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ORIGINAL PAPER

Characterization of Fractionated Soy Proteins Produced by a New Simplified Procedure

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Abstract It was possible to fractionate soy protein into two soy protein isolate fractions (>90% protein) enriched in either glycinin or β-conglycinin by using a new simplified procedure (referred to as the Deak procedure) employing $CaCl_2$ and $NaHSO_3$. The Deak procedure produced fractions with higher yields of solids, protein, and isoflavones, and similar protein purities as well as improved functional properties compared to fractions recovered by established, more complex soy protein fractionation procedures. The Deak glycinin-rich fraction comprised 15.5% of the solids, 24.4% of the protein, and 20.5% of the isoflavones in the starting soy flour, whereas the glycinin-rich fraction of the established procedure (Wu procedure) comprised only 11.6% of the solids, 22.3% of the protein, and 9.6% of the isoflavones. The Deak β-conglycinin-rich fraction comprised 23.1% of the solids, 37.1% of the protein, and 37.5% of the isoflavones in the starting soy flour, whereas the Wu β-conglycinin-rich fraction comprised only 11.5% of the solids, 18.5% of the protein, and 3.3% of the isoflavones. Protein purities were >80% for both fractions when using both procedures. The Wu procedure produced protein fractions with slightly higher solubilities and similar surface hydrophobicities; whereas, the fractions produced by the Deak procedure had superior emulsification and foaming properties and similar dynamic viscosity behaviors.

Keywords Soybeans · Soy protein fractionation · Glycinin · β-Conglycinin · Isoflavones · Functional properties · Protein · Soy protein isolate

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Glycinin and β-conglycinin are the two major storage proteins in soybeans, which are often erroneously classified by their sedimentation coefficients 11S (glycinin) and 7S (β-conglycinin). Not all 7S protein in soybeans is β-conglycinin. Although several procedures have been developed to fractionate these two major storage proteins, there is no commercially viable process to obtain industrial amounts of these individual proteins.

Researchers have attempted to scale-up various laboratory procedures to produce large quantities of glycinin and β-conglycinin proteins so that they can be individually evaluated in clinical trials for health-promoting benefits. Saio and Watanabe [1] developed a laboratory method in which defatted soybean meal was extracted with buffer containing 10 mM $CaCl_2$ to obtain a β-conglycinin-rich extract and a glycinin-rich/fiber residue that was further extracted to obtain a glycinin-rich extract. The purities of the protein fractions were about 60–65% using an ultracentrifugation procedure. The Saio and Watanabe fractionation procedure also used several costly fiber extraction and dilution steps.

Wu et al. [2] refined and scaled up a method developed by Nagano et al. [3] to obtain kg quantities

of the individual soy proteins. The Wu procedure utilized $NaHSO_3$ as a reducing agent, NaCl for salting in protein, and water for salting out protein. This procedure used high salt concentrations and, consequently, large amounts of water were required to dilute the salt concentration when salting out the protein. The fraction yields were low and the procedure was prohibitively expensive and complicated for industrial application. The Wu procedure obtains three protein fractions, a β-conglycinin-rich, a glycinin-rich, and an intermediate fraction (a mixture of the two proteins with a significant amount of lipoxygenase). Richert et al. [4] improved fractional yields but with lower purities and the procedure remained complex.

Preferential binding of glycinin to calcium ions, which is surface charge dependent, has been reported [5, 6]. The number of calcium ions required to precipitate a mole of β-conglycinin is much greater than that required to precipitate a mole of glycinin (164 and 79, respectively) [7]. These observations led us to consider a new simplified procedure (the Deak procedure) to fractionate soy proteins by using $CaCl_2$ as the salt and sulfites as the reducing agent [8]. This simplified two-step procedure yielded two protein products, a glycinin-rich fraction and a β-conglycinin-rich fraction. In that study, we evaluated several combinations of Ca^{2+} and SO_2 concentrations in the forms of $CaCl_2$ and $NaHSO_3$, respectively. We previously identified the ideal combination to be 5 mM $CaCl_2$ and 5 mM SO_2 since these concentrations gave at least 80% protein purities for both the glycinin-rich and β-conglycinin-rich fractions as well as high yields of solids and protein [8]. The objectives of the present study were to characterize the compositional and functional properties of the fractions produced by the Deak procedure and to compare the fractions to those derived from the Wu procedure. We also evaluated eliminating the chilling step prior to precipitating the glycinin-rich fraction.

Experimental Procedures

Materials

Protein fractions were prepared from air-desolventized, hexane-defatted white flakes (soybean variety IA2020) produced with a French Oil Mill Machinery Co. extractor–simulator (Piqua, OH). The flakes contained 57.3% protein and 1,922 μg/g total isoflavones as determined in our laboratory and 93.8 protein dispersibility index (PDI) as determined by Silliker Laboratories (Minnetonka, MN, USA). The flakes were milled until 100% of the material passed through a 50-mesh screen by using a Krups grinder (Distrito Federal, Mexico). The soy flours were stored in sealed containers at 4 °C until used.

Modified Nagano (Wu procedure) Soy Protein Fractionation Procedure

The soy protein fractionation procedure (Fig. 1) utilized as the control in this study has been reported by Wu et al. [2] and is a modification of the procedure of Nagano et al. [3]. About 100 g defatted soy flour was extracted with deionized water at 15:1 water-to-flour ratio and the pH was adjusted to 8.5 with 2 N NaOH. The slurry was stirred for 1 h and centrifuged at 14,300 *g* and 15 °C for 30 min. The protein extract (first protein extract) was decanted and the amount of fibrous residue was determined and sampled. Sufficient $NaHSO_3$ was added to the protein extract to achieve 10 mM SO_2 and the pH was adjusted to 6.4 with 2 N HCl. The slurry was stored at 4 °C for 12–16 h and then centrifuged at 7,500 *g* and 4 °C for 20 min. A glycinin-rich fraction was obtained as the precipitated curd. This fraction was redissolved in deionized water, adjusted to pH 7 with 2 N NaOH, sampled, and stored in sealed containers at –80 °C until freeze-dried.

Sufficient NaCl was added to the supernatant (second protein extract) to obtain 250 mM and the pH was adjusted to 5 with 2 N HCl. The slurry was stirred for 1 h and centrifuged at 14,000 *g* and 4 °C for 30 min. An intermediate fraction (mixture of glycinin and β-conglycinin) was obtained as the precipitated curd and treated as described for the glycinin-rich fraction.

The supernatant (third protein extract) was diluted with deionized water at the ratio of two times the volume of the extract and the pH was adjusted to 4.8. The slurry was centrifuged at 7,500 *g* and 4 °C for 20 min. A β-conglycinin-rich fraction was obtained as the precipitated curd and treated as described for the previous two fractions. The amount of supernatant (whey) was determined and sampled. This procedure was replicated two times.

New Simplified (Deak procedure) Soy Protein Fractionation Procedure

A flow diagram for the Deak procedure is shown in Fig. 2. About 100 g defatted soy flour was extracted with deionized water at 15:1 water-to-flour ratio and the pH was adjusted to 8.5 with 2 N NaOH. The slurry was stirred for 1 h and centrifuged at 14,000 *g* and 15 °C for 30 min. The protein extract (first protein

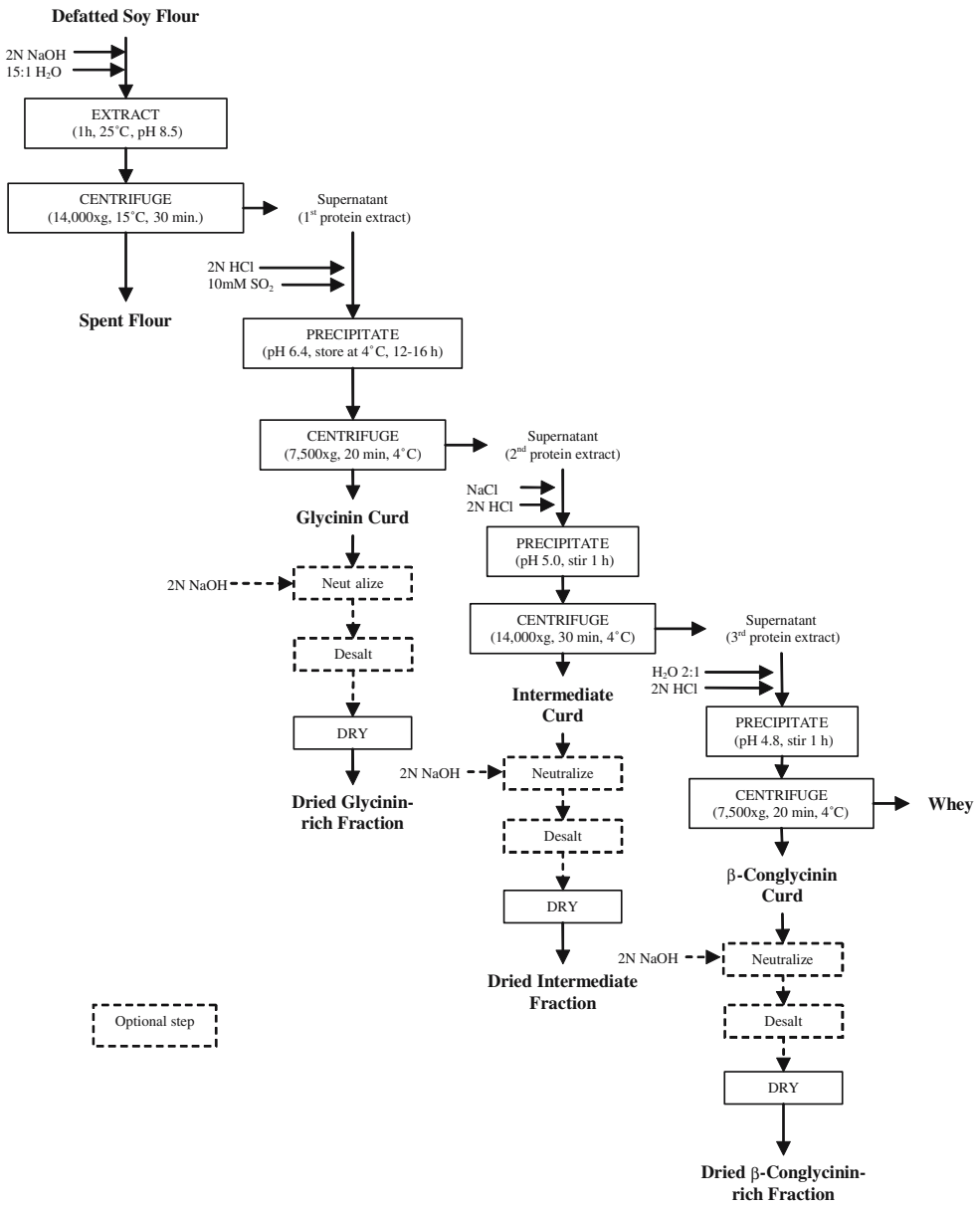

Fig. 1 Flow diagram for the Wu soy protein fractionation procedure

extract) was decanted and the amount of residue was determined and sampled. This extract was combined with sufficient $NaHSO_3$ and $CaCl_2$ to obtain 5 mM SO_2 and 5 mM Ca^{2+}, and the pH was adjusted to 6.4 with 2 N HCl. The slurry was either stored at 4 °C for 12–16 h (referred to as D4C) or stirred for 1 h at ~25 °C (referred to as DRT). The slurry was centrifuged at 14,000 *g* for 30 min at 4 °C for the D4C procedure and at 25 °C for the DRT procedure. A glycinin-rich fraction was obtained as the precipitated curd, which was neutralized and treated as described for the Wu procedure.

The supernatant (second protein extract) was adjusted to pH 4.8 with HCl. The slurry was stirred for 1 h and centrifuged at 14,000 *g* and 4 °C for 30 min. A *β*-conglycinin-rich fraction was obtained as the precipitated curd and treated as previously described. The amount of supernatant (whey) was determined and sampled. Both procedures (D4C and DRT) were replicated twice.

Freeze-drying

All samples were stored at –80 °C until they were placed into a Virtis Ultra 35 (Gardiner, NY, USA) freeze-dryer with shelves cooled to –20 °C. High vacuum was applied while the temperature was held constant until the vacuum dropped to 100 mTorr.

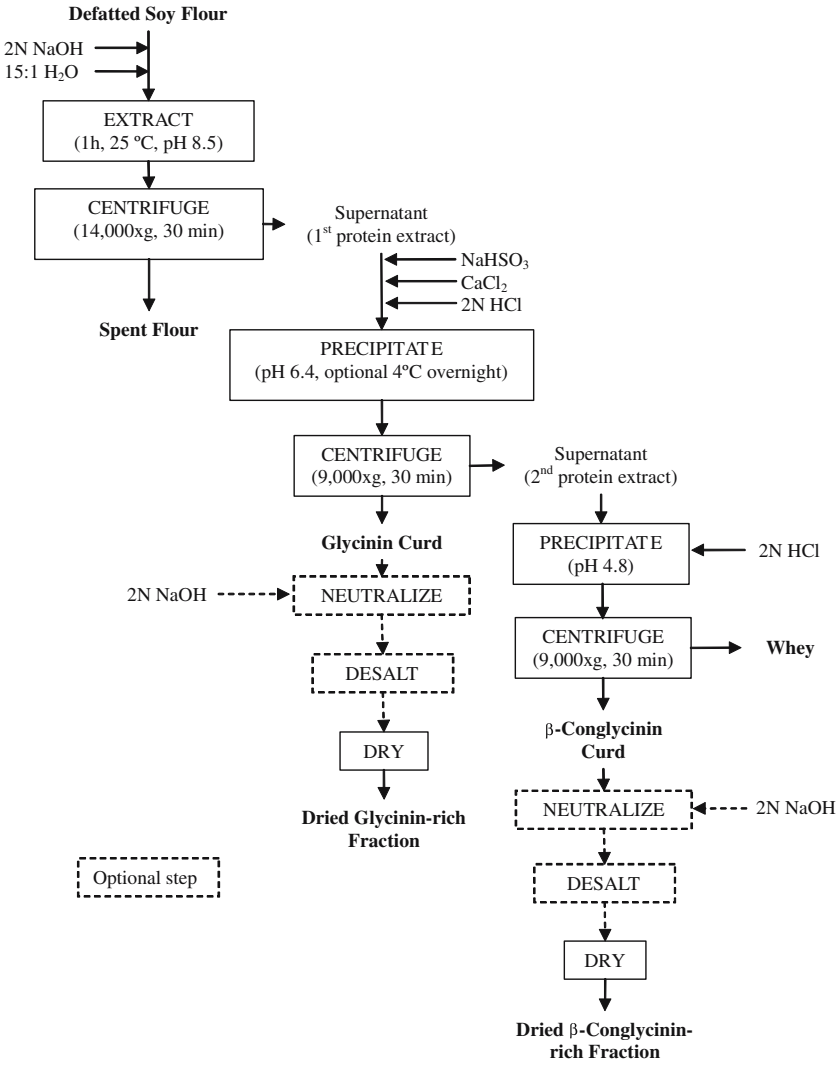

Fig. 2 Flow diagram for the Deak soy protein fractionation procedure

Secondary drying was achieved by heating the freeze-dryer shelves to 26 °C at high vacuum. The complete freeze-drying cycle lasted for 120 h. Samples were placed in sealed containers and stored at 4 °C until they were analyzed.

Proximate Analyses and Mass Balances

Nitrogen contents were measured by the combustion or Dumas method [9] with a Rapid NIII Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). These values were converted to Kjeldahl nitrogen by the conversion formula of Jung et al. [10]. Protein contents were calculated as Nx6.25. Moisture content was determined by oven drying for 3 h at 130 °C [11]. Ash content was measured using American Association of Cereal Chemistry (AACC) methods [12]. Mass balances for solids and protein were determined for all fractions. Yields were calculated as % Yield of a particular component in any given fraction = [(Total concentration for that component in given fraction × mass of the given fraction)/(Total concentration of that component in the starting flour × initial mass of flour)] × 100. All measurements were replicated at least three times.

Protein Profile Analysis

Urea-sodium dodecylsulfate-polyacrylamide gel electrophoresis (urea-SDS-PAGE) was used to quantify the protein component profiles of the fractions using the methods of Rickert et al. [4]. The proteins were identified by comparing gels of our fractions to a pre-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) molecular weight (MW) standard, low range (Bio-Rad Laboratories, Hercules, CA, USA). Glycinin and β-conglycinin subunit bands were confirmed using purified standards produced

according to the methods of O'Keefe et al. [13]. Densitometry was carried out by Kodak one-dimensional (1D) Image Analysis, version 3.5 (Kodak, Rochester, NY, USA) on scanned images produced with a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ, USA). SDS-PAGE results were calculated as % composition where: total storage protein in a given fraction = [(sum of storage protein subunit bands)/(sum of all bands)] × 100; fraction purity = [(sum of subunit bands)/(sum of storage protein bands)]; and subunit composition of a specific protein = [(subunit band)/(sum of subunits for the specific protein)]. All analyses were replicated at least four times.

Isoflavone Yield and Composition

Isoflavones were extracted and analyzed using the methods of Murphy et al. [14]. About 2.5 g of each freeze-dried fraction was extracted with 10 mL acetonitrile, 2 mL 0.1 N HCl, and water. This slurry was stirred for 2 h at 25 °C, filtered, and rotary evaporated at <30 °C. The residue was redissolved in HPLC-grade 80% methanol. Aliquots of these extracts were filtered and analyzed by HPLC within 10 h of extraction. Total isoflavone contents were adjusted for the MW differences and expressed as aglucon contents of the individual isoforms (μg/g). These adjusted contents were also used for yield calculation, where % Yield in a given fraction = [(total isoflavone concentration in a given fraction × mass of the given fraction)/(total isoflavone concentration in the starting flour × initial mass of flour)] × 100. Molar concentrations were used for isoflavone profile analysis. Samples were run in duplicate.

Thermal Behavior

The thermal behavior of the protein fractions was assessed by differential scanning calorimetry (DSC). Samples (15–20 mg) of 10% (w/w, dry basis) dispersions were hermetically sealed in aluminum pans. A sealed empty pan was used as a reference. The samples were heated from 25 to 120 °C at a 10 °C/min heating rate using a SII Exstar 6000 (Seiko Instrument, Inc., Tokyo, Japan). All samples were analyzed at least three times.

Solubility

Solubility was evaluated using the method of Rickert et al. [4]. Protein samples were dispersed (1% w/w, dry basis) in deionized water. The pH was adjusted to 7.0 using 2 N HCl or NaOH. The dispersions were stirred for 1.0 h. Aliquots (25 mL) of the dispersions were transferred to 50-mL centrifuge tubes and centrifuged at 10,000 *g* and 20 °C for 10 min. The protein content of the supernatant was measured by the Biuret method with bovine serum albumin (Sigma, St. Louis, MO, USA) as the reference standard. Protein solubility was calculated as % Solubility = (protein in supernatant/initial total protein) × 100.

Surface Hydrophobicity

Surface hydrophobicity was measured by the method of Wu et al. [2] with 1-anilino–8-naphthalene sulfonic acid magnesium salt monohydrate (ANS, ICN Biomedicals, Inc., Aurora, OH, USA). Protein dispersions prepared as in the solubility test were adjusted to pH 7.0 and centrifuged as previously described. An aliquot of soluble protein was serially diluted to obtain 6.25–100 μg/mL protein with 0.1-M phosphate buffer (pH 7.0) as diluent. Forty-microliter ANS (8.0 mM in 0.01-M phosphate buffer, pH 7.0) was dispersed in 3-mL aliquots of each dilution. Fluorescence intensity (FI) was measured with a Turner Quantech® spectrophotometer (Barnstead Thermolyne, Dubuque, IA, USA) and 440 nm (excitation) and 535 nm (emission) filters. FI was standardized using a solution of 40 μL ANS in 3 mL phosphate buffer as the zero point and 15 μL of ANS in 3 mL methanol assigned an arbitrary value of 80 FI. FIs were plotted versus percentage protein concentration. The slope of the regression line was reported as surface hydrophobicity. Samples were run in triplicate.

Emulsification Properties

Emulsification capacity was measured by the method of Bian et al. [15] with modifications. Twenty-five milliliter of a 2% (w/w, dry basis) sample dispersion was adjusted to pH 7.0 with 2 N HCl or NaOH and transferred to a 400-mL plastic beaker. Soybean oil, dyed with approximately 4 ppm Sudan Red 7B (Sigma, St. Louis, MO, USA), was continuously blended into the protein dispersion at 37-mL/min flow rate with a Bamix wand mixer (ESGE AG Model 120, Mettlen, Switzerland) at the low setting until phase inversion. Emulsification capacity (g oil/g sample) was calculated as amount of oil to cause inversion multiplied by 2. Samples were run at least in triplicate.

Emulsification activity and stability index were determined by the methods of Rickert et al. [4]. Twenty-one milliliter of 2% (w/w, dry basis) sample dispersions adjusted to pH 7.0 were blended with 7 mL refined soybean oil in a 250-mL glass beaker for

1.0 min with the Bamix wand mixer at low speed. Immediately after mixing, the emulsion was diluted 1:1,000 with 0.1% sodium dodecyl sulfate. The absorbance was measured at 500 nm and reported as emulsification activity. After 15 min, the absorbance was measured again. These two absorbance readings were used to calculate emulsification stability index (ESI): ESI (min) = $(A_0/A_0–A_{15})t$, where A_0 and A_{15} are the absorbances at time 0 and 15 min, respectively, and t is the time interval. Samples were run in triplicate.

Foaming Properties

Foaming properties were determined by the methods of Sorgentini et al. [16] with modifications [4]. A 0.5% (w/w, dry basis) sample dispersion was prepared and the pH adjusted to 7.0. A 95-mL aliquot was loaded into a glass column (58.5 cm × 2 cm) fitted with a coarse glass frit at the bottom and N_2 gas was purged through the sample at 100-mL/min flow rate. Time for the foam to reach the 300-mL mark, time for one half of the liquid incorporated into the foam to drain and volume of liquid incorporated into foam were measured. Three parameters were calculated:

$$\text{Foaming capacity (FC)} = V_f(f_r \times t_f)$$

$$\text{Specific rate constant of drainage } (K) = 1/(V_{max} \times t_{1/2})$$

$$\text{Rate of liquid conversion to foam } (V_i) = V_{max}/t_f$$

where V_f = a fixed volume of 300 mL, f_r = the flow rate of the gas, t_f = time to reach V_f, V_{max} is the volume of liquid incorporated into foam, and $t_{1/2}$ is the time to drain one half of the liquid incorporated into the foam. Samples were run in triplicate.

Dynamic Viscosity

A 10% (w/w, dry basis) sample dispersion was prepared at pH 7.0 [4]. The sample was applied to the plate of a RS-150 Rheo Stress (Haake, Karlsruhe, Germany) and shear was applied with a 60-mm 2° titanium cone (C60/2 Ti) over the 10–500/s shear rate range at constant temperature (23 °C). Shear rate (γ) and shear stress (τ) over the course of the analysis combined with the power-law formula were used to determine the consistency coefficient (k) and flow behavior index (n), where $\tau = k\gamma^n$. Using k, n, and γ, apparent viscosity (η) was estimated by the formula $\eta = k\gamma^{n-1}$. Samples were run in triplicate.

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA) and general linear model (GLM). Least significant differences (LSD) were calculated at $P < 0.05$ to compare treatment means by using the SAS system (version 8.2, SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Yields and Proximate Compositions for Soy Protein Fractions

The Nagano fractionation procedure as modified by Wu et al. [2], designated as the Wu procedure, produces three fractions (glycinin-rich, β-conglycinin-rich, intermediate), whereas the Deak procedures produce only two fractions (glycinin-rich, β-conglycinin-rich). The Wu procedure yielded slightly more total solids (41.3%) as the sum of the three fractions than did the Deak procedure with chilling at 4 °C (D4C) (38.6%) and the Deak procedure at 25 °C (DRT) (39.0%) as the sums of the two fractions (Table 1). The total protein yields were also higher for the Wu procedure (67.6%) than for the D4C procedure (61.5%) and the DRT procedure (62.3%). Almost 40% of the total recovered protein occurred in the intermediate fraction (mixture of β-conglycinin and glycinin) of the Wu procedure. The intermediate fraction, not produced in the Deak procedures, is of little use.

Both Deak procedures yielded significantly more solids, protein and isoflavones in the glycinin-rich fractions than the Wu procedure. The protein contents of the glycinin-rich fractions for all procedures were >90%. The ash content of the Wu glycinin-rich fraction was significantly higher than that of either Deak glycinin-rich fractions. The D4C glycinin-rich fraction had the highest isoflavone content, probably because isoflavones are less soluble at low temperatures [17].

More than twice the amount of β-conglycinin-rich fraction was obtained with the Deak procedures compared to the Wu procedure (Table 1). Protein yields for the Deak procedures were also significantly higher. The protein contents of the β-conglycinin-rich fractions for all procedures were >90% (but in all cases, lower in protein content than the glycinin-rich fractions). The Wu β-conglycinin-rich fraction contained very high ash contents due to the higher salt concentrations used.

The Wu intermediate fraction had low protein contents (~80%) not meeting the critical protein con-

Table 1 Yields and compositions (dry basis) of soy protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Yield			Content		
	Solids (%)	Protein (%)	Isoflavone (%)	Protein (%)	Ash (%)	Isoflavone (μg/g)
Glycinin, Wu	11.6[b]	22.3[c]	9.6[c]	96.7[b]	3.9[a]	1591[c]
Glycinin, D4C	15.5[a]	24.4[b]	20.5[a]	98.9[a]	3.2[b]	2547[a]
Glycinin, DRT	15.7[a]	29.9[a]	15.9[b]	96.6[b]	3.0[c]	1942[b]
LSD	1.2	1.8	2.0	0.9	0.2	155
Intermediate, Wu	18.2 ± 1.0	26.8 ± 1.3	20.9 ± 1.2	80.3 ± 1.2	14.3 ± 0.2	2213 ± 130
β-Conglycinin, Wu	11.5[b]	18.5[c]	3.3[c]	92.2[a]	10.1[a]	548[c]
β-Conglycinin, D4C	23.1[a]	37.1[a]	37.5[a]	90.0[b]	6.0[b]	3120[a]
β-Conglycinin, DRT	23.3[a]	32.4[b]	34.8[b]	91.2[a]	5.3[c]	2868[b]
LSD	2.4	1.7	2.5	1.2	0.3	184
LSD[b]	2.2	1.9	2.6	1.4	0.2	192

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step, and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

tent (>90%) for a protein isolate. The ash content was also the highest in the Wu intermediate fraction compared to all other fractions. Considerable amounts of solids, protein, and isoflavones were recovered in this less useful fraction (Table 1).

The yields of solids and protein in the Wu glycinin- and β-conglycinin-rich fractions were similar to values reported in the literature [2, 4]; however, we obtained nearly twice the yield of solids in the Wu intermediate fraction than reported in the literature. The discrepancy was probably due to our soy flour having higher PDI values than used in previous studies of Rickert et al. [4]. The higher PDI values were also responsible for the differences in thermal behavior where we found significant amounts of native protein in the Wu intermediate fraction while both Wu et al. [2] and Rickert et al. [4] found mostly denatured protein. We extracted more solids and protein from our flour resulting in higher yields of solids (glycinin-rich + β-conglycinin-rich + intermediate fractions), 41.3 versus 32.8 [2] and 30.6% [4], but most of the difference was due to the higher yield of intermediate fraction when we used the Wu procedure. The procedures of Wu et al. [2], Rickert et al. [4], and the present study yielded protein and solids for the glycinin-rich and β-conglycinin-rich fractions that were much higher than reported by Nagano et al. [3].

We also obtained higher protein yields in our β-conglycinin-rich fractions and lower protein yields in our glycinin-rich fraction with both Deak procedures than did Saio and Watanabe [1]. Apart from the fact that these studies used different soybeans, the Saio and Watanabe procedure differed from the Deak procedure in that they started by preferentially extracting the β-conglycinin from the flour while we started with alkali extraction, which extracts both glycinin and β-conglycinin. The effects of the procedural differences were apparent in their yields of low-purity fractions (62 and 68% for glycinin-rich and β-conglycinin-rich, respectively) that also resulted in their glycinin-rich fraction yielding a high amount of protein.

Significantly higher yields of isoflavones (58.0 and 50.7% for D4C and DRT, respectively) were obtained with the Deak procedures compared to those with the Wu procedure (33.8%) (Table 1). The Deak procedures yielded over ten times the isoflavones in the β-conglycinin-rich fractions than did the Wu procedure. Two-thirds of the total isoflavones recovered by the Wu procedure were in the intermediate fraction (Table 1).

Protein Composition

The total protein contents of the glycinin-rich fractions were about 90% for all procedures (Table 2) but were higher in the Deak glycinin-rich fractions. The purities of the glycinin-rich fractions were approximately the same for the Wu and D4C procedures (>80%), but about 10% lower for the glycinin-rich fraction recovered by the DRT procedure. The major contaminating β-conglycinin subunit in the glycinin-rich fraction was the β subunit; but, unlike both Deak procedures, the Wu glycinin-rich fraction contained no α' subunits. For all three procedures, there were more acidic than basic subunits in the glycinin recovered in the glycinin-rich

Table 2 Protein compositions and subunit profiles of the protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Storage protein in fraction (%)	β-Conglycinin				Glycinin		
		%	Subunit composition (%)			%	Subunit Composition (%)	
			α′	α	B		A	B
Glycinin, Wu	89.0[a]	16.3[b]	0.0[b]	49.5[a]	50.5[a]	83.7[a]	54.1[b]	45.9[a]
Glycinin, D4C	94.2[a]	19.0[b]	26.9[a]	25.0[c]	48.1[b]	81.0[a]	64.1[a]	35.9[b]
Glycinin, DRT	93.8[a]	28.6[a]	28.0[a]	30.7[b]	41.3[c]	71.4[b]	57.1[b]	42.9[a]
LSD	7.2	5.2	1.5	2.2	2.3	5.2	5.6	5.6
Intermediate, Wu	79.1 ± 2.0	45.3 ± 2.3	23.7 ± 1.2	31.7 ± 2.1	44.6 ± 1.0	54.7 ± 2.3	46.3 ± 4.0	53.7 ± 4.0
β-Conglycinin, Wu	85.2[a]	83.8[b]	28.7[a]	36.7[a]	34.6[a]	16.2[b]	43.5[a]	56.5[c]
β-Conglycinin, D4C	81.9[b]	85.6[a]	27.3[a]	38.0[a]	34.7[a]	14.4[c]	39.8[b]	60.2[b]
β-Conglycinin, DRT	84.3[a]	78.6[c]	29.4[a]	38.5[a]	32.0[b]	21.4[a]	31.9[c]	68.1[a]
LSD	2.2	0.4	2.8	3.6	2.2	0.4	1.8	1.8
LSD[b]	4.5	2.6	3.4	2.1	3.8	2.6	3.7	3.7

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *A* acidic subunits of glycinin, *B* basic subunits of glycinin, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

fractions. The D4C procedure yielded glycinin with more acidic subunits than the DRT procedure.

The β-conglycinin-rich fractions recovered by all three procedures contained more than 80% storage proteins (Table 2). The highest purity was achieved by the Deak procedure with chilling (86% β-conglycinin). The subunit compositions produced by all procedures were approximately the same for β-conglycinin but were significantly different for the contaminant glycinin. The β-conglycinin subunits were nearly evenly distributed among the three subunits. The glycinin contamination was comprised of more basic subunits when using the Deak procedures than the Wu procedure.

About 45% of the storage proteins present in the Wu intermediate fraction was β-conglycinin and the remainder was glycinin. The subunit distribution of the β-conglycinin component in the Wu intermediate fraction was unique in that the principal subunit was β. Similar amounts of acidic and basic subunits were recovered in the glycinin component of the Wu intermediate fraction.

The protein purities of the Deak glycinin-rich fractions (83.7%) were lower than those reported by Nagano et al. [3] (>90%), Wu et al. [2] (84.2–90.5%), and Rickert et al. [4] (85–90%). On the other hand, the protein purity of the Deak β-conglycinin-rich fraction (83.8%) was higher than reported by Wu et al. (73%) and Rickert et al. (68–79%) but lower than reported by Nagano et al. (>90%). When comparing the protein purities of the Deak fractions with those reported by Saio and Watanabe [1], we obtained higher protein purities for both protein fractions and for both procedures, which were probably due to the absence of a reducing agent in their procedure [1].

Isoflavone Composition

The isoflavones commonly found in soybeans are genistein, daidzein, and glycitein, which occur in four forms, the aglycon, the glucoside, the malonylglucoside, and the acetylglucoside isoforms. The glucoside and malonylglucoside predominate in soybeans and soy protein products [14]. The isoflavone profile and isoform distribution are altered during processing [18, 19]. Our soy flour contained 42.9% daidzein, 50.4% genistein, and 6.5% glycitein and the isoform distribution was 3.2% aglucons, 1.8% acetylglucosides, 27.2 glucosides, and 67.6% malonylglucosides.

The fractionation procedure significantly affected the isoflavone distribution of the glycinin-rich fraction (Table 3). The Wu glycinin-rich fraction contained 26.3% daidzein, 61.9% genistein, and 11.8% glycitein. The isoform distribution was also significantly affected. The Wu glycinin-rich fraction contained 30.5% aglycons, about 10 times the amount in the initial flour. At the same time, both glucosides and malonylglucosides decreased (to 16.7 and 44.5%, respectively). Conversions, caused by alkali extraction and β-glucosidase action, of malonylglucosides to glucosides and then to aglycons have been previously reported [17, 18]. The D4C glycinin-rich fraction contained 22.0% daidzein, 64.1% genistein, and 3.9% glycitein. The isoform distribution in the glycinin-rich fraction was also different, containing 24.1% aglycons, 10.8% glucosides, 63.7%

Table 3 Isoflavone profiles (μmol/g) of protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Din	MDin	AcDin	Dein	Glyin	MGly	Glyein	Gin	MGin	AcGin	Gein	Total
Flour	0.73	2.18	0.05	0.13	0.22	0.25	0.00	1.01	2.44	0.08	0.10	7.20
Glycinin, Wu	0.20[a]	0.73[b]	0.03[a]	0.59[b]	0.13[a]	0.22[a]	0.25[a]	0.66[a]	1.69[c]	0.36[a]	0.96[c]	5.92[c]
Glycinin, D4C	0.24[a]	1.42[b]	0.05[a]	0.84[a]	0.09[b]	0.17[b]	0.11[b]	0.69[a]	3.99[a]	0.09[c]	1.34[a]	9.52[a]
Glycinin, DRT	0.22[a]	1.91[a]	0.05[a]	0.60[b]	0.07[b]	0.13[c]	0.08[c]	0.57[a]	2.85[b]	0.17[b]	1.09[b]	7.26[b]
LSD	0.08	0.10	0.13	0.02	0.03	0.01	0.01	0.19	0.05	0.02	0.10	0.28
Intermediate, Wu	0.44	0.90	0.05	1.09	0.25	0.25	0.31	1.31	1.82	0.15	1.60	8.26
β-Conglycinin, Wu	0.07[b]	0.20[c]	0.03[c]	0.33[c]	0.05[b]	0.07[b]	0.09[b]	0.17[b]	0.36[b]	0.07[b]	0.58[c]	2.05[b]
β-Conglycinin, D4C	0.40[a]	2.48[a]	0.05[b]	1.13[a]	0.11[a]	0.22[a]	0.11[a]	1.11[a]	4.20[a]	0.08[b]	1.79[a]	11.68[a]
β-Conglycinin, DRT	0.42[a]	2.25[b]	0.09[a]	0.88[b]	0.12[a]	0.21[a]	0.09[b]	1.13[a]	4.02[a]	0.18[a]	1.33[b]	10.73[a]
LSD	0.06	0.21	0.01	0.19	0.01	0.01	0.02	0.26	0.58	0.04	0.38	1.39
LSD[b]	0.15	0.13	0.07	0.10	0.06	0.02	0.02	0.37	0.30	0.02	0.19	0.82

[a] $n = 2$. Means within a column followed by different superscripts are significant different at $P < 0.05$. *Din* daidzin, *MDin* malonyldaidzin, *AcDin* acetyldaidzin, *Dein* daidzein, *Gly* glycitin, *MGly* malonylglycitin, *Glyein* glycitein, *Gin* genistin, *MGin* malonylgenistin, *AcGin* acetylgenistin and *Gein* genistein. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

malonylglucosides, and 1.41% acetylglucosides. The isoflavone profile of the DRT glycinin-rich fraction was 31.5% daidzein, 49.5% genistein, and 3.9% glycitein. Apparently, chilling to 4 °C favored the recovery of genistein, since the total isoflavone content of the glycinin-rich fraction was higher when precipitated at 4 °C. The isoform distribution was similar to the D4C glycinin-rich fraction.

The fractionation procedure also significantly affected the isoflavone profile and distribution in the β-conglycinin-rich fraction (Table 3). The total isoflavone content of the β-conglycinin-rich fraction obtained with the Wu procedure was about one-fifth that obtained with the Deak procedures. The isoflavone distribution of the β-conglycinin-rich fraction obtained with the Wu procedure was 30.1% daidzein, 57.8% genistein, and 10.7% glycitein. This isoform profile was unique in that this fraction had the highest aglycon (48.7%) and lowest malonylglucoside (30.8%) contents of all fractions recovered, and its glucoside contents were also low (14.1%). The isoflavone profiles and isoform distributions of the β-conglycinin-rich fractions produced by the Deak procedures were similar. Daidzein contents were 34.7 and 33.9%; genistein content, 61.6 and 62.2%; and glycitein content, 2.8 and 3.9% for the Deak procedures (D4C and DRT, respectively). The aglycons were 25.9 and 21.4%; glucosides, 13.8 and 15.6%; malonylglucosides, 59.1 and 60.5% for the Deak procedures (D4C and DRT, respectively). The intermediate fraction produced with the Wu procedure, which contained about 60% of the original isoflavones in the soy flour, had similar isoflavone distribution to the β-conglycinin-rich fraction obtained with the Wu procedure, but was significantly different in isoform distribution (Table 3).

Thermal Behavior

The thermal behaviors of the glycinin-rich and β-conglycinin-rich fractions are shown in Table 4. The peak denaturation temperatures for both the glycinin portions and for the β-conglycinin contaminant were approximately the same for all procedures. The β-conglycinin contamination in the glycinin-rich fractions comprised 2.0–4.0% of the total denaturation enthalpy. The glycinin-rich fraction had the highest total denaturation enthalpy for all three procedures. The glycinin-rich fractions produced by the Deak procedures had significantly higher denaturation enthalpies while containing only slightly more glycinin. Similar trends were also observed by Scilingo and Añón [20, 21], which they attributed to calcium ions stabilizing the glycinin structure through specific ion-protein binding. This explanation was consistent with our proposed mechanism for soy protein fractionation with calcium ions [8]. The denaturation temperature of the β-conglycinin contaminant in the glycinin-rich fraction was lower than for β-conglycinin in the β-conglycinin-rich fraction, probably due to the low concentration of native β-conglycinin in the glycinin-rich fraction.

The peak denaturation temperatures for the β-conglycinin components of the β-conglycinin-rich fractions were approximately the same for all treatments. The glycinin contaminant of the β-conglycinin-rich fraction comprised 0.6–19.3% of the total denaturation enthalpy in this fraction. The Wu procedure produced a β-conglycinin-rich fraction with the highest denaturation enthalpy even though the β-conglycinin contents were similar among all procedures. This was probably

Table 4 Thermal behaviors of protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Denaturation peak temperature (°C)		Enthalpy (mJ/mg of protein)	
	β-Conglycinin	Glycinin	β-Conglycinin	Glycinin
Glycinin, Wu	74.7[a]	89.1[a]	0.32[a]	15.65[b]
Glycinin, D4C	73.3[a]	91.0[a]	0.61[a]	19.23[a]
Glycinin, DRT	72.8[a]	91.3[a]	0.81[a]	19.33[a]
LSD	2.0	2.1	0.55	2.31
Intermediate, Wu	74.8 ± 1.1	93.1 ± 0.5	1.48 ± 0.37	2.91 ± 0.64
β-Conglycinin, Wu	75.1[a]	88.9[c]	10.64[a]	0.06[b]
β-Conglycinin, D4C	75.1[a]	89.8[b]	6.47[b]	0.55[a,b]
β-Conglycinin, DRT	74.7[a]	90.8[a]	4.96[c]	1.19[a]
LSD	1.0	0.9	1.12	0.92
LSD[b]	1.6	1.5	0.93	1.06

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *intermediate* intermediate fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

due to the Deak procedures not producing intermediate fractions, which comprises most of the denatured proteins in the Wu procedure. Apparently, β-conglycinin structure was less affected by calcium ions than glycinin [20, 21].

The Wu intermediate fraction had the lowest total denaturation enthalpy, indicating that some of the protein was denatured. This observation was consistent with Wu et al. [2] and Rickert et al. [4], although they found lower denaturation enthalpies for their intermediate fractions, especially for the β-conglycinin component. We attribute these differences to the higher PDI of our defatted soy flour. The intermediate fraction also had the highest denaturation temperature for its glycinin component, which we attribute to the high salt content in this fraction [20].

Solubility

The fractionation procedure significantly affected the protein solubilities of the different fractions (Table 5). The glycinin-rich fraction obtained with the Wu procedure had slightly higher solubility (88%) although the Deak procedures also gave high solubilities (80–85%). The Wu β-conglycinin-rich fraction also had higher solubility (94%) than the Deak β-conglycinin-rich fractions (70–80%). The differences in protein solubilities among treatments were significantly greater for this fraction than for the glycinin-rich fraction. The higher solubilities observed for the Wu fractions were attributed to the Deak procedures producing only two fractions while the Wu procedure produced an intermediate fraction having low solubility (40%). These lower solubilities can be explained by taking into account their thermal behaviors. The products with lower total enthalpies were also less soluble, probably due to the increased denaturation.

Table 5 Solubilities and surface hydrophobicities of protein fractions prepared by the Wu and Deak procedures

Fraction/Treatment	Solubility (%)	Surface hydrophobicity (dimensionless)
Glycinin, Wu	88.1[a]	160[a]
Glycinin, D4C	85.2[b]	161[a]
Glycinin, DRT	80.5[c]	153[a]
LSD	2.5	39
Intermediate, Wu	39.7 ± 2.1	156 ± 22
β-Conglycinin, Wu	93.8[a]	178[b]
β-Conglycinin, D4C	71.8[c]	226[a]
β-Conglycinin, DRT	80.5[b]	187[b]
LSD	5.1	35
LSD[b]	3.9	39

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

Rickert et al. [4] found no differences in solubility behaviors between their glycinin-rich and β-conglycinin-rich fractions at pH 7.0. In contrast, we found that the Wu laboratory procedure and similar pilot-plant procedure [15] produced β-conglycinin-rich fractions with higher solubility than the glycinin-rich fractions at pH 7.0. Differences in thermal histories of the soy flours used may account for this difference.

Surface Hydrophobicity

The presence of calcium does not prevent the ANS probe from interacting with the proteins [20]. The surface hydrophobicities of the fractions were affected to a lesser extent than were solubilities (Table 5). Apparently, the amount of calcium present in the system did not cause structural changes in the proteins. In contrast, Scilingo and Añón [20] found that calcium-treated soy protein isolates had lower surface hydrophobicities than those that were untreated and attributed the lower hydrophobicities to the formation of soluble aggregates in the presence of calcium.

There were no significant differences in surface hydrophobicities for the glycinin-rich fractions among fractionation procedures. The β-conglycinin-rich fraction obtained by the Deak procedure with chilling had the highest surface hydrophobicity. This observation was consistent with the thermal behavior and solubility of this fraction. The protein precipitated in the β-conglycinin-rich fraction had low denaturation enthalpies and solubilities. In general, the β-conglycinin-rich fractions had high surface hydrophobicities, which was similar to findings of Wu et al. [2] but differed from those of Rickert et al. [4].

Emulsification Properties

The emulsification properties of the protein fractions are shown in Table 6. The emulsification capacities of the Wu fractions were similar to those previously reported [4, 15] with the β-conglycinin-rich fraction having the best emulsification capacity.

The Deak glycinin-rich fractions had significantly higher emulsification capacities than the Wu glycinin-rich fractions. These higher emulsification capacities may be due to less protein denaturation, or more likely, due to calcium-mediated associations between glycinin molecules [7] that introduced structural changes [20] important to emulsification. Emulsification activities and emulsification stability indexes were approximately the same for all glycinin-rich fractions, regardless of procedure used. Probably the structural changes introduced in glycinin in the presence of calcium improved the ability of glycinin to adsorb at the water-oil interface, but did not allow for the flexibility needed to stabilize the emulsion. One possible explanation consistent with our previous observations [8] is that soy protein forms soluble aggregates in the presence of low calcium concentrations [20].

The Deak β-conglycinin-rich fractions had significantly higher emulsification capacities than the Wu β-conglycinin-rich fraction, although the difference among procedures was more dramatic with the glycinin-rich fractions. The Wu β-conglycinin-rich fraction had slightly higher emulsification activity than the Deak β-conglycinin-rich fractions. Emulsification stability index was also significantly affected by the procedure used to fractionate soy protein. These observations agree with our previous values for solubility. Apparently, the presence of calcium ions preferentially affects glycinin [21]. The Wu intermediate fraction had the poorest emulsification properties. The β-conglycinin fractions formed more-stable emulsions with quite high emulsification capacities. These results were not correlated with solubility nor surface hydrophobicity data.

Table 6 Emulsification properties of protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Emulsification capacity (g of oil emulsified/g of product)	Emulsification activity (absorbance at 500 nm)	Emulsification stability index (dimensionless)
Glycinin, Wu	351[c]	0.152[a]	84[a]
Glycinin, D4C	876[a]	0.140[a]	73[a]
Glycinin, DRT	684[b]	0.149[a]	68[a]
LSD	28	0.015	22
Intermediate, Wu	232 ± 29	0.168 ± 0.026	62 ± 26
β-Conglycinin, Wu	586[b]	0.306[a]	194[a]
β-Conglycinin, D4C	678[a]	0.276[b]	192[a]
β-Conglycinin, DRT	647[a]	0.244[c]	151[b]
LSD	35	0.028	38
LSD[b]	30	0.022	32

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

The Deak procedures produced fractions with better emulsification properties.

Foaming Properties

Foaming capacities, stabilities and rates of the fractions are shown in Table 7. Foaming capacity is expressed in mL of foam formed per mL of 0.5% solids dispersion. Foam stability is expressed by K, which is the time for one-half of the liquid to drain from the foam. The smaller that K value, the more stable the foam. Rate of foaming is a measure of speed of foam formation.

The DRT glycinin-rich fraction had the best foaming properties having about 70% higher foaming capacity and was significantly more stable. The Deak fractions foamed five times faster than the Wu fractions. The DRT glycinin-rich fraction was a significantly better foaming agent than the D4C glycinin-rich fraction. The significantly improved foaming properties of the Deak glycinin-rich fractions were partially attributed to the high levels of glycinin acidic polypeptides, which are good foaming agents [22]. In addition, calcium-mediated associations among the different components of this fraction improved film formation. The improved foaming properties of the DRT glycinin-rich fraction were probably due, in part, to the fact that this fraction had significant β-conglycinin contamination. The interaction between glycinin and β-conglycinin components in the glycinin-rich fraction were likely responsible for the improved foaming properties [4]. Our results for the Wu glycinin-rich fraction differed from Bian et al. [15] and Rickert et al. [4] in that our Wu glycinin-rich fractions had lower foaming capacities, foam stabilities and foaming rates but similar solubilities, thermal behaviors, and surface hydrophobicities.

Table 7 Foaming properties of the protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Foaming capacity (mL/mL)	Foaming stability (mL*min)	Rate of foaming (mL/min)
Glycinin, Wu	0.964[c]	0.089[c]	2.0[c]
Glycinin, D4C	1.428[b]	0.075[b]	8.4[b]
Glycinin, DRT	1.654[a]	0.068[a]	10.3[a]
LSD	0.159	0.006	1.9
Intermediate, Wu	0.958 ± 0.059	0.004 ± 0.001	17.2 ± 3.1
β-Conglycinin, Wu	1.069[c]	0.018[b]	12.4[c]
β-Conglycinin, D4C	1.597[b]	0.008[a]	32.0[b]
β-Conglycinin, DRT	1.648[a]	0.007[a]	34.5[a]
LSD	0.124	0.008	2.0
LSD[b]	0.130	0.009	2.3

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

The DRT procedure also produced a β-conglycinin-rich fraction with excellent foaming properties having about 50% more foaming capacity, twice the foam stability, and three times faster foaming than the Wu β-conglycinin-rich fraction. The DRT β-conglycinin-rich fraction also had good foaming capacity, rate and stability. We attributed these differences in foaming properties to more denatured protein in the Wu intermediate fraction as indicated by the thermal analysis data. The intermediate fraction had the best foaming stabilities and rates among all fractions recovered by the Wu procedure [4, 15]. The Wu intermediate fraction had low foaming capacity, the highest foaming stability of all fractions produced, and the highest foaming rate among other Wu fractions. The Wu β-conglycinin-rich fraction had similar foam stabilities to previous reports [4, 15]. In general, the β-conglycinin-rich fractions had better foaming properties than the glycinin-rich fractions (Table 7).

Dynamic Viscosity

Dynamic viscosity is characterized by flow consistency index (K), which is a measure of how much energy the system is taking up in order to flow, and the flow behavior index (n), which is a measure of how closely the system behaves to an ideal Newtonian fluid. There were no significant differences among procedures for the glycinin-rich fractions for any of these two variables (Table 8); however, there were significant differences among procedures for the β-conglycinin-rich fractions. The Wu β-conglycinin-rich fraction had the highest consistency index, was the most viscous, and behaved less like an ideal fluid. The D4C β-conglycinin-rich fraction had higher viscosity than the DRT β-conglycinin-rich fraction. The DRT β-conglycinin-rich fraction also had the greatest glycinin contamination and the lowest viscosity of all fractions tested.

In general, the glycinin-rich fractions were less viscous than the β-conglycinin-rich fractions and the Wu intermediate fraction had intermediate dynamic viscosities. Our results differed from Rickert et al. [4], who found the intermediate fraction to be the most viscous.

Table 8 Dynamic viscosities of protein fractions prepared by the Wu and Deak procedures

Fraction/treatment	Flow consistency index (K = mPa s)	Flow behavior index (n, dimensionless)
Glycinin, Wu	0.010[a]	0.925[a]
Glycinin, D4C	0.011[a]	0.867[a]
Glycinin, DRT	0.010[a]	0.917[a]
LSD	0.008	0.079
Intermediate, Wu	0.167 ± 0.027	0.739 ± 0.051
β-Conglycinin, Wu	0.617[a]	0.471[c]
β-Conglycinin, D4C	0.521[b]	0.585[b]
β-Conglycinin, DRT	0.070[c]	0.789[a]
LSD	0.082	0.058
LSD[b]	0.049	0.067

[a] $n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $P < 0.05$. *Glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *Wu* fractions produced by the Wu procedure, *D4C* fractions produced by the Deak procedure with a chilling step, *DRT* fractions produced by the Deak procedure without a chilling step; and *LSD* least significant difference at $P < 0.05$

[b] Least significant difference for comparing all fractions within a column

The Deak soy protein fractionation procedure, employing $CaCl_2$ and $NaHSO_3$, was a simple and effective means of producing glycinin-rich and β-conglycinin-rich fractions. The fractions contained >90% protein, high levels of isoflavones and >80% protein purity. The fractions produced by the Deak procedure had slightly lower solubilities, similar surface hydrophobicities and dynamic viscosities, and superior emulsification and foaming properties to fractions produced by the Wu procedure.

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