



Enhancing water repellence and mechanical properties of gelatin films by tannin addition

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ABSTRACT

In order to reduce pollution caused by traditional non-biodegradable plastic films, renewable raw materials from plants and wastes of meat industries have been employed in this work. A hydrolysable chestnut-tree tannin was used for gelatin modification. Films of gelatin and gelatin–tannin were obtained by casting at room conditions. Transition temperatures of both gelatin and gelatin–tannin systems were determined by differential scanning calorimetry (DSC). Glass transition temperatures of modified gelatin occurred at higher temperatures than for neat gelatin. Enthalpy and temperature of helix–coil transition decreased when tannin content increased due to variations in the helical structure of gelatin as a consequence of tannin presence in agreement with X-ray analysis. Mechanical and thermal behaviour varied as a function of the content of tannin, showing optimum values for films modified with 10 wt% tannin. The transparency of films was maintained after modification with tannin. Solubility and swelling tests of the films revealed that the presence of tannin reduced the water affinity of gelatin.

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1. Introduction

The new requirements for reducing polymers obtained from fossil resources have promoted the use of renewable resources from animals or plants as proteins or tannins. The protein gelatin has been used as glue since ancient times (Fakirov and Bhattacharyya, 2007), and more recently in photography, and the pharmaceutical, tissue engineering and food industries (Bigi et al., 2000). Despite gelatin being one of the polymers recognized for millennia, there are aspects of its structure and functionality which are still being discussed today (Lukasik and Ludescher, 2006).

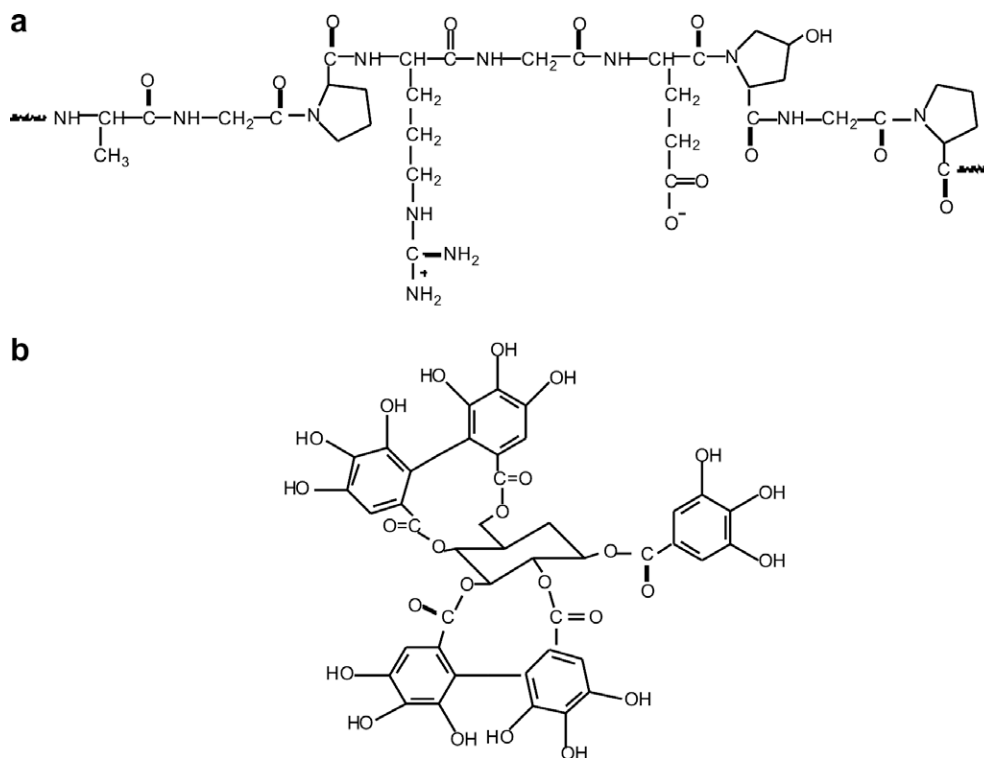
It is well known that gelatin is a protein derived from the chemical degradation of collagen, with average molecular weights from 65,000 to 300,000 g/mol, depending on the grade of hydrolysis. Gelatin contains mainly glycine, proline and 4-hydroxyproline (for pigskin gelatin 33%, 13% and 9%, respectively) (Fakirov and Bhattacharyya, 2007). Chemical structure of gelatin is shown in Scheme 1a. The three-dimensional gel network of gelatin has been defined by several authors using the “fringed micelle” model in which there are microcrystallites interconnected with amorphous regions of randomly coiled segments (Slade and Levine, 1987; Arvanitoyannis et al., 1998). However, this model is not accepted

by other researchers, who propose the existence of local regions of protein quaternary structure, self-limiting in size, which can be triple-helical, only partially triple-helical (Ross-Murphy, 1997) or also include β -turn and β sheet motifs (Prystupa and Donald, 1996). The characteristic triple-helical structure of collagen is lost during gelatin extraction, but can be recovered below the helix–coil transition temperature (Sinha Ray and Bousmina, 2005; Yakimets et al., 2005). This fact is due to a conformational disorder–order transition of the gelatin chains which form thermoreversible networks by associating helices in junction zones stabilized by hydrogen bonds (Bigi et al., 2000).

In solid state, dehydrated gelatin properties depend critically on the thermal history (Fakirov and Bhattacharyya, 2007). In fact, the conformational state of dehydrated gelatin films obtained by casting from gelatin aqueous solution differs when the solvent is evaporated at room temperature or lower or at temperatures above 35 °C (cold- and hot-cast films, respectively). At room temperature, a helical structure is obtained. At temperatures above 35 °C, the conformation of a statistical coil is obtained and films are typically more brittle than cold-cast films and do not show the helix–coil transition temperature (Fakirov and Bhattacharyya, 2007; Mene-galli et al., 1999; Fraga and Willians, 1985).

The tightly bounds (hydrogen bonds and hydrophobic interactions) present in gelatin structure and the polar groups of aminoacids result in brittle materials in dry state with high moisture absorption (Karnnet et al., 2005). Mechanical and barrier

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Scheme 1. Chemical structures of raw materials: (a) gelatin and (b) ellagitannins.

properties have been improved using additives like polyols, carbohydrates of high molecular weight such as starch and chitosan (Arvanitoyannis et al., 1997, 1998; Cao et al., 2009) oligosaccharides and some organic acids (Karnnet et al., 2005). On the other hand, plasticizers like sorbitol (Vanin et al., 2005), hydrophobic plasticizers derived from citric acid (Andreuccetti et al., 2009) or chemical modification with aldehydes (De Carvalho and Grosso, 2004) are also used to modify some of the functional and physical properties of the films (Dalev et al., 2000).

Tannins are polyphenolic compounds of vegetable tissues with different chemical structures depending on their origin. They are usually classified in condensed and hydrolysable tannins (Haslam, 1989). Condensed tannins have flavanol units in their structure, while hydrolysable ones are composed of a molecule of sugar, generally glucose, joined to phenolic acids (Haslam, 1989; Gross, 1992) (Scheme 1b). Besides classical applications of tannins in hide tanning and wine preparation, several new emerging applications are in adhesive industries for replacing hazardous phenol in novolac resins (Peña et al., 2006a,b), for reducing gelation, pressing time and formaldehyde emissions of resole resins (Bisanda et al., 2003; Vázquez et al., 2002; Stefani et al., 2008), or for tannin-based adhesives manufacture (Pizzi, 2006, 2009). On the other hand, because of their antioxidant capacity and astringency properties, and to increase colour intensity, tannins have been also employed in wine preparation. Not only the antioxidant activity of polyphenols, but also other physiological properties (anti-allergenic, anti-inflammatory, antimicrobial, cardioprotective and anti-thrombotic) (Balasaundram et al., 2006) make these compounds a very interesting raw material for the development of green polymeric materials which would not involve the use of toxic components prohibited for food packaging and medical applications.

Tannins tend to have a strong affinity for proline-rich proteins as gelatin (Asquit and Butler, 1986; Taylor et al., 2007). Moreover, the conformational flexibility of tannin is necessary for strong binding (Deville et al., 2007). Protein–tannin interactions were

suggested as a surface phenomenon in which tannin molecules effectively coat the surface of the protein (Spencer et al., 1988). In fact, the very high stoichiometries of tannin–protein precipitates obtained by Kawamoto et al. (1996) and results obtained by Deville et al. (2007) indicate that binding is unlikely to be to specific ligand binding sites on the protein. Frazier et al. (2003) suggested that interactions occur firstly by a synergistic binding in which tannin has an effect on the new binding interactions, and a second stage dominated by less specific binding and aggregation. Two different interactions between gelatin and tannin can be envisaged: hydrogen bonds between hydroxyl groups of tannin and polar groups of gelatin (amide carbonyl of the peptide backbone) and hydrophobic interactions which are important for stabilizing tannin–protein complexes (Yi et al., 2006; Hagerman, 1992). Moreover, Baxter et al. (1997), proposed that salivary proline-rich proteins can interact with pentagalloyl glucose from tannin (an hydrolysable tannin, see Fig. 1) by hydrophobic interactions with

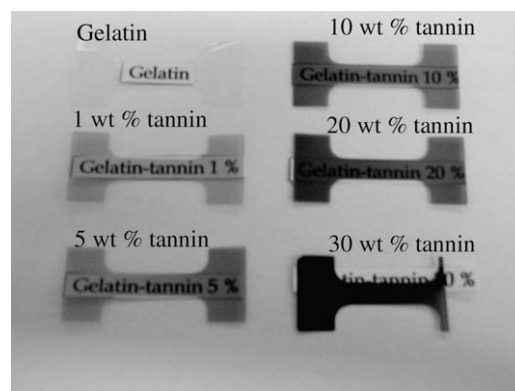


Fig. 1. Transparency on gelatin and gelatin–tannin films. Probes cut for mechanical testing.

the pyrrolidine ring of proline (Baxter et al., 1997). The formation of covalent cross-links between gelatin and tannin it is also possible but only under oxidizing conditions in which quinones react with side chain amino groups of peptides (Strauss and Gibson, 2004).

The use of gelatin and tannins as raw materials for films manufacture could be a good alternative for reducing use of traditional non-biodegradable plastics because of two reasons. Firstly, they are biopolymers obtained from renewable raw materials and their use could reduce petroleum-derived polymers usually employed in foodstuff packaging industry for film manufacture. Secondly, the water solubility of both gelatin and tannins could allow to reducing the amount of organic solvents required for film preparation by casting procedure.

The aim of this work was to prepare gelatin–tannin (GT) modified films in several compositions and to analyse the effect of the addition of different contents of tannin (T) on their physico-chemical and mechanical properties. Thermal characterization and tensile testing were used to characterize the obtained films. Helix–coil transition and glass transition temperatures were measured by differential scanning calorimetry (DSC). The results were compared with those obtained by X-ray analysis. Solubility and swelling tests were carried out to study the water affinity of modified and neat gelatin.

2. Methods

2.1. Materials

Acid pretreated commercial gelatin (G) type A from pigskin (isoelectric point 7) was supplied by Sigma and employed as received without any further treatment. Commercial hydrolysable chestnut-tree tannin (T) was supplied by Agrovin Products (Spain). For solutions and analysis ultrapure water obtained from Millipore purification system (Milli Q) was employed (water resistivity 0.066 $\mu\text{S}/\text{cm}$).

2.2. Preparation of films

Gelatin powder (5 g) was hydrated with 100 mL of distilled water at ambient temperature (about 25 °C) with constant stirring for 30 min. Subsequently, the hydrated gelatin was heated up to 50 \pm 5 °C and different concentrations of tannin (5–30 w/w on dry gelatin basis) were added to the film forming solution. During the tannin addition, the initial pH 5.5 of the solution was adjusted to pH 11 with 1 N NaOH solution in order to prevent the formation of gel. The film forming solution was maintained at 50 \pm 5 °C for 30 min, and subsequently it was poured into polystyrene Petri plates and dried at room conditions (25 °C, 60% RH) to obtain films with an average thickness of 100 \pm 10 μm . Gelatin films containing tannins were labeled as GTX where X corresponds to the weight percentage of tannin. Control gelatin films (G) were prepared in similar conditions without adding tannins. Samples were conditioned at 25 °C and 50% RH in a hydrothermal chamber (Dycometal CCK-81) before testing.

2.3. Total soluble matter

The total soluble matter was calculated as the percentage of film dry matter solubilised during immersion in ultrapure water at 100 °C for 1 h. Conditioned film pieces (0.5 \pm 0.1 g) with an initial moisture content of 12 \pm 1% were placed in nylon filters with a pore diameter of 0.22 μm . Then the filters were sealed and placed in a round-bottomed flask with water at 100 °C. After 1 h, the filters were removed from the flask and dried in air-circulating oven

at 105 °C for 24 h before determining soluble matter. This procedure was repeated twice with each sample and the average value was calculated.

2.4. Swelling experiments

Films were cut into 2.5 \times 2.5 cm². Each film was weighed in air dried conditions, and then immersed in ultrapure water for 2 min at 25 °C. Wet samples were wiped with filter paper to remove excess of liquid and weighed. Measurements were repeated twice for each composition and the average value of swelling was calculated as follows:

$$\text{Swelling (\%)} = 100(w_2 - w_1)/w_1.$$

2.5. Analyses

Calorimetric measurements were performed using a DSC (Mettler DSC822). Sample weights were in the range of 3–5 mg. Thermograms were obtained from samples using sealed aluminium pans to prevent any loss of water during heating at a rate of 10 °C/min in the temperature range of 25–250 °C and under inert atmosphere (10 mL min⁻¹ of nitrogen).

X-ray diffraction patterns (XRD) were collected on a PW1710 X-ray diffractometer equipped with a Cu K α radiation source ($\lambda = 1.5418 \text{ \AA}$) operating at 40 kW and 20 mA. Data were recorded in the range of $2\theta = 2\text{--}40^\circ$ at scanning rate 1°/min.

UV–Vis spectra of obtained films were recorded on a Milton Roy Spectronic Genesys 5 spectrophotometer. The analysis conditions were 200–1100 nm spectral range and plot data were recorded every 1 nm. Measurements were repeated twice.

The thermal stability of films was measured using a TGA/SDTA 851 (Mettler Toledo). Dynamic scans from 30 to 900 °C were carried out at 10 °C/min under helium atmosphere.

Tensile tests were carried out in a Miniature Materials Tester (MiniMat 2000) of Rheometric Scientific. Probes were cut with a pneumatic die cutter according to ASTM D1708-93 standard and the thickness of each film was measured at five random positions using a micrometer (Mitutoyo) with an accuracy of $\pm 0.001 \text{ mm}$.

The test rate was 1 mm/min. For each composition 10 pieces were tested and the average values were calculated.

3. Results and discussion

Gelatin–tannin films turned from light yellow to brownish as tannin content increased (Fig. 1). The transparency of GT films was reduced with increasing tannin content and this was ascribed to some degree of phase separation occurring at high tannin contents, as has been observed by optical microscopy (figure not shown). Similar results were reported for ferulic acid incorporated chitosan–starch blends (Mathew and Abraham, 2008).

For gelatin–tannin samples analyzed by DSC a similar behaviour to that for non-modified gelatin was observed. First and second scans of modified gelatin films and non-modified parent biopolymers (gelatin and tannin) are shown in Fig. 2. In the first scan, water evaporation and helix–coil transition (Apostolov et al., 1998; Yakimets et al., 2005) were observed as endothermic peaks around 100 and 225 °C, respectively. Both transitions disappeared upon re-heating after rapid cooling. The first transition is more likely related to the volatilization of absorbed water (initial moisture content of all samples was about 12 wt%) while the higher temperature transition is related to the relative amount of triple helix in gelatin (Bigi et al., 2004). It is worth noting that the temperature of the second endothermic peak increased from 225 °C up to 240 °C as tannin content increased from 1 to 30 wt%. On

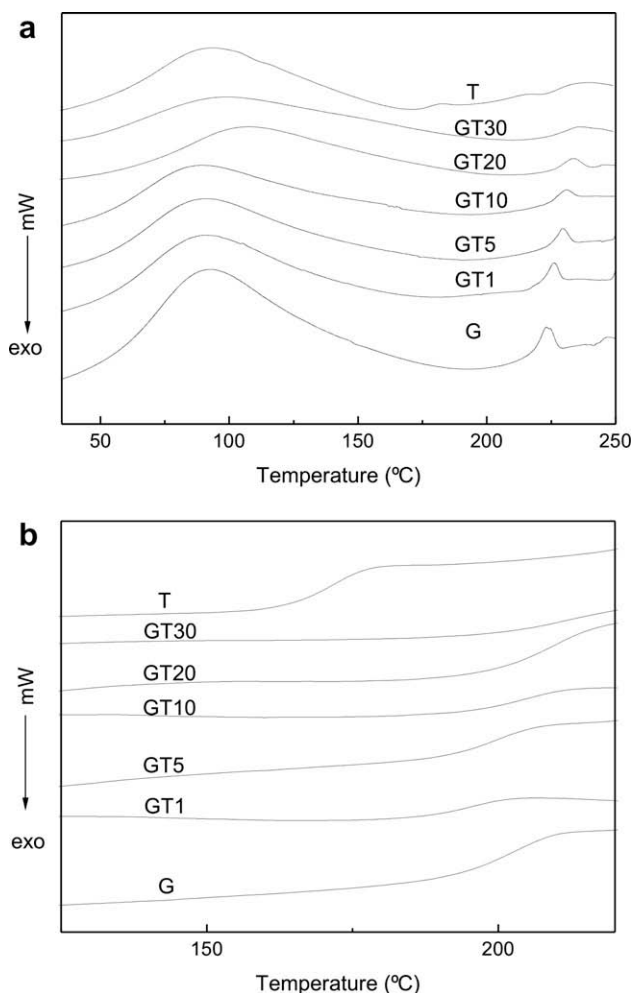


Fig. 2. DSC thermograms of gelatin, chestnut tannin and gelatin–tannin films for T_m , ΔH_m and T_g values evaluation. (a) First scan; (b) second scan.

the contrary, the related enthalpy (ΔH_m) decreased. These results suggest that the presence of tannin alters the formation of the characteristic helical structure of gelatin probably disrupting the hydrogen bonds between gelatin chains by creating new hydrogen interactions between hydroxyl groups of the tannin molecule and polar groups of gelatin (amide carbonyl of the peptide backbone) and hydrophobic interactions. It is also worth noting that the glass transition temperature (T_g) obtained from the second scan (Table 1) increased even though polyphenolic tannin has lower T_g than gelatin (170 and 197 °C, respectively). It can be envisaged that some gelatin–tannin interactions occur involving polar side-chain groups of the gelatin chains, hindering the mobility of gelatin

Table 1

T_g , T_m and ΔH_m values of gelatin, tannin and modified gelatin–tannin films at several tannin contents.

Tannin (wt%)	T_m (°C)	T_g (°C)	ΔH_m (J/g)
0	223 ± 2	197 ± 2	15 ± 1
1	225 ± 3	195 ± 2	9 ± 2
5	229 ± 2	199 ± 2	8 ± 1
10	230 ± 1	202 ± 1	6 ± 1
20	235 ± 2	207 ± 3	5 ± 1
30	239 ± 2	212 ± 2	3 ± 1
100	–	168 ± 3	–

chains, and consequently, the glass transition temperature increases.

UV–Vis transmission spectra of control and gelatin–tannin films are shown in Fig. 3. As the tannin content increased, the transmission peak moved to higher wavelengths, from 280 nm for gelatin to 480 nm for gelatin modified with 30 wt% tannin. This behaviour was previously attributed to interactions between gelatin and phenolic molecules using the same technique (Cao et al., 2007; Kim et al., 2005). UV–Vis results also indicated that optical transparency was similar for all films but decreased for GT30 film.

Fig. 4 shows the X-ray diffractograms for GT films and for neat gelatin and tannin. As can be seen, tannin did not show any peak in DRX patterns which implies an amorphous structure. Gelatin film showed low crystallinity with one diffraction peak around $2\theta = 8.1^\circ$, due to the triple-helical crystalline structure of collagen renatured in gelatin (Itoh et al., 1994). The diffractograms of formulations with low tannin content (i.e. GT1, Fig. 4) were similar to that of neat gelatin. When the tannin content increased, the intensity of the crystal peak of gelatin at 8.1° (2θ) reduced considerably in intensity (in agreement with the reduction in melting enthalpy observed by DSC), while a new peak at about 10.4° was detected. The resulting interplanar spacing of these two peaks (calculated by Bragg's law) were 1.09 and 0.85 nm for $2\theta = 8.1^\circ$ and

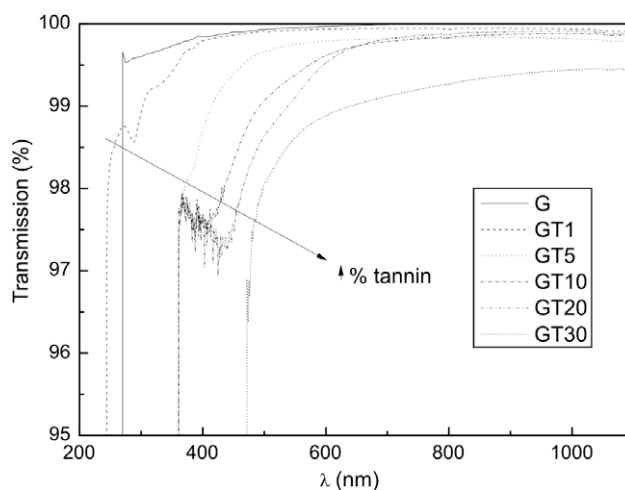


Fig. 3. Evolution of the UV spectra of the gelatin films at various tannin contents.

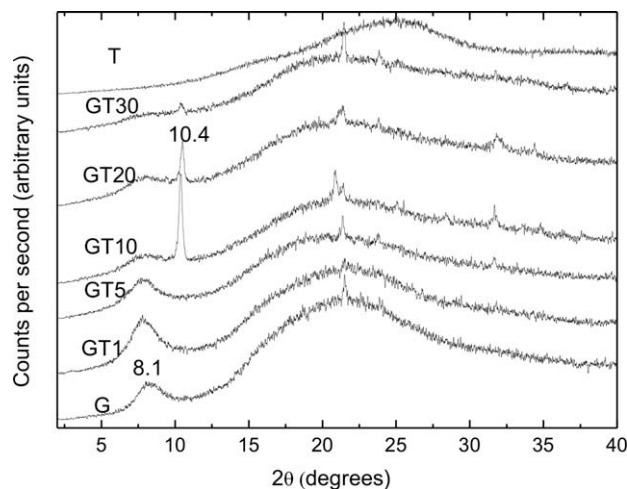


Fig. 4. X-ray diffractograms of gelatin and gelatin modified with several amounts of tannin. Data indicate the angle corresponding to each peak for films.

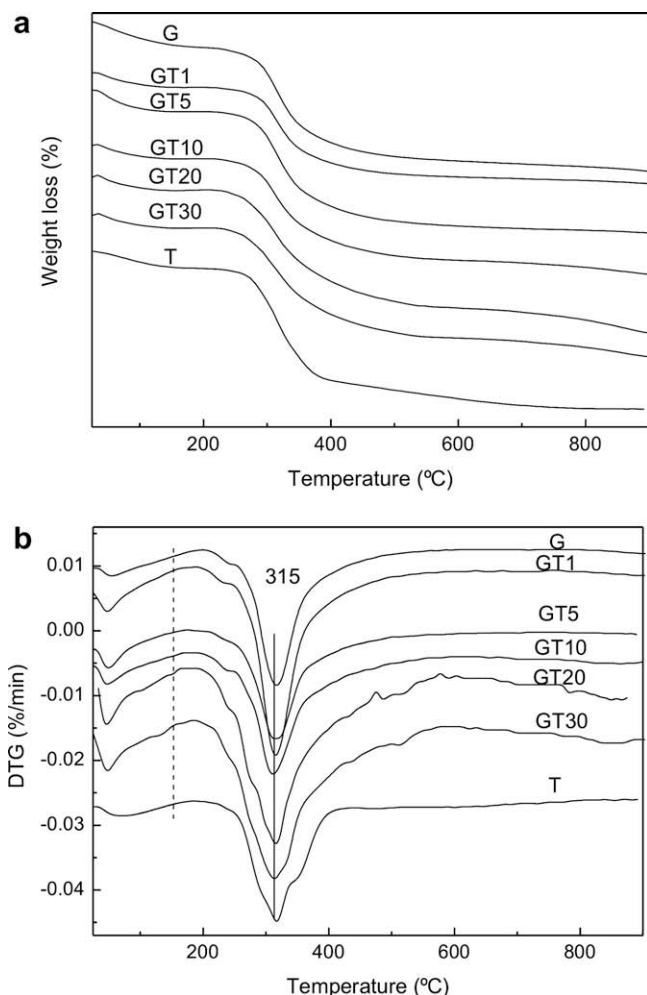


Fig. 5. TG (a) and DTG (b) curves of gelatin, tannin and gelatin–tannin films with several tannin contents (curves have been moved in y-axis).

10.4°, respectively, evidencing that the presence of low tannin contents may induce new crystalline structures in GT materials. At high tannin content, the intensity decrease of the crystal peak at $2\theta = 10.1^\circ$ may be due to the enlarged intermolecular interactions between hydroxyl groups in tannins and NH_2 side-chain groups in gelatin which limit the molecular movements, thus preventing crystallization. These interactions could be associated with the increment observed in T_g values with increasing tannin amount in the formulations.

The curves of weight loss and first derivate related to the rate of weight loss for the parent biopolymers and mixtures are shown in Fig. 5a and b. The experimental curves are vertically displaced to facilitate the analysis. Two different steps can be distinguished in the weight loss curves of all materials. The first one extending from

25 to 150 °C is related to the loss of water, while the second one in the range of 250–400 °C, was associated with the protein chain breakage (helical structure) and peptide bonds rupture (Barreto et al., 2003). For neat tannin, degradation occurred at the same temperature range due to degradation of hydroxyl and ester groups (Garro and Rield, 1997). The residual mass (20 wt%) for neat gelatin (Table 2) is in good agreement with previous works (Martucci et al., 2007; Barreto et al., 2003). When tannin contents increased the residual weights were higher than those obtained for neat tannin and gelatin (around 30 wt%). This result can be explained by taking into account that high temperatures can promote cross-linking reactions between hydroxyl groups and carboxylic acid formed by the polyphenolic chain scission, as in the case of polyamides (Martucci et al., 2007).

Fig. 6 shows modulus, strength and strain at break as a function of tannin content. Compared to the control gelatin film, the elastic modulus and the tensile strength (σ_u) of tannin-incorporated gelatin films increased up to a value of 4200 and 120 MPa, respectively, with the addition of 10 wt% tannin. However, there was a slight reduction in σ_u at higher tannin concentrations. The increment in σ_u value could be linked to the formation of a more stable network due to attractive interactions between gelatin and tannins. When the ratio of tannin/gelatin became too high (i.e. 30 wt% tannin) a slight phase separation took place, which was the main factor responsible for the reduction in σ_u . This finding agrees well with the reduction in the transparency of GT films observed at high tannin concentrations (Fig. 1). The elongation at break (Fig. 6c) decreased from $3.2 \pm 0.1\%$ for the control film to a minimum of $2.5 \pm 0.5\%$ for GT films containing 30% tannins. As reported by other authors for gelatin cross-linked with different aldehydes (De Carvalho and Grosso, 2004), gelatin with ferrulic acid (Cao et al., 2007) and gelatin with glutaraldehyde (Bigi et al., 2001), σ_u increases, while elongation at break decreases for the increase in concentration of the cross-linking agent.

In order to investigate the interactions between water and both G and GT films, water solubility at 100 °C and swelling tests were performed. Solubility results are shown in Fig. 7. Total soluble matter values show that the systems without tannin were completely soluble. When 1 wt% tannin was added, a clear reduction of the solubility (53.3% soluble matter) was observed. The solubility diminished until 20 wt% for the system modified with 10 wt% tannin. Above this tannin concentration, the solubility remained nearly constant as a consequence of the free tannin present in the sample, which was verified by the colour of the solution.

The above reported results are in good agreement with those obtained by swelling tests (Fig. 7). The addition of 10 wt% tannin reduced significantly the swelling of gelatin films in about 52%. When tannin content was higher than 10 wt%, swelling values were overestimated due to the presence of free tannins in the mixtures. Thus the reduction in both total soluble matter and swelling values with increasing tannin content suggest the presence of interactions between tannin and gelatin. As shown for short swelling tests in Fig. 7, the rate of swelling is influenced by hydrophobic and/or hydrogen interactions between gelatin and tannin groups as swelling decreases for higher tannin contents. In general,

Table 2

Weight loss at two temperature ranges defined and residual weight obtained by thermogravimetric analysis of gelatin, tannin and gelatin–tannin films.

System	G	GT1	GT5	GT10	GT20	GT30	T
Weight loss (%) 25–150 °C	14.0 ± 2	10.1 ± 1	11.1 ± 1	10.1 ± 1	10.9 ± 1	10.5 ± 1	8.6 ± 1
Weight loss (%) 250–400 °C	66.0 ± 1	58.6 ± 2	57.9 ± 3	55.7 ± 2	57.2 ± 2	58.2 ± 3	66.1 ± 3
Residual weight (%) 25–900 °C	20.0 ± 3	31.3 ± 2	31 ± 3	34.3 ± 3	31.9 ± 3	31.3 ± 4	25.3 ± 3

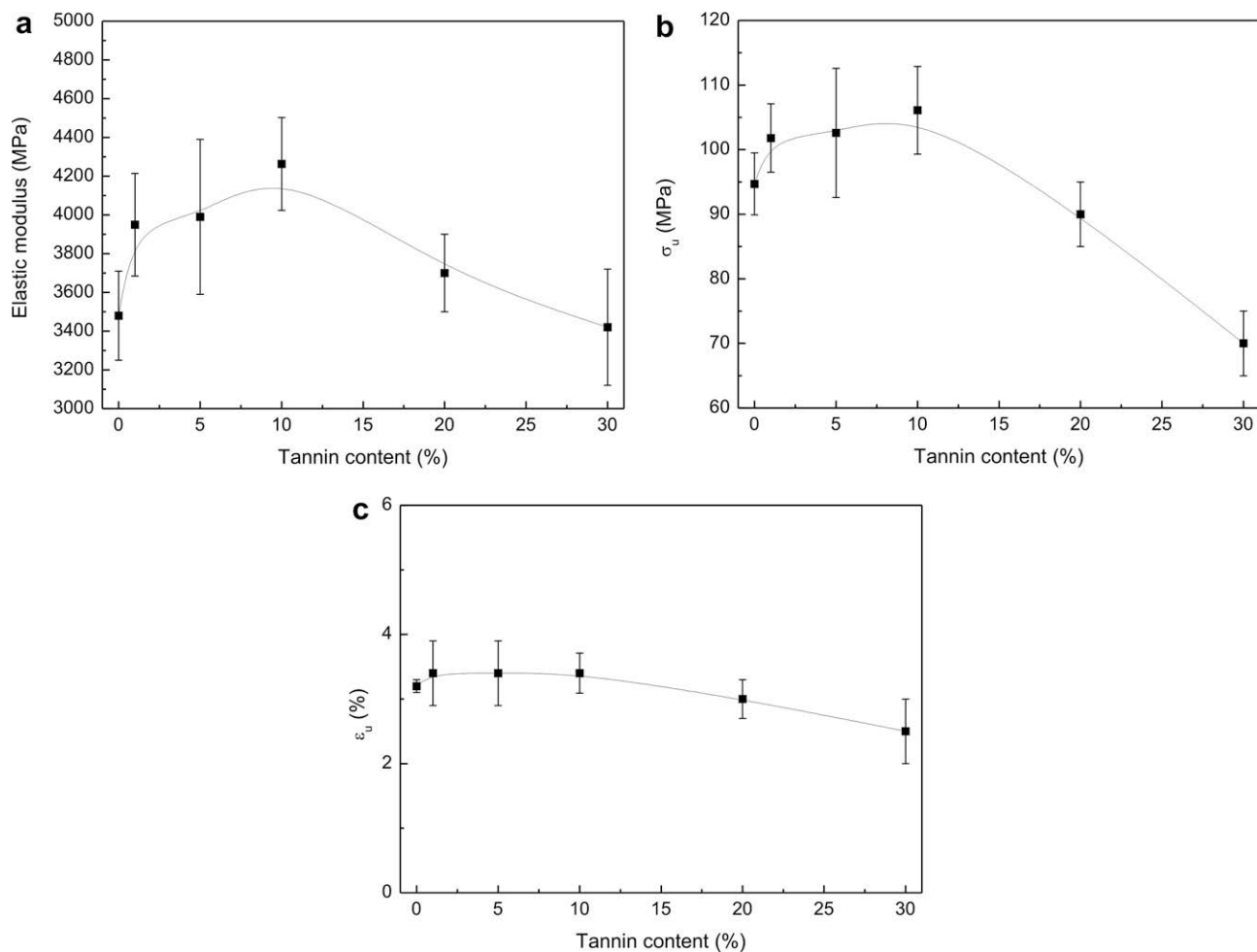


Fig. 6. Elastic modulus (a), tensile strength (b) and elongation at break (c) of gelatin and gelatin–tannin films as a function of tannin contents.

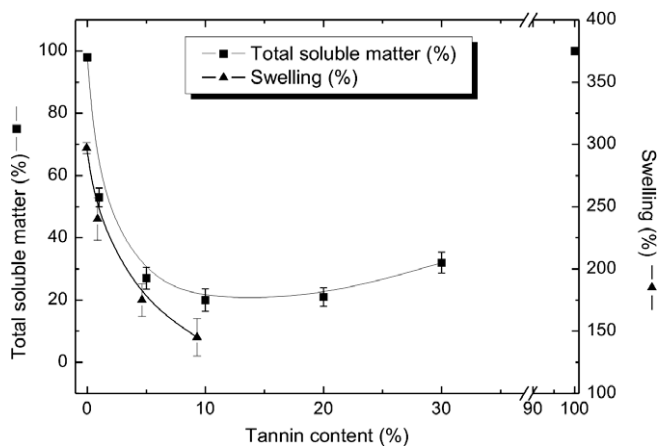


Fig. 7. Percentages of soluble matter, and swelling values of gelatin and gelatin–tannin films.

cross-linking and/or hydrogen interactions with other components decrease water uptake by proteins since polar-side-chain groups become less exposed to bind water (Cao et al., 2007; Kim et al., 2005; Rhim et al., 1998; Bigi et al., 2001). It can be also envisaged that the changes in helical structure due to the tannin–gelatin interactions modify the solubility of the gelatin. In fact, changes

in helical structure have been proved above by the analysis of the enthalpy of the helix–coil transition and X-ray analysis.

The interaction mechanism could be envisaged as a process which occurs in two steps. Firstly, when gelatin is in solution, chains are free and accessible for both solvent and tannin. When casting process starts, water evaporation occurs and interactions (hydrogen and/or hydrophobic interactions) between chains of gelatin and gelatin–tannin take place. As a result of these interactions, a reduction of the crystallinity of neat gelatin and the formation of new ordered structures occur as they have been probed by DSC, X-ray and swelling tests. These changes are influenced by tannin content.

Gelatin is a true alternative to many of today's synthetic materials (Schrieber and Gareis, 2007). Completely biodegradable transparent films for foodstuff packaging for example have already been successfully produced from physically hardened gelatin films (Schrieber and Gareis, 2007). Since one of the major drawbacks in the use of gelatin films in technical applications is their water absorption tendency, any improvement in water resistance as that shown in this work can be highly important.

4. Conclusions

Hydrogen and/or hydrophobic interactions between gelatin and tannin molecules induce changes in the formation of triple helix in gelatin and surely reduced the mobility of the side chains, as reflected in an increment of the glass transition temperature of GT

films. An optimum tannin content around 10 wt% in gelatin–tannin films gives rise to strong, thermally stable, moisture resistant and flexible films as mechanical, thermogravimetric and swelling tests do indicate. Tannins antioxidant and antifungal properties make them suitable modifying agents of gelatin to obtain transparent tailor-made new materials from renewable resources with higher moisture resistance than neat gelatin films.

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