Gelation Properties of Soymilk and Soybean 11S Globulin from Japanese-grown Soybeans

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Received September 18, 1991

A new method to evaluate soymilk for making tofu (soybean curd) is proposed. Dynamic viscoelasticity measurements were carried out to examine the gelation process of soymilk, storage and loss moduli being observed as a function of time after adding glucono-δ-lactone. The gelation curves fitted well with first-order reaction kinetics. The saturated value of the storage modulus correlated well with gel hardness by a curdrometer. The saturated value of the storage modulus was mainly dependent on the concentration of 11S globulin, while the gelation rate was an increasing function of the concentration of glucono-δ-lactone at a fixed 11S globulin concentration.

The production of domestic soymilk has increased since the Japanese government began to promote the reorganization of paddy rice fields in 1978. The yield of soymilk in Japan was 125,600 ton in 1975, 173,900 ton in 1980, 228,300 ton in 1985 and 271,700 ton in 1989. More than 50 percent of the yield has been used for tofu making. Under these circumstances, a stable supply and high quality of domestic soymilk for food processing is required. The properties of soymilk depend not only on the climate and cultivation conditions but also on the soybean variety, and an evaluation of the properties is important for processing purposes.

We investigated a method for evaluating the properties of soymilk for making tofu. The quality of the tofu was measured as its gel hardness with a texturometer or a curdrometer as in previous studies. It has already been demonstrated that the gelation of soymilk with glucono-δ-lactone could be monitored in real time by dynamic viscoelasticity measurements. The dynamic shear modulus is compared with the hardness in the present work.

Since it is known that 11S globulin, the major component of soymilk protein, mainly governs the hardness of tofu gel, and that the gel properties depend strongly on the acidic subunit composition, the gelation properties of 11S globulin were also investigated.

Materials and Methods

Soybeans. Five varieties of soybeans, Enrei, Okushirone, Tamahomare, Akikosho and Tsukuzairai, produced in Japan in 1988 were used as samples. They were stored in sealed bags at 0°C until tested.

Preparation of soymilk. Soy milk for the hardness measurements was prepared as described elsewhere. Six times the weight of water was added to soymilk to simulate the processing of Kinugoshi tofu, the protein content of the soymilk being about 5%.

Soy milk for dynamic viscoelasticity measurements was prepared as described before. Soybeans (5 g) were immersed in 50 ml of deionized water for 18 h at 20°C. The mixture was homogenized in a type of Waring Blender (Nihonseiki Seisakusho, maximum speed for 60 sec) and then in a type of Polytron homogenizer (Tokyo Rika MPC-NS, dial setting 5 for 60 sec). The filtrates through two-fold gauze were used as the soymilk, the protein content being around 3%.

Preparation of 11S globulin. 11S globulin was isolated by the method of Thanh et al. as follows. Soymilk were ground and defatted with n-hexane at 20°C. The resulting powder was immersed in 63 mm tris-(hydroxymethyl)aminomethane–hydrochloric acid (Tris–HCl) at pH 7.8 for 1 h, and then centrifuged for 15 min at 12,000 rpm and 20°C to remove the insoluble fraction. Then 2 N hydrochloric acid was added to lower the pH to 6.6. The extract was dialyzed against Tris–HCl buffer at pH 6.6 for 3 h at 2−3°C, and finally centrifuged for 20 min at 12,000 rpm and 2°C, before the precipitate was directly freeze-dried.

Analysis of subunit composition. The protein content of the soymilk was determined by the micro-Kjeldahl method. Soy milk and 11S globulin samples obtained were examined by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of polyacrylamide was 13%, and Laemmli's buffer system in the presence of 5M urea was used. The relative quantity of the protein subunits was determined by densitometry.

Measurement of gel hardness. The gel hardness was evaluated by the method of Hara and Negishii as follows. Soy milk was boiled for 4 min and then cooled. The solution (25 ml) was poured into a 30-mm-diameter glass tube and mixed with 1 ml of a glucono-δ-lactone (GDL) solution which had been freshly prepared with ice-cold water. GDL was adjusted to 0.4% in the mixture. The tube was allowed to stand for 60 min at 70°C, and cooled in an ice bath and tap water for 60 min each. The gel was then aged at the test temperature for 60 min before measurement. The hardness of the gels was determined by a Neo-Curdimeter (I-to Denki Co., Ltd.), the center of the gel in the tube being vertically compressed with a cylindrical plunger (8 mm dia.) at a compression rate of 3.6 mm/sec. Gel hardness at 20°C was evaluated from the load value at the breaking point. The hardness was calculated by equation 1 to compare to the dynamic shear modulus.

\[ H = Fg/A \] (1)

where \( H \) is the hardness of the gel (Pa), \( F \) is the breaking force in kgf, \( A \) is the cross-sectional area of the plunger (5.02 × 10^-2 m^2), and \( g \) is the acceleration of gravity (9.80 m s^-2).

Dynamic viscoelasticity. The measurement method has been described elsewhere. Soy milk or an 11S globulin aqueous solution (1−5%) was heated in boiling water for 5 min. The dynamic viscoelasticity during the gelation process was measured with a Rheolograph Sol apparatus (Toyoseiki Seisakusho), a block diagram for this instrument being shown.

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† Pa (N m^-2) is the unit of stress in SI units, and 1 Pa is equal to 10 dyne cm^-2 in CGS units. The authors think that the breaking force should be expressed in units of pressure, even though the force in gf has often been used empirically in Japanese food engineering.
Table 1. Protein Content and Subunit Composition of the Soybean Varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>Protein content (%)</th>
<th>α' (%)</th>
<th>α (%)</th>
<th>β (%)</th>
<th>( A_3 ) (%)</th>
<th>( A_4 ) (%)</th>
<th>A (%)</th>
<th>B (%)</th>
<th>11S subunit content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrei</td>
<td>33.7</td>
<td>8.8</td>
<td>13.2</td>
<td>15.4</td>
<td>3.3</td>
<td>0</td>
<td>26.4</td>
<td>22.5</td>
<td>17.6</td>
</tr>
<tr>
<td>Okushirome</td>
<td>32.8</td>
<td>10.5</td>
<td>16.3</td>
<td>13.7</td>
<td>5.3</td>
<td>0</td>
<td>21.1</td>
<td>17.9</td>
<td>14.5</td>
</tr>
<tr>
<td>Tamahomare</td>
<td>31.9</td>
<td>8.2</td>
<td>10.3</td>
<td>12.9</td>
<td>3.4</td>
<td>0.9</td>
<td>24.1</td>
<td>22.4</td>
<td>16.2</td>
</tr>
<tr>
<td>Akiyoshi</td>
<td>33.8</td>
<td>7.3</td>
<td>9.9</td>
<td>12.8</td>
<td>4.7</td>
<td>4.4</td>
<td>20.4</td>
<td>19.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Tsukuiizai</td>
<td>31.4</td>
<td>7.9</td>
<td>12.4</td>
<td>15.5</td>
<td>5.0</td>
<td>6.7</td>
<td>20.9</td>
<td>16.3</td>
<td>15.4</td>
</tr>
</tbody>
</table>

The content of each \( \alpha' \), \( \alpha \), \( \beta \), \( A_3 \), \( A_4 \), A, and B subunit in a protein was determined by densitometry.

Unfortunately, the naming of the acidic subunits in our previous work was wrong, so that \( A_3 \) and \( A_4 \) must be interchanged according to errata details attached at the end of the present paper. As shown in Fig. 2, it is noteworthy that the Tsukuiizai variety contained an \( A_4 \) subunit, while Enrei did not. The Enrei and Okushirome varieties lacked an \( A_4 \) subunit, and Akiyoshi and Tsukuiizai contained an \( A_4 \) subunit. As shown in Table 1, Tamahomare contained a small amount of \( A_4 \), which seems to have come either from the background as an experimental error or from the other protein bands, since Tamahomare is known as an \( A_4 \)-lacking variety.\(^7\)

Both the storage and loss moduli began to rise after a certain time, and the observed curves seem to fit first-order reaction kinetics. Figure 3 shows a typical gelation curve for soy milk, concentration \( C_4 \) of GDL being fixed at 0.4%. Rate of gelation \( k \) was almost the same for 4 varieties, while Tsukuiizai gelled slowly at the same \( C_4 \). The loss modulus showed no significant difference among the varieties as reported previously.\(^7\) However, the saturated storage modulus depended on varietal difference, so that the gelation curves were different among the soybean varieties. The calculated values for saturated storage modulus \( G_{sat} \) and gel hardness by a curdrometer are shown in Table II, \( G_{sat} \) correlating well with the hardness \( r = 0.998 \). Soybeans which form tofu gels with 0.4% GDL and have a breaking strength higher than 100 g/cm² or 9.8 × 10⁴ Pa have been practically evaluated as good enough for making tofu.\(^6\) The linear relationship between \( G_{sat} \) and the hardness implies that those soybean varieties with an over 170 Pa storage modulus at 80°C during dynamic viscoelastic
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Fig. 2. SDS-PAGE Patterns for Soybean Protein from Enrei and Tsukuizairai. α', α, and β are subunits of 7S globulin, and Aγ and Aα are the acidic subunits of 11S globulin. A represents the other acidic subunits and B is the basic subunits of 11S globulin. Electrophoresis was run from the right (−) to the left (+).

Fig. 3. Typical Gelation Curves for Soymilk Prepared from Enrei and Tsukuizairai.

Measuring temperature was 80°C. ●, Experimental storage modulus; —, calculated storage modulus by curve fitting (see the experimental section for kinetic equation 2).

Table II. Saturated Storage Modulus ($G_{sat}$) and Hardness of Soymilk-GDL Gels

<table>
<thead>
<tr>
<th>Variety</th>
<th>$G_{sat}$ (10^{-2} Pa)</th>
<th>Hardness* (10^{-4} Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrei</td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Okushirome</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Tamahomare</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Akiyoshi</td>
<td>2.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Tsukuizairai</td>
<td>1.6</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Hardness [Pa] = 9.80 × (m sec^{-2}) × breaking force [kgf] × cross-sectional area of plunger [m^2]

measured were suitable for tofu processing. The viscoelastic method is convenient for measuring the gelation process in real time, and will shorten the time needed for evaluating the products and improve the efficiency during processing. The direct monitoring of gelation for practical use in tofu-making is also possible if an appropriate instrument can be developed at an attractive price.

The storage modulus was not directly related to the presence or absence of the Aγ subunit. Protein concentration generally affects the gelation characteristics of a protein, although protein content shown in the second column of Table I did not show a strong effect on the storage modulus. The contribution of 11S content (the last column of Table I) to the saturated storage modulus was more important than that of the whole protein content (the second column of Table I). The correlation factor between $G_{sat}$ and the 11S content was 0.87, which is higher than 0.79 for that between $G_{sat}$ and the protein content. Thus, 11S globulin affected the gelation properties of soymilk and the quality of tofu more strongly.

11S globulin was separated from the five varieties of soybeans but, unfortunately, these fractions contained about 10% of an impure component originating from the β-subunit of 7S globulin according to SDS-PAGE patterns. The gelation curves at 80°C for 11S globulin solutions are shown in Fig. 4, protein concentration $C_p$ being 5.0% and $C_p$ being 0.4%. The curves were different according to variety, although rate constant $k$ was almost the same with the same concentration of GDL, except for Tsukuizairai which gelled at a slower rate. The dependence of saturated storage modulus $G_{sat}$ on the 11S concentration in the presence of 0.4% GDL for Enrei and Tsukuizairai is shown

Fig. 4. Gelation Curves for 11S Protein Prepared from Different Varieties of Soybean.

Measuring temperature was 80°C, and concentration of 11S globulin was 5% and that of glucono-d-lactone was 0.4%. ●, Enrei; ▲, Akiyoshi; ■, Tamahomare; ▼, Tsukuizairai; ○, Okushirome; —, calculated storage modulus by curve fitting (see the experimental section for kinetic equation 2).

Fig. 5. Dependence of Saturated Storage Modulus $G_{sat}$ on the Concentration of 11S Globulin in the Presence of 0.4% GDL.

●, Enrei; ▼, Tsukuizairai.
Fig. 6. Dependence of Rate Constant \( k \) on the Concentration of GDL for a 5% 11S Globulin Solution.

\[ k \text{ (sec}^{-1} \text{) } \]
\[ 0 \quad 0.1 \quad 0.2 \quad 0.3 \quad 0.4 \quad 0.5 \]
\[ 0.01 \quad 0.03 \]

\( G_{\text{sat}} \) increased with increasing \( C_p \), the values for \( G_{\text{sat}} \) varying according to variety, although the amount of 11S protein was the same.

The gelation kinetics of the 5% 11S globulin solution were investigated in the presence of various concentrations of GDL for Enrei and Tsukuizuairai (Fig. 6). Rate constant \( k \) increased with GDL concentration. It has been reported that, for the clotting of casein in the presence of rennet, the amount of coagulant (rennet) governed the rate of gelation.\(^{13}\) In this case as well, the amount of coagulant (GDL) governed the rate of gelation of 11S globulin.

The saturated storage modulus was not same for all varieties, even when the 11S concentration, coagulant and other experimental conditions were identical. Nakamura et al.\(^ {9}\) have reported that the hardness of heat-induced 11S globulin gels differed between different varieties at the same concentration of 11S.\(^ {9}\) They also pointed out that the gel hardness increased with the content of an acidic subunit of high molecular weight in total 11S. It is well known that a higher molecular weight fraction in a polymer forms a gel with higher strength than a lower molecular weight fraction.\(^ {14,15}\) The proposal by Nakamura et al.\(^ {9}\) is therefore reasonable. Since the A\(_2\) subunit has the highest molecular weight of the acidic subunits, as shown in Fig. 2, the variety with the highest content of A\(_2\) should form the gel with the highest gel strength. However, our results showed otherwise. For example, the 11S gel prepared from Tsukuizuairai, which has a higher A\(_2\) subunit content than Enrei, was not stronger than that of Enrei. The content of the other acidic subunits in 11S was not determined, and their effect could possibly have been greater even if they had a lower molecular weight. The composition of the acidic subunits of 11S may affect gel properties by interaction between subunits. Our findings lead to the conclusion that the quality of not only heat-induced gels but also of coagulated gels in the presence of GDL is affected by the subunit composition of the 11S protein.

As shown in Fig. 6, differences in subunit composition caused different gelation rates, the gelation rate not being simply governed by either the A\(_3\) or A\(_4\) subunit content nor by the total 11S concentration. It also seems to have been affected by the combination of subunits contained in 11S. Since the gelation rate is important in processing tofu, studies on the effect of subunits on the gelation rate would be valuable from both academic and practical view points. Further investigation is necessary to clarify the detailed relationship between viscoelasticity during the gelation of 11S and the subunit composition.

Acknowledgment. The authors are indebted to Dr. K. Kitamura of the National Agriculture Research Center for his valuable discussions.

References


Errata

K. Nishinari et al., Agric. Biol. Chem., 55, 351—355 (1991). p. 351 left 1.12: A\(_1\), A\(_2\), A\(_3\), and A\(_4\)→AS I, AS II, AS III, and AS IV 1.14: A\(_1\), A\(_2\)→AS I, AS II I.15: A\(_3\)→AS III 1.21: A\(_3\)→AS III Note: AS III in Mori's paper [T. Nakamura, S. Utsumi, and T. Mori, Agric. Biol. Chem., 49, 2733—2740 (1985)] corresponds to our A\(_3\). p. 351 right 1.17: We adopted the naming method proposed by Kitamura et al.,\(^ {10}\) A\(_3\) and A\(_4\) in the present paper being indicated as A\(_3\) and A\(_4\), respectively, by Nielsen et al.,\(^ {11}\) we adopted the naming method proposed by Kitamura et al.,\(^ {10}\) A\(_3\), A\(_4\), and A\(_5\) in the present paper being indicated as A\(_3\), A\(_4\), and A\(_5\), respectively, by Nielsen et al.,\(^ {11}\) p. 352 Table 1: 5th column A\(_2\)→A\(_3\) 6th column A\(_3\)A\(_4\)→A\(_1\)A\(_2\)A\(_3\) p. 353 right 1.10: A\(_1\)→A\(_3\) 1.16: A\(_3\)→A\(_4\) p. 353 Fig. 1: A\(_1\)→A\(_4\) A\(_1\)A\(_2\)A\(_3\)→A\(_1\)A\(_2\)A\(_3\)