Processing Effect on Soybean Storage Proteins and Their Relationship with Tofu Quality

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Tofu was prepared from 13 soybean varieties according to three different methods (bench, pilot, and production methods). Different soybean varieties showed significant differences in storage protein composition (glycinin and β-conglycinin). The β-conglycinin (7S) and glycinin (11S) contents were 7.3−9.9 and 14.1−22.9% on the dry matter basis, respectively. The 11S/7S protein ratio varied from 1.64 to 2.51 among the varieties. Glycinin content and 11S/7S protein ratio of the 13 varieties did not change significantly from soy milk to tofu for the production and pilot methods. Soybean 11S/7S protein ratio positively correlated with the 11S/7S ratio of soy milk and tofu ($0.57 \leq r \leq 0.83$, $p \leq 0.01$). The correlation coefficient depended on the processing method. Processing method affected 7S and 11S protein contents of tofu and their contribution to tofu hardness, yield, and sensory quality. This may explain in part the contradictory findings of the relationships between storage proteins and tofu quality because processing methods differed in various studies.

Keywords: *Tofu quality; soybean protein; processing method*

INTRODUCTION

Tofu is a nutritional, protein gellike soybean product. It is prepared traditionally by coagulating hot soy milk with a coagulant, followed by molding and pressing to form the desirable texture.

Tofu-making is a complex interaction of many factors. These factors include soybean intrinsic characteristics (chemical composition) and processing conditions. Some intrinsic factors affecting tofu yield and quality are not fully understood. It is not uncommon to find conflicting results among studies. Shen et al. (1991) and Schaefer and Love (1992) reported that soybean varieties high in protein content produced tofu with a high yield and firmer texture. However, Wang et al. (1983) and Murphy et al. (1997) found no correlations between soybean protein content and tofu yield. Moreover, Murphy et al. (1997) reported that soybean protein content correlated negatively with tofu hardness and fracturability.

Glycinin and β-conglycinin are the major storage proteins (globulins) in soybeans and soy foods. Glycinin corresponds to the 11S protein, and β-conglycinin is the principal component of the 7S protein (Yamauchi et al., 1991). Their content and ratio vary with soybean varieties and environment (Saio et al., 1969; Hughes and Murphy, 1983; Murphy and Resurreccion, 1984). Because of the different gelation properties of the soybean storage proteins, many researchers have attempted to correlate the proteins with tofu quality, but the results differ greatly. Several studies found that glycinin (11S) and β-conglycinin (7S) had some relationships with tofu texture (Saio et al., 1969; Saio, 1979; Kang et al., 1991; Murphy et al., 1997). The 11S content and 11S/7S protein ratio were reported to correlate positively with tofu gel hardness on the basis of purified soy protein systems (Saio et al., 1969; Saio, 1979; Kang et al., 1991). On the contrary, Utsumi and Kinsella (1985) reported that the 7S protein formed harder gels than the 11S protein. Murphy et al. (1997) made tofu from soybeans and reported a negative relationship between tofu hardness and the 11S/7S protein ratio of food soybeans. Skurray et al. (1980) and Taira (1990) found little correlation between the 11S/7S protein ratio and tofu quality. Thus, the contribution of soybean storage proteins to tofu texture is controversial and needs further investigation.

Processing conditions affect tofu quality (Watanabe et al., 1964; Saio, 1979; Wang and Hesseltine, 1982; Beddows and Wong, 1987a−c; Gandhi and Bourne, 1988; Shen et al., 1991; Sun and Breene, 1991; Cai and Chang, 1997; Hou et al., 1997; Shih et al., 1997). However, how soybean protein compositions change during processing and their relationships to tofu yield and quality have not been reported. Therefore, the objectives of this study were (1) to determine the composition of the major soybean proteins (glycinin and β-conglycinin) in 13 soybean varieties and their products and (2) to investigate relationships between soybean proteins and tofu quality and yield on the basis of three processing methods.

MATERIALS AND METHODS

Materials. Soybeans of 13 cultivars (Proto, T5, Corsoy 79, Vinton, Kato, Hardin, Sturdy, SBB100ND, SBB100SD, Stine 2220, Stine 1590, Stine 0380, and Stine 1570) were obtained from Sinner Brothers and Bresnahan Co. (Casselton, ND). Food grade coagulant (USG Terra Alba $CaSO_4 \cdot 2H_2O$) and modified nigari (a mixture of calcium sulfate and natural nigari) were provided by United States Gypsum Co. (Chicago, IL) and Taiwan Salt Workers (Tainan, Taiwan), respectively. Antifoaming agent (containing 89.5% glycerol fatty acid ester, 8% lecithin, 2% $MgCO_3$, and 0.5% silicon resin) was obtained from Koah Co. (Wakayama, Japan).

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Tofu Processing. Three processing methods (designated bench, pilot, and production methods) with various quantities of soybean used and processing conditions were described as follows.

Bench Method. Soybeans (139 g) were soaked in tap water at 20−22 °C for 8 h to obtain ∼2.1 times their original weight. Soaked beans were ground with 625 mL of water for 4 min at high speed in a blender (model 908-2, Hamilton Beach Co., Washington, NC). The slurry was filtered with a muslin cloth and squeezed manually to obtain filtrate (soy milk). The residue was mixed with water in the amount of weight difference between 765 g and soaked bean weight. The slurry was filtered again to obtain a total of 1000 mL of soy milk. Therefore, the ratio of the total amount of water to raw bean for extracting soy milk was 8:1. After a small amount of the antifoaming agent had been added (∼0.2% of bean weight), soy milk was cooked to boiling in a stainless steel pot (18 cm diameter × 11.5 cm height) with manual stirring and maintained at 92−96 °C for 5 min. When the cooked milk was cooled to 87 °C, calcium sulfate ($CaSO_4 \cdot 2H_2O$ at 2% of raw bean weight, equivalent to 0.29% of cooked soy milk) suspended in 20 mL of water was added. The mixture was stirred with a Caframo stirrer (model RZR1, Caframo Ltd., Wiarton, ON, Canada) equipped with a stainless steel paddle (14 cm length × 1.5 cm width) at 150 rpm for ∼14 s and was poured into a wooden mold (12.5 cm length × 12.5 cm width × 5.5 cm height) lined with a plastic film and a cloth. After the mixture had stood for 8 min, the plastic film was removed and the cloth was folded over the top of the bean curd. The bean curd was pressed sequentially by placing weights of 7.5 lb for 10 min, 15 lb for 10 min, and 22.5 lb for 15 min, which were equivalent to pressures of 21.8, 43.6, and 65.4 g/cm^2 of tofu, respectively. After pressing and weighing, tofu was sampled for chemical analysis, and the rest was stored in water at 4 °C prior to textural analysis and sensory evaluation.

Pilot Method. Tofu was produced using the method of Shih et al. (1997) developed in our laboratory. To facilitate comparisons with the other two processing methods, we describe the procedures in more detail as follows.

Soybeans (900 g) were soaked in tap water at 18−20 °C for 8 h to obtain 2.0−2.1 times their initial weight. Hydrated beans were ground with 5 L of tap water in a high-speed grinder (Chan Shen Machinery Co., Taoyuan, Taiwan), which was equipped with an automatic centrifugal filter to separate soy milk from residue. The soy milk solid content was measured as °Brix on an Auto Abbe refractometer (model 10500, Buffalo, NY) and adjusted to 12 °Brix with tap water. After antifoaming agent had been added, 4.5 L of 12 °Brix raw soy milk was heated to 95 °C on an electric stove with manual stirring and kept hot for 5 min. Soy milk was then cooled to 87 °C with mechanical stirring and mixed with 135 mL of coagulant suspension containing 13.5 g of modified nigari (∼0.32% of cooked soy milk). The soy milk−coagulant mixture was stirred at 285 rpm for 10 s by the above-mentioned Caframo stirrer equipped with a stainless steel paddle (7 cm length × 7 cm width). The mixture was then poured immediately into a cloth-lined wooden mold (25 cm length × 25 cm width × 7 cm height, covered with a sheet of plastic film and a cloth) and allowed to coagulate for 10 min. After coagulation, the plastic film was removed and the cloth was folded over the top of the bean curd. The curd was pressed sequentially at 21.8 g/cm^2 for 10 min, 43.6 g/cm^2 for 10 min, and 65.4 g/cm^2 for 30 min. The weight of freshly formed tofu was recorded after pressing. Tofu was stored in water at 4 °C overnight before analysis.

Production Method. Soybeans were processed into tofu using a commercial automated system (Ta Ti Hsing Machinery Co., Taoyuan, Taiwan) according to the method of Cai et al. (1997). The system was composed of two autosoaking tanks, a soaked bean conveyer, a bean feeder, an autogrinder/milk separator, a raw milk tank, an antifoaming feeder, a direct steam injection cooker, a cooling soy milk−coagulant mixing tank, a filler, a coagulant tank, four tofu trays (40 cm length × 40 cm width × 4.5 cm height, each tray), and two air cylinder presses. The automated machine was also equipped with a computer that can be programmed to control various steps of the processing.

Soybeans (6500 g) were soaked in tap water at 20−22 °C for 9 h to obtain ∼2.2 times their original weight. Soaked beans were ground in the grinder with tap water at the flow rate of 2.5 L/min. This corresponded to a water to raw bean ratio of about 6:1 for extracting solids from soybeans into raw soy milk. After the antifoaming agent had been added, raw milk was transported by a live steam (345 kPa) into the cooker. The soy milk was heated to 98 °C with steam injection and maintained for 1 min before delivery to the mixing tank. When cooled to 87 °C, the cooked milk was mixed at 420 rpm for 10 s with 804 mL of a 13% coagulant suspension containing 115 g of $CaSO_4 \cdot 2H_2O$ (∼0.33% of cooked soy milk). The mixture was then filled onto four tofu trays (each covered with a plastic film and a cloth) and allowed to stand to coagulate for 10 min. The bean curd was then pressed sequentially with the air press gage readings of 1 kg/cm^2 for 10 min, 2 kg/cm^2 for 10 min, and 3 kg/cm^2 for 15 min, which corresponded to 50.7, 101.4, and 152.1 g/cm^2 of tofu, respectively. The press operation removed whey and brought tofu to approximately the height of the tofu tray (4.5 cm). After pressing, the fresh tofu weight was recorded. Tofu was stored in water at 4 °C overnight prior to textural analysis and sensory evaluation.

Tofu Yield and Textural Analysis. Yield of tofu was calculated as the weight (grams) of tofu per 100 g of soybeans used to make tofu. The hardness of tofu was measured using an Instron Universal testing machine (model 1011, Instron Co., Canton, MA). Tofu was cut into cylindrical tofu cakes (5 cm diameter × 1.5 cm height) with a stainless steel cylindrical cutter. Each mold of bench-method tofu was cut into four pieces, three of which were tested for hardness and one was evaluated for sensory quality. Each mold of pilot-method tofu was cut into 16 pieces, and 4 central pieces were used for hardness measurement. For the production method, a sample from the central part of the tofu on each of four trays was taken for texture evaluation. The samples of tofu made by the pilot and production methods were thick enough to cut into top, middle, and bottom portions. Top and middle pieces of tofu were used for textural analysis. A full loading weight of 5 kg was used with the cross-head control at 20 cm/min. The recording chart speed was 20 cm/min. The sample was compressed from 15 to 3.75 mm (75% deformation) with a cylindrical plunger (5 cm in diameter). Hardness was determined as the peak force at 75% deformation using the texture profile analysis curve (Bourne, 1978).

Sensory Evaluation. The sensory quality of tofu was evaluated by visual examination and finger touching using an intensity scale method (Stone and Sidel, 1993). Samples for sensory evaluation were stored in water at 4 °C and warmed to room temperature before evaluation. A panel consisting of five people who were lifelong tofu consumers and familiar with tofu products was selected. The panelists were trained with commercial soft and firm tofu (Hinoichi Premium, House Foods American Corp., Los Angeles, CA) and silken soft and silken extra firm tofu (Mori-nu, Morinaga Nutritional Foods Inc., Torrance, CA) until the sensory score was consistent. Tofu was cut into cubic samples (∼6 cm length × 6 cm width × 4.5 cm height) and placed on a plastic plate with a random number. Replicated treatment samples were evaluated on different days. An intensity score of 7 indicated the highest sensory quality, whereas a score of 1 indicated the lowest quality. Tofu sensory quality was evaluated on the basis of tofu appearance, color, smoothness, and firmness.

Protein Extraction and Analysis. Crude protein of samples was determined according to AOAC Method 955.04 (1990). The extraction procedures of Nash et al. (1974) and Terrill et al. (1992) were modified as follows. Soybean flour and freeze-dried soy milk and tofu (1 g) were defatted in 30 mL of acetone with a mechanical stirrer for 1 h. The content was centrifuged and the pallet was dried in a vacuum oven at <40 °C to dryness. Sodium dodecyl sulfate (SDS, 1%) containing 50 mM 2-mercaptoethanol was added and homogenized in ice water at 9500 rpm for 30 s and at 20500 rpm for 30 s

Table 1. Processing Effect on 7S and 11S Proteins of 13 Soybean Varieties (Production Method)[a]

	7S (%, dry wt basis)			11S (%, dry wt basis)		
variety	soybean	soy milk	tofu	soybean	soy milk	tofu
Proto	9.9 a(b)	12.1 ab(a)	13.1 a(a)	22.9 a(b)	27.7 a(a)	30.5 a(a)
T5	9.8 a(b)	11.5 abcd(a)	11.5 bcde(a)	21.4 ab(b)	25.1 abc(a)	26.8 bc(a)
Corsoy 79	8.6 bc(c)	10.6 def(b)	12.7 ab(a)	16.0 de(b)	20.2 ef(a)	21.2 fg(a)
Vinton	9.9 a(b)	11.3 abcd(a)	12.2 abc(a)	20.2 bc(b)	24.6 bcd(a)	25.8 c(a)
Kato	8.6 bc(b)	11.8 abc(a)	12.1 abcd(a)	18.5 cd(b)	22.8 cde(a)	25.1 cd(a)
Hardin	9.2 ab(b)	11.2 abcde(a)	11.1 cde(a)	15.1 e(b)	18.9 f(a)	19.5 g(a)
Sturdy	8.8 b(b)	12.2 a(a)	11.7 abcde(a)	14.5 e(b)	18.7 f(a)	20.8 fg(a)
SBB100ND	7.3 e(c)	9.8 f(a)	9.2 f(b)	16.8 de(b)	22.2 de(a)	22.0 ef(a)
SBB100SD	7.4 de(b)	10.5 def(a)	11.2 bcde(a)	18.1 cd(b)	25.8 ab(a)	28.0 b(a)
Stine 2220	8.7 bc(b)	10.9 cdef(a)	10.5 def(a)	15.2 e(b)	18.1 f(ab)	20.5 fg(a)
Stine 1590	7.8 cde(b)	11.0 bcde(a)	11.4 bcde(a)	15.9 de(b)	19.2 f(a)	21.0 fg(a)
Stine 0380	8.5 bc(c)	11.1 bcde(b)	12.8 ab(a)	16.8 de(c)	20.5 ef(b)	23.7 de(a)
Stine 1570	8.3 bcd(b)	10.0 ef(a)	10.3 ef(a)	14.1 e(b)	20.1 ef(a)	20.4 fg(a)

[a] Means of four treatment replications. Means within a column followed by different letters are significantly different ($p \leq 0.05$). Means within a row followed by different letters in parentheses are significantly different ($p \leq 0.05$).

with a Tekmar tissumizer (model T25-S1, Tekmar, Cincinnati, OH). The mixture was sonicated at 60 °C for 90 min to extract the proteins. The extract was then centrifuged at 17600*g* for 30 min. The protein content of the supernatant was analyzed according to the method of Bradford (1976).

The protein extract was diluted to 2 mg/mL with distilled water, and 0.5 mL of diluted extract was mixed with 0.5 mL of SDS sample buffer containing 10% 2-mercaptoethanol (Cai et al., 1996). After boiling for 2 min, 40 μL of the cooled solution containing equivalent to 40 μg of protein was loaded onto a gradient gel containing 8−16% polyacrylamide. Electrophoresis was performed in a BioRad Protean II chamber at 100 V for 8 h by SDS−polyacrylamide gel electrophoresis (SDS−PAGE) based on the procedure of Laemmli (1970). At the end of electrophoresis, gels were stained with Coomassie Brilliant Blue R-250. For the quantification of glycinin and β-conglycinin, gels were scanned and analyzed by a Bio-Rad imaging densitometer (model GS-670) equipped with Molecular Analyst/PC Image Analysis software (version 3.11). The determination of glycinin and β-conglycinin and their relative composition was based on the mobility and total area of their subunits (Fontes et al., 1984; Wang and Chang, 1995). The relative amount of glycinin and β-conglycinin was multiplied by percentage of crude protein to obtain 11S and 7S protein contents, respectively. Electrophoresis of the storage proteins in 13 soybean varieties for each processing method and product was performed on the same gel to investigate varietal effect. The protein patterns of soybean, soy milk, and tofu for each variety on the same gel were analyzed to evaluate processing effect.

Data Analysis. Data were analyzed by analysis of variance using the general linear model (SAS, 1990). Duncan's multiple-range test was used to determine difference in processing methods and soybean varieties. Pearson's correlation coefficients were used to determine the degree and significance of association among the various quality attributes. All experiments were performed in four replications.

RESULTS AND DISCUSSION

Major Storage Proteins in Soybeans, Soy Milk, and Tofu. Glycinin and β-conglycinin are the major storage proteins in soybeans. Different soybean varieties had different storage protein compositions (Table 1). SDS−PAGE separated β-conglycinin (α', α, and β subunits) and glycinin (acidic and basic subunits) in soybean and tofu (Figure 1). As observed by band area and density of protein subunits on the gels, cultivars Sturdy (lane 7) and Stine 2220 (lane 10) had the highest β subunit content, whereas cultivars T5 (lane 2) and SBB100SD (lane 9) had the lowest β subunit content. Glycinin and β-conglycinin constituted 58−78% of the extractable protein or 22.4−32.8% of total solids in the soybean seeds, with the highest content given to Proto and the lowest to Stine 1570 (Table 1). These protein contents are comparable to those (55−75% of protein) of 10 soybean varieties reported by Murphy and Resurreccion using rocket immunoelectrophoresis (1984). The content of β-conglycinin (7S) and glycinin (11S) of 13 soybean varieties ranged from 7.3 to 9.9% and from 14.1 to 22.9% on the dry weight basis, respectively.

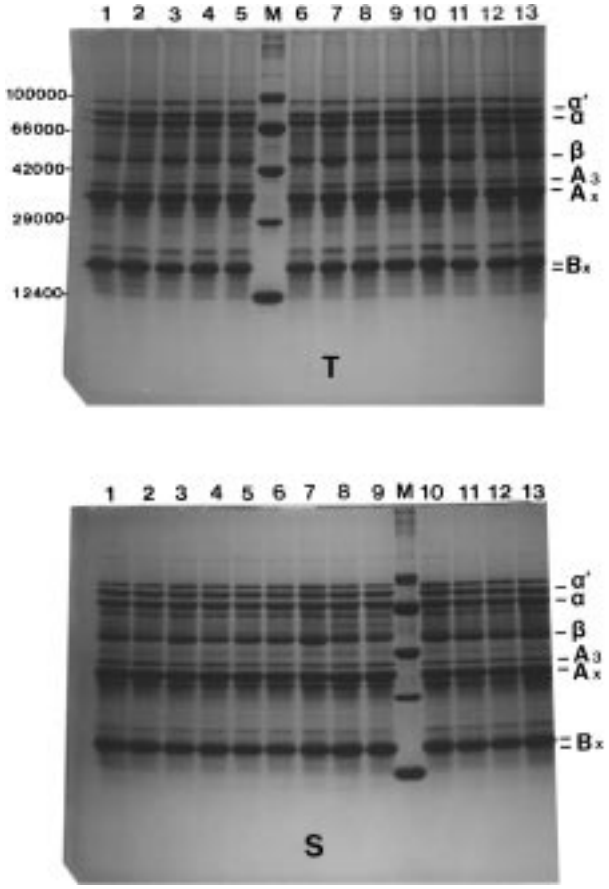

Figure 1. SDS−PAGE protein subunits (polypeptides) of β-conglycinin and glycinin in 13 soybean varieties (S) and their tofu (T). Lanes 1−13 are polypeptides in soybeans and tofu of Proto, T5, Corsoy 79, Vinton, Kato, Hardin, Sturdy, SBB100ND, SBB100SD, Stine 2220, Stine 1590, Stine 0380, and Stine 1570, respectively. Lane M represents protein molecular mass markers (molecular masses are shown on the left). A_3, A_x (A_{1a}, A_{1b}, A_2, and A_4), and B_x (B_{1a}, B_{1b}, B_2, B_3, and B_4) are polypeptides of glycinin (A, acidic; B, basic), and α', α, and β are subunits of β-conglycinin (Fontes et al., 1984).

Table 2. Processing Effect on 11S/7S Protein Ratio of 13 Soybean Varieties[a]

	production method			pilot method			bench method		
variety	soybean	soy milk	tofu	soybean	soy milk	tofu	soybean	soy milk	tofu
Proto	2.32 ab	2.28 ab	2.34 ab	2.32 ab	1.94 ab	1.94 ab	2.32 ab(a)	1.75 a(b)	2.14 ab(ab)
T5	2.18 bc	2.18 bc	2.33 ab	2.18 bc(a)	1.70 abc(b)	1.71 cde(b)	2.18 bc(a)	1.77 a(b)	2.16 a(ab)
Corsoy 79	1.88 cdef	1.92 de	1.67 e	1.88 cdef	1.45 def	1.54 ef	1.88 cdef	1.64 ab	1.79 abcd
Vinton	2.03 bcd	2.19 bc	2.11 bc	2.03 bcd	1.77 abcd	1.82 bcd	2.03 bcd(a)	1.62 ab(b)	2.09 abc(a)
Kato	2.16 bc	1.94 de	2.08 bc	2.16 bc(a)	1.59 bcde(b)	1.65 de(b)	2.16 bc(a)	1.49 bc(b)	1.82 abcd(ab)
Hardin	1.64 f	1.69 efg	1.77 de	1.64 f	1.66 abcde	1.58 ef	1.64 f	1.45 bc	1.89 abcd
Sturdy	1.64 f	1.53 g	1.78 de	1.64 f(a)	1.31 ef(ab)	1.41 fg(b)	1.64 f(a)	1.31 c(b)	1.69 cd(a)
SBB100ND	2.28 ab	2.25 ab	2.39 a	2.28 ab(a)	1.82 abc(b)	1.92 bc(ab)	2.28 ab(a)	1.76 a(b)	2.13 ab(a)
SBB100SD	2.51 a	2.46 a	2.49 a	2.51 a	2.02 a	2.13 a	2.51 a(a)	1.75 a(b)	2.11 abc(ab)
Stine 2220	1.74 def	1.66 fg	2.01 cd	1.74 def(a)	1.17 f(b)	1.25 g(b)	1.74 def	1.34 c	1.58 d
Stine 1590	2.06 bcd	1.75 efg	1.84 cde	2.06 bcd	1.53 cde	1.67 de	2.06 bcd(a)	1.42 bc(b)	1.65 cd(ab)
Stine 0380	1.99 bcde	1.85 def	1.85 cde	1.99 bcde	1.57 cde	1.72 cde	1.99 bcde(a)	1.49 bc(b)	1.76 abcd(ab)
Stine 1570	1.69 ef	2.01 cd	1.99 cd	1.69 ef	1.86 abc	1.70 de	1.69 ef	1.67 ab	1.73 abcd

[a] Means of four treatment replications. Means within a column followed by different letters are significantly different ($p \leq 0.05$). Means followed by different letters in parentheses within a processing method for each row are significantly different ($p \leq 0.05$).

Table 3. Processing Effect on 7S and 11S Proteins of 13 Soybean Varieties (Pilot Method)[a]

	7S (%, dry wt basis)			11S (%, dry wt basis)		
variety	soybean	soy milk	tofu	soybean	soy milk	tofu
Proto	9.9 a(b)	12.1 ab(a)	11.5 a(a)	22.9 a(a)	23.4 a(a)	22.2 b(a)
T5	9.8 a(b)	11.6 abc(a)	12.5 a(a)	21.4 ab(a)	19.6 bcd(a)	21.4 b(a)
Corsoy 79	8.6 bc(b)	11.6 abc(a)	12.9 a(a)	16.0 de(b)	16.8 efg(b)	19.9 bc(a)
Vinton	9.9 a(b)	11.3 abcd(a)	12.1 a(a)	20.2 bc(a)	20.1 bc(a)	21.8 b(a)
Kato	8.6 bc(b)	11.9 abc(a)	12.1 a(a)	18.5 cd(a)	18.9 cde(a)	20.0 bc(a)
Hardin	9.2 ab(b)	10.4 cd(ab)	11.5 a(a)	15.1 e(c)	17.2 efg(b)	18.0 cde(a)
Sturdy	8.8 b(b)	11.9 abc(a)	12.1 a(a)	14.5 e(b)	15.5 fg(ab)	17.0 de(a)
SBB100ND	7.3 e(b)	10.3 cd(a)	10.4 a(a)	16.8 de(b)	18.7 cde(ab)	19.9 bc(a)
SBB100SD	7.4 de(b)	10.7 bcd(a)	11.8 a(a)	18.1 cd(c)	21.6 ab(b)	25.2 a(a)
Stine 2220	8.7 bc(b)	12.5 a(a)	12.4 a(a)	15.2 e(a)	14.6 g(a)	15.5 e(a)
Stine 1590	7.8 cde(b)	11.3 abcd(a)	12.1 a(a)	15.9 de(b)	17.1 ef(b)	20.2 bc(a)
Stine 0380	8.5 bc(b)	11.7 abc(a)	12.1 a(a)	16.8 de(b)	18.3 cde(ab)	20.7 bc(a)
Stine 1570	8.3 bcd(b)	9.7 d(ab)	10.8 a(a)	14.1 e(b)	18.0 cde(a)	18.4 cd(a)

[a] Means of two treatment replications. Means within a column followed by different letters are significantly different ($p \leq 0.05$). Means within a row followed by different letters in parentheses are significantly different ($p \leq 0.05$).

These corresponded to 17.2−23.5 and 36.3−51.3% of total extractable proteins, which are similar to 18.6−29.0% β-conglycinin and 38.1−50.8% glycinin of commercial varieties reported by Murphy and Resurreccion (1984). Proto, T5, and Vinton had the highest 7S protein contents (≥9.8%), whereas SBB100 varieties had the lowest (≤7.4) (Table 1). The 11S protein content of Proto and T5 was the highest (≥21.4%), whereas the 11S content of Stine 1579 was the lowest (14.1%).

The ratio of 11S/7S proteins varied from 1.64 to 2.51 among the soybean varieties (Table 2). SBB100SD, SBB100ND, and Proto had the highest 11S/7S protein ratio (≥2.28), whereas Hardin and Sturdy had the lowest ratio (1.64). The 11S/7S protein ratio of soybean varieties varied greatly in the literature. Taira and Taira (1972) studied 30 cultivars of food soybeans grown in three locations and found the 11S/7S protein ratio ranged from 0.7 to 1.4, with most soybeans in the range of 0.8−1.2. On the other hand, Murphy and Resurreccion (1984) reported the 11S/7S protein ratio of 2.1−3.4 among 12 soybean varieties, and they suggested that the differences in glycinin and β-conglycinin content were due to genetic and environmental differences.

Soybean variety had highly ($p \leq 0.01$) significant effects on the 7S and 11S protein contents and the 11S/7S protein ratio of soy milk and tofu. The 11S/7S ratio of soy milk and tofu produced by the production method ranged from 1.53 to 2.46 and from 1.67 to 2.49, respectively (Table 2). SBB100SD had the highest protein ratio in soy milk and tofu, whereas Sturdy soy milk and Corsoy tofu had the lowest. For the pilot method, SBB100SD also produced the highest protein ratios of soy milk (2.02) and tofu (2.13), but Stine 2220 yielded the lowest ratios of soy milk (1.17) and tofu (1.25). For the bench method, T5 had the highest 11S/7S protein ratio of soy milk (1.77) and tofu (2.16), whereas Sturdy soy milk and Stine 2220 tofu had the lowest (Table 2). The two most important steps in tofu -making are soy milk extraction and coagulation. Hou (1996) found that soybean variety had a significant effect on soy milk solid content and tofu yield and the use of the same solid content of soy milk for coagulation greatly reduced the varietal effect on tofu yield. Sun and Breene (1991) and Cai and Chang (1998) reported that some soybean varieties were more sensitive to changes in coagulation conditions (coagulant concentration and stirring speed and time). This indicated that different varieties may require different coagulation conditions to maximize their tofu yield and quality. Because our three processing methods differed in soy milk extraction and coagulation conditions, varieties could respond differently to the three methods and result in various protein compositions of tofu and soy milk. Therefore, the variations of 7S and 11S protein contents and 11S/7S ratio of soybean varieties in the three processing methods were due to interactions between processing method and soybean variety.

For most of the 13 soybean varieties, the 7S and 11S contents and 11S/7S ratio did not change significantly during processing from raw soy milk to tofu (Tables 1−4). The 7S protein increased from soybean to soy milk or tofu for all three processing methods. No significant changes in 11S/7S protein ratio occurred among soybean, soy milk, and tofu for the production method

Table 4. Processing Effect on 7S and 11S Proteins of 13 Soybean Varieties (Bench Method)[a]

	7S (%, dry wt basis)			11S (%, dry wt basis)		
variety	soybean	soy milk	tofu	soybean	soy milk	tofu
Proto	9.9 a(b)	12.9 a(a)	11.7 a(ab)	22.9 a(a)	22.7 a(a)	24.8 a(a)
T5	9.8 a(b)	12.1 a(a)	11.0 a(ab)	21.4 ab(a)	21.3 ab(a)	23.8 abc(a)
Corsoy 79	8.6 bc(b)	11.7 a(a)	12.1 a(a)	16.0 de(b)	18.9 abcd(ab)	21.5 bcd(a)
Vinton	9.9 a(b)	13.0 a(a)	11.5 a(ab)	20.2 bc(a)	21.2 abc(a)	24.1 abc(a)
Kato	8.6 bc(b)	12.0 a(a)	11.2 a(a)	18.5 cd(a)	17.8 abcd(a)	20.3 def(a)
Hardin	9.2 ab(a)	11.9 a(a)	11.1 a(a)	15.1 e(a)	17.3 bcd(a)	20.7 cdef(a)
Sturdy	8.8 b(b)	11.8 a(a)	11.3 a(a)	14.5 e(b)	15.5 d(b)	19.1 ef(a)
SBB100ND	7.3 e(b)	10.5 a(a)	9.9 a(a)	16.8 de(b)	18.4 abcd(b)	21.1 bcde(a)
SBB100SD	7.4 de(b)	11.6 a(a)	11.4 a(a)	18.1 cd(b)	20.3 abcd(b)	24.2 ab(a)
Stine 2220	8.7 bc(b)	11.4 a(a)	11.5 a(a)	15.2 e(a)	15.4 d(a)	18.0 ef(a)
Stine 1590	7.8 cde(b)	11.4 a(a)	11.4 a(a)	15.9 de(a)	16.2 cd(a)	18.8 ef(a)
Stine 0380	8.5 bc(b)	11.3 a(a)	11.0 a(a)	16.8 de(a)	16.9 bcd(a)	19.3 ef(a)
Stine 1570	8.3 bcd(b)	9.9 a(a)	10.3 a(a)	14.1 e(b)	16.5 bcd(a)	17.7 f(a)

[a] Means of four treatment replications. Means within a column followed by different letters are significantly different ($p \leq 0.05$). Means within a row followed by different letters in parentheses are significantly different ($p \leq 0.05$).

Table 5. Effect of Processing Method on Composition and Recovery of Soy Proteins in Soy Milk and Tofu[a]

method	protein[b] (%)	7S[b] (%)	11S[b] (%)	11S/7S ratio	protein recovery[c] (%)	7S recovery[c] (%)	11S recovery[c] (%)
				Soy Milk			
production	48.2 a	11.1 b	21.8 a	1.96 a	71.4 c	77.4 c	76.1 a
pilot	47.8 a	11.2 ab	18.4 b	1.64 b	76.0 b	84.3 b	69.2 b
bench	46.9 b	11.6 a	18.3 b	1.57 b	81.2 a	94.0 a	74.8 a
				Tofu			
production	50.9 b	11.5 ab	23.4 a	2.04 a	64.5 c	68.9 b	70.0 b
pilot	50.7 b	11.8 a	20.0 c	1.70 c	73.6 b	80.9 a	68.7 b
bench	51.4 a	11.2 b	21.0 b	1.88 b	77.6 a	78.8 a	74.3 a

[a] Data are means of 13 varieties based on two-way ANOVA analysis. Means within a column followed by different letters for each product are significantly different ($p \leq 0.05$). [b] Dry weight basis. [c] From soybeans.

Table 6. Correlation of 7S and 11S Proteins in Soybean, Soy Milk, and Tofu Produced According to Three Methods[a]

		production method		pilot method		bench method	
protein	sample	soy milk	tofu	soy milk	tofu	soy milk	tofu
7S	soybean	0.433**[b]	0.406**	0.138	0.048	0.122	0.332
7S	soy milk		0.492**		0.558**		0.294
11S	soybean	0.868**	0.843**	0.789**	0.609**	0.670**	0.804**
11S	soy milk		0.872**		0.812**		0.821**
11S/7S	soybean	0.774**	0.569**	0.639**	0.831**	0.658**	0.710**
11S/7S	soy milk		0.687**		0.813**		0.779**

[a] Pearson's correlation coefficient. [b] **, significant at $p \leq 0.01$.

(Table 2). The 11S/7S protein ratios of T5, Kato, and Stine 2220 varieties decreased after processing into soy milk and tofu for the pilot method (Table 2). When compared with soybean, soy milk had significantly lower 11S/7S protein ratios for the bench method. There were no significant changes in 7S protein in tofu made by the pilot method and soy milk and tofu made by the bench method among the 13 soybean varieties (Tables 3 and 4).

Effect of Processing Method. In addition to varietal effect, processing method also had significant ($p \leq 0.05$) effects on total protein, 7S and 11S contents, 11S/7S ratios, and protein recoveries of soy milk and tofu (Table 5). When compared with the bench and pilot methods, the production method yielded higher 11S content of soy milk (21.8%) and tofu (23.4%) and higher 11S/7S ratio of soy milk (1.96) and tofu (2.04). Differences in the three processing methods, such as grinding and extraction variables (water-to-bean ratio, hydration ratio, grinding and separation method, sieving/filtering pore size, and additional extraction of okara residue), coagulation conditions (stirring speed and time, propeller type and size, coagulation concentration, and soy milk solid content), and pressing of bean curds (pressure and pressing time), affected the content and composition of soybean proteins. One possible explanation for the higher 11S content is that soybeans with the production method had a higher hydration ratio than pilot and bench methods (2.2 versus 2.0−2.1). The former might render 11S protein more extractable, leading to the higher 11S recovery (76.1%). Higher 11S content led to higher 11S/7S ratio of soy milk; higher 11S content and 11S/7S ratio in soy milk produced higher 11S content and 11S/7S ratio of tofu because they were significantly correlated (Table 6). The higher total protein recovery (81.2%) and 7S protein recovery (94.0%) of soy milk made according to the bench method were due to the higher water-to-bean ratio, longer maceration/extraction time, and the second extraction of okara residue. Beddows and Wong (1987a) also reported that an additional extraction increased solid and protein recoveries. However, the bench method resulted in lower protein content of soy milk, which indicated more nonprotein components were also extracted with proteins. The lower protein recovery of soy milk for the production method was due to incomplete separation of soy milk (producing wetter okara residue).

Tofu made from all three methods contained ~51% protein, ~3−4% higher than soy milk (Table 5). The coagulation and pressing processes removed some carbohydrates and therefore increased protein content on the dry matter basis. Tofu made according to the bench

method had the highest protein recovery, probably due to its higher protein content and more whey proteins retained in bean curds when pressed at lower pressures for a shorter time. However, the 7S protein recovery was reduced from 94 to 79% after soy milk was processed into tofu. Because a higher water-to-bean ratio was used with the bench method, the $CaSO_4 \cdot 2H_2O$ concentration at 0.29% of cooked soy milk may not be high enough to coagulate all 7S protein. We used the same coagulant concentration based on soybean weight for the three processing methods but different water-to-bean ratios and different cooking methods (on the stove for the pilot and bench methods versus direct steam injection for the production method), resulting in different coagulant concentrations in cooked soy milk. Saio and Watanabe (1973) and Tezuka et al. (1995) reported that higher calcium or magnesium concentrations were required for the 7S protein solution or 7S protein-rich soy milk to coagulate than for the 11S solution or normal soy milk. Thus, the lower coagulant concentration used by the bench method may account for greater loss of 7S protein during coagulation as compared to 0.32−0.33% coagulant in cooked soy milk for the pilot and production methods (Table 5). Because of greater loss of 7S during coagulation for the bench method, the tofu had a lower 7S content and a higher 11S/7S ratio when compared with its soy milk. Tofu made according to the bench and pilot methods recovered more 11S globulin from soy milk than the production method, presumably owing to more adequate mixing with coagulant on the smaller scales. Tofu made according to the pilot method had the highest recoveries of protein and globulins form soy milk, which was probably the result of the adjustment of all soy milk solid contents to 12 °Brix before coagulation with the optimum processing procedures of Shih et al. (1997). Shih et al. (1997) studied process optimization for soft tofu on the pilot scale and stated that optimum combinations of four factors were 11.8−12.3% °Brix for soy milk, 0.27−0.32% coagulant of raw soy milk, mixing temperatures of 85−91 °C, and stirring times of 5−11.3 s at 285 rpm.

Correlations of Storage Proteins during Processing. For all three processing methods, the 11S/7S protein ratio was highly ($p \leq 0.01$) significantly correlated to each other among soybean, raw soy milk, and tofu (Table 6). However, the Pearson's correlation coefficient of the 11S/7S protein ratio among them was 0.57−0.83, depending on the processing method and products. The tofu 11S/7S ratio and the soybean 11S/7S ratio had the highest correlation ($r = 0.83$) for the pilot method, whereas they had the lowest correlation ($r = 0.57$) for the production method. The 11S protein had positive correlations ($r = 0.61$−0.87) among soybean, soy milk, and tofu for all three methods. The 7S protein in soybean was not correlated with that of soy milk or tofu made according to the pilot or bench method. Although the correlations for 7S protein among soybean, soy milk, and tofu by the production method were statistically significant, the magnitude of the coefficients (0.41−0.49) was not impressive.

Relationship between Storage Proteins and Tofu Quality. The relationship of the storage protein contents to tofu hardness, yield, and sensory quality (overall score) varied with tofu processing method (Table 7).

Production Method. Tofu sensory quality highly ($p \leq 0.01$) significantly correlated with soybean 11S protein ($r = 0.75$), 11S/7S protein ratio ($r = 0.65$), and total 11S and 7S globulins ($r = 0.70$). The sensory quality also correlated with tofu 11S protein, 11S/7S ratio, and total 11S and 7S globulins (Table 7). Tofu yield had positive correlations ($r = 0.43$−0.63) with the 11S protein, 11S/7S ratio, and total 11S and 7S globulins of soybean and tofu. It should be noted that there were some relationships between tofu hardness and soybean or tofu 7S protein, 11S protein, and their ratio, but the correlation coefficients were <0.44. Soybean 7S protein was not associated with tofu yield and sensory quality. The negative correlations between tofu hardness of middle pieces and 11S protein and 11S/7S ratio were in accordance with those reported by Murphy et al. (1997). The positive correlations between tofu yield and protein content of soybean and tofu agreed with the findings of Shen et al. (1991) and Schaefer and Love (1992).

Bench Method. Tofu sensory quality had positive correlations with soybean and tofu 11S protein, tofu 11S/7S ratio, and soybean and tofu total 11S and 7S globulins (Table 7). Soybean 7S and 11S protein and total 11S and 7S content correlated positively to tofu yield ($r = 0.49$−0.56). Tofu 11S/7S protein ratio was also associated with tofu yield. There were no significant correlations between storage proteins and tofu hardness, which is in agreement with the findings of Skurray et

Table 7. Correlation of 7S and 11S Tofu Protein with Tofu Hardness, Yield, and Sensory Score[a]

soy protein	hardness (top tofu)	hardness (middle tofu)	tofu yield	sensory score
Production Method				
soybean 7S	−0.010	−0.255	0.354	0.269
tofu 7S	0.423*[b]	0.173	−0.030	0.105
soybean 11S	0.269	−0.431*	0.625**[c]	0.754**
tofu 11S	0.376	−0.300	0.513**	0.723**
soybean 11S/7S ratio	0.320	−0.283	0.434*	0.649**
tofu 11S/7S ratio	0.121	−0.439*	0.580**	0.720**
soybean 11S + 7S	0.221	−0.427*	0.615**	0.699**
tofu 11S + 7S	0.431*	−0.207	0.425*	0.639**
soybean protein	0.260	−0.385	0.596**	0.726**
tofu protein	0.414*	−0.257	0.409*	0.620**
Pilot Method				
soybean 7S	0.097	−0.193	0.204	0.646**
tofu 7S	0.103	0.276	−0.136	0.051
soybean 11S	0.696**	0.300	0.102	0.212
tofu 11S	0.657**	0.585**	−0.245	−0.215
soybean 11S/7S ratio	0.719**	0.506**	−0.066	−0.271
tofu 11S/7S ratio	0.521**	0.356	−0.140	−0.210
soybean 11S + 7S	0.605**	0.197	0.140	0.351
tofu 11S + 7S	0.624**	0.618**	−0.266	−0.175
soybean protein	0.671**	0.318	0.055	0.067
tofu protein	0.680**	0.440*	0.023	0.037
Bench Method				
soybean 7S	−0.230	−[d]	0.488*	0.366
tofu 7S	0.371	−	−0.371	−0.234
soybean 11S	−0.174	−	0.518**	0.551**
tofu 11S	−0.165	−	0.312	0.490**
soybean 11S/7S ratio	−0.008	−	0.196	0.347
tofu 11S/7S ratio	−0.312	−	0.458*	0.557**
soybean 11S + 7S	−0.206	−	0.561**	0.556**
tofu 11S + 7S	−0.064	−	0.203	0.403*
soybean protein	−0.151	−	0.485*	0.513**
tofu protein	0.024	−	0.200	0.345

[a] Pearson's correlation coefficient. [b] *, significant at $p \leq 0.05$. [c] **, significant at $p \leq 0.01$. [d] Not available.

al. (1980) and Taira (1990). Tofu 7S, 11S, and total proteins did not have significant correlations with tofu yield. Variations in relationships of tofu yield to soybean and tofu 7S and total 11S and 7S globulin content were probably owing to no correlation of 7S between soybean and tofu (Table 6).

Pilot Method. Except the relationship between tofu hardness of top pieces and tofu total proteins content, those significant correlations between tofu quality/yield and soy proteins for the production and bench methods were not found for the pilot method. In contrast, soybean 11S/7S protein ratio correlated positively with the hardness of tofu made according to the pilot method (Table 7). Both soybean and tofu 11S protein, 11S/7S ratio, and total 11S and 7S protein content had positive correlations with the hardness of tofu top pieces. Tofu 11S and total 11S and 7S content were also associated with the hardness of middle pieces of tofu. On the basis of this processing method, our results were similar to the findings of Saio et al. (1969) that tofu gels prepared from 11S protein and higher 11S/7S protein ratio were harder. Tofu yield and sensory quality did not have any relationship with soybean and tofu 11S protein, 11S/7S ratio, or total 11S and 7S content, but they did have relationships for production and bench methods (Table 7). One of the major steps of the pilot method different from the other two methods was the adoption of adjusting soy milk solid content to the same concentration (12 °Brix) for each variety before coagulation. This significantly reduced varietal effects on tofu yield and smoothness (Hou, 1996). Tofu yield was not related to protein content; similar results were reported by Wang et al. (1983) and Murphy et al. (1997). Tofu sensory quality correlated with soybean 7S but not with tofu 7S, which was attributed to no correlation of soybean 7S and tofu 7S (Table 6). For each processing method in our study, the relationships of tofu hardness, yield, and sensory quality with soybean/tofu total protein content were similar to those with their total 11S and 7S content because 7S and 11S globulins were major components of the total proteins.

Although many researchers have reported that the soy protein composition has an effect on the hardness/firmness of purified soy protein gels, the results are not uniformly predictive for tofu, which is made from soy milk, a more complicated system than the purified protein system. Even in a relatively less pure system of soy protein isolate, Kohyama et al. (1995) found that its gelation rate was much slower than that of the purified 7S and 11S blend under the same 11S/7S ratio and other gelling conditions. The gel formed by soy protein isolate involved both the 7S and 11S types of network, whereas the 11S fraction in the blended protein system was primarily responsible for the gel matrix (Kohyama et al., 1995). Therefore, constituents other than proteins in soy milk may affect the tofu structures and textural properties.

Our results showed that the contribution of soybean storage proteins (glycinin and β- conglycinin) to tofu yield, hardness, and sensory quality depended on the processing method used. Different processing procedures (including making different types of tofu) may account for the discrepancies in reporting relationships between soy protein content and tofu yield/texture among the studies of Shen et al. (1991), Schaefer and Love (1992), Wang et al. (1983), and Murphy et al. (1997). Our study also indicated that the controversy on the relationship of 11S and 7S proteins and their ratio to tofu texture (hardness) could be due to the different processing methods used among researchers (Saio et al., 1969; Saio, 1979; Skurray et al., 1980; Taira, 1990; Murphy et al., 1997).

Conclusion. Processing method had a significant effect on the content of storage proteins (β-conglycinin and glycinin) in tofu. The relationship of these proteins to tofu yield and texture depended on processing method. Unless there is a standardized preparation procedure for making tofu, the role of β-conglycinin (7S protein) and glycinin (11S protein) in influencing tofu quality will differ in various laboratories. Therefore, it is suggestive that one should take tofu preparation method into consideration in interpreting the data on relationship of soybean storage proteins to tofu yield and textural properties.

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