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Physicochemical Properties of Soy Protein: Effects of Subunit Composition

Guangyan Qi,[†] Karthik Venkateshan,[†] Xiaoqun Mo,[†] Lu Zhang,[‡] and Xiuzhi Susan Sun*[,†]

[†]Bio-Materials and Technology Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, United States

[‡]Food Science and Technology, University of California, Davis, California 95616, United States

ABSTRACT: Soy protein elastomer (SPE) exhibits elastic, extensible, and sticky properties in its native state and displays great potential as an alternative to wheat gluten. The objective of this study was to better understand the roles of soy protein subunits (polypeptides) contributing to the functional properties of SPE. Six soy protein samples with different subunit compositions were prepared by extracting the proteins at various pH values on the basis of the different solubilities of conglycinin (7S) and glycinin (11S) globulins. Soy protein containing a large amount of high molecular weight aggregates formed from α' and α subunits exhibited stronger viscoelastic solid behavior than other soy protein samples in terms of dynamic elastic and viscous modules. Electrophoresis results revealed that these aggregates are mainly stabilized through disulfide bonds, which also contributed to higher denaturation enthalpy as characterized by DSC and larger size protein aggregates observed by TEM. Besides, the most viscoelastic soy protein sample exhibited flat and smooth surfaces of the protein particles as observed by SEM, whereas other samples had rough and porous particle surfaces. It was proposed that the ability of α' and α to form aggregates and the resultant proper protein−protein interaction in soy proteins are the critical contributions to the continuous network of SPE.

KEYWORDS: soy protein elastomer, viscoelasticity, electrophoresis, thermal properties, morphology properties, water-absorbing capacity

INTRODUCTION

As a natural polymer, soybean protein possesses unique nutritional and functional properties and has been used extensively as an ingredient in food products such as beverages, whipped toppings, sausages, baking products, and tofu.[1−3] The dominant storage protein in soybean is globulin (50−90%), which has two major components: glycinin (11S) and β-conglycinin (7S). The relative proportion of 11S to 7S ranges from 1:3 to 3:1 depending on the cultivar and growing conditions.[4] 11S globulin is a hexamer with a molecular weight of about 350 kDa and consists of an acidic and a basic polypeptide linked by a disulfide bond.[5] 7S is a trimer with a molecular weight of 150−200 kDa that is composed of three subunits: α', α, and β. These subunits are mainly associated by noncovalent bonds: hydrophobic interaction and hydrogen bonding.[6] However, a little cysteine content was also proved to exist in α' and α subunits, and it can form a small amount of high molecular weight aggregates through disulfide linkage.[7,8] Due to inherent structural differences in 7S and 11S globulin, they perform different physicochemical functions in soy protein. Substantial efforts have been made to study the relationship between each globulin or soy protein having different 7S/11S ratios with the functional properties of soy products such as the texture of tofu and extruded products and the emulsifying, foaming, water-holding capacity, gelling, and solubility properties of the products.[2,9−14]

In our study, an innovative procedure for producing soy protein-based elastomer (SPE) with 39% solid content was discovered. Preliminary results showed that SPE was sticky, elastic, and extensible in its native state, which is similar to the viscoelastic properties of wheat gluten protein. Such soy protein elastomer should have great potential for many applications, such as gluten-free baking products, candy bars, and films. The objective of this research was to study the physicochemical properties of soy proteins with different 7S and 11S ratios, which were extracted at various pH values on the basis of the different solubilities of these two globulin proteins, including electrophoresis, dynamic rheological, thermal, and morphological properties.

MATERIALS AND METHODS

Materials. Defatted soy flour obtained from Cargill (Cedar Rapids, IA) was the starting material. The soy flour contained about 50% protein and 10% moisture with a dispersion index of 90. Sodium chloride (NaCl), urea, and β-mercaptoethanol (β-met) were purchased from Fisher Scientific (Fair Lawn, NJ).

Soy Protein Sample Preparation. Soy flour was dispersed in water at 6.25% solid content with pH 9.5 by using 2 N NaOH. After 2 h of stirring at room temperature, the pH of the slurry was adjusted to a series of pH values (5.1, 5.4, 5.6, 5.8, 6.0, and 6.4) with 2 N HCl to remove carbohydrate and some glycinin proteins by centrifugation at 12000*g*. Then the pH of the supernatant was adjusted to 4.8 with 2 N HCl and centrifuged at 8000*g* to obtain the SPE with 39% solid content. The samples were designated SP5.1, SP5.4, SP5.6, etc.

Electrophoresis (SDS-PAGE). SDS-PAGE of soy protein samples was performed on a 4% stacking gel and 12% separating gel with a discontinuous buffer system according to the method described by Laemmli.[15] A protein sample was mixed with a sample buffer containing 2% SDS, 25% glycerol, and 0.01% bromphenol blue. To study the disulfide bonds in soy protein, SDS-PAGE was carried out under both

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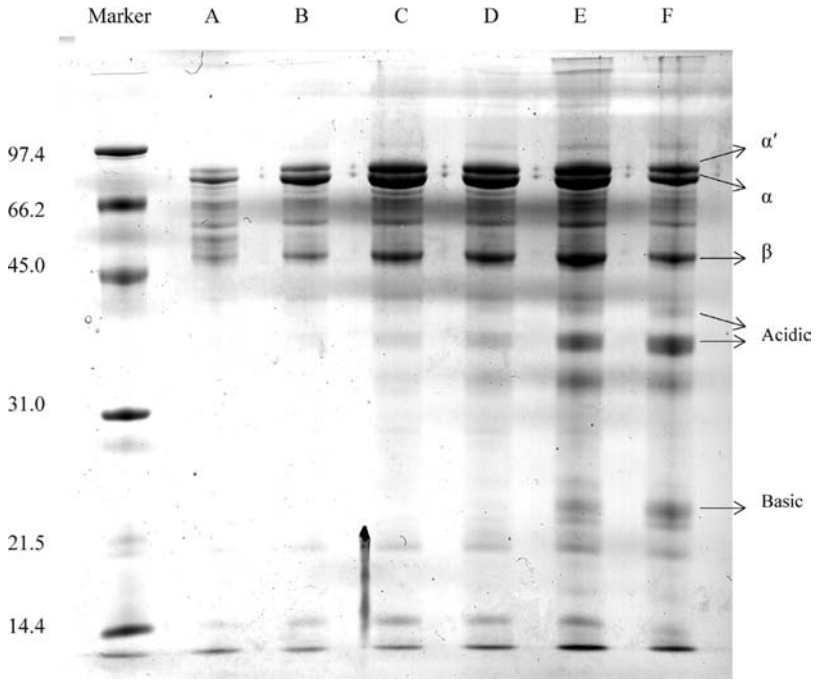

Figure 1. Reducing SDS-PAGE pattern of soy proteins with different subunit ratios in the presence of 2-mercaptoethanol: pH 5.1−4.8 (lane A); pH 5.4−4.8 (lane B); pH 5.6−4.8 (lane C); pH 5.8−4.8 (lane D); pH 6.0−4.8 (lane E); pH 6.4−4.8 (lane F).

reducing (β-met) and nonreducing conditions. A total of 8 μg of protein was applied to sample slots. Molecular weight standards were run with the samples. Electrophoresis was performed at 40 mA and 120 V for 90 min. The gel was stained in 0.25% Coomassie brilliant blue R-250 and destained in a solution containing 10% acetic acid and 40% methanol. Densitometry was obtained by analyzing the gel image using the Kodak 1D Image Analysis software, version 4.6 (Kodak, Rochester, NY).

To study the forces involved in the formation of protein aggregates, sample SP5.4 was treated with NaCl, urea, and β-met. Dried SPE powder (5 mg) was dispersed in 10 mL of 0.2 M citric acid−Na_2HPO_4 buffer (pH 4.8) to make the suspension. Then 4% (dry basis) of NaCl, urea, and 0.02 M β-met were added to the solution. After 2 h of stirring, the treated SP5.4 suspensions were centrifuged at 8000*g* for 15 min, and the precipitated insoluble SPE was lyophilized for the nonreducing electrophoresis.

Dynamic Viscoelastic Measurement. Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) was used to perform the dynamic oscillatory shear testing of soy protein samples, characterizing their viscoelastic properties. A parallel plate head was used with an 8 mm plate diameter and a 1 mm gap. The measurements were performed in a strain-controlled mode wherein the amplitude of shear strain was 0.5% and the frequency range was from 0.01 to 25 Hz. The testing temperature was 40 °C. A thin layer of silicone oil was spread over the circumference of the sample to prevent dehydration of the sample during test. The storage modulus (G'), loss modulus (G''), and complex viscosity (η) were continuously registered.

Differential Scanning Calorimetry (DSC). Thermal denaturation properties of soy proteins were assessed with a differential scanning calorimeter (DSC) (DSC7, Perkin-Elmer, Norwalk, CT) calibrated with indium and zinc. Wet soy protein samples (20 mg) were hermetically sealed in a large-volume stainless pan. Each sample was held at 20 °C for 1 min and then scanned from 20 to 130 °C at a heating rate of 10 °C/min. Peak temperatures (T_d) and denaturation enthalpies (ΔH) were calculated from thermograms.

Transmission Electron Microscopy (TEM). A Philips CM 100 (FEI Co., Hillsboro, OR) TEM was used to observe the microstructure of soy protein samples. The wet soy protein sample were diluted to 1% with deionized water for imaging and sonicated for 10 min in an L&R320 ultrasonic stirrer (L&R Manufacturing Co., Keary, NJ). Diluted samples were absorbed onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Science, Fort Washington, PA) and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Burlington, VT) for 60 s at room temperature.

Table 1. Estimated Polypeptide Content of Soy Proteins with Different Subunit Ratios under Reducing SDS-PAGE

	soy protein fraction distribution (%)					
polypeptide	SP5.1	SP5.4	SP5.6	SP5.8	SP6.0	SP6.4
α'	19.7	22.5	18.0	21.1	15.3	15.2
α	50.8	45.3	50.5	42.1	37.4	20.7
$\alpha' + \alpha$	**70.5**	**67.8**	**68.4**	**63.1**	**52.8**	**35.8**
β	18.0	18.6	12.9	21.9	19.2	17.1
7S ($\alpha' + \alpha + \beta$)	88.5	86.4	81.4	85.0	72.0	52.9
acidic			0.9	6.3	9.1	23.6
basic					4.4	13.2
11S (acidic + basic)			0.9	6.3	13.5	36.8

Scanning Electron Microscopy (SEM). A Hitachi S-3500 N (Hitachi Science System, Ibaraki, Japan) scanning electron microscope was used to observe the surface morphology of soy protein particles. Freeze-dried soy protein sample was affixed to an aluminum stub with two-sided adhesive tape and coated with an alloy of 60% gold and 40% palladium with a sputter coater (Desk II Sputter/Etch Unit, Moorestown, NJ). The SEM of the soy protein samples was performed with operation conditions at an accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

SDS-PAGE Analysis. The reducing SDS-PAGE profiles of soy protein with different subunitratios are shown in Figure 1.

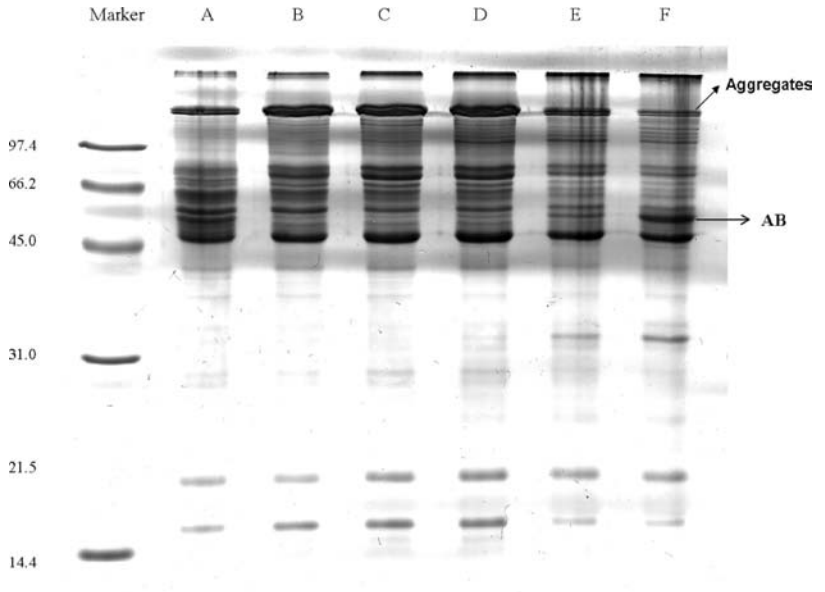

Figure 2. Nonreducing SDS-PAGE pattern of soy proteins with different subunit ratios: pH 5.1−4.8 (lane A); pH 5.4−4.8 (lane B); pH 5.6−4.8 (lane C); pH 5.8−4.8 (lane D); pH 6.0−4.8 (lane E); pH 6.4−4.8 (lane F).

Table 2. Estimated Polypeptide and Aggregates Content of Soy Proteins with Different Subunit Ratios under Nonreducing SDS-PAGE

	soy protein fraction distribution (%)					
polypeptide	SP5.1	SP5.4	SP5.6	SP5.8	SP6.0	SP6.4
aggregates	20.6	25.2	23.5	21.5	19.4	10.6
$\alpha' + \alpha$	8.7	15.5	19.3	17.2	7.3	4.8
β	25.3	27.5	25.2	25.8	29.3	33.5

The components in the soy protein are α', α, and β subunits from 7S and acidic (A_3, A_{1a}, A_{1b}, A_2, and A_4) and basic polypeptides (B_3, B_{1a}, B_{1b}, B_2, and B_4) from 11S. Samples SP5.1 and SP5.4 are mainly 7S subunits without 11S as indicated in Figure 1 (lanes A and B), which suggested the complete separation of 7S and 11S at pH $\leq$5.4. For sample SP5.6, trace bands at 38 and 23 kDa, corresponding to acidic and basic polypeptides of 11S, were observed, and the intensity increased as the pH increased (Figure 1D−F). Thanh and Shibasaki[16] reported that the principal of soy protein globulin fractionation was based on the different solubilities of the proteins. 7S (precipitated at pH 4.0−5.6) and 11S (precipitated at pH 4.4−6.8) can be simultaneously fractionated in the pH range of 6.2−6.4, wherein most of the 7S dissolved but most of the 11S precipitated; then the 7S can be separated by adjusting the pH to 4.8. However, cross-contamination of 7S and 11S always occurred to some extent during fractionation. As to the soy protein extraction in this study, all of the 11S and part of the 7S were precipitated and removed at pH 5.1−5.4, resulting in the pure 7S globulin at pH 4.8, but the 7S protein yield was low (8% wet basis for SP5.4).

At pH range of 5.1−6.4, 7S (52.9−88.5%) globulin dominated in soy proteins, and the percentage of 11S increased from 0.9% for sample SP5.6 to about 37% for sample SP6.4 (Table 1). The α' and β subunits had relatively constant contents of about 18%, whereas the α subunit decreased from 50 to 20% as the pH increased from 5.1 to 6.4. Nakamura et al.[17] reported that the percentages of α', α, and β subunits in native 7S were 37.5, 25, and 37.5%, respectively, which were also similar to the protein composition of soy protein used in this study. Our results demonstrated that the percentages of those three subunits in soy protein samples altered obviously compared to those in native soy protein; it was probably contributed by the different isoelectric points of 7S subunits: 5.2, 4.9, and 5.7−6.0 for α', α, and β, respectively.[18] The rearranged protein subunits ratios could significantly affect the protein's functionality.

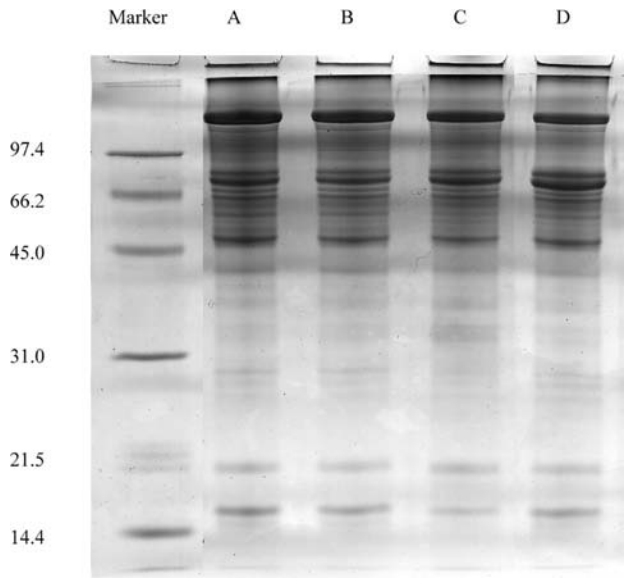

Figure 3. Nonreducing SDS-PAGE pattern of sample SP5.4: control SP5.4 (lane A); SP5.4 treated by NaCl (lane B); SP5.4 treated by urea (lane C); SP5.4 treated by β-met (lane D).

Nonreducing SDS-PAGE in the absence of β-met was performed to study the disulfide linkage in soy proteins (Figure 2). Fairly dense bands with molecular weights of about 120 kDa were

Table 3. Estimated Polypeptide and Aggregates Content of SP5.4 Treated by NaCl, Urea, and β-Met

	soy protein fraction distribution (%)			
polypeptide	control	NaCl	urea	β-met
aggregates	25.2	31.0	27.1	15.0
$\alpha' + \alpha$	15.5	7.8	8.2	22.5
β	27.5	28.4	29.2	29.5

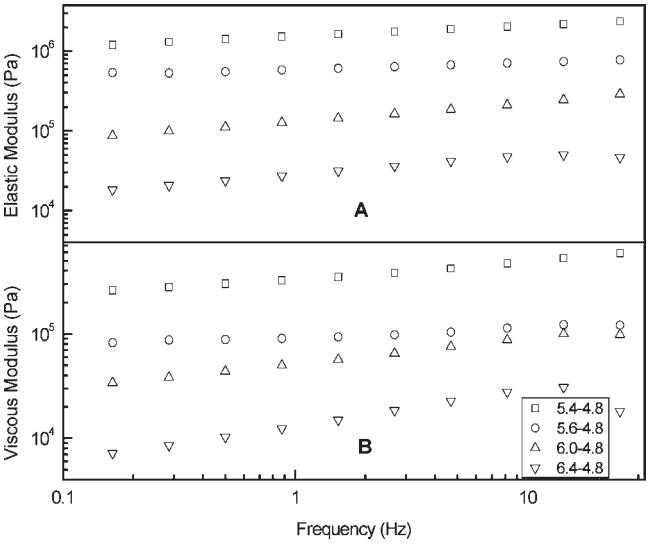

Figure 4. Elastic modulus and viscous modulus of soy proteins with different subunit ratios as a function of shear rate.

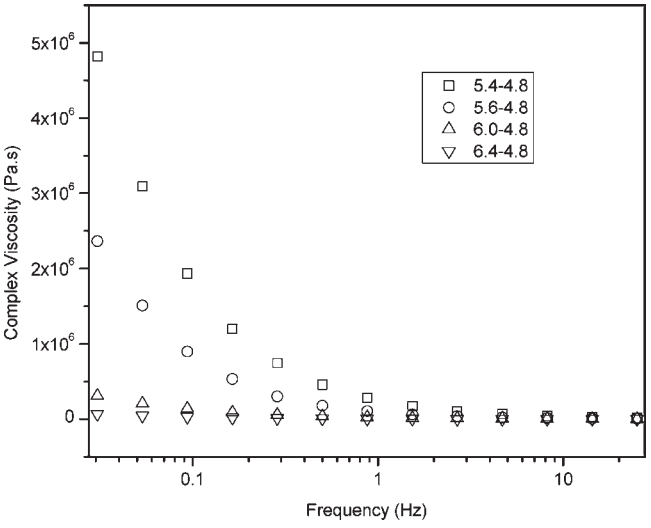

Figure 5. Complex viscosity of soy proteins with different subunit ratios as a function of shear rate.

observed in all SP samples. These protein aggregates were believed to be composed of α' and α subunits, because the $\alpha' + \alpha$ subunits content reduced simultaneously to <20% in nonreducing SDS-PAGE (Table 2) from more than 36% in reducing SDS-PAGE (Table 1). The β subunit content remained in the range of 25−33% compared to 20% under reducing

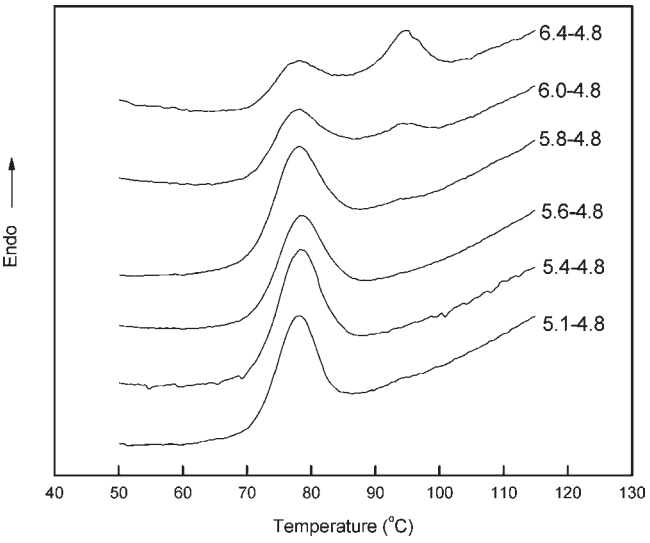

Figure 6. DSC thermogram of soy proteins with different subunit ratios.

Table 4. Denaturation Temperature (T_d) and Total Enthalpy of Denaturation (ΔH_d) of Soy Proteins with Different Subunit Ratios

	T_d (°C)		
pH	7S	11S	total ΔH_d (J/g)
SP5.1	77.80		8.44
SP5.4	78.13		8.67
SP5.6	78.31		8.75
SP5.8	77.97	94.20	7.38
SP6.0	77.97	94.20	5.93
SP6.4	77.97	94.71	5.71

electrophoresis, suggesting that the β subunit did not participate in aggregate formation. Moreover, the band intensity of these aggregates decreased gradually as the pH increased from 5.4 to 6.4. The 11S component was known to incorporate into the soy proteins at pH ≥5.6 (Table 1), and it is possible that 11S subunits interfere with the interaction between α' and α subunits, limiting the protein aggregate formation to some extent as the pH increased. High molecular weight aggregation induced by α' and α was also observed by other researchers,[7,19] but in fairly small quantity. Petruccelli and Anon[7] suggested that both electrostatic interaction and disulfide bonds existed in the aggregates. Besides, the intensity of several weak bands at around 100 kDa increased as the 11S content increased. Those bands could be the disulfide bond-linked polymers caused by freeze-drying or thiol−disulfide exchange in 11S;[20] they faded when reducing SDS-PAGE was performed.

To understand the chemical forces involved in the formation of protein aggregates, sample SP5.4 was treated with 4% NaCl, urea, and 0.02 M β-met and then subjected to nonreducing SDS-PAGE (Figure 3). The percentages of the protein aggregates and polypeptides are shown in Table 3. Sodium chloride-treated SP5.4 showed an increase in aggregates content to 31% compared to 25% in the control, whereas α' and α subunits content decreased to 8% compared to 16% in the control. Sodium chloride

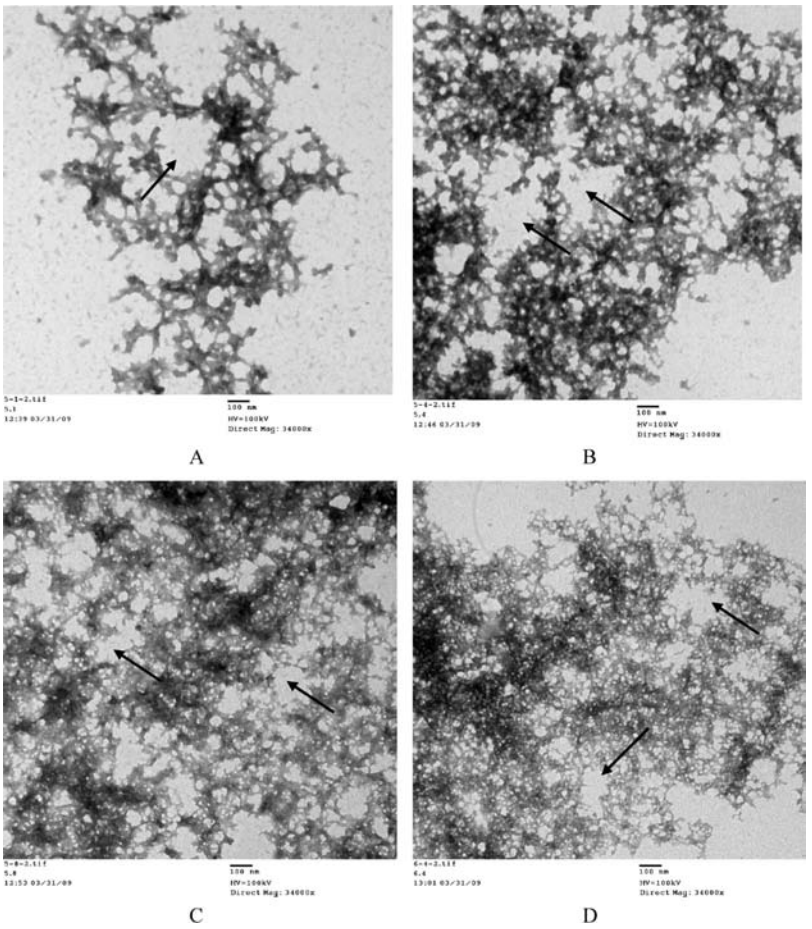

Figure 7. TEM image of soy proteins with different subunit ratios (protein aggregates are indicated by the arrows): pH 5.1−4.8 (A); pH 5.4−4.8 (B); pH 5.8−4.8 (C); pH 6.4−4.8 (D).

reduces electrostatic repulsion among soy protein molecules because of the charge neutralization effects, suggesting that electrostatic interactions are involved in soy protein aggregates formation in some degree. Urea-treated SP5.4 showed similar results: the increase of aggregates contents with concomitant decrease of α' and α subunits content, implying that hydrogen bonding and hydrophobic interactions have limited effects on protein aggregates formation; urea is known to disrupt those interactions in protein. β-Met-treated SP5.4 had reduced protein aggregates of 15%, which indicates that disulfide bonds are involved in the aggregates. Furthermore, almost 40% of sample SP5.4 was solubilized by β-met, demonstrating that the disulfide bonds are also essential in maintaining the protein network.

Dynamic Viscoelasticity. Dynamic rheological measurement is a useful method to study the viscoelastic properties of polymers. It can be carried out at a small strain within the linear viscoelasic region; the modulus curves can be monitored as a function of time and frequency. Figure 4 shows the frequency dependence of the storage modulus (G') and loss modulus (G'') of soy proteins with different subunit ratios. Shear modulus of all soy protein samples exhibited weak frequency-dependent behavior; G' and G'' increased as the frequency increased because of the decreased time for stress relaxation during the shearing with the increased frequency. Sample SP5.4 exhibited much stronger viscoelastic solid behavior than other samples. It had the highest shear modulus (ranging from 4.5×10^5 to 2.4×10^5 Pa for elastic modulus and from 1.6×10^5 to 2.6×10^5 Pa for viscous modulus, respectively) among all of the samples under the same shear condition. Moreover, the elastic modulus predominated over the viscous modulus by an order of magnitude at the frequency range, indicating the more elastic properties of sample SP5.4. Those findings are in agreement with Utsumi et al.,[21] who found that β-conglycinin largely contributed to the gel elasticity, whereas glycinin was related to the gel hardness and unfracturability.

Complex viscosity represents the true viscoelastic characteristics of gels. As with the dynamic modulus, sample SP5.4 had the highest complex viscosity (Figure 5), suggesting that strong intermolecular force existed in proteins. The viscosity of all samples decreased as the frequency increased, revealing the shear thinning properties of soy proteins.

Thermal Properties. DSC is usually used to measure protein denaturation, which significantly affects protein functionality and its application in food. Soy protein thermal denaturation involves unfolding the quaternary, tertiary, and secondary structures, accompanied by extensive uptake of heat. Typical thermal denaturnation peaks for 7S and 11S of soy proteins with different subunit ratios are displayed in DSC thermogram (Figure 6). The endothermic transition peak for glycinin was observed at pH >5.8, which is in agreement with protein composition analysis from SDS-PAGE results. The denaturation temperatures (T_d) of 7S and 11S for all soy proteins were in the similar ranges of

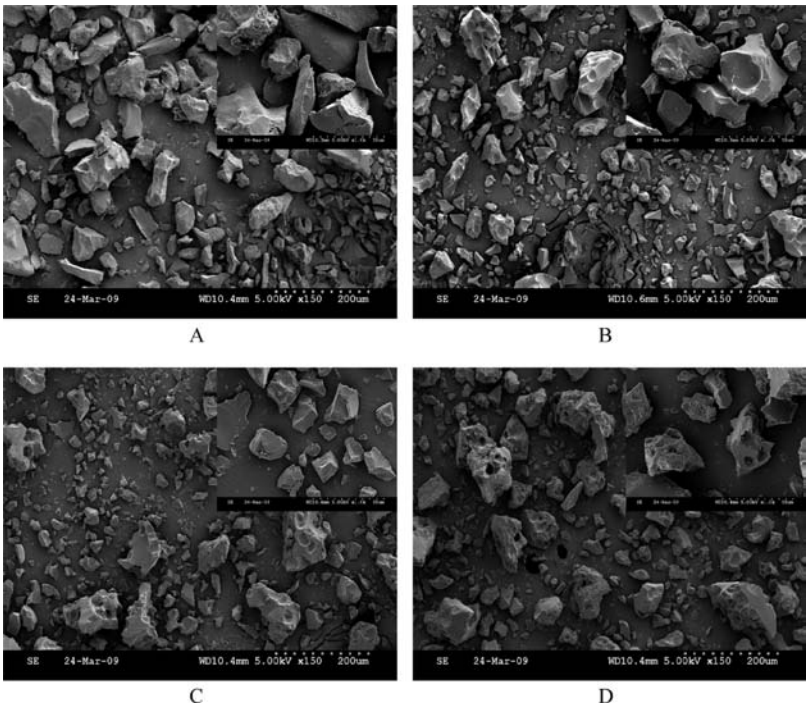

Figure 8. SEM images of soy proteins with different subunit ratios: pH 5.1−4.8 (A); pH 5.4−4.8 (B); pH 5.8−4.8 (C); pH 6.4−4.8 (D).

77.8−78.3 and 94.2−94.7 °C, respectively (Table 4). The total enthalpy (ΔH_d) of soy protein increased as the pH decreased from 6.4 to 5.1; meanwhile, an increase of $\alpha' + \alpha$ content from 36 to 70% was confirmed as well (Table 1). The cysteines in α' and α subunits[8] induced the formation of disulfide-linked aggregation as observed in the nonreducing SDS-PAGE (Figure 2). A higher amount of these aggregates in soy protein at lower pH (Table 2) improved the protein thermal stability.

Morphological Properties. TEM images of soy proteins with different subunit ratios are shown in Figure 7. Branch-like structure and large protein aggregates were observed in sample SP5.1 (Figure 7A). Sample SP5.4 had an increased amount of smaller globular aggregates, and some of them grew into larger irregularly shaped clumps (as indicated by arrows in the image) (Figure 7B). Sample SP5.8 (Figure 7C) displayed a further increase in the number of smaller globular protein aggregates and a further decrease in the size of irregularly shaped clumps. In the case of sample SP6.4, mainly small globular protein aggregates with only a few large-size protein clumps were observed (Figure 7D). As explained previously, high molecular weight aggregates stabilized by disulfide bonds in soy proteins had stronger protein−protein interactions (dynamic viscoelasticity results); consequently, stronger protein−protein interactions could induce larger size protein aggregates as displayed in the TEM images. Therefore, with the decreasing amount of protein aggregates as the pH increased, the number of larger size protein aggregates decreased gradually (Figure 7).

Phase and functionality differences in soy protein samples are known to be partially attributable to several factors, such as protein−protein interactions, protein−water interactions, protein composition, protein solid content, etc.[12,22−24] As evidenced in the nonreducing SDS-PAGE, disulfide bonds played a critical role in maintaining the stability of the soy protein network, and the resultant strong protein−protein interactions might relate tightly to the external physical phases. Water−protein interaction is an important factor in the dehydration, rehydration, solubility, viscosity, gelation, and other important properties of protein products. We proposed that sample SP5.4 had the proper protein−protein interaction, maintaining a certain amount of water inside the protein network (water content = 61.2%), which is vital to the formation of continuous protein phases with viscoelastic properties. As to SP5.1, the initial water content was 65%, indicating the higher water retention capability compared with SP5.4. It was contributed by the stronger protein−protein interaction, as shown in the TEM image (Figure 7A), resulting in more water entrapped in the protein network. Hence, sample SP5.1 exhibited a slightly diluted state with low viscoelastic properties. In contrast, sample SP >5.4 exhibited smaller size protein aggregates (Figure 7C,D), suggesting a weaker protein network. The initial water content of SP5.8 was 58%, indicating a lower water retention capability than that of SP5.4, and its water retention capability was further decreased during storage because water phase separation occurred and formed a water layer on top of the protein sample. Moreover, 11S is known to have greater surface hydrophobicity (lower water hydration properties) than 7S due to the larger number of hydrophobic groups in 11S.[25] Therefore, the weak protein−protein interaction and poor hydration properties in sample SP >5.4 could lead to the clay-state phase without cohesiveness. In brief, proper protein−protein interaction with resultant entraining of certain amount of water molecules is critical to continuous network for SPE with high solid content (39%).

SEM images of soy protein samples with different subunit ratios are shown in Figure 8. All soy proteins exhibited in the form of irregular compact chunks, but with different surface morphology of protein particles. Sample SP5.1 displayed a coarse surface (Figure 8A), whereas the particles of SP5.4 had the smoothest surface with a few dents (Figure 8B). In the case of sample SP5.8 (Figure 8C), a minor amount of 11S was incorporated into protein, and the protein particle surface became much rougher than that of sample SP5.4. As the 11S content continued

increasing in soy protein samples (i.e., at pH 6.4), coarse and fluctuant surfaces with large pores inside were observed (SP6.4, Figure 8D). This suggested that the weak intermolecular interaction in SP6.4 (clay-like sample) had fragile properties compared with the samples (SP <6.4) showing more ductile morphology. These pores might be the air entrapped in the protein or the result of intense water subliming during freezing. What is s more important is that it indicates the morphology structure of the protein extracted at various pH values.

In summary, pure 7S fraction tended to form high molecular weight aggregates mainly from α' and α subunits, which were mainly stabilized by disulfide bonds. Soy protein having a high content of protein aggregates exhibited strong viscoelastic solid behavior (SP5.4). Soy protein containing a large amount of aggregates also contributed to higher protein denaturation enthalpy as characterized by DSC and larger size protein aggregation as observed by TEM. In short, the ability of α' and α to form aggregates through disulfide bonds is a key factor of providing soy protein with strong viscoelastic properties, displaying great potential as an alternative to wheat gluten.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Bio-Materials and Technology Lab, Kansas State University, 101 BIVAP Building, 1980 Kimball Avenue, Manhattan, KS 66506. E-mail: xss@ksu.edu. Phone: (785) 532-4077. Fax: (785) 532-7193.

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