

Heat Treatment To Modify the Structural and Physical Properties of Chitosan-Based Films

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ABSTRACT: This work was focused on studying the changes undergone by heat-treated chitosan films with and without tannic acid addition by monitoring both microstructure and physical properties. Once the films were submitted to different heat treatments, they exhibited higher barrier properties as well as lower water uptake, solubility, and moisture content. These results were also confirmed through X-ray patterns, which changed from the hydrated to the anhydrous conformation, sharper FTIR peaks specifically associated with water, and shift of T_g toward higher temperatures determined by DMA. Moreover, the modifications caused by the curing process at a molecular scale were observed at a structural level by using a TEM technique. FTIR evaluation granted new insights into the interactions between tannic acid and chitosan molecules, before and after the heat curing, especially due to the occurrence of new peaks and changes in the wavenumber region 1550–1750 cm^{-1} .

KEYWORDS: chitosan film, tannic acid, heat treatment, curing, microstructure

■ INTRODUCTION

Chitosan is a biodegradable and biocompatible natural polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine, derived from chitin. Due to its functionality, numerous films,^{1,2} coatings,³ and injectable thermosetting chitosan gels have been developed for the controlled release of drugs and tissue engineering.⁴

Tannic acid (TA) is a high molecular weight polyphenolic compound containing a central carbohydrate (glucose) core, which is esterified into ester by phenol acids (gallic acid). It is also known as hydrolyzable tannin and is mostly extracted from plants and microorganisms; hence, it is fully biodegradable, having exhibited also recognized antioxidant capacity.⁵ According to the literature, tannic acid has the ability to bind with biopolymers such as collagen, gelatin, albumin, and some polysaccharides through noncovalent interactions.⁶

In a previous work, we found that the addition of tannic acid to a chitosan matrix caused a decrease in the water vapor permeability, solubility, and elongation of films, and a modification of the microstructure was demonstrated by X-ray, FTIR, and TEM.⁷ However, the chemical interactions between TA and the chitosan matrix have not been conclusively established and require further investigation.

It is well-known that polymer films show strong temperature- and time-dependent behavior. In general, the curing process is applied to stabilize the synthetic polymer network and its properties; this process can be conducted through heat, UV, and microwave, among others. In the case of hydrocolloid-based films, to achieve the same purpose enzymatic, chemical, and physical treatments have been applied.^{8,9}

Because one of the major problems associated with hydrocolloid-based films is their affinity for water molecules, the curing process could be a promising alternative to overcome their hydrophilic character. Hasimi et al.¹⁰ reported on the effect of thermal treatment on polyvinyl alcohol films to control the release rate of a model drug. According to these

authors heat treatment above the glass transition temperature (T_g) is used as a means of achieving a higher water resistance of the matrices.

Scarce data are available in the literature concerning the structure of the chitosan matrix with and without tannic acid after heat curing. Therefore, this work was focused on (i) studying the changes undergone by the heat-treated chitosan-based films by monitoring both microstructure and physical properties such as thermal stability, color, barrier properties, and water resistance; and (ii) attempting new insights into the interactions between tannic acid molecules and chitosan polymer through FTIR analysis before and after heat treatment and their relationship with chitosan-based film properties.

■ MATERIALS AND METHODS

Reagents. Commercial chitosan from crab shells with a minimum deacetylation degree of 75% was purchased from Sigma (St. Louis, MO). Tannic acid (TA) (analytical grade) was purchased from Anedra (Buenos Aires, Argentina).

Characterization of Tannic Acid. The determination of the tannic acid pK_a was carried out following the procedure described by Shutava et al.⁶ with some modifications. Tannic acid solutions at a concentration of 1.2 mg/mL were prepared, and pH values were adjusted by adding either HCl or NaOH within the range of 2–12 measured using a HACH sensION (U.S.A.) pH-meter. The absorption spectra of UV–vis of the solutions were recorded using a Hitachi U 1900 spectrophotometer (Japan), quartz cells, and water as reference. For each solution the absorbance ratio A_{320}/A_{280} was quantified and was plotted as a function of the pH of the solution; the pK_a corresponded to the inflection point of the curve.

Film-Forming Solution Preparation. A chitosan solution of 1.5% (w/w) was prepared by solubilization in 1.5% (v/v) acetic acid solution as described by Rivero et al.⁷

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The optimum concentration of tannic acid (40 mg TA/g chitosan, selected in a previous work⁷) was incorporated to chitosan solutions.

Film Preparation. 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 constant weight (approximately 24 h). The obtained films were removed from the dish and then submitted to heat treatments at 100, 160, and 180 °C for 15, 30, and 60 min.

Control and cured samples were conditioned in a controlled room at 20 °C and 65% relative humidity (RH). The effect of both temperature and time of the cured matrix was evaluated through the study of the physicochemical and structural properties and the dynamic mechanical behavior of the films.

Film nomenclature used was CH for chitosan films and CHTA for films containing tannic acid. In films submitted to heat treatment, the condition was indicated in each case. Besides, films without any treatment were studied as controls: CH film was used as the main control, whereas CHTA film was used as a secondary control.

From here on heat treatment, curing, or heat curing will be regarded as synonymous.

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

Film Solubility and Water Uptake. Chitosan-based films were cut in 3 × 3 cm pieces to evaluate both water uptake and film solubility. To determine film solubility, samples were weighed and placed in test beakers with 80 mL of distilled water. The samples were maintained under constant agitation for 1 h at 20 °C. The remaining pieces of the films after soaking were dried again in an oven at 105 ± 1 °C to constant weight. Film solubility (percent) was calculated as described in a previous work.⁷

Water uptake was measured by immersion of previously weighed film pieces in 80 mL of distilled water. At initial and after different hydration times (5, 15, 30, and 60 min) samples were recovered and dried with filter paper to remove the excess of surface water and weighed (film wet weight). The water uptake of the films was calculated as

$$\% \text{ water uptake} = \left[\frac{(W_t - W_i)}{W_i} \right] \times 100$$

where W_t is the weight of the swollen sample at time t and W_i is the weight of the dried film. Samples were analyzed at least in triplicate.

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 CIELab scale was used; lightness (L) and chromaticity parameters a^* (red–green) and b^* (yellow–blue) were measured as described in a previous work.⁷ Assays were performed by placing the film samples over the standard. Samples were analyzed in triplicates, recording five measurements at different positions for each sample.

Water Vapor Permeability (WVP). WVP tests were conducted on the basis of a modified ASTM method E96^{11,12} using a specially designed permeation cell that was maintained at 20 °C as described in a previous work.⁷ Each informed value corresponds to at least four determinations; WVP was expressed as $\text{g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$.

Microstructural Studies by Transmission Electron Microscopy (TEM). The morphology of films was examined using a transmission electron microscope (JEM 1200EX II Jeol, Japan) equipped with a digital camera (ESS00W Erlangshen CCD Gatan). Small pieces from the center of the films were prepared according to the method of Rivero et al.⁷

Fourier Transform Infrared (FTIR) Spectroscopy. The FTIR spectra of neat tannic acid and the films were recorded in an IR spectrometer (Vertex 50, Bruker, Germany) in the wavenumber range 4000–400 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution.

Individual microparticles of TA were crushed in an agate mortar. The crushed material was mixed with KBr and compressed to a 7 mm semitransparent disk for 2 min.

X-ray Diffraction. Chitosan-based films were analyzed by X-ray diffraction in an X'Pert Pro P Analytical model PW 3040/60 (Almelo, The Netherlands). Cu $K\alpha$ 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 $2\theta = 0.02^\circ$.

Dynamic Mechanical Analysis (DMA). DMA assays were conducted in dynamic mechanical thermal equipment Q800 (TA Instruments, New Castle, DE) using a clamp tension with a liquid N_2 cooling system. Film probes with a rectangular geometry (30 mm length, 6 mm width, and a thickness values between 40 and 50 μm) were assayed. Amplitude sweeps from 1 to 50 μm at fixed frequency (5 Hz) were performed. Multifrequency sweeps (5, 10, and 15 Hz) at a fixed amplitude (7 μm) from –90 to 200 at 5 °C/min were carried out, with an isotherm of 15 min at –90 °C. Storage (E'), loss (E'') modulus, and $\tan \delta$ (E''/E') curves as a function of temperature were recorded and analyzed using the software Universal Analysis 2000. Temperatures of the relaxation processes associated with glass transition temperatures were determined through the inflection point of the storage modulus E' curve as well as the maximum peak in both the loss modulus E'' and $\tan \delta$ curves.

Statistical Analysis. Systat-software (SYSTAT, Inc., Evanston, IL) 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.

RESULTS AND DISCUSSION

Characterization of Tannic Acid. It is well-known that the properties of tannins may vary strongly depending on the origin.⁶ Consequently, their characterization results are essential to explain the changes produced by TA addition to the chitosan matrix.

The UV–vis absorption spectrum of TA solution exhibited two peaks centered at 210 and 280 nm at acid pH ranging between 1 and 4. With an increase in pH to basic values, the phenolate form gave the absorbance peaks at 230 and 320 nm, and their intensity increased, whereas the intensity of 210 and 280 nm bands decreased. The peak shifts to longer wavelength, 230 and 320 nm in the TA spectra, were assigned to the ionized form of TA molecules as reported by Erel-Unal and Sukhishvili¹³ and Shutava and Lvov.¹⁴ This was the result of the ionization of the phenolic hydroxyl groups of galloyl groups of TA. The ratio of A_{320}/A_{280} sharply increased in the interval of pH from 7.5 to 11. The pK_a value of TA was estimated at 9.1; this value is in agreement with those values reported by Erel-Unal and Sukhishvili.¹³

Study of the Tannic Acid–Chitosan Interaction. In a previous work, the effect of tannic acid addition on the properties and microstructure of chitosan films was demonstrated.⁷ In the present study an insight into the interaction between the polyphenol and chitosan was attempted, because the possible bonds between different parts of these complex molecules have not been elucidated yet.

The pH of the filmogenic solution was 4, and under these conditions the TA remained predominantly in its undissociated form. Thus, hydrogen-bonding interactions were favored. Moreover, the higher stability of TA self-assembly could be a cause of the higher rigidity of the TA molecule, resulting in a higher cooperativity of binding with positively charged groups of chitosan.¹³ Forming bonds probably also include electrostatic interaction between charged sites of chitosan and TA as well as several different types of conjugation between $-\text{NH}_2$

($-\text{NH}_3^+$) and $-\text{OH}$ groups, resulting in the formation of hydrogen-centered bonds.

Figure 1 shows the regions with significant changes in the absorption spectra of chitosan films with different concen-

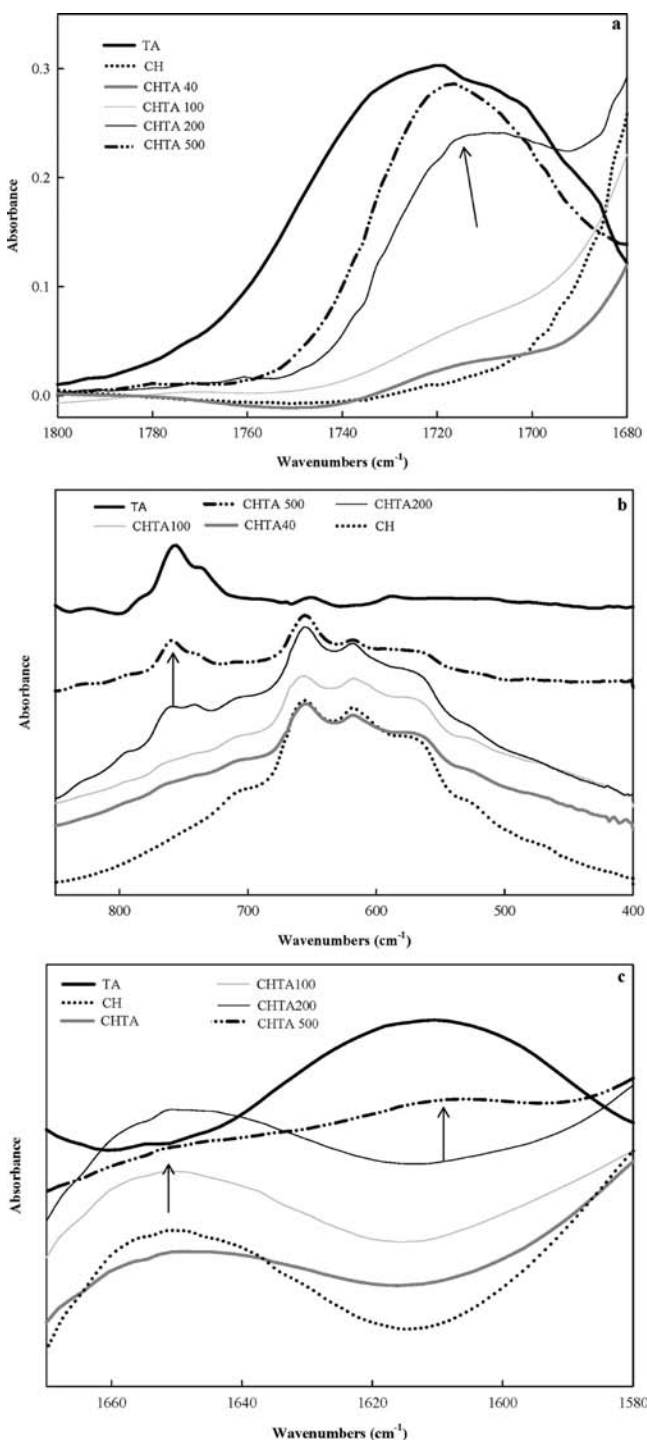


Figure 1. FTIR spectra of CHTA films at different TA concentrations in the wavenumber regions (a) 1800–1680 cm^{-1} , (b) 850–400 cm^{-1} , and (c) 1670–1580 cm^{-1} .

trations of TA. As the exact wavenumber position in IR spectra of biological samples is sometimes difficult to identify, the second-derivative technique was applied as an enhancement resolution method to verify the peak positions. Due to the very

low ratio of TA/CH (40 mg/g) used in this work and with the purpose to clarify the interaction between both compounds, tannic acid concentrations ranging from 40 to 500 mg/g CH were used. Figure 1a shows the spectral window 1800–1680 cm^{-1} , which evidences the microstructure changes caused by the addition of TA to CH matrix. In neat tannic acid FTIR the band at 1720 cm^{-1} corresponds to the $\text{C}=\text{O}$ stretching vibration from the ester groups according to Costa et al.¹⁵ In the case of the spectra of chitosan films formulated with increasing concentrations of TA (CHTA), they exhibited a shoulder peak at wavenumber 1716 cm^{-1} . This shoulder became a well-defined peak with the highest concentrations of tannic acid showing a slight red shift. The occurrence of this band would be a sign of the interaction between chitosan and tannic acid. Similar results were found by Yen et al.¹⁶ working on PCL–TA blends. According to Devi and Maji,¹⁷ tannic acid formed a weak complex with chitosan through hydrogen bonding and hydrophobic association.

As can be seen in Figure 1b the peak located at 759 cm^{-1} , characteristic of the benzene rings, is hardly noticeable, probably as a consequence of the low concentrations of TA used (40 mg/g), but with increasing TA concentrations becomes very evident; according to Li et al.¹⁸ it is indicative that the acid would be immobilized in the CH matrix. With the addition of 500 mg/g TA, the band located at 1650 cm^{-1} , due to the chitosan ($\text{C}=\text{O}$ stretching in amide group, amide I vibration) disappeared, whereas a peak at 1608 cm^{-1} came into view. This fact was consistent with the existence of the band at 1618 cm^{-1} of the neat TA spectrum, assigned to the $\text{C}-\text{C}$ stretching modes of aromatic rings, with possible contribution from the $\text{C}=\text{O}$ modes of H-bonded carbonyl groups (Figure 1c).

Thus, tannic acid–chitosan interactions are complex and take place through electrostatic effects depending on the pH of the filmogenic solution, as well as extensive hydrogen bonds developed throughout the structure, which stabilize the film matrix.

Curing Temperatures of Chitosan-Based Films. The curing temperatures were selected by considering the location of the glass transition temperature (T_g) as a minimum and the degradation temperature of films based on chitosan as a maximum. This last one was determined through DSC from the second scan (279 $^{\circ}\text{C}$, data not shown). Comparable results were obtained by Martínez-Camacho et al.¹⁹ and Zawadzki and Kaczmarek,²⁰ who evaluated chitosan thermal decomposition from thermogravimetric curves. In brief, the minimum temperature assayed was 100 $^{\circ}\text{C}$ on the basis of T_g data for chitosan materials. Accordingly, the heat-curing processes were performed at 100, 160, and 180 $^{\circ}\text{C}$. The selected conditions turned out to be compatible with those proposed by Toffey and Glasser,²¹ who assayed temperatures ranging between 80 and 190 $^{\circ}\text{C}$ for chitosan films. Additionally, Balau et al.²² analyzed the effect of a treatment at 180 $^{\circ}\text{C}$ for 30 min on chitosan film properties.

Dynamic Mechanical Analysis. When control chitosan-based films were scanned by DMA, two relaxations, β and α , were found with increasing temperature (Figure 2). In the case of control CH film, the β relaxation was located around -10 $^{\circ}\text{C}$. Neto et al.²³ and Mucha and Pawlak²⁴ reported that chitosan films exhibited an event associated with β relaxation at around -20 to -10 $^{\circ}\text{C}$, characteristic of the local motion of side groups in chitosan polysaccharide. Other authors²⁴ considered this β relaxation in hydrophilic materials as a

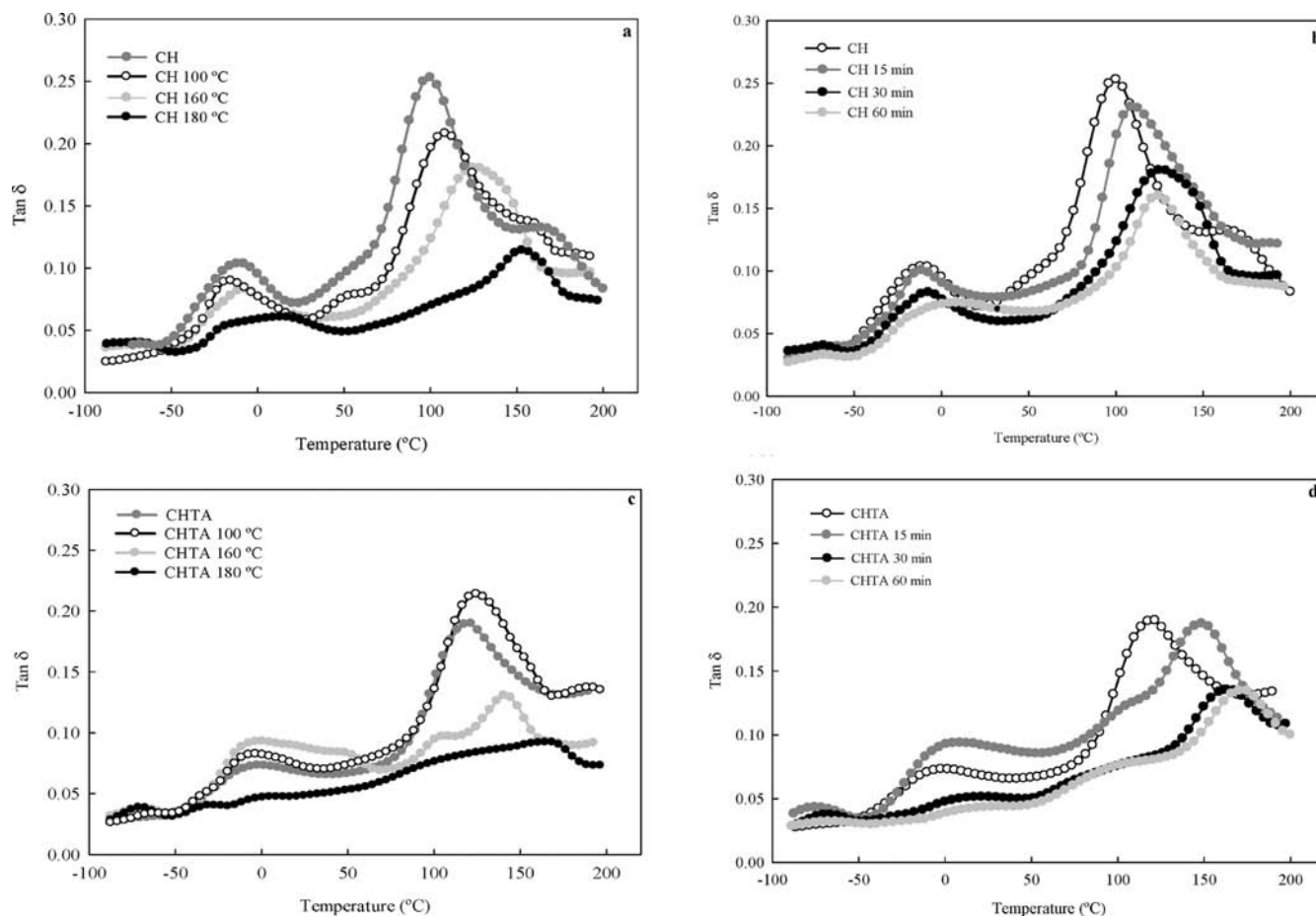


Figure 2. Effect of the temperature and time of curing on dynamic mechanical spectra of (a) CH films cured for 30 min, (b) CH films treated at 160 °C, (c) CHTA films cured for 30 min, and (d) CHTA films treated at 160 °C.

Table 1. Effect of Heat Curing Temperature for 30 min on the Physicochemical Properties of Chitosan-Based Films^a

| curing temp (°C) | film composition | moisture content (g _{water} /100 g _{dry} film) | water uptake (%) | WVP × 10 ¹¹ (g s ⁻¹ m ⁻¹ Pa ⁻¹) | chromaticity parameter b* |
|------------------|------------------|--|------------------|--|---------------------------|
| untreated | CH | 20.67 (0.80) a | 487.63 (23.12) a | 10.82 (0.42) a | 3.89 (0.28) a |
| | CHTA | 21.25 (0.39) a | 641.93 (25.10) b | 9.41 (0.34) b | 4.85 (0.40) b |
| 100 | CH | 22.09 (0.48) a | 385.96 (18.76) c | 5.86 (0.40) c | 5.45 (0.24) c |
| | CHTA | 20.49 (0.87) a | 418.16 (22.81) c | 5.67 (0.58) c | 5.32 (0.08) bc |
| 160 | CH | 15.79 (0.39) b | 226.53 (11.71) d | 5.49 (0.66) c | 27.53 (0.79) d |
| | CHTA | 16.08 (0.71) b | 148.27 (3.36) e | 5.09 (0.48) c | 29.55 (0.19) e |
| 180 | CH | 15.55 (0.10) b | 152.96 (3.26) e | 5.70 (0.44) c | 51.77 (1.04) f |
| | CHTA | 15.05 (0.59) b | 126.34 (7.88) e | 3.57 (0.04) d | 51.06 (1.36) g |

^aThe standard deviations are given in parentheses; values followed by different letters are significantly different ($p < 0.05$).

typical water relaxation, as a consequence of hydroxyl motions favored by water molecules. The second peak in tan δ curves of dynamic mechanical spectra of control CH films corresponded to α relaxation. The temperature at which it took place can be labeled as the dynamic glass transition temperature T_g , despite the frequency tested, and in this case it was found at 100 °C for CH films (Figure 2a).

DMA allowed analysis of the curing process of the chitosan matrix. By increasing the treatment temperature, dynamic T_g shifted to higher values (Figure 2a). This phenomenon is consistent with the cure behavior of thermoset polymers.²¹ As can be seen in Figure 2a, tan δ transitions were progressively

broader and less defined. A similar trend was observed by Toffey and Glaser²¹ for chitosonium acetate films treated at different temperature levels. With regard to the curing time under isothermal conditions for up to 30 min, the longer the heating time, the higher the dynamic T_g . The glass transition temperature remained constant for higher times of curing (Figure 2b). Consequently, 30 min was the selected heating time for further analysis.

In the case of control CHTA, the β relaxation was located around -5 °C, whereas α relaxation was observed at 120 °C in tan δ curves. By comparison of panels a and c of Figure 2, it can be observed that δ transition was better defined in CH films

than in those with tannic acid. Furthermore, the agent addition produced a rise of the dynamic glass transition temperature, whereas the storage modulus turned out to be higher, indicating a more elastic behavior of the material (data not shown), confirming the interaction between both compounds.²⁵ In the same way, Krumova et al.²⁶ stressed that treatment of PVA films with hexamethylene diisocyanate increased the film T_g , this effect being dependent on the agent concentration. As well, Peña et al.²⁷ found that tannin addition to gelatin films produced the T_g rise, attributing this fact to the development of hydrogen bonds in the matrix.

Regardless of both the time and temperature of curing, the presence of tannic acid was evident through the signals associated with the relaxations, which were smoother and less defined (Figure 2c,d). This fact could be explained by considering the slight restriction of the side-chain movements due to the increase in the interaction density.²⁶

Properties of Films Submitted to the Curing Process.

The yellow color of the films was intensified with the heat temperature; the parameter b^* values of films treated at 180 °C became almost twice those obtained at 160 °C and 10 times those obtained at 100 °C (Table 1). According to Martínez Urreaga and de la Orden,²⁸ at temperatures >100 °C the formation of new chromophores causes the rapid yellowing of the materials. Similar results were found by Gennadios et al.,²⁹ working on heat curing of soy protein films.

Table 1 shows the physicochemical and water vapor barrier properties of both control CH and CHTA films and those samples cured at different temperatures for 30 min. The heat treatment of the films improved their barrier properties, obtaining the lowest WVP value at 180 °C for formulations that included tannic acid, which showed a reduction of 62.1% with respect to control CHTA. Meanwhile, heat treatment of CH films led to a decrease in the WVP value of 54.7% on average in relation to control CH films. On the other hand, the moisture contents of the CH and CHTA films were reduced on average by 24.2 and 26.8%, respectively. The last results were obtained through the curing process at the two highest temperatures (160 and 180 °C) as shown in Table 1.

Water uptake of chitosan-based films is shown in Table 1; CHTA films exhibited a higher value than CH samples due to their major capacity to retain water molecules through hydrogen bonds. Films submitted to heat treatment exhibited a water uptake much lower than control samples (Table 1). Taking the cured films into account, those treated at 100 °C showed the highest values of swelling. The films heated at 160 and 180 °C had less capacity for water uptake, the CHTA cured films exhibiting the lowest values (Table 1). Similar results were obtained by several authors.^{30,31} Likewise, Fernández-Saiz et al.³² stressed that the molecular alterations suffered by chitosan films dried at different temperatures led to more yellow and stronger water resistance materials. According to Ritthidej et al.,³³ at elevated temperature, the carboxylic acid would react with the amine group to form an amide and the water would be eliminated. On the other hand, Murray and Dutcher,³⁴ working with chitosan dissolved in 5% acetic acid, found that the heating of chitosan films between 150 and 200 °C caused a decrease in the swelling and a corresponding increase in the degree of acetylation. They stressed that the release of water from the films at elevated temperatures is responsible for these changes. The results suggest that a chemical change occurs in the chitosan films at elevated temperatures, corresponding to a thermally driven conversion of chitosan to a chitin-like material.

A similar explanation was given by Toffey and Glasser,²¹ working on chitosan dissolved in 10% acetic acid solution. It is necessary to highlight that in our case the amount or concentration of acetic acid used to solubilize the chitosan powder was much lower (1.5%) than those used by the aforementioned authors.

Curing treatments reduced the solubility of chitosan-based films in water; the increase in the treatment temperature barely lowered the solubility values, which were <3% in all cases. Working on gelatin–tannin films, Peña et al.²⁷ found that cross-linking reactions may occur between hydroxyl groups and carboxylic acid formed by the polyphenolic chain scission, as in the case of polyamines. Moreover, as was expected, film solubility and water uptake results followed the same trend.

With regard to film solubility in 0.3 M HCl, control chitosan films immersed in the solution were dissolved completely after 24 h under constant stirring, as expected. Instead, CHTA samples under the same conditions did not suffer a total dissolution, and fragments of the film remained undissolved. These results indicate the formation of bonds between CH and TA, in agreement with FTIR findings, especially those related to the band at 1716 cm^{-1} .

After heat treatment, all films submitted to constant stirring in HCl solution for 48 h exhibited only a partial solubility. The fragments of CH and CHTA preserved their structural characteristics and could be retrieved for quantification, resulting in pieces of bigger size in the case of CHTA films. This fact would indicate that the curing process produced a reinforcement of the CH and CHTA networks.

Microstructure Analysis. X-ray diffractograms of control CH films (Figure 3a) exhibited two main peaks located at $2\theta = 11$ and 21° associated with the hydrated (“tendon”) conformation of chitosan.³⁵ The addition of tannic acid changed the pattern toward the anhydrous (“annealed”) crystalline conformation, characterized by peaks located at $2\theta = 15^\circ$ and 21° ; this evolution was studied in a previous work.⁷ Heat curing of CH control films led to a structural change similar to that obtained with the addition of TA agent, regardless of the heating temperature (Figure 3a).

CHTA diffractograms of films cured at different temperatures showed the disappearance of the peak located at $2\theta = 15^\circ$ (Figure 3b). A similar trend was observed by Toffey and Glasser.²¹ These results could indicate the synergic effect of both tannic acid presence and curing treatments.

The curing process caused changes in the FTIR spectral windows associated with the water 3600–3000 cm^{-1} resulting from overlapping of the O–H and N–H stretching vibrations of functional groups engaged in hydrogen bonds (Figure 3a). This region became narrower and sharper as a result of the dehydration process at high temperature after the curing of CH films as well as CHTA samples. Other bands located in the region 1700–1580 cm^{-1} underwent similar modifications due to the curing process compared to the respective controls. Distinctive absorption bands at 1650 cm^{-1} (C=O stretching in amide group, amide I vibration) and 1560 cm^{-1} due to residues of chitin, the latter being associated with the formation of carboxylate groups, were observed. Martínez Urreaga and de la Orden²⁸ and Zawadzki and Kaczmarek¹⁹ assigned the band at 1640 cm^{-1} to the water physically adsorbed (bond water).

The decrease of the band intensity at 1560 cm^{-1} in both heat-treated CH and CHTA films revealed the gradual disappearance of the chitosan amino groups in a chemical reaction in accordance with the results obtained by Martínez Urreaga and

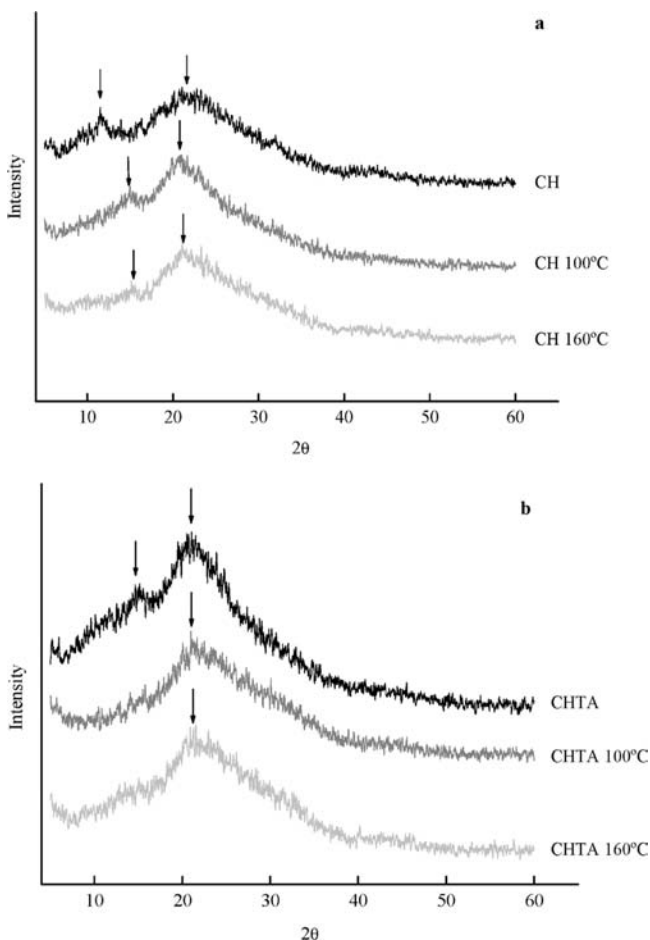


Figure 3. X-ray diffractograms of (a) control and cured CH films and (b) control and cured CHTA films. Samples were cured at 160 °C for 30 min.

de la Orden.²⁸ Also, it was observed that in heated films the band at 1650 cm^{-1} exhibited a relative increase with respect to the band at 1158 cm^{-1} (antisymmetric stretching of the C–O–C bridge), suggesting the increase of new amide and/or imide groups (Figure 4a).

A band located at 1733 cm^{-1} associated with the ester bond was evident in the cured films, the intensity of which increased with the temperature.^{8,19,34}

Nevertheless, this band was less pronounced in the cured CH films with the addition of TA. The appearance of this band hinted that interactions between the components of the matrix took place during the curing process (Figure 4b,c). The higher the temperature, the stronger the developed bonds.

The results obtained by FTIR techniques would explain the behavior of the films in the presence of water as can be seen in Table 1, because the increase of the curing temperature led to intensify the film color, allowed improvement of the water barrier properties, and decreased the solubility and water uptake. The changes in these properties were intensified when the curing process was carried out in the presence of tannic acid.

Figure 5 shows the sample observation by using TEM. Chitosan films were homogeneous, with good structural integrity (Figure 5a). The addition of TA led to the formation of an irregular type structure with randomly distributed clusters (Figure 5b). The posterior curing process yielded a rough

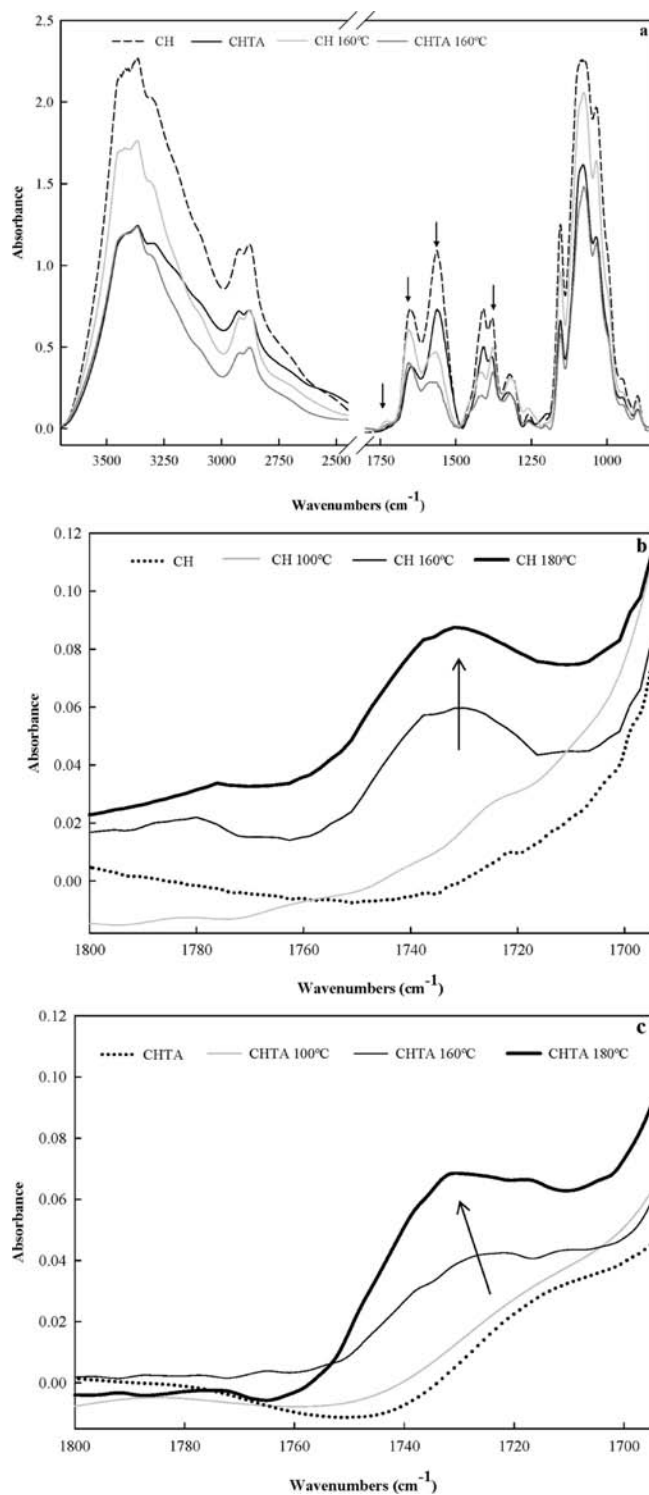


Figure 4. FTIR spectra of (a) control CH and CHTA films and cured CH and CHTA films at 160 °C in the wavenumber region 3700–850 cm^{-1} , (b) control and cured CH at 100, 160, and 180 °C, and (c) control and cured CHTA at 100, 160, and 180 °C, for 30 min in the wavenumber region 1800–1680 cm^{-1} . Samples were cured for 30 min at the mentioned temperatures.

structure, with domains distributed at intervals with some regularity, forming somewhat thicker bands (Figure 5c,d).

The changes suffered by the material, which could be observed by TEM, had a correlation with molecular scale modifications. The change of X-ray pattern of chitosan-based

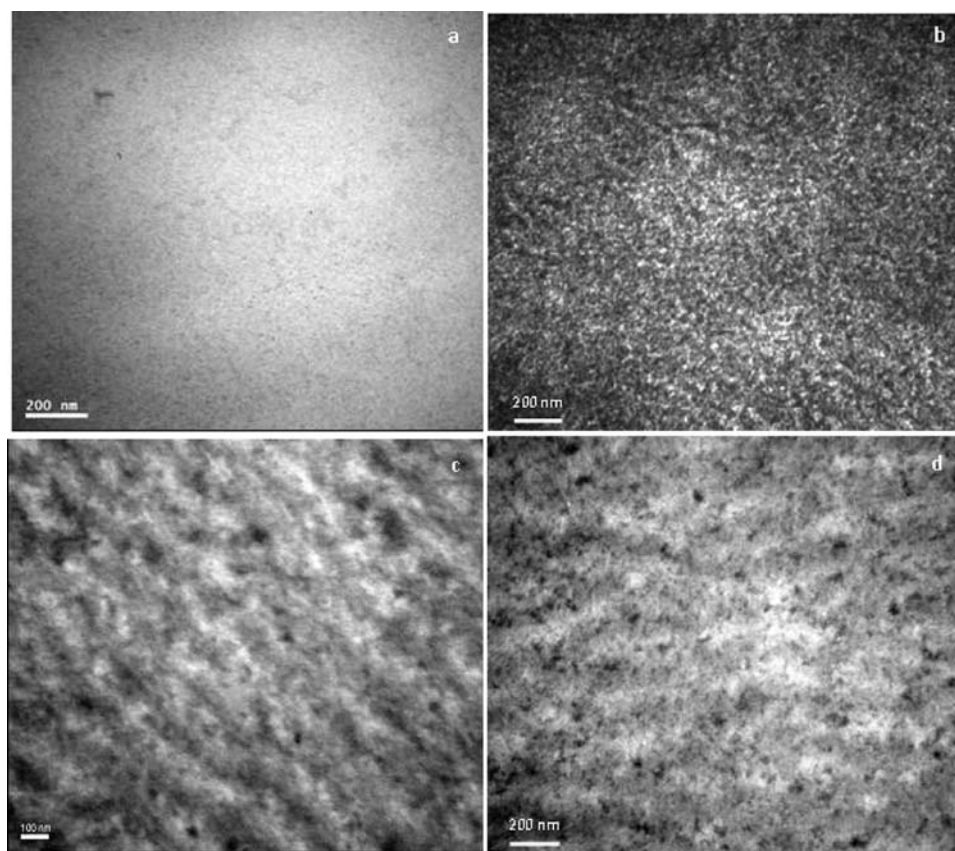


Figure 5. TEM micrographs of (a) control CH, (b) control CHTA, (c) cured CH at 160 °C and (d) cured CHTA films at 160 °C. Scale bars are indicated in each image.

films from the hydrated to the anhydrous conformation indicated the minor affinity of the heat-treated films with the water molecules. Besides, it was also confirmed through sharper FTIR peaks specifically associated with water and shift of T_g toward higher temperatures determined by DMA. Further analysis of the film behavior confirmed that the curing process caused modifications of their properties insofar as they exhibited higher barrier properties as well as lower water uptake, solubility, and moisture content. Moreover, the addition of tannic acid agent, prior to the curing process, made the highlighted modifications of the films even more perceptible, and it acted as an accelerator of the mentioned changes. Thus, DMA, FTIR, and X-ray results allowed us to evaluate the role of water molecules and hydrogen bonding in chitosan-based structures, especially those including TA and submitted to the curing process. The obtained results, mutually supportive, suggested that chemical changes occurred in the chitosan films treated at the highest temperatures, corresponding to a thermally driven conversion from hydrated to anhydrous conformation.

Particular attention should be paid to the tannic acid addition as a cross-linking agent and the curing temperatures to obtain networks with a broad spectrum of properties, ranging from those with chitin-like material behavior to those that could be used as drug release due to their high swelling. The first type of matrix, obtained by curing at the highest temperatures, restricted the motions and led to a higher glass transition temperature and a decrease of the relaxation intensity. In the change, the film treated at the lowest temperature, with and without the presence of TA, evidenced minor changes in T_g and

lower water loss. Understanding the influence of the temperature on the curing process allows the relationship between the microstructure and properties to be established. Knowledge of this dependence would permit making chitosan derivatives with predictable behavior and properties.

The detailed structure of such complexes between TA and CH is complicated owing to possible interaction between different parts of the molecules, which would assert that the interaction phenomena are not due exclusively to hydrogen bonds.

The main event observed by FTIR in films with TA addition was the occurrence of a band at 1716 cm^{-1} , confirming the interaction between CH and TA molecules. On the other hand, in the case of samples submitted to the curing process, the appearance of a band at 1733 cm^{-1} should be a hint of the development of interactions at high temperatures associated with the ester bond. Although promising progress was made in the knowledge of the interactions between these molecules, further studies should be done to draw definitive conclusions.

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