International Journal of Biological Macromolecules 98 (2017) 676–683

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Production and characterization of films based on blends of chitosan from blue crab (*Callinectes sapidus*) waste and pectin from Orange (*Citrus sinensis Osbeck*) peel

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ARTICLE INFO

Article history:
Received 12 October 2016
Received in revised form 11 January 2017
Accepted 1 February 2017
Available online 9 February 2017

Keywords:
Biodegradable films
Edible films
Biopolymers
Extraction
Residue

ABSTRACT

The objective of this study was to develop and characterize films based on blends of chitosan and pectin, produced in laboratory scale, from industrial wastes. The chitosan was obtained by termoalcaline deacetylation of chitin, extracted from blue crab (*Callinectes sapidus*) waste and characterized according to degree of deacetylation (DD) and viscosimetric molecular weight (Mw); and pectin was extracted by conventional heating, from orange (*Citrus sinensis Osbeck*) peel and characterized according to degree of esterification (DE) and molecular weight (Mw). The Ch:P based films were prepared by the casting method in different Ch:P ratios [0: 100, 25:75, 50:50, 75:25 and 100:0], and compared to two controls [0:100 and 100:0], of commercial pectin and chitosan. Glycerol was used as a plasticizer at concentrations of 0.2 g/g macromolecules. The addition of high concentrations of pectin in the formulations resulted in films with high solubility and an increase in moisture. No significant difference ($P > 0.05$) in the degree of swelling (DS) and water vapor permeability (WVP) of the films was observed. Ch:P blend films were less stiff and therefore more elastic and flexible than films based on only one biopolymer. The control films presented better results in terms of color, being brighter and less opaque than other film formulations. These data suggest that chitosan or pectin obtained from agro-industrial waste is a potential matrix to produce biodegradable films for future food applications.

1. Introduction

Limited fossil fuel reserves and the environmental impact caused by the use of non-biodegradable plastic-based packaging, has led to the use of biopolymers recognized as safe for human consumption, such as starches, cellulose derivatives, chitosan/chitin, gums and animal or plant based proteins. These materials offer the possibility of obtaining thin films and coatings to cover fresh or processed foods to extend their shelf life [1]. Some of these biopolymers, such as chitosan and pectin, have excellent film-forming properties.

Chitosan offers real potential for applications in the food industry due to its physic-chemical properties, short time biodegradability, biocompatibility with human tissues, antimicrobial and antifungal activities, non-toxicity [2], as well as the fact that it is produced from renewable resources [3]. Chitosan, a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-b-D-glucan, is a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature, after cellulose [4]. Chitosan has been successfully used to produce packaging material for the preservation of quality of a variety of foods [5], and has a significant potential in the food industry, in view of contaminations that may be associated with food products [2]. Several authors have developed films based on commercial chitosan [6,7], whereas others have produced chitosan in laboratory scale from wastes [8], emphasizing the technological relevance of these studies.

On the other hand, pectin is a complex carbohydrate, whose monomer is galacturonic acid, which contains a variable number

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http://dx.doi.org/10.1016/j.ijbiomac.2017.02.004
0141-8130/

of methyl ester groups and whose percentage of esterified groups is reported as degree of esterification (DE). Depending on the DE, pectin is divided into two major groups: the high methoxyl pectin (HMP), with a DE higher than 50%; and the low methoxyl pectin (LMP), with a DE lower than 50% [9]. Pectin is a hydrocolloid used in the food industry mainly as a gelling, stabilizing, or thickening agent in products such as jams, yoghurt drinks, fruity milk drinks, and ice cream [10]. Usually, research studies on pectin-based films have been carried out using commercial pectin [11] or pectin produced from fruit processing wastes [12].

Nevertheless, films based on pure biopolymers have some limitation such as high solubility of pectin-based films, or poor barrier properties of chitosan-based films. Thus, one alternative to produce films with improved properties is the use of blends, which means to mix chitosan and pectin during film processing. Several studies with blends of chitosan and pectin have demonstrated that there is a high cross-linking potential between both components, imparting some favorable barrier properties for food applications [13]. In this case, polymer–polymer complexes can be formed as a result of inter-chain interactions (electrostatic interactions) when these two macromolecules are mixed in solution [14]. More specifically, hydrogen and/or electrostatic interactions between carboxylate groups of pectin and protonated amino groups of chitosan can occur, forming a more dense and compact matrix.

The objective of this study was to develop and characterize films based on blends of chitosan and pectin, produced in laboratory scale, from industrial wastes. The results of this study can enhance the importance of the involved industrial chains, and contribute to reduce some environmental concerns related to the packaging materials and highly pollutant wastes.

The raw material for chitosan production was blue crab (*Callinectes sapidus*), a crustacean produced abundantly on the northern coast of Colombia. The crab's carapace, claws, chest, legs, entrails and eggs (waste) constitute approximately 80% of the raw material for chitosan production. Moreover, in 2011, Colombia produced 207,727 tons of orange [15], of which 49.6% were used for juice production, and the remainder was waste, composed mainly of peels, from which pectin can be extracted [16].

2. Material and methods

2.1. Material and reagents

The raw materials for chitosan production was blue crab (*Callinectes sapidus*) waste, composed of shells and tongs, supplied by the Mares de Colombia factory, located in Ciénaga (Colombia). For pectin production, the raw material was orange (*Citrus sinensis*) peel, collected in Chimichagua (Colombia).

Commercial pectin and chitosan, lactic acid 2% (v/v), NaOH (5–50% w/v), HCl (1.3N), sodium hypochlorite (0.38% v/v) and other analytical grade reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, SP, Brazil), and glycerol from Labsynth (Labsynth, SP, Brazil).

2.2. Chitosan production and characterization

The raw material was first washed with hot water and dried at 70 °C for 6 h. The dry raw material was then immersed in a 5% (w/v) NaOH solution at 80 °C under continuous agitation for 3 h, in order to remove proteins. The solid precipitate was immersed in 1.3N HCl for 1 h for demineralization. Then depigmentation was performed with 0.38% (v/v) sodium hypochlorite at room temperature under continuous agitation for 1 h. The chitin obtained was deacetylated under alkaline conditions by immersion in 50% (w/v) NaOH at 105 °C for 4 h, followed by extensive water washing to remove excess alkaline solution, and then dried at 50 °C for 6 h [8].

The extracted chitosan was milled to obtain a fine powder with particle size of 150 μm, and characterized for moisture (AOAC 930.15/90) and ash (AOAC 942.05/90) content. The degree of deacetylation (DD) and viscosimetric molecular weight (Mv) was measured by potentiometric [17] and viscosimetric [18] methods, respectively, and the functional groups were identified in an infrared spectra, using an FTIR spectrometer (Thermo, Nicolet 6700 FTIR, MCT detector, range 800–4000 cm^{-1}), using the Attenuated Total Reflectance (ATR) model, according to Chen [19].

2.3. Pectin production and characterization

Pectin was produced in a batch reactor with hot acid solution, according to Durán [20]. Then, 10 g of dry orange peel were treated with 100 mL of water and heated at 80 °C for 10 min for enzyme inactivation; then, the pH of the liquid phase was adjusted to 2.0 using HCl (0.5 M). This suspension was heated at 80 °C and magnetically stirred at 200 rpm for 1 h; next, it was filtered and washed twice with acidified water. Pectin was precipitated with 96% (v/v) ethanol diluted 1:1 in water, stored for 2 h, filtered and washed with ethanol 70% and 96% (v/v), and finally dried at 40 °C.

The degree of esterification of pectin was determined according to Bochek [21]. The galacturonic acid content was determined using m-hydroxydiphenyl (colorimetric method), moisture content was determined gravimetrically at 70 °C, and ash content by oven at 550 °C. Molecular weight was calculated by size exclusion, using an HPLC Agilent 1100 system with a Superose 12 column with 50 mM ammonium acetate eluent solution, at a flow rate of 0.9 mL min^{-1}, and an ELSD (Evaporative Light Scattering Detector), carried out in the laboratory of Complex Carbohydrate Research Center, University of Georgia, USA. The functional groups were identified using an FTIR spectrometer (Thermo, Nicolet 6700 FTIR, MCT detector, range 800–4000 cm^{-1}) using an Attenuated Total Reflectance (ATR) model.

2.4. Chitosan and pectin solution preparation

Chitosan and pectin solutions were prepared following the procedure described by Hoagland and Alvarado [22,3], with slight modifications. Chitosan (2% w/v) was dispersed in 2% (v/v) lactic acid and heated in a water bath at 40 °C for 20 h, under continuous stirring at 500 rpm. Pectin 2% (w/v) was dispersed in water and the solution was heated on a hot plate at 40 °C under continuous stirring (1500 rpm) for 1 h. The dispersions were cooled down to 25 °C and filtered in order to eliminate impurities [3]. Glycerol (0.2 g/g biopolymer) was added while stirring at 500 rpm for 15 min.

2.5. Film-forming solution (FFS)

The FFSs were obtained by mixing chitosan (Ch) and pectin (P) solutions at 0:100; 25:75; 50:50; 75:25 and 100:0 Chitosan:Pectin (Ch:P) weight ratios. The blends were homogenized with a high speed mixer (Ultra-Turrax® IKA T25, Labotechnik, Germany) at 5000 rpm for 10 min. Finally, ultrasound was applied to remove air bubbles from the FFSs and the pH of all preparations was adjusted to 3.5 with a 2% (w/w) lactic acid solution.

2.6. Film production

Films were produced by casting FFSs containing different Ch:P ratios into 12 × 12 cm dishes, and dried in a controlled temperature oven (Marconi MA 035, São Paulo, Brazil) at 30 ± 0.5 °C for 48–72 h. Also, films prepared using commercial chitosan or pectin were used as controls.