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Dissolution Behavior of Soy Proteins and Effect of Initial Concentration

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The solution concentration profiles of soy protein and its components, glycinin and β-conglycinin, as a function of pH and initial concentration have been measured. The concentration profiles generally followed a U-shaped trend with pH, with a minimum at around pH 4−5. Dissolution concentration unexpectedly increased with the initial concentration of the solution, with the increase being approximately proportional to the increase in initial concentration. The reasons for this are not clear. For the initial concentrations studied, β-conglycinin is undersaturated between pH 5 and 7 and remains in solution, while glycinin becomes supersaturated in the same pH range and precipitates. Therefore separation of the two proteins can be achieved.

KEYWORDS: Soy protein solubility; soy flour; protein precipitation; protein separation; glycinin; β-conglycinin.

INTRODUCTION

Soy proteins are widely applied in food products. Recently, there has been increasing interest from manufacturers to produce the separate soy protein fractions: β-conglycinin (∼180 kDa) and glycinin (∼360 kDa). They are alternatively known respectively as the 7S and 11S proteins. The S stands for Svedberg units, which is related to the sedimentation rate of the molecule under centrifugal sedimentation. Values for S usually range from 1 to 200, with units being 10^{-13} s (*1*). These two proteins coexist naturally in the soybean in approximately equal proportion, depending on the cultivar and growing conditions of the bean, and together make up approximately 60% of the total protein in soybeans.

The β-conglycinin molecule is a trimer consisting of various combinations of the α, α′, and β subunits. There are seven possible different β-conglycinin molecules (*2, 3*). The glycinin molecules consist of six subunits, which is a combination of five different types of subunits, each containing one acidic polypeptide and one basic polypeptide (*4*). The exact number of different glycinin molecules is unknown but is large. Therefore β-conglycinin and glyinin are really two heterogeneous groups of molecular species.

The most common method for separating β-conglycinin and glycinin is by extracting protein from defatted soy flour to form an aqueous protein solution, followed by acidification of this solution to between pH 5 and 6, whereby glycinin is preferentially precipitated while β-conglycinin remains in solution (*5*−*10*). β-Conglycinin is subsequently recovered by precipitation or chromatographic means. These methods however, have had limited success as potential larger-scale processes. Furthermore, from a scientific viewpoint, little or no explanation accompanies the published separation methods to rationalize the choice of precipitation conditions.

The prime driving force for separation by precipitation is the differential solubility of the two proteins. However, solubility data are lacking in the literature and there has been no systematic study on the solubility of soy protein and their components. Without these data, it cannot be known how robust the separation method is against practical fluctuations common in large-scale operations. The present study investigates the dissolved concentration of soy proteins as a function of pH and the effect of initial concentration upon them.

MATERIALS AND METHODS

Preparation of Protein Extract. Protein was extracted from defatted soy flour (Cargill Oilseeds Processing) with an aqueous solution at pH 8.5 (with NaOH) containing 15 mM added $Na_2S_2O_5$. The protein content of the soy flour used was 53.5 ± 0.4 wt % as found by combustion of the soy flour and total Kjeldahl nitrogen. Extraction ratios (the ratio of aqueous solvent to soy flour) of 3.3:1, 5:1, 10:1, and 20:1 were used in a 1 L total volume. The suspension was stirred with an overhead impeller in a constant temperature water bath at 21 °C for 1 h. The aqueous protein extract was recovered by centrifuging for 30 min at 9600*g*.

Determination of Protein Content and Composition. The protein content in solution was measured by UV spectrophotometry [absorbance at 280 nm, $E = 1.913$ cm^{-1} (mg/mL)$^{-1}$] with a Pharmacia Biotech Ultrospec 2000 UV/vis spectrophotometer. Protein composition was determined by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) followed by gel density image analysis. A Bio-Rad Mini-Protean 3 system was used with 4−15% Tris-HCl ready gels (Bio-Rad) which gave a 10−250 kDa separation range. Samples were prepared with the Laemmli sample buffer and a Tris−glycine running buffer was used for the electrophoresis (premixed buffers from Bio-

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Table 1. Percentage of Protein Extracted from Soy Flour[a]

extraction ratio	extract concn (mg/mL)	protein extracted (%)
3.3:1	102 ± 9	64 ± 5
5:1	67 ± 3	60 ± 3
10:1	33 ± 1	62 ± 2
20:1	17 ± 0.3	66 ± 1

[a] Protein content in soy flour was 53.5 ± 0.4 wt %. Average of all extractions with 95% uncertainty is shown.

Table 2. Average Percent Protein Composition of the Extracts

extraction ratio	β-conglycinin (%)	glycinin (%)	other proteins (%)
3.3:1	29 ± 7	48 ± 14	23 ± 7
5:1	32 ± 6	50 ± 15	18 ± 6
10:1	30 ± 7	49 ± 15	21 ± 6
20:1	37 ± 7	37 ± 11	26 ± 8
average	30 ± 7	48 ± 14	22 ± 7

Rad). A constant voltage (200 V) was used to run the gels for approximately 35 min. Gels were stained with Bio-Rad BioSafe Coomassie stain and destained with water according to the manufacturer's instructions. Protein composition was quantified by densitometry analysis of the bands with Bio-Rad's Quantity One gel quantification program.

Repeated experiments showed that the 95% confidence interval error of the combined UV spectrophotometer measurement plus SDS−PAGE analysis were ±20% for β-conglycinin measurements, ±30% for glycinin, and ±30% for other proteins that were not β-conglycinin or glycinin.

Determination of Mineral Content. Elemental analysis was conducted by the Analytical Services of the School of Land and Food Sciences at the University of Queensland. The minerals content of the soy flour was found by complete digestion of the flour with nitric acid followed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Mineral contents of solutions were measured directly by ICP-AES. The elements detected were aluminum, boron, calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc.

Determination of Precipitation Kinetics and Protein Solubility. The pH dependence of the dissolved concentration of soy protein between pH 2 and 7 was found by adding small amounts of 1 M HCl to 30 mL of soy protein extract to bring the solution to the desired pH level. The temperature was kept constant at 21 °C in a constant-temperature water bath. The solution at the desired pH was stirred with a magnetic stirrer overnight to ensure equilibrium was reached. At several pH levels, progressive samples were taken to determine the precipitation kinetics. The samples were centrifuged to remove any solids before being analyzed for total protein concentration by UV spectroscopy and for protein fractions by SDS−PAGE and densitometry analysis based on the method used by Wu et al. (*9*); image analysis was carried out with Bio-Rad's Quantity One gel quantification program. Glycinin and β-conglycinin concentrations were determined by multiplying the total protein concentration by the respective protein fraction.

RESULTS AND DISCUSSION

Extraction of Protein from Soy Flour. The average percentage of protein extracted from the soy flour at each extraction ratio is shown in **Table 1**. The percentage of protein extracted from the soy flour was approximately the same for all extraction ratios, whereas one might have expected considerably more protein to be extracted at higher water to flour ratios than at lower ones. Thus the lower extract concentrations for higher extraction ratios seems to be solely due to dilution by the increased amount of solvent present. Interestingly, for all the extraction ratios, only between 60% and 70% of the protein in the soy flour was extracted. Perhaps some of the protein was "locked" in unruptured cells.

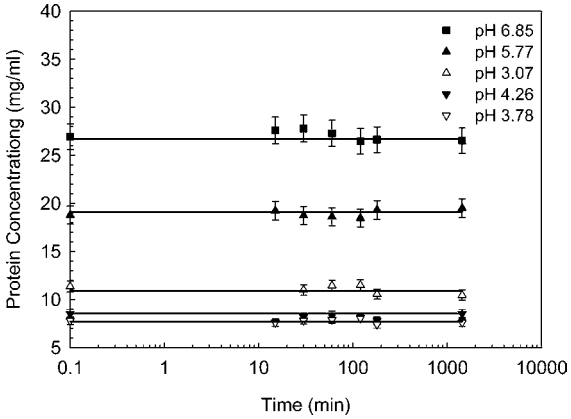

Figure 1. Precipitation kinetics of soy proteins for a 10:1 extract at several pH values.

The protein concentration in solution was followed throughout a 1 h extraction. The extract reached its final concentration within the first 5 min of the extraction process for all of the extraction ratios studied. Therefore, the unextracted protein in the soy flour was not due to the time for extraction. The extraction ratio had little effect on the dynamics of the extraction process. Extraction times of 30 min to 1 h commonly used in the literature are probably excessive.

The compositions of the proteins in the extracts at different extraction ratios are shown in **Table 2**. Within the large experimental errors, the composition at each extraction ratio was approximately constant, that is, the amount of glycinin and β-conglycinin extracted were proportional to the amount of soy flour used regardless of the extraction ratio. Therefore, extraction ratio had no effect on the composition of the resultant extracts. The different glycinin and β-conglycinin concentrations in the extracts were solely due to dilution by the different amounts of solvent present at each extraction ratio.

The inorganic constituents in the 10:1 and 5:1 extracts were analyzed together with the feed analysis. For the majority of the elements analyzed, the percentages of the inorganics extracted were almost the same for the two extraction ratios. Therefore, the different salt concentrations in the extracts were solely due to the different amounts of solvent present at each extraction ratio. The overall low extractions are most likely due to the inorganics being associated with the flour fibers during the extraction process, which were totally digested in nitric acid in the feed analysis.

However, the percentage extracted was different for different minerals, and for some of the minor components (e.g., Al), the percentages extracted were not similar for the two extraction ratios. This was probably due to the smaller concentrations and possibly larger error associated with the analysis of these components. The percentage extracted value was not applicable for sodium as the majority of this element was added into the solution as $Na_2S_2O_5$ and NaOH and was not from the soy flour. As such, the sodium concentration of the two extracts was approximately equal as the amount of added $Na_2S_2O_5$ and NaOH were nominally the same for both extracts.

Precipitation Kinetics. The precipitation kinetics of soy proteins are shown in **Figure 1**. Time zero was when the solution had reached the desired pH with the addition of 1 M HCl. The first sample was taken as soon as possible thereafter, which was around 5−6 s. The solution concentration was followed for 24 h. In all cases tested, the solution had reached

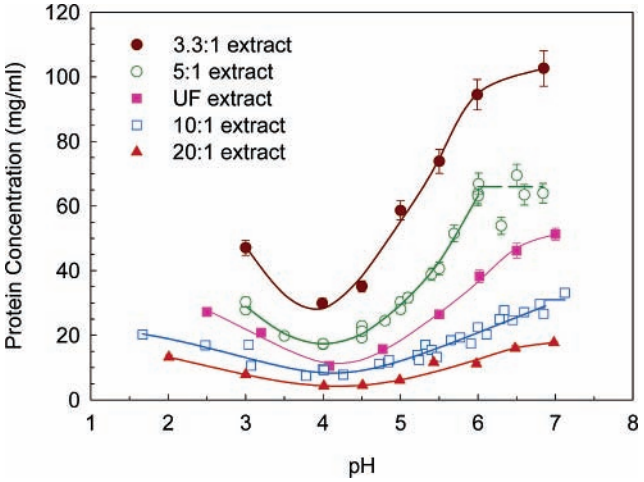

Figure 2. Effect of extraction ratio on residual total protein concentration for different extraction ratios. Initial concentrations are shown by dashed lines.

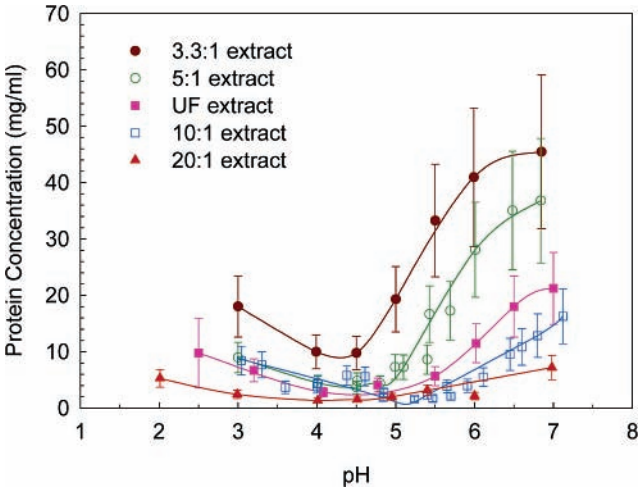

Figure 3. Effect of extraction ratio on residual glycinin concentration for different extraction ratios.

final concentration within the first few seconds. Precipitation rates were very rapid.

Effect of Extraction Ratio on Residual Protein Concentration. The pH−concentration profile of total soy protein is shown in **Figure 2** for each of the extracts from the different exaction ratios. The concentration profiles of glycinin and β-conglycinin are shown in **Figures 3** and **4** respectively. The particular species was present (and detected) in both the aqueous and precipitate phases only when the residual concentrations were below the initial concentration. The initial concentrations are shown by the dashed horizontal lines (**Figures 2** and **4**). Data lying on the dashed lines did not have any significant amounts of that protein in the precipitated phase, that is, they are undersaturated. The undersaturated data, specifically those of β-conglycinin, are included here because they have important implications for the selection of conditions for protein separation. It is this undersaturated region of β-conglycinin that will be exploited for the preferential precipitation of glycinin.

To isolate the effect of initial protein concentration on the final dissolved concentration without changing other parameters, for example, inorganics concentration, a 10:1 extract was concentrated by ultrafiltration (shown as UF extract in **Figures 2**−**4**). A stirred ultrafiltration cell (Amicon model 8400) was used with a 10 000 MW membrane (YM10). This allowed water

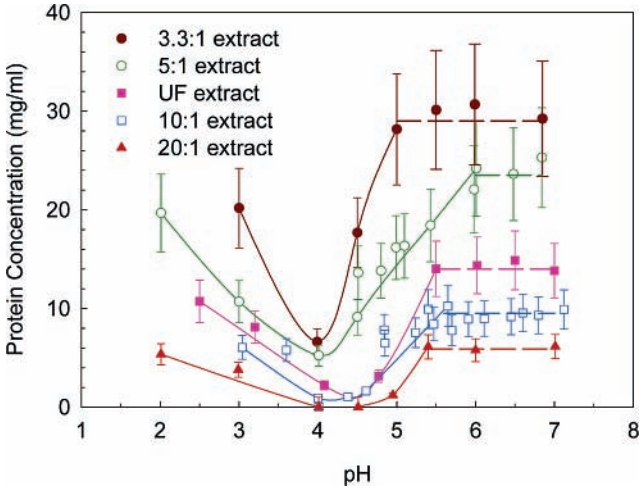

Figure 4. Effect of extraction ratio residual on β-conglycinin concentration for different extraction ratios. Initial concentrations are shown by dashed lines.

and inorganics to pass through the membrane but retained the proteins of interest; thus the protein concentration was increased without increasing the inorganics concentration. The protein concentration of the UF concentrated extract was 51 mg/mL (initial feed = 33 mg/mL). SDS−PAGE and elemental analysis showed that the UF concentrated extract was identical to the 10:1 extract except for the total protein concentration. This material showed dissolved concentration profiles consistent with its initial concentration (**Figures 2** and **4**) and differing from the original 10:1 material.

The dissolved concentration profiles generally followed a U-shaped trend with a minimum at around pH 4−5. This was consistent with observations in the literature (*11*−*14*). The pH at which the concentration profile of β-conglycinin begins to decrease from its initial value appears to vary with extraction ratio (**Figure 4**). The transition point has important implications for the design of the protein separation scheme. However, its experimental value can be strongly influenced by the presence (or absence) of a single data point as well as experimental error.

The pH at which minimum concentration occurred appeared to be slightly lower for extracts from lower extraction ratios (high initial concentration). The profile minimum for the 20:1 extract occurred at about pH 4.5, while that of the 3.3:1 extract was about pH 4.0.

Most surprisingly, the dissolved concentration was apparently higher for extracts from lower extraction ratios, which had higher initial protein concentrations. This is not typical solubility behavior where the solubility should be independent of the initial solution concentration. The present data are therefore, strictly speaking, not thermodynamic solubility data and are instead only dissolved concentrations.

The increase in dissolved concentration was almost proportional to the increase in initial concentration in all cases. For total protein, most of the data collapsed onto a single curve when the dissolved concentration is plotted as a ratio to the initial concentration (**Figure 5**). Only data that were saturation values, that is, below the initial concentration were used. At pH greater than 5, the increase in solubility of the 3.3:1 and 5:1 extracts were slightly larger than proportional to its increase in initial concentration.

This final to initial concentration ratio relationship was not as clear for glycinin (**Figure 6**) and and β-conglycinin (**Figure 7**). There were larger deviations from the average trend. Extracts

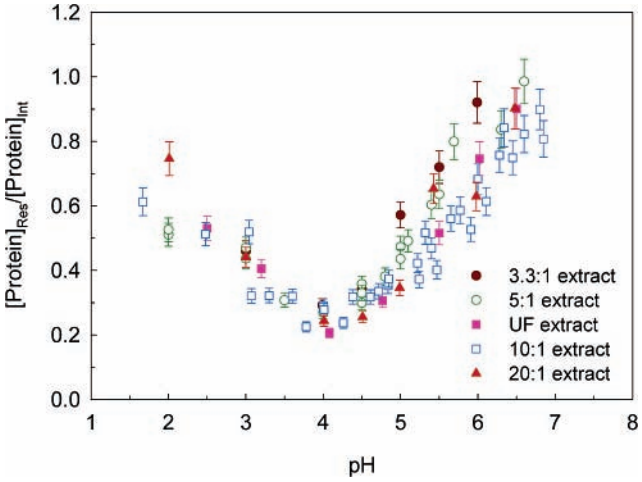

Figure 5. Residual to initial concentration ratio of total soy proteins.

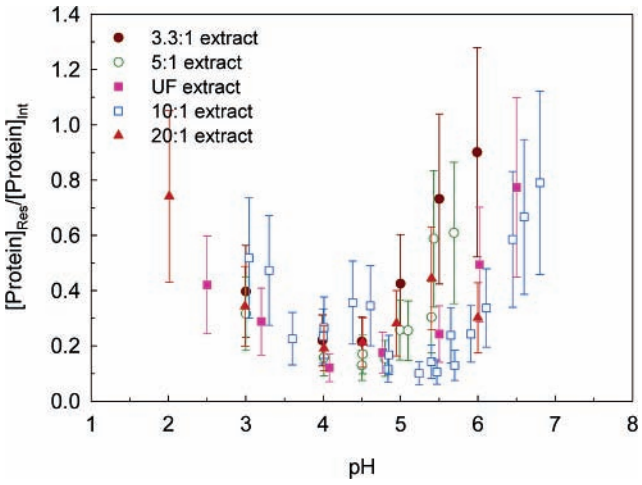

Figure 6. Residual to initial concentration ratio of glycinin.

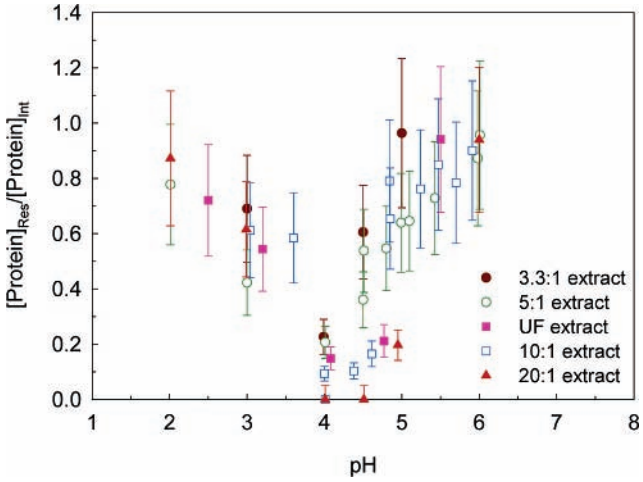

Figure 7. Residual to initial concentration ratio of β-conglycinin.

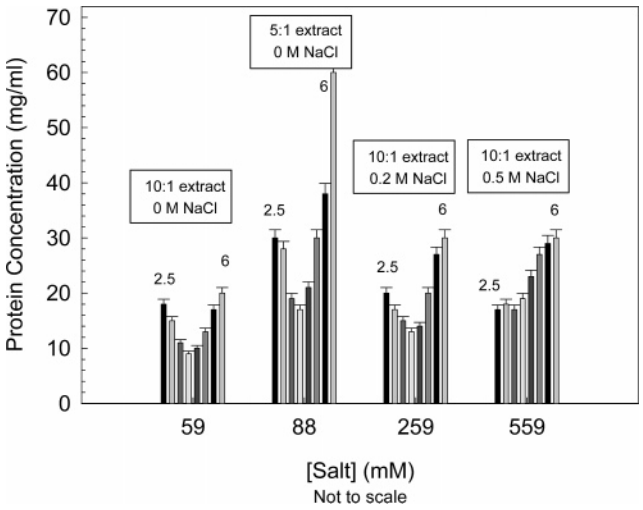

Figure 8. Effect of salt on total protein concentration, each for a range of pH 2.5–6 at intervals of 0.5 pH unit.

of lower extraction ratios generally had higher concentration ratios than the extracts of higher extraction ratio.

The fact that dissolved protein concentration was measured instead of solubility was unexpected. The following discussion attempts to give some explanation for the unexpected measurements. However, the present data cannot be fully explained and a precise understanding is made difficult by the heterogeneous nature of the system. Nonetheless, the empirical data are still very useful for the design of a protein separation scheme.

The likely contributing factors for the unexpected measurements were thought to include (1) the presence of other highly soluble protein fractions and (2) variations in salt concentration for the different extraction ratios. These alone do not fully account for the unexpected measurements. While the focus of this study is on the main protein fractions in soy, glycinin and β-conglycinin, the protein extracts contain between 18% and 26% other protein components, independent of extraction ratio (see **Table 2**). Much of the other protein fraction is whey proteins which cannot be precipitated by acidification (*15*). When these nonprecipitating proteins have been accounted for by subtracting out its concentration, the "corrected" dissolved protein concentration still varied with initial concentration.

The extracts from lower extraction ratios also had a higher concentration of inorganic constituents. Some of these inorganics may have existed as salts. The second suggestion above is that the increasing dissolved concentration with increasing initial concentration may be partly attributed to a salting-in effect due to the inorganic salts in the extracts.

The effect of salt (sodium chloride) on the dissolved concentration was investigated by adding NaCl (to make added 0.2 and 0.5 M solutions) to the protein solutions prior to acidification. **Figure 8** shows the residual concentration of the 5:1 and 10:1 extracts compared to the concentration when NaCl was added to 0.2 and 0.5 M. For clarity, only the labels for the lowest pH (2.5) and the highest pH (6) are indicated on the graph. The intermediate data are at intervals of 0.5 pH unit. The salt concentration shown on the *x*-axis (not to scale) is the total concentration, which includes the all of the inorganics in the extracts and the added NaCl. The solution concentration for the 5:1 extract was clearly much greater than would be expected if the increase weredue solely to the effect of increasing salt concentration, especially at pH below 3.5 and above 4.5.

A similar comparison for glycinin and β-conglycinin when 0.2 and 0.5 M NaCl was added can only be made between pH 3 and 5.2, due to the lack of data at high pH. **Figures 9** and **10** show the data for glycinin and β-conglycinin. Note that no data were available at pH 5.2 for 0.5 M added NaCl.

For glycinin, the increase in the dissolved concentration of the 5:1 extract was small between pH 3.0 and 5.2 and was comparable to the effect of the concentration of inorganics. The

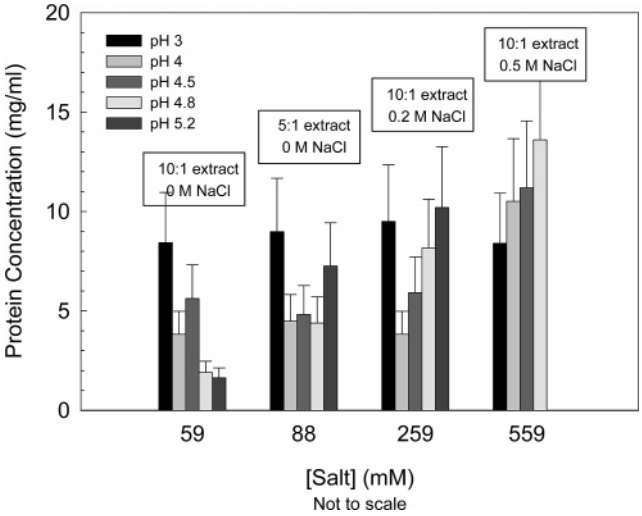

Figure 9. Effect of salt on glycinin concentration.

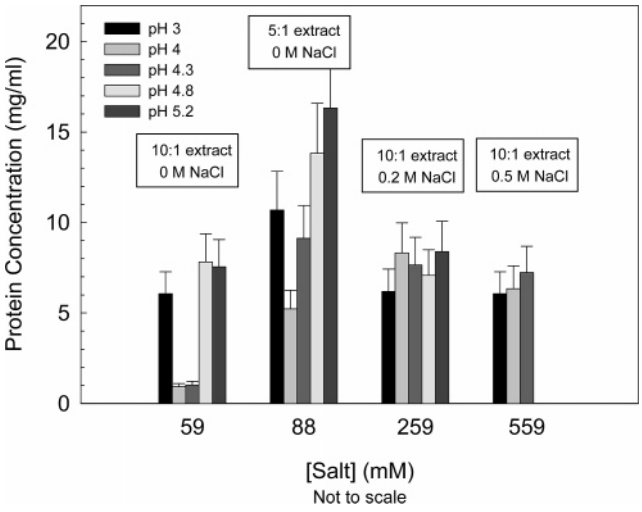

Figure 10. Effect of salt on β-conglycinin concentration.

increase in β-conglycinin concentration was greater than the expected effect of increasing the concentration of inorganics.

The extracts contained a variety of inorganics that might have a greater salting-in effect on the protein than NaCl alone. However, on the basis of the current data, it is unlikely that the presence of inorganics can *fully* explain the effect of extraction ratio on the dissolved protein concentration measurements. Nonetheless, some of the data were consistent with a salting-in effect and therefore a minor salting-in effect cannot be ruled out.

Further hypotheses are presented here as possible explanations. These include (1) nonuniformity in the solubility of the different glycinin and β-conglycinin molecules, (2) nonideal interactions between proteins in solution, and (3) structural changes to molecules of the main protein fractions. These are molecular level considerations that cannot be verified with the protein data presented here alone. No conclusions can yet be drawn regarding the validity of these hypotheses. They are included here for consideration should future data become available.

(1) Nonuniformity in Glycinin and β-Conglycinin Molecules. A possible factor considered as an explanation for the unusual dissolution behavior is heterogeneity within the glycinin and β-conglycinin fractions. In the above analysis, we have considered the proteins to be in three fractions: glycinin, β-conglycinin, and other. As already indicated, these fractions in turn consist of many different types of molecules. The individual molecules may have different solubilities, some of which may be above the initial concentration; that is, the component may be undersaturated. If this is so, then the residual concentration of the fraction could be a function of initial extract concentration and extraction ratio.

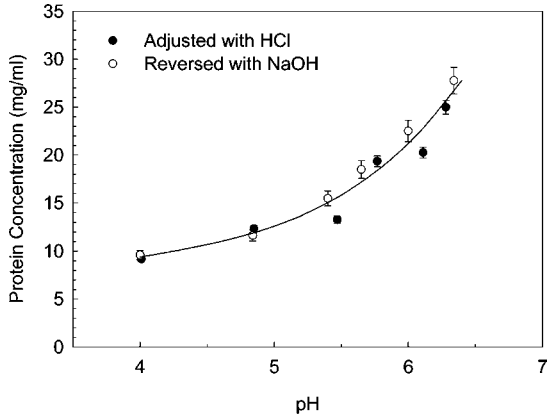

Figure 11. Reversibility of total soy protein precipitation with pH.

Table 3. Conditions for Reversal of Precipitation by Changing pH Experiments

pH adjusted with HCl	pH readjusted with NaOH
3.0	4.0
4.0	4.85
4.85	5.4
5.4	5.65
5.65	6.0
6.0	6.35
6.35	

(2) Protein−Protein Interactions in Solution. The relatively high concentrations of highly soluble whey proteins may increase the dissolution of glycinin and β-conglycinin. One such explanation is if the charges associated with the whey proteins influence the ionic environment of the solution. The presence of these proteins may affect solubility similar to a salting-in effect.

(3) Changes in Molecular Structure. Changes in the molecular conformation alter the protein−protein interactions in solution, which may lead to aggregation and/or precipitation. There are reports in the literature that soy protein solubility is related to molecular conformation (*17*). Both glycinin and β-conglycinin undergo changes in molecular conformation under pH and ionic strength conditions similar to those used in this study (*18*, *19*).

Reversibility of Precipitation. For the purpose of process design, experiments were carried out to determine whether the precipitation process was reversible, that is, whether the protein redissolves upon increasing the pH. Aliquots (50 mL) of protein extract (10:1 extraction ratio) were first equilibrated at pH levels between 3.0 and 6.35. The protein concentration and composition of the solution were measured. The pH change was then reversed (pH increased with the addition of 1 M NaOH). Protein concentration and composition of the final solution were measured and compared with those of the initially equilibrated solution. The conditions for a number of experiments are summarized in **Table 3**.

Figure 11 shows that total soy protein precipitation was reversible when the pH was raised with NaOH. Data for glycinin and β-conglycinin precipitation are shown in **Figures 12** and

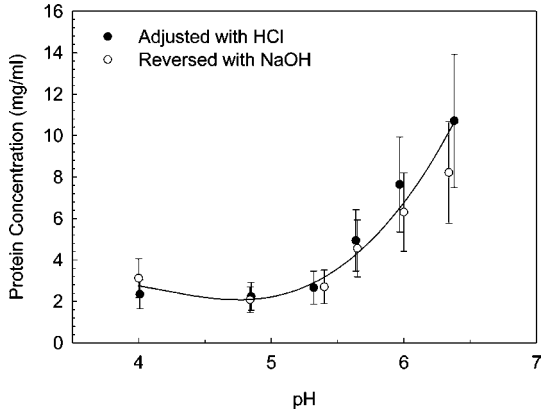

Figure 12. Reversibility of glycinin precipitation with pH.

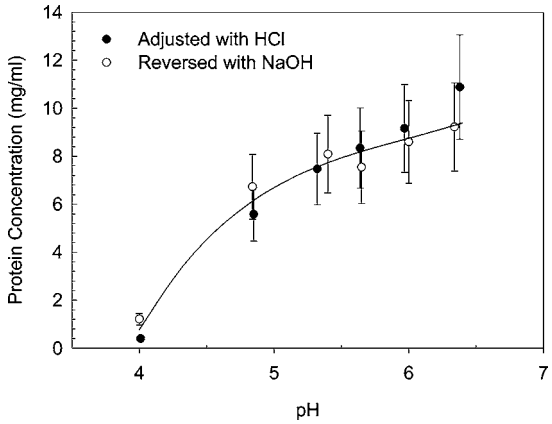

Figure 13. Reversibility of β-conglycinin precipitation with pH.

13. The precipitation of these components was also fully reversible upon raising the pH with NaOH.

As pH was adjusted by using small amounts of highly concentrated HCl and NaOH, it is possible that poor micromixing could cause local denaturation of the protein and affect the measured dissolved protein concentration. Acid and base to adjust pH were always added near the impeller to maximize micromixing and reduce the possibility of denaturation. As the protein concentration in solution was reversible with increases in pH, denaturation on contact with concentrated HCl and NaOH solutions is unlikely.

Protein Separation. The prime driving force for separating glycinin from β-conglycinin by precipitation is the differential solubility. The residual protein concentrations of glycinin and β-conglycinin have been shown in **Figures 3** and **4**. Preferential precipitation of glycinin may be achieved in the region where the glycinin concentration decreases with decreasing pH while β-conglycinin concentration remains constant (at its initial value). In this region, glycinin precipitates while β-conglycinin remains in solution. The initial β-conglycinin concentration is shown by the dashed line. Data lying on the dashed line are the initial concentration and are undersaturated. The lower limit of the separation region is where the dashed line intersects the β-conglycinin residual concentration curve.

The pH ranges for separating glycinin are between pH ~5 and 7. The measured glycinin purity of the separated phase is plotted against glycinin yield in **Figure 14**. pH levels are between 6.4 and 5.6 at intervals of 0.2 pH unit.

The protein yield of the separated phase is between 2% and 40% for extraction ratios of 3.3:1, 5:1, and 20:1. The yield for the 10:1 extraction ratio is significantly higher than the others

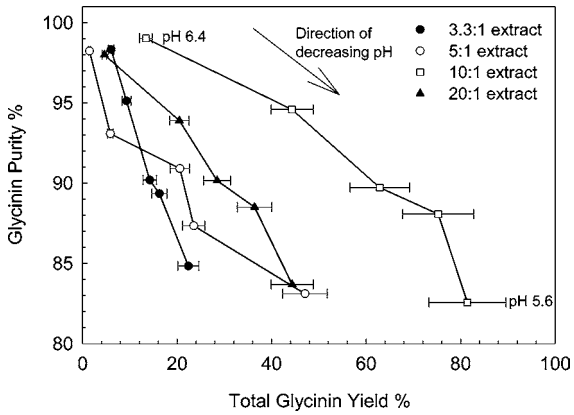

Figure 14. Glycinin purity vs glycinin yield for various extraction ratios at 21 °C for pH 6.4–5.6 at intervals of 0.2 pH unit.

(15−80%). This is because the rate of change of glycinin concentration in this region is much greater for the 10:1 extract than the others. The reason for this is not clear. It could be due to uncertainties in the measured data and requires further confirmation. Using different extraction ratios would lead to different amounts of residual protein after the removal of the glycinin fraction.

Glycinin purity decreases almost linearly as protein yield increases with decreasing pH (**Figure 14**). As little β-conglycinin precipitates within this pH range, the decrease in glycinin purity is due to the precipitation of some of the other minor proteins. The separation process therefore becomes a compromise between purity and yield. Therefore, for reasonable purity, a central final pH range from 6 to 6.2 is recommended.

In conclusion, the precipitation kinetics of soy protein was rapid and therefore precipitation can be a cost-effective means for separating soy proteins, as short precipitation times mean higher throughput for commercial operations.

The concentration profiles generally followed a U-shaped trend with a minimum at around pH 4−5, which is consistent with literature data. However, increasing initial concentration of the aqueous extracts appeared to increase the residual protein concentration at all pH levels, which was unexpected. This was observed for the total protein as well as for glycinin and β-conglycinin. The increase in the total protein residual concentration was approximately proportional to the increase in initial extract concentration. These results were unusual and the reasons for this were not completely understood.

Using extracts from different extraction ratios did not have a large effect on the separation of glycinin and β-conglycinin. The pH range for the preferential precipitation of glycinin was between approximately pH 5 and 7. Therefore, a central pH range (6−6.2) is recommended.

LITERATURE CITED

(1) Snyder, H. E.; Kwon, T. W. Functional properties of soy proteins. In *Soybean utilization*; Van Nostrand Reinhold Co.: New York, 1987; pp 164−186.

(2) Thanh, V. H.; Okubo, K.; Shibasaki, K. Isolation and characterization of the multiple 7s globulins of soybean proteins. *Plant Physiol.* **1975**, *56*, 19−22.

(3) Yamauchi, F.; Sato, M.; Sato, W.; Kamata, Y.; Shibasaki, K. Isolation and identification of a new type of beta-conglycinin in soybean globulins. *Agric. Biol. Chem.* **1981**, *45*, 2863−2868.

(4) Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the acidic and basic subunit complexes of glycinin. *J. Biol. Chem.* **1981**, *256*, 8752−8755.
(5) Howard, P. A.; Lehnhardt, W. F.; Orthoefer, F. T. (A. E. Staley Manufacturing Co.) 7s and 11s vegetable protein fractionation and isolation. U.S. Patent 4,368,151, 1983.
(6) Lehnhardt, W. F.; Gibson, P. W.; Orthoefer, F. T. (A. E. Staley Manufacturing Co.) Fractionation and isolation of 7s and 11s protein from isoelectrically precipitated vegetable protein mixtures. U.S. Patent 4,370,267, 1983.
(7) Bogracheva, T. Y.; Bespalova, N. Y.; Leont'ev, A. L. Isolation of 11s and 7s globulins from seeds of glycine max. *Appl. Biochem. Microbiol.* **1996**, *32*, 429−433.
(8) Nagano, T.; Hirotsuka, M.; Mori, H.; Kohyama, K.; Nishinari, K. Dynamic viscoelastic study on the gelation of 7s globulin from soybeans. *J. Agric. Food Chem.* **1992**, *40*.
(9) Wu, S.; Murphy, P. A.; Johnson, L. A.; Fratzke, A. R.; Reuber, M. A. Pilot-plant fractionation of soybean glycinin and beta-conglycinin. *J. Am. Oil Chem. Soc.* **1999**, *76*, 285−293.
(10) Wu, S.; Murphy, P. A.; Johnson, L. A.; Reuber, M. A.; Fratzke, A. R. Simplified process for soybean glycinin and beta-conglycinin fractionation. *J. Agric. Food Chem.* **2000**, *48*, 2702−2708.
(11) Smith, A. K.; Circle, S. J. Peptization of soybean proteins. Extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. *Ind. Eng. Chem.* **1938**, *30*, 1414−1418.
(12) Shen, J. L. Solubility profile, intrinsic viscosity, and optical rotation studies of acid precipitated soy protein and of commercial soy isolate. *J. Agric. Food Chem.* **1976**, *24*, 784−788.
(13) Virkar, P. D.; Hoare, M.; Chan, M. Y. Y.; Dunnill, P. Kinetics of the acid precipitation of soya protein in a continuous-flow tubular reactor. *Biotechnol. Bioeng.* **1982**, *24*, 871−887.
(14) Renkema, J. M. S.; Gruppen, H.; van Vliet, T. Influence of pH and ionic strength on heat induced formation and rheological properties of soy protein gels in relation to denaturation and their protein composition. *J. Agric. Food Chem.* **2002**, *50*, 6064−6071.
(15) Kinsella, J. E.; Damodaran, S.; German, B. Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New protein foods: Seed storage proteins*; Altschul, A. M., Wilcke, H. L., Eds.; Academic Press: San Diego, CA, 1985; pp 108−172.
(16) George, A.; Wilson, W. Predicting protein crystallisation from a dilute solution property. *Acta Crystallogr.* **1994**, *D50*, 361−365.
(17) Lakemond, C. M. M.; de Jongh, H. H. J.; Hessing, M.; Gruppen, H.; Voragen, A. G. J. Soy glycinin: Influence of pH and ionic strength on solubility and molecular structure at ambient temperatures. *J. Agric. Food Chem.* **2000**, *48*, 1985−1990.
(18) Wolf, W. J.; Briggs, D. R. Studies on the cold-insoluble fraction of the water-extractable soybean proteins. II Factors influencing conformation changes in the 11S component. *Arch. Biochem. Biophys.* **1958**, *76*, 377−393.
(19) Thanh, V. H.; Shibasaki, K. Major proteins of soybean seeds. Reversible and irreversible dissociation of beta-conglycinin. *J. Agric. Food Chem.* **1979**, *27*, 805−809.
(20) Utsumi, S.; Gidamis, A. B.; Mikami, B.; Kito, M. Crystallization and preliminary X-ray crystallographic analysis of the soybean proglycinin expressed in *Escherichia coli*. *J. Mol. Biol.* **1993**, *233*, 177−178.
(21) Utsumi, S.; Takenaka, Y.; Maruyama, N.; Adachi, M.; Mikami, B. Crystallization and x-ray analysis of normal and modified recombinant soybean proglycinins. In *Macromolecular Interactions in Food Technology*; ACS Symposium Series 650; American Chemical Society: Washington, DC, 1996; pp 257−270.
(22) Adachi, M.; Takenaka, Y.; Gidamis, A. B.; Mikami, B.; Utsumi, S. Crystal structure of soybean proglycinin a1ab1b homotrimer. *J. Mol. Biol.* **2001**, *305*, 291−305.

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