Differences in Physical andStructural Properties ofHeat-Induced Gels from Glycinins among Soybean Cultivars

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Heat-induced gels were prepared from glycinins of various soybean cultivars at protein concentrations of 18 to 20%. Textural properties of the gels measured by a compression-decompression test were evaluated by the three-dimensional representation of the gels through factor analysis of the instrumental data and calculation of factor scores for each gel. Differences in gel texture were clearly observed among the soybean cultivars, with Shirotsurunoko gel being the most fracturable and Yamabe-A3 gel the most unfracturable. The most elastic was the gel from Hill and Matsuura gel exhibited the lowest elasticity. The existence of A4 polypeptide also contributed to the textural features of the gels. The gels of A4-containing cultivars were more unfracturable and less elastic compared to those of A4-lacking cultivars. Physical properties of the gels, gel network structure, and polypeptide composition of the glycinin were related each other to some extent. The compressibility which corresponded to the textural attribute of fracturability was related to regularity and/or pore size of network structure of the gels. The acidic polypeptide of A4 seemed to be responsible for whether the gel network was aggregate or strand type, thereby relating to the physical properties of compressibility and resiliency of the gels. The results obtained here suggest that polypeptide composition of the glycinin affects the properties of gel networks and thereby contributes to different physical and textural properties of the gels.

Keywords: heat-induced gels, microstructure, glycinin, acidic polypeptides, basic polypeptides, gelation

Heat-induced gelation and gel properties of proteins are important to the formation of structure and physical properties of many food products. Heating of proteins may give rise to several reactions such as denaturation, association, dissociation and aggregation. Gel formation is a complex process involving such reactions. An absolute prerequisite for gel formation is the interaction between protein molecules in such a way that some kind of a three-dimensional network is formed. Protein gels can be divided roughly into two types: those formed by random aggregation of constitutive molecules and those by strands made from constitutive molecules. Gelation of seed storage proteins and its mechanism have also been investigated. The effects of heating temperature and time (Hashizume & Hermansson, 1991; Bottcher & Foegeding, 1994). In addition, the effects of heating temperature and time (Hashizume & Watanabe, 1979; Beveridge et al., 1984; Ker et al., 1993), fatty acid (Yuno-Ohta et al., 1992) and reagents such as NaSCN, N-ethylmaleimide and 2-mercaptoethanol (Zheng et al., 1992) on gel formation have also been investigated.

Here we report the differences of physical and structural properties of glycinin gels among soybean cultivars. Our specific objectives were to clarify the correlation among physical properties of the gels, heat-induced gel microstructures and polypeptide compositions.

Materials and Methods

Materials The seeds of seven soybean cultivars (Glycine max, var. Shirotsurunoko, Hill, York, Raiden, Suzuyutaka, Matsuura, Yamabe-A3) were supplied by the National Institute of
Agrobiological Resources in Japan. The cultivars were grown in Japan where the seeds of each cultivar developed and matured normally. All other reagents and chemicals were of analytical grade.

Preparation of glycinin Crude glycinin was prepared from acetone powder (Mori et al., 1981) by the method of Thanh et al. (1975). Purified glycinins were dialyzed against 35 mM potassium phosphate buffer (pH 7.6) containing 0.4 M NaCl (standard buffer) before use. Aliquots of the crude extract for sucrose density gradient centrifugation were fractionated by centrifugation on 10 to 30% (w/v) linear sucrose density gradient in the standard buffer at 248,850 g for 18 h at 20°C in a Hitachi RPS 40T rotor. After centrifugation the gradient was divided into 0.4 ml fractions while simultaneously being examined for absorbance at 280 nm with an ISCO density gradient fractionator. Details of the procedure have been described by Mori and Utsunomiya (1979).

Protein determination Protein concentration was determined by the method of Lowry et al. (1951).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Defatted glycinins from seven kinds of soybean cultivars were dialyzed against SDS solution (0.125 M Tris-HCl buffer pH 6.8) containing 6 M urea and 4% 2-mercaptoethanol (2-ME) before electrophoresis. SDS-PAGE was done on slab gels (4.4% stacking gel and 12.5% separating gel) by the method of Laemmli (1970). The gels were stained with Coomassie Brilliant Blue R-250.

HPLC apparatus and chromatographic conditions HPLC analyses were performed to determine the polypeptide composition of each glycinin by Shimadzu liquid chromatography LC-4A (Shimadzu, Ltd., Kyoto, Japan). A Cosmogel DEAE ion exchange column (7.5 mm I.D. ×75 mm, Nacalai Tesque, Kyoto) and guard column of Cosmogel DEAE (Nacalai Tesque) were used for the analysis. Linear gradation of NaCl (0.05 M–0.325 M) was used to elute the polypeptides at the flow rate of 1.0 ml/min. Sodium phosphate buffer (0.1 M sodium phosphate (pH 6.3), 6 M urea, 0.2 M 2-ME) was used in HPCL analysis.

Preparation of gels for scanning electron microscopy (SEM) The purified glycinin in eluting buffer was thoroughly dialyzed against standard buffer just before use. After dialysis, the protein solution was adjusted to the desired protein concentration (18, 19, 20%) with the standard buffer. The protein solution was injected into an aluminum cup (5 mm I.D. ×2.5 mm), the cup was filled, completely degassed and sealed with a silicone sheet on which a steel plate was tightly fixed with clips. The cups were then heated for 30 min at 100°C in a water bath, followed by rapid cooling to room temperature by immersion in tap water. The covering was removed and the gel in each cup was analyzed by SEM.

Scanning electron microscopy A rectangular gel specimen was cut (1×1×2 mm) and fixed in the following solutions; 2% glutaraldehyde, 2% tannic acid and 1% osmium tetroxide. The sample was then dehydrated by immersion in a series of ethanol mixtures of 50%, 70%, 80%, 90%, 95% and 100% and finally immersed in isoamyl acetate. After dehydration, critical-point drying was done in liquid CO₂ in a pressurized chamber. The dried sample was carefully fractured into small pieces to reveal the internal microstructure. The fragments were mounted on an aluminum SEM stub by a small droplet of graphite paste and coated with platinum. All samples were then examined and photographed using a S-4100 Hitachi scanning electron microscope.

Preparation of gels for compression-decompression test Heat-induced glycinin gels in concentrations of 18, 19, 20% were prepared by boiling according to the method of Kang et al. (1991). Briefly, glycinin suspensions were poured into a 2 mm gap between two glass plates equipped with a silicon spacer (2 mm thickness) for scaling, and heated for 30 min. The gel sheets were then removed and cut into 2 cm squares.

Measurement of mechanical properties of gels An uniaxial compression-decompression test was carried out according to the method of Kang et al. (1991) using a KES-FB3 compression tester (Kato Tech Co., Ltd., Japan) equipped with a cylindrical plunger with a cross section of 0.25 cm². The tests were performed at 20°C and at the deformation rate of 1.2 mm/min, and measurement was made by compressing the sample until rupture. Compression work (CW), decompression work (DW), resilience (RS), linearity of the compression process (LC), compressibility (CM), elastic modulus in a practical sense (E) and force (F) were measured. Then, the compression-decompression tests were performed at different levels of deformation up to rupture, where the forces were set at 15, 30 and 60% of the rupture force for small, medium and large deformation levels, respectively. Twenty-eight mechanical parameters, that is seven mechanical parameters (CW, DW, RS, LC, CM, E and F) at four deformation levels (small, medium, large and rupture) were identified. Abbreviations described in the blanket were different from those used in the previous paper (Kang et al., 1991) except E.

Statistical analysis The data set comprising the values taken by sixteen variables, that is, four mechanical parameters (CW, RS, CM and F) at four deformation levels (small, medium, large and rupture), for the seven soybean cultivars was subjected to factor analysis using a multivariate analysis program (Microsoft Systems Co., Ltd., Japan).

Results and Discussion

Polypeptide compositions of glycinin Glycinin consists of acidic and basic polypeptides, the number of which has been identified as varying from three to eight in different soybean cultivars. The molecular weight of the polypeptides is 34,800–45,000 for the acidic and 19,600–22,000 for the basic polypeptides. The acidic polypeptide composition of glycinin of the seven kinds of soybean cultivars was analyzed by SDS-PAGE containing 6 M urea. Figure 1 shows, qualitatively, the difference between the soybean cultivars containing A4 polypeptide and those lacking A4. The three cultivars, Shirotsurunoko, Hill and York had the A4 polypeptide and in the other cultivars as shown in Table 1, A4 polypeptide was detected but was insignificant. The A4 polypeptide was linked to its basic polypeptide counterpart only through noncovalent interactions, which dissociated during the heat treatment. Liberation of A4 polypeptide could result in conformational change of the glycinin molecule and stimulate the formation of soluble aggregates and subsequent polymerization resulting in rapid gel formation (Nakamura et al., 1984b). The contents of A3 polypeptides differed depending on the existence or non-existence of A4 polypeptide in Table 1. Nakamura et al. (1984b) suggested that hardness of heat-induced gels was different among cultivars, depending on percentage of
the A3 polypeptide. As shown in Table 1, the percentages of A3 polypeptide in the A4-lacking cultivars tended to be higher than in those of A4-containing cultivars. Also, Hermansson (1988) reported that A3 polypeptide made a significant contribution to development of the microstructure. In the 7 cultivars used in this study, the A1 and A2 polypeptides were shown to account for the greater part (about 70%, A1 : A2 = 4 : 3) of acidic polypeptide, and this varied slightly among cultivars.

Evaluation of textural characteristics of glycinin gels

The influence of differences in polypeptide composition on texture was investigated. The compression-decompression tests measured the mechanical parameters of the glycinin gels at different levels of compression. A rearranged data set was composed of the values taken for 16 variables: F (force), CW (compression work), CM (compressibility), RS (resiliency) and at small, medium and large compression levels and at rupture. The factor score was obtained at the final step of the factor analysis.

Mechanical attributes that loaded high on factor 1 were compression work and forces of small compression level to rupture. Compressibility of small compression level to rupture loaded high on factor 2, resiliency at rupture on factor 3. As described by Kang et al. (1991), rupture force, compression work, compressibility, and resiliency correspond to hardness, toughness, fracturability, and elasticity, respectively. Therefore, it was construed from the results of factor loadings that factor 1 relates to the hardness and toughness of the gels; factor 2 to the fracturability; and factor 3 to elasticity. Two-dimensional representation of glycinin gels from various soybean cultivars by the factor scores obtained at the last stage of factor analysis is shown in Fig. 2. The X and Y coordinates represent factor 2 (unfracturability) and factor 3 (elasticity) of textural characteristics, respectively. Numbers on the axes correspond to the values of factor scores. T, H, Y, R, S, M and Y denote Shirotsurunoko, Hill, York, Raiden, Suzuyutaka, Matsuura and Yamabe-A3, respectively. B denotes 11S globulin gels from broad beans. The numbers in circles (1, 2 and 3) represent the protein concentrations of 18, 19 and 20%, respectively.

Table 1. Composition of acidic polypeptides of glycinins.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Acidic polypeptides (%)</th>
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<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>Shirotsurunoko</td>
<td>40.6</td>
</tr>
<tr>
<td>Hill</td>
<td>40.8</td>
</tr>
<tr>
<td>York</td>
<td>37.4</td>
</tr>
<tr>
<td>Raiden</td>
<td>42.7</td>
</tr>
<tr>
<td>Suzuyutaka</td>
<td>41.1</td>
</tr>
<tr>
<td>Matsuura</td>
<td>44.3</td>
</tr>
<tr>
<td>Yamabe-A3</td>
<td>40.0</td>
</tr>
</tbody>
</table>

N.D.: not significant.
factor 3 (elasticity) of textural characteristics, respectively. This choice of factors for the axes provided the best visualization of differences in textural characteristics of the gels among the soybean cultivars. The numbers on the axes correspond to the values of the factor scores. As shown in Fig. 2, the textural properties of the gels and their dependence on the protein concentration (18, 19, 20%) were evaluated based on their positions in the diagram. The texture profiles were mainly soybean cultivar-dependent; especially, the differences in fracturability were largely dependent on soybean cultivars. The textural features of Shirotsurunoko, Hill and York, which contain A4 polypeptide were greatly different from those of Raiden, Suzuyutaka, Matsuura and Yamabe-A3, which lack A4 polypeptide, so that these two groups are distributed as two distinctive clusters in the diagram. From these results, it was judged that A4 polypeptide raises the elasticity of the gels and makes them softer and more fracturable. The thermal gelling of glycinin, which is a primary factor of texture, was shown to be affected by polypeptide composition. The gelling might also be affected by factors such as chemical and conformational properties of polypeptides. The results obtained here can be useful for texture control and development of soy gels.

**Fig. 3.** Microstructure (magnification of 100,000×) by SEM of glycinin gels from various soybean cultivars. The SEM micrograph was obtained from the 19% protein concentration gels. R, Raiden; S, Suzuyutaka; M, Matsuura; T, Yamabe-A3; T, Shirotsurunoko; H, Hill; Y, York.
scopy has long been recognized as a valuable tool in relating the detailed structure of foods to properties such as texture. It has been accepted that there is a close relationship between the physical properties of a gel and its network structure. If a gel has a well developed three-dimensional network structure, the gel is more elastic and harder (Kitabatake et al., 1989), while if a gel has a poorly developed structure, specifically an aggregation-type network, it is softer. Network structures of glycinin gels formed by heat treatment, therefore, were analyzed by scanning electron microscopy (Fig. 3, 4). SEM micrographs of globulin gels were obtained at a magnification of 100,000× (Fig. 3) and 50,000× (Fig. 4), respectively, and showed that the thickness of strands constituting the network structure, in typical cases, corresponded to the diameter of glycinin molecules (about 12 nm). As can be seen in Figs. 3 and 4, the network of the gels was composed of a very regular structure (Fig. 3 and 4, M, Y and Y), irregular (Fig. 3 and 4, S and H), or a mixture of both regular and irregular (Fig. 3 and 4, R and T). This showed that the networks fall into roughly two types: strand- (Fig. 3 and 4, R, S, M and Y) and aggregate-type (Fig. 3 and 4, T, H and Y). The degree of pore size in these strand-type and the aggregate-type was large (Fig. 3 and 4, S, T and H), small (Fig. 3 and 4, Y and Y) or a mixture of

![Fig. 4. Microstructure (magnification of 50,000×) by SEM of glycinin gels from various soybean cultivars. The SEM micrograph was obtained from the 19% protein concentration gels. R, Raiden; S, Suzuyutaka; M, Matsuura; Y, Yamabe-A3; T, Shirotsurunoko; H, Hill; Y, York.](image-url)
the two (Fig. 3 and 4, R and M). Size of the network aggregate differed among cultivars and this tendency was not affected by the protein concentration used in this study (18, 19, 20%; data not shown). The relationships among the microstructural properties of gel networks, polypeptide composition and mechanical properties of gels are described below.

**Factors relating to the gel properties** The relationships between the mechanical properties of glycinin gels, gel microstructure and polypeptide composition of various soybean cultivars are presented in Table 2. The mechanical properties of the gels as analyzed by the compression test are also given. Several pieces of information concerning the relationships between the items can be drawn from the data presented. The aggregate-type gels (Fig. 3 and 4, T, H and Y) which contained A4 polypeptide showed lower compressibility (CM), higher cohesive property (LC), and two to three times greater resiliency (RS) than the strand-type gels, which lacked the A4 polypeptide. The A3 polypeptide seems to affect pore size, regularity of network and strand-type gels, which lacked the A4 polypeptide. The A3 polypeptide showed lower compressibility (CM), higher cohesive property (LC) and two to three times greater resiliency (RS) than the A4 polypeptide. The A3 polypeptide, which lacked the A4 polypeptide, exhibited small pore size and high regularity of the gel network. On the other hand, Suzuyutaka, which contains a high level of A3 polypeptide similar to York, exhibited small and medium pore size and a gel network of high regularity. However, the gels of York, which contains A4 polypeptide, exhibited small pore size and high regularity of the gel network. The gel properties, gel microstructure and polypeptide composition of glycinin may be helpful in predicting the mechanical and structural properties of soy protein gels on the basis of their polypeptide compositions.

**References**


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**Table 2.** Relationships among mechanical, microstructural and chemical properties of glycinin gels.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Mechanical parameters at rupture</th>
<th>Gel network</th>
<th>Size of Polypeptide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>RS</td>
<td>CM</td>
</tr>
<tr>
<td>Shirotsurunoko</td>
<td>195.7</td>
<td>11.66</td>
<td>53.33</td>
</tr>
<tr>
<td>Hill</td>
<td>324.5</td>
<td>8.46</td>
<td>67.14</td>
</tr>
<tr>
<td>York</td>
<td>453.3</td>
<td>8.07</td>
<td>72.30</td>
</tr>
<tr>
<td>Raiden</td>
<td>410.3</td>
<td>3.74</td>
<td>83.33</td>
</tr>
<tr>
<td>Suzuyutaka</td>
<td>455.0</td>
<td>4.68</td>
<td>81.41</td>
</tr>
<tr>
<td>Matsuura</td>
<td>557.0</td>
<td>4.04</td>
<td>84.20</td>
</tr>
<tr>
<td>Yamabe-A3</td>
<td>685.3</td>
<td>3.85</td>
<td>88.10</td>
</tr>
</tbody>
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+, ++, +++: irregular, mixture of regular and irregular, regular in the degree of regularity. L, M, S: large, mixture of large and small, small in size. o, ☓ good, better relationships among the items (correlativity of the difference among cultivars). N.D.: not significant.


