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Optimization of extraction and isolation for 11S and 7S globulins of soybean seed storage protein

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Abstract

A new method was developed for extraction and isolation of 7S and 11S fractions from soybean seed, based on methods of Nagano et al., Thanh and Shibasaki [Nagano, T., Hirotsuka, M., & Mori, H. (1992). Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *Journal of Agricultural and food chemistry* 40, 941–944 and Thanh, V. H., & Shibasaki, K. (1976). Major proteins of soybean seeds. A straightforward fraction and their characterization. *Journal of Agricultural and Food Chemistry* 24, 1117–1121]. Optimization of the extraction and isolation of 11S and 7S globulins from soybean seed was investigated under various conditions by the Kjeldahl method and SDS-PAGE. The optimal conditions were as follows: 0.03–0.06 M Tris–HCl buffer (pH 8.5) containing 0.01 M sodium bisulfite as extract solution, extraction twice at 45 °C for 1 h, and with a 1:15 ratio (*w*/*v*) of flour:Tris–HCl. The 11S fraction was precipitated at pH 6.4, and the supernatant, after centrifugation, was adjusted to pH 5.5 to remove the insoluble intermediate fraction by further centrifugation. The supernatant obtained was then adjusted to pH 4.8 to afford the 7S fraction as a precipitate by centrifugation. With the improvements, the protein contents and purities of the isolated 11S and 7S fractions were significantly increased. The contents of all subunits of the isolated 11S and 7S fraction were markedly higher than those by Thanh and Shibasaki method, while the contents of α, β and B_3 were also significantly higher than those by Nagano et al. method.

Keywords: Soybean (*Glycine max* (L.) Merr.); Seed storage protein; 11S and 7S fraction; Extraction and isolation; Optimization

1. Introduction

Soybean protein is one of the important vegetable protein resources due to its functional properties and high nutritional value. Glycinin (11S) and β-conglycinin (7S), the two major storage protein components in soybean, account for approximately 70% of total storage proteins in soybean seed (Mujoo, Trinh, & Ng, 2003; Riblett, Herald, Schmidt, & Tilley, 2001; Silvana & Maria, 1995). Glycinin is a hexameric molecule, and each subunit consists of an acidic (A) and basic (B) polypeptide chain connected by a disulphide linkage. Basically, β-conglycinin is a trimer which consists of α′, α and β subunits without disulphide linkages (Mujoo et al., 2003; Tezuka, Yagasaki, & Ono, 2004; Utsumi & Kinsella, 1985). Five genetic variants of 11S globulin were identified based on the homology of their subunit sequences, which can be divided into group I ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) and group II (A_3B_4, $A_5A_4B_3$) (Badley et al., 1975; Mujoo et al., 2003; Nielson, 1985). It was also demonstrated that the 11S fraction differs noticeably from the 7S fraction in physicochemical, nutritional and functional properties (Maruyama et al., 1999; Prak et al., 2005; Utsumi & Kinsella, 1985).

Several methods have been developed for the preparation of 7S and 11S fractions, including ultracentrifugation,

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fractionation, and reversed-phase high performance liquid chromatography (Garcfa, Torre, Laborda, & Marina, 1997; Hill & Breidenbach, 1974; Nagano, Hirotsuka, & Mori, 1992; Thanh & Shibasaki, 1976; Wu, Murphy, Johnson, Fratzke, & Reuber, 1999; Wu et al., 2000). The methods of Thanh and Shibasaki (1976) and Nagano et al. (1992) seem to be simpler and are able to afford a large-scale preparation of the two major proteins. Therefore, they have been frequently used for preparing 11S and 7S fractions when studying their solubility, heat denaturation, gelling properties, emulsibility, viscoelasticity, foam formation and stabilization, rheological properties, water binding capacity, surface hydrophobicity and structural characteristics (Hou & Chang, 2004; Lehahardt & Gibson, 1983; Mujoo et al., 2003; Nir, Feldman, Aserin, & Garti, 1994; Silvana et al., 1995; Tezuka et al., 2004).

Some modifications were made, based on the method of Thanh and Shibasaki (1976), by use of sodium bisulphite (SBS) instead of the traditional regent, 2-mercaptoethanol (2-ME). Another pH (5.0) adjustment was conducted after the isolation of 11S globulin, and the intermediate product was discarded and solid NaCl was added to the supernatant to a final concentration of 0.25 M. Thus, the purity of isolation of the 7S fraction and the yield of 11S fraction were elevated to above 90% (Nagano et al., 1992). However, the yield of 7S fraction was reduced by more than two-thirds with the method of Nagano et al. (1992). Wu et al. (2000) also reported a simplified process, based on the method of Nagano et al. (1992) for the fractionation of soybean glycinin and β-conglycinin. The method is able to afford a larger quantity of 7S-rich fraction, but at the expense of its purity. Many studies have been reported on yield, protein content, and purities of the isolated fractions. However, little has been done on yields of the corresponding subunits of the isolated fractions. It is of great interest to increase both yields and purities of isolated 11S and 7S fractions, and to evaluate the modifications on the subunits. Therefore, the objectives of this work were to investigate the main factors affecting the extraction and isolation of 7S and 11S fractions, and to obtain both high yields and protein purities of 11S, 7S fractions and their subunits through modification of the methods of Nagano et al. (1992) and Thanh and Shibasaki (1976). The main factors investigated in the present study included extract solution, extracting pH and temperature, duration of extraction, flour-to-water ratio and reducing agent.

2. Materials and methods

2.1. Materials and reagents

Soybean (*Glycine max* (L.) Merr.) seeds of the cultivar "Nannongdahuangdou" were used in this study. The cultivar was planted in July 15, and harvested in October 20, in 2004, in Jiangpu Agriculture Experiment Station, Nanjing, Jiangsu province, PR China. All seeds were stored at 4 ± 0.5 °C, and used within two months. Tris–(hydroxymethyl) aminomethane, 2-ME, SBS, sodium dodecyl sulfate (SDS), acrylaminde, bis-acrylamide, ammonium persulfate (AP), 4-tetramethyl ethylerediamine (TEMED), Coomassie brilliant blue R-250 and bovine serum albumen (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were of analytical grade, purchased from Nanjing Chemical Industry (Nanjing, China).

2.2. Preparation of defatted soybean flour

Soybean seeds were peeled, ground in a Straub grinding mill (model 4E, Straub Co., Philadelphia, PA) to pass through a 60-mesh sieve, and defatted by *n*-hexane extraction (soy flour/hexane = 1:5, *v*/*v*) for 1 h at room temperature. After centrifugation (8000×*g*, 15 min, 4 °C), the supernatant was discarded and the precipitate was extracted twice more. The defatted flour was collected for protein extraction.

2.3. Isolation methods of 7S and 11S globulins

The 7S and 11S globulins were purified from soybean seeds according to the methods of Thanh and Shibasaki (1976) and Nagano et al. (1992), respectively. The fraction yield was expressed as a percentage of the isolated dried fraction weight to defatted soy flour weight. Protein concentration in the samples was determined according to the method of Bradford (1976) using BSA as standard. The protein content was determined by the micro-Kjeldahl method (AACC, 2000b) and a nitrogen to protein conversion factor of 6.25 was used. The analysis was carried out in duplicate, and the values were averaged.

2.4. Optimization of the extracting factors

Five experiments were conducted to optimize the effects of the extracting factors on the isolation efficiency of 7S and 11S globulins. Experiment I was designed to evaluate the influence of extracting solution (pH 7.5 water and pH 7.5 Tris–HCl buffer); Experiment II, studied different pH values (7.5, 8.0, 8.5, and 9.0) of extracting solution; Experiment III studied temperature (25, 35, 45, 55, and 65 °C) of the extracting solution. Experiment IV studied the flour:-Tris–HCl buffer ratio (1:6, 1:8, 1:10, 1:15, and 1:20 *w*/*v*); Experiment V studied the $NaHSO_3$ concentration (0.005, 0.010, and 0.100 M). All other isolating procedures of the five experiments were done according to the method of Nagano et al. (1992).

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

Sodium dodecyl sulfate-polyacrylamide gel eletrophoresis (SDS-PAGE) was performed in a vertical slab gel of 1 mm thickness according to the method of Laemmli (1970). The electrophoretic patterns were analyzed by a scan-

ning densitometer (VersaDco 3000, Bio-Rad) with Quantity-one software (version 4.6). The protein bands of acidic and basic polypeptides of 11S and the subunits of 7S were identified according to the relative mobilities of published photographs of soybean seed SDS-PAGE gels (Mujoo et al., 2003). The relative protein quantity of each subunit (protein band) of 11S and 7S fractions was calculated from its respective percent area on the densitograms against the total area of 11S and 7S. The fraction purity of isolated 7S or 11S was calculated from the total respective percent area of all their subunits against the total 11S and 7S area.

2.6. Statistical analysis

One-factor analysis of variance (ANOVA) and shortest significant ranges (SSR) test at the 5% level were used to evaluate differences among means (SAS 8.2).

3. Results and discussion

3.1. Determination of the types of the extracting solution

Water and 0.03 M Tris–HCl buffer were used as protein extracting solutions, respectively, and the results are listed in Table 1. Compared with water as extracting solution, the yields of the isolated 11S, 7S and 11S + 7S mixture by using 0.03 M Tris–HCl buffer as extracting solution were increased ($p \leqslant 0.05$) by 2.01%, 1.16% and 3.17%, respectively. The purity of the isolated 11S fraction was also significantly increased ($p \leqslant 0.05$), by 2.25%. Thus, 0.03 M Tris–HCl buffer as extracting solution had an advantage over water. The result coincided with previous reports (Nagano et al., 1992; Thanh & Shibasaki, 1976).

3.2. Determination of pH value of extracting solution

The extracting solution pH value in the method of Thanh and Shibasaki (1976) was 8.0. But it is known that the isoelectric point of the basic subunits of 11S globulin is 8.0–8.5 (Silvana et al., 1995). The lower pH value (below 8.0) might be unfavourable for the extraction of the basic subunits. Therefore, it is necessary to examine the pH value of extracting solution further. When the pH value of the extracting solution was adjusted from 7.5 to 9.0, the yields of the isolated 11S, 7S fractions and 11S + 7S mixture, and the protein contents and purities of the isolated 11S and 7S fractions, seemed to increase at first, and then to decrease slightly above pH 8.5 (Table 2). The highest yields of the isolated 11S, 7S fractions and 11S + 7S mixture, the highest protein contents and fraction purities of the isolated 11S and 11S fractions were obtained at pH 8.5. For the 11S fraction, the yields and protein purities between pH 7.5 and 8.0, and the protein content at pH 7.5 were markedly lower, but no significant differences were observed among other pH levels. For the 7S fraction, the yields between pH 7.5 and 8.0 were significantly lower, and no significant differences in the protein content and purity were found among pH levels. The results indicated that the optimum pH of the extracting solution was 8.5.

3.3. Determination of the extracting temperature

Riblett et al. (2001) reported that the initial denaturalizing temperatures for glycinin and β-conglycinin are approximately 82 °C and 68 °C, respectively. Therefore, the extraction temperature range from 25 °C to 65 °C was selected in our experiment. With the temperature rising, the yields of the isolated 11S, 7S fractions and 11S + 7S mixture seemed to increase at first, and then to decrease. The highest yields were obtained at 45 °C. The protein contents of the isolated 11S fraction changed little between 25 °C and 55 °C, but markedly decreased above 55 °C and the purities of both isolated 7S and 11S changed little from 25 °C to 45 °C, but decreased above 55 °C (Table 3). The main reason for the decreases of yield, protein content and purity might be protein denaturalization caused by the

Table 1
The effects of the different types of extracting solutions on yield, protein content and purity of the isolated 11S and 7S fractions of soybean storage proteins[A]

Extracting solution	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
Water	11.1[b]	8.47[b]	19.6[b]	93.12	91.5	89.3[b]	92.0
Tris–HCl buffer	13.1[a]	9.63[a]	22.8[a]	93.85	91.7	91.4[a]	92.7

[A] (a–b): The superscripts following each figure indicate significant differences at 0.05 level.

Table 2
The effects of the pH value of the extracting solution on yield, protein content and purity of the isolated 11S and 7S fractions of soybean storage proteins[A]

pH value	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
7.5	11.1[c]	8.47[c]	19.6[c]	93.12[b]	91.5	89.3[bc]	92.0
8.0	11.9[bc]	8.64[bc]	20.5[b]	93.27[ab]	91.6	90.2[b]	92.1
8.5	12.7[a]	9.59[a]	22.3[a]	94.29[a]	91.8	91.5[a]	92.4
9.0	12.6[a]	9.52[a]	22.2[a]	94.21[a]	91.7	91.4[a]	92.2

[A] (a–c): The superscripts following each figure indicate significant differences at 0.05 level.

Table 3
The effects of extraction temperature on yield, protein content and purity of the isolated 11S and 7S fractions of soybean storage proteins[A]

Temperature (°C)	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
25	11.1^{b}	8.47^{c}	19.6^{c}	93.12^{a}	91.5^{a}	91.0^{a}	92.0^{a}
35	12.0^{ab}	9.29^{b}	21.3^{b}	93.2^{a}	91.8^{a}	90.7^{a}	91.2^{a}
45	13.0^{a}	10.3^{a}	23.3^{a}	93.9^{a}	92.3^{a}	90.6^{a}	91.1^{ab}
55	9.44^{c}	8.71^{bc}	18.2^{d}	92.3^{a}	90.7^{b}	87.2^{b}	89.7^{b}
65	6.48^{d}	2.33^{d}	8.81^{e}	87.2^{b}	89.3^{c}	85.5^{c}	87.3^{c}

[A] (a–e): The superscripts following each figure indicate significant differences at 0.05 level.

rising of temperature. The extracting efficiency was slightly lower for the method of Thanh and Shibasaki (1976) or Nagano et al. (1992), because the protein extraction was done at room temperature. Therefore, the suitable extracting temperature was 45 °C.

3.4. Determination of the flour:buffer ratio

The flour:water (or buffer) ratios of the methods descried by Thanh and Shibasaki (1976) and Nagano et al. (1992) were set at 1:20 and 1:15, respectively. Table 4 shows that the yields, protein contents and purities of isolated 11S and 7S fractions increased by 5.23% and 2.79%, 3.6% and 3.69%, 2.69% and 2.13%, respectively, when the flour:buffer ratio increased from 1:6 to 1:20. This indicated that the rising flour:buffer ratio could increase the protein solubility and raise the yields, protein contents and purities of the isolated 11S and 7S fractions. However, the cost and the container's volume would increase with the raising of the ratio and, thus, its practical application would be limited. As shown in Table 4, the ratio of 1:20 could just markedly increase the yield of the 11S + 7S mixture and the protein content of 11S compared to those with the flour:-buffer ratio of 1:15. Consequently, the preferred flour:buffer ratio was 1:15.

3.5. Determination of the concentration of the reducing agent

The 2-ME and SBS, acting on the disulfide bond of protein and causing the depolymerization of the polymeric compounds, could increase the solubility of protein. 2-ME was used as the reducing agent in the method of Thanh and Shibasaki (1976). It was confirmed that SBS could be used instead of 2-ME, and the protein contents of the 11S globulin fraction were 19% and 41%, respectively, for 2-ME and SBS (Nagano et al., 1992) and a concentration of 0.01 M of SBS was used by some researchers (Silvana et al., 1995; Wu et al., 1999, 2000). The present study indicated that, with the rising SBS concentration, the yields, protein contents, and purities of isolated 7S and 11S increased at first, and then decreased (Table 5). At a concentration of 0.01 M, the yields, protein contents and purities were the highest and, furthermore, the yield of isolated 11S, protein contents and purity of isolated 7S were markedly different from those of the other two concentrations. SBS was preferred to be used at 0.01 M.

Moreover, the discarded insoluble substance obtained by the first centrifugation contained more than 30% protein (data not shown) and the protein was partly lost (see Fig. 1: the insoluble substance ①). In addition, it has been reported that the β subunit is composed of four fractions with iso-

Table 4
The effects of flour-to-water ratio on yield, protein content and purity of the isolated 11S and 7S fractions of soybean storage proteins[A]

Flour:buffer ratio	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
1:6	6.72^{d}	6.39^{d}	13.1^{d}	91.9^{c}	88.6^{d}	88.6^{c}	90.3^{c}
1:8	8.71^{c}	7.60^{c}	16.3^{c}	92.7^{bc}	90.2^{c}	89.1^{b}	91.5^{b}
1:10	10.8^{b}	8.07^{b}	18.8^{bc}	93.1^{b}	91.0^{b}	90.2^{ab}	91.6^{ab}
1:15	11.1^{ab}	8.47^{ab}	19.6^{b}	93.1^{b}	91.5^{ab}	91.0^{a}	92.0^{a}
1:20	12.0^{a}	9.18^{a}	21.1^{a}	95.5^{a}	92.3^{a}	91.3^{a}	92.4^{a}

[A] (a–d): The superscripts following each figure indicate significant differences at 0.05 level.

Table 5
The effects of the concentration of reducing agent SBS on yields, protein contents and purities of 11S and 7S fractions[A]

Concentration (mol/L)	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
0.005	11.7^{b}	9.12^{a}	20.5^{ab}	94.4^{a}	91.1^{b}	94.0^{a}	92.8^{b}
0.010	12.5^{a}	9.31^{a}	21.8^{a}	95.6^{a}	92.7^{a}	92.6^{a}	93.4^{a}
0.100	10.1^{c}	8.98^{b}	19.1^{b}	92.0^{b}	89.9^{b}	92.3^{b}	91.5^{c}

[A] (a–c): The superscripts following each figure indicate the significant differences at 0.05 level.

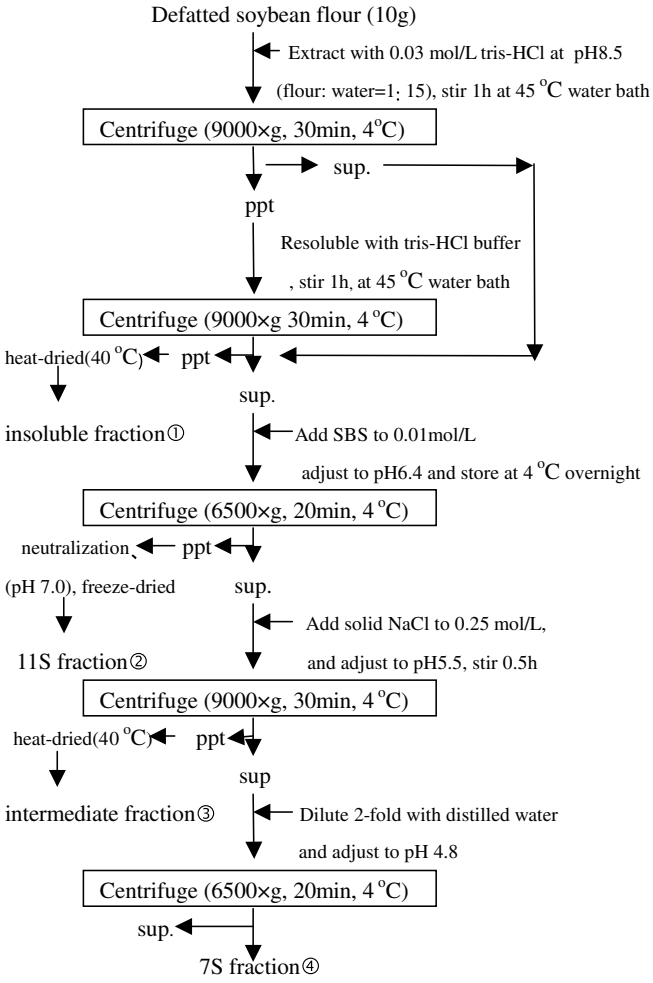

Fig. 1. Schematic diagram for the isolation of 11S and 7S fractions by the improved method (Author: Chun Liu).

electric points ranging from 5.8 to 6.2 (β_1–β_4), whereas α and α′ consist of a single fraction having isoelectric points of 5.2 and 5.3, respectively (Silvana et al., 1995). During the isolating process, adjusting pH value to 5.0 after isolation of the 11S fraction, might cause a proportionate loss of α and α′ as intermediate product (see Fig. 1: the intermediate product ③), and it was necessary to raise the pH value to 5.5 in order to decrease the loss of proteins.

3.6. Operating procedures of the improved method

The scheme for the improved method is presented Fig. 1. Briefly, 10 g defatted soyflour were homogenized (800×*g*, 5 min) with 150 ml of pH 8.5 Tris–HCl buffer (*w*/*v* = 1:15) in a 400 ml beaker by a electromagnetic stirrer, then incubated at 45 °C with stirring at 1000×*g* for 1 h. The extraction solution was centrifuged (9000×*g*, 30 min, 4 °C). The precipitate was treated as described above. The two supernatants were combined, treated with solid SBS to a concentration of 0.01 M, and followed by adjustment of pH value to 6.4 with 2 N HCl. The solution was kept at 4 °C overnight. The precipitate after centrifugation (6500 × *g*, 20 min, 4 °C) was washed three times with pure water and then dissolved in pure water. The solution was adjusted to pH 7.0 with 2 N NaOH, and then dialyzed over water and freeze-dried to obtain the 11S fraction. The supernatant was treated with solid NaCl to 0.25 M and adjusted to pH 5.5 with 2 N HCl, followed by stirring at room temperature for 0.5 h. The solution was centrifuged (9000×*g*, 30 min, 4 °C) and the precipitate was heat-dried to obtain the intermediate product. The supernatant was diluted twofold with pure water, adjusted to pH 4.8 with 2 N HCl, and then centrifuged (6500×*g*, 20 min, 4 °C). The precipitate was processed as the 7S fraction.

3.7. Comparison between the improved method and Thanh and Shibasaki (1976) method and Nagano et al. (1992) method

The methods of Thanh and Shibasaki (1976) and Nagano et al. (1992), as well as our present study, were compared in terms of the yields, protein contents, and purities of the isolated 7S and 11S (Table 6). The yields of isolated 11S, protein contents and purities of isolated 11S and 7S by the improved method were significantly higher than those by the methods of Nagano et al. (1992) and Thanh and Shibasaki (1976), respectively. The yields of 7S and 11S + 7S mixture by the improved method were markedly higher than those by the Nagano et al. (1992) method, but lower than those by Thanh and Shibasaki (1976) method. The differences might be attributed to the fact that the improved procedure increased the protein solubility and decreased the protein content of the abandoned intermediate products. The results from Thanh and Shibasaki (1976) method in this study were proved to be the same as those reported by Thanh and Shibasaki in 1976, whereas those by the Nagano et al. (1992) method were basically the same as reported by Nagano et al. in 1992, only with the lower yield of 7S fraction. Although the yields of 7S and 11S + 7S mixture by Thanh and Shibasaki (1976) were higher, the protein content and purity were

Table 6
The effects of different isolating methods on yield, protein content and purity of 11S and 7S fractions of soybean storage proteins[A]

Method	Fraction yield (%)			Protein content (%)		Fraction purity (%)	
	11S	7S	11S + 7S	11S	7S	11S	7S
Thanh and Shibasaki (1976) method	12.1[a]	17.1[a]	29.23[a]	92.1[c]	84.1[c]	78.6[c]	63.0[c]
Nagano et al. (1992) method	11.1[c]	8.47[c]	19.6[c]	93.1[b]	91.5[b]	89.3[b]	92.0[b]
The improved method	14.4[a]	10.7[b]	25.1[b]	96.3[a]	93.3[a]	92.5[a]	95.5[a]

[A] (a–c): The superscripts following each figure indicate significant differences at 0.05 level.

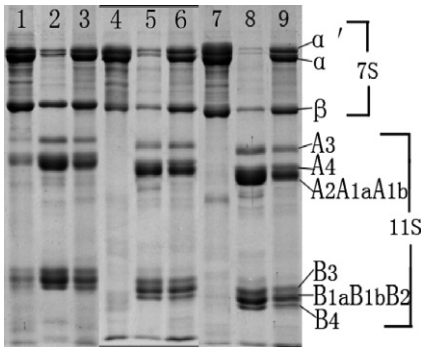

Fig. 2. SDS-PAGE patterns of the total soybean seed protein and fractions (Author: Chun Liu) 1, 2, 3: 7S fraction, 11S fraction, total protein of soybean seed isolated by Thanh and Shibasaki (1976) method; 4, 5, 6: isolated by the Nagano et al. (1992) method; 7, 8, 9: isolated by improved method.

much lower. This might be due to the contamination of isolated 7S by other fractions and a lack of pH adjustment.

The subunit compositions and contents between the 11S and 7S fractions were found to be different among the three methods (Fig. 2). The 7S fraction isolated by Thanh and Shibasaki (1976) method was contaminated by a lot of 11S subunits (approximately 34.6%, including A_3, $A_4A_2A_{1a}A_{1b}$, B_3, $B_{1a}B_{1b}B_2$ and B_4 subunits) (Fig. 3a), and the 7S fraction isolated by the Nagano et al. (1992) method was almost not contaminated by 11S basic subunits (Fig. 3b). The 11S fractions isolated by Thanh and Shibasaki (1976) and Nagano et al. (1992) methods were contaminated by approximately 19.7% and 9.18% 7S subunits (α', α and β), respectively (Fig. 3d and e). These results were consistent with previous studies (Mujoo et al., 2003; Nagano et al., 1992; Thanh & Shibasaki, 1976). The 7S fraction

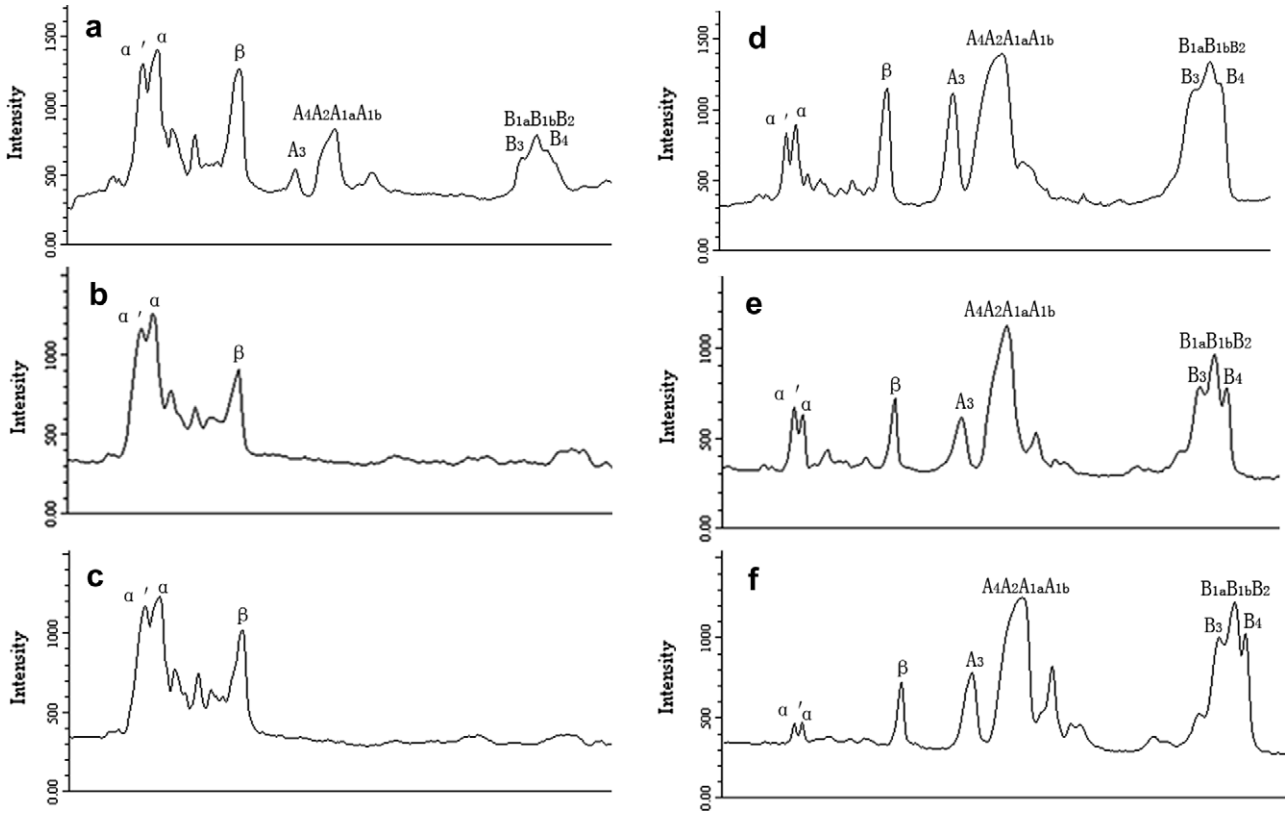

Fig. 3. Densitograms corresponding to SDS-PAGE electrophoresis of the 11S and 7S fractions isolated by three methods, (Author: Chun Liu) (a) 7S fraction isolated by Thanh and Shibasaki (1976) method, (b) 7S fraction isolated by Nagano et al. (1992) method, (c) 7S fraction isolated by the improved method, (d) 11S fraction isolated by Thanh and Shibasaki (1976) method, (e) 11S fraction isolated by Nagano et al. (1992) method, (f) 11S fraction isolated by the improved method.

Table 7
The effects of different isolating methods on subunit contents of the isolated 11S and 7S fractions of soybean storage proteins[A]

Method	11S subunit content (%)					7S subunit content (%)		
	A_3	Ax[B]	B_3	B_1B_2	B_4	α'	α	β
Thanh and Shibasaki (1976) method	14.5^{b}	31.0^{b}	11.6^{c}	12.8^{b}	8.59^{b}	21.0^{b}	22.1^{c}	20.0^{c}
Nagano et al. (1992) method	15.5^{a}	32.6^{ab}	13.4^{b}	17.1^{a}	10.8^{a}	32.5^{a}	33.6^{b}	25.9^{b}
The improved method	15.8^{a}	33.4^{a}	14.9^{a}	17.5^{a}	10.8^{a}	33.2^{a}	35.0^{a}	27.3^{a}

[A] (a–c): The superscripts following each figure indicate significant differences at 0.05 level.
[B] Ax contains $A_4A_2A_{1a}A_{1b}$ subunits.

isolated by the present method was almost un-contaminated by 11S subunits (Fig. 3c), a similar result to Nagano et al. (1992) method. However, the 11S fraction isolated by the present method contained 6.38% 7S subunits (α', α and β), but much lower than that by Thanh and Shibasaki (1976) or Nagano et al. (1992) method, especially for α' and α subunits (Fig. 3f).

With the improvement of extraction and isolation, the protein purities and subunit contents of the isolated 11S and 7S fraction were increased. The contents of all subunits of 11S and 7S fractions isolated by the improved method were markedly higher than those by Thanh and Shibasaki (1976) method while the contents of α, β and B_3 isolated by the present method were significantly higher than those by the Nagano et al. (1992) method, but other subunits increased a little (Table 7).

4. Conclusion

The methods of Thanh and Shibasaki (1976) and Nagano et al. (1992) are the two major methods for extraction and isolation of 7S and 11S fractions from soybean seed. A new method was developed, based on the two methods, through modifications. With the new method, the protein contents and purities of the isolated 11S and 7S fractions were increased significantly and the contents of all subunits of the isolated 11S and 7S fraction were markedly higher than those by Thanh and Shibasaki (1976) method.

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References

AACC, (2000). Crude protein-micro Kjeldhal method. In approved methods of the AACC (Vol-II, 10th ed.). AACC method, 46-13.

Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P., & Stubbs, J. M. (1975). The structure, physical and chemical properties of the soybean protein glycinin. *Biochimica et Biophysica Acta, 412*, 214–228.

Bradford, M. M. A. (1976). Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye biding. *Analytical Biochemistry, 72*, 248–254.

Garcfa, M. C., Torre, M., Laborda, F., & Marina, M. L. (1997). Rapid separation of soybean globulins by reversed-phase high performance liquid chromatography. *Journal of Chromatography A, 758*, 75–83.

Hill, J. E., & Breidenbach, R. W. (1974). Proteins of soybean seeds I. Isolation and characterization of the major components. *Plant Physiology, 53*, 742–746.

Hou, H. J., & Chang, K. C. (2004). Structural characteristics of purified glycinin from soybeans stored under various conditions. *Journal of Agricultural and food chemistry, 52*, 3792–3800.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature, 227*, 680–685.

Lehahardt, W. F., & Gibson, F. T. (1983). Fractionation and isolation of 7S and 11S protein from isoelectrically precipitated vegetable protein mixtures. US Patent, 4, 370, 267.

Maruyama, N., Sato, R., Wada, Y., Matsumura, Y., Goto, H., Okuda, E., et al. (1999). Structure-physicochemical function relationships of soybean β-conglycinin constituent subunits. *Journal of Agricultural and food chemistry, 47*, 5278–5284.

Mujoo, R., Trinh, D. T., & Ng, P. K. W. (2003). Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. *Food Chemistry, 82*, 265–273.

Nagano, T., Hirotsuka, M., & Mori, H. (1992). Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *Journal of Agricultural and food chemistry, 40*, 941–944.

Nielson, N. C. (1985). The structure and complexity of the 11S polypeptide in soybean. *Journal of American Oil Chemists Society, 49*, 2733–2740.

Nir, I., Feldman, Y., Aserin, A., & Garti, N. (1994). Surface properties and emulsification behavior of denatured soy proteins. *Journal of Food Science, 59*, 606–609.

Prak, K., Nakatani, K., Katsube-Tanaka, T., Adachi, M., Maruyama, N., & Utsumi, S. (2005). Structure-function relationships of soybean Proglycinins at subunit levels. *Journal of Agricultural and food chemistry, 53*, 3650–3657.

Riblett, R. C., Herald, T. J., Schmidt, K. A., & Tilley, K. A. (2001). Characterization of β-conglycinin and glycinin soy protein fractions from four selected soybean genotypes. *Journal of Agricultural and food chemistry, 49*, 4983–4989.

Silvana, P., & Maria, C. A. (1995). Soy protein isolate components and their interactions. *Journal of Agricultural and Food Chemistry, 43*, 1762–1767.

Tezuka, M., Yagasaki, K., & Ono, T. (2004). Changes in characters of soybean glycinin groups I, IIa, and IIb caused by Heating. *Journal of Agricultural and Food Chemistry, 52*, 1693–1699.

Thanh, V. H., & Shibasaki, K. (1976). Major proteins of soybean seeds. A straightforward fraction and their characterization. *Journal of Agricultural and Food Chemistry, 24*, 1117–1121.

Utsumi, S., & Kinsella, J. E. (1985). Structure-function relationship in food proteins, subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate proteins. *Journal of Agricultural and Food Chemistry, 33*, 297–303.

Wu, S., Murphy, P. A., Johnson, L. A., Fratzke, A. R., & Reuber, M. A. (1999). Pilot-plant fractionation of soybean glycinin and β-conglycinin. *Journal of American Oil Chemists Society, 76*, 285–293.

Wu, S., Murphy, P. A., Johnson, L. A., Johnson, L. A., Reuber, M. A., & Fratzke, A. R. (2000). Simplified process for soybean glycinin and β-conglycinin fractionation. *Journal of Agricultural and Food Chemistry, 48*, 2702–2708.