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ORIGINAL PAPER

Preparation of Glycinin and β-Conglycinin from High-Sucrose/Low-Stachyose Soybeans

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Abstract A new soybean line, known as high-sucrose/low-stachyose (HS/LS) soybeans, has been developed having elevated sucrose content and reduced content of flatus-causing oligosaccharides, especially stachyose. There is also increased interest in understanding the health benefits, functional properties and potential applications for the two major storage proteins of soybeans, glycinin and β-conglycinin. We evaluated the protein fractionation behavior of a HS/LS soybean line and compared it to normal soybeans when using the three-step Wu procedure, which employs SO_2, NaCl and precipitations at pH 6.4 and 4.8 and a new two-step Deak procedure, which employs SO_2, $CaCl_2$ and precipitations at pH 6.4 and 4.8. Both soybean variety and fractionation procedure significantly affected fraction yields, purities and functional properties. The Wu procedure gave glycinin- and β-conglycinin-rich fractions with 100% purities and high yields of solids (15.4%) and protein (31.7%) from HS/LS soy flour, which were significantly higher than the purities and yields achieved with normal soybeans. The Deak procedure was less efficient in fractionating proteins from HS/LS soybeans than from normal soybeans, producing protein fractions from HS/LS soybeans with purities ranging from 71 to 80%. The Deak procedures yielded products with unique solubilities, surface hydrophobicities, and emulsification and foaming properties.

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Introduction

Soybeans are a good source of high-quality protein, yet <5% of the available soy protein is used for food [1]. Although the health benefits of consuming soy protein, especially β-conglycinin, are becoming recognized, poor functionality, undesirable taste/flavor and flatus-causing indigestible oligosaccharides are significant limitations to consuming higher levels of soy protein ingredients. Recent advances in biotechnology have overcome the normal presence of indigestible sugars by developing a soybean line high in sucrose and low in stachyose contents [known as high-sucrose/low stachyose (HS/LS) soybeans] [2].

HS/LS soybeans enable the production of new soy protein ingredients with unique chemical compositions and functional properties [2, 3], and we recently reported on the properties of soy protein ingredients prepared from defatted soy flour of HS/LS soybeans [4, 5]. In spite of HS/LS soybean flour having similar protein profiles to normal soy flour, we observed that some of the HS/LS protein fractions had higher amounts of β-conglycinin [4]. This and other observations indicated that protein products made from HS/LS soy flour may have unique functional behaviors [5], which prompted us to study the fractionation behavior of HS/LS soybeans and the functionalities of the protein fractions produced.

Producing soy protein fractions enriched in one of the two major storage proteins, glycinin and β-

conglycinin, has been of interest. Recent research has suggested that the β-conglycinin component of soy protein has health benefits [6, 7], more so than glycinin. Several fractionation methods achieving mixed success have been reported. One such method was reported by Wu et al. [8], which was a modification of methods of Nagano et al. [9], and the Wu procedure has been improved and scaled-up to produce kilograms quantities sufficient for human feeding trials [10]. The Wu procedure was based on adjusting the ionic strength of a soluble soy protein extract and isoelectric precipitation. Three fractions were obtained, a glycinin-rich fraction (11.6% solids yield and 84% purity), a β-conglycinin-rich fraction (11.5% solids yield and 84% purity), and an intermediate fraction (18% solids yield as a mixture of the two storage proteins). The Wu procedure is complex, requiring several centrifugation steps and a dilution step, which make the procedure very expensive to conduct on commercial scale.

We recently reported on two new simplified procedures (referred to as the Deak procedures) and characterized the functional properties of the protein fractions produced [11]. The Deak procedures are based on differential precipitation of the storage proteins by means of adjusting the pH of a soluble protein extract in which millimolar amounts of calcium ions and sulfites are used. Only two fractions are obtained, a glycinin-rich fraction (16% solids yield and 81% purity) and a β-conglycinin-rich fraction (23% solids yield and 85% purity). In addition to yield and purity, the functional properties of the protein fractions are important and determine their performance in various food systems and, as a consequence, their values to end users [12]. The objectives of the present study were to evaluate the three different fractionation procedures (two Deak procedures and the Wu procedure) using HS/LS soy flour by determining their yields of solids and protein, purities, and functional properties, and to compare them to those achieved when using normal soybeans.

Experimental Procedures

Materials

Air-desolventized, hexane-defatted HS/LS white flakes were prepared from HS/LS soybeans (Low Stachyose, Lot-980B0001 OPTIMUM, Pioneer a DuPont Company, Johnston, IA, USA) and from normal soybeans (IA2020, Iowa State University) using a French Oil Mill Machinery extractor-simulator (Piqua, OH, USA). The white flakes were milled with a Krupp's grinder (Distrito Federal, Mexico) until all material passed through a 50-mesh screen. The HS/LS defatted soy flour contained 58.3% (dry basis) protein with 95.0 protein dispersibility index (PDI). The flours were stored in sealed containers at 4 °C until used.

Wu Soy Protein Fractionation Procedure

The soy protein fractionation procedure of Wu et al. [8], a modification of the Nagano et al. [9] procedure, was used as the control procedure (Fig. 1). Defatted soy flour (100 g) was extracted with deionized water at 15:1 water-to-flour ratio, the pH was adjusted to 8.5 with 2 N NaOH, and the slurry was stirred for 1 h. After centrifuging the slurry at 14,300*g* and 15 °C for 30 min, the protein extract (first protein extract) was decanted, and the amount of insoluble fiber residue was determined and sampled.

$NaHSO_3$ was added to the protein extract to achieve 10 mM HSO_3^- and the pH was adjusted to 6.4 with 2 N HCl. The resulting 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, which was redissolved in deionized water, adjusted to pH 7 with 2 N NaOH and sampled.

NaCl was added to the supernatant (second protein extract) to achieve 250 mM NaCl, and the pH was adjusted to five 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, a mixture of glycinin and β-conglycinin, was obtained as the precipitated curd and treated as described above.

The supernatant (third protein extract) was diluted with deionized water at 2× the volume of the third protein extract and adjusted to pH 4.8. This slurry was centrifuged at 7,500*g* and 4 °C for 20 min, and the β-conglycinin-rich fraction was obtained as the precipitated curd. This fraction was treated as described above and the amount of supernatant (whey) was determined and sampled. The fractionation procedure was replicated twice.

New Simplified Fractionation Procedure (Deak Procedure)

A flowchart for the Deak procedure [11] is shown in Fig. 2. About 50 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 extract) was

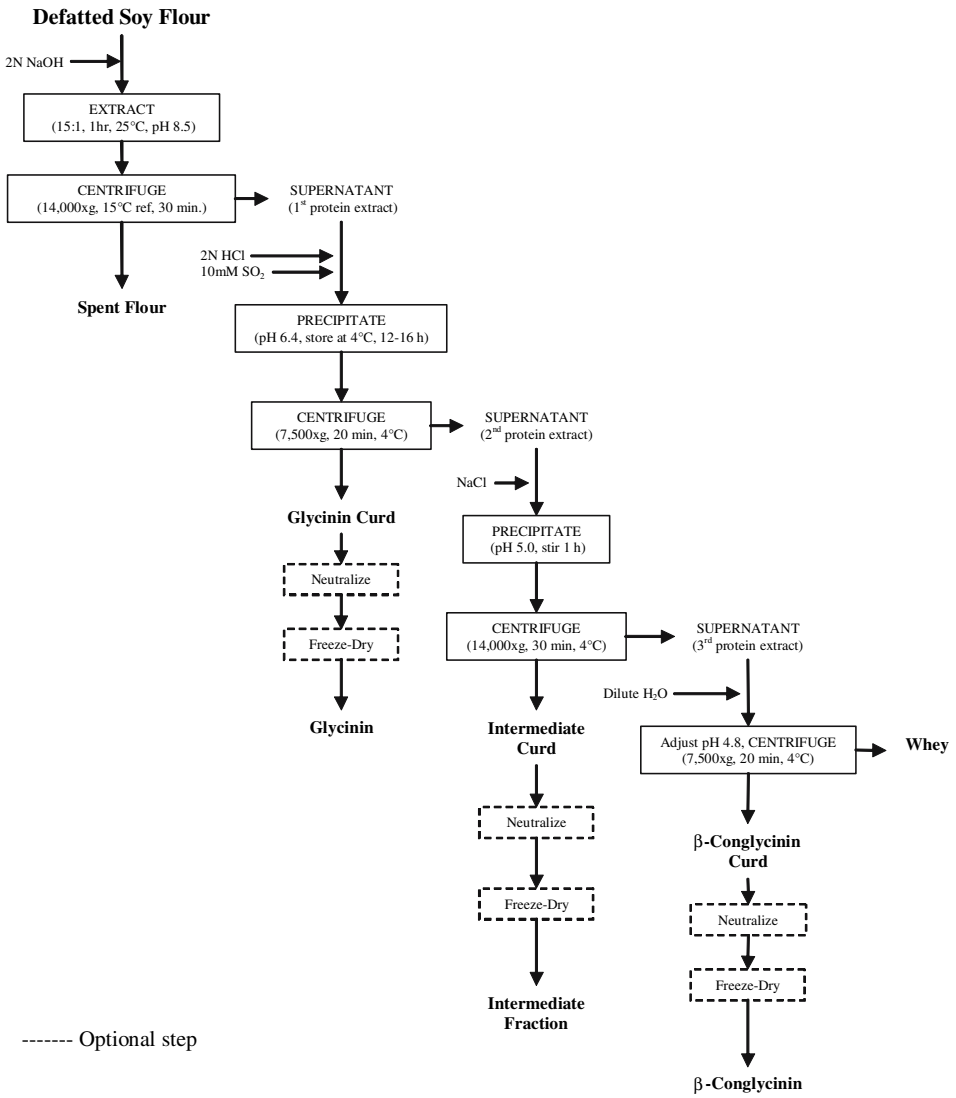

Fig. 1 Three-step soy protein fractionation procedure of Wu et al. [8]

decanted, and the amount of insoluble fiber residue was determined and sampled.

$NaHSO_3$ and $CaCl_2$ were added to the extract to obtain 5 mM HSO_3^- and 5 mM Ca^{2+}. The pH was adjusted to 6.4 with 2 N HCl. In one case, the slurry was stored at 4 °C for 12–16 h (identified as D4C) and, in another case, the slurry was stirred for 1 h at ~25 °C (identified as DRT). In both cases, the slurry was centrifuged at 9,000*g* and 4 °C for 30 min, and the glycinin-rich fraction was obtained as the precipitated curd. The curd was neutralized and treated as described above.

The supernatant (second extract) was adjusted to pH 4.8 with HCl, and the slurry was stirred for 1 h and then centrifuged at 9,000*g* and 4 °C for 30 min to obtain the *β*-conglycinin-rich fraction as the precipitated curd. This fraction was treated as described above, the amount of supernatant (whey) was determined, and the whey was sampled. Both treatments (D4C and DRT) were replicated twice.

Freeze-Drying

All samples were kept at –80 °C until freeze-dried in a Virtis Ultra 35 (Gardiner, NY, USA) freeze-dryer with shelves cooled to –20 °C. High vacuum was then applied and the temperature was held constant until the vacuum dropped to 100 mTorr. Secondary drying was achieved by heating the freeze-dryer shelves to 26 °C at high vacuum. The freeze-drying cycle lasted for 120 h.

Proximate Analyses and Mass Balance

Nitrogen contents were measured using the combustion or Dumas method [13] and a Rapid NIII Analyzer

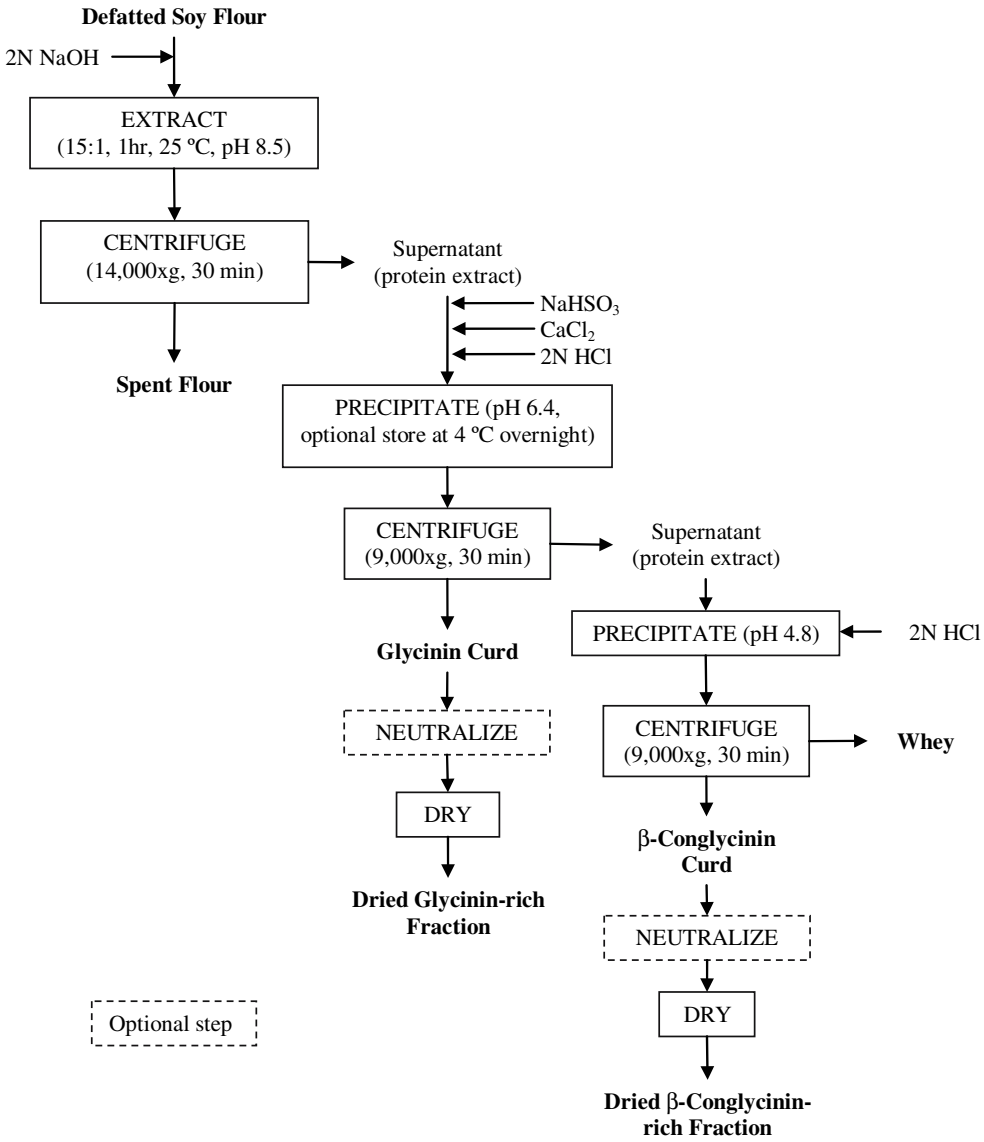

Fig. 2 New soy protein fractionation procedure of Deak et al. [11]

(Elementar Americas, Mt. Laurel, NJ, USA). These values were converted to Kjeldahl nitrogen using the conversion equations of Jung et al. [14]. Protein content was calculated as Nx6.25. Moisture content was determined by oven-drying for 3 h at 130 °C [15]. Ash content was determined using American Association of Cereal Chemistry (AACC) methods [16]. All measurements were in triplicate.

Protein Profile Analysis

Urea-sodium-dodecylsulfate polyacrylamide gel electrophoresis (urea-SDS-PAGE) was performed using the methods of Rickert et al. [10] to determine the protein composition profiles. Protein bands were identified using a prestained sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) low-range-molecular-weight (MW) standard (Bio-Rad Laboratories, Hercules, CA, USA). Glycinin and β-conglycinin subunit bands were confirmed using purified standards produced according to methods of O'Keefe et al. [17]. Densitometry was carried out using the Kodak 1D Image Analysis version 3.5 (Kodak, Rochester, NY, USA) on images scanned with a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ, USA). SDS-PAGE results were calculated as % composition: Total storage protein in a given fraction = [(sum of storage protein subunit bands)/(sum of all bands)] × 100; fraction purity/composition = [(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 measurements were replicated at least four times.

Thermal Behavior

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

Solubility

Protein solubility was determined using the method of Rickert et al. [10] with 1% (w/w dry basis) sample dispersions in deionized water. The pH was adjusted to 7.0 with 2 N HCl or NaOH and the dispersions were stirred for 1 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 contents of the supernatants were measured using the Biuret method with bovine serum albumen (Sigma, St. Louis, MO, USA) as the reference standard. Solubility was calculated as % Solubility = (protein in supernatant/initial protein content) × 100.

Surface Hydrophobicity

Surface hydrophobicity was determined using the method of Wu et al. [8] with 1-Anilino-8-naphtalene sulfonic acid magnesium salt monohydrate (ANS, ICN Biomedicals, Aurora, OH, USA). Protein dispersions prepared as for solubility testing were stirred, adjusted to pH 7.0, and centrifuged. An aliquot of soluble protein (supernatant) was serially diluted to obtain 6.25–100 μg/mL protein with 0.1M phosphate buffer (pH 7.0) as diluent. Forty microliters of ANS (8.0 mM in 0.01 M phosphate buffer, pH 7.0) were dispersed in 3-mL aliquots of each dilution. Fluorescence intensity units (FIU) were measured with a Turner Quantech® spectrophotometer (Barnstead Thermolyne, Dubuque, IA, USA) using 440 nm (excitation) and 535 nm (emission) filters. FIU were standardized using a solution of 40-μL ANS in 3-mL phosphate buffer as the zero point and 15-μL ANS in 3-mL methanol assigned an arbitrary value of 80 FIU. FIUs 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 using the method of Bian et al. [18] with modifications. Twenty-five milliliters of 2% (w/w, dry basis) sample dispersions were adjusted to 7.0 with 2 N HCl or NaOH as needed and transferred to a 400-mL plastic beaker. Soybean oil, dyed with approximately 4 ppm of Sudan Red 7B (Sigma, St. Louis, MO, USA), was continuously blended into the dispersion at 37 mL/min flow rate using a Bamix wand mixer (ESGE AG Model 120, Mettlen, Switzerland) at the low setting until phase inversion was observed. Emulsification capacity (g oil/g sample) was calculated as grams of oil required to cause inversion multiplied by 2. Samples were run at least in triplicate.

Emulsification activity (EA) and emulsification stability index (ESI) were measured using the methods of Rickert et al. [10]. Twenty-one milliliters of 2% (w/w, dry basis) sample dispersions adjusted to 7.0 were blended with 7 mL of 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 1:1000 with 0.1% sodium dodecyl sulfate. Absorbance was measured at 500 nm and recorded as EA. After 15 min, the absorbance was measured again, and the two absorbance readings were used to calculate ESI where ESI (min) = $(A_0/A_0 - A_{15})t$, where A_0 and A_{15} are absorbance at time 0 and 15 min, respectively, and t is the time interval. Samples were run in triplicate.

Foaming Properties

Foaming properties were measured using methods of Sorgentini et al. [19] with modifications [4]. Dispersions of protein fractions (0.5% w/w, dry basis) were prepared and pH adjusted to 7.0. Aliquots (95 mL) were loaded into a custom-designed 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 the volume of the liquid incorporated into the 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} was the volume of liquid incorporated into foam, and $t_{1/2}$ was

the time to drain one half of the liquid incorporated into the foam. 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 using the SAS system (version 8.2, SAS Institute, Cary, NC, USA).

Results and Discussion

Protein Fraction Yields and Proximate Compositions

The Wu fractionation procedure [8] yielded slightly less total solids (34.7%) when all protein fractions were considered. The Deak fractionation procedure with chilling to 4 °C (D4C) yielded 38.5% of the solids and 36.5% of the solids without chilling (DRT) (Table 1). These results differed slightly from our previous findings using normal soybeans [11] where the solids yields for the Wu procedure were slightly higher than for the Deak fractionation procedures.

The total protein yields were higher when using the Wu procedure (69.9%) than were those for D4C (55.0%) and DRT (54.2%), but 23% of the recovered protein in the Wu procedure was in the intermediate fraction, which is much less useful because it is a mixture of denatured proteins and has low protein content. Similar results were obtained in our previous work using normal soybeans [11]. Significantly more protein was recovered in the intermediate fraction when using the Wu procedure, and the Deak procedures yielded more protein when the defatted soy flour was from normal soybeans [11] compared to defatted flour obtained from HS/LS soybeans.

Comparisons of Fractions for Different Fractionation Methods

When comparing the glycinin-rich fractions produced using the three fractionation procedures, the D4C procedure yielded more solids than did the other two procedures, however, the Wu procedure yielded significantly more protein. The protein contents of the glycinin-rich fractions were well above 90% for all three procedures. The ash content for the glycinin-rich fraction of the Wu procedure was higher than the glycinin-rich fractions produced by the other two procedures. The glycinin-rich fraction obtained using the Wu procedure yielded almost 10% more protein from HS/LS flour than from normal flour [11]. The ash contents of the glycinin-rich fractions obtained from HS/LS flour were higher than obtained from normal soy flour [11].

When comparing the β-conglycinin-rich fractions produced by the three fractionation procedures, both Deak procedures yielded significantly more solids and protein. The protein contents of the β-conglycinin-rich fractions were >90% for all treatments. When using the Wu procedure, the protein yield of the β-conglycinin-rich fraction was ~4.0% higher from HS/LS soybeans than from normal soybeans [11].

The Wu procedure also produced an intermediate fraction having ~15% lower protein content than the other two fractions making this fraction much less desirable. The intermediate fraction contained

Table 1 Yields and compositions (dry basis) of soy protein fractions prepared from HS/LS soybeans by the Wu and Deak procedures

Fraction/treatment	Solids yield (%)	Protein yield (%)	Protein content (%)	Ash (%)
Wu glycinin	15.4[b]	31.7[a]	96.4[a]	4.1[a]
D4C glycinin	18.0[a]	25.7[b]	97.3[a]	3.6[b]
DRT glycinin	14.3[b]	25.5[b]	94.7[a]	3.4[b]
LSD	1.8	3.5	4.4	0.3
Wu intermediate	8.8 ± 0.3	15.9 ± 0.7	80.9 ± 0.7	14.8 ± 0.1
Wu β-conglycinin	10.5[c]	22.3[b]	95.6[a]	11.2[a]
D4C β-conglycinin	20.5[b]	29.3[a]	92.2[b]	6.1[b]
DRT β-conglycinin	22.2[a]	28.7[a]	92.0[b]	5.8[b]
LSD	1.1	3.3	1.4	0.5
LSD[a]	1.1	2.4	2.3	0.3

Wu fractions produced by using the Wu procedure; *D4C* fractions produced using the Deak procedure with a chilling step; *DRT* fractions produced using the Deak procedure without a chilling step; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $p < 0.05$

[a] Least significant difference to compare all fractions within a column

considerable amounts of solids and protein with high ash contents. The amounts of solids and protein comprising the intermediate fraction were 9.4 and 10.9% lower, respectively, when using HS/LS soy flour compared to normal soy flour [11].

Protein Compositions

The total storage protein contents of the glycinin-rich fractions for each procedure were different (Table 2). The Wu procedure yielded a glycinin-rich fraction with 10% more storage protein than did the D4C procedure and ~20% more than the DRT procedure. The purity of the glycinin-rich fraction was also affected by the method of fractionation. The Wu procedure yielded a glycinin-rich fraction with 20% higher purity than the D4C procedure and 26.7% more pure than the DRT procedure. The glycinin subunit composition was significantly different for each procedure. The Wu procedure yielded a glycinin-rich fraction with 10.1 and 6.3% more acidic polypeptides than did the glycinin-rich fractions obtained by using the D4C and DRT procedures, respectively. All three procedures gave higher proportions of acidic polypeptides than basic polypeptides in the glycinin component of the glycinin-rich fraction. The subunit compositions of the contaminant β-conglycinin when using the Deak procedures were not different from the Wu procedure (Table 2). Comparing these observations for soy flour from HS/LS soybeans with the results obtained by Deak et al. [11] using soy flour prepared from normal soybeans, the glycinin-rich fraction from HS/LS flour when using the Wu process was more pure, whereas the Deak procedures yielded fractions with similar purities when using normal and HS/LS soy flours.

The β-conglycinin-rich fraction recovered from HS/LS soy flour when using the Wu procedure contained ~20% more storage protein than the same fraction using the Deak procedures. The highest purities were achieved using the Wu procedure followed by the Deak procedures (Table 2). The β-conglycinin subunit composition in the β-conglycinin-rich fraction obtained with the Wu procedure was different than the fractions obtained with the Deak procedures. The Wu procedure yielded a β-conglycinin-rich fraction with no glycinin contamination. The amounts of the contaminant glycinin in the β-conglycinin-rich fraction when using the Deak procedures were higher for HS/LS soy flour than the amounts reported for normal soy flour [11].

The intermediate fraction produced from HS/LS soy flour using the Wu procedure contained ~30% less storage protein than the other two fractions. The intermediate fraction contained nearly equivalent amounts of glycinin and β-conglycinin, but with different subunit compositions. The β subunit was the main component of the β-conglycinin present. Comparing this fraction to the same fraction obtained from normal soybeans [11], HS/LS flour gave an intermediate fraction with less storage protein and different β-conglycinin subunit composition. The Wu procedure was more effective in fractionating glycinin and β-conglycinin from HS/LS soy flour than normal soybeans, while the Deak procedures were more

Table 2 Compositions and subunit profiles (%) of protein fractions prepared from HS/LS soybeans by the Wu and Deak procedures

Fraction/treatment	Storage protein in fraction (%)	β-conglycinin				Glycinin		
		%	Subunit composition (%)			%	Subunit composition (%)	
			α'	A	β		A	B
Wu glycinin	100.0^{a}	0.0^{c}	0.0^{b}	0.0^{b}	0.0^{b}	100.0^{a}	63.6^{a}	36.4^{c}
D4C glycinin	88.9^{b}	20.0^{b}	29.7^{a}	22.4^{a}	48.0^{a}	80.0^{b}	53.5^{c}	46.5^{a}
DRT glycinin	81.5^{c}	26.7^{a}	23.3^{a}	24.2^{a}	52.5^{a}	73.3^{c}	57.2^{b}	42.8^{b}
LSD	0.7	3.1	8.3	3.0	6.3	3.1	2.1	2.1
Wu intermediate	69.6 ± 2.3	51.0 ± 3.6	28.6 ± 1.0	28.0 ± 0.9	43.4 ± 0.2	49.0 ± 3.6	45.1 ± 3.3	54.9 ± 3.3
Wu β-conglycinin	100.0^{a}	100.0^{a}	32.7^{a}	38.5^{a}	28.8^{b}	0.0^{b}	0.0^{b}	0.0^{b}
D4C β-conglycinin	78.2^{c}	73.1^{b}	29.5^{a}	$32.6^{a,b}$	37.9^{a}	26.9^{a}	49.5^{a}	50.5^{a}
DRT β-conglycinn	81.2^{b}	71.9^{b}	30.9^{a}	28.9^{b}	40.1^{a}	28.1^{a}	46.7^{a}	53.3^{a}
LSD	0.3	1.5	10.7	6.4	4.7	1.5	3.4	3.4
LSD[a]	2.1	3.6	6.7	3.5	3.8	3.6	3.6	3.6

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; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *a* acidic subunits of glycinin; *b* basic subunits of glycinin; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $p < 0.05$

[a] Least significant difference to compare all fractions within a column

effective fractionating storage proteins from soy flour prepared from normal soybeans than HS/LS soybeans.

Thermal Behavior

The thermal properties of the glycinin-rich fractions are shown in Table 3. The peak temperature of denaturation was slightly lower for the glycinin portion and slightly higher for the contaminant β-conglycinin portion when using the Wu procedure than were the same fractions obtained using the Deak procedures. The contaminant β-conglycinin comprised 1.6, 2.7, and 6.7% of the total denaturation enthalpy in the glycinin-rich fractions obtained with the Wu, D4C, and DRT procedures, respectively. Although no protein contamination was detected by SDS-PAGE when using HS/LS soy flour in the Wu procedure, some denaturation enthalpy for β-conglycinin was detected. Gel electrophoresis is a less sensitive test, compared to DSC, for detecting the presence of small quantities of contaminant proteins. The glycinin-rich fraction had significantly higher denaturation enthalpy among all fractions in all treatments. The glycinin-rich fractions produced with the Deak procedures had about 2.5 and 2.8 times more denaturation enthalpy than the β-conglycinin-rich fractions using the D4C and DRT procedures, respectively. The denaturation enthalpies and peak temperatures observed for the fractions obtained from HS/LS soy flour were similar to those of soy flour prepared from normal soybeans [11].

The peak denaturation temperatures for the β-conglycinin-rich fractions were approximately the same for all treatments and components with the exception of the contaminating glycinin component. The contaminating component was significantly higher in the DRT procedure than that from the other two procedures (Table 3). The glycinin contaminant comprised 1.6, 13.7, and 24.8% of the total denaturation enthalpy in the β-conglycinin-rich fraction for the Wu, D4C, and DRT procedures, respectively. The denaturation enthalpies of the β-conglycinin-rich fractions prepared from HS/LS soybeans were significantly higher than those prepared from normal soybeans [11]. The Wu procedure yielded a β-conglycinin-rich fraction with the highest denaturation enthalpy.

The intermediate fraction produced using the Wu procedure had the lowest total denaturation enthalpy among all protein fractions indicating substantial denaturation. The β-conglycinin component of the intermediate fraction comprised 26% of the total denaturation enthalpy, while the glycinin component comprised 76%. This 3:1 ratio was not observed by SDS-PAGE (where the proportion was 1 to 1) and was probably due to partial denaturation of the β-conglycinin component recovered in this fraction.

Solubility

The procedure used for fractionation significantly affected the solubilities of the various fractions (Table 4). There were no significant differences in solubility among the glycinin-rich fractions. In our previous study with normal soybeans [11], the Deak procedures yielded glycinin-rich fractions with lower solubilities and the temperature used to precipitate this fraction significantly affected solubility.

The Wu procedure yielded a β-conglycinin-rich fraction having significantly higher solubility. The

Table 3 Thermal behaviors of protein fractions prepared from HS/LS soybeans by the Wu and Deak procedures

Fraction/treatment	β-conglycinin Td (°C)	Glycinin Td (°C)	β-conglycinin enthalpy (mJ/mg)	Glycinin enthalpy (mJ/mg)	Total enthalpy (mJ/mg)
Wu glycinin	74.9[a]	89.5[b]	0.26[b]	15.96[b]	16.21[b]
D4C glycinin	73.8[a,b]	91.5[a]	0.51[b]	18.65[a]	19.16[a]
DRT glycinin	73.5[b]	91.3[a]	1.33[a]	18.62[a]	19.96[a]
LSD	1.3	1.0	0.47	1.70	2.10
Wu intermediate	75.5 ± 0.3	93.6 ± 0.2	1.06 ± 0.13	3.06 ± 0.10	4.08 ± 0.09
Wu β-conglycinin	75.3[a]	90.0[b]	10.33[a]	0.17[c]	10.47[a]
D4C β-conglycinin	75.8[a]	89.8[b]	6.48[b]	1.03[b]	7.50[b]
DRT β-conglycinin	75.3[a]	91.7[a]	5.35[b]	1.77[a]	7.13[b]
LSD	1.4	1.4	1.25	0.37	1.54
LSD[a]	0.9	0.9	0.66	0.85	1.27

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; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *Intermediate* intermediate fraction; *Td* peak denaturation temperature; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $p < 0.05$

[a] Least significant difference to compare all fractions within a column

Table 4 Solubilities and surface hydrophobicities of protein fractions prepared from HS/LS soybeans by the Wu and Deak procedures

Fraction/treatment	Solubility (%)	Surface hydrophobicity (dimensionless)
Wu glycinin	88.9[a]	152[a]
D4C glycinin	92.9[a]	148[a]
DRT glycinin	93.2[a]	154[a]
LSD	5.6	33
Wu intermediate	41.6 ± 0.8	179 ± 5
Wu β-conglycinin	92.8[a]	185[a]
D4C β-conglycinin	75.9[b]	180[a]
DRT β-conglycinin	70.4[b]	130[b]
LSD	6.2	23
LSD[a]	4.1	20

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; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different $p < 0.05$

[a] Least significant difference to compare all fractions within a column

Deak procedures yielded β-conglycinin-rich fractions with 17.0 and 22.8% less solubility than the glycinin-rich fractions produced by the same procedures, respectively. The solubilities of the β-conglycinin-rich fractions prepared from normal soybeans and HS/LS soybeans were similar [11].

The intermediate fraction was the least soluble fraction because it contained more denatured protein as indicated by the low total denaturation enthalpy. Similar conclusions were drawn for the intermediate fraction prepared from flour of normal soybeans [11].

Surface Hydrophobicity

There were no differences in surface hydrophobicities among the glycinin-rich fractions (Table 4). The β-conglycinin-rich fraction prepared using the DRT procedure had the lowest surface hydrophobicity. The fractions obtained from HS/LS soy flour had lower hydrophobicities than the same fractions prepared from normal soybeans [11] with the exception of the intermediate and β-conglycinin-rich fractions prepared by the Wu procedure, which was attributed to less protein denaturation in these fractions.

Emulsification Properties

Emulsification capacity, activity, and stability index results are shown in Table 5. The glycinin-rich fractions produced with the Deak procedures had significantly higher emulsification capacities than the same fraction produced with the Wu procedure. We attributed the higher emulsification capacities of the fractions produced with the Deak procedures to the fractions containing more native-state proteins as indicated by higher denaturation enthalpies. Alternatively, calcium ions may have introduced structural changes [20] to these proteins allowing them to be better surfactants. The emulsification capacities for the glycinin-rich fractions produced from the HS/LS soy flour were lower than those of the same fractions from normal soy flour [11]. EAs and ESIs were similar for the glycinin-rich fractions among all three procedures and were significantly lower than for the β-conglycinin-rich fractions. This phenomenon may have been partially due to the glycinin-rich fractions having lower surface hydrophobicities.

The emulsification capacities of the β-conglycinin-rich fractions produced by the Wu and DRT procedures were significantly higher than for the β-conglycinin-rich fraction produced with the D4C procedure (Table 5). There were no significant differences among treatments for EA and ESI. These findings differed somewhat from our previous findings for fractions produced from normal soybeans [11]. The HS/LS soybeans produced β-conglycinin-rich fractions with the highest EAs and ESIs. This was probably because these fractions also had the highest surface hydrophobicities. The intermediate fraction was a poor emulsifier.

Foaming Properties

Foaming capacity is a measure of foaming efficiency; foaming stability is related to the ability of foam to hold air; and the rate of foaming gives a measure of speed of forming foam. In general, the fractions prepared with the DRT procedure had the best foaming properties (Table 6). The foaming rates for the glycinin-rich fractions were similar for all processes although the fractions made by the DRT procedure foamed twice as fast as did the same fractions made by the Wu procedure. Foaming capacity followed the same order, but the stabilities of the foams formed by the fractions made with the DRT procedure were significantly lower than the same fractions produced by the Wu procedure and similar to the fractions produced by the D4C procedure.

The β-conglycinin-rich fraction produced by the DRT procedure had the best foaming properties. In general, the foams prepared from the β-conglycinin-rich fractions were more stable than those prepared from the glycinin-rich fractions, probably due to their

Table 5 Emulsification properties of protein fractions prepared from HS/LS soybeans 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)
Wu glycinin	307[c]	0.155[a,b]	76[b]
D4C glycinin	618[a]	0.177[a]	103[a]
DRT glycinin	547[b]	0.151[b]	83[a,b]
LSD	62	0.026	22
Wu intermediate	219 ± 5	0.194 ± 0.012	69 ± 6
Wu β-conglycinin	612[a]	0.311[a]	216[a]
D4C β-conglycinin	564[b]	0.301[a]	216[a]
DRT β-conglycinin	633[a]	0.322[a]	240[a]
LSD	41	0.038	147
LSD[a]	36	0.025	73

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; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $p < 0.05$

[a] Least significant difference to compare all fractions within a column

Table 6 Foaming properties of protein fractions prepared from HS/LS soybeans by the Wu and Deak procedures

Fraction/treatment	Foaming capacity (mL/mL)	Foaming stability [k = 1/(mL min)]	Rate of foaming (V_i = mL/min)
Wu glycinin	1.090[c]	0.092[a]	2.3[a]
D4C glycinin	1.300[b]	0.173[b]	4.4[a]
DRT glycinin	1.514[a]	0.164[b]	5.0[a]
LSD	0.083	0.035	2.9
Wu intermediate	1.141 ± 0.062	0.005 ± 0.001	21.9 ± 0.7
Wu β-conglycinin	1.184[c]	0.018[a]	13.7[b]
D4C β-conglycinin	1.396[b]	0.035[b]	14.2[b]
DRT β-conglycinin	1.671[a]	0.012[a]	30.4[a]
LSD	0.186	0.007	4.5
LSD[a]	0.113	0.017	2.6

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; *glycinin* glycinin-rich fraction; *β-conglycinin* β-conglycinin-rich fraction; *LSD* least significant difference at $p < 0.05$

$n = 2$. Means within a column for a specific fraction followed by different superscripts are significantly different at $p < 0.05$

[a] Least significant difference to compare all fractions within a column

higher surface hydrophobicities. The intermediate fraction produced by the Wu procedure had the highest foaming stability with high rate of foaming and low foaming capacity.

When comparing the protein fractions produced from HS/LS soy flour with the same fractions produced from flour of normal soybeans [11], the glycinin-rich fractions prepared from HS/LS soy flour formed less-stable foams with similar foaming capacities and with significantly slower foaming rates with the Deak procedures. The β-conglycinin-rich fractions prepared from HS/LS soy flour had similar foaming properties to the β-conglycinin-rich fractions produced by the Wu and DRT procedures and significantly poorer than produced by the D4C procedure. The intermediate fractions for both flours had similar foaming properties.

The Deak procedures produced higher yields of glycinin-rich and β-conglycinin-rich fractions from HS/LS soybeans than did the Wu process. The Deak procedures were less efficient in fractionating proteins from HS/LS soybeans than from normal soybeans but yielded products with unique solubility, surface hydrophobicity, and emulsification and foaming properties.

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