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Long-term stability of compression-molded soybean protein concentrate films stored under specific conditions

*Emiliano M. Ciannamea, Juan P. Espinosa, Pablo M. Stefani & Roxana A. Ruseckaite**

Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA), Universidad Nacional de Mar del Plata – CONICET, Av. J.B.Justo 4302, B7608FDQ-Mar del Plata, Argentina

* Corresponding author. E-mail: roxana@fi.mdp.edu.ar

ABSTRACT

Post-processing evolution of the functional properties of soybean protein concentrate (SPC) films, plasticized with varying levels of glycerol and processed by compression molding, was examined over a period of 90 days. Films stored in the glassy state (25±2ºC and 65±2% relative humidity) lost glycerol and water over time, as determined by gas chromatography and the decline in moisture content. SPC films plasticized with 40-50% glycerol showed a time-dependent increment of the elastic modulus and the tensile strength. In turn, the elongation, barrier properties, soluble mass and opacity of these films varied marginally with time. By contrast, films with 30% glycerol lost the most moisture and their elongation was reduced significantly, while water vapor permeability slightly increased with aging. The performance of aged films resulted from the balance between plasticizer and water loss, and the progressive replacement of unordered structures by intermolecular hydrogen bonded β-sheets and aggregates.

Keywords: Soy protein concentrate, Films, compression molding, aging, mechanical properties, barrier properties

1. Introduction

Proteins are gaining a place among biopolymers for the development of biodegradable or edible films thanks to their excellent film-forming, gas- and aroma-properties and their adequate mechanical response under low humidity conditions (Debeaufort, Quezada-Gallo, & Voilley, 2002). The main challenge faced by the industry and the academia is to find sustainable solutions to render protein bio-plastics more cost-effective and competitive against conventional plastics. The conversion of proteins into films by usual thermoplastic processing techniques (i.e., thermoforming, extrusion, compression and molding) have favored large-scale commercialization and the competitiveness of protein-based materials (Hernandez-Izquierdo & Krochta, 2008; Klüver & Meyer, 2014). Among plant protein sources, soybean proteins (SPs) are abundant and commercially available as isolates (SPI, 90%> protein content), concentrates (70%> protein content), and flours (about 50% protein content) at a relatively reasonable cost (Singh, Kumar, Sabapathy, & Bawa, 2008). SPs can be processed by thermoplastic technologies with low water content and in the presence of non-aqueous plasticizers (Ciannamea, Stefani, & Ruseckaite, 2014; Cunningham, Ogale, Dawson, & Acton, 2000; Guerrero, Stefani, Ruseckaite, & de la Caba, 2011; Paetau, Chen, & Jane, 1994; Wu & Zhang, 2001). However just like other protein films, thermo-pressed SPs films have shown post-processing physical and/or chemical changes over time under specific temperature and relative humidity (RH) conditions (Xiaoqun Mo & Sun, 2013; Ogale, Cunningham, Dawson, & Acton, 2000). Aging occurs because SPs films are in the glassy state (a non-equilibrium condition) at ambient temperature and tend to approach the thermodynamic equilibrium through time-dependent spontaneous relaxation and molecular rearrangements. In addition, and unlike most synthetic glassy polymers, protein films would suffer chemical

transformations including aggregation and thiol oxidation (Ciannamea, Stefani, & Ruseckaite, 2015; Olabarrieta, Gällstedt, Ispizua, Sarasua, & Hedenqvist, 2006).The durability and predictability of physical and chemical properties over time for wheat gluten and sunflower protein films obtained by thermoplastic processing have been well documented(Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004; Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006; Orliac, Rouilly, Silvestre, & Rigal, 2003; Ullsten, Cho, Spencer, Gällstedt, Johansson, & Hedenqvist, 2009). Nonetheless, scant literature explores such behavior in SP items (Cunningham, Ogale, Dawson, & Acton, 2000; X. Mo & Sun, 2003).

The mechanical properties of extruded vital wheat gluten (WG) sheets changed dramatically during storage at 23ºC and 50% RH for 120 days, mostly due to the loss of glycerol and moisture over time(Ullsten, Cho, Spencer, Gällstedt, Johansson, & Hedenqvist, 2009). Compression molded WG films exposed to 0% RH at 23 ºC for 24 days experienced greater changes in tensile properties than films stored at 50% RH did. At 0% RH, the loss of glycerol and water governed the mechanical behavior of WG films, while differences at 50% RH were not significant due to the high amount of water that still plasticized the matrix(Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004). Thermo-molded sunflower proteins films modified with a variety of plasticizers showed the loss of low-molecular weight plasticizers over time (Orliac, Rouilly, Silvestre, & Rigal, 2003), except for glycerol and triethylene glycol, which remained stable for three months. Regarding soybean proteins, it was found that SPI films obtained by intensive mixing followed by compression molding, evidenced a macroscopic diffusion of the plasticizer after 14 weeks, though their physical properties remained statistically invariable(Cunningham, Ogale, Dawson, & Acton, 2000). Compression-molded SPI plastics added with 1M or 2M urea or glycerol were studied during 60 days. All SPI

plastics became stiffer and brittle during storage; however, those plasticized with glycerol or modified with 2M urea were fairly stable after 60 days (X. Mo & Sun, 2003).

Our current work is focused on the valorization of soybean protein concentrate (SPC, about 70% protein content) as a suitable material for packaging films (Ciannamea, Stefani, & Ruseckaite, 2014). SPC is cheaper than the isolate (SPI, about 90% protein) and can be obtained from the oilcake for non-food applications. SPC has been successfully converted into biodegradable packaging films with an excellent oxygen barrier and adequate tensile properties via intensive mixing followed by compression molding (Ciannamea, Stefani, & Ruseckaite, 2014). The main objective of this work is to determine the temporal stability of SPC films by studying the evolution of their functional properties over three months of storage at 25 ± 2 ºC and 65 ± 2% RH.

2. Materials and methods

2.1. Chemicals. Commercial soy protein concentrate (SPC, 69% protein content, Solcom S110) was supplied byCordis S.A. (Villa Luzuriaga, Buenos Aires, Argentina). Glycerol (Gly, 98%), phosphate buffer solution pH 10, sodium dodecyl sulfate (SDS), urea and sodium azide were purchased from Anedra (Buenos Aires, Argentina). Calcium chloride and 2-mercaptoethanol (2-ME) were obtained from Aldrich (St. Louis, USA). TRIZMA/hydrochloric acid, glycine, Na_2EDTA (Ethylenediaminetetraacetic acid) and trichloroacetic acid (TCA) were supplied by Biopack (Buenos Aires, Argentina).

2.2. Film forming process and storage. SPC films were obtained by compression molding, based on the thermoplastic properties of plasticized-soybean proteins, and following the procedure described in our previous work (Ciannamea, Stefani, &

Ruseckaite, 2014).The SPC films obtained (average thickness: 150 µm) were stored at 25±2ºC and 65±2% RH for 90 daysin a laboratory environmental chamber. Three replications of each film were tested for their statistical analysis. Films were named as M-SPCX, where X indicates Gly concentration (% w/w SPC dry basis).

2.3. Film characterization

2.3.1. Thickness. Thickness (L) was measured by using a manual micrometer (0-25 ± 0.01 mm, Venier, China) at five random locations from three films of each formulation, and averaged.

2.3.2. Equilibrium moisture content (MC). Moisture content was expressed as the percentage of the initial film weight lost, after drying squared-shaped SPC film specimens at 105 ±1 ºC for 24 h in an air-circulating oven (Memmert, Germany). Reported values are the average of three replicates.

2.3.3. Gas-Chromatography analysis. Glycerol content (before and after 90 days of storage) was quantified using an Agilent 7890A gas chromatograph (Agilent, Palo Alto, USA) equipped with a flame ionization detector (GC-FID) and a capillary column Agilent (R) Glycerides (10 m x 320mm x 0.1 mm). Helium was used as a carrier gas (1 cm^3/min) in constant flow mode (3 cm^3/min) with a total GC run time of 21 min. The initial oven temperature was 50°C for 1 min, increased at 20°C/min up to 150ºC and then up to 370ºC at15ºC/min. The detector temperature was 300ºC. Fresh and aged M-SPC film samples (0.5 g) were gridded and extracted with distilled water (10 cm^3) for 24 h. The extract was then diluted 1:10 with methanol and added with 200 ppm of ethyleneglycol as internal standard. The peak areas were reported as a function of the injected concentration, and the calibration curves were obtained by linear regression. Standard solutions of glycerol were prepared in 9:1 methanol-water from 25 up to 125

mg/l. Each value of the calibration curve was the average of five replicates. Reported results are the average of three replicates.

2.3.4. Fourier transform infrared spectroscopy (FTIR).FTIR spectra were recorded on a Thermo Scientific Nicolet 6700 spectrometer (Wisconsin, USA) equipped with an attenuated total reflectance module (ATR) with a diamond crystal. A total of 32 scans at room temperature were performed at a resolution of 4 cm^{-1}, between 4000 and 400 cm^{-1}.

2.3.5.Differential solubility assays. Interactive forces stabilizing the films were assessed by Hager's method (Hager, 1984) with minor modifications (Ciannamea, Stefani, & Ruseckaite, 2014, 2015). Film samples of approximately 150 mg were set into tubes containing 3 cm^3 of water (blank) or denaturizing solution and let stand at 20ºC for 24 h. Extraction buffers were as follows: S4: 0.086 M TRIZMA/HCl, 0.09 M glycine and 4mM Na_2EDTA + 5 mg cm^{-3} SDS and 8 M urea and S5: S4 + 25 $mg.cm^{-3}$ 2-ME. After each extraction, samples were centrifuged at 9000 x g (Labnet International, USA) for 20 min. Protein content in the supernatant was determined by the Biuret method using stock solutions of human albumin (ZLB Behring) as standards. Protein solubility was expressed as grams of soluble protein per 100 g of protein in the film. All determinations were conducted in triplicate.

2.3.6. Opacity. The light absorption of SPC films was measured in a wavelength range from 400 to 800 nm, using a UV-Visible spectrophotometer Agilent 8453 (United States) according to the method described elsewhere(Gontard, Guilbert, & Cuq, 1992). Each film specimen was cut into rectangular strips and placed directly in the spectrophotometer test cell. Air was used as reference. Film opacity was expressed as the area under the absorption curve per thickness unit (arbitrary units/nm).All values reported were from four replicates.

2.3.7. Mechanical testing. Tensile properties were evaluated using an Universal Testing Machine (Instron 4467, Buckinghamshire, England), equipped with a 0.5 kN cell and at a crosshead speed of 3 mm/min. Tensile strength (TS), percentage of elongation at break (ε_b) and Young modulus (E) were calculated following the method described in ASTM D1708-0 on ten micro-tensile specimens of each film formulation.

2.3.8. Barrier properties. Water vapor permeability (WVP) was determined gravimetrically according to ASTM method E96-95. Films were placed on poly(methyl methacrylate) circular cups containing dry $CaCl_2$, with an exposed area of 20 cm^2 (A). The system was placed in a controlled humidity chamber at 65% RH and 25 °C and weighed at 30 min intervals over 10 h. The slope of the mass change Δm (g) vs. t (h) gave the water vapor transmission rate through the film WVTR (g / h). Then, permeability was obtained as:

$$WVP = WVTR \cdot L / [A \cdot S \cdot (HR_1 - HR_2)] \qquad (Kg.m/m^2.s.Pa) \qquad (1)$$

where *L* is the film thickness, *S* is the vapor pressure of pure water at test temperature, and $(HR_1\text{-}HR_2)$ is the relative humidity gradient used in the assay. Three replicates of each film were used for WVP testing.

The oxygen permeability coefficient (OPC) was determined by using an oxygen permeability analyzer Model 8500 Systech Instruments (Oxon, UK), according to the procedure described in ASTM 3985. Film samples with a diameter of 14 ± 0.5 cm were placed in the analyzer at 25 °C. The OPC was calculated from the oxygen transfer rate OTR (cm^3/m^2.day) as:

$$OPC = (OTR \cdot L) / \Delta P \qquad (cm^3.\mu m/(m^2.day.KPa)) \qquad (2)$$

where *L* is the film thickness (μm) and ΔP is the partial pressure gradient of oxygen through the film (kPa).

2.3.9. Statistics. Experimental data were statistically analyzed using the one-way analysis of variance (ANOVA) along with Tukey's tests at 95% confidence interval(α=0.05).

3. RESULTS AND DISCUSSION

3.1. Evolution of plasticizer content and stabilizing interactions

Aged M-SPC films were visually comparable to those freshly prepared, although glycerol migrated toward the surface during storage at 25±2ºC and 65±2 % RH, evidenced by their sticky surface on touching. Similar observations were reported in the past for other plasticized protein films obtained using a variety of processing methods (Anker, Stading, & Hermansson, 2001; Ciannamea, Stefani, & Ruseckaite, 2015; Cunningham, Ogale, Dawson, & Acton, 2000; Orliac, Rouilly, Silvestre, & Rigal, 2003). All M-SPC films reduced their initial weight toward the end of the analyzed storage period (Table 1). This is in agreement with the migration of low-molar mass compounds, i.e., bonded water and glycerol (Anker, Stading, & Hermansson, 2001; Ogale, Cunningham, Dawson, & Acton, 2000; Olabarrieta, Gällstedt, Ispizua, Sarasua, & Hedenqvist, 2006; Sothornvit & Krochta, 2001).

Water loss over time was confirmed by the slight, though significant, decline in MC values (Table 1, $p<0.05$), which indirectly demonstrates glycerol migration(Orliac, Rouilly, Silvestre, & Rigal, 2003). Glycerol level was quantified by subtracting the amount of plasticizer that remained in the film after 90 days of storage from the actual quantity of glycerol measured in the fresh film, determined by GC-FID (Table 1). The films retained more than 85% of the initial plasticizer (Table 1), at any plasticizer level, thereby suggesting that most glycerol molecules had already occupied positions in the protein network linked through hydrogen bonds to C=O amide groups (Lefèvre,

Subirade, & Pézolet, 2005), so aging provoked minor changes in the network structure (Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004; Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006).

The role that water and glycerol play in regulating the time-dependent evolution of protein secondary structures in M-SPC films, which dictates their physical properties, was analyzed by the second derivative of the amide I band (between 1600 cm^{-1}-1700 cm^{-1}) of ATR-FTIR spectra (Lefèvre, Subirade, & Pézolet, 2005). Amide I is representative of the secondary structures of proteins and is governed by the stretching vibration of C=O and, to a lesser extent, by the C–N stretching vibration of the peptide bonds(Ciannamea, Stefani, & Ruseckaite, 2015; Chen & Subirade, 2009). Un-aged films were characterized by the overlapping of peaks, regardless of glycerol level, with prominent features at 1694 cm^{-1}, 1651cm^{-1}, 1645cm^{-1}, 1620 $cm^{-1}$and a shoulder at 1637 cm^{-1} assigned to β-turns, α-helix, unordered structures, intermolecular and extended β-sheet arrangements, respectively (Fig 1) (Lefèvre, Subirade, & Pézolet, 2005; Subirade, Kelly, Guéguen, & Pézolet, 1998). Interestingly, aged films with different glycerol content behaved differently. The second derivative of aged M-SPC50 films superimposed that of the un-aged one, suggesting that secondary protein structures barely change with time. Conversely, aged M-SPC30 films exhibited a retraction and a shift of the band at 1620 cm^{-1} toward lower wavenumber, indicating that part of the aggregates that formed during heat compression revert with time, while others are irreversibly formed through S-S (Subirade, Kelly, Guéguen, & Pézolet, 1998).The evolution of the shoulder at 1637 cm^{-1} toward a more defined band at 1630 cm^{-1},along with the contraction of the band at1645 cm^{-1}, implies a partial reorganization of unordered structures into β-sheet structures (Lefèvre, Subirade, & Pézolet, 2005; Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006; Ullsten, Cho,

Spencer, Gällstedt, Johansson, & Hedenqvist, 2009). This behavior may be correlated with the migration of glycerol and water, as previously reported (Ciannamea, Stefani, & Ruseckaite, 2015). Lefèvre et al. (2005) stated that the loss of plasticizer in β-lactoglobulin films not only reduced the elasticity, but also provided a favorable environment for specific molecular reorganization of polypeptide chains (i.e., β-sheets), which was prevented at a high plasticizer level (Lefèvre, Subirade, & Pézolet, 2005). Overall, the loss of glycerol and water in M-SPC30 films promotes the gradual reduction of disordered structures, in favor of more organized and aggregated β-sheet structures.

Aggregation also involves the rearrangement of intra-molecular disulfide into intermolecular disulfide bonds via sulfhydryl-disulfide interchange reactions (Ciannamea, Stefani, & Ruseckaite, 2015; Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004; Morel, Bonicel, Micard, & Guilbert, 2000; Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006; Ullsten, Gällstedt, Johansson, Gräslund, & Hedenqvist, 2006). The fact that the predominance of disulfide overelectrostatic, hydrogen and hydrophobic interactions contributes to the stability of the protein network in un-aged M-SPC films has been reported in an earlier study (Ciannamea, Stefani, & Ruseckaite, 2014). Figure 2 illustrates the representative protein solubility pattern for aged M-SPC30 in buffer S4 (able to break hydrogen, hydrophobic and electrostatic interactions); in S5, which breaks all the aforementioned plus disulfide bridges; and the S5-S4 difference, representing the only contribution of disulfide bridges. The amount of soluble protein in S4 and S5 was reduced during aging (Fig. 2),suggesting protein aggregation with time. This is associated with conformational changes or protein cross-linking. The age-related role of sulfhydryl-disulfide interchange reaction in protein aggregation was experimentally supported by the

increasing contribution of disulfide bonds, as determined by the increment in the difference of protein solubility between S5-S4 (Fig. 2).

3.2. Tensile, opacity and barrier properties

Fig. 3 a-c provides information about the evolution of the elastic modulus, tensile strength and elongation at break of M-SPC films over time. As aging time increased, a gradual enhancement in film toughness was observed (higherTS and E values, $p<0.05$; Fig 3a,c), due to the lower plasticization of the films caused by glycerol migration (Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004; X. Mo & Sun, 2003; Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006; Orliac, Rouilly, Silvestre, & Rigal, 2003).The elongation at break, in turn, changed marginally, except for a reduction of about 21% ($p<0.05$)registered in M-SPC30 films (Fig 3b). A plausible explanation for this behavior is the development of more rigid β-structures experienced by aged M-SPC30 films (Fig.1). A slight increment in TS and a reduction in ε_b was observed in compression molded sunflower protein films plasticized with glycerol over a three-month period (Orliac, Rouilly, Silvestre, & Rigal, 2003). On the contrary, compression molded SPI based films plasticized with 30 and 35% glycerol did not experience significant changes in TS and ε_b during the same period of time, while elongation was reduced with 40% glycerol, indicating certain plasticizer loss (Cunningham, Ogale, Dawson, & Acton, 2000). All in all aged M-SPC films are still able to meet the requirements for actual applications.

The opacity of M-SPC films did not vary after 90 days of storage ($p> 0.05$, Table 2). This contradicts what was previously observed for casted SPC films aged under similar conditions (Ciannamea, Stefani, & Ruseckaite, 2015), revealing that constancy in such parameters is strongly dependent on the processing method applied.

Moreover WVP values remained stable after 3 months (Table 2, $p>0.05$), except for M-SPC30 films, which registered a slight increase is such parameter with time ($p<0.05$). Nevertheless, the water vapor permeability of M-SPC30 films remained the lowest among the films analyzed. An increased WVP value indicates that water vapor would penetrate easily through the film. An increased WVP value indicates that water vapor could penetrate easily through the film. This finding seems to be contrary to the observed protein aggregation and reorganization into more ordered structures as well as to loss of hydrophilic components (i.e. glycerol). Anker et al (2001) linked the increment in the WVP of aged whey protein isolated films with an increased pore size. In the present case, M-SPC30 films experienced the higher change in the mechanical properties with time, thus the occurrence of pinholes or micro-cracks during manipulation and WVP measurement operation is highly probable. This finding is more likely related to morphological changes in SPC films over time. Anker et al. (2001) related the increment in WVP of aged whey protein isolated films to an increased pore size. In the present case, M-SPC30 films experienced the greatest change in their mechanical properties. As a consequence, the occurrence of pinholes or micro-cracks during manipulation and WVP measurement operation is highly likely.

The OPC of MSPC films increased slightly after 90 days, although differences were only significant for M-SPC40 films ($p<0.05$, Table 2). Like for WVP, OPC would be expected to decrease during aging. However, the decrease in permeability may be compensated by a more permeable structure generated by the loss of plasticizer and protein aggregation (Olabarrieta, Cho, Gällstedt, Sarasua, Johansson, & Hedenqvist, 2006). Despite the raise in OPC after 90 days, all films kept on displaying a good barrier against oxygen, with an OPC below 50 $cm^3 \mu m/(m^2.day.KPa)$.

4. Conclusions

This study reveals the relevance of assessing the stability of plasticized SPC films obtained using compression molding upon storage under specific conditions before proposing any future technological application. The loss of glycerol, protein aggregation, and the evolution to a more organized and cohesive protein network gave experimental evidence of the embrittlement of aged M-SPC films with time. Nonetheless, other properties of interest, such as opacity and WVP, remained almost invariable, attesting the adequate stability of M-SPC films for the timeframe of the study. The high retention rate of glycerol over time (about 85% at any glycerol level), proves that glycerol could be a suitable plasticizer for the thermoplastic processing of SPC into films. Overall, these findings show that M-SPC films plasticized with glycerol can maintain relatively constant and convenient properties for industrial applications, providing a control of the storage conditions as well as the water activity of the foodstuff to be packed.

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References

Anker, M., Stading, M., & Hermansson, A.-M. (2001). Aging of Whey Protein Films and the Effect on Mechanical and Barrier Properties. *Journal of Agricultural and Food Chemistry, 49*(2), 989-995.

Ciannamea, E. M., Stefani, P. M., & Ruseckaite, R. A. (2014). Physical and mechanical properties of compression molded and solution casting soybean protein concentrate based films. *Food Hydrocolloids, 38*(0), 193-204.

Ciannamea, E. M., Stefani, P. M., & Ruseckaite, R. A. (2015). Storage-induced changes in functional properties of glycerol plasticized–Soybean protein concentrate films produced by casting. *Food Hydrocolloids, 45*, 247-255.

Cunningham, P., Ogale, A. A., Dawson, P. L., & Acton, J. C. (2000). Tensile Properties of Soy Protein Isolate Films Produced by a Thermal Compaction Technique. *Journal of Food Science, 65*(4), 668-671.

Chen, L., & Subirade, M. (2009). Elaboration and Characterization of Soy/Zein Protein Microspheres for Controlled Nutraceutical Delivery. *Biomacromolecules, 10*(12), 3327-3334.

Debeaufort, F., Quezada-Gallo, J., & Voilley, A. (2002). Edible films and coatings as aroma barriers. In, (pp. 579-600): CRC Press, Boca Raton, FL.

Gällstedt, M., Mattozzi, A., Johansson, E., & Hedenqvist, M. S. (2004). Transport and Tensile Properties of Compression-Molded Wheat Gluten Films. *Biomacromolecules, 5*(5), 2020-2028.

Gontard, N., Guilbert, S., & Cuq, J.-L. (1992). Edible Wheat Gluten Films: Influence of the Main Process Variables on Film Properties using Response Surface Methodology. *Journal of Food Science, 57*(1), 190-195.

Guerrero, P., Stefani, P. M., Ruseckaite, R. A., & de la Caba, K. (2011). Functional properties of films based on soy protein isolate and gelatin processed by compression molding. *Journal of Food Engineering, 105*(1), 65-72.

Hager, D. F. (1984). Effects of extrusion upon soy concentrate solubility. *Journal of Agricultural and Food Chemistry, 32*(2), 293-296.

Hernandez-Izquierdo, V. M., & Krochta, J. M. (2008). Thermoplastic Processing of Proteins for Film Formation—A Review. *Journal of Food Science, 73*(2), R30-R39.

Klüver, E., & Meyer, M. (2014). Thermoplastic processing, rheology, and extrudate properties of wheat, soy, and pea proteins. *Polymer Engineering & Science*.

Lefèvre, T., Subirade, M., & Pézolet, M. (2005). Molecular Description of the Formation and Structure of Plasticized Globular Protein Films. *Biomacromolecules, 6*(6), 3209-3219.

Mo, X., & Sun, X. (2003). Effects of Storage Time on Properties of Soybean Protein-Based Plastics. *Journal of Polymers and the Environment, 11*(1), 15-22.

Mo, X., & Sun, X. S. (2013). Soy proteins as plywood adhesives: formulation and characterization. *Journal of Adhesion Science and Technology, 27*(18-19), 2014-2026.

Morel, M. H., Bonicel, J., Micard, V., & Guilbert, S. (2000). Protein Insolubilization and Thiol Oxidation in Sulfite-Treated Wheat Gluten Films during Aging at Various Temperatures and Relative Humidities. *Journal of Agricultural and Food Chemistry, 48*(2), 186-192.

Ogale, A. A., Cunningham, P., Dawson, P. L., & Acton, J. C. (2000). Viscoelastic, Thermal, and Microstructural Characterization of Soy Protein Isolate Films. *Journal of Food Science, 65*(4), 672-679.

Olabarrieta, I., Cho, S.-W., Gällstedt, M., Sarasua, J.-R., Johansson, E., & Hedenqvist, M. S. (2006). Aging Properties of Films of Plasticized Vital Wheat Gluten Cast from Acidic and Basic Solutions. *Biomacromolecules, 7*(5), 1657-1664.

Olabarrieta, I., Gällstedt, M., Ispizua, I., Sarasua, J.-R., & Hedenqvist, M. S. (2006). Properties of Aged Montmorillonite–Wheat Gluten Composite Films. *Journal of Agricultural and Food Chemistry, 54*(4), 1283-1288.

Orliac, O., Rouilly, A., Silvestre, F., & Rigal, L. (2003). Effects of various plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. *Industrial Crops and Products, 18*(2), 91-100.

Paetau, I., Chen, C.-Z., & Jane, J.-l. (1994). Biodegradable Plastic Made from Soybean Products. 1. Effect of Preparation and Processing on Mechanical Properties and Water Absorption. *Industrial & Engineering Chemistry Research, 33*(7), 1821-1827.

Singh, P., Kumar, R., Sabapathy, S. N., & Bawa, A. S. (2008). Functional and Edible Uses of Soy Protein Products. *Comprehensive Reviews in Food Science and Food Safety, 7*(1), 14-28.

Sothornvit, R., & Krochta, J. M. (2001). Plasticizer effect on mechanical properties of [beta]-lactoglobulin films. *Journal of Food Engineering, 50*(3), 149-155.

Subirade, M., Kelly, I., Guéguen, J., & Pézolet, M. (1998). Molecular basis of film formation from a soybean protein: comparison between the conformation of glycinin in aqueous solution and in films. *International Journal of Biological Macromolecules, 23*(4), 241-249.

Ullsten, N. H., Cho, S.-W., Spencer, G., Gällstedt, M., Johansson, E., & Hedenqvist, M. S. (2009). Properties of Extruded Vital Wheat Gluten Sheets with Sodium Hydroxide and Salicylic Acid. *Biomacromolecules, 10*(3), 479-488.

Ullsten, N. H., Gällstedt, M., Johansson, E., Gräslund, A., & Hedenqvist, M. S. (2006). Enlarged Processing Window of Plasticized Wheat Gluten Using Salicylic Acid. *Biomacromolecules, 7*(3), 771-776.

Wu, Q., & Zhang, L. (2001). Properties and Structure of Soy Protein Isolate−Ethylene Glycol Sheets Obtained by Compression Molding. *Industrial & Engineering Chemistry Research, 40*(8), 1879-1883.

Figure captions

Figure 1. Second derivative of the ATR-FTIR spectra. Amine I region of un-aged and 90-day aged M-SPC30 and M-SPC50 films.

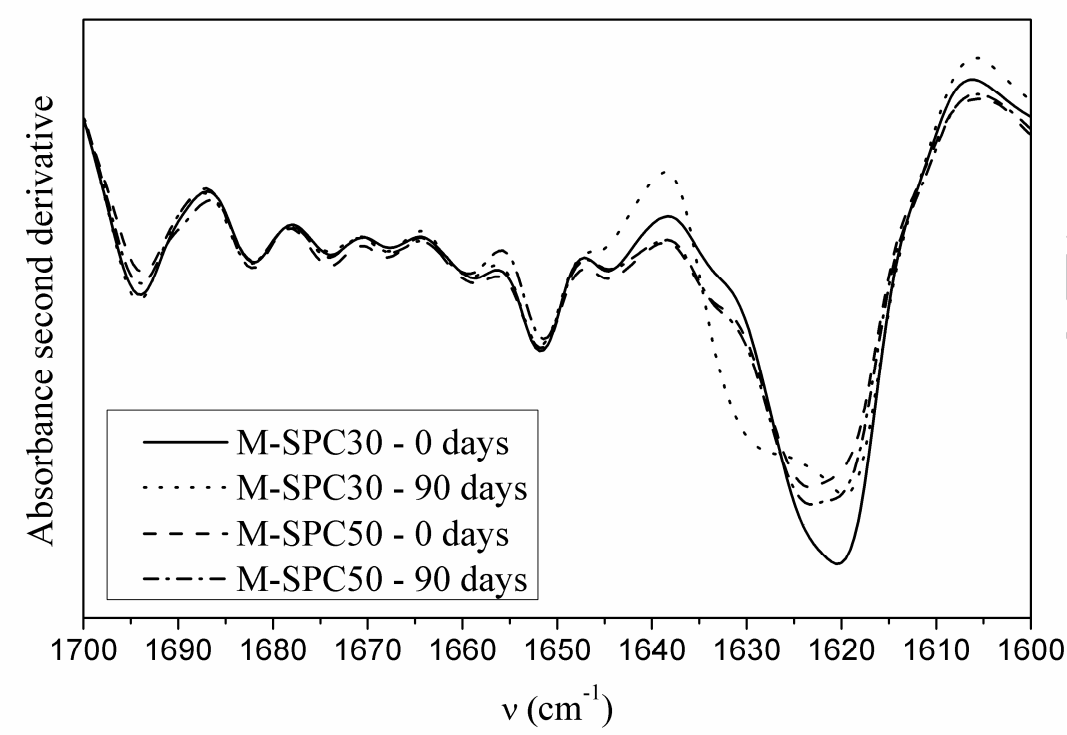
Figure 2. Solubility of M-SPC30 films in different denaturing buffers, fresh and after 90 days of storage. S4: 0.086 M TRIZMA/HCl, 0.09 M glicine, 4mM Na_2EDTA, 5 mg ml^{-1} SDS and 8 M urea; S5: S4 + 25 mg ml^{-1} 2-ME.

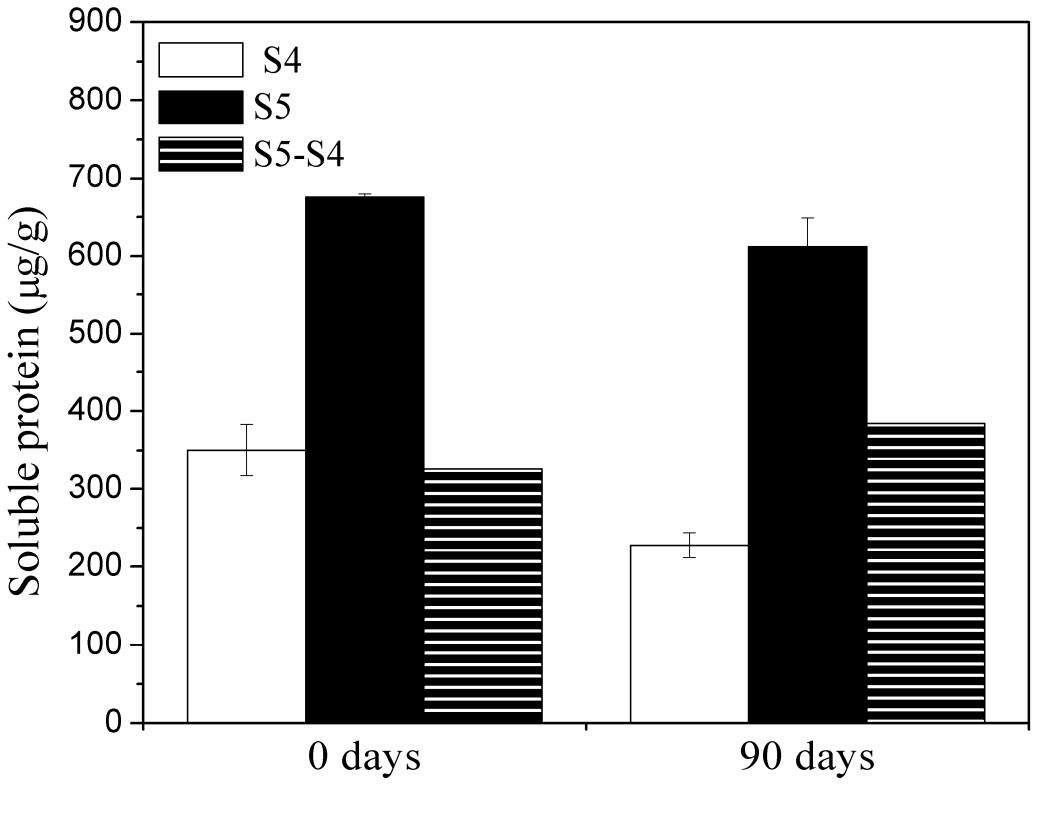
Figure 3. Evolution of mechanical properties of SPC films with time. a) Tensile strength (TS); b) Percentage of elongation at break (ε_b); c) Young modulus (E).

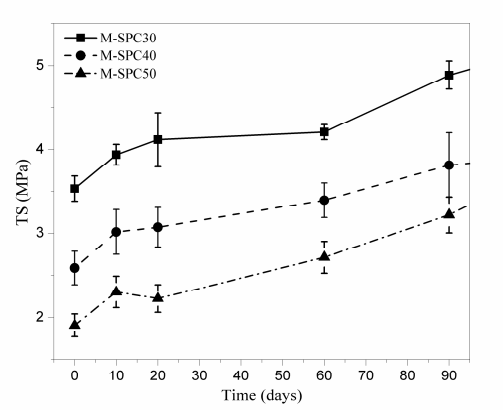
Tables

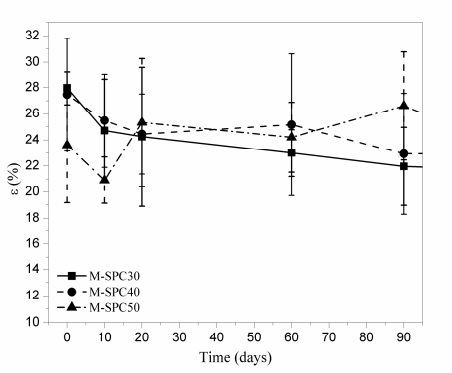
Table 1. Mass loss, residual glycerol concentration and evolution of moisture content (MC) of M-SPC films over time.

Table 2. Evolution of thickness, opacity and barrier properties of M-SPC films over time









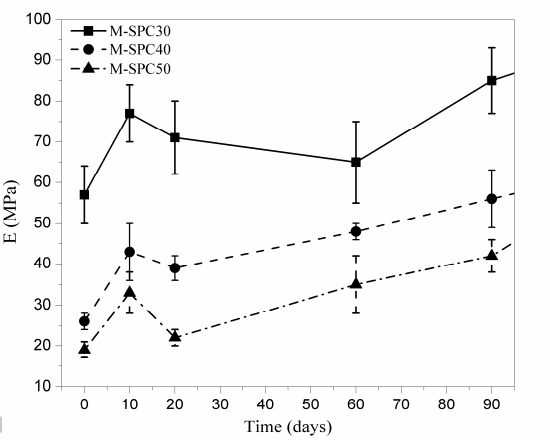


Table 1. Mass loss, residual glycerol concentration and evolution of moisture content (MC) of M-SPC films with time.

	Time (days)	Gly (% w/ w SPC)		
		30	40	50
Mass loss	90	-2.6 ± 0.8	-3.8 ± 0.7	-3.5 ± 0.8
MC (%)	0	22.5 ± 0.5 a	28.7 ± 0.3 a	34.9 ± 0.4 a
	90	20.4 ± 0.7 b	28.6 ± 0.7 a	34.1 ± 0.7 b
Residual Gly content (%)		88.3 %	87.9 %	88.5%

Mean values ± standard deviations. Mean values within the same column followed by the same letter are not significantly different ($p > 0.05$, Tukey's test).

Table 2. Evolution of thickness, opacity and barrier properties of M-SPC films with time

	Time (days)	Gly (%) 30	40	50
Thickness (µm)	0	139 ± 14 a	135 ± 14 a	148 ± 10 a
	90	137 ± 18 a	133 ± 14 a	148 ± 9 a
Opacity (AU.nm)	0	668 ± 4 a	618 ± 7 a	580 ± 18 a
	90	681 ± 12 a	612 ± 11 a	579 ± 2 a
WVP $* 10^{13}$ *(Kg.m/m^2.s.Pa)*	0	1.6 ± 0.0 a	3.00 ± 0.1 a	4.0 ± 0.1 a
	90	1.9 ± 0.1 b	2.9 ± 0.3 a	4.1 ± 0.4 a
OPC (cm^3.µm/(m^2.day.KPa))	0	15.2 ± 2.0 a	27.1 ± 0.0 a	47.4 ± 1.4 a
	90	18.4 ± 8.1 a	38.2 ± 2.5 b	49.2 ± 3.0 a

Mean values ± standard deviations. Mean values within the same column followed by the same letter are not significantly different ($p > 0.05$, Tukey test).

- Compression-molded soy protein concentrate (M-SPC) films lost glycerol and water over time.
- Aging promoted the reorganization of soy protein secondary structure.
- Tensile strength and oxygen permeability were increased in aged films, while opacity and water vapor permeability were statistically unchanged.
- M-SPC films were more satable than casting films under similar storage conditions.