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Physical and Chemical Treatments on Chitosan Matrix to Modify Film Properties and Kinetics of Biodegradation

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Abstract This work was focused on analyzing the effect produced by the addition of tannic acid as a crosslinking agent of chitosan matrix and the influence of the heat treatment applied. Taking into account those aspects relevant for packaging applications, thermal stability, mechanical properties, water resistance and kinetics of biodegradation of the film were monitored. The chemical crosslinking as well as the curing of the matrices have improved the mechanical properties and those related to the water affinity such as solubility, permeability and contact angle. Although both processes had an influence on the extent of the film degradation, these materials conserved their biodegradable character. Moreover, it was observed a synergistic effect of both chemical and physical treatments since the two processes in simultaneous caused further delay in the biodegradation. Consequently, in these materials the access to fungal attack and all those reactions mediated by the presence of water were restricted, which confirmed the higher stability of the matrices submitted to chemical or physical crosslinking.

Keywords: *chitosan film, tannic acid, heat treatment, biodegradation, crosslinking, hydrophilic character*

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1. Introduction

One of the major environmental threats of the synthetic films is their slow degradation rate or even more their non-biodegradable nature. The development of innovative biodegradable materials has been underway for many years, and it continues being an area of interest for many scientists in the field of packaging. Scientific and industrial attention has been focused on the development of both, biodegradable polymers and their derived biocomposites. The current challenge is to design materials with structural and functional stability during storage and use, as well as susceptible to microbial degradation upon disposal with no adverse environmental impact [1].

Chitosan is a biodegradable and biocompatible natural polysaccharide composed of D-glucosamine and N-acetyl-d-glucosamine, derived from chitin. Chitosan applications have been the focus of a wide number of studies, which reported their potential uses in the pharmaceutical, medical and food fields [2-10].

Tannic acid is a gallic ester of D-glucose whose chemical structure has been described in the literature by Isenburg and coworkers [11] and Cao and coworkers [12], among others. While recognizing by its antioxidant capacity due to its multiple phenolic groups, tannic acid may also interact with biological macromolecules as a crosslinker [2,12,13,14]. In a preliminary study, we reported on the ionic crosslinking capacity of tannic acid on chitosan matrix through electrostatic interactions as well as hydrogen bonds [15].

In general, the curing process is a physical crosslinking applied for stabilizing the synthetic polymer network and their properties. Since one of the major problems associated to hydrocolloid-based films is their affinity with water molecules, the curing process could be a promising alternative to overcome its hydrophilic character. In previous work, we confirmed that the curing process caused modifications in film properties and allowed decreasing their moisture susceptibility [16].

Some polymer properties could be altered by crosslinking such as swelling, transport properties, properties, chemical stability and biodegradation rate [17]. To the best of our knowledge, there are not reports that evaluate the biodegradation rate of chitosan films crosslinked with tannic acid and cured by heat treatment.

This work was focused on analyzing the effect produced by the addition of tannic acid as a crosslinking agent of chitosan matrix and the influence of the heat treatment applied. Taking into account those aspects relevant for packaging applications, physical properties such as film thermal stability, mechanical behavior,

barrier properties, solubility and biodegradation kinetic in soil were studied.

2. Materials and Methods

2.1. Reagents

Commercial chitosan from crab shells with a minimum deacetylation degree of 75% was purchased from Sigma (St. Louis, MO, USA). Analytical grade tannic acid (TA) and acetic acid were purchased from Anedra (Buenos Aires, Argentina).

2.2. Film preparation

Chitosan solution of 1.5% (w/w) was prepared by solubilization in 1.5% (v/v) acetic acid solution. Optimum concentration of tannic acid as a crosslinking agent (40 mg TA/g chitosan, selected in previous work [10] was incorporated to chitosan solutions. Films were obtained by casting 20g of filmogenic solutions onto Petri dishes (9 cm diameter) and drying at 37°C in an oven until reaching constant weight (approximately 36 h). The obtained films were removed from the dish and then submitted to a curing treatment at 160ºC for 30 min according to the conditions selected in a preliminary study [16].

Film nomenclature used was CH for chitosan films and CHTA for films containing tannic acid; in films submitted to the curing treatment, the temperature was indicated. Besides, CH and CHTA films without any treatment were studied as primary and secondary controls, respectively.

2.3. Film properties

2.3.1. Physicochemical Properties

The moisture content of the films was determined by measuring their weight loss, upon drying in an oven at 105ºC until reaching constant weight (dry sample weight). Samples were analyzed at least in triplicate. Film color was determined by 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 previous work [15]. Color 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.

2.3.2 Tensile Tests

Tensile strength (TS) and elongation at break (EB) of the films were determined by using a texturometer TA.XT2i-Stable Micro Systems (Surrey, England) equipped with a tension grip system A/TG, according to Rivero and coworkers [15].

The curves of force (N) as a function of the 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 [18]. Each informed value corresponded at least to six determinations.

2.3.3. Water vapor Permeability

Water vapor permeability (WVP) tests were carried out based on a modified ASTM method E96 as described in a preliminary study [15]. After steady-state conditions were reached, eight measurements were performed over 8 h. Each informed value corresponded at least to four determinations.

2.3.4. Film Solubility

To determine film solubility, chitosan-based films samples were cut in 3 × 3cm pieces. The samples were weighed to the nearest of 0.0001g and placed into test beakers with 80 ml distilled water. The films were maintained under constant agitation for 1 hour at 20°C. After soaking, the remained pieces of the films were dried again in an oven at 105 ± 1°C until reaching constant weight. Film solubility was calculated as it was described by Rivero and coworkers [15]. Samples were analyzed at least in triplicate.

2.3.5. Contact Angle Measurement

To determine film hydrophilicity, the surface water contact angle was performed by using a goniometer Ramé-Hart Model 500 (Ramé-Hart Instrument Co., USA) at room temperature. A drop of Milli-Q water was placed on the film surface and the evolution of the droplet shape was recorded with a video camera. An image analysis software (DROPimage Advanced v2.2) was used to determine the contact angle. A minimum of seven measurements, taken at different positions on the film, were carried out. The contact angles were measured on both sides of the drop and averaged.

2.3.6. Thermogravimetric Analysis (TGA)

TGA measurements of chitosan matrix and their modifications were carried out by using a Shimadzu TGA-50 (Japan). Samples (about 5 mg) were heated from 25 to 600ºC at a heating rate of 10ºC min^{-1} under nitrogen atmosphere. The results were plotted as both percentage of weight loss and first derivative of weight loss as a function of temperature. TA-60WS Software version 2.11 was used to analyze the obtained curves.

2.3.7. FT-IR Spectroscopy

The Fourier transform infrared (FT-IR) spectra of films were recorded in an IR spectrometer (Nicolet, iS10 Thermo Scientific, Madison, USA) in the wavenumber range 4000-400 cm^{-1} by accumulation of 64 scans at 4 cm^{-1} resolution. Data were analyzed by using the software Omnic 8 (Thermo Scientific).

2.3.8. Biodegradation in Soil

A series of plastic pots (400 cm^3) were used as soil containers. Natural microflora present in soil was used as a degrading medium. Several circular specimens of 4 cm of diameter with thicknesses within the range of 45-50 μm were put into holders made of a plastic mesh to permit the access of microorganisms and moisture and the easy recovery of the degraded samples. The physicochemical characteristics and the composition of the used soil were: 17-19% moisture, 76-79% ash, 11.5 C/N ratio, 3.5-4.5% organic matter, pH 7 and an electrical conductivity of 0.5-1 mS cm^{-1}. According to the described methodology in ASTM D5988-03 [19], 1 g of compost per 25 g of soil was added. The physicochemical characteristics and composition of the used compost were: 35-40% moisture,

40-45% ash, 7.7 C/N ratio, 15-20% organic matter, pH 6.2 and an electrical conductivity of 1.1 mS cm^{-1}.

The containers were conditioned at a controlled temperature and relative humidity of 20ºC and 50%, respectively, taken as an average of environmental conditions. In all cases, films were sprayed regularly with water. Similar conditions were employed by several authors [20,21,22] evaluating the biodegradation of composite films based on polycaprolactone and lignocellulose fibers. The specimens placed into the holders were buried at 5 cm in depth from the surface in order to ensure the aerobic degradation. Samples were removed from the soil at specific time intervals, and were cleaned with a brush carefully to avoid film structure damages. After that, the specimens were weighed in order to determine the average weight loss (%)

$$\%\ \ Weight\ \ loss = \left[\frac{(W_i - W_t)}{W_i}\right] \times 100 \qquad (1)$$

where, W_i is the initial mass and W_t is the remaining mass at time t. All results were the average of two replicates.

2.3.9. Microstructure Studies by Scanning Electron Microscopy

Studies of the morphology of the degraded films were conducted by scanning electron microscopy technique (SEM) with a FEI model Quanta 200 electron microscope (The Netherlands). For low vacuum SEM, fractured films were mounted vertically onto bronze stubs utilizing a double-sided adhesive carbon tape and examined without any metal or carbon coating using the ESEM, at an acceleration voltage of 15 kV. SEM observations were performed with the following magnifications: 1200, 1600 and 2400.

2.4. 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 level used was 0.05.

3. Results and Discussion

3.1. Tensile Assays

Table 1 shows the parameters from analyzed mechanical profiles obtained from 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 through the biopolymer matrix. The addition of TA increased significantly tensile strength ($p < 0.05$) obtaining materials more resistant without detriment of the elongation (Table 1).

The addition of TA in the formulation (CHTA) prior to the heat treatment of the films led to a significant increase of the stress values ($p < 0.05$) compared to those of CH cured films but no changes were observed in the elongation of the material. This fact would indicate that the curing process produced a reinforcement of both the CH and CHTA networks. The increase of the material strength would be associated with the development of a crosslinked matrix due to the heat treatment. This result could be attributed to the effect of the thermal treatment on the structure stabilization.

Furthermore, the elongation values decreased on average by 16% for cured films compared to their corresponding controls (CH-CHTA) (Table 1).

Table 1. Mechanical properties of films cured at 160°C for 30 min

Films	Stress (MPa)	Elastic modulus* (MPa)	Elongation (%)
CH	52.90 (2.36)[a]	20.37 (1.38)[a]	6.10 (0.04)[a]
CHTA	72.20 (3.27)[b]	39.30 (2.29)[b]	6.02 (0.06)[a]
CH 160ºC	83.62 (0.75)[c]	45.93 (2.17)[c]	5.06 (0.44)[b]
CHTA 160ºC	96.04 (2.99)[d]	52.30 (2.72)[d]	5.16 (0.48)[b]

The values in parentheses correspond to the standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$) between samples.

3.2. Water Transfer Properties and Hydrophilic Character

Contact angle is defined as the angle between the substrate surface and the tangent line at the point of contact of the liquid droplet with the substrate [16]. The contact angle is a good indicator of the relative hydrophobicity or hydrophilicity of a substrate. In the case of high-density-polyethylene (HDPE) the contact angle was 92.4º according to Fávaro and coworkers [23].

Although chitosan film is a hydrophilic material, its wettability could be affected when blending with another material or by the treatment applied as is the case here (physical and chemical crosslinking).

Figure 1 shows contact angles of chitosan based films, exhibiting a value of 88.4° for CH control film. Almeida and coworkers [9] reported a contact angle of 95°, attributing this result to the hydrophobicity of chitosan chains.

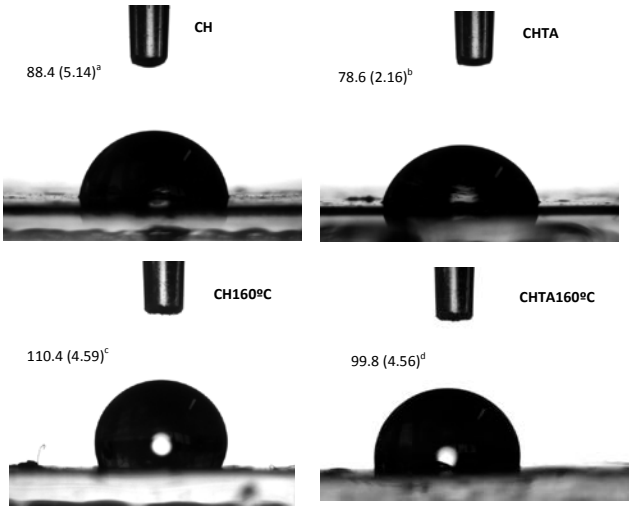

Figure 1. Measurement of static contact angle of a water droplet on a solid surface for CH, CHTA and films cured at 160ºC for 30 min. Different letters indicate significant differences between samples ($p < 0.05$)

The contact angle of the CHTA was lower than CH film value, the reduction being of 11%; these results could be explained by the exposure of the hydrophilic sites of TA molecules on the material surface. In the same way, Jin and coworkers [24] informed values of 78.5 and 62.6º for chitosan and chitosan crosslinked with genipin,

respectively. Zhang and coworkers [14] found a similar trend for gelatin matrices crosslinked with TA and attributed their lower values to the hydrophilic nature of the TA segments. In addition, the cured CH and CHTA samples had the highest contact angle values suggesting that the crosslinking produced during the heat treatment played a significant role in reducing the hydrophilic characteristics of the material surface.

3.3. Structural Changes Induced by Chemical and Physical Crosslinking

Table 2 shows the effect of the different types of crosslinking assayed on the physicochemical and water vapor barrier properties of CH films. Chemical crosslinking with TA as well as the heat treatment of the films (with and without TA) improved their barrier properties. WVP value for formulations crosslinked with tannic acid showed a reduction of 13 % respect to control CH films. Meanwhile, heat treatment of CH films led to a decrease in WVP value of 49.3 % on average in relation to CH films, no significant differences ($p>0.05$) were observed between WVP values of cured CH and CHTA films.

On the other hand, the moisture contents of the CH and CHTA films were reduced on average by 23.6% and 24.3%, respectively.

Table 2. Physicochemical and barrier properties of chitosan films crosslinked with chemical (tannic acid) and physical treatment at 160ºC for 30 min*

	First stage		Second stage		
	T_{max}	Weight mass (%)	T_{max}	Weight mass (%)	Residual mass at 550ºC (%)
CH	96.4[a]	19.35[a]	284.8[a]	43.47[a]	31.03[a]
CHTA	77.9[b]	17.22[b]	284.3[a]	40.68[b]	32.34[b]
CH 160ºC	93.3[a]	14.13[c]	287.5[b]	43.82[a]	34.76[c]
CHTA 160ºC	93.0[a]	15.15[c]	287.4[b]	43.83[a]	34.50[c]

The values in parentheses correspond to the standard deviation. Different letters in the same column indicate significant differences ($p<0.05$) between samples.

The chemical treatment with TA diminished the solubility value in water by 26% while the curing treatments also reduced the chitosan-based film solubility, which was lower than 3%. In addition, the moisture content of films was modify significantly ($p<0.05$) through the heat treatment. Similar findings were informed by Ritthidej and coworkers [25] for chitosan films prepared with different organic acids and treated thermally.

Figure 2 shows TGA curves obtained for CH based films. The observed first stage between 25 and 160°C was related to the loss to water bounded and adsorbed on the structure [26]. These values can be correlated to film moisture content as well as WVP (Table 2). Crosslinked chitosan films presented water loss at the lowest heating temperature. CHTA films tended to retain less water, which turn out to be more volatile than in the case of CH films. These findings indicate how intense the polymer–water interaction was, and if the water molecules were weakly or strongly bound to the chitosan network. Similar results were informed by Beppu and coworkers [27] for chitosan films crosslinked with glutaraldehyde.

The losses in the second stage comprised between 160 and 400ºC were due to complex processes like the dehydration of the polysaccharide rings, and the decomposition of the units of acetylated and deacetylated chitosan [28,29].

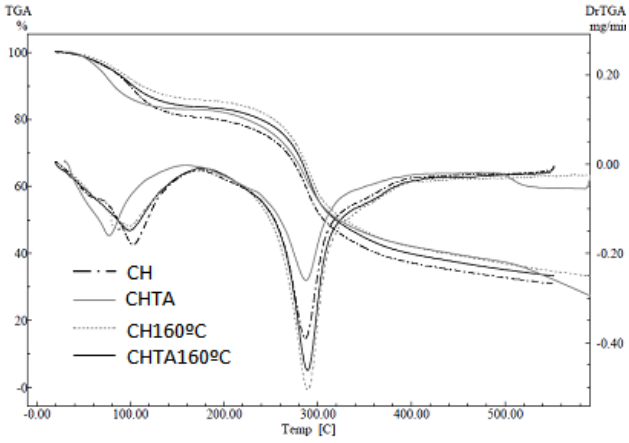

Figure 2. TGA curves and their first derivatives obtained for CH films, CHTA and heat-treated matrices. Samples were cured at 160ºC for 30 min

For CHTA film, no significant differences ($p>0.05$) in the decomposition peak temperatures (detected from the first derivative of the weight loss curves) were observed in relation to CH films (Table 3).

Table 3. Effect of tannic acid addition and heat treatment on (%) loss of chitosan based films obtained by TGA analysis

Film composition	Moisture content (g water/ 100 g dry film)	Solubility** (%)	WVP $\times 10^{11}$ (g/sec m Pa)
CH	20.67 (0.80)[a]	10.57 (0.95)[a]	10.82 (0.42)[a]
CHTA	21.25 (0.39)[a]	7.82 (0.60)[b]	9.41 (0.34)[b]
CH 160ºC	15.79 (0.39)[b]	2.87 (0.06)[c]	5.49 (0.66)[c]
CHTA 160ºC	16.08 (0.71)[b]	2.80 (0.09)[c]	5.09 (0.48)[c]

*Different letters in the same column indicate significant differences ($p<0.05$) between samples.

Aelenei and coworkers [2] reported that CH forms a stable complex with TA. The authors explained that the TGA second stage could also be attributed to the partial decomposition of gallic acid, tannic acid or gallic acid dimers. Peña and coworkers [13] analyzing gelatin matrices containing different contents of tannin, found that residual mass increased with tannins concentration, in accordance with the higher value obtained for CHTA films in this work. On the other hand, the thermograms of the cured films showed the highest decomposition temperature and the % remaining mass, demonstrating the development of a more stable structure (Table 3). This result could be explained considering that high temperatures can promote crosslinking reactions between the hydroxyl and carboxyl groups of the acid formed by the polyphenolic chain scission, as in the case of polyamides [26].

3.4. Soil Biodegradation

Figure 3 shows the evolution of the weight loss of the tested samples, throughout a period of 150 days. Similar values of weight loss were reported by Ludueña and coworkers [20] under analogous assay conditions.

The degradation rate decreased in the presence of tannic acid (CHTA) and further in films submitted to the physical treatment (CH160ºC and CHTA160ºC). This

trend can be explained considering that the matrix, whether through the presence of the crosslinking agent or through the curing process, showed greater stability due to the lower availability of groups susceptible to the microbial attack. This behavior was attributed to changes occurred in the matrix structure by the temperature action, which would be compatible with the conversion of chitosan into a material with characteristics similar to chitin. According to Xu and coworkers [30] since chitosan is rarely present in nature whereas chitin is highly abundant, it is reasonable to observe an increase in the rate of biodegradation of chitosan films with regard to chitin-like material.

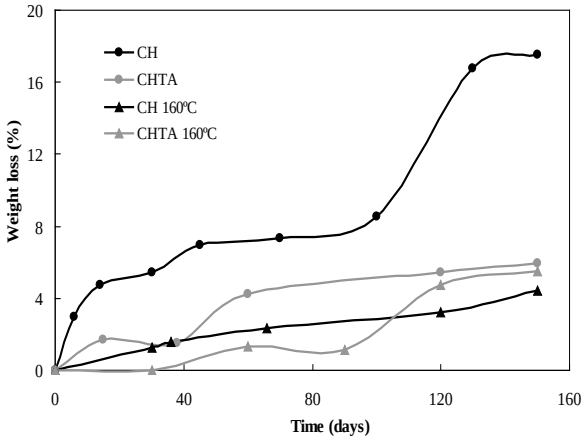

Figure 3. Weight loss (%) of the films buried in soil as a function of the time

Chitosan films were initially transparent, developing a faint yellowish color over time. Film yellow color was intensified with the heat treatment at 160ºC; color difference (ΔE) values increased with the curing process (Figure 4). According to Fernández-Saiz and coworkers [31] the molecular alterations suffered by the material would give as a result a more yellow appearance.

After 30 days of assay, another modification only observed in the case of CH films was the shrinkage of the matrices by approximately 10%. In addition, they turned into brown and brittle. These color and embrittlement changes indicated that films had undergone primary stages of environmental degradation. Similar results were informed by Xu and coworkers [30].

Figure 4 shows the color difference values (ΔE) and the images of films after 1 month of incubation in relation to the film color measured at initial time (t = 0). With the exposition of films to a degrading environment, ΔE values increased significantly ($p<0.05$), this effect being more pronounced in the case of films including tannic acid in the formulation.

Color attributes of samples for exposure times longer than 150 days did not differ significantly ($p> 0.05$). Chromaticity parameter b* was the most affected in the case of both control and cured CH films. However, considering CH films crosslinked with TA and CHTA samples cured at 160ºC, both brightness L* and b* parameter were affected. These results were associated with the intensifying of film brownish color due to the addition of a crosslinking agent (Figure 4).

Figure 5 shows the morphology and cross sections of partially degraded films obtained by SEM after 30 and 150 days of incubation. Regardless of the formulation, it was noted that the attack of soil natural microflora was intensified with exposure time.

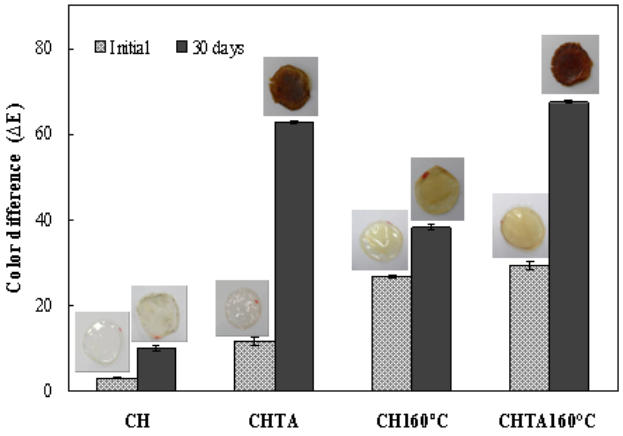

Figure 4. Color difference values of CH, CHTA, and cured films before the incubation (t = 0) and after 30 days of exposure to the degrading soil and macroscopic appearance of the buried CH based films

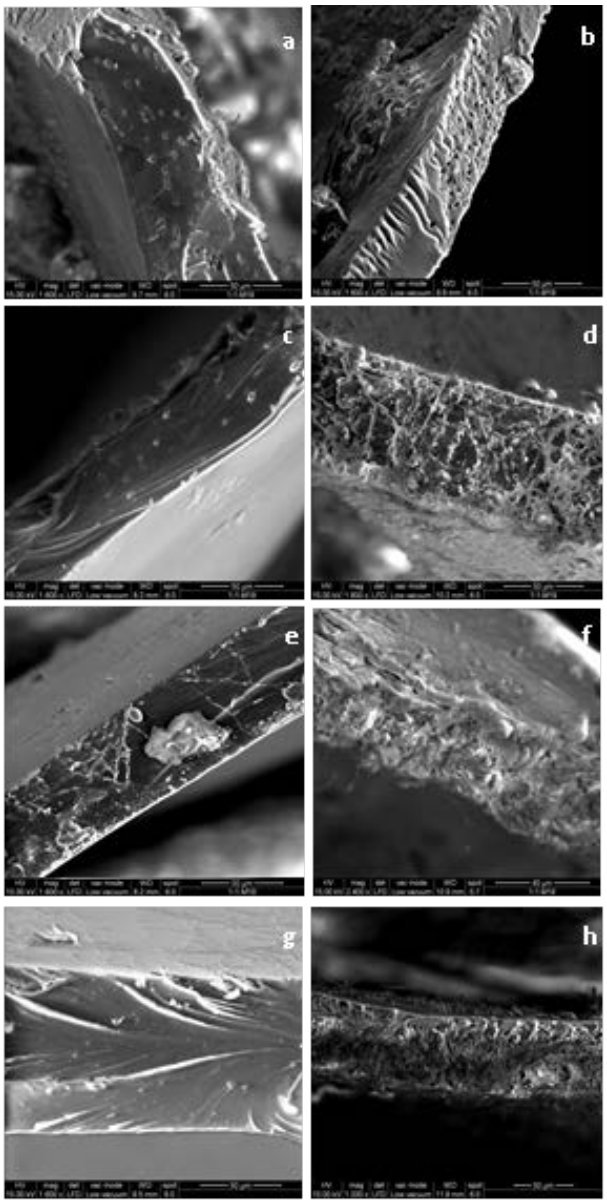

Figure 5. Cross-section micrographs of chitosan based films incubated for 30 and 150 days: **a,b**) chitosan films (CH), **c,d**) chitosan crosslinked with tannic acid (CHTA), **e,f**) CH160ºC films and **g,h**) CHTA160ºC films. Magnification is indicated in the micrographs

Morphological study by SEM was complementary to the weight variation test and visual inspection, as it helped to corroborate the existence of the transformation that was taken place, allowing a detailed assessment of the biodegradation process. The micrographs of CH films revealed the formation of randomly distributed cavities, which were intensified over incubation time (Figure 5a and Figure 5b). These results were in agreement with those reported by Kuo and coworkers [32] who evaluated the biodegradation of chitosan-nylon blends. Zhang and coworkers [33] studied the biodegradation of cellulose-based matrices by SEM at different exposure times and they found a porous structure with obvious signs of degradation due to the presence of fungal mycelium on the film surface.

In the cases less susceptible to microbial attack such as crosslinked films with TA (Figure 5c and Figure 5d) as well as films submitted to curing (Figure 5e, Figure 5f, Figure 5g and Figure 5h), the microscopic observations provided invaluable support to reveal an existing but not yet visible colonization of the samples by filamentous fungi in the early stages (with no visual signs of microbial growth). At this time, it was only possible to detect changes in optical properties and texture. Similar results were reported by Alvarez and coworkers [1]. Wu [34] also used SEM to follow the evolution of the material degradation working under favorable conditions for thermophilic aerobic bacteria development.

Moreover, the diffusion of water in the soil because of the sprayed samples caused the hydrolytic degradation of the material. The matrix ability to absorb water is an important parameter, which significantly influences the biodegradation of a material facilitating the action of the enzymes that convert the polymer into smaller fragments assimilable by the soil microorganisms. Water enters to the polymer matrix producing swelling and rupture of intermolecular hydrogen bridge bonds, causing the hydration of molecules and finally the hydrolysis of unstable linkages.

According to Ludueña and coworkers [20] the main mechanism of chain scission during the biodegradation is a chemical and enzymatic hydrolysis. Consequently, the process of biodegradation is dependent on the availability of water, which promotes the hydrolysis, and microbial attack of the matrix [1,35]. The amorphous nature of the matrix could be considered primarily responsible for the degradation pattern for biodegradable materials.

Several factors influence the hydrolytic degradation of the polymers, including the nature of the functional groups present. There exists a biunivocal correspondence between the transport phenomena that depends on the water presence and the biodegradation kinetic. In other words, there is a direct relationship between the sensitivity of the group to the hydrolysis and the biodegradability of the polymer. In addition, properties such as water solubility and water vapor permeability of the material depend largely on the nature of the hydrophilic functional groups present in the matrix, their number and accessibility. Weian and coworkers [36] and Rimdusit and coworkers [17] explained that the decrease in permeability values was related to the decrease of the biodegradation. Accordingly, in this study the WVP and solubility decreased while biodegradation kinetics of CH films became slower with TA addition as a crosslinker agent as well as with the physical treatment.

In order to visualize the biodegradation at structural level, as an example, Figure 6 shows structural biodegradation of CH films evidenced by FTIR.

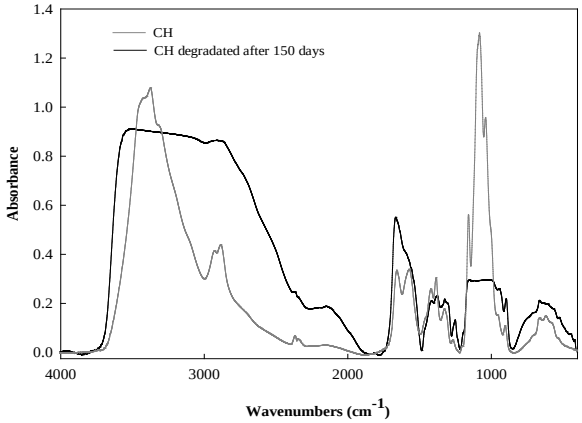

Figure 6. FTIR spectra of CH control films at t=0 and after 150 days of incubation, in the wavenumber region 4000-400 cm^{-1}

The spectrum of CH film (Figure 6) exhibited a broad absorption band in the 3600-3000 cm^{-1} with a maximum at 3460 cm^{-1} due to the overlapping of 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 and coworkers [37] and Ostrowska-Czubenko and Gierszewska-Druzynska [38]. CH 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. 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 [15]. Residues of chitin, attributed to N-H bond of N-acetyl group (amide II) were evidenced by a broad peak at 1581 cm^{-1} (Figure 6).

After 150 days of exposition, CH film FTIR spectrum shows loss of definition or missing bands, demonstrating that this technique can also be used to confirm the biodegradation process. The regions more affected were those related to OH and NH bonds, and glucosamine residues, therefore the distortion of the spectrum could be explained considering the preferential removal of some groups by microbial action.

4. Conclusions

Chemical and physical treatments allowed obtaining a more stable structure, the development of more resistant films similar to chitin-like materials without detriment of the elongation and with better water vapor barrier properties.

The crosslinking and heat treatment of the matrices had an influence on the extent of the biodegradation process,

which confirmed their higher stability. In spite of this fact, the treated materials conserved their biodegradable character. Moreover, it was also observed a synergic effect of both treatments, since a delayed in the biodegradation kinetic was observed in films submitted to both processes. This behavior was undoubtedly related to the greater difficulty to access to moisture of these matrices, due to their higher surface hydrophobicity. Consequently, in these materials the access to fungal attack and all those reactions mediated by the presence of water were restricted.

Acknowledgement

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