Tensile Properties, Barrier Properties, and Biodegradation in Soil of Compression—Molded Gelatin-Dialdehyde Starch Films

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ABSTRACT: Glycerol (Gly)-plasticized gelatin (Ge) films crosslinked with dialdehyde starch (DAS) as environmentally friendly crosslinking agent were successfully produced by compression molding, demonstrating the capacity of gelatin of being transformed into films by using thermoplastic processes. The effect of DAS content on the color, light transmission, total soluble matter (TSM), water uptake (WU), water vapor permeability (WVP), oxygen permeability (OP) as well as biodegradability during soil burial was investigated. The addition of up to 10 wt % DAS (Ge-10DAS) generated transparent films, with reduced TMS, WU, WVP, and OP values but higher extensivity than the uncrosslinked counterpart. Further incorporating DAS into plasticized-gelatin matrix conducted to phase separation with detrimental effect of transparency and tensile properties. DAS-containing films degraded at slow rate than the uncrosslinked counterpart, suggesting that biotic attack during soil burial is restricted by covalent crosslinking points induced by DAS. Ge-10DAS films lost about 28% of their initial mass within the first 8 days of exposure to degrading medium; therefore, the material can be classified as rapidly degradable. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2166–2178, 2009

Key words: gelatin; compression-molding; biodegradation; barrier; mechanical properties

INTRODUCTION

The development of biodegradable films based on biopolymers such as proteins as an alternative to synthetic polymers has increased in recent years as a result of the increasing demand for environmentally friendly products.[1] Gelatin (Ge) is an animal protein derived from the partial hydrolysis of native collagens, which are the most abundant structural proteins found in the connective tissues of mammals and fishes.[2] It can be recovered from the residues in the processing of animal meats[1] or from the pharmaceutical industry.[3]

Considering their transparency, biodegradability, and barrier properties against gases and aroma at low relative humidity[4] gelatin based films may find applications in the packaging sector. These films are usually obtained by casting (wet process) but due to the high cost of preparation from solution,[5] most investigations related to protein-based films are concentrated on the thermomechanical processing techniques.[5,6] The thermal processing ("dry process") relies on the thermoplastic behavior that some proteins and polysaccharides display at low moisture levels in compression molding and extrusion.[5–11] Some studies have documented thermomechanical processing on various proteins such as zein,[12] whey,[9,13] wheat,[5,8] egg-white,[11] and plasticized gelatin.[10] However, and at least at the best of our knowledge, little information is available about thermoprocessing of chemically crosslinked gelatin films.

The main idea of this work was to produce chemically crosslinked gelatin films by compression molding with improved properties as close as possible to those of synthetic polymers commonly used in packaging. Such films present good elongation at break (about 150% or bigger) and rather low tensile strength (about 30–50 MPa).[14] However, gelatin films show two major drawbacks:

i. Poor mechanical properties. Mechanical strength and elongation at break are somewhat lower when compared with similar packages based on synthetic polymers, whereas, fragility is higher.
ii. Low moisture resistance. Their hydrophilic character limits their barrier capacity for

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humidity which is usually accompanied by their water solubility.

Consequently, to achieve our objective, plasticized and crosslinked gelatin films were produced by compression molding technique. The poor mechanical properties were controlled by adding a suitable plasticizer such as glycerol, a common plasticizer for proteinaceous materials.[2] On the other hand, polymeric dialdehyde starch (DAS) was used as crosslinking agent. This reagent is produced by controlled periodate oxidative cleavage of the C(2)–C(3) bond of the anhydroglucose units of native starch.[15] Because dialdehydes such as gutaraldehyde and glyoxal, have been successfully used as crosslinking agent,[4] potential industrial applications of DAS are also based on its crosslinking ability and its low toxicity as reported by Wilson.[16] The efficiency of DAS as crosslinking agent of solution-casting films based on gelatin,[17] soy protein isolate,[18,19] egg-white protein[20] and whey protein,[21] has been documented. Quite recently enzymatic hydrolysates of waste collagen were successfully crosslinked with DAS to produce hydrogels with potential applications in packaging, cosmetic and pharmaceutical products.[22] However, the use of DAS in the production of crosslinked protein films produced by compression molding or extrusion has been poorly investigated. Indeed, the work of Spence et al. [12] on DAS-zein films obtained by compression molding is one of the few studies that have been reported in the last years. Particularly, little information is available about DAS-*cross*-linked gelatin films obtained by thermomechanical processing.

Therefore, this article is focused on the evaluation of the mechanical properties, moisture resistance, barrier properties, and biodegradability during soil burial of glycerol-plasticized gelatin films crosslinked with dialdehyde starch produced by compression molding.

EXPERIMENTAL

Materials

Bovine hide gelatin (Ge) type B was kindly supplied by Rousselot (Argentina), Bloom 150, isoionic point (Ip) 5.3. Dialdehyde starch (DAS), (81.8% starch oxidation; Sigma-Aldrich, St. Louis, MO) was used as received. Glycerol analytical grade (Gly, 98%) was purchased from DEM Chemicals (Mar del Plata, Argentina).

Methods

Preparation of plasticized Ge-DAS films by compression molding

Each film was prepared with 5 g of dry gelatin powder. DAS-containing films were obtained for a variety of DAS percentages (on dry basis of gelatin) varying form 0 to 30 wt %. Before mixing with gelatin, glycerol (30 wt % dry basis of gelatin) was added to DAS to produce a workable blend. This was performed by using a kitchen mixer (M.B.Z., San Justo, Buenos Aires, Argentina) at low speed (i.e. 150 rpm) during 24 h and at room temperature. The blended DAS/Gly was then mixed with gelatin powder at room temperature during 30 min using a kitchen mixer at 150 rpm. Each Ge/DAS/Gly blend was transferred into a stainless steel mould (30 cm $\times$ 30 cm) which was placed in a hydraulic press (E.M.S., Buenos Aires, Argentina). The mold was preheated at 120°C and this temperature was kept during compression step. Pressure of 50 kg/cm^2 was applied during 10 min to obtain homogeneous films. Subsequently, the pressure was released and the plates with the sample between them were cooled down to room temperature. Films were recovered and equilibrated at relative humidity (%RH) depending on the test. The obtained films were named Ge-XDAS where *X* corresponds to the weight percentage of crosslinking agent in the sample. Glycerol-plasticized gelatin films in the absence of DAS (control films) were produced under similar conditions.

Thermogravimetric analysis

Dynamic thermal degradation experiments were carried out using a thermogravimetric analyzer TGA-DTGA Shimadzu 50 (Shimadzu Corp., Japan). Temperature was raised from 25 to 900°C, at a heating rate of 10°C/min and under nitrogen (20 mL/min) to avoid thermo-oxidative degradation.

Opacity

Film opacity was determined according to the method described by Irissin-Mangata et al.[23] on rectangular strips placed in a UV-Visible spectrophotometer test cell directly. 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 recorded curve which was obtained through an integration procedure, and it was expressed as absorbance units per thickness unit (mm).

Water uptake at 75% relative humidity

Water uptake (WU) tests were performed gravimetrically. Samples were dried until constant weight in an oven to remove moisture before testing and this weight was taken as the initial one (m_0). After this, samples were conditioned at 25°C in tightly sealed desiccators containing saturated NaCl solution to ensure 75%RH, according to the procedure described in ASTM E104-95. Samples were removed at specific

intervals (t) and the increment in weight of the specimens (m_t) was measured. The moisture content (w_t) as a function of time t was obtained from the total and partial (water) mass balance over the sample as a function of time:

$$w_t = \frac{m_0 * w_0 + (m_t - m_0) * 100\%}{m_t} \quad (1)$$

where w_t is the moisture content as a function of time (%), m_t is the weight of the sample after exposition, m_0 is the initial weight, and w_0 is the initial moisture content of the samples.

Experimental data were fitted with Peleg's empirical equation[23]:

$$w_t = w_0 + \frac{t}{k_1 + k_2 * t} \quad (2)$$

where w_0 is the initial moisture content and w_t is the moisture content as a function of time, t. The constants k_1 and k_2 are fitting parameters; k_1 (min/(wt % water/wt % solids)) is related to mass transfer, the lower k_1, the higher the initial water absorption rate; k_2 (1/wt % water/wt %solid) is associated to the maximum water absorption capacity, therefore, the lower k_2, the higher the absorption capacity.[25]

Total soluble matter

Total soluble matter (TSM) was expressed as the percentage of film dry matter solubilized after 24 h immersion in distilled water. Rhim et al.[18] proposed two methods, the "wet" and the "dry" method to evaluate the effect of heat-drying the films at 105°C before testing. Both tests were carried out in distilled water (30 mL) and in the presence of sodium azide (0.02%) to prevent the microbial growth. For the dry method, three specimens of each film were weighed (m_h) (±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 (±0.0001 g) to determine their initial dry matter (m_0). The initial moisture content (MC) was calculated from the eq. (3)

$$\text{MC } (\%) = 100 * \frac{m_h - m_0}{m_0} \quad (3)$$

Afterward, the samples were immersed in 30 mL of distilled water stored in 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 following:

$$\text{TSM } (\%) = 100 * \frac{m_0 - m_f}{m_0} \quad (4)$$

For the wet method the heat-drying step was avoided. Three 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 the ones obtained from MC measurements for the same film.

Tensile properties

Tensile tests were carried out on an INSTRON 4467 Universal Test Machine (Darmstadt, Germany) equipped with a 0.5 KN cell, at a crosshead speed of 3 mm/min and at room temperature following the procedure described in ASTM D638-94b. Dog-bone-shaped specimens (30 mm × 4.5 mm × 0.2 mm) were conditioned at 75%HR before testing. The film samples were clamped into the metal grips for tensile testing and stretched at overhead crosshead speed of 3 mm/min. The tensile strength (TS) and the percentage of elongation at break (%E) were calculated as the average of four replicates.

Barrier properties

Water vapor permeability. Water vapor permeability (WVP $\text{Kg} \cdot \text{m}/\text{Pa} \cdot \text{s} \cdot \text{m}^2$) was calculated as:

$$WVP = \frac{(\text{WVT} * e)}{\Delta P} \quad (5)$$

where WVT ($\text{kg}/\text{s} \cdot \text{m}^2$) is the vapor transmission rate through a mean film thickness $e(m)$ and ΔP is the actual difference in partial water vapor pressure between the two sides of film specimens (Pa). The WVP of the films was determined according to ASTM E96-95 desiccant method (ASTM, 1995). The films were fixed on the top of test cells containing a desiccant (silica gel). Test cells were placed in an environmental chamber with controlled temperature and relative humidity 25°C and 65 ± 2%HR. The mass change (±0.0001 g) of the cups versus time was recorded at specific intervals (t) and then plotted. Linear regression was used to calculate the slope of a fitted straight line, which represented the WVT, as follows:

$$\text{WVT} = \frac{\Delta m}{t * A} \quad (6)$$

where Δm is the mass change of the cell test (kg), t is the time (s) and A is the test area (m^2). Permeability was calculated according to:

$$\text{WVP } (\text{Kg} \cdot \text{m}/\text{m}^2 \cdot \text{s} \cdot \text{Pa}) = \frac{\text{WVT}}{S * (\text{RH}_1 - \text{RH}_2)} * e \quad (7)$$

where e is the film thickness (m), S is the saturation pressure (Pa) at the test temperature, RH_1 is the

relative humidity in the test, and RH_2 is the relative humidity inside the cell test. There were at least five repetitions per experiment.

Oxygen permeability. The oxygen permeability (OP) was measured with an oxygen permeation analyzer from Systech Instruments, model 8500 (Metrotec S.A, Spain) on circular samples of each film (14 cm diameter). All experiments were carried out at a pressure of 2.5 atm. Films were clamped in a diffusion chamber at 25°C. Pure oxygen (99.9%) was introduced into the upper half of the sample chamber, while nitrogen was injected into the lower half of the chamber where an oxygen sensor was placed. The gas volumetric flow rate per unit area of the membrane OT (cm^3 $(O_2)/day \cdot m^2$) was continuously monitored until a steady state was reached (OT_∞). Thickness of films was measured at 25°C using a 0–25 mm manual micrometer (±0.01 mm). The films were conditioned at 50%HR before testing. The permeability coefficient (OP) is determined by eq. (8)

$$\mathrm{OP}\ (\mathrm{cm}^3(\mathrm{O}_2)\cdot \mathrm{mm/Pa{\cdot}day{\cdot}m}^2) = \frac{\mathrm{OT}_\infty * e}{\Delta P} = \frac{Q}{\Delta P} \quad (8)$$

where ΔP is the partial pressure gradient across the polymer film (Pa). For a constant ΔP throughout all experiments, OP is proportional to Q ($cm^3(O_2) \cdot mm/day \cdot m^2$).

Scanning electronic microscopy (SEM)

Fracture surfaces were observed on a microscope Phillips 505 (Eindhoven, The Netherlands) with an operating voltage of 15 KV. All specimens were sputter-coated with gold.

Optical microscopy

Transmission optical microscopy (TOM) was performed on the surface of the Ge-XDAS films employing a Leica DMLB (Wetzlar, Germany) microscope, with crossed polarizer, provided with a video camera Leica DC 100.

Indoor soil degradation

The experiment was carried out in a series of plastic boxes (80 cm × 15 cm × 10 cm) containing characterized soil (Pinocha type) and the natural microflora present was used.[26,27] Samples were cut in rectangular shape (2 cm × 3 cm) and then dried until constant weight in an oven to remove the moisture before testing and this weight was taken as the initial one (m_0). Samples were put into containers (cup shape) made of stainless-steel mesh. These holders permit access of microorganisms and moisture, and can be easily pulled out for the retrieval of the degraded samples. The cups were buried at a depth of 8 cm from the surface. The relative humidity was kept around 40% and the temperature was 20 ± 2°C.

Water sorption during soil burial was determined gravimetrically. Samples were removed from the soil at specific intervals (t), carefully cleaned with distilled water, superficially dried with a tissue paper and weighed (m_h). Water uptake (%WS) was quantified by the following equation:

$$\%\mathrm{WS} = \frac{m_h - m_t}{m_0} * 100 \quad (9)$$

where m_0 is the initial mass, m_t is the remaining mass due to biodegradation at time = t and m_h is the humid mass. The values reported are the average of two measurements.

After water sorption determination, samples were dried under vacuum and at room temperature to constant weight. The specimens were weighed on an analytical balance to determine the average weight loss (%WL):

$$\%\mathrm{WL} = \frac{m_t - m_0}{m_0} * 100 \quad (10)$$

where m_0 is the initial mass, m_t is the final mass (after dried) at a predetermined time t. All results are the average of two replicates.

RESULTS AND DISCUSSION

Films preparation

Thermal compression-molding is a simple and conventional method to produce gelatin-based films. However, in this study, the main drawback was to obtain workable Ge-DAS blends. Preliminary work indicated the necessity of blending DAS with glycerol before adding gelatin to rend DAS thermoplastic. It was observed that at least 30%Gly was indispensable to obtain Ge-DAS mixtures with enough flowability to be used in thermopressing process.

The molding temperatures were chosen from exploratory studies. TGA analysis was used to obtain information on the thermal stability of glycerol-plasticized gelatin mixtures and pure DAS. Figure 1 shows the normalized mass loss evolution with temperature of both materials. For pure DAS (Fig. 1), the first step which extended up to 150°C and accounted for about 4% of mass loss was attributed to the retained residual water entrapped in the polysaccharide structure of DAS.[15] The second and third stages (T_{max} = 227°C and 289°C, respectively) were associated with the decomposition of the whole polymeric chain, which can be visualized as a mixture of modified and original starch.[28] Therefore, the most significant weight loss for pure DAS was observed above 200°C. On the other side,

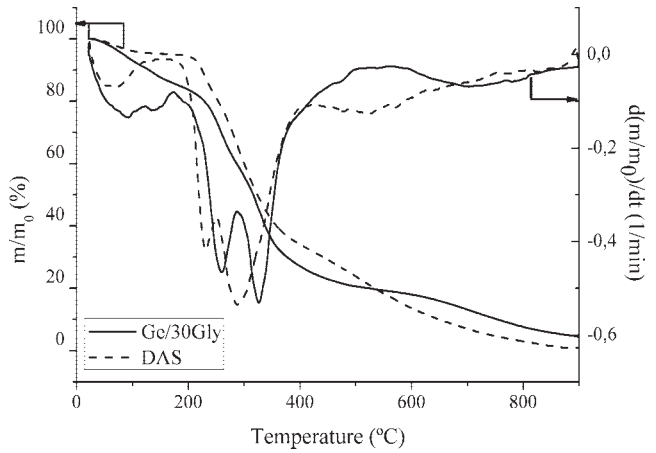

Figure 1 Normalized TG curves obtained in nitrogen atmosphere and at 10°C/min for the glycerol-plasticized gelatin mixture and pure DAS.

plasticized-gelatin control mixture degraded in a gradual process with four stages in the TG curve. The first step, observed up to 170°C, which accounted for about 13% of mass loss was attributed to the volatilization of absorbed and bounded water in gelatin.[29,30] The second stage began at 232°C and gave a maximum degradation rate of 259°C, was attributed to glycerol volatilization (boiling point 289°C). The third and main step, assigned to protein chain thermal decomposition, began at a temperature of 292°C and gave the maximum degradation rate at 333°C.[30,31] The higher temperature step ($T >$ 600°C) was attributed to the decomposition of more thermally stable structures due to crosslinking reactions produced during heating.[29–31] Consequently, the most important mass loss of glycerol-plasticized gelatin mixture was also observed above 200°C. Based on these findings, temperatures in the range of 120 to 140°C were used in compression-molding processing.

Firstly, the mold was preheated at a selected temperature then the temperature was raised in 10°C increments to the desired molding temperature. Results indicated that 120°C was the optimal temperature to preheat the mold and also to produce homogeneous films during the pressing step. A molding temperature higher than 130°C produced dark brown films for all DAS levels, evidencing some extent of thermal degradation of gelatin or DAS. Similar results were observed for soy protein-starch mixtures processed by injection-molding.[31] The compression-molding time was 10 min because lower times were not enough to produce a film and longer times leaded to protein and DAS thermal degradation. The selected conditions allowed one to produce flexible films, indicating that the molding conditions used induced flowability to Ge/DAS/Gly mixtures.

Visual aspect and opacity

All heat-pressed films were stiffer but still workable. Visually, DAS-containing films turned from light yellow to dark brown as DAS content increased from 0 to 30 wt %. Because DAS is a dialdehyde, its crosslinking mechanism is analogous to that of short chain aldehydes such as formaldehyde and glyoxal.[4] Therefore, the increased color intensity may be attributed to formation of conjugated Schiff's bases which are intermediate products of the Maillard reaction.[18] Similar results have been reported for egg-white protein,[16] molded corn zein plastics[17] and whey protein isolate.[21] The rise in color with DAS content in the films could be also related to some extent of thermal degradation of low molecular weight fragments of DAS during compression molding.[15]

The opacity of the films (Table I) presented an increasing tendency when DAS concentration increased. For DAS contents lower than 10 wt %, the films can be considered transparent, according to the values reported by Mali et al.[33] for transparent films. Increasing DAS contents increased considerably the opacity, as it was visually observed. Therefore, it was assumed that the reduction in transparency with increasing DAS level could be a consequence of some degree of phase separation. It is well reported that protein-polysaccharide systems are characterized by limited compatibility between their components.[34,35] In fact, proteins and polysaccharides are normally limitedly compatible. Consequently, phase separation in Ge-DAS mixtures is highly probable.

TABLE I
Opacity, Elongation at Break (*E*%), Tensile Strength (TS) and Total Soluble Matter (TSM) of Ge-DAS Films Plasticized with 30 wt % Glycerol

Sample	Opacity (uA.nm/mm)	*E* (%)	TS (MPa)	TSM1[a] (%)	TSM2[b] (%)
Ge	216.3 ± 15.8	96.9 ± 11.9	3.9 ± 1.0	100.0 ± 0.0	100.0 ± 0.0
Ge-5DAS	290.9 ± 5.1	158.2 ± 23.6	2.7 ± 0.4	34.0 ± 1.5	37.7 ± 1.8
Ge-10DAS	391.7 ± 9.0	111.8 ± 12.1	3.5 ± 0.3	31.2 ± 1.4	35.5 ± 0.9
Ge-30DAS	659.1 ± 20.0	55.3 ± 12.9	1.1 ± 0.5	30.8 ± 1.3	29.7 ± 1.7

[a] TSM1: dry method.
[b] TSM2: wet method.

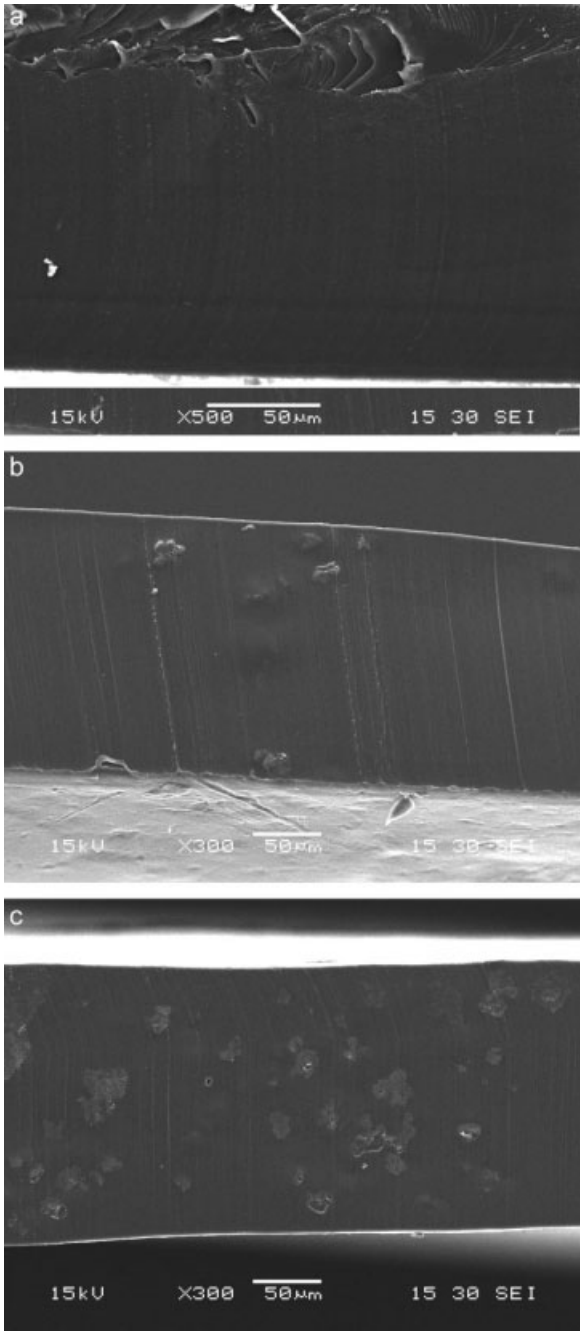

Figure 2 Scanning electron micrographs of the fracture surfaces of glycerol plasticized films. (a) Control Ge; (b) Ge-10DAS, and (c) Ge-30DAS.

To verify this, Ge-DAS and control films were cryo-fractured and the edge of the fracture surfaces was observed by scanning electronic microscopy (SEM). Control gelatin films exhibited a relatively uniform and homogeneous surface [Fig. 2(a)]. A relatively low content of particles into the matrix were observed when DAS content was lower or equal to 10 wt % [Fig. 2(b)]. Phase separation, seems far less likely to have occurred at lower DAS concentration (i.e. 5 wt % DAS), but the size of the dispersed particles was small enough to produce transparent films. For higher amounts of DAS in the mixture (30 wt %), the inhomogeneity of the blend was evident. Larger particles and/or aggregates were observed, even at low magnification [Fig. 2(c)]. A possibility is that aggregation was promoted by segregative interactions ("thermodynamic incompatibility") between protein and polysaccharide[35] or by an ineffective mixing. Furthermore, optical microscopy performed on iodine stained samples showed that gelatin rich domains constitute the continuous phase of the blend and the dispersed phase is mainly constituted by DAS (Fig. 3).

Tensile properties

Table I exhibits tensile strength (TS) and elongation (%E) of the obtained films as a function of DAS content. The addition of DAS resulted in films with lower TS values than control film. TS decreased by $\sim$ 30% with the addition of 5 wt % DAS. This apparently anomalous behavior could be assigned to the fact that the polymeric nature of DAS did not introduce severe restrictions within gelatin matrix as usually occurs with short chain dialdehydes such as formaldehyde or glutaraldehyde.[4,20,36] For DAS content as low as 5 wt %, the crosslinking effect is counterbalanced by the plasticization exerted by the hydroxylated polymeric backbone of DAS, including its ability to attract water to the plasticized protein system. Water is a very effective plasticizer from protein films[14]; therefore, the more hydrophilic the film the higher plasticization is expected. Plasticization reduced the hydrogen interactions between protein chains, thereby increasing the extensibility in Ge-5DAS films by about 60% (Table I).

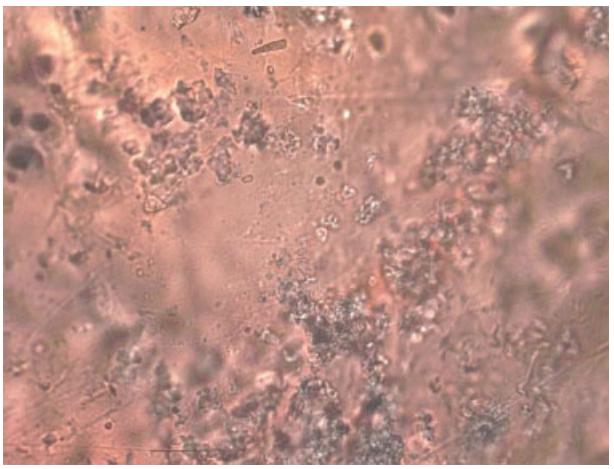

Figure 3 Optical microscopy of Ge-10DAS film stained with iodine solution [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

For Ge-10DAS, TS increased up to 3.5 MPa, which was slightly lower than that of control film. This result is in accordance with a more reticulated material achieved with 10 wt % DAS. Nevertheless, the polymeric nature of DAS did not restrict the extensivity of protein matrix because E value was $\sim$ 15% higher than the value obtained for control film (Table I). Similar results were reported for egg-white-DAS films.[20] It was evident that size and distribution of the particles dispersed into the matrix had a detrimental effect on the mechanical properties of the films, as concluded from the decreased values of TS and elongation at break percent observed for Ge-30DAS films. As particle size decreased (i.e., DAS $<$ 30 wt %) better properties were achieved (Table I).

Clearly, based on opacity and the target mechanical properties of Ge-DAS films, that is, high elongation at break with small drop in TS, Ge-5DAS and Ge-10DAS appear as the best candidates. However, moisture resistance as well as barrier properties must be evaluated to determine the suitability of DAS-containing films as potential packaging materials.

Total soluble matter

Total soluble matter (TSM) of Ge-DAS films was determined to evaluate their integrity in aqueous environment. TSM was calculated by two methods described in the experimental part, according to Rhim et al.[18] The idea of measuring the soluble matter by the "dry" and "wet" methods was to evaluate the effect of drying the samples at 105°C before testing. The results obtained by applying both methods are summarized in Table I. Control films disintegrated almost completely after 24 h soaking. It seems that compression molding at 120°C did not induce significant crosslinking into glycerol-plasticized gelatin matrix. Similar results were reported by Sothornvit et al.[9] for compression-molded whey protein films plasticized with glycerol and without any crosslinking agent. On the other hand, DAS-containing films exhibited substantially lower water solubility than control one, whatever the test method applied (Table I). The wet method resulted in greater TSM values than the dry one, but values became coincident (within the error of the experiment) for DAS contents al least equal to 30 wt %. This finding are in accordance to results previously reported for soy protein isolate-DAS films,[18] egg white-DAS films produced by casting[20] and zein protein-DAS obtained by compression molding.[12] It was also previously shown that an increase in crosslinking in collagenous material provoked a decrease in the water binding capacity, which would have led to a decrease in solubility of the modified films.[1,4] The soluble fraction in Ge-DAS 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 DAS and to the exudation of glycerol out of the film. Consequently, the reduction of TMS values with DAS gave indirect evidence of its crosslinking efficiency.[18,20]

Water uptake at 75% relative humidity

Because one of the major drawbacks in the use of gelatin formulations in technical applications is their water absorption tendency, any improvement in water resistance is highly important. In general, aldehyde-induced crosslinking decreases water uptake by proteins because amino-side chain groups are not available to bind water by hydrogen bonding. Also, due to the development of crosslinks in the protein structure, hydrophilic sites along protein chains become less exposed and, therefore, less accessible to water molecules.[4] On the other hand, thermomechanical processing creates highly crosslinked protein networks.[7] Therefore, the combination of thermomechanical processing combined with aldehyde-crosslinking is expected to enhance the water resistance of gelatin-based films.

Moisture uptake curves of Ge-DAS films are presented in Figure 4. For all samples, moisture absorption was more rapid at the initial stages of storage at 75%RH and then the absorption rate hardly changed, indicating that samples became equilibrated with the storage RH. Analyzing the absorption curves of DAS-modified films a slight reduction in water uptake at equilibrium (WUeq) was verified (Fig. 4, Table II).

Experimental curve data were fitted to eq. (6)[24] and the constants k_1 and k_2, which were derived from the linear fit, are shown in Table II. The coefficients of determination were high (r^2 about 0.99);

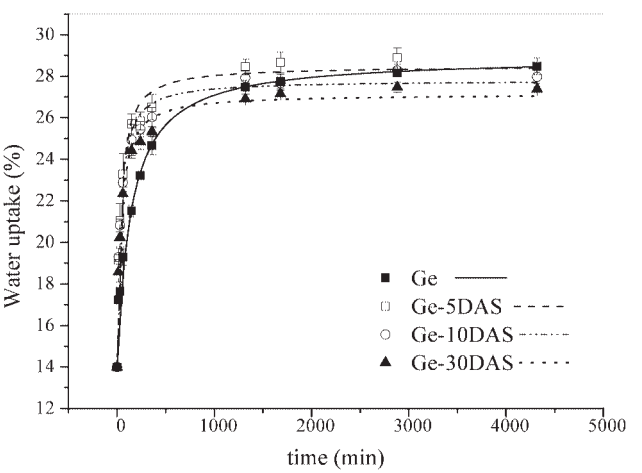

Figure 4 Water uptake of Ge-DAS films plasticized with 30 wt % glycerol as a function of the storage times at 75% relative humidity. Symbols: experimental data, lines: fitted with Peleg's empirical equation.

TABLE II
Peleg Equation Parameters (k_1, k_2), Water Uptake at the Equilibrium (WUeq), Water Vapor Permeability (WVP), and Oxygen Permeability (Q) of Ge-DAS Films Plasticized with 30 wt % Glycerol

Sample	k_1 (min/%)	k_2*10^2 ($\%^{-1}$)	r^2	WUeq (%)	WVP ($kg \cdot m/m^2 \cdot s \cdot Pa$)	Q ($cm^3 \cdot mm/m^2 \cdot day$)
Ge	9.4 ± 1.0	6.7 ± 0.2	0.99	28.7 ± 1.0	$2.5\ 10^{-13} \pm 1.3 \cdot 10^{-14}$	13.3 ± 3.0
Ge-5DAS	2.3 ± 0.2	6.9 ± 0.1	0.99	28.4 ± 0.2	$2.4\ 10^{-13} \pm 3.6 \cdot 10^{-14}$	12.9 ± 2.2
Ge-10DAS	2.3 ± 0.2	7.2 ± 0.1	0.99	27.9 ± 0.4	$1.7\ 10^{-13} \pm 5.4 \cdot 10^{-15}$	12.1 ± 2.6
Ge-30DAS	2.6 ± 0.2	7.6 ± 0.1	0.99	27.0 ± 0.3	$1.6\ 10^{-13} \pm 9.4 \cdot 10^{-15}$	13.3 ± 0.6

this indicates a good agreement between predicted (dashed lines) and experimental (symbols) data, as can be shown in Figure 4. It was found that films with DAS had lower k_1 but higher k_2 values than control film, indicating that these materials absorbed faster but less moisture during storage at 75%RH.[24] Indeed, the curve of control film tended to merge with that of Ge-5DAS at long exposition times. An explanation for this behavior may be proposed based on the structure of Ge-DAS films. For low DAS content there were probably additional hydrophilic groups within the matrix (i.e., hydroxyl groups from glycerol and polymeric DAS backbone) which favored the entrance of water in the early stages of the process. These results support the assumption that the water absorbed by Ge-5DAS is the main thing responsible of the unexpected increment in the elongation at break of such a film (Table I). At longer times, the hydrophilic groups became "saturated" and the decreased maximum moisture absorption with DAS possibly accounted for by the formation of a denser protein network. Additionally, the size and inhomogeneous distribution of the dispersed phase within the matrix might have, at least partially, a contribution to the decrease of water sensitivity of the film, as concluded from the slightly higher k_1 and k_2 values obtained for Ge-30DAS compared with those observed for lower DAS contents (Table II).

Barrier properties

Experimental WVP values measured at 75% RH are reported in Table II. Because water vapor permeability of hydrophilic protein films is affected by film thickness,[22] in this study the films thickness variability was minimized and thicknesses were kept between 200 and 215 μm. Average permeability value for the control gelatin film was about $2.47 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$, which was comparable with that obtained for cold-fish gelatin films producing by casting ($2.58 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$),[36] and lower than that reported for extruded and compressed molded plasticized gelatin films ($24.7 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$, thickness: 197 microns).[10] WVP values of Ge-DAS films decreased when compared with control (Table II). This result is in accordance to TMS values and with the lower water binding capacity of DAS-containing films. The significant decrease in WVP observed for Ge-30DAS could be attributed to the more tortuous path offered by the particles dispersed in the matrix to the water molecules. Ge-DAS films showed WVP values lower to that reported for glycerol plasticized-whey protein isolate crosslinked with DAS ($5.43 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$),[21] glycerol-plasticized alginate films ($6.7 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$)[37] soy protein isolate films crosslinked with DAS ($15.0 \cdot 10^{-13}$–$16.5 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$),[18] compression molded glycerol-plasticized whey protein isolate ($38.0 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$),[9] heat-cured soy protein isolate films ($1.7.0 \cdot 10^{-13}$ $kg \cdot m/m^2 \cdot s \cdot Pa$).[38] However, the values obtained in this work were higher than those reported for enzymatic and chemically crosslinked plasticized-gelatin films ($3.3 \cdot 10^{-14}$ $kg \cdot m/m^2 \cdot s \cdot Pa$ and $4.3 \cdot 10^{-14}$ $kg \cdot m/m^2 \cdot s \cdot Pa$, respectively).[4] With regard to synthetic polymers, Ge-DAS films had higher WVP values compared with those of high-density polyethylene (HPDE) ($2.4 \cdot 10^{-16}$ $kg \cdot m/m^2 \cdot s \cdot Pa$), polyvinyl chloride (PVC) (0.7–$2.4 \cdot 10^{-16}$ $kg \cdot m/m^2 \cdot s \cdot Pa$)[39] and low density polyethylene (LDPE) ($3.6–9.7 \cdot 10^{-16}$ $kg \cdot m/m^2 \cdot s \cdot Pa$).[39,40]

On the other hand, gas permeability of food packaging materials is of great importance for food preservation.[41] The oxygen permeation process was clearly faster in the case of control films and slightly decreased with DAS level. It was observed that the steady-state of oxygen flux was reached more slowly with increasing amounts of DAS. The Q ($cm^3(O_2) \cdot mm/day \cdot m^2$) values in the steady-state are summarized in Table II. The small decrease in average Q could be associated to the higher crosslinking density induced by DAS. This can lead to a lower free volume among protein chains[41] which slightly increases the resistance of the films to oxygen transmission. For the sake of comparison, Q of polyethylene terphtalate (PET), low density polyethylene (LDPE) and cellulose acetate (CA) films tested with the same device in similar conditions were obtained. Q results were 2.5 $cm^3(O_2) \cdot mm/day \cdot m^2$ for PET, 160 $cm^3(O_2) \cdot mm/day \cdot m^2$ for LDPE and 44 $cm^3(O_2) \cdot mm/day \cdot m^2$ for CA.[42] The Q values of Ge-DAS films were lower than those obtained for LDPE and CA, thereby Ge-DAS films could be used in packaging formulation with reduced oxygen permeation requirements.

From tensile properties and opacity results we have chosen Ge-5DAS and Ge-10DAS films as

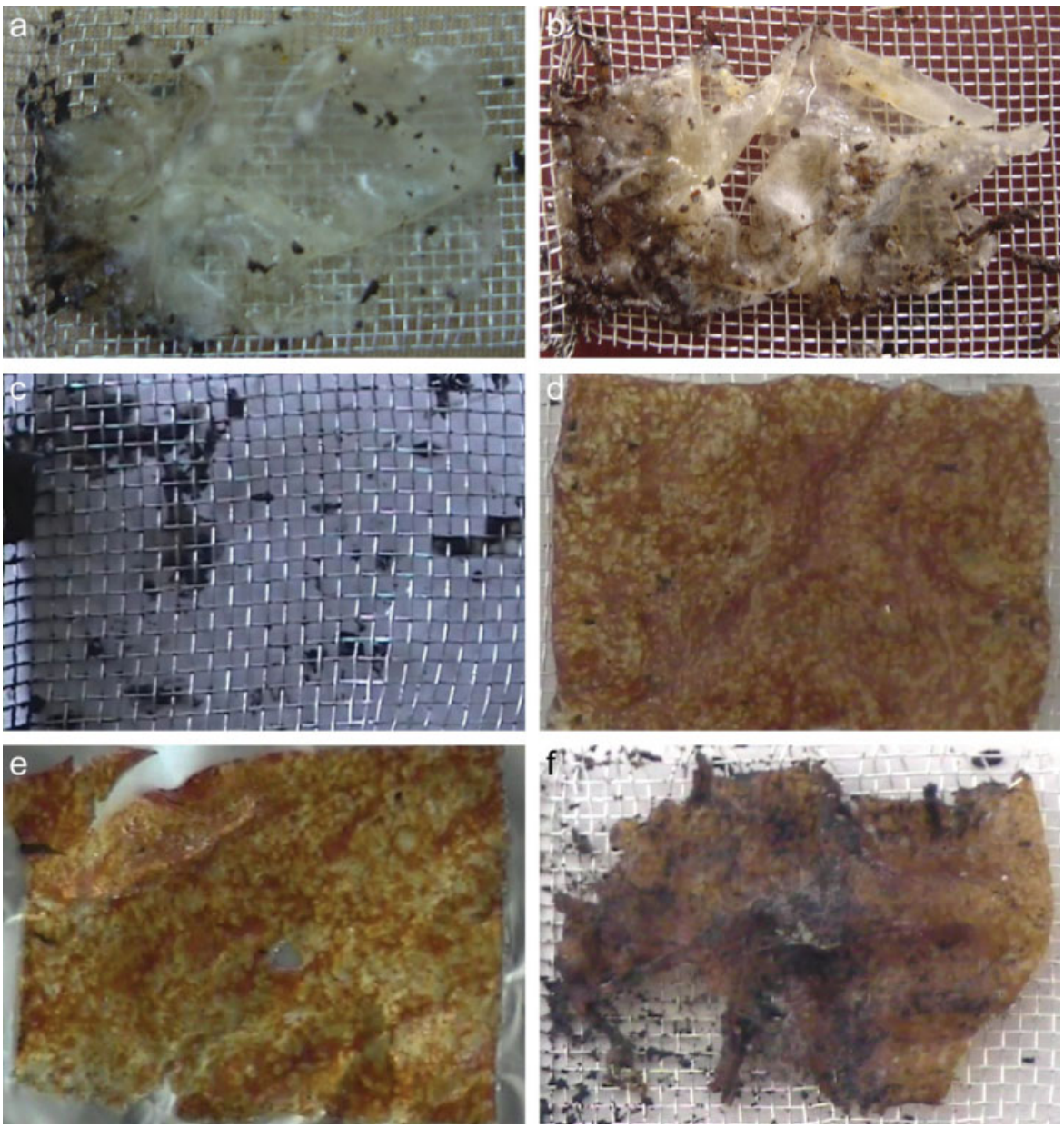

Figure 5 Macroscopic deterioration of control (a–c) and Ge-10DAS (d–f) after different exposition time to the biodegrading medium. (a,d) $t = 5$ days; (b,e) $t = 7$ days (c,f) $t = 14$ days. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

potential candidates for packaging applications. The best results of moisture resistance, TSM, WVP and oxygen permeation were found for Ge-10DAS, therefore, this formulation can be considered as the most suitable for packaging purposes, and therefore, it was selected for evaluating its biodegradability during indoor soil burial experiments.

Indoor biodegradation in soil

To evaluate the susceptibility of Ge-DAS films to aerobic biodegradation, control and Ge-10DAS films were exposed to natural microbial consortium during indoor soil experiments. Mixed undefined microbial population present in the soil microflora was used as degrading medium as it can be considered a realistic approach to the biodegradation process in natural environments. Soil microflora constituted a mixed microbial population (including bacteria, actinomycetes, fungi and protozoa, among others) may act synergistically during degradation and reproduce under naturally occurring conditions. The experiment was carried out up to 15 days. After this time, samples could not be tested any more due to their macroscopic deterioration. It is important to point out that many potential errors may exist in

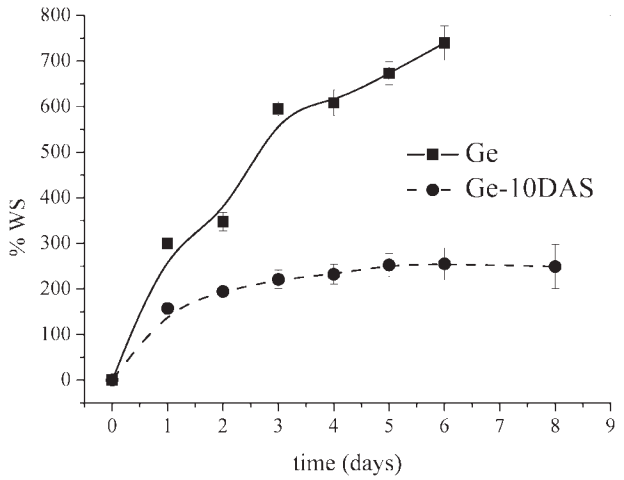

Figure 6 Water uptake as a function of exposure time in soil burial for (■) Ge control film (●) Ge-10DAS film.

gravimetrically measuring weight losses in soil, mainly at the later stages of the experiment. Soil, dirt and occluded biomass are difficult to remove without damaging the samples and may account for errors in determining the residual mass.[43] However, weight loss data were used herein for analyzing qualitatively the effect of DAS-modification on the microbial attack of the produced films.

It is well known that gelatin-based materials are susceptible to the action of enzymes (proteases) which are present in a variety of microorganisms.[1,44] Gelatin is also sensitive to the attack of filamentous fungi under humid conditions.[45,46] Because crosslinking reactions do not involve peptide bonds, crosslinked-gelatin films could be degraded in soil by the same kind of microorganisms possessing proteases. However, some studies reported in the literature highlighted the effect of chemical modifications on the rate and extend of gelatin biodegradation. Goswami and Maiti[47] found that blending gelatin with phenolic resins enhanced its stability against microbial attack from soil. This result was mainly attributed to the biocide activity of phenol from phenolic resin. The biodegradation of chemically modified gelatin films was also analyzed in different environments such as river and lake water and in soil under laboratory conditions.[1,44] It was shown that the rate and extent of biodegradation depended on the type of crosslinking reagent and on the crosslinking density. Similar results were reported for the biodegradation of glutaraldehyde-crosslinked gelatin in soil.[3] On the other hand, according to the studies reported by Spence et al.[12] the biodegrading behavior of DAS depends on the degree of oxidation of the native starch. Authors demonstrated that as the oxidation degree of starch increased from 1 to 90%, the mineralization was reduced from about 60 to 37% at 180th day.

The macroscopic appearance of control (Ge) and Ge-10DAS films (both plasticized with 30 wt %Gly) before and after exposure to degrading medium is shown in Figure 5. During the first 5 days, the specimens suffered swelling due to the absorption of water from the medium [Fig. 5(a,d)]. After this period, the samples lost their initial shape and macroscopic deterioration was evident [Fig. 5(b,e)]. After 14 days of soil burial crosslinked films were difficult to recover because of the high extent of biodegradation [Fig. 5(c,f)]. Therefore, Ge-DAS films can be classified as rapidly degradable materials.[1]

Figure 6 shows the average water absorption calculated from eq. (9) of control and Ge-10DAS films as a function of the exposition time during soil burial. Both materials absorbed enough water to ensure the water bio-availability which favors the microbial attack. The lower water absorption of DAS-containing films was attributed to the lower availability of polar groups due to crosslinking induced by DAS[1,4,13,18,20,44] and agreed well with soluble matter and moisture absorption results.

The average weight loss versus exposition time calculated from eq. (10) is represented in Figure 7. Both materials deteriorated rapidly, being more dramatic for control film. The water intake promotes the entrance of microorganisms present in soil due to the higher water availability within the material, and then biodegradation begins. Results revealed that during the first 12 h, both materials lost about the same amount of mass, due to the leaching of low molecular weight compounds, such as oligomers and plasticizer.[26,27] From this point until the end of the experiment, both materials showed a sustained weight loss (Fig. 7). Nevertheless, control films degraded faster than DAS-containing films. Indeed, after 8 days the weight loss of the control film increased rapidly until reaching a remaining

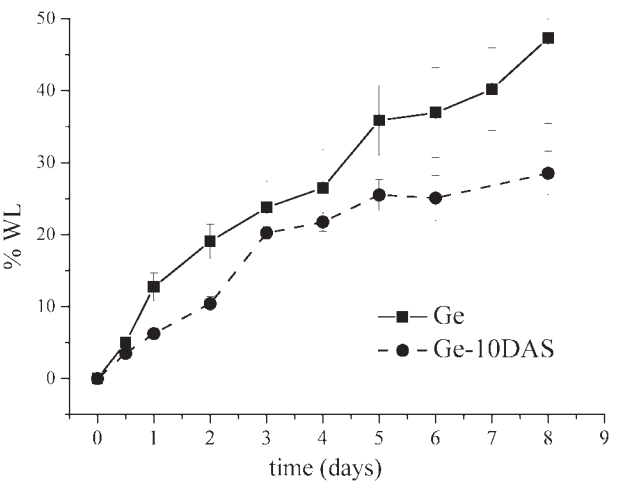

Figure 7 Weight loss as a function of degradation time during soil burial: (■) Ge control film; (●) Ge-10DAS film.

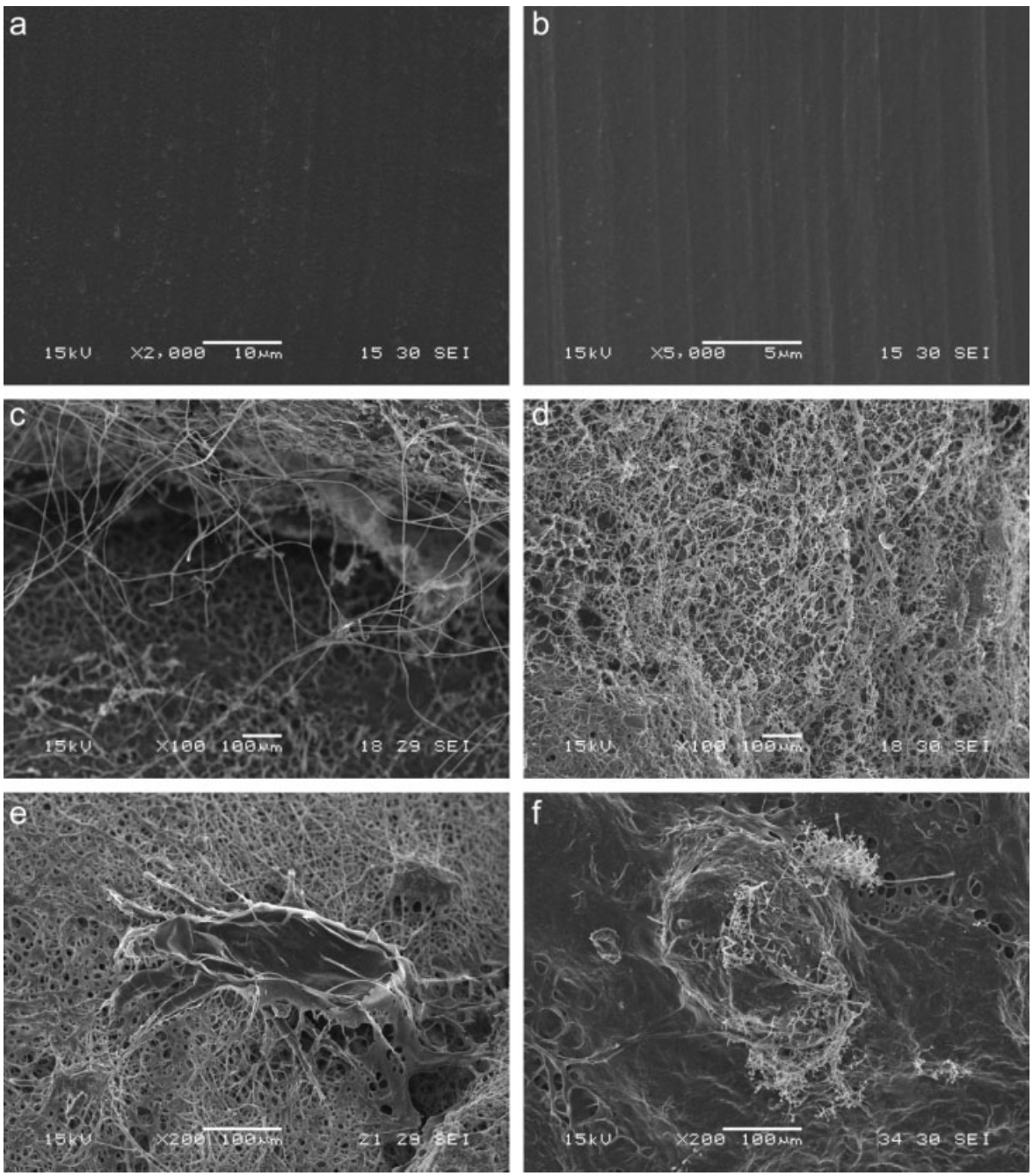

Figure 8 SEM micrographs of the surfaces of the specimens after soil burial from which residues were not washed: (a, b) Ge and Ge-10DAS before biodegradation; (c,d) Ge and Ge-10DAS after 5 days of soil burial; (e,f) Ge and Ge-10DAS after 7 days of soil burial, respectively.

mass of about 48% of the initial one whereas Ge-10DAS film lost about 28% of the initial mass. This difference could be attributed to the presence of covalent crosslinkages in Ge-DAS films, which restrict the entrance of water and microorganisms with proteolytic and oxidative enzymes present in soil, and then the biodegradation rate decreases.[1,44] It seems that covalent crosslinkages are the main responsible of diminishing the biodegradability of Ge-10DAS films in soil.

To visualize the morphological changes due to burial in soil, specimens of Ge-10DAS and control films were collected at different degradation times and SEM micrographs were taken (Fig. 8). At time zero, both samples exhibited a relatively smooth surface with some irregularities attributed to the processing [Fig. 8(a,b)]. SEM micrographs of control and Ge-10DAS films recovered after 5 days after dirt cleaning showed that control and Ge-10DAS films were colonized by filamentous microorganisms such

as fungi and actinomycetes [Fig. 8(c,d)]. After 7 days of exposure to degrading medium control film showed also the presence of larvae and acarus [Fig. 8(e)]. On the contrary, Ge-10DAS suffered the attack by filamentous microorganisms, but no larvae were detected [Fig. 8(f)], suggesting that chemical crosslinking restricted the bio-assimilation of DAS-containing films by insects. These results indicate that Ge-DAS films are less susceptible to microbial attack during soil burial. It is important to remark that fungi can growth under different relative humidity,[45,46] which agrees well with their presence in both specimens. The low water absorption capacity of Ge-DAS films due to crosslinking could explain the absence of larva in the degraded samples.

CONCLUSIONS

This study has demonstrated that glycerol-plasticized gelatin films crosslinked with DAS can be successfully obtained by compression molding with potential as packaging materials. The best properties were found for formulations with DAS content lower or equal to 10 wt %. Higher amounts of DAS conducted to phase separation with detrimental effects on transparency and tensile properties. Crosslinking with DAS up to 10 wt % decreased the solubility and enhanced moisture resistance at 75%RH, giving indirect evidence of the crosslinking efficiency of DAS. A further consequence of a more rigid polymer network was the enhancement in water vapor and oxygen barrier properties of Ge-DAS films. However, the polymeric nature of DAS did not introduce severe restrictions into gelatin matrix as it was confirmed by the higher extensivity of Ge-10DAS film compared with the uncrosslinked counterpart.

Experimental data clearly indicated that biodegradation of Ge-10DAS films during soil burial was quite limited when comparing with control gelatin films. This behavior was attributed to the crosslinking induced by DAS, which reduces the swelling of gelatin network, restricting the penetration of soil microorganism and their enzymes.

Despite the addition of DAS appears to have a negative influence on the biodegradation extent of the produced films, the designed Ge-10DAS films renders them attractive candidates to be applied in biodegradable packaging formulations with reduced oxygen permeation requirements.

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