Effect of Crosslinking on the Properties of Sodium Caseinate Films

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ABSTRACT: Protein films are used as effective lipid, oxygen, and aroma barriers at moderate relative humidity conditions. However, they perform poorly as moisture barriers. The introduction of crosslinks within or between protein chains by enzymatic or chemical modification has been proposed as an alternative means to achieving a stronger polymeric matrix structure, which would result in better functional film properties. In this article, we report the preparation and characterization of sodium caseinate (SC) films crosslinked by glutaraldehyde (GTA) or heat. The crosslinking density increased with GTA content. The thermal stability and tensile modulus and strength increased with GTA content, although films with a low crosslinking density exhibited lower properties than the uncrosslinked sample. Unexpectedly, water vapor permeability and absorption also increased with crosslinking density. The crosslinking of SC was also induced by simple heating. The resulting films showed enhanced thermal, mechanical, and barrier properties compared to the unmodified SC films and even the GTA-crosslinked samples. GTA crosslinking was unable to reduce the high hydrophilicity of the SC films. Thermally induced crosslinking was revealed to be a valid alternative for improving the properties of SC films, without the inherent complications associated with the use of a chemical crosslinking agent. J Appl Polym Sci 116: 18–26, 2010

Key words: films; crosslinking; proteins

INTRODUCTION

Protein films are frequently used as effective lipid, oxygen, and aroma barriers at low to moderate relative humidity (RH) conditions.[1,2] However, because of the inherent hydrophilicity of proteins and the substantial amounts of hygroscopic plasticizers that are usually added to allow manipulation in the final material, protein films perform poorly as moisture barriers.[1,3] The introduction of crosslinks within or between protein chains by physical (radiation or heat), enzymatic, or chemical modification [with a crosslinking agent, e.g., glutaraldehyde (GTA)][4,5] has been proposed as an alternative means to achieving a stronger polymeric matrix structure,[6] which would result in better functional film properties.[6]

Heat treatment can promote the formation of either covalent or noncovalent intramolecular/intermolecular crosslinks.[5] Proteins have the ability to form intermolecular disulfide bonds during heat treatment, which are the most common and well-characterized types of covalent crosslinks in proteins.[4,7] They are formed by thiol–disulfide interchange and by the oxidative coupling of two cysteine residues that are adjacent within a protein matrix to produce disulfide crosslinks.[4,5]

Some advantages in the heat curing of protein films have been reported by several authors; for example, Gennadios et al.[8] showed improvement in the moisture barrier properties of soy protein films, Pérez-Gago et al.[9] reported that heat-cured whey protein films had higher tensile properties than native whey protein films, and other studies have shown that heat curing improves the mechanical toughness and moisture resistance of cast protein films made from wheat gluten, whey protein, and soy protein.[8,10–13] These results suggest that covalent crosslinking, caused by heating treatment, is responsible for water insolubility and higher tensile properties in films and provides opportunities for innovative uses in food protection and preservation.[12] As reported by Han et al.,[5] heat-treated protein-based

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edible coatings could also be used to prevent oil migration from peanuts.

Aldehydes, such as formaldehyde, GTA, and glyoxal, promote intermolecular and intramolecular crosslinking in proteins.[1] Among the aldehydes that can be used to crosslink a protein matrix, GTA has the advantage that it reacts relatively quickly with a large number of available amino groups present in the molecule, covalently linking the protein chains.[2] Moreover, among chemical crosslinking agents, GTA is by far the most widely used because of its high efficiency in the stabilization of protein-based materials (particularly collagen),[5,14,15] relatively low price, ready availability, and high solubility in aqueous solutions.[2] Moreover, GTA crosslinking has been clinically accepted and has many merits despite reports on its cytotoxicity.[2,6,16–19] Because GTA toxicity seems to be related to its release from the material, studies have determined that no GTA release occurs from films reacted with up to 1.5 wt % of the crosslinker.[15] However, Rhim et al.[1] indicated that the inherent toxicity of the aforementioned aldehyde restricts their use for improving the functional properties of protein films and coatings in edible applications. In contrast, Han et al.[5] stated that GTA has generally recognized as safe approval for use as a food additive in the United States.

Nevertheless, protein films crosslinked with GTA could be used to tailor the film properties for nonfood packaging applications, such as packaging for fresh flowers and agricultural mulch. In these applications, the implementation of bioderived packaging materials could result in increased added value.[20]

Although there has been extensive work on gelatin–GTA films,[15,17,21–23] no information is available concerning the effect of chemical crosslinking on caseinate–GTA films. Thus, the aim of this study was to evaluate the effect of GTA crosslinking and heat treatment on the properties of films prepared from sodium caseinate (SC).

EXPERIMENTAL

Materials

SC powder, containing 88.9 wt % protein (with the rest being lactose, lipids, attached moisture, and ashes), was obtained from Lactoprot Deutschland GmbH (Kaltenkirchen, Germany). The average protein molecular weight was 22,600 g/mol.[24] The GTA solution (50 vol %) was obtained from QBS (Buenos Aires, Argentina). The plasticizer used was glycerol (GLY), which was purchased from DEM Chemicals (Mar del Plata, Argentina).

Methods and techniques

Film preparation

SC aqueous solutions with protein concentrations of 2.5% (w/v) were prepared by the dispersion of the SC powder in distilled water with continuous stirring for 3 h at room temperature. Appropriate amounts of GLY were added to achieve a GLY/protein weight ratio of 0.28. Aliquots of GTA aqueous solution (50 vol %) were added to obtain GTA/SC weight ratios of 0.03, 0.05, and 0.10, corresponding to GTA/lysine molar ratios of 0.61, 1.01, and 2.03. Molar ratios were calculated in consideration of the 12.4 mol of potentially reactive amino acid residue (lysine) contained in 1 mol of SC.[25]

Films were prepared according to the usual casting method[26–31]; that is, the solutions were poured into Teflon Petri dishes (diameter = 14 cm) and dried at 35°C for approximately 10 h in a convection oven. After the excess water was evaporated, the obtained films were peeled off from the plates and kept in a closed reservoir at a constant RH and temperature (23 ± 2°C) for 3 days. The films were further characterized and tested.

Characterization of the films

Thickness measurements. The thickness of the films was determined with a 0–25-mm manual micrometer with an accuracy of 0.01 mm. The reported values are the average of four readings taken randomly on each film sample.

Total soluble mass (TSM). TSM was expressed as the percentage of film dry mass solubilized after 24 h of immersion in distilled water. TSM determinations were carried out according to the two different methods proposed by Rhim et al.,[1] the wet and the dry methods. Both tests were carried out in distilled water (30 mL) and in the presence of sodium azide (0.02%) to prevent microbial growth.

For the dry method, three specimens of each film were dried in an air-circulating oven at 105°C for 24 h and then weighed (±0.0001 g) to determine the initial dry mass (m_0). Afterward, the samples were immersed in 30 mL of distilled water at 25°C for 24 h. After this time, the specimens were partially dissolved; the remnant solids were recovered, rinsed with distilled water, and dried in an air-circulating oven at 105°C until they reached a constant weight (m_f). TSM was calculated as follows:

$$\text{TSM}\ (\%) = \frac{m_0 - m_f}{m_0} \times 100 \qquad (1)$$

For the wet method, the first heat-drying step was not performed. Three specimens of each film were weighed and then directly immersed in distilled

water under the conditions described previously. After 24 h of immersion, the samples were oven-dried at 105°C for 24 h to determine the dried remnant insoluble mass (m_f). The m_0 values needed for the TSM calculations were obtained from different specimens cut from the same film and dried at 105°C for 24 h.

Opacity. Film opacity was determined according to the method described by Irissin-Mangata et al.[32] on rectangular strips directly placed in a UV–visible spectrophotometer test cell. The absorption spectrum of the sample was obtained from 400 to 800 nm in a UV–visible spectrophotometer (Shimadzu 1601 PC, Tokyo, Japan). Film opacity was defined as the area under the curve and expressed as Absorbance Units × Nanometers. Measurements were taken in triplicate for each sample.

Thermogravimetric analysis (TGA). TGA measurements were carried out on a Shimadzu TGA-50 thermogravimetric analyzer. Thermal degradation was performed under a nitrogen atmosphere up to 500°C with a heating ramp of 5°C/min. Samples of 4–10 mg were used. The weight loss of the films was calculated with respect to the weight of the samples after moisture evaporation (taken as the sample weight at 105°C) to compare only the thermal degradation of the films, independently of their moisture contents.

Scanning electron microscopy (SEM). The fracture surfaces of the films obtained after they were immersed in liquid air (fragile fracture) were observed with a scanning electron microscope (JEOL, model JSM-6460 LV, Tokyo, Japan). For this purpose, the pieces of the films were mounted on bronze stubs with double-sided tape and then coated with gold before they were observed under the microscope.

Tensile tests. Tensile tests were performed at room temperature (23 ± 2°C) with an Instron universal testing machine (model 8501, Norwood, MA). Bone-shaped specimens were cut from the films with a scalpel according to the ASTM D 1708-93 (1993). A minimum of three films for each composition were prepared, and five specimens from each film were tested. The film samples were clamped into the metal grips for tensile testing and stretched at a crosshead speed of 10 mm/min. The ultimate strength (σ_b), elongation at break (ε_b), and elastic modulus (E) were calculated as described in ASTM D 638-94b (1994).

Moisture sorption. The films, dried at 40°C for 3 days in a vacuum oven, were placed into an environmental chamber maintained at 75% RH and fixed temperature (23 ± 2°C) to obtain water sorption isotherms. Samples were taken out of the chamber at regular time intervals and weighed to a precision of ±0.0001 g. This experiment was performed on four specimens for each sample to ensure the reproducibility of the results. The M_t values of the films as a function of time were obtained from the total mass balances over the sample, as follows:

$$M_t = \frac{(W_t - W_0)}{W_0} \times 100 \tag{2}$$

where M_t is the moisture content of the sample at a fixed time expressed in dry basis (%), W_t is the weight of the sample at a fixed time (g), and W_0 is the initial dry weight of the sample (g). The curves were fitted with the Peleg equation,[33,34] which relates the instantaneous M_t to the initial moisture content (M_0), as shown in Eq. (3):

$$M_t = M_0 + \frac{t}{K_1 + K_2 t} \tag{3}$$

where K_1, K_2, are fitting parameters and t is the time. K_1 is the Peleg's rate factor [h (g water/g solid)/$^{-1}$]. K_2 is the Peleg's capacity parameter (g solid/g water).

The Fick's second law of diffusion was also applied to the results. The expression is as follows[35]:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-D\frac{(2n+1)^2\pi^2 t}{l^2}\right] \tag{4}$$

where M_∞ is the amount of water absorbed at equilibrium, D is the effective diffusion coefficient, and l is the average thickness of the film.

Water vapor permeability (WVP). The water vapor transfer rate [g s^{-1} m^{-2}] through the films was determined gravimetrically with the ASTM E 96-95 (1995). The films were placed in a chamber maintained at room temperature and 64.5% RH for at least 2 days before each test. A fan located inside the chamber was used to move the internal air to ensure uniform conditions at all test locations. During this period, the samples reached equilibrium conditions. After that, the film specimens were mounted onto acrylic cups containing distilled water (100% RH). The weights of the assembled cups were recorded every hour for 6 h. Linear regression was used to fit the data, weight versus time, and to calculate the slope of the resulting straight line in grams per second. Six specimens were tested for each film type. The permeability values are reported as water permeability coefficients [WVPs; g m Pa^{-1} s^{-1} m^{-2}] as follows:

$$\text{WVP} = \Delta W y[A\Delta t(p_2 - p_1)]^{-1} \tag{5}$$

where ΔW is the weight of water absorbed in the cup (g), Δt is the time for weight change (thus, $\Delta W/\Delta t$ is the slope calculated from a plot of cup weight vs time), A is the area of the exposed film (m^2), y is

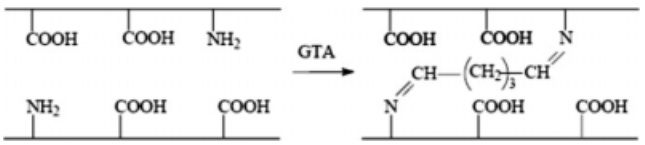

Figure 1 Chemical reaction of SC with GTA.

the film thickness (m), and $p_2 - p_1$ is the vapor pressure difference across the film (Pa), which was calculated on the basis of the chamber temperature and the RHs inside and outside of the cup.

RESULTS AND DISCUSSION

The crosslinking of the caseinate samples with GTA involved the reaction of the free amino groups of the basic amino acids (lysine) that formed the polypeptide chains in casein with the aldehyde groups of GTA to form a Schiff base.[2,20] A visible color change accompanied the reaction between GTA and caseinate because of the formation of an aldimine (Schiff) linkage (—C=N—; see Fig. 1).[15,22,23]

The shrinkage of the films increased and the coloration generated by the Schiff base (yellowish) got more intense as the GTA content increased.[15,22,23] Those were qualitative indications of the increasing crosslinkage of the films.[1] Figure 2 shows the coloration changes in the films as the GTA content increased.

According to literature, GTA tends to homopolymerize in the form of linear chains of various lengths in aqueous solutions.[2,36,37] Thus, and because in our study an aqueous solution of GTA (50 vol %) was used to carry out the crosslinking reaction, we expected that some of the bonds formed between the protein chains by aldehyde crosslinking were relatively long. Moreover, according to Jayakrishnan and Jameela,[2] GTA crosslinking can span through relatively long distances between protein molecules. Thus, bonding with GTA should have two opposite effects: on one hand, the crosslinking of the protein chains should lead to network formation, and on the other hand, linkages formed by homopolymerized GTA might cause an increase in the interspacing between the protein chains. The last effect should act to prevent the formation of both direct rigid covalent bonds and physical bonds between protein chains, such as disulfide bridges and H bonds, that stabilize the control films (non-GTA reacted).

Physical characterization

TSM

TSM of the SC–GTA films was determined to evaluate their integrity in an aqueous environment. Measuring the soluble mass by the dry method allowed us to study the effect of heating the samples (drying was performed at 105°C). Because proteins are susceptible to heat-induced crosslinking,[4,38–40] the heating of the specimens to determine their m_0 values induced the crosslinking of the protein films and also lead to the underestimation of the actual film TSM.[1]

Table I shows the TSM determinations with both the dry and wet methods. The reduction of the soluble mass indicated an increase in the percentage of crosslinking. The TSM values obtained by the dry method were smaller for all of the films, as was observed by other authors,[34] because of the additional crosslinking generated during the drying process at 105°C, which was also evidenced by an increase in the coloration of the films. Moreover, the cohesion of the protein network was better after the drying at 105°C, presumably because of the creation of a larger number of weak and strong bonds (e.g., disulfide bridges) during the thermal treatment, as has been previously reported in the literature.[36,41] The difference between the two methods decreased as the percentage of GTA increased. This was in agreement with the lower amount of amine groups available for the formation of sulfide groups during heating.

The soluble fraction in the SC–GTA films could be principally attributed to the loss of low-molar-mass compounds, such as low-molar-mass polypeptide chains that could not be crosslinked by GTA, and to the exudation of GLY out of the film.[34,42] Orliac et al.[36,41] confirmed this hypothesis, reporting that almost all of the GLY present in thermomolded films produced from sunflower protein isolate was extracted by immersion of the films in water for 24 h.

Figure 2 Optical photographs of the SC films prepared with different GTA concentrations. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Total Soluble Matter of the GTA Crosslinked Films

GTA (%)	Dry method (%)	Wet method (%)
0	25.03 ± 0.75	100 ± 0
3	18.46 ± 1.12	29.98 ± 5.3
5	15.41 ± 1.38	25.39 ± 12.67
10	13.12 ± 0.78	16.66 ± 0.37

SC containing no GTA and heated at 105°C for 24 h [heated sodium caseinate (HSC)] appeared to be a promising material because it was obtained without the addition of chemical crosslinkers and showed reduced water solubility compared with the control SC films (TSM = 25 and 100, respectively).

In considering this behavior, we must indicate that caseins were not susceptible to thermal denaturation at the temperature used for the heating treatment because their restricted formation of disulfide bonds due to the low content of cystine and cystine resulted in increased stability.[43] The thermal stability of casein was also explained by the restraints (high contents of proline and hydroxyprolyne) against the formation of a folded tertiary structure.[44] The exceptional stability of casein makes it possible to boil, sterilize, and concentrate milk without coagulation.[44,45] Because of these advantages, some of the properties of these films were investigated and are reported and discussed in following sections.

Opacity

Transparency is, to a great extent, relevant to the film functionality because of its great impact on the appearance of coating or packaging.[46] Opacity values for the GTA-crosslinked films and HSC films are shown in Table II. Low relative opacity values indicate a transparent film, whereas an increase in these values implies film matrices with greater opacity. Also, opacity is influenced by the film thickness, being higher as the thickness increases. The experimental opacities of the caseinate films without GTA crosslinking ranged between 127 and 158 Au nm; they showed better performance than the wheat gluten films investigated by Gontard[47] (250.4 Au nm), which were measured by the same method. Heated caseinate films showed somewhat higher opacity values (188–224 Au nm) than the control caseinate films but lower than those of GTA-crosslinked films. Regarding the GTA–SC films, the opacity remained almost constant with GTA content when the film thickness was considered, as is shown in the fourth column of Table II.

TGA

Thermal degradation patterns of the HSC films and films prepared with different GTA concentrations are shown in Figure 3. The results are presented from 100°C to eliminate any discrepancy from the initial humidity content in the samples; thus, only the effects introduced by changes in the chemical composition or by thermal treatment on the degradation behavior were considered. According to the literature,[48,49] the residual mass of plasticized protein films does not change much in the range 500–900°C, and thus, TGA was ended at 500°C. Furthermore, the patterns of thermal degradation of the proteins were different from those of comparable synthetic polymers, such as polyamides,[49] with respect to both the kinetics of the degradation process and the residual char. The degradation of the proteins showed final chars much higher than the zero value, which was predicted from the constituent hydrocarbon and amide groups.[50] The skeletal amide and hydrocarbon side chains of many proteins tend to interact with their own kind because of the great difference in their polarities, and it has been suggested that the strong segregation of the hydrogen-bonded amide–amide interactions in proteins promotes char formation.[49]

Additionally, the initial degradation temperature was determined at the onset of the degradative processes, as shown in Figure 3. The TGA curves corresponding to the GTA-crosslinked films showed a shift in the initial degradation temperature of approximately 14°C with respect to that of the SC films and a slightly lower char at 500°C (26.2 and 28.8% for the SC–GTA and SC films, respectively), although these last values were almost independent of aldehyde concentration. The increase in the initial degradation temperature for this kind of materials has been ascribed to the replacement of hydrogen bonds between protein chains by more stable

TABLE II
Opacity of the Crosslinked SC Films

Film	Opacity (AU nm)	Thickness (mm)	Opacity/thickness (AU nm/mm)
SC	142.56 ± 15.55	0.096 ± 0.025	1505.04 ± 405.50
HSC	206.03 ± 18.25	0.108 ± 0.024	1735.46 ± 150.77
GTA3	315.15 ± 54.22	0.123 ± 0.022	2949.26± 389.16
GTA5	340.06 ± 55.84	0.139 ± 0.035	3459.80 ± 494.56
GTA10	348.17 ± 27.92	0.135 ± 0.015	2574.30 ± 362.43

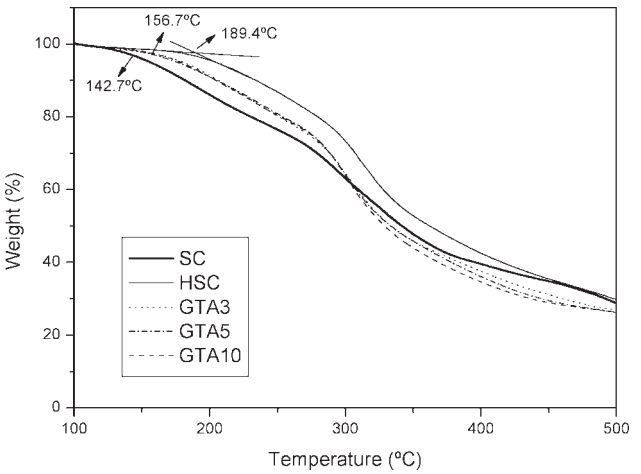

Figure 3 Thermal degradation patterns of the films prepared with different GTA concentrations.

covalent crosslink bonds.[15,21] On the other hand, the HSC sample showed the highest initial degradation temperature (46.7°C higher than the corresponding SC control film) and the lowest degradation rate below 300°C. The char of HSC at 500°C was approximately the same as the SC sample (29.9 and 28.8%, respectively) and slightly higher than the corresponding values of the GTA-crosslinked films (26.2%). Thus, although chemical and heat-induced crosslinking increased the initial degradation temperature of the films, the HSC specimen showed the highest overall thermal resistance.

Mechanical properties

Tensile tests

The mechanical properties of the films are reported in Table III. Increases in E and σ_b were observed as the GTA content increased from 3 to 10 wt %. At the same time, a decrease in ε_b was observed. These results were correlated with the increase in covalent bonds formed between the polypeptide chains as the GTA content increased.

On the other hand, the results obtained for the control SC films were outside this trend. They presented values of modulus and σ_b higher than those of the films made with GTA concentrations lower than 5 wt %. This was explained by the fact that the decrease in the amount of strong interactions (hydrogen bonds) present in the SC[51] were not compensated by the formation of covalent bonds at low degrees of GTA crosslinking. The 10 wt % GTA sample already showed an improvement in the tensile modulus and strength with respect to the uncrosslinked sample, but ε_b was greatly reduced.

The HSC films showed better mechanical properties (modulus and tensile strength) than those samples prepared with up to 5 wt % GTA, and their tensile modulus was similar to the 10 wt % GTA sample. Moreover, these films exhibited the highest ε_b (ε_b = 89.9%) compared to the other formulations, even higher than the corresponding to the SC control films (ε_b = 63.2%). Additionally, the reported values of ultimate strength (Table III) were comparable to the σ_b values obtained by others authors for soy[8,12,13] and gluten proteins[42] subjected to heating treatments and also to the σ_b and elongation reported for heated vicilin-rich proteins.[52] We concluded that the thermal treatment was more effective than GTA crosslinking in enhancing the film tensile properties.

Moisture sorption

To investigate the effect of GTA on the SC films, a water vapor sorption study was conducted at 75% RH and 25°C. Experimental moisture absorption data were fitted with the Peleg empirical equation [Eq. (3)] over the entire time period and also to Eq. (4), which corresponds to the Fickian model, to calculate D. The fitting parameters are reported in Table IV. As shown from the R^2 parameters, the curves resulting from the Peleg fitting process showed good agreement with the experimental data.

The K_1 parameter, inversely associated with the initial moisture absorption rate, revealed that the highest absorption velocity corresponded to the SC films dried at 105°C for 24 h (K_1 = 0.673). The magnitude of D (5.257×10^{-13} m^2/s) confirmed the trend. The control SC films showed intermediate K_1 and D values, and finally, the GTA-crosslinked films presented higher K_1 and lower D values, which were not related to the presence of voids or cracks inside the films, as revealed by SEM examination of the film thicknesses [Fig. 4(c,d)]. There was no clear trend for these two parameters as the GTA content was increased, with K_1 varying between 2.6 and 3.9 and D varying between 2 and 2.316×10^{-13} m^2/s.

The equilibrium M_t values at 75% RH for all of the films are also shown in Table IV. These values, inversely proportional to the K_2 parameter, clearly indicated that the HSC film was the least hydrophilic sample, with the smallest final moisture absorption capacity (21.3%). The crosslinking and

TABLE III
Mechanical Properties of the SC Films with Different GTA Contents

Film	E (MPa)	σ_b (MPa)	ε_b (%)
SC	251.0 ± 20.3	6.3 ± 0.2	63.2 ± 6.0
HSC	412.2 ± 54.3	6.9 ± 0.7	89.9 ± 25.1
GTA3	132.1 ± 20.0	3.3 ± 0.4	58.5 ± 3.5
GTA5	249.8 ± 19.7	5.8 ± 0.2	37.0 ± 7.1
GTA10	444.7 ± 44.6	9.1 ± 0.9	11.3 ± 3.0

TABLE IV
Fitting Parameters of the Peleg Equation and *D* Values for the SC Films with Different GTA Contents

Film	K_1 (min/wt %)	K_2 ($\%^{-1}$)	R^2	D (m^2/s) $\times 10^{13}$	Thickness (mm)	EMC (%)
SC	1.055 ± 0.198	0.0404 ± 0.002	0.992	2.831 ± 0.734	0.089 ± 0.016	25.084± 0.871
HSC	0.673 ± 0.108	0.0445 ± 0.002	0.992	5.257 ± 1.113	0.095 ± 0.014	21.348± 1.042
GTA3	2.646 ± 0.324	0.0362 ± 0.002	0.982	2.045 ± 0.201	0.122 ± 0.025	26.373± 1.184
GTA5	3.939 ± 0.116	0.0350 ± 0.001	0.987	2.002 ± 0.503	0.126 ± 0.040	26.679± 0.485
GTA10	2.675 ± 0.488	0.0400 ± 0.003	0.985	2.316 ± 0.718	0.104 ± 0.004	25.569± 1.054

RH = 75%, temperature = 25°C.

strong bonding generated between the protein chains during the thermal treatment led to a reduced water affinity and final water content and a higher effective diffusivity (there were fewer polar groups in the HSC film than in the SC film that could interact and retain water molecules). This was also observed by Kim et al.[12] for soy protein films after heating treatment.

With regard to the films containing GTA, their EMC (equilibrium moisture content) values were slightly higher than those of the reference sample. A plausible explanation for this unexpected behavior was found again in the reduction of the amount of hydrogen bridges resulting from the introduction of GTA into the protein chains. The crosslinker interfered with the formation of these relatively strong protein interactions, so more polar groups were exposed to water vapor (as compared with the uncrosslinked sample). This increased the water affinity of the films. The interactions between polypeptide chains were so strong that the addition of a hydrophilic plasticizer was required to overcome brittleness and impart some flexibility to the resulting films.[20]

WVP

Table V shows the WVP of the neat SC and GTA-crosslinked samples. Measurements of the HSC films

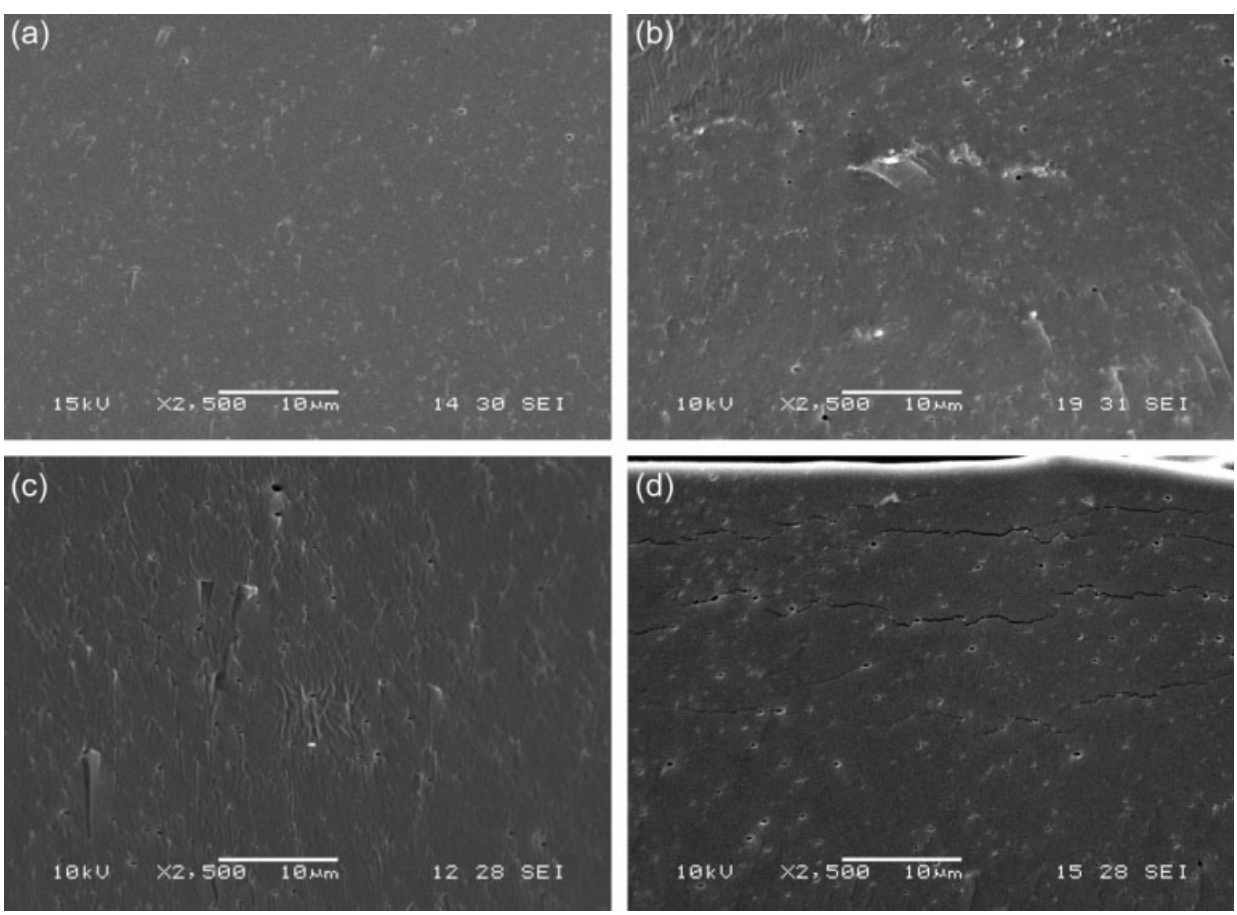

Figure 4 SEM micrographs of selected SC films prepared with different GTA concentrations (cryogenically fractured surface): (a) SC, (b) HSC, (c) GTA3, and (d) GTA5.

TABLE V
WVP of the GTA-Crosslinked SC Films

Film	Thickness (mm)	WVP (m g Pa^{-1} s^{-1} m^{-2}) $\times 10^{10}$
SC	0.089 ± 0.016	7.08 ± 1.19
HSC	0.096 ± 0.012	0.62 ± 0.03
GTA3	0.107 ± 0.016	11.35 ± 2.53
GTA5	0.117 ± 0.009	12.92 ± 1.19
GTA10	0.153 ± 0.059	16.78 ± 3.52

are also included. WVP increased as the crosslinking density increased, opposite to what was initially expected.

Permeability is influenced by the hydrophilic or hydrophobic character of a material, by the presence of voids or cracks, and by the esteric hindrance and tortuosity in the structure.[53] We suggest that the increase in permeability with increased concentration of GTA was due to the molecular structure of the long links formed between the GTA-crosslinked protein chains, which separated the protein molecules through relatively long distances and decreased intermolecular forces (covalent and physical bonds: disulfide bridges and H bridges) along the protein chains. This could have led to an increase in the free volume, facilitating the diffusion of water molecules through the films and, thus, leading to a higher permeability in these samples with respect to those without GTA. However, the most probable reason for this behavior was, unfortunately, the larger number of voids and cracks that existed in the crosslinked samples, as revealed by SEM examination of the film thicknesses [see Fig. 4(c,d)] and surfaces; these were probably formed as a result of the shrinkage experienced by the specimens during film formation. These defects significantly facilitated water vapor transport through the film. Ghanbarzadeh et al.[54] also found an unexpected effect on the WVP values of zein films without plasticizer; these values were greater than those of plasticized films, which was attributed to a poor association of chains and the formation of many voids and cavities in the films.

The HSC films showed WVP values (0.62 m g Pa^{-1} s^{-1} m^{-2}) that were one order of magnitude lower than the neat SC films (7.08 m g Pa^{-1} s^{-1} m^{-2}). This important reduction in WVP was also observed by Kim et al.[12] for soy protein films. This change was attributed to the formation of a large number of strong bonds during the thermal treatment, which made these films the least hydrophilic in this study, as was indicated in the previous section. SEM images of the HSC film thickness [Fig. 4(b)] showed fewer pores than those found in the films prepared with GTA and no evidence of fissures or cracks (as noticed in the GTA-crosslinked films).

Summarizing the behavior of the SC films stored in humid environments, we concluded that the HSC film was a valid option for food packaging because it exhibited a lower hydrophilicity, solubility in water, and WVP than the other studied samples without the toxicity problems associated with the use of GTA. We are continuing to study the optimization of the heat treatment.

CONCLUSIONS

The chemical crosslinking of SC with GTA was studied as a function of GTA content. Physical evidence (TSM and coloration changes due to Schiff base formation) and TGA measurements indicated that the level of crosslinking and thermal stability increased as the GTA content increased. The tensile modulus and strength of the SC films increased with crosslinking level, although only samples prepared with more than 5% GTA showed enhanced behavior compared with the SC control samples (which were not crosslinked). However, the high hydrophilicity, which is the main problem of protein-derived films, was not improved by chemical GTA crosslinking, as revealed by WVP and water vapor absorption measurements. These results are consistent with the formation of a crosslinked but flexible network, which increased chain-segment mobility and, thus, contributed positively to water molecule diffusion. Moreover, voids induced by film contraction during film formation enhanced the WVP.

On the other hand, the behavior of an HSC film was also studied. This specimen showed enhanced physical properties (relatively low moisture sorption and WVP) and tensile properties and improved thermal stability compared to the SC control and GTA–SC samples without the inherent complications associated with the use of GTA (which is toxic at high concentrations). Therefore, crosslinking induced by thermal treatment was revealed to be a valid alternative for improving the properties of SC films.

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