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Loss of AM additives from antimicrobial films during storage

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ABSTRACT

Films based on linear low-density polyethylene (LLDPE) and low-density polyethylene (LDPE) containing linalool or methylchavicol were prepared by extrusion film blowing. Film rolls of LLDPE containing linalool or methylchavicol were stored at ambient temperature for 1 year. Samples of these films were then evaluated for the amount of linalool or methylchavicol retained and for their antimicrobial (AM) activity by the agar disc diffusion assay. In addition, film rolls of LDPE-EVA (LDPE-ethylene vinyl acetate) containing linalool or methylchavicol were stored at 25 and 35 °C. Samples of these films were periodically collected to quantify the amount of linalool or methylchavicol retained as a function of time. For the AM LLDPE films, a decrease in additive retention was observed but there was no statistically significant difference in their AM activity against *Escherichia coli* at the beginning and after 1 year of storage. For the AM LDPE-EVA films, the amount of additive in the film decreased with time and the additive retention in all films tended to deviate from the theoretical first-order decay. These findings suggest that an amount of linalool or methylchavicol that is sufficient to maintain AM activity remained in the polymeric matrix after the storage period. This study confirms the potential use of polymeric films containing basil constituents as AM films for enhancing quality and safety as well as the extension of the shelf life of foods.

1. Introduction

During the past decade, there has been an increasing interest in developing antimicrobial (AM) packaging materials to prevent microbial growth on food surfaces during storage, by a slow release of AM additives onto the food surface. The AM additives that have been mentioned include acid anhydrides, amines, bacteriocins, enzymes, fungicides, metal ions, organic acids and their salts, paraben and plant extracts (Suppakul et al., 2003a; Appendini and Hotchkiss, 2002; Quintavalla and Vicini, 2002; Vermeiren et al., 2002; Kerry et al., 2006; Coma, 2008). Dainelli et al. (2008) reviewed the progress in the area of AM packaging technology and reported that this rapidly emerging technology is expected to grow in the next decade. There is a growing interest in the incorporation of natural AM additives into packaging films to be used as "AM packaging" for the purpose of improving food quality and safety as well as extending shelf life (Becerril et al., 2007; López et al., 2007; Rodríguez et al., 2007; Gutiérrez et al., 2009).

Essential oils are well-known inhibitors of microorganisms (Burt, 2004; López et al., 2005; Di Pasqua et al., 2007; Goñi et al., 2009; Gutiérrez et al., 2010). Basil (*Ocimum basilicum* L.) is one of the oldest identified spices and its essential oils have been used extensively for many years in food flavoring and perfumery. Numerous investigations on basil essential oils have been reported including taxonomy, chemistry and AM activity (Kalemba and Kunicka, 2003). Suppakul et al. (2003b) reviewed the topic of basil essential oils with regards to their chemical composition, their effect on microorganisms, and their possible future use in food preservation or as an AM additive in packaging materials. When focusing on natural plant extracts, basil extract is one of the promising potential AM additives due to its AM activity against a broad spectrum of Gram-positive and Gram-negative bacteria, and yeasts as well as moulds. The principal constituents of basil, namely linalool and methylchavicol, exhibit AM activity against many microorganisms (Suppakul et al., 2003b). These compounds possess "GRAS" status (Suppakul et al., 2003a). They are stable at relatively high temperatures and may therefore have the potential to be incorporated into polymers and used in AM packaging applications. In studies by Suppakul et al. (2006) and Suppakul et al. (2008), linalool or methylchavicol was incorporated into polyethylene-based films. The barrier, optical, physico-chemical and

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thermal properties and the antimicrobial efficacy of the films were investigated. The storage temperature may affect the additive retention in the AM films and therefore their AM activity. However, no published information could be found in the scientific literature in regard to the loss of AM additives and their retained AM activity during film storage. The present study was aimed at determining the effect of time and temperature, at either long-term or accelerated storage conditions, on the retention of basil components that had been impregnated into polyethylene-based films.

2. Materials and methods

2.1. Polymers

The polymers used in the present studies included linear low-density polyethylene (LLDPE, Dowlex 2045 E, Dow Chemical, Australia), low-density polyethylene (LDPE, Alkathene XJF 143, Qenos Pty. Ltd., Australia) and ethylene vinyl acetate copolymer (EVA, Escorene™ Ultra LD 318, ExxonMobil Chemical, USA).

2.2. Antimicrobial additives

The AM additives used in the experiments were linalool (L260-2, Aldrich Chemical Company, Inc., USA) and methylchavicol (AUS-TL 21320, Aurora Pty. Ltd., Australia) with the purity of 97% and 98%, respectively.

2.3. Chemicals

Sodium dihydrogen orthophosphate ($NaH_2PO_4{\cdot}2H_2O$, 30132), di-sodium hydrogen orthophosphate (Na_2HPO_4, 30158.5000) and sodium chloride (NaCl, 10241.AP) were purchased from BDH Chemical Australia Pty. Ltd.

2.4. Media

The media used in the present study were nutrient broth (CM 1) and nutrient agar (CM 3) purchased from Oxoid, USA. Bacteriological agar (RM 250), plate count agar (AM 144), tryptone soya broth (AM 185) were obtained from Amyl, Australia.

2.5. Microorganism

The microorganism used in this research was *Escherichia coli* (FSA 1301), obtained from the Culture Collection of Food Science Australia, Werribee, Victoria, Australia.

2.6. Preparation of AM LLDPE films

Linear low-density polyethylene (LLDPE) films of 45–50 μm in thickness, with and without linalool or methylchavicol, were prepared from LLDPE pellets. Additive-free LLDPE pellets were ground and the powder was doped in linalool or methylchavicol dissolved in isooctane. This AM agent-impregnated powder was used as the master batch. The master batch powder containing linalool or methylchavicol was mixed with virgin 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). Films without linalool or methylchavicol were used as controls and were prepared under similar conditions to the films containing the active agents.

2.7. Additive quantification in AM films

The actual concentration of linalool or methylchavicol 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. Isooctane was used since it was anticipated that an end-use of the films would be for the packaging of hard cheeses that contain predominantly non-polar substances such as fats, etc. The extraction efficiency was checked by periodically analyzing the extract until no further change in the concentration of the AM agent was observed after a period of 18 h extraction. 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, then kept at this temperature for additional 5 min; injector temperature, 250 °C, split ratio, 1:100; FID detector temperature, 300 °C; carrier gas, nitrogen. The linalool and methylchavicol contents of the samples were calculated from prepared standard curves.

2.8. Antimicrobial activity of LLDPE films in solid media

The films were tested for their inhibition against the selected microorganism *E. coli* (Gram-negative bacteria) by using an agar disc diffusion method (Acar and Goldstein, 1986; Parish and Davidson, 1993).

The microorganism used in the microbiological assay was a twice-passaged 15 h culture grown in nutrient broth. Cell densities of 10^6 organisms were calculated and prepared from cultures of approximately 7.50×10^8 CFU mL^{-1} for *E. coli*. Cell densities were estimated from standard curves and confirmed by the "pour plate" method on plate count agar for bacteria (Swanson et al., 1992).

Each film sample was cut into a circle of 5 mm in diameter and sterilized with UV light for 2 min (Cooksey, 2000) prior to being placed on an agar plate surface seeded with 1 mL of test culture consisting of 10^6 organisms. The plates were incubated for 1–2 days at the required temperature for each culture. The clear zone formed around the film disc in the media was recorded as an indication of the inhibition of the microbial species. The evaluation of inhibitory activity was carried out in quadruplicate, by measuring the diameter of the inhibition zone with a Vernier caliper with a precision of 0.02 mm (Mitutoyo, Japan). An average of four diameter measurements, taken 45° apart from each other, was used as the result of each test.

2.9. Long-term storage of AM LLDPE films

Rolls of approximately 100 m films containing linalool or methylchavicol were kept at ambient temperature for 1 year (long-term storage). Samples were then used to evaluate their antimicrobial activity in solid media, as described in the previous section.

For determining the effect of the worst-case storage scenario, film samples taken from the outside and side regions of the rolls were tested for their inhibition of *E. coli* (Gram-negative bacteria) by the agar disc diffusion method (Acar and Goldstein, 1986; Parish and Davidson, 1993). The reason for this is that loss of active agents over time is expected to be greater from the exposed outside and side regions of the roll than from the inside and center regions.

2.10. Preparation of AM LDPE-EVA films

The LDPE-EVA films of 45–50 μm in thickness, with and without linalool or methylchavicol, were prepared from LDPE