Pilot-Plant Fractionation of Soybean Glycinin and β-Conglycinin

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ABSTRACT: A laboratory process for separating glycinin and β-conglycinin from soybean flakes was successfully scaled up to the pilot-plant scale (15 kg soy flakes). Average yields of the glycinin and β-conglycinin fractions were both 9.4% on a dry basis (db). The protein contents of glycinin and β-conglycinin fractions were 92.8 and 97.7% db, respectively. The glycinin and β-conglycinin purities were 90.4 and 72.7% of the protein content, respectively, which were very comparable to those of the laboratory-scale process. The total sulfhydryl plus half cystine content of the glycinin fraction was 37.8 mol/mol protein and 14.8 mol/mol protein for the β-conglycinin fraction. The native glycinin structure loss in the glycinin fraction was negligible. The native β-conglycinin loss in the β-conglycinin fraction was 10%, as estimated by rocket immunoelectrophoresis analysis. Hydrophobicity index value showed that hydrophobic properties of the pilot-plant protein fraction were ordered, from high to low: β-conglycinin fraction > glycinin fraction > intermediate mixture fraction.

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KEY WORDS: β-Conglycinin, glycinin, pilot-plant process, protein, protein physicochemical properties, protein yield and purity, soybean proteins, soybeans, soy protein separation.

Glycinin and β-conglycinin, the two major soybean storage proteins, apparently play different roles in food and nonfood soy protein products due to their different functional properties such as hydrophobicity, solubility, sulfhydryl cross-linking, gelation, and film formation. Wolf *et al.* in 1962 (1) first obtained 91–93% ultracentrifugally pure glycinin (11S) by cryoprecipitation followed by fractionation with ammonium sulfate. They reported 25% yield for the purified glycinin. Factors influencing yield and purity of the glycinin fraction, such as extraction ratio, temperature, pH, salt and sugar content, and reducing reagent (2-mercaptoethanol, 2-ME), have been investigated (2).

Koshiyama (3) reported a procedure for glycinin and β-conglycinin (7S) fractionation. The glycinin fraction was first cryoprecipitated, and then 0.025 M $CaCl_2$ was added to remove the residual cold-insoluble proteins. The 7S fraction, which contained mostly β-conglycinin, was precipitated by adjusting the pH of the supernatant to 4.5. After gel filtration, a homogeneous β-conglycinin fraction was obtained as evaluated by ultracentrifugal analysis, which does not differentiate among different proteins of comparable mass.

Thanh *et al.* in 1975 and 1976 (4,5) developed a straightforward process for glycinin and β-conglycinin separation based on differential solubilities of glycinin and β-conglycinin at pH 6.1–6.6. Tris (THAM) buffer (pH 8.0) containing 10 mM 2-mercaptoethanol (ME) was used to extract soy proteins. Glycinin was separated by adjusting the pH to 6.4 and collecting the precipitate after centrifuging at 2–5°C. β-Conglycinin was precipitated at pH 4.8 and purified by redissolving the precipitate in the 0.03 M Tris buffer and adjusting the pH to 6.2. The β-conglycinin fraction was kept at 3–5°C overnight and obtained after centrifuging to remove undissolved polymerized forms. This particularly useful method for preparing the two soybean globulins has been used for many years, because glycinin and β-conglycinin components can be separated simultaneously. However, cross contamination of proteins in both globulin fractions is a continuing problem. The glycinin fraction was 79% glycinin, 6% β-conglycinin, and 15% other components (6). The β-conglycinin fraction was only 52% β-conglycinin, 3% glycinin, and 45% others. The method of Thanh *et al.* thus requires procedures such as gel filtration and affinity chromatography for purification, which are costly and difficult to scale up.

O'Keefe and co-workers (7) modified the method of Thanh *et al.* to improve the purity of crude β-conglycinin fraction. Two isoelectric precipitations at pH 6.4 and 4.8 were replaced with three precipitations at pH 6.4, 5.3, and 4.8. The purity of the crude β-conglycinin increased, however, at the expense of yield. Nagano *et al.* (8) modified the method of Thanh *et al.* in three ways: (i) soy proteins were extracted by using water at pH 7.5 instead of Tris buffer; (ii) sodium bisulfite was used as a reductant; and (iii) three protein fractions were precipitated at pH 6.4, 5.0, and 4.8 instead of two at pH 6.4 and 4.8. Nagano *et al.* claimed the purities of crude glycinin and β-conglycinin were >90% as measured by densitometer scans of gels from sodium dodecylsulfate-polyacrylamide gel in electrophoresis (SDS-PAGE).

All these separation processes for glycinin and β-conglycinin have been limited to laboratory scale, producing milligram or gram quantities of protein products. A pilot-plant process study, however, is required to generate information needed for plant scale-up and to yield sufficient amounts of

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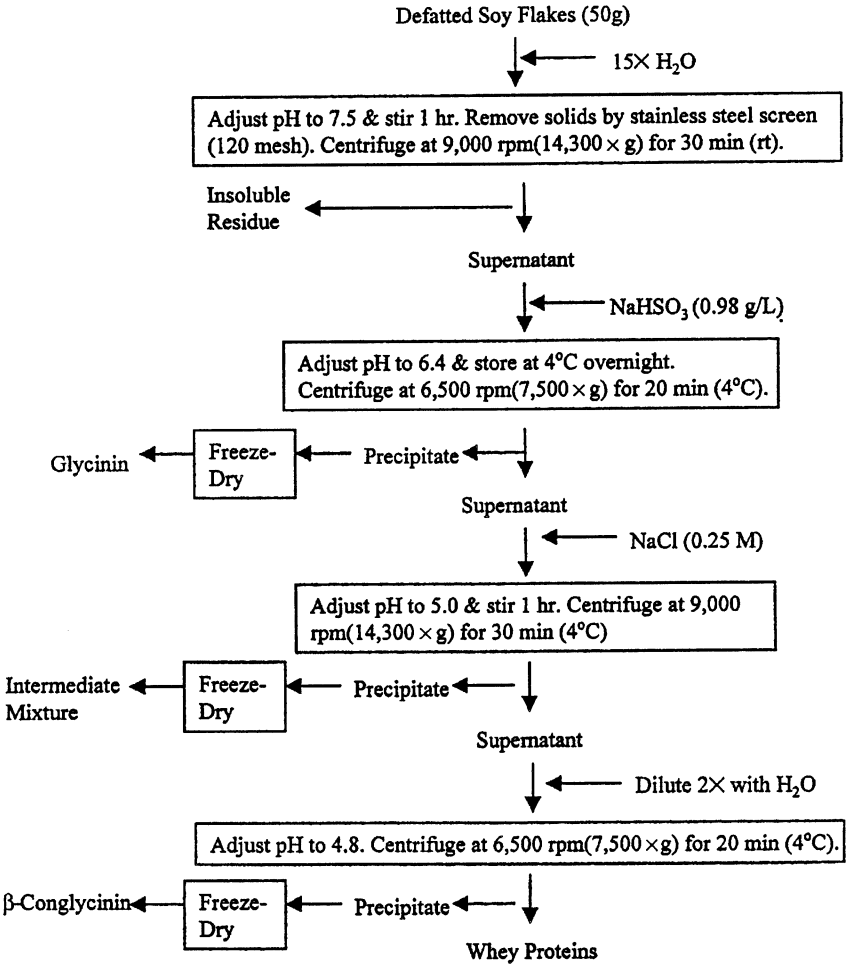
the individual proteins for further food and nonfood utilization studies. We have successfully developed a pilot-plant process, a modification of Nagano's laboratory process, which yields kelogram quantities of glycinin and β-conglycinin fractions from defatted soy flakes. The sulfhydryl and disulfide contents, hydrophobicity, and native structure of glycinin and β-conglycinin fractions produced in the pilot-plant were evaluated.

EXPERIMENTAL PROCEDURES

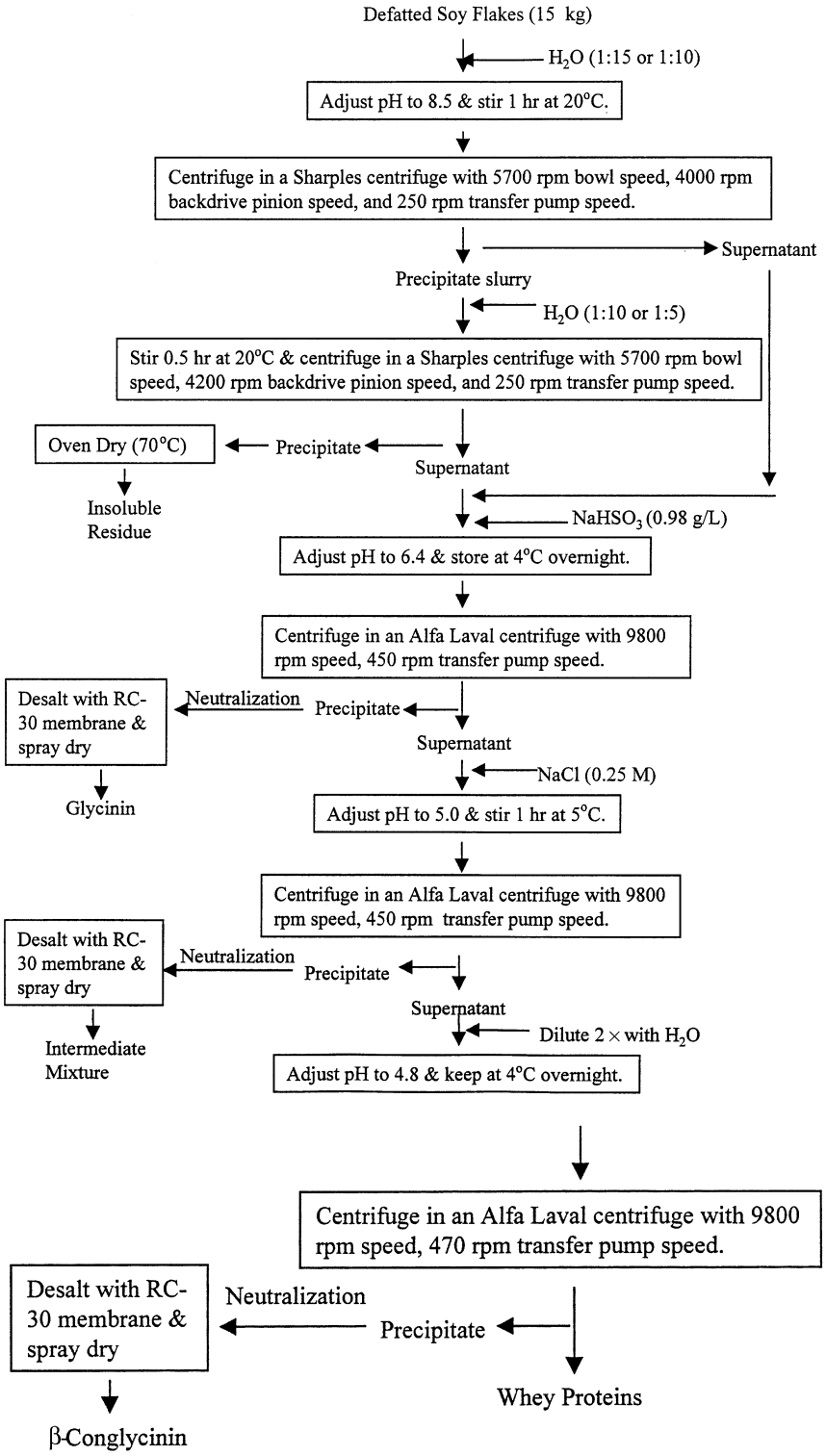
Soy white flakes. Soybean white flakes were produced from IA 3003 (1995 crop, Iowa) and MBS 2795 (1995 crop, Iowa) soybeans in the pilot plant of the Center for Crops Utilization Research, Iowa State University. Whole soybeans were cracked, aspirated to remove hulls, then conditioned by heating cotyledons up to 60°C just prior to flaking. Soy flakes (0.25–0.31 mm thickness) were then extracted with commercial-grade hexane at 65.5°C and a solvent-to-flake ratio of 1.75 to 1 in a batch-advance percolation-type solvent extractor (French Oil Mill Machinery Co., Piqua, OH). The flakes were extracted in five stages. Each extraction stage was 10 min with 4 min of draining between stages and 1 h for final draining. The solvent-laden defatted flakes were unloaded at a slow rate into the desolventizer/toaster (DT) under vacuum at ambient temperature to reduce the hexane content. To produce soybean white flakes with high protein solubility, the remaining solvent was removed by air drying for 24 h at ambient temperature. The defatted flakes were double-bagged in polyethylene bags that were sealed and kept in the cold (2.8°C). The IA 3003 soybean flakes contained 7.8% moisture and 55.1% protein on a dry basis (db). The MBS 2795 soybean flakes contained 11.4% moisture and 53.5% protein (db).

Laboratory protein separation procedure. A modified laboratory method of Nagano *et al.* (8) was performed in duplicate process runs (Scheme 1). Glycinin and β-conglycinin fractions were obtained from 50 g of white defatted soybean flakes and freeze-dried.

Pilot-plant protein separation process. Two extraction steps were used in the pilot-plant process instead of the one extraction step in the laboratory process (Scheme 2). A 200-gallon (740-L) tank with a custom-built chilling system was used for protein extraction. Defatted soybean white flakes were (15 or 20 kg) dispersed at the ratio of flakes to water of



SCHEME 1



SCHEME 2

either 1:15 or 1:10. The pH of the slurry was adjusted to 8.5 with 2N NaOH and stirring. After 1 h of extraction at 20°C, the pH of the slurry had decreased to about 8.0. The insoluble residue was separated by centrifuging with a Sharples P660 decanting centrifuge (Alfa Laval Separation Inc., Warminster, PA) at 5,700 rpm bowl speed, 4,000 rpm backdrive pinion speed, and a Moyno-type transfer pump (Electric Pump, Model 1FFCA SSE SAA, Des Moines, IA) at 250 rpm pump speed. The flake residue was reextracted at a ratio of flakes to water of 1:10 or 1:5. The pH of the slurry remained at about 8.0. The second extraction was carried out for 30 min at 20°C. The insoluble residue was removed by using the Sharples

P660 centrifuge and the transfer pump at the same conditions as previously described.

The two protein extracts were combined and solid sodium bisulfite (calculated to give 10 mM SO_2) was added. Glycinin was precipitated from solution by adjusting the pH to 6.4 with 2 N HCl and storing overnight at 7°C. The cooled protein solution was centrifuged directly. The precipitated glycinin was separated using an Alfa Laval BTPX 205 disc centrifuge at a bowl speed of 9,800 rpm and a transfer pump speed of 540 rpm. Sodium chloride was added to the supernatant to give 0.25 M and the pH of solution was adjusted to 5.0. The solution was stirred for 1 h at 5°C and a mixture of glycinin, β-conglycinin, and other proteins was precipitated and termed intermediate mixture. The precipitated intermediate mixture was separated using the Alfa Laval BTPX 205 centrifuge and the transfer pump at the same conditions as the glycinin fraction separation. The supernatant was diluted to 2× the original volume with chilled tap water (7°C), and the pH of solution was adjusted to 4.8 for β-conglycinin precipitation. The solution was stored at 7°C overnight. The precipitated β-conglycinin was separated with the Alfa Laval BTPX 205 centrifuge at 9750 rpm bowl speed and 500 rpm transfer pump speed.

For 15 kg process, the precipitated glycinin, β-conglycinin, and intermediate mixture fractions were redissolved in an aqueous solution neutralized with 2 N NaOH to pH 7.5. The protein fractions were desalted with a Feed and Bleed Membrane Filtration System (Model SRT-50; North Carolina SRT Inc., Cary, NC) and a 30-KD regenerated cellulose membrane (North Carolina SRT Inc.). Diafiltration was performed until 5× of the original volume of permeate solution was collected. The retentate was dried in an Anhydro Compact Spray-dryer (APV Crepaco Inc., Attleboro Falls, MA) with an air inlet temperature of 180°C and an air outlet temperature of 85°C. Insoluble residue was dried in a tray dryer (National Drying Machinery Co., Philadelphia, PA) at 70°C for 28 h.

Proximate analysis. Protein contents were determined by measuring total nitrogen content using the micro-Kjeldahl method (AOAC methods 988.05 and 960.52) (9). The nitrogen conversion factor of 5.71 was used for glycinin, 6.37 for β-conglycinin, and 6.08 for the intermediate mixture and the flake protein content calculation (10). Moisture contents of soy flakes and protein fractions were determined with a Karl Fischer automatic titrator (ASTM E203-75) (11) (Fisher Scientific Model 392, Pittsburgh, PA), or by drying a 2.00 g sample in a forced-air oven (Fisher Scientific Isotemp 750F) for 3 h at 130°C (AACC method 44-15A) (12). Ash content of protein fractions was determined by using AACC method 08-03 (13).

Urea-SDS-PAGE and gel density image analysis. Urea-SDS-PAGE was performed by using a modified method of Laemmili (14) and Chen (15). A 2.5 mg sample of soybean protein fraction was dissolved in 1.0 mL protein extraction buffer (50 mM THAM, pH 8.0, 5.0 M urea, 0.2% SDS, and 2% 2-mercaptoethanol). The protein solution was diluted 50% by using a 2× sample buffer (125 mM THAM, pH 6.8, 5.0 M urea, 0.2% SDS, 20% glycerol, 2% 2-mercaptoethanol, and 0.01% Bromophenol blue). A quantity of 30 to 40 μg protein was loaded per well. Gradient polyacrylamide 8–18% gel containing gradient urea (3.75–5.45 M) was used. Electrophoresis was performed at 125 V for 6 h. Gels were stained with 0.22% Coomassie Blue G250 solution in methanol/acetic acid/water (50:10:40), and destained with the same solvent without Coomassie Blue. Gels were scanned on an AGFA Arcus II flatbed scanner with reflective mode and 400 lpi output resolution, and analyzed on a Macintosh computer with the software packages of Footlook PS 2.05 and NIH Image 1.61.

Antibody production and rocket immunoelectrophoresis. Polyclonal antibodies of glycinin and β-conglycinin were produced in goats, and the rocket gel immunoelectrophoresis was performed following the method of Murphy and Resurreccion (16).

Free sulfhydryl and disulfide content analysis. The free sulfhydryl group contents of the protein fractions were determined by using a modified DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] method of Nakamura *et al.* (17). Protein samples were dissolved in a 35 mM phosphate buffer (pH 7.4) containing 8 M urea. DTNB reagent (50 μL) was added to 2.0 mL protein samples. The absorbance was read at 412 nm and the molar absorption coefficient of 14,150 was used (18).

Disulfide bonds were determined by using a modified NTSB (2-nitro-5-thio-sulfobenzoate) method (19,20). The soy protein sample was dissolved in 35 mM phosphate buffer at pH 7.0. Guanidine thiocyanate (3 M) was added to the protein solution (>2.5 M) to cleave the disulfide bonds. NTSB reagent was added in the protein solution to react with free sulfhydryls or 1/2 Cys formed by reaction with guanidine thiocyanate and bisulfite, and produced NTB (2-nitro-5-thiobenzoate) which has a maximum absorption at 412 nm. The molar absorption coefficient of 13,700 was used (18). The reaction and all measurements were performed under yellow or subdued light.

Surface hydrophobicity. Surface hydrophobicities of the protein fractions were measured following the method of Sorgentini *et al.* (21). Protein was dissolved in 0.01 M phosphate buffer (pH 7.0) and centrifuged to remove undissolved matter. The protein content of the supernatant was determined by using the biuret method (22). The protein solution was serially diluted with the same buffer to obtain concentrations ranging from 1 to 100 μg/mL. A 100-μL aliquot of 8 mM 8-anilino-1-naphthalene sulfonic acid (ANS) was added to 5 mL of diluted protein buffer solution. Fluorescence intensity (FI) was measured using a fluorescence spectrophotometer (Perkin-Elmer Model 650-15, Norwalk, CT) at an excitation wavelength of 350 nm and an emission wavelength of 525 nm. The initial slope of FI vs. protein concentration plot (calculated by linear regression analysis) was used as an index of protein surface hydrophobicity. Casein was purchased from Sigma, and soy isolate was produced in the pilot-plant with MBS 2795 flakes.

Differential scanning calorimetry analysis. A Perkin-Elmer differential scanning calorimeter (DSC) 7 Series Thermal Analysis System was used to analyze the thermodynamic

properties of the glycinin and β-conglycinin fractions. The heating temperature range was 25–200°C with a heating rate of 10°C/min. A 2.5–3.5 mg sample of dried protein was analyzed.

Statistical analysis. The general linear model and least significant difference (LSD) test at the 5% level were used to separate means.

RESULTS AND DISCUSSION

Comparison of the laboratory method and pilot-plant process. The yields and protein purities of the laboratory- and pilot-plant-scale extraction processes are compared in the Table 1. Approximately 2 kg of each protein fraction was obtained from the 20 kg soy flakes in the pilot-plant process. This pilot-plant process did not utilize the desalting process with the membrane filtration system as was performed in the laboratory-scale process. The process yields of glycinin and intermediate mixture fractions obtained with the pilot-plant process were lower than those obtained from the 50-g laboratory-scale method. The lower yields may be caused by losses during centrifugation, because both the Sharples and the Alfa Laval centrifuges are continuous-type centrifuges rather than closed-batch high-speed laboratory centrifuges. The protein recovery data show similar results for both processes for mass yield. The total protein recoveries of all three fractions were 55.1% with the 50-g laboratory-scale process and 50.6% in the pilot-plant process. Protein mass contents of glycinin and β-conglycinin fractions from the pilot-plant process were 90.4 and 91.0% (db), respectively, which were 5% lower than in the laboratory-scale process. The salt introduced from neutralization prior to spray-drying is probably the cause of the differences in protein content.

Purity of the glycinin fraction as measured by urea-SDS-PAGE and densitometer was higher than that of the β-conglycinin fraction. Purities of the glycinin fraction produced from the laboratory and pilot-plant processes were 95.7 and 84.2% of protein (db), respectively (Fig. 1). β-Conglycinin purities in the crude β-conglycinin fractions were 77.6 and 71.8% of protein, produced from the laboratory and pilot-plant scale processes, respectively. Figure 2 shows the urea-SDS-PAGE gel loaded with protein fractions produced in the laboratory and pilot-plant scale processes. The contaminated glycinin bands were darker in the pilot-plant β-conglycinin fraction than in the laboratory β-conglycinin fraction. Also darker α, α′, and β bands appear in the gel of the pilot-plant glycinin fraction compared with the gel of the laboratory glycinin fraction.

Desalination effects on protein yields and quality. A 15-kg batch of soybean white flakes was used in triplicate process runs with desalination steps of all protein fractions. The mean yield was 9.4 ± 0.2% (db) for the glycinin fraction, 4.9 ± 0.2% (db) for the intermediate mixture fraction, and 9.4 ± 1.6% (db) for the β-conglycinin fraction (Table 2). The β-conglycinin fraction had a larger deviation because that fraction had undergone the greatest number of processing steps. The yields of the protein fractions were generally lower than for the 20-kg process (Table 1), probably because the desalting steps were added in 15-kg pilot-plant process.

Means of protein recoveries have a pattern similar to the means for yields of glycinin, β-conglycinin, and intermediate mixture fractions. The total recovery of the three protein fractions was 41.2 ± 2.3%. The protein mass contents of glycinin, β-conglycinin, and intermediate mixture fractions were 92.8 ± 0.6, 97.6 ± 0.1, and 85.8 ± 0.6% (Table 2), respectively, and were much higher than compared to the 20-kg process without desalination.

The average glycinin purity was markedly higher with inclusion of the desalination process, which increased purity from 84.2 to 90.5% (Table 3, Fig. 1). The average purity of β-conglycinin in the β-conglycinin fraction was 72.7% of protein, which was close to the 71.8% achieved without desalination. The ash content with desalination was reduced from 4.46 to 2.88% for the glycinin fraction from 9.95 to 2.72% for the β-conglycinin fraction, and from 13.61 to 2.73% for the intermediate mixture fraction.

Effect of extraction ratio on protein extraction. Three ex-

TABLE 1
Effects of Extraction Process Scale-Up on the Yield, Protein Recovery, and Content of Soy Protein Fractions

	Extraction fractions		
	Glycinin	β-Conglycinin	Intermediate mixture
Yield (%, db)[a]			
Laboratory process (50 g)[b]	12.9 ± 0.1	9.8 ± 0.3	10.1 ± 0.2
Pilot-plant process (20 kg)	11.2	10.9	9.1
Protein recovery (%, db)			
Laboratory process (50 g)[a]	22.4 ± 0.3	17.2 ± 0.6	14.5 ± 0.8
Pilot-plant process (20 kg)	18.8	18.6	13.2
Protein content (%, db)			
Laboratory process (50 g)[a]	94.7 ± 0.9	96.7 ± 0.6	79.4 ± 1.0
Pilot-plant process (20 kg)	90.4	91.0	77.4

[a]db, Dry basis.
[b]$n = 2$.

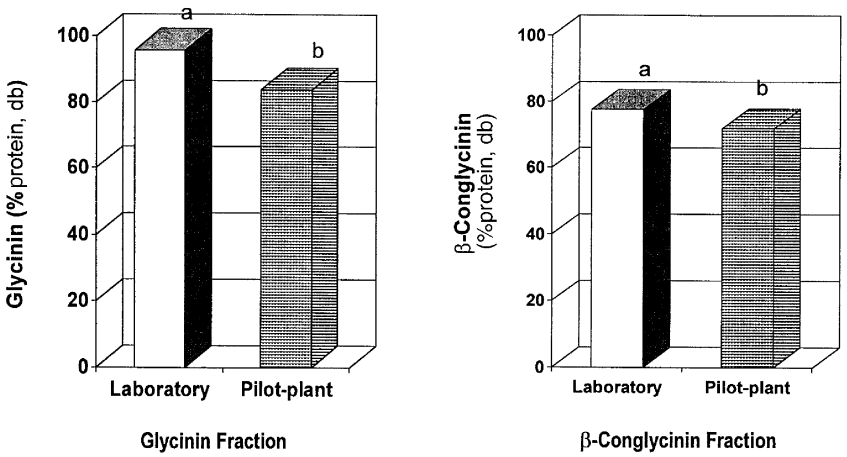


FIG. 1. Protein purities of fractions produced in the laboratory- and pilot-plant-scale (20 kg) processes. Different letters are significantly different at $P < 0.05$. db, Dry basis.

traction ratios (1:25, 1:20, and 1:15) of soy flakes to water were used in the pilot-plant process. The amounts of insoluble residue increased and the protein contents of the residue decreased as the extraction ratio decreased, but the total protein content in the insoluble residue increased from 36.1 to 41.5% (db) (Table 2). The yields and protein contents of the crude glycinin and the intermediate mixture fractions were not affected by extraction ratio. The purities of the glycinin fraction were the same for all three extraction ratios. However, the yield of β-conglycinin fraction increased from 7.7 to 10.7% (db) (Table 2), and the β-conglycinin purity in the β-conglycinin fraction decreased from 75.6 to 70.4% (Table 3), even though the protein content in the β-conglycinin fraction did not change. Therefore, the major effects of extraction ratio were on yields and qualities of the insoluble residue and the β-conglycinin fraction.

Protein physicochemical properties. Sulfhydryl and disulfide bonds of protein play an important role in protein gelation and plastic formation. Even though soybean varietal differences may account for the variability of sulfhydryl values, the protein preparation method is the major cause of sulfhydryl variability (23), especially adding bisulfite during extraction, diafiltration, and drying. Table 4 presents the free sulfhydryl and free sulfhydryl plus 1/2 Cys in the glycinin and β-conglycinin fractions produced in our pilot-plant process. Assuming that the MW of glycinin is 350 KD and β-conglycinin is 180 KD (24), the free-SH groups in the glycinin fraction had a lower μmol/g protein mean value but a higher mol/mol protein mean value than in the β-conglycinin fraction. The glycinin free sulfhydryl groups constituted 0.63 ± 0.14 mol-SH/mol protein; this was in good agreement with the results of Wolf (23), 0.4–0.5 -SH/mol glycinin after his glycinin fraction was dialyzed against water to remove 2-ME and freeze-dried. The sulfhydryl plus 1/2 Cys in the glycinin fraction was significantly greater than in the β-conglycinin fraction, which was expected based on the amino acid analyses in the literature. The mean of free sulfhydryl plus 1/2 Cys content in the glycinin fraction was 37–38 mol/mol protein. This value was very close to Wolf's estimate of 41 mol/mol protein (23). The β-conglycinin fraction contained a total free sulfhydryl plus 1/2 Cys group of 14–15 mol/mol protein, which was larger than the amino acid analysis prediction value of 4 mol/mol protein (10). The difference between the reference value and our results may reflect the impurity of our protein fractions.

Denaturation influences protein solubility, emulsifying, foaming, and further processing properties. The processes of protein separation and purification are factors causing protein denaturation. The degree of denaturation of the protein structure was evaluated by rocket immunoelectrophoresis. The

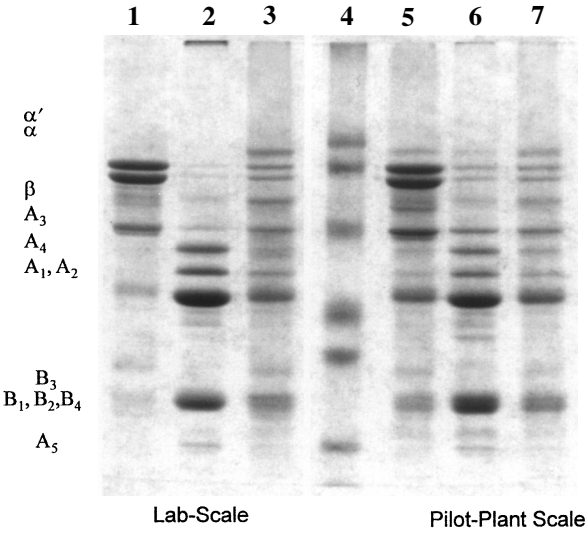


FIG. 2. Urea-SDS-PAGE gel loaded with protein fractions (30–40 μg/lane). Lane 1; β-conglycinin; Lane 2; glycinin; Lane 3; intermediate mixture from the lab-scale process; Lane 4; MW standard; Lane 5; β-conglycinin; Lane 6; glycinin; and Lane 7; intermediate mixture from the pilot-plant process. SOS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoreses.

TABLE 2
Yields, Protein Recoveries, and Contents of Soy Protein Fractions Produced from Pilot-Plant Processes Including a Desalting Step

	Extraction fractions			
	Glycinin	β-Conglycinin	Intermediate mixture	Insoluble residue
Yield (%, db)[a]				
15 kg process #1	9.4	7.7	5.2	44.5
15 kg process #2	9.2	9.9	4.8	49.5
15 kg process #3	9.5	10.7	4.8	53.3
Protein recovery (%, db)				
15 kg process #1	16.4	14.1	8.3	36.1
15 kg process #2	15.9	18.0	7.6	38.9
15 kg process #3	16.2	19.5	7.7	41.5
Protein content (%, db)				
15 kg process #1	93.2	97.7	86.2	43.5
15 kg process #2	93.0	97.5	85.1	42.1
15 kg process #3	92.1	97.7	86.2	41.7

[a]For abbreviation see Table 1.

TABLE 3
Protein Purities (% of protein) of Soy Protein Fractions Produced in the Pilot Plant[a]

	Glycinin in glycinin fraction	β-Conglycinin in β-conglycinin fraction
15 kg Process #1	90.9 ± 0.5 a	75.6 ± 0.8 a
15 kg Process #2	90.0 ± 0.2 a	72.1 ± 0.7 b,c
15 kg Process #3	90.6 ± 1.1 a	70.4 ± 2.3 c

[a]Data for % of protein that are in the same column with different letters are significantly different at $P < 0.05$.

mean for native glycinin structure in three 15-kg pilot-plant glycinin fractions was 82.8 ± 0.7% (Table 5). Compared to 83.9 ± 3.9% glycinin in the glycinin fraction (based on protein mass content and urea-SDS-PAGE results), the loss of glycinin native structure was negligible. All β-conglycinin contaminants in the glycinin fractions were in the denatured form. The mean of the native β-conglycinin structure was 60.9% in the β-conglycinin fraction, but 71.0% β-conglycinin in the β-conglycinin fraction was calculated based on protein content and urea-SDS-PAGE results. These calculations indicated that about 10% β-conglycinin in the β-conglycinin fraction had lost its native structure. The average glycinin content in the β-conglycinin fraction was 15.7%, whereas the native glycinin structure as measured by the rocket gel was only 7.8%; about one-half of the glycinin content in the β-conglycinin fraction was denatured. In the intermediate mixture fraction, the average glycinin content was 44.1% and the β-conglycinin content was 22.9%. However, the average native glycinin structure was 12.1% and native β-conglycinin structure was only 0.8% in the intermediate mixture fraction. Most of the glycinin and β-conglycinin present in the intermediate fraction appeared to be denatured.

The native glycinin and β-conglycinin structures, as measured by immunoreactivity, in the pilot-plant glycinin fractions were the same as those in the 50-g scale process (Table 5). About 10% native structure in glycinin was lost during the laboratory process. Freeze-drying in the laboratory method is the most likely reason for glycinin denaturation, as compared to spray-drying of the pilot-plant process. Most of the glycinin and β-conglycinin present in the laboratory-scale intermediate fractions appeared to be denatured similarly to the pilot-plant products. One-half of glycinin content in the laboratory-scale β-conglycinin fractions had lost its native structure. However, the native β-conglycinin content of the laboratory-scale β-conglycinin fraction was similar to the β-conglycinin content calculated from protein content and urea-SDS-PAGE results. Diafiltration with water to remove salt and excess bisulfite ions in the pilot-plant process may have caused additional denaturation of β-conglycinin.

TABLE 4
Free-Sulfhydryl and Disulfide Groups in the Protein Fractions Produced from 15-kg Pilot-Plant Processes[a]

	Free -SH group		Total free -SH plus 1/2 Cys groups		Reference
	μmol/g protein	mol/mol protein[b]	μmol/g protein	mol/mol protein[b]	1/2 Cys res./ mol protein
Glycinin fraction	1.79 ± 0.38	0.63 ± 0.14	108 ± 1	37.8 ± 0.3	41[c]
β-Conglycinin fraction	2.15 ± 0.22	0.39 ± 0.04	82 ± 2	14.8 ± 0.3	4[d]

[a]$n = 3$.
[b]Assume the MW of glycinin is 350,000 D and MW of β-conglycinin is 180,000 D.
[c]Reference 23; res., residue.
[d]Reference 10.

TABLE 5
Native Protein Contents of the Soy Protein Fractions

	Native glycinin structure (%)	Glycinin (%)	Native β-conglycinin structure (%)	β-Conglycinin (%)
15-kg Pilot-plant process[a]				
Glycinin fraction	82.8 ± 3.9	83.9 ± 0.7	0.0 ± 0.0	4.4 ± 0.5
β-Conglycinin fraction	7.8 ± 5.6	15.7 ± 3.4	60.9 ± 7.4	71.0 ± 2.6
Intermediate mixture	12.1 ± 4.0	44.1 ± 7.1	0.8 ± 1.4	22.9 ± 1.3
50-g Lab-scale process[b]				
Glycinin fraction	81.3 ± 3.0	90.6 ± 0.3	0.0 ± 0.0	1.8 ± 0.4
β-Conglycinin fraction	4.9 ± 0.0	9.9 ± 0.7	73.1 ± 11.0	75.1 ± 3.3
Intermediate mixture	14.6 ± 1.3	53.8 ± 0.5	0.0 ± 0.0	15.2 ± 0.1

[a]$n = 3$.
[b]$n = 2$.

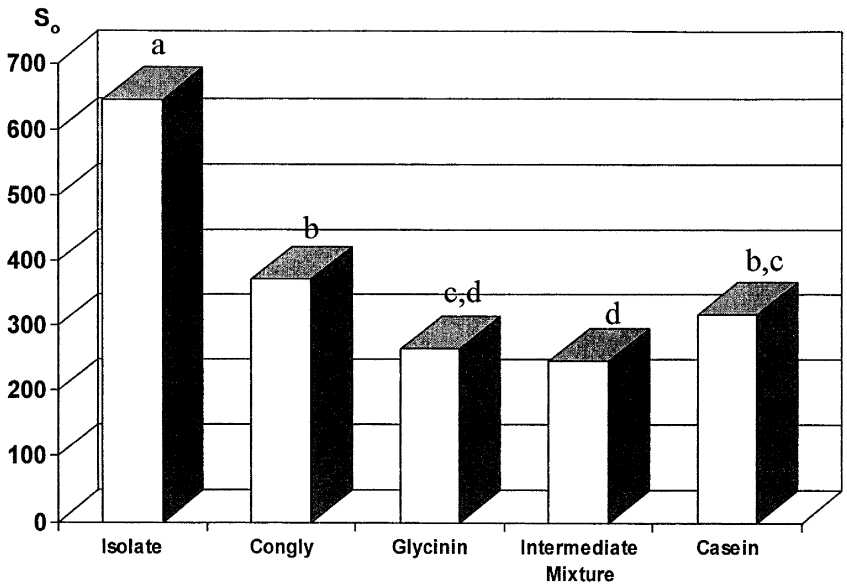


FIG. 3. Surface hydrophobicity (S_o) of pilot plant proteins. Different letters are significantly different at $P < 0.05$.

Surface hydrophobicity is another important parameter related to protein solubility, foaming, emulsifying, and gel formation. Surface hydrophobicity of protein depends on amino acid composition and structural configuration, including unfolding of protein native structure. The pilot-plant β-conglycinin fraction had a significantly higher hydrophobic index than that of glycinin and intermediate mixture fractions (Fig. 3). The amino acid composition and partial denaturation of β-conglycinin structure contributed to the higher hydrophobicity. Although more glycinin and β-conglycinin structures were denatured in the intermediate mixture, lower total protein, glycinin, and β-conglycinin contents in the intermediate mixture fractions may have been a major cause of the lower hydrophobicity.

Hydrophobicity indices for soy protein isolate and casein are also presented in Figure 3 for comparison with glycinin and β-conglycinin fractions. The very high apparent surface hydrophobicity of soy isolate may be due to the high salt content. High salt concentrations in protein solutions can increase protein-ANS interactions (25). The surface hydrophobicity of pilot-plant glycinin and β-conglycinin fractions was similar to commercial casein.

Only a single endothermic peak was observed in the glycinin samples produced either from laboratory or pilot-plant-scale processes in DSC analysis. The endothermic peak temperature of glycinin ranged from 170–180°C. Two endothermic peaks (β-conglycinin and glycinin) were observed in the β-conglycinin fractions for both scales of processes. The endothermic peak temperature of β-conglycinin was 150–160°C.

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