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Physical and functional characterization of active fish gelatin films incorporated with lignin

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ABSTRACT

In order to provide gelatin films with antioxidant capacity, two sulphur-free water-insoluble lignin powders (L_{1000} and L_{2400}) were blended with a commercial fish-skin gelatin from warm water species at a rate of 85% gelatin: 15% lignin (w/w) (G–L_{1000} and G–L_{2400}), using a mixture of glycerol and sorbitol as plasticizers. The water soluble fractions of G–L_{1000} and G–L_{2400} films were 39.38 ± 1.73% and 46.52 ± 1.66% respectively, rendering radical scavenging capacity (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS assay)) of 27.82 ± 2.19 and 15.31 ± 0.88 mg vitamin C equivalents/g film, and ferric ion reducing ability (FRAP assay) of 258.97 ± 8.83 and 180.20 ± 5.71 μmol Fe^{2+} equivalents/g film, respectively. Dynamic oscillatory test on film-forming solutions and Attenuated Total Reflectance (ATR)-FTIR spectroscopy study on films revealed strong lignin-induced protein conformational changes, producing a noticeable plasticizing effect on composite films, as deduced from the study of mechanical (traction and puncture tests) and thermal properties (Differential Scanning Calorimetry, DSC). The gelatin films lose their typical transparent and colourless appearance by blending with lignin; however, the resulting composite films gained in light barrier properties, which could be of interest in certain food applications for preventing ultraviolet-induced lipid oxidation. Lignin proved to be an efficient antioxidant at non-cytotoxic concentrations, however, no remarkable antimicrobial capacity was found.

1. Introduction

Gelatin has been one of the most studied biopolymers on account of its film-forming ability and its usefulness as an outer film to protect food from drying and exposure to light and oxygen (Arvanitoyannis, 2002). Fish gelatins exhibit good film-forming properties, yielding transparent, nearly colourless and highly extensible films (Avena-Bustillos et al., 2006; Carvalho et al., 2008; Gómez-Estaca, Montero, Fernández-Martín, & Gómez-Guillén, 2009; Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006; Zhang, Wang, Herring, & Oh, 2007). Furthermore, enriching gelatin films with antioxidants and/or antimicrobial substances will extend the functional properties of these biodegradable films and provide an active packaging biomaterial. Because of "clean labelling" concerns, there is growing interest in using natural compounds, such as polyphenolic plant extracts (Gómez-Guillén, Ihl, Bifani, Silva, & Montero, 2007) or α-tocopherol (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2008) in the formulation of active fish gelatin films.

Lignin, most commonly derived from wood, is largely thrown off as a waste product in pulp and paper industries. It is a complex polydisperse natural polymer made up of phenyl-propane (C6–C3) units that bind cellulose fibres together, thus hardening and strengthening the plant cells. Lignin derivatives have been incorporated as fillers in different synthetic polymer matrices to develop lignin-based materials with improved physical properties (Cui, Xia, Chen, Wei, & Huang, 2007; Feldman, Lacasse, & Beznaczuk, 1986; Kadla & Kubo, 2003; Mishra, Mishra, Kaushik, & Khan, 2007). During the last decade, a great deal of research was devoted to the development of lignin-containing biopolymeric materials, on account of its renewable, non-toxic and biodegradable character (Ban, Song, & Lucia, 2007; Baumberger, Lapierre, Monties, Lourdin, & Colonna, 1997; Chiellini, Cinelli, Fernandes, Kenawy, & Lazzeri, 2001; Julinová et al., 2010; Li & Sarkanen, 2002; Vengal & Srikumar, 2005; Wu, Wang, Li, Li, & Wang, 2009). Lignin has been referred to as a plasticizing agent in composite films with starch (Wu et al., 2009). However, in composites prepared using adipic acid-modified starch microparticles within a corn-starch matrix, addition of lignin produced higher tensile strength and lower elongation capacity (Spiridon, Teaca, & Bodirlau, 2011). Similarly, lignin acted as a reinforcing agent with cellulose (Rohella, Sahoo, Paul, Choudhury, & Chakravortty, 1996) or polyethylene oxide

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(Kadla & Kubo, 2003), in all cases providing adequate miscibility with the polymer. In addition, the incorporation of small amounts of lignin into polypropylene films has been shown to stabilize the composite material against photo-oxidation (Kosikova, Demianova, & Kakurakova, 1993). The hydrophobic nature of lignin has been also shown to produce strong reduction of water absorbency and transparency in starch-based films (Ban et al., 2007).

Due to their complex polyphenolic nature, lignins can exert antioxidant (radical scavenging capacities) (Dizhbite, Telysheva, Jurkjane, & Viesturs, 2004; Lu, Chu, & Gau, 1998; Pan, Kadla, Ehara, Gilkes, & Saddler, 2006; Satoh et al., 1999; Ugartondo, Mitjans, & Vinardell, 2008) and antimicrobial properties (Dong et al., 2011), thus opening up the possibility of new potential applications. As a result of the molecular complexity of lignins, it becomes difficult to assign the antioxidant efficacy to specific structural components, compared to the activities of chemically defined tannins and flavonoids (Sakagami et al., 2005). The radical scavenging activity of lignins is influenced by structural features, such as the presence of phenolic hydroxyl groups, methoxy groups, π-conjugation systems as well as the molecular weight, heterogeneity and polydispersity (Dizhbite et al., 2004). Only a few studies have reported the cytotoxic effects of lignins. A good correlation between cytotoxicity and some features such as carbohydrate content and polydispersity has been reported; the lignins with higher polydispersity and lower carbohydrate content are the most cytotoxic (Ugartondo et al., 2008). Lignin derivatives have been shown to be effective antioxidants at concentrations that are not harmful to normal human cells, thus furthering their possible use in the formulation of active food packaging biomaterials (Núñez-Flores et al., 2012; Ugartondo et al., 2008). In this sense, the appearance, protein quality and oxidative stability of salmon fillets subjected to high pressure processing were enhanced by the combined use of a gelatin–lignin film similar to the one characterized in the present study (Ojagh, Núñez-Flores, López-Caballero, Montero, & Gómez-Guillén, 2011).

The antimicrobial properties of lignins have been reported previously in the literature, both in model system and in experimental animals, for example, from hydrolysates of several lignocellulosic materials (ethyl acetate extracts) (Cruz, Domínguez, Domínguez, & Parajó, 2001), lignin-related structures from alkaline extractions (Oh-Hara et al., 1990), kraft-lignins (Dizhbite et al., 2004) and to a lesser extent from lignosulphonates (Núñez-Flores et al., 2012). The origin of lignin might influence their antimicrobial properties. Thus, Oh-Hara et al. (1990) reported that the antimicrobial activity induced by commercial lignins was much lower than that induced by fractions of pine cone extracts obtained by successive alkaline extractions (and then recovered as acid precipitates at pH 5). By comparing the spectra, these authors found that some pine cone extract include more alkenic double bonds and fewer OCH3 than commercial alkali-lignin, while a coumaryl type of lignin structure could be responsible for the antimicrobial activity.

The aim of the present work was to produce antioxidant fish gelatin films by mixing gelatin with two types of lignin, and to characterize structural, mechanical, optical, and thermal properties of the composite active material. In order to establish the harmlessness and potential functionality in food packaging applications, cytotoxicity, radical scavenging capacity and antimicrobial capacity of lignin were also tested.

2. Materials & methods

2.1. Materials

Commercial type A warm-water fish gelatin was supplied by Rousselot S.A.S. (Puteaux, France). For comparison purposes, two sulphur-free water-insoluble commercial lignin powders were used: Protobind 2400 (L_{2400}) and Protobind 1000 (L_{1000}) (Granit Recherche & Developpement SA, Lausanne, Switzerland). According to manufacturer's specifications, both lignins aqueous suspensions presented pH ~ 4, number average molecular weight ~1000 Da and particle size <210 micron, differing in bulk density (~0.55 kg/l in L_{2400} *vs* ~0.30 kg/l in L_{1000}) and in softening temperature (~130 °C in L_{2400} *vs* ~200 °C in L_{1000}). Glycerol and sorbitol were obtained from Panreac (Barcelona, Spain). All other reagents used were of analytical grade. The 2,4,6-tripyridyl-s-triazine, the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical, Vitamin C and the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical were purchased from Sigma–Aldrich (St. Louis, MO, USA). The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) salt was supplied by Promega Biotech Ibérica (Madrid, Spain).

2.2. Preparation of films

The gelatin–lignin film forming solution (FFS) was prepared by dissolving the fish gelatin in distilled water (3.4% w/v) at 40 °C, adding sorbitol (15 g/100 g gelatin) and glycerol (15 g/100 g gelatin) as plasticizers. The lignin powder was added to a final concentration of 0.6% w/v in the FFS. This concentration was selected according to previous experiments. The mixture was stirred at 40 °C for 15 min and was alkalinized to ~pH = 11 to obtain a good blend with total solubility. The films were made by casting an amount of 40 ml over a plate of 12 × 12 cm^2 and drying at 45 °C in a forced-air oven for 15 h to yield a uniform thickness of ~100 ± 10 μm. Films were conditioned over a saturated solution of KBr in desiccators for 4 d.

2.3. Viscoelastic properties of film forming solutions

Dynamic oscillatory study of the film-forming solutions was carried out on a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle 4°, gap 0.15 mm). Cooling and heating from 30 to 2 °C and back to 30 °C took place at a scan rate of 1 °C/min, a frequency of 0.5 Hz, and a target strain of 0.5%. The elastic modulus (G'; Pa), viscous modulus (G''; Pa) and phase angle (°) were plotted as functions of temperature in the heating ramp from 2 to 30 °C. At least two determinations were performed for each sample. The experimental error was less than 6% in all cases.

2.4. Film thickness

Film thickness was measured using a digital micrometer (Mitutoyo, model MDC-25M, Kanagawa, Japan), averaging nine different locations.

2.5. Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of the films were determined using a TA.XT.plus Texture analyser (SMS, Surrey, UK). The samples were cut into rectangles (20 mm width and 50 mm length), fixed on the grips of the device with a gap of 20 mm, and tractioned at a speed of 1 mm/s. Results of TS and EAB were average of five determinations, and expressed as N/m^2 and %, respectively.

A puncture test was performed to determine the breaking force and the breaking deformation of the films. Films were placed in a cell 5.6 cm in diameter and punched to the breaking point using the same texture analyser, with a round-ended stainless-steel plunger 3 mm in diameter at a cross-head speed of 60 mm/min. Breaking force was expressed in N and breaking deformation in %, according to Sobral, Menegalli, Hubinger & Roques (2001). All determinations are the means of at least five measurements.