $t^{1/2}$ will produce a straight line that passes through the origin and the diffusion coefficient can be obtained from the gradient of the line.

A precise expression for the long-term migration of an additive is as follows [39, 50]:

$$m_t/m_\infty = 1 - \sum_{n=0}^{\infty} \{8/[(2n+1)2\pi^2]\exp[-(2n+1)2\pi^2 Dt/l^2]\} \quad (2)$$

The following equation has been developed as an approximation to the precise long-term migration equation above and has been extensively used in the literature to characterize additive migration from polymer films:

$$m_t/m_\infty = 1 - (8/\pi^2)\exp(-\pi^2 Dt/l^2) \quad (3)$$

Figure 6 shows theoretical plots of m_t/m_∞ versus time for both the approximate and the exact solutions to the diffusion equation for an "idealized" system [39, 50] that have been calculated for

the long-term period where the diffusion coefficient was taken to be 1 × 10-12 m2 s-1 and film thickness was 50 μm [40]. It is apparent that the two functions remain almost convergent for values of m_t/m_∞ down to *ca.* 0.5 [40]. It has therefore been suggested that the definition of the short-term/long-term boundary, *b*, in the analysis can be amended such that the boundary may be shifted from *b* = 0.6 [50] to *b* = 0.5 with little consequence in the theoretical analytical result. Such a shift can be affected for the purposes of producing a simultaneously enhanced goodness of fit of the data that lie in the short-term and in the long-term time domains [40].

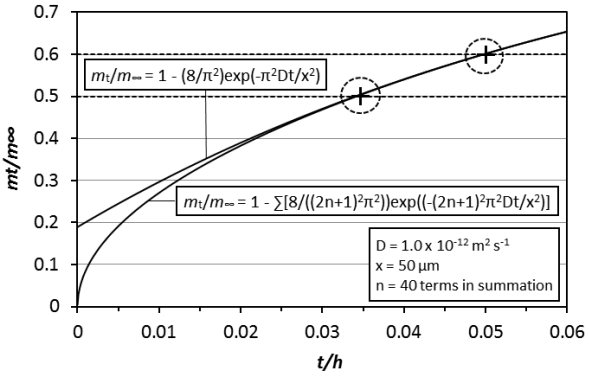

Figure 6. Theoretical plots of m_t/m_∞ versus time for the exact and approximate solutions to the diffusion equations that pertain to an "idealized" system.

The application of the latter technique can be clearly seen in Figure 7 where the long-term and short-term migration of thymol from LDPE/EVA extrusion-blown films into 95% v/v ethanol/water at 10°C has been analyzed [40]. A shift in the boundary between the short- and long-term migration data produces a better fit to the data in each case. Consequently a more reliable estimate of the diffusion parameters such as *D* = 2.84 × 10-14 m2 s-1 and k_d = 8.54 × 10-5 s-1 for the migration of thymol into 95% v/v ethanol/water at 10°C can be extracted upon the analysis of the data.

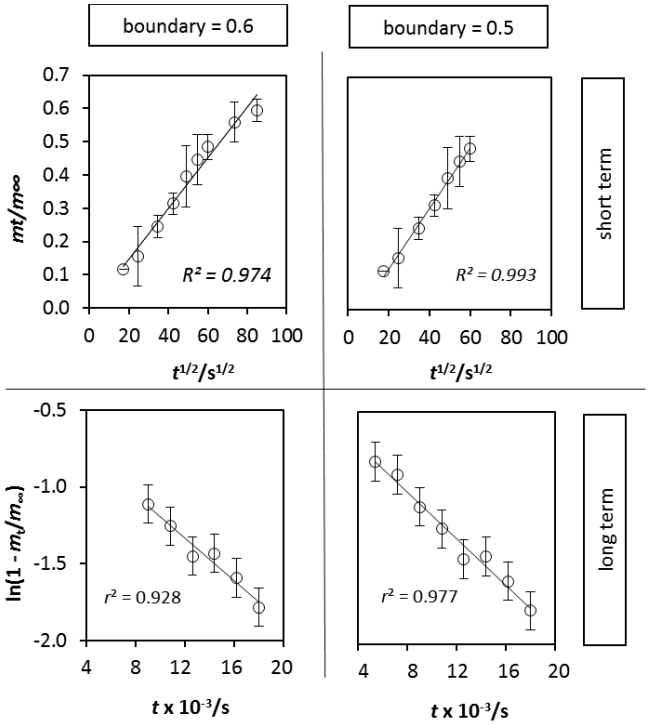

Figure 7. Plots of: (i) m_t/m_∞ versus $t^{1/2}$ for the short-term release and (ii) ln(1 – m_t/m_∞) versus time for the long-term release of thymol from LDPE/EVA extrusion-blown films into 95% v/v ethanol/water at 10°C where the boundary conditions are either b = 0.6 or b = 0.5.

The importance of making reliable measurement of AM migration will become even more critical in the future if AM systems that are readily biodegradable after their use as packaging are to be produced from materials that may be derived from renewable resources. An example of one such potential system is the biodegradable aliphatic polyester thermoplastic starch (APTPS) substrate that is coated with an AM agent dispersed within a mixture of MC, HPMC and PEG. An analysis of the migration of the AM agent carvacrol from such a coating into the food simulant isooctane is shown in Figure 8.

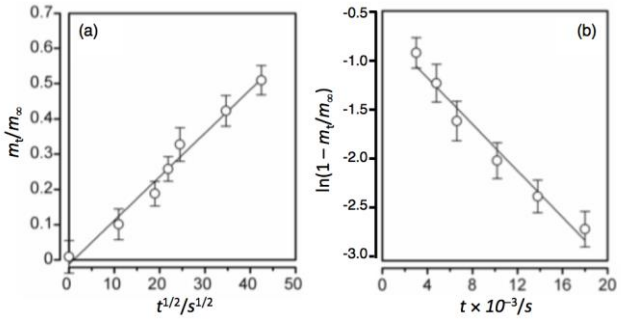

Figure 8. Plots of: (a) m_t/m_∞ versus $t^{1/2}$ and (b) ln(1 – m_t/m_∞) versus *t* for the migration of carvacrol from MC-HPMC coatings on APTPS films into isooctane at 25°C.

The limited flexibility in determining the boundary as described above was invoked in the analysis in order to produce the best possible estimates of the diffusion coefficient and kinetic data

associated with the release [38]. These data are necessary in designing systems where the rate of dissipation of the AM agent during the lifetime of the film is such that it is effectively zero at the time when the material is to be disposed of in landfill.

Conclusion

The future delivery of fresh and uncontaminated foods to the world's population will increasingly depend on the ability of materials science and engineering to deliver intelligent materials that can retard the food spoilage that occurs due to micro-organisms.

Traditional packaging materials such as polyethylene formulations can include AM agents in their masterbatches and these agents can act to control the proliferation of spoilage microorganisms. Consumer preference as well as demand has prompted the search for natural AM agents that can be used in active packaging systems. However, these are volatile materials whose severe loss from the polymer matrix during thermal processing must be overcome if their incorporation into materials is to be commercially viable. Nonetheless, there has been some advances in this area such as the use of a co-additive such as EVA that helps to retain the AM additive or lubricants in the extrusion stage of film production that decrease the friction and help to disperse the additive within the polymer matrix whilst enhancing the retention. The use of encapsulating technologies such as inclusion compound formation may also be a way in which this problem can be overcome in the future.

Consumer demand and decreasing world supplies of petroleum has also led to the search for packaging materials that can be produced from renewable resources. Such naturally derived materials are claimed to be more readily biodegradable than the typical synthetic packaging materials that have been used traditionally. The biodegradability of many of these materials in certain environments is still under widespread investigation. The future challenge in this field of materials science will be to produce food-safe packaging materials that: (i) can be derived economically from natural and renewable sources, (ii) contain natural AM systems whose lifetimes in the active packaging are such that they will not affect the final biodegradation of the materials in the environment and (iii) will biodegrade in the environment in a way that produces useable products such as methane that can be collected for energy recovery.

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