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Effects of high pressure on functional properties of soy protein

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Abstract

Simultaneous measurement of the effects of low soy protein concentration, pH and high pressure treatment at room temperature on solubility, emulsifying properties and rheological properties (loss modulus, G'') of soy protein isolate (SPI) were evaluated. Central composite rotatable designs (2^3) were employed over two pH ranges (2.66–4.34 and 5.16–6.84) with SPI concentration (0.32–3.68%) and pressure (198–702 MPa) as the other independent variables. The surface responses were obtained for protein solubility, emulsifying activity index (EAI) and G''. The samples with the highest effect on protein solubility, EAI and G'' values were evaluated, as well, by electrophoresis and free sulphydryl determination. The pH was the main factor that affected protein solubility, with solubility at a maximum at pH < 3 or pH > 6. Increasing SPI concentration and decreasing/increasing the pH away from the isoelectric point both caused a reduction in EAI. Loss modulus (G'') was found to increase with SPI concentration in both pH ranges.

Keywords: Soy protein isolate (SPI); High pressure; Functional properties; pH; Loss modulus

1. Introduction

Soy protein is the predominant commercially available vegetable protein in the world and may be the most inexpensive source of protein for nutritional or technological uses. Soy protein isolate (SPI) contains at least 90% protein and it is widely used as an ingredient in meat products, baby foods, beverages and bread products. Its nutritional value is linked mainly to β-conglycinin and glycinin globulins; however, these proteins exhibit differences in their functional properties.

Proteins represent a most important class of technological functional ingredients because they possess a range of dynamic functional properties, (sensorial, kinaesthetic, hydration, surface and rheological/textural) that can show versatility during processing and improve quality attributes of foods (Kinsella, Rector, & Phillips, 1994). Vojdani (1996) reported that proteins have two important properties that are responsible for their various functional properties; these are solubility and hydrodynamic properties. Solubility is the result of the surface-active properties of proteins, as well as foaming, emulsification, fat- and flavour-binding properties. Hydrodynamic properties of proteins influence viscosity and gelation. The pH has a strong influence on both these aspects of protein functionality because of its control over the ionisation state of ionisable groups of the protein molecule (Petruccelli & Añon, 1996).

In addition to its applications for food preservation, high-pressure treatment can also be used to modify the functional properties of food components, mainly proteins (Messens, Van Camp, & Huyghebaert, 1997). It is known that high pressure can affect protein conformation and can lead to protein denaturation, aggregation or gelation, depending on the protein system, the applied pressure, the solution conditions, and the magnitude and duration

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of the applied pressure treatment (Galazka, Dickinson, & Ledward, 2000). Therefore, in recent years some researchers have studied the potential to modify functional properties of soy protein and their products using high-pressure technology (Apichartsrangkoon, 2003; Kajiyama, Isobe, Uemura, & Noguchi, 1995; Molina & Ledward, 2003; Molina, Papadopoulou, & Ledward, 2001; Puppo et al., 2004; Zhang, Li, Tatsumi, & Isobe, 2005).

Kajiyama et al. (1995) reported an improvement in the emulsifying activity of soy milk after high-pressure treatment. Molina et al. (2001) also reported that pressure treatment at neutral pH could improve the emulsifying activity of soy protein, but that solubility was not greatly influenced by high pressure. Apichartsrangkoon (2003) used a three-way factorial design to evaluate the influence of pressure, temperature and holding time on rheological properties of soy protein gels. It was found that the rheological properties were more affected by temperature than by pressure. Molina and Ledward (2003) studied the effects of combined high pressure and heat treatment on textural properties of soy gels. Puppo et al. (2004) observed an increase in protein surface hydrophobicity and aggregation; a reduction of free SH, and a partial unfolding of 7S and 11S fractions were observed in high pressure-treated SPI samples at pH 8. Changes in secondary structure were also detected, which led to a more disordered structure. Zhang et al. (2005) studied the effects of high-pressure treatment on the modifications of soy protein in soy milk, using various analytical techniques. Electrophoretic analysis showed the change of soy protein clearly and indicated that soy proteins were dissociated by high-pressure into subunits, some of which associated to aggregate and became insoluble.

Despite the existing literature describing high pressure treatment of soy protein, there are very limited data available on the simultaneous measurement of the effects of low soy protein concentration, pH and high-pressure treatment at room temperature on solubility, emulsifying properties and rheology, relating to the exploration of alternative processing conditions for novel applications of SPI.

2. Materials and method

2.1. Extraction of native soy protein isolate

Soy protein isolate (SPI) was prepared from defatted flour (Prosam R®, Bunge Alimentos S.A., Brazil) by alkaline extraction (pH 8), followed by precipitation at pH 4.5, with some modifications, as described in Takeiti (2002). The isoelectric precipitate was dispersed in deionised water, neutralised and freeze-dried.

2.2. Preparation of samples

Aliquots of 50 ml of protein dispersions (w/v) of SPI, at certain concentration values, and different pH values, were used, as shown in Table 1. The pH adjustment was by addition of 2 N of HCl or 2 N NaOH solutions. The prepared aliquots were sealed in Cryovac® plastic bags and then pressurised.

Table 1
Definition and coded levels for central composite design (2^3)

Independent variable	Coded levels				
	−1.68	−1	0	1	1.68
Low pH					
SPI concentration (%)	0.32	1.00	2.00	3.00	3.68
pH	2.66	3.00	3.50	4.00	4.34
Pressure (MPa)	198	300	450	600	702
Near-neutral pH					
SPI concentration (%)	0.32	1.00	2.00	3.00	3.68
pH	5.16	5.50	6.00	6.50	6.84
Pressure (MPa)	198	300	450	600	702

2.3. High pressure treatment

Samples of protein solutions were subjected to high pressures as shown in Table 1 for 20 min at ambient temperature, using a Stansted 'Food Lab' high pressure 'rig' (Stansted Fluid Power Ltd., Stansted, Essex, UK).

2.4. Statistical analysis

Two 2^3 central composite designs, each with a star configuration (six axial points) and three central points, totalling 17 assays were used to detect the optimum concentration of SPI, pH and pressure for maximum values of protein solubility, EAI and G'', as shown in Table 1. The experiments were carried out in a randomised way. The distances of axial points were ± 1.68, calculated as shown in Rodrigues and Iemma (2005). The data were treated with the aid of Statistica 5.5 software from Statsoft Inc. (3225 East 13th Street, Tulsa, OK, 74104, USA). Protein solubility, EAI and G'', were fitted to a second-order model equation and examined in terms of the goodness of fit. ANOVA was used to evaluate the adequacy of the fitted model. The R-squared value provides a measure of how much of the variability in the observed response values can be explained by experimental factors and their interactions.

2.5. Analytical procedures

2.5.1. General

All samples are measured for protein solubility, emulsifying activity index and rheological measurement (determination of G'') as described below. The samples from both pH ranges having the highest protein solubility, EAI and G'' were further analysed to determine free sulphydryl content and by electrophoresis.

2.5.2. Protein solubility

Protein solubility was determined at the original pH of each sample, as described by Morr et al. (1985). The pro-

tein content of each sample was adjusted to 1% of protein (w/v) with deionised water and centrifuged at 20,000*g* for 30 min at 4 °C and the resulting supernatant fraction was filtered through Whatman no.1 filter paper. The percentage of solubility of the protein in each sample was determined in duplicate using a bicinchoninic acid protein assay kit by sigma for standard 2.1 ml assay protocol at 562 nm.

2.5.3. Emulsifying activity

Emulsifying activity index (EAI) was measured following Molina et al. (2001) and Pearce and Kinsella (1978). Dispersions of 0.5% (w/v) of SPI in water were homogenised with corn oil (1:3) using a Turrax® homogeniser at 10,000 rpm for 30 s. An aliquot of 1 g of emulsion was diluted (1/500) with 0.1% SDS solution and the absorbance was measured at 500 nm.

2.5.4. Rheological measurement

Dynamic oscillatory measurements were performed on a RTI Controlled Stress Rheometer, using a 50 mm diameter parallel plate system with 1 mm gap setting at a temperature of 25 ± 0.3 °C. Frequency sweeps were performed over the range 0.1–10 Hz at torque of 0.5 m Nm. As G'' is the main component of G^* this value was used to compare the results. As $G' \times$ frequency and $G'' \times$ frequency plots showed the same tendency (data not shown), just the frequency of 1.667 Hz was used to plot G'' in surface response graphics.

2.5.5. Free sulphydryl content

Free sulphydryl (SH) content of selected samples was determined as described by Beveridge, Toma, and Nakai (1974) and Hardham (1981). Ellman's reagent was prepared by dissolving 4 mg of 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB) in 1 ml of Tris–Glycine buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per litre, pH 8.0). Ten milligrammes of sample were dissolved in 10 ml of 8 M urea in Tris–Glycine buffer and incubated for 30 min at room temperature with 100 μl of Ellman's reagent. Absorbance was measured at 412 nm in an UV/VIS/NIR Perkin–Elmer Lambda Spectrometer controlled via the UV Winlab® software. Results were expressed as the means of duplicate analyses.

2.5.6. Polyacrylamide gel electrophoresis

Native PAGE polyacrylamide gel electrophoresis was carried out in a gel with 4.5–10% w/v concentration gradient following Laemmli (1970). Separation gel solution was prepared with 1.5 M Tris buffer (pH 8.8). Protein samples were standardised to 0.1% protein with deionised water. The standardised protein solutions were diluted 1:1 with sample buffer (1.5 g Tris, 2 mg bromophenol blue, 10 ml glycerol per 100 ml, adjusted to pH 6.8 with 5 M HCl). Fifteen microlitres of each sample were loaded into each channel in the gel.

Electrophoresis was conducted with tank buffer (0.025 M Tris, 0.192 M glycine, pH 8.6–8.7) at 500 V, 50 mA, until the blue band reached the bottom of the gel (approximately 60 min). The staining method was adopted from Puppo et al. (2004); gel was immediately stained with the staining solution (Coosmasie blue 0.05%, ethanol 25%, acetic acid 10%) overnight (minimum 12 h). The stained gel was then destained (destaining solution of acetic acid 7%, ethanol 40%, water 53%).

3. Results and discussion

3.1. Protein solubility

The regression coefficients for the model that predicts protein solubility at low pH, as a function of SPI concentration, pH and pressure, show that only the mean, pH linear term and pH quadratic term were statistically significant ($p < 0.05$). Therefore, the non-significant terms were ignored and a second-order model (Eq. (1), $R^2 = 0.89$) describing the protein solubility as a function of pH was established. The pure error calculated from the central points was very low (about 0.13%) according to the total sum of squares, indicating good reproducibility of the experimental data. Based on the *F*-test, the model was predictive ($p < 0.05$), since the calculated regression *F*-value was 56.6, which was 15 times higher than the Table value of 3.74. Therefore, the coded model expressed by Eq. (1) was used to generate contour diagrams for protein solubility at low pH.

$$\text{Protein solubility} = 11.91 - 18.92\ \text{pH} + 7.54\ \text{pH}^2 \qquad (1)$$

The regression coefficients for the model predicting protein solubility at near-neutral pH reveal that the mean, SPI concentration, pH linear term and pH quadratic terms were statistically significant factors ($p < 0.05$). The terms that were not statistically significant were ignored and a second-order model (Eq. (2), $R^2 = 0.88$), describing the protein solubility as a function of SPI concentration and pH, was established. The pure error calculated from the central points was low (about 0.71%) according to the total sum of squares, indicating good reproducibility of the experimental data. Based on the *F*-test the model was predictive, since the calculated *F*-value of regression was 31.4, which is about 9 times higher than the table value of 3.41. Therefore, the coded model, expressed by Eq. (2), was used to generate contour diagrams for protein solubility in the near-neutral pH range.

$$\begin{aligned}\text{Protein solubility} = {} & 56.93 + 22.07(\text{pH}) \\ & - 11.62(\text{SPI concentration})^2 \\ & - 8.01(\text{pH})^2 \end{aligned} \qquad (2)$$

Fig. 1 shows the influence of the studied variables on protein solubility. The highest protein solubility value was for pH < 3 or pH > 6. The pH was the dominant influence on protein solubility in the low pH range (i.e., pH 2.66–4.34), regardless of SPI concentration or the high-pressure treatment used. SPI concentration was a factor at near-neutral pH, with the highest solubility being in the range of 1.5–

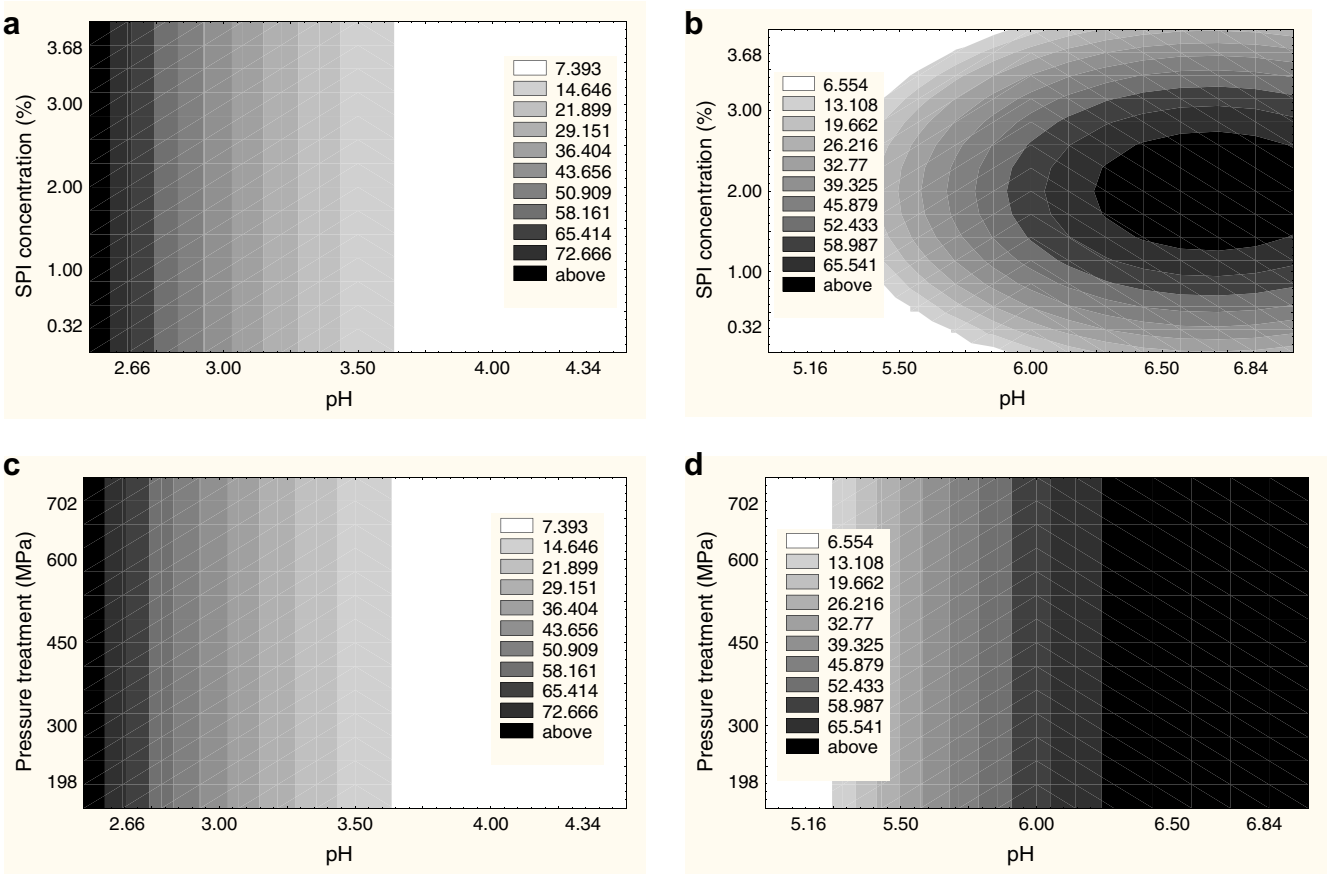

Fig. 1. Contour diagrams for protein solubility as a function of: (a) pH and SPI concentration at pH 2.66–4.34, (b) pH and SPI concentration at pH 5.16–6.84, (c) pH and high-pressure treatment at pH 2.66–4.34, and (d) pH and high-pressure treatment at pH 5.16–6.84.

3% concentration. If pH > 3.5 or pH < 5.5, then protein solubility was very low, which can be attributed to the known phenomenon of protein aggregation near the isoelectric point (pH 4.2–4.6) (Hettiarachchy & Kalapathy, 1999).

As observed in Fig. 1 and Eqs. (1) and (2), pressure treatment did not significantly influence protein solubility, which has been reported previously by other researchers (Molina et al., 2001; Puppo et al., 2004; Apichartsrangkoon, 2003). The highest solubility within the low pH range was 53% (pH 2.66, 2% SPI concentration, 450 MPa) that it is about 11.5% higher than the solubility of untreated samples (47.5%). In the near-neutral pH range, the highest solubility was 65.1% (pH 6.84, 2% SPI concentration, 450 MPa), an approximate 37% increase over an untreated sample.

3.2. Emulsifying activity

All of the studied variables had some statistically significant effect on EAI. At low pH, only the pressure linear term was not statistically significant ($p < 0.05$) and, at near-neutral pH, it is apparent that only the pressure linear term and SPI concentration by pressure interaction term were non-significant ($p < 0.05$). The non-significant terms were ignored and second-order models were established for low pH (Eq. (3), $R^2 = 0.86$) and near-neutral pH (Eq. (4), $R^2 = 0.89$) ranges.

$$\begin{aligned}\text{EAI} = {} & 92.06 - 24.14(\text{SPI concentration}) + 64.54(\text{pH}) \\ & + 22.81(\text{SPI concentration})^2 + 30.40(\text{pH})^2 \\ & + 26.32(\text{pressure})^2 - 50.82(\text{SPI concentration})(\text{pH}) \\ & + 21.49(\text{SPI concentration})(\text{pressure}) \\ & - 35.40(\text{pH})(\text{pressure})\end{aligned} \tag{3}$$

$$\begin{aligned}\text{EAI} = {} & 85.06 - 39.01(\text{SPI concentration}) - 47.40(\text{pH}) \\ & + 33.57(\text{SPI concentration})^2 + 12.76(\text{pH})^2 \\ & - 13.19(\text{pressure})^2 + 15.45(\text{SPI concentration})(\text{pH}) \\ & - 14.34(\text{pH})(\text{pressure})\end{aligned} \tag{4}$$

In both cases the pure error calculated from the central points was very low (<0.18%) according to the total sum of squares, indicating good reproducibility of the experimental data. Based on the F-test the model was predictive ($p < 0.05$) since the calculated regression F-values were higher than the appropriate Table values ($5.92 > 3.44$ at low pH and $10.1 \gg 3.29$ at near-neutral pH). Therefore, the coded models expressed by Eqs. (3) and (4) were used to generate the contour diagrams shown in Fig. 2 for

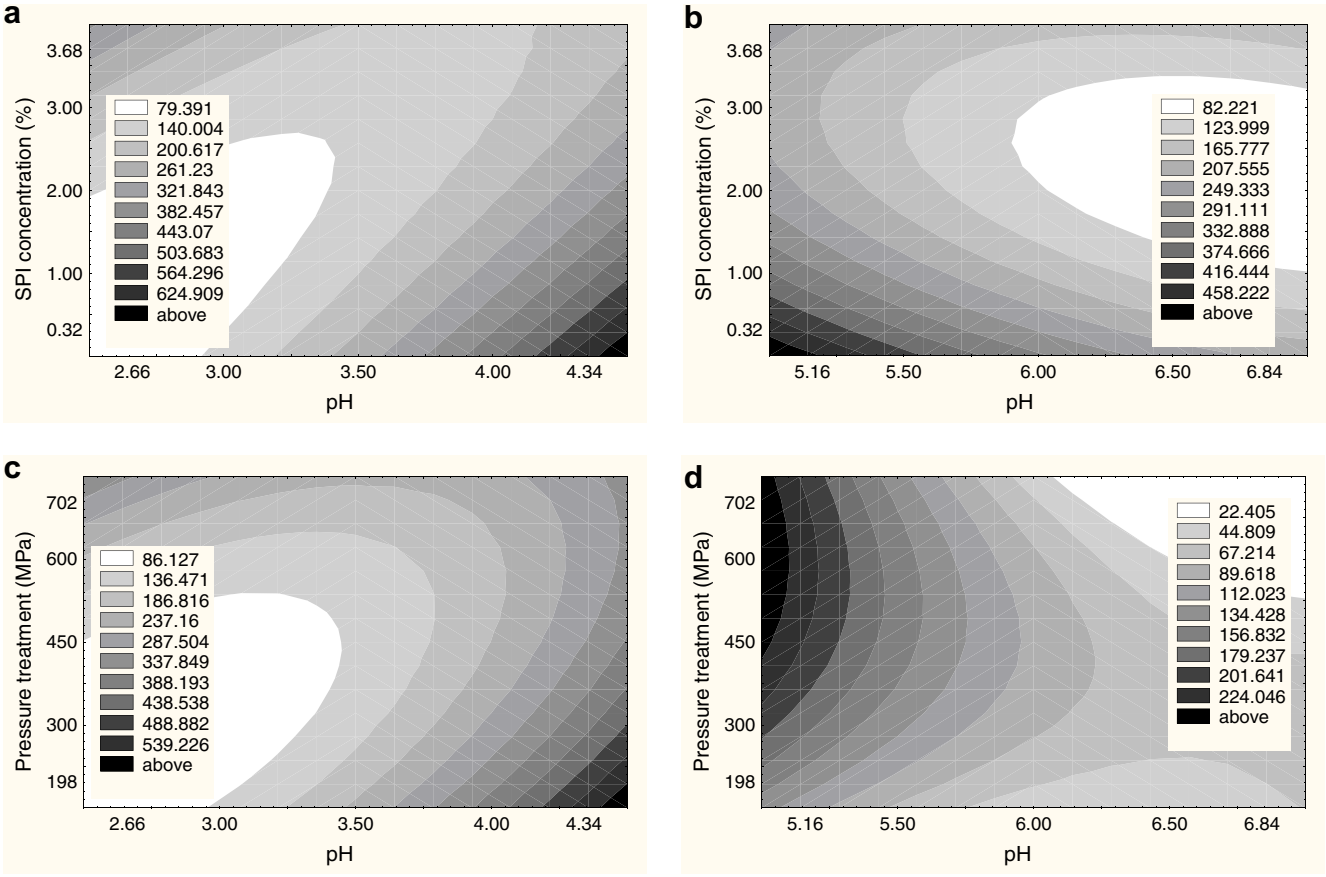

Fig. 2. Contour diagrams for EAI as a function of (a) pH and SPI concentration at pH 2.66–4.34, (b) pH and SPI concentration at pH 5.16–6.84, (c) pH and high-pressure treatment at pH 2.66–4.34, and (d) pH and high-pressure treatment at pH 5.16–6.84.

EAI. From these contour diagrams, it is apparent that EAI would be highest with a combination of the lowest SPI concentration, pH near the isoelectric point and the lowest pressure treatment. Indeed, the observation with respect to pressure treatment agrees with the work of Dickinson and James (1998), who measured the effect of high pressure on properties of emulsions made with pure milk proteins and found that high pressure treatment reduced the emulsifying capacity of β-lactoglobulin and that the extent of the reduction increased with increasing pressure. However, it is also worth noting that, at near-neutral pH (5.16), high-pressure treatment improved the emulsifying activity of soy protein, which agrees with the findings of Molina et al. (2001).

3.3. Loss modulus

Loss modulus or viscous component (G'') was the dominant component of rheological measurements, showing a viscous flow behaviour in all studied samples. Results showed that G'' was influenced in some way by all of the studied variables, with a positive correlation between both protein concentration and high-pressure treatment and the value of G''.

The analysis of the regression coefficients at low pH shows that the pressure quadratic term and the interaction SPI concentration by pressure are not statistically significant ($p < 0.05$); therefore these terms were ignored in the second-order model (Eq. (5), $R^2 = 0.93$) describing G'' as a function of SPI concentration, pH and pressure. The pure error calculated from the central points was low (about 2%) according to the total sum of squares, indicating good reproducibility of the experimental data. Based on the F-test the model was predictive since the calculated regression F-value of 16.7 was about 5 times higher than the Table value of 3.29.

$$G'' = 9.84 + 0.09(\text{SPI concentration}) + 0.12(\text{pH}) + 0.22(\text{pressure}) - 0.17(\text{SPI concentration})^2 - 0.23(\text{pH})^2 - 0.11(\text{SPIconcentration})(\text{pH}) + 0.10(\text{pH})(\text{pressure}) \quad (5)$$

The linear terms of SPI concentration, pH and pressure and also the pressure quadratic term were not statistically significant ($p < 0.05$) for G'' response at near neutral pH. These terms were therefore ignored in the second-order model (Eq. (6), $R^2 = 0.79$) describing G'' as a function of SPI concentration, pH and pressure. The pure error calcu-

lated from the central points was low (about 0.86%) according to the total sum of squares, indicating good reproducibility of the experimental data. Based on the *F*-test the model was predictive since the calculated *F*-value of regression was 8.27 and was about 2.5 times higher than the listed 3.20. The coded models expressed by Eqs. (5) and (6) were used to generate the contour diagrams for G'' shown in Fig. 3.

$$G'' = 8.30 + 0.45(\text{SPI concentration})^2 + 0.57(\text{pH})^2 + 0.64(\text{SPI concentration})(\text{pH}) - 1.04(\text{SPIconcentration})(\text{pressure}) - 1.02(\text{pH}) \times (\text{pressure}) \quad (6)$$

3.4. Effects on free SH content

Fig. 4 shows the free SH content of untreated SPI and of selected samples from both pH ranges showing the highest protein solubility, EAI and G''. The SH free content in untreated SPI (7.41 μmol/g of protein) was lower than the 10 μmol/g of protein found by Liu, Xiong, and Butterfield (2000), but was higher than the 3.82 μmol/g of protein found by Takeiti (2002). Free SH contents are lower in all the samples in comparison with initial SPI, just by preparation, so we can conclude that pH has a strong effect on the aggregation of these samples. After high-pressure treatment, all the sam-

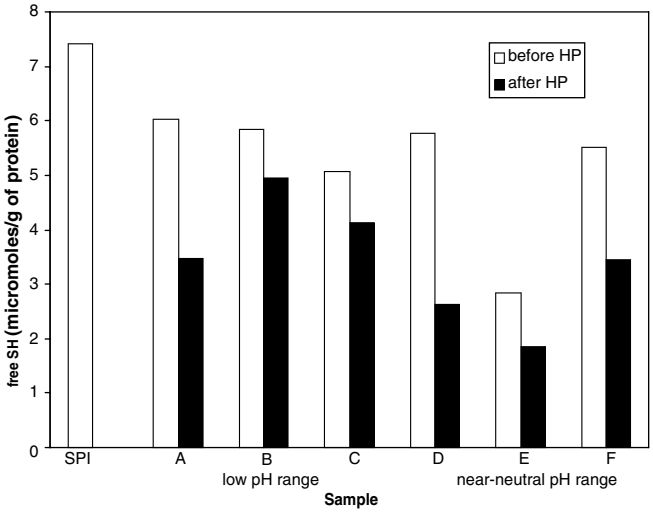

Fig. 4. Free SH content (μ mol/g of protein) of SPI and of selected samples before and after high-pressure treatment ($A = 1\%$ SPI, pH 4.00, 300 MPa; $B = 2\%$ SPI, pH 2.66, 450 MPa; $C = 2\%$ SPI, pH 3.50, 702 MPa; $D = 1\%$ SPI; pH 5.50, 600 MPa; $E = 0.32\%$ SPI, pH 6.00, 450 MPa; $F = 2\%$ SPI, pH 6.84, 450 MPa).

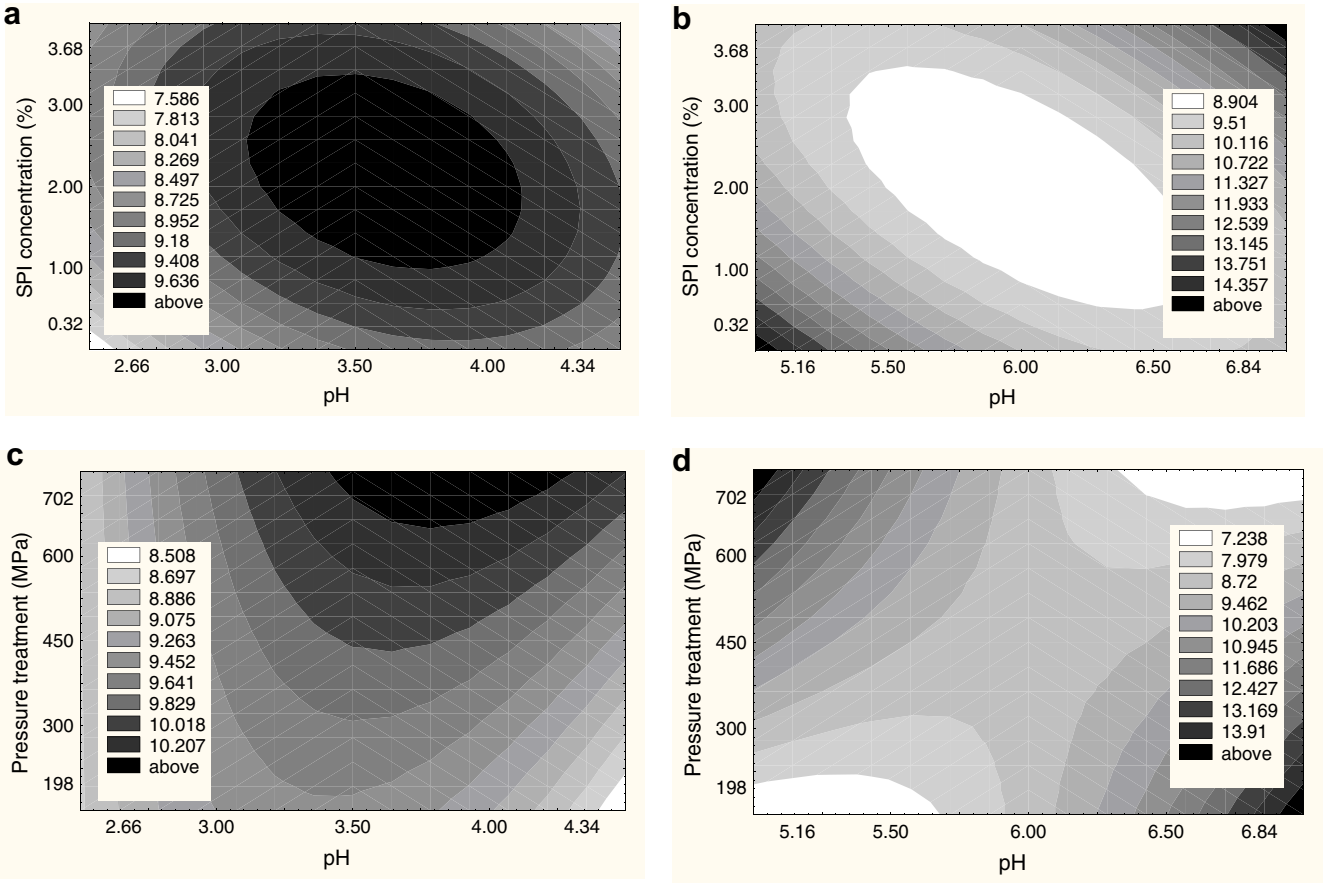

Fig. 3. Contour diagrams for loss modulus (G'') as a function of (a) pH and SPI concentration at pH 2.66–4.34, (b) pH and SPI concentration at pH 5.16–6.84, (c) pH and high-pressure treatment at pH 2.66– 4.34, and (d) pH and high-pressure treatment at pH 5.16–6.84.

ples exhibited lower SH contents. Galazka et al. (2000) and Puppo et al. (2004) suggested that this occurred due to the formation of S–S bonds through SH/S–S interchange reactions. Kajiyama et al. (1995) also reported that, whatever the soy protein concentration, SH content was consistently lower after pressure treatment than before.

3.5. Electrophoresis

Native PAGE electrophoresis, before and after high-pressure treatment of the same selected samples, as analysed for free SH content, is shown in Fig. 5. All samples appeared to have changes in the native PAGE profiles after high pressure treatment. In general, band A (marked in Fig. 5) remained intact in all the samples, regardless of pressure treatment conditions or sample composition. The untreated samples in the low pH range showed distinctive bands at the start of the gel. As the pH increased (Sample 11–3) the amount of more mobile smaller units seemed to decrease in quantity. When proteins approached the p*I* of 4.60, the proteins started to aggregate. At the lowest pH of 2.66 (sample 11), the electrostatic charges prevented the formation of aggregates, resulting in fewer bands at the start of gel, probably indicating the presence of smaller and more mobile units in sample 11. Puppo et al. (2004) suggested that, under acidic conditions, high mobility species observed in 11S would dissociate during pH treatment (not observed in 7S). After high-pressure treatment, most of the distinctive bands disappeared, especially in samples 14 and 3. It seems that the pressure treatment could have dissociated the proteins, breaking the aggregates into smaller units. However, at the lowest pH of 2.66 (sample 11), the loss of the larger unit appears to be minor. This could suggest that, at extreme low pH, the effect of pH is stronger than the effect of high-pressure (comparing samples 11 and 3). This result reinforces the lower efficacy of high-pressure treatment at lower pH.

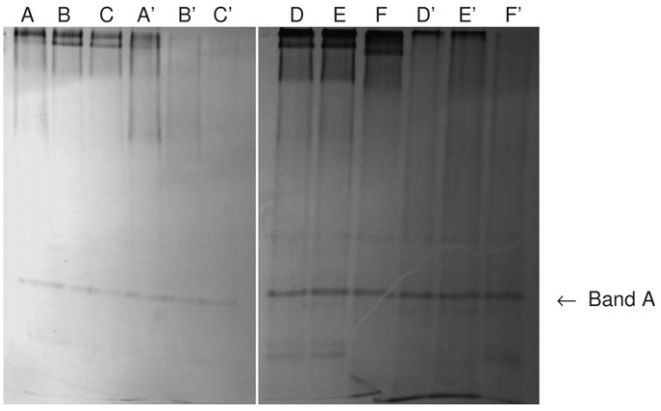

Fig. 5. Native PAGE of selected samples before and after high-pressure treatment ($A = 2\%$ SPI, pH 2.66 and $A' =$ sample A after 450 MPa treatment; $B = 2\%$ SPI, pH 3.50 and $B' =$ sample B after 702 MPa treatment; $C = 1\%$ SPI, pH 4.0 and $C' =$ sample C after 702 MPa treatment; $D = 0.32\%$ SPI, pH 6.00 and $D' =$ sample D after 450 MPa treatment; $E = 2\%$ SPI, pH 6.84 and $E' =$ sample E after 450 MPa treatment; $F = 1\%$ SPI, pH 5.50 and $F' =$ sample F after 600 MPa treatment).

In the near-neutral pH range, all of the samples, before treatment, displayed large amounts of aggregation at the top of the gel with sample 5 showing the highest intensity. Sample 5 had a lower pH than the other 2 (samples 9 and 12), which was approaching the pI of soy protein, of around 4.50. The proteins at this pH have less overall electrostatic charge, resulting in more aggregation, which explains the darker band in sample 5 and appears to have slightly more smaller mobile units as well. After treatment, the larger units at the start of gel virtually disappeared, with no evidence of new bands forming elsewhere. The samples 9 and 12, after treatment, showed a small degree of aggregation, probably suggesting that some form of aggregates were still intact or formed after treatment.

SDS-PAGE profiles (data not shown) of these selected samples, before and after high-pressure treatment, showed no visible changes before and after high-pressure treatment. This result is in accordance with Apichartsrangkoon (2003) who found no changes between control samples of soy protein concentrate and high pressure-treated (200–800 MPa) samples at ambient temperature when analysed by SDS-PAGE.

4. Conclusions

Protein solubility was strongly influenced by pH, although, in the near-neutral pH range, the SPI concentration also influenced protein solubility. Emulsifying activity was influenced in some way by SPI concentration, pH and pressure treatment. In both pH ranges, increasing the protein concentration caused a reduction in EAI. In the low pH range the EAI values were highest with low pressure treatments whereas, in the near-neutral pH range, the highest EAI was at the middle range of pressure treatment and lower pH.

G'' (loss modulus or viscous component) was the dominant component of rheological measurements in both pH ranges analysed, showing a viscous flow behaviour in all studied samples. G'' was influenced in some way by SPI concentration, pH and pressure, although the overall effects were low. The protein concentration had a positive influence on G'' in both pH ranges and the increase of high pressure supported the increase of G'' in the low pH range.

The analysis of some selected samples in both pH ranges showed that, after high pressure treatment all the samples exhibited lower values of SH content in comparison with those before treatment, suggesting some changes in structure that were confirmed by native PAGE electrophoresis.

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