Preparation and characterization of plasticized gelatin films cross-linked with low concentrations of Glutaraldehyde

J. F. Martucci, A. E. M. Accareddu & R. A. Ruseckaite

Journal of Materials Science
Full Set - Includes `Journal of Materials Science Letters'

ISSN 0022-2461

J Mater Sci
DOI 10.1007/s10853-011-6167-3

Springer

Your article is protected by copyright and all rights are held exclusively by Springer Science+Business Media, LLC. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Springer

Author's personal copy

J Mater Sci
DOI 10.1007/s10853-011-6167-3

Preparation and characterization of plasticized gelatin films cross-linked with low concentrations of Glutaraldehyde

J. F. Martucci · A. E. M. Accareddu · R. A. Ruseckaite

Received: 15 August 2011 / Accepted: 29 November 2011
© Springer Science+Business Media, LLC 2011

Abstract Thermal and mechanical properties as well as moisture resistance and water vapor barrier properties of films from bovine gelatin added with D-sorbitol (30 wt%) as plasticizer and cross-linked with low amounts of glutaraldehyde (GTA, from 0 to 2 wt%) were investigated to determine their suitability as barrier layers for flexible packaging materials. Results revealed that free amino side chain groups of gelatin decreased with GTA, confirming the occurrence of cross-linking between GTA and gelatin. The extent of cross-linking reaction in the presence of D-sorbitol was lower compared with the unplasticized counterpart suggesting that plasticizer hampers GTA to react. The glass transition temperature (T_g) as measured from differential scanning calorimetry (DSC) increased with GTA concentration owing to the formation of more reticulated materials while the incorporation of D-sorbitol led to a small reduction in this parameter due to plasticization. Increasing GTA concentration from 0 to 1 wt% provoked the enhancement of elastic modulus from 3.7 ± 0.2 to 4.9 ± 0.2 GPa. These values reduced significantly by the addition of D-sorbitol, whereas elongation at break improved in about 150%. The optimum formulation for the intended purpose was that containing 1 wt% GTA and 30 wt% D-sorbitol since it exhibited the best set of properties: total soluble mater reduced from 100 to 16%, moisture absorption decreased from 1854.1 ± 85 to 210.4 ± 8%, water vapor permeability at 65% relative humidity improved from 2.42 ± 0.27 to 0.94 ± 0.06 × 10^{-14} kg m Pa^{-1} s^{-1} m^{-2}, with minor reduction in opacity and with the additional benefit of releasing only 5% of the initial GTA content.

J. F. Martucci · A. E. M. Accareddu · R. A. Ruseckaite (✉)
Research Institute of Material Science and Technology (INTEMA), National Research Council (CONICET), Mar del Plata University, Juan B. Justo 4302, 7600 Mar del Plata, Buenos Aires, Argentina
e-mail: roxana@fi.mdp.edu.ar

Introduction

Nowadays research attention has turned to the use of biogenic polymers as raw materials to produce biodegradable plastic films due to several advantages connected with their natural and renewable origin, abundance, low cost, and inherent eco-compatibility [1, 2]. Proteins are interesting biopolymers since they have specific structures derived from the α-amino acid composition which confer a wide range of functional properties [2]. Gelatin (Ge) is a water-soluble protein derived from animal sources, obtained from the hydrolysis of bone-collagen or connective tissues skin of mammalian and fish [2–4]. The acceptance of gelatin as "Generally Recognized as Safe" (GRAS) substance in the area of food additives by the U.S. Food and Drug Administration (FDA) together with its excellent film-forming ability, gel-forming properties around 35 °C [5], excellent versatility due to its alpha-amino acid composition, abundance, low cost, and biodegradability make gelatin an attractive protein in the design and development of functional films with potential application in the food sector. Gelatin films generally have good oxygen barrier properties at low or intermediate relative humidity and satisfactory mechanical properties [2–4, 8–16]. However, these beneficial characteristics are counterbalanced by the hydrophilic nature of gelatin which imparts high-moisture sensitivity to the derived films compromising their broader application [2, 3, 5–15].

Numerous attempts have been undertaken to improve and modulate gelatin film properties to meet comparable performance to that of the conventional non-biodegradable

Published online: 23 December 2011

Author's personal copy

plastics used in food packaging. Accordingly, one approach to improve the overall performance of gelatin-based materials is by introducing covalent bonds between protein chains by cross-linking. Cross-links in proteins can be created by a number of ways, namely by physical, or chemical routes [4, 5]. Aldehyde-based cross-linking agents (including formaldehyde, glyoxal, and glutaraldehyde) have shown great efficiency to improve thermal, mechanical, and moisture resistance of gelatin items by introducing covalent stable bonds between gelatin chains [1, 3, 5, 8, 14–18]. Glutaraldehyde (GTA) has been extensively used for stabilizing collagen and gelatin due to its excellent efficiency, short-reaction time and low cost, which enables achieving strength, and water resistance of the obtained structure [5, 8, 12, 14, 16] reducing its toxic effect when used at very low concentration [14, 15]. It is well reported that GTA reacts with the lateral residues of amino acids, particularly with the e-NH_2 functional groups of lysine and hydroxylysine, forming bonds similar to those of Schiff bases [1, 5].

Although other alternative cross-linking agents such as starch dialdehyde [9, 10, 29], genipin [19], and natural phenolic compounds [20] seem to be preferable in order to reduce the toxicity risks, they cannot be compared to GTA in collagen and gelatin stabilization [21, 22]. GTA cross-linking has been clinically acceptable despite some problems related to the polymerization of GTA monomers in solution leading to heterogeneous cross-linking and toxicity [14, 21, 22] which make questionable its use in food contact materials. The risk of cytotoxicity during biodegradation of GTA-containing materials can be diminished by lowering GTA concentration [14, 21]. It has been found that GTA contents as low as 0.5 wt% were enough to enhance the water resistance and thermal stability of gelatin-GTA films, with minor GTA release [14]. Progressive cross-linking of collagen membranes with low GTA contents (i.e., 0.01 wt% for 1 h followed by 0.05 wt% for 7 h) reduced degradation of the polyanionic membranes combined with a low immune response [21]. However, the introduction of covalent links between gelatin chains dramatically reduced the film extensibility due to the strong cohesiveness of Ge-GTA films. A large number of articles have described the effects of plasticizers on the physical, chemical, and functional properties of gelatin films [2, 4, 6, 7, 9–11, 23–25]. The addition of hydrophilic plasticizers helps to decrease the inherent brittleness of cross-linked gelatin films by reducing intermolecular forces, increasing the mobility of polymeric chains, and improving their flexibility [4]. Gelatin films from different sources have been successfully plasticized with several compounds including glycerol [2, 9–11, 23, 24], D-sorbitol [2, 23, 25], ethylene glycol, diethylene glycol, triethylene glycol, ethanolamine, diethanolamine, and triethanolamine [23], among others.

Although GTA has been widely used to stabilize gelatin films for biomedical applications [14, 19, 21, 22], only a few studies on GTA-gelatin film formulations for food packaging are available [2, 7, 8], probably due to safety considerations [14]. Therefore, there is a necessity of studying the effect of low concentrations of GTA on the relevant properties of plasticized and unplasticized gelatin films. Accordingly, this study analyses the influence of the addition of GTA (0–2 wt%) and D-Sorbitol (30 wt%) as plasticizer on some properties of gelatin films, including opacity, tensile properties, and water solubility. In the context of the intended application of these films, GTA release to a simulant medium as well as water vapor permeability were also measured. This last information is scarcely found in the literature for GTA-stabilized gelatin films and it is critical because it may serve as a guide for the future application of these materials in food packaging.

Materials and methods

Materials

Bovine gelatin (Ge) (type B, 200 Bloom) was kindly provided by Rousselot (Buenos Aires, Argentina). Glutaraldehyde (GTA), commercialized as 50 wt% aqueous solutions, was purchased from QBS (Buenos Aires, Argentina) and was used as cross-linking agent in various weight proportions (on dry gelatin basis) without any further purification. D-Sorbitol monohydrate (S) and phosphate buffer solution pH 7.0 were purchased from Anedra (Buenos Aires, Argentina).

Preparation of gelatin films

Gelatin films were produced by casting from their film-forming solutions (FFS) [8]. Plasticized or unplasticized cross-linked gelatin films were obtained by hydrating 10 g of gelatin powder in 100 mL of distilled water for 30 min and then dissolved at 50 ± 2 °C under stirring for 30 min. D-sorbitol (S) (30 wt% on dry gelatin basis) was added as plasticizer and the solution was held at 35 ± 2 °C under stirring for further 30 min. Appropriate amounts of 50 wt% GTA aqueous solution (from 0.1 to 2 wt% on dry gelatin basis) were added to the FFS under stirring at 35 ± 2 °C. The resulting mixture was adjusted to pH 6 with NaOH and then, stirred for additional 30 min at 35 ± 2 °C. The produced FFS were then cast onto Teflon-coated Petri dishes (diameter 10 cm) and disposed on a leveled horizontal surface to maintain constant area and uniform thickness. After drying at 35 °C in a convection oven for 24 h, the resulting films were manually peeled off from the plates. Films were kept in a desiccator at room temperature before

Author's personal copy

testing to prevent any further change before the experiments. Plasticized and unplasticized cross-linked gelatin films were labeled as Ge-xGTA/S and Ge-xGTA, where x defines the percentage of GTA added and S indicates the presence of D-sorbitol.

Measurements

Thickness

The film thickness was used to assess mechanical, optical, and barrier properties and it was measured with a 0–25 mm manual micrometer (Venier, China) with an accuracy of ±0.01 mm. The reported values are the average of four readings taken randomly on each film sample.

Cross-linking extent

The extent of the amino groups involved in the cross-linking reaction was determined by UV–visible spectroscopy by using ninhydrin (2,2-dihydroxy-1,3-indanedione, NHN), in order to estimate the amount of free amino groups remaining after the chemical cross-linking reaction [19]. Ninhydrin forms a purple complex (Ruhemann's purple) with the α-amino functionality of proteins. The absorbance of the solution measured at 570 nm (the wavelength of the blue–purple color) is proportional to the amount of free amino groups left after the reaction with the cross-linking agent. Cross-linked gelatin films were dried under vacuum at room temperature until constant weight. A precise amount of sample (100 ± 5 mg) was heated with ninhydrin solution (0.5 wt%) for 20 min. The absorbance of this solution was recorded on a Shimadzu 1601 PC spectrophotometer at 570 nm (Tokyo, Japan). The percentage of free amino groups was expressed by the following equation:

$$\left[\left((\text{NHN reactive amine})_{g} - (\text{NHN reactive amine})_{rg}\right) / (\text{NHN reactive amine})_{g}\right] \times 100 \quad (1)$$

where $(\text{NHN reactive amine})_g$ is the total amount of amino groups in the gelatin film, and $(\text{NHN reactive amine})_{rg}$ is the amount of free amine groups present in the gelatin film after the cross-linking reaction.

Network density

The presence of covalent bonds within gelatin films affects their water uptake (WU) and it is indicative of the cross-linking reaction efficiency. Gelatin samples ($1 \times 3 \times 0.02$ cm^3) were dried under vacuum and then weighed (w_d) before immersion in distilled water (ASTM D570-81) at 20 °C. After different periods of time (t), the wet samples were recovered, blotted with tissue paper to remove the water excess on the surface and then, weighed again (w_t). The WU was calculated as:

$$\text{WU}\ (\%) = 100\,(w_t - w_d)/w_d \quad (2)$$

Triplicate measurements of WU were conducted for each type of film, and the average was reported as the result.

In order to estimate the network cross-linking density, the number-average molecular weight of chain segments between two cross linking points (M_c) was estimated by using the Flory–Renher equation [26]:

$$M_c = \rho V_1(\phi_g^{1/3} - 2\phi_g/f) / [\chi\phi_g^2 + \ln(1-\phi_g) + \phi_g] \quad (3)$$

where ρ is the density of the dry gelatin determined by pycnometry, V_1 is the solvent molar volume, χ is the polymer–solvent interaction parameter taken from the literature ($\chi = 0.49 \pm 0.05$) [27] and ϕ_g is the volume fraction of the swollen gelatin. This parameter was estimated from the experimental equilibrium water uptake (WU_{eq}) values, assuming the following relationship:

$$\phi_{g=} w_0\rho_{w\,/\,[}w\rho_{g-}W_{0(\rho-}\rho_{w)]} \quad (4)$$

where w_0 is the initial weight of the dry sample; w is the weight of the swollen sample; ρ_w is the density of the water which was measured after removing the excess liquid on the sample surface by a filter paper and ρ is the density of the dry and uncross-linked gelatin [27].

Thermal properties

Differential scanning calorimetry (DSC) analysis was performed by using a Perkin Elmer calorimeter model Pyris 1. Sample weights were in the range of 5–7 mg and all runs were performed from room temperature up to 250 °C, under nitrogen atmosphere and a heating rate of 5 °C min^{-1}.

DRX analysis

X-ray diffraction patterns were recorded by using a Phillips PW1700 diffractometer equipped with CuK α radiation source ($\lambda = 0.1546$ nm), operating at 45 kV and 30 mA as the applied voltage and current, respectively. The incidence angle was varied between 2° and 50° at a scanning rate of 1 °C min^{-1}.

GTA release

The release of GTA from gelatin cross-linked films was evaluated by the spectrophotometric method reported elsewhere [14]. Basically, a known weight of gelatin film

Author's personal copy

was immersed in 3 mL of phosphate buffer solution (pH 7.4) at 25 °C. After a determined time, 7 mL of a 0.1 M glycine solution were added to the buffer solution and the absorbance at 436 nm was measured. GTA concentration in this solution was determined through comparison with a calibration curve. Results were the average of three replicates.

Optical properties

The visible light-barrier properties of films were determined by measuring their light absorption at wavelength ranging from 400 to 800 nm, with a UV–visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan), according to the method described by Irissin-Mangata et al. [28] on rectangular strips placed directly in the spectrophotometer cell. Air was used as reference. Film opacity was defined as the area under the recorded curve that was obtained through an integration procedure, and it was expressed as absorbance units per thickness unit (mm).

Total soluble matter (TSM)

TSM was determined as the percentage of sample dry matter solubilized after 24 h immersion in distilled water at pH 7. Rhim et al. [29] proposed two ways of measuring TSM to evaluate the thermal effect during prior heat-drying at 105 °C, the ''wet'' and the ''dry'' method. For the dry method, four samples (2×2 cm^2) from each type of film were weighed (m_h) ($\pm$ 0.0001 g) and subsequently dried in an air-circulating oven at 105 °C for 24 h. After this time films were recovered and reweighed ($\pm$0.0001 g) to determine their initial dry matter (m_0). The initial moisture content (MC) was calculated from the Eq. 5 as follows:

$$\text{MC } (\%) = 100 \times (m_h - m_0)/m_0 \quad (5)$$

Samples were then immersed in 30 mL of distilled water and stored in an environmental chamber at 25 °C for 24 h. After this time, specimens were recovered, rinsed with distilled water, and dried in an air circulating oven at 105 °C until reaching constant weight (m_f). The TSM was calculated as follows:

$$\text{TSM } (\%) = 100 \times (m_0 - m_f)/m_0 \quad (6)$$

For the wet method the heat-drying step was skipped. Four specimens of each film were weighed (m_h) and then directly immersed in distilled water and incubated as above described. After 24 h samples were oven dried at 105 °C during 24 h, to determine the remnant un-soluble matter (m_f). Initial dry matter values needed for TSM calculations were those obtained from MC measurements for the same film. Results are the average of three replicates.

Mechanical properties

Tensile tests were carried out on an INSTRON 4467 Universal Testing Machine (Darmstadt, Germany) equipped with a 0.5 KN cell, at a crosshead speed of 10 mm min^{-1} at room temperature by following the procedure described in ASTM D638-94b. Dog-bone shaped specimens ($30 \times 4.5 \times 0.2$ mm^3) were conditioned at 43% RH in a humidity chamber before testing. The tensile strength (TS) and the percentage of elongation at break (ε%) were calculated as the average of six replicates.

Water vapour permeability (WVP)

WVP was measured using a modified method described by ASTM E96-95. The film was sealed on a glass permeation cup containing silica gel (0% RH) using silicone vacuum grease and a rubber band to hold the film in place. The cups were then placed at 25 °C in a desiccator containing distilled water. The cups were weighed at 5 h intervals over a 48 h period. WVP of films was calculated as follows:

$$\text{WVP}(\text{Kg} \times \text{m} \times \text{s}^{-1} \times \text{Pa}^{-1} \times \text{m}^{-2}) = \frac{w}{A \times t \times \Delta P} \times e \quad (7)$$

where w is the weight gain of the cup (Kg) at time t (s); e is the film thickness (m); A is the exposed area of film (m^2); ΔP is the vapour pressure difference across the film (Pa). Three replicates of each film were used for WVP testing.

Statistical analysis

Values obtained in all these experiments were statistically analyzed by one-way analysis of variance (ANOVA). Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The level of significance was $p < 0.05$.3

Results and discussion

Film formation: confirmation of cross-linking reaction

Films cross-linked with GTA were obtained by treating gelatin FFS with GTA and 30 wt. % sorbitol. It was verified in preliminary studies that at least 10 wt% gelatin was required to obtain freestanding films by drying the FFS at 35 $\pm$ 2 °C. At low gelatin concentration (i.e., 5 wt%) the cohesive strength seems to be insufficient to form strong bonds at such temperature. The pH of the reaction medium was monitored during the cross-linking reaction since this parameter affects the protein charge which in turn influences the solubility and the type of interactions involved in

Author's personal copy

the network formation [30]. A pH value slightly higher than the isoelectric point, i.e., $pI_{gelatin}$ 5.1, ensures a certain amount of free amino groups in gelatin chain able to react with GTA and also restricts GTA auto-condensation which takes place at highly alkaline conditions [5, 8, 14, 16]. Consequently, pH value was kept near 6. D-Sorbitol is a polyhydric alcohol with a high-boiling point (bp 296 °C), water-soluble, polar, non-volatile, and protein-miscible. These properties make sorbitol a suitable plasticizer for using with a compatible water-soluble polymer such as gelatin [23, 25].The amount of sorbitol added to the film-forming solution was fixed at 30 wt%, because lower plasticizer percentages gave rise to films excessively brittle and susceptible to cracking, especially under low RHs and temperatures, where the biopolymer was in a glassy state [13]. On the contrary, for sorbitol contents higher than 30 wt%, phase separation was verified suggesting that gelatin and plasticizer are not thermodynamically compatible in the whole composition range, in accordance with results reported by Sobral et al. [25] for films based on bovine and pig skin gelatin blended with sorbitol. Reaction between gelatin and GTA was carried out at 35 ± 2 °C, in order to control the reaction rate [16] and restrict the reorganization of gelatin strands into a collagen-like structure [5, 8, 17]. For similar reasons drying temperature was maintained higher or equal than gelation point (about 35 °C) [5, 8, 17] to obtain almost completely amorphous films [17].

The visual aspect of un-plasticized Ge-GTA films is depicted in Fig. 1. Gelatin control film with no cross-linking was in general, practically colorless. After cross-linking with GTA, light yellow to dark brown color films were obtained as GTA level increased from 0 to 2 wt%. The increased color intensity confirmed the formation of conjugated Schiff's bases which are intermediate products in the Maillard reaction [8, 16, 29]. This result agreed well with those reported for bovine and porcine gelatin–GTA [5, 8, 14], bovine gelatin–dialdehyde starch [9], fish gelatin–GTA [3], and soybean protein isolate-based films cross-linked with dialdehyde starch [29], among others.

In order to verify the Ge-GTA cross-linking reaction, ninhydrin test was performed on control and GTA-treated gelatin films and the cross-linking degree was calculated from Eq. 1. Cross-linking densities were also estimated by calculating the number-average molecular weight of chain segments between two cross-linking points, M_c, according to Eq. 3. The cross-linking degree increased from 75 ± 2% for 0.5 wt% GTA up to 92 ± 3% for 1 wt% GTA, while M_c dropped from 3780 ± 110 to 1434 ± 548 Da (Table 1). The slight increment in the reaction extent with further additions of GTA (i.e., 97% for 2 wt% GTA) (results not shown) suggested that at GTA ≥ 2 wt% most of the amino groups have already reacted and the final conversion was reached. Similar results were reported by Fraga and Williams [17] for cold water-fish gelatin cross-linked with formaldehyde. The authors described this effect as similar to the arrest of the reaction due to vitrification in thermosetting systems.

The incorporation of 30 wt% sorbitol into Ge-GTA FFS gave rise to more flexible cross-linked films. Plasticizers are usually added to a polymeric matrix before drying, just to overcome brittleness. Plasticizers have the ability of increasing film flexibility by reducing the internal hydrogen bonding between polymer chains while increasing free volume in the macromolecules [4, 6, 9, 23–25]. For low-GTA concentrations (i.e., Ge-0.5GTA/S), M_c value was 8034 ± 482 Da (Table 1), representing 35% of cross-linking, much lower than the value found for unplasticized films, which accounted for 75% of cross-linking. These results suggest that sorbitol seems to interfere in the cross-linking reaction between Ge and GTA by reducing the sites available for GTA to react. However, the increase in GTA content up to 1 wt% led to more reticulated materials when comparing with the un-plasticized counterpart (99% vs. 92% cross-linking, respectively). It was postulated that at such GTA concentration, cross-linking reaction proceeded with a simultaneous reduction in the plasticizer–gelatin interactions due to the restrictions imposed by cross-links, leaving the plasticizer entrapped into the Ge-GTA network.

Opacity

The light barrier properties of Ge-GTA films with and without plasticizer expressed as opacity values are summarized in Table 1. Lower opacity values indicate higher transparency. For un-plasticized films, opacity significantly increased ($p < 0.05$) with GTA. Only the Ge-0.5GTA film can be considered transparent according to the values

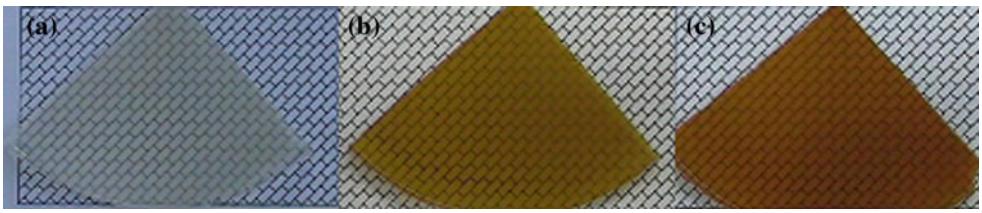

Fig. 1 Visual aspect of GTA cross-linked gelatin films **a** 0 wt% GTA, **b** 0.5 wt% GTA, **c** 1 wt% GTA

Author's personal copy

Table 1 Number average molecular weight of chain segments between two cross-linking points (M_c), cross-linking density, thickness, and opacity of Ge-GTA films

Sample name	M_c (Da)	Cross-linking density (%)	Thickness (mm)	Opacity (Ua mm)
Ge	37283 ± 5780[a]	0 ± 0[a]	0.21 ± 0.03[a]	38.9 ± 5.0[a]
Ge-0.5GTA	3780 ± 110[b]	75 ± 2[b]	0.20 ± 0.02[a]	243.6 ± 6.4[b]
Ge-1GTA	1434 ± 548[c]	92 ± 3[c]	0.20 ± 0.04[a]	437.5 ± 5.2[c]
Ge/S	31540 ± 6050[b]	0 ± 0[a]	0.18 ± 0.02[a]	108.5 ± 10.0[d]
Ge-0.5GTA/S	8034 ± 482[d]	35 ± 7[d]	0.21 ± 0.03[a]	242.5 ± 12.1[b]
Ge-1GTA/S	525 ± 60[c]	99 ± 5[c]	0.23 ± 0.04[a]	488.3 ± 33.1[c]

nd not determined

Any two means in the same column followed by the same *letter* are not significantly ($p > 0.05$) different according to Tukey test

reported by Mali et al. [31] for transparent films. In general terms, films with sorbitol showed higher opacity values ($p < 0.05$) compared to un-plasticized gelatin film, except for 0.5 wt% GTA. Since film thickness was almost invariable (Table 1) the higher opacity of gelatin blended with sorbitol could be a consequence of some degree of phase separation [25]. If the particle size of the dispersed phase is higher than the wavelength of visible light, when light passes through the sample light scattering occurs and transmission is reduced, thereby increasing opacity. These light absorbent films could be useful barriers to reduce light-induced lipid oxidation and consequently improving food quality.

Thermal properties

Figure 2 shows DSC thermograms (first and second scan) of samples stabilized at room temperature and 43% RH. The thermogram of the unplasticized gelatin film from room temperature up to 200 °C (Fig. 2a), was typical of partially crystalline materials [13]. Gelatin structure can be visualized as a block copolymer built up from triads of α-amino acids with glycine at every third position (soft blocks) and triads of hydroxyproline, proline, and glycine (rigid blocks) [17]. The devitrification of each block can be associated with a glass transition temperature (T_g). In mammalian gelatins the rigid blocks prevail over the soft ones and consequently the high temperature T_g represents the overall glass–rubber transition of the material [17]. In addition, if some triple-helix regeneration takes place, characteristic melting transition (T_m) will be evident. In the present case, the thermogram obtained during the first scan displayed a T_g occurring around 60 °C (Table 2), and second heat capacity change with endothermic relaxation at around 90 °C. Fraga and Williams [17] observed a T_g at 120 °C and a broad less pronounced T_g at 190 °C for fish gelatin. Moisture absorption shifted these values and at 20 wt% of moisture content T_gs were located at −30 and +80 °C, respectively. Similarly, absorbed moisture can

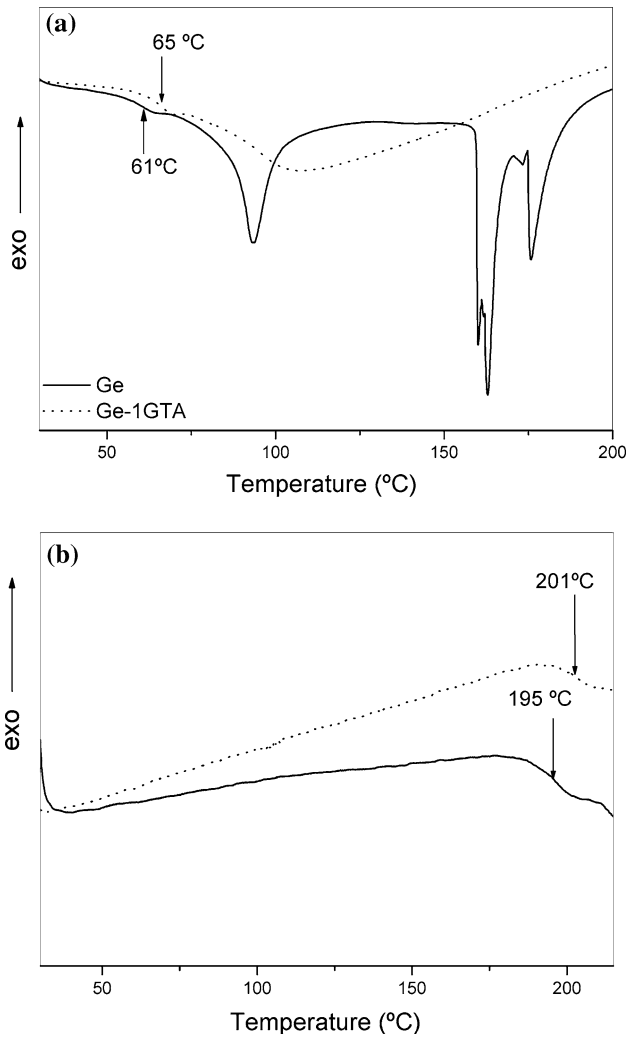

Fig. 2 Thermograms of Ge and Ge-1GTA films **a** first scan from ambient temperature up to 250 °C, **b** second scan of samples quenched from the melt and re-heating from ambient temperature up to 25 °C (Heating rate: 10 °C min^{-1} and N_2 atmosphere)

plasticize gelatin film and therefore, the first glass transition was associated with gelatin soft blocks and the second one was associated with gelatin rigid blocks [1, 17].

Author's personal copy

The complex superimposition of thermal events above 150 °C was ascribed to the superposition of different events including the melting of partially regenerated triple-helix and other thermal events related to rigid blocks [1, 13, 17]. The presence of partially regenerated collagen in unplasticized gelatin films was confirmed by the presence of a distinctive diffraction band at $2\theta = 8.1°$ (Fig. 3) according to data reported elsewhere [15]. The addition of 1 wt% GTA, slightly shifted the lower temperature T_g to higher values (with an associated enthalpy of relaxation), followed by a broad band corresponding to volatiles evaporation (particularly absorbed water and not reacted GTA, $b.p._{GTA\ 50\%} =$ 101 °C) (Fig. 2a). Cross-linked films did not evidence melting behavior in accordance to XRD analysis. The presence of covalent cross-links prevents the reordering into a triple helix, adopting an amorphous morphology (Fig. 3). Only films containing 0.1 wt% GTA showed a low intensity diffraction peak at $2\theta = 8.1°$. Such behavior was similar to results reported by Watanabe et al. [32]. The authors, who demonstrated that cross-linking degrees higher than 80% restricted the possibility of triple helix regeneration giving rise to amorphous materials.

After quenching from the melt and re-heating, the high temperature T_g was more visible, being around 195 °C for control Ge and from 198 to 201 °C for Ge-GTA films (Fig. 2b; Table 2). At high-GTA levels the high temperature T_g was superimposed with thermal degradation around 230 °C. As it could be expected, the addition of D-sorbitol displaced the T_g to lower values as compared to those of the unplasticized films (Table 2), in accordance to its plasticizing effect.

Tensile properties

The use of films for packaging strongly depends on their favorable mechanical, moisture resistance, and barrier properties. The loss of mechanical integrity in films and coatings due to poor mechanical properties reduces their effectiveness as barrier materials [14]. Figure 4 shows representative stress–strain curves obtained from tensile tests and calculated parameters are summarized in Table 2.

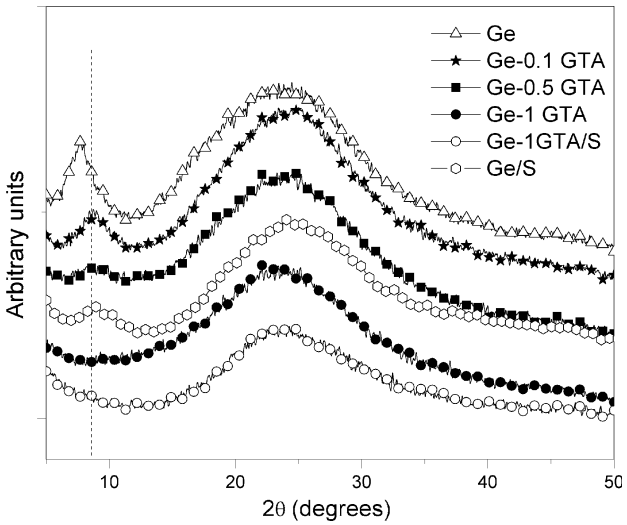

Fig. 3 X-ray diffraction patterns of pure gelatin and Ge-GTA films

As a general trend, at strain values lower than 10% the stress increased rapidly with strain and the slopes were in the elastic region defining the elastic modulus. It is evident that films with high GTA content and no plasticizer exhibited a typical brittle fracture behavior characterized by high-tensile strength, low-elongation percentage and no yield point, in accordance with the development of a more rigid structure as the amount of GTA increases. Films treated with GTA levels higher than 1 wt% were unable to be tested owing to their extreme brittleness, even after conditioning at 43% RH. Table 2 lists tensile strength (TS), elongation at break (ε%), and elastic modulus (E) for GTA cross-linked films unplasticized and plasticized with D-sorbitol. Increasing GTA content from 0 to 1 wt% induced significant increment in E and TS values ($P < 0.05$), in agreement with the observed increment in T_g while elongation at break experienced a small but statistically significant reduction ($p < 0.05$). For gelatin-based films modified by glutaraldehyde, Bigi et al. [15] observed a significant increase in tensile strength from 1 to 3.5 MPa with a decrease in elongation at break from 40 to 10% as the glutaraldehyde concentration increased from 0.05 to 2.5%.

Table 2 Thermal and tensile properties of Ge-GTA films

Sample name	Glass transition temperature, T_g (°C)		Tensile properties 43 ± 2% RH; T: 23 ± 2 °C		
	T_{g1}	T_{g2}	E (GPa)	ε(%)	TS (MPa)
Ge	61	195	3.7 ± 0.2^{a}	30.1 ± 0.4^{a}	75.2 ± 12.6^{a}
Ge-0.5GTA	62	198	4.7 ± 0.2^{b}	2.1 ± 0.8^{a}	76.1 ± 19.6^{a}
Ge-1GTA	65	201	4.9 ± 0.2^{b}	1.3 ± 0.3^{b}	55.9 ± 15.3^{b}
Ge/S	58	180	1.1 ± 0.1^{c}	20.7 ± 3.2^{c}	45.9 ± 2.5^{c}
Ge-0.5GTA/S	60	182	0.7 ± 0.1^{d}	30.4 ± 2.2^{d}	33.3 ± 4.7^{d}
Ge-1GTA/S	66	200	$1.5 \pm 0.3^{c,e}$	30.4 ± 11.7^{d}	33.9 ± 2.4^{d}

Any two means in the same column followed by the same *letter* are not significantly ($p > 0.05$) different according to Tukey test

Author's personal copy

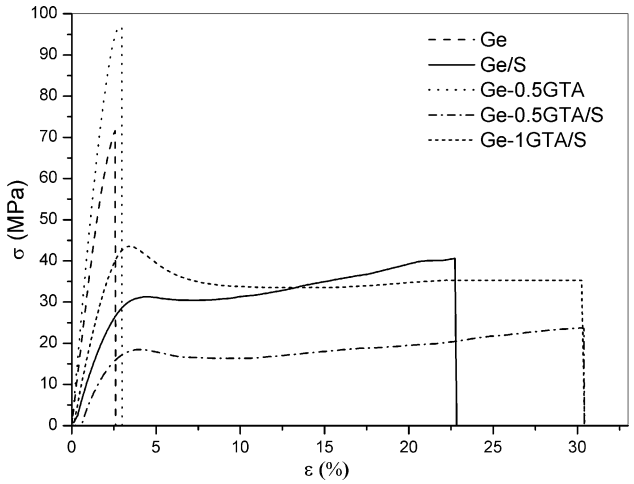

Fig. 4 Representative stress–strain curves of plasticized and unplasticized gelatin films (Ge and Ge/S) and GTA-cross-linked gelatin films (Ge-GTA and Ge-GTA/S). All samples were stabilized at 43 ± 2% RH and 23 ± 2 °C before testing

On the other hand, GTA-treated films plasticized with 30 wt% D-sorbitol exhibited yield-like deformation similar to thermoplastics (Fig. 4). After the yield point, a large deformation region was observed, indicating that films were ductile with an increased extensively. The addition of D-sorbitol reduced both E and TS values ($p < 0.05$) owing to the decrease in gelatin intermolecular interactions as already discussed in the literature [23, 25]. Interestingly, the addition of 0.5 wt% GTA induced an unfavorable effect on E value (Table 2) when comparing with non-cross-linked films. The low cross-linking extent achieved by such films (c.a. about 35%) let the plasticizer to expand the network displaying a strong plasticizing effect and water bonding capacity. Water is a very effective plasticizer for protein matrix [4, 9, 27] since the more hydrophilic the film the greater plasticization is expected. Plasticization reduced the hydrogen interactions between protein chains, thereby increasing the extensibility of Ge-0.5GTA/S films by about 66% compared to Ge/S films (Table 1). Further additions of GTA increased E value up to 1.5 ± 0.3 GPa, in agreement with the increased cross-linking density. Tensile properties of Ge-0.5GTA/S films were comparable with values reported by Rivero et al. [24] for gelatin films plasticized with 20% glycerol. When compared to synthetic polymers usually applied in food packaging, Ge-0.5GTA/S films exhibited analogous TS values but much lower ε% than low density polyethylene films [33].

Total soluble matter and water uptake

Total soluble matter (TSM) and water absorption were measured to evaluate the integrity of Ge-GTA films in moist environments. The determination of TSM through the ''dry'' and ''wet'' methods allows evaluating the effect of drying the samples at 105 °C before testing. The results obtained by applying both methods are summarized in Table 3. Gelatin films without GTA disintegrated almost completely after 24 h soaking, and no significant differences ($p > 0.05$) in TSM values measured by both methods were observed. Consequently, drying conditions did not induce significant cross-linking into gelatin matrix, as previously observed for gelatin films plasticized with glycerol [9]. Conversely, the addition of GTA to gelatin-based films provoked statistically lower ($p < 0.05$) water solubility; dissolution degree decreased with increasing GTA level from 0 to 1 wt%. This result confirms that covalent cross-links enhanced the stability of gelatin films in wet environments in consistency with the observation of other authors for protein systems [9, 10, 29]. The soluble fraction in plasticized cross-linked gelatin films could be mainly attributed to the loss of low molar mass compounds, such D-sorbitol and short-chain polypeptides that could not be linked to the network [9, 10].

Experimental moisture sorption curves would offer suitable in-use performance to predict the in-use behavior of modified gelatin films at a given relative humidity environment. Figure 5 illustrates the water uptake as a function of the immersion time for different GTA contents. For all compositions the water absorption was rapid in the initial zone ($t < 150$ min). Beyond this time the absorption rate slowed down until reaching a plateau corresponding to the water uptake at the equilibrium (WU_{eq}). For gelatin with no cross-linking, a few minutes in water provoked considerable absorption (i.e., WU_{eq} 286 wt% after 30 min) followed by hydrolysis as already reported [7]. The addition of increasing amounts of GTA had a beneficial effect on water uptake as revealed by a significant reduction in WU_{eq} with GTA (Fig. 5; Table 3). Blending with D-sorbitol induced an adverse effect in such property for GTA varying from 0 to 0.5 wt% (Fig. 5; Table 3). For no cross-linked films, the addition of a highly hygroscopic plasticizer such as D-sorbitol increased significantly the water sensitivity of films ($p < 0.05$). By cross-linking with 0.5 wt% GTA, moisture absorption improved but the obtained value was still higher than in the absence of plasticizer (Table 3).

The low cross-linking degree achieved by Ge-0.5GTA/S films (c.a. 35%) allowed the plasticizer to expand the network showing a strong plasticizing effect and water binding capacity. This result supports the assumption that the water absorbed by Ge-0.5GTA/S is the main responsible of the reduction in the elastic modulus and the increment in the elongation at break of this film. The substantial moisture resistance showed by Ge-1GTA/S film means that at such GTA concentration cross-linking prevail

Author's personal copy

Table 3 Total soluble matter (TSM), water absorption at the equilibrium (WUe), GTA release, and water vapor permeability (WVP) of GTA-cross-linked gelatin films

Sample name	*TSM[1] (%)	*TSM[2] (%)	WU_{eq} (%)	GTA release (%GTA_0, 24 h)	WVP $*10^{14}$ (Kg m Pa^{-1} s^{-1} m^{-2}) 0:65% RH
Ge	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	1341.1 ± 91.7^{a}	0.0 ± 0.0^{a}	17.3 ± 1.3^{a}
Ge-0.5GTA	27.4 ± 1.3^{b}	29.4 ± 2.5^{b}	521.1 ± 46.3^{b}	1.0 ± 0.4^{b}	*nd
Ge-1GTA	16.2 ± 1.5^{c}	18.4 ± 1.4^{c}	351.1 ± 35.4^{c}	6.9 ± 0.6^{c}	*nd
Ge/S	97.1 ± 1^{d}	100.0 ± 0.0^{a}	1854.1 ± 85.4^{d}	0.0 ± 0.0^{a}	2.42 ± 0.27^{d}
Ge-0.5GTA/S	27.4 ± 0.4^{db}	38.2 ± 0.3^{e}	710.0 ± 10.6^{e}	2.1 ± 0.5^{e}	1.23 ± 0.02^{e}
Ge-1GTA/S	15.6 ± 0.2^{c}	16.6 ± 0.4^{c}	210.4 ± 8.6^{f}	5.0 ± 0.3^{f}	0.94 ± 0.06^{f}

* TSM^1 dry method, * TSM^2 wet method

Any two means in the same column followed by the same *letter* are not significantly ($p > 0.05$) different according to Tukey test

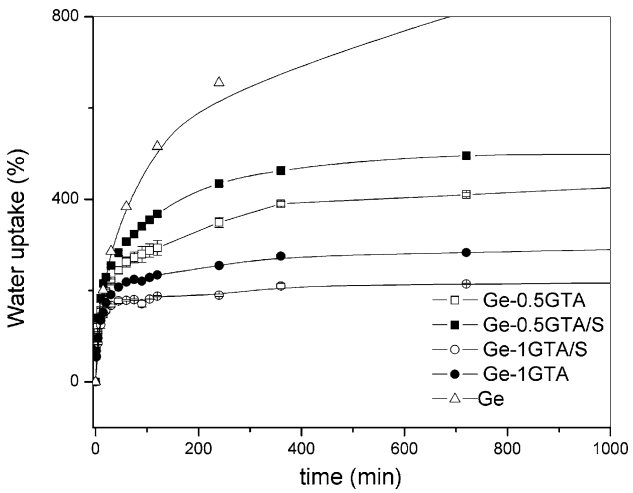

Fig. 5 Water uptake curves of cross-linked gelatin films plasticized and unplasticized by D-sorbitol as a function of the immersion time

over plasticization in accordance with observations above-reported for tensile behavior.

GTA release

The potential of Ge-GTA films as food contact materials is mostly limited by the potential release of GTA to foodstuff. In order to evaluate this possibility, films were subjected to a GTA release study by immersing them in a buffer solution for 24 h at room temperature. Results are summarized in Table 3. The highest amount of GTA released was about 7% of the initial concentration for films containing 1 wt% GTA, and this value was reduced with decreasing GTA content. A similar behavior was evidenced in plasticized cross-linked gelatin films, exhibiting lower values of GTA release for the highest GTA content due to the slightly higher cross-linked degree of such films (Table 1). No further GTA release was verified after storing in a buffer solution up to 72 h. The slow GTA release in this medium arose from the reversibility of Schiff bases and the depolymerization of GTA polymers formed during cross-linking reaction [5, 14, 16]. The results reported herein differ from those found by Bigi et al. [14] who determined no GTA release for pig skin gelatin films containing GTA concentrations smaller than 2 wt%. This discrepancy could be due to differences in film-forming conditions, such as gelatin source, drying temperature, the presence of plasticizer, etc. Silva et al. [34], characterized GTA–cross-linked chitosan membranes in simulated body conditions. Authors reported that all the cells culture in contact with GTA–chitosan membranes (0–20% GTA–chitosan amino groups molar ratio) displayed normal morphology after 72 h with a viability percentage around 80%, signifying that these materials did not exert a significant cytotoxic effect over the cells. Similar trend was reported for collagen cross-linked with GTA [22]. Since GTA toxicity seems to be related to its release from the material, it is important to point out that GTA release was small ($p > 0.05$) in gelatin films plasticized with sorbitol (Table 3), suggesting that Ge-GTA/S films could be considered safe materials for food contact applications.

WVP

The water vapor barrier ability of D-sorbitol plasticized gelatin films improved appreciably ($p < 0.05$) compared with those of unplasticized gelatin films (Table 3). Mali and co-workers [31] reported that WVP depends on many factors including the ratio between crystalline and amorphous areas in the polymer structure, polymeric chain mobility, and specific interactions between the functional groups of the polymer and the permeating gases in the amorphous zone. Therefore, WVP values were related to the occurrence of hydrogen-bonding interactions between gelatin and sorbitol, leading to a denser matrix able to hinder the transfer of water molecules through the film. The presence of some D-sorbitol crystals could also offer a tortuous path for the diffusion of gases. However, the

Author's personal copy

results reported herein differ from those data reported by Sobral et al. [25], who found that the addition of increasing amounts of D-sorbitol to bovine and porcine gelatin increased WVP values of the resulting films. Authors stated that plasticizers modified the molecular organization of the protein network and increased the free volume resulting in a less dense network and finally on a higher permeability to water.

The incorporation of 0.5 wt% GTA was enough to reduce significantly ($p < 0.05$) WVP values ($1.23 \pm 0.02 \times 10^{-14}$ kg m Pa^{-1} s^{-1} m^{-2} vs. $2.42 \pm 0.27 \times 10^{-14}$ kg m Pa^{-1} s^{-1} m^{-2} for Ge-0.5GTA/S and Ge/S films, respectively). For films with 1 wt% GTA the reduction in WVP was around 38%, indicating an increase in film hydrophobicity due to the consumption of polar amino groups from gelatin through cross-linking with GTA, in agreement with the increased cross-linking density [7, 29]. Consequently, gelatin chains became less mobile due to the restrictions imposed by cross-linking reducing the water diffusivity through the protein matrix leading to a decrease in WVP. On the other hand, WVP values of unplasticized Ge-GTA films showed large dispersion between samples obtained from the same film, limiting the possibility of reporting realistic values of such property. This was linked to the difficulty in obtaining Ge-GTA film without pores or cracks. Ge-GTA films showed WVP values lower than those obtained for Ge-DAS plasticized films ($2.4–1.6 \times 10^{-13}$ kg m Pa^{-1} s^{-1} m^{-2}) [7] in accordance with the better performance of GTA as cross-linking agent. WVP values obtained in this work ($1.23–0.94 \times 10^{-14}$ kg m Pa^{-1} s^{-1} m^{-2}) were in the same order than those reported for enzymatic and chemically cross-linked plasticized gelatin films (3.3 and 4.3×10^{-14} kg m Pa^{-1} s^{-1} m^{-2}, respectively) [18]. With regard to synthetic polymers, Ge-GTA films had higher WVP values than those of high-density polyethylene (HPDE) (2.4×10^{-16} kg m Pa^{-1} s^{-1} m^{-2}), polyvinyl chloride (PVC) ($2.4–0.7 \times 10^{-16}$ kg mm^{-2} s^{-1} Pa^{-1}) and low density polyethylene (LDPE) ($10–7 \times 10^{-16}$ kg m Pa^{-1} s^{-1} m^{-2}) [35].

Conclusions

At the light of the obtained results it can be concluded that gelatin films cross-linked with 1 wt% GTA and plasticized with 30 wt% of D-sorbitol are the most suitable formulation for the intended applications, in terms of thermal, optical, tensile, solubility, and barrier properties with low level of GTA release, which ensure its performance as food contact materials. When compared with no cross-linked plasticized gelatin films, WVP decreased around 38%, the elongation at break increased approximately to 150%, solubility decreased from 100 to 16% and water absorption improved from $1854.1 \pm 85.4\%$ to $210.4 \pm 8.6\%$ with a release of GTA as low as 5% of the initial amount. Further work must be undertaken in order to analyze the possibility of using these films in the food packaging sector. Migration behavior to food stimulants is under study, in order to evaluate the conformity of the obtained films with the current and future international regulations.

Acknowledgements The authors thank the National Research Council, Argentina (CONICET) and ANPCYT (PICT 1791) for their financial support and express their gratitude to Rousselot Argentina for providing the bovine gelatin used in this study.

References

1. Chiellini E, Cinelli P, Corti A, Kenawy ER (2001) Polym Deg Stabil 73:549
2. Vanin FM, Sobral PJA, Menegalli FC, Carvalho RA, Habitante AMQB (2005) Food Hydrocolloid 19:899
3. Chiou B-S, Avena-Bustillos RJ, Bechtel PJ, Jafri H, Narayan R, Imama SH, Glenn GM, Orts WJ (2008) Eur Polym J 44:3748
4. Gennadios A (2002) In: Gennadios A (ed) Soft gelatine capsules en protein-based films and coatings, Chapter 1, 1st edn. CRC Press, Boca Raton
5. Farris S, Song J, Huang Q (2010) J Agric Food Chem 58:998
6. Bergo PVA, Sobral PJA (2007) Food Hydrocolloid 21:1285
7. Al-Hassan AA, Norziah MH (2012) Food Hydrocolloid 26:108
8. Martucci JF, Ruseckaite RA, Vazquez A (2006) Mater Sci Eng A 435–436:681
9. Martucci JF, Ruseckaite RA (2009) J Appl Polym Sci 112:2166
10. Martucci JF, Ruseckaite RA (2010) J Food Engin 99:377
11. Pereda M, Ponce AG, Marcovich NE, Ruseckaite RA, Martucci JF (2011) Food Hydrocolloid 25:1372
12. Alves PMA, de Carvalho RA, Moraes ICF, Luciano CG, Bittante AMQB, Sobral PJA (2011) Food Hydrocolloid 25:1751
13. Sobral PJA, de Carvalho RA, Moraes ICF, Habittante AMQB, Monterrey–Quintero ES (2011) Ciência e Tecnologia de Alimentos 31:372
14. Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N (2001) Biomaterials 22:763
15. Bigi A, Borghi M, Cojazzi G, Fichera AM, Panzavolta S, Roveri N (2000) J Therm Anal Cal 61:451
16. Robinson ID (1964) J Appl Polym Sci 8:1903
17. Fraga AN, Williams RJJ (1985) Polymer 26:113
18. de Carvalho RA, Grosso CRF (2004) Food Hydrocolloid 18:717
19. Chen Y-S, Chang J-Y, Cheng C-Y, Tsai F-J, Yao C-H, Liu B-S (2005) Biomaterial 26:3911
20. Peña C, De la Caba K, Eceiza A, Ruseckaite RA, Mondragón I (2010) Bioresour Technol 101:6836
21. Azami M, Rabiee M, Moztarzadeh F (2010) Polym Compos 31:2112
22. Scotchford CA, Cascone MG, Downes S, Giusti P (1998) Biomaterials 19:1
23. Cao N, Yang X, Fu Y (2009) Food Hydrocolloid 23:729
24. Rivero S, García MA, Pinotti A (2010) Innov Food Sci Emerg Technol 11:369
25. Sobral PJA, Menegalli FC, Hubinger MD, Roques MA (2001) Food Hydrocolloid 15:423
26. Flory PJ, Rehner JJ (1943) Swelling Chem Phys 11:521
27. Patil RD, Mark JE, Apostolov A, Vassileva E, Fakirov S (2000) Eur Polym J 36:1055

Author's personal copy

28. Irissin-Mangata J, Bauduin G, Boutevin B, Gontard N (2001) Eur Polym J 37:1533
29. Rhim J-W, Gennadios A, Weller CL, Cezeirat C, Hanna MA (1998) Ind Crop Prod 8:195
30. Phillips LG, Whitehead DM, Kinsella J (1994) In: Structure-function properties of food proteins, Chapter 10. Academic Press, New York
31. Mali S, Grossmann MVE, García MA, Martino MN, Zaritzky NE (2004) Carbohyd Polym 56:129
32. Watanabe K, Tezuka Y, Ishii T (1997) Macromolecules 30:791
33. Smith SA (1986) In: Bakker M (ed) The Wiley encyclopedia of packaging technology. Wiley, New York, p 514
34. Silva RM, Silva GA, Coutinho OP, Mano JF, Reis RL (2004) J Mater Sci: Mater Med 15:1105
35. Morillon V, Debeaufort F, Blond G, Capelle M, Voilley A (2002) Crit Rev Food Sci 42:67