Soybean cultivars impact quality and function of soymilk and tofu

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Abstract: The functional properties of soymilk and tofu prepared from Benning and Danbaekkong soy cultivars were identified. The protein content in soymilk was significantly higher for Danbaekkong, at 46.4 g kg\(^{-1}\), than for Benning at 42.0 g kg\(^{-1}\). The 11S/7S globulin ratios of Danbaekkong and Benning were estimated at 0.93 and 0.38, respectively. Soymilk from Danbaekkong was more non-Newtonian (\(n = 0.69\)) than soymilk from Benning (\(n = 0.84\)). Tofu prepared from Danbaekkong was significantly harder, chewier and gummier than tofu from Benning. Differences in protein content, protein ratio, viscosity, textural properties and color of soymilk can be applied as indicators of quality and functionality in soy foods such as tofu.

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INTRODUCTION

The soybean market for the manufacture of tofu and related products is expanding, and contracts with grower or seed companies to buy specific soybean cultivars on the basis of seed color, protein content and other characteristics are common. Soy food manufacturers are using tailored soy fractions that are high in specific functional traits. Soybean breeders and processors generally believe that high protein, large-seed soybeans result in better quality tofu and create higher yields.\(^1\) Significant variations among soybean varieties are observed in terms of protein and oil contents, size, amount of water absorbed at complete hydration, protein concentration of soymilk, fresh tofu yield, tofu protein content and tofu hardness. High protein cultivars generally produce a higher protein content tofu than low protein cultivars.\(^2\) Although evidence exists for genotypic differences in soymilk and tofu characteristics,\(^3\) functional differences are not typically associated with origin of beans from the USA or Japan.\(^4\)

The protein content in soybean seed is approximately 40–45 g kg\(^{-1}\) on a dry-weight basis. The two main seed storage proteins in soybean are 7S globulin (β-conglycinin) and 11S globulin (glycinin). These two storage proteins account for approximately 700 g kg\(^{-1}\) of the total protein in soybean seed, and differ in gel forming abilities.\(^5\) The concentrations of these two components differ among soybean varieties, and differences in 7S, 11S and the 11S/7S ratio influence the quality of soy-food products.\(^6\)–\(^8\) For example, the 11S/7S ratio in soymilk affects the textural properties of tofu.\(^9\)

Tofu, a product manufactured by curdling soymilk with calcium or magnesium chloride, is a highly hydrated, gelatinous product. The texture of tofu is smooth, firm and coherent, but not hard or rubbery. Since tofu is prepared from soymilk, the physical properties of tofu curd likely depend on the properties of the soymilk. Specifically, the protein in soymilk may play an important role in the physical properties of tofu.\(^10\) Chemical composition of different soybean varieties also affects tofu yield and quality.\(^11,12\) Soybean cultivars significantly influence tofu yield and quality\(^13\) and manipulation of the 11S/7S ratio between 1.6 and 3.2 has different effects on tofu yield and quality, depending on cultivar.\(^14\)

‘Benning’ soybeans (Glycine max (L.) Merr.) result from breeding programs conducted at the Georgia Agricultural Experiment Station, with a release date of January 1996. Benning soybeans are significantly disease and nematode resistant, and have high production yields.\(^15\) ‘Danbaekkong’ is a medium to small size Korean soybean cultivar used for tofu and soymilk production. The raw soybean seeds contain high crude protein and fiber as well as excellent tofu quality, but have a low production yield.\(^16\) Identification of the specific traits responsible for functional properties would markedly increase the value-added potential for soybeans and enhance the ability to process tailored soy ingredients. The objectives of this study were to identify qualitative and functional properties of soymilk and tofu of Benning

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and Danbaekkong by comparison of protein content, protein subunit composition and amount, viscosity, and textural properties. This information will facilitate the development of methods to quantify potential lines in a breeding program.

EXPERIMENTAL

Plant materials
Danbaekkong and Benning soy seeds were obtained from the Crop and Soil Science Department, University of Georgia (Athens, GA, USA), and stored at 4°C prior to use.

Preparation of soymilk and tofu
Soybeans (100 g) were soaked in 500 g of tap water for 16 h at room temperature. Soaked seeds were drained and then chopped in a food processor at room temperature. The ground soybeans were homogenized for 16 h at room temperature. Soaked seeds were drained and then chopped in a food processor at room temperature. The ground soybeans were homogenized in 500 g of distilled water with 0.3 g kg⁻¹ antifoam A (Sigma Chemical Co., St Louis, MO, USA) for 1 min at room temperature (Pro Homogenizer 300A, 30 mm diameter, Pro Scientific Inc., Oxford, CN, USA). Homogenates were heated on a hot plate set to approximately 90°C for 15 min, while being stirred. Homogenates were immediately strained for 10 min through two layers of cheese cloth suspended over a stainless steel pan. Filtration was facilitated by placing a weight of 5.4 kg over the homogenate. The soymilk was stored at 4°C until analysis.

To make tofu, 147 mL of soymilk was heated to 95°C, while being stirred on a hot plate. After the desired temperature was reached, the hot plate was turned off and the soymilk was stirred and cooled to around 90°C. An aliquot of 1.2 mL of a stock 200 g kg⁻¹ MgCl₂ solution was added to the soymilk to give a final MgCl₂ concentration of 4 g kg⁻¹ in the soymilk. After adding coagulant, the soymilk was left for 2 min, transferred to an acrylic mold (26 mm × 65 mm × 63 mm), and lined with one layer of cheese cloth. The cheese cloth was folded over the soy curd, and pressing for 2 min was facilitated by a 3.1 kg weight.

Protein determination
The protein concentration in the soymilk was assayed using the BCA standard method. A calibration curve for the assay was made with bovine serum albumin (Bio-Rad, Hercules, CA, USA). An aliquot consisting of 50 μL of sample or standard was mixed with 2 mL of standard working reagent in a test tube. After incubation, a 200 μL aliquot was transferred to a micro plate reader (MPR model 550, Bio-Rad Inc.). Absorbance was read at 540 nm, and the protein content was calculated using MPR software. Soymilk was diluted 1:100 with deionized water prior to protein analysis.

Analysis of subunit composition
SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a Phastsystem according to manufacturer specifications (Amersham Pharmacia Co., Uppsala, Sweden). So that the same amount of protein would be added to the gel, samples were diluted to a constant protein concentration prior to boiling in sample buffer, and a 5 μL aliquot was run on a PhastGel gradient gel, 80–250 g L⁻¹.

The gel was stained with Coomassie Brilliant Blue R250™ according to manufacturer specifications (Amersham Pharmacia Co.). The staining intensity of the bands on SDS-PAGE was measured by densitometry with a scanner (model GS-670, Bio-Rad Inc.), and the subunit composition was estimated by calculating the percent total area.

Viscosity analysis of soymilk
The rheological properties of soymilk were analyzed using a rheometer (SR-5000, Rheometric Scientific Inc., Piscataway, NJ, USA). A steady rate ramp test was performed at 4°C to determine viscosity data, with initial and final rates of 0 and 50 s⁻¹, respectively, and a measurement time of 300 s per cycle. The resulting viscosity data were fitted using the power law \( \sigma = K \gamma^n \), where \( \sigma \) is the shear stress, \( K \) is the consistency index, \( \gamma \) is the shear rate and \( n \) is the flow behavior index. Analysis was performed on \( n \) and \( K \) values of the up cycle.

Texture profile of tofu
Textural properties of tofu were evaluated with a texture analyzer (TAXT2, Stable Micro Systems, Godalming, Surrey, UK) fitted with a 25 kg load cell. Cylindrical samples of tofu (13 mm diameter, 10 mm height) were compressed by a flat plate (4 cm × 4 cm) to 50% deformation. The parameter settings and operation of the instrument were accomplished using Texture Expert® software version 2.12 (Texture Technologies, Scarsdale, NY, USA). The test mode was set to 'texture profile analysis'. Textural properties including hardness, cohesiveness, adhesiveness, gumminess and chewiness were calculated from the curve according to definitions given by Bourne. The pre-test, test and post-test speeds were set to 2, 2 and 4 mm s⁻¹, respectively. Each test was repeated a total of four times at room temperature.

Color and moisture content
Color values of soymilk and tofu were measured with a CR-300 Minolta Chroma Meter™ (Minolta Camera Co. Ltd., Osaka, Japan). This instrument is a color analyzer with an 8 mm diameter measuring area and glass light-projection tube for liquid. The \( L_a^*, b^* \) color scale was used to measure color: \( L \) (0, dark black) to \( L \) (100, white), \( +a^* \) (red) to \( -a^* \) (green), and \( +b^* \) (yellow) to \( -b^* \) (blue). The instrument was standardized using a standard white tile with \( L = 97.30, a^* = 0.04 \) and \( b^* = 1.65 \). The moisture content of tofu was determined by drying about 3 g of freshly prepared tofu at 90°C for 8 h in a vacuum oven.
Statistical analysis
Results from three replicate experiments were analyzed by the general linear model procedure (SAS software version 6.12 SAS Institute Inc., Cary, NC, USA). The differences of means among the samples were resolved by confidence intervals using a Duncan test, and the level of significance was set at \( P \leq 0.05 \).

RESULTS AND DISCUSSION
Protein content of soymilk
The protein content of soymilk from Danbaekkong was significantly higher than that of soymilk from Benning (\( P \leq 0.05 \)), with values of 46.4 g kg\(^{-1}\) and 42.0 g kg\(^{-1}\), respectively. The range of protein content in soymilk from 17 cultivars was 45.1–50.9 g kg\(^{-1}\), and the range of protein content in beans and flakes from three soy cultivars was 35.0–51.7 g kg\(^{-1}\). Generally, the higher the protein content of the soybean, the higher the protein in the resulting soymilk and tofu. \(^{24}\) Cai \textit{et al}. \(^{11}\) showed that high soybean protein content resulted in tofu with higher yield and with improved texture. Shen \textit{et al}. \(^{12}\) reported that soybean varieties higher in protein content produced tofu with firmer and springier texture.

Subunit composition of soymilk protein
SDS-PAGE analysis of soymilks (Fig 1) illustrated that the protein bands were similar in the two soybean cultivars in that there was no unique absence or presence of subunits between them. The molecular weights of the \( \alpha \), \( \alpha \)′, \( \beta \) subunits of \( \beta \)-conglycinin are approximately 76,000, 72,000, and 53,000, respectively. The acidic polypeptides, \( A_3 \), \( A_{1,2,4} \) and basic polypeptides of glycine have molecular weights of 43,000, 40,000 and 20,000, respectively. \(^{26}\) The results illustrated in Table 1 show that soymilk from Danbaekkong had larger amounts of glycine (11S), whereas soymilk from Benning had larger amounts of \( \beta \)-conglycinin (7S). Thus, the ratio of 11S/7S was higher in Danbaekkong soymilk than in Benning soymilk (0.93 and 0.38, respectively). Glycinin and \( \beta \)-conglycinin are largely responsible for the functional characteristics of soy protein ingredients, for example, solubility in beverages, viscosity in gravy, gelling properties in tofu, foaming in whipped toppings, and emulsification in hot dogs. \(^{27}\) Glycinin contains a higher concentration of sulfur-containing amino acids, such as methionine and cysteine, than other protein subunits which are important to the quality of tofu. \(^{28}\) Moreover, 11S (glycinin) contains two \(-\)SH groups and 20 S–S bonds per molecule; however, 7S (\( \beta \)-conglycinin) does not contain \(-\)SH groups, and has two S–S bonds per molecule. Thus, ionic interactions and disulfide bonds were involved in the formation of 11S globulin gel, and hydrogen bonding was an important factor in 7S globulin gel formation. \(^{29}\)

Viscosity of soymilk
Based on a steady rate ramp test, soymilk was evaluated across a range of shear rates from 0 to 50 s\(^{-1}\). The \( n \) values of soymilks from Danbaekkong and Benning were significantly different (\( P \leq 0.05 \)) at 0.69 and 0.84, respectively (Table 2). The \( K \) values of soymilk from Danbaekkong and Benning were significantly different (\( P \leq 0.05 \)) at 0.14 and 0.03, respectively. Rickert \textit{et al}. \(^{27}\) reported that a mixture of glycine and \( \beta \)-conglycinin showed 3- or 20-fold higher apparent viscosity than that of \( \beta \)-conglycinin or glycine alone, respectively. \(^{27}\) Of the two subunits, \( \beta \)-conglycinin displayed a higher viscosity than glycine. Glycinin gels were more elastic than \( \beta \)-conglycinin gels. \(^{30}\)

![Figure 1. SDS-PAGE of soymilk proteins separated on 80–250 g L\(^{-1}\) gradient gel using a Phastsystem and stained with Coomassie Brilliant Blue. Lane 1 is molecular weight standards; lane 2 depicts peptides separated from Benning; lane 3 depicts peptides separated from Danbaekkong. Tentative identification based on molecular weight is indicated in the legend.](image-url)

Table 1. Composition of glycine (11S) and \( \beta \)-conglycinin (7S) protein subunits in soymilk

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>( \alpha )' and ( \alpha )</th>
<th>( \beta )</th>
<th>( A_3 )</th>
<th>( A_{1,2,4} )</th>
<th>Basic</th>
<th>7S</th>
<th>11S</th>
<th>11S/7S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danbaekkong</td>
<td>34.7</td>
<td>7.5</td>
<td>1.7</td>
<td>22.3</td>
<td>15.4</td>
<td>42.2</td>
<td>39.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Benning</td>
<td>40.7</td>
<td>13.4</td>
<td>3.9</td>
<td>4.0</td>
<td>9.9</td>
<td>54.2</td>
<td>17.8</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Protein content (g kg\(^{-1}\)) of soymilk: Danbaekkong 46.40 ± 0.94; Benning 42.04 ± 1.38.
**Texture profile of tofu**

The texture profile analysis results of the tofu are summarized in Table 3. The properties of hardness, chewiness and gumminess of Danbaekkong tofu were significantly higher than those of Benning tofu \((P \leq 0.05)\), whereas there were no significant differences in springiness and cohesiveness between Danbaekkong and Benning. Wang et al.\(^4\) reported significant variation among soybean varieties of both US and Japanese soybeans in the hardness of resultant tofu, and also found a negative correlation with moisture content.\(^4\) However, the tofu moisture contents of Danbaekkong and Benning were 76.9 and 77.5 g kg\(^{-1}\), respectively, and not significantly different \((P \leq 0.05)\). Thus, moisture content is not the likely cause of differences in the texture of tofu. In contrast, the ratio of 11S/7S is likely to have the greatest effect on tofu texture, since the Danbaekkong 11S/7S ratio was 0.93 while the Benning 11S/7S ratio was 0.33. Earlier, Saio et al.\(^9\) had found that gel made from 11S protein isolated from defatted soybean meal displayed greater hardness, cohesiveness and springiness than that made from 7S protein. Tofu from crude glycinin is harder than tofu from crude β-conglycinin, and the difference was attributed to higher numbers of disulfide bonds.\(^11\) Cai et al.\(^31\) reported that curd produced from soybeans, chickpeas and fava beans displayed a better texture, with higher texture scores for hardness, springiness and cohesiveness, than curds produced from lentils and mung beans, due to the greater amounts of 11S globulin and lower amounts of 7S globulin present. Furthermore, seed protein was positively correlated with tofu hardness and firmness, while seed oil, free sugar, sucrose and remainder content were generally negatively correlated with tofu quality parameters.\(^12\)

**Color of soymilk and tofu**

The color of soymilk and tofu varied with soybean cultivars (Table 4). Depending on the cultivars, \(L\) values (lightness), \(a^*\) values (redness) and \(b^*\) values (yellowness) of soymilk and tofu were 71.7 to 93.2, -1.45 to -2.32 and 10.9 to 17.8, respectively. In the soymilk, Benning showed significantly higher \(L\) values and lower \(b^*\) values \((P \leq 0.05)\). However, no significant effect on \(a^*\) values was observed \((P \leq 0.05)\). The \(L\), \(a^*\) and \(b^*\) values for tofu were slightly higher than those in soymilk, but the trends of differences between Benning and Danbaekkong for \(L\), \(a^*\) and \(b^*\) values were the same. Tofu and soymilk made from Benning were whiter and less yellow than those from Danbaekkong. This tendency is in agreement with a study\(^12\) which reported that seed protein content showed a weak, but significant, negative correlation with seed \(L\) value and tofu \(L\) value. The color measurement can be one of the criteria for assessing the quality of soymilk and tofu. Generally, a white or light yellow (creamy white) color is a desirable tofu characteristic.\(^32\)

**CONCLUSION**

The two soybean cultivars, Danbaekkong and Benning, vary in composition, quality and functionality of soymilk and tofu. In particular, the soybean protein of soymilk may contribute to the physical properties of tofu. Soymilk from Danbaekkong had higher protein content, a larger amount of glycinin (11S), and a lower \(n\) value than soymilk produced from Benning. In the case of tofu, Danbaekkong tofu showed increased hardness, chewiness and gumminess than

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**Table 2. Rheological properties of soymilk**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Consistency index K</th>
<th>Flow behaviour index n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danbaekkong</td>
<td>0.14(^a)</td>
<td>0.69(^a)</td>
</tr>
<tr>
<td>Benning</td>
<td>0.03(^b)</td>
<td>0.84(^b)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means within the same column with different letters are significantly different \((P \leq 0.05)\).

**Table 3. Texture profile analysis of tofu**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Hardness (N m(^{-1}))</th>
<th>Springiness (mm)</th>
<th>Chewiness (N m(^{-1}) · mm)</th>
<th>Cohesiveness</th>
<th>Gumminess (N m(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danbaekkong</td>
<td>292.4(^a)</td>
<td>0.74(^a)</td>
<td>122.5(^a)</td>
<td>0.56(^b)</td>
<td>165.3(^a)</td>
</tr>
<tr>
<td>Benning</td>
<td>198.8(^b)</td>
<td>0.77(^a)</td>
<td>78.0(^b)</td>
<td>0.51(^b)</td>
<td>101.5(^b)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means within the same column with different letters are significantly different \((P \leq 0.05)\).

\(^c\) Textural parameters from 50% deformation of tofu.

Moisture content (g kg\(^{-1}\)):

- Danbaekkong 76.9 ± 0.64;
- Benning 77.5 ± 0.17.
Benning tofu. The differences between Danbaekkong and Benning products are most likely due to their differing protein content and composition, especially the 11S/7S ratio. Soymilk with higher protein content, higher 11S, lower n value and lower L and b² values produced tofu with greater hardness, chewiness and gumminess. Quality parameters such as color and viscosity parameters in soymilk reflect quality parameters such as texture profile in tofu.

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