Structural and functional properties of soy protein isolate and cod gelatin blend films

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Abstract

The structure-function relationship of composite films obtained from soy protein isolate (SPI) and cod gelatin was studied. Films with different ratios of SPI:gelatin (0, 25, 50, 75, 100% w/w) plasticized by a mixture of glycerol and sorbitol, were prepared by casting. Regardless of the soy protein concentration, the thickness and water vapor permeability of the composite films diminished significantly as compared to pure gelatin films. The formulation containing 25% SPI: 75% cod skin gelatin had the maximum force at the breaking point which was 1.8-fold and 2.8-fold of those of 100% gelatin and 100% SPI films, respectively. Moreover, this formulation offered high percent deformation values as the 100% gelatin film and relatively low water vapor permeability as the 100% SPI film. While all the films exhibited high water solubility, a slight reduction of the protein released in water was observed with increasing soy contents. DSC analyses revealed that gelatin was completely denatured in all the films, while the soy proteins maintained most of their native conformation. The FTIR analysis revealed the presence of 25% SPI to produce gelatin conformational changes, self aggregation of gelatin α -chains as well as intermolecular associations through C=O bonds between gelatin and SPI proteins.. Regarding appearance, all the films were translucent, but the yellowish color (b^*) increased with increasing proportions of soy proteins.

Keywords: edible films, soy proteins, cod gelatine, structural properties, mechanical properties, water vapour permeability, FTIR.

1. Introduction

The production of biodegradable materials from renewable biopolymers, such as proteins, appears as an interesting alternative, at least for some applications, to synthetic polymers whose accumulation contributes greatly to environmental pollution. The functionality of protein films are determined by their microstructure, which varies significantly depending on the initial protein structure and the preparation method (Denavi, Tapia, Añón, Sobral, Mauri & Menegalli, 2008). All the aspects of protein structure (primary, secondary, tertiary and quaternary) determine primarily the ability of protein chains to interact between them and also with other components of the formulation considered. The type and concentration of the interactions involved in the stabilization of the proteinaceous matrix (covalent –disulfide bonds- and non-covalent –hydrogen bonds, electrostatic, hydrophobic) is determined by the amino acid composition and the molecular weight of the protein (which vary significantly for different proteins) and by the experimental parameters used in film preparation. The protein-protein interactions involved in film formation determine the degree of cross-linking and the hydrophilic-hydrophobic character of the films, and correlate with their physicochemical, mechanical and barrier properties (Mauri & Añón, 2006 and 2008).

The aim of preparing films from mixtures of structurally different proteins is to obtain composite materials in which each component provides a determined functional property. (Damodaran & Paraf, 1997; Sabato, et al., 2001; Barreto, Pires & Soldi, 2003; Longares, Monahan, O’Riordan & O’Sullivan, 2005, Chambi & Grosso, 2006; Güçbilmez, Yemenicioğlu & Arslanoğlu, 2007; Cao, Fu & He, 2007). In particular, soy proteins and gelatin are very different regarding their origin, structure and amino acid composition. Soy proteins, of vegetal origin, are formed by a mixture of albumins and globulins, 90% of which are storage proteins with globular structure, mainly formed by 7S globulins, or β -conglycinin, and 11S globulin, or glycinin. Globulin 7S is a trimer of molecular mass 150-200 kDa formed by four subunits, from which α (72 kDa), α' (68 kDa) and β (52 kDa) are the most important while the γ subunit (with a molecular mass similar to β) is a minor component. All these subunits have similar amino acid

sequences, and are poor in cysteine, methionine and tryptophan. On the other hand, the 11S globulin or glycinin is an hexamer of molecular mass 300-380 kDa, in which each subunit is composed of an acidic polypeptide (acid pI, ~ 35 kDa) and a basic polypeptide (basic pI, ~ 20 kDa) linked by a disulfide bond, with a cysteine, methionine and tryptophan content higher than that of 7S globulin (Nielsen, 1985a and b; Thanh & Shibasaki, 1977; Staswick, Hermedson, & Nielsen, 1984). On the other hand, gelatin is a mixture of polypeptides derived from hydrolysis of collagen contained in bones and skins. The collagen rod, composed of three α -chains of around 100 kDa each, can be solubilized in acid without altering its original triple-helix configuration. Subsequent thermal treatment cleaves hydrogen and covalent bonds; leading to destabilization of the triple helix by means of a helix-to-coil transition (Djabourov et al., 1993) and conversion into soluble gelatin, which has a definite molecular weight distribution pattern corresponding to the α -chains and its oligomers (Ledward, 1986). As shown in previous studies, cod skin gelatin contains α -chains, β - and γ -components (covalently linked α -chain dimers and trimers, respectively), and also an appreciable amount of higher molecular weight polymers as well as fractions of molecular weight lower than that of α -chains (Gómez-Guillén, Turnay, Fernández-Díaz, Ulmo, Lizarbe & Montero, 2002). According to the same study cod skin gelatin is mostly composed of Gly (34%), iminoacids (Pro+Hyp, 16%) and Ala residues (10%). As for most gelatins derived from type I collagen, cysteine and tryptophan were absent, and the content of Tyr residues was below 1%.

The structural differences between soy and gelatin proteins are reflected in their functionality, consequently, in the characteristics of the films they form, although both proteins may exhibit good film-forming properties. SPI films have been reported to be rather brittle and coloured, and to have relatively poor mechanical properties (Ghorpade, Li, Gennadios & Hanna, 1995; Rhim, Gennadios, Handa, Weller & Hanna, 2000; Kim, Weller, Hanna & Gennadios, 2002; Mauri, et al., 2008). On the other hand, fish gelatin form transparent, weakly colored, and highly extensible films (Jongjareonrak, Benjakul, Visessanguan & Tanaka, 2006; Avena-Bustillos et al., 2006; Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007; Zhang, Wang, Herring & Oh, 2007; Carvalho et al., 2008). However, the main drawback of cod gelatin films is

their low water resistance and their extremely high solubility (Piotrowska, Kolodziejska, Januszevska-Jozwiak & Wojtasz-Pajak, 2005; Pérez-Mateos, Montero & Gómez-Guillén, 2009).

Taking into account the best characteristics of soy and gelatin proteins it can be speculated that their combination would lead to improved films as compared to those formed by each material individually. In a recent article, Cao et al. (2007) described the diverse functional properties of composite films made from soy protein isolate and type B- bovine-bone gelatin, suggesting that complex films have an increased range of applications due to the different mechanical and barrier properties of their components.

The goal of the present work was to study the formation and the characteristics of composite films obtained from cod skin gelatin and soy protein isolates mixed in different proportions, and to analyze the structure-function relationship of such mixtures.

2. Materials and methods

2.1. Soy protein Isolate (SPI) preparation.

Soy protein isolate was prepared from defatted low-heat soybean meal produced by Bunge-Ceval SA (Brazil) as described by Petruccelli & Añón (1995). Protein content of SPI was 83.82% $\pm$ 0.14 % protein on a dry basis, determined by the Kjeldahl technique (Nx5.7).

2.2. Fish skin gelatin.

Cod (*Godus morhua*) skin gelatin was obtained using a mild acid pre-treatment followed by overnight extraction by water at 45°C, as reported in Gómez-Guillén & Montero (2001). The protein content of the dry gelatin powder was 84.4 % $\pm$ 0.23 (N x 5.4) as determined with a Nitrogen analyzer LECO FP-2000 (Leco Corporation, St Joseph, MI) calibrated with EDTA (Dumas method following A.O.A.C. 992.15) (A.O.A.C. 2000).

2.3. Preparation of films

Five groups of composite films with different ratios of soy protein isolate to gelatin (0:100, 25:75, 50:50, 75:25 and 100:0) were prepared. Aqueous solutions of 4 % soy protein isolate and/or gelatin (w/v) were prepared in 250 mL Erlenmeyer flasks with 1.5 % plasticizers (0.75 % glycerol + 0.75 % sorbitol). Dry proteins were dissolved in distilled water with stirring, at room temperature in the case of soy protein and heating at 60 °C in a water bath in the case of gelatin. Once they were completely dissolved, pH was adjusted to 10.5 using 2N NaOH for both proteins, based on previous findings about the effect of pH on the properties of soy protein films (Mauri et al, 2006 and 2008). Then protein solutions were mixed at the different ratios shown in Table 1. 40 mL of the film-forming solution were poured on plexiglass plates (11.5 x 11.5 cm) and were dehydrated at 45°C in an oven with air flow and circulation (Binder FD 240, Tuttlingen, Germany) for 18-20h. Dry films were conditioned at room temperature and 58% relative humidity in desiccators with saturated solutions of NaBr for two days, and were subsequently peeled from the casting surface for characterization analyses. The nomenclature of films is shown in Table 1.

2.4. Thickness

Film thickness was measured with a digital micrometer (Mitutoyo MDC-25M, Kanagawa, Japan). For each film, values obtained at nine different positions were averaged.

2.5. Water solubility and protein soluble matter

Films solubility was determined in triplicate, according to the method proposed by Gontard, Guilbert & Cuq (1992). Three pieces of film (2 cm in diameter, about 0.25 g total) were immersed in 50 mL distilled water and the system was gently shaken at room temperature (22-25 °C) for 24 hours. Then, the samples were filtered through a filter paper (Whatman 1). The unsolubilized fraction was dried in a forced air oven (105 °C, 24 hours) in order to determine the water soluble matter as a percentage of the initial weight.

The protein content of the soluble fraction was measured with a Nitrogen analyzer LECO FP-2000 (Leco Corporation, St Joseph, MI) calibrated with EDTA (Dumas method following

A.O.A.C. 992.15) (A.O.A.C. 2000). Results are the average of three determinations and are expressed as percent protein solubilized with respect to 100 g of film (Nx5.7 for SPI; Nx5.4 for cod gelatin; N x 5.5 estimated for the mixture of SPI and cod gelatin).

2.6. Electrophoretic analysis (SDS-PAGE)

Aliquots of the water soluble fractions of the films (15 μ L) were mixed with 200 μ L of loading buffer (12.5% SDS, 25% mercaptoethanol, 50 mM Tris-HCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 10 % glycerol and 0.002% bromophenol blue) until reaching a final concentration of 2 mg/mL of protein. Samples were heat-denatured 5 min at 90 $^{\circ}$ C and analyzed by SDS-PAGE according to Laemmli (1970) using 7.5% gels in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA) at 30 mA/gel. The loading volume was 20 μ L in all lanes. Protein bands were stained with Coomassie Brilliant Blue (R-250, Merck). An aqueous solution of 30% (v/v) methanol and 10% v/v acetic acid was used for destaining, and an aqueous solution of 5% (w/v) glycerol and 10% (v/v) acetic acid was used as storage solution. Two mixtures of known proteins covering different molecular weight ranges (Amersham Biosciences, Buckinghamshire, England)-were used for reference. The high molecular weight standard contained myosin (220 kDa), α_2 -macroglobulin (170 kDa), β -galactosidase (116 kDa), transferrin (76 kDa), and glutamic acid dehydrogenase (53 kDa). The low molecular weight standard contained phosphorilase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α -lactalbumin (14.4 kDa).

2.7. Light absorption

The light barrier properties of films were determined by measuring their light absorption at wavelengths ranging from 690 nm to 280 nm, using a UV-1601 spectrophotometer (Model CPS-240, Shimadzu, Kyoto, Japan). The transparency of the films was calculated by the equation $T = \text{Abs}_{600}/x$, where Abs_{600} is the absorbance at 600 nm and x is the film thickness (mm).

2.8. Fourier transform infrared spectroscopy (FTIR).

Pieces of film 2 cm in diameter were sandwiched between two KBr disks. FTIR spectra were recorded from wavenumber 400 to 4000cm⁻¹ in a Bruker IFS28 spectrometer (Bruker Banner Lane, Coventry, UK). Absorbance spectra were obtained with a spectrum of the KBr disk as background. In all, 50 interferograms were compiled for each spectrum.

2.9. Differential Scanning Calorimeter

A Polymer Laboratories Rheometrics Scientific DSC (Church Stretton, UK) was used for these studies. Temperature and heat flow calibration of the equipment was carried out according to ASTM Standards. Lauric acid and indium were used as temperature standards; the latter was also used as a heat-flow standard. SPI, cod gelatin and films were studied by DSC, in order to determine the conformational state of both proteins. Hermetically sealed aluminum pans containing 10-15 mg of samples were prepared. The capsules were scanned at 10°C/min over the range 30 -150 °C. An empty double capsule was used as a reference.

2.10. Water vapour permeability measurements

Water vapor permeability was determined following the method described by Sobral et al. (2001). Samples were cut from each film and mounted on plastic cups (permeation area = 15.90 cm²) containing silica gel, and then the cups were placed in desiccators with distilled water. Weights were measured every hour for 6 hours. Water vapor permeability was calculated from the equation $WVP = w \times t^{-1} A^{-1} \Delta P^{-1}$, where w is the weight gain (g), x is the film thickness (mm), t is the time of gain (h) and ΔP is the difference of partial vapor pressure of the atmosphere with silica gel and pure water (2642 Pa at 22°C). Results were expressed as g mm h⁻¹ cm⁻² Pa⁻¹. All tests were made in triplicate.

2.11. Mechanical properties

Films were fixed in a 5.6 cm diameter cell and perforated to breaking-point using a texturometer (Instron 4501, Instron Engineering Corp., Canton, MA, U.S.A.) with a round-ended stainless steel plunger (Ø=3 mm). Cross-head speed was 60 mm/min and a 100 N load-cell was used. Breaking

force was expressed in N and breaking deformation in %, according to Sobral, Menegalli, Hubinger & Roques (2001). All determinations are the means of at least five measurements.

2.12. Color

Films were placed between two steel dishes with a hole of 5.7 cm diameter. The color of films was determined with a tristimulus colorimeter (HunterLab D25 A-9, Hunter Associates Laboratory Inc., Reston, VA., USA) using the CIE Lab scale (C/2°) where L^* , a^* and b^* are the parameters that measure lightness, redness and yellowness, respectively. A standard white plate with reflectance values of $L^* = 93.68$, $a^* = -0.69$, $b^* = -0.88$, was used as reference. The result was the average of five measurements taken at ambient temperature at different points on the sample.

2.13. Statistical analysis.

Data were subjected to one-way analysis of variant (ANOVA) using SPSS computer program (SPSS 14, SPSS INC., Chicago, Illinois, USA), means were compared using Bonferroni test with level of significance set at $p < 0.05$. Pearson or Spearman's rho correlations ($p < 0.01$ or 0.05) were calculated to analyze dependence of film characteristics on the ratio of soy protein isolate :gelatin.

3. Results and Discussion

The thickness, water content, water solubility and soluble protein content of the films obtained are shown in Table 2. It can be observed that all the films containing soy protein isolate (regardless of its proportion) had a similar thickness, which in all cases was significantly thinner than those of films made from gelatin alone. It can be also observed that film containing only soy proteins exhibited water content significantly higher than that of the film containing only gelatin, while composite films had intermediate water contents without significant differences among them. It is interesting to note that in spite of the use of the same total protein mass and drying conditions for preparing all films, those containing gelatin had a higher thickness in spite of their lower water content. This indicates a higher compaction degree of the film matrix when

soy proteins are present, suggesting a different degree of molecule unfolding or cross-linking within the protein network of the film.

Solubility properties

All the films exhibited a water solubility above 80% (Table 2), gelatin films being slightly ($p \leq 0.05$) more soluble than SPI films. This would indicate a poor water resistance, although for some applications could be interesting, for example, as a carrier of bioactive compounds. The solubility tended to decrease with increasing the SPI proportion in the composite films, although differences were found not significant ($p > 0.05$). It is worth noting that the water insoluble film fraction in the composite films did not preserve the initial integrity but appeared as a viscous mass, mostly due to the presence of swelled aggregated gelatin, and this might account for a considerable high variability in the results. In the case of films made from pure gelatin, the results obtained agree with those reported by Carvalho, et al. (2008) for films elaborated from two differently processed Atlantic halibut skin gelatins, as well as by Piotrowska, et al. (2005) and Pérez-Mateos, et al. (2009) for cod skin gelatin films, but were considerably higher than those reported for bovine gelatin films (around 30 %) (Bertan et al., 2005; Gómez-Estaca, Montero, Fernández-Martín, Gómez-Guillén, 2008). It is known that, compared to mammalian gelatins (Norland, 1990; Montero et al., 1990), cold water fish gelatins are characterized by a lower content in intra- and interchain covalent crosslinks mainly involving lysine and hydroxylysine residues as well as aldehyde derivatives, which may be responsible for such a higher film solubility. On the other hand, solubility values for pure soy films were higher than those reported by other authors (Stuchell & Krochta, 1994; Kunte, Gennadios, Cuppett, Hanna & Weller, 1997; Cho & Rhee, 2004,). Denavi et al. (2008) have shown that the solubility of films prepared by casting from native soy protein isolates using glycerol as plasticizer was significantly affected by drying conditions. They also reported solubilities higher than 80% when films were dried at low temperatures and low relative humidities (conditions also used in the present work), and attributed this finding to the fact that proteins are not denatured under these conditions thus not favoring the interactions between chains (including thiol/disulphide -

SH/SS- interchange reactions) and facilitating the subsequent solubility of the films. The same explanation may apply to the findings of the present study.

While no significant differences in solubility change of films were observed, it was verified that the amount of protein released from the films upon water solubilization (Table 2) was significantly higher ($p \leq 0.05$) in the pure gelatin film, as well as in the composite films where SPI did not exceed 50%. The high compaction of all the films containing soy proteins would be expected to hamper the protein-solvent contact thus reducing the solubility of these films as compared to 100% gelatin ones. However, this fact did not explain the behavior of composite films, all of which had a similar thickness that was notably lower than that of 100% gelatin films. Thus, in accordance to Voutsinas, Cheung and Nakai (1983), the lower protein solubility in the films with the highest SPI ratio (i.e. 100S:0G and 75S:25G) was mainly attributed to the higher surface hydrophobicity of soy proteins as compared to gelatin ones.

Electrophoretic analysis

The SDS-PAGE analysis of the protein fraction released upon film water solubilization is shown in Figure 2. A clear difference between 100% SPI and 100% G films was observed, so much in the type of bands as in their intensity. As expected, gelatin soluble fractions exhibited molecular weights notably higher than those of soy proteins ones. Moreover, the bands corresponding to the gelatin polipeptides appeared with a considerably higher intensity than the soy protein bands, which was in agreement to the higher amount of soluble protein in the gelatin films than in the SPI films. The protein released from gelatin films showed bands that could be identified mainly as α_1 - and α_2 -chains (~100 KDa), b-components (~200 KDa) and higher molecular weight aggregates, with a distribution similar to that of the gelatin originally used for film preparation (Gómez-Guillén et al., 2002). This finding indicates that all fractions, including those of very high molecular weight, are soluble in water, which is in accordance with the high film water solubility. Proteins released from SPI films upon water solubilization included lower molecular weight components (<80 KDa) that may be attributed to the polypeptides that constitute the two mains globulins present in SPI, i.e. acidic polypeptide chains (A: 37-40 KDa)

and basic polypeptide chains (B: 9.9-20 KDa), and the AB subunit (~60 KDa) of glycinin globulin (11S), and subunits α' (83-57 KDa), α (76-57 KDa) and β (53-42 KDa) of β -conglycinin (7S). Notably, soluble aggregates of higher molecular weight, previously found in other studies (Mauri et al, 2006), were not observed among soy polypeptides in the present work. Regarding the composite films, the SPI bands exhibited a similar intensity in all formulations irrespective of the SPI proportion in the film. On the contrary, gelatin molecules showed a noticeable decrease in the intensity of the different bands when 25% gelatin was replaced by 25% SPI in the formulation of the composite films (25S:75G). This strongly suggested some gelatin aggregation in the presence of a low amount of soy proteins, which would hinder its solubilization in water. This event, however, could not be clearly evidenced by determining the film water solubility or the soluble protein, since no significant differences were found. As the SPI ratio increased, gelatin fractions were hardly visible, in part due to molecule aggregation, but also to a dilution effect, as a result of the reduced gelatin ratio. This was especially apparent in the 75S:25G formulation, in agreement with the significantly lower values of soluble protein found.

Water vapour permeability

The WVP of the films is shown in Figure 3. It can be observed that the value of this measurement is significantly lower for soy films than for gelatin ones, and that the presence of soy proteins in composite films determines a reduction of WVP regardless of the amount of soy added, which is clearly related to the lower thickness of films containing soy proteins as well as to the more surface hydrophobic nature of the latter ones. As reported previously for other hydrophilic films (pectin, amylose, cellulose ethers, sodium caseinate and soy proteins), WVP increases with film thickness (McHugh, Avena-Bustillos & Krochta, 1993; Ghorpade, et al., 1995). In the present work, the replacement of 25% cod gelatin by SPI (25S:75G) was enough to induce a film matrix compaction which remained unaltered by increasing the soy protein level.

Mechanical properties

The puncture force and deformation of the studied films as measured in compression assays are shown in Figures 4a and 4b, respectively. Gelatin films exhibited greater deformation (at least 10-fold higher since gelatin films did not break under the conditions assayed) and greater breaking force (1.8-fold higher) than that of soy ones. Regarding composite films, it can be observed that deformation increases progressively with gelatin proportion in the mixture, and that 50S:50G and 25S:75G samples exhibit breaking forces higher than those of films made from gelatin alone. Cao et al. (2007) reported that the mechanical properties in the tensile test of composite films made from type B- bovine-bone gelatin and soy isolate improved progressively with increasing gelatin proportions, but traction resistance, elongation and Young module of films prepared from gelatin alone were higher than those of all the composite films. The higher breaking force observed in the current study for 50S:50G and 25S:75G films suggests a reinforcement of the film matrix probably induced by a certain degree of protein cross-linking involving both gelatin and SPI proteins.

Differential scanning calorimetry

The thermograms of films as determined by DSC are shown in Figure 1. It can be observed that cod gelatin is completely denatured in all films, while soy proteins are only partially denatured and conserve part of their native structure. The two endotherms in the thermograms of films containing soy proteins correspond to the thermal denaturation of 7S and 11S globulins. These denaturation temperatures are shift to higher values than those usually informed for these proteins (to 78 and 92 °C), which is due to the low water content of the film (Hägerdal & Martens, 1976; Mauri et al., 2006). The denaturation heat values associated to these endotherms were lower than those of native proteins and similar to those reported previously (Mauri et al., 2006) for similar soy protein isolate film-forming solutions at pH 10.5, showing that the drying step at 45 °C does not contribute to soy globulins denaturation. Although not pronounced, there is a tendency to a slight upward shift of denaturation temperatures with increasing gelatin proportions (3 and 7 °C for the 25S:75G sample as compared to the 100S:0G sample), partially

due to the lower water content of these films. This is accompanied by a small increase of denaturation heat (the difference being 2.9 J per gram of soy protein in the samples mentioned above). When the contribution of each fraction to the denaturation heat is analyzed separately, the 11S fraction seems to be the one presenting a lower denaturation degree with increasing gelatin proportions in the film (its denaturation heat increases), while the presence of gelatin does not modify the state of the 7S fraction, whose denaturation heat stays approximately constant in all the formulations (including that formed from the soy protein isolate alone).

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of cod skin gelatin films (0S:100G), soy protein isolate films (100S:0G), and their corresponding mixtures (25S:75G and 50S:50G) are shown in Figure 5. The gelatin film exhibited a sharp absorption band between 3550 and 3510 cm^{-1} , which corresponds mainly to stretching vibrations of OH groups from adsorbed water molecules due to the relatively high hygroscopic nature of the film (Yakimets, Wellner, Smith, Wilson, Farhat, & Mitchell, 2005). A similar band was also evident in the gelatin-SPI composite films, largely linked to the presence of gelatin. On the contrary, the SPI film showed a broad absorption range between 3500 and 3200 cm^{-1} , which as reported by Guan, Qiu, Liu, Hua, & Ma (2006) and Nanda, Rao, Kar, & Nayak (2007), will correspond mainly to free –OH and amines N-H stretching of the soy protein isolate in the film.

The infrared spectra of blends films revealed noticeable changes to occur in the 1700-1650 cm^{-1} region, as compared to the pure gelatin and SPI films. Regarding gelatin containing films, changes in this frequency range are indicative of changes in collagen or gelatin secondary structure involving the amide I region (Muyonga, Cole, & Duodu, 2004). The spectrum of the cod gelatin film has been described in a previous work, where the gelatin was mixed with increasing proportions of sunflower oil, revealing noticeable lipid-protein interactions (Pérez-Mateos, et al., 2009). As reported previously, the main peaks observed at 1687 and 1656 cm^{-1} in

the cod gelatin film were related to the predominance of helices of aggregated collagen-like peptides and, to a lesser extent, of random coils.

On the other hand, the pure SPI film showed main relevant peaks at 1656, 1676 and 1687 cm^{-1} . A previous study (Kurose, Urman, Otaigbe, Lochhead, & Thames, 2007) revealed well defined peaks at 1658 and 1625 cm^{-1} in a film made with commercially available SPI, representing the overlap of signals from α -helix and random segments, and the contribution of β -sheets in the secondary structure, respectively. These peaks were reported to increase with increasing glycerol concentrations, by inducing activation of molecular chain motion. On the contrary, no absorption band at around 1650-1658 cm^{-1} could be detected in another dried commercial SPI (Nanda, et al., 2007), with no reported addition of glycerol or sorbitol. It is clear that intrinsic properties of the biopolymer and conditions used for film preparation (for example the use of certain plasticizers) are essential in determining the prevalence of secondary structure within the film. The SPI film in the present work contained 37.5% (g/100 g protein) of plasticizers (glycerol plus sorbitol), which according to Kurose, et al. (2007) may have promoted the appearance of the peak at 1656 cm^{-1} associated to α -helix structures. Similarly, the peaks at 1676 and 1687 cm^{-1} in the SPI film, which were also present to a lesser extent in the gelatin film spectra, may be related to interactions with the plasticizer through C=O bonds. The extremely high pH (10.5) used for dissolving the SPI for film formation, very far from the isoelectric point (≈ 4.5), may have induced a certain degree of soy protein unfolding favoring the interaction between the protein and the plasticizers, as was verified in DSC studies.

The gelatin-SPI mixtures, especially in the 25S:75G film, induced an increase of previously existing peaks in gelatin or SPI films, as well as the appearance of new ones, which could be indicative of protein-protein interactions between both polymers. The higher intensity of the peaks at 1687 and 1656 cm^{-1} , especially as compared to the pure gelatin film, denoted increased aggregation of gelatin polypeptide α -chains with a concomitant increase in the amount of disordered random coils, the latter largely coming from SPI fractions. The peak at 1676 cm^{-1} was clearly visible in the 25S:75G film and, to a lesser extent, also in the 50S:50G film. Such an FTIR event was scarcely appreciable in the pure gelatin film where it was present in the form of

a slight trace shoulder. Muyonga, et al. (2004) reported a peak at 1675 cm^{-1} that they ascribed to the presence of β -turns in fish gelatin films. In agreement with these authors, and considering the low SPI proportion in the 25S:75G film, the increase in the intensity of this peak in the present study may reveal gelatin conformational changes induced by the presence of the SPI. The appearance of peaks at 1702 cm^{-1} in the 25S:75G film, and to a lesser degree at 1697 cm^{-1} in the 50S:50G film, denoted an increment in inter-molecular associations through C=O bonds, likely due to gelatin cross-linking favored by esterification reactions with SPI lateral reactive groups. Similar peaks have been previously reported to occur in spectra from pigskin gelatin with added lauric acid (Djagny, Wang, & Xu, 2001) or in similar cod skin gelatin films added with different concentrations of sunflower oil (Pérez-Mateos et al., 2009). The higher intensity and frequency of appearance of the peak at 1702 cm^{-1} in the 25S:75G films, as compared to the 50S:50G film, clearly suggested that the gelatin-SPI interaction was stronger in the former.

From all the above findings, the replacement of 25% gelatin by SPI in the formulation of composite films led to gelatin conformational changes producing a double effect: it favoured the self-aggregation of gelatin polypeptide α -chains, as well as a certain degree of interaction between gelatin and SPI proteins. This may explain why the resulting film matrix was more compact, as revealed by the reduced thickness, and gave rise to stronger and less deformable films. Cao et al. (2007) reported that interactions between the amidogen and carboxyl of SPI and gelatin might determine the improved mechanical properties of composite films, proposing the hydrogen bonding as a main mechanism responsible for those interactions. To this respect, our FTIR study revealed a more strong interaction via C=O bonds. The higher degree of compactness together with the more hydrophobic nature of the SPI proteins also caused the WVP to decrease in the composite films. When the ratio of the SPI in the film was increased, both gelatin cross-linking and gelatin-SPI interactions occurred to a lower extent, probably as a result of the higher amount of SPI present in the matrix forming a network apart from gelatin, as observed previously by DSC. This strongly suggests that, beyond a certain level, the SPI excess may act as simple filler in the gelatin network, making films to be less resistant, and especially less deformable, as being this the most striking feature of SPI films. Bearing also in mind the

relatively low molecular weight of SPI proteins and their globular nature, in contrast to the gelatin polypeptides, this may explain why increasing the soy protein level did not significantly affect the thickness of the composite films or the WVP.

Light barrier properties

Regarding the light barrier properties of films, Figure 6 shows the spectroscopic scanning of the films at wavelengths between 690 and 280 nm. All the spectra exhibited maxima at 290 nm and 295 nm, which were much more noticeable with increasing soy protein contents (0-100 %S), and minimum absorption at <280 nm. It is known that proteins exhibit light absorbance around 280 nm mainly due to the presence of hydrophobic residues from tyrosine and tryptophan. A slight shift of the typical maximum absorption for these amino acids residues was observed, which may be attributed to the extremely high pH (10.5) which may cause the dissociation of the carbonyl and amino groups. The increase in the absorbance of films with increasing SPI proportions may be largely attributed to the exposure of a higher number of hydrophobic residues which are present in the SPI. The transparency level of the films at 600 nm was not significantly ($p>0.05$) modified due to the rate of S:G (Table 3).

The Hunter color parameters (L^* , a^* y b^*) of the films are shown in Table 3. Soy films are yellowish (b^+) while those made from gelatin are colorless. The color difference among samples is a consequence of the formulation. As the proportion of soy in the formulation increases, there is an increase in the yellowish color (b^* is higher) and the films are more clear (L^* increases), these changes being significant for films formed with soy proportions higher than 50%. Since no changes in a^* are observed, differences are mainly evidenced in the intensity of the yellowish tone.

Conclusions

The polydisperse nature of gelatin molecules with the presence of unfolded fractions of high molecular weight results in a film of considerable thickness, with extremely high deformability. When the soy proteins (which preserves partially their globular structure) were introduced in

low concentration (25S:75G), gelatin aggregation as well as gelatin-SPI interactions were induced, leading to a notable reinforcement of the composite film. As the proportion of soy protein increased, the gelatin matrix approached saturation, in such a way that the formation of an independent soy matrix would reduce the mechanical properties (both breaking force and deformation) of the film.

Furthermore, soy proteins, which are well known to have surface hydrophobicities higher than those of gelatin, would be also responsible for the better water vapor barrier properties of the composite films. Although some physical properties have been improved (mainly breaking force and WVP), composite films are still highly water sensitive, so that they are far from being yet a real alternative to the plastic synthetic ones. However, they could be an excellent carrier of active components to be used for food preservation or for designing functional foods.

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Table legends

Table 1. Naming system for films according to the proportion (w/w) of each protein.

Table 2. Thickness, water content and water solubility of films obtained with different proportions of soy protein isolate (S) and cod gelatin (G).

Table 3. Color parameters (L^* , a^* , and b^*) of films formed with soy protein isolate and cod gelatin.

Figure legends

Figure 1. DSC thermograms of films with different soy proteins:gelatin ratios.

Figure 2. SDS-PAGE gel show results for water soluble fraction of the films (Lanes 1 & 7= MW standards; 2 = 0S:100G; 3 = 25S:75G; 4 = 50S:50G; 5= 75S:25G; 6 =100G:0S)..

Figure 3. Water vapor permeability of films obtained with different soy protein:cod gelatin proportions.

Figure 4. Mechanical properties as determined by the puncture test of films formed with SPI and cod gelatin: a) breaking force (N); b) deformation (%).

Figure 5. FTIR spectra of cod skin gelatin films (0S:100G), soy protein isolate films (100S:0G), and their corresponding blends (25S:75G and 50S:50G).

Figure 6. Light barrier properties of films with different ratios of soy protein isolate and cod gelatin