Characterization of 7S and 11S Globulins in Soybean Varieties Differing in Seed Size and Their Effects on the Properties of Soybean Curd

Sun-Lim Kim, Han-Mo Koo¹, Se-Cheol Chun², Jung-Tae Kim, Min-Young Kim², Hee-Youn Chi, Eun-Hye Kim², Hyun-Bok Kim³, Mi-Jung Kim, Bo-Ram Seo², Eun-Young Kang², Su-Hyun Seo², and Ill-Min Chung²⁸

National Institute of Crop Science, Rural Development Administration, Suwon, Gyeonggi 441-857, Korea

Abstract The ratio between 11S and 7S globulins was greater in large seed size varieties (LSSVs) of soybean than in small seed size varieties (SSSVs) or medium seed size varieties (MSSVs) but did not differ between MSSVs and SSSVs. The cysteine and methionine contents of 11S globulins were greater than those of total seed proteins and 7S globulins. The acidic monoamino monocarboxylic amino acids were the most abundant class of amino acid in soybean seed (27.2%) and soybean curd protein (29.3%). Isolated 7S and 11S fractions were analyzed by HPLC. Of the 12 peaks detected, 4 constituted 64.1% of the proteins of the SSSVs, 65.6% of the proteins of the MSSVs, and 70.5% of the proteins of the LSSVs. The 11S/7S globulin ratio was related to the yield and hardness of soybean curd. The MSSVs had the greatest yield of soybean curd, but the soybean curd hardness of the MSSVs was greater than that of the SSSVs. These results show that the 11S/7S ratio and color of soybean seeds can be used to predict the yield, hardness, and color of soybean curd.

Keywords: soybean, seed storage protein, 7S, 11S, amino acid, soybean curd

Introduction

Soybean (Glycine max L.) has long been an important dietary protein source in Korea and other Asian countries (1,2). Soybean consumption has increased during the past decade because of increased recognition of the health benefits of soy foods (3-5). Soybean curd is a highly hydrated and gelatinous foodstuff that is prepared by precipitation of heated soybean milk with coagulants (6-8). Texture and yield are important determinants of consumer and producer acceptance of soybean curd and are determined by interactions among parameters such as soybean cultivar, growth environment, and processing conditions (9-13). High protein soybean cultivars were known to be produced much protein content soybean curd than low protein cultivars (14). Moreover, the present evidence shows dissimilar with genotypic in soymilk and soybean curd characteristics (15).

7S Globulin (β-conglycinin) and 11S globulin (glycinin) constitute 70% of the protein in soybean seeds (16). The ratio between 7S and 11S globulins affects the nutritional quality of soybean protein and is important for the production of soy foods. 11S Globulin is more nutritious than 7S globulin because it contains more sulfur-containing amino acids. The ratio of 11S to 7S globulin is used as an indicator of protein quality (17,18).

hydrogen bonds, whereas the gelation force of 11S globulin is due to disulfide and hydrogen bonds (19,20). The number of disulfide bonds that can be formed with 7S globulin is limited because it contains only 2 to 3 cysteine

The gelation force of 7S globulin is mainly due to

groups; in contrast, 11S globulin contains 6-37 sulfhydryl and disulfide groups per mole of protein (21). The 7S globulin has 6 isomers, each of which is composed of 3 discrete protein subunits, α' , α , and β , with Mw of 80, 76, and 50 kDa, respectively, while the 11S globulin has both acidic and basic subunits with a Mw of 27-37 kDa (22). Gels prepared from 11S protein are firmer than those prepared from 7S protein, and the 11S content and 11S/7S ratio is positively correlated with sovbean curd firmness (23-25). However, other researchers found that the 7S globulin formed firmer soybean curd than the 11S globulin and did not detect a significant correlation between the 11S/7S ratio and soybean curd firmness (26).

Soybean curd quality depends not only on processing conditions but also on processing skills (4). It is well known that coagulation of soymilk is the critical step in the soybean curd-making process because it entails a complex interaction of soybean chemical composition, soymilk boiling temperature, soybean curd coagulant type and concentration, and coagulation temperature and time (27).

In Korea, soybean seeds are traditionally classified into small (<15 g/100 seeds), medium (15-24 g/100 seeds), or large (>24 g/100 seeds). Small-seeds are mainly used for making soybean sprouts, and medium- and large-seeds are used for making soybean curd, soymilk, soy sauce, and traditional Korean fermented soy pastes such as cheonggukjang and meju (28,29). Soybean breeders and processors generally believe that soybean varieties with large seeds and high protein content make better quality soybean curd and give greater yields; however, the relationships between quality, yield, and soybean seed size have not been scientifically validated.

To effectively select soybean lines that produce highquality soybean curd, it is necessary to understand the

College of Industrial Science, Kongju National University, Yesan, Chungnam 340-702, Korea

²College of Life and Environment Science, Konkuk University, Seoul 143-701, Korea

³National Institute of Agriculture Science and Technology, Rural Development Administration, Suwon, Gyeonggi 441-100, Korea

^{*}Corresponding author: Tel: +82-2-450-3730; Fax: +82-2-446-7856 E-mail: imcim@konkuk.ac.kr (Ill-Min Chung) Received July 20, 2007; accepted October 8, 2007

sources of genetic variation between lines and to identify easily quantifiable traits that affect the quality of soybean curd. It is important to characterize the proteins of soybean varieties because protein composition, especially that of the 7S and 11S globulins, is important for the preparation of foodstuffs from soybean.

Materials and Methods

Soybean seeds Soybean varieties were grown and harvested in 2005 at the National Institute of Crop Science, Suwon, Korea. Seeds were classed as small (<15 g/100 seeds), medium (15-24 g/100 seeds), or large (>24 g/100 seeds). Eighteen soybean varieties (6 small-seed, 6 medium-seed, and 6 large-seed varieties) of similar protein content ($40\pm1\%$) were selected (Table 1). Prior to analysis for chemical composition and isolation of 7S and 11S globulins, soybean seeds were milled to flour using a cyclone mill and defatted with hexane using an automatic fat extraction system (Gerhardt Soxtherm 2000; Hoffmannstr, Bad Salzuflen, Germany).

Isolation of 7S and 11S globulins The 7S and 11S globulin fractions were isolated from defatted soybean flour according to the method of Thanh and Shibasaki (22) with slight modifications. Defatted soybean flour is extracted with 0.03 M Tris buffer (pH 8.0) containing 0.01 M mercarptoethanol at room temperature for 1 hr. Whole soybean (WS) protein extract was adjusted to pH 6.4 with 2 N HCl. The 11S globulin was collected by centrifugation, and dialyzing the extract at pH 6.4 for 3 hr at 4°C. A

crude 7S globulin fraction was separated from the supernatant that resulted in this supernatant by adjusting the pH to 4.8. The prepared 7S globulin was washed with Tris buffer (pH 6.2) and then dispersed in the standard buffer by adding 2 N NaOH until the pH reached 7.6, followed by adjustment of the pH back to 6.2. The solution was kept at 4°C overnight. A trace of precipitate was removed by centrifugation at 15,000×g. The 7S and 11S globulins were then freeze-dried and stored at 4°C until analysis (Fig. 1).

Protein determination Soybean proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). β-Mercaptoethanol was added to the proteins as a reducing agent. Soybean flour (50 mg) was dispersed into 10 mL of 100 mM HEPES-sodium buffer (pH 8.0), vortexed twice for 10 sec at an interval of 5 min, and then heated to 98°C in a water bath for 5 min. The heated mixture was vortexed for 10 sec and then centrifuged for 20 min at 15,000×g. Immediately after centrifugation, the supernatant was applied to a Trisglycine SDS-PAGE gel with a loading volume of 2 mL. SDS-PAGE was performed using 4-20% Tris-glycine gradient gels (Tefco Co., Ltd., Tokyo, Japan). Electrophoresis was carried out at a constant voltage of 200 V in a vertical electrophoresis system (KE021; Koma, Seoul, Korea). The running buffer (pH 8.4) consisted of 25 mM Tris, 190 mM glycine, and 0.1% SDS. Gels were stained with See-band Forte protein staining solution (Gene Bio-Application Ltd., Kfar Hanagid, Israel) for 1 hr and destained twice with water/methanol/acetic acid solution

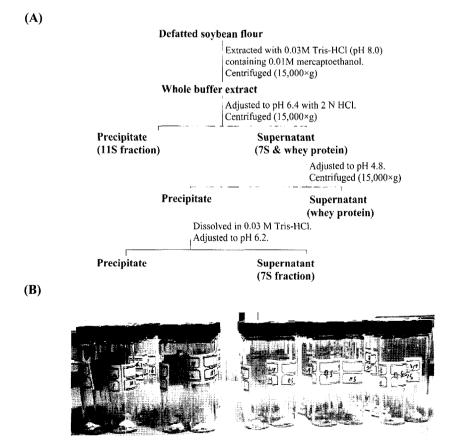


Fig. 1. Flow diagram for simultaneous isolation of soybean globulins (A) and freeze-dried 7S and 11S globulins (B).

(60/30/10, v/v) for 30 min. Protein Mw marker II (Tefco Co.) was used as a Mw marker and bromochlorophenol blue was used as a tracking dye.

Amino acid analysis About 0.3 g of each sample was weighed and mixed with 5 mL 6 N HCl. Hydrolysis was maintained for 24 hr at 110°C in test tubes that were flushed with nitrogen gas. Afterwards, the samples were diluted to 100 mL with Milli-Q water and filtered through 0.45 µm syringe filters (Waters, Millipore, Milford, MA, USA). One mL of each hydrolysate was placed in an autosampler bottle and injected into an amino acid autoanalyzer (Hitachi L-8800; Hitachi Ltd., Tokyo, Japan). Amino acid concentrations of samples were calculated with reference to an amino acid standard (Ajnomoto-Takara Co., Kyoto, Japan).

High performance liquid chromatography (HPLC) analysis HEPES sodium buffer (100 mM, pH 8.0) was used to extract total seed proteins and dissolve freeze-dried 11S and 7S globulins. Soybean seed proteins were separated with a reverse-phase C₁₈ column (4.6×250 mm, 5 μm, Phenomenex Inc., Torrance, CA, USA) and a Millenium³² HPLC workstation system (Waters). The column temperature was 25°C and the mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (eluent A) and 0.1% TFA in acetonitrile (eluent B). The flow rate was 1 mL/min and a 90 min gradient of 20-45% acetonitrile was followed by elution with 45% acetonitrile for 20 min. Spectra were monitored at 210 nm. The injection volume for all samples was 20 μL.

Preparation of soybean curd Soybean curd was made using an automated system (model DY-800; Daeryuk Engineering Co., Seoul, Korea) consisting of a grinder, soymilk tank, electronic steam boiler, coagulant tank, soybean curd trays, and air cylinder presses. Soybean (4 kg) were soaked in water for 9 hr and then ground with the addition of tap water. After adding antifoaming agent, raw soymilk was transferred to the steam cooker where it was heated to 98°C for 1 min before it was transferred to the mixing tank. When the soymilk had cooled to 87°C, 4 g/kg of MgCl₂ was added. The mixture was cooled and allowed to coagulate for 10 min. The coagulated curd was air pressed at 1 kg/cm² for 10 min to a thickness of about 10 cm. After pressing, the weight of the soybean curd was recorded and it was stored in water at 4°C overnight prior to texture

analysis. The yield of soybean curd was expressed as the weight (kg) of fresh soybean curd produced per kg of soybean.

Texture profile and color value The texture of the soybean curd was determined by texture profile analysis (TPA) at room temperature using a TA-XT2 texture analyzer (Stable Micro System Ltd., Godalming, England). Three soybean curd samples were cut from a central part of the soybean curd using a wire cutter in the vertical plane. The soybean curd samples were sliced into 10 mm thick sections for texture analysis. The sections were compressed twice to 30% of their original height and the TPA curves were used to calculate hardness, springiness, gumminess, cohesiveness, adhesiveness, and chewiness using the software provided with the texture analyzer. Color values of soybean and soybean curd were measured with a color and color difference meter (CR-200; Minolta Chromameter, Tokyo, Japan). The L, a*, b* color scale was used (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 title with the following Lab color values: L=97.38, $a^*=-0.02$, and $b^*=1.66$.

Statistical analysis All measurements were conducted in triplicate. Data were analyzed using SAS version 8.0 for Windows (Statistical Analysis Systems Institute Inc., Raleigh, NC, USA). Duncan's multiple range test was used to determine differences between soybean varieties. A probability of 0.05 or less was regarded as significant.

Results and Discussion

Electrophoresis profile of soybean protein The weights of globulin fractions and their ratios are shown in Table 1. The 7S and 11S globulins constituted 26.6-27.1 and 42.5-43.7%, respectively, of the total protein content of the various varieties. The 7S and 11S globulin contents did not differ between varieties. However, the ratio between 11S and 7S globulins was greater in large seed size varieties (LSSVs) than in small seed-size varieties (SSSVs) or medium-seed size varieties (MSSVs) but did not differ between MSSVs and SSSVs.

The SDS-PAGE profiles of whole seed proteins and the 7S and 11S globulin fractions are presented in Fig. 2. Some previous reports indicate that the composition of the subunits of the 7S and 11S globulins differs between

Table 1. Classification of soybean varieties according to seed size with mean values for 100-seed weight, 7S and 11S globulin content, and 11S/7S ratio

Small (<15 g)	'Anpyeongkong', 'Danbaegkong', 'Kwangankong', 'Sojinkong', 'Somyeongkong', 'Sowonkong'	11.2±2.0	26.6±2.1"	42.5±2.7"	1.45±0.15 ^{b1})
Medium (15-24 g)	'Daepungkong', 'Jangsukong', 'Jinpumkong', 'Mallikong', 'Muhankong', 'Sinpaldalkong2'	19.5±2.6	27.1±1.9 ^a	43.1±2.9 ^a	1.59±0.17 ^{uh}
Large (>24 g)	'Bokwangkong', 'Hwangkeumkong', 'Jangwonkong', 'Jangyeobkong', 'Sodamkong', 'Taekwangkong'	25.9±1.5	26.8±2.0°	43.7±2.5 ^a	1.63±0.11 ^a

¹⁾Column values with the same superscript letters are not significantly different at $p \le 0.05$.

138 S. -L. Kim et al.

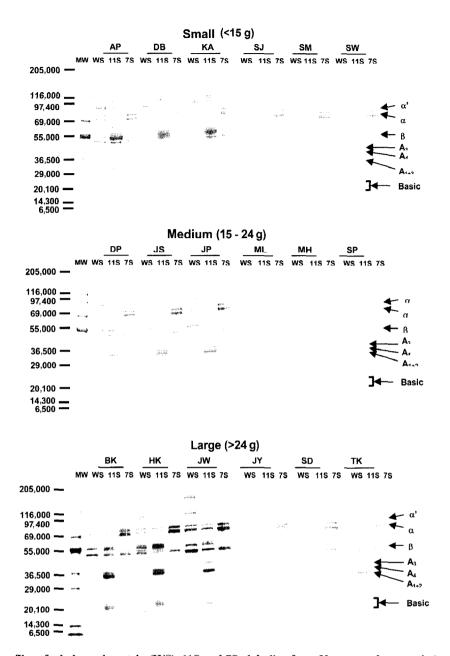


Fig. 2. SDS-PAGE profiles of whole seed protein (WS), 11S and 7S globulins from Korean soybean varieties. α' , α , and β indicate subunits of 7S globulin (β -conglycinin). A₃, A₄, A₁₊₂, and basic indicate polypeptides of 11S globulin (glycinin). Lane MW, Mw marker. AP, 'Anpyeongkong'; DB, 'Danbaegkong'; KA, 'Kwangankong'; SJ, 'Sojinkong'; SM, 'Somyeongkong'; SW, 'Sowonkong'; DP, 'Daepungkong'; JS, 'Jangsukong'; JF, 'Jinpumkong'; ML, 'Mallikong'; MH, 'Muhankong'; SP, 'Sinpaldalkong2'; BK, 'Bokwangkong'; HK, 'Hwangkeumkong'; JW, 'Jangwonkong'; JY, 'Jangyeobkong'; SD, 'Sodamkong'; TK, 'Taekwangkong'.

soybean varieties and that the 11S and 7S content of soybean varieties is a predictor of soybean curd texture because the 11S and 7S globulins have different gelling properties (6,25-27,30-33). However, the results of studies are not consistent because there is no standard method for isolating and quantifying soybean proteins or for processing soybean curd. Therefore, quantification of soybean protein fractions is an appropriate and efficient method of correlating soybean protein characteristics with soybean curd texture.

The electrophoresis patterns of 3 soybean varieties were determined and the polypeptide subunits of the 7S and 11S globulins separated well. The 7S globulin separated into subunits α' and α and the 11S globulin separated into acidic and basic subunits. The whole seed protein extracts

encompassed a wide range of molecular masses, but different varieties had similar electrophoretic characteristics. However, the density of the a subunit of the SSSVs was slightly less than that of the MSSVs and LSSVs.

Amino acid composition The amino acid compositions of whole soybean, 7S globulin, 11S globulin, and soybean curd are presented in Table 2. 11S Globulin contained more cysteine and methionine than total seed protein and 7S globulin. Marcone (34) reported that 11S globulin contains higher levels of essential amino acids such as tryptophan, methionine, lysine, histidine, phenylalanine, valine, and isoleucine than 7S globulin. Our study showed that the 11S globulin had higher methionine and phenylalanine levels

than 7S globulin, but lower valine and lysine levels than 7S globulin. The concentrations of isoleucine, aspatic acid, arginine, histidine, phenylalanine, and tyrosine were greatest in whole seed proteins; the concentrations of valine, serine, threonine, glutamic acid, and lysine were greatest in 7S globulins; and the concentrations of alanine, glycine, leucine, cysteine, methionine, and proline were greatest in 11S globulins.

The amino acid composition of the total seed proteins of the various sovbean varieties was similar. The level of glutamic acid was higher than that of other amino acids. The acidic monoamino monocarboxylic acids (glycine, alanine, valine, leucine, and isoleucine) were the most abundant and constituted 27.2% of total amino acids. The acidic monoamino dicarboxylic acids (glutamic and aspartic acid) constituted 26.9% of total amino acids, and the basic diamino monocarboxylic acids (lysine and arginine) constituted 15.6% of total amino acids. However, all soybean varieties had a low content of sulfur-containing amino acids. It is well known that soybean is deficient in sulfur-containing amino acids, and they are considered the first limiting amino acids of soybean (17,35,36). The nutritional and functional qualities of proteins are generally determined by their amino acid contents and nitrogen solubility (21). For this reason, several breeding strategies (including genetic engineering) are currently being undertaken to increase the sulfur-containing amino acid content of soybean.

It was reported that variations in the composition of

hydrophobic amino acids such as isoleucine, proline, phenylalanine, leucine, and valine may be the primary cause of differences in soybean protein functionality and physical characteristics (37). Creighton (38) reported that valine, alanine, leucine, and isoleucine have no reactive groups and therefore do not interact with water, but they interact with each other in the core of proteins and help to stabilize protein conformation. In our study, the 11S globulin fraction contained considerably more hydrophobic amino acids than the 7S fraction. Therefore, the textural properties of sovbean curd prepared from varieties with high 11S content or high 11S/7S ratios were expected to differ from those of sovbean curd prepared from varieties with high 7S content or low 11S/7S ratios. However, the level of hydrophobic amino acids did not differ much between varieties. The concentration of glutamic acid was greater than that of other amino acids in soybean curd and in soybean.

The acidic amino acids were the most abundant amino acids and constituted 29.3% of the amino acids in soybean curd. As shown in Fig. 3, the ratio of soybean curd to soybean amino acids varied. Levels of histidine, isoleucine, leucine, methionine, phenylalanine, tyrosine, and valine were increased by the soybean curd process. The soybean curd/soybean amino acid ratios of isoleucine, valine, and tyrosine were greater than those of leucine, histidine, methionine, and phenylalanine. The concentrations of 5 essential amino acids (isoleucine, leucine, methionine, phenylalanine, and valine) increased during the processing

Table 2. Amino acid composition of whole soybean, isolated globulins 7S and 11S, and soybean curd¹⁾

Amino acids ²⁾		7	S Globul	in	11	S Globu	lin	1 Whole soybean			Soybean curd		
Ammo	acius	S	M	L	S	M	L	S	М	L	S	М	L
	Ala	6.29	6.06	5.41	5.92	6.58	6.79	5.31	5.78	5.23	4.89	5.32	4.814)
MMA	Gly	10.18	9.50	8.41	9.92	11.43	12.02	6.41	6.19	5.65	5.69	5.69	5.11
	lle	1.10	1.90	2.21	2.68	2.60	2.42	3.89	3.60	4.31	5.45	5.04	6.03
	leu	2.05	3.67	3.75	12.31	11.90	11.54	7.38	6.82	8.12	7.75	7.12	8.53
	Val	5.02	5.63	5.65	4.93	5.02	4.99	4.19	4.39	4.41	5.51	5.68	5.31
Sum oj	^c MMA	24.64	26.76	25.43	35.76	37.53	37.76	27.18	<i>26.78</i>	27.72	29.29	28.85	29.79
OAA	Ser	6.37	6.07	6.22	6.15	6.13	6.15	5.60	5.71	5.64	5.15	5.25	5.18
UAA	Thr	9.43	9.09	8.90	8.23	8.63	8.43	4.55	5.40	4.82	4.41	5.11	4.68
Sum o	f OAA	15.8	15.16	15.12	14.38	14.76	14.58	10.15	11.11	10.46	9.56	10.36	9.86
SCAA	Cys	0.53	0.40	0.33	1.36	1.03	1.06	0.61	0.52	0.51	0.07	0.10	0.12
SCAA	Met	0.67	0.60	0.51	1.72	1.73	1.79	0.98	0.81	0.83	1.05	0.87	0.79
Sum of	SCAA	1.2	1.00	0.84	3.08	2.76	2.85	1.59	1.33	1.34	1.12	0.97	0.91
MDA	Glu	20.30	19.70	21.08	20.06	19.60	19.43	16.85	17.01	16.89	16.21	16.49	16.38
MDA	Asp	8.01	7.41	7.82	3.33	3.31	3.55	10.01	10.07	9.91	9.31	9.27	9.12
Sum oj	f MDA	28.31	27.11	28.9	23.39	22.91	22.98	26.86	27.08	26.8	25.52	25.76	25.50
DMA	Lys	9.42	9.10	9.06	6.41	5.72	5.16	5.92	6.68	6.51	5.86	5.41	6.34
DIVIA	Arg	9.32	8.77	9.05	4.81	4.62	4.92	9.90	9.11	8.62	9.70	8.91	8.41
Sum oj	F DMA	18.74	17.87	18.11	11.22	10.34	10.08	15.82	15.79	15.13	15.56	14.32	14.75
	His	2.85	2.90	2.82	2.71	2,91	2.87	2.90	3.01	2.82	2.96	3.02	2.88
AAA	Phe	1.03	1.20	1.11	1.69	1.77	1.72	5.12	4.80	5.39	5.23	5.38	6.01
$\Delta \Delta \Delta \Delta$	Pro	5.45	6.60	6.94	7.14	6.41	6.61	6.25	6.19	6.12	6.13	6.03	5.99
	Tyr	1.98	1.40	0.73	0.63	0.61	0.55	4.13	3.91	4.22	4.64	5.31	4.32
Sum o	f AAA	11.31	12.10	11.60	12.17	1 1.70	11.75	18.4	17.91	18.55	18.96	19.74	19.20

The value is the means of 3 replicates. S, Small size (<15 g); M, medium size (15-24 g); L, large size (>24 g) soybean varieties.

²⁾MMA (monoamino monocarboxylic acid), OAA (oxy amino acid), SCAA (sulfur containing amino acid), MDA (monoamino dicarboxylic acid), DMA (diamino monocarboxylic acid), AAA (aromatic amino acid).

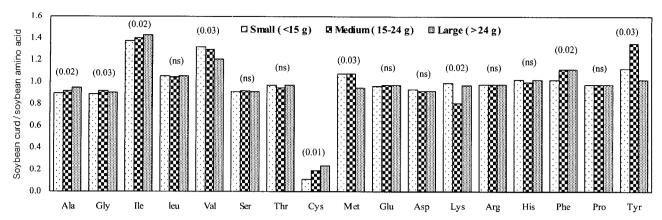


Fig. 3. The soybean curd/soybean amino acid ratios of small-, medium-, and large-seed varieties. Soybean curd/soybean amino acid ratios were calculated by as follows: Soybean curd/soybean amino acid ratio=(amino acid composition of soybean curd)/(amino acid composition of soybean seed). Parentheses indicated the values of LSD at p < 0.05.

of soybean for soybean curd production and those of alanine, glycine, serine, cysteine, aspartic acid, lysine, arginine, glutamic acid, threonine, and proline decreased during the processing of soybean for soybean curd production. The level of cysteine decreased to a greater extent during the processing of soybean for soybean curd production than that of other amino acids and the level of methionine changed little during the processing of soybean for soybean curd production. It is suggested that the amino acid composition of soybean is directly related to the composition and nutritional value of soybean curd.

HPLC analysis of total seed protein, 11S and 7S globulins As shown in Fig. 4, weak hydrophobic polypeptides were eluted before strong hydrophobic polypeptides (39). The chromatogram of 7S globulin was composed of nine peaks (peak 1 and 5-12), whereas that of the 11S globulin was composed of 10 peaks (peak 1-7, 9, 10, and 12). The relative contributions of the areas under the individual peaks to the total area under the peaks differed between varieties (Table 3). Peak 9, the main component of 11S globulin, was the most abundant peak in soybean seeds (28.2-35.1%), whereas the area of peak 8, the main component of 7S globulin, constituted 2.5-4.1% of the total area in soybean. Peak 2, 3, and 4 were detected only in the 11S globulin fractions and peak 8 was detected only in the 7S globulin fractions. Peak 1, 3, 9, and 12 were the main protein peaks, comprising 64.1% of the total area under the peaks of SSSVs, 65.6% of the total area under the peaks of MSSVs, and 70.5% of the total area under the peaks of LSSVs. Other peaks such as peak 5-7, peak 10, and peak 11 were minor contributors to the 11S and 7S fractions and their contributions varied between varieties. These results show that the composition of soybean seed proteins varies according to seed size.

Yield and quality of soybean curd The yields and texture profiles of soybean curd are presented in Table 4. Soybean seeds differ phenotypically in respect of coloration, size, shape, luster, and permeability but the relationships between differences in protein composition between varieties and soybean curd yields and quality are unclear. Soybean curd yield and quality depend not only on processing parameters

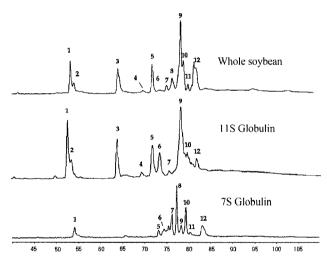


Fig. 4. HPLC chromatogram profiles of whole seed proteins, 11S and 7S globulins separated from the Korean, *Glycine max* cv. Daepungkong soybean variety. The chromatogram was obtained using a Jupiter C_{18} 4.6×250 mm (5 mm) column, 0.1% TFA in water (eluent A) and 0.1% TFA in acetonitrile (eluent B). The flow rate was 1 mL/min and a 90 min gradient of 20-45% acetonitrile was applied with monitoring at 210 nm.

but also on processing skills because the processing of soybean for soybean curd production involves the complex interaction of many parameters (4,23,40). Because of the rapid development of the world soybean curd market, reproducible research results on a mass production scale are necessary (4). Murphy *et al.* (41) suggested that soybean curd preparation should be conducted on a mass production scale when evaluating soybean varieties for soybean curd production. Therefore, in our study, we reduced variation by using an automatic soybean curd processing system.

The yields of soybean curd prepared from MSSVs were significantly greater than those of SSSVs and LSSVs but there was no significant difference between the yields of SSSVs and LSSVs. Moisture content was correlated with yield of soybean curd (r=0.953, p<0.01). The moisture content of soybean curd affects the yield of soybean curd because soybean curd is highly hydrated. The yield of

Table 3. Comparison of the relative contributions of individual protein peaks to the total area under the peaks of soybean varieties of different seed sizes¹⁾

	Soybean seed size									
Peaks	Small (<15 g)	Medium (15-24 g)	Large (>24 g)	Remarks						
1	13.1±1.7	9.7±0.3	11.4±0.8	11S, 7S						
2	5.4 ± 0.8	5.7 ± 0.7	4.5 ± 0.6	118						
3	10.6 ± 0.6	9.4 ± 1.2	11.2 ± 0.5	11S						
4	2.1 ± 0.2	2.2 ± 0.1	1.8 ± 0.1	118						
5	8.2 ± 1.1	7.6 ± 0.6	8.7 ± 0.6	118, 78						
6	1.8 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	11S, 7S						
7	3.1 ± 0.5	3.5 ± 0.4	4.5 ± 0.3	11S, 7S						
8	4.1 ± 0.5	3.6 ± 0.6	2.5 ± 0.1	7S						
9	28.2 ± 2.6	33.2 ± 1.9	35.1 ± 2.2	11S, 7S						
10	8.3 ± 0.4	6.8 ± 0.5	3.9 ± 0.3	11S, 7S						
11	2.9 ± 0.3	3.7 ± 0.5	2.0 ± 0.5	11S, 7S						
12	12.2 ± 1.1	13.3 ± 1.6	12.8 ± 1.3	11S, 7S						

¹⁾Means±SD, n=3.

soybean curd per unit of raw soybean is an important aspect of the evaluation of soybean varieties for suitability as soybean curd cultivars.

Although soybean curd can be made from soybean of various sizes, most soybean curd producers prefer large soybean with yellow hila and high protein content because they maintain that they consistently produce high-quality soybean curd. However, this claim has not been subjected to scientific validation. Soybean curd prepared from soybean of different seed sizes differed in hardness, gumminess, and chewiness, but not in springiness or cohesiveness. Soybean curd prepared from LSSVs was significantly harder than soybean curd prepared from SSSVs or MSSVs. Texture determines the quality and acceptance of soybean curd. Wang and Chang (42) reported that the hardness of sovbean curd was significantly affected by sovbean variety and found that it had a negative correlation with moisture content. However, in our study, hardness was positively (but not significantly) correlated with moisture content (r=0.405, p<0.05).

Figure 5 and 6 depict the relationships between 11S/7S ratios and yields and hardnesses of soybean curd, respectively. There was a significant quadratic relationship between 11S/7S globulin ratio and soybean curd yield (y=-1.9114x² +6.0426x-2.4015, R^2 =0.5032, p<0.05) and a significant linear relationship between 11S/7S globulin ratio and soybean curd hardness (y=63.207x+153.76, R^2 =0.7012, p<0.01). These results show that the 11S/7S globulin ratio has an important effect on the yield and texture of soybean

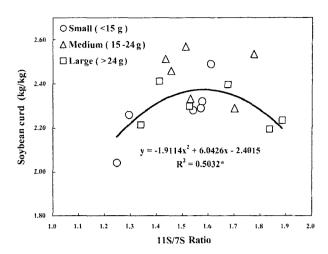


Fig. 5. Relationship between 11S/7S globulin ratio and the yield of soybean curd. *p<0.05.

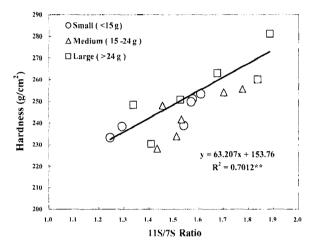


Fig. 6. Relationship between 11S/7S globulin ratio and the hardness of soybean curd. **p<0.01.

curd. The 11S/7S ratios of the MSSVs and LSSVs were more favorable for soybean curd yields than those of the SSSVs, but the hardness of soybean curd made from them was significantly greater than that of soybean curd made from the SSSVs. These results show that soybean variety significantly influences soybean curd yield and quality and that the effect of the 11S/7S ratio on soybean curd yield and hardness depends on the variety. These results are in agreement with those of several other studies (6,25,43).

Color values for soybean seeds and soybean curd are shown in Table 5. The L* values of soybean seeds were 51.04 for the SSSVs, 52.52 for the MSSVs, and 53.15 for

Table 4. Yields, moisture content, and texture profiles of soybean curd

				Texture parameters						
Seed size	Soybean curd yield (kg/kg)	Moisture content (%)	Hardness (N/m)	Springiness (mm)	Gumminess (N/m)	Cohesiveness (N/m)	Chewiness (N/m)			
Small (<15 g)	2.28	82.1"	244.2ª	0.92^{a}	129.5 ^a	0.47 ^a	119.6 ^{a1)}			
Medium (15-24 g)	2.46 ^b	83.4 ^b	246.7 ^a	0.92^{a}	106.1 ^b	0.47^{a}	97.3 ^h			
Large (>24 g)	2.31 ^a	82.3 ^a	257.3 ^h	0.94^{a}	128.1 ^a	0.46^{a}	120.4 ^a			

¹⁾Column values with the same superscript letters are not significantly different at $p \le 0.05$.

142 S. -L. Kim et al.

Table 5. Color values of soybean seed and soybean curd¹⁾

Condition	Soy	bean s	eed	Soybean curd			
Seed size	L	a	b	L	a	b	
Small (<15 g)	51.04 ^a	5.48 ^a	19.82ª	81.07 ^a	0.08^{a}	12.71 ^{a2)}	
Medium (15-24 g)	52.52 ^a	5.51 ^a	19.49^{a}	81.61 ^{ab}	0.06^{a}	11.97^{ab}	
Large (>24 g)	53.15^{b}	5.48^{a}	19.20^{a}	82.06^{b}	-0.08^a	11.58^{b}	

¹⁾L, Lightness; a, redness; b, yellowness.

the LSSVs. The L values (lightness) of LSSVs were significantly greater than those of the other varieties, but a* and b* values did not differ among cultivars. L* values were increased by the processing of soybean for soybean curd production, and those of soybean curd made from LSSVs were significantly greater than those of soybean curd made from SSSVs or MSSVs. Although the a* and b* values were decreased by the processing of soybean for soybean curd production, there were no differences between cultivars. These results indicate that the color value of soybean seeds affects the color value of soybean curd, especially the L* value. LSSVs produced lighter colored soybean curd. These results show that 11S/7S ratios and color values of soybean seeds can be used to consistently predict the yield, hardness, and color values of soybean curd. However, further investigations are required because the SSSVs are mainly used for soybean sprouts at present.

Acknowledgments

This research was supported by a Technology Development program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

References

- Shen, CF, de Man L, Buzzel RI, de Man JM. Yield and quality of tofu as affected by soybean and soymilk characteristics: Glucono delta lactone coagulant. J. Food Sci. 56: 109-112 (1991)
- Sun N, Breene WM. Calcium sulphate concentration influence on yield and quality of *tofu* from five soybean varieties. J. Food Sci. 56: 1604-1607 (1991)
- Rajni M, Dianne TT, Perry KWN. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chem. 82: 265-273 (2003)
- Cai TD, Chang KC. Characteristics of production-scale tofu as affected by soymilk coagulation method: Propeller blade size, mixing time, and coagulant concentration. Food Res. Int. 31: 289-295 (1998)
- Sexton PJ, Naeve SL, Paek NC, Shibles R. Sulfur availability, cotyledon nitrogen: Sulfur ratio, and relative abundance of seed storage proteins of soybean. Crop Sci. 38: 983-986 (1998)
- Cai T, Chang KC. Processing effect on soybean storage proteins and their relationship with *tofu* quality. J. Agr. Food Chem. 47: 720-727 (1999)
- 7. Wang HL, Swain EW, Kwolik WF. Effect of soybean varieties on the yield and quality of *tofu*. Cereal Chem. 60: 245-248 (1983)
- Schaefer MJ, Love J. Relationships between soybean compounds and tofu texture. J. Food Quality 15: 53-66 (1992)
- Beddows CG, Wong J. Optimization of yield and properties of silken tofu from soybeans. II. Heat processing. Int. J. Food Sci. Tech. 22: 23-27 (1987)
- Kim Y, Wicker L. Soybean cultivars impact quality and function of soymilk and tofu. J. Sci. Food Agr. 85: 2514-2518 (2005)

- 11. Shih MC, Hou HJ, Chang KC. Process optimization for soft *tofu*. J. Food Sci. 62: 833-837 (1997)
- Lee JY, Choe EO. Effects of various food materials on texture characteristics and on freeze-drying of whole-tofu (Chundubu). Food Sci. Biotechnol. 12: 285-289 (2003)
- Mujoo R, Dianne TT, Perry KW Ng. Evaluation of soybean varieties for soymilk and tofu production potential using laboratorydeveloped procedures. Food Sci. Biotechnol. 11: 470-476 (2002)
- Sun N, Breene WN. Calcium sulfate concentration influence on yield and quality of *tofu* from five soybean varieties. J. Food Sci. 56: 1604-1607 (1991)
- Shin MC, Yang KT, Kuo SJ. Quality and antioxidative activity of black soybean tofu as affected by bean cultivar. Food Sci. 67: 480-484 (2002)
- Utsumi S. Plant food protein engineering. Vol. 36, pp. 89-208. In: Advanced Food Nutrition Research. Kinsella JE (ed). Academic Press, San Diego, CA, USA (1992)
- 17. Peter JS, Paek NC, Shibles RM. Effects of nitrogen source and timing of sulfur deficiency on seed yield and expression of 11S and 7S seed storage proteins of soybean. Field Crop Res. 59: 1-8 (1998)
- Paek NC, Imsande J, Shoemaker RC, Shibles R. Nutritional control of soybean seed storage protein. Crop Sci. 37: 498-503 (1997)
- Adachi M, Chunying H, Utsumi S. Effects of designed sulfhydryl groups and disulfide bonds into soybean proglycinin on its structural stability and heat induced gelation. J. Agr. Food Chem. 52: 5717-5723 (2004)
- Keun EH, Lee SI, Oh SS. Effect of enzymatic hydrolysis of 7S globulin, a soybean protein, on its allergenicity and identification of its allergenic hydrolyzed fragments using SDS-PAGE. Food Sci. Biotechnol. 15: 128-132 (2006)
- Kinsella JE. Functional properties of soy proteins. J. Am. Oil Chem. Soc. 56: 242-258 (1979)
- Thanh VH, Shibasaki K. Major proteins of soybean seeds. A straight forward fraction and their characterization. J. Agr. Food Chem. 24: 1117-1121 (1976)
- Delia AS, Wagner JR. Comparative study of foaming properties of whey and isolate soybean protein. Food Res. Int. 35: 721-729 (2002)
- Kang LJ, Matsumura Y, Mori T. Characterization of texture and mechanical properties of heat-induced soy protein gels. J. Am. Oil Chem. Soc. 68: 339-345 (1991)
- Saio K, Kamiya M, Watanabe T. Food processing characteristics of soybean 11S and 7S. Part I. Effect of difference of protein components among soybean varieties on formation of tofu-gel. Agric. Biol. Chem. 33: 1301-1308 (1969)
- Taira H. Quality of soybeans for processed foods in Japan. J. Agr. Res. Quality 24: 224-230 (1990)
- Cai TD, Chang MC, Shih HJ, Hou HJ, Ji M. Comparison of bench and production scale methods for making soymilk and *tofu* from 13 soybean varieties. Food Res. Int. 30: 659-668 (1997)
- Kang SE, Rhee JH, Park C, Sung MH, Lee I. Distribution of poly-γ-glutamate (γ-PGA) producers in Korean fermented foods, cheonggukjang, doenjang, and kochujang. Food Sci. Biotechnol. 14: 704-708 (2005)
- Jang EK, Seo JH, Park SC, Yoo BS, Lee SP. Characterization of mucilage produced from the soild-state fermentation of soybean grit by *Bacillus firmus*. Food Sci. Biotechnol. 16: 722-727 (2007)
- Utsumi S, Kinsella JE. Structure-function relationship in food proteins: Subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate proteins. J. Agr. Food Chem. 33: 297-303 (1985)
- Tezuka M, Taira H, Igarashi Y, Yagasaki K, Ono T. Properties of tofus and soymilks prepared from soybeans having different subunits of glycinin. J. Agr. Food Chem. 48: 1111-1117 (2000)
- Kohyama K, Sano Y, Doi E. Rheological characteristics and gelation mechanism of tofu. J. Agr. Food Chem. 43: 1808-1812 (1995)
- Yagasaki K, Kousaka F, Kitamura K. Potential improvement of soymilk gelation by using soybeans with modified protein subunit composition. Breeding Sci. 50: 101-107 (2000)
- Marcone MF. Possible nutritional implications of varietal influence on the 7S/11S seed globulin ratios in amaranth. Plant Food Hum. Nutr. 54: 375-380 (1999)
- 35. Coates JB, Mederiros JB, Thanh VH, Nielsen NC. Characterization

²⁾Column values with the same superscript letters are not significantly different at $p \le 0.05$.

- of the subunits of β -conglycinin. Arch. Biochem. Biophys. 243: 184-194 (1985)
- Fukushima D. Recent progress of soybean protein foods: Chemistry, technology, and nutrition. Food Res. Int. 7: 323-351 (1991)
- Riblett AL, Herald TJ, Schmidt KA, Tilley KA. Characterization of β-conglycinin and glycinin soy protein fractions from four selected soybean genotypes. J. Agr. Food Chem. 49: 4983-4989 (2001)
- Creighton TE. Proteins: Structures and Molecular Properties. 2nd ed. WH Freeman and Company, New York, NY, USA. p.7 (1993)
- 39. Mujoo R, Trinh DT, Perry KW. Characterization of storage proteins in different soybean varieties and their relationship to *tofu* yield and texture. Food Chem. 82: 265-273 (2003)
- Kohyama K, Nishinari K. Rheological studies on the gelation process of soybean 7S and 11S proteins in the presence of gluconodelta-lactone. J. Agr. Food Chem. 41: 8-14 (1993)
- Murphy PA, Chen HP, Hauck CC, Wilson LA. Soybean protein composition and *tofu* quality. Food Technol.-Chicago 51: 86-88, 110 (1997)
- Wang CCR, Chang SKC. Physicochemical properties and tofu quality of soybean cultivar 'Proto'. J. Agr. Food Chem. 43: 3029-3034 (1995)
- 43. Tay SL, Xu GQ, Perera CO. Aggregation profile of 11S, 7S, and 2S coagulated with GDL. Food Chem. 91: 457-462 (2005)