The Role of Composition and Content of Protein Particles in Soymilk on Tofu Curding by Glucono-\(\delta\)-lactone or Calcium Sulfate

SHUN-TANG GUO AND TOMOTADA ONO

ABSTRACT: The effects of composition and content of protein particles on tofu curding were investigated by using soymilk prepared from mixtures of glycinin-rich and \(\beta\)-conglycinin–rich soybeans. The breaking stress of tofu curds increased with the increase of protein particle content in soymilk. The soymilk from glycinin-rich soybeans had a high protein particle content and formed harder tofu curds. There is a significant positive correlation between the content of protein particles and the breaking stress of tofu curds. It is suggested that the increase of the quantity of protein particles reinforces the combination among protein particles during the tofu curding, thus forming a stronger network of tofu curds.

Keywords: soymilk, soybean curd, tofu, protein particles, glycinin, \(\beta\)-conglycinin

Introduction

Soybeans have been utilized for food since ancient times, for example, soymilk and tofu (soybean curd), which are important dietary sources of protein and lipids. Tofu is a curd-like traditional food made from soymilk by the addition of a coagulant. In traditional tofu processing, magnesium chloride or calcium sulfate is used as a coagulant, but recently glucono-\(\delta\)-lactone (GDL) has also been widely used to make packed tofu with good texture and an extended shelf life.

The textural characteristics including hardness, springiness, cohesiveness, brittleness, and so on, are important in evaluating tofu quality and as determinants of consumers. In the addition to the effects in tofu processing, the effect of the protein content and composition in soybeans or soymilk on tofu texture is significant. Glycinin and \(\beta\)-conglycinin are major storage proteins in the soybean protein components and correspond to the 11S and 7S fractions, respectively (Yamauchi and others 1991). Saio and others (1969) reported that the tofu gel made from 11S protein was significantly harder than that from 7S protein, and that 11S gel also had a greater cohesiveness and elasticity than 7S. The 11S/7S ratio in soymilk strongly affected the textural properties of tofu (Saio 1979; Murphy and others 1997). These results led to the suggestion that tofu texture is possibly improved by using soybeans with high glycinin content or a high 11S/7S ratio.

Several studies have reported that the mechanisms of gelation on the basis of purified glycinin, \(\beta\)-conglycinin, or soy protein isolated in view of intermolecular binding forces (Utsumi and Kinsella 1985; Kohyama and Nishinari 1993; Kohyama and others 1995). However, soymilk as a hydrocolloid, which contains proteins, lipids, minerals, and so on, is a much more complex system than the soy protein solution. The protein in soymilk coagulated and formed tofu curds when added to the coagulant. The amount of coagulant required was correlated to the phytate content, pH, 7S protein content, 11S/7S ratio, titratable acidity, and original calcium content in soymilk (Liu and Chang, 2004). The mechanisms of tofu curding should be different from those of the gelation of soy protein isolate. The state of these proteins was very complex as a result of the interaction between protein and protein or other components in the soymilk. Ono and others (1991) studied the protein state and fractionated the protein in soymilk as particulate fraction and soluble fraction. The protein particles were larger than 40 nm in diameter, and the soluble fraction was smaller than 40 nm. Additionally, the particulate protein aggregated at a lower concentration of the coagulant than the nonparticle protein as a soluble protein (Ono and others 1993). The lipids were incorporated into the coagulum by conjugating the protein particles during the soymilk curding (Guo and others 1999). The breaking stress value of the tofu curd was dependent upon the amount of protein particles in the soymilk (Tezuka and others 2000). While increasing the 11S/7S ratio in soymilk, the protein particle content and the glycinin content of the particles also increased (Guo and others 2002). The incorporation of lipids occurred at a lower concentration of coagulant when the quantity of protein particles in soymilk were increased as well as the glycinin ratio in the particulate protein (Guo and others 2002). Therefore, it was considerable that there was a difference in the coagulation of proteins during different states of soymilk.

In our study, the effects of the protein particles on tofu curding were investigated by rheological measurement with the objective being to estimate the role of protein in different states of soymilk in the tofu curding process.

Materials and Methods

Materials

The soybeans (\textit{Glycine max L.}) used in this study included 1 variety (Tatikogane) and 2 lines having different proportions of glycinin to \(\beta\)-conglycinin. One line had a low content of glycinin (\(\beta\)-conglycinin–rich), which was bred as Tosan 205 from Tamahomare (Yagasaki and others 1997). The other line lacked \(\alpha\) and \(\alpha\)’ subunits of \(\beta\)-conglycinin (glycinin-rich) and was bred as Yumeminori (Ogawa and others 2000). These soybean seeds were harvested in Japan in 2001 and were stored at 5°C until used. Calcium sulfate or GDL was used as the coagulant, and other chemical agents were of the highest purity available and were used without further purification.
Preparation of the soymilk

The mixtures of the β-conglycinin–rich and glycinin-rich soybeans were made with the ratio of 4:0, 3:1, 2:2, 1:3, and 0:4, respectively, in weight. As a result, 5 shares of soymilk were different in protein composition but had the same protein content. Soybean seeds (20 gram) of β-conglycinin–rich and glycinin-rich soybeans mixed at different ratios were soaked in deionized water for 16 h at 4°C. The swollen beans were ground into a homogenate with 120 mL water using an Oster blender (Oster Co., Milwaukee, Wis., U.S.A.), and the homogenate was then filtered through a defatted cotton sheet. The filtrate was heated in a water bath for 5 min above 95°C and then quickly cooled to 20°C in ice water. The filtrate was used for the following experiments as soymilk. The protein and phytate content of the samples of soymilk prepared with β-conglycinin–rich soybean were 3.5% and 0.21%, respectively; while with glycinin-rich soybeans were 3.2% and 0.23%, respectively.

Preparation of soymilk containing various contents of protein particles

Each sample of soymilk, having various contents of protein particles but having the same content of total protein, was diluted with a soluble fraction of each soymilk. The soluble fraction was obtained from each soymilk by removing protein particles using centrifugation at 156000 × g for 30 min at 20°C (Ono and others 1991), and then the protein content was adjusted by ultra filtration using a YM-10 membrane (molecular cut-off 100000) to make the same level in each soymilk. The ratios of protein particles were 1, 3/4, 1/2, 1/4, and 0 (without particles) × that in the original soymilk. These total protein contents were the same in each soymilk.

The protein content was determined by the Bradford method (1976). The relative particulate protein contents were determined by the difference of the protein concentrations of the samples of soymilk before and after centrifugation at 156000 × g for 30 min at 20°C and converted to a percentage against the value of the protein content of each sample.

Preparation of tofu curds and texture analysis

Tofu curds were prepared and described as below. Soymilk (50 mL) was poured into a beaker. A 10% GDL or CaSO4 suspension freshly prepared was added to the soymilk at 20°C. The GDL or CaSO4 concentration was adjusted to 0.3%. The mixture of soymilk and coagulant was immediately distributed into 2 plastic syringes of 25 mL (20 mm diameter and 200 mm height). The samples with added GDL or CaSO4 were allowed to stand in a 90°C or 70°C water bath for 1 h each, respectively. Then, the coagulated soymilk (tofu curds) was cooled at room temperature and stored in a refrigerator at 10°C overnight for tofu curd aging. Before the texture analysis, the samples were allowed to stand at room temperature for 1 h.

The tofu curds in the syringes were carefully injected and cut to a columned sample of 13 mm height (20 mm dia), and the hardness of the tofu curds (breaking stress) was then measured with a FUDOH rheometer RT-2002 (Fudohkogyo Co. Ltd., Tokyo, Japan) using a disk adapter of 25 mm diameter. The moving speed of the adapter was 2 cm/min. The breaking stress was defined by the quotient of stress and transection area, when the sample was broken under the compression of the adapter of the rheometer. The value of the breaking stress was calculated by the analysis software of the instrument. Each tofu sample was measured 3 times.

Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a vertical slab gel of 1 mm thickness using an alkaline discontinuous buffer (Laemmli 1970). A broad range of molecular weight standard proteins was made from bovine serum albumin (66000 Da), ovalbumin (45000 Da), β-casein (contaminated with small α-casein) (24000 Da), and lysozyme (13000 Da).

The protein bands stained with Coomassie brilliant blue G-250 in the gel were scanned with a HP Desk Scan II instrument (Hewlett-Packard Co., Palo Alto, Calif., U.S.A.), and then the band intensities were analyzed using Scion image PC software (Scion Co., Frederick, Md., U.S.A.). The relative ratio of glycinin and β-conglycinin was obtained by these treatments.

Statistical analysis

Each experiment was conducted in duplicate. Analysis of variance of the data and path analysis were performed using a Statistical Analysis System package SAS 6.12. When differences were observed, the least significant difference was tested to analyze the significance of the treatment.

Results and Discussion

Texture characteristics of the tofu curds made from mixed soybeans with different protein compositions

The major storage proteins of soybeans consist of glycinin containing acidic (A) and basic (B) peptides, and β-conglycinin containing α′, α, and β subunits (Mori and others 1981; Staswick and others 1984). In this study, 2 kinds of soybean seeds with different protein compositions were used to make the tofu curds, and their SDS-PAGE patterns are shown in Figure 1. Compared with the Tatikogane, a conventional soybean (Lane 4), the glycinin ratio occupied 79.9% of the total protein and the ratio of glycinin/β-conglycinin was 4.0 for a glycinin-rich soybean, Yumeminori (Lane 3), which lacks α′ and α subunits. While acidic (A) and basic (B) peptides (subunits) decreased in β-conglycinin-rich soybeans (Lane 2), the glycinin ratio occupied 31.1% and the ratio of glycinin/β-conglycinin was 0.377.

The breaking stress of these tofu curds made with GDL coagulant was determined and shown in Figure 2. The breaking stress (1.9 × 10^3 N/m2) of tofu made from β-conglycinin–rich soybeans was lower than that (2.9 × 10^3 N/m2) of Tatikogane with a normal protein composition. In contrast to the β-conglycinin–rich soybeans, the tofu

Figure 1—SDS-PAGE patterns of the proteins in soymilk. Lane 1, molecular weight standard; lane 2, β-conglycinin-rich (Tosan 206); lane 3, glycinin-rich (Yumeminori); lane 4, Tatikogane. The molecular weight standard proteins are bovine albumin (66000 Da), ovalbumin (45000 Da), β-casein (24000 Da), and lysozyme (13000 Da).
made with glycinin-rich soybeans showed a greater breaking force (6.4 × 10³ N/m²) than those of β-conglycinin–rich and Tatikogane soybeans. Similar results were also observed in the tofu curd derived from glycinin-rich soybeans coagulated by calcium sulfate, but these were much harder than those from β-conglycinin–rich and Tatikogane soybeans, in spite of the fact that the effects of the calcium salt differed from the GDL in the mechanisms of curd formation and stabilization (Kohyama and others 1993). These results were consistent with those showing that crude 11S protein gel is much harder than crude 7S protein gel made using calcium sulfate or GDL (Saito and others 1969). Cai and Chang (1999) reported that the glycinin content and 11S/7S protein ratio of soybean varieties do not change significantly from soymilk to tofu during the production and pilot methods, and the 11S/7S ratio of soymilk and tofu is positively correlated with the 11S/7S protein ratio of soybean seeds. Consequently, our results indicated that the soybean varieties with the higher glycinin/β-conglycinin ratio made the tofu curds harder.

The protein in soymilk exists in soluble and particulate states having different sizes in diameter. The particulate (more than 40 nm in diameter) protein is formed through the association of subunits of glycinin and β-conglycinin during soymilk processing (Ono and others 1991; Guo and others 1997). In our previous study (Guo and others 2002), it was found that the protein particle content and the glycinin ratio in particles increased linearly with the increase of glycinin content in soymilk. Samples of soymilk were made from mixtures of 0/1, 1/3, 2/2, 3/1, and 4/0 ratios of glycinin-rich and β-conglycinin–rich soysbeans, and then tofu curds were made from these samples with the addition of GDL or CaSO₄. Their particulate protein content and breaking stress were measured and are shown in Figure 3. The particulate protein content increased from 35.6% to 78.3%, which was proportional to the glycinin content in the soymilk as well as the soymilk in the previous report (Guo and others 2002). As the particulate protein content was higher, the breaking stress of the tofu curd was also higher with the addition of either GDL or CaSO₄.

The soymilk samples, having various particulate protein content and the same protein concentration, were made using concentrated soymilk, which had removed the particulate protein from glycinin-rich soymilk. The tofu curds were then prepared from these samples and shown in Figure 4. The tofu curds coagulated either by calcium sulfate or GDL had a shapely guise and a hard texture as well as an increase of the particulate protein content in the soymilk. On the other hand, the soymilk having lower than 20% of particulate protein content formed an extremely soft curd, that is, the coagulum could not form a shapely curd and became distorted and broken up by their weight when the tofu curds were extruded from the tube device. This result indicates that the tofu curd formation depends on the ratio of the particulate protein in the soymilk. Therefore, when increasing the glycinin content, more protein particles will exist simultaneously in the soymilk with which harder tofu curds with higher breaking stress can be obtained.

**The effects of particulate protein content on the formation of tofu curds**

Breaking stress was an important parameter evaluating the quality of the tofu curds; it directly reflected the texture of the tofu. Kohyama and Nishinari (1993) reported that 7S and 11S gels induced by GDL had similar storage modules, but did not show the same breaking stress, strain, or energy. Furthermore, to show the effects of particulate protein content in soymilk on the formation of tofu curds, soymilk samples with different subunit compositions were adjusted to the various particulate protein contents by dilution using soymilk removed from particulate protein and then used to prepare the tofu curds. The breaking stresses of tofu curds are shown in Figure 5. The breaking stress of each tofu curd prepared from the soymilk samples with different ratios of β-conglycinin/glycinin (except β-conglycinin–rich soymilk, 4/0 [Figure 5a], 3/1, and 4/0 [Figure 5b]) of β-conglycinin–rich; glycinin-rich soybean) was increased as the protein particle content increased. The good square regression relationships were found between the breaking stress of tofu and the content of protein particles in soymilk (Y = ax² + bx + c, R² = 0.9707 = 0.9999; Y, breaking stress; x, particulate protein content), especially soymilk with a high ratio of glycinin/β-conglycinin, where the correlation coefficient is close to 0.999. This result indicated that the breaking stress of tofu curds depends on the protein particle content in the soymilk. The protein particles lead protein to form a harder gel network. In our previous study, we proposed that the protein particles combined with oil bodies 1st during the soymilk coagulation by adding coagulant (Guo and others 1999). Therefore, it is thought that the gelation and formation of tofu

![Figure 2—Comparison of the breaking stress of tofu curds prepared by soybean varieties with different protein composition.](image1)

![Figure 3—Comparisons of protein particle content in soymilk and the breaking stress of tofu curds prepared by mixture glycinin-rich and β-conglycinin-rich soybeans.](image2)
curds are formed based on contact between protein particles and oil bodies with the more particulate proteins.

On the other hand, the trend of increasing the breaking stress of tofu curd induced by the increasing of the protein particle content was decreased gradually with the increase of the ratio of β-conglycinin to glycinin. It is known that the ratio of 11S/7S is negatively correlated with the amount of coagulant required for making tofu with the highest breaking stress (Liu and Chang 2004). The increasing effect of protein particle content on breaking stress of tofu curd was not significant when the ratio of β-conglycinin–rich soybean was higher than 75% (Figure 5a, 4.0, and Figure 5b, 3:1 and 4:0, of β-conglycinin–rich:glycinin-rich soybean). Part of the reasons for this is probably caused by the fact that the 0.3% coagulant added was not enough to form a protein network when increasing the ratio of β-conglycinin and glycinin in the soymilk. On the other hand, we also observed that the content of particulate protein decreased in β-conglycinin–rich soymilk; most of the content of particulate protein was only 35% in soymilk prepared using β-conglycinin–rich soybeans, which is a lower percent than that of normal soymilk. Consequently, it was considered that the conjugation of protein particles in β-conglycinin–rich soymilk must be smaller than that of normal soymilk.

However, the value of the breaking stress of the gel corresponds with the interaction forces of the protein intermolecular, hydrogen bonds, hydrophobic interaction, and also charge–charge interaction, which also may be involved in the network structure formed by heating (Nakamura and others 1984; Mori and others 1986). The SH content in soymilk increased during the 1st 2 or 3 min by heating at 100°C (Ohata and others 1989). Shimoyamada and others (2000) reported that the surface hydrophobicity of soymilk increased during a shorter heating time, thiol-disulfide exchange reactions and hydrophobic interactions were complexly related with a three-dimensional network of the freeze-gel of soymilk. But the stabilizing mechanism of tofu curds induced by coagulant was different from that of heat-induced soy protein gels. Kohyama and others (1995) considered that the formation of disulfide bonds is very slow at the low-pH region, and the contribution of disulfide bonds on the tofu is negligible. They speculated that the gelation mechanism consists of the following steps: the hydrophobic regions of the protein molecules in the native state are located inside and exposed to the outside by heat denaturation. The denatured soy protein is negatively charged, so that protons with GDL or calcium ions added neutralized the net charge. As a result, the hydrophobic interaction of the neutralized protein molecules becomes more predominant and induces aggregation. Ono and others (1999) report that 50% of proteins exist as particles of more than 40 nm in diameter in both unheated soymilk and soymilk (heated). But the protein particles in soymilk formed from a combination of components degraded the original particles and a part of soluble protein by heating. Additionally, the protein particles precipitated at a lower calcium concentration than that of the soluble protein (Ono and others 1993). Therefore, it is considered that the protein particles rearrangement was mainly due to the denaturation by heat treatment, the particles having SH groups and hydrophobic regions exposed in the soymilk. When the coagulant was added to the soymilk, the pH decreased and the net charge of the protein particles was neutralized. The hydrophobic interaction occurred between the protein particles 1st and formed a network for tofu curd formation. The increase of quantity of protein particles reinforced the combination between protein particles and formed a stronger framework.

### Contribution of glycinin/β-conglycinin and the relative protein particle content on the tofu curding

| Table 1 | shows the ratios of glycinin/β-conglycinin (x1), the proportions of glycinin and β-conglycinin in protein particles (x2), the relative protein particle content (x3), and the coefficient (r) of the correlation relation of these factors (x1) compared with the breaking stress of the tofu (y). There were significant positive correlations between the above factors and the breaking stress of tofu (y). The lipid incorpor-
Effect of protein particles on tofu curd formation  

Table 1—Analysis of correlation between protein composition of soymilk, relative content of protein particle, and breaking stress of tofu curd induced by GDL or CaSO₄

<table>
<thead>
<tr>
<th>Variable/Repeat</th>
<th>Soybean protein composition: β-conglycinin (x₁)</th>
<th>Protein composition in particle glycycin/β-conglycinin (x₂)</th>
<th>Relative content of protein particle (%) (x₃)</th>
<th>Breaking stress of tofu curd from GDL(y₁) N/m²</th>
<th>Breaking stress of tofu curd from CaSO₄(y₂) N/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.377</td>
<td>0.777</td>
<td>35.6</td>
<td>2.824</td>
<td>1.319</td>
</tr>
<tr>
<td>2</td>
<td>0.641</td>
<td>0.974</td>
<td>41.8</td>
<td>4.604</td>
<td>3.742</td>
</tr>
<tr>
<td>3</td>
<td>1.19</td>
<td>1.537</td>
<td>52.4</td>
<td>6.002</td>
<td>5.072</td>
</tr>
<tr>
<td>4</td>
<td>2.597</td>
<td>2.144</td>
<td>70.97</td>
<td>8.264</td>
<td>6.001</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3.22</td>
<td>78.3</td>
<td>9.519</td>
<td>7.031</td>
</tr>
</tbody>
</table>

Correlation coefficient of factors (rₓᵧ)

\[r_{x_1} = 0.993^{**}\]
\[r_{x_2} = 0.947^{*}\]
\[r_{x_3} = 0.985^{*}\]
\[r_{y_1} = 0.967^{**}\]
\[r_{y_2} = 0.951^{**}\]
\[r_{y_1} = 0.992^{**}\]
\[r_{y_2} = 0.938^{*}\]

Degree of freedom is 3. Every correlation coefficient is close to or arrives at the most significant level (rₓᵧ = 0.959), expressed by ** except the correlation coefficient of glycycin/β-conglycinin in soybean (x₁) and breaking stress of tofu curd from CaSO₄ (y₂) is distinct level (rₓᵧ = 0.878), expressed by *.

Conclusions

Ono and others (1993) reported the properties of the soluble protein and particulate protein. Compared with the soluble protein, the particulate protein was more sensitive to Ca²⁺ and H⁺, namely, and the particulate protein aggregated at a lower concentration of the coagulant than that of the soluble protein. Guo and others (1999, 2002) reported that during soymilk curding induced by a coagulant, protein particles conjugated with lipid droplets prior to the soluble protein and then aggregated. However, soluble proteins 1st formed particles and then aggregated in the presence of the coagulant. According to the above findings, tofu curd can be speculated as follows: the protein in the soymilk was denatured by heating and rearranged to particulate and soluble proteins, the net charge of the protein particles can be neutralized by adding coagulant, the hydrophobic interaction occurs among the protein particles. Then, protein particles and lipid droplets are formed producing a network for tofu curd formation. The breaking stress of tofu curd was harder and harder with the increase of the protein particle content. At the same time, the soluble protein formed the new particles and incorporated them to that network. Lastly, a curd with good water-hold capacity was formed.

Acknowledgments

We express our appreciation to Dr. Koji Takahashi and Kazuhiro Yagasaki (Natl. Inst. of Crop Science, Japan, and Nagano Central Agricultural Experimental Station) for material, and to X.M. Zhang and J.Y. Han (College of Food Science and Nutrition Engineering, China Agricultural Univ.) for considerable assistance. A part of this work was supported by financial aid from the research project “Selective breeding for high-quality products and development of new products” in the Ministry of Agriculture, also from the Forestry and Fisheries of Japan, and from the project “The chemical and processing properties of soybeans lacking one or more specific subunits” from the Natl. Nature and Science Foundation of China.

References


