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Development and characterization of bilayer films of FucoPol and chitosan

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ABSTRACT

Bilayer films of FucoPol and chitosan were prepared and characterized in terms of optical, morphologic, hygroscopic, mechanical and barrier properties, to evaluate their potential application in food packaging. Bilayer films have shown dense and homogeneous layers, and presented enhanced properties when comparing to monolayer FucoPol films. Though, a high swelling degree in contact with liquid water (263.3%) and a high water vapour permeability (0.75×10^{-11} mol/ms Pa), typical of polysaccharide films, was still observed. However, they presented a low permeability to O_2 and CO_2 (0.47×10^{-16} mol m/m^{2}s Pa and 5.8×10^{-16} mol m/m^{2}s Pa, respectively). Tensile tests revealed a flexible and resistant film with an elongation at break of 38% and an elastic modulus of 137 MPa. The studied properties, in particular the excellent barrier to gases, impart these bilayer films potential to be used in packaging of low moisture content products, as well as in multilayered hydrophobic/hydrophilic/hydrophobic barriers for food products with a broader range of water content.

1. Introduction

Primary packaging, defined as "a sales unit to the final user or consumer at the point of purchase" (European Parliament and Council Directive, 1994) tends to be the most visible aspect of packaging (Barlow & Morgan, 2013). The use of synthetic non-biodegradable polymers for primary packaging was tremendous in the last century, mainly because they are low-cost and present good mechanical and thermal properties, and are good barriers to gases, aroma compounds and microorganisms. Though primary packaging is mandatory for food preservation and protection, the intensive use of synthetic plastics created serious environmental problems because they are non-biodegradable and non-renewable materials.

This severe problem can be minimized using biodegradable natural and bio-based polymers instead of the non-biodegradable synthetic ones (Muscat, Adhikari, Adhikari, & Chaudhary, 2012; Siracusa, Rocculi, Romani, & Rosa, 2008).

As such, the attention has been driven to the search of alternative materials for use in food packaging. Bio-based films are usually designed from biodegradable, non-toxic and edible polymers (e.g. polysaccharides and proteins) and lipids (Galgano et al., 2015). The use of blends and multi-layers of those materials are strategies for new composite materials development, with properties that turn them potential synthetic polymers substitutes (Debeaufort, Quezada-Gallo, & Voilley, 1998; van den Broek, Knoop, Kappen, & Boeriu, 2015).

Polysaccharides obtained from plant, algae, animal and microbial origin (e.g. starch, alginate, chitosan, gellan gum) have been widely used for edible and/or biodegradable films development (Khwaldia, Arab-Tehrany, & Desobry, 2010; Song, Murphy, Narayan, & Davies, 2009; van den Broek et al., 2015). Such films are usually poor barriers to water vapour but good barriers to gases.

FucoPol, one of the microbial polymers referred in the literature, is a fucose-rich exopolysaccharide produced by the bacterium Enterobacter A47 (Alves, Freitas et al., 2010; Torres et al., 2011). It is a high molecular weight heteropolysaccharide composed of neutral sugars (fucose, galactose, glucose), an acidic sugar (glucuronic

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acid) and acyl groups (acetate, succinate and pyruvate). Glucuronic acid, together with succinate and pyruvate, are responsible for the anionic character of the biopolymer (Freitas et al., 2011). FucoPol has film forming capacity and its films have been reported to be transparent, with brown tone, hydrophilic with high permeability to water vapour and good barrier properties to gases (CO_2 and O_2) (Ferreira et al., 2014).

Chitosan is derived from chitin, which is the most abundant natural amino polysaccharide and the second most abundant biopolymer in nature. Crustacean shells, a food industry waste, are one of the chitin main sources (Ravi Kumar, 2000). Chitosan is a copolymer of β-(1-4)-2-cetamido-D-glucose and β-(1-4)-2-amino-D-glucose units, with the latter usually exceeding 60%. It possesses a cationic character, antimicrobial properties and film forming capacity (Elsabee & Abdou, 2013). Chitosan films have a selective permeability to gases (CO_2 and O_2) and good mechanical properties, but are highly permeable to water vapour that limits their use in food packaging applications.

The improved properties obtained by the combination of different hydrocolloids have been reported for several systems. Blends and bilayer films of chitosan and anionic polymers have been reported to have improved mechanical and barrier transport properties comparing to single component based films. This fact was attributed to the formation of polyelectrolyte complexes through electrostatic interactions between the protonated amino groups of chitosan and the negatively charged side-chain groups in the other biopolymer at the operating pH (Elsabee & Abdou, 2013; Luo & Wang, 2014; Nowzari, Shábanpour, & Ojagh, 2013).

Improvement in mechanical properties, better performance in terms of water vapour permeability and lower water solubility have been reported for blends and bilayer films of chitosan with starch, pectin or alginate (Jindal, Kumar, Rana, & Tiwary, 2013; Luo & Wang, 2014; Xu, Kim, Hanna, & Nag, 2005), gelatin (Rivero, García, & Pinotti, 2009) or whey (Kurek, Galus, & Debeaufort, 2014), comparing to chitosan stand-alone films.

Some authors reported difficulties in total solubilization of one of the polymers in specific conditions and formation of insoluble complexes between polymers in blends preparation (Ferreira, Nunes, Delgadillo, & Lopes-da-Silva, 2009). Otherwise, bilayer systems are reported to have better water vapour barrier properties than blend films (Kurek et al., 2014; Rivero et al., 2009). In this context, the aim of the present study is to develop bilayer films in combination with chitosan in order to enhance the properties of FucoPol films. The films were characterized in terms of their optical, hygroscopic, surface, mechanical and barrier properties, envisaging their potential use in food-packaging applications.

2. Materials and methods

2.1. Materials

FucoPol was produced and purified as described by Ferreira et al. (2014). The freeze dried FucoPol was analysed in terms of chemical composition and average molecular weight. Commercial medium molecular weight chitosan (deacetylation degree of 75–85%) was purchased from Sigma (USA). Citric acid monohydrate was purchased from VWR chemicals—BDH Prolabo (UK). Glycerol (analytical grade) was used as plasticizer and purchased from Sigma (USA).

2.2. FucoPol chemical composition

FucoPol dried samples (5 mg) were hydrolyzed (2 h at 120 °C) with trifluoroacetic acid (TFA) (0.1 ml TFA 99%), and the hydrolysate was used for the identification and quantification of the constituent sugar monomers by ion chromatography (HPIC), using a CarboPac PA10 column (Dionex), equipped with an amperometric detector. The separation was performed at 30 °C with a gradient of NaOH (0.018–0.025 mol l^{-1}) and CH_3COONa (0–0.17 mol l^{-1}). Fucose, glucose, galactose and glucuronic acid (Sigma, USA) were used as standards at concentrations between 0.005 and 0.1 g l^{-1}.

The acid hydrolysates were also used for the identification and quantification of acyl groups by HPLC with an Aminex HPX-87H column (BioRad) coupled to a UV detector.

The analysis was performed using sulphuric acid (0.005 mol l^{-1} H_2SO_4) as eluent, at a flow rate of 0.6 ml min^{-1} and a temperature of 30 °C. Acetate, pyruvate and succinate (Sigma, USA) were used as standards at concentrations between 0.015 and 1.0 g l^{-1}.

2.3. FucoPol average molecular weight

The EPS averages molecular weight (Mw) were determined by size exclusion chromatography-multi-angle laser light scattering (SEC-MALLS—Wyatt Technology Corporation Dawn Model). The FucoPol solutions (2 g l^{-1}) were prepared in 0.1 M Tris–HCl, NaCl (0.2 mol l^{-1}), pH 8.1 buffer, which was also the SEC mobile phase. The SEC columns (PL aquagel-OH mixed 8 μm, 300 × 7.5 mm) were equilibrated for 24 h before running the analysis at a flow rate of 0.7 ml min^{-1} at room temperature. Each analysis was conducted in duplicate. Signals from MALLS were recorded in parallel and treated with Astra (V 4.73.04) in order to follow the purity and molecular mass distribution of the polysaccharide. A dn/dc of 0.190 ml g^{-1} was adopted to calculate the Mw.

2.4. Films preparation

FucoPol filmogenic solutions were prepared by dissolving freeze dried FucoPol in distilled water (1.5% w/w) under stirring, at room temperature, until complete dissolution. Then, citric acid (50% $w_{citric\ acid}/w_{dried\ polymer}$) was added and the solution was left under stirring for complete homogenization.

Chitosan films were prepared by dissolving chitosan in an acetic acid (1% w/w) solution, at a concentration of 1.5% (w/w). After stirring overnight at ambient temperature, glycerol (50% $w_{glycerol}/w_{dried\ polymer}$) and citric acid (50% $w_{citric\ acid}/w_{dried\ polymer}$) were added, followed by stirring for complete homogenization.

Air bubbles were removed under vacuum, and the solution was transferred to Teflon petri dishes and left to dry at 30 °C, during 24 h.

Bilayer films were prepared by a two-step coating technique. Firstly, the FucoPol solution, prepared as described above, was cast onto a Teflon petri dish and then dried at 30 °C until a firm but still adhesive surface was obtained. Then, the chitosan solution, prepared as described above, was cast on the top of FucoPol film and both layers were dried at 30 °C during 24 h.

2.5. Morphological characterization

The morphology of the bilayer films was evaluated by scanning electron microscopy (SEM) using a FEG-SEM JEOL JSM7001F (Oxford) equipment, with the acquisition system JEOL software PC-SEM. Samples were coated with chromium sputtering (Quorum Technologies, Q150TES) to enable the observation of surface and cross section. For cross-section observation, the films were cryofractured by immersion into liquid nitrogen.