# **Emulsifying Properties of Acidic Subunits of Soy 11S Globulin**

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The emulsifying properties of the acidic subunits (AS11S) isolated from soy glycinin (11S) have been studied. The isolated AS11S existed in solution mainly as a dimer species. Circular dichroic analysis indicated only a slight increase in aperiodic structure and no significant difference in  $\beta$ -sheet structure when compared with those of soy 11S. At similar experimental conditions, the emulsifying properties of AS11S were superior to those of soy 11S and heat-denatured 11S. Emulsions prepared with 1% AS11S remained very stable without any visible oil separation for more than a month under gentle agitating conditions, whereas those prepared with 1% 11S collapsed and separated into phases within 2–3 days. The AS11S-stabilized emulsions were very stable below 0.15 M ionic strength. Studies on the rate of adsorption and surface tension reduction at the air–water interface showed that AS11S was significantly more surface active than soy 11S. It is proposed that, because the mass fraction of acidic subunits in soy 11S is ~60% and it is relatively easy to separate the acidic subunits from soy 11S, it may be industrially feasible to develop an economical process to isolate functional acidic subunits for use in emulsion-based food products.

**Keywords:** Soy proteins; glycinin; acidic subunits of soy 11S; emulsifying properties; emulsion stability

## INTRODUCTION

Soy protein is increasingly being used as a functional ingredient in processed foods. Because of its excellent heat-induced gelling properties, it is being used extensively in texturized protein products and as binder and emulsifier in comminuted meat products. However, although soy protein isolate functions reasonably well as an emulsifier in concentrated emulsions, such as meat emulsions, its use as a surface active agent in dilute emulsion and foam type products is very limited.

The emulsifying properties of soy proteins have been extensively studied (Tornberg, 1978; Stone and Campbell, 1980; McWatters and Holmes, 1979). At low bulk concentrations, adsorption of soy proteins at the oilwater interface is relatively low compared to that of casein. For instance, whereas an oil-in-water emulsion can be produced using a 0.05% casein solution (Liu et al., 1999), it is not possible with a 0.05% solution of soy proteins. However, a concentrated solution of soy protein produces a viscous emulsion by forming a cohesive film with good rheological properties. Flint and Johnson (1981) reported that at all pH values below that of the isoelectric point of the protein and up to pH 6.5, soy protein forms a skinlike cohesive film around an oil droplet. At pH > 7.4, however, formation of such a film was not evident. Thus, although soy protein may function satisfactorily as an emulsifier in the pH range below 6.5, it may not do so at higher pH.

The less-than-satisfactory surface activity of soy protein might be related to the quaternary structure of its major protein components, that is, 7S and 11S globulins, and compact tertiary structures of the subunits of these globulins. For instance, the 11S globulin is a hexamer. Each subunit consists of a basic polypeptide (~20 kDa) and an acidic polypeptide (~35 kDa) linked together via a disulfide cross-link (Staswick et al., 1984). On the other hand, the 7S globulin is a trimer made up of  $\alpha'$  (~72 kDa),  $\alpha$  (~68 kDa), and  $\beta$  (~52 kDa) subunits (Nielsen, 1985a,b). It is likely that the poor lipophilicity of these soy globulins might be due to burial of a majority of surface hydrophobic residues at the subunit interfaces in the quaternary structures. In other words, individually, the subunits of 7S and 11S globulins may possess good lipophilicity, but this is lost as a result of quaternary structure formation.

In the present study, we isolated the acidic subunits of 11S globulin and studied its emulsifying properties. We report that the isolated acidic subunits display excellent emulsifying properties when compared to those of intact soy 11S.

### MATERIALS AND METHODS

Isolation of Acidic Subunits of Soy 11S Globulin. Soy 11S was isolated from a low-heat-treated and defatted soy meal obtained from Central Soya Co. (Chicago, IL) as described by Thanh and Shibasaki (1976). The acidic subunits (AS11S) of soy 11S globulin were separated from intact 11S according to the method of Damodaran and Kinsella (1982). Briefly, a 0.5% solution of soy 11S in 30 mM Tris-HCl buffer (pH 8.0) containing 10–20 mM  $\beta$ -mercaptoethanol was heated at 85–90 °C for 30 min, followed by centrifugation for 10 min at 10000g and at 4 °C. The supernatant, which contained only AS11S, was filtered through a 0.45  $\mu$ m Millipore membrane (Millipore Corp., Bedford, MA) and lyophilized.

**Solubility.** The pH solubility profile of AS11S isolate was determined by measuring the turbidity of a 0.1% protein solution at 500 nm at various pH values as described by Kitabatake et al. (1985). Briefly, the protein (0.1%) was dissolved in 10 mM Tris. The pH was adjusted to various values by incremental addition of 2.5 N HCl. After each addition of HCl, the pH and the volume of HCl added were recorded and the percent transmittance of the solution at 500

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nm was measured. The transmittance values were corrected for the dilution caused by the volume of HCl added.

**Gel Permeation Chromatography (GPC).** The apparent molecular weight of the AS11S preparation was determined by GPC on a Sephacryl S-100 gel column (98.5  $\times$  1.6 cm). The column was eluted with 30 mM Tris-HCl buffer (pH 8.0) at a rate of 0.5 mL/min. The column fractions were analyzed for protein by measuring absorbance at 280 nm.

**Circular Dichroism (CD).** Far-UV CD spectroscopy measurements were made using a 0.1 cm path length cell in a computerized spectropolarimeter (On-Line Instrument Systems, Jefferson, GA). The instrument was calibrated using  $d^{-}(+)$ -10-camphorsulfonic acid. Ten scans of each sample (0.012% protein solution) were averaged, and the mean residue ellipticity ( $\theta$ ) values, expressed as degree-cm<sup>2</sup> dmol<sup>-1</sup>, were calculated using a mean residue molecular weight of 115. The spectra were corrected for the buffer (10 mM phsophate buffer, pH 7.5) baseline. The secondary structure estimates were calculated using the CDESTIMA software developed by Chang et al. (1978).

**SDS**–**PAGE.** SDS–PAGE was performed using a discontinuous buffer system as described by Laemmli (1970) on a 10% slab gel. Protein samples were mixed directly with the SDS–PAGE sample buffer solution containing 5%  $\beta$ -mercaptoethanol, 2% SDS, 5% glycerol, and 0.05% bromphenol blue. About 50  $\mu$ g of protein was loaded per well. The gel electrophoresis was carried out at 120 V constant voltage. The gel was stained with 0.1% Coomassie Brilliant Blue-R250. Molecular weight marker proteins were run along with test samples.

**Emulsion Preparation.** Oil-in-water emulsions were prepared by vigorously stirring canola oil (16.6% v/v) with protein solution using a magnetic stirrer, followed by sonication in a Branson 450 sonifier using a microtip horn. The protein solutions (0.1–1.0%, w/v) were prepared in 10 mM Tris-HCl buffer (pH 7.0) containing 0.02% NaN<sub>3</sub>. In all emulsion preparations, an energy input of 50 W (setting 2 on the sonifier) and an emulsification time of 3 min were used as standard conditions. During emulsification, the temperature of emulsions was kept constant at 25 °C by circulating water from a thermostated water bath. In experiments related to the effect of ionic strength on emulsion stability, the ionic strength of 10 mM Tris-HCl buffer was varied by adding NaCl.

**Determination of Protein Load at the Emulsion Interface.** An aliquot (1 mL) of freshly prepared emulsion was centrifuged in a Marathon 21K/R centrifuge (Fisher Scientific Co., Pittsburgh, PA) at 11800 rpm for 15 min at 25 °C. The bottom aqueous phase, which was transparent and apparently free of emulsion particles, was removed using a syringe, and its protein content was determined according to the Lowry et al. (1951) method. The adsorption load,  $\Gamma$  (amount adsorbed in mg/m<sup>2</sup> surface area), was calculated as

$$\Gamma = (C_{\rm i} - C_{\rm f}) V_{\rm p} / \mathbf{a} \tag{1}$$

where  $C_i$  and  $C_f$  are the initial and final protein concentrations (mg/mL) in the aqueous phase,  $V_p$  is the volume of the aqueous phase in emulsion, and **a** is the total interfacial area of the emulsion.

The interfacial area **a** was calculated on the basis of the Mie theory of light scattering, according to which the interfacial area of an emulsion is 2 times its turbidity. The turbidity, *T*, of an emulsion is given by T = 2.303 A/4, where *A* is the absorbance and 4/3 is path length. The interfacial area **a** of emulsions was determined by measuring the turbidity at 500 nm. The emulsifying activity index (EAI) of proteins was determined from the relation (Pearce and Kinsella, 1978)

$$EAI = 2T/(1 - \phi)c \tag{2}$$

where  $\phi$  is the volume fraction of the oil and *c* is the protein concentration in mg/mL.

**Particle Size Analysis.** The average size of emulsion droplets in emulsions was determined using a particle size analyzer (Model PSA 2010, Brinkmann Instruments Inc., New



**Figure 1.** SDS–PAGE profile of soy 11S (column 2) and isolated AS11S (column 3). Column 1 is molecular weight markers.

York). Prior to measurement, the emulsions were sufficiently diluted with 0.1% SDS to meet the particle number density requirement of the instrument. The average diameter  $d_{32}$  (based on volume to surface area) of emulsion particles was directly obtained from the instrument data output.

**Emulsion Stability.** An aliquot (5 mL) of each emulsion taken in a capped glass vial was kept in constant gentle agitation in a TubeRocker (American Dade, Miami, FL) placed inside an incubator maintained at 25 °C. The tube rocker speed was set at ~10 cycles per minute. Aliquots (0.5 mL) were withdrawn at different time intervals over a period of 1-10 days. These aliquots were accurately diluted with 0.1% SDS to give an absorbance value of <0.7 at 350 nm. The turbidity spectra were recorded in the visible range from 400 to 700 nm in a Beckman DU-60 spectrophotometer. The emulsion stability was analyzed according to the method of Reddy and Fogler (1981)

$$\frac{N_0}{N_t} = \frac{1}{r_0^3} \left[ \frac{\lambda^{m_t - m_0} r^{m_0 - 1}}{T_0 / T_t} \right]^{3/m_t - 1}$$
(3)

where  $N_0$  and  $N_t$  are the initial number concentrations of emulsion droplets at zero time and at time *t*, respectively;  $T_0$ and  $T_t$  are turbidities at t = 0 and t = t, respectively;  $m_0$  and  $m_t$  are the slopes of a plot of ln *T* versus ln  $\lambda$  of an emulsion (where  $\lambda$  is the wavelength) at zero time and at time *t*, respectively;  $r_0$  is the initial average droplet radius.

**Adsorption Studies.** The kinetics of adsorption of <sup>14</sup>C-labeled AS11S and 11S proteins at the air-water interface was studied using a radiotracer method (Xu and Damodaran, 1993). The proteins were radiolabeled by reductive methylation of amino groups with [<sup>14</sup>C]formaldehyde at pH 7.0 as described in detail elsewhere (Jentoft and Dearborn, 1979; Xu and Damodaran, 1992). The time-dependent increase of concentration of <sup>14</sup>C-radiolabeled proteins at the air-water interface was monitored using a radiotracer method as described elsewhere (Xu and Damodaran, 1992, 1993). The time-dependent increase of surface pressure was monitored using the Wilhelmy plate method using a thin sand-blasted platinum plate (1 cm width) hanging from an electrobalance (Cahn Instruments, Co., Escondido, CA). Both surface concentration and surface pressure solution.

### **RESULTS AND DISCUSSION**

Figure 1 shows the SDS–PAGE profile of soy 11S globulin and the isolated AS11S. The AS11S sample was practically free of any basic subunit contamination, indicating that the procedure employed for the isolation



**Figure 2.** pH solubility profiles of isolated AS11S ( $\bigcirc$ ) and unheated soy 11S ( $\bigcirc$ ) in 10 mM Tris. The protein concentration was 0.1%.



**Figure 3.** Elution profile of AS11S (bottom panel) on Sephacryl-S100 gel permeation column. The top panel represents molecular weight versus elution volume of standard proteins.

of AS11S cleaved the disulfide linkage between the acidic and basic subunits and caused precipitation of the basic subunits. Although separation of the acidic subunits from the oligomeric structure of 11S was carried out at 85–90 °C, which may have caused denaturation, the isolated AS11S showed excellent solubility above pH 6.0 and below pH 3.0 and minimum solubility in the pH range 4.5–5.0, suggesting that the isoelectric point of AS11S is in the neighborhood of 4.8 (Figure 2). On the other hand, the pH solubility profile of unheated soy 11S in 10 mM Tris showed a broader minimum solubility in the pH range 4.5–6.0, suggesting that the isoelectric point of soy 11S in 10 mM Tris lies somewhere between 5.5 and 6.0.

To determine if the isolated AS11S existed in solution as monomers or in an oligomeric state, its apparent molecular weight was determined by GPC. The elution profile of AS11S on a Sephacryl S-100 column is shown in Figure 3. The elution profile contained a major peak corresponding to a molecular weight of  $\sim$ 78.5 kDa and a minor shoulder corresponding to a molecular weight of  $\sim$ 54.8 kDa. These molecular weights do not correspond to the molecular weights of individual acidic subunits of soy 11S. Soy 11S globulin contains at least four different acidic subunits, namely A<sub>1</sub> (A<sub>1a,1b</sub>), A<sub>2</sub>, A<sub>3</sub>,



**Figure 4.** CD spectra of AS11S (-), unheated soy 11S (· · ·), and heated 11S (- - -). In the case of heated soy 11S, the heat treatment was similar to that used for the isolation of AS11S but without the presence of  $\beta$ -mercaptoethanol.

and A<sub>4</sub>A<sub>5</sub> (Nielsen, 1985a,b). Among these, A<sub>1</sub>, A<sub>2</sub>, and  $A_3$  are the predominant ones. The  $A_5$  is a small polypeptide with only  $\sim$ 97 amino acid residues (Utsumi, 1992). The molecular weight of  $A_1$  and  $A_2$  is  $\sim$ 32–33 kDa, and that of  $A_3$  is ~36 kDa. The  $A_4A_5$  exists as a complex with a molecular weight of  $\sim$ 40 kDa (Utsumi, 1997). On a mass basis, the amount of  $A_4A_5$  in soy 11S is very low compared to the other acidic subunits. Thus, the major protein species with an apparent molecular weight of 78.5 kDa in the elution profile (Figure 3) might be a dimer of the type  $A_1$ - $A_3$  or  $A_2$ - $A_3$ . The higher than expected molecular weight, that is, 78.5 kDa instead of  $\sim$ 69 kDa, of this dimer species might be due to an increase in the Stokes radius of the subunits as a result of partial unfolding during separation from soy 11S at 85-90 °C. The minor species with an apparent molecular weight of 54.8 kDa might be a dimer of  $A_4$  (~30 kDa) with  $A_1$  or  $A_2$ . Alternatively, it is guite likely that the 78.5 and 54.8 kDa species were actually denatured  $A_1$ ,  $A_2$ , and  $A_3$  with a large Stokes radius. Because separation of proteins in gel filtration chromatography reflects the Stokes radius, these unfolded subunits may permeate through the column as high molecular weight species. For example, according to Stokes law

$$R \propto M^{1/3} \tag{4}$$

where R is the hydrodynamic radius of the globular protein and M is its molecular weight. Using this relationship, it can be shown that for a 36 kDa protein to behave like a 78.4 kDa species in a gel permeation column, its hydrodynamic radius should increase only by 1.3 times its original radius. A 30% increase in the hydrodynamic radius is quite possible in thermally denatured globular proteins. Thus, the GPC results suggest that the isolated AS11S exists either as monomers or possibly as dimers, but certainly not as higher order oligomers, in solution.

The CD spectra of unheated soy 11S, heated soy 11S, and isolated AS11S are shown in Figure 4, and the secondary structure estimates are given in Table 1. The secondary structure contents of both heated 11S and AS11S were almost identical with a random coil content of ~35% and a  $\beta$ -sheet content of ~61–62%. In contrast,

 Table 1. Secondary Structure Contents (Percent) of

 AS11S and Unheated 11S Globulin

structure	unheated 11S	heated 11S	AS11S
α-helix	9.0	4	0
$\beta$ -sheet	65.0	61.0	62.5
$\beta$ -turns	0.0	0	2.0
aaperiodic	26.0	35.0	35.5



**Figure 5.** Time-dependent increase of surface concentration (A) and surface pressure (B) of AS11S ( $\bigcirc$ ) and native soy 11S ( $\triangle$ ) at the air–water interface at 25 °C. The initial protein concentration in the bulk phase was 1.5  $\mu$ g/mL in 10 mM phosphate buffer containing 0.1 M NaCl.

the unheated 11S contained only ~26% random coil. It is not known whether the  $\alpha$ -helix structure content of unheated soy 11S belongs to the acidic subunits or to the basic subunits. Nevertheless, the data in Table 1 indicate that the conditions employed for isolating AS11S from 11S did not cause massive changes in the acidic subunits at the secondary structure level, especially the  $\beta$ -sheets. The tertiary structure of the acidic subunits, however, might have been altered under the severity of the conditions used.

Surface Activity. The surface activities of unheated soy 11S and AS11S were determined by studying the kinetics of adsorption and surface pressure evolution at the air-water interface using <sup>14</sup>C-labeled 11S and AS11S. Figure 5 shows the time-dependent increase of surface concentration ( $\Gamma$ ) and surface pressure ( $\Pi$ , i.e., the net reduction in surface tension) during adsorption of AS11S and unheated 11S from a 1.5 µg/mL bulk protein solution to the air-water interface. The rate of adsorption was faster and the equilibrium  $\Gamma$  value (after 20 h of adsorption) was higher for AS11S than those for 11S. Similarly, the rate of increase as well as the final surface pressure value for AS11S was much higher than that for 11S. The  $\Gamma_{eq}$  of ~1.6–1.7 mg·m<sup>2</sup> for AS11S is similar to the values for  $\beta$ -casein and  $\alpha$ -casein reported by Anand and Damodaran (1996) under similar experimental conditions. These results indicate that AS11S readily adsorbed to the air-water interface and



**Figure 6.** Variation of  $\ln(N_0/N_d)$  as a function of storage time of emulsions formed by AS11S ( $\Box$ ), native 11S ( $\odot$ ), and heated 11S ( $\triangle$ ). The initial protein concentration in the aqueous phase of the emulsion was 1.0%, and the volume fraction of the oil phase was 16.6% (v/v). The data points represent an average of triplicate measurements.



**Figure 7.** Effect of protein concentration on the stability of AS11S-stabilized emulsions:  $(\Box) 0.1\%$ ;  $(\diamond) 0.2\%$ ;  $(\bigcirc) 0.5\%$ ;  $(\triangle) 1.0\%$ . The oil volume fraction was 16.6% (v/v). The data points represent an average of triplicate measurements.

reduced its surface tension to a greater extent than soy 11S. Although the data shown in Figure 5 relate to differences in surface activities of AS11S and soy 11S at the air-water interface, it is reasonable to expect similar behavior at the oil-water interface of emulsions.

Emulsifying Properties. The stability of the AS11S-, unheated 11S-, and heated 11S-stabilized oil-in-water emulsions was analyzed using eq 3, and the results are shown in Figure 6. The 11S was heated under conditions similar to those used for isolating AS11S, that is, at 85-90 °C for 30 min. However, no  $\beta$ -mercaptoethanol was used. The rate of decrease of the number of emulsion particles in the AS11S-stabilized emulsion followed firstorder kinetics, whereas it was not the case with the unheated 11S- or heated 11S-stabilized emulsions. The AS11S-stabilized emulsions were very stable compared to those stabilized by 11S. Visual observation of the emulsions also confirmed that oil separation and the extent of flocculation/aggregation were extensive in the 11S-stabilized emulsion, whereas no oil separation occurred in the AS11S-stabilized emulsion even after 10 days of storage.

The EAI values of AS11S and 11S were determined by measuring the turbidity of emulsions formed by 1% (w/v) protein solutions and an oil volume fraction of 16.6% under identical emulsification conditions. The EAI of AS11S was 349 cm<sup>2</sup> mg<sup>-1</sup>, whereas those of native 11S and heated 11S were about 220 and 255 cm<sup>2</sup> mg<sup>-1</sup>, respectively. These results further confirm that AS11S possesses better emulsifying "ability" and "stability" than soy 11S.

Figure 7 shows the effect of protein concentration on the stability of AS11S-stabilized emulsions. The stability increased with increasing protein concentration. The

 Table 2. EAI, Adsorption Density, and Average Droplet

 Size (d<sub>32</sub>) Values of AS11S-Stabilized Emulsions at

 Various Bulk Concentrations<sup>a</sup>

protein concn (%)	EAI (cm <sup>2</sup> mg <sup>-1</sup> )	% adsorbed	adsorption density (mg m <sup>-2</sup> )	d <sub>32</sub> (μm)
0.1	1988	90.1	5.03	$3.12 \pm 1.78$
0.2	1838	94.4	5.44	$0.80\pm0.14$
0.5	755	93.5	13.24	$0.83\pm0.18$
1.0	349	73.6	28.65	$0.84 \pm 0.18$

<sup>*a*</sup> The data represent an average of triplicate measurements. The  $d_{32}$  values are expressed as mean  $\pm$  SD.

emulsion prepared with 1% AS11S concentration was stable for more than a month at 25 °C. No visible oil separation occurred even after a 1 month storage period. The EAI and adsorption density values of freshly prepared emulsions at different protein concentration levels are given in Table 2. In the bulk concentration range of 0.1-0.5%,  $\sim 90-95\%$  of total proteins were adsorbed to emulsion particles. At 1.0%, however, only  $\sim$ 73% of the total proteins were adsorbed and the rest remained in the serum phase of the emulsion. The EAI of the protein decreased with increase of bulk protein concentration, although the drop in EAI was more precipitous at 0.5% than at 0.2%. The adsorption density, on the other hand, was similar at 0.1 and 0.2% protein concentrations but increased significantly from 5.44 mg m<sup>-2</sup> at 0.2% to  $\sim$ 13.24 mg m<sup>-2</sup> at 0.5%. The data tentatively suggest that at 0.1-0.2% bulk concentration, AS11S forms a saturated monolayer coverage around emulsion particles. The saturated monolayer coverage is in the range of 5.0-5.5 mg m<sup>-2</sup>. At 0.1%protein concentration, the average size of the emulsion particles produced is  $\sim$ 3.12  $\pm$  1.78  $\mu$ m. This might be due to limited availability of protein to create emulsion particles with a large surface area-to-volume ratio. At 0.2% protein concentration, there is enough protein to form smaller emulsion particles with a saturated monolayer film. At higher bulk concentrations, the particle size reaches a limiting value of  $\sim 0.84 \,\mu m$ , but, because more than enough protein is available in the bulk phase, formation of a multilayer protein film occurs. The drop in EAI and the increase in adsorption density at higher bulk concentrations are essentially due to multilayer formation and the absence of creation of new interfacial area. The increase in the stability of the emulsion with increasing protein concentration (Figure 7) might be attributed to the protein load. A high adsorption density implies formation of a thick membrane-like protein layer on the oil droplets, which effectively resists coalescence (Britten and Giroux, 1993). The protruding polypeptide chains in this thick layer will also impart steric stabilization of the emulsion against coalescence.

Because the emulsion produced by a 0.5% AS11S solution was reasonably stable enough to monitor the relative effects of additives on its stability, a protein concentration 0.5% was selected to study the effect of ionic strength on the stability of AS11S-stabilized emulsions. The effect of ionic strength on time-dependent changes in  $d_{32}$  of AS11S-stabilized emulsions at 0.5% protein concentration and at pH 7.0 is shown in Table 3. The average droplet sizes of the freshly formed emulsions were almost the same, that is, 0.80–0.84  $\mu$ m in the ionic strength range 0.05–0.50. During storage under agitating conditions, however, the average droplet size increased with storage time as the ionic strength was increased. The increase in droplet size during

Table 3. Effect of Ionic Strength on Droplet Size  $(d_{32})$  during Storage of Emulsions Made with 0.5% (w/v) AS11S at pH 7.0

ionic - strength	droplet size <sup><i>a</i></sup> (µm)					
	0 h	20 h	44 h	68 h	140 h	
0.05	$0.84 \pm 0.19$	$\textbf{0.87} \pm \textbf{0.24}$	$\textbf{0.88} \pm \textbf{0.25}$	$\textbf{0.88} \pm \textbf{0.22}$	$\overline{0.90\pm0.26}$	
0.10	$0.83 \pm 0.18$	$0.90\pm0.26$	$0.97 \pm 0.34$	$0.99 \pm 0.52$	$1.00\pm0.58$	
0.15	$0.79 \pm 0.13$	$0.90\pm0.25$	$0.92\pm0.27$	$0.96 \pm 0.30$	$1.06\pm0.51$	
0.20	$0.84 \pm 0.19$	$0.93 \pm 0.27$	$0.94 \pm 0.29$	$1.05\pm0.53$	$2.01\pm2.07$	
0.50	$0.81\pm0.16$	$\textbf{0.88} \pm \textbf{0.24}$	$0.91\pm0.27$	$0.95\pm0.34$	$2.33\pm2.33$	

<sup>*a*</sup> Data represent mean  $\pm$  SD based on triplicate measurements.

storage was more significant at ionic strength 0.2 and above. For instance, in the ionic strength range 0.05– 0.15, the size of oil droplets increased from  $\sim$ 0.8 to  $\sim$ 1.06  $\pm$  0.5  $\mu$ m over a storage period of  $\sim$ 140 h. However, at 0.2 and 0.5 ionic strengths, the average size increased from 0.8 to 2.01  $\pm$  2.07 and 2.33  $\pm$  2.33  $\mu$ m, respectively, over the same storage period. It should be noted that the standard deviation was much larger for the 0.2 and 0.5 ionic strength samples than for the 0.05–0.15 ionic strength samples, suggesting that polydispersity was very large in the former compared to that in the latter. This can be attributed to a greater extent of coalescence of oil droplets at ionic strengths 0.2 and 0.5. The stability of emulsion particles at 0.05–0.15 ionic strength against coalescence might be due to electrostatic repulsion. Above 0.15 M ionic strength, charge screening by counterions may decrease electrostatic repulsion and increase hydrophobic attractive interactions between emulsion particles, leading to coalescence.

The data presented here clearly show that the acidic subunits of soy 11S possess excellent emulsifying properties compared to those of soy 11S. Emulsions prepared with 1% AS11S solutions remained very stable without any visible oil separation for more than a month under gentle agitating conditions, whereas those prepared with 1% 11S collapsed and phase separated within 2-3days. The AS11S-stabilized emulsions were very stable below 0.15 M ionic strength. Because the mass fraction of acidic subunits in soy 11S is  $\sim$ 60% and it is relatively easy to separate the acidic subunits from soy 11S, it may be industrially feasible to develop an economical process to isolate functional acidic subunits for use in emulsionbased food products. In addition, development through genetic engineering of soybean varieties that bear only the 11S fraction or the acidic subunits of 11S as the main storage protein may also increase the scope of utilization of soy proteins in emulsion and possibly in foam-type food products.

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