Simplified Process for Soybean Glycinin and β-Conglycinin Fractionation

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A simplification of the pilot-plant scale modified Nagano method yielding two protein fractions, glycinin and β-conglycinin, by pH adjustment and ultrafiltration membrane separation was developed and compared with our pilot-plant-scale modified Nagano procedure and with a soy protein isolate pilot-plant procedure as our reference process. Two protein fractions, glycinin and β-conglycinin, were produced from our simplified process and compared to the three protein fractions, glycinin, β-conglycinin, and an intermediate protein mixture, produced with the modified Nagano method. The pilot-plant yields of glycinin, β-conglycinin, and intermediate mixture fractions from the modified Nagano method were 9.4, 10.3, and 4.8% [dry basis (db)], respectively. The yield of glycinin fraction of the simplified method was 9.7% (db), and it had a protein content and purity similar to those obtained with the modified Nagano method. The yield of the β-conglycinin fraction was 19.6% (db), which was twice that of the modified Nagano process. The protein content of β-conglycinin was 91.6% (db), and the purity was 62.6% of the protein content, which was 9% lower in purity than the modified Nagano method. Process optimization of the simplified method suggested the best operating conditions for the membrane filtration system were 20−25 psi inlet pressure and 200−250 L/min ultrafiltration recirculation speeds.

Keywords: *Pilot-plant protein fractionation; glycinin; β-conglycinin; soy protein separation; ultrafiltration*

INTRODUCTION

Soy protein isolate has been produced on a large scale for many years, and the process has been progressively improved (Mustakas and Sohns, 1979; Moulton and Wang, 1982). The commercial production of soy protein isolate for industrial uses began in 1935. Typically, soy proteins are extracted with water over a pH range of 6.8−9.0 and at temperatures of 55−60 °C for 15 min to 2 h depending on the manufacturer. Proteins in the extract are precipitated by lowering the pH to between 4 and 5 and then separated from the whey by using a centrifuge and spray-dried. However, little published information can be found on larger scale separation processes for the individual soy storage proteins, glycinin and β-conglycinin. We successfully produced kilogram quantities of the individual soy storage protein fractions in the pilot plant (Wu et al., 1999) by using a modification of Nagano's method (Nagano et al., 1992). Three protein fractions, glycinin, an intermediate protein mixture, and β-conglycinin, were produced by using water as an extraction buffer and sodium bisulfite as a reducing agent and adjusting the pH to 6.4, 5.0, and 4.8 for precipitating the three protein fractions, respectively. The yields of glycinin and β-conglycinin were 9.4 and 10.3% [dry basis (db)], respectively. If we could eliminate the intermediate protein mixture, the yields of glycinin and β-conglycinin fractions would significantly increase and the economics of individual soy storage protein fractionation would be more attractive.

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Industrial membrane processes for producing purified and concentrated soy protein were reported by Lawhon et al. (1977). Rather than acid precipitation of the proteins, ultrafiltration (UF) and diafiltration were used to produce soy protein with >90% protein content. The protein extract was pasteurized by heating at 65 °C for 30 min and prefiltered with a 100 μm membrane. The protein extract was circulated across a high-performance noncellulosic second-generation membrane in a UF system. The retentate was spray-dried as soy protein isolate. The permeate was processed with a cellulose-based reverse-osmosis (RO) membrane in an RO system. The RO products were freeze-dried, and the process water was recycled for subsequent extractions. A 50% soy isolate yield, based on soy flour mass, was reported (Lawhon et al., 1979, 1981). Using UF and RO techniques, all of the solubilized protein could be removed without generating whey-like byproducts, thus avoiding disposal problems while increasing the yield of protein. The membrane-processed soy proteins were very soluble and had different functionalities compared to the acid-precipitated product (Lawhon et al., 1979).

Here, we report an alternate procedure producing two fractions, glycinin and β-conglycinin, utilizing a pilot-plant UF membrane system. The protein yields and purities were compared with the pilot-plant results of our modified Nagano method and with traditional pilot-plant soy protein isolate production.

MATERIALS AND METHODS

Soybean White Flakes. Soybean white flakes were produced from MBS 2795 (1995 crop, Iowa) soybeans extracted with hexane and desolventized at ambient temperature in the

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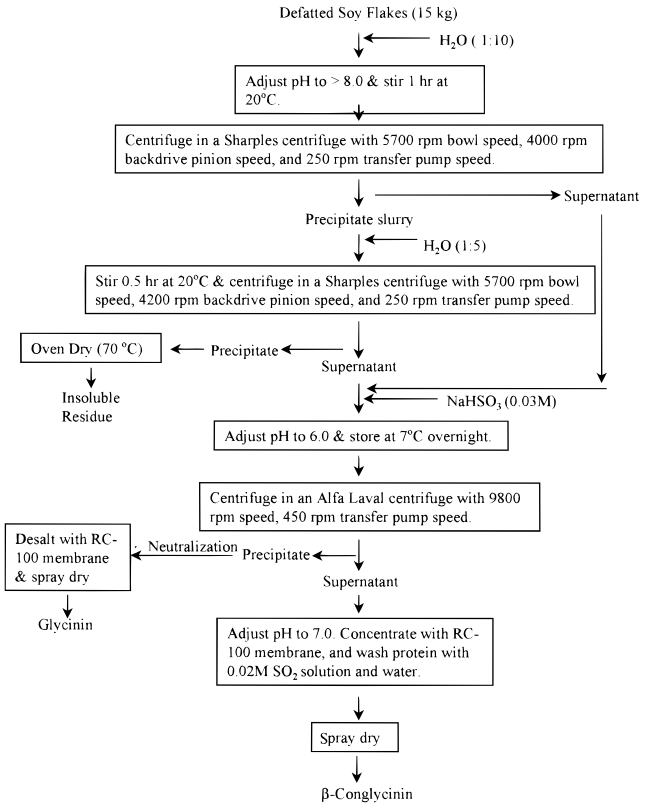

Figure 1. Simplified glycinin and β-conglycinin pilot-plant separation process for 15 kg of defatted soy flakes.

pilot plant of the Center for Crops Utilization Research, Iowa State University (Wu et al., 1999).

Chemicals. All chemical reagents were from Fisher Scientific Co., Inc. (St. Louis, MO).

Simplified Membrane Pilot-Plant Procedure. Fifteen kilograms of defatted white soybean flakes was dispersed at a 1:10 ratio of flakes to water in a 200-gal (740 L) tank with a custom-built chilling system (Figure 1). The pH of the slurry was adjusted to >8.0 with 2 N NaOH and stirred. After 1 h of extraction at 20 °C, the pH of the slurry had decreased to ≅7.5. The insoluble residue was separated by centrifugation with a Sharples P660 decanting centrifuge (Alfa Laval Separation Inc., Warminster, PA) at 5700 rpm bowl speed and 4000 rpm backdrive pinion speed; feeding was performed with a Moyno-type feed pump (Electric Pump, model 1FFCA SSE SAA, Des Moines, IA) at 250 rpm pump speed. The flake residue was re-extracted at a 1:5 ratio of flakes to water. The pH of the slurry remained at 7.5. 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 feed pump at the same conditions as previously described.

The two protein extracts were combined, and solid sodium bisulfite was added to give 30 mM SO_2. Glycinin was precipitated from solution by adjusting the pH to 6.0 with 2 N HCl and storing the solution overnight at 7 °C. The precipitated glycinin in the cold solution was separated using an Alfa Laval BTPX 205 disc-type centrifuge (Alfa Laval Separation Inc.) at 9800 rpm bowl speed and 540 rpm feed pump speed. The precipitated glycinin fraction was 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 an RC-100 membrane (100-kDa molecular weight cutoff regenerated cellulose membrane, 20 ft^2) (North Carolina SRT Inc.). Diafiltration was performed at a 300 L/min recirculation speed, 44 psi inlet membrane pressure, and 16 psi outlet membrane pressure until 5 times the original volume of permeate solution was collected.

The supernatant, containing β-conglycinin, was adjusted to pH 7.0 with 2 N NaOH. The protein solution was concentrated with the Feed and Bleed membrane filtration system and an RC-100 membrane. UF was carried out at 200 L/min recirculation speed. Inlet pressure of membrane at 20−25 psi, outlet pressure at 6−7 psi, and feed temperature of 25 °C were used. After the total mass of protein solution was concentrated to 20 kg, diafiltration was performed at the same recirculation speed. Three original volumes of bisulfite solution, at 20 mM SO_2 and pH 7.0, and two original volumes of distilled water were used to wash the protein fraction.

UF Process Optimization for β-Conglycinin Recovery by the Simplified Process. Three experiments were conducted to optimize the UF regenerated-cellulose RC-100 membrane process for β-conglycinin recovery from the β-conglycinin supernatant. Five UF recirculation speeds (100, 150, 200, 250, and 300 L/min) were evaluated in the first process optimization experiment. A second optimization experiment was designed to investigate the influence of recirculation speeds of 150−300 L/min, at 50 L/min intervals, on permeate solids while the membrane pressure was kept at a constant 20 psi. A third optimization experiment was designed to compare the influence of 200 and 250 L/min recirculation speeds on the purity of the β-conglycinin fraction. After removal of the glycinin precipitate, the pH 7.0 supernatant was concentrated using the UF system until the total mass of the retentate was 20 kg. Sodium bisulfite solution (as 20 mM SO_2) at 3 times the protein solution mass and water at 3 times the protein solution mass were used to wash the UF retentate.

Both glycinin and β-conglycinin retentates were dried in an Anhydro compact spray-dryer (APV Crepaco Inc., Attleboro Falls, MA) with 180 °C air-inlet temperature and 85 °C air-outlet temperature. Insoluble residue was dried in a tray dryer (National Drying Machinery Co., Philadelphia, PA) at 70 °C for 28 h.

Modified Nagano Pilot-Plant Procedure. Fifteen kilograms of defatted white soy flakes was used to produce glycinin, β-conglycinin, and intermediate protein mixture fractions using our modified Nagano method on a pilot-plant scale (Figure 2) (Wu et al., 1999). A 200-gal (740 L) tank with a custom-built chilling system was used for protein extraction. Fifteen kilograms of defatted soybean white flakes were dispersed at the ratio of flakes to water of either 1:15 or 1:10. The pH of the slurry was adjusted to 8.5 with 2 N NaOH and stirring. After 1 h of extraction at 20 °C, the pH of the slurry had decreased to ∼8.0. The insoluble residue was separated by centrifugation with a Sharples P660 decanting centrifuge (Alfa Laval Separation Inc.) at 5700 rpm bowl speed, 4000 rpm backdrive pinion speed, and 250 rpm pump speed for a Moyno-type transfer pump (Electric Pump, model 1FFCA SSE SAA). The flake residue was re-extracted at a ratio of flakes to water of 1:10 or 1:5. The pH of the slurry remained at ∼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 (Alfa Laval Separation Inc.) at a bowl speed 9800 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 the 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 times 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. The precipitated glycinin,

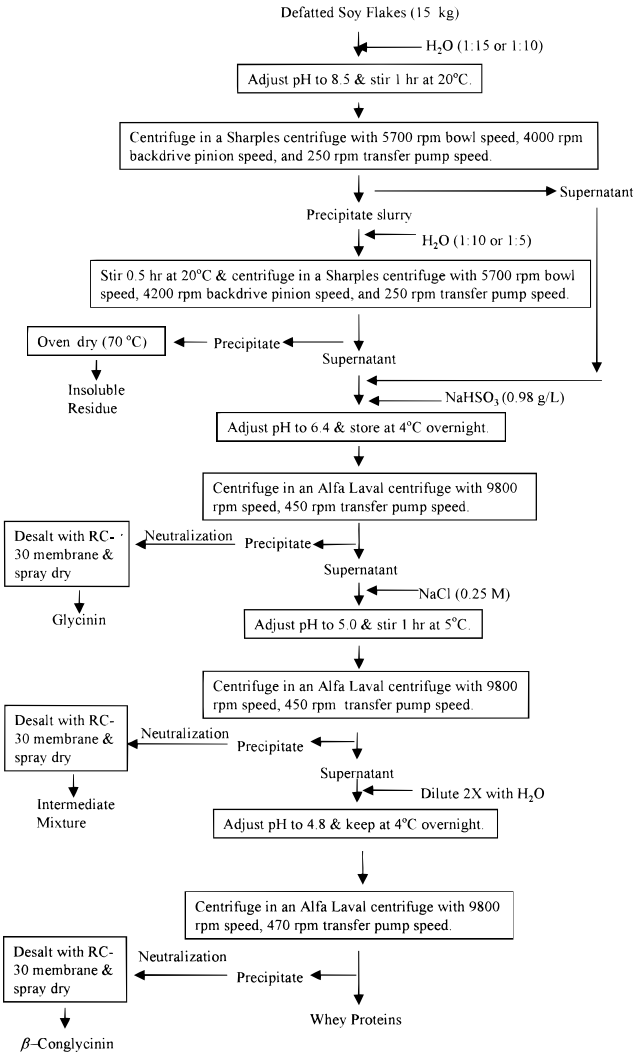

Figure 2. Modified Nagano glycinin and β-conglycinin pilot-plant separation process (Wu et al., 1999).

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.) and a 30-kDa regenerated-cellulose membrane (North Carolina SRT Inc.). Diafiltration was performed until 5 times the original volume of permeate solution was collected. The retentate was dried in an Anhydro compact spray-dryer (APV Crepaco Inc.) with an air-inlet temperature 180 °C and an air-outlet temperature 85 °C. Insoluble residue was dried in a tray dryer (National Drying Machinery Co., Philadelphia, PA) at 70 °C for 28 h.

Pilot-Plant Procedure for Soy Protein Isolate Production. Traditional soy protein isolate was produced using a pilot-plant method developed by the Center for Crops Utilization Research, Iowa State University, to compare with the glycinin and β-conglycinin protein products from the simplified fractionation process and the modified Nagano process. All of the equipment, Sharples and Alfa Laval centrifuges, Moyno-type feed pump, and spray-dryer, used the same setting, temperature, and speed as described above for the simplified fractionation process. Fifteen kilograms of MBS 2795 defatted white soy flakes was used to extract protein (Figure 3). The first extraction was with a 1:10 ratio of flakes to water, and the second extraction was with a 1:5 flakes-to-water ratio. The pH of the extraction slurry was adjusted to 8.6 with a 2 N NaOH solution, and each extraction was performed at 60 °C for 30 min. After centrifugation with a Sharples P660 decanting centrifuge to remove the insoluble residue, the pH of supernatant was adjusted to 4.5 with 2 N HCl solution. The

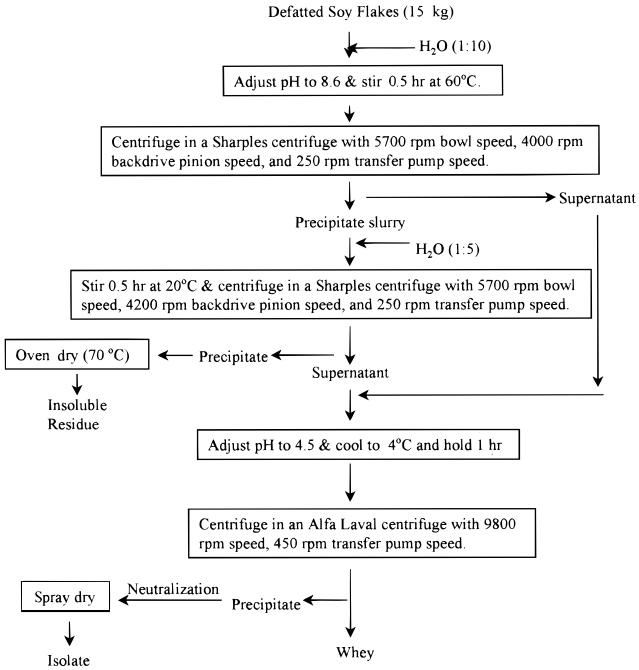

Figure 3. Soy protein isolate pilot-plant production process.

solution was cooled to 4 °C and held for 1 h. After separation of the whey with the Alfa Laval PTPX 205 disc centrifuge, the soy protein precipitate was dispersed in water, neutralized with 2 N NaOH to pH >7.5, and spray-dried in the Anhydro compact spray-dryer.

Proximate Analysis. Protein contents were determined by measuring total nitrogen content using the micro-Kjeldahl method (AOAC, 1990, methods 988.05 and 960.52). 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 (Wolf, 1978). Moisture contents of soy flakes and protein fractions were determined by drying a 2.00 g sample in a forced-air oven (Fisher Scientific, Isotemp 750F, Pittsburgh, PA) for 3 h at 130 °C (AACC, 1983a, method 44-15A). Ash content of protein fractions was determined by using AACC method 08-03 (AACC, 1983b).

Urea−SDS−PAGE and Gel Density Image Analysis. Urea−sodium dodecyl sulfate−polyacrylamide gel electrophoresis followed by gel density image analysis was used to evaluate glycinin, β-conglycinin, and lipoxygenase contents in the protein fractions (Wu et al., 1999).

Statistical Analysis. The general linear model and least significant difference (LSD) test at the 5% level were used to evaluate differences among means (SAS 6.3, Cary, NC, 1999).

RESULTS AND DISCUSSION

Two protein fractions, glycinin and β-conglycinin, were produced with our simplified process and compared to three protein fractions, glycinin, β-conglycinin, and an intermediate protein mixture, produced from the modified Nagano procedure. In our new method, glycinin was produced by precipitation at 7 °C, pH 6.0, and β-conglycinin was produced by precipitation at pH 7.0 and by using a UF membrane system in our simplified process. The modified Nagano protein fractions, glycinin, intermediate mixture, and β-conglycinin, were precipitated at pH 6.4, 5.0, and 4.8, respectively.

The yield of the glycinin fraction in the simplified method was 9.7% (db), which was significantly higher than that obtained by using the modified Nagano method (Table 1). This increased yield is most likely due to fractionation of proteins into the glycinin fraction of

Table 1. Yields and Protein and Ash Contents of Soy Protein Fractions Produced in Pilot-Plant Processes[a]

	modified Nagano method	simplified method
yield (%, db)		
glycinin	9.4b	9.7a
β-conglycinin	10.3b	19.6a
intermediate mixture	4.8	na
protein content (%, db)		
glycinin	92.6a	91.2b
β-conglycinin	97.6a	91.6b
intermediate mixture	85.7	na
ash content (%, db)		
glycinin	3.11a	2.68b
β-conglycinin	2.61b	4.96a
intermediate mixture	2.67	na

[a] $n = 2$. Data in the same row with different letters are significantly different at $p < 0.05$

the simplified method that formerly were part of the intermediate fraction in the modified Nagano method. We previously reported that the two storage proteins in the intermediate fraction are more denatured than in the glycinin and β-conglycinin fractions (Wu et al., 1999). The simplified method appears to be less sensitive to native protein structure in controlling protein mass distribution. Average protein content in the glycinin fraction was 91.2%, and the ash content was 2.68%. Both results were significantly lower than the results of the modified Nagano method due to the shorter desalinization periods used in the modified Nagano process and the difference in UF membrane filters, a 100-kDa cutoff membrane in the simplified method and a 30-kDa membrane in the modified Nagano method.

The simplified method produced only one other protein fraction, β-conglycinin. Sodium bisulfite, introduced in the UF washing solution, facilitated non-β-conglycinin protein removal and eliminated the pasteurization step usually used in soy protein isolate production because bisulfite has antibacterial properties (Davidson and Juneja, 1990). The yield of the β-conglycinin fraction was higher than that produced from the modified Nagano method. The average yield of the β-conglycinin fraction from the simplified method was 19.6%, almost double the amount obtained from the modified Nagano method (Table 1). These yields were higher than the sum of the β-conglycinin and the intermediate protein mixture fractions of the modified Nagano method. Again, we may be observing fractionation of parts of the intermediate fraction of the modified Nagano process into the β-conglycinin fraction of the simplified method as discussed above for glycinin yield differences. Average protein content of the β-conglycinin fraction from the simplified method was 91.4% (db) and was significantly lower than that obtained when using the modified Nagano method. The ash content in the β-conglycinin fraction was significantly higher than in the modified Nagano method, thus contributing to the lower protein content. During the desalting step of the modified Nagano method, five original volumes of distilled water were used to wash the β-conglycinin fraction. Only two original volumes of distilled water were used to wash the β-conglycinin fraction in the simplified method because of time and labor limitations, and this change in washing is the probable cause of higher ash content and lower protein content of this fraction.

The glycinin purity, using the simplified method, was 92.8% protein, which was significantly higher than that produced with the modified Nagano method (Table 2). This difference is most likely due to denatured glycinin, found in the intermediate fraction of the modified Nagano process and now appearing in the glycinin fraction of the simplified process (Wu et al., 1999). The β-conglycinin and lipoxygenase contaminants in the glycinin fractions were not different for the two methods. The intermediate fraction of the modified Nagano method contained glycinin, β-conglycinin, and significant amounts of the lipoxygenases. With no intermediate fraction produced when using the simplified method, average β-conglycinin purity was 62.6% protein and significantly lower than the 71.3% β-conglycinin purity from the modified Nagano method. The glycinin and lipoxygenase contaminations in the β-conglycinin fraction were significantly higher than obtained when using the modified Nagano process because we no longer have the intermediate fraction (Table 2).

The fraction mass recoveries of the two processes and soy isolate are compared in Table 3. The total mass recoveries of the protein fractions were highest for the simplified method, followed by the modified Nagano method, and traditional soy isolate had the lowest mass recovery. The mass recovery of soy isolate in our pilot plant was lower than the 30−40% reported by others (Circle and Smith, 1978). The mass recoveries of the insoluble residues produced from both the simplified and the modified Nagano methods were not significantly different (Table 3). Almost half of the soy flake mass remained as insoluble residue. The simplified method did not yield an acid whey, but rather a permeate produced by the UF concentration step. The mass recovery in the permeate fraction after concentration was 20.0% (db), which was significantly lower than the mass recovery of whey produced when using the modified Nagano method (24.8%, db) but higher than the whey produced when using the traditional soy isolate process. The mass difference between acid whey and permeate reflected the differences in mass recoveries in the protein fraction yields. The total mass recoveries from the UF washings were 3.2% with the modified Nagano method and 6.2% with the simplified method. The different size of membrane MW cutoff was a major cause of the mass difference in the two processes. The sodium bisulfite added during diafiltration also contrib-

Table 2. Protein Purities of Soy Protein Fractions Produced in Pilot-Plant Processes[a]

	protein purity (% of protein)					
	glycinin		β-conglycinin		lipoxygenase	
	modified Nagano method	simplified method	modified Nagano method	simplified method	modified Nagano method	simplified method
glycinin	90.3b	92.8a	4.5c	4.5c	0.2d	0.5d
β-conglycinin	18.0d	25.4c	71.3a	62.6b	1.0f	4.0e
intermediate mixture	46.9a		27.2b		6.8c	

[a] $n = 2$. Data in the same row with different letters are significantly different at $p < 0.05$.

Table 3. Percentage Mass Recovery (db) of Fractions Produced from Pilot-Plant Processes ($n = 2$)

	modified Nagano method	simplified method	traditional soy isolate method
protein fractions	24.4 ± 0.8	29.2 ± 0.9	21.5 ± 0.4
insoluble residue	51.4 ± 2.7	46.9 ± 5.8	80.0[a]
whey	24.8 ± 0.9		16.3[a]
UF permeates			
UF concentration		20.0 ± 0.1	
glycinin wash	0.9 ± 0.2	1.4 ± 0.0	
intermediate mixture wash	1.2 ± 0.2		
β-conglycinin wash	1.1 ± 0.2	4.8 ± 0.0	

[a] $n = 1$.

Table 4. Percentage Protein Recovery (db) of Fractions Produced from Pilot-Plant Processes ($n = 2$)

	modified Nagano method	simplified method	traditional soy isolate method
protein fractions	42.5 ± 1.3	49.8 ± 1.6	32.2 ± 0.5
insoluble residue	40.2 ± 1.8	35.9 ± 4.0	56.2[a]
whey	7.2 ± 2.6		4.8[a]
UF permeates			
UF concentration		5.1 ± 0.4	
glycinin wash	0.5 ± 0.0	0.7 ± 0.1	
intermediate mixture	0.4 ± 0.1		
β-conglycinin wash	0.4 ± 0.0	1.7 ± 0.1	

[a] $n = 1$.

uted to the mass increase in the UF wash fractions of the simplified process.

Total protein recoveries of the fractions followed the same pattern as the total mass recoveries (Table 4). The protein recovery from our simplified method was 49.8% (db), which was significantly higher than those of the modified Nagano method and the traditional soy isolate production. The insoluble residues of the simplified and modified Nagano processes contained significant amounts of protein. The protein recovery of the insoluble residue fractions ranged from 33.1 to 41.5% (db) but was not different between the processes. The acid whey from the modified Nagano method comprised 7.2% of the total protein (Table 4), which was not significantly higher than the concentrated permeate fraction produced from the simplified method. The amount of protein recovery in the concentrate permeate fraction from our simplified method was very similar to the amount of protein in the acid whey of the traditional soy isolate process. Little protein was lost in the UF washing. In the simplified method, 2.4% protein was lost during washing, but it was higher than loss in the modified Nagano process. The average total protein recoveries of duplicate runs were similar: 93.2% (db) for the simplified process, 91.2% for the modified Nagano process, and 93.2% for the traditional soy isolate process.

The mass and protein recoveries of traditional soy protein isolate production were significantly lower than literature values. Soybean quality may be a major factor for the low recoveries. The variety of soybean, crop production year, and soybean storage time and condition are very important factors influencing protein extraction rate (Murphy and Resurreccion, 1984; Murphy et al., 1997). The soybean variety we used in the current study was MBS 2795, 1995 crop. The defatting process was performed in October 1996, and soy isolate production occurred in November 1997. The long storage time for these soybeans and defatted flakes, even at 5 °C, may have resulted in a lower protein extraction rate, although the soybean variety may have an effect. Unfortunately, we did not have additional MBS 2795, 1995, crop soybeans remaining to test this hypothesis. However, we attempted to partially test this hypothesis by preparing soy isolate with fresher soybeans. The same pilot-plant soy isolate process and equipment conditions for duplicate soy isolate production runs using IA20020 soybean, 1996 crop, were performed. The soybean defatting process was performed in December 1996. The isolate was produced in March 1997. The average protein isolate mass yield of duplicate processes was 41.5% (db), and the protein content of the soy isolate was 92.2% (db) with the mean protein recovery of 61.7% (db). These results match the other pilot-plant and commercial results very well (Mustakas and Sohns, 1979). The age of the MBS soybean material at isolate production time was the probable cause of the low soy isolate yields.

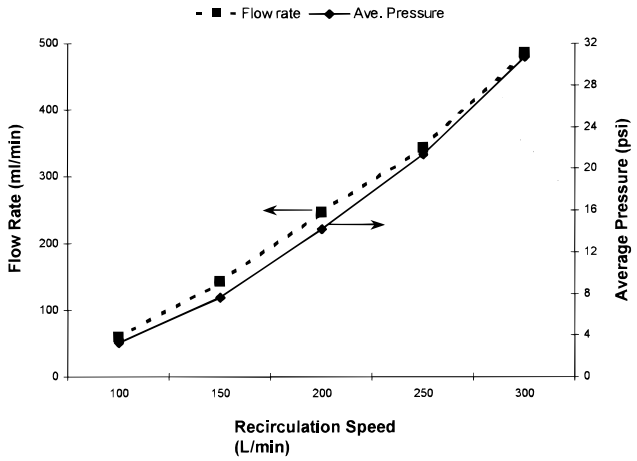

Figure 4. Effect of UF recirculation speed on flow rate and membrane pressure for pilot-plant production of glycinin and β-conglycinin.

Process Optimization. In the evaluation of the UF recirculation speed experiment, permeate flow rate was observed to increase exponentially as recirculation speed increased following this exponential function: $y = 58.496x^{1.2983}$, where y = flow rate and x = recirculation speed with an R-square of 0.9993 (Figure 4). The average pressure on the membrane increased exponentially as recirculation speed increased. The solids content in the permeate dropped dramatically from 1.56 to 0.51% between 100 and 150 L/min recirculation speeds (Figure 5). The solids content of the permeate decreased further from 0.51 to 0.26% as the recirculation speed increased from 150 to 200 L/min. The protein content in the permeate followed the same pattern as percent solids recovery. At flow rates >200 L/min, the solids and protein contents in the permeate did not change with increasing recirculation speed. A urea−SDS−PAGE gel represents the protein distribution patterns of permeate samples (Figure 6). At higher recirculation speeds (250 and 300 L/min), clear α', α, and β bands of β-conglycinin appeared on the gel, indicating that more β-conglycinin came through the UF membrane. When the recirculation speed was decreased from 250 to 150 L/min, smaller amounts of β-conglycinin passed through the UF membrane but other lower MW proteins were able to pass through as well. The amounts of glycinin, lipoxygenases, and other proteins in the permeate markedly increased at a recirculation speed of 100 L/min. The β-conglycinin bands were less intense

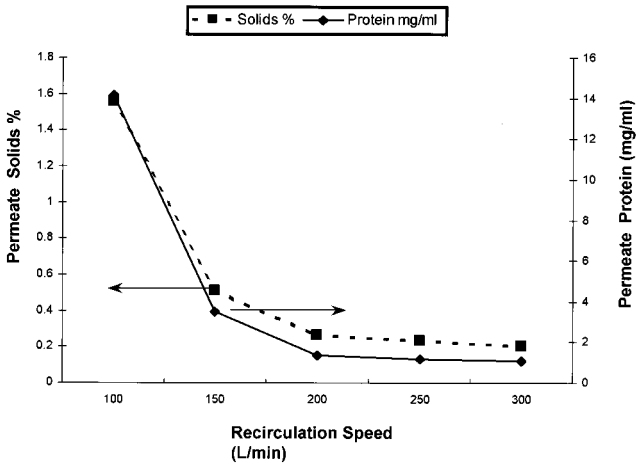

Figure 5. Effect of UF recirculation speed on permeate solids and protein contents for pilot-plant production of glycinin and β-conglycinin.

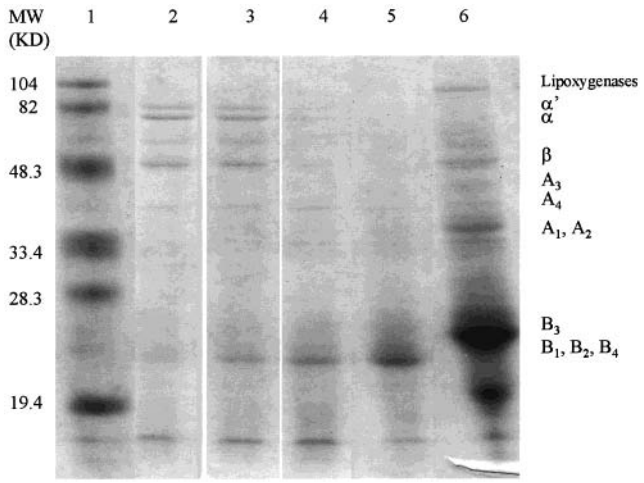

Figure 6. Urea−SDS−PAGE of protein permeate samples obtained from UF with RC-100 membrane at different recirculation speeds (RS): (lane 1) MW standards; (lane 2) 300 L/min RS; (lane 3) 250 L/min RS; (lane 4) 200 L/min RS; (lane 5) 150 L/min; (lane 6) 100 L/min RS. α, α′, and β are peptide bands of β-conglycinin. A and B are the respective acidic and basic peptides of glycinin. Thirty micrograms of protein was loaded per well.

compared to the other protein bands at 100 L/min. These data indicate that β-conglycinin can be best purified with UF recirculation speeds between 100 and 250 L/min without significant losses of the desired protein. Although higher recirculation speeds, up to 300 L/min, can reduce fouling of the membrane, the lower recirculation speeds of 100 and 150 L/min result in lower membrane pressure and allow molecular size to become the major mechanism for the molecular membrane passage. However, very slow recirculation speeds of 100−150 L/min are unacceptable in real practice because excessive time would be required to complete the process run.

Increases in the recirculation speed at a constant 20 psi membrane pressure caused increased flow rates. The flow rate increased ~12% for each 50 L/min incremental increase in recirculation speed between 100 and 250 L/min. However, the solids and protein contents in the permeate were not significantly different. These data indicated the membrane pressure, rather than the recirculation speed, controls the solids and protein contents in the permeate.

Table 5. Protein Purities of Soy Protein Fractions Produced in UF Processes Using Different Recirculation Speeds[a]

	protein purity (% of protein)		
	before UF	200 L/min	250 L/min
UF retentate			
glycinin	32.2a	23.9c	26.9b
β-conglycinin	57.0c	66.8a	63.2b
permeate from concentration			
glycinin		49.9b	57.0a
β-conglycinin		7.7b	17.3a
permeate from bisulfite wash			
glycinin		45.9b	53.8a
β-conglycinin		15.6b	24.1a
permeate from water wash			
glycinin		48.7a	36.3b
β-conglycinin		40.6b	54.8a

[a] $n = 2$. Data in the same row with different letters are significantly different at $p < 0.05$.

The protein distributions of the retentates and the permeates before and after the washing steps were evaluated by the density image analysis of urea−SDS−PAGE to evaluate the effect of 200 and 250 L/min UF membrane recirculation speeds on β-conglycinin purity. The pH 7.0 supernatant had a protein distribution of 57.0% β-conglycinin and 32.2% glycinin (Table 5). The β-conglycinin fraction increased to 63.2% of protein and glycinin fraction decreased to 26.9% in the UF retentate when a recirculation speed of 250 L/min was used. The β-conglycinin distribution increased to 66.8% and glycinin decreased to 23.9% in the UF retentate when a recirculation speed of 200 L/min was used. These distributions were significantly different for the two recirculation speeds. The recirculation speed of 200 L/min was better than the 250 L/min speed because the permeates had higher concentrations of glycinin and β-conglycinin at 250 L/min. The 250 L/min recirculation speed produced a 20 psi membrane pressure, whereas the 200 L/min speed produced 14 psi (Figure 4). Therefore, the higher pressure apparently facilitated passage of both glycinin and β-conglycinin through the membrane. The water washing removed large amounts of salts and large amounts of glycinin and β-conglycinin. Significantly higher proportions of β-conglycinin were in the permeate from the water washing than in the permeates of concentration and bisulfite washing steps (Table 5). The large fraction of β-conglycinin that passes through the membrane under these conditions was not expected. Therefore, long water washing times for the UF membrane are not recommended. The results of the optimization experiments suggest a recirculation speed of 200 L/min with ~15 psi membrane pressure will result in acceptable purity and protein content for the β-conglycinin fraction. However, we selected a recirculation speed of 250 L/min with 20 psi membrane pressure in our simplified process because of the costs of time and labor in a pilot-plant size production operation.

Our fractionation method could simplify the process for producing glycinin and β-conglycinin products. Only one additional ultrafiltration step was required for the simplified method compared the traditional soy isolate process (Figures 1 and 3). The simplified method produced similar amounts of glycinin and doubled the β-conglycinin mass produced compared to the yields for the modified Nagano method. Although the β-conglycinin purity was 9% lower than that obtained with the modified Nagano method, the higher protein yields and

lower production costs, because there are fewer production steps, would be attractive to the manufacturer.

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