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Physicochemical properties of high pressure treated wheat gluten

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Hydrated wheat gluten samples with moisture contents of about 62.5% w/w were subjected to high pressure treatment. The experiments followed a three way factorial design which treated pressures at 200, 400, 600 and 800 MPa, temperatures at 20, 40 and 60°C and times of 20 and 50 minutes as the variables. The treated gluten samples were analysed for structural modifications by texture profile analysis (TPA) to measure hardness, initial moduli and solubility in sodium dodecyl sulphate and sodium dodecyl sulphate plus 2-mercaptoethanol. There was a strong correlation between hardness and the elastic modulus at (55%) strain ($r=0.98$). Results obtained revealed that, at 20°C and 40°C, pressure could alter gluten structure but at these temperatures disulphide cross-linking only became significant when samples were held at 800 MPa for 50 minutes. Treatments at the highest temperature (60°C) markedly increased hardness and the degree of disulphide bonding, especially in the pressure range of 400 to 800 MPa ($p \leq 0.001$).

INTRODUCTION

Pressure induced unfolding of proteins, and subsequent aggregation leads to the formation of gels, which may ultimately affect the textural quality of a food (Defaye *et al.*, 1995). Pressure-induced gels of water-soluble proteins differ from those induced by heat, being glossier, smoother, softer and having greater elasticity (Mertens *et al.*, 1993). Oligomeric proteins are usually dissociated by pressure (< 200 MPa) and unfolding of single-chain proteins occurs beyond 300 MPa (Masson, 1992). Globular proteins may yield modified conformations after pressure treatment (Defaye *et al.*, 1995). Due to chemical modification, e.g. thiol oxidation and subsequent aggregation, such pressure-induced denaturation can be irreversible (Masson, 1992). Tauscher (1995) stated that pressure modifies the quaternary structure by destroying hydrophobic interactions, the tertiary structure by reversible unfolding and the secondary structure via irreversible unfolding. As a consequence, oligomeric proteins may be reassembled into complex systems (Gross, 1994).

Non-covalent linkages, such as hydrophobic interactions, hydrogen bonds and electrostatic effects, may be altered by high pressure, but covalent bonds are believed to be largely unaffected (Heremans, 1992; Cheftel, 1992). Pressure will thus lead to unfolding, or partial denaturation and dissociation of polymeric structures into subunits, as well as ionisation of acid groups on amino acid side chains (Carter *et al.*, 1978). In many cases aggregation to a gel network or precipitate may occur (Ledward, 1995).

Techniques for assessing the extent of protein modification following denaturation include electrophoresis, differential scanning calorimetry, circular dichroism and nuclear magnetic resonance spectroscopy. Gluten, however, is difficult to study using many of these standard techniques since it is not readily soluble and does not have a large enthalpy change associated with its denaturation (Eliasson *et al.*, 1980).

Texture Profile Analysis (TPA) may be used as an index of protein modification or denaturation for such systems and both Ram and Nigam (1983) and Szczeniak and Hall (1975) have shown that TPA can distinguish very clearly between 'strong' and 'weak' wheat flour doughs. Montejano *et al.* (1985) compared torsion failure testing with Instron TPA using eight heat-induced protein gels and found a high correlation between the shear stress at failure and the TPA hardness as well as the true shear strain at failure and the TPA cohesiveness.

In the absence of pressure, high temperature is usually required to bring about the denaturation of gluten through the modification of its disulphide

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linkages (Schofield *et al.*, 1983). The present work was undertaken to determine the effect of various pressure/heat treatments on the texturisation of wheat gluten and to evaluate the potential of such processes to generate novel textures and products.

MATERIALS AND METHODS

Vital wheat gluten

Commercial vital wheat gluten (Cerestar Deutschland GmbH, Barby, Germany) having the following composition was used: moisture 9.0±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, starch 12.4±0.13% determined using the modified enzyme method described by Karkalas (1985).

Preparation of hydrated wheat gluten

The gluten was mixed with 1.5 times its weight of distilled water in a Morton Z blade mixer (Morton Machine Co. Ltd., Wishaw Scotland) operated at high speed for 100 seconds and then low speed for a further 200 seconds.The resultant hydrated gluten had a moisture content of about 62.5% w/w, which is similar to the moisture content of freshly isolated gluten (Schofield *et al.*, 1983). The gluten doughs were then divided into 100 g portions and rolled tightly into a cylinder shape so as to fit in a lubricant-free condom. The samples were then treated at the appropriate temperature, pressure and time, and removed from the condom for subsequent analysis.

High pressure treatments

The 100 g gluten samples were subjected to the pressures/temperature/time regimes using a prototype Stansted 'Food lab' high-pressure apparatus (Stansted Fluid Power Ltd., Stansted, Essex, UK) as described by Defaye *et al.* (1995). The pressure cell was maintained at the appropriate temperature by circulating water. The target pressure was achieved within 2 minutes of the sample being placed in the cell (temperature equilibration occurred during pressure treatment). The rate of pressure increase was about 250 MPa/min. During this high pressure treatment an adiabatic increase in temperature occurs. At ambient temperature, the monitored cell temperature increased by about 15°C in the first 4 min on treatment to 800 MPa but decreased to the set equilibrium value in less than 5 min. (Defaye *et al.*, 1995).

Texture profile analysis (TPA)

Texture Profile Analysis (TPA) of the gluten samples was performed using a Stable Micro Systems (version 3.7 G) texturometer. Rheological compression measurements were made using a sample with dimensions of 30 mm diameter and 15 mm thickness. The tests were carried out to a final strain of 55% at a test speed of 1 mm/sec using a 50 mm diameter flat probe. Two parameters from the TPA analysis were recorded: the hardness and the modulus of elasticity. The hardness is the force necessary to attain a given deformation corresponding to the first major peak (Brennan, 1980). The initial modulus is derived as initial stress/initial strain, where initial stress is the average force over the range 0.5 to 1.5 sec divided by the contact area and the initial strain is the strain at the 1.5 sec point of the curve (User Guide, Stable Micro Systems, Haslemere, Surrey, 1993).

Statistical analysis

The experiment was set for three-way factorial design (4×3×2) (Cochran *et al.*, 1957), which was comprised of three treatment combinations as follows: four levels of pressure, i.e. 200, 400, 600 and 800 MPas, three levels of temperature i.e. 20, 40 and 60°C, and two holding times 20 and 50 min. Multiple comparisons were made using Scheffe's test (John, 1971; Ouppadissakul, 1984). Response surface methodology RSM (Box *et al.*, 1978) was used to study the effect of three independent variables. Eight TPA measurements were performed for each combined treatment as well as for the native untreated gluten. All statistical data were computed using the Statistical Analysis System (SAS) (SAS Institute Inc. Cary North, Carolina).

Solubility test

Two grams of treated gluten samples with a moisture content of 62.5% w/v were stirred in 40 ml of 2% w/v sodium dodecyl sulphate (SDS) solution and 2% w/v SDS plus 2% 2-mercaptoethanol (v/v) overnight at room temperature (25°C). The solutions were then centrifuged at 34 000×g for one hour and the supernatants dialysed against distilled water and subsequently freeze-dried. The total nitrogen contents of the samples were analysed using the Leco apparatus (Laboratory Equipment Company, Michigan).

Electrophoretic analysis

Sodium dodecylsulfate 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 5, 10 μg of extracted samples were applied to each well. The extracted samples were prepared in both reduced/dissociated conditions (2-mercaptoethanol and 6M urea) and non-reduced conditions. Fixing of the protein patterns was by immersion in 12% w/v trichloroacetic acid for 1 h and subsequnt staining was accomplished using Coomassie brilliant blue G-250 (Neuhoff *et al.*, 1988).

RESULTS AND DISCUSSION

The design of this experiment used pressures in the range 200 to 800 MPa and temperatures 20 to 60°C, as these two parameters effectively define the limits of the high pressure equipment used. Preliminary experiments suggested that a holding time of 20 minutes could induce structural changes in some of the samples while after 50 min little further change in structure occurred.

The gels formed were very unlike heat-processed gluten, having a more marked elastic character (Table 1). The values of moduli of elasticity were high, since these were the measurements derived from a 'Young's modulus' which in turn was two to three times larger than the usual shear modulus (Muller, 1973). The data at higher pressures (600–800 MPa) agreed with the shear moduli found in the previous investigation (Apichartsrangkoon *et al.*, 1997) and, at lower pressure (200–400 MPa), results were in accord with work reported by Inda *et al.* (1991).

There were high positive correlations between the hardness of the samples and their moduli of elasticity ($r = 0.98$) ($p \leq 0.001$). This indicated that either hardness or elasticity could be used as an index of structural modification, although hardness appears to be slightly the more sensitive parameter (Table 1). Thus, this single point measurement can be used as an index for the structural characterisation of the gluten samples.

Figure 1(a) illustrates the effect of pressure and temperature at time 20 minutes; the response hardness

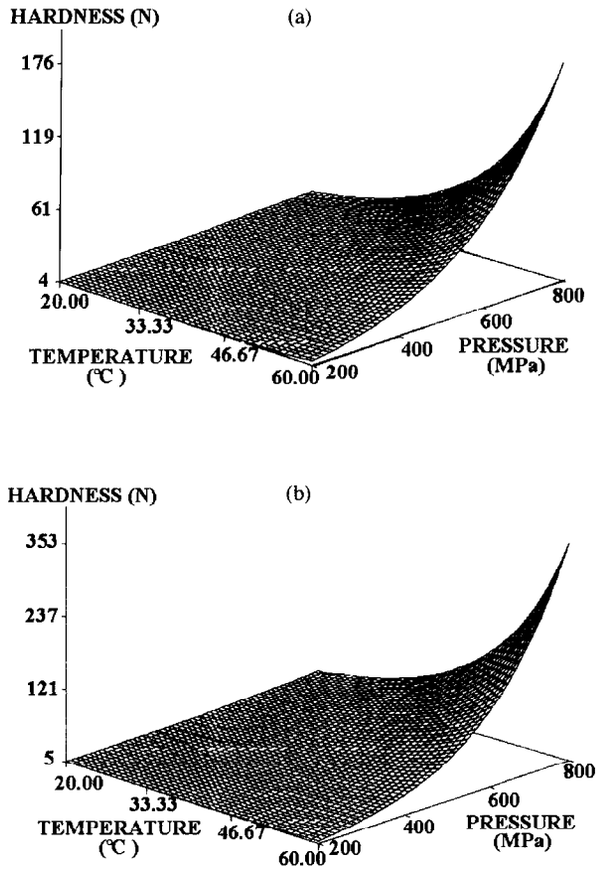

Fig. 1. The effect of pressure and temperature on response hardness at (a) 20 min and (b) at 50 min.

Table 1. Comparison of means of hardness and initial moduli estimated using Scheffe's test ; Minimum significant difference = 2.59 (Hardness) and 2.69 (Initial moduli)

Treatment combinations	Pressure (MPa)	Temperature (°C)	Time (min)	Mean of Hardness (N)	Mean of Initial Moduli (kN/m^2)
Control	0.1	20	0	6.08 ± 1.25^{a}	4.16 ± 1.10^{A}
1	200	20	20	6.32 ± 1.13^{a}	5.14 ± 0.30^{A}
2	200	20	50	6.07 ± 1.12^{a}	4.47 ± 0.95^{A}
3	200	40	20	6.00 ± 1.00^{a}	4.37 ± 0.90^{A}
4	200	40	50	5.01 ± 0.47^{a}	3.86 ± 1.19^{A}
5	200	60	20	6.34 ± 0.84^{a}	4.72 ± 1.08^{A}
6	200	60	50	$7.97 \pm 0.76^{a,b}$	5.16 ± 0.61^{A}
7	400	20	20	6.99 ± 1.05^{a}	5.61 ± 0.29^{A}
8	400	20	50	10.19 ± 1.38^{b}	$6.62 \pm 0.31^{B,A}$
9	400	40	20	$8.06 \pm 1.57^{b,a}$	4.84 ± 1.42^{A}
10	400	40	50	9.67 ± 0.77^{b}	$6.12 \pm 1.19^{B,A}$
11	400	60	20	25.24 ± 5.69^{f}	12.65 ± 2.74^{C}
12	400	60	50	67.31 ± 6.50^{m}	36.48 ± 5.10^{G}
13	600	20	20	$11.28 \pm 0.48^{c,b}$	$6.43 \pm 1.10^{B,A}$
14	600	20	50	19.82 ± 3.61^{e}	11.27 ± 1.20^{C}
15	600	40	20	28.77 ± 2.97^{g}	$14.75 \pm 2.55^{C,D}$
16	600	40	50	45.61 ± 1.70^{k}	25.01 ± 2.07^{F}
17	600	60	20	101.09 ± 7.29^{q}	61.11 ± 6.00^{H}
18	600	60	50	163.54 ± 9.61^{s}	97.19 ± 7.58^{M}
19	800	20	20	15.91 ± 2.05^{d}	$9.06 \pm 0.63^{B,C}$
20	800	20	50	38.78 ± 4.36^{h}	18.90 ± 1.82^{E}
21	800	40	20	74.53 ± 4.08^{n}	38.63 ± 3.77^{G}
22	800	40	50	96.24 ± 6.89^{p}	62.29 ± 8.56^{H}
23	800	60	20	132.86 ± 7.32^{r}	91.05 ± 9.39^{K}
24	800	60	50	224.27 ± 9.32^{t}	161.29 ± 13.90^{N}

(Means of Hardness and Initial Moduli having the same superscripts are not significantly different $p \leq 0.05$)

values were increased sharply at high pressures and temperatures whereas the hardness values at lower pressures and temperatures were much lower. There were similar effects of pressure and temperature on hardness for samples treated at 50 minutes (Fig. 1(b)).

The equation for Fig. 1(a) and Fig. 1(b) is as follows

$$\log \text{hardness} = 3.0334 + 1.2578X_1 + 0.7286X_2 + 0.1986X_3 - 0.1661X_1^2 + 0.2168X_2^2 + 0.4475X_1X_2 + 0.1219X_1X_3 + 0.0287X_2X_3$$

(X_1 = pressure, X_2 = temperature, X_3 = time)

($p \leq 0.05$)

The data thus tended to be condensed at lower levels of measurements. This suggests that the structure of gluten was slightly modified by mild treatments, but modification developed sharply with the more severe treatments.

Figure 2 and Table 1 show the interaction between pressure and temperature at the two holding times (20&50 minutes). It is apparent that the hardness values of the gluten samples treated at ambient temperature (20°C) for 20 minutes were only slightly changed, but were significantly affected at the same pressures when treated for 50 minutes, these increases becoming more marked with increasing pressure ($p \leq 0.001$). Thus, at 20°C, pressure only significantly modified the gluten structure at the higher pressure levels and the effect was time-dependent. Solubility studies supported this conclusion in that samples treated at 200–400 MPa and 20°C showed little change in the total amounts of SDS soluble nitrogen (Table 2). It is suggested that the structure of gluten gel is aggregated by hydrogen bonding and hydrophobic interactions, possibly as a result of the high amounts of glutamine and the presence of nonpolar side chains (He *et al.*, 1990; Wrigley *et al.*, 1988). However, after treatment at 800 MPa and 20°C for 50 min, the amount of SDS-soluble nitrogen was significantly decreased. This structure appeared to be stabilised by disulphide bonding since the initial solubility

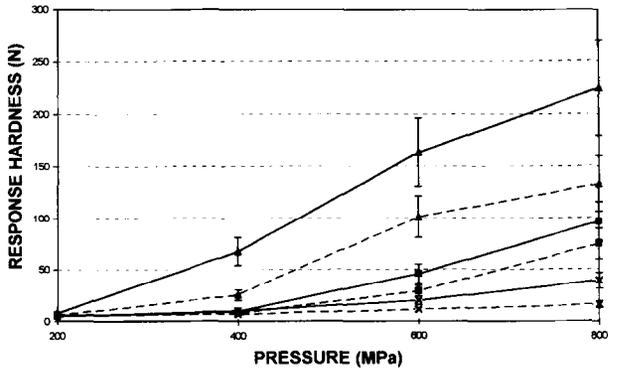

Fig. 2. The effects of interaction between pressure and temperature at two different times on hardness of gluten samples; the dotted lines are at 20 minutes and the solid lines at 50 minutes (temperature, x; 20°C, ■; 40°C and ▲; 60°C).

Table 2. The solubility as estimated by nitrogen contents of treated gluten samples in 2% SDS and 2% SDS plus 2% 2-mercaptoethanol. Values are the means of three replications

Treatment combinations			Nitrogen contents of gluten samples	
Pressure (MPa)	Temperature (°C)	Time (min)	Soluble in 2% SDS (%)	Soluble in 2% SDS plus 2-mercaptoethanol (%)
Control	20	0	93.8 ± 3.60	98.4 ± 1.11
200	20	50	93.4 ± 2.01	98.8 ± 3.61
400	20	50	82.0 ± 2.29	
600	20	50	73.0 ± 5.33	
800	20	50	65.7 ± 4.65	97.3 ± 2.04
200	60	50	87.7 ± 5.37	96.3 ± 4.78
400	60	50	55.8 ± 4.24	
600	60	50	31.5 ± 4.87	
800	60	50	18.2 ± 2.22	97.5 ± 1.52

values were restored in the presence of 2-mecaptoethanal, a well established disulphide bond breaker. This conclusion is supported by Galazka and Ledward (1995), who found that pressure-treated bovine serum albumin and β-lactoglobulin at 800 MPa for 20 minutes gave rise to some soluble aggregates which were believed to be due to the formation of disulphide bonds. In addition, Gomes *et al.* (1997) found that thiol group oxidation at the active site was the major reason for the loss in activity of papain seen on pressure treatment.

It is worth noting that pressure treatment for 20 and 50 minutes at 600 MPa and 400 MPa for 50 minutes at 20°C (i.e. treatments 13, 14 and 8 in Table 1) significantly increased the hardness compared to the control samples ($p \leq 0.05$). This suggests that some cross-linking has occurred, and there is some evidence of disulphide bond formation since the SDS solubilities decreased significantly for these pressure treatments (Table 2).

It is apparent from Table 1 that several treatments have similar effects; e.g. treatments 1 to 6 and 7 to 9 do not differ significantly from the control ($p \leq 0.05$) suggesting that, at 60°C, pressures greater than 200 MPa are needed to induce textural changes and, at 40°C, pressures of 400 MPa may bring about significant changes (treatments 9 and 10). As expected, at 60°C, the effects of both pressure and time were most marked (Table 1). At 40°C the structure started to be modified at 400–600 MPa and the two holding times show significant differences in the TPA hardness ($p \leq 0.001$) (Fig. 2 and Table 1). In gluten samples treated for 50 minutes at 400 MPa, the hardness was significantly different from the control, whereas the difference was not significant after only 20 minutes of treatment. This suggests that the longer holding time at this pressure and temperature gave rise to the formation of some additional bonding.

At 60°C, the effect of time on the 'gel' hardness was highly significant ($p \leq 0.001$) at all pressures from

400–800 MPa (Table 1). These results all correlate well with the increased extent of disulphide bond formation with increasing 'severity' of treatment (Table 2).

Marked losses of SDS-soluble nitrogen are seen in all severely treated samples; however, in all cases, the addition of 2-mercaptoethanol restored the levels of soluble nitrogen to those seen for the control material (Table 2). Since thiol oxidation is favoured in the presence of oxygen by both increasing temperature and pressure (Gomes *et al.*, 1997), it is perhaps not surprising the two parameters work synergistically to increase 'texturisation.' Such a chemical change (oxidation) will be very time-dependent, further supporting the major role of disulphide bond formation in the texturisation of this type of sample.

Jeanjean and Feillet (1980) and Hoseney *et al.* (1987) found that disulphide bonds are the major bonds involved in developing the final gluten structure after heat treatment. The rupture or formation of both hydrophobic and hydrogen bonds are, however, over the ranges studied, affected differently by pressure and temperature (Defaye *et al.*, 1995). Thus hydrophobic bonds readily break under pressure but, up to 60°C, increase in strength with increasing temperature while hydrogen bonds are little affected by pressure but readily break on heating. Thus, if as seems likely, the networks set up in the disulphide bonded aggregates are additionally modified by the presence of hydrophobic interactions and hydrogen bonds, a difference will be found between the heat set systems (favouring the formation and retention of existing hydrophobic interactions as the system cools) and pressure-treated systems (which favour the formation of new hydrogen bonds on removal of the pressure).

Figure 3.1 illustrates the SDS-PAGE electrophoregrams of gluten samples treated at 20 and 60°C; similar electrophoregrams are seen for the native samples, and those pressure-treated at 200 to 800 MPa at ambient temperature. Only samples H and I, pressure-treated at 600 and 800 MPa at 60°C, display loss of some of the protein bands. This tends to confirm that high pressure at 60°C makes the gluten less soluble in SDS due to formation of covalent, presumably disulphide bonds (Galazka *et al.*, 1995). Since the addition of the reducing agent, 2-mercaptoethanol, which disrupts disulphide bonds, solubilised the high molecular weight proteins, the electrophoregrams were not treatment sensitive in the solvent (Fig. 3.2).

Figure 3.3 shows the electrophoregrams of samples treated with SDS/6M urea, which appears to disrupt some of the hydrogen-bonding of the protein aggregates produced at high temperature and pressure to yield fragments of high molecular masses (eg 116 kDa). This may be due to the disruption of hydrogen bonds to give the distinct bands observed in samples D to I in Fig. 3.3. Samples B and C gave bands which were essentially the same as to those of the control (A). This suggests that the hydrogen bonds (which are urea-sensitive) are

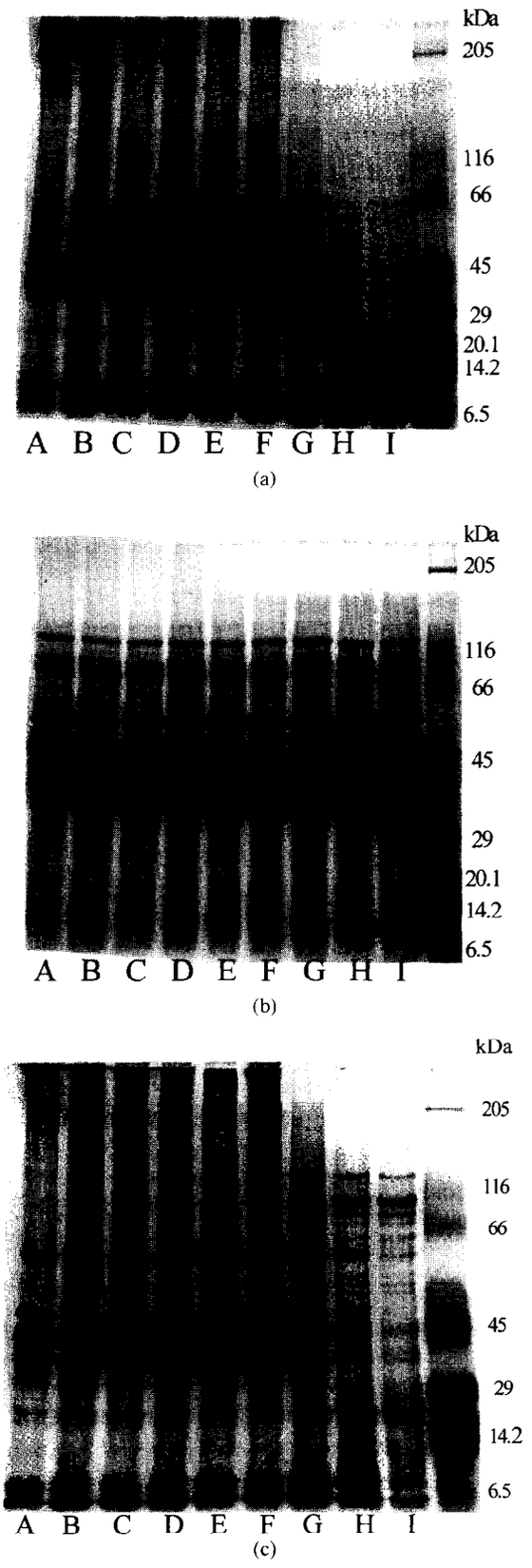

Fig. 3. The electrophoregrams (SDS-PAGE) of high pressure treated samples; A, control samples; B-E, pressure-treated for 200–800 MPa at ambient temperature; F-I, pressure-treated for 200–800 MPa at 60°C. The samples were dissolved in 2% SDS (Fig. 3.1), 2% SDS plus 2% 2-mercaptoethanol (Fig. 3.2) and 2% SDS plus 6M urea (Fig. 3.3).

involved in the association of subunits in the pressure-denatured gluten (Jeanjean and Feillet, 1980).

CONCLUSION

This study has shown that the number of disulphide bonds stabilising the gluten structure in these products increases with time, temperature and pressure. Temperature, over the range of conditions studied, having the greatest effect (Table 2 and Fig. 1). However, the final structures achieved, using pressure or pressure and heat, are very different from those induced by heat alone (Mertens and Niklaas, 1993). This suggests that, although the effects of pressure and temperature may be synergistic, the high pressure used must have a key role in the changes which are occurring. This work suggests considerable scope for the development of novel textured products by high pressure treatment of gluten material.

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