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Effects of AOT reverse micelle on properties of soy globulins

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ABSTRACT

This study was focused on the influence of AOT reverse micelle on physical–chemical properties of 7S and 11S globulins from soy proteins, and compared with aqueous buffer extraction. The results showed that the contents of the surface hydrophobicity and SH groups of 7S and 11S globulins and SS bonds of 11S globulin, using AOT reverse micelle extraction, were augmented, and SS bonds of 7S globulin decreased. The thermal and rheological properties of 7S and 11S globulins extracted using the two methods were studied by differential scanning calorimetry (DSC) and rheometery. It was found that the peak denaturation temperature and heat of transition of 7S and 11S globulins with aqueous buffer extraction were different from that with AOT reverse micellar extraction. The AOT reverse micelle did not affect the gel properties of 11S globulin, while it influenced 7S globulin's. Hardness, springiness, gumminess, adhesiveness and chewiness of 7S globulin from AOT reverse micelle were lower than that from aqueous buffer extraction, but gumminess was higher.

1. Introduction

Soybean proteins constitute an interesting alternative due to their high nutritional value, functional properties and low cost. The soybean proteins contain four globulins, namely, 2S, 7S, 11S and 15S proteins. These protein fractions are characterized by their sedimentation coefficients. The percentage content of 2S, 7S, 11S and 15S globulins was found to be 15%, 34%, 41.9% and 9.1%, respectively (Fukushima, 1991). 7S and 11S globulins were shown to have different surface hydrophobicity, contents of sulfhydryl (SH) groups and disulfide (SS) bonds and, thermal and rheological properties, which could be due to the differences in their aggregation process (Mohamed, 2002; Tay, Xu, & Perera, 2005; Utsumi, Matsumura, & Mori, 1997). Adequate modification on soy proteins isolate may improve their functional properties, so detailed investigation of 7S and 11S globulins is necessary to elucidate the surface hydrophobicity, SH groups, SS bonds, thermal properties and rheological properties of soybean proteins.

Functional properties of 7S and 11S globulins change with structural changes. The effect of ionic strength, pH, heat and water on surface hydrophobicity, SH groups, SS bonds, thermal and rheological properties of 7S and 11S globulins has been widely studied (Hua, Cui, Wang, Mine, & Poysa, 2005; Mohamed & Xu, 2003). Adequate modification on the structure of soy proteins has brought out the following outstanding functional properties that the native soy proteins do not possess: surface hydrophobicity, solubility, emulsifying properties, thermal and rheological properties, etc. The structural changes of 7S and 11S globulins depend on the treatment method, which may thus affect the functional properties of the soy proteins. Using physical (heat or mild alkali treatment), chemical (ionic strength, pH, etc.), high pressure treatments and enzymatic treatments (Hua et al., 2005; Mohamed & Xu, 2003; Tang, Chen, Li, & Yang, 2006), the textural properties of 7S and 11S globulins have been considered in detail. While very limited information is available on the effect of the reverse micelles on surface hydrophobicity, SH groups, SS bonds, and thermal and rheological properties of 7S and 11S globulins.

A considerable volume of work has been published on the proteins dissolved in reverse micelles. These can also be utilized to recover proteins from the aqueous phase on a large scale through phase–phase extraction (Stephanie, Thorsten, Alan, Pawel, & Walter, 1991). Moreover, using micelles for the simultaneous production of oil and proteins from soybean is attractive since soybeans represent one of the major oilseeds for producing edible oils (Leser & Luisi, 1989).

In reverse micelles, the main driving forces responsible for the solute distribution between the organized assembly and the organic medium are considered to be hydrophobic effects, hydrogen

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bonding interactions and electrostatic interactions, which may affect the structure of the proteins (Correa, Durantini, & Silber, 1998). Besides other factors, such as pH and ionic strength in reverse micelles (Matzke, Creagh, Haynes, Prausnitz, & Blanch, 1992, may also affect the structure of the proteins. The most conventional reverse micellar system studied was with bis (2-ethylhexyl) sodium sulfosuccinate (AOT) as an amphiphic surfactant and with isooctane as an organic solvent. The structural changes of 7S and 11S globulins dissolved in AOT/isooctane reverse micelles have been studied using FTIR (Zhao, Chen, Xue, & Lee, 2008), but not much work has been reported about the effect of AOT/isooctane on the surface hydrophobicity, SH groups, SS bonds and, thermal and rheological properties of 7S and 11S globulins. These characteristics play important roles in determining the gel properties (Utsumi et al., 1997). So it is important to study the effect of the reverse micelles on surface hydrophobicity, SH groups, SS bonds and, thermal and rheological properties of 7S and 11S globulins from soybean.

In this paper, the dry-addition method was used to dissolve proteins in AOT reverse micellar solution. The objective of the work was to investigate the effect of AOT/isooctane reverse micellar system on the surface hydrophobicity, SH groups, SS bonds and, thermal and rheological properties of 7S and 11S globulins by DSC, rheometery, fluorescence probe, etc. We have compared the surface hydrophobicity, SH groups, SS bonds and, thermal and rheological properties of 7S and 11S globulins in the reverse micellar solution extraction with that of aqueous buffer (pH 7.5) solution extraction.

2. Materials and methods

2.1. Materials

AOT was bought from Sigma Chemical Company (>98%). Bicinchoninic Acid (BCA) protein assay kit was purchased from USA Pierce Company. All the other reagents were of analytical grade. Soybean (No. 8) was obtained from the local market, Beijing. The soy flour was sieved through a 100 mesh screen to enrich the protein contents; the soy flour consisted of 37.53% protein, 7.99% humidity, and 20.75% oil; and the soy flour was defatted as the material for aqueous buffer extraction of proteins. All the values are given in wt.% of the total weight of the flour.

2.2. AOT reverse micellar extraction

AOT reverse micelle was used to isolate protein from soy flour (Vassiliki, Aristotelis, & Athanasios, 1993). First a stock solution of 0.05 M AOT was obtained by solubilizing AOT in isooctane. The water content was determined periodically with Karl-fischer reagent in the monophasic area of the phase diagram. The total volume of water was adjusted by the ratio $W_0 = [H_2O]/[AOT]$ using phosphate buffer of pH 7.5 containing 0.05 M KCl, $W_0 = 18$. Then the forward extraction was performed by adding soybean flour to the AOT system (1:20, w/w). Solubilization was conducted in a magnetically agitated Erlenmeyer flask for 30 min at 45 °C. The resulting mixture was centrifuged at 6000 rpm for 10 min, and the clear supernatant was used for the next extraction step. 200 ml of this solution was carefully laid on 200 ml of 1 M KCl phosphate buffer (50 mM), pH 7.5, kept in a magnetically agitated Erlenmeyer flask at ambient temperature for 1 h. The resulting mixture was centrifuged at 4000 rpm for 10 min. Then two phases, namely oil phase and aqueous phase, were separated by centrifugation at 3700 rpm for 5 min, respectively. The aqueous phase was stored for the experiment. The protein content in the aqueous phase was measured using the BCA method (microplate procedure, microplate reader 550: Japan Bio-RAD Company) (Smith et al., 1985).

2.3. Aqueous buffer solution extraction

For the preparation of the crude extract in an aqueous buffer solution (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992), the defatted soybean flour was dispersed in 50 mM phosphate buffer (1:20, w/w) (pH 7.5) and stirred at room temperature for 2 h. The suspension was then centrifuged at 12,000 rpm at 20 °C for 20 min. The supernatant was used for further investigations. Note that centrifugation of the micellar suspension needs as much less energy, speed and time as centrifugation of the aqueous suspension.

2.4. Isolation of 7S and 11S globulins

The 7S and 11S globulin fractions were isolated by the modified method of Nagano et al. (1992). The protein solution with reverse micellar extraction method was dialyzed before 7S and 11S globulins were isolated. Dry sodium bisulfite (SBS) was added to the supernatant (1.00 g/l SBS), the pH was adjusted to 6.4 with 0.2 M HCl, and the mixture was kept in an ice bath overnight (for about 20 h). The following preparation procedure was performed at 4 °C. The dispersion was centrifuged at 10,000 rmp for 10 min. The precipitate (11S fraction) was suspended in 50 mM phosphate buffer (pH 7.5), and then purified with ammonium sulfate. The insoluble fraction was obtained by centrifugation at 8000 rmp for 15 min, the precipitate was suspended in 1 mM phosphate buffer (pH 7.5), dialyzed and lyophilized. Salt concentration was then adjusted to 0.25 M with solid NaCl. The pH of the supernatant was then adjusted to pH 5.0 with 0.2 M HCl. After 1 h, the insoluble fraction was removed by centrifugation at 12,000 rmp for 10 min. The supernatant was diluted 2-fold with ice-cold water, adjusted to pH 4.8 with 0.2 M HCl, and then centrifuged (10,000 rmp, 10 min). The obtained precipitate, namely the 7S globulin fraction, was dissolved in phosphate buffer (pH 7.5). The purity process of 7S globulin was the same as that of the 11S globulin purified with ammonium sulfate, dialyzed before freeze-drying process. Protein content of this lyophilized powder was 95.43%, which was determined by the BCA method (Smith et al., 1985).

2.5. SDS–PAGE

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to examine the purity of 7S and 11S globulin isolates. SDS–PAGE was performed according to Laemmli (1970), using 12.5% homogenous gels and 4.5% stacking gels, respectively. A buffer system containing 2 M Tris-base, pH 8.8, with 0.15% SDS, for the separation gel and 0.027 M Tris-base, 0.38 M glycine, pH 8.3, with the addition of 0.15% SDS, for the running buffer, was used. 7S and 11S globulins samples (10 mg/ml) were treated with a sample buffer containing 10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5% SDS and 0.01% bromophenol blue. The samples were heated for 5 min in a boiling water bath, in the presence or absence of 5% β-mercaptoethanol (ME), and cooled. Five microliter sample solutions of 7S and 11S globulins were loaded. The gel was stained with 2.5% Coomassie Brilliant Blue R-250 in water–methanol–acetic acid (4:5:1, v/v/v) and destained with water–methanol–acetic acid (10:4:1, v/v/v).

Low MW markers which were used, included rabbit phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), carbonic anhydrase (31.0 kDa) and lysozyme (14.4 kDa).

2.6. Differential scanning calorimetry (DSC)

The thermal properties of 7S and 11S globulin samples were examined using a TA DSCQ10 thermal analyzer (TA Instruments,

New Castle Delaware 19720 USA). The thermal denaturation analysis procedure was according to that of Tang et al. (2006). Approximately 2.0 mg of 7S and 11S globulin samples was weighed in an aluminium liquid pan and to it 10 μl of 0.05 M phosphate buffer (pH 7.0) was added. The pan was hermetically sealed and heated at 25–120 °C at a rate of 5 °C/min. A sealed empty pan was used as a reference. Onset temperature (T_m), peak transition or denaturation temperature (T_D), and enthalpy of denaturation (ΔH) were calculated by an instrument software after manually setting the starting and ending points of the peak. All the experiments were conducted in duplicate and the coefficient of variation was in the range from 0.2% to 0.6% for T_m and T_D, and 5% to 10% for ΔH. In all the cases, the sealed pans containing samples and buffers were equilibrated at 25 °C for more than 6 h.

2.7. Surface hydrophobicity (H_0)

The surface hydrophobicity was determined using 1-anilino-8-naphthalene-sulfonate (ANS) as a fluorescence probe (Kato & Nakai, 1980). 7S and 11S globulins were resolved in a 0.1 M phosphate buffer, pH7.0, at a concentration of 4 mg/ml and the protein concentration was determined by the BCA method (Smith et al., 1985). To 4.5 ml protein solution, 0.5 ml of ANS solution (1.25 mmol/L, in 0.1 mol/L phosphate buffer, pH 7.0, was added, and kept at room temperature for 2 h. Fluorescence intensity (FI) was measured at 374 nm (excitation) and 485 nm (emission), scan speed 2400 nm/min, and slit width 5 nm with a Perkin–Elmer 2000 fluorescence spectrometer (Perkin–Elmer Corp. Norwalk, CT, USA). All the determinations were performed in triplicate.

2.8. SH groups and SS bonds

Ellman's reagent was used to determine the SH groups and SS bonds (Beveridge, Toma, & Nakai, 1974). For SH groups, 0.5 ml of 10 mg/ml 7S and 11S globulins was added to 2.5 ml of 8 M urea in Tris–glycine buffer (10.4 g Tris, 6.9 g glycine and 1.2 g EDTA per liter, pH 8.0, denoted Tris-Gly) and 0.02 ml of 4 mg/ml Ellman's reagent (5.5′-dithiobis-2nitrobenzoic acid in Tris-Gly). For SS bonds, 0.2 ml of 10 mg/ml 7S and 11S globulins, 1 ml of 10 M urea in Tris-Gly and 0.02 ml of 2-meraptoethanol were incubated at 25 °C for 1 h. After an additional 1 h incubation with 10 ml of 12% trichloroacetic acid (TCA), the tubes were centrifuged at 5000 rmp/min for 10 min. The precipitate was resuspended twice in 5 ml of 12% TCA and centrifuged to remove 2-mercaptoethanol. The precipitate was dissolved in 3 ml of 8 M urea in Tris-Gly and the color was developed with 0.05 ml of Ellman's reagent. The solution added to Ellman's reagent without protein was used as a reference. After 5 min, the absorbance was measured at 412 nm on a spectrophotometer

$$\text{Calculation}: \quad \mu MSH/g = \frac{73.53 A_{412} \cdot D}{C} \tag{1}$$

where A_{412} value indicates the absorbance at 412 nm; C indicates the concentration of sample, the unit was mg/ml; and D indicates the dilution factor, D = 3.02/0.5 for SH groups, and D = 3.05/0.2 for total (SH groups + reduced SS bonds).

2.9. Rheological properties of 7S and 11S globulins

Rheological properties of 7S and 11S globulins were measured with a rheometer using parallel plate geometry (Xu, Bietz, Felker, Carrierem, & Wirtz, 2001). The edge of the plates was sealed with mineral oil to prevent sample moisture evaporation. To induce gel formation, 15 ml of 100 mg/ml 7S and 11S globulins was consecutively heated from 25 to 95 °C at a heating rate of 0.5 °C/min, kept at 95 °C for 1 h, and chilled in an ice bath for 30 min, and stored overnight at 4 °C. Gels were equilibrated to room temperature for 2 h before being compressed with a cylinder probe in a texture analyzer (RT-2002D, Rherotech Co., UK). The gels were compressed twice with 50% compression; the operating conditions of the apparatus were: cylindrical probe with 2.5 mm diameter, pre-test speed 5.0 mm/s, the uniaxial compression at the rate of 1.0 mm/s, and applied force 5.0g.

The textural parameters of 7S and 11S globulins could be calculated with the textural profile analysis (TPA) curve (Fig. 1), including hardness at 50% of deformation, springiness, cohesiveness, adhesiveness, gumminess and chewiness (Tang et al., 2006). Hardness was determined by peak force (g) during the first bite, H_1/0.02, g. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve (dimensionless), A_2/A_1. Springiness was determined as a ratio of the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal, H_2, the unit was mm. Gumminess was obtained by multiplying hardness and cohesiveness, the unit was Pa mm. Chewiness was obtained by multiplying hardness, cohesiveness and springiness, the unit was N. Adhesiveness was determined by the area under the first curve during the carrier down, A_3/0.02, the unit was J.

Each measurement was repeated at least six times with different samples. The relative errors were all within the range of ±12%.

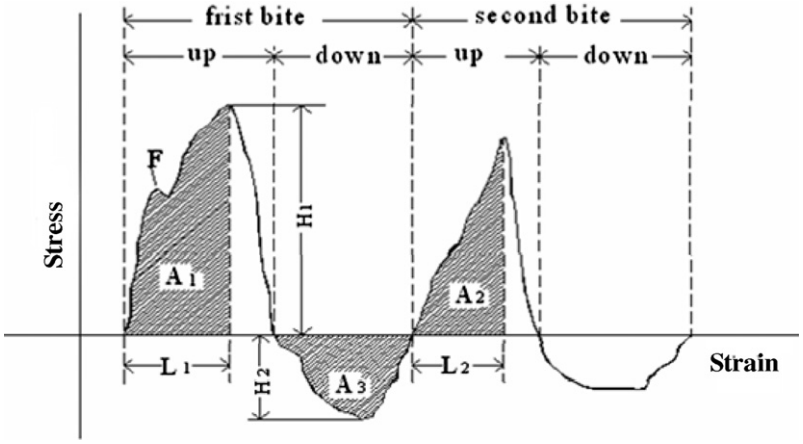

Fig. 1. Typical textural profile analysis (TPA) curve of 7S and 11S globulins.

2.10. Statistical analysis

The data reported in all the tables are an average of triplicate observation and subjected to one-way analysis of variance (ANOVA) using SAS software (v.8.2, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Isolation of 7S and 11S globulins

The experiment showed that it was impossible to directly isolate 7S and 11S globulins of soybean proteins with AOT reverse micellar extraction by Nagano et al. (1992) method. The ionic strength (1 M) in the backward extraction of proteins with AOT reverse micelle extraction would affect the isoelectric point of 7S and 11S globulins, which would lead to the hindrance of the separation of 7S and 11S globulins. According to Koshiyama (1972) method, when the ionic strength was more than 0.5, the pH was adjusted to 2.0, the 11S globulin would be completely separated. However, it was difficult to properly isolate 7S and 11S globulins of soybean proteins by this method.

It could be concluded from the experiment that the backward extraction of proteins with AOT reverse micellar extraction dialyzed at 4 °C (24 h) was suitable to isolate 7S and 11S globulins. 1 mol 11S globulin molecule consisted of at least 20 disulfide bonds, 0.0082 M SBS was not enough to break all the disulfide bonds. Therefore, 0.0094 M SBS was used, which facilitated the separation of 11S globulin. The amount of reducing reagent was required to be in favor of obtaining a good purity of 11S globulin and a high yield of 7S globulin (Fig. 2). In addition, 2S fraction from soybean proteins was attributed to the small molecular protein. When 7S and 11S globulins were separated at isoelectric point, 2S fraction could be dispersed in suspension, and was removed by centrifuging and dialyzing. The further effects of AOT reverse micelle on 2S fraction are still being studied.

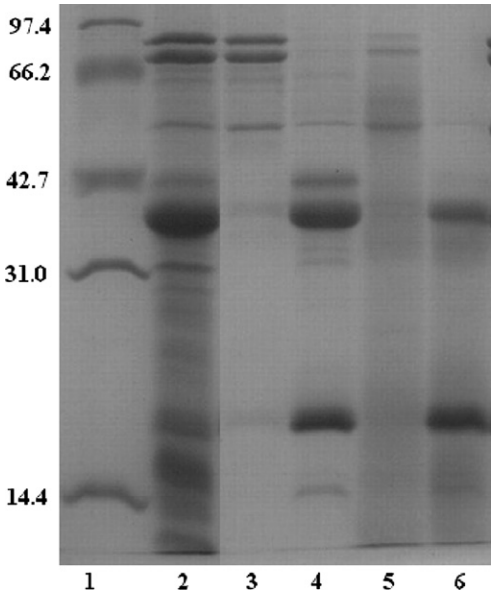

Fig. 2. SDS–polyacrylamide gel electrophoresis of 7S and 11S globulin fractions. Lane 1: molecular weight markers; lane 2: SPI; lanes 3 and 4: 7S and 11S globulins by using aqueous buffer extraction; lanes 5 and 6: 7S and 11S globulins by using AOT reverse micellar extraction.

3.2. Thermal denaturation, surface hydrophobicity, SH group and S–S bond

DSC was performed on the protein isolates to investigate their thermal stability. The thermal stability of protein functionally indicated its resistance to aggregation in response to heating (Horax, Hettiarachchy, Chen, & Jalaluddin, 2004). Fig. 3 shows DSC-thermograms of 7S and 11S globulins using AOT reverse micelle and aqueous buffer extraction. The onset temperature (T_m), peak denaturation temperature (T_D) and heat of transition or enthalpy (ΔH) of 7S and 11S globulins with the two extraction methods are shown in Table 1. T_D is the temperature at which a transition occured and was a measure of thermal stability, while ΔH was an indication of hydrophobic/hydrophilic interactions and compactness of the proteins (Ma & Harwelkar, 1991). Two endothermic peaks were observed on the thermograms which corresponded to the thermal denaturation of 7S and 11S globulins with the two extraction methods, respectively. It was found that T_D (99.51 and 101.02 °C, respectively) of 11S globulin using aqueous buffer and AOT reverse micelle extraction was in agreement with the previously published work (Tang et al., 2006). But T_D (97.41 and 98.47 °C, respectively) of 7S globulin was not supported (Tang et al., 2006). Moreover, Hua et al. (2005) reported that such variations might be attributed to the differences between soy cultivars, kind of denaturants, condition of solubilization, etc. The transition heat (ΔH) was used to monitor the proportion of the protein that did not denature during the process (Biliaderis, 1983). For 11S globulin using the two extraction methods, ΔH, T_m and T_D were not significantly different; for 7S globulin using AOT reverse micelle extraction, ΔH (12.24 J/g), T_m (94.24 °C) and T_D (98.47 °C) were higher than that using aqueous buffer extraction1 (ΔH 9.68 J/g; T_m 92.12 °C; T_D 97.41 °C). It was observed that the structures of 7S and 11S globulins were somewhat affected by AOT reverse micelle.

Hydrophobicity is one of the most important structure-related factor influencing the functional properties of protein and surface hydrophobicity, and is significantly correlated with protein gelation property (Jackman & Yada, 1989). ANS fluorescence probe method was widely adopted in researches to determine the surface hydrophobicity due to its simplicity and high sensitivity. As could be seen in Table 1, the surface hydrophobicity for 7S and 11S globulins with the two extraction methods measured by the ANS fluorescence probe was significantly different. 7S and 11S globulins (98.67 and 104.92, respectively) using AOT reverse micelle extraction were more hydrophobic than that using the aqueous buffer extraction (91.42 and 98.67, respectively), which showed an increase of 7.25 and 21.05, respectively. It was interesting to note that 7S globulin was more hydrophobic than 11S globulin by using

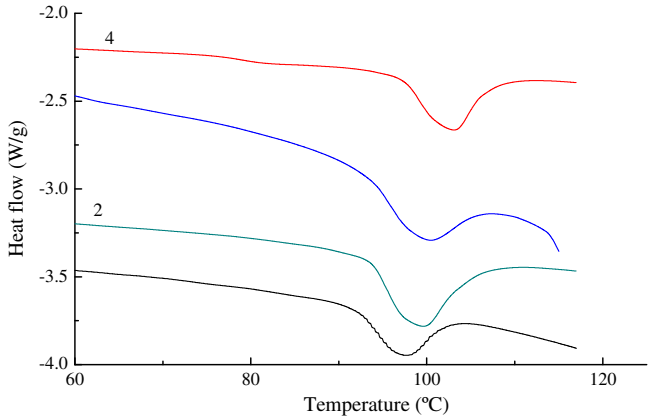

Fig. 3. DSC-thermograms of 7S and 11S globulins.

Table 1
Enthalpy of denaturation, onset temperature, the peak temperature, surface hydrophobicity, SH groups and SS bonds of 7S and 11S globulins[a]

Globulin	Enthalpy of denaturation (ΔH, J/g)	Onset temperature (T_m, °C)	The peak temperature (T, °C)	Surface hydrophobicity (H_0)	SH groups (μM/g)	SS bonds (μM/g)
7S[b]	9.68 ± 0.38[d]	92.12 ± 1.22[d]	97.41 ± 1.15[d]	91.42 ± 3.66[e]	5.09 ± 0.03[d]	40.32 ± 0.12[d]
7S[c]	12.24 ± 0.41[d]	94.24 ± 0.98[d]	98.47 ± 0.88[d]	98.67 ± 3.11[e]	1.36 ± 0.01[e]	38.42 ± 0.11[e]
11S[b]	18.66 ± 0.57[d]	93.16 ± 1.53[d]	99.51 ± 1.02[d]	83.87 ± 2.86[e]	3.62 ± 0.03[d]	43.52 ± 0.14[e]
11S[c]	19.85 ± 0.61[d]	93.41 ± 1.01[d]	101.02 ± 1.03[d]	104.92 ± 6.86[d]	1.23 ± 0.01[e]	46.37 ± 0.14[d]

[a] Means ± standard deviations of triplicate analyses.
[b] Globulin using aqueous buffer extraction.
[c] Globulin using AOT reverse micellar extraction.
[d–e] Indicate significant ($P \leqslant 0.05$) difference within a column.

aqueous buffer extraction, but the result was opposite to that of AOT reverse micelle extraction. It indicated that the AOT reverse micelle might influence the surface hydrophobicity of 7S and 11S globulins. Further studies were needed to investigate the mechanisms for the variation in surface hydrophobicity.

SH groups and SS bonds significantly influenced the functional properties of proteins and played an important role in the formation of relatively rigid structures, such as protein gels or doughs. SH group and SS bond contents are presented in Table 1. It was obvious that the contents of total SH groups (5.09 μM/g) and SS (40.32 μM/g) bonds of 7S globulin, and SH groups (3.62 μM/g) of 11S globulin using aqueous buffer extraction were more than that using AOT reverse micelle extraction (the contents of SH groups and SS bonds for 7S globulin were 1.36 μM/g and 38.42 μM/g, respectively, and the content of SH groups for 11S globulin was 1.23 μM/g), while the content of SS bonds (43.52 μM/g) for 11S globulin using aqueous buffer extraction was lower than that using AOT reverse micelle extraction (46.37 μM/g). The data indicated that the SH groups and SS bonds might interchange because of the different treatment methods. At least four factors contributed to the different distribution in SH and SS of 7S and 11S globulins. Firstly, the unusual properties of water localized in the interior of reverse micelles (Matzke et al., 1992) could bring about stronger interaction with the charged groups of SH and SS on surface of the neighboring 7S and 11S globulin molecules than by the aqueous buffer extraction. Secondly, the interaction of AOT with 7S and 11S globulins could change the structures of 7S and 11S globulins. Thirdly, the contact of 7S and 11S globulin molecules with isooctane in the process of solubilization could also change the structures of 7S and 11S globulins. Last but not the least, the state of water and water-head group interaction in AOT/isooctane microemulsion could also change the structures of 7S and 11S globulins (Christopher, Yarwood, Belton, & Hills, 1992). Further more, solvent (such as, isooctane) of a low dielectric constant and the co-effect of these factors had changed the protein environment. The structures of 7S and 11S globulins changed when the environment of the proteins changed, the results agreed with the study of Nguyen, Baldwin, Cohen, and Prusiner (1995).

3.3. Rheological properties of 7S and 11S globulins

Fig. 4A shows that the hardness of 7S globulin with the two extraction methods was lower than that of 11S globulin, the result was in agreement with the study of Hua et al. (2005) and Tang et al. (2006). The hardness of 11S globulin using the two extraction methods was not significantly different. Moreover, the hardness of 7S globulin with aqueous buffer extraction was increased by 33.71% compared with that with AOT reverse micelle extraction. It indicated that the microenvironment of AOT reverse micelle might have an effect on the conformation of 7S and 11S globulins, and affected the gel hardness of 7S and 11S globulins. Yagasaki, Takagi, Sakai, and Kitamura (1997) supported these results.

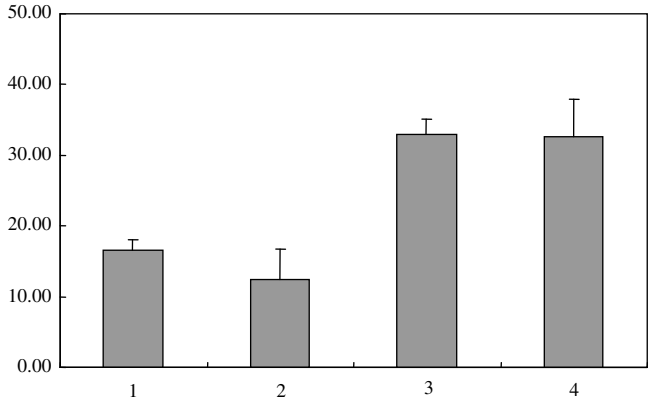

Fig. 4A. Hardness of 7S and 11S globulins. 1 and 3: 7S and 11S globulins by using aqueous buffer extraction. 2 and 4: 7S and 11S globulins by using AOT reverse micellar extraction.

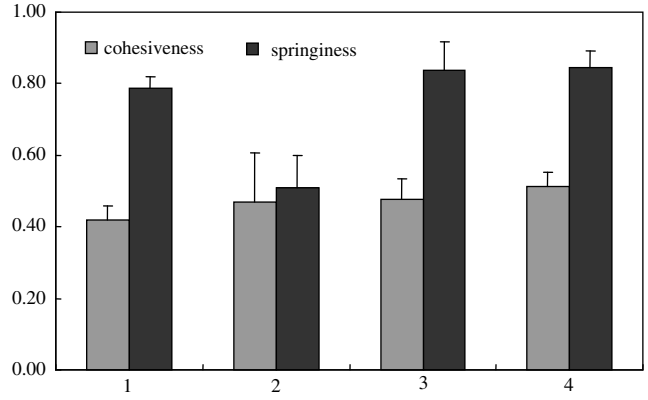

Fig. 4B. Cohesiveness and springiness of 7S and 11S globulins. 1 and 3: 7S and 11S globulins by using aqueous buffer extraction. 2 and 4: 7S and 11S globulins by using AOT reverse micellar extraction.

Cohesiveness and springiness of 7S and 11S globulins are shown in Fig. 4B, cohesiveness of 7S globulin and cohesiveness and springiness of 11S globulin using the two extraction methods were not significantly different, and springiness of 7S globulin with aqueous buffer extraction was higher by 35.44% than that with AOT reverse micelle extraction. The results indicated that the AOT reverse micellar system might influence the springiness of 7S and 11S globulins. The reasons were attributed to the special micro-structure of reverse micelle.

Gumminess and adhesiveness of 7S and 11S globulins are shown in Fig. 4C. By this experiment, it was observed that gumminess and adhesiveness of 7S and 11S globulins using the two

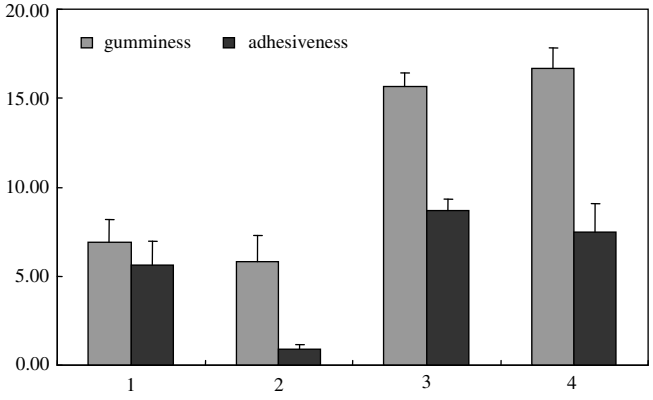

Fig. 4C. Gumminess and adhesiveness of 7S and 11S globulins. 1 and 3: 7S and 11S globulins by using aqueous buffer extraction. 2 and 4: 7S and 11S globulins by using AOT reverse micellar extraction.

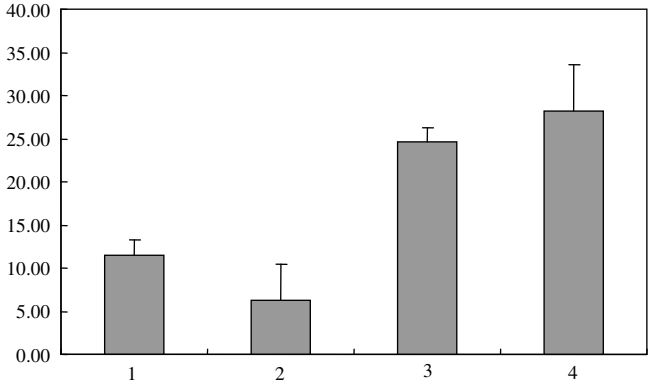

Fig. 4D. Chewiness of 7S and 11S globulins. 1 and 3: 7S and 11S globulins by using aqueous buffer extraction. 2 and 4: 7S and 11S globulins by using AOT reverse micellar extraction.

extraction methods were significantly different. Gumminess and adhesiveness of 7S globulin with aqueous buffer extraction were higher than that with AOT reverse micelle, especially adhesiveness, which showed an increase of 84.36%. Gumminess of 11S globulin using aqueous buffer extraction was lower than that using AOT reverse micelle, which showed a reduction of about 6%, while the adhesiveness to be contrary showed an increase of about 14.17%. The reasons were the same as mentioned above.

The chewiness was affected by the hardness, springiness and cohesiveness. As shown in Fig. 4D, the chewiness of 7S globulin with the two extraction methods was lower than that of 11S globulin, the result was in agreement with the study of Tang et al. (2006). The chewiness of 7S globulin using aqueous buffer extraction was higher than that using AOT reverse micelle extraction, which showed an increase of about 45.28%. However, the chewiness of 11S globulin using aqueous buffer extraction was lower, which showed a reduction of about 14.28%.

Murphy, Chen, Hauck, and Wilson (1997) thought that there was a relationship between the content of 7S and 11S globulins, 11S/7S ratio, A_3, $A_{1a}A_{1b}A_2$ subunits of 11S globulin and α, α, β subunits of 7S globulin and rheological properties of the protein. In addition, Utsumi and Kinsella (1985) reported that 7S and 11S globulins of soybean produced gels with different formation mechanisms and forces involved in the gel formation. The stabilizing forces of the gels were suggested to be hydrogen bonding, hydrophobic interaction and, ionic and SS bonds. The AOT reverse micellar system might be involved in the gel formation process, because the reverse micelle would influence the structural changes of 7S and 11S globulins.

4. Conclusion

Experimental results showed that AOT reverse micelle would affect surface hydrophobicity, SH groups, SS bonds and, thermal and rheological properties of 7S and 11S globulins from soybean. The surface hydrophobicity of 7S and 11S globulins using AOT reverse micelle was augmented, sulfhydryl (SH) groups increased, disulfide (SS) bonds of 7S globulin decreased, while disulfide bonds of 11S globulin increased. The thermal and rheological properties of 7S and 11S globulins using the two extraction methods were studied by differential scanning calorimetry (DSC) and rheometery. It was found that the peak denaturation temperature (T_D 97.41 and 99.51 °C) and heat of transition or enthalpy (ΔH 9.68 and 18.66 J/g) of 7S and 11S globulins with aqueous buffer extraction were different from that with AOT reverse micellar extraction (T_D 98.47 and 101.02 °C; ΔH 12.24 and 19.85 J/g). Gel properties of 11S globulin using AOT reverse micelle and aqueous buffer extraction, namely, hardness, springiness, gumminess, adhesiveness, gumminess and chewiness, were not significantly different. Moreover, the AOT reverse micelle could influence the gel properties of 7S globulin. Hardness, springiness, gumminess, adhesiveness and chewiness of 7S globulin were lower than that with aqueous buffer extraction, which decreased by 33.71%, 35.44%, 84.36%, 14.17% and 45.28%, respectively, but gumminess increased by 6%.

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References

Beveridge, T., Toma, S. J., & Nakai, S. (1974). Determination of SH- and SS-groups in some food proteins using Ellman's reagent. *Journal of Food Science, 39*, 49–51.
Biliaderis, C. G. (1983). Differential scanning calorimetry in food research: A review. *Food Chemistry, 10*, 239–265.
Christopher, D. J., Yarwood, J., Belton, P. S., & Hills, B. P. (1992). A Fourier transform infrared study of water-head group interactions in reversed micelles containing sodium bis(2-ethylhexyl) sulfosuccinate (AOT). *Journal of Colloid and Interface Science, 152*, 465–471.
Correa, N. M., Durantini, E. N., & Silber, J. J. (1998). Binding of nitrodiphenylamines to reverse micelles of AOT in *n*-hexane and carbon tetrachloride: Solvent and substituent effects. *Journal of Colloid and Interface Science, 208*, 96–103.
Fukushima, D. (1991). Recent progress of soybean protein foods: Chemistry, technology, and nutrition. *Food Reviews International, 7*, 323–351.
Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004). Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science, 69*, 114–118.
Hua, Y. F., Cui, S. W., Wang, Q., Mine, Y., & Poysa, V. (2005). Heat induced gelling properties of soy protein isolates prepared from different defatted soybean flours. *Food Research International, 38*, 377–383.
Jackman, R. L., & Yada, R. Y. (1989). Ultraviolet absorption and fluorescence properties of whey-potato and whey-pea protein composites. *Canadian Institute Food Science and Technology Journal, 22*, 252–259.
Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe methods and its correlation with surface properties of proteins. *Biochimica et Biophysica Acta, 624*, 13–20.
Koshiyama, I. (1972). Purification and physico-chemical properties of 11S globulin in soy bean seeds. *International Journal of Peptide and Protein Research, 4*, 167–176.
Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–686.
Leser, M. E., & Luisi, P. L. (1989). The use of reverse micelles for the simultaneous extraction of oil and proteins from vegetable meal. *Biotechnology and Bioengineering, 34*, 1140–1146.
Ma, C. Y., & Harwelkar, V. R. (1991). Thermal analysis of food proteins. Advanced Food Nutrition Research. *35*, 317–366.

Matzke, S. F., Creagh, . A. L., Haynes, L. C., Prausnitz, J. M., & Blanch, H. W. (1992). Mechanisms of protein solubilization in reverse micelles. *Biotechnology and Bioengineering, 40*, 91–102.

Mohamed, A. A. (2002). Effect of corn oil and amylase on the thermal properties of native soy protein and commercial soy protein isolate. *Food Chemistry, 78*, 291–303.

Mohamed, A. A., & Xu, J. Y. (2003). Effect of ionic strength and pH on the thermal and rheological properties of soy protein–amylopectin blend. *Food Chemistry, 83*, 227–236.

Murphy, P. A., Chen, H. P., Hauck, C. C., & Wilson, L. (1997). Soybean protein composition and tofu quality. *Food Technology, 51*(1), 86–88.

Nagano, T., Hirotsuka, M., Mori, H., Kohyama, K., & Nishinari, K. (1992). Dynamic viscoelastic study on the gelation of 7S globulin from soybeans. *Journal of Agricultural and Food Chemistry, 40*, 941–944.

Nguyen, J., Baldwin, M. A., Cohen, F. E., & Prusiner, S. B. (1995). Prion protein peptides induce α-helix to β-sheet conformational transitions. *Biochemistry, 34*, 4186–4192.

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartener, F. H., Provenzano, M. D., et al. (1985). Measurement of protein using bichinchoninic acid. *Analytical Biochemistry, 150*, 76–85.

Stephanie, R. D., Thorsten, B., Alan, H. T., Pawel, P., & Walter, N. (1991). Interfacial transport processes in the reverse micellar extraction of proteins. *Journal of Colloid and Interface Science, 145*, 33–50.

Tang, C. H., Chen, Z., Li, L., & Yang, X. Q. (2006). Effects of transglutaminase treatment on the thermal properties of soy protein isolates. *Food Research International, 39*, 704–711.

Tay, S. T., Xu, G. Q., & Perera, C. O. (2005). Aggregation profile of 11S, 7S and 2S coagulated with GDL. *Food Chemistry, 91*, 457–462.

Utsumi, S., Matsumura, Y., & Mori, T. (1997). Structure function relationships of soy proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 257–291). New York: Marcel Dekker.

Utsumi, S., & Kinsella, J. (1985). Forces involved in soy protein gelation: Effect of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, and 11S, and soy isolate. *Journal of Food Science, 50*, 1279–1282.

Vassiliki, P., Aristotelis, X., & Athanasios, E. E. (1993). Proteolytic activity in various water-in-oil microemulsions as related to the polarity of the reaction medium. *Colloid Surfaces B, 1*, 295–303.

Xu, J., Bietz, J. A., Felker, F. C., Carrierem, C. J., & Wirtz, D. (2001). Rheological properties of vital wheat gluten suspension. *Cereal Chemistry, 78*(2), 181–185.

Yagasaki, K., Takagi, T., Sakai, M., & Kitamura, K. (1997). Biochemical characterization of soybean protein consisting of different subunits of glycinin. *Journal of Agriculture and Food Chemistry, 45*, 656–660.

Zhao, X. Y., Chen, F. S., Xue, W. T., & Lee, L. T. (2008). FTIR spectra studies on the secondary structures of 7S and 11S globulins from soybean proteins using AOT reverse micellar extraction. *Food Hydrocolloids, 22*, 568–575.