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Antioxidant and antimicrobial properties of ethylene vinyl alcohol copolymer films based on the release of oregano essential oil and green tea extract components

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ABSTRACT

Polymer films with antioxidant and antimicrobial properties were manufactured for the active packaging of food products. Green tea extract (GTE) and oregano essential oil (OEO) were incorporated in an ethylene–vinyl alcohol copolymer (EVOH). Release studies of the main active components of the films into three food simulants, 3% acetic acid, 10% ethanol and 50% ethanol, were conducted at 4 °C and 23 °C. The total antioxidant activity at equilibrium was measured in the food simulants and the antimicrobial capacity of the films was tested *in vitro* against *Listeria monocytogenes*, *Escherichia coli* and *Penicillium expansum*. The results suggest that the release kinetics depend on the affinity between the active agents and the food simulants. In general, the fastest diffusion was obtained when films were exposed to 50% ethanol and that effect was concurrent with greater antioxidant efficiencies. The films also showed microbial growth inhibition in liquid media and in vapour phases, demonstrating that the developed films show strong potential for development as active food packaging films.

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1. Introduction

Traditional food packaging methods do not always provide the required shelf-life to allow a satisfactory commercialization of foods, particularly perishable fresh or minimally-processed products (Quintavalla and Vicini, 2002). Active packaging with antioxidant or antimicrobial properties can improve fresh food stability and potentially offer shelf-life extension to certain foods. Current trends in active packaging include the incorporation of active natural agents into the polymeric packaging materials (Gomez-Estaca et al., 2014; Suppakul et al., 2003). In these developments, the time-controlled release of such substances into the food has the potential to provide prolonged food safety and quality. Polymers are very attractive in active packaging applications since the well-established mass transport properties (permeation, sorption and migration) can constitute the mechanism of action of the active packaging system. However, such release requires certain control, especially to initiate the action at the correct time. Recently, ethylene–vinyl alcohol copolymers (EVOH) have been explored as an active inner coating on diverse substrate films (Muriel-Galet et al., 2013, 2012b). In these systems, the high affinity between EVOH and water, which results in severe plasticization of the copolymer when it is exposed to foods with high water activity, was used as a triggering mechanism for the film activity.

Green tea is considered to be a healthy product with its consumption linked to lower incidences of various pathological conditions due to its reported anti-inflammatory, anti-tumour, antioxidative and antimicrobial properties (Bansal et al., 2013). The chemical composition of green tea is complex and the activity of its components can vary significantly with climate and seasons. The most important bioactive constituents of green tea include polyphenols or flavonoids such as catechins, alkaloids such as caffeine, amino acids, volatiles and minerals (Bansal et al., 2013; Davidson et al., 2005). It is known that the mixture of catechins is responsible for the high antioxidant effect of green tea, and other reports have shown that different types of catechins are responsible for its overall antimicrobial activity (Hara, 2001). Due to the advantageous properties and classification as a food additive by the European Union (EU), green tea extracts (GTEs) have been incorporated in different food packages in order to extend product shelf-life based on their inherent antioxidant properties (Lopez-de-Dicastillo et al., 2012b). However, reports on the use of GTEs as antimicrobial agents for food-packaging applications are limited.

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Nevertheless, the thermal degradation of catechins is a limiting factor to be considered on active packaging applications (Wang et al., 2006).

Oregano essential oil (OEO) is recognised as a natural antimicrobial agent with a strong potential for food preservation and is classified as a food additive by the EU. Similarly to GTE, the chemical composition of OEO can vary significantly between season, climate, and geographical location. Several studies have described the wide range of antimicrobial effects of OEOs against food-borne pathogens (Klein et al., 2013; Moore-Neibel et al., 2013). Several studies have reported the composition of OEO which commonly includes a mixture of *p*-cymene, α-terpinene, thymol and carvacrol with the latter two components representing the main antimicrobial constituents that can damage the outer membrane of many types of bacteria (Kulisic et al., 2004). In addition to the antimicrobial characteristics, some studies have reported antioxidant effects of these compounds that protected lard products from oxidation processes (Amorati et al., 2013; Kulisic et al., 2004). The used of OEOs as food additives is limited because of their intense aroma that may impart unacceptable organoleptic properties to some food products. Further improvements to the beneficial antimicrobial and antioxidant properties with simultaneously minimizing any adverse sensory effects could be achieved with the combined use of these with other natural compounds. Also, the volatile character of the most relevant constituents of OEO needs to be considered in the preparation of packaging materials to obtain an efficient method of incorporation (Efrati et al., 2014; Suppakul et al., 2003).

The aim of this study was to develop EVOH films that simultaneously provide protection against oxidation and microbial deterioration of food products. Films were prepared by casting procedure which can be considered as a simulation of industrial film coating by printing procedures (Muriel-Galet et al., 2013). After the incorporation of GTE and/or OEO in EVOH, the characterization of the release of these agents into different food simulants was performed. Finally, the *in vitro* antioxidant activity and the antimicrobial activity of the systems against Gram-positive *Listeria monocytogenes*, Gram-negative *Escherichia coli* and fungal species *Penicillium expansum* were also studied.

2. Materials and methods

2.1. Materials, chemicals and reagents

Ethylene vinyl alcohol copolymer with a 29% molar content of ethylene (EVOH29, Soarnol DT2904RB®), was kindly provided by the Nippon Synthetic Chemical Company (Osaka, Japan). Chemical agents 1-propanol, oregano essential oil (*CAS No*: 8007-11-2), reagent-grade absolute ethanol, acetic acid, 2,2-diphenyl-1-picrylhydrazyl (95% free radical) and carvacrol were purchased from Sigma (Madrid, Spain).The green tea extract was supplied from Plantextrakt (The Nature Network, Baceiredo S.L., Vitoria, Spain). Caffeine, catechin, epigallocatechin were purchased from Fluka Biochemika (Barcelona, Spain). Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was purchased from Sigma (Madrid, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

2.2. Film preparation

Film samples were prepared according to a process previously reported (Muriel-Galet et al., 2012a). Briefly, EVOH29 was dissolved in a 50% v/v 1-propanol/water mixture at 50 °C. The GTE and OEO were added to the film-forming solution at a concentration of 5% w/w with respect to the dry polymer weight. The films were prepared by firstly spreading the solution on a flat surface with a coating bar and then evaporating the solvents with hot air. Films of EVOH29 without active compounds, with 5% w/w GTE (EVOH29-GTE), with 5% w/w OEO (EVOH29-OEO) and with 5% w/w of each, GTE and OEO (EVOH29-GTE+OEO) were prepared. The average film thickness of the film samples was20 ± 2 μm (individually measured before each experiment). Samples were stored under dry conditions in aluminium foil until use.

2.3. Differential scanning calorimetry

The thermal properties were determined with a Mettler Toledo Differential Scanning Calorimetry (DSC) 1 instrument. The glass transition (*Tg*) and melting (*Tm*) temperatures and the melting enthalpy (ΔHm) were calculated using STAR^{e}Evaluation Software (Mettler Toledo). (ΔHm) values were corrected to account the percentage of polymer actually introduced in the pan. Film samples ofEVOH29, EVOH29-GTE, EVOH29-OEO, and EVOH29-GTE + OEO were dried for 1 week in a desiccator over P_2O_5 before analysis. Thermograms were obtained by heating samples encapsulated in 40 μL crucibles from 25 °C to 220 °C, cooling to 25 °C, and a second heating to 220 °C. All heating and cooling rates were 10 °C min^{-1}.

2.4. Optical properties

The film colour was determined using a Konica Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). The films were placed on the surface of a standard white plate and three measurements were taken of each sample. The measurements were repeated in triplicate. The parameters L^* (black (0) to white (100)), a^* (green (−) to red (+)) and b^* (blue (−) to yellow (+)) were obtained and the polar coordinates, the chroma C^* and the hue angle $h°$ calculated.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}; \quad h^o = arctan\left(\frac{a^*}{b^*}\right) \tag{1}$$

2.5. Quantification of GTE and OEO components

The concentrations of epicatechin, catechin and caffeine, that are the main relevant compounds with antimicrobial and antioxidant activity of GTE, were determined using high performance liquid chromatography (HPLC). A Shimadzu GCU-20A5 HPLC instrument equipped with a 250 × 4.6 mm, 5 μm column and UV–visible absorbance detector set at 220 nm was used. The mobile phase was acetonitrile/water 10:90% v/v containing 0.1% phosphoric acid at 1 mL min^{-1} flowrate, the temperature was 23 °C and the injection volume was 15 μL. The concentration of carvacrol, the major component of OEO, was determined by gas chromatography using a Shimadzu GC-2100 Plus instrument (Shimadzu, Australia) under the following conditions: DB-5 capillary column (30 m × 0.32 mm, and 0.50 mm); column temperature, 80–120 °C at 5 °C/min; injector temperature, 250 °C; FID temperature, 300 °C; split ratio, 1/100; carrier gas, N_2; flow rate, 2.0 mL/min; and volume injected,1μL. Calibration for each active agent of OEO and GTE was performed by injecting known amounts of each compound into the respective HPLC or GC instruments.

2.6. Release into food simulants

The release of the active components of GTE and OEO from EVOH29-GTE + OEO into three food simulants was carried out at 4 °C and 23 °C. Food simulants A, B and D1 were selected according to the European Regulations: A, 10% v/v ethanol as an aqueous food simulant; B, 3% v/v acetic acid as an acidic food simulant

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