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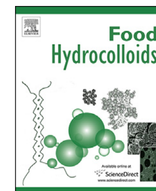

Development of active films of chitosan isolated by mild extraction with added protein concentrate from shrimp waste

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ABSTRACT

Chitosan prepared through a mild process using 10% NaOH during deacetylation and a protein concentrate (PCc) obtained from shrimp waste was used to develop active films. Film forming solutions were prepared by solubilizing chitosan with lactic acid without plasticizers addition. The structural properties of films, as observed by optical microscopy, Cryo-SEM, DSC and FTIR, were strongly affected by the addition of the PCc. Films with added PCc were much more tensile resistant but less deformable upon perforation. The presence of PCc also improved film barrier to the light. The films showed good antioxidant and antimicrobial properties, enhanced by the incorporation of PCc. The significance of these films lies in the fact that proteins do not usually appear to enhance antimicrobial properties, and therefore this is especially useful for food applications.

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1. Introduction

The incorporation of antioxidant and antimicrobial agents into edible films is one of the landmark advances in food technology today. The need for new antimicrobial packaging with low migration of active substances remains a major safety concern because of food spoilage. For environmental reasons it is increasingly desirable for at least the packaging to be biodegradable and where possible from low-cost, sustainable sources (Conte, Angiolillo, Mastromatteo, & Nobile, 2013).

In this context, shrimp waste remains a valuable source of chitin, proteins and carotenoids, which are bioactive substances in great demand by the food and pharmaceutical industries (Mezzomo, Maestri, dos Santos, Maraschin, & Ferreira, 2011; Pérez-Santín, Calvo, López-Caballero, Montero, & Gómez-Guillén, 2013; Rødde, Einbu, & Vårum, 2008). Chitosan is obtained by N-deacetylation of chitin, and the isolation process basically involves deproteinization and demineralization (Gildberg & Stenberg, 2001). The process has been extensively studied, and this article places special emphasis on the influence of molecular weight and degree of N-acetylation, and also the influence of the processes and conditions under which this polymer is prepared on physical, chemical and biological properties (Kumirska, Weinhold, Thöming, & Stepnowski, 2011). Traditional methods generally involve significant consumption of reagents and therefore appear to be technically less eco-friendly, and moreover are not very effective in the recovery of protein from the exoskeletons (Valdez-Peña et al., 2010).

Chitosan has recently attracted more interest due to its antimicrobial properties, biodegradability, biocompatibility and film forming ability (Devlieghere, Vermeulen, & Debevere, 2004). Most studies on chitosan active packaging have focused on improving active properties (Feng, Du, Li, Hu, & Kennedy, 2008; Yu et al., 2013). In this connection, protein concentrates and carotenoids from shrimp waste are known to possess strong antioxidant activity (Manni, Ghorbel-Bellaaj, Jellouli, Younes, & Nasri, 2010; Pérez-Santín et al., 2013; Sowmya, Rathinaraj, & Sachindra, 2011). Furthermore, Arancibia et al. (2014) found also noticeable antimicrobial properties in caroteno-protein concentrates recovered from shrimp processing residues (head and exoskeleton). Multifunctional proteins containing antimicrobial sub-domains have been identified in some crustacean groups; some of these proteins have been characterized in vivo as part of the animals' immune system (Rosa & Barracco, 2010). Much attention is being paid to improving

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the stability and functionality of crustacean polysaccharide-protein complexes for the development of products (Romero, Verwijlen, Guerrero, & Vermant, 2013). The incorporation of chitosan with an astaxanthin-rich shrimp protein concentrate was found to increase the antioxidant and antimicrobial properties of the resulting coating solution (Arancibia et al., 2014).

The objective of the present investigation was to use a mild-processed chitosan and a protein concentrate obtained from shrimp (*Litopenaeus vannamei*) processing waste, for the development and characterization of active edible films from marine origin with antioxidant and antimicrobial properties.

2. Materials and methods

2.1. Recovery of film forming material

The protein concentrate was prepared from frozen shrimp (*L. vannamei*), kindly provided by Angulas Aguinaga Burgos (Burgos, Spain), as described in a previous work (Arancibia et al., 2014). Briefly, shrimp waste (cephalothorax and exoskeleton) were suspended in water (1:1 ratio w/v) and subjected to autolysis by incubation at 40 °C for 4 h with constant stirring. After thermal inactivation at 80 °C for 20 min, the suspension with the autolysed residues was kept at 2 °C overnight. An organic extraction was carried out using an acetone:ethanol mixture (1:1 v/v) at 40 °C for 2 h with constant stirring, with a waste suspension:solvent ratio of 1:3 (w/v). After 3 h decanting, three well-defined phases were formed. The intermediate phase, with an intense orange colour, opaque and viscous appearance, was filtered through cheesecloth to separate the solid residue, consisting of chitinous material. The liquid phase was further subjected to organic extraction under the same conditions as described above. The resulting aqueous phase was centrifuged (10,000 × g − 30 min −5 °C), the supernatant was discarded and the pellet was lyophilized, and constituted the protein concentrate ***PCc***.

Mild-processed chitosan was obtained from chitinous material, following a demineralization using lactic acid (75.6 g/L), ratio 1:3 (w/v) for 36 h at 21 °C. The residue was then collected and the protein was removed through an enzymatic hydrolysis with Viscozyme® L (Sigma–Aldrich Quimica, S.L. Spain) (pH 4.5, 50 °C) followed by hydrolysis with Alcalase® (Sigma–Aldrich Quimica, S.L. Spain) 2.4 L (pH 8.5, 50 °C), using the pH-stat (TIM 856, Radiometer Analytical, Villeurbanne Cedex). The enzyme inactivation was carried out at 90 °C by 10 min. Removal of acetyl groups from the chitin was achieved by using 10% NaOH solution, ratio 1:7 (w/v) for 72 h at 100 °C with constant stirring. The resulting chitosan ***Ch*** was washed to neutrality with distilled water.

2.2. Chitosan characterization

The viscosity average molecular weight (Mv) of chitosan was calculated from experimental intrinsic viscosity [η] (mL/g) of chitosan in 0.1 M acetic acid and 0.2 M sodium chloride solution measured by a Cannon–Fenske glass capillary viscometer (Cannon Instrument Co., USA; size 50) at 25 °C utilizing the Mark-Houwink–Sakurada–Staudinger equation: $[\eta] = K_m M v^a$, where $K_m = 1.81 \times 10^{-3}$ cm^3 g $a = 0.93$. The mean of four replicates was taken for the calculation (Roberts & Domszy, 1982).

The deacetylation degree (DD) was determined by the method of Miya, Iwamoto, Yoshikawa, and Mima (1980). Considering the −OH band at 3450 cm^{-1} as a reference, the acetyl content (%) was determined from the ratio of absorbance: (A_{1655} cm^{-1}/A_{3450} cm^{-1}) × 100/1.33, which was calculated by baseline drawing for these absorbances (Domard & Rinaudo, 1983).

2.3. Film preparation

A starter chitosan solution and a protein concentrate solution were prepared separately. Chitosan solution (2% w/w) was made using chitosan synthesized in our laboratory and dissolved in 0.15 M lactic acid solution (pH 3.2). The protein concentrate recovered from a shrimp waste was dispersed in 0.15 M lactic acid solution (1% w/w) and, sonicated (Q700, Qsonica, CT, USA) for 1 min at 100% amplitude. The chitosan filmogenic solution was prepared by blending the chitosan starter solution (2% w/w) with 0.15 M lactic acid solution (pH 3.2) ratio of 1:1. The final concentration of chitosan in the filmogenic solution was 1% (w/w). The blended chitosan-protein filmogenic solution was prepared with a ratio 1:1 w/w of each starter solution at a final concentration of 1% (w/w) of chitosan and 0.5% (w/w) of caroteno-protein concentrate.

The viscosity of the filmogenic solutions was determined at 25 ± 0.1 °C using a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) with cone-plate geometry (cone angle 4°, gap 0.15 mm), at a constant shear rate of 0.5 s^{-1}. Results were averages of five determinations and were expressed as mPa.s.

The films were prepared casting 25 g of each filmogenic solution into methacrylate plates (120 × 120 mm) (Plexiglas® GS Röhm GmbH & Co. KG, Darmstadt, Germany). Plates were dried in a ventilated oven (FD 240 Binder, Tuttlingen, Germany) for 12 h at 45 ± 0.5 °C. All films were conditioned at 58.0 ± 0.2% RH for 4 days prior to analysis. Finally the samples were called as **Ch-F** for chitosan films and **ChPCc-F** for composite chitosan-protein concentrate films.

2.4. Film characterization

2.4.1. Thickness

It was measured using a micrometre (MDC-25M, Mitutoyo, Kanagawa, Japan), averaging the values of 4–6 random locations in 15 films for each treatment.

2.4.2. Microstructure

Micrographs of the film surface were obtained with a stereomicroscope (Stereomicroscope Stereo Zoom L2S8 APO, Leica Microsystems, Switzerland) and an optical microscope Nikon (AFX-IIA, equipped with camera Nikon mod. FX35W Nikon Corporation, Japan). Low temperature scanning electron microscopy (cryo-SEM) (Oxford CT1500 Cryosample Preparation Unit, Oxford Instruments, Oxford, England) was used to examine representative film cross section and surface. Samples were mounted with an optical coherence tomography (OCT compound Gurr®) and mechanically fixed onto the specimen holder and cryo-fractured after mounted as described by Gómez-Guillén, Ihl, Bifani, Silva, and Montero (2007).

2.4.3. Differential scanning calorimetry DSC

Calorimetric analysis of films were performed using a differential scanning calorimeter (DSC) model TA-Q1000 (TA Instruments, New Castle, DE, USA) previously calibrated by running high purity indium (melting point, 156.4 °C; melting enthalpy, 28.44 J/g). Samples of around 10–15 mg of films were weighed within ±0.002 mg by an electronic balance (Model ME235S Sartorius, Goettingen, Germany) and then tightly encapsulated in aluminum hermetic pans. An empty pan was used as reference. They were scanned under dry nitrogen purge (50 mL/min) between 5 and 400 °C at a heating rate of 10 °C/min. Peak temperatures (T_{peak}, °C) and denaturation enthalpies (ΔH) were measured at least in triplicate, the last data being normalized to dry matter content (J/g_{dm}) after desiccation of each particular capsule. Samples were dried under silica before analyses.

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