



Crosslinking capacity of tannic acid in plasticized chitosan films

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ABSTRACT

Plasticized and unplasticized chitosan films with tannic acid addition were developed. The crosslinking capacity of tannic acid was studied by evaluating both the structural modification produced on chitosan films and its effect on physicochemical, barrier and mechanical properties. Likewise the changes of these properties during the storage were analyzed; formulations containing tannic acid increased 29% tensile strength whereas decreased 24% water vapor permeability. Film solubility decreased at 100 °C, confirming that exposure at high temperatures facilitates the crosslinking process. The presence of tannic acid and glycerol simultaneously showed a synergic effect on the studied properties, all of which presented intermediate behavior in relation to those determined with the addition of either acid or plasticizer. The effect of the storage was also relevant since the films evidenced their tendency to a more stable structure due to the reorganization toward an anhydrous conformation, as was demonstrated by X-ray diffraction and FTIR analysis.

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1. Introduction

Chitosan is an attractive polymer for a number of uses since it is non-toxic and renewable, two properties which are extremely important in the biomaterial development. Chemical and biochemical reactivity of chitosan is strongly related to the free primary amine groups distributed regularly through its molecular chain that allow to customize its functionality. Thus chitosan applications have been the focus of a wide number of studies which reported their potential uses in the pharmaceutical, medical and food fields (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997; Arvanitoyannis, Nakayama, & Aiba, 1998; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004).

Polymer network can be modified by crosslinking treatments (Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001; Chambi & Grosso, 2006; Sabato et al., 2000). Crosslinking is an important step in the preparation of hydrocolloid films to ensure their stability and mechanical resistance (Mathew & Abraham, 2008). Crosslinking modifications occur mainly through two different reactions either Schiff base or Michael-type adducts (Ravi Kumar et al., 2004).

Glutaraldehyde, the most studied chemical agent, is synthetic and cytotoxic (Bigi et al., 2001; Sung, Huang, Huang, & Tasi, 1999) whereas transglutaminase, which is commonly used for enzymatic

reactions, is expensive (Carvalho & Grosso, 2004). Nowadays natural alternatives are been studied such as genipin (Bigi, Cojazzi, Panzavolta, Roveri, & Rubini, 2002), hydroxycinnamic (Rice Evans, Miller, & Paganga, 1996), citric (Reddy & Yang, 2010), ferulic and tannic acid (Cao, Fu, & He, 2007), as well as proanthocyanidin (Kim, Nimni, Yang, & Han, 2005).

Tannic acid, a gallic ester of D-glucose is recognized by its antioxidant capacity due to their multiple phenolic groups that can interact with biological macromolecules (Aelenei, Popa, Novac, Lisa, & Balaita, 2009).

To the best of our knowledge, the use of tannic acid to crosslink chitosan-based films has hardly been reported; thus this could be an alternative to achieve better film properties.

The development of plasticized and unplasticized chitosan films with the addition of tannic acid was carried out. The crosslinking capacity of tannic acid was studied by evaluating both the structural modification produced on the chitosan films and its effect on physicochemical, barrier and mechanical properties. Likewise the changes of the abovementioned properties during the storage were analyzed.

2. Materials and methods

2.1. Reagents

Commercial chitosan from crab shells with a minimum deacetylation degree of 85% and Mw of 1474 kDa was purchased from Sigma (St. Louis, MO, USA).

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Glycerol (analytical grade), used as plasticizer, was provided by J.T. Baker (Xalostoc, México). Tannic acid (analytical grade) was purchased by Anedra (Buenos Aires, Argentina).

2.2. Film-forming solution preparation

Chitosan solution of 1.5% (w/w) was prepared by solubilization in 1.5% (v/v) acetic acid solution under stirring for 24 h, as it was described in a previous work (Rivero, García, & Pinotti, 2009).

A screening of glycerol (P) and tannic acid (TA) was assayed in order to select the optimum concentrations. Glycerol was added as plasticizer to chitosan solution; the concentrations tested were: 0.25, 0.5, 0.75, 1 and 1.5% (g glycerol/100 ml solution).

Different amounts of crosslinking agent, tannic acid (20, 40, 60, 80 mg TA/g chitosan), were incorporated to plasticized and unplasticized chitosan solutions to select the optimum concentration.

2.3. Film preparation

Plasticized and unplasticized chitosan films with and without TA were prepared by casting 20 g of filmogenic solutions onto Petri dishes (9 cm diameter) and drying at 37 °C in an oven until reaching constant weight (approximately 24 h). The ratio of film-forming solution weight to acrylic plate area was maintained in order to guarantee a constant film thickness. The obtained films were removed from the dish and stored for 48 h prior to the determinations of structural, physicochemical, barrier and mechanical properties. The films were conditioned in a controlled room during 30 days (final time) at 20 °C and 65% relative humidity (RH) to evaluate the effect of the storage time on the properties studied.

Film nomenclature used was for chitosan films CH; plasticized chitosan films CHP; chitosan films containing tannic acid CHTA and films containing both tannic acid and glycerol CHPTA. Besides letter I was added for initial time and F for stored samples.

2.4. Physicochemical characterization

2.4.1. Film solubility in water

Samples of 2 cm × 2 cm were cut from each film, weighed to the nearest of 0.0001 g and placed into test beakers with 80 ml deionized water. The samples were maintained under constant agitation for 1 h at 20 °C or at 100 °C. The remained pieces of the films after soaking were dried again in an oven at 105 ± 1 °C to constant weight. Film solubility (%) was calculated as follows:

$$\% \text{ Solubility} = \left[\frac{\text{initail dry weight} - \text{final dry weight}}{\text{initail dry weight}} \right] \times 100$$

All samples were analyzed at least in triplicate.

2.4.2. Moisture content

Film moisture contents were determined by measuring their loss of weight, upon drying in an oven at 105 ± 1 °C until reaching constant weight (dry sample weight). Samples were analyzed at least in triplicate and results were expressed in grams of water per 100 g of sample.

2.4.3. Color

Film color was determined using a Minolta colorimeter CR 400 Series (Osaka, Japan) calibrated with a standard ($Y=93.2$, $x=0.3133$, $y=0.3192$). The CIE Lab scale was used; lightness (L) and chromaticity parameters a^* (red–green) and b^* (yellow–blue) were measured. Color differences ΔE were also calculated (Rivero et al., 2009). Assays were performed by placing the film samples over the standard. Samples were analyzed in triplicates, recording four measurements for each sample.

2.5. Water vapor permeability

Water vapor permeability (WVP) tests were conducted based on a modified ASTM (1995) method E96 using a specially designed permeation cell that was maintained at 20 °C as it was described in a previous work (Rivero, García, & Pinotti, 2010). After steady-state conditions were reached, eight measurements were performed over 8 h. Each informed value corresponded at least to four determinations.

2.6. Microstructural studies by transmission electron microscopy

The morphology of films was examined using a transmission electron microscope (JEM 1200EX II Jeol, Japan) equipped with a digital camera (ES500W Erlangshen CCD Gatan). Small pieces from the center of the films were prepared according to Denavi et al. (2009).

2.7. X-ray diffraction

Plasticized and unplasticized chitosan films with and without tannic acid were analyzed by X-ray diffraction in an X'Pert Pro P Analytical Model PW 3040/60 (Almelo, The Netherlands). The CuK α radiation (1.542 Å), operated at room temperature, was generated at 40 kV and 30 mA, and the relative intensity was recorded in the scattering range of (2θ) 3–60° with a step size 2θ 0.02°.

2.8. Mechanical properties

The tensile test of the preconditioned films was performed at room temperature in a texturometer TA.XT2i—Stable Micro Systems (England) as it was described in a previous work (Rivero et al., 2009) using a tension grip system A/TG at a constant rate of 1 mm/s. Film probes of 6 cm length and 0.7 cm width were used.

To evaluate if the crosslinking process had been developed in a preferential direction, CHTA films were casted onto rectangular plates (10 cm × 20 cm) and longitudinal and transverse probes relative to the major dimension were cut and tested.

The curves of force (N) as a function of deformation (mm) were automatically recorded by the Texture Expert Exceed software. The elongation at break, EB (% of the original probe length) and the tensile strength (MPa) were also calculated according to the ASTM D638-01 method (2001). Each informed value corresponded at least to six determinations.

2.9. FTIR spectroscopy

The Fourier transform infrared (FTIR) spectra of the films were recorded in an IR spectrometer (Vertex 50, Brooker, Germany) in the wavenumber range 4000–400 cm⁻¹ by accumulation of 60 scans at 4 cm⁻¹ resolution. For each film formulation the spectra were obtained at initial time and after stored for 30 days. The samples were conditioned at 20 °C and 65% relative humidity (RH).

2.10. Statistical analysis

Systat-software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for all statistical analysis. Analysis of variance (ANOVA), linear regressions and Fisher LSD mean comparison test were applied. The significance levels used were 0.05 and 0.01.

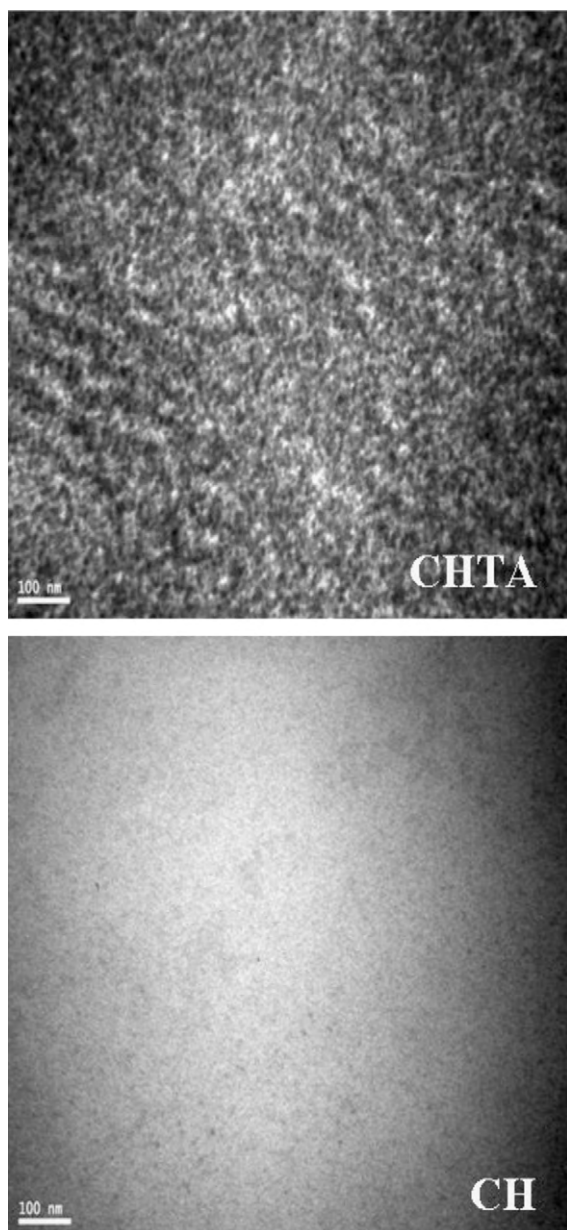


Fig. 1. TEM micrographs of: (a) tannic acid crosslinked chitosan films (CHTA) and (b) chitosan films (CH). Magnification: 100 nm between marks.

3. Results and discussion

3.1. Tannic acid and glycerol concentration screening

The tannic acid effect on chitosan film morphology was studied by TEM. Fig. 1 shows that chitosan films were homogeneous, with smooth surfaces and good structural integrity whereas those containing TA presented irregular and compact surface, with particulate-like structure without any phase separation. The irregularity of the films would be either an indicator of the crosslinking points or the structural reinforcing of chitosan matrix and, consequently, good mechanical properties such as high resistances at break would be expected. The effect of tannic acid addition on mechanical and barrier properties of the chitosan films is shown in Table 1. The addition of 20 and 40 mg TA/g chitosan caused significant differences ($P < 0.05$) in both properties (Table 1) with regard to the other concentrations assayed. In spite of these results, a concentration of 40 mg TA/g chitosan allowed to obtain significantly

Table 1

Tannic acid concentration screening: mechanical and water vapor barrier properties.

Tannic acid addition to CH films (mgTA/g chitosan)	Water vapor permeability $\times 10^{11}$ (g/s m Pa)	Tensile strength (MPa)
0	10.14 \pm 0.39 ^a	73.83 \pm 5.37 ^a
20	8.95 \pm 0.02 ^c	92.13 \pm 4.70 ^b
40	8.35 \pm 0.73 ^b	95.03 \pm 4.20 ^b
60	9.44 \pm 0.28 ^a	73.02 \pm 1.27 ^a
80	9.18 \pm 0.42 ^a	75.04 \pm 1.73 ^a

Different letters within columns indicate significant differences ($P < 0.05$).

lower WVP values than 20 mg TA/g chitosan, and that is why the concentration selected for further analysis was 40 mg/g chitosan.

Glycerol addition, which is a typical plasticizer for hydrophilic-based films, changes the mechanical pattern of chitosan films from a rigid material to a flexible one. The well known effect of glycerol concentration was observed. Elongation at break increased from 18.3% for unplasticized chitosan films to 54% for those that contained 0.5 g glycerol/100 g solution. However, for higher plasticizer concentrations, EB reached a plateau at 72%. This could be associated to the saturation of the film matrix with glycerol; although plasticizer migration was not detected.

Considering the water vapor permeability, glycerol content of 0.5 g/100 g solution increased slightly WVP in relation to unplasticized CH films (from 1.01×10^{-10} to 1.13×10^{-10} g m⁻¹ s⁻¹ Pa⁻¹). Likewise, higher concentrations of the plasticizer led to a significant ($P < 0.05$) increase in WVP of 38 and 93%, for 0.75 and 1.5 g glycerol/100 g solution, respectively. Thus, according to the mechanical and barrier properties results, the plasticizer concentration selected for further assays was 0.5 g/100 g solution.

3.2. Physicochemical properties

The visual properties of a film finally determine the consumer acceptability of the packaged products. Polysaccharide films are typically colorless, though those of CH may exhibit a slightly yellow appearance. The yellowness of the plasticized chitosan films indicated by b^* (not shown) and ΔE values may be due to an oxidative reaction produced when they were stored for a long period (Table 2). This tendency was more marked for tannic acid–chitosan blends. Trezza and Krochta (2000), working with whey protein-based films found that the intensity of the yellowness increased with storage time.

Table 2 shows that the color differences of CH films did not differ ($P > 0.05$) between initial and final time significantly. The addition of both TA and plasticizer led to the major color changes ($P < 0.05$) since both agents impart color due to their tendency to oxidize, yielding brownish films.

Water solubility is an important factor to select the possible applications of biopolymer films. CH films presented lower water solubility values in relation with other hydrocolloid-based films such as cellulose derivatives (Pinotti, García, Martino, & Zaritzky, 2007). The tannic acid addition decreased film solubility at 20 °C, while the glycerol incorporation to either chitosan or tannic acid–chitosan blends (CHP and CHPTA) increased it (Fig. 2). This fact could be attributed to the crosslinking of the matrix by TA and the hydrophilic character of the plasticizer, respectively.

At 100 °C, the water solubility of CH films increased compared with that obtained at 20 °C; in this case the addition of plasticizer did not affect it significantly ($P > 0.05$) (Fig. 2). On the other hand, formulations containing TA decreased the film solubility at 100 °C, since exposure at high temperatures facilitates the crosslinking process. According to Kubota, Kikuchi, Mizuhara, Ishihara, and Takita (1993) N-acetylation with some crosslinking agents could be formed on the chitosan molecule in the presence of water; the decrease of hydrophilic groups led to the decrease in the percentage

Table 2
Physicochemical characterization of chitosan-based films: effect of tannic acid and plasticizer addition.

Film composition	Water vapor permeability $\times 10^{11}$ (g/s m Pa)		Film color differences (ΔE)		Moisture content (g water/100 g film)	
	Initial	Final	Initial	Final	Initial	Final
CH ^a	10.14 \pm 0.39	7.68 \pm 0.48	3.01 \pm 0.42	3.12 \pm 0.19	18.12 \pm 0.99	9.73 \pm 0.76
CHP ^b	11.30 \pm 1.42	9.92 \pm 0.28	3.35 \pm 0.21	4.15 \pm 0.28	31.44 \pm 1.03	21.24 \pm 1.16
CHTA ^c	8.34 \pm 0.73	5.79 \pm 0.36	5.69 \pm 0.35	11.62 \pm 0.90	17.48 \pm 0.79	11.94 \pm 0.72
CHPTA	8.72 \pm 0.51	6.26 \pm 0.42	5.93 \pm 0.56	17.24 \pm 0.57	34.54 \pm 1.38	19.04 \pm 0.85

^a CH: 1.5% (w/v).

^b P: 0.5% (w/w) glycerol.

^c TA: 40 mg tannic acid/g chitosan (selected concentration).

of water solubility and dissolution of treated films. In summary, by tailoring film formulations, the water solubility of CH films could be altered to meet the requirements of specific applications.

As it can be visualized in Table 2, plasticizer addition increased moisture content of films, due to its hydrophilic character, while tannic acid did not affect it. For all tested formulations, the moisture content of chitosan-based films decreased during the storage.

As it was previously described, plasticization increased WVP of CH films. In contrast, the incorporation of tannic acid caused a significant decrease ($P < 0.05$) to a value of $8.34 \times 10^{-11} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ (Table 2). This tendency was even more pronounced during the storage, which would be an indicator of the reorganization of the film matrix. According to Ou, Wang, Tang, Huang, & Jackson (2005) crosslinking restricts the movement of molecules by formation of a more tight structure leading to a lower WVP.

The WVP results obtained for CHPTAI at initial time and stored CHPTAF samples indicated that the tannic acid had much higher influence than the plasticizer addition on the film matrix. A significant ($P < 0.05$) increase in WVP of CHPTAF was observed with reference to CHTAF samples (Table 2).

3.3. X-ray diffraction

For film materials, an insight into microstructure is critical since it determines their barrier and mechanical properties; thus, films were studied by X-ray diffraction. Ogawa, Yui, & Okuyama (2004) studying structures of several chitosan solubilized in different acids have found different conformations. These authors proposed three forms: noncrystalline, hydrated (“tendon”) and anhydrous (“annealed”) crystalline. The hydrated crystalline structure reflects at 2θ 10° (or peaks at around 8° and 12°), while the anhydrous structure at 2θ 15°. In our work, CHI films exhibited a similar behavior. Fig. 3a shows reflections at 2θ 22.9, 18.3, 16.3, 11.6 and 8.4 at initial time, in agreement with the findings of Ritthidej, Phaechamud, & Koizumi (2002) and Zhong and Xia (2008). According to Wang, Shen, Zhang, & Tong (2005) the last two peaks correspond to the

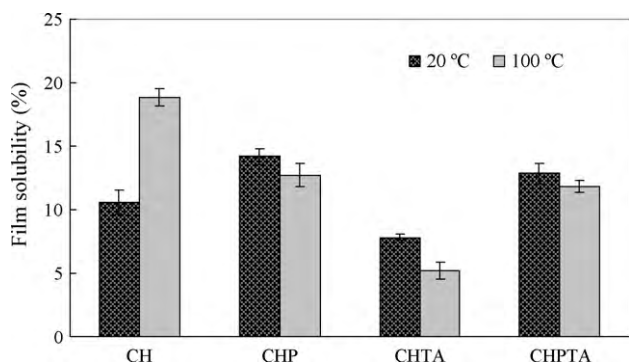


Fig. 2. Effect of tannic acid and glycerol addition on chitosan water film solubility at 20 and 100 °C. Error bars correspond to standard deviations. TA: 40 mg tannic acid/g chitosan.

hydrated crystalline structure, while the broaden peak around 2θ 23° indicates the existence of an amorphous structure.

During the storage unplastitized chitosan films (CHF) showed a slight shift to higher 2θ values in relation to CHI (Fig. 3a and Table 3). These facts would indicate that acetic acid was spontaneously removed from the chitosan matrix accompanied by water molecules. Ogawa et al. (2004) attributed this shift to a gradual conformational evolution toward the anhydrous polymorph of chitosan. A similar trend was reported by Brown et al. (2001).

Films containing plasticizer, tannic acid or a combination of both exhibited similar X-ray diffraction patterns. All of them kept their characteristic peaks respect to those obtained at initial time but a shift of these peaks was observed during the storage (Table 3). The magnitude of these displacements was different depending on the

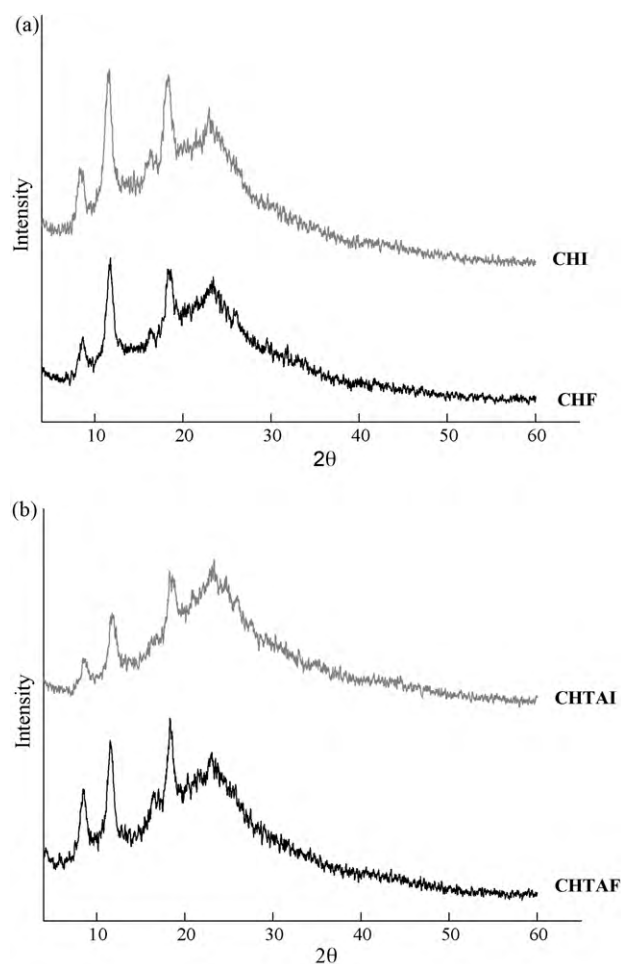


Fig. 3. X-ray diffractograms of: (a) chitosan films (CHI) and (b) tannic acid crosslinked chitosan films (CHTA) at initial time and after stored for 30 days at controlled conditions.

Table 3

Displacement of X-ray pattern peaks of stored CH films with tannic acid and plasticizer addition.

Peaks location of CHF	Displacement of peaks X-ray (%) ^a		
	CHTAF	CHPF	CHPTAF
8.64	0.92	6.48	3.47
11.76	1.19	4.08	3.74
16.30	0.12	7.73	1.47
18.38	0.11	1.63	1.63
23.44	1.54	9.22	2.56

^a Relative to peak location of chitosan films after the storage (CHF).

additive used. The addition of TA shifted the peaks toward lower 2θ values (Fig. 3b and Table 3) while with the plasticizer incorporation the effect was far more marked. X-ray diffraction patterns obtained with the addition of both TA and glycerol, showed a lower percentage of displacement of the aforementioned peaks than in the case of plasticized chitosan films, as can be visualized in Table 3. These results could be attributed to a synergic effect of TA and glycerol.

3.4. Mechanical properties

CHI films, characterized as rigid materials, exhibited a high tensile strength (73.8 MPa) and low elongation at break values (17.7%). As it was abovementioned the mechanical pattern of chitosan films was changed by the addition of the plasticizer toward one own of flexible or ductile materials characterized by medium tensile strength and high deformation (Fig. 4).

The mechanical assays of films formulated with TA were performed in both transverse and longitudinal directions. The obtained results were independent of the measuring direction, which could be indicative of the homogeneity of the crosslinking process in the film matrix.

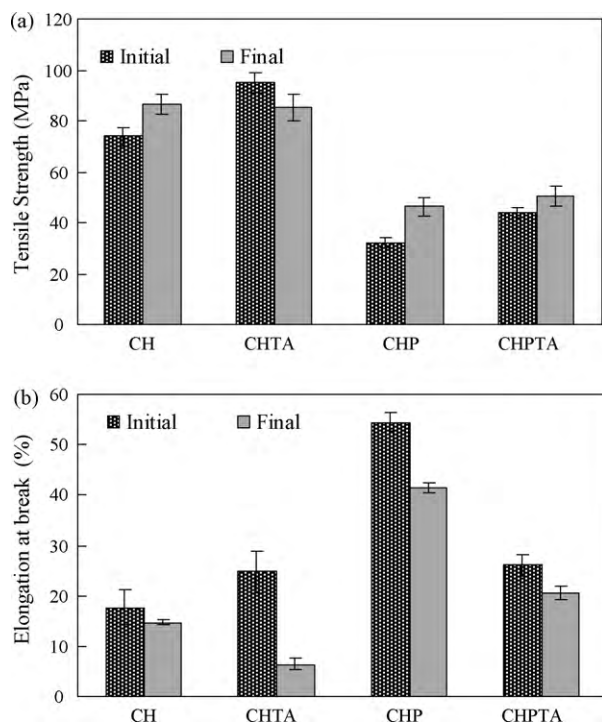


Fig. 4. Mechanical properties of chitosan films (CH) with the addition of tannic acid (CHTA), glycerol as plasticizer (CHP) and tannic acid and plasticizer (CHPTA), at initial time and after stored for 30 days at controlled conditions: (a) tensile strength and (b) elongation at break. Error bars correspond to standard deviations.

Analyzing the film behavior at the initial time, the tensile test results suggested that the mechanical properties of the tannic acid–chitosan blends were superior to those of neat chitosan due to the reinforcement effect of the crosslinker throughout the biopolymer matrix. The addition of TA increased tensile strength significantly ($P < 0.05$), around 29% but film flexibility was not affected (Fig. 4a and b). A similar trend was reported by both Cao et al. (2007) working on gelatin film crosslinked with tannic acid, and Mathew and Abraham (2008) for starch–chitosan blends with ferulic acid. However, comparing films containing both additives (CHPTAI) with those without plasticizer (CHTAI) a marked decrease in the tensile strength was observed even though a similar elongation value was obtained.

Since water is the most ubiquitous plasticizer in hydrophilic films, a correlation between elongation and film moisture content was found; the higher the water content the higher the elongation. The addition of glycerol (CHPI) decreased (on average) 49% tensile strength while it increased at least 76% elongation at break and 80% moisture content of the films.

Regardless of the storage time, tannic acid addition did not affect tensile strength of chitosan films; this could be attributed to the effect of the crosslinker on the structure stabilization, while for unplasticized and plasticized chitosan films tensile strength increased during storage (CHF and CHPF). In all cases, EB decreased significantly ($P < 0.05$), this effect being more marked in unplasticized films containing tannic acid (Fig. 4b). These results were in agreement with the reduction of moisture content and the structural reorganization previously mentioned (Table 2). A similar explanation was attempted by Suyatma, Tighzert, & Copinet (2005) studying the effect of hydrophilic plasticizers on mechanical properties of chitosan films.

3.5. Infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) reveals information about the molecular interactions of chemical components and is an useful technique to supplement microstructural characterization of composite films. As the exact wavenumber position in IR spectra of biological samples is sometimes difficult to be identified the second derivative technique is applied as an enhancement resolution method to verify the peak positions.

The spectrum of non-plasticized CHF film (Fig. 5a) exhibited a broad absorption band in the $3600\text{--}3000\text{ cm}^{-1}$ with a minimum at 3460 cm^{-1} results from overlapping of the O–H and N–H stretching vibrations of functional groups engaged in hydrogen bonds. Bands at 2867 , 2912 , 1419 , and 1263 cm^{-1} , belonging to symmetric and antisymmetric CH_2 vibrations of carbohydrate ring, and the band at 1336 cm^{-1} , characteristic of –OH, – NH_2 , –CO groups, measure the extent of N-acetylation, according to the findings reported by Brugnerotto et al. (2001) and Ostrowska-Czubenko and Gierszewska-Druzynska (2009). CHF spectrum also exhibited the distinctive absorption bands at 1653 cm^{-1} (C=O stretching in amide group, amide I vibration) and 1581 cm^{-1} (– NH_2 bending in non-acetylated 2-aminoglucose primary amine). Absorption bands at 1158 cm^{-1} (antisymmetric stretching of the C–O–C bridge) and 1031 cm^{-1} (skeletal vibrations involving the C–O stretching), characteristics of the chitosan saccharide structure were observed (Pawlak & Mucha, 2003; Smitha, Sridhar, & Khan, 2005).

Other peaks were located at wavenumbers of 1730 cm^{-1} , implying the ester linkages and 896 cm^{-1} referred to the C–O–C of the pyranose ring (Pasanphan & Chirachanchai, 2008). Besides, with the storage time, the spectrum of chitosan (CHF) films showed a split into several separate peaks in the wavenumber ranges $1458\text{--}1562$ and $1637\text{--}1650\text{ cm}^{-1}$, compared with CHI spectrum.

Residues of chitin, attributed to N–H bond of N-acetyl group (amide II) were evidenced by a broad peak at 1556 cm^{-1} at initial

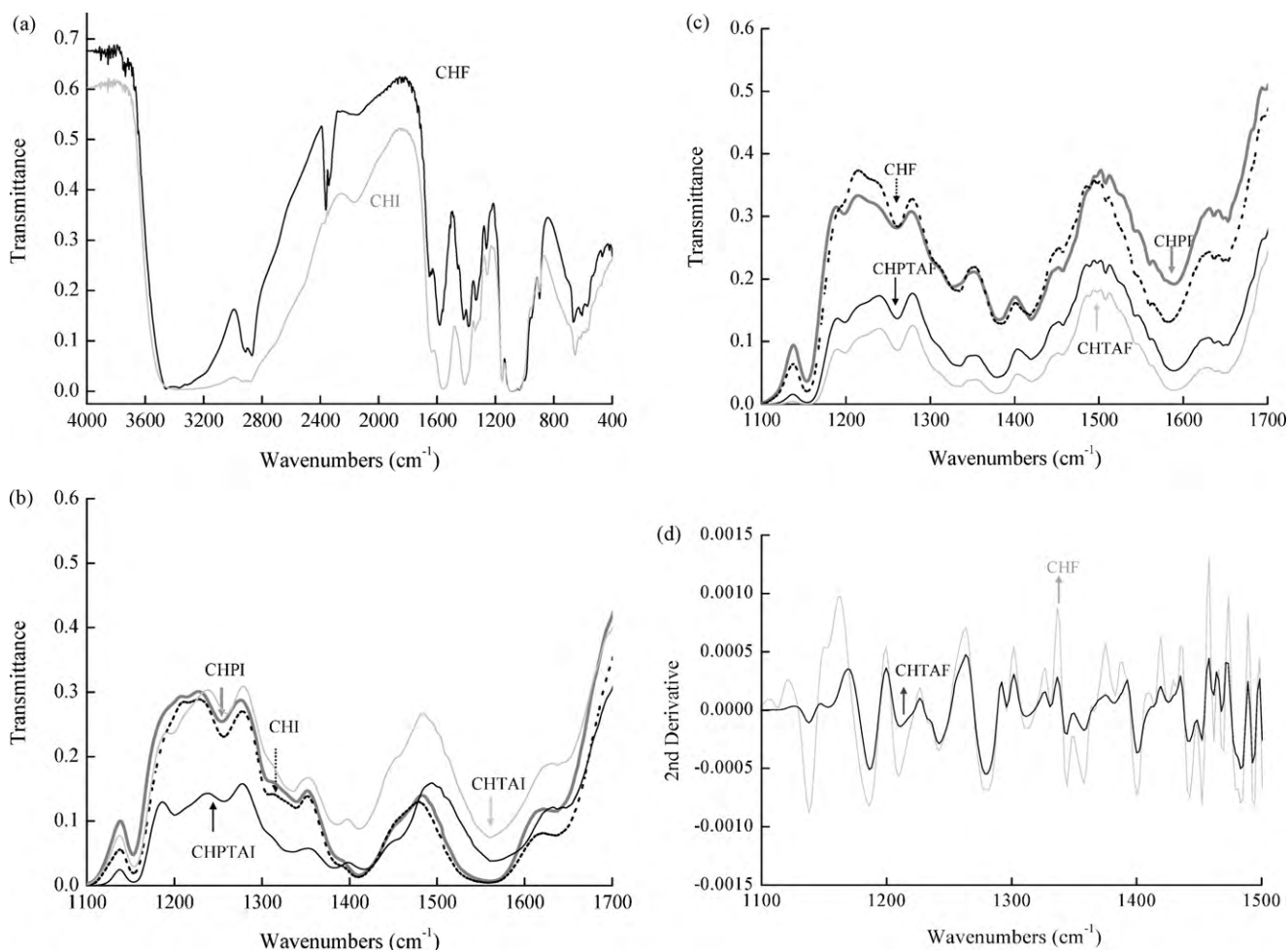


Fig. 5. FTIR spectra of: (a) chitosan films at initial time (CHI) and after 30 days of storage (CHF), (b) chitosan films with (CHPI) and without plasticizer (CHI), tannic acid crosslinked chitosan films with (CHPTAI) and without plasticizer (CHTAI), at initial time, (c) chitosan films with (CHPF) and without plasticizer (CHF), tannic acid crosslinked chitosan films with (CHPTAF) and without plasticizer (HTAF), after stored for 30 days and (d) second derivatives of CHF and CHTAI films after storage.

time (CHI) which appeared as a sharp peak at higher wavenumber (1581 cm^{-1}) in the stored samples (CHF). Similar results were informed by Yamamoto, Kawada, Yui, & Ogawa (1997) due to the spectrum of the chitosan aged sample changed to those own of the anhydrous polymorph (Fig. 5b and c).

As it can be visualized in Fig. 5a, in CHI spectrum some of the abovementioned exhibited a red shift (compared with CHF), among them 1255 , 1340 , 1411 cm^{-1} and others a blue shift like those located at 2873 and 2929 cm^{-1} . These last displacements can be explained by the dehydration of methyl groups in the lateral chain and by conformation changes of the main chain (Gu & Wu, 2007).

In the case of CHTAI films, the spectra turned out to be similar to those obtained for CHF films. Nevertheless, the absorption peaks decreased in intensity at constant wavenumber (Fig. 5c); likewise the second derivative technique put in evidence this result (Fig. 5d). According to Aelenei et al. (2009) characteristic bands of the tannic acid were either attenuated or covered (as shoulders underneath other bands), which represents a proof that the tannic acid was included in the chitosan matrix or that it was found in a very small amount in the blend.

Again, as it was observed in the case of CH films, both peaks of the spectra of CHTAI films, located at 2868 and 2915 cm^{-1} at initial time underwent a displacement in their positions to lower wavenumbers, to 2854 and 2910 cm^{-1} , respectively, during the storage.

Samples of CH with plasticizer (CHPI and CHPF) are visualized in Fig. 5b and c, which shows that the spectra of CHP films fol-

lowed the shape of those obtained for unplasticized films (CHI and CHF); absorption intensities being hardly affected by the presence of glycerol. The major changes in plasticized chitosan films during the storage (CHPI and CHPF spectra) can be visualized in the $2800\text{--}3000\text{ cm}^{-1}$ region; the peaks located at 2889 and 2935 cm^{-1} corresponding to initial time suffered a shift toward 2877 and 2923 cm^{-1} , respectively (Fig. 5b and c).

Spectra of CHPTAF showed the synergistic effect of both components glycerol and tannic acid, since intermediate absorption intensity peaks (between CHPF and CHTAI) were obtained (Fig. 5c). The marked peak displacement at wavenumber ranged between 2800 and 3000 cm^{-1} , attributed to the plasticizer was attenuated by the presence of the tannic acid.

FTIR technique results exhibited a similar trend to those found for all the studied properties and moreover, these findings supported that the values obtained with TA and glycerol addition were intermediate between those obtained with the incorporation of either the acid or the plasticizer.

4. Conclusions

The use of the optimal concentration of glycerol, which was determined by a screening, in chitosan-based films, improved the mechanical properties of the films and allowed to obtain the best WVP value. A microstructural analysis showed the major displacements in the peaks of X-ray diffraction patterns and in the FTIR

spectra at high wavenumbers in comparison with the unplasticized chitosan films.

The addition of the optimum content of tannic acid, obtained through a concentration screening, to the chitosan matrix led to form a more rigid structure because it acted as a crosslinking agent. The increase of tensile strength and the decrease of elongation as well as WVP indicated the compaction of the chitosan matrix. Likewise tannic acid incorporation decreased the film solubility with the temperature, the higher the temperature the lower the solubility. Results obtained through X-ray attributed this gradual evolution of the matrix toward to anhydrous polymorph, correlated to the equilibrium moisture and the blue shifts observed in FTIR spectra.

The presence of tannic acid and glycerol in the chitosan films simultaneously, showed a synergic effect that was supported by the intermediate values obtained for all the analyzed properties with respect to those determined with the addition of either the acid or the plasticizer.

The effect of the storage was also relevant given that the studied films had tendency to a more stable structure due to the reorganization toward an anhydrous conformation. For this reason, the moment at which the determinations are made have to be taken into account since film properties are not static and could have not been reached the equilibrium state yet.

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