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Wool keratin film plasticized by citric acid for food packaging

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ABSTRACT

Wool keratin (natural resource) and citric acid (effective preservative) were mixed in water to produce a transparent film for application in active packaging. This film showed excellent biocidal effect, high elongation value (600%) and little loss of keratin after immersion in water. The capability of citric acid to bind keratin macromolecules by hydrogen bonds is probably responsible for the improvement of film's extensibility. On the other hand, the study of FT-IR spectrum allows understanding that the presence of citric acid in aqueous solution enhances the content of alpha helix structure in the film, with a reduction in the amount of side chain and disordered conformations in the macromolecular structure. Carrot shelf-life was qualitatively improved with this film in comparison with a commercial film for preserving food. Consequently, this film can have possible application for food packaging as a substitute of synthetic polymers replacing them with a natural, environmental friendly and renewable resource.

1. Introduction

Innovative packaging materials that can extend the shelf-life, maintaining consumer safety, reducing losses to the production sector and being environmental friendly, has been the focus of extensive efforts. Particularly, biopolymers such as lipids, proteins, polysaccharides and mixtures thereof, have been investigated to enhance the properties of films as packaging materials (Caro, Medina, Díaz-Dosque, López, & Abugoch, 2016). There is a current trend to incorporate active agents into packaging materials, to maintain the quality and to enhance the safety of packaged foods (Fabra, López-Rubio, & Lagaron, 2016). In food technology, active packaging with antibacterial property is a concept based on incorporating an antimicrobial compound inside the packaging material or using a packaging material with inherent antimicrobial properties to reduce or inhibit microbial growth (Appendini & Hotchkiss, 2002).

Traditionally, antimicrobial agents are directly mixed into the initial food formulations, but this direct addition may change the taste of the food and result in the inactivation or evaporation of active agents with rapid migration into the bulk of the foods; consequently, antimicrobial activity is rapidly lost. As antimicrobial packaging materials must contact the surface of food, the antimicrobial agents could spread out to its surface, enhancing the shelf-life and safety of packaged food (Long, Joly, & Dantigny, 2016).

Keratin is a natural protein extracted from wool or other natural resource (Fraser, MacRae, & Rogers, 1972). In different works it has been used to produce nanofibers (Aluigi, Corbellini, Rombaldoni, Zoccola, & Canetti, 2013; Varesano, Vineis, Tonetti, Sanchez Ramirez, & Mazzuchetti, 2014; Varesano et al., 2015) because of its special absorption properties of metal ions like Cu (Aluigi, Tonetti, Vineis, Tonin, & Mazzuchetti, 2011), Cr (Aluigi et al., 2012; Aluigi et al., 2009), dyes like methylene blue (Aluigi, Rombaldoni, Tonetti, & Jannoke, 2014) and volatile organic compounds like formaldehyde (Aluigi et al., 2009) and because of its possible applications as scaffolds for human body (Tachibana, Furuta, Takeshima, Tanabe, & Yamauchi, 2002; Tachibana, Kaneko, Tanabe, & Yamauchi, 2005) thanks to its biodegradability (Yamauchi, Maniwa, & Mori, 1998). Unfortunately, keratin has not good mechanical properties, so it is necessary to use it in blend with a synthetic and not biodegradable polymer (Aluigi et al., 2011; Aluigi et al., 2009).

On the other hand, it is possible to find several works where citric acid was employed with natural polymers as compatibilizer and plasticizer to improve the mechanical properties of films. In fact, citric acid has properties to form stronger hydrogen bonds with hydroxyl groups to prevent recrystallization and to enhance the interactions between molecules in polymers as polyvinyl alcohol, starch and polycaprolactone (Ghanbarzadeh, Almasi, & Entezami, 2011; Jiugao, Ning, & Xiafoei, 2005; Ortega-Toro, Collazo-Bigliardi, Talens, & Chiralt, 2016; Reddy & Yang, 2010; Shi et al., 2008). In addition, citric acid has antibacterial properties (In, Kim, Kim, & Oh, 2013; Pundir & Jain, 2011)

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and it is used in many different products to preserve different types of food (Pundir & Jain, 2011; Soccol, Vandenberghe, Rodrigues, & Pandey, 2006). The aim of this work is to obtain a film produced by casting using citric acid and keratin in aqueous solution, to use it in food industry as an active packaging because of its antimicrobial activity and plasticizer property. The film was analyzed by means of TGA, DSC and FT-IR. Moreover, strength tension, elongation at break, antibacterial efficiency, transparency and loss of weight after immersion in water were measured. Finally, the carrot shelf-life preserved with the keratin–citric acid film was qualitatively evaluated and compared with one of the commercial films for food packaging.

2. Materials and methods

2.1. Keratin extraction and purification

Keratin was extracted from wool by sulfitolysis with sodium metabisulfite (Aluigi et al., 2007). Preliminarily, the wool fibers were cleaned by Soxhlet extraction with petroleum ether to remove fatty matter and washed with distilled water. An amount of 15 g of cleaned fibers were cut into snippets and treated with 300 ml of a solution containing urea (8 M) and sodium metabisulfite (0.5 M), adjusted to pH 6.5 with sodium hydroxide (5 N), under shaking for 2 h at 65 °C. The mixture was filtered with 30 μm and then 5 μm pore-size filters, and the keratin aqueous solution obtained was dialyzed against distilled water with a cellulose tube (3500-Da molecular weight cutoff) for 3 days at room temperature, changing the distilled water frequently. The keratin solution was frozen and then lyophilized with a Heto PowerDry PL3000 freeze dryer to obtain soluble and pure keratin.

Citric acid (99%) was purchased from Sigma Aldrich. Citric acid and keratin were dissolved in distilled water to obtain a final concentration of 15 wt.% of keratin and 10 wt.% of citric acid. The solution was shaken until dissolution by means of a magnetic stirrer ensuring its completed homogeneity. After that, 3 ml of solution was poured in a polyethylene bowl and dried at 20 °C and 65%RH to form a film by casting. The film was separated from the support and cut in a rectangular form of 1 cm × 2 cm. The same volume of solution and bowl dimension was maintained for each test to standardize the production process.

2.2. UV–vis (transparency and immersion in water)

In order to measure the keratin–citric acid film transparency, an analysis employing UV–Visible Spectrometer (Lamba 35 PerkinElmer) between 200 and 700 nm was carried out. In addition, sample pictures of film were taken with a camera.

Samples (0.1 g) were immersed in 5 and 20 ml of water up to 170 h in order to verify the water stability. The water after film immersion was qualitative analyzed in UV–Visible spectrometer between 200 and 700 nm. The intensity of peaks were measured at different immersion times. In addition, gravimetric analyses at different times (between 5 min and 2 h) were carried out measuring the loss of weight in the dried samples; the quantification of protein in solution was realized with Bio-Rad Protein Assay (Micro Assay) based on the Bradford method. The Protein Assay Dye Reagent Concentrate with one standard (bovine serum albumin) were purchased from Bio Rad.

The dry weight was measured using the moisture analyzer DBS 60-3 equipped with a Halogen quartz glass heater 400 W. The measurements were carried out in Drying mode AUTO (standard drying), where the drying temperature was set at 105 °C and the drying process was finished automatically when the present weight loss remained constant for 30 s.

In addition, the pH of water after film immersion was measured with a PC 8 Instrumental Bench (pH/mV/COND/TDS/°C) – CARLI Biotec equipped with a polymer electrode type (Basic Pro pH).

2.3. Thermal treatments

Some samples were heated in an oven to study the effect of annealing on the mechanical properties of film. These were subjected to heat treatments in air at different temperatures (80, 100 and 120 °C) for one hour. Samples were conditioned for at least 24 h at 20 °C and 65% RH before and after each heat treatment.

2.4. TGA and DSC analysis

The thermogravimetric analysis was done with a Mettler Toledo TGA-DSC. About 2.5 mg of film was put in a 70 ml aluminum oxide crucible for the analysis. The calorimeter cell was flushed with nitrogen at 70 ml min^{-1}. The TGA data were elaborated with a Mettler Toledo STARe system. The run was performed from 30 to 500 °C with a heating rate of 10 °C min^{-1}. Derivative thermogravimetry (DTG) was used to identify the temperature of maximum mass-loss rates.

Differential scanning calorimetry (DSC) was performed with a Mettler Toledo DSC calorimeter calibrated by an indium standard. The calorimeter cell was flushed with 100 ml min^{-1} nitrogen. The run was performed from 30 to 450 °C, at the heating rate of 10 °C min^{-1}. The mass was close to 2.5 mg of sample.

2.5. Mechanical tests and thickness measurement

In order to evaluate tensile strength and elongation at break an Instron (USA) 5500R tensile testing machine, configured with a 10 N load cell with a velocity of 10 mm min^{-1} was used.

Scanning electron microscopy (SEM) instrument was used to make a measurement of thickness in seven different samples on eight different points for each of them. Measurements were carried out with a LEO (Leica Electron Optics) 435 VP SEM. Operative parameters were 15 kV acceleration voltage and 30 mm working distance. Specimens were sputter-coated with gold before SEM analysis in an Emitech K550 Sputter Coater with a current of 20 mA for 5 min in rarefied argon at 20 Pa.

2.6. FT-IR analysis

IR spectra were recorded with a Thermo Nicolet Nexus spectrometer by an attenuated total reflection technique with a Smart Endurance accessory (equipped with a diamond crystal ZnSe focusing element) in the range from 4000 to 550 cm^{-1} with 50 scans and 4 cm^{-1} band resolution. Ommic 6.2 software (by Thermo Electron) was used to perform attenuated total reflection baseline correction. Tests were performed on several parts of each sample to evaluate the citric acid distribution homogeneity.

2.7. Antibacterial test

Antibacterial tests were performed employing the gram positive *Staphylococcus aureus* by using AATCC 100 Test Method. In the procedure was used 1 ml of bacteria standardized inoculum containing 1.5–3 × 10^5 CFU ml^{-1} on the sample. After one-hour 100 ml of sterile buffer were added on the sample, the buffer was then diluted 10 times and pleated on Petri dishes with suitable agar. The Petri dish was incubated at least 24 h at 37 °C, then the bacteria colonies were counted. Bacterial reduction is calculated as $((B - A)/B) * 100$, where A is the number of colonies of the sample with the film and B is the number of colonies of the sample without film (reference).

2.8. Qualitative assessment of carrot shelf-life

Carrots pieces were stored on the laboratory bench at 20 °C of temperature and 65% of humidity. In order to realize the quality assessment of carrot shelf-life, the different pieces of carrot were packed