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Nanocrystal-reinforced soy protein films and their application as active packaging

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ABSTRACT

Soy protein isolate (SPI) films reinforced with starch nanocrystals (SNC) were developed by simple casting method. The films were transparent and homogeneous. The opacity and degree of crystallinity increased with the amount of nanocrystals. Moisture content, total soluble matter and swelling in water were evaluated, showing a marked effect on SNC additions. As the amount of SNC increased, the films exhibited lower affinity for water. Moreover, mechanical properties were determined showing that SPI-SNC reinforced films became more resistant and less elongable as SNC amount increased. With the incorporation of a considerable amount of reinforcements, a marked variation was observed in these properties. In addition, assays performed demonstrated that β-cyclodextrins (β-CD)-containing SPI-SNC films were able to sequester cholesterol when brought into contact with cholesterol rich food such as milk. This effect was more marked as the amount of β-CD into the films increased. The methodologies developed in this work allowed yielding biodegradable films with optimized physical and mechanical properties which were assayed as active food coating.

1. Introduction

Synthetic polymers, specifically plastics, are the main packaging material on high demand since they offer versatile solutions for several needs variety of purposes. Despite this, conventional packaging polymers are being questioned due to increasing environmental concerns.

Nowadays, a great number of research studies focus on solving the problems produced by plastic waste in order to obtain environmental friendly materials. For this reason, several studies examine the possibility of substituting the traditional petroleum-based plastics by biodegradable and low-cost materials with similar properties (Famá, Goyanes, & Gerschenson, 2007; Jiménez, Fabra, Talens, & Chiralt, 2012a, 2012b). At present, the biopolymer concept is emerging. Biodegradable materials are associated with the use of renewable raw materials such as proteins and polysaccharides extracted from agricultural and animal co-products and by-products, marine or microbial sources. These materials can be environmentally degraded (via exposure to soil optimum moisture, microorganisms and oxygen) into simple substances (water and carbon dioxide) and biomass. In particular, the isolated soy protein (SPI) as raw material has shown advantages over other sources due to its exceptional film-forming properties, low cost (for its extensive production in our country and for being isolated from oil industry waste) and good barrier properties to oxygen, aromas and lipids under intermediate moisture conditions (Gennadios, Weller, & Testin, 1993). This type of proteins also produces softer, more transparent and flexible films compared to those derived from other sources (Guilbert, Gontard, & Cuq, 1995).

As in conventional packaging, bio-based materials must exhibit a number of important properties, including containment and protection of food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation and loss of volatile aromas and flavors, maintaining the sensory quality and safety of food (Roy, Saha, Kitano, & Saha, 2012).

On the other hand, materials developed from natural polymers are commonly associated with poor properties, often having less important properties compared to those of commodity polymers. As opposed to most synthetic plastics used as a packaging material, most of the currently available bio-plastics do not fulfill the requirements of food packaging especially in terms of barrier and

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mechanical properties (Hendrix, Morra, Lee, & Min, 2012). Modification techniques are being developed to achieve the required property combinations for specific applications. The chemical structure of biopolymers offers possibilities for chemical reactions. The modification strategies of the starting material should enhance water resistance, the barrier effect against the flow of gases and the mechanical properties (to avoid loss by rupture or break of the material). It should also allow the incorporation of active ingredients, promote adhesion to the surface of food and increase its stability in storage conditions.

Nanocomposites are being used for the development of films with suitable properties as coating material. Improvements in the properties of the final reinforced nanocomposite material, not achieved in the individual component and not conferred by the sum of its properties, involve the synergistic effect produced by the combination of both: the matrix (film precursor) and the reinforcement. This effect arises from the presence of a reinforcement-matrix interface with own physical properties, increased by the high surface/volume ratio showed by the nanomaterials. Their incorporation into polymeric matrices yields composites with excellent properties as compared to those of conventional microcompounds without impairing density, transparency and film processability (Gao, Dong, Hou, & Zhang, 2011). Various examples of nanomaterials used as reinforcement in natural matrices are reported in the literature, in which improvements in their mechanical properties or a decrease in the total amount of water soluble matter are described. These improvements derive from the strong interactions between the matrices and the nanoreinforcements (Kristo & Biliaderis, 2007). Furthermore, particular important improvements in gas- and water-vapor barrier properties have also been obtained for similar films.

Usually, the reinforcements employed in films of this type are classified according to their dimensions in lamellar, fibrillar and particulates. The most studied nano-reinforcements are the particulates such as nanocrystals formed from semi-crystalline polysaccharides (cellulose, starch and chitin). These nanocrystals are ideal candidates for processing high performance materials, even in low concentrations (D. Chen, Lawton, Thompson, & Liu, 2012; Dufresne, 2010; Lin, Huang, Chang, Anderson, & Yu, 2011; Pires, Neto, Alves, Oliveira, & Pasquini, 2013) since they present excellent mechanical properties by reinforcing ability, abundance, low weight, biodegradability and high surface area. When nanocrystals produced from sources of polar characteristics (such as polysaccharides) are used as a reinforcement of materials based on starch or proteins, the chemical structure of the matrix and filler is similar. These structural similarities promote good miscibility and strong interfacial adhesion between both components (Lin et al., 2011). Reports on the improvements in films reached with starch nanocrystals (SNC) are described (Chen, Cao, Chang, & Huneault, 2008; García, Ribba, Dufresne, Aranguren, & Goyanes, 2011; Kristo & Biliaderis, 2007). These formed materials could be used as packaging. In particular, active food packaging is currently one of the most studied areas, stressing the development of new techniques capable of improving conservation and food quality in terms of their interaction with the packaging. According to Regulation (CE) N 450/2009 of the European Union Commission on active and intelligent materials (EU, 2009, pp. 1–11), an active food packaging comprises the group of materials intended to prolong life or maintain or improve the condition and quality of the packaged food. These containers are designed to incorporate specific components that can absorb or released substances from the packaged food. The absorber substance systems involves oxygen, carbon dioxide, ethylene, water, odors and flavors or other undesirable substances (Restuccia et al., 2010).

On the other hand, a low cholesterol diet is recommended to prevent medical conditions such as atherosclerosis, thrombosis or cardiovascular diseases. The easiest and most efficient way of controlling cholesterol levels is through diet. For this reason, there is a wide range of commercially available food low in cholesterol and fat, such as partial or completely skimmed milk.

In general, cyclodextrins (CD) are cyclic oligosaccharides comprising six (α-CD), seven (β-CD), eight (γ-CD) or more glucopyranose units joined by alpha-(1, 4) linkages. The hydrophobic cavity of β-CD can form inclusion complexes with a wide range of organic guest molecules mainly through weak interactions, such as Van der Waals, dipole–dipole and hydrogen bonding forces, while the hydrophilic surface allows dissolution of the CD in water. A report describes cholesterol/β-CD inclusion complexes in proportions ranging from 1:1 to 1:3 (Yamamoto, Kurihara, Mutoh, Xing, & Unno, 2005). Some reports document cholesterol-retaining systems based on the use of cyclodextrins in food like milk (Alonso, Cuesta, Fontecha, Juarez, & Gilliland, 2009; López-de-dicastillo, Catalá, Gavara, & Hernández-Muñoz, 2011; Tahir et al., 2013), butter (Jung, Kim, Yu, Ahn, & Kwak, 2005; Kim, Jung, Ahn, & Kwak, 2006), cream (Shim, Ahn, & Kwak, 2003), egg (Chiu, Chung, Giridhar, & Wu, 2004) and cheese (Bae, Kim, & Kwak, 2008; Han, Kim, Ahn, & Kwak, 2008).

This article describes the methodologies developed to yield SPI-based biodegradable films reinforced with SNC, which showed optimized physical and mechanical properties. The products were assayed as active food coating with cholesterol sequester properties.

2. Materials and methods

2.1. Materials

The following chemicals were used: soy protein isolate (SPI) SUPRO *E* with 90% protein on fat-free, dry-weight basis (donated by The Solae Company, Argentina), glycerol (Gly) (Taurus, Argentina), sulfuric acid (Cicarelli, Argentina), sodium hydroxide and calcium chloride (Cicarelli, Argentina), unmodified regular corn starch containing approx. 73% amylopectin and 27% amylose (Sigma, USA), cholesterol 99% (Sigma, USA) and β-ciclodextrin (β-CD) (Roquette Freres, France).

2.2. Synthesis of SNC

The SNC synthesis was developed following a method described in the literature which comprises acid hydrolysis of native corn starch (Y. Chen et al., 2008). To this, an aqueous dispersion of 22 g of starch in 150 mL of 3.16 M H_2SO_4 solution was prepared. The reaction proceeded for 5 days at 40 °C and 100 rpm. The nanocrystal purification was performed through five washes with 500 mL of water and successive filtrations to neutral pH. Then, SNC were sonicated in 500 mL of water and stored in the refrigerator. To determine SNC concentration, the dispersion was vigorously shaken and three extractions of 1 mL each were taken, dried in an oven and weighed. The amount of SNC per milliliter of dispersion was calculated as an average of three determinations.

2.3. Preparation of nanocrystal-reinforced films

The starch nanocrystal-reinforced soy protein films (SPI-SNC) were obtained by the casting method. 0.25 g of SPI was dispersed in 30 mL of water and 100 mL of Gly as plasticizer (50% w/w with respect to SPI) was added. This dispersion was stirred for 30 min and the required amount of SNC dispersion was added. Different films were prepared with 0; 2; 5; 10; 20 and 40% of SNC (in mass with respect to SPI). Prior to each addition, SNC dispersion was stirred and sonicated for 30 min. SPI dispersion including SNC was stirred a further 30 min, poured into plastic Petri dishes and dried