International Journal of Biological Macromolecules 101 (2017) 643–652

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Properties of gelatin-based films incorporated with chitosan-coated microparticles charged with rutin

Ilyes Dammak*, Ana Mônica Quinta Barbosa Bittante, Rodrigo Vinicius Lourenço, Paulo José do Amaral Sobral

Department of Food Engineering, FZEA, University of São Paulo, Avenida Duque de Caxias Norte 225, 13635-900 Pirassununga, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 4 November 2016
Received in revised form 19 March 2017
Accepted 28 March 2017
Available online 30 March 2017

Chemical compounds studied in this article:
Rutin (PubChem CID: 5280805)
Glycerol (PubChem CID: 753)
Chitosan (PubChem CID: 71853)

Keywords:
Encapsulation
Chitosan
Microparticles

ABSTRACT

The aim of this study was development an active film based on gelatin incorporated with antioxidant, rutin carried into microparticles. The complexation between oppositely charged lecithin and chitosan was applied to prepare the chitosan-coated microparticles. The generated microparticles had an average size of 520 ± 4 nm and a span of 0.3 were formulated by a rotor-stator homogenize at the homogenization speed 10,000 rpm. Composite films were prepared by incorporating chitosan-coated microparticles, at various concentrations (0.05, 0.1, 0.5, or 1% (based on the weight of the gelatin powder)) in the gelatin-based films. For the prepared films, the results showed that obtained physicochemical, water vapor barrier, and mechanical were compared with native gelatin film with a slight decrease for chitosan concentration higher than 0.5%. The microstructure studies done by scanning electron microscopes, revealed different micropores embedded with oil resulting from the incorporation of the microparticles into the gelatin matrix. Moreover, the calorimetric results were comparable to those of gelatin control film with T_g value 45 °C and increased crystallinity percentage with increasing incorporation of microparticles. This original concept of composite biodegradable films may thus be a good alternative to incorporate liposoluble active compounds to design an active packaging with good properties.

1. Introduction

Lipid oxidation, besides microbial growth, is the main cause of food putrefaction particularly for lipids rich in poly-unsaturated fatty acids. To avoid oxidation and prolong food shelf-life, antioxidants have been commonly used as food additives for decades [1].

Antioxidants directly applied into food may be limited, such that, once the action of the antioxidants is neutralized by the reaction with food composites, the protection ends and the food quality reduces quickly [2,3]. Owing to the general inducement of the lipid oxidation process from the surface of foodstuffs, many efforts have been put together to incorporate antioxidants into food packaging to control the oxidation of food product, with the prospect that food package might delay the oxidation and prolong the shelf-life of food by a controlled diffusion of incorporated antioxidant into the foodstuff [4–6].

Direct incorporations of antioxidants, such as green tea antioxidants, curcuma antioxidants extracts, and resveratrol, into biopolymer films formed by the blending of different polysaccharides or proteins have been cited in the literature [7,8]. However, the problem of physical and chemical stability of incorporated antioxidants into the films was not fulfilled for a long term. The antioxidants were found released rapidly from the films, without being active for long time of storage. According to Gómez-Estaca et al. [2], the inclusion of antioxidants in the matrix of microparticles before being incorporated into the film matrix, might increase the tortuosity of the diffusion path and would be one of the valid solutions for decelerating the releasing process of antioxidants from the film. Indeed, the microparticles avoids the release (or loss) of antioxidants.

Besides the function of carrying and preserving antioxidants, polymers such as chitosan can improve the mechanical, thermal, and hindering properties of the films. Indeed, the water repellent feature of the chitosan polymer often make them recommended as a moisture barrier, but, they are also useful for antioxidants encapsulation due to their capacity of retention and slow release [9]. The inclusion of chitosan resulted in enhanced barrier properties and better mechanical properties as detected by de Moura et al.

* Corresponding author.
E-mail addresses: dammakilyes@usp.br, dammakilyes@hotmail.fr (I. Dammak).

http://dx.doi.org/10.1016/j.ijbiomac.2017.03.163
0141-8130/

[10] in hydroxypropyl methylcellulose films. Bonilla and Sobral [11] have shown that chitosan and gelatin form a very homogeneous blend with a good filmogenic character. Eça et al. [12] have reviewed literature works on films and edible coatings containing antioxidants in different forms (extracts, essential oils, and pure compounds) and found that several researches about this theme are being developed. Chitosan nanoparticles are natural materials with excellent physicochemical properties, which are environmentally friendly, and bioactive [13]. Several bio-nanocomposite films have been developed by incorporating chitosan nanoparticles into polymeric matrices [14,15]. Indeed, Hosseini et al. [15] studied the effect of different amounts of chitosan nanoparticles added to fish gelatin film forming solutions, and the results indicated that the barrier and mechanical properties of the resulting composite films have been further improved after formation of nanocomposites. Additionally, nanocomposites with antimicrobial function are highly useful to minimize the growth of post-processing contaminant microorganisms, extending shelf life of food and improving food safety [16]. Therefore, the incorporation of chitosan-coated microparticles into the gelatin films may change the microstructure, barrier, mechanical, thermal, color and light transmission properties of the prepared films. As a result, the inclusion of antioxidants into microparticles coated with chitosan, and then into polymer matrices might bring about a two-fold advantage: the properties enhancement of the film, and the provision of longer antioxidant properties of the food package.

The aim of the present study was to understand the effect of chitosan-coated microparticles, incorporation on the properties of the gelatin-based film and therefore on its capacity to protect encapsulated antioxidants.

2. Materials and methods

2.1. Materials

Pigskin gelatin's powder, Type A (bloom 260, moisture content = 9.98%) was provided by Gelnex (Itá, Brazil). Anhydrous glycerol was supplied from Labsynth® (São Paulo, Brazil). Refined soybean oil was supplied from Cargill Agricola S.A. (São Paulo, Brazil). Soy lecithin (300 M, Caramuru) was bought from Prolabo (São Paulo, Brazil). Medium molecular weight chitosan (practical grade, batch STBF3507V, degree of deacetylation: 75–85%, viscosity: 200–800 cps) was supplied by Sigma-Aldrich (São Paulo, Brazil). Rutin hydrate (purity ≥ 94.0%) was purchased from Sigma-Aldrich (São Paulo, Brazil). The water used was of Milli-Q quality with conductivity at 25 °C of 0.056 μS/cm.

2.2. Interfacial tension measurements

Interfacial tensions at the oil-water interface was measured in a dynamic mode at 25 °C, by Du Nouy ring method using a tensiometer (Attension Sigma 702, Espoo, Finland). In those measurements, the aqueous phases were chitosan/lecithin solutions as continuous phases of emulsions at pH 3. The oil phase was soybean oil containing 0.1% rutin. All of the measurements were carried out in triplicate with an immersion depth of 20 mm.

2.3. Preparation of chitosan-coated microparticles

The continuous phase was prepared by adding lecithin and chitosan to the water with different concentrations tested in this study. The dispersed phase was prepared by dissolving rutin in soybean oil at a concentration of 0.1% (w/w) with heating at 90 °C for 1 h. Solution was cooled to room temperature before subsequent experiments. The weight ratio of dispersed phase to continuous phase of 1:9 was homogenized using a rotor-stator homogenizer (Ultra-Turrax® IKA T25, Labotechnik, Germany) at 10,000 rpm for 15 min.

To select the optimal lecithin concentration, we added different concentrations of lecithin to the continuous phase, without addition of chitosan, to rich the minimal oil droplet size.

To study the effect of chitosan addition, we prepared different aqueous solutions of chitosan with final pH 3.0 (by adding acetic acid 1 M). Then we added the chitosan solutions to the continuous phase.

2.4. Preparation of film-forming solutions

To prepare the film-forming solutions, 5 g of pigskin gelatin's powder Type A (Gelnex, Brazil) dissolved into 80 mL of distilled water, then temperature increased to 70 °C using a hot water bath. The mixture was stirred for 30 min at this temperature. After cooling to 37 °C, glycerol (Merck, Darmstadt, Germany) was added (30 g per 100 g of the gelatin powder) to film-forming solutions, acting as a plasticizer. To study the effect of chitosan-coated microparticles incorporation into films, five concentrations of chitosan-coated microparticles solutions were incorporated to get equivalent concentrations of 0.05%, 0.1%, 0.5%, 1% and 2% (g chitosan/100 g of gelatin powder).

Then, distilled water was added to complete to the final volume of 100 mL. Finally, the film-forming solutions were homogenized at 10,000 rpm with an Ultra-Turrax (IKA T25, Labotechnik, Germany) for 5 min [17,18].

To remove the air bubble from film-forming solutions, due to the high-speed homogenization, vacuum condition was applied during 15 min with a diaphragm pump (model ME 1, Vacuubrand, Brazil).

2.5. Preparation of films

To prepare films, around 25 mL of film-forming solutions were cast into polystyrene Petri dishes (150 × 15 mm, Pleion Co., São Paulo, Brazil), and dried in a ventilated climatic chamber (MA 035, Marconi, Brazil), at temperature and relative humidity fixed at 30 ± 1 °C and 40 ± 2%, respectively, for 12 h. Before characterization, all the film samples were conditioned at 25 ± 0.2 °C and 58 ± 1% of relative humidity, for at least 7 days.

2.6. Characterization of chitosan-coated microparticles

2.6.1. Determination of mean droplet size and size distribution

The mean droplet size and size distribution were examined with the multi-sample analytical centrifuge LUMiSizer (LUM GmbH, Germany), which permits the intensity of the transmitted near infrared light (λ = 734 nm) to be measured as a function of time and position over the total sample length simultaneously, which allow particle size characterization and equally the particles dispersity of the dispersion.

The mean particle size measured is the diameter of a hypothetical particle that represents the total number of particles in the samples. The volume-surface diameter ($d_{3,2}$) denotes the average size, based on the specific surface per unit volume [19].

These mean particles sizes were defined by Eq. (1),

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where n_i is the number of particles and d_i is the particle diameter both in each size class. The specific surface corresponds to the developed area of fat globules dispersed normalized by the volume of the lipid phase expressed as m^2/mL.