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Active starch-gelatin films for shelf-life extension of marinated salmon

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ABSTRACT

Biodegradable active films were obtained by casting, using glycerol plasticized oxidized corn starch (OS) and bovine gelatine (BG) blends (1:1 mass ratio), with and without ethyl lauroyl arginate (LAE) as antimicrobial agent (1.3 g LAE/100 g polymer). Water vapour barrier capacity and colour of the films conditioned at 53 or 88% relative humidity were determined. Both LAE incorporation and high RH promoted film browning, coherently with the progression of Maillard reactions between amino groups of gelatine or LAE and carbonyl groups of oxidized starch. These compounds imparted antimicrobial properties to the films with and without LAE, both exhibiting antilisterial activity in *in vitro* tests. Packaging of marinated salmon samples in these films greatly reduced the total viable counts, which remained below the legal limit after 45 storage days at 5 °C. Nevertheless, films were not effective at controlling weight loss of salmon samples during the cold storage.

1. Introduction

Active packaging protects food products against chemical contaminants, oxygen, moisture, mechanical damage or deterioration caused by spoilage microorganisms (Rhim, Park, & Ha, 2013; Sung et al., 2013). Although petroleum-derived synthetic plastics have been commonly used for food packaging, their serious environmental impact has driven current research towards biobased/biodegradable food packaging materials (Byun & Kim, 2014). Of the biodegradable polymers, starch is one of the most promising materials due to its ready availability, low cost and food compatibility. Nonetheless, starch films have some drawbacks, mainly associated with their highly hydrophilic nature (Du et al., 2008; Ortega-Toro, Jiménez, Talens, & Chiralt, 2014).

Different strategies have been used to improve properties of starch films, such as blending with other polymers o chemical modification (Masina et al., 2016). Starch oxidation promotes hydrophobicity of starch chains (-OH number reduction) through the oxidative cleavage of the C-2 and C-3 bond of the anhydroglucose units, giving rise to di-aldehyde starch (DAS) (Du et al., 2008; Yu, Chang, & Ma, 2010). DAS can act as a crosslinking agent when blended with proteins through the condensation reaction between carbonyl and amino groups (Azeredo & Waldron, 2016), thus improving the film functionality (Martucci & Ruseckaite, 2009; Rhim, Gennadios, Weller, Cezeirat, & Hanna, 1998). Starch-gelatin blend films exhibited good mechanical performance (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015; Fakhouri et al., 2013). They are food contact materials and could be used as carriers of antimicrobials to obtain active films. Promotion of crosslinking in the matrix by starch oxidation could favour the film ability to control the antimicrobial release (De Oliveira Pizzoli et al., 2016), while enhancing the film water vapour barrier capacity.

LAE (*N*-α-lauroyl-*l*-arginine ethyl ester monohydrochloride) is a potent antimicrobial agent, derived from lauric acid, L-arginine, and ethanol, with a wide spectrum of antimicrobial activity (Muriel-Galet, López-Carballo, Hernández-Muñoz, & Gavara, 2014), reported to cause cell growth inhibition or death by increasing the permeability of the cell membrane, as a consequence of the membrane protein denaturation (Rodriguez, Seguer, Rocabayera, & Manresa, 2004). It is considered as GRAS (Generally recognized as safe) by the FDA with a maximum dose of up to 200 ppm (Kang et al., 2014), and accepted as food additive (E243, in Europe) for several food products (Hawkins, Rocabayera, Ruckman, Segret, & Shaw, 2009; Higueras, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013).

Fish and seafood products are highly perishable mainly due to microbial spoilage (Aubourg et al., 2007), which make them good candidates for preservation by using antimicrobial packaging. Moreover, they are highly susceptible to *Listeria* spp. contaminations, which represent a huge problem for food safety, due to the

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high resistance of the bacteria under refrigeration conditions (Cornu et al., 2006) and the high mortality rate associated with listeriosis (Scallan et al., 2011). Therefore, development of effective strategies to reduce the initial contamination levels and to inhibit the growth of these bacteria in fish products is essential. Antimicrobial packaging containing LAE may be a good means of facing up to this issue, since LAE has been successfully proven to be effective at controlling the growth of *Listeria moncytogenes* in different food matrices, such as roasted turkey (Jiang, Neetoo, & Chen, 2011), frankfurters (Porto-Fett et al., 2010), cheese (Soni, Desai, Oladunjoye, Skrobot, & Nannapaneni, 2012) or cooked cured ham (Stopforth, Visser, Zumbrink, Van Dijk, & Bontenbal, 2010). The application of LAE for the purposes of salmon preservation has been reported to require a higher dose than the maximum allowed, 200 ppm, since its partitioning into the lipid phase reduces the molecules available for direct contact with bacteria (Kang et al., 2014). Its inclusion in a food compatible polymer matrix could mitigate this problem, improving the effectiveness of the active compound through its controlled release to the potentially contaminated product surface.

The aim of the present study was to characterize antimicrobial films based on oxidized corn starch and bovine gelatin, by analysing the effect of LAE incorporation on the film functional properties, as well as to study the effectiveness of the films at controlling microbial growth (with special emphasis on the antilisterial activity) in marinated salmon and at extending its shelf life.

2. Materials and methods

2.1. Materials

Corn starch (Roquette Laisa España, S.A.), bovine gelatin type A (BG) (Sancho de Borja, S.L., Zaragoza, Spain), ethyl-lauroyl-arginate (LAE) at 10 % wt in ethanol (Vedeqsa, Lamirsa, Terrassa, Spain) were used. Sodium periodate was supplied by Sigma-Aldrich (Madrid, Spain). Glycerol, magnesium nitrate and potassium chloride were supplied by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain). Microbiological products were supplied by Scharlab (Barcelona, Spain). *Listeria innocua* (CECT 910) was supplied by Colección Española de Cultivos Tipo (CECT, Burjassot, Valencia, Spain).

2.2. Film preparation

Starch was oxidized according to the procedure described by Wang et al. (2015), using sodium periodate. Starch (10 % wt.) and oxidant were dispersed in distilled water at 1:1 M ratio, with respect to the glucose units (solution pH 3.5). Reaction occurred at 35 °C for 4 h, under magnetic stirring in the dark. Afterwards, dispersion was vacuum filtered to stop the reaction. The filtrate was washed three times with distilled water at 8000 rpm (Ultraturrax T25, Janke and Kunkel, Germany) for 30 s and vacuum filtered. Moisture content of the oxidized starch (OS) was determined gravimetrically.

Films were prepared with a 1:1 mass ratio of OS dry solids and BG, using glycerol (0.25 g/g dry polymer blend) as plastizicer, with (OS:BG:LAE) and without LAE (OS:BG). LAE was added at 0.013 g/g of dry polymer blend. The LAE concentration was fitted in order not to exceed the legal limit (200 ppm, Kang et al., 2014) when released into the food. OS was dispersed in distilled water (2 % wt.) and then gelatinized under stirring at 99 °C for 1 h. BG was dispersed in distilled water (2 % wt.) under magnetic stirring at 40 °C. OS and BG dispersions were mixed and glycerol was added. LAE was afterwards added to this dispersion. Formulations were finally vacuum degasified.

The films were obtained by casting the mass of film-forming dispersion containing 1.5 g of solids per casting plate (15 cm diameter). After drying for 48 h at 45% RH and 25 °C, the films were peeled off and conditioned for one week at either 53 or 88% RH, using saturated solutions of magnesium nitrate or potassium chloride, respectively. These conditions were chosen because 53% RH is a common storage condition for the films, whereas 88% can be the equilibrium value in the films when applied to food products with intermediate a_w values.

2.3. Film characterization

2.3.1. Moisture content and water vapour permeability (WVP)

Moisture content (g of water per 100 g of dry film) was gravimetrically determined (six replicates) by desiccation in a convection oven (60 °C, 24 h) and subsequent equilibration in desiccator containing P_2O_5, until constant weight.

Water vapour permeability was determined using the standard method (ASTM E96-95), and considerations of McHugh, Avena-Bustillos, and Krochta (1993) for hydrophilic films. Circular film samples were fitted to 3.5 cm diameter Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) containing distilled water and then put into desiccators with the controlled RH at 5 °C. Six replicates were made per film formulation and RH gradient (53–100% and 88–100%). The cups were weighed every 1.5 h, for 24 h. WVP values were determined as previously described (Atarés, Bonilla, & Chiralt, 2010) from the slope of the weight loss *vs.* time relationship when the steady state was reached.

2.3.2. Optical properties: transparency, colour and gloss

The film CIE-L*a*b* colour coordinates (lightness, Lab*; chrome, Cab* and hue, hab*; D65 illuminant/10° observer) and internal transmittance (Ti) were obtained from the reflectance spectra measured on both black and white backgrounds, by using a spectrocolorimeter (CM-3600d, Minolta Co., Tokyo, Japan), as previously described by Atarés et al. (2010). The infinite reflectance (R_∞) of the films were obtained by applying the Kubelka–Munk theory for multiple scattering (Hutchings, 1999). Measurements were taken on the free film surface with six replicates per formulation and conditioning conditions.

The film gloss was measured at a 60° incidence angle, following the ASTM standard D-523 (ASTM., 1999), using a flat surface gloss meter (Multi-Gloss 268, Minolta Co., Tokyo, Japan). Measurements were taken on the free film surface with 15 replicates per formulation.

2.3.3. In vitro antimicrobial activity of the films

The antimicrobial activity of the films against *Listeria innocua* (CECT 910) was analysed. The strain, which was initially kept frozen in TSB with 30% glycerol, was regenerated by inoculating a loopful in 10 mL TSB. After incubation (24 h at 37 °C), 10 μl were transferred into 10 mL TSB, which was incubated for 24 h at the same temperature to obtain the culture with exponential growth phase. Tubes with 10 mL of TSB were inoculated with 10^4 CFU/mL of *L. innocua*. Film samples (stored for 1 week and 5 months), 5.3 cm in diameter, were introduced into the inoculated tubes, using inoculated tubes without film as control. Tubes were incubated at 37 °C for 0, 5 and 24 h and bacterial counts were performed. Palcam agar was used as the specific medium for *Listeria*. All of the tests were run in duplicate.

2.4. Preparation and characterization of salmon samples

Fresh salmon, purchased in a local supermarket, was marinated