Food Hydrocolloids 25 (2011) 389–397

Contents lists available at ScienceDirect

Food Hydrocolloids

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

β-Conglycinin and glycinin soybean protein emulsions treated by combined temperature–high-pressure treatment

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ARTICLE INFO

Article history:
Received 9 March 2010
Accepted 2 July 2010

Keywords:
β-Conglycinin
Glycinin
Temperature–high-pressure treatment
Emulsifying properties
Emulsion rheology

ABSTRACT

Most studies on functionality of soybean proteins have been made with total protein isolates, with the drawback to limit the knowledge of phenomena due to the important complexity of protein composition. In this study we have tried to better understand the behavior of soy emulsions by using their two partially purified fractions: β-conglycinin (7S) and glycinin (11S). Furthermore, we have assessed the combined effect of temperature (20–60 °C) and high pressure (0.1–600 MPa) on physicochemical, microstructural and rheological properties of oil-in-water emulsions prepared with 7S or 11S proteins at 7% (w/v). Our results show that 7S and 11S emulsions behaved differently under the combined treatments and that 7S protein was responsible for the global properties of soybean emulsions, whereas 11S proteins exerted a negligible effect. From 400 MPa and at 60 °C, we have noticed for 7S emulsions an increase of flocculation and gelation, largely confirmed by confocal microscopy due to aggregation between adsorbed and aqueous 7S proteins. Globally we have evidenced that temperature reinforces the effect of high pressure and that the threshold to obtain some changes is 400 MPa. The very different behavior of 7S and 11S proteins in emulsions under treatments could help to orientate their commercial use as function of planed treatments.

1. Introduction

The two major components of storage soybean proteins, glycinin and β-conglycinin (11S and 7S globulins), largely influence their functionality in food stuff (Kinsella, 1979; Renkema, Knabben, & Van Vliet, 2001; Wagner & Añón, 1990). Particularly, their composition, structure, denaturation and aggregation degree are of high significance. These proteins can be prepared in the form of soybean protein isolates (SPI) that are mainly formed by an equivalent proportion of β-conglycinin and glycinin. These proteins may be partially purified by different methods, based in differential precipitation for bench and pilot scale production (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992; Wu, Murphy, Johnson, Fratzke, & Reuber, 1999; Wu, Murphy, Reuber, & Fratzke, 2000). As these proteins have exhibited own functionalities (*e.g.* solubility, emulsification, gelation), they were planed to be incorporated as partially purified fractions in food systems (Rickert, Johnson, & Murphy, 2004).

High-pressure (HP) technology is increasingly used to microbiologically stabilize food without the use of additives and permits a response for consumers in terms of high quality, minimally processed, and additive-free food (Galazka & Ledward, 1995; Gould, 1995; Knorr, 1999, 2000, chap. 2). In contrast to thermal processing, HP does not affect small molecules such as amino acids, vitamins and flavor compounds (O'Reilly, Kelly, Murphy, & Beresford, 2001). However, HP has a disruptive effect on the tertiary and quaternary structure of the globular proteins (Puppo et al., 2004; Tedford, Smith, & Schaschke, 1999) and non-covalent bonds of protein are altered. Consequently, HP technology adequately controlled can be used to modify functional properties of food components (Dickinson & James, 1998, Montero, Fernández-Díaz, & Gómez-Guillén, 2002).

Several studies were focused on HP effects on soybean components. Jung and Mahfuz (2009) have analyzed the effect of HP on oil and protein extraction from full fat soybean, finding that HP processing decreased solubility, and water and oil holding capacity of protein. Puppo et al. (2004) have studied HP effects on isolated soybean proteins and observed that HP disrupts their tertiary and quaternary structure. Treatments with HP higher than 200 MPa exert important effects at pH 8: changes in secondary structure to a more disordered one, accompanied by protein aggregation, especially the 11S fraction. Tang and Ma (2009) have studied the effects of HP on aggregation phenomena of soybean proteins and described the formation of both insoluble and soluble

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doi:10.1016/j.foodhyd.2010.07.005

aggregates; moreover, at 600 MPa soluble aggregates may be formed at the expense of insoluble ones. Aggregation might be due to disulfide bridges formed during sulfhydryl-disulfide interchange (Galazka, Dickinson, & Ledward, 1999; Galazka, Smith, Ledward, & Dickinson, 1999; Hayakawa et al., 1996). The change in protein structure is a pH-dependent phenomenon, inducing also dissociation at acidic pH (Puppo et al., 2004).

HP treatment on neutral soybean proteins improved emulsifying activity of these proteins (Molina, Papadopoulou, & Ledward, 2001). The formation of an interfacial film resistant to depletion-flocculation phenomenon with high interfacial protein concentration has been reported (Puppo et al., 2005). All these studies have been performed in protein solution and very few deal with the impact of such treatments directly applied on emulsions stabilized with soy proteins. In this sense, we have recently studied the effect of temperature plus high-pressure (T–HP) treatment on the stability of emulsions prepared with an aqueous dispersion (70 g/L) of soybean protein isolate (Puppo et al., 2008). We have found that the size and aggregation of oil droplets are not altered by the combined T–HP treatment. These emulsions do not flocculate nor coalesce, but they exhibit a significant increase of the apparent viscosity after 400 and 600 MPa. This phenomenon was attributed to gelation of non-adsorbed soybean proteins, facilitated by their high concentration and improved by the thermal treatment. Pressures equal to or higher than 400 MPa combined with heating, conduct to dissociation of the protein aggregates. However, we were not able to conclude about the key protein (7S or 11S) that was responsible of that increase of viscosity. Consequently, it is of real importance to study the influence of this combined treatment, temperature plus high-pressure (T–HP) on stability of emulsions prepared with these two partially purified proteins (7S and 11S) to better understand and control the stability of emulsions made with soybean proteins as emulsifying agent. For this reason, the objective of this work was to study the influence of T–HP processing on physicochemical and rheological properties of emulsions prepared with native soybean β-conglycinin and glycinin.

2. Materials and methods

2.1. Preparation of 7S and 11S soybean protein fractions

Partially purified globulins β-conglycinin (7S) and glycinin (11S) were obtained according to the method of Nagano et al. (1992). Defatted soybean flour was dispersed in distilled water (1:15 w/w), adjusted to pH 8.0 with 2 N NaOH, stirred at room temperature for 2 h and centrifuged at 10,000 × g for 20 min at 4 °C. Dry $NaHSO_3$ was added to supernatant (0.98 g $NaHSO_3$/L), the pH was adjusted to pH 6.4 with 2 N HCl and the mixture was kept overnight at 4 °C. The resulting dispersion was centrifuged at 6500 × g for 15 min at 4 °C. The precipitate (11S fraction) was suspended in distilled water, adjusted to pH 7.8 with 2 N NaOH, dialyzed against distilled water and freeze dried. Solid NaCl was added to supernatant (0.25 mol/L) and pH was adjusted to 5.0 with 2 N HCl. After 1 h, the insoluble fraction was removed by centrifugation (9000 × g for 30 min at 4 °C). The supernatant was diluted 2-fold with cold water and pH adjusted to 4.8 with 2 N HCl. Centrifugation at 6500 × g for 15 min at 4 °C was carried out. The washed precipitate (7S fraction) was suspended in distilled water and adjusted to pH 7.8 with 2 N NaOH and dialyzed against distilled water before freeze drying process. Protein content of fractions, determined by Kjeldahl method, were 85.9 ± 1.4 for 7S and 88.2 ± 2.1 for 11S (N × 6.25).

2.2. Preparation of emulsions

Oil-in-water emulsions (25 mL) were prepared with sunflower seed oil and aqueous dispersion of 7S and 11S fractions (70 g/L) with an oil volume fraction (ϕ) of 0.3. The composition of the aqueous phase used was buffer 50 mM Tris–HCl pH 8.0. The two phases were premixed for 30 s at 20,000 rpm with a polytron PT 3000 (Kinematica, Switzerland) equipped with a 12 mm diameter head. Homogenization of emulsions was performed with a high-pressure valve Stansted FPG 7400 (Stansted Fluid Power Ltd., Stansted, UK) at 25 bar with a recirculation of 1.7 min.

2.3. Thermal and high-pressure treatment (T–HP) of emulsions

High-pressure processing of emulsions was carried out in a 3.0 L reactor unit (ACB Pressure Systems, Nantes, France) equipped with temperature and pressure regulator device. Before pressure processing, 25 mL of each emulsion was vacuum conditioned in a polyethylene bag (La Bovida, France). Emulsions were HP processed at 20 (7S-20 and 11S-20) or 60 °C (7S-60 and 11S-60) (±2 °C). Temperature during treatment was controlled to avoid overheating of emulsions. Samples were subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min. The level of pressure was reached at 3.4 MPa/s and released instantaneously. Conditions of HP processing were selected in accordance to previous experiments (Puppo et al., 2004). Emulsions treated at 0.1 MPa during 10 min at 20 or 60 °C were used as controls.

2.4. Confocal laser scanning microscopy (CLSM)

One hundred μL of fresh emulsions was introduced inside the adhesive gene frame onto a glass slide. Emulsion samples were covered with the glass cover slip previously treated with the fluorescent probes: BODIPY® 665/676 (=E, E)-3,5-bis-(4-phenyl-1, 3-butadienyl) 4,4-difluoro-4-bora-3a-diaza-s-indacene for lipid and ALEXA Fluor 488 (carboxylic acid succinimidyl ester) for protein detection, respectively. Five μg of BODIPY® and 2 μg of ALEXA were dissolved into 100 μL of dimethylformamide (DMF). Four μL of this dispersion was expanded in all the surface of each cover slip. Slips were then dried at 50 °C. Covered slides were left overnight at 4 °C to favour probe diffusion into emulsions. Oil droplets became fluorescent with BODIPY® probe (red color at 633 nm), while the green matrix corresponds to the aqueous protein dispersion in which are dispersed the oil droplets. Protein is able to be fluorescent with ALEXA (543 nm) probe. The simultaneous labelling of two or more components of foods with probes which are specific for each component permitted us a more detailed analysis of emulsion structure. A Carl Zeiss Axiovert 135M (Carl Zeiss, Oberkochen, Germany) confocal microscope with a LSM 410 software was used. Microscope was employed with an objective of 63×, fitted with green (543 nm) and red (633 nm) lasers and mounted with a LP570 and RG665 filters.

2.5. Optical microscopy (DIC)

The destabilization process of emulsions, especially flocculation process, was investigated by differential interference contrast microscopy (DIC). One mL of each sample was diluted in 20 mL of 50 mM pH 8 Tris–HCl buffer. Two droplets of diluted emulsions were placed onto a microscopy glass slide and inside the adhesive gene frame device (ABHene House, Epsom, UK). The system was covered with a glass cover slip and immediately observed. A Zeiss Axioskop 2 (Carl Zeiss, Oberkochen, Germany) microscope was used.

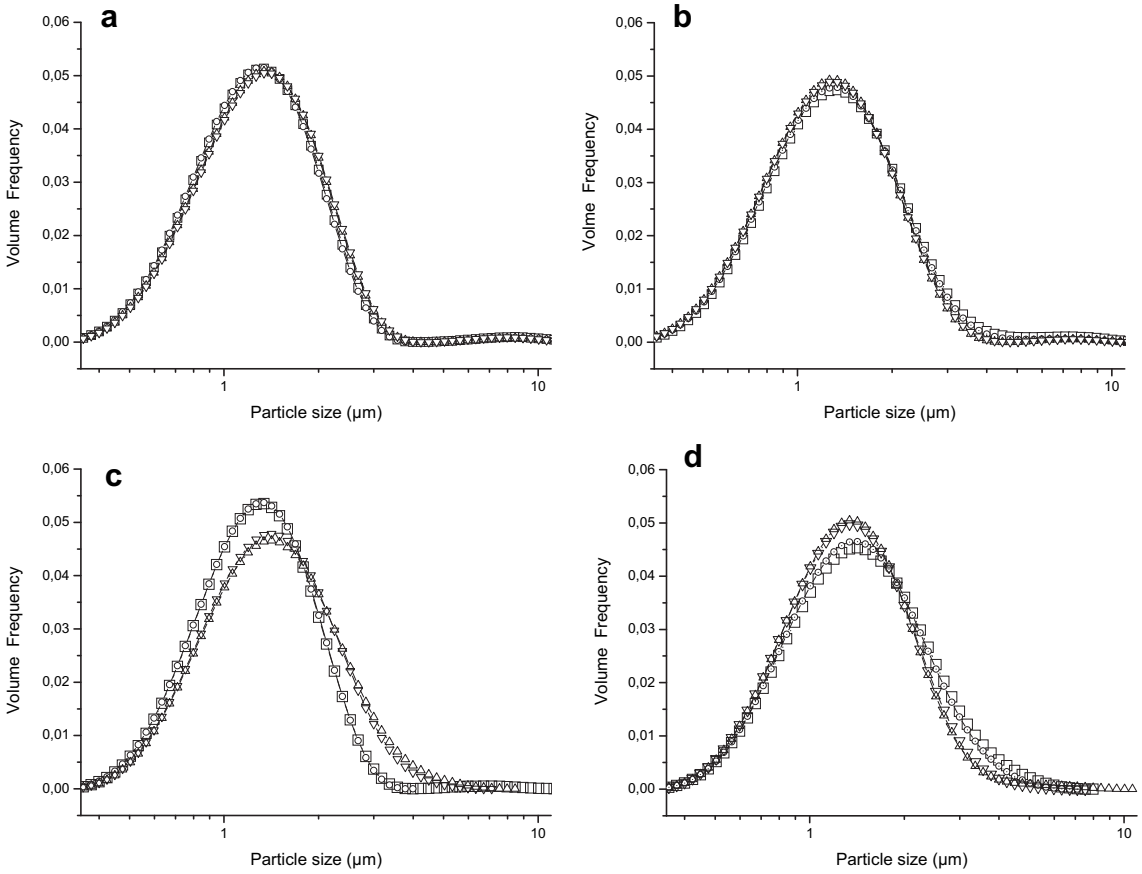


Fig. 1. Droplet size distribution (volume frequency) of 7S emulsions (a, c) and 11S emulsions (b, d). Oil-in-water emulsions (30/70) with aqueous protein dispersion of 70 g/L. HP values were 0.1 (square), 200 (circle), 400 (up triangle) and 600 MPa (down triangle). Temperature during HP treatment was 20 °C (a, b) or 60 °C (c, d).

2.6. Droplet size distribution

After T–HP treatment 0.5 mL of emulsion was diluted in 11.5 mL of 0.05 M pH 8.0 Tris–HCl buffer with or without 0.1 g/L SDS. Droplet size distribution was estimated by laser light diffraction ($\lambda = 658$ nm) in a high definition particle size analyzer (Saturn Digisizer 5200, Micrometrics Instrument Corporation, Atlanta, USA). The mean diameter weighted in volume, $d_{4,3}$, and the volume-surface average diameter, $d_{3,2}$ of emulsion droplets, both expressed in μm, were determined. The specific surface area (Sv), was calculated according to Walstra (1983):

$$\mathrm{Sv} = 6\phi/d_{3,2}\left(\mathrm{m}^2/\mathrm{mL\ emulsion}\right)$$

where ϕ is the oil volume fraction and $d_{3,2}$ the volume-surface average diameter of the particles suspended in SDS buffer.

2.7. Flocculation index (FI)

The flocculation index (FI) was calculated by the ratio between the $d_{4,3}$ droplet sizes in the buffer without SDS to the $d_{4,3}$ in the 0.1 g/L SDS buffer:

$$\mathrm{FI} = d_{4,3}\ \text{of flocs}/d_{4,3}\ \text{of droplets}$$

Table 1
Droplet size ($d_{4,3}$) and flocculation index (FI) of emulsions prepared with dispersions of 7S and 11S proteins (7 g/L protein) in Tris–HCl buffer 50 mM pH 8. Emulsions were immediately subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min at 20 and 60 °C.

7S-20	$d_{4,3}$ (μm)	FI	11S-20	$d_{4,3}$ (μm)	FI
0.1	1.3^{a}	1.02^{a}	0.1	1.3^{a}	1.10^{a}
200	1.2^{a}	1.02^{a}	200	1.3^{a}	1.08^{a}
400	1.3^{a}	1.04^{a}	400	1.2^{a}	1.04^{b}
600	1.3^{a}	1.04^{a}	600	1.2^{a}	1.04^{b}
7S-60			11S-60		
0.1	1.2^{a}	1.00^{a}	0.1	1.4^{a}	1.14^{a}
200	1.2^{a}	0.99^{a}	200	1.4^{a}	1.11^{a}
400	1.4^{b}	1.12^{b}	400	1.3^{b}	1.06^{b}
600	1.4^{b}	1.10^{b}	600	1.3^{b}	1.06^{b}

Results are mean values of triplicate analysis. Means within a column (7S/11S-20 or 7S/11S-60) with same superscripts are not significantly different ($p < 0.05$).

Table 2
Apparent viscosity (η_{app}) and flow index of emulsions prepared with dispersions of 7S and 11S proteins (7 g/L protein) in Tris–HCl buffer 50 mM pH 8. Emulsions were immediately subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min at 20 and 60 °C.

7S-20	η_{app} (Pa s)		Flow index		11S-20	η_{app} (Pa s)		Flow index	
0.1	45	9^{a}	0.88	0.04^{a}	0.1	5	1^{a}	1.13	0.02^{a}
200	47	2^{a}	0.87	0.01^{a}	200	15	1^{b}	1.00	0.01^{a}
400	258	18^{b}	0.72	0.02^{b}	400	28	3^{c}	0.94	0.02^{b}
600	302	4^{c}	0.71	0.00^{c}	600	27	1^{c}	0.96	0.00^{c}
7S-60					11S-60				
0.1	35	2^{a}	0.83	0.07^{a}	0.1	7	1^{a}	1.12	0.02^{a}
200	42	2^{a}	0.89	0.00^{a}	200	6	1^{a}	1.15	0.04^{a}
400	304	22^{b}	0.74	0.01^{b}	400	28	2^{b}	0.95	0.01^{b}
600	340	8^{c}	0.72	0.00^{c}	600	36	5^{b}	0.93	0.02^{c}

Results are mean values of duplicate analysis. Means within a column (7S/11S-20 or 7S/11S-60) with same superscripts are not significantly different ($p < 0.05$).

Table 3
Percentage of adsorbed proteins (AP) and protein interfacial concentration (Γ) for emulsions prepared with dispersions of 7S and 11S proteins (7 g/L protein) in Tris–HCl buffer 50 mM pH 8. Emulsions were immediately subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min at 20 and 60 °C.

7S-20	AP (%)	Γ (mg/m^2)	11S-20	AP (%)	Γ (mg/m^2)
0.1	28.8 ± 0.2^a	7.6 ± 0.1^a	0.1	30.5 ± 0.7^a	8.6 ± 0.1^a
200	31.2 ± 0.6^a	8.3 ± 0.8^a	200	34.0 ± 0.7^b	9.5 ± 0.2^a
400	33.7 ± 0.4^b	8.9 ± 0.1^a	400	33.2 ± 0.2^b	8.6 ± 0.2^a
600	32.8 ± 0.4^b	8.7 ± 0.1^b	600	35.4 ± 0.6^b	9.3 ± 0.2^b
7S-60			11S-60		
0.1	27.1 ± 0.2^a	7.4 ± 0.1^a	0.1	29.0 ± 0.1^a	9.6 ± 0.1^a
200	28.2 ± 0.7^a	7.7 ± 0.2^a	200	28.8 ± 1.0^a	9.5 ± 0.5^a
400	32.0 ± 0.4^b	8.8 ± 0.1^b	400	29.0 ± 1.0^a	9.6 ± 0.4^a
600	30.5 ± 0.5^b	8.4 ± 0.1^b	600	29.9 ± 0.4^a	9.9 ± 0.1^b

Results are mean values of triplicate analysis. Means within a column (7S/11S-20 or 7S/11S-60) with same superscripts are not significantly different ($p < 0.05$).

2.8. Viscosity of emulsions

Apparent viscosity of emulsion was determined by flow measurements through shear stress vs. shear strain assays. Measurements were performed at 20 °C in a Rheometer AR1000 (TA Instrument, New Castle, UK) equipped with a cone–plate sensor system (60 mm diameter, angle 1.58°). Herschel–Bulkley model was applied in the analysis of apparent viscosity and flow index.

2.9. Interfacial protein concentration (Γ)

Oil droplets were washed from the non-adsorbed proteins according to the method described by Patton and Huston (1986). Two mL of fresh emulsion was diluted into 2 mL sucrose solution (500 g/L in 0.1 M pH 7 Tris–HCl buffer). Two mL of this mixture were carefully deposited at the bottom of a centrifuge tube containing 10 mL of the sample buffer solution. The tubes were centrifuged at 3000 × g during 2 h at 10 °C. After centrifugation, two phases were observed: the creamed oil droplets at the top of the tube and the aqueous phase of the emulsion at the bottom. The tubes were frozen at −20 °C and then cut to separate the phases. Adsorbed proteins at the creamed phase were desorbed by adding 20 mL of 1% SDS and the dispersion was then centrifuged at 10,000 × g during 20 min at 10 °C. The bottom aqueous phase, that contained the non-adsorbed proteins, was carefully extracted with the aid of a micropipette. Adsorbed and non-adsorbed protein concentration was determined by the method used by Markwell, Haas, Bieber, and Tolbert (1978). Interfacial protein concentration was calculated as:

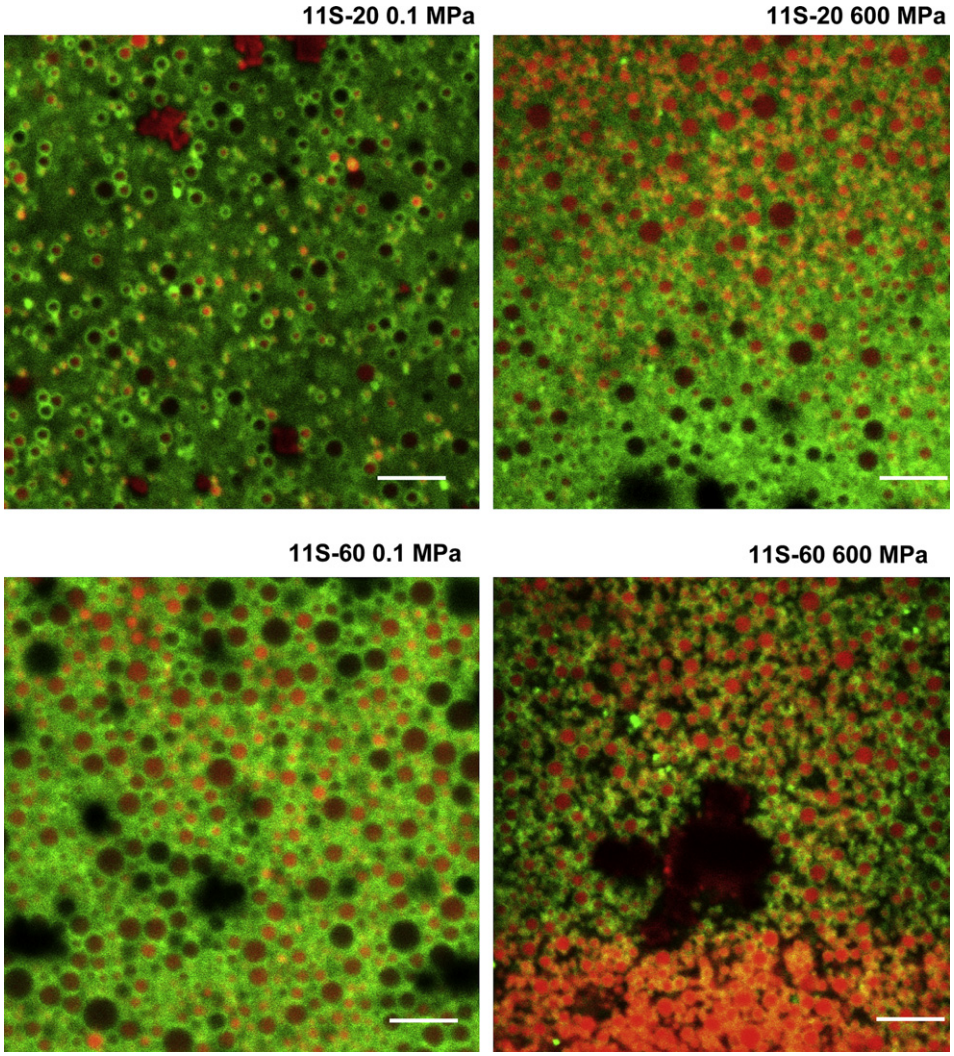


Fig. 2. Confocal laser scanning microscopy of emulsions (CLSM). Fluorescence images of oil-in-water (30/70) emulsions non-HP treated (0.1 Pa) and HP treated (600 MPa) at 20 and 60 °C, previously prepared with 11S proteins (7 g/L protein). Images were obtained at 20 °C. Magnification: 63×. The bar accounts for 15 μm. Fat globules: red particles, protein network: green particles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

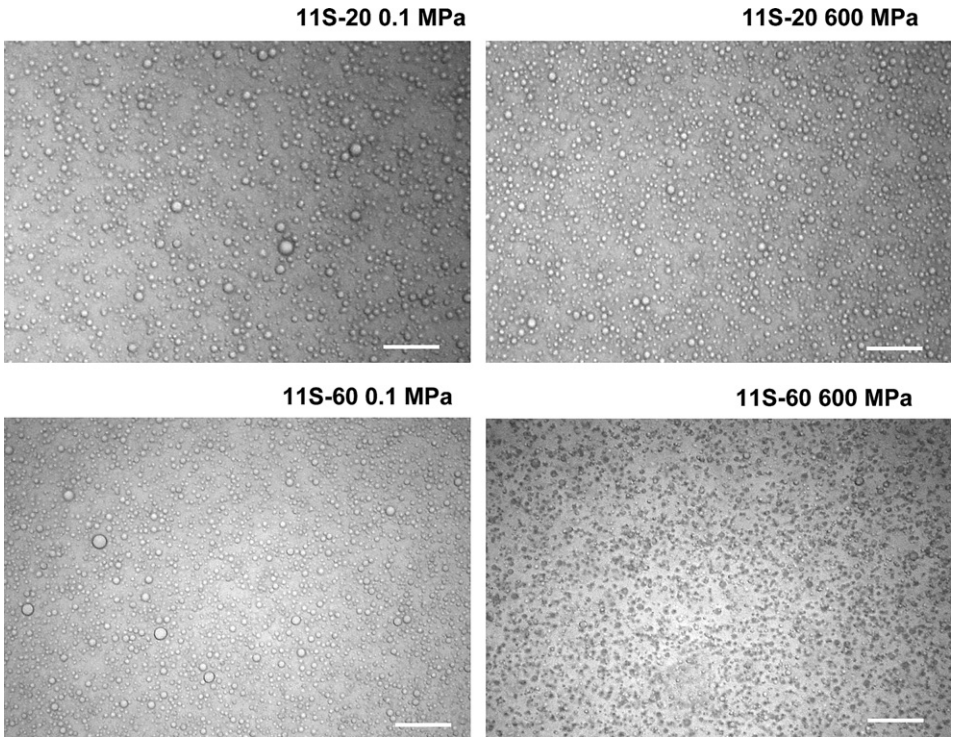


Fig. 3. Optical microscopy of emulsions (DIC). Optical images of oil-in-water (30/70) emulsions diluted (1:20) in Tris–HCl buffer 50 mM pH 8 without SDS to preserve droplet aggregation. Non-HP treated (0.1 Pa) and HP treated (600 MPa) emulsions, both treated at 20 and 60 °C, were previously prepared with 11S proteins (7 g/L protein). Assays were performed at 20 °C. Magnification: 40×. The bar accounts for 20 μm.

$$\Gamma\left(\mathrm{mg/m^2}\right) = \text{adsorbed protein concentration}\ (\mathrm{mg/mL\ emulsion})/\mathrm{Sv}\left(\mathrm{m^2/mL\ emulsion}\right)$$

Adsorbed protein percentage (AP %) was calculated as the adsorbed protein respect to initial protein concentration.

2.10. Interfacial protein composition

Nature of adsorbed and non-adsorbed proteins at the interfacial film was analyzed by SDS-PAGE under non-reducing conditions. Continuous and stacking gels of 100 g/L and 40 g/L of acrylamide, respectively, were prepared. A buffer system, containing 2 M pH 8.8 Tris-base–1.5 g/L SDS for the separating gel and 0.027 M Tris-base–0.38 M glycine-pH 8.3–1.5 g/L SDS for the running buffer were used. Coomassie brilliant Blue was used as colorant agent. Low molecular weight markers (Biorad SDS-calibration kit) used included phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). Percentage of proteins was analyzed using the Bio-Rad Imaging System with the 1-D Analysis Software.

2.11. Statistical analysis

Results were subjected to a one-way analysis of variance according to the general linear model procedure with least-square means effects. Multiple range tests were applied to determine which means were significantly different ($p < 0.05$) according to Fisher's Least Significant Differences (LSD). Statistical analysis was carried out using SYSTAT software (SYSTAT, Inc., Evanston, IL, USA).

3. Results and discussion

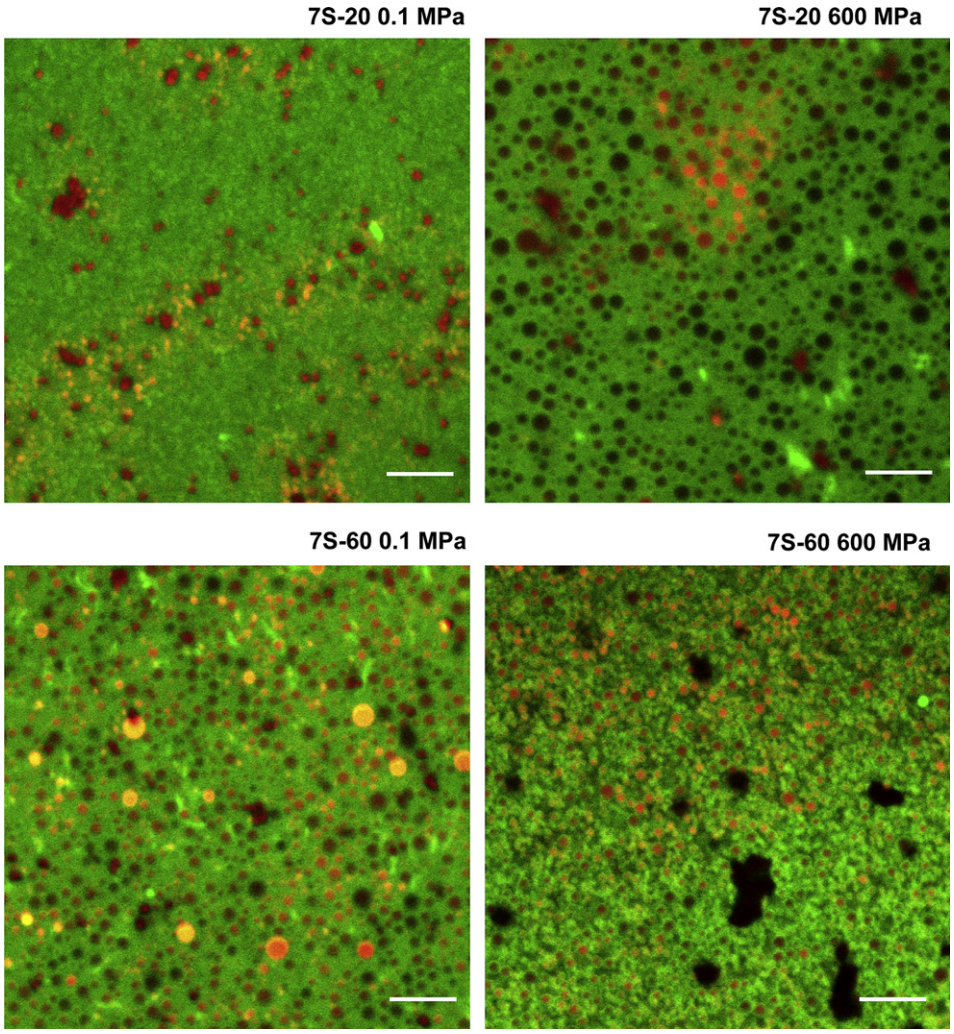
3.1. Droplet size distribution and flocculation behavior

The droplets formed with both 7S and 11S protein fractions exhibited a monomodal distribution of size in presence of SDS (data not shown), characteristic of soybean protein emulsions (Puppo et al., 2005).

In the absence of deflocculating agent, 7S and 11S control emulsions presented at 20 °C a similar size distribution to that obtained in the presence of SDS (Fig. 1a and b). This behavior suggests that bridging flocculation was not occurring in control samples. At 60 °C (Fig. 1c and d), treatment at 400 and 600 MPa produced in 11S emulsions a shift of particle size distribution towards small values, whereas for 7S emulsions the opposite effect was observed. Values of droplet size ($d_{4,3}$) are shown in Table 1. This behavior was also reflected in the flocculation index (FI) (Table 1), this parameter decreased in 11S emulsions at both temperatures, while increased in 7S ones at 60 °C only.

Neither the HP treatment at 20 °C nor the heating at 0.1 MPa affected the droplet size distribution, however, pressurizing at 60 °C, modified this distribution; these effects seemed to be due to simultaneous application of treatments. Boonyaratanakornkit, Park, and Clark (2002) stated that the combination of pressure and temperature, under certain values, has a protective effect on protein unfolding, but, over those values, protein unfolding is favored. For β-galactosidases from *E. coli* and *Aspergillus oryzae* these values are 200–300 MPa and 50 °C.

Results obtained in our previous work show that T–HP treatment on emulsions prepared with SPI had no effect on flocculation behavior (Puppo et al., 2008). Consequently, the results obtained in the present paper with 7S and 11S emulsions confirm that the blend between the two proteins had balanced the two opposite



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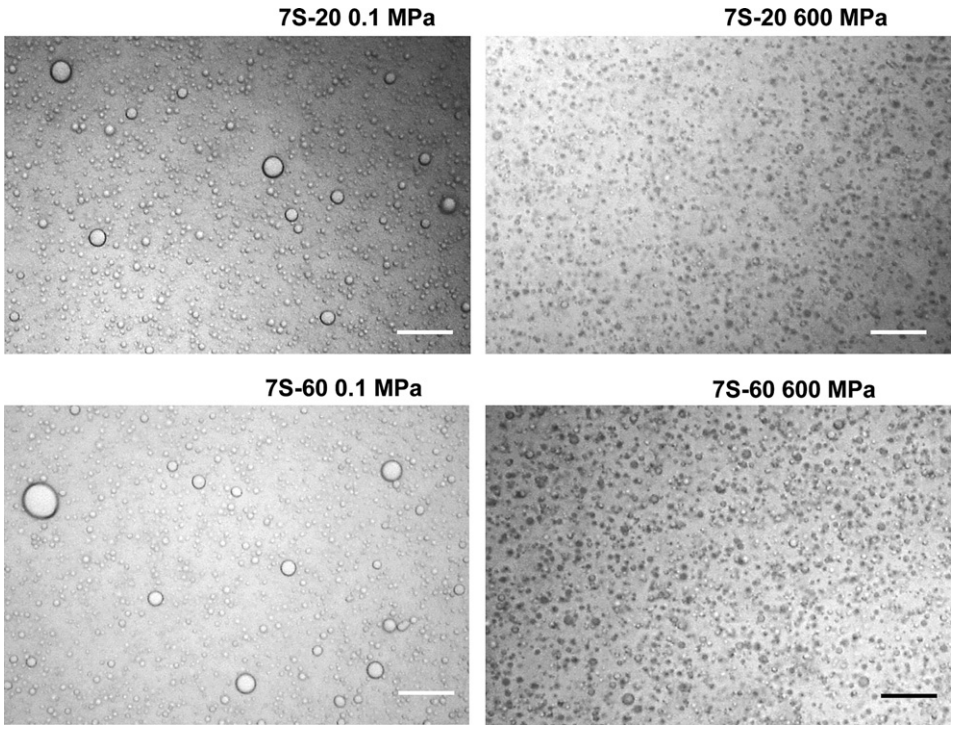
effects and masked the real effect observed with partially purified and separately used 7S and 11S.

3.2. Viscosity of emulsions

The shear rate vs. shear strain curves of the T–HP emulsions prepared with 7S and 11S soybean protein fractions presented beyond the yield stress, a pseudo-plastic flow behavior (data not shown). The Herschel–Bulkley model was applied to experimental data. Table 2 shows values of apparent viscosity (η_{app}) and flow index of emulsions. It must be first noticed that, whatever the treatment used (T–HP), emulsions made with 11S presented η_{app} much lower than emulsions made with 7S. Furthermore, values obtained were very similar to those obtained previously for emulsions made with soybean protein isolates (Puppo et al., 2008). Consequently we can assess, thanks to these results, that rheological properties of emulsions made with soybean protein isolates are mainly due to the 7S fraction.

Despite the important differences of rheological behaviors between 7S and 11S emulsions, the η_{app} increased for both emulsions at pressure equal or higher than 400 MPa at 20 and 60 °C. Viscosity of 11S emulsions increased with HP 4 and 5.7 fold at 20 °C and 60 °C, respectively; while for 7S emulsions the increase was 5.7 and 9.7 folds at the same temperatures. We have to notice that the values obtained for 7S emulsions (258–340 Pa s) corresponded to the formation of a gel-like matrix. As we have observed at the same time, for this type of emulsions, an increase of flocculation during HP treatment, we can assess that the formation of this gel could be due to the concurrent aggregation of 7S protein in the aqueous phase and between adsorbed 7S proteins at the interfaces of the oil droplets. According to Speroni et al. (2009), HP treatment of 7S and 11S solutions conduced to a gel-like network formation for the former and did not modify rheological properties of the second. Consequently in this study the important increase of viscosity during T–HP treatment of emulsions could be due to the formation of a gel in the aqueous phase and/or interactions between 7S proteins in the aqueous phase or adsorbed at the interface, owing to HP-induced conformational changes in adsorbed polypeptides. These interactions can reinforce the gel strength. The increase of oil droplet flocculation could be a signature of that mechanism.

In the case of 11S, we have observed a limited increase of emulsion viscosity under T–HP treatment accompanied with a decrease of flocculation. The increase of viscosity may be linked to the interactions between 11S proteins in the aqueous phase. This confirms the results of Speroni et al. (2009) showing that 11S



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solutions (100 g/L) exhibited a very poor increase of elastic modulus (G') under combined treatment at 600 MPa and temperature between 20 and 75 °C.

Speroni et al. (2009) found that the effect of HP at 20 °C on viscosity of aqueous protein dispersions was significant on 7S, but it was negligible on 11S, despite the degree of denaturation. Furthermore, we previously observed similar viscosity levels and comparable evolution with T–HP treatments for emulsions made with soybean proteins without attributing the cause to one or other protein fraction (Puppo et al., 2008). Thank to this study we can now assess that it the 7S fraction of soybean proteins that is responsible for the rheological properties emulsions made with soybean proteins.

3.3. Interfacial protein adsorption

Whatever the treatment or sample, the general proportion of protein adsorption is around 30% (Table 3), signifying that protein concentration remaining in the aqueous phase was of about 50 g/L.

At 20 °C HP provoked an increase in adsorbed protein percentage (AP %) in both 7S and 11S emulsions, whereas at 60 °C HP induced an increase only in 7S emulsions (Table 3). Interfacial protein concentration followed almost the same behavior as AP%. Since values of interfacial protein concentration were high even though in control samples, it is not probable that oil surface was available to attach more protein, and we assume that the observed increase is due to protein coming from aqueous phase that is associated and/or aggregated to the previously formed film. This aggregation may be consequence of HP-induced unfolding, contributing to the formation of a secondary film. This phenomenon reflected interactions between proteins belonging to the interfacial film and to the continuous phase, reinforcing the statements resulting from the analysis of viscosity.

3.4. Microstructure of emulsions

Emulsions prepared with 11S at 20 °C (0.1 MPa) showed a brilliant and well defined interfacial film around droplets dispersed in a continuous phase, stabilized by proteins present in the aqueous phase (Fig. 2). Pressure treatment (600 MPa) seems to not provoke changes in the microstructure of the emulsion as studied by CLSM. In the same way thermal treatment (60 °C) did not change also the emulsion microstructure. However, the combined effect T–HP (60 °C–600 MPa) produced a micro separation of phases, possibly due to protein aggregation in the aqueous phase in such conditions. The same evolution is ascertained by DIC microscopy, with a visual change of the microstructure of the emulsions only for the 60 °C–600 MPa treatment (Fig. 3).

In 7S emulsions (Fig. 4) a homogeneous protein network around the oil droplets was observed at 20 °C. No changes were detected after heating at 60 °C by CLSM, neither by rheological determinations (Table 2). Pressure treatment (600 MPa) at 20 °C enhanced the formation of a more structured protein matrix, with protein aggregation around oil droplets. The combined effect T–HP enhanced the extent of protein aggregation. These more structured emulsions also presented very high apparent viscosities (Table 2). The high apparent viscosity of SPI emulsions treated at pressures higher than 400 MPa at 20 °C and 60 °C, found in a previous work (Puppo et al., 2008), can be now attributed mainly to the 7S fraction. Using DIC microscopy we observed equally important microstructural changes with 600 MPa and combined 60 °C–600 MPa treatments. Furthermore, we can also observe for these two samples an important flocculation of oil droplets (Fig. 5), confirming the increase of flocculation index as calculated in Table 1.

3.5. Composition of the interfacial proteins

The nature of the adsorbed and non-adsorbed proteins at the interface was analyzed by SDS-PAGE under non-reducing

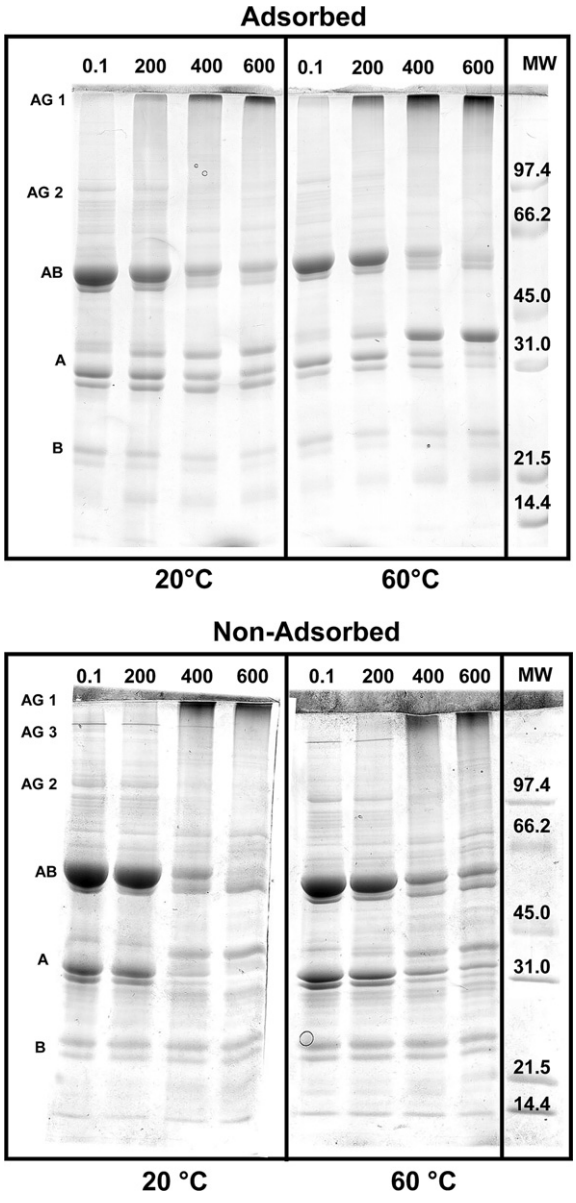

Fig. 6. SDS-PAGE profile of proteins adsorbed and non-adsorbed at the oil-water interface derived from 11S emulsions. Proteins were extracted with 10% SDS sample buffer. Emulsions were treated at different pressures (HP treatment): 0.1, 200, 400 or 600 MPa; and at distinct temperatures (T treatment): 20 °C or 60 °C. MW: molecular weight standard markers.

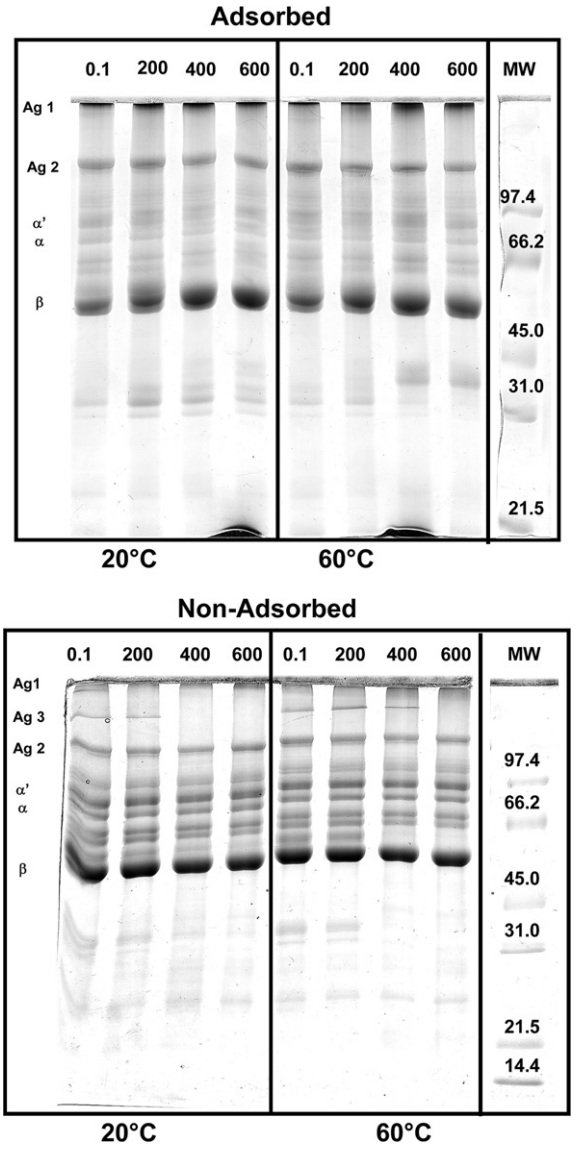

Fig. 7. SDS-PAGE profile of proteins adsorbed and non-adsorbed at the oil-water interface derived from 7S emulsions. Proteins were extracted with 10% SDS sample buffer. Emulsions were treated at different pressures (HP treatment): 0.1, 200, 400 or 600 MPa; and at distinct temperatures (T treatment): 20 °C or 60 °C. MW: molecular weight standard markers.

conditions (Figs. 6 and 7). Fig. 6 shows the electrophoretic profiles of proteins belonging to 11S emulsions treated with HP at 20 and 60 °C. For 20 °C, it appears that AB subunit and A polypeptides, well adsorbed without HP treatment, were involved in an aggregation process at the interface from 400 MPa, since they disappeared gradually from the adsorbed profiles. These proteins (AB and A) also disappeared from the non-adsorbed profiles with HP treatment. At 60 °C, the AB subunit desorption due to HP treatment was intensified and rendered the A polypeptide as the main adsorbed among 11S proteins, specifically the 43 kDa peptide also observed in the SPI over 400 MPa (Puppo et al., 2008). Furthermore an increase of the intensity of AG1 band (non entering proteins) due to the HP treatment was detected in the adsorbed and the non-adsorbed patterns, indicating that whatever the location of the proteins (aqueous phase or interface) the HP treatment induced the aggregation of this type of proteins. The AB subunit dissociation prior to their aggregation as a result of high-pressure treatment, cannot be ruled out. This hypothesis is based on the fact that in the electrophoretic profiles was only detected an increase of polypeptide A, without the consequent increase of the polypeptide B which must be involved in the formation of aggregates.

For the 7S emulsion (Fig. 7) it was observed in the non-adsorbed protein a decrease in intensity of AG3, after HP treatment at 400 and 600 MPa. This aggregate was absent in SPI emulsions treated under the same conditions (Puppo et al., 2008). It is likely that this protein is concerned by the aggregation processes assumed above. AG2 aggregate was present in a high proportion in both adsorbed and non-adsorbed phases of 7S emulsion, while in SPI emulsions was mainly forming the interfacial film. Furthermore, no appreciable changes on protein composition in the aqueous phase and at the interface were noticed as function of temperature and HP treatment. We can assess that distribution of β-conglycinin

polypeptides in the adsorbed and non-adsorbed phases is more resistant to T–HP treatment than the glycinin one. Physicochemical changes of 7S emulsions due to combined T–HP treatment cannot be explained by interfacial composition of proteins. However, this study can help us to determine what types of subunits are involved in the formation of aggregates due to T–HP treatment.

4. Conclusions

The aim of this study was to better understand the behavior of soy emulsions under a combined effect of temperature (20–60 °C) and high pressure (0.1–600 MPa) by using their two partially purified fractions: β-conglycinin (7S) and glycinin (11S). We have particularly focused our study on the physicochemical, microstructural and rheological properties of oil-in-water emulsions.

Our results demonstrate that globally 7S emulsions undergo flocculation and gelation processes from 400 MPa and that this effect is reinforced by the heat treatment at 60 °C, whereas 11S emulsions are poorly impacted by the combined treatments. This results permit us to assess that 7S fraction is responsible for the physicochemical properties of soybean protein, as 7S emulsions shows similar behavior than total soy protein emulsions under the same combined treatments. Furthermore emulsions made with 11S fraction, not modified by this type of treatments, could be used for very intensive heating or pressure treatments.

Acknowledgements

This work was supported by the Ecos-Sud Project of France and SECyT of Argentine (N° A01B04). Authors thank Brigitte Metro and Alain Riaublanc (INRA – LEIMA) for the assistance in CLSM analysis.

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