Food Hydrocolloids 33 (2013) 85–91

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Cold-set gelation of high pressure-treated soybean proteins

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ARTICLE INFO

Article history:
Received 28 September 2012
Accepted 1 March 2013

Keywords:
Soybean proteins
Cold-set gelation
High pressure

ABSTRACT

The ability of soybean proteins to form cold-set gels, using high pressure (HP) processing as denaturing agent and calcium incorporation was evaluated. Different protein preparations were assayed: soybean protein isolate (SPI), a β-conglycinin enriched fraction (7SEF) and a glycinin enriched fraction (11SEF). 7SEF formed aggregated gels with low water holding capacity whereas 11SEF did not form self-standing gels. SPI formed the better gels: ordered and with high water holding capacity. SPI gels were relatively soft and their visual aspect, rheological and texture properties, and water holding capacity depended on HP level (400–600 MPa), $CaCl_2$ concentration (0.015–0.020 mol L^{-1}) and protein concentration (85–95 g L^{-1}), thus gels with different characteristics may be obtained. The gels comprised a three dimensional network stabilized by non-covalent interactions with spaces filled of proteins in aqueous solution. The results indicate that it is possible to use HP and subsequent calcium incorporation to form self-standing cold-set gels from soybean proteins. These gels may be of interest to incorporate heat-labile compounds or probiotics during the gelation step.

1. Introduction

Soybean represents a very important crop due to its agronomic features – such as ability to grow in a wide range of soils, high yield – and its oil and protein content. Because of their functional properties, low cost, availability and high nutritional value, soybean proteins are extensively used in food manufacturing. These are mainly composed of the storage globulins β-conglycinin, of *ca.* 180 kDa, made up of three polypeptides (α, α′ and β) and glycinin, of *ca.* 360 kDa, made up of six subunits AB, (Badley et al., 1975; Thanh & Shibasaki, 1977). These proteins may be prepared as soybean protein isolates (with approximately equal quantities of glycinin and β-conglycinin) or partially purified glycinin or β-conglycinin fractions, with a higher proportion of each protein.

Gelation is an important functional property of soybean proteins (Nagano, Hirotsuka, Mori, Kohiyama, & Nishimari, 1992). Heat-induced gelation has been widely studied. For this phenomenon to take place, proteins are heated (80–95 °C) so that they are denatured and so that they can establish a three-dimensional network, provided that the protein concentration is higher than a critical value (Renkema & van Vliet, 2004). Gelation of soybean proteins may also be carried out at a low temperature, provided that proteins are pre-denatured (by thermal processing) at a concentration lower than the critical value, and that certain conditions (increase in ionic strength, decrease in pH or calcium concentration) are further modified, at low temperature (Maltais, Remondetto, Gonzalez, & Subirade, 2005). Cold-set gelation allows heat-labile or volatile compounds to be added during the gelation step without losing flavor or other properties (Weijers, van de Velde, Stijnman, van de Pijpekamp, & Visschers, 2006). Cold-set gels were assayed as bioactive compounds delivery devices. Cold-set gels showed they might be useful for transporting bioactive molecules through the gastrointestinal tract and for delivering them in the small intestine (Maltais, Remondetto, & Subirade, 2009).

The processing and composition conditions affect final properties of cold-set gels. Maltais et al. (2005) found that calcium concentration was an important factor; an ordered, filamentous gel was obtained at low concentration, while a disordered, phase-separated or aggregated gel was obtained at higher concentration. Lu, Lu, Yin, Cheng, and Li (2010) found that the variation of preheating temperature generated different protein species that, in the presence of calcium, produced gels that showed different dynamic viscoelasticity.

High pressure (HP) is an emerging technology focused on food preservation, but it may also be applied in order to modify functional properties of ingredients. HP denatures soybean proteins:

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http://dx.doi.org/10.1016/j.foodhyd.2013.03.001

glycinin is 100% denatured after a 400 MPa treatment, while β-conglycinin conserves about a 30% of native structure after a 600 MPa treatment. This reflects their different sensitivity to HP. These denatured proteins form species whose molecular weights depend on HP level (Puppo et al., 2004; Speroni, Jung, & de Lamballerie, 2010).

The aim of this work was to analyze if HP-induced alterations may provide to soybean proteins the ability to form gels after calcium addition at low temperature. These gels should be of great interest to manufacturers of innovative functional foods.

2. Materials and methods

2.1. Preparation of soybean protein isolate, glycinin-enriched fraction and β-conglycinin-enriched fraction

Soybean protein isolate (SPI) was prepared by isoelectric precipitation from defatted soybean flour (ADM, Decatur, USA) according to Speroni, Añón, et al. (2010) and Speroni, Jung, et al. (2010). SPI contained mainly β-conglycinin and glycinin in similar proportions and small quantities of lectin and Kunitz trypsin inhibitor; its protein content was 86.6% (Speroni, Añón, et al., 2010; Speroni, Jung, et al., 2010).

Glycinin-enriched fraction (11SEF) and β-conglycinin-enriched fraction (7SEF) were prepared by differential precipitation, using the same flour as the one used for SPI, according to Speroni, Añón, et al. (2010) and Speroni, Jung, et al. (2010). Extraction at pH 8.0 was followed by precipitation at pH 6.4 in the presence of $NaHSO_3$, at 4 °C during 12 h. The dispersion was centrifuged and the precipitate was suspended in distilled water, adjusted to pH 8.0, and then dialyzed and freeze-dried to obtain 11SEF. Solid NaCl was added to the supernatant (0.25 mol L^{-1}) and the pH was adjusted to 5.0. After 1 h, the insoluble intermediate mixture was removed by centrifugation. The supernatant was 2-fold diluted with cold water and the pH was adjusted to 4.8. Centrifugation was carried out and the precipitate was suspended in distilled water, adjusted to pH 8.0, and dialyzed before freeze-drying to obtain 7SEF. 7SEF contained β-conglycinin in a high proportion (75%), a low proportion of glycinin (25%) and a protein content of 84.5%. 11SEF was almost fully composed of glycinin and a protein content of 93.0% (Speroni, Añón, et al., 2010; Speroni, Jung, et al., 2010).

2.2. Protein dispersions

Dispersions of SPI, 7SEF and 11SEF and mixtures of 7SEF-11SEF in a 63 and 37, 50 and 50, 37 and 63, 25 and 75% proportion of β-conglycinin and glycinin respectively were prepared in distilled water at 9.0% w/v and pH 8.0. Dispersions of SPI in distilled water at 8.5% w/v and pH 8.0 were also prepared.

2.3. High pressure treatment

Dispersions of SPI, 7SEF and 11SEF and their mixtures underwent HP treatment at 200, 400, or 600 ± 5 MPa for 10 min in a 2.0 L reactor unit model FPG 9400:922 (Stansted Fluid Power Ltd, United Kingdom) equipped with temperature and pressure regulation. Prior to pressure processing, protein dispersions were vacuum conditioned in polyethylene bags. A mixture of propylene glycol and water (30:70) was used as pressure-transmitting medium. The target pressure was reached at 6.5 MPa s^{-1} and released at 20 MPa s^{-1}. The adiabatic heating was manifested as an increase in temperature that reached a maximum at 600 MPa. In that case, a transient increase was verified and temperature of reactor reached up to 33.5 °C.

2.4. Gel formation

Calcium chloride was added from a stock solution of 1 mol L^{-1}, prepared from $CaCl_2$ dihydrate (Sigma, St Louis, USA), to HP-treated protein dispersion and immediately magnetically stirred. Samples were centrifuged at 2000 × *g* for 30 s at 20 °C to separate foam. Dispersions were deposited in acrylic cylinders of 14 mm diameter and stored for 10 h at 4 °C. The bottom of the cylinders was removable so that gels could be removed by a piston before being tested.

Along this work samples were identified by the figures of protein concentration (% w/v)-calcium concentration (mol L^{-1})-HP level (MPa). By example 9.0–0.020–600 means that the sample contained 9.0% w/v protein, was treated at 600 MPa and 0.020 mol L^{-1} $CaCl_2$ was then incorporated.

2.5. Water holding capacity

Portions of gels of about 1 g were centrifuged at 19,000 × *g* for 20 min at 4 °C. Supernatant was separated and the pellet was weighted. Water loss was determined by weighing before and after centrifugation. Water holding capacity was expressed as percent of the initial water remaining in the gel after centrifugation.

2.6. Color

Color of gels was determined using a Minolta Chroma meter (CR 300, Minolta Chroma CO., Osaka, Japan). The instrument was standardized using a white calibration plate. A CIE Lab color scale was used, and L^* (lightness), a^* (related with the red (positive values) − green (negative values) opposition) and b^* (related with the yellow (positive values) − blue (negative values) opposition) were determined. Since some gels were transparent, the determinations were carried out on 5 mm thick portions of gels laid on a white surface so that the effect of transparence could be standardized.

2.7. Small deformation rheology

Dynamic rheological measurements (storage modulus (G') and loss modulus (G'') *vs.* frequency) were carried out using a serrated plate-and-plate geometry (35 mm diameter, 1 mm gap) using a Controlled Stress Rheometer Haake RS 600 (Thermoelectron, Karlsruhe, Germany). Frequency of oscillations ranged from 0.01 to 10 Hz. The linear viscoelasticity region was determined through stress sweep tests at a fixed frequency (1 Hz), all samples were analyzed at 1 Pa, which was within the linear range. Before starting the corresponding measurement, samples were allowed to rest for 10 min after positioning the sample on the sensor system. Temperature was 20 °C.

2.8. Texture profile analysis

Texture profile analysis was performed on gels using a TA.XT2 Texture analyzer (Stable Micro Systems Ltd., Surrey, United Kingdom) in the compression mode. Compression was exerted by a cylindrical probe with a flat section (75 mm diameter) at a displacement speed of 0.5 mm s^{-1}. The force (N) at 20% compression was measured. Since the gels were relatively soft and differed in strength depending on the formulation and HP treatment, their shape was modified after being removed from the cylinder. This means that the softest gels showed an increase in the area of the top face of the gel cylinder and a decrease in height. This fact was accompanied by the exposure of a higher area to the probe in the cases of the softest gels. In order to normalize this measurement,

the force at 20% compression (maximum force) was divided by the area of the gel at the moment of 20% compression. Thus, maximum force/area was used as indicator of gel hardness.

The measurements of gel adhesivity were performed with two compression cycles. Gel adhesivity was calculated as the negative force *vs*. displacement area (N mm) obtained after the first compression cycle, representing the work necessary to pull the compressing plunger away from the sample.

2.9. Molecular characterization using sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Proteins were extracted from gels (9.0–0.015–600 and 9.0–0.020–600) in a two-step sequence. The first one with distilled water to separate the molecules that were not part of the matrix (1:2 gel:water). The second step consisted of exposing the pellet of centrifugation from the first step (15,000 × *g* for 15 min at 10 °C) to one of the following solvents: Saline buffer (0.0325 mol L^{-1} K_2HPO_4, 0.0026 mol L^{-1} KH_2PO_4, 0.4 mol L^{-1} NaCl, pH 8.0); Saline buffer + SDS 10 g L^{-1}; Saline buffer + SDS 10 g L^{-1} + 6 mol L^{-1} urea; or Saline buffer + SDS 10 g L^{-1} + β-mercaptoethanol 5% v/v (in a boiling bath for 10 min), at 1:2 pellet:solvent ratio. The supernatants resulting from the two steps were treated with a buffer containing 0.125 mol L^{-1} Tris–HCl, 200 mL L^{-1} glycerol, 10 g L^{-1} SDS, 0.5 g L^{-1} bromophenol blue at pH 6.8, and were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out using a separating gel (120 g L^{-1} polyacrylamide) and a stacking gel (40 g L^{-1} polyacrylamide). These gels were prepared in a 0.375 mol L^{-1} Tris–HCl, 1 g L^{-1} SDS, pH 8.8 buffer, in a mini slabs system (Bio-Rad Mini-Protean II Model). Protein molecular weights were estimated using low MW markers (Pharmacia, Amersham, England) that included phosphorylase b (94.0 kDa), albumin (67.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa). Gels were fixed and stained with R-250 Coomasie blue (2 g L^{-1}) in water/methanol/acetic acid (5:5:2) overnight and distained with 25% v/v methanol and 10% v/v acetic acid. Gels images were acquired with a scanner HP Scanjet G2710.

2.10. Statistical analysis

Each treatment was performed at least in triplicate. The statistical analysis was completed using the Origin software (OriginLab Corporation, Northampton, MA, USA). Analyses of variance were conducted. Differences between the sample means were analyzed by Tukey's test at an α level of 0.05.

3. Results and discussion

3.1. Gel formation

The ability to form gels of HP-treated and calcium-added (0.015 and 0.020 mol L^{-1}) dispersions (9.0% w/v) of SPI, 7SEF, 11SEF and mixtures of 7SEF and 11SEF was evaluated. Three HP levels were assayed on SPI: 200, 400 and 600 MPa. After carrying out the treatment at 200 MPa, calcium addition brought about the insolubilization of proteins and did not form a space-filling network. After carrying out the treatments at 400 and 600 MPa, self-standing gels were formed from SPI after calcium addition. These phenomena may be explained by the degree of denaturation achieved by the proteins after those treatments (27.8, 80.6 and 84.3% for 200, 400 and 600 MPa respectively, Speroni, Añón, & de Lamballerie, 2010). It seems that the unfolding achieved after 200 MPa did not allow the exposure of enough reactive sites to bridge the polypeptides in a stable, ordered matrix, and consequently precipitated after calcium addition. When higher degrees of denaturation were achieved, polypeptides could align and were able to establish a network when calcium was added. These data indicate that, provided that a high degree of denaturation is reached, HP may be used as a denaturing process for cold-set gelation of soybean proteins.

Proteins in 11SEF samples, treated at 400 and 600 MPa, were insolubilized after calcium addition, and rapidly settled. Since glycinin solubility is very sensitive to calcium concentration, lower concentrations were assayed for this sample. After adding 0.005 or 0.010 mol L^{-1}, protein also settled and no network was formed, which indicated that it was not feasible to form gels from HP-treated 11SEF in our experimental conditions. Self-standing gels were obtained from 7SEF samples, treated at 400 and 600 MPa. The gels obtained from 7SEF had a low water holding capacity that was evidenced by a leak of liquid, and a high proportion of insoluble protein that was manifested as an opaque and phase-separated aspect. In order to evaluate the effect of protein composition on the gel formation ability, samples with 63; 50; 38 and 25% of β-conglycinin, created by mixture of 7SEF and 11SEF (total protein concentration 9.0% w/v), were assayed (600 MPa and 0.020 mol L^{-1} calcium). No gels were formed from the samples with 25 and 38% of β-conglycinin. Gels were obtained from the mixtures containing 50 and 63% of β-conglycinin. These gels were opaque and lost liquid (26 and 12% respectively of gel mass was separated as an aqueous solution after centrifugation, carried out at 19,000 × g for 20 min at 4 °C). These characteristics may be a consequence of phase separation induced by the mixing of 7SEF and 11SEF in those proportions. The gels resulting from SPI properly retained water and they seemed to have a low proportion of insoluble protein, as they were quite transparent. Considering that both the gels resulting from SPI and those from the mixture 50:50 have a similar proportion of glycinin and β-conglycinin but were very different, we hypothesize that some protein species that were present in SPI may be lost during fractions purification; those species would be needed to obtain a good gel. These species may belong to the fraction discarded at pH 5.0, during 7SEF and 11SEF preparation, as an insoluble intermediate mixture of β-conglycinin and glycinin.

Taking into account that 11SEF and the mixtures containing high proportion of glycinin did not form gels, we conclude that when glycinin was HP-treated in absence of β-conglycinin, the HP-induced aggregates tended to insolubilize after calcium incorporation rather than to form a network. It is possible that in the presence of high proportion of β-conglycinin, HP induced the formation of protein species that, in the calcium presence, tend to establish a network. Speroni, Añón, et al. (2010) and Speroni, Jung, et al. (2010) analyzed by size exclusion chromatography the protein species formed from calcium-added soybean proteins after HP treatment. They found that the species formed in the β-conglycinin presence (7SEF and SPI) were bigger and presented a broader distribution of molecular weights than those formed in 11SEF. These data suggest a differential effect of HP on the two major storage proteins. The species formed from glycinin alone seem to have low ability to interact via calcium bridges, whereas β-conglycinin and/or glycinin, in the presence of β-conglycinin, form species highly capable of interacting via calcium bridges and forming a network.

After analyzing the results on water retention, visual aspect of gels and convenience of obtaining protein preparations, we came to the conclusion that the best sample to continue the study with was SPI. Thus, gels were prepared from SPI by combining protein concentration of 8.5 or 9.0% w/v, HP treatment of 400 or 600 MPa and calcium concentration of 0.015 or 0.020 mol L^{-1}. Several properties of these gels were evaluated in order to characterize them.

3.2. Water holding capacity

The gels exhibited high values of water holding capacity. Only at 0.020 mol L^{-1} and 8.5% w/v, minimum calcium:protein ratio, the values were significantly lower than 100% ($p < 0.05$) (Table 1). Taking into account that these values were obtained under very intense centrifugation conditions, we concluded that SPI proteins after HP treatment and calcium addition have a great capacity of interact with water. The results also indicate that the ratio between protein and calcium plays an important role on syneresis. Maltais et al. (2005) found that high calcium concentration promotes protein–protein association at expense of water–protein association, in cold-set gels made from heat-denatured soybean proteins. Kohyama, Sano, and Doi (1995), preparing tofu from heat-denatured soybean proteins and $CaSO_4$, also reported that high $CaSO_4$ concentration promoted the formation of too much large aggregates and syneresis. Our results indicate that under several conditions of HP level, protein and calcium concentrations, gels with excellent water retention may be obtained from SPI.

3.3. Color and visual aspect

The values of L^* and b^* depended on calcium concentration. For the same pressure level and protein concentrations, the highest values of L and b corresponded to the lowest calcium concentration. The value of a^* seemed to be independent of the gel composition or the HP level (Table 1).

Turbidity was observed in some samples. Under given HP level and protein concentration, samples were more turbid when calcium concentration was 0.020 mol L^{-1}. The most opaque samples were those corresponding to 8.5–0.020–400, followed by 8.5–0.020–600. On the other hand, the most translucent samples corresponded to 9.0–0.015–600. The turbidity in samples as 8.5–0.020–400 and 8.5–0.020–600 largely impacted the visual aspect of gels. Turbidity resulted from the protein insolubility and the presence of disordered aggregates (Hermansson, 1986), and was possibly responsible for changes in L^* and b^*. At a given calcium:protein ratio, turbidity was higher at 400 than at 600 MPa, suggesting that the highest the HP level, the highest the protein solubility. It is possible that these insoluble aggregates have interacted with smaller amounts of water leading to the decrease in water holding capacity of 8.5–0.020–400 and 8.5–0.020–600. Our results indicate that the ratio between calcium and protein concentrations and the HP level exerted an important effect on solubility, structure of the protein species and visual aspect of gels.

Añón, de Lamballerie, and Speroni (2012) found that HP treatment enhanced solubility of previously calcium-added 1% w/v soybean protein dispersions. In our case, protein and calcium were more concentrated, and calcium was added after HP treatment but this "solubilizing" effect of HP was also manifested. These findings indicate that HP-treated soybean proteins tolerate higher concentrations of calcium keeping high values of solubility in a wide range of protein and calcium concentrations. Improved solubility of calcium enriched soybean proteins may be achieved by HP processing under different operational conditions.

Table 1
Water holding capacity (WHC), color parameters (L^*, a^*, and b^*) of cold-set gels obtained from calcium-added and high pressure-treated soybean protein isolates.

	WHC	L^*	a^*	b^*
8.5–0.015–400	99.9 ± 0.2^{b}	66.4 ± 4.1^{bc}	3.4 ± 0.5^{a}	28.9 ± 2.3^{d}
8.5–0.020–400	95.4 ± 1.3^{a}	54.9 ± 1.8^{ab}	3.3 ± 1.1^{a}	14.6 ± 2.8^{ab}
8.5–0.015–600	99.9 ± 0.1^{b}	57.8 ± 1.3^{b}	3.8 ± 0.4^{a}	23.3 ± 0.1^{cd}
8.5–0.020–600	94.6 ± 1.6^{a}	53.6 ± 2.3^{ab}	2.8 ± 0.1^{a}	10.8 ± 2.1^{a}
9.0–0.015–400	99.8 ± 0.2^{b}	60.2 ± 3.6^{bc}	3.6 ± 1.1^{a}	26.5 ± 2.8^{cd}
9.0–0.020–400	99.7 ± 0.3^{b}	53.3 ± 2.8^{ab}	2.0 ± 0.3^{a}	14.1 ± 0.5^{ab}
9.0–0.015–600	99.5 ± 0.4^{b}	53.8 ± 0.7^{ab}	3.3 ± 1.4^{a}	19.5 ± 1.1^{bc}
9.0–0.020–600	99.9 ± 0.1^{b}	50.1 ± 0.8^{a}	1.9 ± 0.5^{a}	11.9 ± 2.1^{a}

Values, expressed as averages ± standard deviation, within a column with same superscripts are not significantly different ($p < 0.05$).

3.4. Small deformation rheology

The storage module (G') was chosen as indicator of gel strength. The highest value corresponded to 9.0–0.020–600, while the lowest to 8.5–0.015–400 (Fig. 1, panel a). Protein concentration was a main factor in affecting the gel strength, since for the same HP level and calcium concentration, the samples containing higher protein concentration exhibited the highest G' values ($p < 0.05$). These results agree with those of Renkema and van Vliet (2004) that showed that G' values of heat-induced gels from soybean proteins greatly increased with small increases in protein concentration. Moreover, increases in calcium concentration or HP level (at constant protein concentration) were accompanied by increases in G' values ($p < 0.05$) in several cases. It is remarkable that a small difference in denaturation degree (80.6 *vs*. 84.3%, for 400 and 600 MPa respectively) produced a significant change in the rheological behavior. As examples of this effect, G′ increased from 12.5 to 57.2 Pa (8.5–0.020) and from 24.7 to 61.8 (9.0–0.015) when pressure level increased from 400 to 600 MPa. This result suggests that treatments at 400 or 600 MPa may induce different unfolding and/or the formation of different protein species with a different exposition of reactive sites. Our results suggest that these different protein species have diverse capability to interact in a matrix, instead of exhibit similar degree of denaturation.

The cold set gels, thermally pre-denatured, obtained by Maltais et al. (2005) exhibited higher values of G' than our gels (for 9.0% w/v protein and 0.020 mol L^{-1} calcium G' at 1 Hz was 2191 Pa). Such difference indicates that thermal denaturation provides protein species with improved abilities to interact between them, which results in a stronger structure. This behavior may arise from the difference in the degrees of denaturation achieved with heat or HP and in the nature of interactions established between denatured polypeptides in both cases. According to Maltais et al. (2005) after carrying out the heating (30 min at 105 °C) both glycinin and β-conglycinin were completely denatured. In the case of HP treatment, after 600 MPa, total proteins of SPI exhibited a denaturation degree of 84.3%, but the denaturation degree of β-conglycinin was only 70% (Speroni, Añón, et al., 2010; Speroni, Jung, et al., 2010). It is possible that this partial unfolding of the proteins hampered the exposition of all reactive sites, thus, the HP induced gels had lower G′ values than heat-induced ones. Beyond the number of interactions that may be established between proteins due to different degrees of denaturation, the kind of interactions that heat and HP favor are not the same. Heat promotes hydrophobic interactions and disulfide formation that are very important in stabilizing heat-induced SPI aggregates (Petruccelli & Añón, 1995), while HP promotes the formation of hydrogen bonds (Boonyaratanakornkit, Park, & Clark, 2002). Thus, SPI proteins exhibit different structures and abilities to establish a network in the presence of calcium depending on the nature of the denaturing treatment. Thus, the number and nature of attractive and repulsive interactions between polypeptides may conduct to different rheological behavior.

The values of tan δ (G''/G') show that G' was higher than G″ for all formulation and HP level assayed. The gel exhibiting the most elastic character was 9.0–0.015–600, and that of most viscous character was 8.5–0.015–400 (Fig. 1, panel b). Taking into account the translucent aspect of the gels obtained at 9.0–0.015–600, it is possible that in this case, proteins formed fine strands that

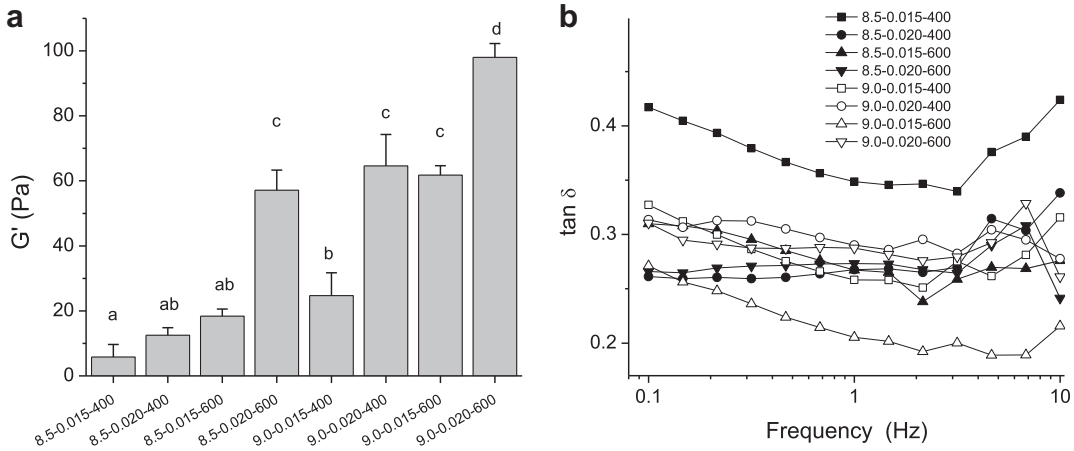

Fig. 1. Small deformation rheology of cold-set gels obtained from calcium-added and high pressure-treated soybean protein isolates. Elastic modulus (G') and tan δ *vs*. frequency as function of protein concentration, calcium concentration and high pressure level. G' values are expressed as averages ± standard deviation (a), tan δ values are shown as average curves (b). Different superscripts indicate that means are significantly different ($p < 0.05$).

homogeneously filled the space. This well ordered strands may be responsible for the high elastic character of these gels. It is interesting to compare the gel obtained at 9.0–0.015–600 (high elastic character reflected as a low tan δ, and low G' value) with that obtained at 9.0–0.020–600 (low elastic character reflected as a higher tan δ, and high G' value): it may be possible that the insoluble aggregates that appeared in 9.0–0.020–600 that provided turbidity, associated themselves with a lower amount of water resulting in a coarse and phase-separated system, with its spaces filled with a liquid solution of proteins. This structure would result hard but would present an increased viscous behavior due to the presence of regions filled with that whey. On the other hand, the high viscous character and low G' of 8.5–0.015–400 may result from the low degree of protein denaturation (incomplete unfolding) and low protein and calcium concentrations. This condition proves unfavorable for proteins to interact between them or between them through calcium bridges. Further studies would be needed in order to better understand these relationships between microstructure and rheology, microscopy may be useful.

3.5. Texture profile analysis

Fig. 2 shows the hardness and adhesivity of gels formed from SPI. The hardest gels (which presented the highest maximum force/area) were 9.0–0.015–600 and 9.0–0.020–600. The differences between the values of this parameter resulting from other experimental conditions were not of great magnitude, which suggests that the impact of composition and HP level was small on this texture parameter. On the other hand, adhesivity was function of calcium concentration; the gels obtained with 0.015 mol L^{-1} exhibited values of the adhesivity higher than zero ($p < 0.05$), for every HP level and protein concentration. On the other hand, gels that contained 0.020 mol L^{-1} of calcium were not adhesive. The sample that showed the highest adhesivity was 8.5–0.015–400. It is possible that the high viscous character and the low values of G' and G'' allowed the samples to deform and remain attached to the probe for a longer period of time. Adhesion can be described in terms of the sum of two contributions: surface energy (type and strength of bonding) and cohesive energy (viscoelastic and plastic deformation within the adhesive), Dobraszczyk (1997). This researcher worked with wheat flour dough and reported that adhesivity was controlled by the bulk rheological properties of the system, having observed an inverse correlation between G′ and stickiness. It is possible that our soybean protein gels have a similar behavior, in which the viscoelastic deformation governs the adhesivity.

3.6. Analysis of the interactions that established the matrix

A two-step extraction of proteins was carried out on gel portions of 9.0–0.015–600 and 9.0–0.020–600. Based on the results

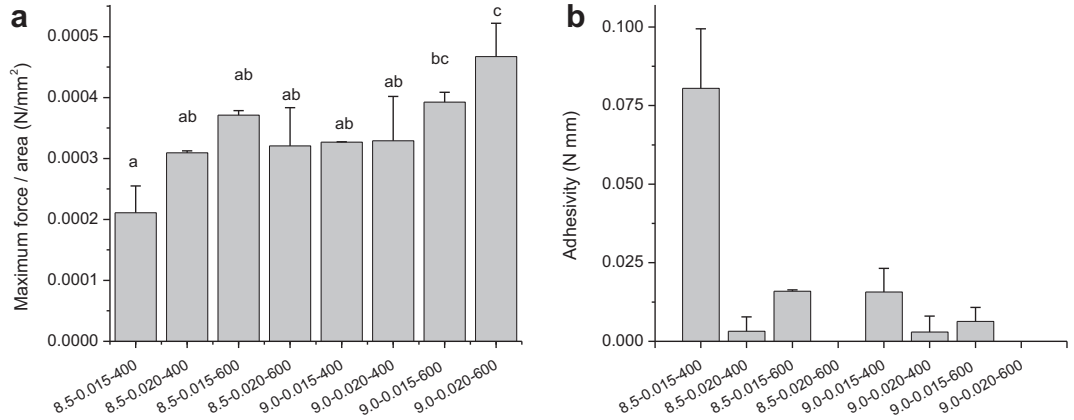

Fig. 2. Texture profile analysis of cold-set gels obtained from calcium-added and high pressure-treated soybean protein isolates. Maximum force/area (a) and adhesivity (b) as function of protein concentration, calcium concentration and high pressure level. Values are expressed as averages ± standard deviation. Different superscripts indicate that means are significantly different ($p < 0.05$).

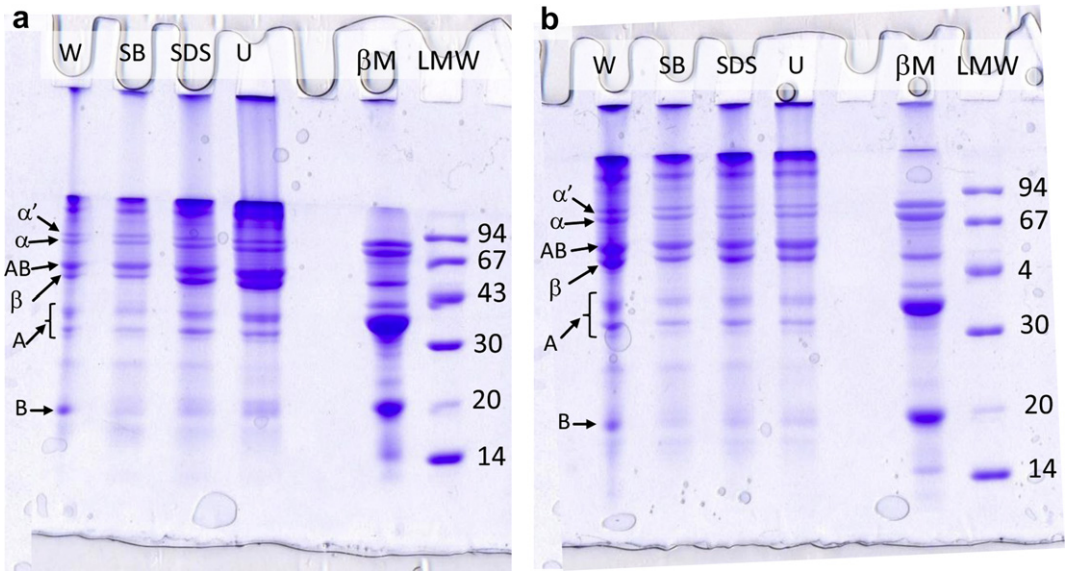

Fig. 3. SDS-PAGE profile of proteins extracted from cold-set gels obtained from calcium-added and high pressure-treated soybean protein isolates. Proteins were extracted in a two-step sequence: Water + saline buffer (lane SB), water + saline buffer with sodium dodecyl sulfate (lane SDS), water + saline buffer with sodium dodecyl sulfate and urea (lane U), or water + saline buffer with sodium dodecyl sulfate and β-mercaptoethanol (lane βM), a sample of the water-extracted proteins was also analyzed (lane W). Soybean protein isolates (9.0% w/v protein) were treated at 600 MPa and 0.015 (a) or 0.020 (b) mol L^{-1} $CaCl_2$ was incorporated. Soybean polypeptides and subunits are indicated in lane W. Low molecular weight markers were also included (lane LMW).

discussed previously, two samples were selected to analyze the likely interactions that stabilize the gel matrix. The samples selected were those in which there were probably stronger matrices formed with a larger number of interactions between polypeptides. Water was used as solvent during the first step, whereas other solvents that disturbed interactions between polypeptides were used during the second one. The supernatants of extractions were analyzed by SDS-PAGE (Fig. 3). In the supernatants of water extraction, all polypeptides and subunits from soybean storage proteins were found in the electrophoresis gel. This result suggests that the system consisted of a three dimensional network coexisting with a liquid phase in which proteins were soluble and/or dispersed but not attached to the matrix. The existence of these regions full of an aqueous solution may explain the relative high values of tan δ that indicated an important viscous character.

The polypeptide profiles obtained after extraction with Saline buffer; Saline buffer/SDS, Saline buffer/SDS/Urea were similar to each other. After exposition to Saline buffer/SDS/Urea no pellet remained after centrifugation. This indicates that the whole sample was solubilized. Taking into account these results, we conclude that gel matrix was stabilized by non-covalent interaction apparently of electrostatic and hydrophobic nature. By comparing the profiles obtained after water extraction with those obtained from the other solvents, we observed that the relative amount of free A and B polypeptides from glycinin were higher in the water extraction. These results suggest that the free A and B polypeptides were less involved in the matrix than the other polypeptides, and agree with the fact that 11SEF did not form gel as showed above. When protein extraction was carried out with Saline buffer/SDS/β-mercaptoethanol, some aggregates of high molecular weight disappeared, indicating that they were stabilized by disulfide bonds; and the AB subunit disappeared as well, increasing the intensity of free A and B bands. No differences were observed in polypeptide profiles between the two calcium concentrations assayed. The results indicate that most of the interactions that establish these gels were relatively weak, and this may be the reason why low values of G' and maximum force/area were observed above.

Puppo and Añón (1998) found that heat-induced gels made from SPI and $CaCl_2$ were completely insoluble in distilled water, and also reported that covalent bonds (disulfide bonds) were responsible for the stabilization of the structure. Glycinin alone at pH 7.6 (Renkema, Knabben, & van Vliet, 2001), and also in the presence of $CaCl_2$, at pH 8.0 (Speroni, Añón, et al., 2010; Speroni, Jung, et al., 2010) is able to form gels after thermal treatment. Taking into account these data and our results, in which HP-denatured glycinin did not gelify in the presence of $CaCl_2$, we concluded that glycinin, despite achieving 100% of denaturation with both kinds of treatments, generated different species with different ability to establish a matrix. Heat-denatured glycinin provided to SPI the capability of forming a more structured network, possibly due to its capacity to establish disulfide bonds.

4. Conclusions

The results point out that cold-set gels can be formed from soybean proteins, using HP as denaturing treatment and calcium incorporation. The best protein preparation to obtain cold-set gels was SPI. The properties of gels depended on protein concentration, calcium concentration and the level of applied HP. Cold-set gels were less hard than those obtained by thermal pretreatment and had excellent water holding capacity. Unlike what occurs after gelation by heat treatment, the matrix of the cold-set gel was basically stabilized by non-covalent interactions. An important amount of protein remained retained in the gel matrix but not forming part thereof. This structural difference is the cause of the textural characteristics of the gels obtained. These gels may be useful for certain applications, such as incorporating other components that may be thermolabile.

Acknowledgments

The authors wish to thank Natalia Szerman, Sergio Vaudagna, Claudio Sanow from ITA- INTA – Castelar, for allowing the use of the HP equipment.

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