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Analysis of the Amino Acids of Soy Globulins by AOT Reverse Micelles and Aqueous Buffer

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Abstract The 7S and 11S globulins from soybean proteins using reverse micelle and aqueous buffer extraction methods were characterized by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and scanning electron microscope (SEM), and their amino acid compositions were also evaluated. SDS-PAGE did not show electrophoretic differences between 7S and 11S globulin subunits with two extraction methods. SEM analysis showed that the AOT reverse micelle processing of 7S and 11S globulins induced a reduction of droplet size. Some individual amino acid contents of 7S and 11S globulins using two extraction methods were different, some were similar. In all the samples, the glutamic acid, aspartic acid, and leucine were the dominant amino acids while the cystine and methionine were the first-limiting amino acids. The proportion of essential amino acids to the total amino acids (E/T) of the 7S globulin from aqueous buffer and reverse micelles was similar. While significant differences were obtained in the proportion of E/T of the 11S globulin.

Keywords Reverse micelles · Soybean · 7S and 11S globulins · Amino acids

Introduction

As a food group, legumes make a major contribution to the human diet as a good source of protein, while soybean is one of the main legume crops. The use of soy protein products as functional ingredients is gaining increasing acceptance in food manufacturing due to their high nutritional value, functional properties and low cost.

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The soybean proteins contain four types of globulins, namely, 2S, 7S, 11S, and 15S proteins which are characterized by their sedimentation coefficients. The percentage contents of 2S, 7S, 11S, and 15S were 15%, 34%, 41.9%, and 9.1%, respectively [1]. Because the 7S and 11S globulins greatly impact on the nutritional value and quality of soybean protein products, the two storage proteins have been studied extensively, and their primary and crystal structures have also been determined [2, 3]. Soybean storage proteins, like many globulins from other species, characteristically have a good balance of the essential amino acids required by humans and animals. The 7S and 11S globulins have different amino acid contents, which may be due to the effect of molecular structures on amino acid compositions and sequences [3, 4]. Adequate modification on soy proteins has brought out the changes of different amino acid contents that the native soy proteins do not possess and improve their functional and nutritional properties [5, 6]. So, a detailed investigation of 7S and 11S globulins is necessary to elucidate the amino acids of soybean proteins.

The effect of different treatments on the amino acids of soybean proteins has widely been studied [5, 7, 8]. The different amino acid content of treated soy proteins may well have different functional properties and protein digestibility compared with the native soy proteins [8]. The change of the amino acid compositions from 7S and 11S globulins depends on the different treatment methods, including physical (heat or mild alkali treatment), chemical (acylation, phosphorylation, and deamidation), high pressure treatment, and enzymatic hydrolysate methods, which may, thus, affect the functional and nutritional properties of soy proteins [5, 8].

While very limited information is available on the effect of the reverse micelle system on the amino acid compositions of 7S and 11S globulins. A considerable volume of work has been published on the proteins dissolved in reverse micelles [9, 10]. It can also be utilized to recover proteins from the aqueous phase on a large scale through phase–phase extraction [11]. Moreover, the use of reverse micelles for the simultaneous production of oil and proteins from soybean is attractive since soybean represents one of the major oilseeds for producing edible oils [12].

In reverse micelles, the main driving forces responsible for the solute distribution between the organized assembly and the organic medium are considered to be mainly hydrophobic effects, hydrogen bonding interaction and electrostatic interaction, which may affect the physical–chemical properties of proteins [13]. Besides other factors, pH and ionic strength in reverse micelles [9] may also affect the physical–chemical properties of proteins. The most conventional reverse micellar system studied was with bis(2-ethylhexyl) sodium sulfosuccinate (AOT) as amphiphilic surfactant and with isooctane as organic solvent. The structure changes of 7S and 11S globulins from AOT reverse micelles have been quite extensively investigated [14], which can influence the functional properties of 7S and 11S globulins [15]. However, information about the effect of AOT reverse micelles on the amino acid compositions of 7S and 11S globulins is still very limited. The amino acids play important roles in determining nutritional and digestibility properties of protein products [16]. So, it is important to study the effect of the reverse micelles on the amino acid compositions 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 reverse micellar system on the amino acid compositions of 7S and 11S globulins for assessing the level of amino acids and soybean protein quality. Comparison was made between the amino acid compositions of 7S and 11S globulins from aqueous buffer and reverse micelle extraction methods.

Materials and Methods

Materials

AOT was bought from Sigma Chemical Company (>98%). Bicinchoninic acid (BCA) protein assay kit was purchased from USA Pierce Company. All other reagents were of analytical grade. Soybean (tube no. 8) was obtained from the China Academy of Agricultural Sciences (Beijing, China). The soy flour was sieved through a 100 screen; flour composition was as follows: total protein, 37.53% (by Kjeldhal, $N\times6.25$); fat, 20.75%; humidity, 7.99%; and ash, 6.5% [17]. All values are given in wt.% of the total weight of the flour.

AOT Reverse Micellar Extraction

AOT reverse micelles were used to isolate protein from soy flour [18]. Stock solution of 0.05 M AOT was obtained first by AOT mixing with isooctane and stirring at room temperature. 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 pH 7.5 containing 0.05 M KCl, $W_0=18$. Then the forward extraction was prepared by adding soybean flour to the AOT reverse micellar 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 6,000 rpm for 10 min; the clear supernatant solution was named the forward extraction and used for the next extraction step. Two hundred milliliters of this solution was carefully laid on 200 ml of 1 M KCl phosphate buffer (50 mM) at pH 7.5 and conducted in a magnetically agitated Erlenmeyer flask at ambient for 1 h. The resulting mixture was centrifuged at 4,000 rpm for 10 min. Then two phases were separated, namely oil phase and aqueous phase. The aqueous phase was named the backward extraction, and stored until the next experiment. The protein content in the aqueous phase was measured with BCA method (microplate procedure, microplate reader 550: Japan Bio-Rad Company) [19].

Aqueous Buffer Solution Extraction

The proteins were isolated by modified method of Nagano et al. [20]. The defatted soybean flour was dispersed in 50 mM phosphate buffer (1:20, *w*/*w*), adjusted to pH 7.5 with 2 M NaOH, and stirred at 45 °C for 2 h. The resulting mixture was then centrifuged at 12,000 rpm at 20 °C for 20 min. The supernatant solution was used for further investigation. It was worthwhile to note that the centrifugation of reverse micellar suspension needed less energy, speed, and time than the aqueous suspension.

Isolation of 7S and 11S Globulins

The 7S and 11S globulin fractions were isolated by modified method of Nagano et al. [20]. The protein solution with AOT reverse micellar extraction was dialyzed at 4 °C for 24 h to remove impurities and excess reagents. 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 ice bath overnight (about 20 h). The following preparation procedure was performed at 4 °C. The dispersion was centrifuged at 10,000 rpm for 10 min. The clear supernatant solution was used for preparing 7S globulin. The precipitate (11S fraction) was

suspended in 50 mM phosphate buffer (pH 7.5) then purified with ammonium sulfate. The insoluble fraction was obtained by centrifugation at 8,000 rpm for 15 min; the precipitate was suspended in 1 mM phosphate buffer (pH 7.5), dialyzed, and lyophilized. The 11S globulin powder was obtained.

For the preparation of 7S globulin, the salt concentration of clear supernatant was 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 rpm 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 at 10,000 rpm for 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, dialyzed before freeze-drying process. Protein content of this lyophilized powder was 95.43% determined by the BCA method [19].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to examine the purification of 7S and 11S globulins. SDS-PAGE was performed according to the method of Laemmli [21], 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 at pH 8.3 with the addition of 0.15% SDS, for the running buffer, were used. The 7S and 11S globulins (10 mg/ml) were treated with 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, cooled. 5 μl sample solutions of 7S and 11S globulins were loaded. The gel was stained in 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 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).

Scanning Electron Microscopy

The 7S and 11S globulin samples were mounted on metal stubs with double-sided adhesive tape. Then the samples were sputtered with 150 Å thick layer of gold in Bio-Rad sputter apparatus. The images were taken by scanning electron microscope (SEM; KYKY 2800, China). Then, the structure of 7S and 11S globulins was observed by SEM.

Analysis of Amino Acid Profiles

Amino acid analyses were carried out on an automatic amino acid analyzer (L8500A, Japan) with ion-exchange chromatography column (4.6×60 mm i.d.) and removing ammonia column (Φ4.6×40 mm). The buffering system with 0.2 M citric acid sodium (pH 2.2) was used as a mobile phase. Ninhydrin (Sigma Chemical Co., St Louis, MO, USA) was used for the post-column derivatization of amino acids, and absorption was measured at 546 nm. Proline was simultaneously detected at 436 nm. The column was held at 57 °C and the post-column reaction coil at 137 °C. Amino acids were eluted by a gradient control from buffer solution at 0.4 ml/min. A 20 μm sample was applied to the column. Ninhydrin was pumped at a constant flow rate of 0.35 ml/min. Norleucine (Sigma Chemical Co., St Louis, MO, USA) was added in the

hydrolysate as an internal standard. Amino acids were quantified by comparing areas under the curve with those of the internal standard (norleucine).

The 7S and 11S globulin samples (0.0500 g) were hydrolyzed in Pyrex tubes (20 cm× 150 mm) in 10 mL of 6 M HCl at 110 °C for 22–24 h in vacuum (below 10 mmHg) for all amino acids except sulfur amino acids with the usual precautions described previously [22]. Each hydrolysate was dried under vacuum on a rotary evaporator at 60 °C. The dried mass was washed twice with distilled water, evaporated to dryness between washings, and made an appropriate volume with 0.2 M citric acid sodium buffer solution (pH 2.2). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate by methods described previously [23]. The sample solution was passed through a 0.45 μm cellulose acetate filter prior to analysis. Methionine and cysteine were measured after oxidation with performic acid, followed by acid hydrolysis [23, 24]. Tryptophan could not be measured because of its degradation during acid hydrolysis. All determinations were carried out in two replications for each experiment.

The contents of amino acids were calculated as follows [25, 26]:

$$X = \frac{c \times \frac{1}{50} \times F \times V \times M}{m \times 10^9} \times 100 \tag{1}$$

The X indicated the content of amino acid in sample, the unit was g/100 g; the c indicated the concentration of amino acid in sample determination solution, the unit was nmol/50 μL; the F indicated the dilution factor; the V indicated the fixed volume of hydrolyzed sample, the unit was ml; the M indicated the molecular weight of amino acid; the m indicated the mass of sample, the unit was g; the 1/50 indicated the content of amino acid in per milliliter determination solution, the unit was nmol/ml; the 10^9 indicated the conversion coefficient of sample content unit.

The amino acid score was calculated according to the scoring pattern, as suggested by FAO/WHO [27]. The amino acid concentration of the tested protein was compared with the scoring pattern and expressed as grams of amino acid/16 gN in the test protein/g of amino acid/16 gN in the scoring pattern. Essential amino acid (EAA) index was calculated according to Oser [25], using the amino acid composition of whole egg protein published by Hidvegi and Bekes [26]. The essential amino acid score was calculated as following:

$$\text{Essential amino acid score} = \frac{\text{g of essential amino acid in 100g of the test protein}}{\text{g of essential amino acid in 100g FAO/WHO (1991) reference pattern}} \tag{2}$$

Statistical Analysis

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

Results and Discussion

Isolation of 7S and 11S Globulins

The results showed that it was impossible to directly isolate 7S and 11S globulins of soybean proteins with AOT reverse micellar extraction by Nagano et al. method [20]. The

ionic strength (the ionic concentration was 1 M) in backward extraction of proteins with AOT reverse micelle extraction would affect the isoelectric of 7S and 11S globulins, which would hinder the separation of 7S and 11S globulins. According to the method of Koshiyama [28], when ionic strengthen was more than 0.5, the pH should be adjusted to 2.0, the 11S globulin would be completely separated. However, it was difficult to isolate 7S and 11S globulins properly of soybean proteins by this method.

It could be concluded from experiment that the backward extraction of protein 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 down all disulfide bonds. Therefore 0.0094 M SBS was used, which facilitated the separation of 11S globulin. The amount of reducing agent was required to give a good purity of 11S globulin and a high yield of 7S globulin (Fig. 1). SDS-PAGE analysis showed that aqueous buffer and reverse micelles treated 7S and 11S globulins mainly presented the typical soybean protein subunits of β-conglycinin (7S): α′, α, and β and glycinin (11S): A and B.

SEM Images of 7S and 11S Globulins

Compared with SEM images of 7S and 11S globulins using aqueous buffer extraction (Fig. 2a, b), the size of the aperture for 7S and 11S globulins using AOT reverse micelle extraction was smaller (Fig. 2c, d). The aperture size means the particle size of pore in proteins. The resulted indicated that the reverse micelles could affect the aperture sizes of 7S and 11S globulins. Because the 7S and 11S globulin proteins and AOT surfactant contained the positive and negative charges, which could result in hydrophilicity or hydrophobicity interaction between surfactant and protein in reverse micelle solution (pH 7.5). The structure of proteins would be changed [29]. At the same time, the interaction would lead to protein/surfactant complexes, which were different from those of the native proteins [30]. So the size of protein dissolved in reverse micelles might be affected by AOT. In addition, the W_0 value in reverse micellar system would influence on the aperture size of

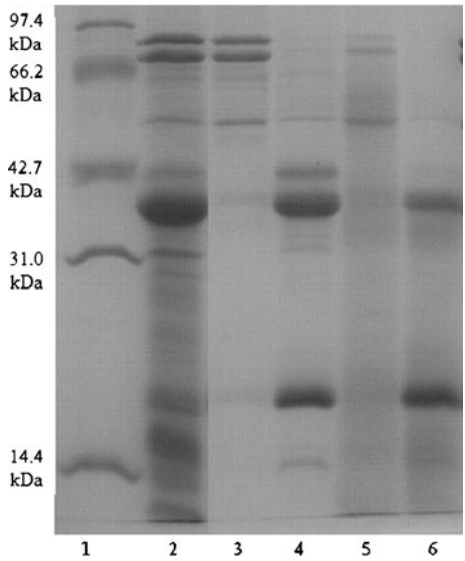

Fig. 1 SDS-polyacrylamide gel electrophoresis of 7S and 11S globulin fractions. *Lane 1*, molecular weight markers; *lane 2*, SPI; *lanes 3* and *4*, 7S and 11S globulins by aqueous buffer extraction; *lanes 5* and *6*, 7S and 11S globulins by AOT reverse micellar extraction

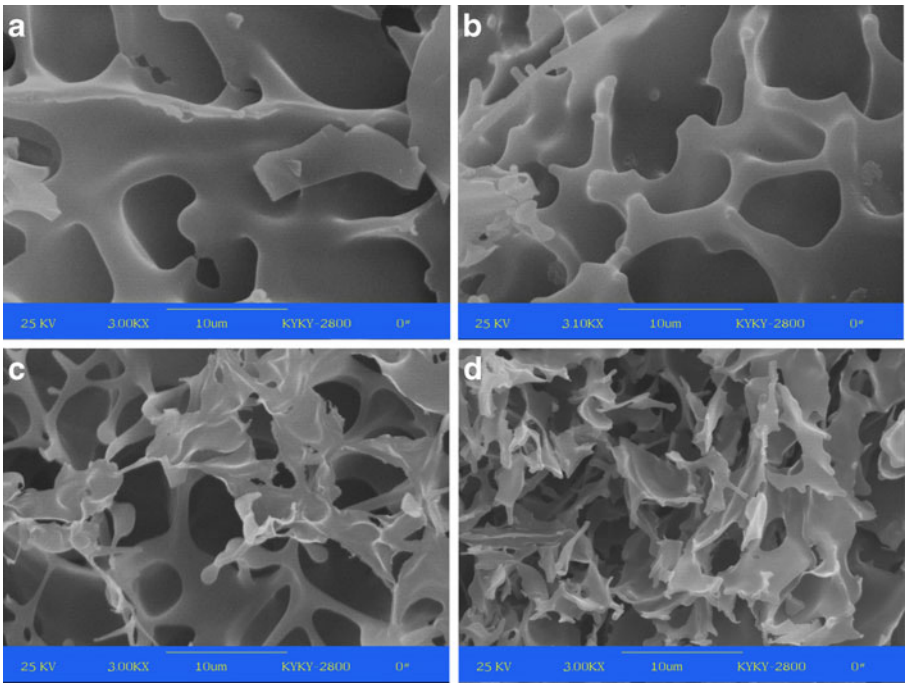

Fig. 2 Scanning electron microscope of 7S and 11S globulins. **a**, **b** 7S and 11S globulins by aqueous buffer extraction. **c**, **d** 7S and 11S globulins by AOT reverse micellar extraction

the solubilized protein molecules. There was a strong relation between the incorporated protein size and the reverse micelle size, when the micelle size was comparable to the protein dimension, the structure of proteins was more affected by the water content [31]. Other factors in reverse micelles, such as pH value and ion strength, etc., would affect the aperture size of 7S and 11S globulins.

Analysis of Amino Acid Compositions of 7S and 11S Globulins

The amino acid profiles of the 7S and 11S globulins from soybean using AOT reverse micelles and aqueous buffer extraction methods are showed in Table 1. The 7S and 11S globulins using two extraction methods were very similar in amino acid compositions (g/100 g of protein). But the contents of some individual amino acids for 7S and 11S globulins with two extraction methods were different. The side chains of some protein-bound amino acids of 7S and 11S globulins in AOT reverse micellar system could react chemically with each other or other molecules, resulting in a decrease or increase in nutritive value. Gayler and Sykes [32] reported that the change of microenvironment around the 7S and 11S globulin molecules could cause the changes of amino acid contents. At least four factors contributed to the changes of amino acid contents of 7S and 11S globulins. Firstly, the unusual properties of water localized in the interior of reverse micelles [7] could bring about stronger interaction with the charged groups of amino acids on surface of the neighboring 7S and 11S globulin molecules than by the aqueous buffer extraction. Secondly, the interaction of AOT and proteins (7S and 11S globulins) could change not only the constituents of 7S and 11S globulins, but also the amino acid

Table 1 Amino acid compositions of 7S and 11S globulins by aqueous phase and AOT reverse micelle extraction (g/100 g protein)

Amino acid	Globulins with aqueous buffer extraction		Globulins with AOT reverse micelle extraction	
	7S	11S	7S	11S
Nonessential amino acids (NEAA)				
Aspartic acid	11.31±0.01	10.73±0.01	11.30±0.02	11.75±0.02
Serine	4.40±0.01	4.44±0.01	4.38±0.01	5.17±0.01
Glutamic acid	20.78±0.02	18.59±0.05	22.24±0.01	21.98±0.03
Glycine	2.64±0.05	3.68±0.01	2.18±0.01	4.17±0.01
Alanine	2.66±0.02	3.17±0.02	2.29±0.02	3.48±0.01
Cystine	0.90±0.01	1.48±0.01	0.55±0.01	1.42±0.01
Tyrosine	2.77±0.02	3.32±0.01	2.58±0.01	3.62±0.01
Proline	4.37±0.02	4.69±0.01	4.47±0.02	4.98±0.01
Arginine	7.45±0.03	6.51±0.03	7.93±0.04	6.69±0.01
Histidine	2.41±0.01	2.38±0.01	2.22±0.01	2.55±0.01
Total NEAA	59.6	58.99	60.14	65.81
Essential amino acids (EAA)				
Leucine	7.33±0.01	6.86±0.03	7.40±0.03	7.75±0.02
Valine	3.51±0.01	4.03±0.02	3.00±0.02	4.05±0.01
Phenylalanine	5.43±0.01	4.66±0.02	5.58±0.02	5.21±0.03
Lysine	5.52±0.02	4.56±0.01	5.65±0.01	4.93±0.03
Isoleucine	3.78±0.02	3.54±0.01	3.77±0.03	3.70±0.03
Methionine	0.56±0.01	1.09±0.01	0.26±0.01	1.04±0.01
Threonine	2.15±0.03	3.05±0.01	1.59±0.02	3.27±0.02
Total EAA	28.28	27.79	27.25	29.95
EAAI	0.31	0.64	0.11	0.93

compositions of 7S and 11S globulins. Thirdly, the interaction between proteins (7S and 11S globulins) and isooctane in AOT reverse micellar system could also result in the transferring among amino acids [13]. Last but not the least, the state of water and water-head group interaction in AOT/isooctane micro-emulsion could also change the amino acid compositions of 7S and 11S globulins [33]. Furthermore, solvent (such as, isooctane) of low dielectric constant and the co-effect of these factors had changed the protein environment, which either made the 7S and 11S globulins change the contents of the amide acids. The amino acids of 7S and 11S globulins changed with environment of proteins changed, the results agreed with the study of Correa et al. [13].

In the 7S globulin using two extraction methods, the dominant amino acids were glutamic acid and aspartic acid. In comparison, the glutamic acid proportion of the total amino acid content (22.24%) in AOT reverse micelles was higher than in aqueous buffer solution (20.78%) while the proportion of aspartic acid was very similar (11.31% and 11.30%, respectively). The proportion of agrinine, leucine, lysine and phenylalanine was in the range of 5–8%; that of tyrosine, proline, threonine, histidine, serine, valine, isoleucine, alancine, and glycine varied from 2% to 5%. The lowest proportions were in the sulfur containing amino acids, cystine (0.90% in aqueoues buffer and 0.55% in AOT reverse micelles) and methionine (0.56% in aqueoues buffer and 0.26% in AOT reverse micelles)

(Table 1). The results were consistent with the previous data of these amino acids [23, 34, 35]. The cysteine and methionine contents of 7S globulin with AOT reverse micelle extraction were remarkably lower than that of aqueous buffer extraction. The loss of cysteine and methionine could be related to the special microenvironment of AOT reverse micelle. The relative contribution of serine, glycine, alanine, tyrosine, isoleucine, valine, threonine, and histidine of 7S globulin using aqueous buffer extraction was higher compared with that of AOT reverse micelle extraction, while the contribution of agrinine, proline, leucine, phenylalanine, and lysine was lower. The proportion of EAA to the total amino acids (E/T) for 7S globulin using aqueous buffer extraction was significantly higher than using AOT reverse micelle extraction, suggesting that the 7S globulin in aqueous buffer solution had more nutritional amino acid pattern than that of 7S globulin in AOT reverse micelles (Table 1). However, the difference in total amino acid content of 7S globulin using two extraction methods was not significant.

Like 7S globulin, in the 11S globulin, the highest proportion in all amino acids was the glutamic acid, the proportions of the total amino acid content were 18.59% (aqueous buffer extraction) and 21.98% (AOT reverse micellar extraction), respectively. Aspartic acid came next, the proportions of the total amino acid content using aqueous buffer and reverse micelle extraction and were 10.73% and 11.75%, respectively. The results indicated that the glutamic acid and aspartic acid of 11S globulin using two extraction methods were the most abundant amino acids. By Comparison with the 11S globulin using the aqueous buffer extraction, it was the most interesting that the contents of glutamic acid and aspartic acid were significantly higher than that of AOT reverse micellar extraction. The proportion of agrinine, leucine, lysine, valine, serine, and phenylalanine of 11S globulin was in the range of 4–8%; that of tyrosine, proline, threonine, histidine, isoleucine, alancine, and glycine varied from 2% to 4%. The lowest amino acid amounts among the amino acid residues were methionine (1.09% in aqueous buffer solution and 1.04% in AOT reverse micelles) and cysteine (1.48% in aqueous buffer solution and 1.42% in AOT reverse micelles) (Table 1). The results agreed with the study of Zarkadas et al. [24] and Fathi Nasri et al. [8]. The contents of some individual amino acids in the 11S globulin using AOT reverse micellar extraction also increased for each amino acid analyzed, except for the contents of valine, cystine, and methionine.

The proportion of EAA of total amino acid content from 7S globulin using aqueous buffer extraction was 27.79% and increased to 29.95% using AOT reverse micelle extraction (Table 1). The proportion of EAA to the total amino acids (E/T) for 11S globulin in aqueous buffer solution was significantly lower than in AOT reverse micelles, suggesting that the 11S globulin using aqueous buffer extraction have less nutritional amino acid pattern than using AOT reverse micelle extraction (Table 1). However, the total content of amino acids in the AOT reverse micellar system was also significantly higher than in the aqueous buffer solution. Be compared with the content of amino acids of 7S and 11S globulins using aqueous buffer extraction, the content of total amino acids in AOT reverse micellar system increased 5.98%, indicating that the AOT reverse micelles could improve the content of total amino acids of 11S globulin from soybean.

The essential amino acid index of 7S and 11S globulins with aqueous buffer extraction was 0.31 and 0.64, respectively, corresponding to 0.11 and 0.93 using AOT reverse micelle extraction, respectively. There was a dramatic decrease for EAAI of 7S globulin by AOT reverse micelle extraction, but a dramatic improvement for EAAI of 11S globulin. These results revealed that the11S globulin using AOT reverse micelle extraction was a very good source of amino acids.

Effect of Processing Methods on the Levels of Amino Acid Profiles

The essential amino acid profiles of the 7S and 11S globulins with two extraction methods investigated were compared with those of the FAO/WHO [27] reference pattern, and the results were summarized in Table 2. To assess the potential food value of the soybean proteins as the source of amino acids, the levels of essential amino acids (valine, cystine, methionine, threonine, phenylalanine, tyrosine, isoleucine, leucine, histidine, lysine, and tryptophan) of soybean proteins had to be compared with FAO/WHO [27] requirement pattern.

The first-limiting amino acids in soybean proteins were, depending on the source, the sulfur amino acids containing cystine and methionine [23, 24]. In the 7S and 11S globulins using two extraction methods investigated in the present study, cystine and methionine were the 1st limiting amino acids with respect to protein quality (Table 2). The chemical score (CS) index for 7S globulin using aqueous buffer and AOT reverse micellar extraction was 42 and 23, respectively; for 11S globulin, 73 and 70, respectively. The 2nd-limiting amino acid was threonine. The lowest CS value of 23 for cystine and methionine was found in 7S globulin using AOT reverse micellar extraction; for the remaining samples, the CS index varied in the range of 42–73.

The total quality of protein could be expressed by an EAA index. The lowest, 78, was found for the 7S globulin with AOT reverse micelle extraction; the highest, 86, was for the 11S globulin with AOT reverse micellar extraction, while values for the remaining samples were broadly similar at 79–80.

Conclusions

The 7S and 11S globulins using aqueous buffer and AOT reverse micellar extraction were mainly composed of α′, α, β, A and B subunits. In Comparing with 7S and 11S globulins using aqueous buffer extraction, the size of the aperture of 7S and 11S globulins using AOT reverse micelle extraction was smaller. The results suggested that AOT reverse micelles could not affect the subunit bonds of 7S and 11S globulins but influence the particle size of proteins.

Table 2 Amino acid score of 7S and 11S globulins by aqueous phase and AOT reverse micelle extraction

Index	Amino acid	Globulins with aqueous buffer extraction		Globulins with AOT reverse micelle extraction	
		7S	11S	7S	11S
CS	Leu	105	98	106	111
	Val	70	81	60	81
	Phe+Tyr	137	133	136	147
	Lys	100	83	103	90
	Ile	95	88	94	92
	Met+Cys	42	73	23	70
	Thr	54	76	40	82
EAA		80	79	78	86

CS chemical score index, *EAA* essential amino acid index

Be compared with amino acid compositions of 11S globulin using aqueous buffer extraction, the content of total amino acids using AOT reverse micelle extraction increased 5.98% but 7S globulin was similar. For 7S and 11S globulins, the contents of main amino acids in aqueous buffer solution were lower than in AOT reverse micelles. Essential amino acids contents as a proportion of total amino acids for 7S and 11S globulins using aqueous buffer extraction were 28.28% and 27.29%, respectively, while were 27.25% and 29.95% using AOT reverse micellar extraction. It was concluded that the proportion of E/T in 7S globulin using two extraction methods was similar. However, the proportion of E/T of 11S globulin using AOT reverse micelle extraction was significantly higher as compared with aqueous buffer extraction. According to Zarkadas et al. [23, 24] and Grieshop et al. [33], soybean was a good source of amino acids. the 1st-limiting amino acids were cystine and methionine (CS 42 and 73 for 7S and 11S globulins in aqueous buffer, 23 and 70 for 7S and 11S globulins in AOT reverse micelles) and the 2nd threonine (CS 54 and 73 for 7S and 11S globulins in aqueous buffer, 40 and 82 for 7S and 11S globulins in AOT reverse micelles). The present investigation suggested that soybean proteins using AOT reverse micelle extraction could be suitable for human consumption as a more superior source of protein nutrition.

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