JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9171–9180 9171
DOI:10.1021/jf101425j

Heat-Induced Changes Occurring in Oil/Water Emulsions Stabilized by Soy Glycinin and β-Conglycinin

Maneephan Keerati-u-rai and Milena Corredig*

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Glycinin and β-conglycinin are the two major proteins in soy protein isolate, and their emulsifying behavior was the subject of this study. These proteins form a thin layer of 30−40 nm when adsorbed at the interface. Microcalorimetric experiments showed that the thermal transitions of these proteins in the emulsion were very similar to those of the proteins in solution. The results also suggested that molecular rearrangements occurred during adsorption of β-conglycinin, as an endothermic transition peak appeared at high temperature when this protein was present at the interface. In general, β-conglycinin exhibited greater emulsifying activity than glycinin, confirming previous reports. Heating at 95 °C for 15 min caused a decrease in solubility of glycinin, and interactions between the oil droplets, with an increase in the apparent viscosity, shear thinning behavior, and droplet particle size distribution of the emulsions. While, similar behavior was noted in b-conglycinin after heating at both 75 and 95 °C. Furthermore, the order of processing affected the subunits' composition at the interface. Heating the solution before emulsification caused a higher protein load at the interface and with all of the subunits present. On the other hand, when heating was carried out after homogenization, the basic glycinin polypeptide and the β subunit of β-conglycinin were absent from the interface, suggesting that heat-induced complexes between these subunits formed and remained soluble in the unadsorbed phase.

KEYWORDS: Glycinin; β-conglycinin; soy proteins; oil in water emulsions; heat treatment; emulsifying properties

INTRODUCTION

Glycinin and β-conglycinin are the two main storage proteins found in soybeans, accounting for >70% of the total seed proteins (*1*). Glycinin, commonly referred to as 11S, because of the sedimentation behavior of this fraction during ultracentrifugation, is a hexamer with a molecular mass of 300−380 kDa, depending on its polypeptide composition. An acidic chain linked with a disulfide bond to a basic polypeptide constitutes the glycinin's monomer. Five major subunits have been identified for glycinin (*2*, *3*), and it has been suggested that the subunit composition may affect the emulsifying ability of the isolated protein (*4*). Another major protein present in a soy protein isolate is β-conglycinin. It is a trimeric glycoprotein with a molecular mass of 150−200 kDa, composed of three different subunits in various combinations: α′, α, and β (*5*).

Many studies have shown that β-conglycinin has good emulsifying properties, as it adsorbs readily at the interface because of its structure and the presence of glycosylated groups of the α and α′ subunits of β-conglycinin (*6*, *7*). In particular, when compared to other soy fractions, β-conglycinin is a better emulsifier than soy protein isolate, followed by the glycinin fraction (*8*). The smaller molecular mass and higher flexibility of β-conglycinin, as well as the high number of hydrophobic patches compared to those of glycinin, are the reasons for the greater emulsifying activity (*7*). In addition, it has been suggested that the α′ and α subunits are better emulsifiers than the β subunit because of the larger extension regions present in α′ and α compared to β, which contains only the core structure (*9*).

As heating is a widespread unit operation for processing of protein-based foods, the effect of heat treatment has also been evaluated for soy proteins in solution (*10*−*13*). It is known that glycinin is more heat stable than β-conglycinin (*6*, *13*) and that heating of soy protein isolates induces interactions between glycinin and β-conglycinin's subunits. In particular, the formation of heat-induced soluble complexes between beta subunit of β-conglycinin and the basic subunit of glycinin has been reported (*12*, *14*). Heating of glycinin in isolation causes the aggregation of the basic subunits (*12*−*15*), whereas thermal treatment induces the dissociation of β-conglycinin subunits above 70 °C into its monomers (*11*).

It has been previously reported that when treating soy protein isolates at high temperatures, proteins dissociate but the stability of the emulsions prepared with native proteins is superior to that of emulsions prepared with heated proteins (*16*). More recently, it has been demonstrated that heating increases the surface load of emulsions prepared with soy protein isolates and that the composition at the interface varied depending on the temperature of heating and if heating was carried out before or after homogenization (*17*). However, very little has been reported on the effect of heating on emulsions prepared with fractions containing isolated β-conglycinin or glycinin.

*Corresponding author [e-mail mcorredi@uoguelph.ca; phone (519) 824-4120, ext, 56101; fax (519) 824-6631].

Published on Web 07/27/2010

The purpose of this study was to determine which of the two main proteins present in soy protein isolate (i.e., glycinin or β-conglycinin) plays a major role in determining the emulsifying behavior of soy proteins when soy proteins are used to prepare oil in water emulsions. The effect of heat treatment was investigated to evaluate changes in the state of the protein and the composition at the interface and the bulk behavior of the emulsions. In addition, emulsions prepared with heated protein solutions were compared to heated emulsions prepared with unheated solutions, to determine if the order of processing may change the properties of the emulsions when the isolated fractions are used to stabilize the oil/water interface.

EXPERIMENTAL PROCEDURES

Isolation of Soy Protein Fractions. Defatted soy flakes (provided by The Solae Co., St. Louis, MO, with a dispersion index of 90) were suspended in ultrapure water (Barnstead International, E-pure D4641, Dubuque, IA) in 1:15 ratio (w/v). After adjustment of the pH to 8.0 with 2 M NaOH, the suspensions were stirred for 2 h at room temperature (*18*). The insoluble fraction was separated by centrifugation at 9000*g* for 30 min at 20 °C (Beckman Coulter model J2-21, Fullerton, ON, Canada). Sodium bisulfite (0.98 g/L) was added to the soluble fraction, and the solution pH was adjusted to 6.4 with 1 M HCl. After overnight storage at 4 °C, the suspension was centrifuged at 7000*g* for 20 min at 4 °C (Beckman Coulter). This isolation procedure does not result in pure fractions, but enriched soybean fractions with >85% purity (see below). A fraction rich in glycinin was recovered in the precipitate and was resolubilized with ultrapure water by adjusting the slurry to pH 7.5 with 2 M NaOH. The supernatant was adjusted to pH 5.0 with 1 M HCl, after the addition of 0.25 M NaCl, and stirred for 1 h in an ice bath. The insoluble residue was separated by centrifugation at 9000*g* for 30 min at 4 °C; the remaining supernatant was diluted with cold ultrapure water in a 2:1 ratio (v/v) and adjusted to pH 4.8 with 2 M HCl. A β-conglycinin-rich fraction was recovered in the precipitate after centrifugation at 7000*g* for 20 min at 4 °C. The precipitate was resolubilized to slurry with ultrapure water and adjusted to pH 7.5 with 2 M NaOH. The two β-conglycinin- and glycinin-rich fractions were dialyzed at 4 °C overnight against ultrapure water and freeze-dried. Freeze-dried protein was stored at −20 °C until needed. Protein contents of all fractions were determined using the combustion method for nitrogen (Leco FP-528, Mississauga, ON, Canada) using N × 6.25 factor for calculation (approved method 46-30 AACC, 2000). The purity of all the fractions was assessed using SDS-PAGE and scanning densitometry (see below) based on values of glycinin and β-conglycinin polypeptide bands. The glycinin fraction showed 90% purity, whereas β-conglycinin showed 85.7% purity. Both enriched fractions were also compared with native soy protein isolate prepared by isoelectric precipitation as previously described (*17*).

Emulsion Preparation. Fractions rich in glycinin and β-conglycinin were suspended in 50 mM sodium phosphate, pH 7.4, at various concentrations, ranging from 0.5 to 3.0% (w/v), and stored overnight at 4 °C to fully hydrate. The protein dispersions were mixed with 10% soybean oil (w/w) (Sigma-Aldrich, St. Louis, MO), prehomogenized using a shear dispersing unit (PowerGen 125, Fischer Scientific, Ottawa, ON, Canada) for 1 min, and immediately homogenized using a microfluidizer (110S model, Newton, MA) for five passes with overall pressure at 300 kPa. Fresh emulsions were either used for analysis or stored at 4 °C to follow storage stability. All experiments were performed in triplicate.

Thermal Treatment. Thermal treatment of protein solutions and emulsions was carried out at two different heating temperatures, 75 or 95 °C, for 15 min using a water bath (Thermo Haake, DC10 model, Sigma-Aldrich, St. Louis, MO). These temperatures were chosen on the basis of the denaturation temperature of β-conglycin and glycinin as measured by micro-DSC (*17*). Emulsions were prepared either with heated or unheated protein dispersions. Heating was conducted by placing 30 mL of protein solutions or emulsions in polypropylene tubes in a water bath at the desired heating temperature. Samples were heated for 15 min and then immediately removed from the water bath and cooled in an ice bath to room temperature. Then, heated protein solutions were immediately used to prepare the emulsions. When heating was conducted after homogenization, the emulsions were prepared with unheated protein dispersions and then heated at the desired temperature immediately after emulsification. Emulsion samples were then cool to room temperature using ice bath.

Particle Size Measurement. Static light scattering (Mastersizer 2000S, Malvern Instruments Inc., Westborough, MA) was employed to measure the droplet size distribution and the average particle size ($D_{4,3}$) as a function of protein concentration under various conditions (i.e., unheated, heated at 75 or heated 95 °C for 15 min before and after emulsification). Refractive indices of 1.47 and 1.33 were used for the dispersed phase (soybean oil) and the water phase, respectively. The analyses were performed with freshly made emulsions and after storage at 4 °C for 1 week to assess any changes that may have occurred in the particle size distribution.

Differential Scanning Calorimetry. Protein samples were diluted to 5 mg/mL with 50 mM sodium phosphate buffer, pH 7.4. The thermal transitions were measured using a microcalorimeter (VP-DSC, Microcal Inc., North Hampton, MA). Proteins solutions were held at 20 °C for 15 min prior to heating from 20 to 115 °C with a 1 °C/min scan rate and then cooled to 20 °C. The instrument contains two measuring cells, a sample and a reference cell. Sodium phosphate buffer was used as reference when protein solutions were measured, whereas for the oil-in-water emulsions, an emulsion containing the same volume fraction of oil but stabilized by a small molecular weight emulsifier (Tween 80, 2.0%) was used in the reference cell. Enthalpy (ΔH) and denaturation temperature midpoint (T_d) were calculated using Origin software version 7.0.

Emulsion Viscosity. The flow behavior of freshly made emulsions was measured using a controlled stress rheometer (AR1000, TA Instruments, New Castle, DE) with a cone (2° angle, 40 mm diameter) and plate geometry. A shear rate ramp was performed at constant temperature (25 °C). The power law ($\tau = K(\gamma)^n$) was used to analyze the data, where τ is defined as shear stress, K as the flow consistency index, γ as shear rate, and n as the flow behavior index. The values of K and n were determined for each replicate sample, and statistical analysis was performed for three independent replicate experiments.

Thickness of Interfacial Layer. To have an indication of the protein behavior once adsorbed at the oil/water interface, experiments were performed by adsorbing the protein fractions on polystyrene particles and then determining their change in diameter using dynamic light scattering. Latex microspheres (polystyrene) with a mean diameter of 0.26 μm (with ≤3% of variation in size uniformity) (Duke Scientific Corp., Palo Alto, CA) were diluted with filtered (0.22 μm filters, Fisher Scientific) 50 mM sodium phosphate buffer, pH 7.4, in a 1:64 ratio of suspension to buffer. The diluted latex suspension was then mixed with various protein concentrations ranging from 0.06 to 1.4% using a 1:1 ratio of protein suspension to diluted latex suspension. The mixture was gently inverted and left at room temperature for 30 min. The mixture (5 μL) was then diluted in 3 mL of filtered buffer, and the apparent diameter was measured. The diameter of latex particles without protein was used as reference to determine the change in size.

Protein Solubility and Protein Composition at Interface; SDS-PAGE Analysis. Protein solubility was defined as the protein remaining in solution, after centrifugation at 10000*g* for 15 min at 25 °C. The protein fractions (1% (w/v)) were suspended in 50 mM sodium phosphate, pH 7.4, stirred for 1 h at room temperature, and then stored overnight at 4 °C. After equilibrating to room temperature, the dispersions (1 mL) were then centrifuged (10000*g* for 15 min) at 25 °C using an Eppendorf centrifuge (Brinkmann Instruments, Westbury, NY) to separate any insoluble residue. After centrifugation, the soluble material was removed, and 210 μL of electrophoresis extraction buffer was added to the pellet. The total protein dispersion and the soluble fraction (i.e., the centrifugal supernatant) were analyzed using DC Protein assay (Bio-Rad) with BSA as a standard. Solubility was calculated as the amount of protein in the centrifugal supernatant/initial protein content (*21*). The solution and the soluble phases were then loaded (5 μL) to determine any differences in the composition of subunits after heating and centrifugation. The loading was not adjusted for protein, to better identify differences between the original solution and the serum phase. Traces of insoluble protein were dissolved in electrophoresis buffer to determine, at least qualitatively, if any polypeptides selectively precipitated after heating.

In addition to the proteins present in the soluble and insoluble phases in soy protein suspensions, the differences between the proteins present at the

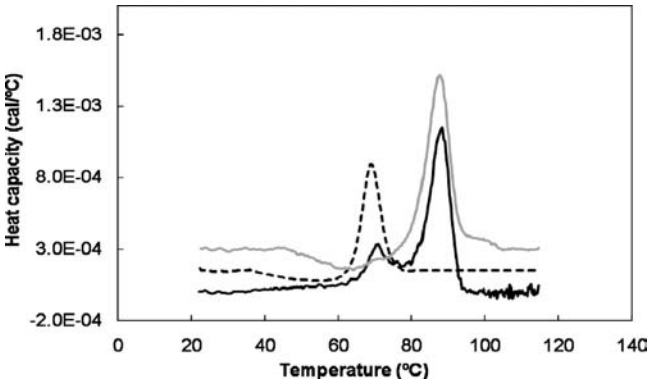

Figure 1. Thermal transitions measured by microcalorimetry for soy protein isolate (solid line), glycinin (gray line), and β-conglycinin (dashed line) suspended in 50 mM sodium phosphate, pH 7.4.

interface and in the unadsorbed phases were also analyzed by gel electrophoresis. The analyses were carried out under both reducing and nonreducing conditions to determine whether disulfide linkages were involved in the stabilization of the aggregates. To separate the protein present at the interface from the unadsorbed fraction, the emulsions were centrifuged at 10000*g* (Optima LE-80K, Beckman Coulter, Brea, CA) at 25 °C for 45 min. The cream layer was then carefully removed from the top layer and dried on filter paper (Whatman no. 1, Fisher Scientific, Ottawa, ON, Canada), and then resuspended in 50 mM sodium phosphate buffer, pH 7.4, to the initial volume fraction of oil. Meanwhile, the serum phase was withdrawn using a syringe and filtered through a 0.22 μm filter (Millipore, Billerica, MA) to remove excess oil. Aliquots (200 μL) were used for the analysis.

Samples (200 μL for dispersions, soluble fractions, centrifugal supernatants of emulsions, resuspended creams or traces of pellet in the Eppendorf tubes) were treated with 210 μL of extraction buffer, containing 50 mM Tris-HCl, 5 M urea, 1% SDS, and 4% 2-mercaptoethanol, pH 8.0. After 1 h of incubation at room temperature, the samples were diluted with 210 μL of electrophoresis buffer containing of 125 mM Tris-HCl, 5 M urea, 1% SDS, 20% glycerol, and 4% 2-mercaptoethanol, pH 6.8. In electrophoretic analysis in nonreducing condition, 2-mercaptoethanol was replaced by water in the extraction and the electrophoresis buffer. The samples for electrophoresis were then heated at 95 °C for 5 min. After cooling to room temperature, the samples containing fat were centrifuged (Eppendorf, Brinkmann Instruments, Westbury, NY) at 10000*g* for 10 min to separate the oil phase before loading of the aqueous extract.

Aliquots (5 μL) of all protein samples were loaded onto 12.5% polyacrylamide gel with 4% stacking gel in Bio-Rad mini-protein electrophoresis (Bio-Rad, Mississauga, ON, Canada) for protein separation. The volume was kept constant to better understand relative changes between emulsions and resuspended creams or between solutions and soluble fractions. The amount of protein loaded was approximately 40 μg for β-conglycinin and 55 μg for glycinin, as measured by DC protein assay. Gels were fixed and stained using a Coomassie blue R-250 stain solution (45% methanol, 10% acetic acid, and 0.10% Brilliant Blue Blue R-250, Fischer Biotech), followed by destaining using 45% methanol, 45% ultrapure water, and 10% acetic acid solution. Gels were then scanned using a SHARP JX-330 scanner (Amersham Biosciences, Baie d'Urfe, PQ, Canada). The density of each protein subunit was analyzed using ImageMaster 1D, version 2.0 (Phamacia Biotech, Amersham Biosciences) to determine any differences in the protein migration.

Statistical Analysis. All experiments were carried out in triplicate (three separate solutions or emulsions), and the data are reported as the average and standard deviation.

RESULTS AND DISCUSSION

Microcalorimetry of Glycinin- and β-Conglycinin-Enriched Fractions. Soy protein isolate contains two major protein fractions, glycinin and β-conglycinin. These two fractions have two distinct endothermal transition peaks, as shown in **Figure 1**. The thermal transitions of the isolated β-conglycinin and glycinin

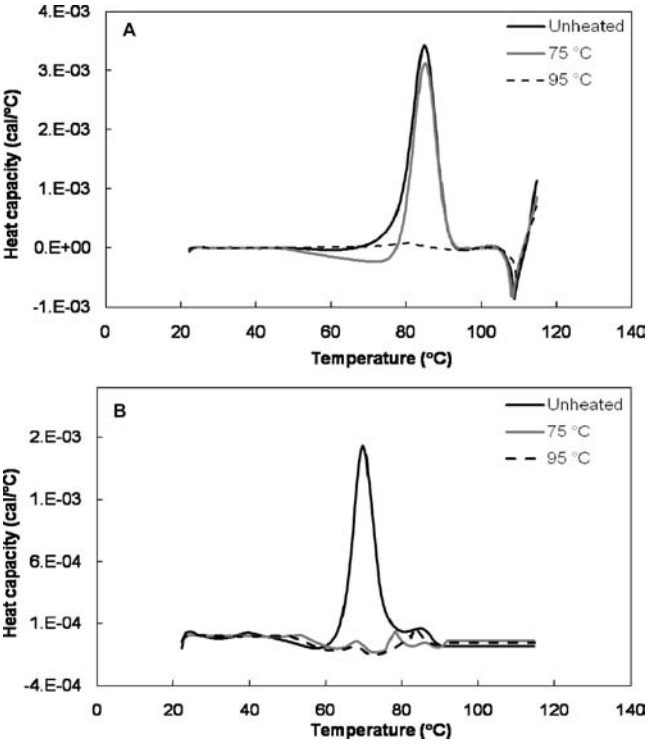

Figure 2. Thermal transitions measured by microcalorimetry for glycinin (**A**) and β-conglycinin (**B**) suspended in 50 mM sodium phosphate, pH 7.4. Samples were heated at 75 or 95 °C for 15 min.

compared to those of soy protein isolate suspensions are summarized in **Figure 1**. Whereas soy protein isolate showed two thermal transition peaks, the other two fractions exhibited only one thermal peak with maximum temperatures of denaturation of 85 and 69 °C for glycinin and β-conglycinin, respectively. These temperatures of denaturation are in agreement with those previously reported in other studies in low ionic strength buffers (*9*, *17*).

To determine the effect of the time−temperature regimens used in this study, the thermal transitions of the solutions were also assessed after the enriched protein fractions had been heated at the two different temperatures (75 or 95 °C) for 15 min (**Figure 2**). The protein solutions were therefore first heated and then subjected to microcalorimetric analysis. In glycinin solutions (**Figure 2A**) after heating at 75 °C, there was no significant change in the maximum denaturation temperature of the protein. However, there was a small but statistically significant decrease in the enthalpy after heating at this temperature. It has been previously suggested that some structural changes may occur to glycinin after heating at subdenaturation temperatures (*10*, *13*), and these results confirm these suggestions. On the other hand, when glycinin solutions were heated at 95 °C for 15 min, no residual thermal transition was measured by microcalorimetry (**Figure 2A**), confirming that changes in conformation (i.e., quaternary structure) occurred at this temperature. In β-conglycinin solutions (**Figure 2B**) the thermal transition peak was no longer observed in both heating regimens, confirming that heating at 75 °C for 15 min was sufficient to cause extensive conformational changes.

Protein Solubility. Changes in the proteins' solubility were defined as the amount of protein loss after centrifugation at 10000*g*. **Table 1** summarizes the solubility of the two protein solutions after heating at 75 or 95 °C for 15 min. Whereas, β-conglycinin solution did not show changes in the solubility with heating, in the case of glycinin, heating at 95 °C for 15 min showed a statistically significant change in the solubility of the protein.

Differences in subunit composition in the soluble and insoluble phases were also observed by analyzing the soluble and insoluble proteins using SDS electrophoresis. **Figure 3** summarizes the electrophoretic migration of the various subunits in the samples before and after heating. Both glycinin and β-conglycinin fractions showed high solubility in the buffer solution, and all of the polypeptides were recovered in the supernatant. Only traces of pellet were recovered, and there was no selective precipitation of a particular subunit in the samples, either before or after heating.

In glycinin solutions, small amounts of acidic and basic polypeptides were recovered in the insoluble fraction after heating, with darker bands after heating at 95 °C compared to samples heated at 75 °C. After heating at 95 °C, about 30% of the protein was insoluble (**Table 1**; **Figure 3A**). It is important to note that in the microcalorimetry experiments (**Figure 2A**), there was a significant decrease of the area under the thermal transition after the solution had been heated at 75 °C for 15 min. It has been previously suggested (*13*) that structural changes occur in glycinin after heating at 73 °C, leading to an increase in the number of exposed hydrophobic sites and aggregation with subsequent cooling. These structural changes, promoted by heating at 75 °C, however, did not result in a loss of solubility. Small differences were shown in the electrophoretic pattern of the soluble fraction. On the other hand, the nonreducing electrophoretic pattern of protein heated at 95 °C changed substantially, with less AB fraction (just above the 45 kDa marker) migrating in the gel (**Figure 3C**) and with much more protein in the stacking part of the gel. This difference noted in **Figure 3C** suggests that the newly formed aggregates associated via disulfide linkage (**Figure 3B**).

Table 1. Protein Solubility (Percent of the Original Protein in the Solution) for 1% Glycinin and β-Conglycinin Solution before and after Heating at 75 or 95°C for 15 min[a]

	unheated	heated at 75 °C for 15 min	heated at 95 °C for 15 min
β-conglycinin	98 ± 3.5	96 ± 1.1	100.0 ± 2
glycinin	97.7 ± 4	94 ± 1	70.7 ± 5.5

[a] Values are average ± standard deviation of three replicate experiments.

On the other hand, β-conglycinin solutions remained soluble even after heating at both 75 °C or 95 °C for 15 min (**Figure 3**). These results confirm previous reports on the solubility of β-conglycinin with heating (*4*, *9*). Previous authors have attributed the solubility of β-conglycinin to the extension regions of the α and α′ subunits (*4*, *9*). It is important to note that microcalorimetry data clearly showed that β-conglycinin molecules showed structural changes after heating at both temperatures probed (**Figure 2B**). The electrophoretic patterns in both reducing and nonreducing conditions showed that all subunits (α, α′, β) were present in serum, with no significant changes after heating (**Figure 3B**,**D**). The heat-induced aggregates of β-conglycinin remain soluble, despite the structural changes shown by other authors and attributed to the extension regions of β-conglycinin (*9*, *11*, *12*).

Thickness of Interfacial Layer. Very little detail is available on the behavior of soy proteins when they are adsorbed at the interface. Although some differences may occur in the adsorption behavior of the proteins onto solid interfaces instead of oil interfaces, the thickness of the layer formed by the proteins when adsorbed onto polymer latex particles was measured. The thickness of the adsorbed layer was determined by the changes in the diameter of the polymer latex particles after adsorption of

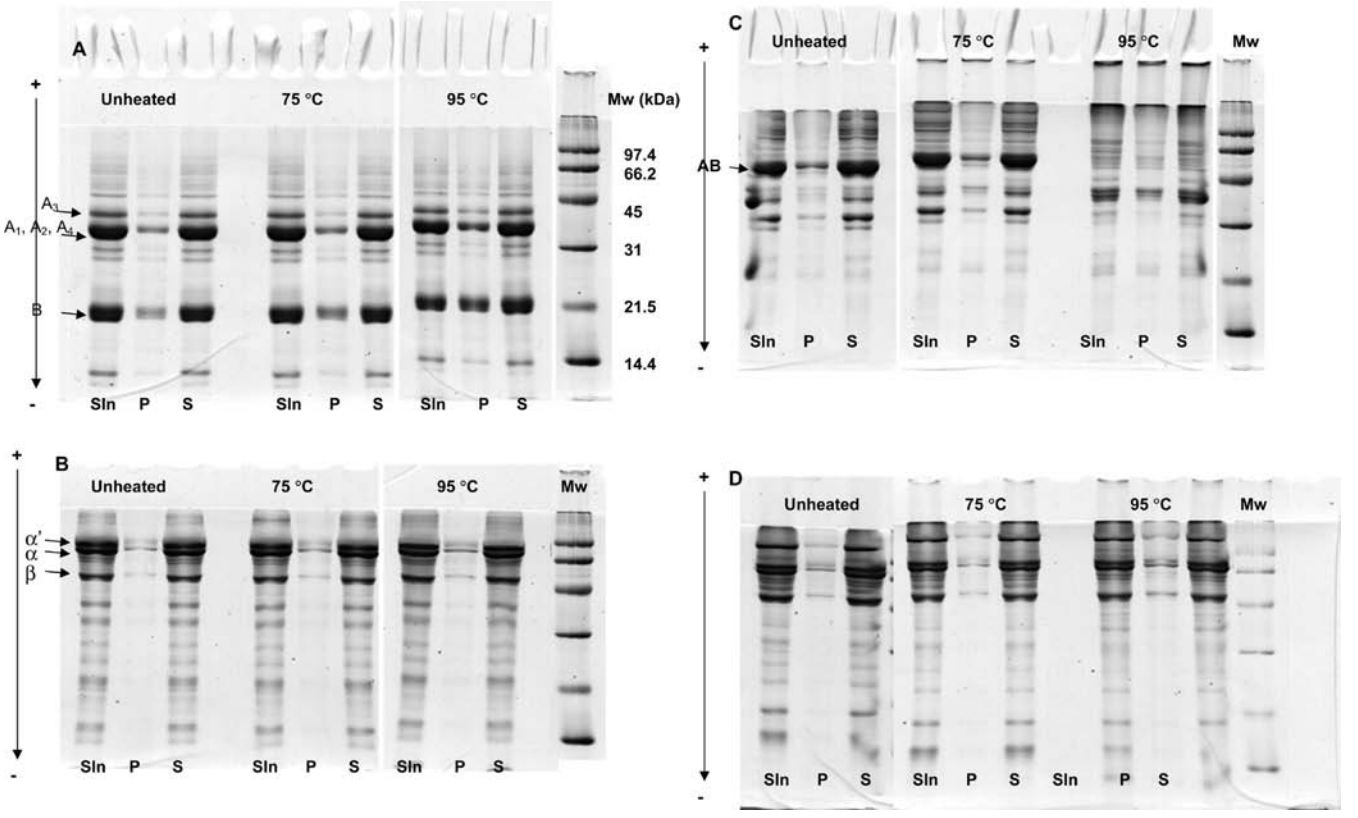

Figure 3. Electrophoretic analysis under reducing conditions (**A**, **B**) and nonreducing conditions (**C**, **D**) of 1% glycinin (**A**, **C**) and 1% β-conglycinin (**B**, **D**). Solutions (Sln), precipitate (P), or soluble phase (S) after centrifugation. Molecular weight (MW) markers: phosphorylase B, 97400 Da; BSA, 66000 Da; ovalbumin, 45000 Da; carbonic anhydrase, 31000 Da; soybean trypsin, 21500 Da; lysozyme, 14400 Da. Subunits are identified. Samples were unheated or heated at 75 or 95 °C for 15 min.

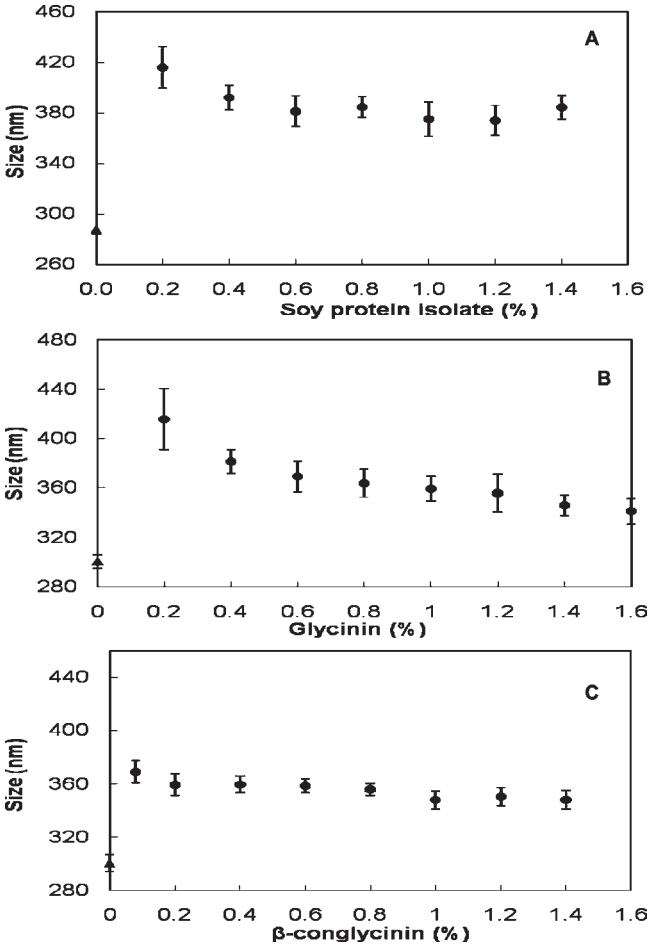

Figure 4. Apparent diameter of latex particles after addition of soy protein isolate (**A**), glycinin (**B**), or β-conglycinin (**C**) at various concentrations. Measurements were carried out using dynamic light scattering. Values are the average of triplicate experiments; bar represents standard deviation. ▲, initial size of latex particles.

β-conglycinin and glycinin fractions. Whereas in the case of emulsion droplets more than enough protein needs to be present in solution before a monomodal distribution of particle size can be obtained, and it is challenging to measure a "core" droplet size, in the case of latex particles, the core size of the particle is the initial size of the latex, and when the particles are dissolved in a protein solution, adsorption occurs. The particle diameter will increase by an amount corresponding to the protein layer thickness. **Figure 4** compares the thickness of the interface formed by β-conglycinin and glycinin isolates with soy protein isolate.

In all cases, at low protein concentration the apparent diameter was high and then the value decreased, because of bridging of the proteins between two or more particles, until reaching a plateau, when enough protein was present to fully cover the surface of the latex particles. To measure the thickness, the values at plateau were taken. The thickest layer (about 45 nm) was obtained when soy protein isolate was used, followed by glycinin (37 nm) and β-conglycinin (31 nm). These values indicate that the unheated protein fractions adsorbed at the interface formed a layer corresponding to that of the unheated oligomers. These dimensions are in agreement with the data on radius of gyration reported for soy proteins measured using multiangle laser light scattering (*22*) and with measurements of sizes carried out by scanning electron microscopy (*23*). To confirm that adsorption of the soy proteins was indeed the cause of the change in size,

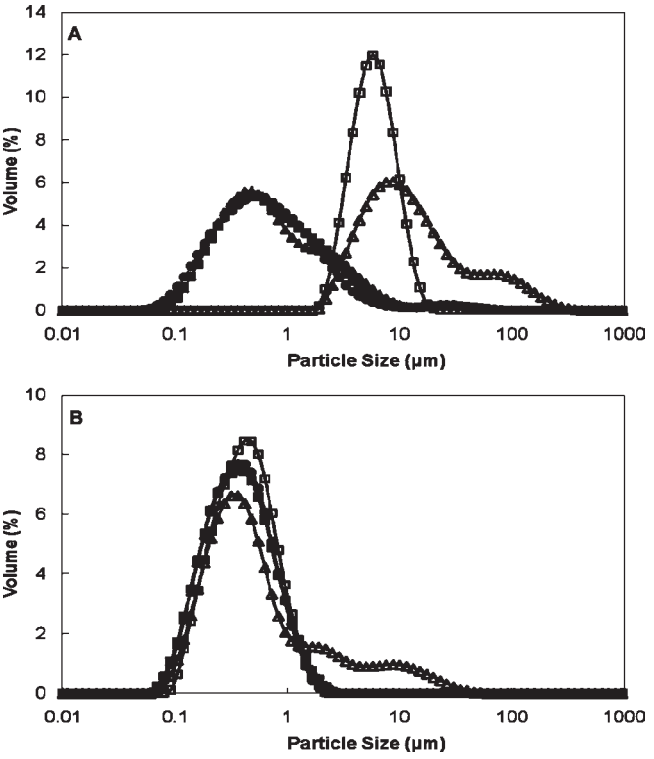

Figure 5. Particle size distribution of emulsions measured by integrated light scattering. Emulsions were prepared using 10% oil and either 2% glycinin (**A**) or 1% β-conglycinin (**B**). Emuslions were unheated (●) or and heated at 75 °C for 15 min (□, ■) or 95 °C for 15 min (△, ▲). Open symbols represent solutions heated before homogenization, and solid symbols represent emulsions prepared with unheated protein solution and heated after homogenization.

the overall charge of the particles was measured. After the addition of the soy protein fractions, indeed, the overall charge of the particles changed from −60 to −15 mV for the soy protein samples.

Heated proteins could not be accurately measured with this method, because of the instability of polymer latex to heating (when the tests were conducted on the latex particles with protein already adsorbed on) and the bridging of the protein aggregates when heated solutions were used in the adsorption studies. These results were not surprising, as it has been previously shown using transmission electron microscopy that heating significantly increases the thickness of the adsorbed soy protein layer forming large aggregates at the interface (*17*).

Emulsifying Behavior of Soy Protein Fractions. The effect of varying protein concentration and heating conditions on the average droplet size and the size distribution of the emulsions, as measured by static light scattering, is summarized in **Figure 5** and **Table 2**. By looking at the changes occurring in the emulsion droplet sizes with the amount of protein used, it is possible to determine the emulsifying properties of the protein.

In emulsions prepared with unheated solutions (unheated, **Table 2**) the changes in $D[4,3]$ clearly show that a higher amount of glycinin was needed to create emulsions with a small average diameter and with minimal changes after 1 week of storage, compared to the amounts necessary to make small droplets when β-conglycinin is used. These results are in agreement with previous literature (*8*, *20*) as it has been previously reported that β-conglycinin has better emulsifying properties than glycinin when the two protein fractions are tested under the same conditions. Emulsions prepared with unheated β-conglycinin showed a

Table 2. Average Particle Size (D[4, 3]) of Oil Droplets Stabilized by Glycinin or β-Conglycinin for Unheated Emulsions and for Emulsions Heated at 75 or 95 °C for 15 min (after Homogenization), as a Function of Protein Concentration[a]

	glycinin		β-conglycinin	
sample	freshly made	after 1 week	freshly made	after 1 week
unheated				
1.0%	17.9 ± 10.0	19.7 ± 8.7	0.5 ± 0.02	0.7 ± 0.3
1.5%	7.5 ± 5.7	10.0 ± 0.8	0.4 ± 0.02	0.4 ± 0.02
2.0%	2.3 ± 1.0	3.5 ± 2.2	0.3 ± 0.03	0.3 ± 0.01
2.5%	1.4 ± 0.5	2.3 ± 1.6	0.3 ± 0.1	0.3 ± 0.01
3.0%	1.0 ± 0.8	1.8 ± 0.9	n/a	n/a
heated at 75 °C 15 min				
1.0%	18.5 ± 10.1	27.7 ± 13.2	0.5 ± 0.03	0.5 ± 0.03
1.5%	12.8 ± 6.3	11.6 ± 5.8	0.4 ± 0.02	0.4 ± 0.02
2.0%	2.6 ± 1.9	4.7 ± 2.1	0.3 ± 0.01	0.3 ± 0.01
2.5%	2.0 ± 0.4	2.8 ± 1.3	0.3 ± 0.02	0.3 ± 0.02
3.0%	1.0 ± 0.4	1.3 ± 0.7	n/a	n/a
heated at 95 °C 15 min				
1.0%	22.0 ± 11.8	22.0 ± 11.2	0.5 ± 0.03	0.5 ± 0.1
1.5%	12.1 ± 11.0	12.0 ± 10.4	0.4 ± 0.02	0.4 ± 0.01
2.0%	3.7 ± 0.3	3.8 ± 1.0	0.3 ± 0.01	0.3 ± 0.01
2.5%	2.0 ± 0.5	2.7 ± 0.5	0.3 ± 0.02	0.3 ± 0.01
3.0%	0.8 ± 0.4	0.8 ± 0.4	n/a	n/a

[a] Measurements were carried out after homogenization and after 1 week of storage at 4 °C. Results are the average of three independent experiments (three separate emulsion experiments). Errors are also indicated. Experiments on β-conglycin were not performed (n/a) with 3% protein as 1% was already sufficient to create a stable system.

small average size (around 0.3−0.4 μm) and no changes after 1 week of storage at 4 °C; on the other hand, even with 3% of glycinin, the emulsion showed an average particle size of > 1 μm (**Table 2**, unheated emulsions).

Figure 5A illustrates the particle size distribution for emulsions prepared with 2% glycinin. Unheated emulsions showed a broad monomodal distribution, with an average droplet size (D[4,3]) of 2.32 ± 1 μm (**Figure 5A**; **Table 2**). After 1 week of storage at 4 °C, the size remained similar (with no significant differences in the average size distribution). However, the droplet diameters of glycinin emulsions were statistically significantly higher than those of emulsions containing β-conglycinin (**Table 2**). In other words, much less protein was necessary to obtain a monomodal size distribution in emulsions prepared with β-conglycinin than with glycinin. These results clearly showed that the two proteins have very different interfacial properties, with β-conglycinin being a better emulsifier than glycinin.

Despite the available studies of the emulsifying properties of soy protein fractions, which have shown that β-conglycinin provides better emulsifying properties than glycinin (*19*−*21*), the changes occurring during heating and, more importantly, which of these two proteins influences the emulsifying behavior when present together in soy protein isolates are still not fully understood. It is important to note that both proteins showed good solubility (see **Table 1**), and only glycinin solutions heated at 95 °C for 15 min substantially decreased their solubility.

Figure 5 shows the effect of the order of heating on the particle size distribution of the emulsions. The order of heating was of great importance in determining changes in the particle size distribution for emulsions containing glycinin, whereas fewer differences was noted for β-conglycinin samples (**Figure 5**). Heating of glycinin solutions before homogenization resulted in emulsions with much larger oil droplets than those of emulsions heated after homogenization (**Figure 5A**). Heating after homogenization of glycinin emulsions in fact did not show significant

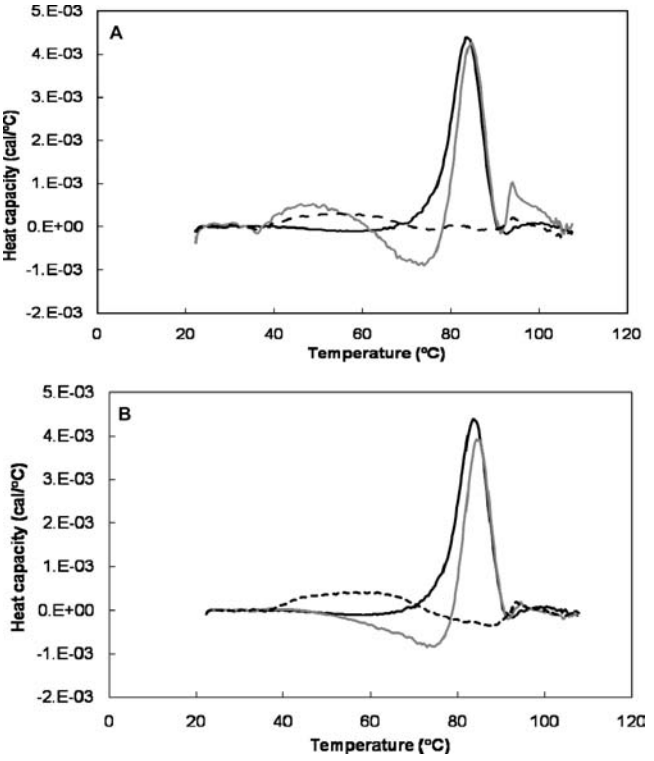

Figure 6. Thermal transition of glycinin-stabilized emulsions (2% glycinin, 10% oil) unheated (solid black line), heated at 75 °C for 15 min (solid gray line), or heated at 95 °C for 15 min (dashed line). Emulsions were prepared with heated glycinin solutions (**A**) or with unheated protein solution and heated after homogenization (**B**).

differences in the particle size distribution (**Figure 5A**; see also **Table 2**). Heating of β-conglycinin solutions before homogenization caused a significant change in the particle size distribution only after heating at 95 °C for 15 min (**Figure 5B**). No changes were shown in emulsions heated after homogenization when prepared with β-conglycinin (**Figure 5B**; **Table 1**).

When these findings were compared with those reported previously for emulsions stabilized with soy protein isolates (*17*), where heating of the protein solution prior to emulsification induced an increase in the average emulsion droplet size, it was possible to conclude that glycinin plays a major role in the heat-induced destabilization of soy protein emulsions.

Microcalorimetry of Proteins Adsorbed at the Interface. Microdifferential calorimetry was used to observe if any structural changes occurred when the proteins were adsorbed at the interface. **Figure 6** illustrates the thermal transitions of glycinin-stabilized emulsions before or after heating, with heating applied before or after homogenization. A standard emulsion stabilized with Tween 80 was used as reference, and the thermograms of the reference were subtracted from the sample simultaneously. Unheated emulsions showed one thermal transition peak with a maximum at about 90 °C, as already shown for the solutions. These results confirmed what had already been reported for soy protein isolate stabilized emulsions, that the thermal transitions did not change upon protein adsorption at the interface. When glycinin was heated in solution at 75 °C and the emulsion was prepared with the heated protein, the thermal transition peak of glycinin was still conserved (**Figure 6A**). However, an endothermic transition also started to occur at about 40 °C, followed by an exothermic transition at about 70 °C (most likely because of some aggregation and precipitation), and the main transition peak was observed at a temperature of > 90 °C. The thermal behavior of

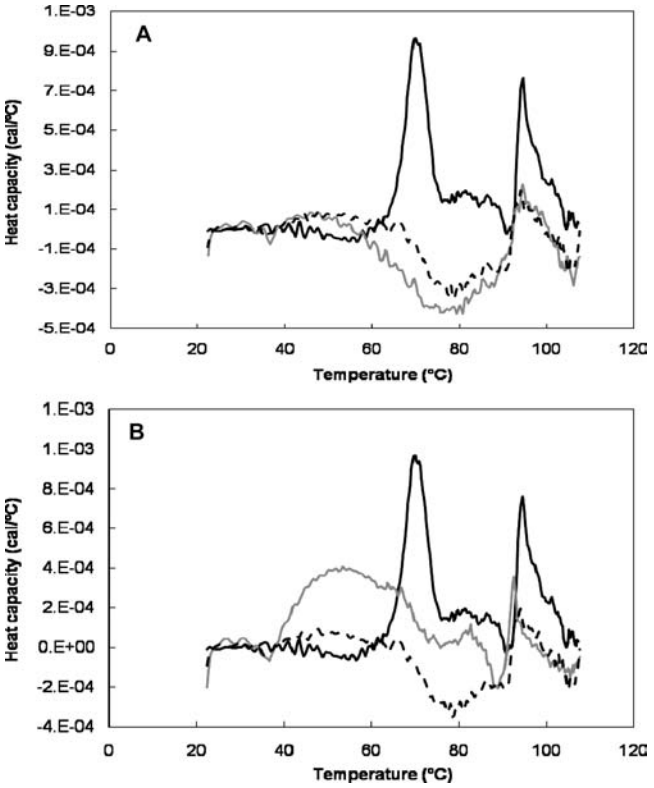

Figure 7. Thermal transition of β-conglycinin-stabilized emulsions (1% protein, 10% oil) unheated (solid black line), heated at 75 °C for 15 min (solid gray line), or heated at 95 °C for 15 min (dashed line). Emulsions were prepared with heated glycinin solutions (**A**) or with unheated protein solution and heated after homogenization (**B**).

the glycinin emulsion was different when the emulsion was prepared with the unheated protein solution, and then the emulsion was heated at 75 °C for 15 min. In this case, there was no early endothermic transition at 40 °C (**Figure 6B**), suggesting that different heat-induced rearrangements may occur when the protein is heated once already adsorbed at the interface, compared to when glycinin is heated in solution and then adsorbed at the interface. It is unlikely that the early thermal transitions may be caused by the presence of traces of β-conglycinin in the glycinin fraction, as they were not present in **Figure 6B**. It is certainly possible that heat-induced structural changes occurring in solution may cause different types of aggregates when adsorbed at the interface. Once adsorbed at the interface, the proteins will orient themselves to minimize the free energy, but this will depend on their original conformation. After heating at 95 °C for 15 min, both heated emulsions and emulsions prepared with heated solutions showed no transition peaks during microcalorimetry, but a very broad endothermic transition starting at 40 °C in both systems was observed (**Figure 6**).

The thermal transitions measured for β-conglycinin emulsions (before or after heating) are shown in **Figure 7**. Emulsions prepared with unheated β-conglycinin showed a thermal transition at 69 °C. This temperature corresponds to the denaturation peak for β-conglycinin in solution (**Figure 7**). A peak at temperatures of >95 °C was also noted in all samples, unheated and heated emulsions. A similar transition profile was also observed in a previous study with emulsions with soy protein isolate (*17*). This peak occurred only when β-conglycinin was present and was therefore attributed to a structural change occurring to the β-conglycinin structure at the interface. Despite no differences noted in the particle size distribution of emulsions prepared with heated β-conglycinin, either in solution or after homogenization, the thermal transition behavior of the emulsions was different for samples heated at 75 °C: When heating was applied to the solution (**Figure 7A**), an exothermic transition was shown at temperatures >60 °C, because of aggregate formation. An exothermic transition was also noted after heating at 95 °C for 15 min. On the other hand, when heating was carried out when β-conglycinin was adsorbed at the interface (**Figure 7B**), after heating at 75 °C, a small endothermic transition at 50 °C was noted, suggesting partial denaturation of the protein at the interface.

Table 3. Effect of Protein Concentration on the Flow Behavior of Unheated Emulsions Prepared with Different Concentrations of Glycinin and β-Conglycinin[a]

sample	K (Pa·s)	n
2.0% glycinin	$(3.8 \pm 0.05) \times 10^{-2}$	0.75 ± 0.14
2.5% glycinin	$(2.6 \pm 0.03) \times 10^{-2}$	0.77 ± 0.10
3.0% glycinin	$(1.5 \pm 0.01) \times 10^{-2}$	0.82 ± 0.06
1.0% β-conglycinin	$(3.6 \pm 0.00) \times 10^{-3}$	0.93 ± 0.04
1.5% β-conglycinin	$(3.5 \pm 0.00) \times 10^{-3}$	0.93 ± 0.03
2.0% β-conglycinin	$(5.2 \pm 0.00) \times 10^{-3}$	0.92 ± 0.05

[a] Values of consistency index (K) and flow behavior index (n) are the average of three independent experiments.

Flow Behavior of Emulsions. The flow behavior of the emulsions is influenced by the droplets' size and the interactions between the proteins (either adsorbed or unadsorbed). The flow behavior of unheated emulsions prepared with enough protein to obtain a monomodal distribution of droplet sizes was tested, and results are summarized in **Table 3**. It is possible to hypothesize that in the case of glycininin emulsions, the shear thinning behavior observed with the increase in protein concentration is due mostly to interactions occurring between droplets (most likely, bridging flocculation). A similar shear thinning behavior was shown for soy protein isolate stabilized emulsions (*17*). In this study, a non-Newtonian shear thinning behavior was shown for the emulsions prepared with 2−3% glycinin, most likely because of the strong interactions and bridging occurring between the emulsion droplets, even at these protein concentrations (**Table 3**). In fact, even at these concentrations, a larger particle size of oil droplet was noted than in the β-conglycinin emulsion (**Table 2**).

In the case of β-conglycinin-stabilized emulsions, significantly higher values for the flow index (n) were measured compared to glycinin emulsions, indicating that these emulsions showed a behavior close to Newtonian. The consistency index of the emulsions prepared with β-conglycinin was also much lower than that of emulsions prepared with glycinin. The larger oil droplet size, a higher extent of flocculation, and a higher amount of protein are all contributing to the higher consistency index of glycinin emulsions compared to those prepared with β-conglycinin (**Table 3**). In this case also, as for the particle size distribution data, it was possible to conclude that the glycinin fraction in soy protein isolate played a major role in imparting the shear thinning behavior previously described for soy protein stabilized emulsions (*17*).

Tables 4 and **5** summarize the flow behavior of the emulsions stabilized by the two protein isolates. Only heating after homogenization is shown as these emulsions showed a monomodal distribution of particle sizes. When heating was carried out before homogenization (i.e., emulsions were prepared with heated solutions), there was a high extent of aggregation of the oil droplets (see **Figure 5**), and this would account for a non-Newtonian behavior of the emulsions and high viscosity. An increase in the flow consistency index and a decrease of the n value

Table 4. Effect of Heat Treatment (Applied after Homogenization) on the Flow Behavior of 10% Oil Emulsions Prepared with Different Concentrations of Glycinin[a]

sample	K (Pa·s)	n
2.0% glycinin		
unheated	$(3.8 \pm 0.05) \times 10^{-2}$	0.75 ± 0.14
heated at 75 °C for 15 min	$(9.7 \pm 0.1) \times 10^{-2}$	0.51 ± 0.1
heated at 95 °C for 15 min	$(1.8 \pm 0.1) \times 10^{-1}$	0.40 ± 0.02
2.5% glycinin		
unheated	$(3.2 \pm 0.03) \times 10^{-2}$	0.73 ± 0.10
heated at 75 °C for 15 min	$(1.1 \pm 0.06) \times 10^{-1}$	0.46 ± 0.14
heated at 95 °C for 15 min	$(1.7 \pm 0.04) \times 10^{-1}$	0.39 ± 0.01
3.0% glycinin		
unheated	$(1.5 \pm 0.01) \times 10^{-2}$	0.82 ± 0.06
heated at 75 °C for 15 min	$(8.5 \pm 0.02) \times 10^{-2}$	0.47 ± 0.03
heated at 95 °C for 15 min	$(1.5 \pm 0.010) \times 10^{-1}$	0.41 ± 0.01

[a] Values of consistency index (K) and flow behavior index (n) are the average of three independent experiments.

Table 5. Effect of Heat Treatment (Applied after Homogenization) on the Flow Behavior of Emulsions (10% Oil) Prepared with Different Concentrations of β-Conglycinin[a]

sample	K (Pa·s)	n
1.0% β-conglycinin		
unheated	$(3.8 \pm 0.001) \times 10^{-3}$	0.93 ± 0.04
heated at 75 °C for 15 min	$(2.1 \pm 0.03) \times 10^{-1}$	0.34 ± 0.04
heated at 95 °C for 15 min	$(2.1 \pm 0.06) \times 10^{-1}$	0.30 ± 0.04
1.5% β-conglycinin		
unheated	$(3.5 \pm 0.001) \times 10^{-3}$	0.93 ± 0.03
heated at 75 °C for 15 min	$(1.9 \pm 0.06) \times 10^{-1}$	0.36 ± 0.07
heated at 95 °C for 15 min	$(1.8 \pm 0.02) \times 10^{-1}$	0.35 ± 0.03
2.0% β-conglycinin		
unheated	$(5.2 \pm 0.002) \times 10^{-3}$	0.95 ± 0.05
heated at 75 °C for 15 min	$(1.6 \pm 0.02) \times 10^{-1}$	0.39 ± 0.04
heated at 95 °C for 15 min	$(1.5 \pm 0.02) \times 10^{-1}$	0.38 ± 0.04

[a] Values of consistency index (K) and flow behavior index (n) are the average of three independent experiments.

(flow behavior index) with heating temperature were shown in all emulsions. At all protein concentrations, heating temperature increased the consistency index and dramatically shifted the samples to a non-Newtonian behavior. This effect with increasing temperature occurred for both glycinin and β-conglycinin fractions, indicating that heat-induced flocculation occurred, although this flocculation was not noted when particle size was measured by light scattering under diluted conditions (no significant changes in the average size of the particles). It is possible to hypothesize that with heating, protein−protein interactions increase and aggregates form in solution. These protein aggregates then caused depletion flocculation and an increase in the interactions between the droplets when measured undiluted. In emulsions containing β-conglycinin, there was no difference between heating at 75 and 95 °C, as most heat-induced changes already occurred at 75 °C. On the other hand, there was a significant difference in the consistency index and flow behavior index depending on heating temperature in glycinin-stabilized emulsions as most of the denaturation occurred during the heating at 95 °C for 15 min.

Protein Composition at Interface. Electrophoretic analysis was carried out to determine if specific subunits were adsorbed at the interface and if the distribution changed depending on the heating temperature or the order of the process. **Figures 8** and **9** show the polypeptide patterns of emulsions, cream, and serum phases for unheated and heated emulsions prepared with glycinin and β-conglycinin, respectively. For unheated emulsion (**Figures 8** and **9**, first three lanes), approximately 50% of glycinin or

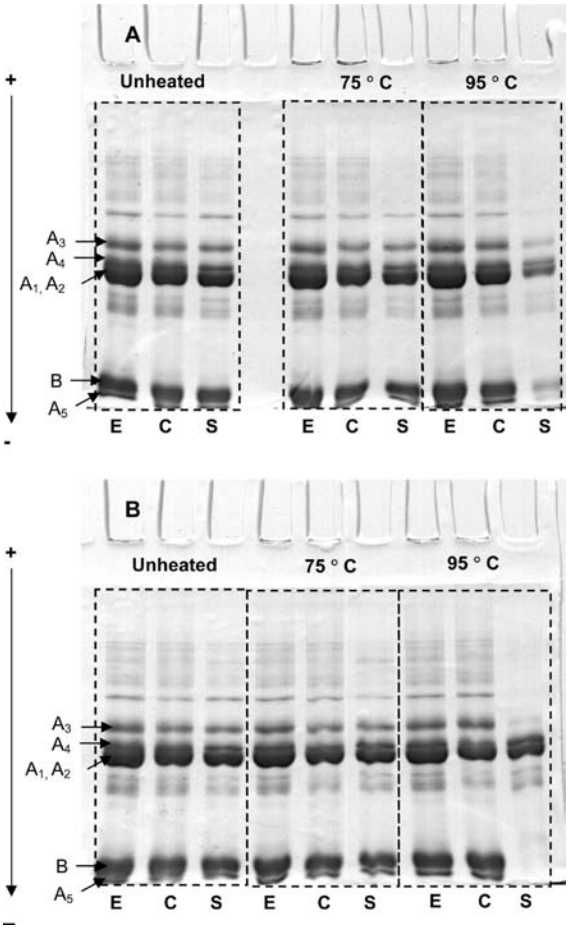

Figure 8. Electrophoretic migration of the protein present in the emulsion (E), cream (C), and serum (S) phases. Emulsions were prepared with 2% glycinin and analyzed either unheated or heated at 75 or 95 °C for 15 min. Emulsions were prepared with heated protein solutions (**A**), or emulsions were heated after homogeneization (**B**).

β-conglycinin was present in the cream phase, as determined by scanning densitometry of the cream and the emulsion lanes. In the unheated emulsions, all polypeptide chains were recovered both in the cream and in the serum phases, indicating that there was no selective adsorption of protein subunits.

When the effect of heating temperature on the adsorption of glycinin at the interface is examined (**Figure 8**), it is clear that heating at 75 °C, either before (**Figure 8A**) or after homogenization (**Figure 8B**), did not show significant changes in the distribution of the subunits in the cream or serum phase. Therefore, despite the changes observed earlier in glycinin solutions or emulsions at this subdenaturing temperature, no differences were shown in the composition of the protein in the serum or adsorbed at the interface. On the other hand, when emulsions were heated at 95 °C, whereas all of the glycinin subunits were present at the oil interface, the basic glycinin chain was not recovered in the serum phase, indicating preferential adsorption of the interface for this subunit once heated at 95 °C. It is important to note that approximately 30% of the protein was insoluble when solutions were heated at this temperature (**Table 1**), and the pellet was not analyzed in this case. The loss of the basic polypeptide in the serum phase was noted in both systems, emulsions prepared with heated solutions (**Figure 8A**) or emulsions heated after homogenization (**Figure 8B**).

The effect of thermal treatment on the distribution of polypeptides between the adsorbed and the unadsorbed phases was

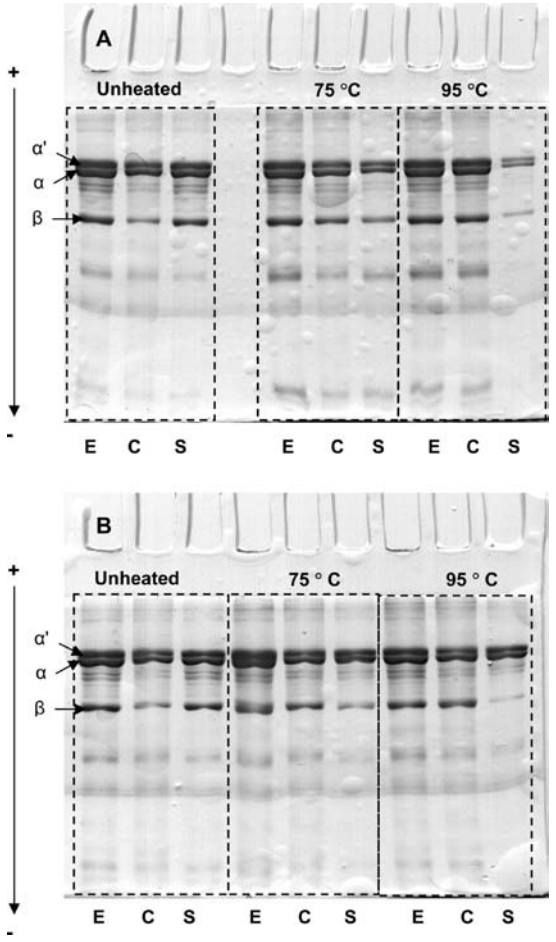

Figure 9. Electrophoretic migration of the protein present in the emulsion (E), cream (C), and serum (S) phases. Emulsions were prepared with 1% β-conglycinin and analyzed either unheated or heated at 75 or 95 °C for 15 min. The subunits are identified. Emulsions were prepared with heated protein solutions (**A**), or emulsions were heated after homogeneization (**B**).

also determined for β-conglycinin emulsions (**Figure 9**). A smaller amount of β subunits of β-conglycinin was found in the cream phase compared to the serum phase in unheated β-conglycinin emulsions. It has been previously shown that the α and α′ subunits of β-conglycinin play an important role in the emulsifying ability of this protein, because of their difference in the extension region compared to the β subunit (*9*). The results shown in **Figure 9** for unheated emulsions seem to confirm this difference in the functionality of the subunits. After heating at 75 °C, fewer β subunits were recovered in the serum phase, for both types of emulsions, made with heated solutions (**Figure 9A**) or with heated emulsion (**Figure 9B**). When the solution was heated at 95 °C before homogenization (**Figure 9A**), very little β-conglycinin was left unadsorbed after homogenization, suggesting that the heat-induced aggregates formed in solution, adsorbed at the interface. On the other hand, when the emulsion was heated at 95 °C after homogenization (**Figure 9B**), there was a selective adsorption of the β subunit of β-conglycinin compared to the α and α′ subunits, still present in the unadsorbed phase.

Conclusions. This study clearly confirmed previous reports that β-conglycinin has better emulsifying properties than glycinin. The emulsions prepared with glycinin showed larger particle size distribution and higher susceptibility to aggregation with heating. However, β-conglycinin emulsions also showed an increase in shear thinning behavior after heating, with a destabilization that seemed to be induced by a depletion flocculation mechanism. Experimental evidence on latex particles suggested that when adsorbed at the interface, both soy protein fractions formed a layer at the interface of about 30−40 nm depending on the protein. Heating caused changes in the supermolecular structure of soy proteins, and some changes in functionality were noted for glycinin at subdenaturing temperature. Whereas the order of heating strongly affected the particle size and the stability of glycinin emulsions, it showed less effect for β-conglycinin emulsions. When the present results on purified protein fractions are compared with those previously published on emulsions prepared with soy protein isolate (*17*), it is also possible to conclude that glycinin plays a major role in determining the behavior of soy protein isolate-stabilized emulsions.

LITERATURE CITED

(1) Wolf, W. J. Soybean proteins: their functional, chemical and physical properties. *J. Agric. Food Chem.* **1970**, *18*, 969–976.
(2) Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the acidic and basic subunit complexes of glycinin. *J. Biol. Chem.* **1981**, *256*, 8752–8755.
(3) Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the cysteines which link the acidic and basic components of the glycinin subunits. *J. Biol. Chem.* **1984**, *259*, 13431–13435.
(4) Maruyama, M.; Prak, K.; Kotoyama, S.; Choi, S.; Yagasagi, K.; Ishomoto, M.; Utsumi, S. Structure−physicochemical functioan relationships of soybean glycinin at subunit levels assessed by using mutant lines. *J. Agric. Food Chem.* **2004**, *52*, 8197–8201.
(5) Thanh, V. H.; Shibasaki, K. Major proteins of soybean seeds. Reversible and irreversible dissociation of β-conglycinin. *J. Agric. Food Chem.* **1979**, *27*, 805–809.
(6) Utsumi, S.; Matsumura, Y.; Mori, T. Structure−function relationships of soy proteins. In *Food Proteins and Their Applications*; Damodaran, S., Park, A., Eds.; Dekker, New York, 1997; pp 257−288.
(7) Bernard, E. C.; Grandison, A. S.; Lewis, M. J. Emulsifying properties of soy protein isolate fractions obtained by isoelectric precipitation. *J. Sci. Food Agric.* **2001**, *81*, 759–763.
(8) Molina, E.; Papadapoulou, A.; Ledward, D. A. Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. *Food Hydrocolloids* **2001**, *15*, 263–269.
(9) Maruyama, N.; Sato, R.; Wada, Y.; Matsumura, Y.; Goto, H.; Okuda, E.; Nakagawa, S.; Utsumi, S. Structure−physicochemical function relationships of soybean β-conglycinin constituent subunits. *J. Agric. Food Chem.* **1999**, *47*, 5278–5284.
(10) Mill, E. N. C.; Marigheto, N. A.; Wellner, N.; Fairhurst, S. A.; Jenkins, J. A.; Mann, R.; Belton, P. S. Thermally induced structural changes in glycinin, the 11S globulin of soya bean (*Glycine max*): an in stiu spectroscopic study. *Biochim. Biophys Acta* **2003**, *1684*, 105–114.
(11) Iwabuchi, S.; Watanabe, H.; Yamauchi, F. Observations on the dissociation of β-conglycinin into subunits by heat treatment. *J. Agric. Food Chem.* **1991**, *39*, 34–40.
(12) German, B.; Damodaran, S.; Kinsella, J. E. Thermal dissociation and association behavior of soy proteins. *J. Agric. Food Chem.* **1982**, *30*, 807–811.
(13) Lakemond, C. M. M.; de Jongh, H. H. J.; Hessing, H.; 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.
(14) Renkema, J. M. S.; Lakemond, C. M. M.; de Jongh, H. H. J.; Gruppen, H.; van Vliet, T. The effect of pH on heat denaturation and gel forming properties of soy proteins. *J. Biotechnol.* **2000**, *79*, 223–230.
(15) Sorgentini, D. A.; Wagner, J. R.; Anon, M. C. Effect of thermal treatment of soy protein isolate on the characteristics and structure−function relationship of soluble and insoluble fractions. *J. Agric. Food Chem.* **1995**, *43*, 2471–2479.
(16) Palazolo, G. G.; Mitidieri, F. E.; Wagner, J. R. Relationship between interfacial behaviour of native and denatured soybean isolates and

microstructure and coalescence of oil in water emulsions − effect of salt and protein concentration. *Food Sci. Technol. Int.* **2003**, *9*, 409–411.

(17) Keerati-u-rai, M.; Corredig, M. Heat-induced change in oil-in-water emulsion stabilized with soy protein isolate. *Food Hydrocolloids* **2009**, *23*, 2141–2148.

(18) Nagano, T.; Hirotsuka, M.; Mori, H.; Kohyama, K.; Nishinari, K. Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *J. Agric. Food Chem.* **1992**, *40*, 941−944.

(19) Wu, S.; Murphy, P. A.; Johnson, L. A.; Reuber, M. A.; Fratzke, A. R. Simplified process for soybean glycinin and β-conglycinin fractionation. *J. Agric. Food Chem.* **2000**, *48*, 2702–2708.

(20) Bian, Y.; Myers, D. J.; Dias, K.; Lihono, M. A.; Wu, S.; Murphy, P. A. Functional properties of soy proteins produced using pilot-plant scale process. *J. Am. Oil Chem. Soc.* **2003**, *80*, 545–549.

(21) Rickert, D. A.; Johnson, L. A.; Murphy, P. A. Functional properties of improved glyicnin and β-conglycinin fraction. *J. Food Sci.* **2004**, *69*, 303–311.

(22) Keerati-u-rai, M.; Corredig, M. The effect of dynamic high pressure homogenization on the aggregation state of soy proteins. *J. Agric. Food Chem.* **2009**, *57*, 3556–3562.

(23) Lam, M.; Paulsen, P.; Corredig, M. Interactions of soy protein fractions with high methoxyl pectin. *J. Agric. Food Chem.* **2008**, *56*, 4726–4735.

Received for review January 16, 2010. Revised manuscript received June 8, 2010. Accepted June 28, 2010. This research was partly funded by the Ontario Ministry of Research and Innovation, The Solae Co., and the Natural Sciences and Engineering Council through a graduate scholarship for M.K.