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Effects of high pressure on rheological properties of soy protein gels

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Abstract

Hydrated soy concentrate of 80% moisture content was subjected to pressures of 200, 400, 600 and 800 MPa at 20 and 60 °C for 20 and 50 min. The treated samples were subsequently analysed for viscoelastic behaviour and nitrogen solubility and their SDS-PAGE electrophoretic patterns were recorded. It was found that the rheological properties were more affected by temperature than pressure. The shape of the G' and G'' data, as a function of frequency, changed little with temperature and/or pressure treatment. The chemical studies suggest that some limited disulphide bond formation occurs in temperature- and pressure-induced gel systems.
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Keywords: High Pressure; Soy concentrate; Viscoelastic gel

1. Introduction

Soy is a major source of protein in most vegetarian foods and high pressure treatment is a recently commercialised process which may be used with advantage in these systems. The major advantages would be that, in comparison with heat set gels, high pressure induces less browning and generally gives a softer and smoother texture (Apichartsrangkoon, Bell, Ledward, & Scholfield, 1999; Apichartsrangkoon, Ledward, Bell, & Brennan, 1998).

Soy protein consists of two main components, β-conglycinin, with molecular mass of 180 kDa, and glycinin, with molecular mass of 360 kDa. Upon heating, soy gel is primarily aggregated by hydrophobic interactions (Sheard, Fellows, Ledward, & Mitchell, 1986), since the 11S and 7S globulins contain about 39 and 41% w/w hydrophobic amino acid residues (Damodaran, 1996). With increasing soy concentration, disulphide bond formation does though become a major factor in stabilising the gel structure. 7S and 11S globulins both have two or more active free SH groups (Zayas, 1997) and hydrophobic groups may also become exposed during treatment of the protein; thus both types of linkage may contribute to the final gel structure (Fukushima, 1980).

Although work has been reported on the effects of different pressure temperature combinations on gluten gels (Apichartsrangkoon et al., 1998, 1999) little work has been done on soy protein. Dumoulin, Ozawa, & Hayashi (1997) found that a soy protein solution (17% w/w) pressure treated at 400 and 500 MPa, at −5–50 °C for 30 min, gave gels with increasing hardness with increasing pressure and temperature but the pressure-induced gels were far softer and more deformable than heat-induced gels formed at atmospheric pressure.

In view of the great potential of high pressure to manipulate protein structures and thus generate novel gel textures, it was thought of interest to study the effects of high pressures on the rheological and chemical characteristics of concentrated soy protein (20%) systems in the further development of novel vegetarian foods of improved (textural) quality.

2. Materials and methods

2.1. Soy protein specification

Commercial soy protein concentrate (DURA-GRIP, Bemis Company, Inc., Crossett, Arkansas, USA) of the following composition was used: moisture 9.1±0.06%, ash 4.1±0.05%, lipid 0.85±0.06%, extracted as described in AOAC (1990), protein 75.6±0.87% (N × 6.25) determined by Kjeldahl method, starch 7.78±1.01% (Karkalas, 1985).

2.2. Preparation of the samples

Commercial soy-concentrate, with pH 7.06, was mixed with 3.8 times its weight of deionised water in a

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Nomenclature

G' Storage Modulus
G'' Loss Modulus

Morton Z blade mixer (Morton Machine Co. Ltd., Wishaw, Scotland) operated at high speed for 100 s and then low speed for a further 200 s. The resultant hydrated soy concentrate had a moisture of 81% w/w, which is the amount of water that soy can hold to form a firm, resilient and self-supporting gel at room temperature without any mealy appearance.The soy doughs were divided into 50 g portions and palced in polypropylene bags. The samples were then treated at the appropriate temperature and pressure for 20 or 50 min. The dynamic rheological properties were measured at 25 °C immediately after such treatments.

2.3. High pressure treatments

The 50 g of soy samples were subjected to pressures in the range 200–800 MPa at temperatures of 20, 40 and 60 °C for 20 or 50 min, as described previously (Defaye, MacDougall, & Tester, 1995). The pressure cell was maintained at the appropriate temperature by circulating water and the pressure was applied within 2 min; thus temperature equilibration occurred concomitantly with pressure treatment. The rate of pressure increase was about 250 MPa/min. During high pressure treatment, an adiabatic increase in temperature occurs. At ambient temperature, the monitored cell temperature increased by about 15 °C in the first 3 min after achieving 800 MPa but decreased to the initial temperature over the next 4 min. Proportionately smaller increases were observed at lower pressures and higher temperatures.

2.4. Rheological measurement

A controlled stress rheometer (Stress Tech Rheometer, Reologica Instruments AB, Lund, Sweden) was used to measure the dynamic viscoelastic properties of the high pressure treated soy samples. In order to ensure all measurements were carried out within the linear viscoelastic region, first stress amplitude sweeps were performed (data not shown). Based on these results, a stress amplitude of 100 Pa was chosen. A parallel plate measuring geometry was used (20 mm diameter) with a gap width of 2 mm. Samples were loaded onto the rheometer and allowed to equilibrate to the measuring temperature (25±1 °C) for 10 min. Excess sample was trimmed off and a thin layer of silicone oil applied to the exposed free edges to prevent moisture loss. Storage (G') and loss (G'') moduli were obtained over the frequency range 0.01–10 Hz.

2.5. Solubility analysis

Three grams (±0.2) of high pressure-treated soy concentrate were stirred overnight in 40 ml of 2% w/v sodium dodecyl sulphate (SDS) solution and 2% w/v SDS plus 2% v/v 2-mercaptoethanol at room temperature (25 °C). The solutions were then centrifuged at 34,000 g for 1 h, the supernatants dialysed against distilled water and subsequently freeze-dried. The percentage of nitrogen in each sample was determined by the thermal combustion method (Leco apparatus, Laboratory Equipment Company, Michigan).

2.6. Electrophoretic analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a gel with a 7.5–15% w/v concentration gradient (Laemmli, 1970). Gel solutions were diluted with 1.5 M Tris buffer (pH 8.8) and 10 μg of extracted sample were applied to each well. The extracted sample buffers were prepared in both reduced (2-mercaptoethanol) and non-reduced conditions. Fixing of the protein was done by immersion in 12% w/v trichloroacetic acid for 1 h and subsequent staining was accomplished using Coomassie brilliant blue G-250 (Neuhoff, Arold, Taube, & Ehrhardt, 1988).

2.7. Statistical analysis

The design of the experiments was three-way factorial, with the variables of pressure (200, 400, 600 and 800 MPa), temperature (20 and 60 °C) and holding time (20 and 50 min). After subjecting the samples to the various treatments, the dynamic rheological measurements were carried out on four replicate samples from two separate experiments ($n = 8$). Native (untreated) samples were analysed at the same time.

The Statistical Analysis System (SAS Institute Inc., Cary North, Carolina, 1995) software programme was used for statistical data analysis.

3. Results and discussion

3.1. Dynamic viscoelastic properties of pressurised soy protein

Soy protein exhibited (assessed using small sinusoidal stresses) some structural modifications through the pressure/temperature/time regimes studied. Fig. 1 shows how the shear moduli change as a function of frequency for soy samples pressure treated for 50 min at 20 °C

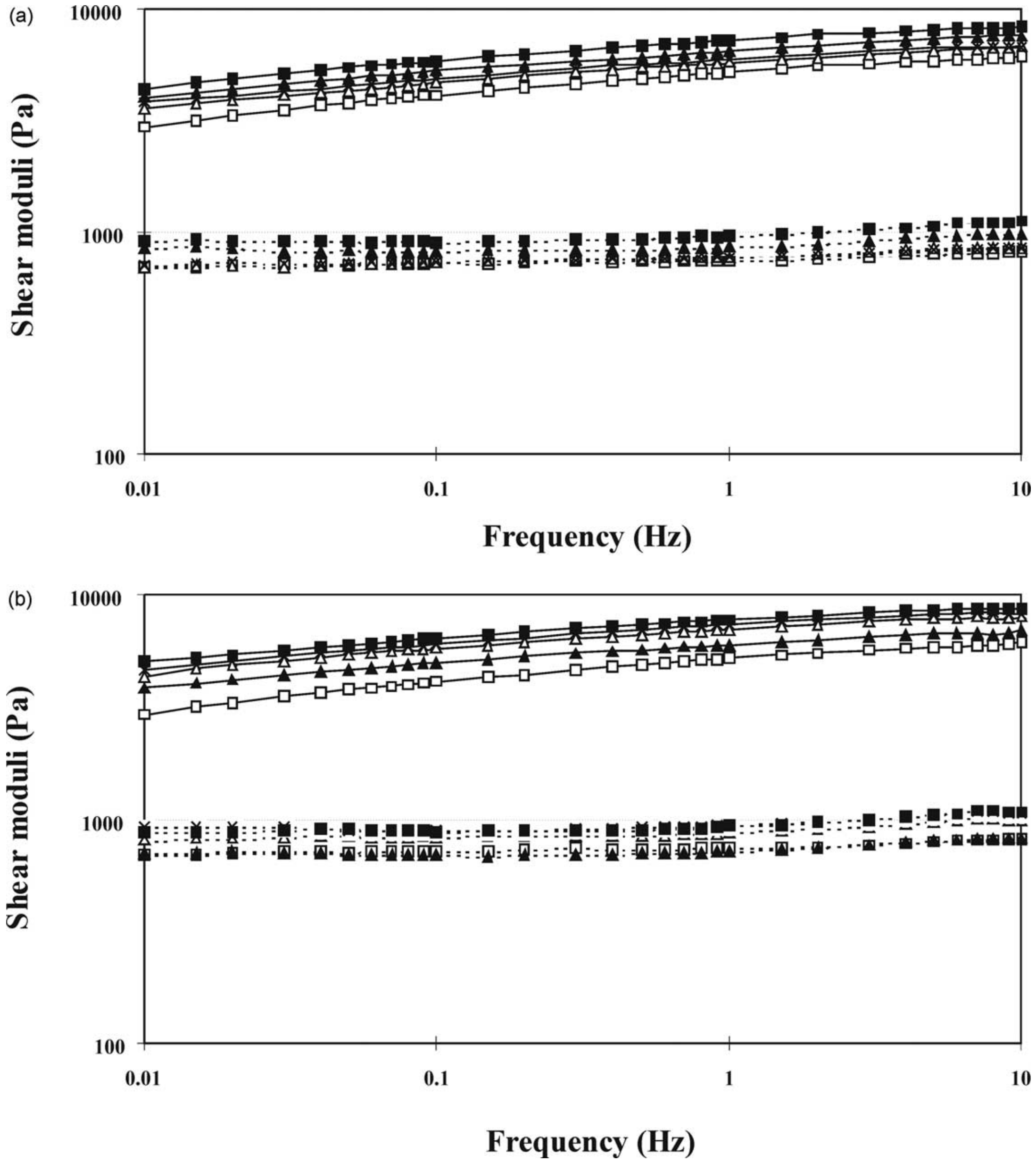

Fig. 1. The initial plots of shear moduli as a function of frequency for high pressure treated soy samples for 50 min at 20 °C (a) and 60 °C (b); *G*′ for the solid lines; *G*″ for the dotted lines; □ for the *G*′ and *G*″ control samples; △ for the *G*′ and *G*″ pressure treated at 200 MPa; × for the *G*′ and *G*″ pressure-treated at 400 MPa; ◆ for the *G*′ and *G*″ pressure-treated at 600 MPa; ■ for the *G*′ and *G*″ pressure-treated at 800 MPa.

(Fig. 1a) and 60 °C (Fig. 1b), respectively. Both storage and loss moduli increase little with increasing pressure, but temperature has slight effect on the storage moduli, and even less effects on the loss moduli. The storage moduli (at both temperatures) are more frequency dependent than the corresponding loss moduli and the loss tangents (tan δ) are low, illustrating predominantly solid-like response with an elastic network (Ross-Murphy, 1984).

Further considerations of structural modification by temperature and pressure regimes, with statistical analysis for *G*′ and *G*″ values, at a frequency of 1 Hz, are summarized in Table 1.

Table 1 shows the values of *G*′ and *G*″ at frequency 1 Hz. It is seen that all treatments give significantly greater *G*′ than the control or native samples. Treatments at low temperature (20 °C) have similar *G*′ values; i.e. treatments 1, 2, 5, 6 and 9 are distributed among the different pressure levels from 200 to 600 MPa. Treatments at the higher temperature (60 °C) yield similar *G*′ values; i.e. 3, 4, 7 and 16 are also distributed over even broader pressure regions (200 and 800 MPa). The intermediate values of the *G*′ are given by treatments 10, 11, 14 and 15, all in the high pressure regions (600 and 800 MPa). These results indicate that temperature causes rheological modification of soy samples more than

Table 1
Multiple comparison of storage (G') and loss (G'') moduli, at frequency 1 Hz of high pressure-treated soy concentrate

Treatment combinations	Pressurisation conditions			Viscoelastic properties	
	Pressure (MPa)	Temperature (°C)	Time (min)	Means of G' (kPa)	Means of G'' (kPa)
Control	0.1	20	0	4.73±0.27a	0.72±0.03a
1	200	20	20	5.24±0.18b	0.75±0.04a
2	200	20	50	5.45±0.32b,c	0.76±0.05a
3	200	60	20	6.71±0.51e	0.87±0.07b,c
4	200	60	50	7.11±0.37e,f	0.89±0.03c
5	400	20	20	5.37±0.28b,c	0.74±0.03a
6	400	20	50	5.51±0.38b,c	0.75±0.05a
7	400	60	20	6.90±0.44e,f	0.87±0.05b,c
8	400	60	50	7.27±0.56f	0.99±0.09d
9	600	20	20	5.26±0.41b	0.74±0.09a
10	600	20	50	6.16±.0.4d	0.79±0.1a,b
11	600	60	20	6.39±0.28d	0.80±0.04a,b
12	600	60	50	7.26±0.29f	0.93±0.02c,d
13	800	20	20	5.61±0.32c	0.81±0.08b
14	800	20	50	6.25±0.42d	0.84±0.1b
15	800	60	20	6.16±0.45d	0.83±0.06b
16	800	60	50	6.85±0.42e	0.90±0.04c

Means of G' and G'' having the same letters are not significantly different ($P \leqslant 0.05$), estimated using Duncan's Multiple Range Test.

does pressure. The G'' values, at frequency 1 Hz, are much less variable than those found for the G', the highest values are found for treatments 8 and 12 (Table 1). Overall G'' are substantially lower than those G' values by almost one log cycle. These are indicative of gel behaviour found with some weak viscoelastic character (Ross-Murphy, 1984; Bell, 1989).

Since there are only minor variations between treatments for G'' with little frequency dependence and the shape of the plots for both G' and G'' is only little changed (Fig. 1a and b), it is suggested that all treatments give rise to solid-like behaviour due to the formation of cross-links, which might possibly be due to disulphide bonds or hydrophobic interactions, which are stabilised on moderate heating (Messens, Van Camp, & Huyghebaert, 1997); however, hydrogen bonds may also be important, since these are favoured in pressure treated system (Galazka & Ledward, 1995).

Overall, both G' and G'' values show that increasing temperature gives rise to greater changes than pressure. This may be related to the 7S globulins, which are easily denatured at temperatures of 67 °C at atmospheric pressure (Hermansson, 1978), whereas 11S globulins exhibit a higher denaturation temperature of 84.5 °C. In general, both 11S and 7S globulins contain more than 60% of β-sheet structures which are more stable than α-helices (Damodaran, 1996).

3.2. Protein solubility and electrophoretograms of pressurised soy samples

The solubility of all treated soy samples in 2% w/v SDS plus 2% 2-mercaptoethanol was essentially complete (>98%, data not shown); thus, only the soluble nitrogen contents of samples in 2% w/v SDS are shown (Table 2).

Table 2 shows that the soluble protein contents of the pressurised samples at 200 and 800 MPa and temperatures of 20 and 60 °C for 50 min are high and in the range of 85–90%. The variations in solubility between treatments were not significantly different, suggesting that such treatments lead to little change in the degree of disulphide bonding. Since the soy protein was completely solubilised in the presence of SDS plus 2-mercaptoethanol, which is a disulphide bond breaker, some limited disulphide bonding may occur in all samples; however, additional disulphide bonding is not introduced during the pressure/temperature treatments, although interchange may occur. Gomes and Ledward (1996) found that disulphide bonds are formed by pressure with moderate heating.

Table 2
The solubility, as estimated by nitrogen contents of high pressure-treated soy samples in 2% SDS (values are the means of three replications)

Pressurisation conditions		Nitrogen contents of soy samples	
Pressure (MPa)	Temperature (°C)	Time (min)	Soluble in 2% SDS (%)
Controls	20	0	88.0±2.9
200	20	50	89.2±4.5
200	60	50	90.7±7.6
800	20	50	86.9±3.6
800	60	50	85.2±9.2

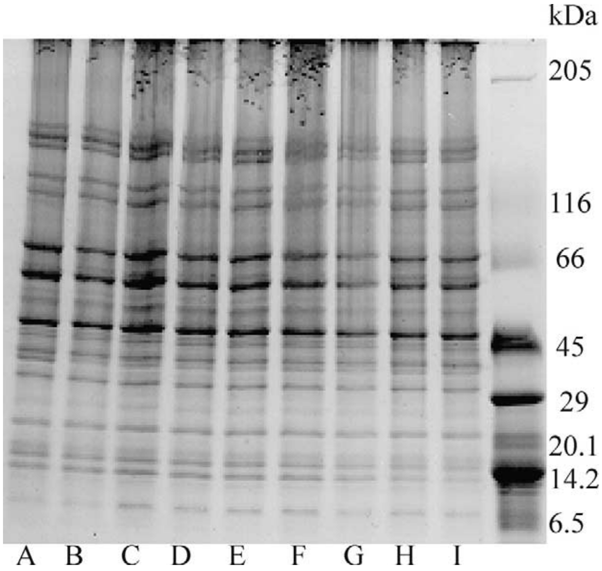

Fig. 2. The electrophoregrams (SDS-PAGE) of high pressure-treated soy samples for 50 min; A control samples; B–E pressure-treated with 200–800 MPa at ambient temperature; F–I, pressure-treated with 200–800 MPa at 60 °C; the samples were dissolved in 2% SDS.

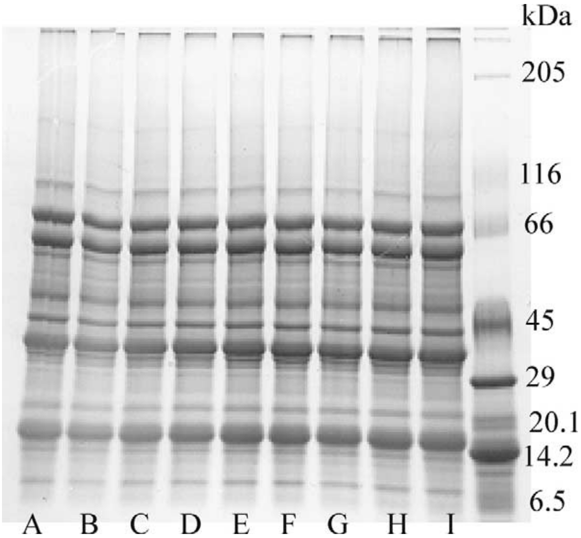

Fig. 3. The electrophoretograms (SDS-PAGE) of high-pressure treated soy samples for 50 min; A control samples; B–E pressure-treated with 200–800 MPa at ambient temperature; F–I, pressure-treated with 200–800 MPa at 60 °C; the samples were dissolved in 2% SDS plus 2% 2-mercaptoethanol.

Figs. 2 and 3 illustrate the SDS-PAGE electrophoregrams of soy samples treated at 20 and 60 °C, which are similar to those obtained for the control samples, and those pressurised at 200 to 800 MPa at ambient temperature. Only samples G–I pressurised at 400 to 800 MPa at 60 °C, display some slight reduction in the intensity of some bands, especially in the low molecular mass regions (14.2 kDa). However, addition of the reducing agent (2-mercaptoethanol) solubilised the aggregates, suggesting that the electrophoregrams were not treatment-sensitive in these solvents.

Wolf (1972) stated that conditions which favour soy protein–solvent interactions, such as the presence of urea, detergents, alkaline or acid pH, cause dissociation into subunits, the monomer forms being stable at an ionic strength of 0.5 M and pH 7.6. Rheological measurements showed that, in the high temperature regions, only temperature influences the storage moduli, and it may be that, as suggested by the electrophoregrams, this is due to some strengthening of the structures by disulphide bonds. However the solubility data (Table 1) indicate that such bonding is not extensive. Saio, Kamiya, and Watanabe (1971) found changes in the amounts of sulphydryl groups in tofu to be important for the physical properties of the 11S but not of the 7S gels. Thus, they concluded that intermolecular S–S bonds were involved in the protein network of the 11S fraction. Koshiyama (1971) also confirmed that the 7S globulin has a very low sulphur content and disulphide bridges do not appear in the binding between subunits. However disulphide bonds do appear to participate in the binding between pairs of acidic and basic subunits of the 11S globulin, which contains 48 sulphur atoms (Kitamura, Takagai, & Shibasaki, 1976).

4. Conclusions

Soy protein shows modification by pressure and temperature treatments. The effect of temperature is greater than that of pressure, but pressure at ambient temperature still has some effects, despite the shapes of the G' and G'' curves seeming to change little with the temperature/pressure treatments. These results accord with Baird (1981) who found that there were only slight changes in the rheological properties of heat treated soy isolate doughs at high concentration (20–25% w/w).

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