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ARTICLE

Effect of Dynamic High Pressure Homogenization on the Aggregation State of Soy Protein

Maneephan Keerati-u-rai and Milena Corredig*

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

Although soy proteins are often employed as functional ingredients in oil−water emulsions, very little is known about the aggregation state of the proteins in solution and whether any changes occur to soy protein dispersions during homogenization. The effect of dynamic high pressure homogenization on the aggregation state of the proteins was investigated using microdifferential scanning calorimetry and high performance size exclusion chromatography coupled with multiangle laser light scattering. Soy protein isolates as well as glycinin and β-conglycinin fractions were prepared from defatted soy flakes and redispersed in 50 mM sodium phosphate buffer at pH 7.4. The dispersions were then subjected to homogenization at two different pressures, 26 and 65 MPa. The results demonstrated that dynamic high pressure homogenization causes changes in the supramolecular structure of the soy proteins. Both β-conglycinin and glycinin samples had an increased temperature of denaturation after homogenization. The chromatographic elution profile showed a reduction in the aggregate concentration with homogenization pressure for β-conglycinin and an increase in the size of the soluble aggregates for glycinin and soy protein isolate.

KEYWORDS: High pressure homogenization; soy proteins; glycinin; β-conglycinin; micro-DSC; SEC-MALLS; average molecular mass

INTRODUCTION

Soy proteins are increasingly gaining importance as functional ingredients because of the health benefits associated with their consumption as well as their low cost compared to other protein ingredients (*1*). Isolated soy protein (SPI) ingredients are composed mainly of two protein fractions: glycinin and β-conglycinin. These two proteins account for 65−80% (depending on seed genotype) of the total seed protein. Glycinin is a hexamer with two symmetric trimers stacked on top of one another, with a molecular mass of approximately 300−380 kDa. The individual glycinin monomer is constituted of an acidic and a basic polypeptide linked by a disulphide bridge (*2*). Glycinin is a heterohexamer with subunits arranged in five different combinations, classified into 2 major groups, group I (A1aB1b, A1bB2, and A2B1a) and group II (A3B4 and A5A4B3) (*3*). The other major protein present in SPI is β-conglycinin, with a molecular mass of approximately 180−200 kDa. It is a heterogeneous trimeric glycoprotein, composed of three subunits, α, α′, and β with an estimated molecular weight of 67, 71, and 50 kDa, respectively (*4*).

The pH and ionic strength strongly affect the conformational changes of soy proteins and as a consequence their processing functionality (*5*, *6*). At neutral pH, glycinin is in its hexameric form, while, at acidic pH, the protein is present mostly as a trimer. In addition, it has been hypothesized that a low ionic strength environment causes rearrangements in the glycinin structure with an increased exposure of the basic polypeptides (*5*). At pH greater than 7.0, soy proteins show improved solubility (*5*). Although changes in molecular structure and alteration of protein solubility have been reported, it is still not clear what state of aggregation is present for soy proteins before and after processing. It has been recently shown that soy proteins in solution show an average size of about 50 nm, with larger aggregates present at acidic pH (*6*). Any change in protein structure and aggregation state will have a great impact on their functional properties (*7*, *8*).

Using thermal analysis, it is possible to follow thermodynamic changes occurring to the proteins and to derive information on their structural changes as a function of temperature. Soy protein isolates exhibit two distinct thermal transition peaks, attributable to the thermal denaturation of the two major soy proteins, glycinin and β-conglycinin. Glycinin has denaturation temperatures ranging from 80 to 98 °C and β-conglycinin from 70 to 78 °C (*5*, *9*−*12*). The denaturation temperature, which varies depending on pH, ionic strength, composition of subunits, pressure, and protein concentration, is an indication of the thermal stability of the proteins.

Physical treatments such as high pressure homogenization involve high shear forces, which may cause structural changes and denaturation of proteins. High pressure treatment modifies protein conformation by affecting hydrogen and hydrophobic interactions, disrupting the tertiary and/or quaternary structure of most globular protein. Depending on the

*Corresponding author. Tel: 1 519 824 4120 ext 56101. Fax: 1 519 824 6631. E-mail: mcorredi@uoguelph.ca.

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conditions, the changes can be reversible (*13*, *14*). The effect of static high pressure treatment on soy proteins' structure and their functional properties has been reported. Zhang et al. (*19*) reported the formation of insoluble aggregates in soy milk after high pressure treatment. High pressure processing enhances the emulsifying properties of SPI by increasing the amount of protein adsorbed on the oil−water interface, especially for the β-subunit of β-conglycinin and the acidic polypeptides of glycinin (*15*). High pressure improves the emulsifying activity index of SPI but not the emulsifying stability (*16*). It has been shown that, at neutral pH, high pressure treatment causes structural changes, glycinin aggregation, and partial unfolding of β-conglycinin (*17*). The solubility of fractions rich in glycinin decreases as a function of pressure (*16*). Although many studies have reported the effect of static high pressure on the functional properties (i.e., emulsifying properties) of soy proteins, yet very little is understood on the effect of dynamic high pressure homogenization on the protein aggregates themselves (*9*, *16*, *18*, *19*). During dynamic homogenization, the emulsion undergoes high shear forces as well as high pressure, whereas under static conditions, proteins mainly experience changes in hydrostatic pressure.

Homogenization under high pressure is an important unit operation to obtain a finely dispersed emulsion (*20*). The process involves not only the mechanical force to disrupt the fat globules/oil droplets into uniform dispersions but also causes conformational changes to the proteins, which adsorb on the surface of the oil droplets. The present work focused on understanding if high pressure homogenization caused changes to the soy protein dispersions per se. Microdifferential scanning calorimetry was employed to identify the effect of high pressure on soy protein conformational changes, by observing the denaturation temperature (T_d) or changes in the transition peak (ΔH). In addition, size exclusion chromatography coupled with a multiangle laser light scattering detector was used to observe the changes in the molecular mass and aggregation state of soy proteins as a function of dynamic pressure.

MATERIALS AND METHODS

Defatted soy flakes (donated by The Solae Company, with a dispersion index of 90) were used to prepare the various protein isolates. Soy protein isolate (SPI) was prepared by suspending the soy flakes in 100 mM Tris-HCl buffer at pH 8.0 in a 1:10 ratio (w/v). After centrifugation at 12,000*g* for 30 min at 10 °C, the extract was adjusted to pH 4.8 with 2 M HCl, refrigerated at 4 °C for 2 h, and centrifuged at 12,000*g* for 30 min at 10 °C (Beckman Coulter, Model J2-21, Follerton, CA, USA). The precipitated protein was washed with 10 mM sodium acetate buffer at pH 4.8 (suspended to 1:8 ratio (w/v)) and subsequently centrifuged, as described above. The SPI slurry was resolubilized in ultrapure water (Barnsted, Iowa, USA), and the final pH was adjusted to 7.5 with 2 M NaOH. The fraction was dialyzed overnight at 4 °C and freeze-dried. Protein contents of all fractions were determined using the Dumas combustion method for nitrogen (Leco FP-528 Missisuaga, Ontario, Canada) using the Nx6.25 factor for proteins (approved method 46-30 AACC, 2000).

Fractions rich in glycinin and β-conglycinin were also prepared suspending defatted soy flakes in ultrapure water in a 1:15 ratio (w/v) and adjusting the pH to 7.5 with 1 M NaOH. After stirring for 1.5 h, the insoluble fraction was removed by centrifugation at 9000*g* for 30 min at 20 °C (Beckman Coulter Model J2-21, Fullerton, CA, USA). The extracted protein was adjusted to pH 6.4 with 1 M HCl and stored overnight at 4 °C. The protein suspension was centrifuged at 7,000*g* for 20 min at 4 °C. The glycinin recovered in the precipitate was resolublized with ultrapure water and adjusted to pH 7.5. 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. Insoluble material was then separated by centrifugation at 9,000*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 1 M HCl. A β-conglycinin rich fraction was then separated by centrifugation at 7,000*g* for 20 min at 4 °C. The precipitate was resolubilized with ultrapure water and adjusted to pH 7.5.

SPI, β-conglycinin and glycinin fractions were dialysed at 4 °C overnight against ultrapure water and freeze-dried. Freeze-dried protein was stored at −30 °C until needed.

The purity of all the fractions was assessed using SDS−PAGE. Protein solutions (200 μL) were centrifuged (Brinkmann Instruments, Westbury, NY) at 25 °C and 10,000*g* for 15 min to separate any insoluble residue. The supernatants and precipitates were carefully collected, and the extraction buffer (210 μL, 50 mM Tris-HCl, 5 M urea, 1% SDS, and 4% 2-mercaptoethanol, pH 8.0) was added. After 1 h of incubation at room temperature, electrophoresis buffer was again added (210 μL, 125 mM Tris-HCl, 5 M Urea, 1%SDS, 20% Glycerol, 4% 2-mercaptoethanol, pH 6.8). The samples were heated at 95 °C for 5 min and centrifuged (Brinkmann Instruments, Westbury, NY) at 10,000*g* for 10 min and loaded (5 μL) onto a 12.5% polyacrylamide gel with 4% stacking gel in Bio-Rad mini-protein electrophoresis (Bio-Rad, Mississauga, ON) for protein separation. Gels were fixed and stained using Biorad Comassie blue R-250 stain solution (45% methanol, 10% acetic acid, and 0.10% Blue R-250), followed by destaining using 45% methanol, 45% ultrapure water, and 10% acetic acid solution, and then the gel was scanned using a SHARP JX-330 scanner (Amersham Biosciences, Quebec).

Protein fractions were suspended in 50 mM sodium phosphate buffer at pH 7.4 to a final concentration of 10 mg/mL and stored overnight at 4 °C. The suspensions were then passed through a Microfluidizer (Model 110S, Microfluidizer, Newton, USA) for 3 passes at two different pressures, 26 and 65 MPa. Samples were then analyzed within a few hours using differential scanning microcalorimetry (micro-DSC) and multiangle laser light scattering (MALLS), after appropriate dilution.

The degree of protein denaturation as a function of thermal stability was determined using a VP-DSC microcalorimeter (Microcal Incorporated, North Hampton MA). Protein samples were diluted to 5 mg/mL with 50 mM sodium phosphate buffer, pH 7.4. Solutions were held at 20 °C for 15 min prior to heating from 20−115 °C with a 1 K/min scan rate and then cooled to 20 °C at the same rate. Enthalpy (ΔH) and denaturation temperature midpoint (T_d) were analyzed using Origin version 7.0.

Soy proteins solutions were filtered through 0.45 μm filters (low protein binding, Millipore, Fisher Sci.) and diluted to various concentrations from 0.25 to 5 mg/mL before injecting into a size exclusion HPLC system. The proteins were analyzed under native conditions, using 50 mM sodium phosphate at pH 7.4 as mobile phase. The buffer was triple filtered (0.2 and 0.1 μm filters, Millipore, Billerica, MA, USA) and degassed using an in line degassing system (SpectraSystems, ThermoFinnigan, San Jose, CA, USA). Aliquots (100 μL) were injected into two gel filtration columns connected in series (Biosep 4000 and 3000, 5 mm Phenomenex, Torrance CA, USA) and eluted at room temperature (25 °C) at 0.5 mL/min. These columns have a nominal exclusion volume of 2000 and 700 kDa, respectively. The separation system was connected to a HPLC (SpectraSystems, ThermoFinnigan, San Jose, CA, USA), consisting of a degasser, a pump (P-2000), an auto sampler, and a UV detector. Radius and molecular mass parameters were determined by on line detection with a refractive index (RI) (Optilab Rex, Wyatt Tech., Santa Barbara, CA) and multi angle light scattering detector (Dawn EOS, Wyatt Tech.). Values of weight mass average and radius of gyration were calculated using a refractive index increment of 0.18 (*21*). Absolute molecular masses and radius of gyration (R_g) were determined using the ASTRA software (version 5.1.9.1, Wyatt Tech.) with RI as a concentration detector. Molecular mass averages were calculated as $M_w = [\Sigma c_i M_i/\Sigma c_i]$, where c_i is the concentration of polymer at an elution volume i, and M_i is the mass at i. In addition, the root-mean-square radius of gyration was calculated as $\langle R_g^2 \rangle_z^{0.5}$ $(= [\Sigma(c_i M_i \langle R_g^2 \rangle_i^{0.5})/\Sigma(c_i M_i)]$. The radius of gyration R_g can be defined

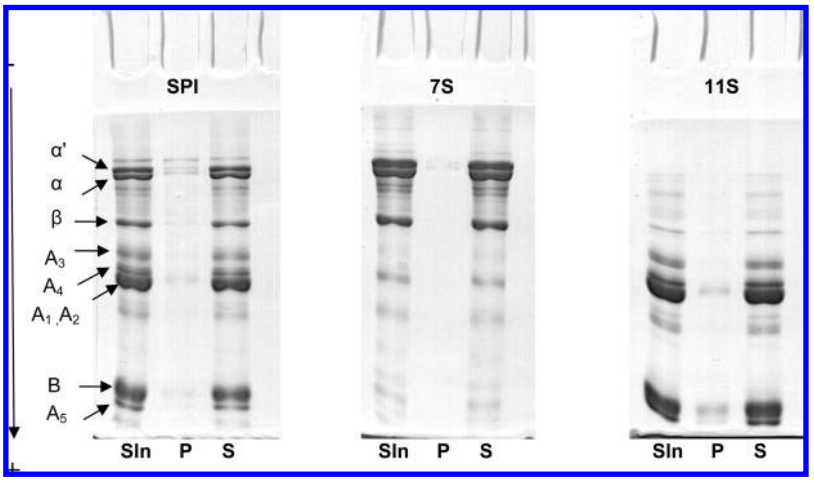

Figure 1. SDS−PAGE electrophoresis of isolates of soy protein (SPI), β-conglycinin (7S), and glycinin (11S), dispersed in 50 mM sodium phosphate, pH 7.4. Solution (Sln), supernatant (S), and pellet (P) were collected after centrifugation at 12,000*g* for 15 min.

as the distribution of the volume elements of the molecule with respect to the square of the distance from its center of gravity.

Concentrations of 1−2 mg/mL, for SPI and 7S, and 0.25−0.5 mg/mL, for 11S, were considered for the calculations and statistical analysis, as these concentrations gave the best ratio of light scattering to concentration detector signal. Three separate replicate experiments (i.e., homogenization treatments) were performed. Statistical analysis was carried out using the general linear model procedure in SAS (version 8.1, Cary, NC). Means and mean comparisons were calculated using the least-squares mean procedure.

RESULTS AND DISCUSSION

All fractions prepared were soluble prior to homogenization. **Figure 1** shows the electrophoresis pattern for the various protein isolates (10 mg/mL) in solution, before and after centrifugation. All protein subunits remained in the soluble phase after centrifugation, and very little protein was present in the pellet fraction.

The thermal transitions for the various protein solutions before high pressure homogenization are summarized in **Figure 2**. The endothermic transition peaks indicated that structural changes were associated with temperature. The enthalpy of the transition was defined as the amount of energy required to denature proteins and quantified as the area under the curve (*22*). SPI solutions exhibited two distinct endothermic transitions, attributed to the two main fractions present in the isolate: β-conglycinin and glycinin. The β-conglycinin showed a thermal transition at 68 °C, whereas glycinin had a thermal transition at 85 °C (**Table 1**). This was confirmed by microcalorimetric measurements for the β-conglycinin and glycinin enriched fractions, which showed only one endothermic transition at about 69 and 84 °C, respectively. These results are in agreement with previously reported data (*23*). Values derived from thermal analysis are summarized in **Table 1** for the solutions before and after homogenization. An increase in the peak temperature was observed in the soy protein isolate, as the temperature of the first peak changed from 68.3 ± 0.10 to 69.2 ± 0.17 °C after treating with high pressure. A similar shift in the peak temperature was also shown for the fractions enriched in β-conglycinin and glycinin. These significant changes of T_d after pressure may suggest that structure rearrangements occur during high pressure homogenization (*24*). The changes in enthalpy of denaturation were not discussed, as these changes were within dilution errors (i.e., slight differences in concentration of the protein could affect the total area of the peak).

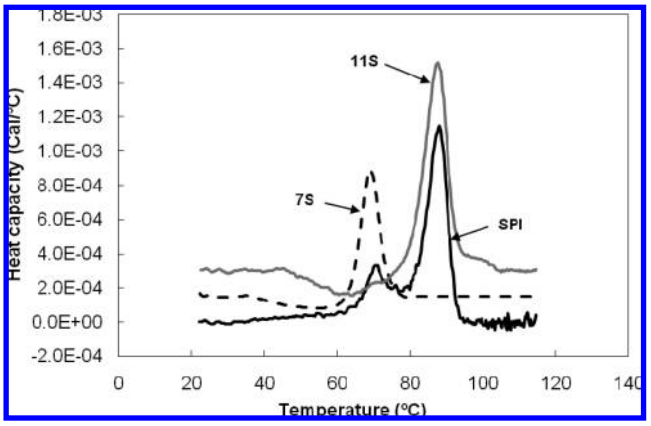

Figure 2. Endothermic transitions measured by microcalorimetry for the soy isolate (SPI), β-conglycinin (7S), and glycinin (11S) fractions (5.0 mg/mL) suspended in 50 mM sodium phosphate at pH 7.4.

Previous studies using static high pressure reported an effect of pressure greater than 150 MPa on the thermal stability of glycinin (*8*, *25*), as the hydrostatic pressure treatment caused an unfolding and subsequent aggregation of glycinin. Similar studies reported partial destabilization of both β-conglycinin and glycinin fractions after treatment with 400 and 600 MPa, at pH 8 (*17*, *25*). The results in **Table 1** indicate that dynamic high pressure homogenization increases the stability of the supramolecular structures, as shown by the increase in T_d. In the case of glycinin, the highly rigid structure with disulfide linkages between the acidic and basic subunits as well as the hydrogen bonding between the trimers may assist with stabilizing its conformation (*26*). It is possible to conclude that the high mechanical forces during dynamic homogenization may induce rearrangements and aggregation.

To better understand the aggregation state of the proteins as affected by high pressure homogenization, size exclusion chromatography was also performed on the soy protein fractions, and the molecular mass of the eluted peaks was measured using static light scattering. SEC-MALLS is an effective method to characterize the changes in the molecular mass of biopolymers. In particular, several studies have reported the heat-induced changes of globular proteins measured using SEC-MALLS (*21*, *27*−*29*).

Figure 3 shows the chromatography profiles for the various soy protein isolates in solution, before homogenization. In all cases, the peaks were not well separated, indicating the

Table 1. Effect of high Pressure Homogenization on the Endothermic Transition Peak (T_d) and Enthalpy (ΔH) for the Various Soy Protein Fractions[a]

	soy protein i solate		β-conglycinin		glycinin	
sample	T_d (°C)	ΔH (Cal/°C)	T_d (°C)	ΔH (Cal/°C)	T_d (°C)	ΔH (Cal/°C)
no treatment	68.33 ± 0.10	0.001 ± 0.0002	68.96 ± 0.17	0.005 ± 0.0002	84.30 ± 0.06	0.009 ± 0.0003
	85.26 ± 0.04	0.006 ± 0.0004				
26 MPa	69.20 ± 0.18	0.001 ± 0.0004	69.46 ± 0.12	0.005 ± 0.0013	84.86 ± 0.05	0.011 ± 0.0008
	86.43 ± 0.14	0.005 ± 0.0007				
65 MPa	69.14 ± 0.17	0.001 ± 0.0003	69.93 ± 0.06	0.004 ± 0.0005	84.72 ± 0.12	0.007 ± 0.0014
	86.43 ± 0.32	0.007 ± 0.0010				

[a] Values reported are the averages and standard deviations of three replicate experiments.

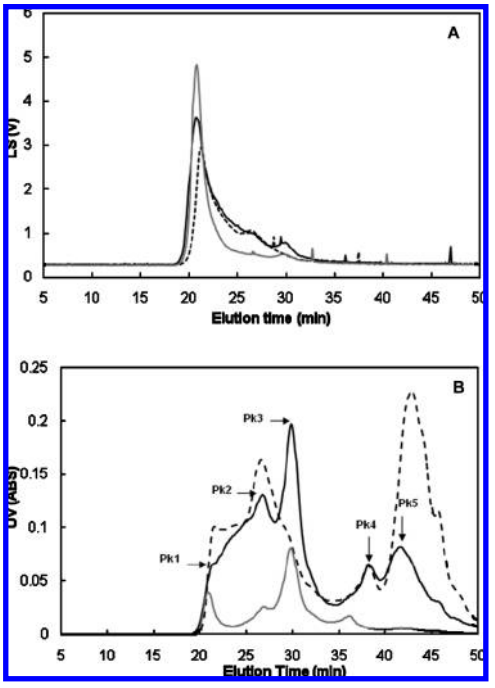

Figure 3. Light scattering (90° response) (**A**) and UV (280 nm) (**B**) profiles for untreated soy protein isolate (solid line) (2 mg/mL), β-conglycinin (dashed line) (2 mg/mL), and glycinin (gray line) (0.5 mg/mL).

polydispersity of molecular sizes. However, using light scattering detection, it was possible to quantify the molecular mass for each slice of the chromatogram and estimate the molecular mass data for the various elution regions. The signal collected from the light scattering detector is shown in **Figure 3A**. All of the protein fractions showed a large peak eluting at about 20 min. From the UV signal, it is evident that glycinin shows the lowest protein concentration in the aggregate peak (**Figure 3C**, peak 1), meaning that glycinin had the largest particles in this peak, compared to those of the SPI and β-conglycinin fractions. The elution profile for β-conglycinin (**Figure 3B**) shows a polydisperse population of protein aggregates, starting at 20 min and decreasing in UV intensity only after 32 min of elution. The elution profile of the soy protein isolate (SPI) showed a combination of the characteristic elution from β-conglycinin and glycinin. Although five peaks were identified in the chromatogram (**Figure 3B**), the peaks eluting after 35 min contain small molecular mass molecules and therefore are not of interest in this study. Three regions were further analyzed for molecular mass data, the first peak eluting at 20 min (peak 1), the second eluting at 27 min (peak 2), and the third eluting at 30min (peak 3). It is possible to identify peak 3 as unique to the glycinin fraction, as the glycinin samples eluted mostly in peak 1 (aggregated fraction) and peak 3. However, β-conglycinin also showed a distinct elution peak in the intermediate region (peak 2). Similar elution patterns were observed by Floury et al. (*8*); however, in the present work, the aggregated peak (peak 1) is better identified using the light scattering detector.

The refractive index and the light scattering signals were used to calculate the average molecular mass using the refractive index increment (dn/dc) and is 0.18, which is independent of the amino acid composition (*30*). As described above, the three major peaks were considered in the M_w and R_g calculations for both the soy protein isolate and glycinin fractions, while only the first two peaks were used in the calculations of β-conglycinin fractions. The average molecular mass of each fraction as well as the radius gyration are summarized in **Table 2**.

Figure 4 illustrates the differences in the molecular mass for the untreated protein fractions. Glycinin showed the highest values (see also **Table 2**) in the first and second elution peak, as 26×10^6 and 1.8×10^6 Da, respectively. The radius of gyration of the protein fractions isolated in peaks 1 and 2 was 39.7 ± 3.2 and 35.0 ± 2.9 nm, respectively. The third peak of the glycinin fraction showed an average mass of 485,000 Da, slightly higher than the reported molecular weight for glycinin, ranging between 300,000 and 380,000 Da (*3*, *31*). In addition, the radius of gyration showed an average of 26.9 ± 2.6 nm for the protein eluting in peak 3. These results would suggest that the majority of glycinin proteins are present in solution as large aggregates. It has been previously reported that large aggregates of glycinin form during purification (*32*). Conversely, the first elution peak for β-conglycinin showed aggregates with a M_w at 1.8×10^6 Da, and a second fraction (peak 2) with an average molecular mass of 454,000 Da, corresponding to a value approximately two times higher than the molecular weight of β-conglycinin reported in the literature of about 180,000 to 200,000 Da (*4*). It has been previously demonstrated that β-conglycinin can reversibly assemble to hexameric structures at neutral pH at low ionic strength (*23*): Koshiyama (*34*) reported the presence of a dimer (9S) with a molar mass of 370 kDa using ultracentrifugation. In addition, Maruyama et al. (*33*) demonstrated that the α and α′ subunits of β-conglycinin have a tendency to form hexamers or aggregates at neutral pH (*33*, *34*). The radius of gyration for the aggregated β-conglycinin was significantly lower (23.2 ± 0.3 nm) compared to the radius of peaks 1 and 2 of soy protein isolates or glycinin.

Table 2. Effect of High Pressure Homogenization on the Average Molecular Mass (M_w) and Radius of Gyration (R_g) for Each Eluted Peaks of Soy Protein Fractions[a]

		peak1		peak2		peak3	
sample	treatment	M_w(10^5Da)	R_g (nm)	M_w(10^5Da)	R_g (nm)	M_w (10^5Da)	R_g (nm)
soy protein isolate	no treatment	33.9 ± 11.3^{a}	38.5 ± 3.0^{c}	8.75 ± 1.00^{b}	29.3 ± 1.3^{c}	$4.56 \pm 0.07^{a,b}$	27.3 ± 0.4^{b}
	26 MPa	127.8 ± 18.3^{b}	$34.8 \pm 4.0^{b,c}$	11.21 ± 1.06^{b}	23.5 ± 4.5^{b}	$4.69 \pm 0.07^{b,c}$	19.6 ± 5.5^{a}
	65 MPa	111.5 ± 15.2^{b}	31.5 ± 2.3^{b}	11.56 ± 0.30^{b}	21.9 ± 1.5^{b}	4.21 ± 0.13^{a}	17.9 ± 1.4^{a}
glycinin	no treatment	261.2 ± 4.5^{c}	39.7 ± 3.2^{c}	$18.39 \pm 2.42^{c,d}$	35.0 ± 2.9^{d}	$4.85 \pm 0.20^{b,c}$	26.9 ± 2.6^{b}
	26 MPa	347.3 ± 23.3^{d}	38.6 ± 1.1^{c}	16.56 ± 4.57^{c}	$33.1 \pm 4.9^{c,d}$	$5.11 \pm 0.38^{c,d}$	24.0 ± 4.6^{b}
	65 MPa	344.2 ± 33.0^{d}	36.9 ± 1.6^{c}	20.97 ± 3.87^{d}	$31.9 \pm 3.4^{c,d}$	5.58 ± 0.40^{d}	$23.6 \pm 3.3^{a,b}$
β-conglycinin	no treatment	17.9 ± 0.98^{a}	23.2 ± 0.3^{a}	4.54 ± 0.13^{a}	$17.4 \pm 0.5^{a,b}$		
	26 MPa	25.41 ± 8.7^{a}	24.1 ± 4.9^{a}	4.74 ± 0.22^{a}	$17.3 \pm 4.4^{a,b}$		
	65 MPa	32.4 ± 11.2^{a}	21.1 ± 2.1^{a}	4.37 ± 0.23^{a}	14.5 ± 3.1^{a}		

[a] Values represent the least square means. $n = 3$. Data in the same column with different letters are significantly different at $p < 0.05$.

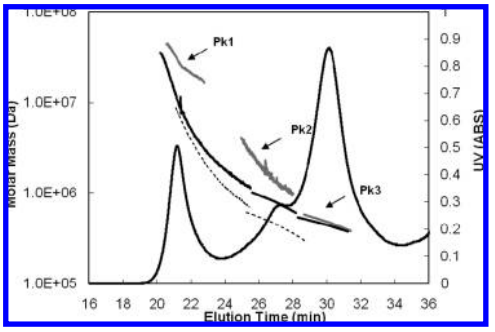

Figure 4. Average weight molecular mass of untreated glycinin (gray line), β-conglycinin (dashed line), and soy protein isolate (solid line). A representative UV elution pattern for the soy protein isolate is also shown.

Lastly, in the soy protein isolate solutions, the aggregated peak (peak 1) had a M_w of 3.3×10^6 Da, while peaks 2 and 3 were 875 and 456 kDa, respectively. An isolate of soy protein is usually composed of glycinin and β-conglycinin in a ratio between 1.48 to 1.12 depending on genotype and variety (*35*). The values of molecular mass calculated for the soy protein isolate solutions are in agreement with our findings for the isolated β-conglycinin and glycinin fractions (**Table 2** and **Figure 4**). The average radius values for peak 1, 2, and 3 also reflect the presence of mixed proteins in the soy protein isolate, with a radius of 38.5 ± 3.0, 29.3 ± 1.3, and 27.3 ± 0.4 nm, respectively. These values are smaller than the average diameters for soy protein isolates measured by Lam et al. (*6*) using scanning electron microscopy and image analysis.

The effect of high pressure homogenization was determined by observing the differences in the average molecular mass of the various protein fractions before and after homogenization. **Figure 5** illustrates the effect of high pressure on the UV elution profiles. A difference in the intensity in the elution profiles was observed for β-conglycinin and soy protein isolate solutions (**Figure 5A**,**B**). No differences in the elution pattern were shown between the two pressure levels (26 and 65 MPa) applied (data not shown). However, the elution profile for glycinin did not show significant differences with high pressure homogenization, meaning that the concentration of protein in the eluted peaks did not change (**Figure 5C**).

An increase of the M_w of glycinin was noted for the aggregate peak (peak 1), with a significant change for peaks 2 and 3 when homogenizing at 65 MPa. No significant changes were found for the radius of gyration with homogenization (**Table 2**). The results suggested that aggregation of glycinin occurred during high pressure homogenization. An increase in the aggregation of glycinin was also reported previously using size exclusion chromatography for protein solutions subjected to pressures greater than 150 MPa (*8*). It is possible to hypothesize that during high shear homogenization, partial disruption and rearrangements occur in the supramolecular structure of glycinin, and although the radius of gyration does not seem to increase, the molecular mass significantly increases, suggesting the formation of more spherical aggregates.

However, the average molecular mass and radius of β-conglycinin solutions did not change with high pressure homogenization. The chromatographic elution, however (**Figure 5B**), showed a reduction in the concentration of the aggregate peak (peak 1), indicating a decrease in the concentration of protein injected. This may suggest that large aggregates formed but were not recovered after filtration of the β-conglycinin solution.

When observing the elution of soy protein isolate (**Figure 5A**), it is also possible to notice a decrease in the UV signal for pressure treated solutions. In addition, there was a dramatic increase in the M_w of peak 1 from 3.4×10^6 to 12.7×10^6 Da with homogenization at 26 MPa. In the soy protein isolate solutions, there was a significant decrease in R_g for the three fractionated peaks, as a function of homogenization pressure, confirming our hypothesis that high shear forces during high pressure homogenization cause disruption of the supramolecular structures and further rearrangement in aggregates of higher molecular weight. Disruption of soy protein isolates during high pressure has been previously reported also using SDS−PAGE (*18*, *25*).

This study demonstrated for the first time that dynamic high pressure homogenization causes changes in the supramolecular structure of soy proteins. The changes in the average molecular mass of the aggregates seemed to suggest a partial disruption of the structures, with formation of aggregates not significantly different in radius (apart from SPI samples) but with higher molecular mass. Most changes occurred already at 26 MPa, with no further changes using higher pressure of homogenization. By observing the changes occurring to the β-conglycinin and glycinin enriched fractions, it is possible to distinguish between the contributions of the two proteins in the soy protein isolate. While in the case of β-conglycinin, no changes occurred to the molecular weight average of the peaks after high pressure homogenization, although less protein was recovered in the soluble phase, it was very clear that glycinin was significantly affected by high pressure homogenization, causing the formation of soluble

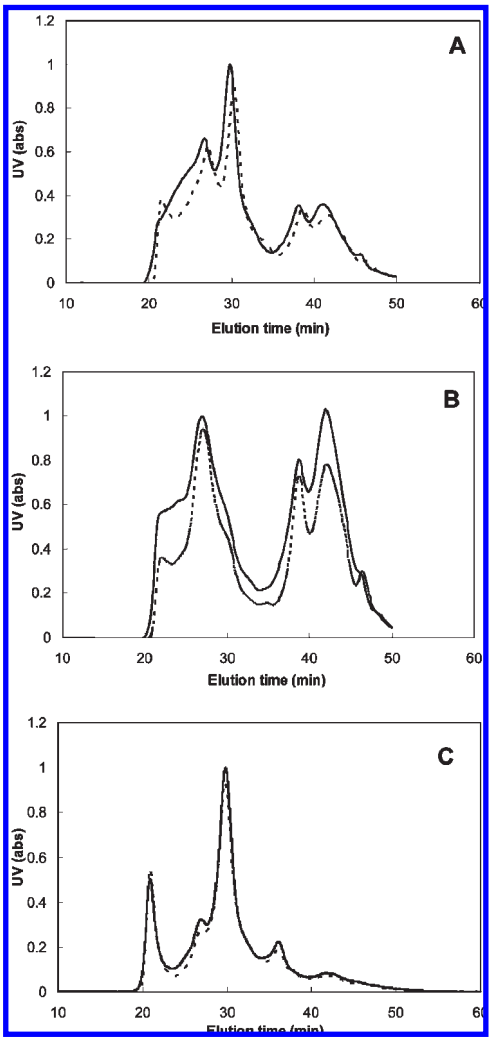

Figure 5. Representative UV elution profiles of soy protein isolate (**A**), β-conglycinin (**B**), and glycinin (**C**) before (solid line) and after (dashed line) high pressure homogenization at 65 MPa.

aggregates with higher average molecular mass and a radius of about 39 nm. The behavior of soy protein isolate solutions reflects that of the two protein fractions. The present results seem to suggest that glycinin is more subjected to the formation of soluble aggregates than β-conglycinin. Further studies will evaluate the composition in each fraction and determine if these supramolecular rearrangements have an effect on the functional properties of the proteins.

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