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Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture

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Abstract

The contribution of the total soybean proteins, the storage proteins [glycinin (11S) and β-conglycinin (7S) fractions] and their respective subunits to tofu yield and texture was studied. Protein contents were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry and reversed phase-high performance liquid chromatography (RP-HPLC), for seven soybean varieties, and correlated to tofu yield and texture. Results from SDS-PAGE, coupled with densitometry, showed that the 11S fraction proteins ($r=0.863$, $P<0.01$), the α′ polypeptide of 7S ($r=0.917$, $P<0.01$) and the basic polypeptide of 11S ($r=0.775$, $P<0.01$) appeared to each affect tofu yield; however, no relationship between storage protein fractions and tofu firmness was observed. On the other hand, the RP-HPLC profiles of the total soybean proteins and the 11S and 7S fractions indicated relationships between tofu firmness and the 11S fraction, the 7S fraction, and their ratio. The inverse correlation ($r=-0.832$, $P<0.01$) between peak 7 of the 7S fraction and tofu firmness seems to point toward its important role in defining tofu quality.

Keywords: Soybean; Tofu; Glycinin; β-conglycinin; Tofu yield and firmness; SDS-PAGE; RP-HPLC

1. Introduction

Soybean seeds have a protein content of 35–40% on a dry weight basis, which makes them a relatively inexpensive source of protein for human consumption (Derbyshire, Wright, & Boulter, 1976). Soybeans have been transformed into various forms of soy foods, tofu being the one most widely accepted throughout the world.

Tofu making involves the complex interactions of many factors. These influencing factors include intrinsic characteristics, such as soybean total protein content (Schaefer & Love, 1992; Shen, DeMan, Buzell, & DeMan, 1991), the contents of the two major storage protein components, glycinin (11S) and β-conglycinin (7S), and the 11S to 7S ratio (Saio, Kamiya, & Watanabe, 1969). In addition, processing conditions affect tofu quality. Concentration and type of the coagulant used influence the tofu texture and yield (Hou, Chang, & Shih, 1997; Sun & Breene, 1991).

Soybean storage proteins are composed of 11S and 7S fractions, accounting for about 40% and 30% of the total seed proteins, respectively (Utsumi, 1992; Utsumi, Matsuma, & Mori, 1997). Their contents have been shown to vary with soybean variety and environment (Cai & Chang, 1999; Hughes & Murphy, 1983; Murphy & Resurreccion, 1984; Saio et al., 1969; Wolf, Babcock, & Smith, 1961). Due to differences in the gelation properties of soybean storage protein fractions, many researchers have attempted to correlate these proteins with tofu quality, but results have differed greatly. The 11S content and 11S/7S protein ratio have been reported to correlate positively with tofu gel firmness on the basis of purified soy protein systems (Kang, Matsumura, & Mori, 1991; Murphy, Chen, Hauck, & Wilson, 1997; Saio et al., 1969). On the other hand, a few researchers found that the 7S protein formed firmer gels than the 11S protein (Utsumi & Kinsella, 1985), and others found little correlation between the 11S/7S protein ratio and tofu quality (Skurray, Cunich, & Carter, 1980; Taira, 1990). Tofu characteristics have also been reported to be cultivar-dependent, and manipulation of

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the 11S/7S ratio may not result in substantial effects on tofu yield and quality (Cai & Chang, 1999).

Glycinin consists of six subunits, each made up of an acidic (A) polypeptide chain (MW ~ 35,000) linked by disulfide bonding to a specific basic (B) polypeptide chain (MW ~ 20,000). Beta-conglycinin is a trimer which consists of α′ (MW ~ 72,000), α (MW ~ 68,000) and β (MW ~ 52,000) subunits (Mori, Utsumi, Inaba, Kitamura, & Harada, 1981; Nielson, 1985a, 1985b; Staswick, Hermodson, & Nielson, 1984). The 11S peptide has five genetic variants which are divided into group-I ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) and group-II (A_3B_4, $A_5A_4B_3$) based on the homology of their subunit sequences (Nielson, 1985b; Staswick, Hermodson, & Nielson, 1981). Group II is further classified into two subgroups, normally IIa ($A_5A_4B_3$) and IIb (A_3B_4) (Yagasaki, Takagi, Sakai, & Kitamura, 1997). Heterogeneity in the 11S fraction, both among soybean varieties (Kitamura, Toyokawa, & Harada, 1980; Mori et al., 1981) and within single varieties (Utsumi, Inaba, & Mori, 1981) has been reported. It is known that about 20% of Japanese soybean varieties lack the $A_5A_4B_3$ subunit (Harada, Toyokawa, & Kitamura, 1983), which in heat-induced gels was indicated to be related to gel firmness (Fukushima, 1991). A soybean variety lacking the A_3B_4 subunit has also been reported (Kitamura, Ishimoto, & Kainuma, 1983). Group I subunits were also found to be related to firmness of tofu (Tezuka, Taira, Igarashi, Yagasaki, & Ono, 2000). Therefore, decreasing 11S/7S ratios and the structural changes caused by a relative lack of 11S subunits are thus expected to influence the food processing properties of soybeans.

Published results reveal that soybean storage proteins, whose contents vary among different varieties, have a significant role in determining the yield and quality of tofu, which are important factors influencing acceptability of tofu by producers/consumers. Their contribution is still varied and controversial and is in need of further investigation. The present study was, therefore, designed to elicit an understanding of the roles of soybean proteins, protein fractions and subunits to tofu yield and texture. This information can, in turn, help in developing methods to modify firmness and smoothness of tofu to meet specifications for desired products.

2. Materials and methods

2.1. Sample preparation

Seven soybean cultivars (Vinton-81, S-20F8, HP-204, IA-2034, Steyer, IA-2020, S-2020) harvested in the year 2000 were obtained from Michigan soybean growers. The seeds were ground in a Micro-Mill grinder (Bel-Art Products, Pequannock, NJ, USA) to a coarse powder, followed by grinding in a Udy cyclone sample mill with a 0.5 mm screen (Udy Corporation, Fort Collins, CO, USA) to a fine powder. The powder was then defatted using hexane. Solvent was evaporated at room temperature and dried-defatted soybean meal stored at 4 °C until analysis.

2.2. Chemicals and reagents

All chemicals and reagents were either from Sigma chemical company (St. Louis, MO) or of analytical grade.

2.3. Moisture and protein contents

Moisture content was determined by the standard AACC procedure (AACC, 2000a). Protein content was determined by the micro-Kjeldahl method (AACC, 2000b).A nitrogen to protein conversion factor of 6.25 was used.

2.4. Extraction of total soybean proteins

Twenty milligrammes of defatted soybean flour were extracted with 500 μl of 0.03 M Tris [Tris(hydroxymethyl)aminomethane] buffer (pH 8.0) containing 0.01 M β-mercaptoethanol (β-ME) for 1 h with vortexing every 10 min. Samples were then centrifuged at room temperature for 20 min at 11,000×*g*; the supernatant contained the total soybean proteins.

2.5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein content of the supernatant was analyzed according to the Bradford method (Bradford, 1976). The protein extract was diluted to 4 mg/ml with distilled water, and 20 μl of the diluted extract were mixed with 20 μl of SDS-sample buffer (0.15M Tris–HCl, pH 6.8, 4%w/v SDS, 5%v/v β-ME) and heated at 96 °C for 3 min. Twenty μl of the solution cooled to room temperature (20 °C), containing 40 μg of protein, were loaded onto a gradient gel containing 8–16% polyacrylamide. SDS-PAGE was performed (Cai & Chang, 1999) in a vertical electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) at 100 V constant voltage for 1 h, followed by 125V constant voltage until the tracking dye migrated to the bottom edge of the gel (6 h). Gels were stained with Coomassie Brilliant Blue R-250 (0.05%, w/v) in methanol-acetic acid-water (25:10:65 v/v/v) and destained in the same solution without the dye.

Myosin (MW 205,000), β-galactosidase (MW 116,000), phosphorylase-b (MW 97,000), fructose-6-phosphate kinase (MW 84,000), bovine serum albumin (MW 66,000), glutamic dehydrogenase (MW 55,000),

ovalbumin (MW 45,000), phosphate dehydrogenase (MW 36,000), carbonic anhydrase (MW 29,000), trypsinogen (MW 24,000), α-lactalbumin (MW 14,200) and aprotinin (MW 6500) were used as standards to estimate the molecular weight ranges of polypeptides in each fraction, as well as to identify the subunits of the major soybean proteins, i.e., 11S and 7S.

2.6. Quantification of protein fractions from total protein extracts by densitometry

For quantification of 11S and 7S fractions and their respective subunits, the gels were rinsed after destaining and the protein bands subjected to densitometric analysis. The electrophoretic patterns were analyzed by a transmittance/reflectance scanning densitometer (GS 300, Hoefer Scientific Instruments, San Francisco, CA) with GS 365W software. Relative mobilities from published photographs of soybean seed SDS-PAGE gels (Cai & Chang, 1999; Fontes, Moreira, Davies, & Nielson, 1984; Ji, Cai, & Chang, 1999; Sathe, Lillrey, Mason, & Weaver, 1987; Yagasaki et al., 1997) were used to identify the protein bands that were the acidic and basic polypeptides of 11S and the subunits of 7S. The relative protein quantity of each subunit (protein band) was calculated from their respective percent area on the densitograms against the total percent area, which was equivalent to 40 μg protein, and the values were further converted to percentage of soy meal protein.

2.7. Preparation of crude 11S and 7S

Glycinin and β-conglycinin fractions were extracted from defatted soybean flour according to the method of Thanh and Shibasaki (1976). The samples were then freeze-dried and stored at 4 °C until analysis.

2.8. SDS-PAGE of 11S and 7S protein fractions

Twenty milligrammes of crude 11S fraction or 7S fraction were dispersed in 500 μl of 0.03 M Tris buffer (pH 8.0) for 15 min with vortexing every 5 min. The extract was then centrifuged for 10 min at 11,000×g. Twenty microlitres of extract were mixed with 20 μl of SDS-sample buffer, heated at 96 °C for 3 min and a 10 μl aliquot was electrophoresed as described earlier.

2.9. Reversed phase-high performance liquid chromatography (RP-HPLC) of total proteins, 11S and 7S fractions

Chromatography was performed on a Millenium 2010 HPLC workstation, consisting of a Waters 600E multisolvent delivery system (Waters, Milford, MA, USA), a temperature control module, and a 996-photodiode-array detector. Separations were performed at 60 °C on a Phenomenex 300 RP, Jupiter C-18 column (4.6×250 mm, 5 m particle diameter; Phenomenex, Torrance, CA, USA). The Phenomenex security guard with the same packing material served as the guard column.

The samples used for chromatography were prepared in the following manner. The supernatants obtained after extraction of total soybean proteins with 0.03 M Tris buffer (pH 8.0) and 0.01 M β-ME were dialyzed against distilled water. The freeze-dried 11S and 7S fractions were reconstituted in 0.03 M Tris buffer (pH 8.0). The extracts were filtered through a 0.45 μm filter (Millipore, MA, USA) and 20 μl aliquots were used for injection. The elution conditions used were according to the method of Peterson and Wolf (1992).

The solvents used were A: water (distilled water filtered through Milli-Q-Plus, Millipore) with 0.1% trifluoroacetic acid (TFA), and B: HPLC grade acetonitrile with 0.1% TFA. The solvents were purged with helium at the rate of 8 ml min^{-1} during the run. The solvent flow rate was 1 ml min^{-1}. The column was equilibrated for 20 min with 20% acetonitrile prior to injection. A 90 min gradient of 20–45% acetonitrile in water was followed by elution with 45% acetonitrile for 20 min, yielding a 110 min chromatogram (Table 1). The gradient programme was monitored at 190–310 nm and the detector output plotted at 210 nm and transmitted simultaneously to the computer for data storage and graphic representations.

2.10. Tofu processing and evaluation of tofu yield and firmness

Tofu was prepared according to the procedure described in Mujoo, Trinh, and Ng (2002).Tofu yield was expressed by weight in kilogrammes of fresh tofu produced per kilogramme of dry soybeans. Texture analysis for firmness (hardness) was carried out according to methodology reported in Mujoo et al. (2002).

2.11. Analyses of data

Pearson's correlation coefficients were used to determine the degree and significance of association among the various quality attributes (yield and firmness) of tofu and the 11S and 7S fractions and their respective protein subunits.

Table 1
Solvent gradient for RP-HPLC

Min	Solvent A	Solvent B
0	80	20
90	55	45
110	55	45

3. Results and discussion

3.1. Moisture and protein contents

The moisture and protein contents of the soybeans studied and their tofu yield and firmness are listed in Table 2. The moisture contents of different soybean varieties were between 9.04% and 9.71%. On a dry weight basis, the protein content of defatted soybean meal of the different varieties ranged from 42.9 to 49.6%. There was no clear relationship between tofu yield and soybean protein content; however, the firmness of tofu prepared from these varieties decreased as the protein content of the soybean decreased (Table 2). These results are in general agreement with other findings, wherein it has been reported that soybean varieties high in protein content produced tofu with high yield and firmer texture (Schaefer & Love, 1992; Shen et al., 1991).

3.2. SDS-PAGE profile of total soybean proteins

Electrophoretic patterns of total soybean proteins from seven different soybean varieties on SDS-polyacrylamide gradient gel are shown in Fig. 1. The protein bands were similar among all the soybean varieties. The molecular weight values of the α′, α and β-subunits of the 7S fraction were approximately 80,000, 75,000 and 50,000, respectively. The subunit with the molecular weight of 36,000 is an acidic A_3 polypeptide and the group of polypeptides near the molecular weight of about 34,000 is a major group of acidic polypeptides (A_{1a}, A_{1b}, A_2, A_4). The resolution in this region was not clear enough to detect several components separately. The acidic polypeptide A_5 located near the bottom of the gel had a molecular weight of about 10,000. The cluster of protein bands with molecular weight values of approximately 15,000 are basic components of the 11S fraction. The protein band found slightly above the basic components is the B_3 polypeptide of a basic subunit of the 11S fraction. These results are consistent with earlier reports (Fontes et al., 1984; Thanh, Okuba, & Shibasaki, 1975).

Table 2
Moisture and protein contents of seven soybean varieties, and tofu yield and firmness

Variety	Moisture (%, d.b.)	Protein (%, d.b.)	Tofu yield (kg/kg soybean)	Tofu firmness (N)
Vinton-81	9.67	49.6	2.93	10.02
S-20F8	9.40	49.1	2.69	9.91
HP-204	9.06	48.5	3.20	8.53
IA-2034	9.66	47.9	3.22	8.19
Steyer	9.71	47.9	3.14	7.97
IA-2020	9.19	45.9	3.43	7.84
S-2020	9.04	42.9	2.90	6.93

3.3. SDS-PAGE profile of 11S and 7S fractions

Fig. 2 shows the electrophoretic patterns of 11S and 7S protein fractions from seven soybean varieties. The polypeptide subunits of 11S and 7S proteins were well separated by SDS-PAGE. On the gel, the 7S protein fraction was separated into subunits α′, α and β. The 11S protein fraction was separated into acidic and basic subunits that were identified based on earlier reports (Cai & Chang, 1999; Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992; Wang & Chang, 1985). The qualitative profiles of the proteins of each of the fractions appeared to be similar among soybean varieties.

3.4. Quantification of 11S and 7S proteins and their subunits by densitometry

Densitometric analysis was used to quantify the two major storage proteins, 11S and 7S, and the subunits of 11S (acidic and basic subunits) and 7S (α′, α and β) of the soybean varieties separated on SDS-PAGE shown in Fig. 1 (Table 3). The 11S content of the varieties studied ranged from 19.5 to 23.1% and those of 7S varied from 10.0 to 12.7%. Variety S-20F8 had the lowest 11S content (19.5%) while IA-2020 had the highest (23.1%). Vinton-81 had the lowest 7S protein content (10.0%), whereas IA-2034 had the highest (12.7%). These values are similar to the 14.1–22.9% for

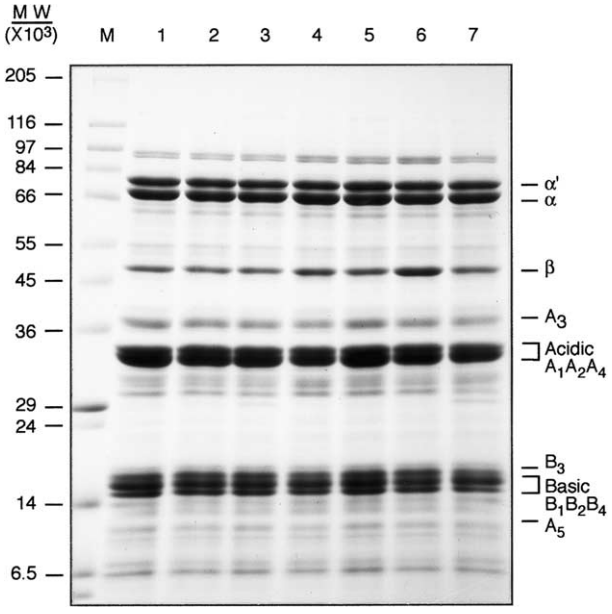

Fig. 1. SDS-PAGE profile of total proteins from seven soybean varieties. Lane 1: Vinton-81; 2: S-20F8; 3: HP-204; 4: IA-2034; 5: Steyer; 6: IA-2020; 7: S-2020. Lane marked M contains standard molecular weight markers.

11S content, though a bit higher than the 7.3–9.9% for 7S content, reported in literature for 13 different soybean varieties (Cai & Chang, 1999).The ratio of 11S/7S proteins varied from 1.63 to 2.05 among the varieties. The 11S/7S protein ratio of soybean varieties varies greatly in literature. One report showed that the 11S/7S protein ratio ranged from 2.1 to 3.4 in 12 soybean varieties (Murphy & Resurreccion, 1984), while a second study reported the 11S/7S protein ratio ranged from 1.64 to 2.51 in 13 soybean varieties (Cai & Chang, 1999). These authors suggested that the differences in 11S and 7S contents were due to both genetic and environmental differences.

3.5. Relationship between storage protein contents and tofu yield and firmness

The total 11S fraction ($r=0.863$, $P<0.01$), the α′ polypeptide ($r=0.917$, $P<0.01$) of the 7S fraction and the basic subunits of 11S ($r=0.775$, $P<0.05$) were each positively correlated with tofu yield. There was no significant correlation between total storage protein fractions and tofu firmness, which is in general agreement with other earlier findings (Skurray et al., 1980; Taira, 1990). The positive correlation between the soybean 11S protein and tofu yield were in accordance with those reported by Cai and Chang (1999). Their results showed that the contribution of soybean storage proteins to tofu quality and hardness depended upon the tofu processing method used, since the processing method also had a significant effect on the content of storage proteins. Different processing procedures may account for the discrepancies reported in relationships between soy protein content and tofu yield/texture among the various studies (Cai & Chang, 1999; Murphy et al., 1997; Schaefer & Love, 1992; Shen et al., 1991; Wang, Swain, & Kwolik, 1983).

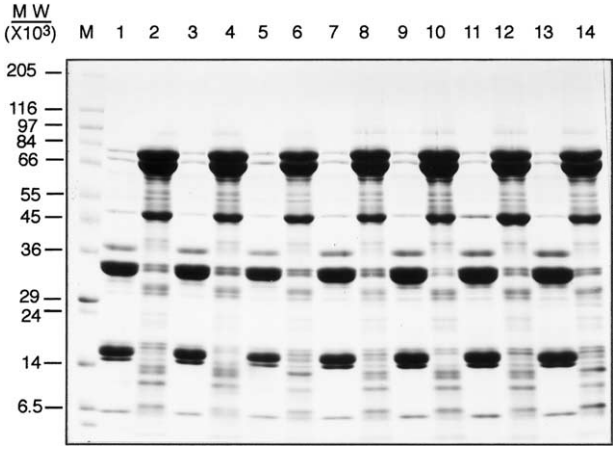

Fig. 2. SDS-PAGE profile of 11S and 7S protein fractions from seven soybean varieties. Lanes 1 and 2: 11S and 7S protein fractions from Vinton-81; 3 and 4: S-20F8; 5 and 6: HP-204; 7 and 8: IA-2034; 9 and 10: Steyer; 11 and 12: IA-2020; 13 and 14: S-2020. Lane marked M contains standard molecular weight markers.

Many researchers have reported that protein composition has an effect on the firmess of purified soy protein gels, although the results are not predictive for tofu made from soymilk, a more complex system than the purified protein system. In a relatively less pure system of soy protein isolate, it was found that the gelation rate was much slower than that of a purified 7S and 11S, blend even when using the same 11S/7S ratio as in other gelling conditions. The gel formed by soy protein isolate involved both the 7S and 11S types of networks, whereas the 11S fraction in the blended protein system was primarily responsible for gel matrix (Kohyama, Murata, Tani, Sano, & Doi, 1995). Therefore, constituents other than proteins in soymilk may also affect the tofu structure and textural properties.

3.6. RP-HPLC profile of total proteins, 11S and 7S fractions

Fig. 3 shows the representative RP-HPLC chromatograms of total proteins, and the 11S and 7S fractions for variety Vinton-81. The total soybean proteins resolved into 10 major peaks, labelled peaks 1–10 (Fig. 3A). In order to identify the 11S and 7S peaks, partially purified fractions were separately chromatographed. The 11S fraction separated into six major peaks and eluted as peaks 1–4 and peaks 8 and 9 of the total proteins (Fig. 3B). On the other hand, the 7S fraction resolved into four peaks, which eluted at the positions of peaks 5,

Table 3
Percent of total protein of different protein fractions and subunits separated by SDS-PAGE and quantified by densitometry of seven soybean varieties

Variety	11S	7S	α′	α	β	Acidic	Basic
Vinton-81	20.6	10.0	3.06	4.55	2.41	9.69	10.9
S-20F8	19.5	10.5	2.96	4.96	2.62	8.69	10.8
HP-204	20.6	10.7	3.23	5.16	2.36	9.68	10.9
IA-2034	20.7	12.7	3.50	5.16	4.02	8.67	12.0
Steyer	21.0	10.4	3.22	4.74	2.39	9.92	11.1
IA-2020	23.1	11.3	3.49	5.25	2.50	10.29	12.8
S-2020	20.2	10.8	3.04	5.06	2.69	9.13	11.0

6, 7 and 10 of the total proteins (Fig. 3C). The results reported by Peterson and Wolf (1992) indicated that, of the two major storage proteins, 11S began to emerge first, but 7S began to elute before 11S was completely eluted. RP-HPLC profiles show that some of the 11S and 7S subunits are adjacent peaks, which may be due to the known dimerization and dissociation occurring between 7S and 11S protein fractions (Murphy, 1984). These results suggest that some of the 11S polypeptides with low surface hydrophobicity elute earlier, followed by the intermediate hydrophobic components of 7S and, finally, by the highly hydrophobic components of 11S and 7S.

Qualitative comparisons, based on the presence or absence of peaks, were made to detect differences among varieties. However, it was found that the RP-HPLC elution profiles of the seven different varieties were very similar. Differences in size and shape of peaks, or in retention time for a given peak, were not apparent from the chromatograms. Buehler, McDonald, Van Toai and Martin (1989) also did not find any apparent differences in the RP-HPLC elution profiles of the proteins of 12 common U.S. Midwest soybean varieties.

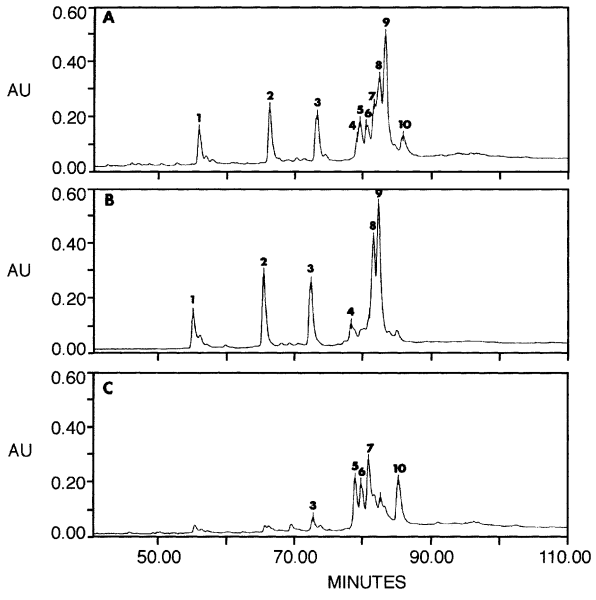

Fig. 3. RP-HPLC profile of proteins from Vinton-81 soybean variety. A: total soybean proteins; B: 11S fraction; C: 7S fraction. AU, Absorbance units.

In an attempt to identify a more consistent method for differentiation of soybean varieties, peak area percentages were calculated for peaks, and compared to detect quantitative differences among the varieties. Peak area percentage computes the area of selected peaks relative to the total area of all peaks in that chromatogram. The sum of the areas of peaks identified as 11S and 7S peaks was taken to represent the total 11S and 7S content and their ratio was calculated. Evaluations were made to determine if any relationships existed between different peaks, 11S content, 7S content, their ratio, and the tofu yield and firmness. Peak area percentages of peaks 1 through 10 for total proteins from the seven different soybean varieties are presented in Table 4. Peaks 2, 3, 5, 7, 8 and 9 constituted the major peaks, with peak area percentages that ranged from 6.59 to 11.3%, 8.91 to 11.2%, 6.53 to 11.2%, 6.84 to 12.0%, 17.4 to 20.3%, and 23.6 to 32.2%, respectively, for the varieties studied. Peaks 5 and 7 are components of the 7S fraction whereas peaks 8 and 9 are major components of the 11S fraction. Other peaks, i.e. peak 1 (range of 3.22–6.29% for the studied varieties), peak 4 (0.00–1.72%), peak 6 (3.93–7.73%) and peak 10 (2.26–8.98%) are minor contributors to 11S and 7S fractions and also showed percentage variations among different varieties. Table 5 lists the contents of 11S and 7S fractions calculated from mean percentage areas of different varieties, and these ranged from 64.9% to 77.8% and from 21.8% to 35.1%, respectively. The 11S/7S ratios ranged from 1.85 to 3.57.

3.7. Correlation of storage proteins with tofu yield and firmness

The correlations of different peaks of total proteins, 11S and 7S protein fractions and their ratios to tofu yield and texture were calculated (Table 6). Peak 5, separated from total proteins, showed significant correlation ($P<0.05$) with tofu yield ($r=0.741$) and peak 6 showed correlation ($P<0.05$) with tofu firmness ($r=-0.761$). Soybean 7S content showed negative correlation ($P<0.01$) with tofu firmness, with a value of

Table 4
Mean peak area percentages of different peaks of total soybean proteins separated by RP-HPLC

Variety	Peak1	Peak2	Peak3	Peak4	Peak5	Peak6	Peak7	Peak8	Peak9	Peak10
Vinton-81	6.15	10.4	10.9	1.60	7.33	5.23	6.84	19.9	28.1	3.67
S-20F8	4.32	11.3	9.83	1.01	6.53	3.93	8.22	19.2	32.2	3.13
HP-204	5.54	10.5	10.0	1.72	7.49	6.64	7.52	20.3	27.6	2.71
IA-2034	3.22	6.59	10.6	1.03	9.37	7.38	9.38	17.4	26.0	8.98
Steyer	6.29	9.86	10.54	0.89	11.2	4.96	11.0	19.4	23.6	2.26
IA-2020	3.31	9.13	11.2	0.95	11.1	7.00	9.08	20.0	24.6	3.66
S-2020	4.81	7.56	8.91	0.0	8.47	7.73	12.0	18.6	27.4	4.57

$r = -0.823$. Soybean 11S content ($P < 0.05$) and the 11S/7S ratio ($P < 0.01$) were also significantly correlated with tofu firmness, showing values of $r = 0.820$ and $r = 0.861$, respectively. However, peak 7 of the total proteins had an inverse correlation with tofu firmness ($r = -0.832$, $P < 0.05$). Peak 7 comprises one of the components of the 7S fraction of soybean proteins, as identified from chromatographic separation of 11S and 7S fractions. These results, therefore, indicate that 7S content, 11S content and 11S/7S ratio each appear to be associated with tofu firmness. However, peak 7 of the 7S fraction shows the most significant negative correlation. The correlations between 11S protein content and tofu firmness, and between the 11S/7S ratio and tofu firmness, were similar to findings reported by several other workers (Cai & Chang, 1999; Ji et al., 1999).

3.8. Mean peak area percentages of different peaks in 11S and 7S fractions

When the 11S and 7S protein fractions of soybean were separated by RP-HPLC, the 11S fraction resolved into six peaks and the 7S fraction separated into four peaks, with peak 3 of the 11S fraction eluting in minute quantities along with the 7S fraction. The mean peak area percentages of different peaks of the 11S and 7S fractions varied among different varieties (Tables 7 and 8). Peaks 1, 2, 3 and 4, the early eluting peaks of the 11S fraction, ranged in their mean peak area percentages from 8.95 to 10.4, 14.6 to 15.4, 12.6 to 15.2 and 3.01 to 4.72%, respectively. The major and late eluting peaks, which eluted later than the 7S fraction, i.e. peaks 8 and 9, varied from 22.0 to 27.0% and from 31.5 to 34.0%, respectively (Table 7). On the other hand, the mean peak area percentages for peaks 5, 6, 7 and 10 of the 7S fraction ranged from 19.5 to 24.7, 4.23 to 9.03, 33.5 to 46.5% and 12.4 to 26.8%, respectively, for the varieties studied (Table 8). Peak 7 constituted the major peak of the 7S fraction. Peak 3 (1.75–7.84%) eluted in minor quantities along with the 7S fraction in all the varieties.

3.9. Correlation between 11S and 7S fraction peaks and tofu yield and firmness

Peak 1 ($r = 0.741$, $P < 0.05$) and peak 3 ($r = 0.874$, $P < 0.01$) of the 11S fraction each correlated significantly with tofu yield. The major peak of the 7S fraction, i.e. peak 7, showed a negative relationship with tofu firmness ($r = -0.809$, $P < 0.05$) and peak 10 appeared to positively correlate with tofu firmness ($r = 0.880$, $P < 0.01$). This inverse correlation between peak 7 of the 7S protein fraction was also observed for peak 7 separated from the total proteins. Since peak 7 comprises a major portion of the 7S fraction, the results seem to point toward a negative association of the 7S fraction with tofu firmness. A higher 7S fraction content means a proportionally lower 11S fraction content, which results in a softer tofu gel.

Table 5
Mean peak area percentages of 11S and 7S fractions of soybean proteins separated by RP-HPLC, and their ratio

Variety	11S	7S	11S/7S
Vinton-81	76.9	23.1	3.33
S-20F8	77.8	21.8	3.57
HP-204	75.6	24.4	3.10
IA-2034	64.9	35.1	1.85
Steyer	70.6	29.4	2.40
IA-2020	69.2	30.9	2.24
S-2020	67.3	32.7	2.05

Table 6
Correlation coefficients of different RP-HPLC peaks, protein fractions and protein ratios with tofu yield and firmness ($n = 7$)

Peak	Tofu yield	Tofu firmness
5	0.741*	−0.634
6	0.558	−0.761*
7	0.046	−0.832*
9	−0.812*	0.646
7S	−0.518	−0.823*
11S	−0.507	0.820*
11S/7S	−0.561	0.861**

* $P < 0.05$.
** $P < 0.01$.

Table 7
Mean peak area percentages of the RP-HPLC peaks comprising the 11S protein fraction of seven soybean varieties

Variety	Peak 1	Peak 2	Peak 3	Peak 4	Peak 8	Peak 9
Vinton-81	9.47	14.6	14.0	3.74	25.9	32.4
S-20F8	9.16	15.3	12.6	3.66	25.2	34.0
HP-204	9.66	14.8	13.5	4.19	26.4	31.5
IA-2034	10.4	15.1	14.7	3.92	23.1	32.9
Steyer	9.99	15.0	14.1	4.72	23.6	32.6
IA-2020	9.85	15.4	15.2	4.36	22.0	33.2
S-2020	8.95	15.2	13.0	3.01	27.0	32.8

Table 8
Mean peak area percentages of the RP-HPLC peaks comprising the 7S protein fraction of seven soybean varieties

Variety	Peak 3	Peak 5	Peak 6	Peak 7	Peak 10
Vinton-81	7.84	19.5	9.03	33.5	21.6
S-20F8	3.08	21.4	6.51	36.1	26.8
HP-204	5.63	21.7	4.92	39.5	16.5
IA-2034	2.86	22.9	4.54	46.8	14.2
Steyer	4.83	19.5	5.52	42.1	18.6
IA-2020	1.75	24.7	4.23	45.0	14.7
S-2020	5.34	24.3	7.60	42.6	12.4

The positive correlations between 11S content and 11S/7S protein ratio with tofu gel firmness have been reported previously (Cai & Chang, 1999; Ji et al., 1999; Kang et al., 1991; Saio et al., 1969). Recently, breaking stress values of tofu curds prepared from soybeans having different subunits of 11S have been reported by Tezuka et al. (2000), who found that breaking stress values of tofu curds prepared from soybeans having group I 11S subunits were higher than those without, and that those tofu curds contained more protein particles (polypeptides of varying sizes obtained through centrifugation) (Ono, Choi, Ikeda, & Odagiri, 1991). These protein particles form the core of tofu curd upon coagulation (Ono, Katho, & Mothizuki, 1993). This suggests that the firmness of tofu is dependent upon the number of protein particles in the soymilk, which in turn is determined by the proportion and structure of the 11S subunits in the soybean.

Texture is considered a direct consequence of microstructure, determined by chemical composition and physical forces (Stanley & Tung, 1976). Both 11S and 7S proteins form gels upon heating and the addition of coagulant. Electrostatic interactions and disulfide bonds in the 11S proteins are important in the formation of three-dimensional networks of the protein gel, whereas hydrogen bonding and hydrophobic interactions are important for 7S protein gel networks (Utsumi & Kinsella, 1985). Heating causes dissociation of both 7S and 11S proteins, enabling them to interact with each other with preferential association of 11S basic subunits with 7S β-subunits to form soluble macrocomplexes in soy isolate gels (Utsumi & Kinsella, 1985). When the 11S/7S ratio is increased, more covalent bonds can be produced through disulfide bonding, since 11S proteins contain more total cysteine groups than 7S proteins. Therefore, it is assumed that stronger molecular forces (covalent bonding) increase tofu firmness.

4. Conclusions

Most previous studies seem to point towards a relationship between the soybean 11S protein fraction and tofu textural properties. Our current study also indicated that the 11S protein fraction, and the 11S/7S ratio are both good indicators for these properties, based on total protein analysis. In addition, strong negative relationships were found between peak 7 of the total proteins and tofu firmness, and between peak 7 of the 7S protein fraction separated by RP-HPLC and tofu firmness. An inverse correlation between this peak of the 7S fraction and tofu firmness seems to indicate a role of the 7S fraction in determining tofu firmness. Further work is needed to characterize this peak fraction of β-conglycinin (7S) in order to fully understand its role in defining tofu texture.

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