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Antifungal films based on starch-gelatin blend, containing essential oils

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ABSTRACT

The antimicrobial activity of essential oils (EO) is very well-known and it has been reported that incorporating them into edible films based on biopolymers extends the food's shelf-life. In this study, cinnamon, clove and oregano EO, at 25% with respect to the polymer, were incorporated into glycerol plasticized starch-gelatin blend films (ratio 1:1) in order to elucidate their effect on the physical (barrier, mechanical and optical), structural and antifungal properties of the films. Whereas EOs exhibited no significant effect on tensile behavior in the case of casting films conditioned at 53% relative humidity and 25° C, the EO compounds did significantly reduce the water vapor and oxygen permeability of the films. Likewise, the EOs increased the films' transparency but reduced their gloss. Despite the fact that about 60% of the incorporated EOs were lost during the film drying step, they exhibited antifungal activity against the two tested fungal species, *Colletotrichum gloesporoides* (CG) and *Fusarium oxysporum* (FOG), as revealed by the in vitro agar diffusion method.

1. Introduction

Starch is widely available from renewable sources and has a great ability to form edible films with reasonable mechanical resistance, which can be used to coat food products in order to prevent moisture loss or to protect them from oxidation. Starch films are transparent, tasteless, odorless and have very good oxygen barrier properties. Nevertheless, some drawbacks must be improved, such as the water sensitivity and retrogradation phenomena, both giving rise to changes in the film barrier and mechanical properties during storage (Cano, Jiménez, Cháfer, González, & Chiralt, 2014; Jiménez, Fabra, Talens, & Chiralt, 2012, 2013). Blending starch with other polymers with different compatibility, such as chitosan or sodium caseinate (more compatible) or poly-ε-caprolactone (non-compatible), using different compatibilizers, can be a useful strategy by which to improve the properties and stability of starch-based films (Averous & Boquillon, 2004).

Starch-gelatin blend films have been studied by different authors (Al-Hassan & Norziah, 2012; Fakhouri et al., 2013) who report that these blends offer advantages in terms of oxygen and water vapor barrier properties, mechanical behavior and optical parameters (Wang, Rakotonirainy, & Papua, 2003). Even though gelatin may improve the performance of starch-based materials, both polymers are highly hydrophilic, which limits the water vapor barrier capacity of the blend films.

Recently, Acosta, Jiménez, Cháfer, González-Martínez, and Chiralt (2015), have reported the benefits of the addition of lipids on the starch-gelatin blend films with different polymer ratios. They report that cassava starch films with 50% gelatin exhibited highly adequate properties for the purposes of food coating or packaging. Gelatin blending gave rise to more resistant, harder and more extensible films than pure starch material. In this blend, although lipid incorporation improved the film's stretchability and water barrier capacity, it enhanced oxygen permeability. In this sense, essential oils (EOs) are interesting lipids that can be used in

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starch-gelatin blend films, due to their antimicrobial and antioxidant capacity which adds functionality to the film materials. Likewise, some essential oils have been effective at reducing the oxygen permeability of different polymer films in line with their oxygen scavenging activity (Bonilla, Talón, Atarés, Vargas, & Chiralt, 2013).

The antibacterial activity of EOs has been reported by many authors (Alves-Silva et al., 2013; Burt, 2004), but few studies analyse the antifungal effect of these compounds (Alves-Silva et al., 2013; Avila-Sosa et al., 2012; Perdones, Sánchez-González, Chiralt, & Vargas, 2012; Perdones, Vargas, Atarés, & Chiralt, 2014; Roselló, Sempere, Sanz-Berzosa, Chiralt, & Santamarina, 2015; Saggiorato et al., 2012; Sanchez-González, Cháfer, Chiralt, & González-Martínez, 2010). The EOs affect the microbial cells via different mechanisms, including interactions with the phospholipid bilayer of the cell membrane, the disruption of enzyme systems and compromising the genetic material of bacteria (Burt et al., 2007). The use of EOs as food preservatives is limited by their strong flavour, but embedding them into a polymer matrix represents an alternative means of reducing their sensory impact while the diffusion of the antimicrobials to the product may be modulated (Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López, 2013). Nevertheless, the non-polar nature of the EO components requires the homogenization techniques in order to incorporate them into the aqueous film forming dispersions (FFD) of hydrocolloids, such as starch or gelatin. During the drying of the FFD cast to obtain the films, a part of the EO is lost as the water evaporates, the amount depending on the stability of the initial emulsion and the extension of the coalescence and creaming phenomena that occur in this step (Perdones et al., 2014; Sanchez-Gonzalez, González-Martínez, Chiralt, & Chafer, 2011), which affects the final bioactivity of the film. Likewise, the presence of a lipid dispersed phase and the potential interactions of lipid molecules with the polymer also affect the film's functional properties (Chiralt, Talens, Monedero, & Fabra, 2015).

The aim of this study was to analyze the effect of three different essential oils (cinnamon bark, clove and oregano) on the functional properties of cassava starch-bovine gelatin (ratio 1:1) blend films, in terms of the tensile, barrier, optical and structural properties, as well as their antifungal effect against two fruit fungal pathogens (*Colletotrichum gloesporoides:* CG and *Fusarium oxysporum f.sp. gladiolo*: FOG) through in vitro tests.

2. Materials and methods

2.1. Raw materials

Cassava starch, with 9.28 wt% amylose and an amylose:amylopectin ratio of 1:9.8, was produced by Asia CO, LDT (Kalasin, Thailand) and purchased by Quimidroga SA (Barcelona, Spain). Bovin skin type A gelatin (Bloom 220–240°), used in combination with starch, was supplied by Sancho de Borja, S.L. (Zaragoza, Spain), and glycerol, used as plasticizer, was provided by Panreac Química, SA (Barcelona, Spain). Essential oils (oregano, clove and cinnamon bark) were acquired from Herbes del Moli (Benimarfull, Alicante, Spain). Table 1 shows the main components of EO used in this study and their boiling point. Stock culture of *Colletotrichum gloesporoides* (CG), isolated from citrus trees from Valencia (Spain), was provided by the Mediterranean Agroforest Institute (Universitat Politécnica of Valencia, Spain). *Fusarium oxysporum f.sp. gladiolo* (FOG) CECT 2868 was supplied by the Colección Española de Cultivo Tipo (CECT, Burjassot, Spain). These were preserved frozen in Agar Potato Dextrose (PDA, Scharlab, Barcelona, Spain), then incubated at 25 °C until sporulation, and were used after 7 days of active growth.

2.2. Preparation of film-forming dispersions

The film forming dispersions were prepared from a 2% (w/w) polymer aqueous solution, using the same proportion of cassava starch (S) and gelatin (G). Every formulation contained glycerol as plasticizer (polymer:glycerol ratio of 1:0.25). Furthermore, three more formulations were prepared by adding essential oils as antimicrobial agents (polymer:essential oil ratio of 1:0.25). So, four formulations were considered with the following codes: SG for the control starch-gelatin blend films, SG-C, SG-Cl and SG-O, respectively for films containing cinnamon, clove, and oregano essential oils.

Firstly, starch and gelatin dispersions were prepared separately. Starch aqueous dispersions were maintained at 95 °C for 30 min to induce starch gelatinization. Meanwhile, gelatin was dissolved in water at 40 °C. Then, the glycerol was added to the dispersion blend and homogenized by using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 1 min and 20,500 rpm for 3 min at 95 °C under vacuum, in agreement with previous studies (Jiménez et al., 2012). In these conditions, air incorporation was minimized and the oil droplet size was reduced mainly in the second step at higher speed. For formulations containing essential oils, these were added prior to the homogenization step.

Controlled volumes of film-forming aqueous dispersions (equivalent to 1.5 g of solids) were cast into leveled Teflon® casting plates (15 cm diameter) and dried at 25 °C and 45% RH for 48 h. Then, they were peeled intact from the plates and conditioned at 53% RH and 25 °C in a chamber with magnesium nitrate-6-hydrate saturated solution (Panreac Química, S.A., Barcelona, Spain). In this way, the film application under intermediate relative humidity and room temperature was simulated.

2.3. Characterization of the films

2.3.1. Microstructure

The microstructural analysis of the films was carried out by scanning electron microscopy (SEM), using a JEOL microscope model JSM-5410 (Japan). Prior to testing, films were equilibrated in desiccators with P_2O_5 in order to remove any water present in the samples. SEM observations were carried out by considering the surfaces and cross sections of film samples. Films were frozen in liquid nitrogen, and then cryofractured to observe the cross section. Samples were fixed on copper stubs, gold coated to make them conductive (for 1.5 min) and observed directly with an accelerating voltage of 10 KV.

2.3.2. Water vapor permeability

The water vapor permeability (WVP) of films was determined following the gravimetric method ASTM E96-95 (1995) for a 53–100% relative humidity gradient at 25 °C by using Payne permeability cups (Payne, Elcometer SPRL, Hermelle/s Argenteau, Belgium) of 3.5 cm diameter. The RH gradient of 53–100% of the cabinet was obtained using oversaturated solutions of $Mg(NO_3)_2$ and distilled water, respectively. The weight of the cup was measured every 2 h using an analytical balance (±0.00001 g). The water vapor transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area. Water vapor permeability (WVP) values were obtained, in quadruplicate, according to previous studies (Jiménez et al. 2012).

2.3.3. Oxygen permeability

The oxygen permeability (OP) of the films was analyzed in film samples (50 cm^2) by using an Oxtran system (Mocon, Minneapolis,