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Biopolymeric matrices made of carrageenan and corn starch for the antioxidant extracts delivery of Cuban red propolis and yerba mate

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ABSTRACT

The design of biopolymeric matrices for the delivery of bioactive compounds constitutes a useful strategy to prevent the spoilage of food products. In the current work, carrageenan–starch films with antioxidant extracts of Cuban red propolis and yerba mate were prepared by casting. The morphological analysis by SEM showed a more homogeneous structure for the yerba mate films in comparison with the propolis ones. The incorporation of the natural extracts affected the dynamic-mechanical behavior of the films, whereas their crystallinity degrees were maintained. FTIR analysis showed stronger interactions of the polymer matrix with the propolis extract than with the yerba mate one. The films exhibited differences in their mechanical properties; higher tensile strength values were obtained for the yerba mate films than for the propolis samples. However, the last films exhibited higher elongation at break. Both matrices showed good stability of the active compounds along 6 months of storage at 75% RH and 23 °C. After this time, the samples showed an increase in their DPPH scavenging activity. The release behavior of the phenolic compounds from the films in an aqueous medium was assayed finding significant differences ($p < 0.05$) between release rates of both extracts.

1. Introduction

Polyphenols-rich natural extracts could be considered as an effective way for preventing lipid oxidation of foods or act as a functional additive [1]. Propolis, a natural product produced by the honeybees, has been used for thousands of years in folk medicine of several countries. The chemical composition of propolis is very complex and differs greatly depending on its geographical and botanical origin [2]. Studies referred to Cuban red propolis have been carried out finding that it contain a wide variety of substances including flavonoids, phenolic acids and their esters, terpenes, sesquiterpene quinones, coumarins, steroids and amino acids [3]. Yerba mate dried and minced leaves are used to prepare a highly consumed tea-like beverage in several South American countries. This plant contains many bioactive compounds such as polyphenols, xanthines, saponins, amino acids, minerals and vitamins [4]. Both extracts have demonstrated strong free radical-scavenging activity attributed mainly to their high content of phenolic compounds [5,6].

Natural polymers constitute an actual alternative for diminishing the use of non-degradable and non-renewable materials in the packaging industry. These materials have the advantages of being abundant, renewable, low cost and biodegradable [7,8]. Polysaccharides and proteins have been used to develop eco-friendly matrices for edible films and coatings applications in the food industry. Starches and carrageenans are commonly employed because are known for their good film forming properties. Different ratios of starch alone, carrageenan alone and blends of the two polysaccharides have been assayed [9,10]. Tanner, Draper, Getz, Burnett and Youngblood [11], working with weight ratios of starch to carrageenan between 1.5:1 and 4:1, found that ratios from 2:1 to 3:1 yielded to forming films with good handling properties.

A feasible way to improve the functionality of the films is the incorporation of active compounds (e.g. antioxidant or antimicrobial agents); the use of natural compounds instead of synthetic additives is preferred due to the association of the last ones with adverse effects on human health [12]. Several works on biopolymer-based films with natural antioxidants have been reported [13–16]. However, as it is well known, the structure and the physicochemical properties of the films may be affected by its composition, thus the possible interactions between the active compounds and the biopolymeric matrix should be evaluated. In this topic, our research offers an important contribution.

In the current work, carrageenan–starch films with extracts of propolis and yerba mate were developed. The effect of each extract

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on the film microstructure was evaluated as well as it release kinetic from the films into an aqueous media. To our knowledge, it is the first time that active antioxidant films are developed using carrageenan–starch blends as a supporting matrix for propolis and yerba mate extracts.

2. Materials and methods

2.1. Cuban red propolis and yerba mate extracts

The hydro-alcoholic extract of Cuban red propolis (17.9 g dry solids/100 g extract) was supplied by the Estación Experimental Apícola (Habana, Cuba). Yerba mate extract (3 g dry solids/100 g extract) was prepared according to the previously optimized methodology by Deladino, Anbinder, Navarro and Martino [17]. Briefly, a blend of 10 g of commercial yerba mate (Las Marías, Corrientes, Argentina) and 100 mL of distillated water was placed in a thermostatic bath (Viking, Argentina) at 100 °C for 40 min. After this time, samples were filtered and cooled.

2.2. Polyphenols content and antioxidant activity

Total polyphenols content was determined by the Folin–Ciocalteu method [18]. Briefly, 2 mL of Na_2CO_3 (2 g/100 mL) (Anedra, Argentina) were mixed with 200 μL of the sample and 200 μL of Folin–Ciocalteu reagent (Anedra, Argentina, 1:1 diluted). After 30 min, sample absorbance was measured at 725 nm in a spectrophotometer (Shimadzu UV-mini 1240, Japan). The calibration curves were performed using chlorogenic acid (Fluka, USA) for yerba mate extract and gallic acid (Sigma Aldrich, USA) for propolis extract.

Antioxidant activity of the yerba mate and propolis extracts at different concentrations was tested according to the method described by Brand-Williams et al. [19]. A volume of 100 μL of each sample was mixed with 3.9 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH·) ethanol solution (25 mg DPPH·/L). Absorbance was determined at 517 nm until the reaction reached a plateau. Antioxidant activity was expressed as the DPPH· inhibition percentage calculated with the following equation:

$$\text{DPPH}\cdot\ \text{inhibition}\ (\%) = ((A_b - A_s)/A_b) \times 100 \quad (1)$$

where A_b is the absorbance of the blank and A_s is the absorbance of the sample.

2.3. Preparation of the active films

Carrageenan–starch films with extracts of propolis or yerba mate were produced by casting. The native corn starch was supplied by Unilever (Barcelona, Spain). The kappa-carrageenan (molecular weight 798 g mol^{-1}) was supplied by Cases & Cases (Habana, Cuba). The polymer concentrations for the preparation of the films were chosen from literature data [10,11]. Film-forming solutions were prepared by dissolving of native corn starch (4 g/100 mL) and κ-carrageenan (2 g/100 mL) in distillated water under continuous stirring at 90 °C for 10 min. Then, each extract was added (6 mL/100 mL) and the blends were stirred with a vertical agitator (IKA Labortechnik, Germany) for 3 min. After this stage, the mixtures were cooled to 70 °C and glycerol (1 g/100 mL) was added as plasticizer. Finally, 10 g of films-forming blend were poured on polystyrene Petri plates and then dried at 60 °C until constant weight (around 2 h). The films obtained were removed from the plates and these were conditioned in desiccators at 75% RH and 23 °C until use. Samples without active compounds were prepared as described above for control purposes.

2.4. Characterization of the films

2.4.1. Film thickness and morphological analysis

The thicknesses were measured using a digital micrometer Elcometer A300 FNP 23 (UK). Fifteen measurements were randomly taken at different locations for each specimen and the mean value was reported.

The morphological analysis was performed by scanning electron microscopy (SEM) using a JEOL JSM 6360 equipment (Japan). The samples were attached to stubs using a two-sided adhesive tape, coated with a layer of gold (40–50 nm) and then examined using an accelerating voltage of 25 kV. Cross-cuts of the films were obtained by cryofractured immersing the samples in liquid nitrogen.

2.4.2. X-ray diffraction analysis

Films were analyzed using an X'Pert Pro equipment (The Netherlands) provided with a copper anode and a detector operating at 40 kV and 30 mÅ. The samples were scanned with 2θ varying from 3° to 60°. Crystallinity degree (%) of the films was calculated as the ratio between the area of absorption peaks and the total diffractogram area [20].

2.4.3. Differential scanning calorimetry (DSC)

The equipment used was a DSC Q100 controlled by a TA 5000 module (TA Instruments, New Castle, USA), provided with a quench-cooling accessory under a N_2 atmosphere (20 mL/min). Samples (around 6 mg) were placed in aluminum pans hermetically sealed; an empty pan was used as reference. Samples were heated from −100 to 200 °C at a heating rate of 10 °C/min. Glass transition temperatures (T_g) were obtained from the thermograms, using the Universal Analysis V1.7F software (TA Instruments, USA).

2.4.4. Dynamic mechanical analysis (DMA)

DMA assays were conducted in a dynamic-mechanical thermal equipment Q800 (TA Instruments, New Castle, USA) using a tension clamp with a liquid N_2 cooling system. Film probes with a rectangular geometry (6 mm width and 30 mm length) were assayed. An amplitude sweep from 1 to 20 μm at a fixed frequency (5 Hz) was performed. Multi-frequency sweeps (1, 3, 5, 10 and 15 Hz) at fixed amplitude (5 μm) from −100 to 200 °C at 5 °C/min were carried out. Storage modulus (E') and $\tan\delta$ (E''/E') curves as a function of the temperature were analyzed using the software Universal Analysis 2000. The relaxation temperatures, associated to the dynamic glass transition temperatures, were determined from the tan δ curves.

2.4.5. Fourier transform infrared spectrometry (FTIR)

FTIR analysis of the antioxidant extracts and the films were carried out using a Nicolet IS-10 equipment (Thermo Scientific, USA). Disks (7 mm) were obtained by milling 1 mg of sample with 100 mg of KBr and were then analyzed between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. The spectral analysis was performed with the Omnic version 8.1 software (Thermo Scientific).

2.5. Storage stability of the films

The films were equilibrated at 75% RH and 23 °C for 5 days, in glass desiccators with NaCl supersaturated solution, before being tested at initial time. After this stage, these were maintained under the same conditions during 6 months. The samples were analyzed at initial time and after storage in terms of their mechanical properties, total polyphenols content and antioxidant activity.