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Structural Characteristics of Purified β-Conglycinin from Soybeans Stored under Four Conditions

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Four storage conditions including adverse conditions [84% relative humidity (RH), 30 °C], mild conditions (57% RH, 20 °C), cold conditions (4 °C), and uncontrolled ambient conditions were used for storing soybeans. The storage time was 9 months for the adverse conditions and 18 months for the other three conditions. β-Conglycinin was purified and characterized with respect to its molecular properties. After storage under the adverse conditions, β-conglycinin showed no significant changes in total sugar content, surface hydrophobicity, free SH and SS bonds, and amino acid composition within 6 months; however, it showed a significant decrease in surface hydrophobicity and a significant increase in total free SH and total SH including SS content after 6 months. Analysis of the secondary structure showed a significant increase in α-helix content, but a significant decrease in β-sheet content after 3 months. For the other three conditions, no significant changes occurred to the structures of β-conglycinin when compared to the control. The molecular mass of β-conglycinin remained in the range of 199−212 kDa for all conditions during the entire storage periods.

KEYWORDS: Soybeans; β-conglycinin; 7S protein; storage; structure

INTRODUCTION

Soy proteins consist of discrete groups of polypeptides that have a wide range of molecular size. A typical ultracentrifuge pattern of water-extractable soy proteins has four major fractions designated 2S, 7S, 11S, and 15S on the basis of their sedimentation rates. The 7S and 11S fractions are the major proteins in soybeans, which comprise ∼70% of storage proteins. The 7S fraction is a complex mixture of proteins, including at least three major proteins: β-conglycinin, γ-conglycinin, and basic 7S globulin. β-Conglycinin is the most prevalent of these three and accounts for ∼30−35% of the total seed protein, which is used interchangeably with 7S protein because it is the major 7S protein. In addition to conglycinin, hemagglutinin, β-amylase, and lipoxygenase are minor proteins in the 7S fraction (*1*). β-Conglycinin is a trimer protein with a molecular mass of 150−200 kDa, in which four subunits are identified: three major subunits (α, α′, and β) and one minor subunit (γ) (*2*). Due to the various methods used by researchers, there are some differences regarding molecular mass of the subunits of β-conglycinin in the literature. The molecular masses for the α′, α, and β subunits have been reported in the ranges of 58−83, 58−77, and 42−53 kDa, respectively (*2*).

β-Conglycinin exhibits molecular heterogeneity, in which seven molecular species are isolated and their subunit compositions are identified as α′ββ, αββ, αα′β, ααβ, ααα′, ααα, and βββ (*3*). β-Conglycinin is a glycoprotein and undergoes a complicated association−dissociation phenomenon in response to changes in ionic strength and pH. At neutral pH and an ionic strength of >0.5, β-conglycinin is a 7S-form globulin, but it aggregates to become a 9S form at an ionic strength of <0.2 (*4*). The change of ionic strength affects β-conglycinin's thermal dissociation−association behavior. β-Conglycinin aggregates directly when heating at 0.5 ionic strength, whereas it dissociates into subunits upon heating at a near zero ionic strength (*5*).

The gel-forming ability of soy proteins is one of their most important functional properties in the food application. Glycinin and β-conglycinin show differences in gelation mechanism and in their gel properties. The gel formed by β-conglycinin is transparent in contrast to the turbid gel formed by glycinin. The hydrogen bonding and hydrophobic interactions are the major forces for forming β-conglycinin gel (*6*). In a purified protein system, β-conglycinin gel has a lower water-holding capacity, a lower tensile value, and a lower hardness and can expand less on heating than glycinin gel (*7*). In a mixed system, β-conglycinin largely contributes to the elasticity of the gel, whereas glycinin is related to hardness and unfracturability of the gel (*8*).

Hydrogen bonds, hydrophobic interactions, and disulfide bonds have been reported to participate in the polymerization of isolated 7S globulin during storage (*9*). The effects of whole soybean storage under certain conditions on protein physicochemical properties have been reported by researchers, including decreases in the nitrogen solubility index (NSI), decreases in the extractability of glycinin and β-conglycinin, and changes in the subunit composition of glycinin (*10*, *11*). We have recently reported the structural changes of purified glycinin as affected by storage (*12*). The structural changes of β-conglycinin during

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storage under various conditions have not been reported. This paper's objective is to investigate how storage of whole soybean under various conditions altered the structural characteristics of purified β-conglycinin.

MATERIALS AND METHODS

Soybeans and Chemicals. The soybean cultivar used in this study is Proto from Sinner Brothers and Bresnahan (Cassleton, ND). 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). The 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 the Sigma Chemical. The standards of amino acids were from Pierce (Rockford, IL).

Storage of Soybeans. Four storage conditions, including (a) adverse conditions, 84% relative humidity (RH), 30 °C for 9 months; (b) mild conditions, 57% RH, 20 °C for 18 months; (c) cold conditions, 4 °C in a walk-in cooler for 18 months; and (d) uncontrolled temperature conditions, in an uncontrolled-temperature garage for 18 months, were used. The details of storage methods were described in Hou and Chang (*13*). For the temperature- and humidity-controlled conditions, soybean (120 g) was sealed in a can (size 303 × 406), in which 40 mL of saturated salt solution was placed in a cup with a lid, which were punctured to make many holes, and stored in a temperature-controlled incubator. Saturated sodium bromide solution was used for 57% RH and 20 °C, and saturated potassium chloride solution was used for 84% RH and 30 °C (*14*). At the end of the storage, samples were taken from the cans. The 84% RH samples were spread in the room to air-dry further to <10% moisture content. Samples were placed into plastic bags and stored in a freezer (−20 °C) until analysis.

Isolation of β-Conglycinin (7S). Soybean flour ground by a Straub grinding mill (model 4E, Straub Co., Philadelphia, PA) and run through a 60-mesh sieve was defatted by *n*-hexane extraction. The procedures for removing lipid from flour were according to our previous paper (*12*). Isolation of β-conglycinin was conducted using the fractionation method reported by Bogracheva and others (*15*) with slight modifications. The crude β-conglycinin was washed twice with ice-cold water and then dispersed in proper volumes of 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 into solution. A trace of precipitate was removed by centrifugation. The isolated β-conglycinin was further purified through chromatography.

Purification of β-Conglycinin (7S). Crude β-conglycinin was purified further through a concanavalin A (Con A) affinity column followed by a gel filtration, according to the method of Wolf and Nelsen (*16*). The detailed running conditions of both columns were described in Hou and Chang (*12*). When the β-conglycinin went through a Con A-Sepharose 4B column (2.6 × 19 cm), which is a glycoprotein, it was retained in the stationary phase. β-Conglycinin was eluted off the affinity column with the Tris-HCl buffer containing 0.1 M α-methyl-D-mannopyranoside. The major β-conglycinin fractions were checked by SDS−gel electrophoresis and concentrated in an Amicon ultrafiltration cell (model 8400, Danvers, MA) with a Diaflo membrane of 10000 molecular mass cutoff to ∼5 mL and then applied to a gel filtration column.

A Sephacryl S-300 superfine gel filtration column (2.6 cm × 95 cm) with a molecular mass fractionation range of 10,000−1,500,000 Da was packed. The β-conglycinin was eluted at 4 °C with the standard buffer at a flow rate of 20 mL/h. Fractions of 5 mL were collected, and the absorbance at 280 nm was measured. The molecular mass of protein was calculated from a calibration curve using molecular markers; the details were described in Hou and Chang (*12*). Fractions of the major peak were pooled and analyzed by electrophoresis (SDS-PAGE) for purity and dialyzed against distilled water in the cold (4 °C) for 3 days with at least three changes and then lyophilized for further molecular characterizations.

Determination of Protein Content. The protein content of crude β-conglycinin was determined according to the biuret method (*17*). The concentration of purified β-conglycinin, which was used for characterizing amino acid composition, surface hydrophobicity, and sulfhydryl/disulfide bonds, was determined using the Lowry method (*18*) due to a low concentration. Bovine serum albumin was used for establishing standard curves in both methods, respectively.

Crude Protein Yield and Protein Extractability. Extracted or purified proteins in solutions were determined according to the biuret method. Crude protein in soybean powder was determined according to the Kjeldahl method of the AOAC (*19*). Crude β-conglycinin yield was determined by dividing the protein content of isolated β-conglycinin by total protein content of soybean dry matter and expressed in percent. Protein extractability was expressed on the basis of total protein extracted from the dried soybean.

SDS−Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE was used to examine the purity of isolated β-conglycinin and to quantify the relative amount of its subunits. The quantification of β-conglycinin 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). The detailed procedures were described in Hou and Chang (*12*).

Sugar Content. The sugar content in purified β-conglycinin was determined by using the phenol−sulfuric acid method (*20*) according to our previous paper (*12*). Glucose was used to generate a standard curve for estimating the sugar content. The sugar content in protein was expressed as grams per 100 g of protein.

Analysis of Sulfhydryl and Disulfide Contents. The free sulfhydryl group (SH) and total sulfhydryl group (SH + SS) contents of purified β-conglycinin were determined using the 5,5′dithiobis(2-nitrobenzoic acid) (DTNB) reagent. The procedures for determining the surface free SH, total free SH, and total SH (including S−S content) contents had been described in Hou and Chang (*12*). The disulfide group (SS) content was obtained by subtracting total free SH from total SH, dividing the difference by 2. All types of sulfhydryl groups and SS contents were expressed as micromoles per mole of protein, using the molecular mass of 200 kDa for β-conglycinin.

Surface Hydrophobicity (H_0). The details for measuring the surface hydrophobicity of purified β-conglycinin were determined using a fluorescence probe, 1-anilinonaphthalene-8-sulfonic acid (ANS) (*12*).

Differential Scanning Calorimetry (DSC). DSC was used to assess the degree of denaturation of isolated proteins. 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 baseline, was identified. The temperature at the peak maximun (T_p), generally used to indicate heat denaturation of protein, was recorded.

Amino Acid Analysis. The amino acid composition of purified β-conglycinin was analyzed according to the Pico-Tag method as modified by Chang and co-workers (*21*).

Protein Secondary Structure. The secondary structures of purified β-conglycinin were determined using a circular dichroism (CD) spectropolarimeter (Jasco J-710, Jasco Corp.). Freeze-dried protein was redissolved in 35 mM potassium phosphate buffer, pH 7.6, and was centrifuged at 3000*g* to remove any insoluble residue that otherwise would interfere with UV absorption during spectroscopic determinations of the secondary structures. The concentration used for CD measurements was 2−4 mg/mL, using a quartz cell with a path length of 20 mm. The detailed procedures were described in Hou and Chang (*12*). Four protein samples from 0, 3, 6, and 8 months of storage under the adverse conditions and three protein samples from the three other storage conditions for 18 months were selected for analyzing their secondary structures.

The protein concentrations were determined according to the Bradford dye-binding method (*22*). The molarity of peptide bonds of

Table 1. Isolation Yield of Crude β-Conglycinin, Sugar Content, and Surface Hydrophobicity of β-Conglycinin Purified from Soybeans under Various Conditions[a]

storage conditions	crude protein yield[b] (%)	sugar content[c] (%)	surface hydrophobicity (cps × 10^6)
control	12.59 ± 0.38a	4.27 ± 0.03c	3.41 ± 0.29bc
84% RH, 1 month	11.61 ± 0.12b	4.73 ± 0.06ab	3.14 ± 0.05cd
84% RH, 2 months	9.28 ± 0.49c	4.65 ± 0.03b	3.04 ± 0.13de
84% RH, 3 months	5.74 ± 0.11d	4.58 ± 0.09b	3.71 ± 0.09b
84% RH, 4 months	5.54 ± 0.41de	4.67 ± 0.19b	2.74 ± 0.14ef
84% RH, 5 months	4.97 ± 0.57de	4.79 ± 0.11ab	2.86 ± 0.17def
84% RH, 6 months	5.12 ± 0.38de	4.83 ± 0.12a	3.01 ± 0.07de
84% RH, 7 months	1.63 ± 0.21f	4.89 ± 0.04a	3.03 ± 0.04de
84% RH, 8 months	1.45 ± 0.32f	4.77 ± 0.12a	2.54 ± 0.05f
84% RH, 9 months	0.19 ± 0.11g	NA[d]	2.12 ± 0.26g
57% RH, 18 months	12.15 ± 0.37a	4.27 ± 0.11c	3.39 ± 0.32bc
4 °C, 18 months	12.51 ± 0.22a	4.45 ± 0.16bc	4.09 ± 0.44a
ambient, 18 months	12.23 ± 0.15a	4.30 ± 0.07c	3.40 ± 0.26bc

[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 β-conglycinin yield was calculated on the basis of the total protein of dried soybeans. [c] The sugar content was based on dried purified β-conglycinin. [d] Sample was not available for determination.

β-conglycinin was calculated by the using molecular weight of 200 kDa and mean amino acid molecular weight of 116, according to Utsumi and co-workers (*8*). The secondary structure of β-conglycinin 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 (*23*). Four secondary structures including α-helix, β-sheet, β-turns, and unordered structures were calculated.

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 (*24*). Significant differences between group means were analyzed by Duncan's multiple-range test ($p = 0.05$).

RESULTS AND DISCUSSION

Isolation and Purification of β-Conglycinin. Under the adverse conditions, the total protein extractability of soybean declined along with the storage time (*25*, *26*). The yield of crude β-conglycinin showed a decreasing trend for the soybeans that were stored in the adverse conditions with storage months (**Table 1**). The yield of crude β-conglycinin decreased from 12.6% of the total protein in soybeans at time 0 to 0.2% after 9 months. The yield of crude β-conglycinin decreased after 2 months and after 6 months of storage. The reduction of protein yield was significant with storage time. Only a small amount of crude β-conglycinin was still extractable in the 7 and 8 month samples. For the soybeans that were stored in the other selected conditions, the yield of crude β-conglycinin remained at ~12% and was not significantly different from that of control (**Table 1**). After purification through chromatographic columns, the protein content of purified β-conglycinin measured with the biuret method was ~95.2%. The reason for the <100% protein content might be due to the carbohydrate moiety in the β-conglycinin. β-Conglycinin is a glycoprotein, and its α′, α, and β subunits contain 2, 2, and 1 carbohydrate moieties, respectively (*2*).

Figure 1 shows the electrophoresis pattern of β-conglycinin purified from stored soybeans under various conditions. The subunits α′, α, and β could be identified from the gel, and their molecular masses were 78, 74, and 50 kDa, respectively, which were in good agreement with those of Riblett and co-workers

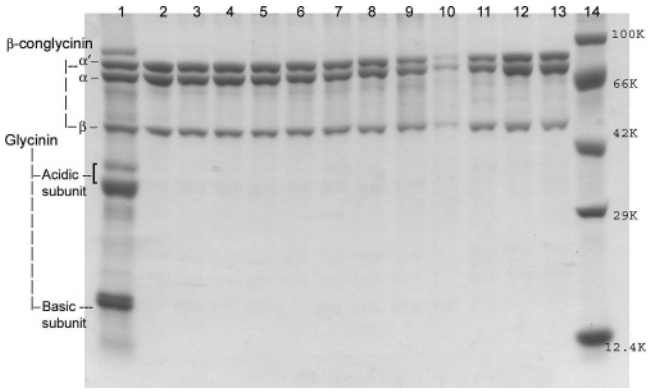

Figure 1. SDS-PAGE analysis of glycinin purified after gel filtration from the soybeans stored in various conditions: lane 1, control before purification; lane 2, control, 0 months; lane 3–10, 84% RH, 30 °C, 1–8 months; lane 11, 57% RH, 20 °C, 18 months; lane 12, cold, 4 °C, 18 months; lane 13, uncontrolled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

(*27*). Researchers have reported different molecular masses for β-conglycinin subunits. Beachy and co-workers (*28*) reported 83 and 77 kDa for the α′ and α subunits and 52−53 kDa for the β subunit. Thanh and Shibasaki (*2*) reported 59−57 kDa for the α′ and α subunits and 42−44 kDa for the β subunit. The differences might be due to various soybean genotypes and estimating methods used in different laboratories. The purities of β-conglycinins as calculated on the basis of the subunits α′, α, and β in **Figure 1** ranged from 93 to 98%. The results of SDS-PAGE indicated that the purification through affinity and gel filtration chromatography could efficiently eliminate most of the contaminants in the protein fractions.

Freeze-drying was used to preserve the purified protein before molecular characterizations. It is necessary to preserve proteins because several months were needed to purify proteins from all bean samples. Wolf and Nelsen reported that dialysis and freeze-drying promoted the dissociation of 15S glycinin to 11S proteins (*29*). However, the effect of freeze-drying on the secondary structures of the isolated β-conglycinin has not been reported in the literature. In our study, dialysis and freeze-drying of the β-conglycinin caused only a trace of precipitation during rehydration. Freeze-drying could change the protein structures of some proteins (*30*). However, the secondary structure perturbation of proteins during freeze-drying is mostly reversible after rehydration (*30*). Therefore, freeze-drying might have minimum effects on the secondary structures of β-conglycinin after rehydration before structural characterization.

Molecular Mass. Figure 2 shows the typical gel filtration elution pattern of β-conglycinins after passage through a Con A-Sepharose 4B column. The main peak is β-conglycinin with a molecular mass of 199−212 kDa, which is in agreement with that of Koshiyama (*31*), who reported molecular masses of 180−210 kDa for 7S globulins in soybeans. Thanh and Shibasaki (*32*), however, reported a smaller molecular mass of 150−175 kDa for the 7S protein. The differences might result from different estimating methods used, because the molecular mass is very sensitive to small variations in the K_{av} value. A small variation in K_{av} results in a large change in molecular mass. The molecular mass of β-conglycinin obtained from the gel filtration column agreed with that obtained from the sum of molecular mass of subunits by SDS-PAGE. Storage conditions had no effect on the molecular mass of β-conglycinin.

Sugar Content. The total sugar contents in β-conglycinin purified from the soybeans stored under the adverse conditions

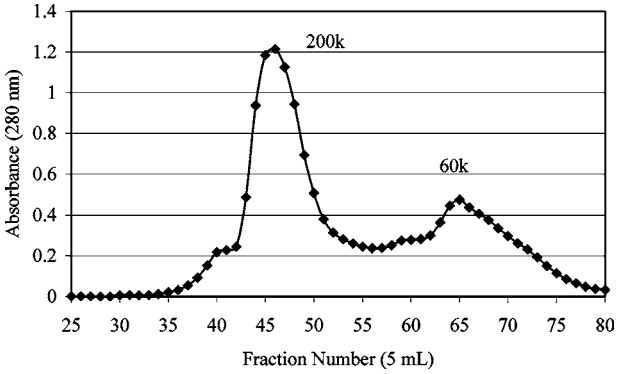

Figure 2. Gel filtration elution pattern for β-conglycinin, which was purified using a Con A-Sepharose 4B column. Fractions 43–50 were pooled, dialyzed, and used for molecular characterization.

(84% RH, 30 °C) for up to 8 months and under the other selected conditions for 18 months are also shown in **Table 1**. The total sugar content increased significantly but with small differences. The sugar content that was found in this study was consistent with previous reports by other researchers. Fukushima (*33*) reported 4.3% of carbohydrate measured as glucose in the 7S proteins. Koshiyama (*34*) reported ~4.8% of hexose in the 7S proteins. The sugar content in the β-conglycinin purified from soybeans in the other three conditions for 18 months did not show significant changes and that was close to that of the β-conglycinin isolated from the initial soybeans (**Table 1**). The main carbohydrate bound in the structure of β-conglycinin is mannose (*2*).

Sulfhydryl and Disulfide Contents. Table 2 shows that the surface free SH content in β-conglycinin was only 0.1−0.2 mol/mol for the first 6 months of storage and then increased significantly to 0.4 mol/mol after 7 months. In contrast to the changes in surface free SH, the internal free SH contents showed a significant decrease in 8 months. The results in **Table 2** indicated that most of the total free SH in β-conglycinin located in the inside region as internal free SH before storage of soybeans. After 8 months, most of the internal free SH became exposed to the surface of the protein molecule, which was reflected by the dominant surface free SH content in the eighth month. The total SH (including S−S content) in β-conglycinin increased significantly during the first month of storage and remained at the same level without significant changes until 6 months; it showed a significant increase in the seventh and eighth months. The trend of change caused by storage in the disulfide (S−S) content was similar to that in the total SH content. The S−S content increased significantly from about 1.2 to 1.7 within 1 month of storage, remained stable until 6 months, and then increased significantly to about 2 and 3 mol for the seventh and eighth months, respectively. The increases in disulfide contents indicated that the β-conglycinin molecule tended to aggregate together through intramolecular disulfide bond formation, because the molecular mass of the purified protein remained unchanged during storage. As the storage time increased, more disulfide bonds formed due to the interchanges of SH/SS groups.

Isolated 7S globulin has a very low sulfhydryl and disulfide content. Fukushima (*35*) reported that one 7S globulin molecule contains no free SH groups and two S−S bonds. Koshiyama (*34*) reported four half-cystine residues in a 7S protein molecule and suggested that all of the SH groups in the 7S protein may exist in the form of S−S bonds, because no free SH groups were found in the protein by the method used for measuring SH groups. By analyzing the amino acid composition of the subunits of β-conglycinin, Thanh and Shibasaki (*32*) found no half-cystine residues in the subunits. Coates and co-workers (*36*) reported a small amount of cysteine residues, ranging from 0.76 to 2.32 residues per mole, in each subunit of β-conglycinin. The SH and S−S groups of β-conglycinin found in the present study were in good agreement with the literatures values.

Table 2 also shows the sulfhydryl and difulfide contents of β-conglycinin purified from the soybeans stored under other selected conditions for 18 months. The surface free SH content was not significantly different among the three conditions after storage; it showed a significant increase, however, when compared with that from the control soybeans. The internal free SH content (0.12) of β-conglycinin in the mild conditions of 57% RH and 20 °C was lower than that in the cold conditions (0.20) and in the uncontrolled ambient conditions (0.19). On the contrary, the total SH and S−S contents for the mild conditions were higher than for the cold and ambient conditions. The results indicated that the β-conglycinin from the soybeans that were stored in 57% RH at 20 °C formed more disulfide bonds in the molecule than those from soybeans stored in the cold and in the ambient conditions (**Table 2**). The value of S−S content in the mild conditions for 18 months was close to that in the adverse conditions for the first 6 months. The β-conglycinin from the soybeans stored in the cold and ambient conditions for 18 months had a disulfide content similar to that from the control soybeans.

Amino Acid Composition. The amino acid composition of the β-conglycinin purified from the soybeans stored in the adverse conditions for 7 months did not have significant differences with storage time, indicating the primary structure of β-conglycinin might have remained unchanged during storage in the adverse conditions. The amino acid compositions for β-conglycinin purified from the soybeans stored under other selected conditions for 18 months also were not significantly different as compared to the control soybeans of 0 months. The results indicated that amino acid composition was not affected by storage condition or storage time.

Surface Hydrophobicity. The effect of soybean storage under the adverse conditions on the hydrophobicity of β-conglycinin is reported in **Table 1**. Except for the 3 months, the surface hydrophobicity of β-conglycinin showed a decreasing trend with storage time. The hydrophobicity significantly decreased after 9 months of storage. Why the soybeans stored for 3 months had the highest hydrophobicity in β-conglycinin was unknown.

The effect of storage of soybeans under the other three selected conditions on the hydrophobicity of β-conglycinin is also reported in **Table 1**. Except for the cold (4 °C) conditions, the β-conglycinin from the other two conditions and from the control had similar hydrophobicities. The reason for the higher surface hydrophobicity in the β-conglycinin from the cold (4 °C) conditions was not known. The results suggested that soybean storage in the conditions of the mild (57% RH, 20 °C), the cold (4 °C), and the uncontrolled ambient temperature for up to 18 months had no effect on the surface hydrophobicity of β-conglycinin molecule.

Secondary Structure. β-Conglycinin from various storage conditions and storage times had similar CD patterns from 260 to 190 nm. The typical CD spectrum for the 0 month sample showed troughs at 216 nm, but for the other three samples troughs were seen at 210 nm.

The estimated secondary structures of β-conglycinin from various storage conditions are shown in **Table 3**. For the β-conglycinin from the adverse conditions, its α-helix content

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

storage conditions	surface free SH	internal free SH	total free SH	total SH (including S–S)	S–S content (in cystine)
control	0.06 ± 0.01e	0.22 ± 0.02a	0.28 ± 0.02c	2.61 ± 0.23d	1.16 ± 0.10d
84% RH, 1 month	0.11 ± 0.01d	0.14 ± 0.02bc	0.25 ± 0.03d	3.56 ± 0.31c	1.65 ± 0.15c
84% RH, 2 months	0.09 ± 0.01de	0.12 ± 0.01c	0.21 ± 0.02e	3.72 ± 0.02c	1.76 ± 0.01c
84% RH, 3 months	0.10 ± 0.02de	0.24 ± 0.02a	0.34 ± 0.02b	3.59 ± 0.15c	1.62 ± 0.07c
84% RH, 4 months	0.12 ± 0.03d	0.18 ± 0.02b	0.30 ± 0.03c	3.64 ± 0.02c	1.67 ± 0.01c
84% RH, 5 months	0.13 ± 0.01cd	0.22 ± 0.03a	0.35 ± 0.03b	3.59 ± 0.11c	1.62 ± 0.04c
84% RH, 6 months	0.17 ± 0.01c	0.11 ± 0.01c	0.28 ± 0.02c	3.61 ± 0.11c	1.67 ± 0.05c
84% RH, 7 months	0.25 ± 0.03b	0.11 ± 0.02c	0.36 ± 0.02b	4.26 ± 0.32b	1.95 ± 0.15b
84% RH, 8 months	0.40 ± 0.02a	0.08 ± 0.01d	0.48 ± 0.01a	6.43 ± 0.45a	2.98 ± 0.22a
57% RH, 18 months	0.15 ± 0.01cd	0.12 ± 0.01c	0.27 ± 0.01c	3.82 ± 0.10c	1.77 ± 0.05c
4 °C, 18 months	0.10 ± 0.03d	0.20 ± 0.01ab	0.30 ± 0.01c	2.51 ± 0.10d	1.10 ± 0.05d
ambient, 18 months	0.13 ± 0.03cd	0.19 ± 0.02b	0.32 ± 0.02b	2.72 ± 0.16d	1.20 ± 0.07d

[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. All contents were determined as cysteine equivalent, except S–S content in cystine equivalent. Data were calculated on the basis of a β-conglycinin molecular weight of 200 kDa.

Table 3. Secondary Structure of β-Conglycinin Purified from Soybeans Stored under Various Conditions[a]

	secondary structure (%)				
storage conditions	α-helix	β-sheet	β-turn	unordered	total
control, 0 months	13.2b	37.9a	24.7	23.0	98.9
84% RH, 30 °C, 3 months	15.3a	34.5c	24.9	24.2	99.0
84% RH, 30 °C, 6 months	15.1a	35.6b	24.8	23.7	99.2
84% RH, 30 °C, 8 months	15.1a	34.4c	24.7	24.1	98.3
57% RH, 20 °C, 18 months	12.9b	35.8b	24.2	22.5	95.5
cold, 4 °C, 18 months	12.5bc	37.1a	23.8	22.1	95.6
ambient temp, 18 months	12.3c	38.4a	24.2	22.6	97.6

[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.

Table 4. Comparison of Thermal Denaturation Properties of β-Conglycinin Purified from Soybeans Stored under Various Conditions[a]

storage conditions	denaturation temp, T_d (°C)	peak temp, T_p (°C)
control, 0 months	73.8 ± 0.3	79.8 ± 0.4
84% RH, 30 °C, 3 months	72.9 ± 0.3	78.6 ± 0.5
84% RH, 30 °C, 6 months	73.6 ± 0.5	79.8 ± 0.3
84% RH, 30 °C, 8 months	73.0 ± 0.4	79.0 ± 0.5
57% RH, 20 °C, 18 months	73.5 ± 0.2	79.4 ± 0.3
cold, 4 °C, 18 months	73.0 ± 0.6	78.8 ± 0.7
ambient temp. 18 months	73.2 ± 0.5	79.2 ± 0.6

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

showed an increase from 0 to 3 months and then remained stable until 8 months; the β-sheet content, on the other hand, showed a decrease from 0 to 3 months and then also remained at the same value until 8 months. The β-turn and unordered contents were stable during the storage time. For the β-conglycinin from the other three selected conditions, its secondary structures had no significant differences, except for the β-sheet content in the mild conditions (**Table 3**). The small differences among the data might result partly from the slight differences in the total content of secondary structures. Johnson (*37*) suggested that the sum of secondary structure should be between 96 and 105%.

The secondary structures of β-conglycinin have been reported inconsistently by researchers. Koshiyama and Fukushima (*38*) reported secondary structures of 7S soybean globulins composing of 5% α-helix, 35% β-structure, and 60% random coil. Ishino and Kudo (*39*) reported that 7S globulin contains 20% α-helix, 23% β-structure, and 57% random coil. Argos and others (*40*) predicted the secondary structures of β-conglycinin using amino acid sequence data and reported that it was composed of 25% α-helix, 25% β-strand, 42% β-turn, and 8% random coils. Wang and Damodaran (*41*) found 6% helix, 62.5% β-sheet, 2% β-turn, and 29.5% random coils in native 7S globulin. Fukushima (*42*) reported that β-conglycinin contains 10% α-helix, 33% β-structure, and 57% disordered structure. These reports along with the findings in the present study suggest that α-helical structure is not the main structure in β-conglycinin. Instead, the β-structure is the main secondary structure. The structural variations reported in the literature might be partly due to the molecular heterogeneity of β-conglycinin and/or various reference spectra used by researchers in different laboratories. Furthermore, isolation techniques may contribute to differences in the conformation of proteins.

Storage of soybeans in the adverse conditions had effects on the molecular conformation of β-conglycinin, because its α-helix and β-sheet contents were altered. A negatively linear correlation between protein hydrophobicity and helical structure was measured using ellipticity $[\theta]_{220}$ of the protein, indicating a tendency of the higher helical structure, the lower protein hydrophobicity and, thus, the lower extent of exposure of hydrophobic sites of the protein molecule (*43*). Therefore, the β-conglycinin molecules from the soybeans under the adverse conditions for 8 months might have a lower extent of exposure of the interior hydrophobic regions because of a significant decrease in the surface hydrophobicity of protein (**Table 1**). This speculation was supported by the findings of Koshiyama and Fukushima (*38*), who reported an increase in α-helix contents of 7S and 11S proteins with SDS treatment that could decrease protein surface hydrophobicity.

DSC Analysis. For soy proteins, DSC is usually used as an indicator for protein denaturation that affects significantly the functionality and thus their applicability in the food systems. The thermograms of the purified β-conglycinin each showed only one sharp peak, which demonstrated the purity of proteins. **Table 4** shows the approximate T_d and T_p of β-conglycinin from soybeans stored under various conditions. For all of the conditions, the denaturation temperatures of β-conglycinin were in the range of 72.9−73.8 °C with no significant differences. The peak temperatures were also not significantly different in the range of 78.6−79.8 °C. The thermal properties of β-conglycinin found in this study were higher than that reported in

the literature. Nagano and co-workers (*44*) reported a denaturation temperature of 64 °C and a peak temperature of 70 °C for 7S globulin. Riblett and others (*27*) reported a peak temperature range from 75.8 to 77.1 °C for β-conglycinin from various genotypes of soybeans. Similarly to what occurred in glycinin, the differences might be because the β-conglycinin purified in the present study is purer and the protein concentration used in DSC is also higher.

Conclusion. The extractability of β-conglycinin from soybean stored in the adverse conditions decreased dramatically after 2 and 6 months; beyond 6 months, almost all β-conglycinin became unextractable due to structural changes. In the adverse conditions, the structural properties of purified β-conglycinin from soybean stored for 6 months had insignificant changes in total sugar content, surface hydrophobicity, free SH and SS bonds, and amino acid composition; however, β-conglycinin showed a significant decrease in surface hydrophobicity and a significant increase in total free SH and total SH including SS content after 6 months of storage. The secondary structure of β-conglycinin showed a significant increase in α-helix content, but a significant decrease in β-sheet content in the adverse conditions for 3 months. The denaturation temperature of purified β-conglycinin analyzed from DSC did not show changes during storage for all four selected conditions. The molecular mass of purified β-conglycinin maintained in the range of 199−212 kDa for all four selected conditions during the entire storage duration of 18 months. For the soybeans stored under the other three conditions for up to 18 months, the β-conglycinin showed no significant changes in all structural characteristics selected for analysis.

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