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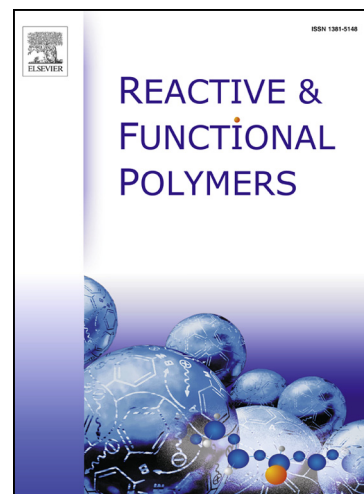
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**Biopolymeric matrices made of carrageenan and corn starch for the antioxidant  
extracts delivery of Cuban red propolis and yerba mate**

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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.

**Keywords:** Active films; Natural antioxidants; Carrageenan; Starch; Release.

## 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 on the film microstructure was evaluated as well as its 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 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 distilled 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<sub>2</sub>CO<sub>3</sub> (2g/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, Cuvelier and Berset [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:

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

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  $798 \text{ g mol}^{-1}$ ) 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 (4g/100 mL) and  $\kappa$ -carrageenan (2 g/100 mL) in distilled water under continuous stirring at  $90 \text{ }^\circ\text{C}$  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  $70 \text{ }^\circ\text{C}$  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  $60 \text{ }^\circ\text{C}$  until constant weight (around 2 h). The films obtained were removed from the plates and these were conditioned in desiccators at 75% RH and  $23 \text{ }^\circ\text{C}$  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  $2\theta$  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<sub>2</sub> 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<sub>2</sub> cooling system. Film probes with a rectangular geometry (6 mm width and 30 mm length) were assayed. An amplitude sweep from 1 to 20  $\mu\text{m}$  at a fixed frequency (5 Hz) was performed. Multi-frequency sweeps (1, 3, 5, 10 and 15 Hz) at fixed amplitude (5  $\mu\text{m}$ ) 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  $\tan \delta$  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  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ . 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.

### 2.5.1. Mechanical properties

The mechanical properties were evaluated using a TA.TX2i Stable Micro Systems texture analyzer (England) in accordance with ASTM D882 [21]. Five sample strips (0.5 x 6 cm) of each formulation were cut and clamped between tensile grips. Force (N) and deformation (mm) were recorded during extension at 5  $\text{mm min}^{-1}$  and with an initial distance between the grips of 40 mm. Tensile strength was calculated dividing the maximum force by film cross-section area. Elongation at break was calculated as the percentage of increment of the probe length. Elastic modulus was obtained from the slope of the linear part of the stress-strain curve.

### 2.5.2. Active compounds content and antioxidant activity

Films samples (0.5 g) were immersed in 10 mL of distilled water and were then placed in an orbital shaker at 25 °C and 125 rpm for 24 h (Orbit Environ Shaker, Lab-Line Instruments, USA). Aliquots of the supernatant were removed and the polyphenols content and the antioxidant activity were determined as described in Section 2.2.

## 2.6. Release kinetics of the extracts in aqueous medium

Film pieces of 5 x 5 cm were supported with a mesh to improve the contact from both sides with the release medium. The samples were immersed in a volume of 120 mL of distilled water and were then placed in an orbital shaker at 25 °C and 125 rpm. Aliquots of 200  $\mu$ L of supernatant were removed at different times. The percentage of polyphenols released was calculated with the following equation:

$$\text{Polyphenols released (\%)} = \left( \frac{m_t}{m_\infty} \right) \times 100 \quad \text{Eq. 2}$$

where  $m_t$  is the mass of polyphenols quantified at each time  $t$  and  $m_\infty$  is the mass of polyphenols loaded in the films. Both  $m_t$  and  $m_\infty$  were determined by Folin-Ciocalteu method (Section 2.2).

Besides, the fitting of the release profiles of both extracts to the empirical models of power law (Eq. 3), diffusion-relaxation (Eq. 4) and first order (Eq. 5) [22, 23] was evaluated, using the corrected correlation coefficient ( $R^2_{\text{cor}}$ ) and the residual analysis (SYSTAT software, USA).

$$\frac{m_t}{m_\infty} = kt^n \quad \text{Eq. 3}$$

$$\frac{m_t}{m_\infty} = k_d t^m + k_r t^{2m} \quad \text{Eq. 4}$$

$$\frac{m_t}{m_\infty} = e^{-k_1 t} \quad \text{Eq. 5}$$

In Eq. 3,  $k$  is a constant related to structural and geometric characteristics of the matrix and  $n$  is the transport exponent indicating the type of release mechanism involved. In Eq.4  $k_d$ ,  $k_r$  and  $m$  are constants. The first term represents the diffusional contribution and the second term represents the case-II relaxation contribution. In Eq. 5,  $k_1$  is the first-order release



constant. Eq. 3 and Eq. 4 are only valid for the first 60% of the release curve, which means that  $m_t/m_\infty$  is  $< 0.6$  [22, 23].

## 2.7. Statistical analysis

Analysis of variance (ANOVA) and mean comparisons were carried out using the SYSTAT INC. (Evanston, USA). Unless indicated, a level of 95% of confidence ( $\alpha=0.05$ ) was used.

## 3. Results

### 3.1. Polyphenols content and antioxidant activity of the extracts

The extracts of yerba mate and propolis showed a polyphenols content around 10 mg chlorogenic acid equivalent/mL and 22 mg gallic acid equivalent/mL, respectively. Fig. 1 shows the relationship between the total polyphenols content and the antioxidant activity towards the DPPH•. For both extracts, DPPH radical scavenging activity increased proportionally to their polyphenols content until reaching a plateau in which the antioxidant activity became independent of the concentration.

The polyphenols concentration necessary to reduce the initial amount of DPPH radical to 50%, called efficient concentration ( $EC_{50}$ ), was extrapolated from the linear part of the Fig. 1. The lower  $EC_{50}$  values correspond to a higher antioxidant activity [19]. The extracts of yerba mate and propolis showed  $EC_{50}$  values around 0.12 and 0.66 mg/mL, respectively. Although the propolis extract exhibited higher polyphenols content than the yerba mate one, this last exhibited a higher antioxidant activity. This behavior could be attributed to differences in the chemical composition of the extracts; aqueous extract of yerba mate is rich in phenolic acids and their esters [24, 25], whereas the hydro-alcoholic extract of Cuban red propolis has a wide variety of flavonoids compounds [3]. According to Brewer [1] both phenolic acids and flavonoids act as antioxidants by trapping free radicals, but the last compounds can also chelate metals.

### 3.2. Carrageenan-starch films with natural antioxidant extracts

The formulations used allowed to obtain films with good handling properties. All samples were flexible, translucent and showed the characteristic color given by each extract. In

preliminary experiments carried out with higher concentrations of extracts, the films obtained exhibited strong aroma and unpleasant appearance. The mean thicknesses were around  $90.6 \pm 13.1$  for the yerba mate films and  $110.1 \pm 5.6 \mu\text{m}$  for the propolis films.

### 3.2.1. Carrageenan-starch films with propolis extract

#### 3.2.1.1. Morphological aspect and crystallinity

Fig. 2 shows SEM micrographs of the surface and the cross-sections of the control and active films. Control films showed a homogenous surface without cracks, whereas the propolis films showed a slightly rougher surface. The cross-section images of both films showed multilayer structure, as was observed by López, Lecot, Zaritzky and García [26] for unplasticized acetylated corn starch films. Bodini, Sobral, Favaro-Trindade and Carvalho [27] and Pastor, Sánchez-González, Cháfer, Chiralt and González-Martínez [28], working with films based on gelatin and hydroxypropyl methylcellulose, found that the addition of propolis extract provoked both more irregular structure and changes in the porosity of the matrix [27, 28].

Diffraction patterns of the control and propolis films showed crystalline and amorphous zones and peaks located around  $2\theta = 5.6, 17.2, 22.2$  and  $26.3^\circ$  corresponding to a B-type pattern (Fig. 3). According to Zobel [29] native corn starch generally present a A-type crystalline structure with sharp diffraction peaks at  $2\theta = 15.3^\circ, 17.5^\circ$  and  $23.1^\circ$ . However, several authors have reported that in the films from gelatinized starch a B-type structure is developed [30, 31]. On the other hand, non-significant differences were found between the crystallinity degrees of control and propolis films, obtaining values around 11%. Crystallinity is an important factor which influencing the end-use properties of the biopolymeric matrices. According to Mali, Grossmann, Garcia, Martino and Zaritzky [32] this property is dependent of both the processing conditions and the characteristics of the polymers. Crystallinity degrees higher than 10% have been reported as suitable to obtain films with optimal mechanical and thermal characteristics [20].

### 3.2.1.2. Thermal properties and dynamic mechanical behavior

$E'$  (storage modulus) and  $\tan \delta$  curves as a function of the temperature are shown in Fig. 4. To temperatures below than 50°C, propolis films exhibited higher  $E'$  values than control ones indicating higher stiffness (Fig. 4a). From  $\tan \delta$  curve (Fig. 4b), control and propolis films showed a relaxation peak around -41 °C associated to the glass transition of the glycerol-rich phase. Moreover, a broad peak around 50 °C was also observed, associated to the starch-rich phase. According to Mathew and Dufresne [33] the peak to higher temperature may be attributed to the rearrangement of amorphous amylopectin chains in the presence of moisture.

By DSC analysis (Fig. 4c), two glass transition temperatures were found for the control and propolis films. The first ( $T_{g1}$ ) was observed around -68.5 °C for both films; whereas, the second ( $T_{g2}$ ) was observed to -3.1 °C for the control samples and to -6 °C for the propolis ones (Table 1). However, this difference was not statistically significant ( $p>0.05$ ). Besides, an endotherm between 150 and 180 °C was found for both samples, which could be due to the melting process of the crystalline phase of the films as detected by X-ray diffraction (Fig. 3). In complementary studies by Modulated Temperature DSC, the total heat flow was separated into the part that responds to heating rate (reversing heat flow) and the part that responds to absolute temperature (non-reversing heat flow). Similar  $T_g$  values were obtained from the signal of reversing heat flow.

The glass transition temperatures obtained by DSC were lower compared with the relaxation temperatures obtained by DMA. These differences can be attributed to the properties evaluated by each technique. DMA provide information concerning molecular relaxation processes and mechanical properties both as a function of the time and the temperature. While DSC technique is typically used to assess the enthalpic changes associated with the phase transitions as function of the temperature [34, 35].

### 3.2.1.3. Analysis of interactions by FT-IR

The carrageenan-starch films with and without propolis extract were analyzed by FT-IR (Fig. 5). The more significant changes in the spectra of the films appeared in the region of 1250–900  $\text{cm}^{-1}$ . According to Kačuráková and Wilson [36] in this zone starches and

carrageenans showed bands that are sensitive to conformational changes. Control films (without extract) showed signals at 1157, 1079 and 1043  $\text{cm}^{-1}$  attributed to vibrations of glycosidic linkage of the biopolymers. The bands found at 1263, 925 and 848  $\text{cm}^{-1}$  were attributed to stretching mode of the S=O groups characteristic of the building units of the carrageenan. Similar signals were found by Pereira, Sousa, Coelho, Amado and Ribeiro-Claro [37] in spectra of  $\kappa$ - and  $\iota$ -carrageenan obtained by FTIR and FT-Raman. The spectra of the propolis films showed significant changes in the position of the bands above-mentioned (Fig. 5). A shift of the signals attributed to the glycosidic linkage was observed from 1157, 1079 and 1043  $\text{cm}^{-1}$  for control films to 1150, 1096 and 1036  $\text{cm}^{-1}$  for propolis films, respectively. Moreover, the band located at 925  $\text{cm}^{-1}$  was highly reduced and shifted to 905  $\text{cm}^{-1}$ . Likewise, the signals located to 1263 and 848  $\text{cm}^{-1}$  were shifted to 1296 and 821  $\text{cm}^{-1}$ , respectively. This behavior could indicate an interaction between the propolis extract and the carrageenan-starch matrix. As it is well known, the positions of absorption bands in the IR spectrum give information about the presence or absence of specific functional groups in a molecule; a whole spectrum constitutes a “fingerprint” that can be used to determine the identity of the sample [36].

In addition, the films showed signals corresponding to the stretching of the aromatic rings due to the presence of phenolic compounds from the propolis extract (Table 2). Several authors have reported that the Cuban red propolis contain a wide variety of flavonoids, phenolic acids and their esters [3].

### 3.2.2. Carrageenan-starch films with yerba mate extract

#### 3.2.2.1. Morphological aspect and crystallinity

SEM micrographs of the yerba mate films showed homogenous surface without cracks as control ones, which suggest that the yerba mate extract was integrated into the carrageenan-starch matrix, i.e. a good compatibility. However, some multilayer zones typical of starch films were observed, as well [26].

The diffractograms of the yerba mate films showed crystalline and amorphous zones and a B-type pattern [30]. The crystallinity degree of the carrageenan-starch matrix was not

affected by the addition of the yerba mate extract, showing similar values than the films without extract (around 11%).

### 3.2.2.2. Thermal properties and dynamic mechanical behavior

The yerba mate films showed higher storage modulus values than the control ones, almost in all range of temperatures assayed (Fig. 4a). As above mentioned, control films exhibited a behavior characteristic of partially miscible systems with plasticizer-rich and polymer-rich phases (Fig. 4b). For yerba mate films, the peak associated to the glycerol-rich phase appears to higher temperatures ( $-27\text{ }^{\circ}\text{C}$ ) than for control ones, whilst the peak attributed to the starch-rich phase was not observed (Fig. 4b). These facts may indicate a higher miscibility of all matrix components and are agree with the SEM observations (Fig. 2).

DSC thermograms of the control and yerba mate films exhibited two glass transition temperatures around  $-68$  and  $-6\text{ }^{\circ}\text{C}$  (Fig. 4c). Non-significant differences ( $p>0.05$ ) were found between the  $T_{g1}$  and  $T_{g2}$  values of both films (Table 1). Similar  $T_g$  values were obtained from the signal of reversing heat flow by Modulated Temperature DSC.

In addition, an endotherm between  $150$  and  $180\text{ }^{\circ}\text{C}$  was detected which could be due to the melting process of the crystalline phase of the films as observed by X-ray diffraction analysis (Section 3.2.2.1).

### 3.2.2.3. Analysis of interactions by FT-IR

FTIR spectra of the carrageenan-starch films with and without yerba mate extract showed a similar pattern suggesting that strong interactions between the yerba mate extract and the biopolymeric matrix did not take place (Fig. 5). Both spectra showed signals around  $1157$ ,  $1079$  and  $1043\text{ cm}^{-1}$  attributed to vibrations of glycosidic linkage of the biopolymers. The bands found at  $1263$ ,  $925$  and  $848\text{ cm}^{-1}$  were attributed to stretching mode of the S=O groups characteristic of the building units of the carrageenan [37].

Besides, the films showed signals corresponding to the stretching of the aromatic rings due to presence of phenolic compounds from the extract (Table 2). As it has been reported, the aqueous extracts of yerba mate are rich in phenolic acids including caffeic and chlorogenic acids and their isomers [24, 25].

### 3.3. Storage stability of the films

#### 3.3.1. Mechanical properties

The mechanical properties of the films are shown in Table 3. At initial time, control and yerba mate films showed a similar behavior: low elongation at break (%) and high values of tensile strength and elastic modulus. Whereas, propolis films exhibited higher elongation at break (%) and lower values of tensile strength and elastic modulus compared with control samples. This behavior could be attributed to the strong interaction between the propolis extract and the polymer matrix as observed by FTIR analysis. Similar observations were reported by Bodini, Sobral, Favaro-Trindade and Carvalho [27] and Pastor, Sánchez-González, Cháfer, Chiralt and González-Martínez [28]. These authors stressed that the propolis extract dispersion into the polymer matrix may also produce areas of discontinuity, reducing the resistance of the matrix to fracture and leading to decreased tensile strength and elastic modulus of the films.

After storage, yerba mate films did not show significant changes in their mechanical properties, values around 46.1 MPa, 2.4% and 26.1 MPa were obtained for the tensile strength, elongation at break (%) and elastic modulus, respectively. In contrast, the films with propolis extract exhibited a strong reduction in the elongation (from 12.9 to 1.5%) and an increase in the values of elastic modulus (from 10.4 to 19.9 MPa) and tensile stress (from 25.7 to 33.9 MPa). This behavior could be due to changes in the film crystallinity as reported Mali, Grossmann, García, Martino and Zaritzky [20]

#### 3.3.2. Chemical stability

Fig. 7 shows the chemical stability of the active films stored at 75% RH and 23 °C during 6 months. The films with yerba mate extract did not show significant changes in their polyphenols content along storage; however, the scavenging activity toward DPPH radical was increased around 50%. The propolis films showed a strong increase in both their polyphenols content and antioxidant activity after storage. This behavior could be attributed to possible modifications in the qualitative profile of the phenolic compounds present in the extracts. Pinelo, Manzocco, Nuñez and Nicoli [38] explained that the strong tendency of

polyphenols to undergo polymerization reactions, whereby the resulting oligomers possess larger areas available for charge delocalization and thus, increase the antioxidant activity.

#### 3.4. Release kinetics of the extracts in aqueous simulant medium

Fig. 6 shows the release profiles of the extracts of propolis and yerba mate in aqueous medium. According to ANOVA, the release kinetic of both extracts showed significant differences ( $p < 0.05$ ). As it is well known, several factors may influence the release rates of active compounds from biopolymeric matrices including the chemical composition of the active compounds, polymer–active compound interactions, matrix structure and physical state and surrounding medium conditions [39, 40]. The yerba mate films exhibited a faster polyphenols release rate than the propolis films. After 30 min, the recovery percentage of yerba mate polyphenols was around 70%, whereas that the recovery for propolis polyphenols was only 52%. Therefore, power law (Eq. 3) and diffusion-relaxation models (Eq. 4) could no longer be applied after 30 min because these models are valid up to 60% release [22, 41]. For the yerba mate films, the remaining amount of polyphenols was fully released after total disintegration of the films at 2 h. While, only an 85% of the propolis polyphenols were released from the remaining particles of the propolis films after 6 h. This behavior could be explained based on FTIR results. The strong interaction between propolis extract and the polymeric matrix could avoid the total release of the propolis extract.

The first-order model (Eq. 5) showed the best fitting ( $R^2_{\text{cor}} = 0.98$ ) for both types of films and is represented in Fig. 6. According to Costa and Sousa Lobo [42] the systems following first-order release profile, release the active compound in a way that is proportional to the amount remaining in its interior, in such way, that the amount released by unit of time diminish.

## 4. Discussion

Carrageenan-starch films from both extracts constitute a useful alternative for potential application as food packaging material. As expected, the differences between both extracts allow obtaining films with different features (e.g. color, appearance, mechanical properties, antioxidant level and release behavior in water). This fact is interesting from the industrial



point of view, because different applications could be found for each type of film. A more homogeneous film surface was obtained when the aqueous extract of yerba mate was used. Whereas, the use of the hydro-alcoholic extract of propolis led to films with rougher surface. The solvent influence have been reported previously; Gonçalves, Tomé, Garcia, Brandão, Mendes and Marrucho [14] stressed that due to the hydrophobic character of the hydrocarbon chains present in some extract compounds it is conceivable that substances to precipitate from they forming rougher surface. Similar results were found by Pastor, Sánchez-González, Cháfer, Chiralt and González-Martínez [28]. Additionally, the last authors group found that the coarser structures caused by the propolis extract presence can greatly contribute to improving the water barrier properties of the films.

On the other hand, slight differences in the thicknesses of the propolis and yerba mate films were observed. These results could be attributed to both a solvent effect and to the dry matter content of each extract. Propolis extract, with higher dry matter content, led to films with higher thickness as compared with yerba mate ones. Peng, Wu and Li [43], evaluating the effect of different concentrations of green and black tea extracts on the properties of chitosan films, found that the thicknesses of the films increased when a greater dry solid amount of the tea extracts was added.

FT-IR spectra of the propolis films showed noticeable differences as compared with control ones indicating a strong interaction between the extract and the polymeric matrix. This fact is positive from the mechanical point of view because led to films with suitable mechanical properties. Several authors have reported that the mechanical response of the films is mainly affected by their composition, crystallinity degree, polymer-active compound interaction, moisture content, among others [9, 32]. The chemical composition of the natural extracts is very complex and it is very difficult establish that kind of groups from extract compounds can interact with the polymer units. However, similar observations were reported by other authors suggesting that some interactions between the aromatic rings (C=C) of the polyphenols and the polymer functional groups (e.g. CH<sub>2</sub>OH, OH, and sulfate groups) may take place [43-45]. Pastor, Sánchez-González, Cháfer, Chiralt and González-Martínez [28] stressed that propolis components (resins, balsams containing flavonoids, phenolic acids or their esters) have polar characteristics and can interact with the



hydrophilic groups along the backbone of the polymer molecules. These interactions can result in stronger interfacial adhesion, which leads to a more efficient resistance to the mechanical stress.

X-ray diffraction, DSC and DMA results agreed in a stronger influence of the starch on the overall microstructural properties of the carrageenan-starch films. In this sense, the B-type crystalline pattern and the characteristics of starch-rich films were observed. This facts are in agreement with the reported by Larotonda [46] and Lafargue, Lourdin and Doublier [9]. Release studies were useful to shows that the delivery of the active compounds from the biopolymeric matrices was not prevented. As it is well known, the polymer matrix can affect the biodisponibility and the functionality of the active agents. The films exhibited a fast release rate of the extracts into water, which is an unfavorable medium taking into account the hydrophilic character of the biopolymers used. A similar behavior was also observed by Pinheiro, Bourbon, Vicente and Quintas [47] working with films based on hydrophilic polymers and antioxidants. López-de-Dicastillo, Gómez-Estaca, Catalá, Gavara and Hernández-Muñoz [15] analyzed the lipid stability of brined sardines packaged with antioxidant active films based on ethylene vinyl alcohol copolymer (EVOH). These authors found that the films improved sardine stability by decreasing the peroxide index and the malondialdehyde content.

## 5. Conclusions

Active films based on carrageenan-starch blends containing natural antioxidants were obtained. The addition of the natural extracts provoked changes in the microstructure of the films mainly in their dynamic-mechanical behavior. The yerba mate extract led to films more homogeneous than the propolis extract. However, both films exhibited optimal mechanical properties. The yerba mate films maintained their polyphenol content along storage, whereas the propolis films showed a strong increase in their active compounds amount. The release profile in water depended on the active compound and the microstructure of the films. The first order kinetic model showed a good fit to release experimental data. The incorporation of Cuban red propolis and yerba mate extracts into

biopolymeric blends constitutes a useful strategy for the development of new active films with antioxidant properties.

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**Fig. 1** DPPH radical inhibition percentage vs. the total polyphenols content of the extracts of propolis (o) and yerba mate (●)

**Fig. 2** SEM micrographs of the control and active films (magnification: 810x). The images on the left-side were taken on the surface and the pictures on the right-side on the cross-section

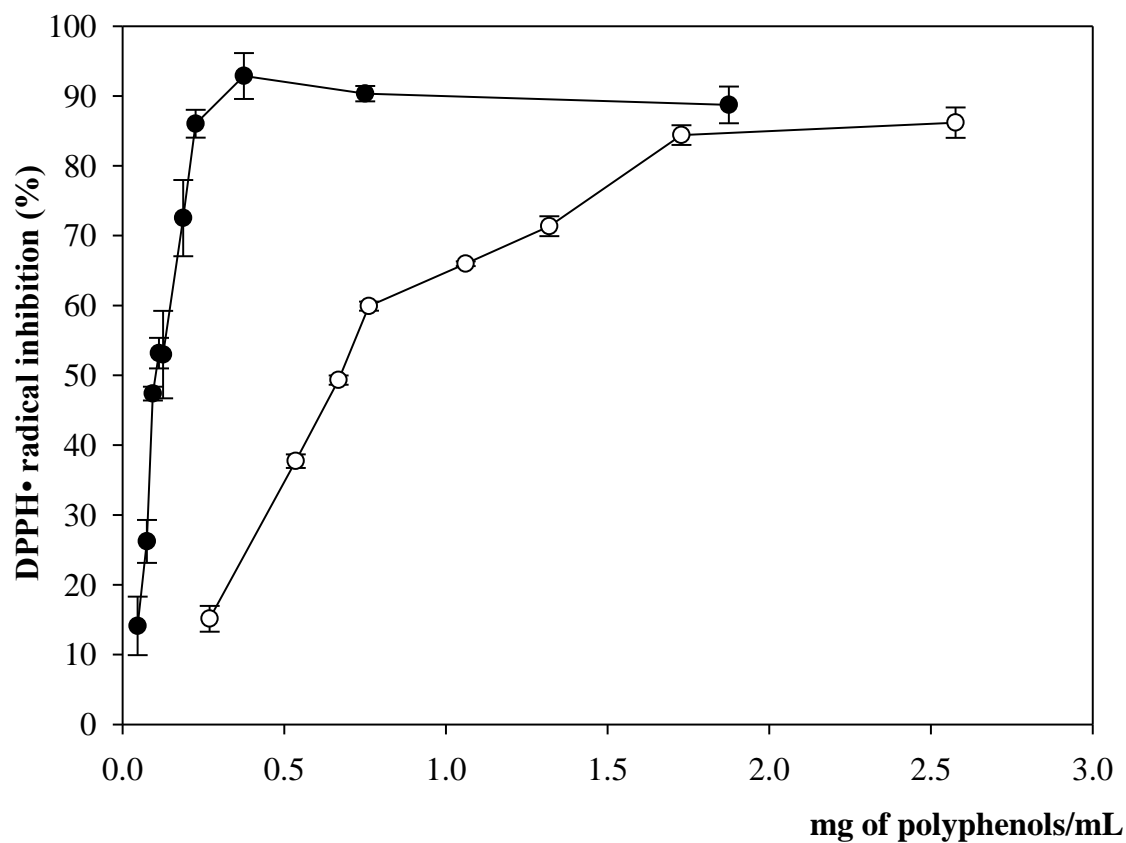
**Fig. 3** X-ray diffraction patterns of the carrageenan-starch films with and without the extracts of yerba mate and Cuban red propolis

**Fig. 4** DMA and DSC analysis of control and active carrageenan-starch films: (a) Storage modulus ( $E'$ ), (b)  $\tan \delta$  curves and (c) DSC thermograms.

**Fig. 5** FTIR spectra of the carrageenan-starch films with and without the extracts of yerba mate and propolis

**Fig. 6** Release kinetics of the extracts of propolis (o) and yerba mate (●) in aqueous medium. Symbols represent experimental data, while lines corresponding to the predicted values by the first-order kinetic model (Eq. 5)

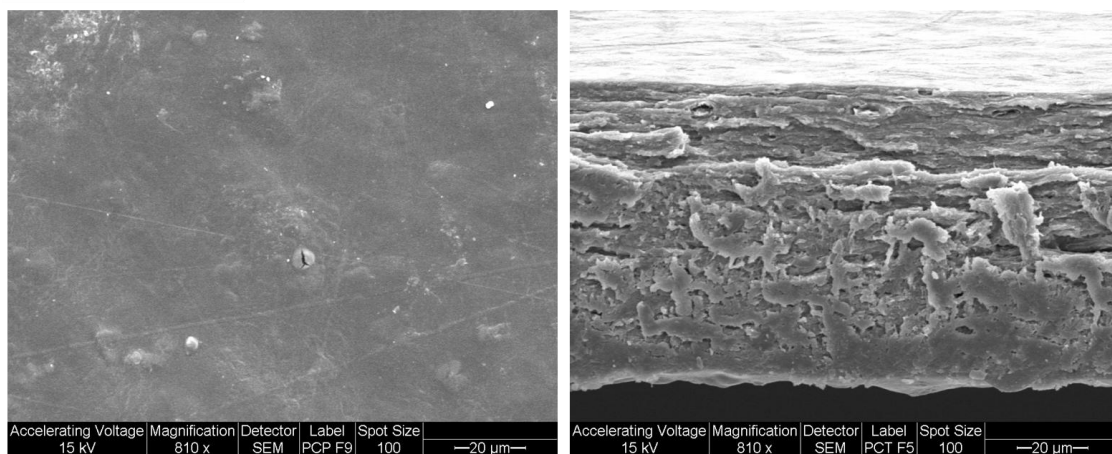
**Fig. 7** Chemical stability of the films stored at 75% RH and 23 °C during 6 months



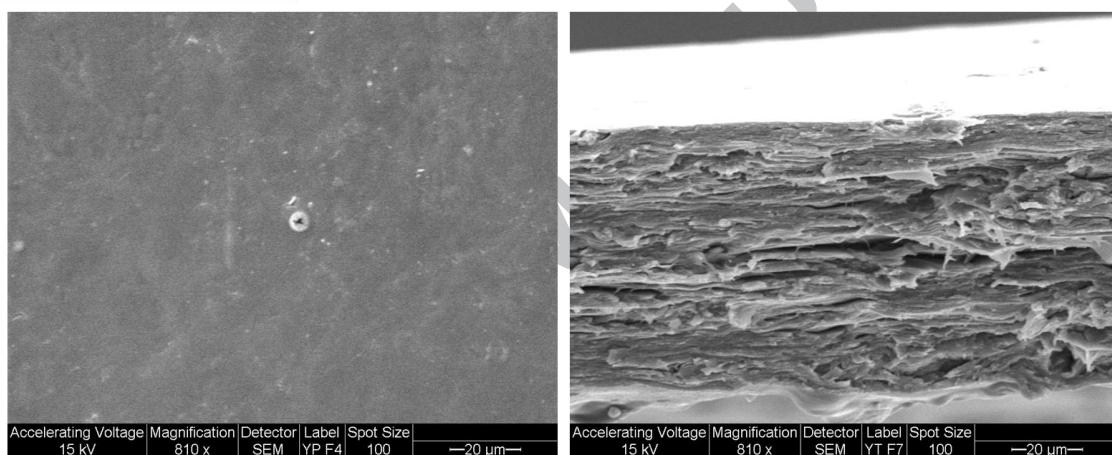
**Fig. 1** DPPH radical inhibition percentage vs. the total polyphenols content of the extracts of propolis (o) and yerba mate (●)



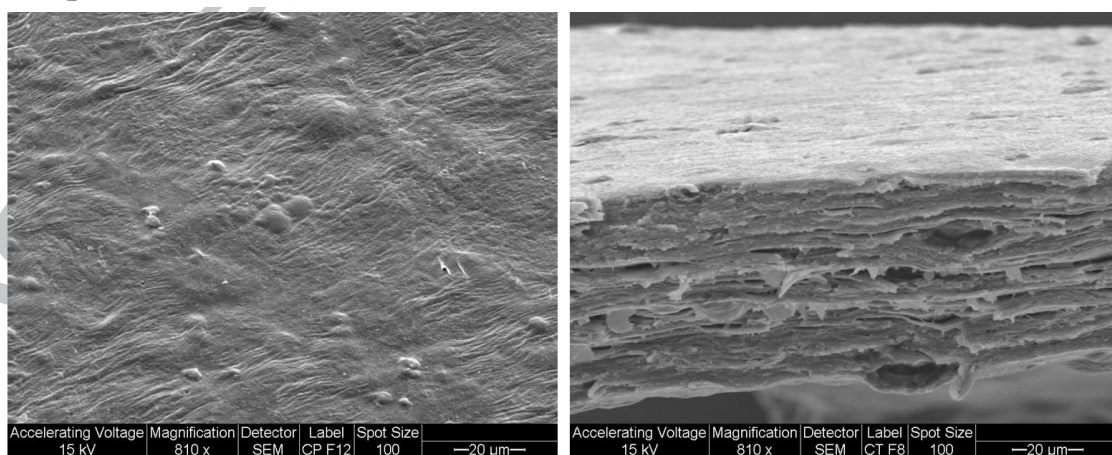
### Control films



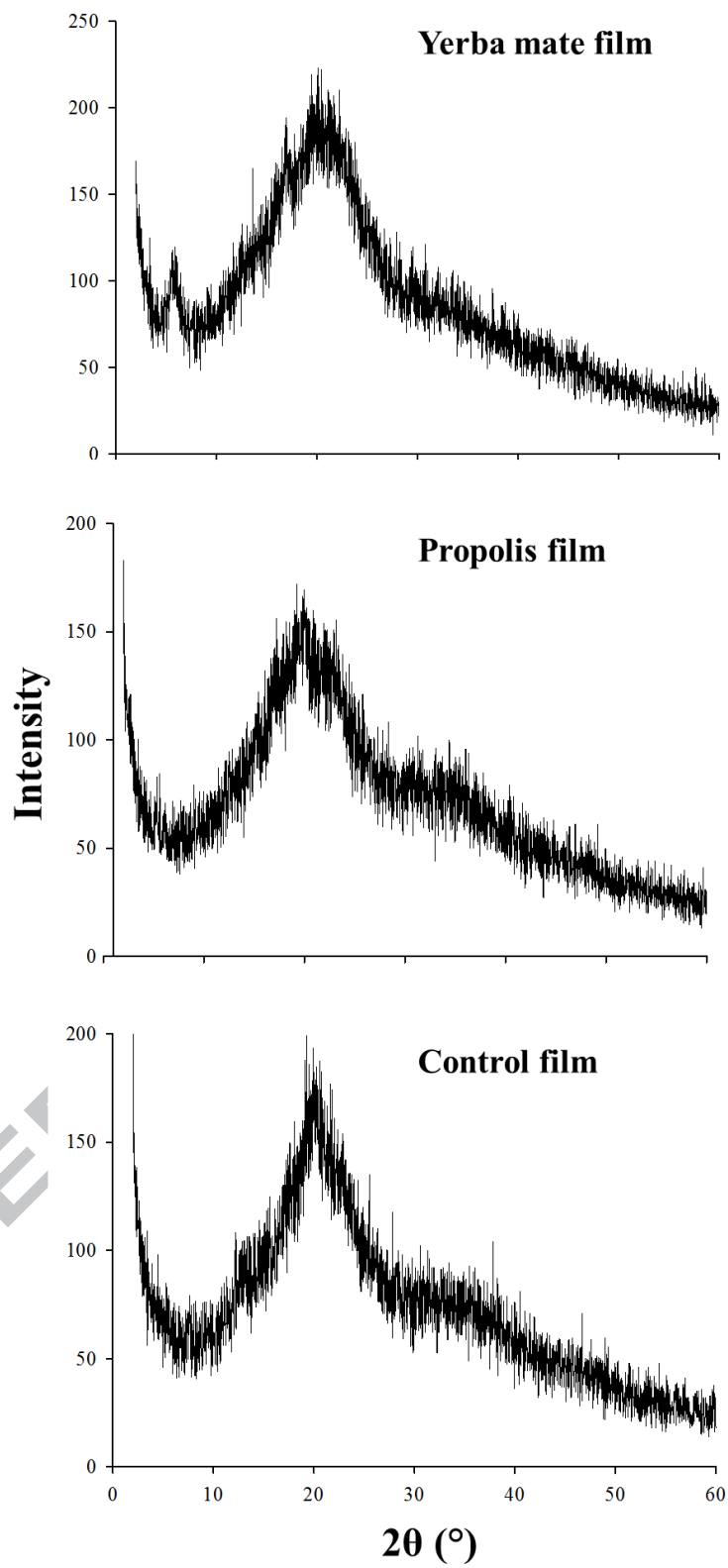
### Yerba mate films



### Propolis films

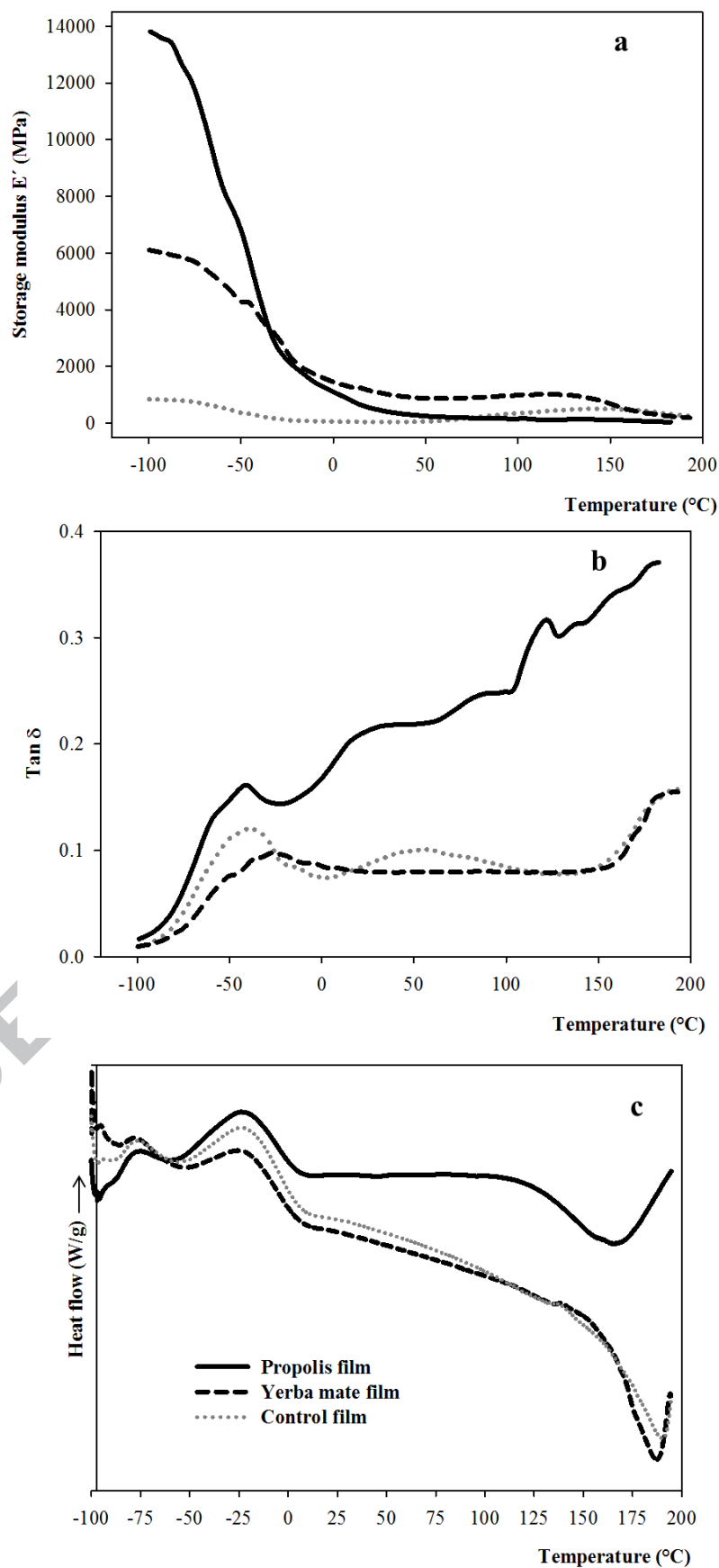


**Fig. 2** SEM micrographs of the control and active films (magnification: 810x). The images on the left-side were taken on the surface and the pictures on the right-side on the cross-section

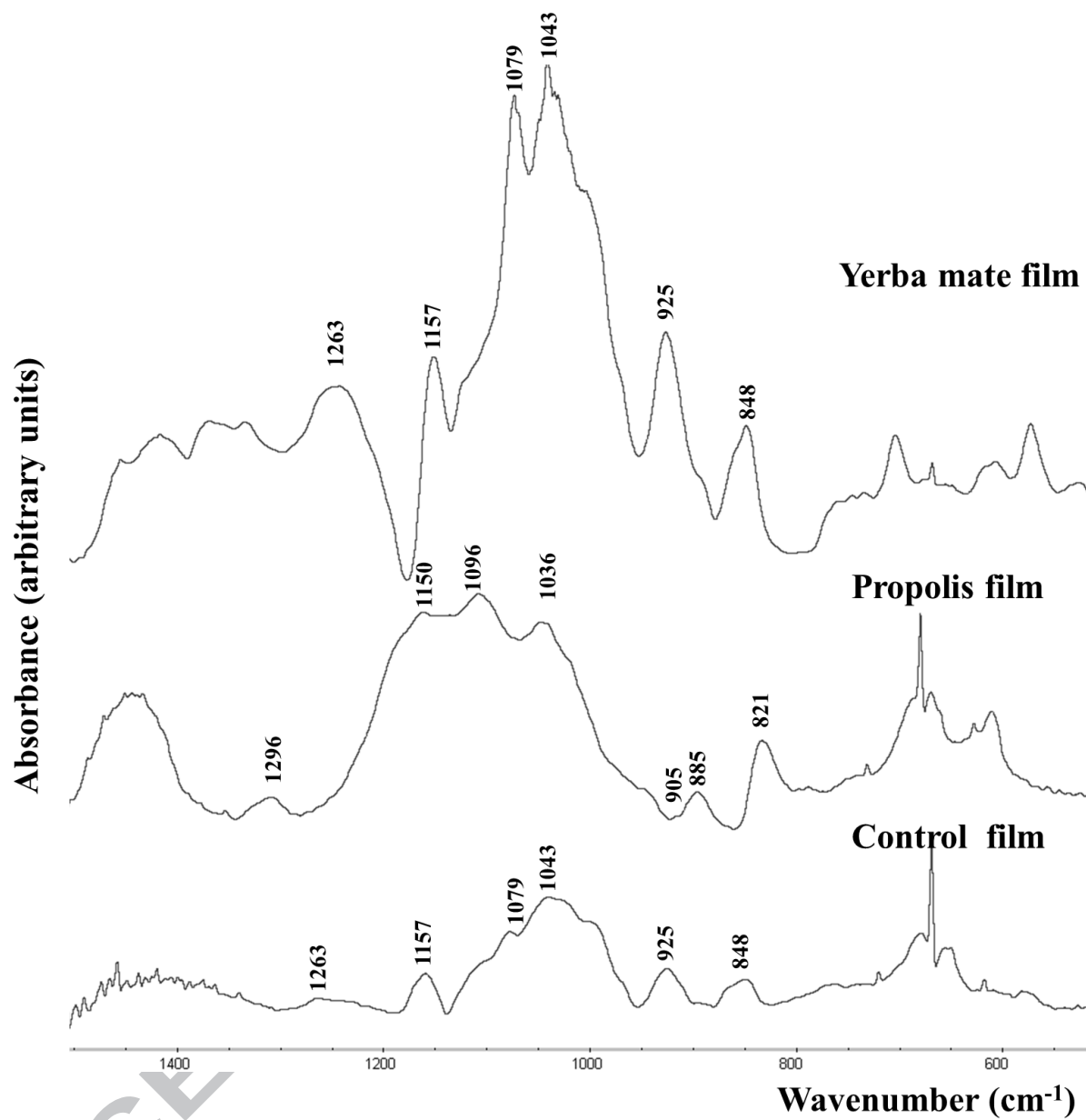


**Fig. 3** X-ray diffraction patterns of the carrageenan-starch films with and without the extracts of yerba mate and Cuban red propolis

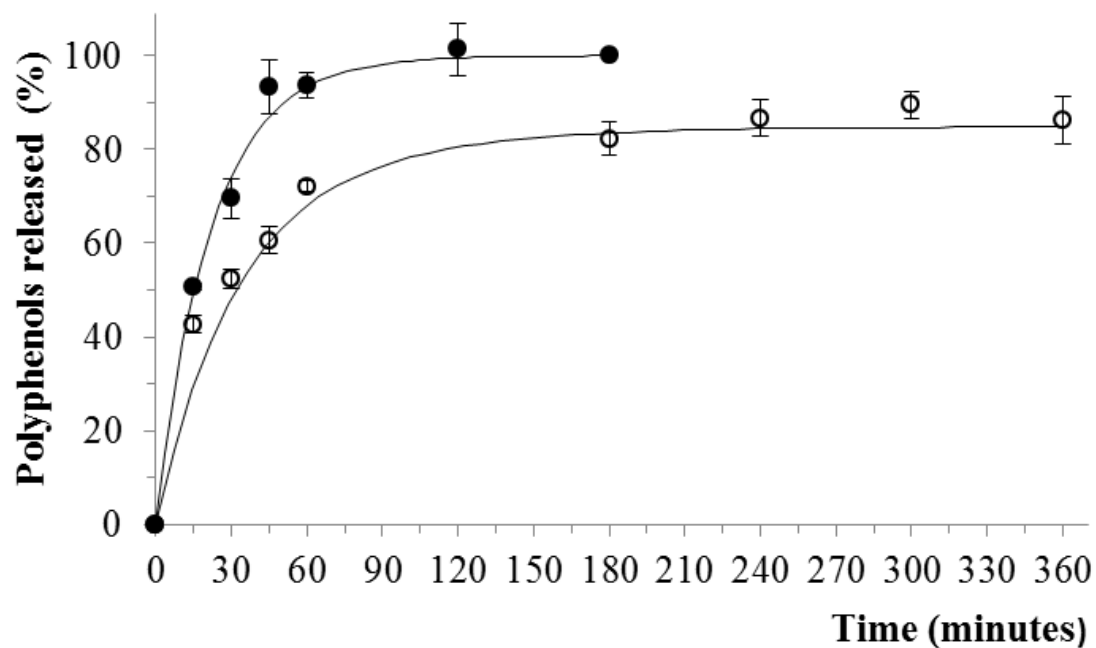




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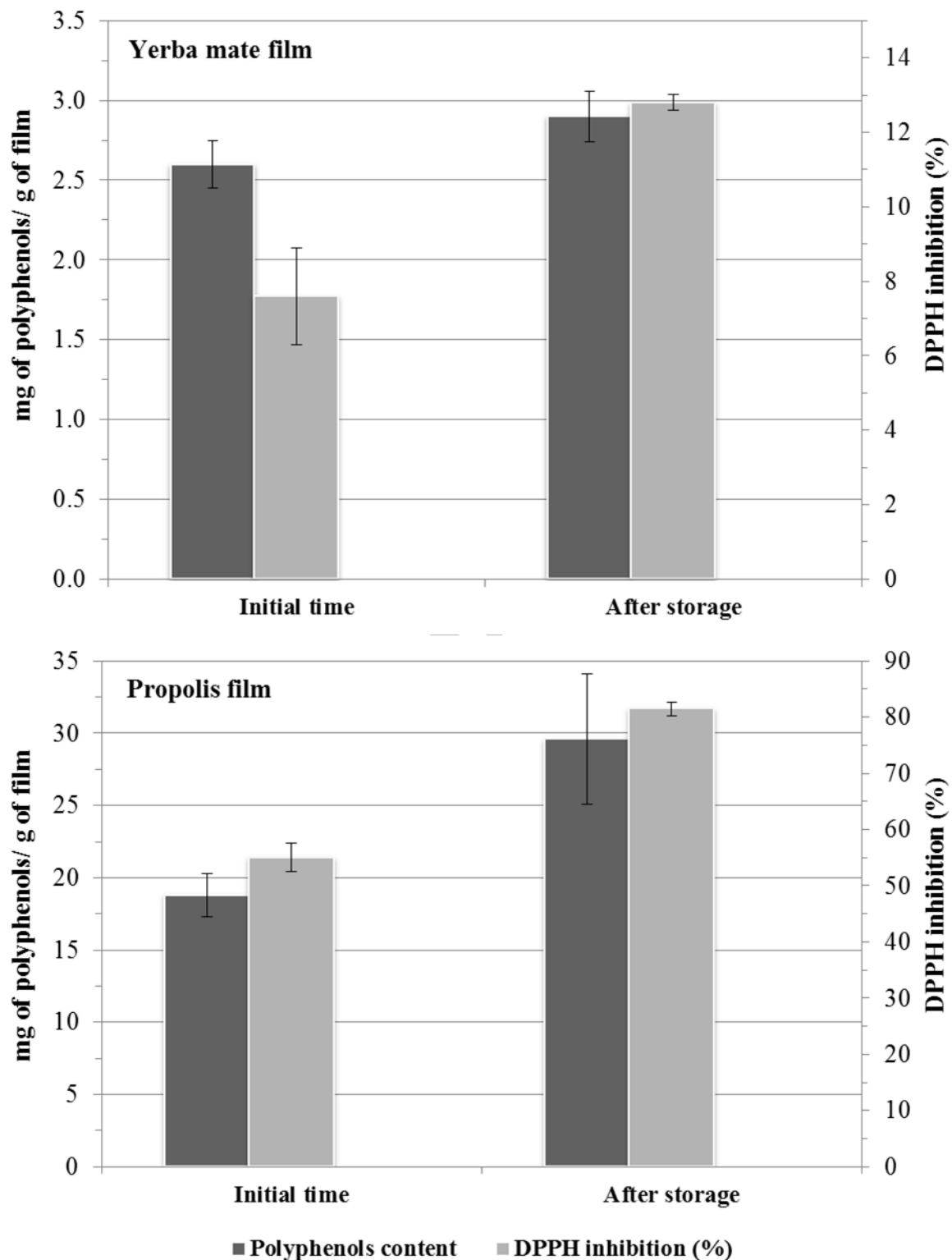


Fig. 7 Chemical stability of the films stored at 75% RH and 23 °C during 6 months.

Table1. Glass transition temperatures obtained by differential scanning calorimetry

Samples	T <sub>g1</sub> (°C)			T <sub>g2</sub> (°C)		
	T <sub>on</sub>	T <sub>mid</sub>	T <sub>end</sub>	T <sub>on</sub>	T <sub>mid</sub>	T <sub>end</sub>
Control film	-73.2	-68.5	-62.7	-14.8	-3.1	3.5
Propolis film	-72.8	-68.4	-63.8	-15.8	-6.0	2.9
Yerba mate film	-73.2	-68.4	-65.1	-16.1	-6.4	1.9

T<sub>on</sub>, T<sub>mid</sub> and T<sub>end</sub> are the onset, midpoint and endset temperatures, respectively. Maximum standard deviation 0.5 °C

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Table 2. Main functional groups identified by FTIR in the extracts of yerba mate and propolis

Propolis extract		Yerba mate extract	
Signals (cm <sup>-1</sup> )	Functional group	Signals (cm <sup>-1</sup> )	Functional group
1630	C = O; C = C stretching	1520	C = C stretching
1507	C = C stretching	1270	C–O stretching
1160	C–O stretching; C–OH bending	1160	C–O stretching; C–OH bending
1430	C–H bending	1070	C–C stretching; C–OH bending

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Table 3. Mechanical properties of the carrageenan-starch films without and with extracts of yerba mate and propolis

Sample	Tensile strength (MPa)	Elongation at break (%)	Elastic modulus (MPa)
Control films	53.3 ± 9.3	2.3 ± 1.1	32.1 ± 0.6
Yerba mate films	47.9 ± 2.3	2.2 ± 0.2	34.2 ± 6.3
Propolis films	25.7 ± 0.1	12.9 ± 0.3	10.4 ± 0.5

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