JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structural Characteristics of Purified Glycinin from Soybeans Stored under Various Conditions

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Soybeans were stored in 84% relative humidity at 30 °C (adverse conditions) for 9 months and in 57% relative humidity at 20 °C, cold (4 °C), and an uncontrolled ambient garage for 18 months. Glycinin was isolated and purified; its structural properties were characterized. The purified glycinin from soybean in the adverse conditions was associated with a significant amount of sugar and showed reductions in hydrophobic interactions after 3 months; the total free sulfhydryl content in glycinin decreased, but the intramolecular disulfide bonds increased; the α-helix content of secondary structure slightly increased, but the β-sheet content decreased. The structure of glycinin purified from the other three conditions showed no significant changes for 18 months of storage when compared to the control. The molecular mass of glycinin remained in the range of 313−340 kDa during the whole storage period for the four conditions.

KEYWORDS: Soybeans; glycinin; 11S protein; storage; structure

INTRODUCTION

Soybeans have become popular with consumers since the U.S. FDA approved a health claim for processed foods containing soy proteins in 1999. It has been estimated that ∼60% of processed foods contain ingredients that are from soybeans. Soy proteins have long been known to have a good nutritional quality and excellent physicochemical properties that can be applied in a wide range of food products. Soy proteins constitute ∼35−45% of the total solids in beans on a dry basis. Around 90% of total proteins in soybeans are globulin and are extractable with water or dilute salt solutions. The glycinin, also called soy 11S protein, accounts for approximately a third of the total seed proteins and is generally simple protein. Glycinin has been considered as a hexamer protein with a molecular mass of 300−380 kDa; each subunit is composed of an acidic polypeptide (A_n) with a molecular mass of ∼35 kDa and a basic polypeptide (B_n) with a molecular mass of ∼20 kDa. The acidic and basic polypeptides are linked together by a single disulfide bond shown as $A_n-S-S-B_n$ (*1*). Researchers discovered that the pH and ionic strength of solutions could influence the molecular structure of glycinin. At pH 7.6 and at an ionic strength of 0.5, glycinin is a hexamer complex (11S), whereas it dissociates from a hexamer into a trimer (7S) at pH 3.8 and an ionic strength of 0.03 (*2*). Glycinin in a trimer form becomes more sensitive to heat due to a decreased denaturing temperature (*3*).

The gel-forming ability of soy proteins is one of their most important functional properties in food applications, especially for a gelling type of food such as tofu. The gel formed by glycinin is a turbid gel, in contrast to the transparent gel formed by β-conglycinin (soy 7S protein). Researchers have demonstrated that hydrogen bonding, van der Waals interactions, hydrophobic interactions, and disulfide bonds are the major forces involved in glycinin gel (*4*). In a purified protein system, glycinin gels have a higher water-holding capacity, a higher tensile value, and a higher hardness and can expand more on heating than β-conglycinin gels (*5*). In a mixed system, glycinin is responsible for the gel matrix structure; it is related to hardness and unfracturability of the gels, whereas β-conglycinin largely contributes to the elasticity of the gels (*6*).

Researchers have found that soy 11S and 7S proteins became difficult to extract from soybean under adverse condition (85% relative humidity, 35 °C) (*7*). A storage study of isolated soy proteins has been documented in the literature. Hoshi and coworkers (*8*) stored 7S and 11S globulins at 50 °C under 96 and 11% relative humidity for up to 45 days and reported that the redispersibility of both proteins under 96% relative humidity drastically decreased in a few hours, whereas it did not decrease under 11% relative humidity for up to 45 days. Storage of whole soybean under various conditions has negative effects on the physicochemical properties of proteins, including decreases in nitrogen solubility index (NSI), decreases in extractability of glycinin and β-conglycinin, and changes in the subunit compositions of glycinin (*7*, *8*). However, the structural properties of glycinin purified from whole soybean that was stored under various conditions have not been fully investigated. The objective of this study was to provide an understanding of how glycinin structure changed during various conditions of storage.

MATERIALS AND METHODS

Soybeans and Chemicals. The soybean cultivar Proto (Sinner Brothers & Bresnahan, Cassleton, ND) was used in this study.

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10.1021/jf035072z CCC: $27.50
Published on Web 05/15/2004

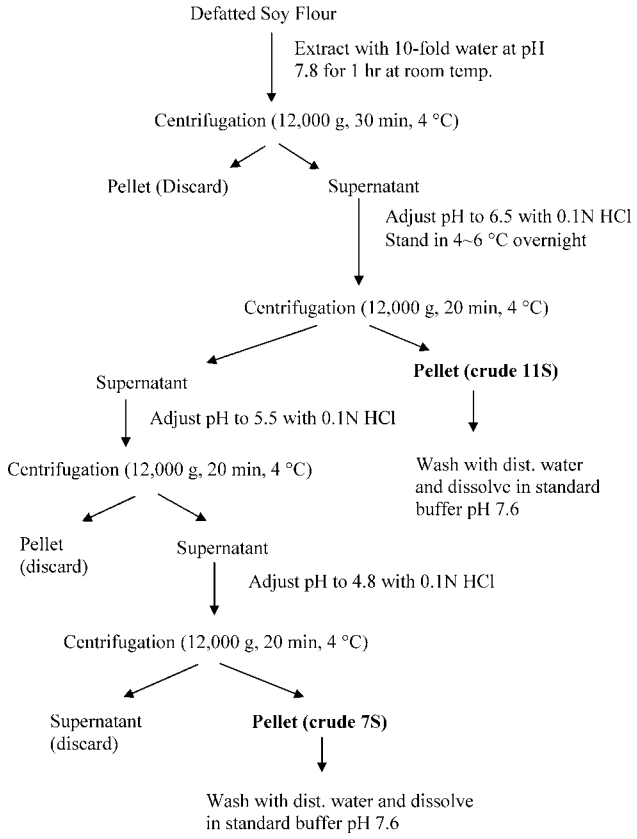

Figure 1. Scheme of isolation of glycinin (11S) and β-conglycinin (7S) from defatted soy flour.

Chemicals used in this study were of reagent grade from Fisher Scientific (Pittsburgh, PA) and Sigma Chemical (St. Louis, MO). The columns and resins used for affinity (Con A-Sepharose 4B) and gel filtration (Sephacryl S-300 high-resolution) chromatography were from Amersham Pharmacia Biotech (Piscataway, NJ). Gel electrophoresis was performed in a Bio-Rad Protean II chamber (Bio-Rad Laboratory, Hercules, CA). An MW-GF-1000 molecular mass marker kit, consisting of carbonic anhydrase (29,000), bovin serum albumin (66,000), alcohol dehydrogenase (150,000), β-amylase (200,000), apoferritin (443,000), thyroglobulin (669,000), and blue dextran (2,000,000) was from Sigma Chemical. The standards of amino acids were from Pierce (Rockford, IL).

Storage of Soybeans. Soybeans were stored in four sets of environmental conditions: adverse conditions (84% relative humidity, 30 °C), mild conditions (57% relative humidity, 20 °C), cold conditions (3−4 °C in a walk-in-cooler), and uncontrolled ambient conditions (in a garage in Fargo, ND). These conditions were selected to represent an adverse, a mild, a good, and a natural environment, because soybeans after harvest might be stored and/or transported in various environmental circumstances before processing. Soybeans stored in a freezer (−18 to −20 °C) served as the control. The detailed procedures for storing soybeans in selected relative humidity and temperature environment for various storage periods were according to our previous paper (*9*). At the end of each storage period, soybeans were placed in a −20 °C freezer until analysis.

Isolation of Glycinin (11S) from Soybeans. Soybean seeds were ground in a Straub grinding mill (model 4E, Straub Co., Philadelphia, PA) to pass through a 60-mesh sieve and were defatted by *n*-hexane extraction (soy flour/hexane = 1:5, v/v) for 1 h at room temperature. After centrifugation (8000*g*, 15 min), the supernatant was discarded and solids were extracted two more times. The defatted flour was collected for soy protein extraction.

Isolation of glycinin was conducted with a fractionation method reported by Bogracheva and co-workers (*10*) with slight modifications. The scheme for isolating glycinin from defatted soy flour is presented in **Figure 1**. The crude glycinin was washed twice with ice-cold water and then dispersed in proper volumes of a standard buffer (2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, 0.4 M NaCl, 0.2% NaN_3, pH 7.6) to redissolve in the solution. A trace of precipitate was removed by centrifugation. The isolated glycinin was either further purified through chromatography or dialyzed against water at 4 °C for 3 days with at least three changes and lyophilized for future analysis.

Purification of Glycinin (11S). Crude glycinins were purified further through a concanavalin A (Con A) affinity column followed by a gel filtration, according to the method of Wolf and Nelsen (*11*). A Con A-Sepharose 4B column (2.6 × 19 cm) was packed and equilibrated with a Tris-HCl buffer (20 mM, pH 7.4, containing 0.5 M NaCl) at 4 °C. A 5−6 mL crude glycinin solution containing 100−200 mg of protein, which had been dispersed in the standard buffer, was applied to the Con A-Sepharose 4B column. Glycinin was eluted with the Tris-HCl buffer at a flow rate of 20 mL/h, whereas the 7S protein of the contaminants was retained in the column. Fractions (9.5 mL) were collected until the absorbance at 280 nm returned to the baseline. The major glycinin fractions were checked by SDS−gel electrophoresis and concentrated in an Amicon ultrafiltration/concentrator (with Diaflo membrane of 10000 molecular weight cutoff) to ~5 mL and then applied to a gel filtration column.

A Sephacryl S-300 superfine gel filtration (2.6 × 95 cm) column with a molecular mass fractionation range of 10,000−1,500,000 Da was packed. Glycinin was eluted with the standard buffer described above at 4 °C to keep it in the native structure at a flow rate of 20 mL/h. Fractions of 5 mL were collected, and the absorbance at 280 nm was measured. The column was calibrated with a MW-GF-1000 molecular mass marker kit as described under Soybeans and Chemicals. A calibration curve was established by plotting the log of molecular mass versus the K_{av} of proteins. The K_{av} was calculated from $(V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume, V_0 is the void volume, and V_t is the total volume of the packed bed. Blue dextran (2000 kDa) was used to measure the void volume. Fractions of the major peak were analyzed by electrophoresis (SDS-PAGE) for purity and dialyzed against distilled water in a cold (4 °C) room for 3 days with at least three changes and then lyophilized for further use.

Determination of Protein Content. Protein content of crude glycinin was determined according to the biuret method (*12*). Protein content of the purified glycinin that was used for characterizing amino acid composition, surface hydrophobicity, and sulfhydryl/disulfide bonds was determined using the method of Lowry and co-workers (*13*) due to a low concentration level. Bovine serum albumin (Sigma) was used for establishing standard curves in the biuret and Lowry methods, respectively.

SDS−Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE (*14*) was used to examine the purity of purified glycinin and to quantify the relative amount of its subunits. A discontinuous acrylamide gradient gel, 16 cm length × 16 cm width × 0.15 cm thick slab gel, was prepared. The 4-cm stacking gel was a nonrestrictive gel with 4.0% acrylamide−bisacrylamide (40% T, 4.5% C). The 12-cm separating gel was a linear gradient gel with concentration varying from 8 to 16%.

The protein sample (10 μL, 1 mg/mL) was applied to the gel and run at a constant current of 20 mA/gel until the tracking dye reached the bottom of the gel. The gel then was stained for 8 h according to the paper by Kwanyuen and Wilson (*15*) and destained to remove the background dye. The quantification of glycinin subunits was performed using a Bio-Rad imaging scanning densitometer (model GS-670) and analyzed with Molecular Analyst/PC Image Analysis software (version 3.11). Molecular mass marker proteins, including α-actinin (100,000), bovine serum albumin (66,000), actin (42,000), carbonic anhydrase (29,000), and cytochrome *c* (12,400), were used to establish a standard curve of log molecular mass versus mobility for estimating sample molecular mass.

Glycosylation of Glycinin. Glycosylation of purified glycinin was determined by its total sugar contents according to the method of Dubois and co-workers (*16*). The freeze-dried purified proteins were dissolved in a Tris buffer (20 mM, pH 8) to become solutions with protein concentrations ranging from 1 to 2 mg/mL. The absorption of the yellow-orange color was measured at 480 nm against the reagent blank. The amount of total sugar was calculated against a standard curve, which was prepared using glucose as the reference sugar.

Analysis of Sulfhydryl and Disulfide Contents. The free sulfhydryl group (SH) and total sulfhydryl group (total SH including disulfide bonds) content of purified glycinin was determined using the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) reagent according to the method of Felker and Waines (*17*) and Chang and co-workers (*18*). For determining total free sulfhydryl content, glycinin solution (2−3 mg/mL) was added to a 9 M urea−0.1% EDTA solution (prepared freshly) to unfold protein structure. Subsequently, the DTNB solution was added for color reaction, and then the absorbance was read at 412 nm. The surface free sulfhydryl (SH_S) content was determined using the same procedures without adding the denaturant, 9 M urea−0.1% EDTA solution. The SH value after subtracting the surface SH from the total free SH value was defined as the internal free SH. The total sulfhydryl including S−S bonds (total SH) were determined by using the same method (with urea) but after reduction of the proteins with sodium borohydride ($NaBH_4$). Reagent blank and sample blank were initially prepared to correct the color from reagents and protein solution. The total free, surface free, and total sulfhydryl (after reduction) contents were calculated using a standard curve and converted to micrograms of cysteine by multiplying by a factor of 121/103. A standard curve using reduced glutathione was prepared. The disulfide group (SS) content was obtained by calculation as (total SH − total free SH)/2. All types of sulfhydryl groups and SS contents were expressed as moles per mole of protein, using the molecular mass of 340,000 for glycinin.

Surface Hydrophobicity (H_0). Surface hydrophobicity of purified proteins was determined using a fluorescence probe, 1-anilinonaphthalene-8-sulfonic acid (ANS), according to the method of Kato and Nakai (*19*). Fluorescence intensity (FI) was measured at wavelengths of 365 nm (excitation) and 520 nm (emission) using a SPEX Fluorolog 1680/0.22m double spectrometer. The surface hydrophobicity was calculated by linear regression analysis using the slope of the straight line obtained by plotting the FI as a function of the protein concentration. The slope is a function of the number of binding sites and of the nature and amount of the groups forming these sites that determine both the affinity and the energy transferred to the probe.

Differential Scanning Calorimetry (DSC). The use of DSC to study thermal denaturation and thermodynamics of proteins has been widely adopted by researchers. For soy proteins, DSC is usually used as an indicator for protein denaturation that affects significantly functionality and thus their applicability in the food system. The method used for DSC in this study was that of Arress and co-workers (*20*). DSC thermograms were recorded using a Saiko Instrument model SII 2200 calorimeter. The heating rate was 2 °C/min from 40 to 110 °C. Protein sample (45 mg) of 10% (w/v) solution in 35 mM potassium phosphate buffer, pH 7.6, was hermetically sealed in aluminum pans; an empty pan was used as reference. Peaks indicating an endothermic heat flow were obtained. The denaturation temperature (T_d), obtained from the intercept of the extrapolated slope of the peak and the baseline, was identified. The temperature at the peak maximum (T_p), generally used to indicate heat denaturation of proteins, was recorded as well.

Amino Acid Analysis. The amino acid composition of purified glycinin was analyzed according to the Pico-Tag method as modified by Chang and co-workers (*21*). Glycinin was sealed in an ampule tube under vacuum (<50 μmHg) and hydrolyzed at 110 °C (±1 °C) for 24 h. After derivatization, the dried sample was analyzed using a Waters Associates (Milford, MA) HPLC system with a Pico-Tag column (15 cm × 3.9 mm, Waters Associates). Amino acids were identified using amino acid standards and quantified by calculating the peak areas obtained from the HPLC chromatograms. The results were expressed as the percentage of each amino acid weight to the protein weight.

Secondary Structure of Glycinin. Secondary structures of purified glycinin were determined using a circular dichroism (CD) spectropolarimeter (Jasco J-710, Jasco Corp., Japan). Freeze-dried glycinin was dissolved in 35 mM potassium phosphate buffer (pH 7.6) and was centrifuged to remove any insoluble residues. The CD spectra were scanned using a quartz cell with a path length of 20 mm. The scan interval for CD spectra was 260−178 nm. Spectra were recorded as averages of 32 spectra using a scan speed of 50 nm/min, a bandwidth of 1.0 nm, a response time of 0.25 s, and a step resolution of 0.2 nm. The mean residue ellipticity (θ) was expressed as deg cm^2 dmol^{-1}.

Table 1. Isolation Yield of Crude Glycinin and Total Sugar Content and Surface Hydrophobicity of Glycinin Purified from Soybeans under Various Conditions[a]

storage conditions	crude glycinin yield[b] (%)	total sugar content[c] (%)	surface hydrophobicity (cps × 10^6)
control	14.62 ± 0.21a	0.19 ± 0.01f	2.68 ± 0.08bcd
84% relative humidity, 1 month	14.22 ± 0.65a	0.21 ± 0.01f	3.02 ± 0.03ab
84% relative humidity, 2 months	11.77 ± 0.46b	0.27 ± 0.01e	2.75 ± 0.24abc
84% relative humidity, 3 months	8.81 ± 0.61c	0.32 ± 0.02de	3.15 ± 0.07a
84% relative humidity, 4 months	8.75 ± 0.24c	0.35 ± 0.03cd	2.60 ± 0.03cd
84% relative humidity, 5 months	7.17 ± 0.06d	0.45 ± 0.01b	2.83 ± 0.10bc
84% relative humidity, 6 months	5.47 ± 0.41e	0.39 ± 0.01c	2.76 ± 0.30bcd
84% relative humidity, 7 months	0.47 ± 0.21f	1.04 ± 0.12a	2.42 ± 0.06d
84% relative humidity, 8 months	0.05 ± 0.06g	NA[d]	1.33 ± 0.31e
84% relative humidity, 9 months	0.00 ± 0.00g	NA	NA
57% relative humidity, 18 months	14.05 ± 0.07a	0.30 ± 0.01d	2.49 ± 0.11d
4 °C, 18 months	14.51 ± 0.12a	0.21 ± 0.01f	2.61 ± 0.16bcd
ambient, 18 months	14.23 ± 0.15a	0.20 ± 0.01f	2.67 ± 0.13bcd

[a] Data are expressed as mean ± standard deviation and are the mean of three replicates. Means with different letters within the same column are significantly ($p < 0.05$) different. [b] The crude glycinin yield was calculated on the basis of the total protein of dried soybeans. [c] The sugar content was based on dried purified glycinin. [d] Sample was not available for determination.

The protein concentrations were determined by the Bradford dye-binding method. The molarity of the peptide bonds of glycinin was calculated using a molecular mass of 340,000 and a mean amino acid molecular weight of 112 based on the report of Utsumi and co-workers (*6*).

The CD spectra of glycinin could be used to estimate the relative secondary structures using the reference CD spectra of proteins with known secondary structures. The secondary structure of glycinin in this study was estimated using a computer program called SELCON that was provided by Jasco Corp. and originated by the method of Sreerama and Woody (*22*). Four secondary structures were calculated: α-helix, β-sheet, β-turns, and unordered.

Statistical Analyses. Soybean samples from all storage conditions were extracted at least in triplicate. Purified proteins were analyzed at least in duplicate. All data were analyzed by using analysis of variance (ANOVA) in the General Linear Models procedure of the Statistical Analysis Systems software package (*23*). Differences between group means were analyzed by Duncan's multiple-range test. Statistical significance was set at a 0.05 probability level.

RESULTS AND DISCUSSION

Isolation of Glycinin. A significant decrease of the yield of crude glycinin from the soybeans stored under adverse conditions is shown in **Table 1**. The decrease of glycinin yield was consistent with the decrease in the total protein extractability (*9*). The yield of crude glycinin decreased from 14.6% of the total proteins at time zero to 0.05% after 8 months. At the end of storage (9 months), no glycinin could be extracted using water and pH adjustment. The yield decrease in the crude glycinin indicated that more glycinin became denatured and unextractable during storage time and remained in the residues. The yield of crude glycinin from the soybeans stored under other selected conditions for 18 months had no substantial changes from the control (~14%). The purified glycinin contained 98.5−99.5% protein, indicating the purification scheme was efficient to remove contaminants.

Purity of Glycinin. Figure 2 depicts the electrophoresis pattern of glycinin purified from soybeans stored under various conditions. According to Hirano and co-workers (*24*) and Kagawa and Hirano (*25*), the acidic polypeptides including A_3, A_x (A_{1a}, A_{1b}, A_2, and A_4), and A_5 could be identified from the

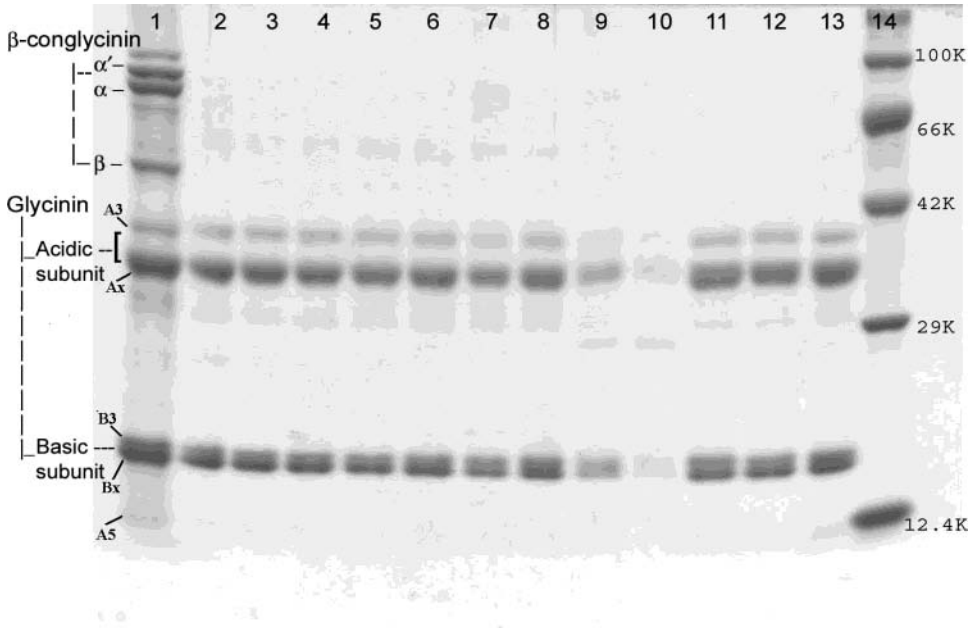

Figure 2. SDS-PAGE analysis of glycinin purified after gel filtration from soybeans stored under various conditions: (lane 1) control before purification; (lane 2) control, 0 months; (lanes 3–10) 84% relative humidity, 30 °C, 1–8 months, respectively; (lane 11) 57% relative humidity, 20 °C, 18 months; (lane 12) cold, 4 °C, 18 months; (lane 13) uncontrolled ambient temperature, 18 months; (lane 14) molecular markers.

gel (**Figure 2**). The basic polypeptides could be separated into one minor B_3 band and a major B_x band (B_{1a}, B_{1b}, B_2, and B_4). The acidic A_x and the basic B_x polypeptides are two prominent bands in the pattern of glycinin. The A_3, A_x, and A_5 acidic polypeptides had molecular masses of 40, 34, and 12 kDa, respectively; the basic polypeptides (B_x) had a molecular mass of ~17 kDa (**Figure 2**). The molecular masses of the acidic and basic polypeptides of glycinin found were consistent with those of Riblett and co-workers (*26*). The acidic and basic polypeptides have been reported with molecular masses of 37-44 and 17−22 kDa, respectively (*27*). The A_5 is a small polypeptide, which is composed of 97 amino acids with a molecular mass ~11 kDa (*28*). The molecular mass of the A_5 polypeptide in this study was close to that reported by Kagawa and Hirano (*25*).

The purity of glycinin was quantified using the densitometric data analyzed by an imaging densitometer. The purities of glycinin determined by acidic and basic polypeptides presented as in **Figure 2** were between 90 and 98%. The purities of glycinins from the soybeans in the adverse conditions for 7 and 8 months (lanes 9 and 10, respectively) were lower than others, which were 93 and 90%, respectively. The lower purity resulted from the appearance of a 26 kDa band. We do not know the origin of this band. There were no differences in glycinin purity for three selected conditions (lanes 11−13). The glycinin purity indicated that the purification through affinity and gel filtration chromatography could efficiently eliminate most of the contaminants.

Molecular Mass. A typical gel filtration elution pattern for glycinin after passage through a Con A-Sepharose 4B column is shown as **Figure 3**. There were two peaks, a minor peak and a major peak, shown on the elution pattern. The molecular masses corresponding to the minor peak and the major peak were 690 and 340 kDa, respectively. The analysis of SDS−electrophoresis showed that the subunit patterns of these two peaks were identical. Therefore, the major peak was predominantly glycinin (11S), and the minor peak was 15S protein. The results confirmed the findings of Wolf and Nelsen (*11*) that an aggregate of two glycinin molecules forms a 15S protein in the solution. The molecular mass of glycinin from the adverse conditions (84% relative humidity, 30 °C) for up to 8 months did not show significant differences, which remained in the range of 313−340 kDa. The glycinin from the three selected conditions for up to 18 months also had a similar molecular mass (320−330 kDa). These results indicated that storage of soybeans under various conditions, no matter adverse or good conditions, had no effect on the molecular mass of the purified glycinin.

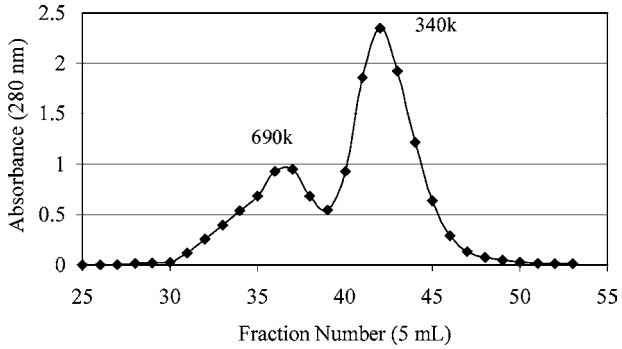

Figure 3. Gel filtration elution pattern of glycinin, which was purified by a Con A-Sepharose column.

Sugar Content in Glycinin. The total sugar content in glycinin that was purified from the soybeans stored under the adverse conditions (84% relative humidity, 30 °C) for 7 months is also shown in **Table 1**. The total sugar content increased gradually along with the storage months in the adverse conditions. The total sugar content increased slightly from 0.2 to 0.45% in the first 6 months and then increased rapidly to >1% for the seventh month. Glycosylated protein molecules might have been preferentially extracted as their water solubility was expected to increase when they became glycosylated. At any rate, the results indicated that more carbohydrate became associated with purified glycinin as soybeans aged, especially during the late period of storage, which might be one of the reasons resulting in the decrease of glycinin purity as checked by SDS-PAGE (**Figure 2**).

Table 2. Sulfhydryl and Disulfide Contents (Moles per Mole of Protein) of Glycinin from Soybeans Stored under Various Conditions[a]

storage conditions	surface free SH	internal free SH	total free SH	total SH[b] including S–S	S–S content[c]
control	0.15 ± 0.02d	1.67 ± 0.01a	1.82 ± 0.03a	32.17 ± 0.50d	15.18 ± 0.24d
84% relative humidity, 1 month	0.15 ± 0.01d	1.59 ± 0.03b	1.74 ± 0.04b	34.56 ± 0.64c	16.41 ± 0.30c
84% relative humidity, 2 months	0.16 ± 0.04d	1.50 ± 0.05c	1.66 ± 0.09c	34.93 ± 0.30c	16.64 ± 0.10c
84% relative humidity, 3 months	0.14 ± 0.01d	1.42 ± 0.06d	1.56 ± 0.07d	35.18 ± 0.50bc	16.81 ± 0.22bc
84% relative humidity, 4 months	0.22 ± 0.01c	1.38 ± 0.07cd	1.60 ± 0.08cd	34.90 ± 0.90c	16.65 ± 0.41c
84% relative humidity, 5 months	0.27 ± 0.03bc	1.20 ± 0.04e	1.47 ± 0.07e	36.38 ± 0.64b	17.45 ± 0.28b
84% relative humidity, 6 months	0.28 ± 0.01b	1.13 ± 0.04e	1.41 ± 0.05e	38.08 ± 0.61a	18.33 ± 0.28a
84% relative humidity, 7 months	0.37 ± 0.02a	0.32 ± 0.01f	0.69 ± 0.02f	38.31 ± 0.65a	18.65 ± 0.32a
57% relative humidity, 18 months	0.12 ± 0.03d	1.41 ± 0.09b	1.53 ± 0.12b	32.39 ± 0.30d	15.43 ± 0.09d
4 °C, 18 months	0.13 ± 0.02d	1.58 ± 0.10ab	1.71 ± 0.12ab	33.76 ± 0.35c	16.17 ± 0.12c
ambient, 18 months	0.17 ± 0.01d	1.51 ± 0.07b	1.68 ± 0.08b	33.83 ± 0.82c	16.07 ± 0.37c

[a] Data are expressed as mean ± standard deviation and are the mean of three replicates. All contents were expressed as cysteine equivalents except S–S, in cystine equivalent. Data were calculated on the basis of a glycinin molecular mass of 340,000 Da. Means with different letters within the same column are significantly ($p < 0.05$) different. [b] Total SH contains all free and disulfide cysteine. [c] S–S content was obtained from the difference of total SH and total free SH and divided by 2.

Glycinins that were purified from the cold and the uncontrolled ambient conditions contained the same sugar content as the glycinin in the control (**Table 1**). The total sugar content of the glycinin from the mild conditions for 18 months increased to 0.3%, which was equivalent to that of the glycinin from the adverse conditions for 3 months. The total sugar content of glycinin observed in this study was lower than that reported by Fukushima (*29*), who found ~0.8% carbohydrate (sugar) in 11S protein. This might have resulted from a higher glycinin purity obtained in this study.

Sulfhydryl and Disulfide Contents. The surface sulfhydryl content, the internal sulfhydryl content, the disulfide content (S−S), the total sulfhydryl content, and the half-cystine content of purified glycinin from soybeans stored under adverse conditions for 7 months are summarized in **Table 2**. The surface SH contents of glycinin remained low and ranged from 0.15 to 0.37 mol/mol of protein; a significant increasing trend beyond 3 months of the adverse storage was observed, even though the value was low. In contrast to the surface SH contents, the internal SH contents showed a significant decreasing trend with the adverse storage time. After 7 months, the internal SH content reduced from 1.67 to 0.32, which is an 80% reduction. A decrease in internal SH but an increase in SH and S−S content suggested that the originally internal cysteine residues, which were usually buried in the inside region of a native glycinin molecule, became more exposed on the surface. Some of the internal SH may form disulfide bridges on the surface during storage. The disulfide bonds in glycinins increased from 15.2 to 18.7 mol/mol of protein after 7 months of storage, which indicated a sign of protein aggregation through intramolecular disulfide bond formation. The findings of a decrease in total free SH content with a concomitant increase in the amount of total SH and S−S during storage were in good agreement with Hong (*30*), who observed the similar trends in soy protein after storage in 80% relative humidity and 30 °C.

The literature values for surface SH and free SH groups of glycinin tend to be low and variable. The contents range from 0 to 1.3 mol of SH/mol of protein for surface SH and from 1.2 to 1.7 mol of SH/mol of protein for internal SH (*31*−*33*). Various soybean materials and different determination methods used by the researchers may explain the variations in the literature. The values of surface and free sulfhydryl (SH) contents for the glycinin purified from the control soybeans fall into the ranges of literature values. For total SH content of fully reduced glycinin, researchers have reported various content, ranging from 33 to 48.9 mol/mol of protein (*33*−*35*). The value of 32−38 half-cystine residues (total SH) found in this study was in the lower end of the literature range. The discrepancy with the literature values may be due to different raw mateials, preparation procedures, and molecular masses of glycinin estimated in this study.

Table 3. Amino Acid Composition of Glycinin Purified from Soybeans Stored in 84% Relative Humidity and 30 °C for up to 7 Months[a]

amino acid	storage month: control (0)	1	2	3	4	5	6	7
Asx[b]	13.02a	11.98c	12.29bc	11.92c	11.98c	13.26a	12.80b	11.28d
Glx[c]	21.29	21.48	21.93	20.72	21.20	22.86	22.84	20.13
Ser	5.87	5.34	5.31	5.96	5.66	5.23	5.18	4.81
Gly	4.18	3.94	3.96	3.76	3.88	4.05	4.23	4.33
His	2.88b	2.60b	1.77d	2.04c	2.74b	2.11c	2.26c	3.78a
Arg	9.73	10.16	9.98	11.62	11.18	9.78	9.64	11.44
Thr	3.84	4.02	4.01	4.56	4.19	3.97	4.05	5.60
Ala	4.05b	4.34a	4.23b	3.98b	3.33c	3.44c	3.40c	4.34a
Pro	5.66	5.71	5.86	5.95	6.03	5.91	6.21	6.01
Tyr	3.24	3.31	3.27	3.10	3.33	3.30	3.31	2.95
Val	4.22	4.47	4.22	4.13	4.17	4.01	4.18	4.23
Met	1.30	1.35	1.32	1.31	1.32	1.20	1.15	1.20
Cys[d]	1.14d	1.23c	1.24c	1.25c	1.24c	1.29b	1.36a	1.36a
Ile	4.10	4.38	4.27	4.01	4.24	4.13	4.07	3.78
Lue	6.52	7.15	6.91	6.53	6.54	6.59	6.53	6.47
Phe	4.45	4.75	4.80	4.67	4.53	4.63	4.61	4.69
Lys	4.40	4.50	4.35	4.63	4.56	4.63	4.58	4.22
Trp[e]	ND[f]	ND	ND	ND	ND	ND	ND	ND

[a] Data are expressed as percentage (grams per 100 g of protein) and are the mean of two replicates. Means with different letters within the same row are significantly ($p < 0.05$) different. [b] Asx = Asp + Asn. [c] Glx = Glu +Gln. [d] Cysteine was determined from the total SH content measurement. [e] Trptophan was not determined. [f] Not determined.

The sulfhydryl and disulfide content of glycinin purified from the soybeans stored under three selected conditions for 18 months are also reported in **Table 2**. The surface free SH contents had no significant difference after storage in the selected conditions. The differences in internal free SH content for the selected conditions were not significant; however, the internal free SH contents showed slight decreases compared with that from the initial soybeans. The total SH (containing S−S cystine) content of glycinins showed slight differences among the three selected conditions for 18 months (**Table 2**).

Amino Acid Composition. The amino acid composition of glycinin purified from the soybeans stored in 84% relative humidity and 30 °C for 7 months is shown in **Table 3**. The results indicated that the effect of storage on amino acid composition of glycinin was not significant. Individual amino acid content varied along with the storage time, but there was

Table 4. Amino Acid Composition of Glycinin Purified from Soybeans Stored under Various Conditions for 18 Months[a]

amino acid	storage conditions: control	57% relative humidity, 20 °C	cold (4 °C)	uncontrolled ambient temp
Asx[b]	13.02a	12.99a	10.79c	11.87b
Glx[c]	21.29	22.91	20.03	21.14
Ser	5.87ab	4.80c	5.02bc	6.00a
Gly	4.18	3.92	3.97	4.06
His	2.88a	2.01b	2.12b	1.98b
Arg	9.73	9.62	11.69	9.95
Thr	3.84	4.01	4.97	3.70
Ala	4.05ab	3.56b	4.50a	3.49b
Pro	5.66	5.96	5.87	6.27
Tyr	3.24	3.45	3.48	3.43
Val	4.22	4.18	4.38	4.67
Met	1.30	1.35	1.34	1.35
Cys[d]	1.14b	1.15b	1.20a	1.20a
Ile	4.10	4.00	4.27	4.18
Lue	6.52	6.09	6.55	6.93
Phe	4.45	4.55	4.74	4.63
Lys	4.40	4.30	4.77	4.77
Trp[e]	ND[f]	ND	ND	ND

[a] Data are expressed as percentage (grams per 100 g of protein) and are the mean of two replicates. Means with different letterss within the same row are significantly ($p < 0.05$) different. [b] Asx = Asp + Asn. [c] Glx = Glu +Gln. [d] Cysteine was determined from the total SH content measurement. [e] Trptophan was not determined. [f] Not determined.

no specific trend. Soy major storage proteins have polymorphism. The small differences in amino acid composition may be due to differential extraction of various polymorphic forms. The results suggested that the glycinin's primary structure did not change during storage under adverse conditions for 7 months. Glutamic acid and aspartic acid contents were high in the protein, because glutamine and asparagine were hydrolyzed and counted together, respectively. Except for glycine, arginine, and alanine, the amino acid content of glycinin measured in this study was in good agreement with that of Wolf and Nelsen (*11*). Compared with Fukushima (*36*), the agreement is good except for lysine, phenylalanine, and leucine. In the literature, variations in individual amino acid content of glycinin are very substantial, because various cultivars and different methods are used. Various soybean genotypes have significant differences in amino acid composition that are related to the gel-forming properties of soy proteins (*26*).

The amino acid composition of the glycinins purified from other selected conditions for 18 months is reported in **Table 4**. The amino acid compositions among the conditions were not significantly different and were close to that of glycinin from the control soybeans of 0 months. The results indicated that storage conditions selected in this study for up to 18 months did not affect glycinin's amino acid composition.

Surface Hydrophobicity. Hydrophobicity is the tendency of nonpolar solutes to adhere to one another in an aqueous environment. A molecule of a globular protein such as soy glycinin in its native state is compactly folded; the hydrophobic amino acid side chains are located in the interior of the subunit molecule to form a hydrophobic region, whereas the hydrophilic ones are located on its surface, accessible to water. The native soy proteins, therefore, are water-soluble because the molecular surface is covered with amino acids that are relatively hydrophilic. Hydrophobicity of a protein has been a useful structural property used by researchers to predict its functionality such as solubility, emulsifying ability, and viscosity (*19*, *37*). Due to the propensity of nonpolar amino acids to position themselves in the interior of the native protein molecules in solution, only a portion of them participates in the hydrophobicity measured. Therefore, the hydrophobicity measured in this study would be only the surface or effective hydrophobicity that does not directly correlate with the total hydrophobicity. The surface hydrophobicity manifested by a protein is one of the structural characteristics to evaluate the change in protein conformation.

The surface hydrophobicity of the glycinin purified from the soybeans stored in the adverse conditions for 8 months is shown in **Table 1**. For the first 6 months, the surface hydrophobicity in glycinin showed changes, but the magnitudes were not significant. When the storage time was extended to 7 and 8 months, however, the surface hydrophobicity decreased significantly. The results suggested that the glycinin conformation from the soybeans under the adverse conditions had a tendency to fold its structure, which might result in an increase in glycinin's disulfide bonds (**Table 2**). The finding of the decreasing trend in hydrophobicity of glycinin with storage time was parallel with the increasing trend in total sugar content after storage (**Table 1**). The moiety of carbohydrates made glycinin more hydrophilic.

The surface hydrophobicity of glycinin from soybeans under other selected conditions is also reported in **Table 1**. The glycinin from the condition of 57% relative humidity at 20 °C showed the lowest hydrophobicity among these three conditions for 18 months; however, the magnitude was not statistically significant. For the mild condition (57% relative humidity, 20 °C), the glycinin with a lower surface hydrophobicity was related to a higher glycosylation (**Table 1**). The results suggested that glycinins from soybeans stored in the conditions of the mild (57% relative humidity, 20 °C), the cold (4 °C), and the uncontrolled ambient temperature could retain their surface hydrophobicity for up to 18 months.

Secondary Structures. Circular dichrosim has been considered as a sensitive technique for measuring a protein's secondary structure, especially for proteins and polypeptides in solution. The CD of a protein is primarily the CD of the amide chromophore. Thus, protein secondary structure as measured by the CD counts of the amide−amide interactions that are a somewhat different number from counting residues in X-ray diffraction structures (*38*). The CD spectra of the glycinins purified from the adverse conditions were in a similar shape but with slight differences at the minimum ellipticity. The spectra showed troughs for 0, 3, 6, and 8 months at 216, 214, 210, and 207 nm, respectively. The estimated secondary structures of glycinins from various conditions are presented in **Table 5**. The α-helical structure with ∼15% was the minor structure comparing to the two β-structures (∼60% together) and the random coil. The α-helix content showed a slight increasing trend (from 14.88 to 16.60) for the adverse conditions (84% relative humidity, 30 °C) for 0, 3, 6, and 8 months, whereas the β-sheet content had a decreasing trend (from 36.66 to 33.10). The β-turn and unordered content remained relatively stable with storage time. The glycinin from the mild conditions (57% relative humidity, 20 °C) also showed an increase in α-helix and a decrease in β-sheet (**Table 5**). The glycinins from the cold and the uncontrolled ambient temperature conditions had no significant changes in secondary structures as compared with the control beans before storage.

The sum of secondary structures presented in **Table 5** is between 96 and 103%, which agrees with what Johnson (*38*) suggested. The secondary structures of glycinin are reported

Table 5. Secondary Structure of Glycinin Purified from Soybeans Stored under Various Conditions[a]

storage conditions	secondary structure (%)				
	α-helix	β-sheet	β-turn	unordered	total
control, 0 month	14.88c	36.66b	24.91b	22.66c	99.11
84% relative humidity, 30 °C, 3 months	15.27bc	35.96bc	24.20b	21.16d	96.59
84% relative humidity, 30 °C, 6 months	16.07ab	35.18c	24.05b	21.30d	96.60
84% relative humidity, 30 °C, 8 months	16.60a	33.10d	24.92b	24.60b	99.22
57% relative humidity, 20 °C, 18 months	15.43b	33.79d	24.72b	25.62a	99.56
cold, 4 °C, 18 months	15.63b	36.32b	26.38a	24.63b	102.96
ambient temp, 18 months	14.97c	37.64a	24.32b	22.91c	99.84

[a] Data are expressed as percentage and are the mean of two replicates. Means with different letters within the same column are significantly ($p < 0.05$) different.

inconsistently by a wide range by researchers. 11S proteins with 5.1−20% α-helix, 17−64.5% β-structure, and 27−63% random coil have been reported (*39*−*41*). By using amino acid sequence data, glycinin with 25% α-helix, 25% β-strand, 42% β-turn, and 8% random coils had been reported (*42*). The molecular heterogeneity of glycinin has been an obstacle for researchers to understand its three-dimensional structure for a long time. Until recently, by using the protein composed of six subunits (A_3B_4), glycinin has been crystallized and its three-dimensional structure was unveiled by X-ray analysis (*43*). Subsequently, the secondary structures of glycinin were calculated to contain 8% α-helix, 36% β-structure, and 56% random coils (*44*).

These previous reports along with the findings in the present study suggest that α-helical structure is not the main structure in glycinin. Lakemond and co-workers (*2*), however, reported that glycinin predominantly consists of α-helical structure on the basis of comparing their CD spectra with reference spectra of Johnson (*38*). The contradictions in the literature might result from various reference spectra used by researchers in different laboratories. In addition, there is no common computer program for predicting the secondary structures of glycinin.

The result of an increase in α-helix contents after 8 months (**Table 5**) indicated that soybean storage under the adverse conditions had influences in the molecular conformation of glycinin. Kato and co-workers (*45*) reported a negatively linear correlation between protein surface hydrophobicity and helical structure that was measured using ellipticity $[\theta]_{220}$ for the ovalbumin and lysozyme, indicating a tendency of the lower helical structure, the greater extent of exposure of hydrophobic sites (higher surface hydrophobicity) of the protein molecule. Therefore, the glycinin molecule from the adverse conditions after 8 months might have lower extent of exposure of the interior hydrophobic regions because of a significant decrease in hydrophobicity of protein (**Table 1**). This speculation is supported by the findings of Koshiyama and Fukushima (*46*), who reported an increase in α-helix contents of 7S and 11S protein with SDS treatment.

Analysis of Differential Scanning Calorimetry. The thermograms of the purified glycinin show only one sharp peak, demonstrating the purity of proteins. The approximate T_d and T_p of glycinin from soybeans stored under various conditions are reported in **Table 6**. The denaturation temperatures, ranging from 93.8 to 94.9 °C, had no significant differences for the glycinin from the adverse conditions for up to 8 months, even though they showed small changes. The differences in the peak temperature of glycinin also were not significant. The T_p of glycinin remained in the range of 96.1−98.2 °C. For the other three selected conditions, the T_d and T_p of glycinin remained stable after 18 months. The thermal properties of glycinin found in this study were in agreement with those of Nakamura and co-workers (*47*), who found that the thermal denaturation temperature appeared between 89 and 99 °C and exhibited a peak at 94 °C. A peak temperature of ∼95.5 °C for 11S protein has been reported (*48*). The differences between our study and others might be partly due to the use of different raw materials and extraction methods. Soy major proteins possess different polymorphism, which may affect denaturation properties.

Table 6. Comparison of Thermal Properties of Glycinin Purified from Soybeans Stored under Various Conditions[a]

storage conditions	denaturation temp, T_d (°C)	peak temp, T_p (°C)
control, 0 months	94.9 ± 0.2	98.2 ± 0.3
84% relative humidity, 30 °C, 3 months	94.5 ± 0.4	97.4 ± 0.5
84% relative humidity, 30 °C, 6 months	94.4 ± 0.3	98.1 ± 0.2
84% relative humidity, 30 °C, 8 months	93.8 ± 0.5	96.1 ± 0.5
57% relative humidity, 20 °C, 18 months	94.6 ± 0.3	98.0 ± 0.4
cold, 4 °C, 18 months	94.8 ± 0.5	98.3 ± 0.4
ambient temp, 18 months	94.4 ± 0.2	97.9 ± 0.6

[a] Data are expressed as mean ± standard deviation and are the mean of two replicates.

Conclusion. The structures of purified glycinin from soybean stored under adverse conditions showed changes after 3 months; however, the glycinin from soybean stored under the other three conditions had no significant changes after 18 months compared with the control. Glycinin from soybean in the adverse conditions associated with a significant amount of sugar and showed decreases in hydrophobic interactions after 3 months of storage. The interchange reactions between SH and S−S in a glycinin molecule increased due to a decrease in total free SH content but an increase in SS content. On the contrary, the hydrogen bondings within the glycinin molecule might decrease because of an increase in α-helix structure but a decrease in the β-sheet structure. The molecular mass of purified glycinin remained in the range of 313−340 kDa after storage for all conditions.

Changes in glycinin structure due to the adverse storage may negatively affect soy product quality, especially for tofu that forms a gel matrix from glycinin. Coagulation is the most important step to affect tofu quality. The coagulant requirements for making tofu from Proto decreased with long-term storage of soybean (*49*). The reason might be partly due to the compositional (*50*) and structural changes of glycinin and beta-conglycinin during soybean stoarge. The stable structure found in glycinin that was purified from the other selected conditions after 18 months indicated that soybeans maintained their good qualities for making tofu (*51*).

ACKNOWLEDGMENT

We thank Dr. Steven Meinhardt of the NDSU Biopolymers Service Center for assistance in the determination of the secondary structures by circular dichroism.

LITERATURE CITED

(1) Nielsen, N. C. The structure and complexity of the 11S polypeptides in soybeans. *J Am. Oil Chem. Soc.* **1985**, *62*, 1680−1686.

(2) Lakemond, C. M. M.; deJongh, 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 temperature. *J. Agric. Food Chem.* **2000**, *48*, 1985−1990.

(3) Lakemond, C. M. M.; deJongh, H. H. J.; Hessing, M.; Gruppen, H.; Voragen, A. G. J. Heat denaturation of soy glycinin: influence of pH and ionic strength on molecular structure. *J. Agric. Food Chem.* **2000**, *48*, 1991−1995.
(4) Utsumi, S.; Kinsella, J. E. Forces involved in soy protein gelation: Effect of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S and soy isolate. *J. Food Sci.* **1985**, *50*, 1278−1282.
(5) Saio, K.; Watanabe, T. Differences in functional properties of 7S and 11S soybean proteins. *J. Texture Stud.* **1978**, *9*, 135−157.
(6) Utsumi, S.; Matsumura, Y.; Mori, T. Structure−function relationships of soy proteins. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Dekker: New York, 1997; pp 257−291.
(7) Saio, K.; Kobayakawa, K.; Kito, M. Protein denaturation during model storage studies of soybeans and meals. *Cereal Chem.* **1982**, *59*, 408−412.
(8) Murphy, P. A.; Chen, H. P.; Hauck, C. C.; Wilson, L. A. Soybean protein composition and tofu quality. *Food Technol.* **1997**, *51*, 86−88, 110.
(9) Hou, H. J.; Chang, K. C. Interconversions of isoflavones in soybeans as affected by storage. *J. Food Sci.* **2002**, *67*, 2083−2089.
(10) 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.
(11) Wolf, W. J.; Nelsen, T. C. Partial purification and characterization of the 15S globulin of soybeans, a dimer of glycinin. *J. Agric. Food Chem.* **1996**, *44*, 785−791.
(12) Gornall, A. G.; Bardawill, J.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751−766.
(13) Lowry, O. H.; Rosebrough, N. J.; Lewis, A.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.
(14) Laemmli, U. K. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **1971**, *227*, 680−685.
(15) Kwanyuen, P.; Wilson, R. F. Optimization of Coomassie staining for quantitative densitometry of soybean storage proteins in gradient gel electrophoresis. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1251−1254.
(16) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1959**, *28*, 350−356.
(17) Felker, P.; Waines, G. Colorimetric screening assay for cystine plus cysteine in legume seed meals. *Anal. Biochem.* **1978**, *87*, 641−644.
(18) Chang, K. C.; Marshall, H. F.; Satterlee, L. D. Sulfur amino acids stability: hydrogen peroxide treatment of casein, egg white, and soy isolate. *J. Food Sci.* **1982**, *47*, 1181−1183.
(19) Kato, A.; Nakai, S. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochim. Biophys. Acta* **1980**, *624*, 13−20.
(20) Arress, E. L.; Sorgentini, D. A.; Wagner, J. R.; Anon, M. C. Electrophoretic, solubility, and functional properties of commercial soy protein isolates. *J. Agric. Food Chem.* **1991**, *39*, 1029−1032.
(21) Chang, K. C.; Skauge, L. H.; Satterlee, L. D. Analysis of amino acids in soy isolate and navy beans hydrolyzates using precolumn derivatization with phenylisothiocyanate and reverse-phase high performance liquid chromatography. *J. Food Sci.* **1989**, *54*, 756−757.
(22) Sreerama, N.; Woody, R. W. A self-consistent method for the analysis of protein secondary structure from circular dichroism. *Anal. Biochem.* **1993**, *209*, 32−44.
(23) SAS. *SAS User's Guide: Statistics*, 6th ed.; SAS Institute Inc.: Cary, NC, 1996.
(24) Hirano, H.; Kagawa, H.; Kamata, Y.; Tamauchi, F. Structural homology among the major 7S globulin subunits of soybean seed storage proteins. *Phytochemistry* **1987**, *26*, 41−45.
(25) Kagawa, H.; Hirano, H. Identification and structural characterization of the glycinin seed storage protein A_7 subunit of soybean. *Plant Sci.* **1988**, *56*, 189−195.
(26) Riblett, A. L.; Herald, T. J.; Schmidt, K. A.; Tilley, K. A. Characterization of β-conglycinin and glycinin soy protein fractions from four selected soybean genotypes. *J. Agric. Food Chem.* **2001**, *49*, 4983−4989.
(27) Kitamura, K.; Tagagi, T.; Shibasaki, K. Subunit structure of soybean 11S globulin. *Agric. Biol. Chem.* **1976**, *40*, 1837−1844.
(28) Hirano, H.; Fukazawa, C.; Harada, K. The primary structures of the A_4 and A_5 subunits are highly homologous to that of the A_3 subunit in the glycinin seed storage protein of soybean. *FEBS Lett.* **1985**, *181*, 124−128.
(29) Fukushima, D. Recent progress of soybean protein foods: chemistry, technology and nutrition. *Food Rev. Int.* **1991**, *7*, 323−351.
(30) Hong, T. L. Processing quality of soybeans for tofu-making and its assessment. Ph.D. Dissertation, National Chung-Hsing University, Taichung, Taiwan, 1994.
(31) Draper, M.; Catsimpoolas, N. Disulfide and sulfhydryl groups in glycinin. *Cereal Chem.* **1978**, *55*, 16−23.
(32) Nakamura, T.; Utsumi, S.; Kitamura, K.; Harada, K.; Mori, T. Cultivar differences in gelling characteristics of soybean glycinin. *J. Agric. Food Chem.* **1984**, *32*, 647−651.
(33) Wolf, W. J. Sulfhydryl content of glycinin: effect of reducing agents. *J. Agric. Food Chem.* **1993**, *41*, 168−176.
(34) Kella, N. K. D.; Kinsella, J. E. Anomalous effect of denaturants on sulfitolysis of protein disulfide bonds. *Int. J. Pept. Protein Res.* **1986**, *28*, 586−592.
(35) Simard, C.; Boulet, M. Distribution of sulfhydryl groups and disulfide bridges in the protein fractions of soybean, fababeans and rapeseed. *Can. Inst. Food Sci. Technol. J.* **1978**, *11*, 45−49.
(36) Fukushima, D. Internal structure of 7S and 11S globulin molecules in soybean proteins. *Cereal Chem.* **1968**, *45*, 203−224.
(37) Nakai, S.; Li-Chan, E.; Hirotsuka, M.; Vazquez, M. C.; Arteaga, G. Quantitation of hydrophobicity for elucidating the structure activity relationships of food proteins. In *Interactions of Food Proteins*; Parris, N., Bardord, R., Eds.; ACS Symposium Series 454; American Chemical Society: Washington, DC, 1991; pp 42−58.
(38) Johnson, W. C., Jr. Protein secondary structure and circular dichroism: a practical guide. *Proteins: Struct., Funct., Genet.* **1990**, *7*, 205−214.
(39) Koshiyama, I.; Hamano, M.; Fukushima, D. A heat denaturation study of the 11S globulin in soybean seeds. *Food Chem.* **1980−1981**, *6*, 309−322.
(40) Ishino, K.; Kudo, S. Conformational transition of alkali-denatured soybean 7S and 11S globulins by ethanol. *Agric. Biol. Chem.* **1980**, *44*, 537−543.
(41) Wang, C. H.; Damodaran, S. Thermal gelation of globular proteins: influence of protein conformation on gel strength. *J. Agric. Food Chem.* **1991**, *39*, 433−438.
(42) Argos, P.; Narayana, S. V. L.; Nielsen, N. C. Structural similarity between legumin and vicilin storage proteins from legumes. *EMBO J.* **1985**, *4*, 1111−1117.
(43) Adachi, M.; Kanamori, J.; Masuda, T.; Yagasaki, K.; Kitamura, K.; Mikami, B.; Utsumi, S. Crystal structure of soybean 11S globulin: Glycinin A3B4 homohexamer. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 7395−7400.
(44) Fukushima, D. Recent progress on biotechnology of soybean proteins and soybean protein food products. In *Proceedings of the Third International Soybean Processing and Utilization Conference*; Kourin Publishing: Tsukuba, Tokyo, Japan, 2000; pp 11−16.
(45) Kato, A.; Tsutsui, N.; Matsudomi, N.; Kobayashi, K.; Nakai, S. Effects of partial denaturation on surface properties of ovalbumin and lysozyme. *Agric. Biol. Chem.* **1981**, *45*, 2788−2760.

(46) Koshiyama, I.; Fukushima, D. Comparison of conformations of 7S and 11S soybean globulins by optical rotatory dispersion and circular dichroism studies. *Cereal Chem.* **1973**, *50*, 114−121.
(47) Nakamura, T.; Utsumi, S.; Mori, T. Effects of temperature on the different stages in thermal gelling of glycinin. *J. Agric. Food Chem.* **1985**, *33*, 1201−03.
(48) Utsumi, S.; Nakamura, T.; Harada, K.; Mori, T. Occurrence of dissociable and undissociable soybean glycinin. *Agric. Biol. Chem.* **1987**, *51*, 2139−2144.
(49) Liu, Z. S.; Chang, K. C. Development of a rapid titration method for predicting optimal coagulant concentration for filled tofu. *J. Agric. Food Chem.* **2003**, *51*, 5214−5221.
(50) Liu, Z. S.; Chang, K. C. Effect of soymilk characteristics and cooking conditions on coagulant requirements for making filled tofu. *J. Agric. Food Chem.*, in press.
(51) Hou, H. J.; Chang, K. C. Yield and textural properties of tofu as affected by the changes of phytate content during soybean storage. *J. Food Sci.* **2003**, *68*, 1185−1191.

Received for review September 19, 2003. Revised manuscript received March 14, 2004. Accepted April 1, 2004. Presented at the IFT Annual Meeting, Anaheim, CA, June 15−19, 2002. Published with the approval of the Director of the North Dakota Agricultural Experiment Station. An NSF-ND EPSCOR Doctoral Fellowship grant, USDA-CSREES-NRI CGP 2001-10853, North Dakota Soybean Council, and NDSU Hatch Project 1905 provided funding for this project.

JF035072Z