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Dynamic viscoelastic behaviour of high pressure treated gluten–soy mixtures

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Abstract

Hydrated gluten and soy mixtures with concentrations of gluten–soy = 20:80, 40:60, 60:40 and 80:20% were subjected to high pressure treatment at 700 MPa for 50 min at 20 and 60 °C. The treated samples were subsequently analysed for viscoelastic properties and electrophoretic patterns. A quadratic canonical polynomial model was used for the mixture design. Following high pressure treatment, the samples formed solid-like gel structures. In general, in the gels having high concentrations of gluten, both storage and loss moduli tended to increase with increasing pressure and temperature whereas, in the gels having high concentrations of soy, both moduli appeared to increase only slightly with increasing severity of the treatments. These results meant that the combined effect of temperature and pressure was much greater on the large complex gluten molecule than on the smaller soy globulins.

Keywords: High pressure; Rheology; Soy concentrate; Viscoelasticity; Wheat gluten

1. Introduction

High pressure can affect protein conformation and lead to denaturation, aggregation or gelation, depending on the system, e.g. type of protein, pH, ionic strength, the applied pressure and temperature, and duration of the pressure treatment.

High pressure effects on proteins are mainly related to the rupture of non-covalent interactions within protein molecules and to the subsequent reformation of intra and intermolecular bonding (Cheftel, Cuq, & Lorient, 1985). Many quaternary structures show complex behaviour, such as dissociation, followed by aggregation of subunits (Masson, Arciero, Hooper, & Balny, 1990) or precipitation upon pressure treatment (Morild, 1981). Secondary structure changes occur at very high pressure and lead to irreversible denaturation. For example, irreversible denaturation of chymotrypsinogen, induced at 760 MPa, is characterised by a dramatic change in α-helix and β-sheet contents (Wong & Heremans, 1988).

High pressure treatment of foods can be used to create new products from existing sources or to obtain meat analogues from vegetable/cereal proteins as presented in this research.

2. Materials and methods

2.1. Vital wheat gluten and soy protein concentrate

Commercial wheat gluten (Cerestar Deutschland GmbH, Barby, Germany), having the following composition, was used: pH 6.00, moisture 11.0±0.03%, ash 0.66±0.01%, lipid 4.44±0.1%, as determined by Approved Methods of the AACC (1983), protein 70.5±0.42% (N×5.7) determined by Kjeldahl method, starch 12.4±0.13%, determined using the modified enzyme method (Karkalas, 1985).

Commercial soy protein concentrate (DURA-GRIP, Bemis Company, Crossett, AR, USA), having the following composition, was used: pH 7.06, moisture 9.1±0.06%, ash 4.1±0.05%, lipid 0.85±0.06% extracted by ether and alcohol as described in the AOAC (1990) procedure, protein 75.6±0.87% (N×6.25), determined by Kjeldahl method, starch 7.78±1.01% (Karkalas, 1985).

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2.2. Preparation of hydrated gluten–soy mixtures

The mixtures of gluten and soy concentrate, with the proportions of gluten:soy concentrate = 20:80, 40:60, 60:40 and 80:20%, were hydrated to moisture contents of 63–74% w/v. Each hydrated mixture was mixed thoroughly in the Morton Z blade mixer (Morton Machine, Wishaw, Scotland, UK), operated at high speed for 100 s and then low speed for a further 200 s. These mixtures were treated at a pressure of 700 MPa for 50 min at 20 and 60 °C, except for the control. These conditions were selected on the basis of the results obtained with the individual components (Apichartsrangkoon, Ledward, Bell, & Brennan, 1998; Apichartsrangkoon, Ledward, Bell, & Gilmour, 1998; Apichartsrangkoon, Bell, Ledward, & Schofield, 1999).

2.3. High pressure treatments

The 50 g gluten–soy mixtures were subjected to pressures of 700 MPa for 50 min at 20 and 60C. 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 700 MPa but decreased to the initial temperature over the next 4 min. Proportionately smaller increases were observed at lower pressures and higher temperatures, as observed previously (Defaye, Ledward, MacDougall, & Tester, 1995).

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 gluten–soy mixtures. In order to ensure that all measurements were carried out within the linear viscoelastic regions, a stress amplitude was first selected (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 non-volatile silicone oil was applied to the exposed free edges to prevent moisture loss. Storage (G') and loss (G'') moduli were obtained over the frequency range of 0.01–10 Hz.

2.5. 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

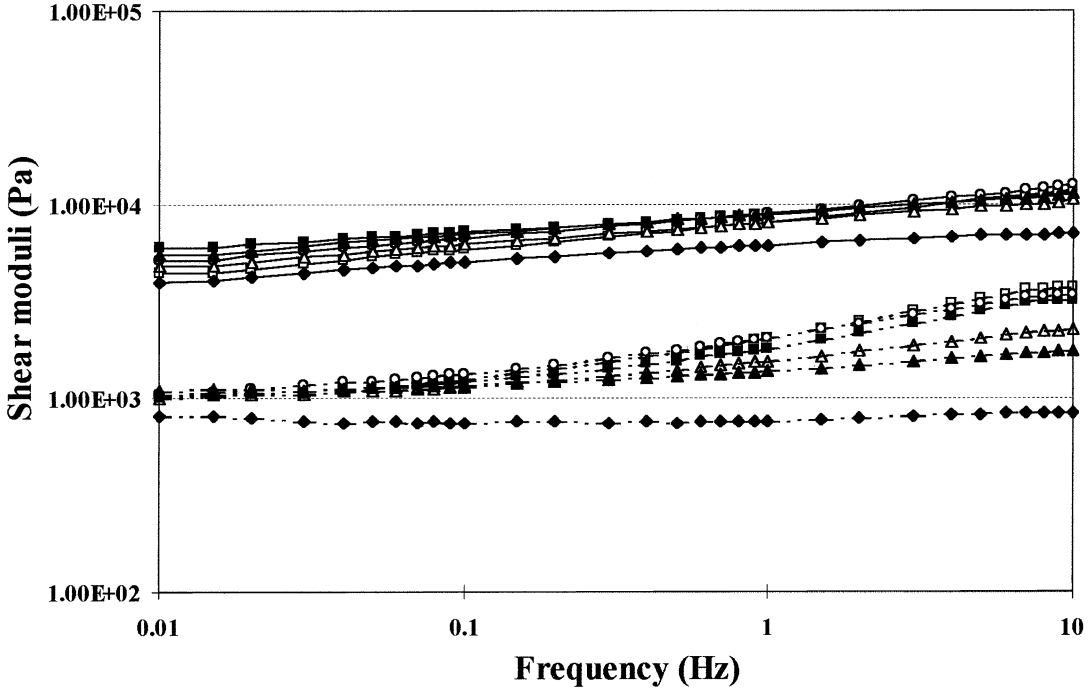

Fig. 1. The initial plots of shear moduli as a function of frequency for high pressure treated gluten–soy mixtures at 700 MPa for 50 min at 20 °C; the solid lines are for the storage modulus (G'), the dotted lines for the loss modulus (G''), ■, 100% gluten; □, 80:20% gluten–soy mixtures; ○, 60:40% gluten–soy mixtures; △, for 40:60% gluten–soy mixtures; ▲, 20:80% gluten–soy mixtures; ◆, for 100% soy samples.

8.8) and 10 μg of extracted samples were applied to each well. The extracted sample buffers were prepared in both the reduced (2-mercaptoethanol) and non-reduced conditions. Fixing of the protein patterns was 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).

2.6. Statistical analysis

For dynamic rheological measurements, linear regression analysis was used to determine the slopes and intercepts (at zero or log 1 frequency) of all G' or G'' plots as a function of frequency. This does not mean to imply that a 'straight line fit' was representative of the rheological function, but was only used as an indicator for processing the data.

Multiple regression analysis (Proc Reg, SAS, 1995) was used to fit a quadratic canonical polynomial model described by Cochran and Cox (1957) for the soy–gluten mixture design as follows:

$$Y_i = \beta_{1i}\,\mathrm{G} + \beta_{2i}\,\mathrm{S} + \beta_{12i}\,\mathrm{G\,S} + \delta_{12i}\,\mathrm{G\,S(G-S)}$$

where Y = a predicted dependent variable (either slopes or intercepts of G', G''), β_{1i}, β_{2i}, β_{12i} and δ_{12i} = corresponding parameter estimates for each linear and cross-product term for i sets of treatments, G = gluten and S = soy concentrate.

Statistical Analysis System (SAS Institute, Cary, NC, 1995), software programme was used for data analysis.

3. Results and discussion

3.1. Rheological properties of pressurised gluten–soy mixtures

The effects of pressure on the individual gluten and soy protein were previously evaluated (Apichartsrangkoon, Ledward, Bell, & Brennan, 1998; Apichartsrangkoon, Ledward, Bell, & Gilmour, 1998; Apichartsrangkoon et al., 1999). Since the effects were very different it is of interest to study the behaviour of mixtures of these two proteins following the same treatments. Figs. 1 and 2 illustrate the effect of pressure on the storage (G') and loss (G'') moduli as a function of frequency (0.01–10 Hz) for gluten–soy mixtures after treatment at 700 MPa for 50 min at 20 and 60 °C. It is seen that temperature has substantial influence on the shear moduli. At the lower temperature (20 °C), the storage and loss moduli are clearly divided into two distinct groups and, except for G' of the 100% soy samples, the other G' closely mirror the G' of the 100% gluten samples, which are far more frequency-dependent than the soy samples. The loss moduli are slightly different from the storage moduli in that, although the 100% gluten, and mixtures of 80:20% gluten–soy and 60:40% gluten–soy have the same shape for G'',

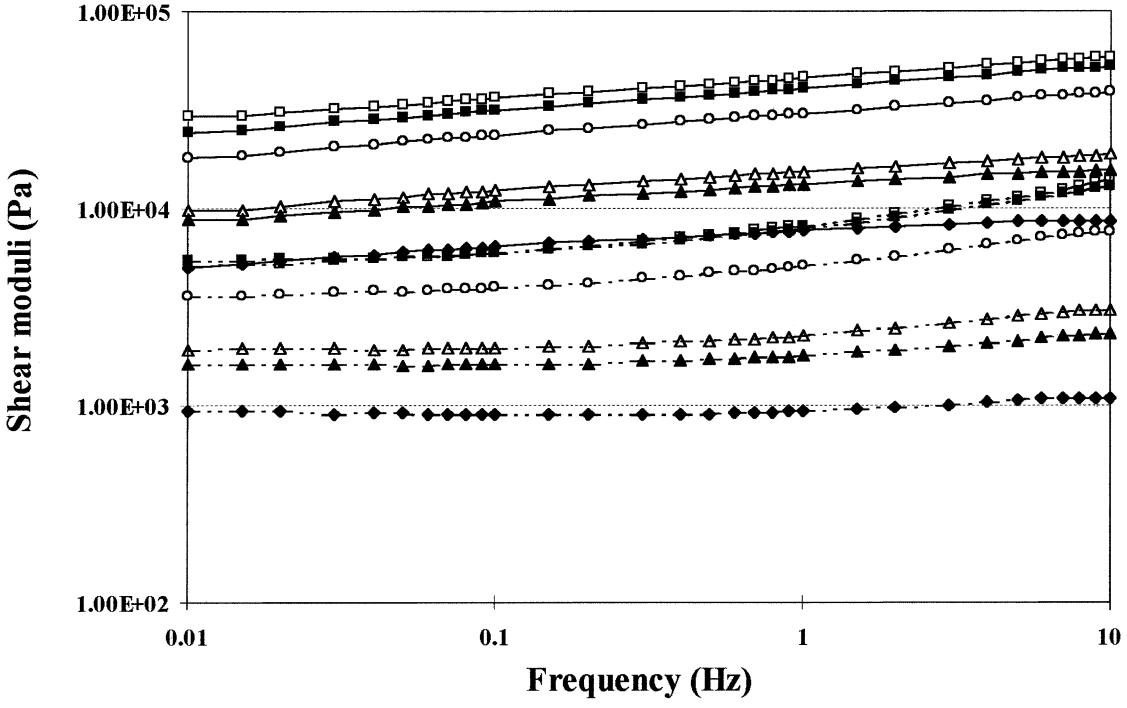

Fig. 2. The initial plots of shear moduli as a function of frequency for high pressure treated gluten–soy mixtures at 700 MPa for 50 min at 60 °C; the solid lines are for the storage modulus (G'), the dotted lines for the loss modulus (G''), ■, 100% gluten; □, 80:20% gluten–soy mixtures; ○, 60:40% gluten–soy mixtures; △, for 40:60% gluten–soy mixtures; ▲, 20:80% gluten–soy mixtures; ◆, for 100% soy samples.

mixtures of 40:60% gluten–soy and 20:80% gluten–soy have a similar G'' pattern to G'' of 100% soy, these samples have the least frequency dependence. The tan δ values, which are the ratios of loss (G'') to storage moduli (G'), are low, for all the soy rich samples, but the samples containing high amounts of gluten have higher tan δ values at frequencies in the range 1–10 Hz (Fig. 1).

At the higher temperature (60 °C), both moduli are greater and the tan δ values are very much lower than those found for the low temperature treatments. After treatment at 60 °C the G' and G'' plots can be divided into three groups: 100% gluten, 80:20% gluten–soy and 60:40% gluten–soy are similar as are the 40:60% gluten–soy and 20:80% gluten–soy mixtures. G' and G'' of the100% soy samples are much lower than the rest and their shapes are much flatter (Fig. 2). These are an indication that solid-like gel structures form, following high temperature and pressure treatments. In general, in the gels having high concentrations of gluten (80:20% gluten–soy and 60:40% gluten–soy), both moduli tend to increase with increasing temperature whereas, in the gels having high concentrations of soy, both moduli appear to increase only slightly with increasing temperature. These results mean that the combined effect of temperature and pressure is much greater on the large complex gluten molecule than on the smaller soy globulins. These results also strongly suggest that there is no interaction between the two proteins, the soy merely serving to dilute the gluten network. Thus, there is evidence that the high pressure has more influence on gluten than soy (Apichartsrangkoon, Ledward, Bell, & Brennan, 1998; Apichartsrangkoon, Ledward, Bell, & Gilmour, 1998; Apichartsrangkoon et al., 1999). This is not surprising, since gluten is a complex mixture of more than 100 protein components, some of which are rods and others are globular structures (Schofield, 1994). The soy glycinin, which consists of 64% β-sheet structure, is quite stable to temperature and pressure.

To more precisely characterise the storage and loss moduli, a statistical analysis was carried out. Fig. 3 shows the mean values ($n=8$) of the G' and G'' ln intercepts (log 1 frequency) of the pressurised gluten–soy mixtures after treatment at 700 MPa for 50 min at temperatures of 20 and 60 °C. The range of G' ln intercepts varies with temperature; these, including the control, have a broad range in the gluten-rich samples, but the range is much narrower in the soy-rich samples. The G' ln intercept for the control (no treatment) has its lowest values in the gluten-rich samples and increases with the addition of soy, up to the addition of 60–80% soy. All values of the G' ln intercepts increase after pressurisation at 700 MPa and ambient temperature, but the effect in the soy-rich samples is very small. The G' ln intercepts of high temperature (60 °C)-treated samples, rich in gluten, increase still further but the increase gradually decreases as the proportion of soy protein increases. The G'' ln intercepts follow the same pattern as that of the G', but have lower values in every data set.

It is interesting to note that the G' and G'' ln intercepts of the control samples are similar in the gluten rich samples, but are much further apart in the soy-rich samples. In the pressurised samples, the distances between G' and G'' ln intercepts are greater in the gluten-rich samples than the soy. In the soy-rich samples, the lines of G' and G'' are almost parallel to one another.

Considering the slopes of the G' and G'' against frequency plots of the gluten–soy mixtures after treatment at ambient, 60 °C and the controls (Fig. 4), the patterns of the slopes are similar to those of the ln intercepts, there is a wide range in the gluten-rich samples and a very narrow range in the soy-rich samples. In general, the slopes of G'' are greater than those of G' in the systems containing up to 40 or 60% gluten, after which the

Table 1
Quadratic canonical polynomial models for each of the dependent rheological attributes containing different proportions of high pressure treated (700 MPa/50 min) gluten (G) and soy (S)[a]

Rheological attributes	Predictive models	Treatment conditions
G'/1n mean intercept	6.22 G + 8.48 S + 5.34 G S	Control
G'/1n mean intercept	8.996 G + 8.79 S + 0.62 G S	20 °C
G'/1n mean intercept	10.63 G + 8.83 S + 1.32 G S	60 °C
G'/mean slope	0.25 G + 0.06 S + 0.05 G S	Control
G'/mean slope	0.11 G + 0.05 S + 0.23 G S	20 °C
G'/mean slope	0.10 G + 0.04 S + 0.14 G S	60 °C
G''/1n mean intercept	5.76 G + 6.54 S + 4.96 G S	Control
G''/1n mean intercept	7.498 G + 6.73 S + 1.87 G S	20 °C
G''/1n mean intercept	9.02 G + 6.77 S + 1.48 G S	60 °C
G''/mean slope	0.36 G + 0.03 S − 0.05 G S + 0.18 G S (G–S)	Control
G''/mean slope	0.19 G + 0.03 S + 0.23 G S + 0.23 G S (G–S)	20 °C
G''/mean slope	0.14 G + 0.03 S + 0.04 G S + 0.11 G S (G–S)	60 °C

[a] G = 1 − S; R^2 adjusted, in the range of 0.98–0.99; $P \leqslant 0.05$. Only the G''/mean slope attributes were significantly affected by the GS (G–S) interaction.

slopes of G'' become less than those of G'. The controls have the highest slope values and these progressively decrease with increasing soy protein content. The predictive equations for the ln intercept and slope are illustrated in Table 1.

Overall, it is apparent that the ln intercepts of both the G' and G'' values belong to different groupings, especially when temperature is incorporated. This means that both high temperature and pressure increase the strength of G' and G'', of all the gluten–soy mixtures,

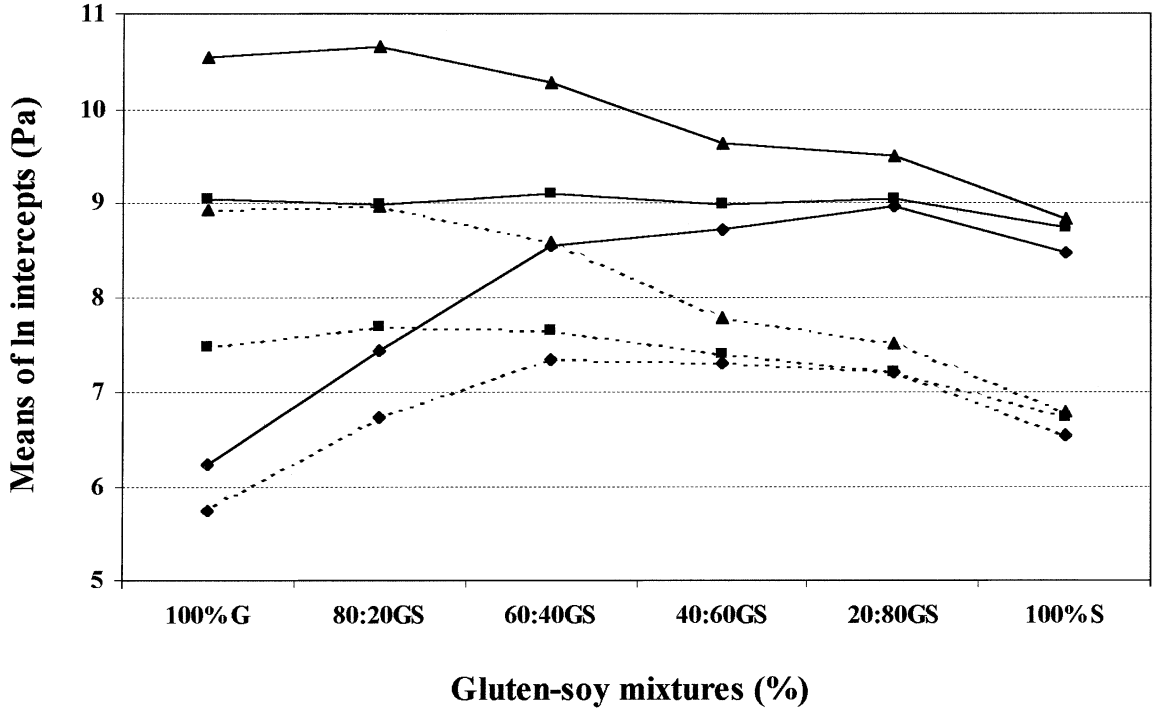

Fig 3. Means of log intercepts of shear moduli of high pressure treated gluten–soy mixtures after treatment at 700 MPa for 50 min; the solid lines are for storage modulus (G'), the dotted lines for loss modulus (G''); υ, untreated samples; ν, pressure treated at 20 °C; σ, pressure treated at 60 °C.

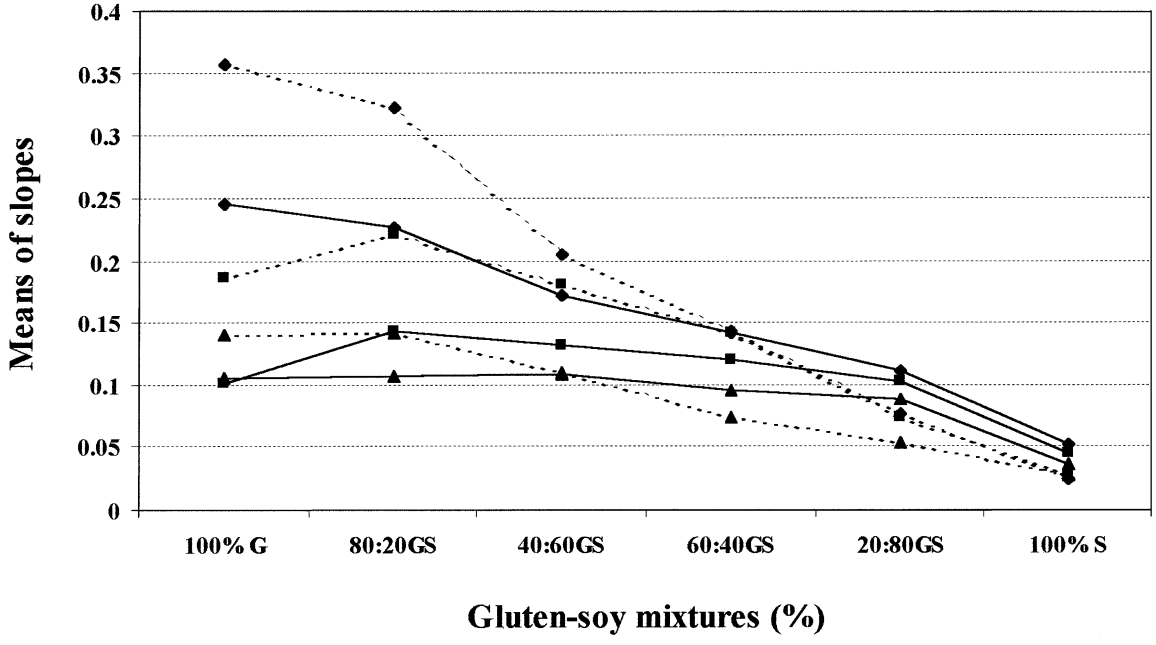

Fig 4. Means of log slopes of shear moduli of high pressure treated gluten–soy mixtures after treatment at 700 MPa for 50 min; the solid lines are for storage modulus (G'), the dotted lines for loss modulus (G''); ◆, untreated samples; ■, pressure treated at 20 °C; ▲, pressure treated at 60 °C.

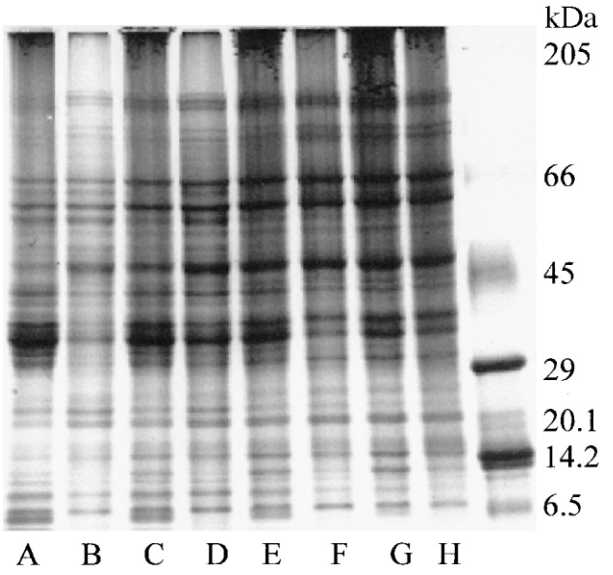

Fig 5. The electrophoregrams (SDS-PAGE) of high pressure treated gluten–soy mixtures at 700 MPa/50 min/60 °C ; A and B are control and treated samples of gluten–soy 80:20%; C and D are control and treated samples of gluten–soy 60:40%; E and F are control and treated samples of gluten–soy 40:60%; G and H are control and treated samples of gluten–soy 20:80%; the samples were dissolved in 2% SDS.

and especially of those mixtures having high gluten concentrations, as with the gluten samples. These observations suggest that, following pressurisation at 60 °C, the structures of the gluten–soy mixtures were very firm, with solid-like behaviour and a high permanent cross-link density (Bell, 1989; Mitchell, 1980). Since the ratios of G''/G' were just less than 1, 'weak gel' structures were probably formed (Ross-Murphy, 1984). Catsimpoolas and Meyer (1970) stated that, at high soy protein concentration, disulphide bonds enhance gelation. It is widely accepted that disulphide bonds play a fundamental role in gluten structure, since they affect intra and/or inter molecular bonding of branched and non-branched glutenin polymers. This was confirmed by the chemical analysis.

3.2. Electrophoretic characterisation of pressurised gluten–soy mixtures

Figs. 5 and 6 show the SDS-PAGE electrophoregrams of gluten–soy mixtures treated at 700 MPa for 50 min at 60 °C. It is seen that the pressure-treated samples, D, F, H and in particular B, which contains a high proportion of gluten, display loss of some protein bands (Fig. 5). These results suggest that the mixtures containing high concentrations of gluten, after treatment at 60 °C for 50 min, become less soluble in SDS, due to the formation of disulphide bonds, since the addition of the reducing agent, 2-mercaptoethanol, which ruptures disulphide bonds, solubilised the aggregates so that the electrophoretic patterns were similar to those of the controls (Fig. 6).

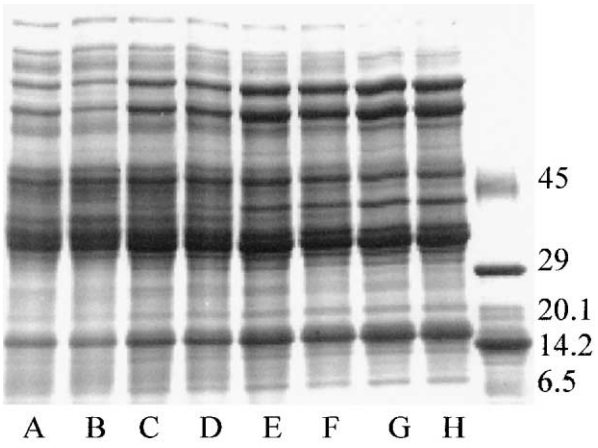

Fig 6. The electrophoregrams (SDS-PAGE) of high pressure treated gluten–soy mixtures at 700 MPa/50 min/60 °C ; A and B are control and treated samples of gluten–soy 80:20%; C and D are control and treated samples of gluten–soy 60:40%; E and F are control and treated samples of gluten–soy 40:60%; G and H are control and treated samples of gluten–soy 20:80%; the samples were dissolved in 2% SDS plus 2% 2-mercaptoethanol.

4. Conclusions

The variations of structural modification of the gluten–soy mixtures are mainly due to the gluten rather than soy protein. This is not surprising, since gluten has a more complex structure than soy and the limited water content for the soy system might cause incomplete hydration and thus restrict the mobility of polypeptide chains, inhibiting further modification or denaturation (Damodaran, 1996). Disulphide bonds contributed to the final structure of gluten-soy mixtures treated at high temperature; however, other non-covalent bonds must also be involved.

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