Electrophoretic, Solubility, and Functional Properties of Commercial Soy Protein Isolates

Estela L. Arrese,† Delia A. Sorgentini,‡ Jorge R. Wagner,† and Maria C. Añón*,†

Centro de Investigación y Desarrollo en Criotecnologia de Alimentos (CIDCA), Facultad de Ciencias Exactas, UNLP, Calle 47 y 116, 1900 La Plata, Argentina, and Cátedra de Quimica Biológica, Facultad de Ciencias Exactas, UNLP, Calle 47 y 115, 1900 La Plata, Argentina

The effect of protein composition and degree of protein denaturation on the solubility, water-imbibing capacity (WIC), viscosity, and gelation capacity of commercial soy protein isolates was studied. It was found that the degree of denaturation may affect protein solubility, but very denatured proteins with high solubility were also detected. Isolates containing completely denatured proteins showed low gelation capacity. This characteristic is closely related to the relative amounts of the 7S and 11S proteins, since β-7S subunit and basic 11S polypeptide were present in decreased concentrations in the soluble protein fraction. Isolates with a high degree of denaturation and intermediate solubility values presented the maximal WIC. Results confirmed that the apparent viscosity of soy protein dispersions is intimately related to WIC.

INTRODUCTION

One of the most popular plant protein sources to serve as an ingredient in food formulation is soy protein. The commercial preparation of soy proteins causes physical and chemical changes that affect their functional properties (Kinsella, 1979). These changes occur to a varying extent; thus, each preparation has to be evaluated with regard to functional properties. Globulins glycinin (11S) and β-conglycinin (7S) are the major components of soy isolates. The former has an estimated molecular weight (MW) of 309 000–393 000 and consists of acidic polypeptide chains (A) (MW 37 000–40 000) and basic polypeptide chains (B) (MW 19 900–20 000) (Nielsen, 1985), while 7S has a trimeric structure having a molecular weight of 140 000–170 000 and consists of subunits α′ (83 000–57 000), α (76 000–57 000), and β (53 000–42 000) (Brooks and Morr, 1985). These two globulins have different structures and molecular properties (Derbyshire et al., 1976; Kinsella et al., 1985) and different functional properties (Saio and Watanabe, 1978). The aim of this study was to determine (1) the degree of protein denaturation and the relative amounts of the different protein species and (2) the incidence of the structural features of the proteins on the solubility, water-imbibing capacity, viscosity, and gelation capacity of commercial soy protein isolates.

MATERIALS AND METHODS

Materials. Nineteen commercial soy isolates were utilized; 1–15 were produced by Sanbra S. A. and 16–19 by Ralston Purina. They are isolates 1–4, Proteinmax 90 HG, differents lots; isolates 13 and 14, Proteinmax MP, differents lots; isolates 15 and 16, Supro 500 E, differents lots; isolate 17, Supro 610; isolate 18, Supro 515; and isolate 19, Supro 590. These isolates exhibited the following characteristics: range of pH of 1% isolate dispersions in distilled water was 6.8–7.0; the total protein content expressed as wet basis was 83–90% and moisture 5.0–6.5%.

Methods. *Solubility.* Isolate dispersions (1% w/v) were prepared in distilled water, stirred magnetically at 20 °C for 1 h, and then centrifuged at 12100*g* for 20 min. Aliquots from the supernatant were taken for determination of the contents of soluble protein. Protein solubility was expressed as the ratio of soluble to total protein. Total protein of samples was determined by the Kjeldahl method (N × 6.25) and the soluble protein by the biuret method (Gornall et al., 1949).

Degree of Denaturation. Differential scanning calorimetry (DSC) was used to assess the degree of denaturation of commercial protein isolates. DSC thermograms were recorded on a Du Pont Model 910 calorimeter. Heating rate was 10 °C min^{-1}. Samples (15–20 mg) of 20% (w/v) dispersions in distilled water were hermetically sealed in aluminum pans; a double empty pan was used as reference. After DSC analysis, the pans were punctured and the dry matter content was determined by drying overnight at 105 °C. Peaks indicating an endothermic heat flow were obtained for scans of some samples. To determine peak areas, baselines were constructed as shown in Figure 1 and the area obtained by using a Morphomat 34 Zeiss image analyzer. The enthalpy of total denaturation (ΔH_T) was calculated according to the method of Arntfield and Murray (1981), and the values were expressed in calories per gram of dry weight basis. Triplicate samples were evaluated by DSC.

Electrophoresis (SDS–PAGE). Slab sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the discontinuous buffer system of Laemmli (1970) at a gel concentration of 12.6% using Pharmacia gel electrophoresis apparatus GE-2/4. Gel slabs were fixed and stained simultaneously in a solution of methanol, acetic acid, and water (5:5:2) and 0.1% Coomassie Brilliant Blue R-250. Molecular weights of the protein bands were estimated by means of a MW-SDS-70L kit (Sigma Chemical Co.). Analyses were done in duplicate. Sample preparation for SDS–PAGE was carried out as follows: (a) 30 mg of protein isolate was dissolved in 30 mL of 0.086 M Tris, 0.09 M glycine, and 4 mM EDTA, pH 8 buffer, containing 8 M urea (urea buffer); (b) 30 mg of protein isolate was shaken in 30 mL of distilled water at 20 °C for 1 h and centrifuged (12100*g*, 4 °C, 20 min). The dispersions (a) and the supernatants (b) were mixed with an equal volume of SDS–PAGE sample buffer containing 5% (v/v) 2-mercaptoethanol. About 50 μg of protein was applied to each gel slot. For protein quantification by densitometric scanning, gels were scanned at 570 nm (reference 405 nm) by using Shimadzu dual-wavelength TLC scanner CS-910. The areas corresponding to each band were measured by using image analyzer Morphomat 34 Zeiss. For this purpose, each peak was determined by vertical lines perpendicular to the baseline of the densitogram.

Total 7S and 11S percentages were calculated as the sum of the areas of their subunits/polypeptides with respect to the total area of the densitogram.

The quantitative estimation of each subunit/polypeptide of

† CIDCA.
‡ Cátedra de Quimica Biológica.

0021-8561/91/1439-1029$02.50/0

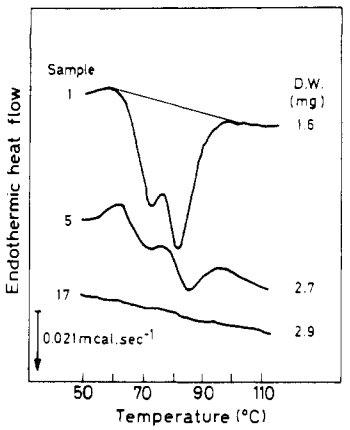

Figure 1. Differential scanning calorimetry (DSC) thermograms of 20% (w/v) commercial soy protein isolates in distilled water with different degrees of denaturation. Sensitivity was 0.021 mcal s^{-1} and heating rate 10 °C min^{-1}. Baseline is shown in sample 1. DW, dry weight.

7S and 11S proteins was calculated as the percentage of the area of the subunit polypeptides with respect to the total 7S or 11S area.

Apparent Viscosity. Apparent viscosities (η_{app}) were measured in isolate dispersions between 2.5 and 14% (w/w). Measurements were carried out at 20 °C in a Haake Rotavisco RV2 viscometer using a Sensor system NV and a rotor speed varying from 0 to 128 rpm in 2 min. Apparent viscosity at 128 rpm was calculated as

$$\eta_{app} = GS/n \quad \text{(centipoise)}$$

where G is an instrument factor (centipoise/scale grade min), S is the scale value, and n is the rotor speed (rpm).

Imbibed Water Ratio. Ratios of total to imbibed (T/I) water were calculated (Urbanski et al., 1983) as

$$\frac{T}{I} = \frac{\text{g of total water/g of dispersion}}{\text{g of imbibed water/g of dispersion}} = \frac{1-P}{P(\text{WIC})}$$

where P is grams of isolate per gram of dispersion and WIC is grams of water imbibed per gram of soy protein isolate.

Gel Viscosity. Gel properties of the isolates were measured in 10% (w/w) dispersions in distilled water, heated at 80 °C for 30 min and then cooled overnight at 4 °C. Gel viscosity was measured at 25 °C in a Brookfield viscometer RVT using the Helipath stand with the T spindle series at 5 rpm. Values were expressed in poises.

Water-Imbibing Capacity (WIC). The WIC of soy protein isolates was determined by using a modification of the Baumann apparatus (Torgensen and Toledo, 1977). This apparatus consists of a funnel connected to a horizontal capillary. A 50-mg sample was dusted on a wetted filter paper which was fastened to a glass filter placed on top of the funnel filled with water. The apparatus was kept at 20 °C. The uptake of water by the sample at equilibrium was read in the graduated capillary and expressed as milliliters of water imbibed per gram of isolate. Determinations were performed in duplicate.

RESULTS AND DISCUSSION

Solubility and Denaturation Degree. Three thermograms corresponding to isolates having marked differences in their degrees of denaturation are shown in Figure 1. Peaks corresponding to endothermic transitions are attributed to 7S (T_{max} 74 °C) and 11S (T_{max} 83 °C) proteins (Hermansson, 1978). The ΔH value represents a valuable parameter in assessing the degree of denaturation of plant proteins (Hermansson, 1978; Murray et al., 1981). Thus, isolate 17 is completely denatured (ΔH_T = 0 cal/g) and isolate 5 is partially denatured. Values of total enthalpy of denaturation (ΔH_T) of all samples are shown in Table I. Isolates having maximal ΔH_T values present solubility values greater than 50%, while those

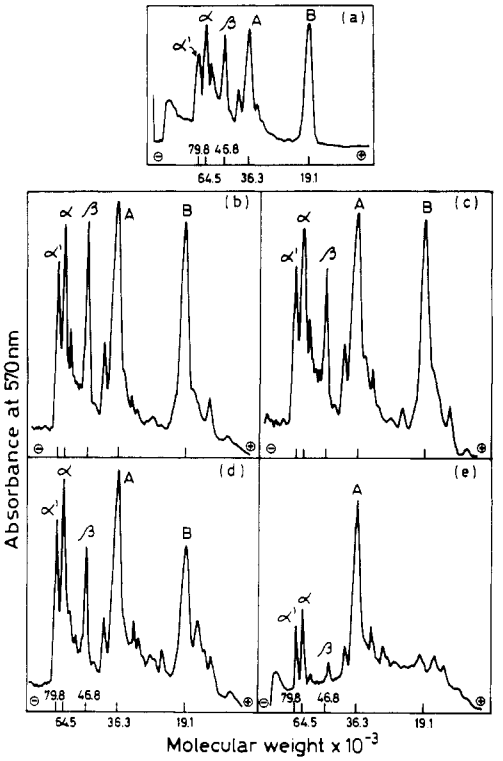

Figure 2. Densitometric scans of the electrophoretic patterns: (a) Sample 1 solubilized in urea buffer; (b, c, d, e) soy isolates 4, 11, 14, and 17 water-soluble fractions, respectively.

Table I. Solubility and Total Denaturation Enthalpy (ΔH_T) of Commercial Soy Isolates

soy isolates	solubility, %	ΔH_T, cal/g	soy isolates	solubility, %	ΔH_T, cal/g
1	83.6	2.85	11	59.0	0.30
2	73.8	2.53	12	83.8	0.10
3	54.7	2.30	13	45.5	0
4	57.8	2.16	14	27.7	0
5	68.1	0.60	15	29.7	0
6	61.0	0.65	16	27.9	0
7	44.0	0.45	17	24.8	0
8	36.3	0.30	18	20.6	0
9	58.0	0.085	19	36.5	0
10	48.1	0.081			

isolates with ΔH_T = 0 cal/g have solubilities below 50%. Bearing in mind that denaturation of globular proteins leads to unfolding of the polypeptide chain, hydrophobic groups located in the interior of the molecule would be exposed for interaction with water, thus leading to a decrease in solubility. Therefore, solubility could serve as an index of denaturation for these proteins. At variance with this, some isolates with a high degree of denaturation (5, 6, 9, 11, and 12), also have high solubilities (Table I). These cases, already reported by Shen (1976) and Hermansson (1986), indicate that it is not feasible to correlate low solubility with protein denaturation in commercial soy protein isolates. Therefore, determinations of solubility as a single index parameter are not a sensitive indicator of denaturation. Other aspects should be analyzed to understand the cause of this behavior. For instance, it would be interesting to study the superficial hydrophobicity of different isolates as well as the presence of water-soluble aggregates.

Relative Composition of Different Protein Species. The proportion of 7S and 11S proteins present in commercial isolates was evaluated by means of SDS-PAGE of samples solubilized in urea buffer. The densitogram corresponding to sample 1 is shown in Figure 2a. All other isolates showed similar electrophoretic patterns. Molec-

Table II. Quantitative Estimation of 7S Protein Subunits and 11S Protein Polypeptides of Water-Soluble Fractions

		7S			11S	
group[a]	isolates	%α′	%α	%β	%A	%B
I, $\Delta H > 2$	1–4	20.8–24.0	27.4–37.0	39.0–52.9	48.1–60.1	39.9–51.9
II, $0 < \Delta H < 1$	5–9, 11, 12	25.4–33.0	36.3–46.9	21.1–37.8	39.9–60.3	39.9–60.1
III, $\Delta H \simeq 0$	10, 13–16, 18, 19	20.6–38.8	39.6–52.3	14.3–38.4	61.9–83.3	16.7–38.1
IV, $\Delta H = 0$	17	33.8	52.6	13.6	94.2	5.8

[a] Groups I–IV correspond to groups of soy isolates exhibiting the same type of electrophoretic pattern.

Table III. Water-Imbibing Capacity of Soy Protein Isolates

soy isolates	WIC[a]	soy isolates	WIC[a]
1	5.8	11	11.6
2	6.2	12	6.7
3	7.8	13	7.3
4	5.2	14	7.6
5	12.2	15	8.8
6	11.0	16	7.8
7	10.6	17	6.4
8	9.4	18	8.1
9	12.8	19	8.0
10	7.3		

[a] WIC was expressed as milliliters of water imbibed per gram of isolate.

ular weight estimates of subunits α′, α, and β of 7S and A and B polypeptides of 11S were 79 800, 64 500, 46 800, 36 300, and 19 100, respectively. The percentages of each protein in each isolate were similar in all samples. Average values corresponding to 7S were 36.8 ± 2.3%, while those of 11S were 47.3 ± 2.3%. Quantitative estimations of each subunit/polypeptide in all samples were as follows: α′, 24.6–31.6%; α, 39.4–44.6%; β, 28.7–33.5%; A, 40.0–49.3%; B, 50.3–60.0%. Nevertheless, the electrophoretic pattern of proteins extracted in distilled water was not the same in all samples. Four different types of characteristic patterns were observed (Figure 2b–e) corresponding to groups I, II, III, and IV (Table II). The quantitative estimation of each subunit in all samples is shown in Table II. It should be noted that, while the degree of denaturation of different isolates increased, the β-subunit proportion corresponding to 7S in the water-soluble fraction decreased. Similarly, the proportion of 11S basic polypeptides also diminished in completely denatured isolates. On the basis of these results, it may be suggested that the β-7S subunit B-11S polypeptides of the completely denatured isolates form water-insoluble aggregates. On the other hand, isolates with a high proportion of native protein show a low content of β-B aggregates.

Water-Imbibing Capacity. The WIC of soy protein isolates is shown in Table III. As it can be seen, isolates 5–9 and 11, which have a high degree of denaturation (ΔH_T = 0.085–0.65 cal/g), showed a larger WIC than both samples with a high proportion of native proteins and samples containing completely denatured proteins (ΔH_T = 0 cal/g). This fact indicates that a certain unfolding of the polypeptide chains is necessary to obtain a matrix capable of absorbing or retaining water. Furthermore, it can be observed that isolate 12 with a degree of denaturation comparable to that of sample 8, 9, or 11 evidences a low WIC. This different behavior may be correlated to the high solubility of isolate 12 (84%); the other isolates showed intermediate values (40–60%). On the other hand, isolates with ΔH_T = 0 cal/g showed both low solubility and low values of WIC. Thus, it might be suggested that extreme values of either solubility or degree of denaturation would lead to the formation of protein isolates with low WIC.

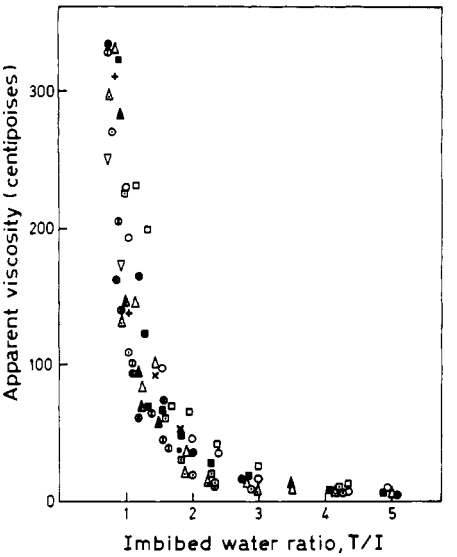

Figure 3. Relationship between apparent viscosity and imbibed water ratio (T/I) for commercial soy protein isolates. T = grams of total water per gram of dispersion; I = grams of imbibed water per gram of dispersion. Soy isolates: (○) 1; (×) 2; (△) 3; (□) 4; (●) 5; (◎) 6; (+) 7; (▲) 8; (▲) 9; (⊗) 10; (▽) 11; (■) 12; (⊡) 13; (▲) 15; (•) 17; (⊕) 18.

Relationship between Viscosity and WIC. Water-imbibing capacities of soy sodium proteinate, sodium caseinate, and whey protein concentrate have been related to viscosity by Hermansson (1975). Urbanski et al. (1983) suggested that the factor controlling flow characteristics of solid particles is the ratio between imbibed water and the liquid or remaining water. They also found that all points fell on the same curve when the apparent viscosity was plotted against the imbibed water ratio. This relationship for some commercial isolates is shown in Figure 3. When T/I was below 1.5, slight variations in the imbibed water ratio correspond to large variations of the apparent viscosity. Thus, for values lower than 25% free water (T/I = 1.33), the internal friction was markedly increased, leading to an increase in the apparent viscosity. On the other hand, when the amount of free water was more than 75% of the total water (T/I = 4.0), the apparent viscosity always reached the minimum value. The apparent viscosity in all samples behaved in the same way when the imbibed water ratio was changed. Consequently, previous treatments undergone by the different isolates during processing have a marked effect on the WIC. Thus, dispersions of similar viscosity can be obtained from two isolates of different WICs by varying the T/I ratio.

Gel Viscosity. Gel viscosities of dispersions (10%) of commercial isolates are shown in Figure 4. The highest gel-forming ability induced by heating of soybean proteins corresponds to isolates having a higher proportion of β-7S subunits and B-11S polypeptides which are soluble in water (groups I and II, Table II). Conversely, the samples corresponding to groups III and IV show very low or no gelation capacity under the conditions studied. Hermansson

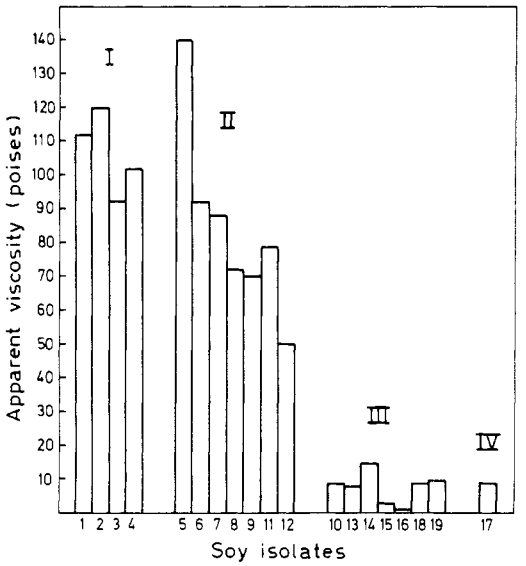

Figure 4. Gel viscosity of 10% w/w commercial soy protein isolates in distilled water, heated at 80 °C for 30 min and cooled at 4 °C overnight. I–IV correspond to soy isolate groups in Table II.

(1986) reported that heat-induced gel formation is a complex process involving several different reactions, in which association and dissociation reactions are of utmost importance for the onset of gelation. Heating caused dissociation of both 7S and 11S globulins, which subsequently interacted with each other. Utsumi et al. (1984) demonstrated that basic 11S polypeptides have a high affinity for the β-7S subunit, thus forming soluble macrocomplexes. They also suggested that such changes might occur under the conditions of thermal gelation of soy proteins. It has been found in this study that heat-induced gelation capacity in the commercial isolates analyzed is related to soluble β-subunit B polypeptides content. This means that the water-soluble fraction contains 7S and 11S proteins, the β subunits and B polypeptide of which may interact, producing macrocomplexes that lead to gel formation. Hence, isolates showing a greater proportion of β-subunit B polypeptide will exhibit the highest gelation capacity and will also produce gels with greater viscosity. Isolates corresponding to groups I and II show the above-described characteristics. Isolates of groups III and IV contain water-insoluble aggregates in which β subunits and B polypeptides participate. The gelation capacity was almost nil in these isolates, since it was observed that subunits previously aggregated do not contibute to the gelation process.

CONCLUSIONS

The degree of denaturation reached by soy proteins in preparing isolates is an important factor that affects functional properties such as solubility, water absorption, viscosity, and capacity for gel formation.

Commercial isolates showing a high capacity for both water imbibition and heat-induced gelation are those in which proteins have a high degree of denaturation without important aggregation between β-subunit B polypeptides.

ACKNOWLEDGMENT

This investigation was supported by the Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET), Secretaria de Ciencia y Técnica (SECyT), and Comision de Investigaciones Cientificas de la Provincia de Buenos Aires (CIC). E.L.A. is a Research Fellow of CIC. M.C.A. and J.R.W. are Career Investigators of CONICET. We acknowledge Teresa Quattrini for the transcription of the manuscript.

LITERATURE CITED

Arntfield, S. D.; Murray, E. D. The influence of processing parameters on food protein functionality I. Differential scanning calorimetry as an indicator of protein denaturation. *Can. Inst. Food Sci. Technol. J.* **1981**, *4*, 289–294.

Brooks, J. R.; Morr, C. V. Current aspects of soy protein fractionation and nomenclature. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1347–1354.

Derbyshire, E.; Wright, D. J.; Boulter, D. Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **1976**, *15*, 3–7.

Gornall, A. C.; Bardawill, C. J.; David, M. M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751–766.

Hermansson, A. M. Functional properties of proteins for foods: flow properties. *J. Texture Stud.* **1975**, *5*, 425–439.

Hermansson, A. M. Physico-chemical aspects of soy proteins structure formation. *J. Texture Stud.* **1978**, *9*, 33–58.

Hermansson, A. M. Soy protein gelation. *J. Am. Oil Chem. Soc.* **1986**, *63*, 658–666.

Kinsella, J. E. Functional properties of soy protein. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242–251.

Kinsella, J. E.; German, B.; Damodaran, S. Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New Protein Foods*; Academic Press: New York, 1985; Vol. 5.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* **1970**, *227*, 680–685.

Murray, E. D.; Myers, C. D.; Barker, L. D.; Maurice, T. J. Functional attributes of proteins. A noncovalent approach to processing and utilizing proteins. In *Utilization of protein resources*; Stanky, D. W., Murray, E. D., Lee, D. H., Eds.; Food and Nutrition Press: Westport, CT, 1981.

Nielsen, N. C. The structure and complexity of the 11S polypeptides in soybeans. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1680–1686.

Saio, K.; Watanabe, T. Differences in functional properties of 7S and 11S soybean proteins. *J. Texture Stud.* **1978**, *9*, 135–157.

Shen, J. L. Solubility profile, intrinsic viscosity, and optical rotation studies of acid precipitated soy protein and of commercial soy isolate. *J. Agric. Food Chem.* **1976**, *24*, 784–787.

Torgensen, H.; Toledo, R. T. Physical properties of proteins preparations related to their functional characteristics in comminuted meat systems. *J. Food Sci.* **1977**, *42*, 1615–1620.

Urbanski, G. E.; Wei, L. S.; Nelson, A. J.; Steinberg, M. P. Flow characteristics of soybean constituents controlled by ratio of total to imbibed water. *J. Food Sci.* **1983**, *48*, 691–694.

Utsumi, S.; Damodarán, S.; Kinsella, J. E. Heat-induced interactions between soybean proteins: preferential association of 11S—Basic subunits and β-subunits of 7S. *J. Agric. Food Chem.* **1984**, *32*, 1406–1412.

Received for review June 4, 1990. Revised manuscript received October 12, 1990. Accepted January 14, 1991.