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Release of cinnamon essential oil from polysaccharide bilayer films and its use for microbial growth inhibition in chilled shrimps

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ABSTRACT

Active biodegradable bilayer films based on agar and sodium alginate were developed by the incorporation of cinnamon essential oil in the upper layer. The release of both cinnamaldehyde and eugenol from the agar and alginate bilayer films into water at 4 °C and 20 °C did not increase significantly from 9 h onwards, and consequently increasing values of antioxidant activity measured by ferric reducing ability and radical scavenging capacity were observed until 9 h in both cases. Agar bilayer films showed an antimicrobial activity approximately 1.4-fold higher than the alginate bilayer films, although both of them were effective against all the microorganisms tested in this study, especially against *Photobacterium phosphoreum*. Both agar and alginate bilayer films allowed to reduce significantly the microbial growth, including the pathogenic bacteria *Listeria monocytogenes*, in peeled shrimps during the chilled storage without a negative impact on the organoleptic properties.

1. Introduction

Nowadays there is an increasing interest in biodegradable packaging materials due to environmental considerations related to nonbiodegradable petrochemical-based plastics. These biodegradable packaging materials are biopolymers that come from recycling materials or renewable sources, and mainly include naturally occurring polysaccharides and proteins from plant and animal origin, as well as those synthesized chemically from naturally derived monomers such as lactic acid (Bordes, Pollet, & Avérous, 2009). Regarding polysaccharides, their wide variety of structures provides films with a large range of properties. Agar and alginate are polysaccharides extracted from marine algae with good film-forming properties. Packaging materials with high mechanical strength and moderate water resistance have been obtained from agar (Phan, Debeaufort, Luu, & Voilley, 2005). Furthermore, agar seems a good matrix to obtain active biodegradable packaging films since allows the release of antioxidant and antimicrobial compounds when active compounds are incorporated (Gimenez, López de Lacey, Pérez-Santín, López-Caballero, & Montero, 2013). Water insoluble films have been obtained from alginate, since this polysaccharide has the ability to react with polyvalent metal cations to produce strong gels and reduce the water vapor permeability of the films (Pavlath, Gosset, Camirand, & Roberton, 1999). This property of crosslinking allows alginate to be used as a drug controlled release vehicle in drug delivery systems (Wang, Hu, Du, & Kennedy, 2010).

The incorporation of essential oils in polymeric matrices to give antimicrobial and antioxidant properties has been widely studied. Among them, cinnamon essential oil (EO) has demonstrated a high and broad-spectrum antimicrobial activity (Chao, Young, & Oberg, 2000). The use of biodegradable films with EO is a promising technology to fish and meat preservation and some studies have been published in recent years. For example, cinnamon EO has been incorporated to alginate-calcium and chitosan coatings to maintain the quality of northern snakehead and trout fillets (Lu, Ding, Ye, & Liu, 2010; Ojagh, Rezaei, Razavi, & Hosseini, 2010). However, one limitation to the use of EO in food preservation is the persistence of the strong aroma that could affect the organoleptic properties of the foodstuff (Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2011).

The term bilayer film was defined as two hydrocolloid layers casted one over another (Rivero, García, & Pinotti, 2009). According to these authors, bilayer chitosan-gelatin films showed better mechanical and water vapor barrier properties than the corresponding composite films. Furthermore, Thu, Zulfakar, and Ng (2012) have reported that bilayer hydrocolloid film based on alginate can be exploited as slow-release wound healing vehicle, in which the

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drug-free lower layer acted as a rate-controlling membrane. Moreover, the use of bilayer films could allow the incorporation of essential oils to polymeric matrices in effective doses, reducing the negative sensory repercussions when the EO is added in the upper layer. To our knowledge there are no references about this subject.

The objective of this study was to develop alginate and agar bilayer films with cinnamon EO and to evaluate the release of antioxidant and antimicrobial compounds from both matrices, together with the antimicrobial effect of these films in peeled shrimps inoculated with *Listeria monocytogenes*. Furthermore, the sensory impact of the application of these films with cinnamon EO on chilled peeled shrimps was assessed.

2. Material and methods

2.1. Preparation of bilayer films

Both agar and alginate bilayer films were prepared by a two-step coating technique, and the formulations are summarized in Table 1. This method involves forming one film (lower layer) and after drying, the polymer solution of the second layer (upper layer) is poured directly on top of the lower dried layer. For the lower layers, agar (Goldagar, Hispanagar, Burgos, Spain) and sodium alginate (Manuel Riesgo, Madrid, Spain) were dissolved in distilled water (1 g/100 mL) at 90 °C and 70 °C, respectively, for 30 min under continuous stirring. Glycerol was used as plasticizer (1 g/100 mL). In the case of alginate film forming solution, the cross-linking of the polymer was induced by the addition of $CaCl_2$ (Panreac, Barcelona, Spain) to the filmogenic solution (0.07 g/g of sodium alginate) in order to obtain insoluble films. The lower layers of agar and alginate bilayers were made by casting 25 g of agar and alginate film forming solution, respectively, on polystyrene Petri plates (12 cm × 12 cm), drying afterward at 45 °C in a forced-air oven for 12 h (FD 240, Binder, Tuttlingen, Germany). For the upper layers, agar and alginate were dissolved as described for the lower layers. Cinnamon EO (*Cinnamomum zeylanicum*; Isabrubotanik S.A., Ambato, Ecuador) was added at 2 g/100 mL and glycerol was used as plasticizer at 0.5 g/100 mL since the essential oil has plasticized effect. In the case of alginate film forming solution, the cross-linking of the polymer was induced by the addition of $CaCl_2$ as described for the lower layer. Both filmogenic solutions were sonicated for 1 min at 100% amplitude to emulsify the EO. Finally the agar and alginate film forming solutions were cast by pouring 25 g above the dried lower layers, and the composites were then dried again in the forced-air oven at 45 °C for 12 h.

2.2. Release of cinnamaldehyde and eugenol in water

The release of cinnamaldehyde and eugenol from agar and alginate bilayer films was determined following the method described by Giménez, Gómez-Guillén, López-Caballero, Gómez-Estaca, and Montero (2012) with some modifications. The release was determined during 72 h in water, which is considered as an aqueous food simulant by European law (EC, 1997), at both 20 °C (room temperature) and 4 °C (temperature for chilled storage of food). Pieces of the agar and sodium alginate bilayer films (9 cm^2) were placed in beakers with 15 mL of distilled water. The undisclosed films were removed at different time intervals. The remaining solution was filtered through Whatman no. 1 filter paper and used to analyze both the cinnamaldehyde and eugenol released from the film matrices, as well as the antioxidant capacity.

The cinnamaldehyde and eugenol released from the film matrices after 0.25, 9, 24 and 72 h in water at 4 °C and 20 °C was quantified using a GC–MS system (Agilent Technologies, USA) composed of a gas chromatograph with an integrated auto-sampler and a splitless injector, coupled to an inert mass spectrometer. The aqueous samples were diluted with hexane (1:100) and vigorously shaked in vortex for 5 min. After phase separation, 10 μL of an internal standard (2-octanone in hexane) was added to 500 μL of the organic phase. The injection volume was 0.2 μL. Chromatographic separation was performed on a column HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, USA). Injector was operated at 230 °C. Helium was employed as carrier gas, with a constant flow of 1 μ/min. The oven temperature profile was as follows: start at 40 °C and hold for 5 min, increase to 160 °C at a rate of 8 °C/min, increase to 220 °C at a rate of 20 °C/min and hold for 3 min. The mass spectrometer was operated in the electron impact mode (EI) and quadrupole mass filter. The ionization energy was 70 eV and the transfer line was 230 °C. Selected ion monitoring (SIM) was applied for quantitative analysis of 2-octanone, cinnamaldehyde and eugenol. Results were expressed as mg cinnamaldehyde per g of film and mg eugenol per g of film based on a standard curve of cinnamaldehyde and eugenol, respectively (Sigma–Aldrich, Madrid, Spain).

2.3. Antioxidant activity of the bilayer films

The antioxidant activity released from the agar and sodium alginate bilayer films during 72 h in water at 4 °C and 20 °C was monitored by both FRAP and ABTS assays. The ABTS radical [2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), Sigma–Aldrich] scavenging capacity was determined according to a modified version of the method of Re et al. (1999). Results were expressed as mg Vitamin C Equivalent Antioxidant Capacity (VCEAC) per g of film based on a standard curve of vitamin C (Sigma–Aldrich). All determinations were performed at least in triplicate.

FRAP (ferric reducing ability of plasma) is a measurement of the reducing ability of samples and was performed according to the method described by Pulido, Bravo, and Saura-Calixto (2000). Results were expressed as μmol $FeSO_4 \cdot 7H_2O$ equivalents/g of film based on a standard curve of $FeSO_4 \cdot 7H_2O$ (Sigma, Aldrich). All determinations were performed at least in triplicate.

2.4. Antimicrobial activity of the bilayer films

The antimicrobial activity of the bilayer films with cinnamon EO was evaluated by the disk diffusion method in agar against 8 microorganisms. The species, selected because of its importance in human health (either lactic acid bacteria or pathogens) or for being responsible for food spoilage, were obtained from the Spanish Type Culture Collection (CECT): *Photobacterium phosphoreum* CECT 4192, *Staphylococcus aureus* CECT 240, *Salmonella choleraesuis* CECT 4300, *Escherichia coli* CECT 515, *Pseudomonas fluorescens* CECT 4898, *L. monocytogenes* CECT 4032, *Vibrio parahaemolyticus* CECT 511T, *Shewanella putrefaciens* CECT 5346T.

The agar and sodium alginate bilayer films with cinnamon EO were aseptically cut into 10 mm diameter discs and then placed on the agar petri dishes previously seeded with the corresponding

Table 1
Formulation of agar and alginate bilayer films.

	Biopolymer (g)	Glycerol (g)	$CaCl_2$ (g)	EO (g)	Distilled water (mL)
Agar					
Lower layer	1	1	–	–	100
Upper layer	1	0.5	–	2	100
Alginate					
Lower layer	1	1	0.07	–	100
Upper layer	1	0.5	0.07	2	100