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Effect of high-pressure treatment on emulsifying properties of soybean proteins

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Abstract

Modifications of emulsifying properties of soybean protein isolates (SPI) by high-pressure processing have been studied. SPI solutions at 10 g/l in two pH conditions: alkaline (pH 8: SPI8) and acidic (pH 3: SPI3) were treated by high-pressure at various pressure levels (200, 400 and 600 MPa for 10 min at 10 °C). Oil-in-water emulsions (30/70) were prepared with untreated and high-pressure treated SPI3 and SPI8. Emulsifying properties (oil droplet size, flocculation, interfacial protein concentration and composition) were evaluated.

Pressure processing of SPI8 from 200 MPa induced a reduction of droplet size and an increase of depletion flocculation, not observed with SPI3. Bridging flocculation decreased and percentage of adsorbed proteins increased when pressure was applied, whatever the pH conditions. High-pressure treatment induced more ability to proteins, and particularly β-7S and A-11S polypeptides, to be adsorbed at the oil–water interface. At pH 3, high-pressure processing seemed to improve emulsifying properties that have declined due to acidification.

Keywords: Soybean protein isolates; High-pressure treatment; Emulsifying properties; Emulsion rheology

1. Introduction

Applied directly to proteins, high-pressure causes the formation of new bonds leading to a modified structure that presents altered functional properties as compared to native proteins (Molina, Papadopoulou, & Ledward, 2001). These modifications can lead to aggregation and, under appropriate conditions, to gelation or precipitation (Galazka, Dickinson, & Ledward, 2000a; Molina, Defaye, & Ledward, 2002). Furthermore, interfacial properties of proteins have been modified by high-pressure (Galazka et al., 2000a): foaming properties of pressure-treated β-lactoglobulin (Galazka, Ledward, & Varley, 1997) were improved, while worst emulsifying properties, in comparison with the untreated protein, were observed (Dickinson & James, 1998, 1999; Galazka, Dickinson, & Ledward, 1996; Galazka, Dickinson, & Ledward, 2000b).

The use of soybean protein isolates (SPI) as functional ingredients in food formulation is attractive both because of their high functionality and nutritional value. Emulsifying properties of soybean proteins were widely studied (Puppo & Añón, 1999; Kim, Renkema, & van Vliet, 2001; Mitidieri & Wagner, 2002; Roesch & Corredig, 2003). Mitidieri and Wagner (2002) observed that emulsions prepared with native SPI presented a high resistance to coalescence. It is well known that soybean proteins decrease interfacial tension between water and oil. However, because of their globular structure, they could not adequately unfold and adsorb at the interface, but form a structured interfacial film which acts as a physical barrier to coalescence (Roesch & Corredig, 2003).

Adequate modifications on SPI may improve their functional properties. Emulsifying properties of soybean proteins would be improved by using emergent technologies such as high-pressure. Only limited data have been reported about the effect of high-pressure on structural and functional

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properties of soybean proteins. High-pressure treatment at neutral pH improved the emulsifying activity but not the stability of emulsions made with soybean proteins. On the other hand, self-supporting gels of SPI and 7S and 11S fractions were obtained at high-pressure range of 300–700 MPa with improved water holding capacity (Molina et al., 2002).

In a previous work (Puppo, Chapleau, Speroni, de Lamballerie, Añón & Anton, 2004) we studied the effect of high-pressure on structural properties of SPI. At pH 8, high-pressure produced an increase in the surface hydrophobicity and in the denaturation degree, specially at pressures higher than 200 MPa. At 600 MPa, only 20% of native conformation corresponding to the 7S fraction was detected. Formation of soluble aggregates of high molecular mass stabilised by disulfide bonds and non-covalent interactions was also observed. At acidic pH (pH 3), high-pressure produced an increase in solubility, surface hydrophobicity and denaturation degree and a decrease in free sulfhydryl content of proteins. Aggregation phenomenon, due to protein–protein interactions favoured near the pI, was observed at pH 3. Soluble fraction only contained dissociated polypeptides of molecular mass <66 kDa stable to high-pressure treatment.

These changes in protein structure observed in our previous work could affect interfacial and emulsifying properties of soybean proteins. Therefore, the objective of the present work was to study the influence of high-pressure treatment on the emulsifying properties of soybean proteins in native state (pH 8) and at acidic pH (pH 3).

2. Materials and methods

2.1. Preparation of soybean protein samples

SPI were prepared from defatted flour manufactured by Bunge-Ceval S.A. (Brazil). An alkaline extraction from the flour (pH 8.0), followed by precipitation at the isoelectric point (pI = 4.5) was carried out according to Puppo, Lupano, and Añón (1995). The isoelectric precipitate was dispersed in distilled water and adjusted to pH 8.0 with 2 N NaOH. The dispersion thus obtained was lyophilized. Protein content of SPI was determined by microkjeldahl method using a colorimetric method for protein detection (Nkonge & Murray Ballance, 1982), was 83.0 ± 1.6 (N×5.8).

For high-pressure processing, dispersions of SPI of 10 g/l protein, at pH 3 (50 mM glycine), SPI3, and pH 8 (50 mM Tris–HCl), SPI8, were prepared.

2.2. High-pressure processing

High-pressure processing was carried out in a 3 l reactor unit (ACB Pressure Systems, Nantes, France) equipped with temperature and pressure regulator device. Prior to pressure processing, 50 ml of SPI dispersions (SPI3 and SPI8) were vacuum conditioned in a polyethylene bag (La Bovida, France). Temperature during treatment was controlled to avoid the overheating of protein. Conditions of high-pressure processing were chosen in accordance to Chapleau and de Lamballerie-Anton (2003) treatment. SPI dispersions (10 g/l protein) were subjected to high-pressure treatment at 200, 400 and 600 MPa (± 7 MPa) for 10 min. The level of pressure was reached at 6.5 MPa/s and released at 20 MPa/s. Temperature of transmitting medium in the vessel was settled at 20 °C (± 2 °C) during pressure processing.

2.3. Emulsion preparation

Oil-in-water emulsions (40 ml) were prepared with sunflower oil and SPI dispersions (10 g/l) (SPI3 and SPI8) with an oil volume fraction (ϕ) of 0.3. 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. Homogenisation of emulsions was carried out with a high-pressure valve Stansted FPG 7400 homogeniser (Stansted Fluid Power Ltd, Stansed, UK) at 120 bars with a recirculation of 3 min.

2.4. Droplet size distribution

Immediately after homogenization, 0.5 ml of emulsion was taken and diluted in 11.5 ml of 0.05 M pH 8 Tris–HCl buffer with 1% 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) and determined using the $d_{4,3}$ and $d_{3,2}$ indexes. $d_{4,3}$ represents the mean diameter weighted in volume, while $d_{3,2}$ is the volume-surface average diameter of the emulsion droplets, both expressed in micrometres. The specific surface area, Sv, was calculated according to Walstra (1983)

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

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

2.5. Flocculation behaviour

Bridging flocculation (FI). The Flocculation Index (FI) was calculated by the ratio between the $d_{4,3}$ in the buffer without SDS and the $d_{4,3}$ in the 1% SDS buffer.

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

Depletion flocculation. Depletion flocculation phenomenon can be analysed by flow measurements through viscosity versus shear stress assays. A first step consisted in disruption of flocs present in the emulsion by application of a strain of 10 Pa during 2 min. Thereafter, the emulsion was left 15 min to enhance droplet interactions. Finally an increasing shear stress from 0.002 to 10 Pa in a 6 min period

was applied to the emulsions for floc disruption (Bower, Washington, & Purewall, 1996). Flocs become aligned with the shear field which decreases their resistance to flow. Viscosity versus shear stress was recorded to follow the extent of floc disruption. Measurements were performed at 20 °C in a Haake Rheostress RS75 equipped with a plate/cone sensor system (60 mm diameter, 2.09° angle).

2.6. Protein adsorption

The oil droplets were washed from non-adsorbed proteins according to the method described by Patton and Huston (1986). Two millilitres of fresh emulsion was diluted in 2 ml of sucrose solution (500 g/l in 0.1 M pH 7 Tris–HCl buffer). Two millilitres of this mix 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 so as 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 were determined by the method used by Markwell, Haas, Bieber, and Tolbert (1978). Interfacial protein concentration was calculated as:

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

2.7. Protein composition of the interfacial film

The nature of adsorbed and non-adsorbed proteins at the interfacial film was analyzed by SDS-polyacrylamide gel electrophoresis. Continuous and stacking gels of 10 and 3.5% of acrylamide, respectively, were prepared. A buffer system containing 2 M Tris–base, pH 8.8 containing 0.15% SDS for the separating gel and 0.027 M Tris–base, 0.38 M glycine pH 8.3 with the addition of 0.15% SDS, for the running buffer were used. Coomassie Brilliant Blue was used as colorant agent.

Low MW 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).

2.8. Statistical analyses

Three measures were conducted for the following characteristics: average droplet diameter, interfacial protein concentration and rheology. Results were subjected to a one-way analysis of variance according to the general linear model procedure with least-square means effects. Multiple range test was applied to determine which means were significantly different according to Fisher's Least Significant Differences (LSD). Statistical analysis was carried out using Statgraphics plus version 2.1 software (Statistical Graphics Corp., Princeton NJ, USA).

3. Results

3.1. Droplet size distribution

Droplet size distribution and average droplet size ($d_{3,2}$) of the different SPI emulsions are shown respectively in Fig. 1 and in Table 1. Before high-pressure treatment, SPI8 emulsions presented lower $d_{3,2}$ than those prepared with SPI3: 1.2 versus 1.5 μm. Emulsions prepared with SPI8 were characterized by a distribution of oil droplets in

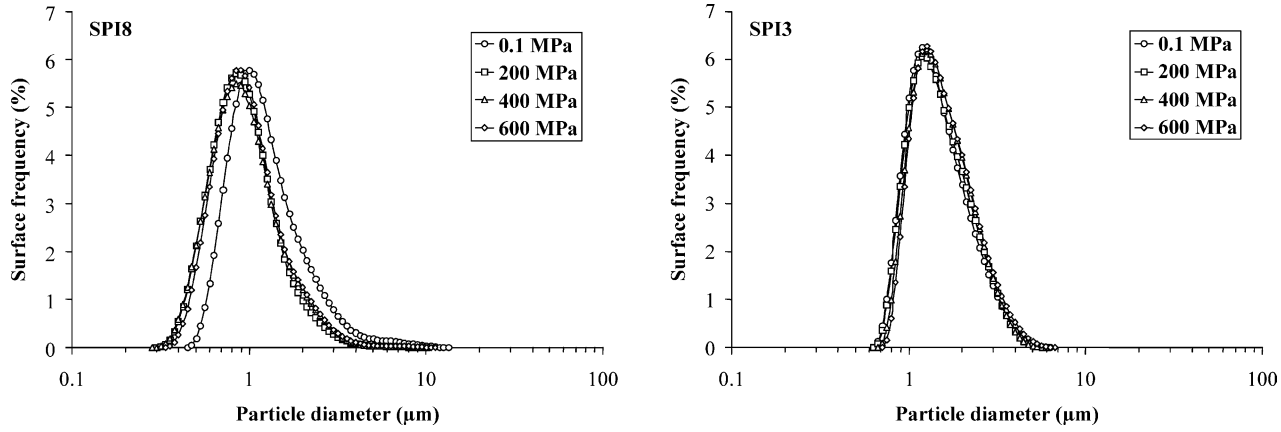

Fig. 1. Droplet size distribution (surface frequency) of SPI8 (Tris–HCl buffer 50 mM pH 8) or SPI3 (glycine–HCl buffer 50 mM pH 3) emulsions. SPI dispersions (10 g/l protein) were previously subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min. Oil-in-water emulsions (30/70) with SPI at 10 g/l were diluted with SDS (1%) to promote dissociation of aggregation.

Table 1
Droplet size ($d_{3,2}$), flocculation index (FI), % adsorbed proteins, and interfacial protein concentration (Γ) of SPI8 (Tris–HCl buffer 50 mM pH 8) or SPI3 (glycine–HCl buffer 50 mM pH 3) emulsions

	$d_{3,2}$ (μm)	FI	% adsorbed proteins	Γ (mg/m^2)
SPI8				
0.1 MPa	1.20 ± 0.05^{a}	6.7 ± 0.3^{a}	63.6 ± 2.2^{a}	3.03 ± 0.01^{a}
200 MPa	0.86 ± 0.05^{b}	6.1 ± 0.3^{b}	68.9 ± 1.7^{b}	2.46 ± 0.08^{b}
400 MPa	0.92 ± 0.02^{b}	4.8 ± 0.1^{c}	76.4 ± 2.4^{b}	2.87 ± 0.11^{ab}
600 MPa	0.91 ± 0.07^{b}	4.5 ± 0.4^{c}	77.5 ± 1.4^{b}	2.84 ± 0.20^{ab}
SPI3				
0.1 MPa	1.49 ± 0.02^{a}	4.4 ± 0.1^{a}	60.9 ± 2.8^{a}	3.69 ± 0.05^{a}
200 MPa	1.45 ± 0.04^{b}	3.3 ± 0.0^{b}	63.7 ± 2.1^{b}	3.74 ± 0.01^{a}
400 MPa	1.57 ± 0.05^{c}	1.5 ± 0.1^{c}	64.0 ± 1.7^{c}	4.03 ± 0.01^{b}
600 Mpa	1.57 ± 0.02^{c}	1.8 ± 0.1^{d}	70.1 ± 0.2^{c}	4.47 ± 0.05^{c}

SPI dispersions (10 g/l protein) were previously subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min. Results are mean values of triplicate analysis. Means within a column (SPI3 or SPI8) with same superscripts are not significantly different ($p<0.05$).

the range of 0.3–10 μm, whereas SPI3 emulsions presented a narrower distribution.

After high-pressure treatment of proteins, a shift in droplet populations towards smaller size (<1 μm) was detected for SPI8 emulsions whereas no differences were observed for SPI3 emulsions (Fig. 1). The decrease in droplet size was about of 25–30% and was significantly different from 200 MPa for SPI8 emulsions. In the case of SPI3 emulsions, changes in the droplet size between untreated and pressured samples were small whatever the pressure assayed.

3.2. Flocculation behaviour

3.2.1. Bridging flocculation

In the presence of a deflocculation agent (SDS), untreated SPI8 emulsions presented a droplet size distribution (calculated in volume) with a great percentage of particles of 1.5 μm and a minor fraction of particles of 8 μm (Fig. 2). After high-pressure treatment the fraction of 8 μm decreased and the maximum of the first population was shifted towards 1 μm. Volume frequency of SPI3 emulsions presented a similar behaviour to that observed in surface frequency, whatever the pressure treatment. These observations corroborate the results presented above.

Without a deflocculation agent (SDS), we observed the presence of three populations of droplets: 10, 40 and 1 μm in order of importance. The appearance of the two higher populations without SDS addition suggests the presence of flocculated oil droplets in all the emulsions prepared with both SPIs. As depletion flocculation is not likely to occur due to the intense flow occurring in the liquid in the optical

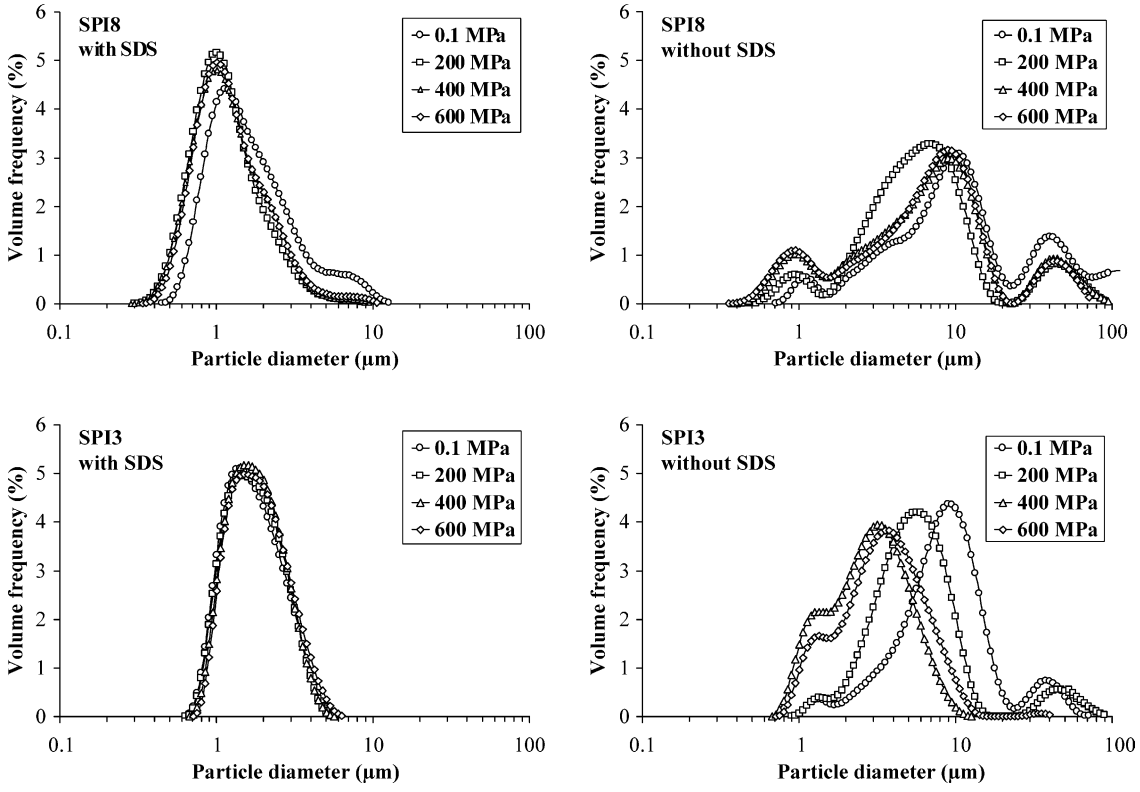

Fig. 2. Droplet size distributions (volume frequency) of SPI8 (Tris–HCl buffer 50 mM pH 8) or SPI3 (glycine–HCl buffer 50 mM pH 3) emulsions. SPI dispersions (10 g/l protein) were previously subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min. Oil-in-water emulsions (30/70) with SPI at 10 g/l were diluted with SDS (1%) to promote dissociation of aggregation or were diluted without SDS.

cell, it is expected that bridging flocculation of droplets was observed in SPI emulsions.

We can remark that for SPI3, the high-pressure treatment of proteins clearly eliminated higher populations and shifted the population of 10 μm towards to 3–5 μm depending the extent of the treatment. For SPI8, we only observed a shift of all the populations towards smaller sizes in response to the high-pressure treatment of the proteins. Based on these results we have calculated an flocculation index (FI) presented in Table 1. The FI values of emulsions corresponding to 400 and 600 MPa treated SPI8 dispersions decreased slightly (maximum 32% at 600 MPa) as compared to the untreated dispersion. The FI values of emulsions obtained with high-pressure treated SPI3 dispersion decreased more intensely: 25, 67 and 59% at respectively 200, 400 and 600 MPa as compared to the control, suggesting an important decrease in floc sizes.

3.2.2. Depletion flocculation

Fig. 3 shows viscosity of emulsions as function of shear stress application. The principle of the measure was first to eliminate any flocculation (bridging an depletion) an intense strain. Then the reformation of droplet aggregates is highly influenced by depletion. Modification of emulsion flocs can then be characterised by viscosity variation measurements.

We can observe that untreated emulsions made at pH3 presented a higher flocculation level than emulsions prepared at pH 8. SPI8 emulsions have a very weak viscosity (0.005–0.02 Pa s) all over the range of shear. This behaviour demonstrated the absence of depletion flocculation. SPI3 emulsions showed, first, a high viscosity (20–30 Pa s) at low shear stress, and, second, a rapid drop of viscosity as the shear stress was increased (from 0.2 to 0.3 Pa). A complete disruption was detected from 1 Pa. The high apparent viscosity observed at low shear stress demonstrated the presence of a flocculated system. The initial viscosity is dependent on the size of droplet aggregates and, consequently, on the number of droplets concerned with these aggregates. Furthermore, the stress value corresponding to the drop of apparent viscosity gave an estimation of the strength to apply to break the flocs.

Pressurization of proteins at pH 8 induced an increase of depletion flocculation in corresponding emulsions. We can observe that viscosity at low shear stress rose as a function of the pressure level. Furthermore, the shear stresses necessary to start the deflocculation increased also with pressure level. Initial viscosity as well as shear stress value for deflocculation for SPI8 emulsions were much lower than those observed for SPI3 emulsions, whatever the pressure level. This indicates that the extent and the forces between droplets are weaker at pH 8 than at pH 3. All emulsions presented the same aggregation state after shear stress treatment. At pH 3, no differences were found between emulsions prepared with untreated and high-pressure treated proteins.

3.3. Protein adsorption

Interfacial protein concentration (Γ) and percentage of adsorbed proteins without high-pressure treatment were similar whatever the pH (Table 1). In emulsions prepared with SPI8, Γ slightly decreased with previous high-pressure treatment of proteins. This is linked with the associated increase of interface area (decrease of $d_{3,2}$). A different behaviour was observed for SPI3 emulsions, where Γ slightly increased for emulsions prepared with proteins treated at pressures higher than 200 MPa. In this case, associated interface area did not change whatever the pressure. On the other hand, high-pressure treatments increased the percentage of adsorbed proteins, whatever the pH value.

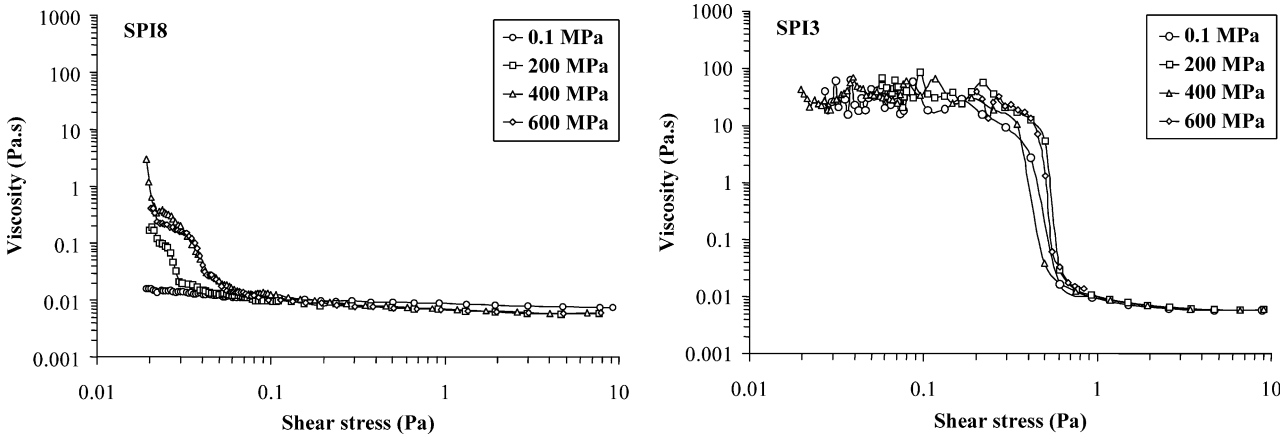

Fig. 3. Viscosity versus shear stress of SPI8 (Tris–HCl buffer 50 mM pH 8) or SPI3 (glycine–HCl buffer 50 mM pH 3) emulsions. Results obtained after application during 2 min of a strain of 10 Pa and then a release for 15 min. SPI dispersions (10 g/l protein) were previously subjected to high-pressure treatment at 200, 400 and 600 MPa (±7 MPa) for 10 min. Oil-in-water emulsions (30/70) with SPI at 10 g/l were diluted with SDS (1%) to promote dissociation of aggregation or were diluted without SDS.

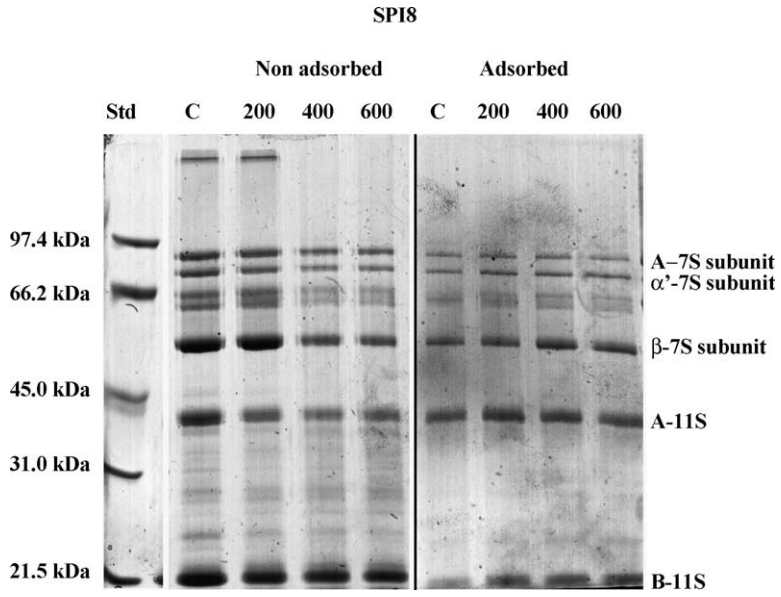

Fig. 4. SDS-PAGE profile of adsorbed and non-adsorbed at the oil–water interface SPI8 from untreated and high-pressure treated (200, 400 or 600 MPa for 10 min). Electrophoresis carried out with stacking and running gel, respectively, at 3.5 and 10% of polyacrylamide. Oil-in-water emulsions 30:70 prepared with 1% protein (w/v). SPI fractions were stained with Coomassie Blue. MW: Molecular weight, Std: Standard marker, C: Control.

3.4. Composition of interfacial proteins

The nature of adsorbed and non-adsorbed proteins was analysed by SDS-PAGE under reducing conditions (Figs. 4 and 5). Untreated and high-pressure treated SPI8 and SPI3 mainly presented the typical soybean protein subunits of β-conglycinin (7S): α', α and β, and glycinin (11S): A and B.

Interface composition of emulsions prepared with native isolate (SPI8) is shown in Fig. 4. For non-adsorbed proteins (Fig. 4), the composition of proteins includes α'-, α- and β-7S subunits of MM of 92, 80 and 55 kDa, and A- and B-11S polypeptides of 42 and 22 kDa, respectively. A soluble aggregate of MM of 220 kDa was observed for the control and 200 MPa treated samples. This aggregate disappeared at major pressures, and it was not adsorbed at the interface

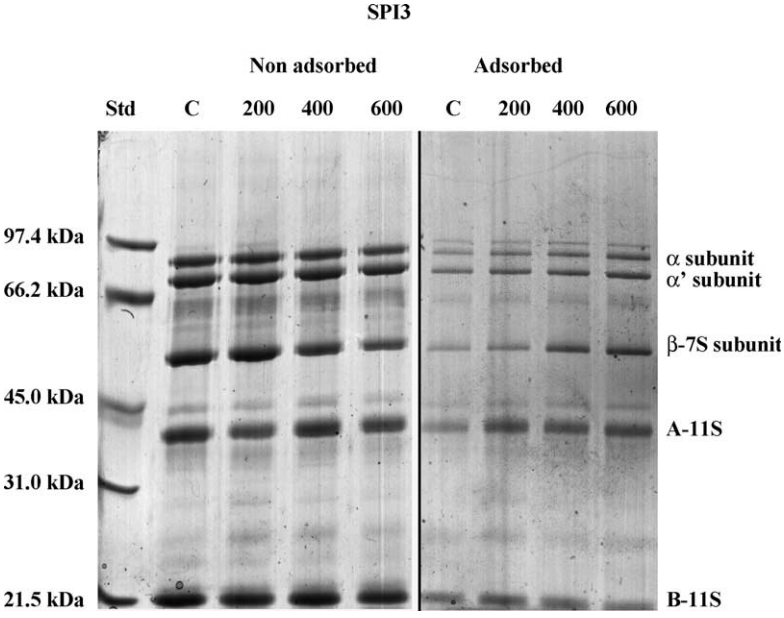

Fig. 5. SDS-PAGE profile of adsorbed and non-adsorbed at the oil–water interface SPI3 from untreated and high-pressure treated (200, 400 or 600 MPa for 10 min). Electrophoresis carried out with stacking and running gel, respectively, at 3.5 and 10% of polyacrylamide. Oil-in-water emulsions 30:70 prepared with 1% protein (w/v). SPI fractions were stained with Coomassie blue. MW: Molecular weight, Std: Standard marker, C: Control.

(Fig. 4). The intensity of non-adsorbed β-7S and A-11S polypeptides decreased while the same polypeptides in the cream phase increased at very high pressures (400 and 600 MPa). At the interface, a band of 62 kDa was observed for the control sample, whereas the pressurised protein emulsions presented in this region three bands of 60, 62 and 64 kDa.

Fig. 5 shows for SPI3 the same non-adsorbed protein composition than that observed for SPI8 emulsions. A decrease of the intensity of β subunit was observed after pressure treatments at 400 and 600 MPa. A different protein composition was detected for adsorbed SPI3 proteins: a new band of 91 kDa was detected (Fig. 5). Previous high-pressure treatment of SPI3 proteins produced more adsorption of β-7S and A-11S polypeptides, specially at 400 and 600 MPa. This selective interfacial adsorption could be due to distinct changes produced by high pressure on both polypeptide structure via modification of non-covalent interactions.

4. Discussion

We have clearly demonstrated that previous high-pressure treatment of soybean proteins alters their emulsifying properties and that these modifications depend on pH value. At pH 8, the major alterations provoked by the treatment are a decrease of the droplet size, an increase of the depletion flocculation and of the percentage of adsorbed proteins. We have previously evidenced (Puppo et al., 2004) that high-pressure treatment induces physicochemical changes of soybean proteins at pH 8: an increase of surface hydrophobicity, a partial unfolding of 7S and 11S fractions, and an aggregation of proteins, specially of the 11S fraction. Such physicochemical modifications are coherent with the results obtained in the present study. The increase of surface hydrophobicity combined with a partial denaturation and a more disordered structure are able to provide a better potentiality for the adsorption at the oil–water interface. This could explain the decrease of droplet size and the increase of the percentage of adsorbed proteins as the high-pressure treatment is improved. Furthermore, under turbulent conditions occurring during homogenisation, convection movements favour the displacement of more dense materials and then adsorption of aggregates predominates (Walstra, 1983). We can assess that the soluble aggregates generated by high-pressure treatment of SPI8 are likely to be projected and adsorbed at the oil–water interface during homogenisation. This is in accordance with the increase of the percentage of adsorbed proteins observed in our study.

Molina et al. (2001), observed also an improvement of the emulsifying activity index of 7S and 11S fractions of soybean proteins at pH 7.5 when a previous high-pressure treatment was applied between 200 and 600 MPa.

On the other hand, in this study, the presence of soluble aggregates generated during high-pressure treatment could also explain, in case of non adsorption, the reinforcement of the depletion flocculation phenomenon by the creation of a depleted volume in the emulsion.

Concerning the results obtained at pH 3, we recently demonstrated that acidic treatment of soybean proteins conducted to a drop of solubility from 85% at pH 8 to 60% at pH 3 (Puppo et al., 2004). Consequently, the decrease of pH before high-pressure treatment has an initial denaturing effect. We have observed that this acidic treatment induced an increase of droplet size, a slight decrease of bridging flocculation and an important improvement of depletion flocculation. It is likely that at pH 3 the drop of solubility and the presence of non adsorbed aggregates could explain these effects on emulsion characteristics.

At this pH, the impact of high-pressure treatment on emulsifying properties of soybean proteins is clearly focused on a reduction of bridging flocculation and on an increase of adsorbed proteins. We have demonstrated in a previous study (Puppo et al., 2004) that high-pressure treatment induced an increase of solubility and an increase of surface hydrophobicity of SPI3. We can suggest that the increase of surface hydrophobicity leads to a better potential adsorption of proteins at the oil–water interface. This is corroborated by the associated increase of the percentage of adsorbed proteins. The decrease of bridging flocculation could be influenced by the increase of percentage of adsorbed proteins as bridging is often caused by the adsorption on two different droplets during emulsion formation due to the lack of proteins at the interface. However, this is not the unique explanation as for SPI8 emulsions we observed an important increase of the percentage of adsorbed proteins associated with only a slight decrease of bridging flocculation.

5. Conclusion

Our results indicate that high-pressure treatment improves the emulsifying activity of SPI8. Emulsions prepared with high-pressure treated SPI8 showed a smaller droplet size and an increase of the percentage of adsorbed proteins. On the other hand, the same emulsions presented an increase of depletion flocculation and a slight decrease of bridging flocculation, as compared to untreated samples. For SPI3, the acidification contributes to a first modification of emulsifying properties: increase of droplet size and of depletion flocculation. The high-pressure treatment results on an increase of adsorbed proteins and on a decrease of a flocculation.

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