Characterization of β-Conglycinin and Glycinin Soy Protein Fractions from Four Selected Soybean Genotypes

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The β-conglycinin and glycinin fractions of soy protein were isolated from Macon, Ohio FG1, Enrei, and IL2 genotypes that were grown under the same environmental conditions. The soy protein fractions were evaluated to determine whether chemical composition and gel-forming properties were related. Amino acid analyses suggested that the hydrophobic residues may be the primary cause of differences in soy protein gel characteristics as the storage moduli increased with higher percentages of hydrophobic residues. Reversed-phase high-performance liquid chromatography profiles revealed variations in the composition of each fraction that corresponded to differences observed among the storage moduli. The gel-forming properties may be related to more than just protein content, such as the amount and type of amino acid in the fraction.

Keywords: *glycinin, β-conglycinin, soybean genotypes, protein characterization*

INTRODUCTION

Approximately 40% of the world's soybean supply is produced in the U.S. (*1*). The U.S. exports more than 33% of its soybeans, of which, about half are purchased by the Asian market (*2*). Growers in the U. S. plant two types of soybeans, oil/meal and food grade. The oil/meal beans are grown primarily for the U. S. market. The soy used as a food ingredient is typically in the form of flour, concentrate, isolate, or oil. The soy ingredients are highly sought after because of their functionality, nutritional properties, low cost, and abundance (*3*). Further growth in the soy market is anticipated because of the 1999 FDA ruling that approved the use of the label claim that soybeans can lower cholesterol.

Composition (*4*) and conformation (*5*) are responsible for a protein's functionality. Compositional differences that may alter functionality include the ratio of protein fractions (*6*), variations in subunit concentrations within fractions (*7*), or differences in amino acid profiles (*8*). Soy proteins have four major water-extractable fractions (2S, 7S, 11S, and 15S) that can be isolated on the basis of their sedimentation coefficients. The 7S (β-conglycinin) and 11S (glycinin) proteins represent the majority of the fractions within the soybean (*9*).

Differences in gel strength among glycinin fractions from various genotypes are related to variations in the makeup of the acidic subunits (*7*). Soy protein functionality is partly dependent on the β-conglycinin-to-glycinin ratio, which can vary among genotypes (*6*). Tanteeratarm et al. (*10*) determined that this ratio was dependent on soybean maturity; as soybeans mature, the concentration of glycinin increased at a greater rate than did the concentration of β-conglycinin. The differences in composition and structure between β-conglycinin and glycinin are exhibited in both nutritional and functional properties. Glycinin contains more methionine and cysteine per unit than β-conglycinin (*11*). Yamauchi et al. (*12*) reported that glycinin was a better gel former, although β-conglycinin was shown to possess greater emulsifying properties than glycinin.

Detailed information regarding specific properties of soybeans may provide growers and processors an edge that would increase the marketability of U.S. soybeans to countries where soy foods, such as tofu, are popular. The purpose of this research was to provide information about the chemical characteristics and gel-forming properties of β-conglycinin and glycinin among four soybean genotypes grown in Kansas.

MATERIALS & METHODS

Kansas State University Soybean Breeding Program supplied soybeans from four varieties (*Glycine max* vars. Macon, Ohio FG1, Enrei, and IL2) that were grown under the same controlled environmental conditions in 1997.

Isolation of β-Conglycinin and Glycinin Soy Protein Fractions. Soy protein fractions were isolated using a modified technique discussed by Kwanyuen et al. (*6*). Modifications included performing the initial extraction at pH 8.0 instead of 7.0 and adjusting the pH of the β-conglycinin and glycinin solutions to 7.0 with 2 N HCl prior to freeze-drying. Lyophilization was performed using a freeze-dryer (FTS Systems, Inc., Stone Ridge, NY) equipped with a tray dryer (Dura-Stop, model TDS-4A) and a condenser module (Dura-Dry, model FD-20−54B).

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the method of Kwanyuen et al. (*6*) using the Bio-Rad (St. Louis, MO) mini protean II apparatus. Proteins (30 μg) were loaded into a Bio-Rad linear 10−20% gradient polyacrylamide gel, and proteins were separated using 135 V of power at 4 °C. Electrophoretic patterns of β-conglycinin and glycinin were determined using Sigma gel software version 1.1 (Jandel Scientific, Chicago, IL). Molecular weights were estimated by means of a prestained Protein Ladder (Life Technologies, Rockville, MD).

Protein Analysis. Nitrogen content of β-conglycinin and glycinin fractions was determined using the LECO nitrogen

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analyzer (LECO Corporation, St. Joseph, MI) (*13*). Approximately 135 mg of freeze-dried protein was loaded into a ceramic boat sample holder. Instrument output was in % nitrogen and was converted to % protein using a conversion factor of 6.25 (AOAC, 1996). Protein content was determined on a dry weight basis. Glycine was used as the protein blank for calibration.

Amino Acid Analysis. Protein solutions were prepared using isolated β-conglycinin and glycinin fractions. Approximately 50 mg of protein was added to 10 mL of 0.01 M KH_2PO_4 buffer, pH 6.8. Protein solutions were filtered through a 0.2 μm nylon filter (Fisher Scientific, St. Louis, MO), and absorbance measurements ($\lambda = 280$ nm) were used to determine protein concentrations. Triplicate samples of β-conglycinin and glycinin from each of the four genotypes were prepared and pooled utilizing 1 mg of protein from each replicate. This procedure gave an average amino acid composition of each fraction from each genotype.

Amino acid analysis was performed on an amino acid analyzer with an Applied Biosystems model 420A derivitizer (Perkins-Elmer, Foster City, CA) and a 130A separation system (Perkins-Elmer) using PTH amino acid standards from Pierce (Rockford, IL). Prior to analysis, each sample was flushed with nitrogen and then hydrolyzed with 6 N HCl for 24 h at 100 °C. Tryptophan was not included in the analysis because the hydrolysis process used to cleave the other amino acids caused excessive degradation of tryptophan (*14*).

Differential Scanning Calorimetry (DSC). Calorimetry was conducted with a DSC-4 Robotic System (Perkins-Elmer Corp., Norwalk, CT). Onset temperature (T_o), peak temperature (T_p), and enthalpy (ΔH) were measured using the DARE DSC data collection system (version 2.04, Hardwich, England). Approximately 11 mg of 10% protein solutions was sealed hermetically into standard aluminum pans (*15*). Samples were scanned from 30 to 110 °C at 10 °C/min. A sealed, empty pan was used as a reference, and measurements were performed in a nitrogen atmosphere flushed at 50 mL/min.

Surface Hydrophobicity. Reversed-phase high-performance liquid chromatography (RP-HPLC) was used to separate β-conglycinin and glycinin on the basis of the hydrophobicity method of Woo and Ahan (*16*). A Hewlett-Packard (Pittsburgh, PA) 1100 liquid chromatograph equipped with a diode ray detector and HP ChemStations (version 6.0) data acquisition system was used. All reagents were HPLC grade (Fisher Scientific, St. Louis, MO). The flow rate was 1.0 mL/min. An injection volume of 20 μL was loaded, and separations were performed at 30 °C on a Luna column (C_{18}, 5 μm particle size, 250 × 4.6 mm I. D.). The binary solvent system consisted of solvent A, water, and solvent B, a mix of 60% water and 40% acetonitrile, (each with 0.1%, v/v, trifluoroacetic acid). The multistep linear gradient was continuous from 0% solvent B at time 0 to 100% solvent B at 30 min, held at 100% B for 15 min, and then returned to initial conditions for 0% solvent B.

Preparation of Soy Protein Gels. Soy protein gels were formed using β-conglycinin and glycinin from the four genotypes. Gel preparation was performed using a modify method from Yao et al. (*17*). Modifications included use of 0.3 M Tris-HCl, pH 8 containing 14% (w/v) protein. A volume of 5 mL of each solution was placed into a 5 cm diameter Petri dish and covered. Each sample was heated at 90 °C for 30 min and cooled to and stored at 4 °C for 24 h.

Rheology. Storage modulus (G') and loss modulus (G'') of β-conglycinin and glycinin soy protein gels were determined using a rheometer (VOR, Bohlin Rheology, Lund, Sweden). Ocillatory testing was carried out using a parallel plate geometry (*18*) with a 30 mm radius and a 4.308 g cm torsion bar. The gap between the plates was set at 3.1 mm, and the rheometer was equilibrated to 4 °C before testing. The samples were removed from the Petri dish and placed directly onto the lower plate and allowed to equilibrate for 10 min at 4 °C. A strain sweep was performed to determine the linear viscoelastic region, and the strain was set at 0.0016. The dynamic rheological parameter was measured for each sample in the frequency range from 0.1 to 10 Hz.

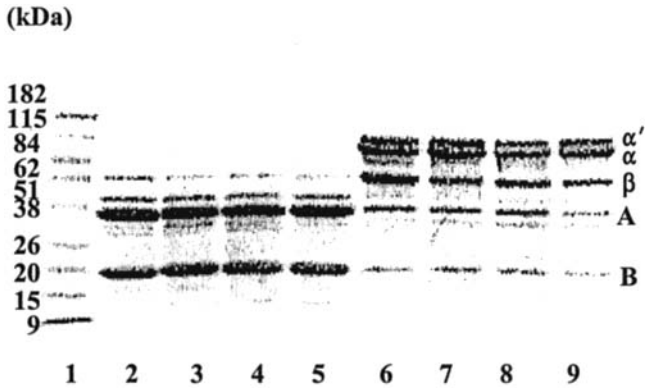

Figure 1. Electrophoretigram of glycinin (lanes 2−5) and β-conglycinin (lanes 6−9) fractions from soybean varieties Enrei, Macon, Ohio FG1, and IL2, respectively. The first lane contains the molecular weight standard with the molecular weights listed in kDa. Glycinin acidic and basic subunits are A and B, respectively. The β-conglycinin subunits are labeled α', α, and β.

Statistical Analysis. Soy protein β-conglycinin and glycinin were isolated in triplicate from each of four genotypes grown under the same environmental conditions. Three subsamples from each isolation were used for each test procedure. A randomized complete block design was used to analyze the data using the Statistical Analysis System (version 6.12, SAS7 Institute Inc., Cary, NC 1996). A two-way analysis of variance (ANOVA) was used to determine genotype effects in the General Linear Model (GLM). The least significant difference (LSD) test was applied to determine significant differences among the treatment means at $p \leq 0.05$.

RESULTS AND DISCUSSION

Electrophoresis. Figure 1 depicts β-conglycinin (lanes 6−9) and glycinin (lanes 2−5) isolated from each genotype. Enrei, Macon, FG1, and IL2 are represented in this order in lanes 2−5 and 6−9, respectively. The β-conglycinin subunits α, α', and β have molecular weights of 84 000, 72 000, and 51,000, respectively, which are in agreement with Petruccelli and Añón (*5*) and Ji et al. (*19*). The β-conglycinin bands constitute 85%, whereas the remaining 15% was glycinin contamination, as suggested by molecular weight distribution of the protein in these lanes.

The two most prominent glycinin bands are the acidic (A) and basic (B) subunits. The acidic and basic subunits have a molecular weight range of 36 000−40 000 and 18 000−20000, respectively, which are in agreement with results reported by Kella et al. (*20*). According to densitometric data, these bands constitute 92% of glycinin. The band at approximately 51 000 is the β subunit of β-conglycinin, a contaminant accounting for 8% of protein in glycinin. Wolf (*21*) also reported the presence of the β-conglycinin β subunit as a contaminant in glycinin. A low-intensity band at 15 000 was observed in glycinin, which was at levels below the detection limit of the densitometric instrument.

Protein Analyses of β-Conglycinin and Glycinin Soy Protein Fractions. Protein contents of β-conglycinin and glycinin are presented in Table 1. Among the β- conglycinin fractions, IL2 exhibited a significantly lower protein content compared to that of the other genotypes. Among the glycinin fractions, IL2 had the significant higher protein content of −96%, while Macon glycinin had significantly lower protein content of −79%. In general, glycinin was present in higher concentra-

Table 1. Comparison of Chemical, Rheological, and Thermal Properties of β-Conglycinin and Glycinin Fractions from the Soybean Genotypes Enrei, IL2, Macon, and Ohio FG1[a]

fraction	genotype	protein (%)	T_o (° C)[b]	T_p (° C)[c]	ΔH (cal/g)[d]	G' (kPa)[e]
β-conglycinin[f]	Enrei	78.61 ± 0.81[d]	68.87 ± 0.32[c]	75.85 ± 0.25[d]	2.35 ± 0.01[b]	4.44 ± 0.04[c]
	IL2	73.04 ± 1.91[e]	71.65 ± 0.41[b]	77.08 ± 0.14[b]	2.07 ± 0.03[e]	2.87 ± 0.04[e]
	Macon	88.90 ± 2.15[b]	68.80 ± 0.38[c]	75.75 ± 0.39[d]	2.16 ± 0.01[d]	4.84 ± 0.07[b]
	Ohio FG1	83.95 ± 1.25[c]	68.28 ± 0.29[d]	76.30 ± 0.23[c]	2.31 ± 0.02[c]	3.31 ± 0.19[d]
glycinin[f]	Enrei	87.11 ± 1.98[d]	82.83 ± 0.31[b]	91.32 ± 0.25[b]	4.37 ± 0.45[c]	4.31 ± 0.17[c]
	IL2	96.70 ± 0.50[b]	82.25 ± 0.43[b]	90.97 ± 0.26[bc]	5.15 ± 0.08[b]	3.26 ± 0.45[d]
	Macon	78.87 ± 0.87[e]	81.60 ± 0.25[b]	90.58 ± 0.10[c]	5.05 ± 0.01[b]	6.38 ± 0.44[b]
	Ohio FG1	93.41 ± 1.70[c]	81.43 ± 0.30[b]	89.88 ± 0.18[d]	4.40 ± 0.18[c]	3.64 ± 0.28[d]

[a] Means with the same letter in the same column for the same fraction are not significantly different ($p \leq 0.05$). [b] The onset temperature is represented by T_o. [c] The peak temperature is represented by T_p [d] The enthalpy is represented by ΔH. [e] The storage modulus is represnted by G'. A frequency of 5 Hz was used to compare the statistical significance of the storage moduli. [f] The β-conglycinin and glycinin fractions were not compared statistically to one another.

tions compared to that of β-conglycinin, which is in agreement with Tanteeratarm et al. (*10*).

Although electrophoresis is a useful tool in protein characterization, the densitometric data on SDS-PAGE did not account for impurities from nonprotein material. According to the protein data (Table 1), all fractions contained some nonprotein material. These materials were likely composed of carbohydrates, water, and lipids (*22*). Pernollet and Mossé (*23*) reported that the β-conglycinin subunits consisted of approximately 4−5% carbohydrate. Kakalis and Baianu (*24*) used ^{13}C NMR to analyze β-conglycinin soy proteins and observed an intense peak in all spectra that were believed to be from nonprotein material.

Sources in the literature indicated that variety and isolation protocol may account for the purity of the soy protein fraction recovered. Iwabuchi and Yamauchi (*25*) analyzed several soy protein fractions and reported that glycinin contained approximately 85% protein and 15% nonprotein material and β-conglycinin consisted of about 55% protein and 45% nonprotein material, although Kohyama and Nishinari (*26*) reported that β-conglycinin contained 92% protein and glycinin contained 95% protein.

Amino Acid Analysis. The Ohio FG1 β-conglycinin contained a greater percentage of alanine and proline, while being relatively high in leucine compared to IL2 and Macon β-conglycinin (Table 2). The Enrei β-conglycinin had a higher percentage of leucine, whereas Macon β-conglycinin had a low percentage. The Enrei and Ohio FG1 β-conglycinin had more valine and isoleucine compared to Macon and IL2 β-conglycinin. The IL2 β-conglycinin was low in valine, leucine, and isoleucine.

The Enrei glycinin contained the highest percentage of alanine (Table 3). The glycinin isolated from Macon and Enrei contained higher percentages of isoleucine, proline, leucine, and valine. Compared to Enrei glycinin, Macon glycinin had higher percentages of leucine and phenylalanine and contained twice as much lysine. Keshavarz and Nakai (*27*) reported that in addition to the hydrophobic amino acids, the nonpolar portion of the amino acid lysine participates in interactions that are essential in gel formation.

Variations in the composition of hydrophobic amino acids such as isoleucine, proline, phenylalanine, leucine, and valine may be the primary cause of differences in soy protein functionality and physical characteristics as exhibited in Macon. Creighton (*14*) reported that hydrophobic interactions are among the primary forces responsible for protein conformation and are highly significant in the stabilization of proteins with compact tertiary structures such as glycinin. The researcher

Table 2. Amino Acid Composition of the β-Conglycinin Fraction of Soy Protein Isolated from Four Soybean Genotypes Expressed as the Percent of Residues Per Mole of β-Conglycinin Protein

amino acid	Macon (mol %)	Enrei (mol %)	IL2 (mol %)	Ohio FG1 (mol %)
alanine	5.56	5.43	5.43	7.31
arginine[a]	8.76	5.74	9.51	6.36
asparagine + aspartic acid	9.87	6.95	7.80	2.61
cysteine	0.94	0.60	0.84	0.60
glutamine + glutamic acid	23.75	13.40	20.44	12.21
glycine	6.04	4.22	5.50	5.11
histidine	1.60	1.26	2.18	1.56
isoleucine	3.88	6.52	3.76	5.99
leucine	0.98	19.23	9.54	16.20
lysine	9.02	5.05	7.68	6.25
methionine	1.06	0.63	0.30	1.34
phenylalanine	6.68	9.86	5.75	9.22
proline	7.11	8.80	7.44	11.72
serine	7.94	4.53	7.78	4.35
threonine[a]	0.00	0.00	0.00	0.00
tyrosine	3.33	3.44	2.80	4.26
valine	3.48	4.33	3.25	4.90

[a] Note: Arginine and threonine peaks exhibited poor resolution. As a result, arginine values are artificially high, and threonine values are artificially low.

Table 3. Amino Acid Composition of the Glycinin Fraction of Soy Protein Isolated from Four Soybean Genotypes and Expressed as the Percent of Residues Per Mole of Glycinin Protein

amino acid	Macon (mol %)	Enrei (mol %)	IL2 (mol %)	Ohio FG1 (mol %)
alanine	5.40	8.25	5.92	6.01
arginine[a]	4.99	5.23	8.12	10.78
asparagine + aspartic acid	2.74	2.59	12.33	9.90
cysteine	1.30	1.83	2.46	2.71
glutamine + glutamic acid	8.95	11.96	18.67	16.98
glycine	5.32	7.67	8.11	7.76
histidine	1.34	1.37	1.95	1.86
isoleucine	6.68	7.07	2.70	3.07
leucine	18.59	13.60	7.15	8.28
lysine	10.75	5.27	6.04	6.00
methionine	1.10	2.23	1.13	0.96
phenylalanine	11.25	7.27	5.20	4.84
proline	9.05	10.19	7.18	7.38
serine	3.91	4.58	6.64	7.06
threonine[a]	0.00	0.00	0.00	0.00
tyrosine	3.47	3.97	3.01	2.70
valine	5.14	6.91	3.37	3.72

[a] Note: Arginine and threonine peaks exhibited poor resolution. As a result, arginine values are artificially high, and threonine values are artificially low.

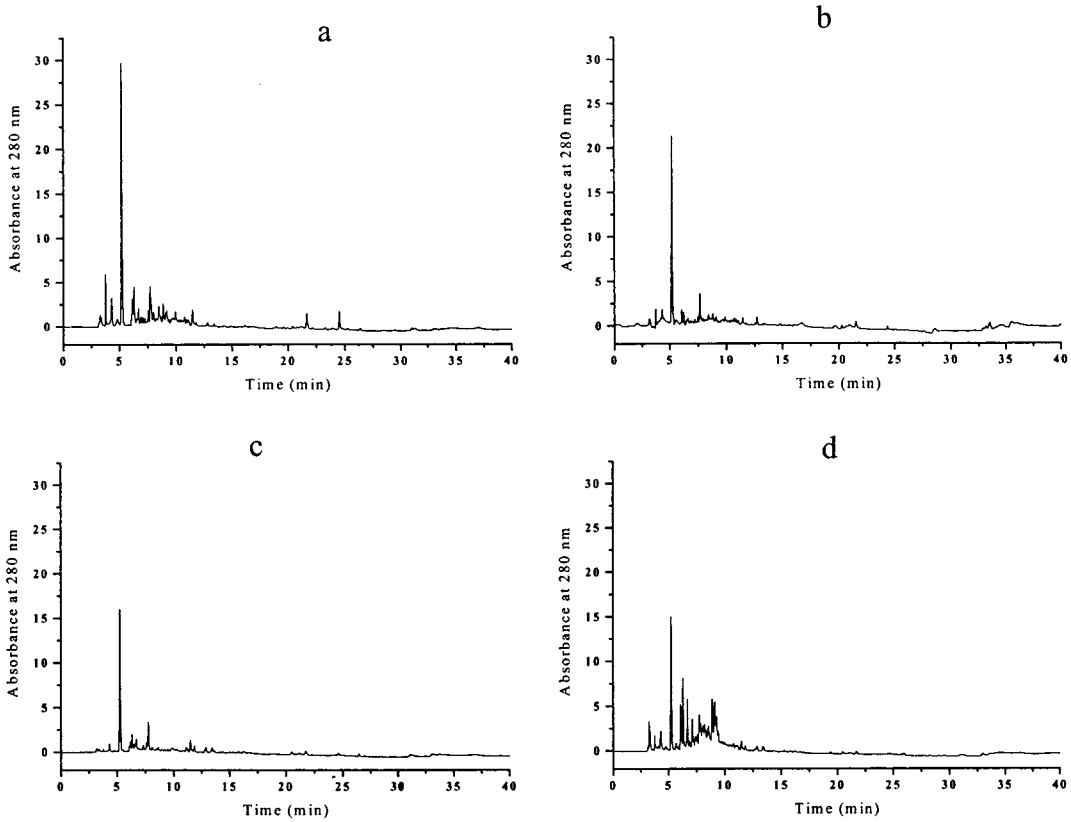

Figure 2. Reversed phase high performance liquid chromatography profiles of the β-conglycinin soyprotein fractions (a, Macon; b, Enrei; c, Ohio FG1 d. IL2).

further elucidated that valine, alanine, leucine, and isoleucine have no reactive groups and, as a result, do not interact favorably with water, although these amino acids are known to interact with each other in the core of a protein, thus stabilizing the conformation.

Differential Scanning Calorimetry. The approximate T_o of Enrei, FG1, and Macon β-conglycinin was 68 °C (Table 1), which was about 4 °C lower than that of IL2 β-conglycinin. Differences in T_o values may indicate slight conformational differences among β-conglycinin from various genotypes. This could reflect differences in amino acid composition that subsequently impacts the protein structure and inherent thermal stability. IL2 β-conglycinin had the lowest protein concentration, so the increased thermal stability may be the result of other nonprotein constituents in the sample. John and Shastri (*28*) reported that carbohydrates may increase T_o because of their ability to bind water. No significant differences in T_o (−82 °C) occurred among the glycinin fractions.

Among β-conglycinin fractions, IL2 exhibited a significantly higher shift in the T_p (−77 °C) than the other varieties, while in the glycinin fractions, Enrei and IL2, the T_p (∼91 °C) was significantly higher than that in FG1. Kinsella et al. (*29*) reported T_p values of 75 and 90 °C for β-conglycinin and glycinin, respectively. Kitabatake et al. (*30*) reported T_p values of 76.5 °C for β-conglycinin and 93.3 °C for glycinin. Byun et al. (*31*) reported T_p of soy protein fractions, isolated from the variety Hwangkeum, of 79 and 95 °C, corresponding to the β-conglycinin and glycinin, respectively. Differences in T_p may be due to different genotypes studied and isolation methods used.

The Enrei β-conglycinin exhibited significantly higher enthalpy values compared to than those of β-conglycinin from the other genotypes (Table 1), which suggested that more energy was necessary to drive the thermal denaturation reaction. The IL2 β-conglycinin was the least endothermic. Compared to Macon glycinin and IL2 glycinin, Enrei glycinin and Ohio FG1 glycinin were significantly less endothermic. All glycinin fraction enthalpies were approximately 2-fold greater than those of the β-conglycinin. This may reflect differences in conformation stability. Glycinin possess a higher percentage of sulfur-containing amino acids that potentially contribute to S−S bonds that strengthen and stabilize protein structure. The DSC data for glycinin reported by Sessa and Nelsen (*32*) were similar to the enthalpy data in the present study.

Surface Hydrophobicity. Comparison of RP-HPLC profiles for β-conglycinin (Figure 2a−d) revealed differences in hydrophobicities, which were indicated by elution times and peak intensities. All four β-conglycinin fractions exhibited the most intense peak at a retention times (T_R) of about 5 min (Figure 2a−d). The intensities of this peak were very similar for Ohio FG1 and IL2 β-conglycinin, whereas this peak had an increase for Enrei β-conglycinin and doubled in intensity for Macon β-conglycinin.

Peaks common to all glycinin fractions were at T_R = 22 and 24.5 min (Figure 3a−d). The peak at T_R = 22 min was most intense in Macon glycinin and Enrei glycinin. The peak at 24.5 min was most intense in Macon glycinin, followed by that in Enrei glycinin. Both peaks had low intensities in Ohio FG1 glycinin and IL2 glycinin.

The β-conglycinin protein components appeared to have lower surface hydrophobicity than did the glycinin protein components. The majority of β-conglycinin peaks eluted between 3 and 10 min, whereas the majority of

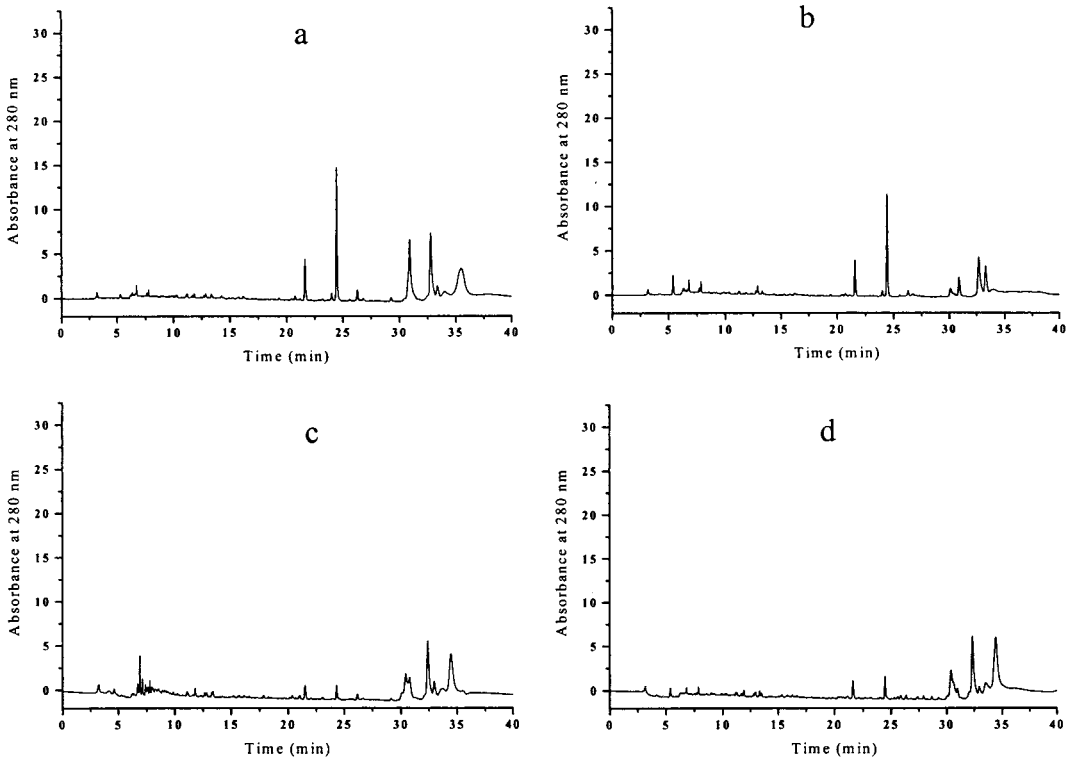

Figure 3. Reversed phase high performance liquid chromatography profiles of the glycinin soy protein fractions (a, Macon; b, Enrei; c, Ohio FG1; d, IL2).

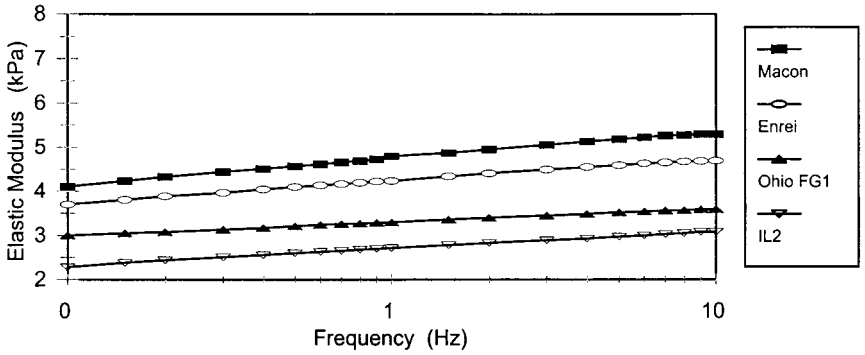

Figure 4. A representative rheogram comparing 14% soy protein gels prepared with β-conglycinin from the genotypes Macon, Enrei, Ohio FG1, and IL2.

glycinin protein peaks eluted between 22 and 35 min. Longer glycinin retention times indicated that these proteins possessed a higher affinity for the Luna column than β-conglycinin proteins; thus, they had greater surface hydrophobicity than β-conglycinin. Soy protein analysis performed by Peterson and Wolf (*33*) revealed that proteins with higher surface hydrophobicity contained acidic and basic proteins of glycinin. The differences between RP-HPLC profiles of β-conglycinin and glycinin proteins may be related to the genotype i.e., amino acid profiles.

The ratio of intensities of these peaks within β-conglycinin and glycinin may be of greater importance than a single peak. The glycinin ratios for Macon and Enrie were approximately 1:3 and 1:2, respectively, whereas the glycinin ratio was 1:1 for both Ohio FG1 and IL2.

Rheology. The rheograms of gels prepared with β-conglycinin and glycinin (not shown) showed that G' was greater than G'', there was no $G'-G''$ crossover, and G' had a slight frequency dependence from 1 to 10 Hz. Telis and Kieckbusch (*34*) classified gels of this type as "physical". With the exception of Enrei, glycinin gels were more elastic than β-conglycinin gels obtained from the same genotype.

The G' values of all β-conglycinin gels exhibited frequency dependence (Figure 4) and were significantly different from each other (Table 1). Gels prepared with Macon β-conglycinin exhibited the most elastic behavior with a G' value 40% higher than that in IL2 β-conglycinin gels. IL2 β-conglycinin formed the least elastic gels, which may have been a result of low levels of hydrophobic amino acids. The RP-HPLC data corresponded to rheological data. The differences between specific peaks and peak intensities among soybean genotypes may be associated with differences in gelation behavior of protein fractions. Oomah et al. (*35*) found that different soybean genotypes had various amounts of early- and late-eluting proteins that corresponded to the amount of low- and high-surface hydrophobic proteins, respectively. Macon β-conglycinin had the most intense peak at $T_R = 5$ min in the profile (Figure 2a). This peak may account for a protein that is critical to

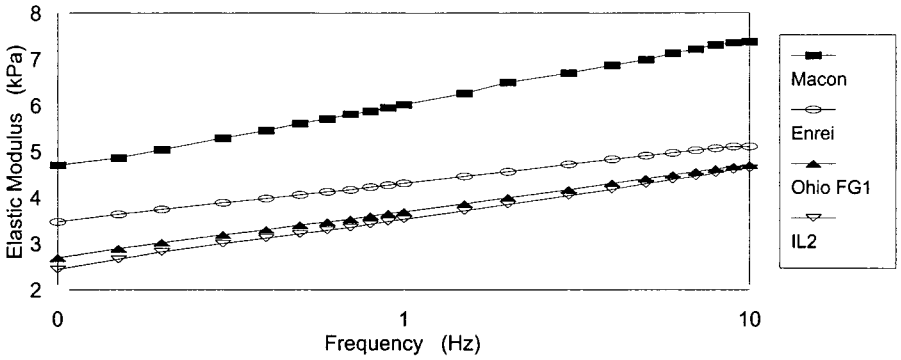

Figure 5. A representative rheogram comparing 14% soy protein gels prepared with glycinin from the genotypes Macon, Enrei, Ohio FG1, and IL2.

the production of elastic gels. Macon β-conglycinin also had small peaks at $T_R = 22$ and 24.5 min, which were associated more with glycinin. These peaks may be responsible for the higher storage modulus of gels prepared using Macon β-conglycinin. Protein analysis of the IL2 β-conglycinin revealed that this fraction had a significantly lower protein concentration. Additionally, IL2 β-conglycinin's peak at $T_R = 5$ min had the lowest intensity of all the genotypes measured (Figure 2d).

The gels prepared with glycinin were frequency-dependent (Figure 5). The Macon glycinin gels were approximately 32% more elastic than gels formulated with Enrei glycinin and approximately 47% more elastic than gels prepared with Ohio FG1 glycinin and IL2 glycinin (Table 1). Rheological data corresponded to RP-HPLC peak intensities (Figure 3) at $T_R = 22$ and 24.5 min with glycinin. These peaks were more intense in Macon glycinin. Enrei glycinin had peaks with the next highest intensity and a G' second only to that of Macon glycinin. Those two peaks were low in intensity in Ohio FG1 glycinin and IL2 glycinin, which both formed gels with a low G'. These data suggested that Macon glycinin was more hydrophobic per molecule than glycinin isolated from the other genotypes and, therefore, might form a better gel. Thus, amino acid variations may have been responsible for rheological differences among the fractions. As cited earlier, lysine does play a role in gel strength. The lysine content in Macon was higher than the other fractions which may partially account for the higher G'. Saio and Watanabe (*36*) reported that glycinin gels were firmer than β-conglycinin gels. The authors attributed this to a larger density of S−S bonds and larger aggregate formation in glycinin gels.

Chemical characteristics and gel-forming properties of β-conglycinin and glycinin among the four genotypes suggested that Macon exhibited a stronger, more elastic gel. Thus, compared to the other varieties examined, Macon possessed protein functionality attributes important in foods that require a gel structure. These important attributes may be related to more than just protein content, such as the amount and type of amino acid in the fraction. The information reported in this study may be valuable to food industries that purchase soybeans on the basis of functional criteria such as gel-forming ability.

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LITERATURE CITED

(1) Liu, K. *Soybeans in Chemistry, Technology, and Utilization*; Chapman and Hall: New York, 1997.
(2) Liu, K.; Orthoefer, F.; Thompson, K. The case for food-grade soybean varieties. *J. Food Technol.* **1995**, *6* (5), 593−599.
(3) Zayas, J. F. *Functionality of Proteins in Food*. Springer-Verlag: Berlin, Germany. **1997**.
(4) Murphy, P. A.; Chen, H.-P.; Hauck, C. C.; Wilson, L. A. Soybean protein composition and tofu quality. *Food Technol.* **1997**, *51* (3), 86−110.
(5) Petruccelli, S.; Añón, M. C. Partial reduction of soy protein isolate disulfide bonds. *J. Agric. Food Chem.* **1995**, *43*, 2001−2006.
(6) Kwanyuen, P.; Wilson, R. F.; Burton, J. W. Soybean protein quality in Emerging technologies, current practices, quality control, technology transfer, and environmental issues,. In *The Proceedings of the World Conference on Oilseed and Edible Oils Processing*; Kosceoglu, S. S., Wilson, R. F., Eds.; AOAC Press: Champaign, IL, 1998; Vol. 1.
(7) 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.
(8) Serretti, C.; Schapaugh, W. T., Jr.; Leffel, R. C. Amino acid profile of high seed protein soybean. *Crop Sci.* **1994**, *34*, 207−209.
(9) Wagner, J. R.; Sorgentini, D. A.; Añón, M. C. Thermal and electrophoretic behavior, hydrophobicity, and some functional properties of acid-treated soy isolates. *J. Agric. Food Chem.* **1996**, *44*, 1881−1889.
(10) Tanteetatarm, K.; Wei, L. S.; Steinberg, M. P. Effect of soybean maturity on storage stability and process quality. *J. Food Sci.* **1989**, *54* (3), 593−597.
(11) Kitamura, K. Genetic improvement of nutritional and food processing quality in soybean. *Jpn. Agric. Res. Quart.* **1995**, *29*, 1−8.
(12) Yamauchi, FF. Yamagishi, T.; Iwabuchi, S. Molecular understanding of soybean protein. *Food Rev. Int.* **1991**, *7*, 283−322.
(13) *Official Methods of Analyses*, 16th ed; Association of Official Analytical Chemists: Washington, DC, 1996.
(14) Creighton, T. E. *Proteins Structures and Molecular Properties*, 2nd ed; W. H. Freeman and Company, New York 1993.
(15) Petruccelli, S.; Añón, M. C. pH-induced modifications in the thermal stability of soybean protein isolates. *J. Agric. Food Chem.* **1996**, *44*, 3005−3009.
(16) Woo, K.-L.; Ahan, Y.-K. Determination of protein amino acids as benzylthiocarbamyl derivatives compared with phenylthiocarbamyl derivatives by reversed-phase high-performance liquid chromatography, ultraviolet detection and precolumn derivatization. *J. Chromatogr.* **1996**, *740*, 41−50.

(17) Yao, J. J.; Tanteeratarm, K.; Wei, L. S. Effects of maturation and storage on solubility, emulsion stability and gelation properties of isolated soy proteins. *J. Am. Oil Chem. Soc.* **1990**, *67* (12), 974−979.
(18) Puppo, M. C.; Añón, M. C. Effect of pH and protein concentration on rheological behavior of acidic soybean protein gels. *J. Agric. Food Chem.* **1998**, *46*, 3039−3046.
(19) Ji, M. P.; Cai, T. D.; Chang, K. C. Tofu yield and textural properties from three soybean cultivars as affected by ratios of β-conglycinin and glycinin proteins. *J. Food Sci.* **1999**, *64* (5), 763−767.
(20) Kella, N. K. D.; Barbeau, W. E.; Kinsella, J. E. 1986. Effect of oxidative sulfitolysis of disulfide bonds of glycinin and solubility, surface hydrophobicity, and in vitro digestibility. *J Agric. Food Chem. 34*, 251−256.
(21) Wolf, W. J. RP-HPLC for varietal identification in cereals and legumes. In *High Performance Liquid Chromatography of Cereal and Legume Proteins*; Kruger, J. E., Bietz, J. A., Eds.; American Association of Cereal Chemists, Inc.: St. Paul, MN, 1994**;** pp 190−200.
(22) Erdman, J.; Johnaon, L. A.; Liener, I. E.; Lusas, E. W.; Wolfe, W. J.; Sipos, E. F.; Smith, K. J. Soy Protein Council(http://www.spcouncil.org, accessed 1999).
(23) Pernollet, J. C.; Mossé, J. Structures and location of legume and cereal seed storage proteins. *Annu. Proc. Phytochem. Soc. Eur.* **1983**, *20*.
(24) Kakalis, L. T.; Baianu, I. C. High-resolution carbon-13 nuclear magnetic resonance study of the soybean β-conglycinin storage protein fraction in solution. *J. Agric. Food Chem.* **1990**, *38*, 2126−2132.
(25) Iwabuchi, S.; Yamauchi, F. Determination of glycinin and β-conglycinin in soybean proteins by immunological methods. *J. Agric. Food Chem.* **1987**, *35*, 200−205.
(26) Kohyama, K.; Nishinari, K. Rheological studies on the gelation process of soybean β-conglycinin and glycinin proteins in the presence of glucono-ä-lactone. *J. Agric. Food Chem.* **1993**, *41* (1), 8−14.
(27) Keshavarz, E.; Nakai, S. The relationship between hydrophobicity and interfacial tension of proteins. *Biochim. Biophys. Acta* **1979**, *576*, 269−279.
(28) John, A.; Shastri, P. N. Studies on food macromolecules by differential scanning calorimetry: A critical appraisal. *J. Food Sci. Technol.* **1998**, *35* (1), 1−14.
(29) Kinsella, J. E.; Damodaran, S.; German, B. Physiochemistry and Function of Oilseed Proteins. In *New Protein Foods*; Altschul, A. M., Wilcke, H. L., Eds.; Academic Press: Orlando, FL, 1985; Vol, 5, Chapter V, p 108−171.
(30) Kitabatake, N.; Tahara, M.; Doi, E. Thermal denaturation of soybean protein at low water contents. *Agric. Biol. Chem.* **1990**, *54* (9), 2205−2212.
(31) Byun, M.; Kang, I.; Hayahsi, Y.; Matsumura, Y.; Mori, T. Effect of γ-irradiation on soya bean proteins. *J. Sci. Food Agric.* **1994**, *66*, 55−60.
(32) Sessa, D. J.; Nelsen, T. C. Thermal stability of soy storage proteins in cracked beans hydrated with a variety of salts. **1994**, *27* (6), 556−563.
(33) Peterson, R. E, and Wolf, W. J. Scale-up from analytical to preparative reversed-phase high performance liquid chromatography of soybean glycinin. *J. Chromatogr.* **1988**, *444*, 263−268.
(34) Telis, V. R. N.; Kieckbusch, T. G. Viscoelasticity of frozen/thawed egg yolk. *J. Food Sci.* **1997**, *62* (3), 548.
(35) Oomah, B. D.; Voldemg, H.; Fregeau-Reid, J. A. Characterization of soybean proteins by HPLC. *Plant Foods Human Nutr.* **1994**, *45*, 251−263.
(36) Saio, K.; Watanabe, T. Differences in functional properties of 7S and 11S soybean proteins. *J. Texture Stud.* **1978**, *9*, 135−157.

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