Dynamic Viscoelastic Properties of Heated Gluten/Soy Protein Gels

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ABSTRACT: Hydrated gluten, soy concentrate, and mixtures of both were heated at 90 °C for 0.5 to 6 h. All heated samples were analyzed for viscoelastic properties and SDS-PAGE electrophoretograms. Results of the rheological analysis showed storage (G') and loss (G'') moduli of gluten increased sharply as a result of heating and the shapes of both shear moduli were significantly different from the unheated samples. In the soy system, both shear moduli increased slightly upon heating and the shapes were similar to the unheated samples. The linkages found in the heated systems were disulfide bonds as shown by the electrophoretograms.

Keywords: rheology, soy concentrate, viscoelasticity, wheat gluten

Introduction

Both gluten and soy protein are extensively used as basic components for vegetarian food products especially in many Asian countries. Therefore, the rheological characteristics of these proteins and their mixtures undergoing heat modification could be an interesting area to study.

Gluten, which is a mixture of more than 100 heterogeneous polypeptide components, is composed of 2 main storage proteins, gliadins and glutenins. Glutenins with molecular mass of 69 to 88 kDa based on SDS-PAGE (Anderson and others 1988) are responsible for elastic behavior, whereas gliadins with molecular mass of 30 to 50 kDa (Tatham and others 1990) are responsible for viscous flow properties. When gluten is heated, both rheological properties storage (G') and loss (G'') moduli are increased, indicating an increase in the number of rheologically effective cross-links (Schofield and others 1984). Allenburrow and others (1990) observed that the G' of heated hydrated-gluten increased steadily up to about 60 °C when it began to increase, and at about 90 °C it increased dramatically. Consistent with these observations, heating results in a decrease in gluten extractability (Schofield and others 1983; Weegels and others 1994a). Electrophoresis and gel-filtration chromatographic analysis of the extracted protein showed that glutenin is affected at a lower temperature than gliadin and that the α-gliadins, which are deficient in sulphur amino acids, are not affected. The increase in extractability that occurs when reducing agents are added to the extraction implies that heat-induced changes in gluten's rheological and extractability properties are related to changes in disulfide bonding (Jeanjean and others 1980; Wrigley and others 1980; Schofield and others 1983; Weegels and others 1994).

Soy protein is also composed of 2 main components, β-conglycinin with molecular mass of 180 kDa and glycycin with molecular mass of 360 kDa (Liu 1997). Baird (1981) found that on heating 20 and 25% soy isolate "doughs" up to 75 °C, G' and G'' of the "doughs" showed only slight increases with increasing temperature. The shape of G' and G'' against frequency plots was suggestive of solid-like behavior that did not change with temperature, which indicated there was little tendency for the protein molecules to undergo cross-linking on heating. In contrast, a lower concentration, 15% "dough," which is a dispersion, exhibited values of G' and G'' that increased with increasing temperature above 50 °C.

Attempts have been made to measure the rheological properties of low concentration (8 to 16%) soy protein slurries which form gels on heating at 70 to 100 °C, but little work has been reported on high concentration (> 18%) soy gels. This study is concerned with heat-treated high concentration of viscoelastic gel systems.

Materials and Methods

Vital wheat gluten and soy protein concentrate

Commercial wheat gluten, Cerestar Deutschland GmbH, (Barby, Germany) having the following composition was used: moisture 11.0 ± 0.03%, ash 0.66 ± 0.01%, lipid 4.44 ± 0.1% as determined by Approved Method of the AACC (1983), protein 70.5 ± 0.42% (N × 5.7) determined by Kjeldahl method, and starch 12.36 ± 0.13% determined using the modified enzyme method described by Karkalas (1985).

Commercial soy protein concentrate, DURA-GRIP, Bemis Co., Inc., Crossett, (Ark., U.S.A.) having the following composition was used: moisture 9.1 ± 0.06%, ash 4.1 ± 0.05%, and lipid 0.85 ± 0.06% extracted by ether and alcohol as described in AOAC procedure (AOAC 1990); protein 75.64 ± 0.87% (N × 6.25) determined by Kjeldahl method; and starch 7.78 ± 0.12% (Karkalas 1985).

Preparation of hydrated gluten, soy samples, and gluten-soy mixtures

Hydrated gluten pH 6.00 and soy samples pH 7.06 with moisture content of 62.5% v/w and 80.84% v/w respectively were mixed separately in the Morton Z blade mixer (Morton Machine Co. Ltd., Wishaw, Scotland) operated at high speed for 100 s and then at low speed for an additional 200 s. Each 50 g of gluten or soy dough was fitted in polypropylene bags and heated in a water-bath at 90 °C for 0.5, 1, 2, 3, 4, 5, and 6 h. The mixtures of gluten and soy samples with the ratios, gluten:soy concentrate = 20:80%, 40:60%, 60:40%, and 80:20% were hydrated to moisture contents of 63 to 74% w/w, which were adjusted to comply with the moisture contents of the individual component and subsequently mixed as described above. Each 50 g of hydrated mixture was heated at 90 °C for 4 h, which was adequate time for complete denaturation of these proteins as found for the individual components (Apichartsrangkoon and others 1998a, 1998b, 1999).
Rheological measurement

A controlled stress rheometer (Stress Tech Rheometer; Reologica Instruments AB, Lund, Sweden) was used to measure the dynamic viscoelastic properties of the heat-treated gluten, soy concentrate, and gluten-soy mixtures. In order to ensure that all the measurements are carried out within the linear viscoelastic regions, first stress amplitude sweeps were performed (Figure 1 and 2). Based on these results, a stress amplitude of 100 Pa was chosen. A parallel plate (20 mm dia) measuring geometry was used with a gap width of 2 mm. Samples of 25 mm dia were approximately cut for thickness of 2 mm and subsequently loaded onto the rheometer and allowed to equilibrate to the measuring temperature ($25 \pm 1 ^\circ C$, for 10 min). Excess samples were trimmed off, and a thin layer of 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 to 10 Hz.

Solubility analysis

Three grams ($\pm 0.2$) of heated gluten and soy samples 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 ($25 ^\circ C$). The solutions were then centrifuged at 34,000 $\times$ g for 1 h, and the supernatants dialyzed against distilled water and subsequently freeze-dried. The percentage of nitrogen in each sample was determined by Leco apparatus (Laboratory Equipment Co., Mich., U.S.A.).

Electrophoretic analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a gel with a 7.5 to 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 samples were applied to each well. The extracted sample buffers were prepared in both reduced (2-mercaptoethanol) and nonreduced 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 and others 1988).

Results and Discussion

Viscoelastic properties of gluten, soy, and gluten-soy mixtures after heat treatment

Figure 3 illustrates the shear moduli of gluten samples treated at $90 ^\circ C$ from 0.5 to 6 h as a function of frequency (0.01 to 10 Hz). It is seen that, not unexpectedly, the shear moduli of the unheated samples are much lower than those of the heated ones. Samples treated for 0.5 h had developed some structure compared to the unheated. Both the storage and loss moduli of those treated from 1 to 6 h exhibit similar relationships. It is of interest to note that the shear moduli for the unheated samples have low tan $\delta$ values (ratios of $G''/G'$) at low frequency, but the tan $\delta$ values increase with increasing frequency. This is an indication of less solid-like behavior as the speed of oscillation increases. After heat treatment, even for 0.5 h, the tan $\delta$ values are much lower than for the unheated samples, which is evidence of permanently cross-linked density, however it is still a 'weak viscoelastic gel' system (Bell 1989; Apichartsrangkoon and others 1999).

Figure 4 shows the shear moduli as a function of frequency (0.01 to 10 Hz) for soy samples treated at $90 ^\circ C$ for 0.5 to 6 h. Compared to gluten, heated soy protein exhibits lower val-
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Table 1—The solubility as estimated by nitrogen contents of heat treated gluten samples in 2% SDS and 2% SDS plus 2% 2-mercaptoethanol. Values are the means ± S.D. of 3 replications.

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Soluble nitrogen of gluten samples</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Soluble in 2% SDS (%)</td>
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<tr>
<td>Unheated</td>
<td>93.8 ± 3.6</td>
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<tr>
<td>0.5</td>
<td>30.1 ± 3.9</td>
</tr>
<tr>
<td>1</td>
<td>29.6 ± 1.2</td>
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<tr>
<td>2</td>
<td>18.9 ± 2.6</td>
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<tr>
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<td>19.8 ± 1.1</td>
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<tr>
<td>6</td>
<td>14.9 ± 2.4</td>
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</table>

Table 2—The solubility as estimated by nitrogen contents of heat treated soy samples in 2% SDS. Values are the means ± S.D. of 3 replications.

<table>
<thead>
<tr>
<th>Heat treated conditions of soy samples</th>
<th>Soluble in 2% SDS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unheated</td>
<td>88.0 ± 2.9</td>
</tr>
<tr>
<td>0.5</td>
<td>88.5 ± 4.2</td>
</tr>
<tr>
<td>1</td>
<td>88.2 ± 6.1</td>
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<tr>
<td>2</td>
<td>87.2 ± 4.8</td>
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<tr>
<td>4</td>
<td>85.9 ± 1.2</td>
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<tr>
<td>6</td>
<td>88.4 ± 2.7</td>
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Solubility indexes of heat treated gluten and soy samples

Table 1 illustrates soluble nitrogen contents as a function of treatment time for gluten samples heated at 90 °C for 0.5 to 6 h. Up to 2 h treatment, there is a marked loss of SDS soluble nitrogen but after this time there is little further loss of solubility. These indicate that the anionic detergent (SDS) has only a very limited ability to extract the heated gluten structure. The unextracted components appear to be because of disulfide cross-link formation in the heated samples, since the addition of disulfide bond breaker, 2-mercaptoethanol totally dissolved all samples.

Table 2 shows the soluble nitrogen contents of soy samples treated for different times at 90 °C. There are no significant differences between treatment times and the unheated samples, the soluble nitrogen contents being in the range of 85 to 88 %. In the presence of 2% SDS plus 2% 2-mercaptoethanol over 99% of the nitrogen became soluble (data not shown), again suggesting some covalent disulfide bonds are present in the samples, though the numbers do not apparently increase on heating.
Electrophoretic characterization of heat-treated gluten, soy samples, and gluten-soy mixtures

Figure 6 and 7 display the electrophoretograms of heat-treated gluten samples dissolved in 2% SDS and 2% SDS plus 2% 2-mercaptoethanol respectively. Figure 6 illustrates some loss of bands (G to I) for samples heat treated for 5 to 6 h. In the presence of the reducing agent 2-mercaptoethanol, all bands were recovered so that the electrophoretograms were similar to the unheated ones (Figure 7), confirming that disulfide bonds formed at the longer heating times.

Figure 8 and 9 show the electrophoretic patterns of heat-treated soy samples solubilized in 2% SDS and 2% SDS plus 2-mercaptoethanol.
Conclusion

Heat treatment of gluten, soy concentrate, and mixtures of both at 90 °C for 0.5 to 6 h brings about large changes in the rheological characteristics of these systems, especially higher cross-link density in the gluten-rich samples than in soy-rich samples were found. Viscoelastic gel structures were formed after heating and behave as weak gel systems since the storage and loss moduli were in the same decade (Ross-Murphy 1988). Thus, there is considerable evidence that gluten is modified to a greater extent by heat than the soy samples used in the present study. The formation of covalent disulfide cross-links are important in the heat-treated systems as shown by the soluble nitrogen contents as well as the electrophoretograms. This work suggests there is considerable scope for the development of vegetarian textured products especially with gluten-soy mixtures.

References

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