Cross-linked soy protein as material for biodegradable films: Synthesis, characterization and biodegradation

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ABSTRACT

The modification of soy protein isolate (SPI) with different amounts of a naturally occurring cross-linking agent (genipin, Gen) and glycerol used as plasticizer was carried out in this work. The films yielded were cast from heated and alkaline aqueous solution of SPI, glycerol and Gen and then dried in an oven. Total soluble matter, water vapor permeability and mechanical properties were improved by adding small amounts of Gen. These properties were not significantly affected ($P \geqslant 0.05$) by additions exceeding 2.5% (w/w of SPI). The opacity and cross-linking degree were linearly increased with the addition of Gen, whereas the swelling ratios in water were decreased. All the films were submitted to degradation under indoor soil burial conditions and the weight loss of the films was measured at different times. This study revealed that the film biodegradation time can be controlled or modified from at least 14 to 33 days. The tests performed showed the potential of Gen to improve the SPI film properties, in which the possibility of employing such new films as biodegradable food packaging was raised.

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1. Introduction

In recent years the interest in the use of biodegradable materials for coating, packaging (Singh et al., 2007; Sorrentino et al., 2007), agriculture and medicine (drug encapsulation for controlled release) (Park et al., 2005; Sosnik, 2007) has notably increased.

The development of biodegradable polymer coatings from natural materials (renewable sources) reduces the need of synthesizing petroleum-based polymers, eliminating the negative effects produced on the environment. In particular, food packaging represents a potential new market for such materials. The main purpose of the food coatings centers on the preservation and protection of processed food and raw material, during processing, manufacturing, handling and storage (Singh et al., 2007). At the present, natural materials are not usually use for coating developments due to their poor mechanical properties. Despite this, soy protein-based edible films have received considerable attention due to their excellent film-forming abilities, low cost, and barrier properties against oxygen, lipid and aroma permeation under low to intermediate humidity conditions (Gennadios et al., 1993; Kim et al., 2002; Monedero et al., 2010). This type of proteins produces smoother, clearer and more flexible films compared to those from other sources (Guilbert et al., 1995). However, due to its inherent hydrophilic nature, this material presents two major disadvantages: fragility in the wet state and poor properties of moisture barrier. These effects can be minimized using physical, chemical or enzymatic treatments including: blending with hydrophobic additives such as neutral lipids, fatty acids or waxes (Rhim et al., 1999; Rhim, 2004); changing drying conditions (Denavi et al., 2009); enzymatic treatment with horseradish peroxidase (Stuchell and Krochta, 1994); heat curing (Gennadios et al., 1996); UV irradiation (Gennadios et al., 1998); and cross-linking. The ε-amino group of lysine was considered the primary reactive site between proteins and cross-linkers (Nayudamma et al., 1961). The cross-linkers most extensively used for proteins are aldehydic compounds such as glutaraldehyde (Park et al., 2000; Bigi et al., 2001; Marquié, 2001), formaldehyde (Marquié, 2001) and glyoxal (Vaz, 2005), and epoxy (Patil et al., 2000) and phenolic compounds (Strauss and Gibson, 2004). However, the cytotoxicity of these compounds restricts their use for food covering.

Recently, a new natural cross-linker, genipin (Gen), about 10,000 times less cytotoxic than glutaraldehyde (Song and Zhang, 2009; Yuan et al., 2007), has been used. The colony-forming assay also showed that the proliferative capacity of cells after being exposed to Gen was approximately 5000 times greater than that of cells exposed to glutaraldehyde (Song and Zhang, 2009; Sung et al., 1999). This novel cross-linker is obtained from the enzymatic hydrolysis of *Genipa* with β-glucosidase (Fujikawa et al., 1987). *Genipa* is extracted from the fruit of a type of jasmine called *Gardenia Jasminoides Ellis.* The dark blue pigments obtained by

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spontaneous reaction of Gen with amino acids or proteins have been applied to the development of food dyes (Touyama et al., 1994).

Gen has been employed to stabilize chitosan-forming gels (Muzzarelli, 2009; Yao et al., 2004) for controlled release of drugs improving their mechanical properties and thermal stability, and decreasing their swelling in water. The gelation modification of aqueous soy protein isolate (SPI) dispersion in the presence of Gen and the use of the resulting SPI gel were investigated for the controlled release of bovine serum albumin as a model protein drug in simulated gastric and intestinal media (Song and Zhang, 2009). The possibility of stabilizing gelatin films by cross-linking with Gen has been studied with different concentration solutions. The cross-linking of gelatin reduces significantly the swelling in physiological solution and enhances the thermal stability of the samples (Bigi et al., 2002).

The cross-linking mechanism of the reaction between Gen and amine-containing polysaccharides (such as chitosan) (Muzzarelli, 2009) or proteins (such as SPI) is pH dependent. Under acidic and neutral conditions, a nucleophilic attack occurs by the amino groups on the olefinic carbon atom, followed by the opening of the dihydropyran ring and attacked by the secondary amino group on the newly formed aldehyde group.

The aims of this study were to evaluate the physical and chemical effects produced in films by the modification of a material obtained from natural sources, such as SPI and different amounts of Gen as cross-linker. Hence, the opacity, cross-linked degree, total soluble matter, moisture content, surface and mechanical properties, swelling in water and water vapor permeability of the films yielded were investigated to assess the potential of Gen for improving SPI film properties. In addition, the degradation study under indoor soil burial conditions was performed to evaluate the possible use of such new films in biodegradable food packaging and attempt to contribute to replacing the conventional petroleum-derived plastics, adding more value to this abundant agricultural resource.

2. Materials and methods

2.1. Materials

SPI Supro 500E with 90% protein on a fat-free, dry weight basis was kindly provided by The Solae Company, Argentina. Glycerol (Gly), calcium chloride and sodium hydroxide were acquired from Cicarelli (Santa Fe, Argentina). Gen was provided by Wako (Japan); and 2-iminothiolane, dithiothreitol and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Film preparation

SPI films were prepared by casting dissolving SPI powder under constant stirring in distilled water (8.33 g/100 mL water). Gly, a plasticizer, was added at 50% (w/w) of SPI and the pH was adjusted to pH 9 with 0.5 M NaOH. The dispersions were magnetically stirred in beakers for 30 min at room temperature. 0.4% w/v Gen solution was obtained by dissolving 0.1 g of Gen powder in 25 mL of water. Different volumes of this solution were then added to SPI dispersions to obtain the final SPI-Gly-Gen mixture with 0%; 0.1%; 1%; 2.5%; 5%; 7.5% and 10% (w/w of SPI) of Gen. The dispersions were heated in a water bath with continuous magnetic stirring at 70 °C for 2 h, and kept at room temperature for 15 min. All dispersions were poured into plastic plates (polypropylene) and dried in an oven with air circulation at 65 °C for 12 h. After that, films were removed and conditioned for 48 h at 25 °C and 50% relative humidity (RH) before measuring their properties.

2.3. Opacity measurements

The area under the absorbance curve from 400 to 800 nm was taken as the opacity (O) of the films (Cho et al., 2004). Film specimens were cut into rectangles (0.8×2 cm^2) and placed in the spectrophotometer cell. A spectrum of each film was recorded using a spectrophotometer (Shimadzu model UV-2101PC). It was expressed as absorbance unit (AU) × nm/unit thickness (μm) and determined in duplicate.

2.4. Film thickness

The thickness was determined as the average of 10 measurements for each sample with a hand-held micrometer (Schwyz model ESP1-0001PLA, Schwyz, Swiss). The average film thickness was used for assessing opacity, water vapor permeability and mechanical properties.

2.5. Determination of cross-linking degree (CL%)

The extent of cross-linking of SPI films was measured in duplicate by a spectrophotometric method (Tyllianakis et al., 1993). The amount of ε-amino groups before (m_0) and after (m_1) cross-linking reactions was determined. Equal masses of different films were washed with distilled water and reacted with 4 mL of 2-iminothiolane (Taut's reagent) 40 mM solution. After several washes, the films were then reacted with 4 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent) 0.5 mM solution to quantify the sulfhydryl groups generated from the reaction of amino groups with Taut's reagent. The quantification was carried out through the extinction coefficient of 2-nitro-5-thiobenzoic acid (TNB) (14150 M^{-1}cm^{-1}), produced in the solution by the cleavage of DTNB, at 412 nm. The base line was taken following the procedure described with no Taut's solution. The percentage of cross-linking was then calculated by Eq. (1).

$$CL\% = [(m_0 - m_1)/m_0] \times 100 \tag{1}$$

2.6. Moisture content (MC)

Moisture content (MC) was determined according to a method described (Rhim et al., 1998). Film samples were weighted (W_0) into glass dishes, dried in an air-circulating oven at 105 °C for 24 h and weighted again (W_i). Moisture content for each film was determined in quadruplicate by Eq. (2).

$$MC = [(W_0 - W_i)/W_0] \times 100 \tag{2}$$

2.7. Total soluble matter (TSM)

Total soluble matter (TSM) was determined according to a method described (Rhim et al., 1998). Dry and soluble matters were measured on different films from each cast film trying to avoid cross-linking by heating the samples prior to incubation in water. Four weighted samples of each film were directly immersed in beakers (50 mL) containing 30 mL of distilled water. Traces of sodium azide were also added to inhibit microbial growth. The beakers were covered and stored in an environmental chamber at 25 °C for 24 h with occasional stirring. The insoluble matter was then separated and dried in an oven at 105 °C for 24 h (W_f) to determine the solubilized dry matter by Eq. (3). Initial dry matter values needed for TSM calculations were those obtained from MC measurements for a film with the same mass (W_i). The reason for using different film specimens to measure initial and soluble dry film matter is that the proteins are susceptible to heat-induced cross-linking and this effect would decrease the

TSM of the films (Gennadios et al., 1996). The measurements for each type of film were obtained in quadruplicate.

$$\text{TSM} = [(W_i - W_f)/W_i] \times 100 \qquad (3)$$

2.8. Swelling ratio (S)

The swelling characteristics of the different specimens were studied in triplicate at different times. All the films were weighted (W_d) and immersed in 30 mL of deionized water for specific time intervals at room temperature. The samples were removed from the swelling medium, wiped with a piece of paper to absorb excess water on the surfaces and reweighted (W_s). The swelling ratios of the samples were calculated from Eq. (4).

$$S = [(W_s - W_d)/W_d] \times 100 \qquad (4)$$

2.9. Mechanical properties

Stress–strain curves for each specimen (25×100 mm) were recorded; tensile strength (TS) and elongation at break (E) were determined according to ASTM D882-02, 2002 An Instron Universal Testing Machine (model 3342, Norwood, MA, USA) equipped with a 500 N capacity cell was used with an initial grip separation of 100 mm and crosshead speed of 0.5 mm/s. Four replicates were tested for each sample.

2.10. Water vapor permeability (WVP)

The water vapor permeability (WVP) was determined in duplicate for all films according to the desiccant method described in the ASTM standard method (ASTM E96M-10, 2010). Each film of 38.5 cm^2 (without physical defects such as cracks, bubbles or pinholes) was sealed onto an aluminum permeation cup (50 mm in diameter and 17 mm in depth) containing dry $CaCl_2$ (0% RH) with silicone vacuum grease and a ring to hold the film in place. The side in contact with the casting plate surface was exposed inside the test cups. Once the films were held, the test cells were then placed in a humidity chamber at (70 ± 2) %RH and 25 °C. The permeability cups with the films were weighted at intervals of one hour during 12 h. Linear regression was used to calculate the slope of a fitted straight line in a graph of variation of mass versus time. The water vapor transmission rate (WVTR) (kg s^{-1}m^{-2}) and the WVP (kg m Pa^{-1} s^{-1} m^{-2}) were calculated from Eqs. (5) and (6) respectively:

$$\text{WVTR} = F/A \qquad (5)$$

$$\text{WVP} = (\text{WVTR} \times e)/[S_p \times (RH_1 - RH_2)] \qquad (6)$$

where F is the slope of the graph of variation of mass versus time (kg s^{-1}), A is the test area (cup mouth area), e is the film thickness (m), S_p is the saturation pressure (Pa) at the test temperature, RH_1 is the relative humidity in the humidity chamber, and RH_2 is the relative humidity inside the cell test.

2.11. SEM and IR analysis

To study the surface structures, the materials were observed by a scanning electron microscope (SEM Model LEO 1450VP). The samples were examined under low vacuum.

Fourier transform infrared spectra (FTIR) for SPI-Gly and SPI-Gly-Gen films were obtained on a Nicolet 5-5X C FTIR spectrometer by diffuse reflectance.

2.12. Indoor soil degradation

The biodegradability of the films was analyzed by burying equal masses of each dried film in a characterized soil using a method previously described in the literature (Martucci et al., 2009).

Samples were cut into rectangular pieces (2×3 cm^2), dried in an oven at 105 °C for 12 h and weighted (W_0). By using plastic boxes ($100 \times 20 \times 15$ cm^3), the samples were buried into an iron mesh (to allow the access of microorganisms and moisture and to ease the removal of the degraded samples) at 8 cm depth from the soil surface in order to ensure aerobic conditions of degradation. The characteristics of the soil included: pH 6.6; total organic matter: 17.06%; organic carbon: 9.90%; total nitrogen: 0.823%; C:N ratio: 12.0; NO_3^-:87.5 ppm; P: 40.4 ppm; Ca^{2+}: 40.0 meq/100 g; Mg^{2+}: 4.50 meq/100 g; Na^+: 0.13 meq/100 g; K^+: 1.76 meq/100 g; E.S.P. (exchangeable sodium percentage): 0.3%; saturation extract (electrical conductivity): 1.8 dS/m. The microbiological analysis showed abundance of nitrifying microorganisms: 360 bact/g; abundance of cellulolytic microorganisms: 16×10^7 bact/g; abundance of N_2-fixing microorganisms: 14×10^5 bact/g; abundance of ammonifying microorganisms: 36×10^6 bact/g; CO_2 production: 2.88 mg CO_2/g/ 8 days.

The assay was performed at 21 ± 2 °C and 48 ± 4% RH by adding water periodically. Soil moisture fluctuation was followed gravimetrically by using the oven drying method (ASTM D2216-10, 2010). Samples were removed from the soil at different times, cleaned several times with distilled water to remove the remaining soil and dried in an oven at 105 °C for 12 h. All the determinations were performed in triplicate. The dry specimens were weighted (W_t) in order to determine the average weight loss (%WL) by Eq. (7).

$$\%\text{WL} = [(W_0 - W_t)/W_0] \times 100 \qquad (7)$$

2.13. Statistical analysis

Data for each test were statistically analyzed. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between means. Turkey test was used for comparing mean values. Differences between means were considered significant when $P \leqslant 0.05$.

3. Results

3.1. Synthesis, characterization and properties

The preparation of SPI films using different concentrations of Gen (0%; 0.1%; 1%; 2.5%; 5%; 7.5% and 10% w/w of the mass of SPI) and glycerol as a plasticizer was carried out by "casting". Thicknesses from 55 to 65 μm were obtained. The films were flexible and the coloration varied from yellowish (film without Gen) to dark blue (films with Gen) and both color intensity and opacity linearly increased with the increase of Gen added (Figs. 1 and 2, respectively). The dark blue color of the films is resulted from the chemical cross-linking of Gen with the amino groups on the SPI macromolecular chains (Song and Zhang, 2009). Gen forms blue pigments upon spontaneous reaction with amino groups (Touyama et al., 1994).

To confirm the cross-linking reaction, analysis of FTIR was carried out. The characteristic adsorption peaks of SPI were observed at 3000–3500 (O–H stretching; N–H bending), 2925 (C–H stretching), 1668 (amide I, C=O stretching), 1548 (amide II, N–H bending), and 1238 cm^{-1} (amide III, C–N stretching). After the cross-linking with Gen, the absorption of the band at around 1668 cm^{-1} showed a relative increase due to the formation of new amide linkages between SPI and Gen. In addition, the relative increase in the C–H stretching at 2925 cm^{-1} and the appearance of the following bands: C–O stretching at 1048 cm^{-1}, C–N stretching (amide III) at 1238 cm^{-1} and C–H bending (out of plane) of C=C at 854 cm^{-1}, were observed. Fig. 3 shows the FTIR spectra.
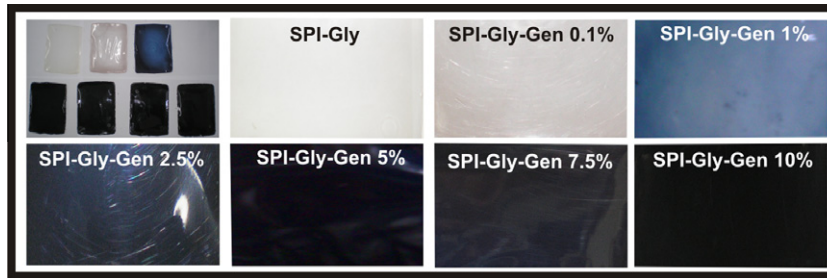
Fig. 1. Macroscopic appearance of the films synthesized.

Further evidence for the cross-linking reaction was provided by the quantification of free amine groups. These amounts were determined using both Ellman's and Taut's reagents by spectrophotometric quantification (Tyllianakis et al., 1993).

Considering that the cross-linker reacts with the protein matrix through amino groups, the cross-linking degrees were calculated taking into account the percentage of amino groups unreacted after the cross-linking reaction with respect to the total amount of amino groups contained in the protein matrix. Fig 4 shows the linear relationship formed between the amounts of Gen added to the reactions and the CL% yielded.

To evaluate the effect of the cross-linking, different film properties such as moisture content, total soluble matter, water vapor permeability, tensile strength and elongation at break were evaluated. Table 1 shows the results.

It was observed that MC values did not vary significantly ($P \geqslant 0.05$). However, TSM values showed a marked variation since the film with 0.1% (w/w of SPI) of Gen already showed difference and the sample with only 1% (w/w of SPI) of Gen revealed a decrease of TSM of approximately 45% with respect to the control film without cross-linker. This effect is particularly important since such materials, when used as coating, could be more resistant in the wet state. When the films were kept in contact with water, they initially showed softening and a subsequent swollen state. In addition, it was observed that in all cases swelling increased sharply in the first five minutes; then it begun to decline since part of the components was probably lost by solubilization during the assay. Higher cross-linking degree (CL%) allows the formation of three-dimensional, rigid and less expandable structures with a smaller capacity of softening and swellability. A similar tendency was obtained for Gen cross-linked gelatin films (Bigi et al., 2002).
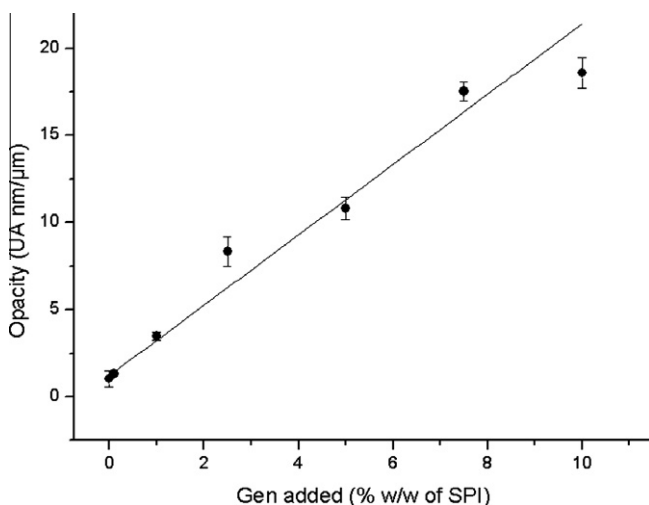
Fig. 5 displays the swelling variation in water of each film as a function of time.

The reduction in swelling ratio values of cross-linked SPI films was considered an indirect evidence of SPI-Gen cross-linking reaction.

Mechanical properties were evaluated by tensile strength (TS) and elongation at break (E) from the stress–strain curves of each film. As observed from Table 1, TS values increase until they reach a limit value at 1% of Gen (w/w of SPI). Larger additions of Gen do not vary significantly ($P \geqslant 0.05$) the TS values. In cross-linked films, E values (Table 1) show an increase when Gen additions are fewer than or equal to 1%. An increase in Gen concentration leads to a diminution in E values becoming into a less deformable material.
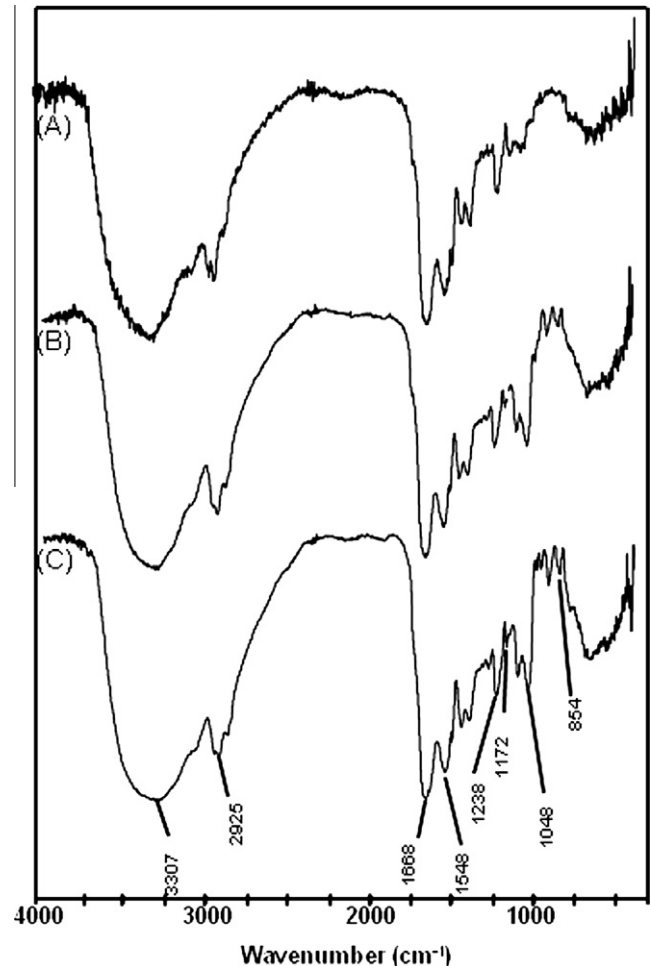

Fig. 2. Linear relationship between opacity and the amount of Gen added.

Fig. 3. FTIR spectra of SPI-Gly films, with (A) 0%; (B) 5% and (C) 10% (w/w of SPI) of Gen.

WVP was evaluated for each sample, showing relatively low permeability values. The effect of Gen amount is inversely proportional to WVP values with additions up to 1% (w/w of SPI) of Gen, remaining constant with larger additions.

The superficial structures of the films were visualized by SEM micrographs (Fig. 6). As seen, the formation of aggregates is likely to derive from the presence of major cross-linking density zones. The higher amount of Gen added to the films yielded products with more aggregates.

3.2. Biodegradation tests

Biodegradation tests were carried out by burying the films into the soil under indoor conditions for 33 days. Pictures of the samples recovered are shown in Fig. 7.

The macroscopic examination revealed that the degradation of the different films in soil strongly depends on the cross-linking degree. Any minimal degree of modification by cross-linking allowed a faster biodegradation. For example, the film with no modification almost completely degraded in 14 days, whereas the SPI-Gly-Gen 10% remained unaltered after 33 days. This dependence can be attributed to the hindering effect of the chemical networks on the enzymatic degradation (Martucci et al., 2009). As demonstrated in the swelling assays, the water absorption of most cross-linked films is lower than that of the less cross-linked. This effect produces a decrease in the bio-availability of water inside

the matrices and could explain the fact that in most cross-linked films, microbial attack, proteolytic enzyme action, and hydrolysis occur to a lesser extent.
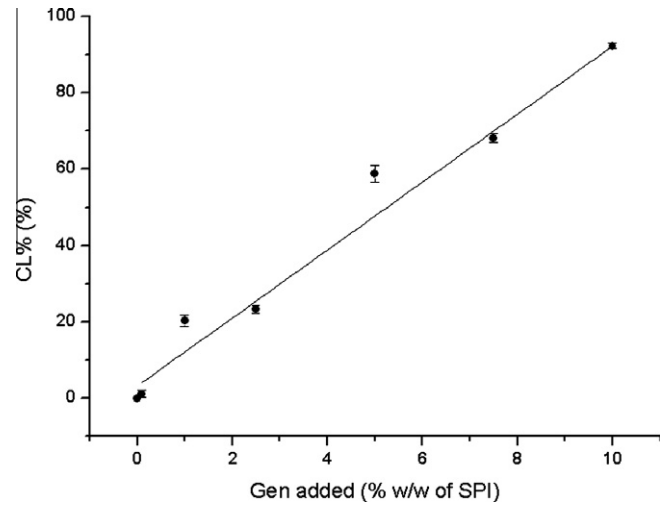

Fig. 4. Linear relationship between the amount of Gen and the percentage of cross-linking.

Table 1
Moisture content (MC), total soluble matter (TSM), water vapor permeability (WVP), tensile Strength (TS) and elongation at break (E) of SPI films with 0%; 0.1%; 1%; 2.5%; 5%; 7.5% and 10% (w/w of SPI) 19 of Gen added.

Film	Thickness (µm)	MC (%)	TSM (%)	WVP ($\times 10^{-10}$ g m Pa^{-1} s^{-1} m^{-2})	TS (MPa)	E (%)
SPI-Gly	58.33 ± 7.13[a]	27.07 ± 4.19[a]	64.66 ± 3.47[c]	2.41 ± 0.04[b]	3.22 ± 0.10[a]	22.53 ± 5.02[bc]
SPI-Gly-Gen 0.1%	62.25 ± 4.43[a]	28.37 ± 2.29[a]	51.58 ± 2.36[b]	2.22 ± 0.09[ab]	3.28 ± 0.16[a]	26.71 ± 4.77[cd]
SPI-Gly-Gen 1%	60.54 ± 6.53[a]	26.03 ± 2.79[a]	35.14 ± 4.42[a]	1.88 ± 0.10[ab]	4.16 ± 0.38[b]	45.84 ± 0.25[e]
SPI-Gly-Gen 2.5%	59.23 ± 7.25[a]	24.74 ± 3.41[a]	33.94 ± 3.81[a]	1.72 ± 0.11[a]	4.46 ± 0.04[b]	36.86 ± 0.46[de]
SPI-Gly-Gen 5%	61.17 ± 3.38[a]	25.60 ± 3.22[a]	34.08 ± 3.12[a]	1.81 ± 0.16[ab]	4.60 ± 0.11[b]	12.14 ± 4.46[ab]
SPI-Gly-Gen 7.5%	65.34 ± 4.22[a]	25.43 ± 2.28[a]	35.20 ± 2.16[a]	1.80 ± 0.20[ab]	4.52 ± 0.13[b]	3.22 ± 0.89[a]
SPI-Gly-Gen 10%	61.42 ± 3.15[a]	26.14 ± 3.17[a]	34.19 ± 3.43[a]	1.89 ± 0.21[ab]	4.58 ± 0.09[b]	2.79 ± 1.48[a]

Any two means in the same column followed by the same letter are not significantly ($P \geqslant 0.05$) different according to Turkey test.
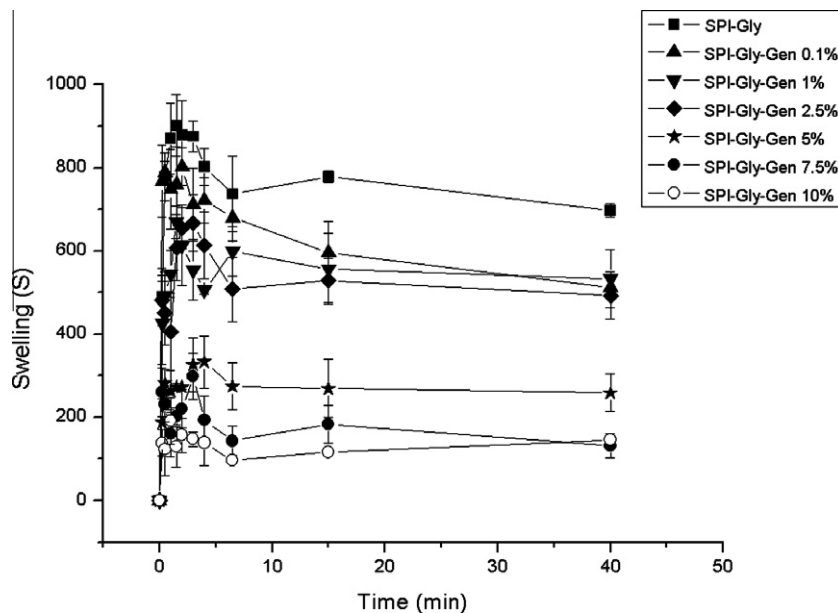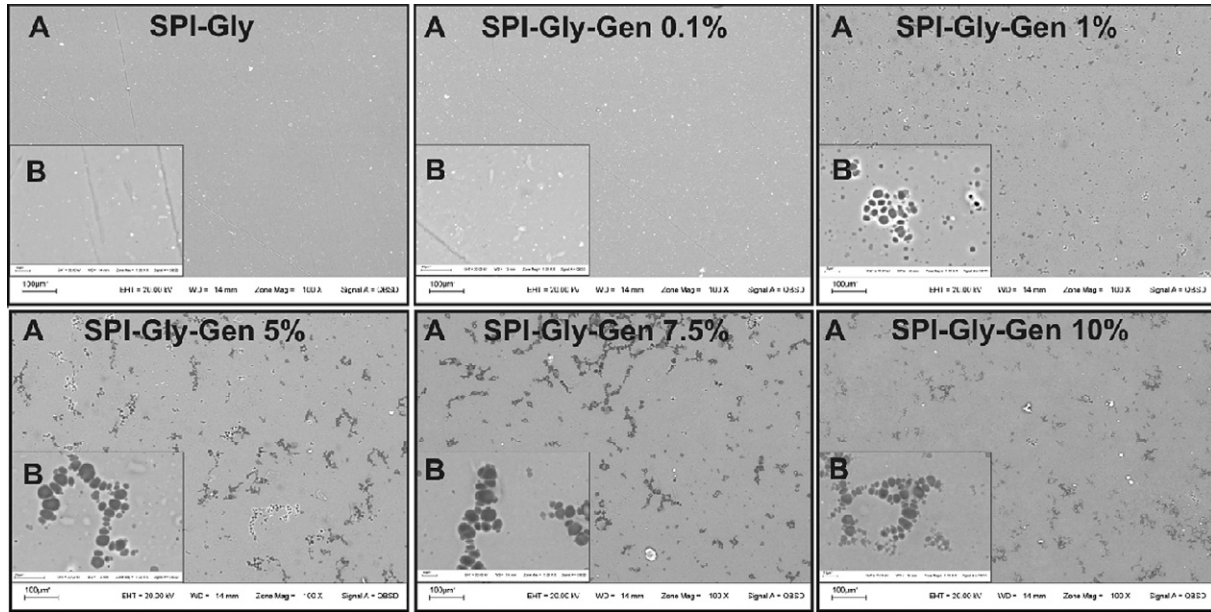

Fig. 5. Swelling variation in water vs. time.

Fig. 6. Scanning electron micrographs (SEM) of SPI-Gly uncross-linked and cross-linked with different amounts of Gen films. Magnification: (A) 100×; (B) 1000×.
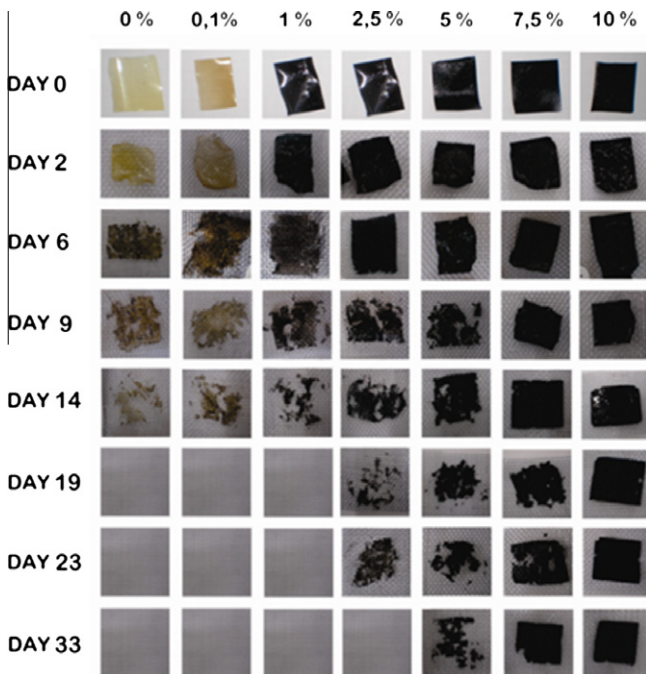

Fig. 7. Macroscopic appearance of the buried SPI films with 0%; 0.1%; 1%; 2.5%; 5%; 7.5% and 10% (w/w of SPI) of Gen after 0, 2, 6, 9, 14, 19, 23 and 33 days.

Fig. 8. Weight loss (%WL) of the films buried in soil as a function of time.

It was found that all materials absorb water (in a larger or lesser extent), losing their initial shape and structural integrity afterwards. After the 23rd incubation day a characteristic decomposition smell appeared. The moisture of the soil was maintained below the saturation moisture (57 ± 5%) to minimize the possibility of solubilization of the matrices. Fig. 8 shows the weight loss as a function of time.

4. Discussion

According with the results of CL%, S, TS, E, TSM and WVP, two groups of products can be distinguished: first, films with 0%; 0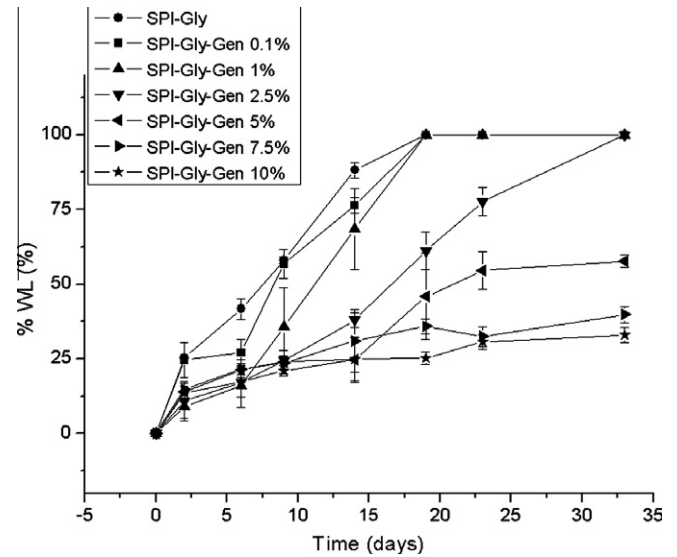.1% and 1% (w/w of SPI) of Gen and a second group with 2.5%; 5%; 7.5% and 10% (w/w of SPI) of Gen. In connection with the first group, a considerable variation in these properties was found with small additions of Gen since these caused an increase in CL%, E and TS and a decrease in S and WVP.

The specimens from the second group showed that additions of cross-linking agent in amounts equal or major to 2.5% increased the cross-linking degree and sharply decreased the swelling in water; a notable reduction in E values was observed, however, some of their properties, including TS, TSM and WVP, kept constant. The fact that some properties do not vary with large additions of Gen can be explained by the different type of cross-links formed when small and large amounts of Gen are added. The cross-linking reaction of proteins can take place intermolecularly (between protein structures) and/or intramolecularly (within protein structures) (Park et al., 2000). Small Gen additions could produce intermolecular cross-linking since the reactive amino groups are those that are more available, while large additions

form intramolecular cross-linking since the Gen enters the protein structures as the amino groups of the periphery are occupied. Therefore, concentrations up to 1% increase the intermolecular cross-linking degree, while higher amounts increases the intramolecular cross-linking degree.

The reduction in TSM with additions up to 1% (w/w of SPI) of Gen has been probably produced because the protein structures (for example globulins belonging to the 7S and 11S fractions) are now linked forming a three-dimensional network by the intermolecular cross-linking. This can be also considered as an evidence of SPI-Gen cross-linking. With this amount of Gen, the materials lead to a limiting value of TSM where the only component that is solubilized is a portion of the plasticizer which is soluble in water. Greater additions of cross-linker do not significantly change ($P \geqslant 0.05$) the TSM due to the fact that protein structures are now attached one to another by intermolecular cross-linking, therefore the amount of soluble material is not affect. Intramolecular cross-linking formation does not prevent the dissolution of glycerol.

In the TS determinations, two different behaviors were also observed, since the intermolecular cross-linking affects this magnitude increasing with low amounts of Gen and remaining constant with higher amounts. Similarly, E values show an increase when Gen additions are fewer than or equal to 1% probably due to the fact that small Gen additions break some intrinsic protein interactions forming more expansible networks. However, an increase in Gen concentration leads to a diminution in E since the formation of new cross-linking produces a more rigid matrix in which the movement between the chains is restricted. These measurements suggest that the mechanical properties of the films are enhanced with small additions of Gen. Thus, it can be concluded that the film with 1% (w/w of SPI) presents the best mechanical properties.

WVP values were substantially lower than those reported in the literature (Cho et al., 2007; Kim et al., 2002; Rhim et al., 1998). This decrease probably derives from the thermal treatment followed during drying under alkaline conditions, causing the disruption of the quaternary structure of proteins accompanied by a partial protein denaturation (unfolding) (Mauri and Añón, 2006). Denaturation of soy proteins promoted the intra and intermolecular cross-linking of amino acid residues, as well as the formation of disulfide cross-links and hydrophobic bonds (Kim et al., 2002), resulting in a decrease in WVP values (Denavi et al., 2009).

Analyzing the effect on WVP caused by chemical cross-linking with Gen, it was observed that the values decreased since the film with 2.5% (w/w of SPI) of Gen diminished approximately 29.5% of WVP with respect to the film without Gen. This decrease can be attributed to an increase in the film density generated by the higher intermolecular cross-linking degree into the matrices. WVP values of films between 2.5% and 10% (w/w of SPI) of Gen did not vary significantly ($P \geqslant 0.05$) due to the fact that the intramolecular cross-linking yielded with this amount of Gen did not probably avoid the permeation of water since the water penetrates through the matrix by the spaces between the protein structures.

The best properties for the SPI-Gly films were obtained with the aggregate of 1% (w/w of SPI) of Gen since TS and E were maxima while WVP and TSM were minimal.

5. Conclusions

This study showed the preparation of new biodegradable materials through the casting methodology from cross-linking of SPI with different amounts of a non-toxic cross-linker (Gen). These materials acquired a dark blue color and became opaque with the increase in cross-linking. The efficiency of the cross-linking reaction was evidenced by FTIR and by the determination of the cross-linking degree. Several tests were performed to show the potential of Gen to improve the SPI film properties, mainly the substantial decrease in solubility, the swelling in water and the WVP. In addition, mechanical properties were improved with small amounts of Gen, increasing TS and E values although larger amounts of Gen produced a decrease in E values but with no significant changes in TS. Biodegradation studies revealed that the biodegradation time of the films can be controlled or modified from at least 14 to 33 days. These effects may increase the applicability of such films in biodegradable packaging or other industrial applications. The use of natural biodegradable materials with improved properties such as cross-linked soy protein provides a valuable opportunity to replace conventional petroleum-derived plastics, adding more value to this vast agricultural resource.

Acknowledgements

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