Functional Properties of Soy Protein Fractions Produced Using a Pilot Plant-Scale Process

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ABSTRACT: Soy proteins fractionated by the modified Nagano process (Nagano method) and a simplified pilot-plant process (CCUR method) were studied for their functional properties, including solubility, viscosity, emulsification, and foaming. The functional properties of the three fractions produced by the Nagano method—glycinin (11S), β-conglycinin (7S), and an intermediate fraction (IM)—were studied under a selected range of pH, ionic strengths, and protein concentrations. The 11S fraction was more soluble than the 7S at pH 2–3, whereas the 7S was more soluble than 11S at pH 5–6. Adding NaCl changed the solubility of both fractions at pH 4–5 compared to a neutral pH. Other functional properties were related to solubility in the 7S and 11S fractions. The CCUR method yielded only two fractions, 11S and 7S, and the functionality of those fractions was tested at a neutral pH. The solubility of the CCUR samples was slightly higher at extreme pH levels compared to 11S and 7S fractions from the Nagano method at a neutral pH. The relationship between solubility and other functional properties was clearer in CCUR samples. These results indicate that the simplified pilot-scale CCUR fractionation process can influence the functional properties of the protein fractions.

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KEY WORDS: Functional properties, pilot process, soy protein fractions.

Soy proteins have been used in a variety of food applications for many years. Some of the reasons for their use include their relatively low cost and availability compared to other competing food ingredients. The primary reason for the use of soy proteins, however, is their wide range of functional properties that help to stabilize food systems as well as provide sensory properties such as texture that consumers demand. Soy proteins are used not only in food applications but also in nonfood applications, such as plastics and adhesives (1,2). These functional properties, which are inherent in the protein or imparted through modification, include water solubility, emulsification, viscosity, foaming, and gel and film formation. Research is now being carried out to allow the major fractions of soy protein, glycinin (11S) and β-conglycinin (7S), to become commercially available. If these protein fractions are to be used in food and nonfood applications, however, their functional properties will have to be characterized in order to choose the appropriate application. The Center for Crops Utilization Research (CCUR) at Iowa State University modified the Nagano procedure (3,4) of soy protein fractionation and, using pilot-plant processing, produced three soy protein fractions: 11S, 7S, and an intermediate mixture (IM). A simplified pilot-plant process was also developed to eliminate the IM (5). Processing treatments, including choice of raw materials, method of oil removal, pH, and salts added during protein isolation or fractionation, have been known to affect the functional properties of the final product (6–10). Since the processing method can influence functional properties, it is important to study the functional properties of protein fractions derived from new or modified separation processes, such as the ones derived from these two processes. The functional properties evaluated in this study were solubility, viscosity, emulsification, and foaming.

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EXPERIMENTAL PROCEDURES

Materials. The soybean cultivar MBS 2795 (1995 crop, Iowa) was used throughout the entire process of soy protein isolate and fraction production at the CCUR pilot plant. The modified Nagano process was carried out on a 15-kg sample of soybean seeds (4). Three fractions were separated from the process: glycinin (11S-N), β-conglycinin (7S-N), and an intermediate mixture (IM-N). Protein and ash contents of the fractions processed were analyzed, and the functionality of each of these fractions was tested at different pH levels, ionic strengths, and temperatures. The simplified pilot-plant process developed at CCUR (5) produced only two fractions: glycinin (11S-CCUR) and β-conglycinin (7S-CCUR). Except for solubility, the functionality of these fractions was tested at a neutral pH and zero ionic strength. In all functionality analyses, each experiment was replicated three times.

Methods. (i) Solubility. A modified method adapted from the procedure described by Vojdani (11) was used for solubility testing. From samples produced by the modified Nagano method, 5 mL of a 0.1% protein suspension in water was added to 50-mL Oakridge tubes and adjusted to the desired pH or ionic strength. The pH was adjusted from 2–10 using 0.1 M hydrochloric acid (HCl) or sodium hydroxide (NaOH). The suspension was mixed at 120 rpm (Versa-Bath® Model S #224, Fisher Scientific, Fairlawn, NJ) for 1 h at room temperature.

Samples were centrifuged at 32,500 × *g* for 30 min. The protein content in the supernatant was determined by the biuret method. The percentage of soluble protein was calculated based on the total protein in the sample, estimated using the micro-Kjeldahl method (g N × 6.25) (12).

(ii) Viscosity. A protein suspension was prepared using a Waring® blender (model 31BL92; Dynamics Corp., New Hartford, CT) at low speed. Variations in parameters were tested using the modified Nagano samples. To demonstrate the effect of protein concentration on viscosity, suspensions were made with 5, 7, and 9% protein. For other experiments (temperature and pH), a suspension was made with 7% protein. The suspension was centrifuged at low speed (3,200 × *g*) to remove air bubbles and allowed to stabilize for 2 h. Viscosity was measured using a capillary viscometer (Cannon Instrument Company, State College, PA). The effect of temperature was studied at 5, 25, and 50°C. For experiments other than those comparing temperature, the chosen temperature was 25°C. Except for the experiment studying the effect of pH (3–9), samples were kept at a neutral pH (≃6.7).

(iii) Emulsification capacity (EC). A 2% protein suspension (25 mL) was placed in a 500-mL beaker and the suspension was blended continuously at high speed (*ca.* 12,000 rpm) with a hand-held mixer (Bamix®, Mettlen, Switzerland). Soybean oil was added continuously at a flow rate of 1 g/s until the inversion point of oil-in-water to water-in-oil was reached. The amount of oil added (by weight) was recorded. EC was calculated as the maximum amount of oil that could be emulsified by 1 g of protein. For the modified Nagano method samples, EC was measured at three different pH levels. The CCUR samples were tested only at the natural pH of the samples.

(iv) Emulsification activity (EA). A 2% protein suspension (21 mL) was blended with 7 mL of soybean oil for 1 min in the microcontainer of a Waring® blender (110 mL size) at low speed. The emulsion was diluted 1000-fold with 0.1% SDS, and the absorbance was measured at 500 nm. The diluted emulsion was incubated at 95°C in a water bath. The emulsion absorbance was measured every 15 min for 60 min, starting from time 0. The emulsification activity index (EAI) was calculated using the formulas explained by Pearce and Kinsella (13).

(v) Foaming. A 0.5% protein suspension was added to a glass column with a fritted glass disk at the bottom. Nitrogen (N_2) gas at a flow rate of 100 mL/min was added to the column. Data were obtained and tabulated according to the method described by Sorgentini *et al.* (6).

RESULTS AND DISCUSSION

Solubility. Solubility is considered the most important functional attribute for soy protein because of its significant influence on other functional properties. Fractions were tested for their solubilities at pH ranges from 2 to 10. All samples showed an inverted bell-shaped trend in solubility with the change in pH (Fig. 1). Solubility was high at the extreme ends

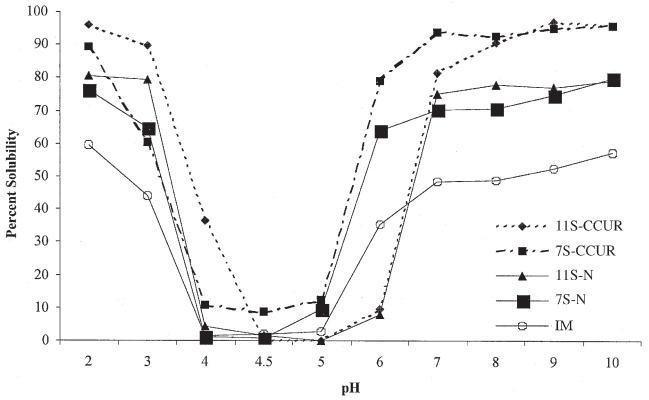

FIG. 1. Percent solubility of protein at different pH levels. Each point represents an average of three data points. 11S, glycinin; 7S, β-conglycinin; CCUR, Center for Crops Utilization method; N, Nagano method; IM, intermediate fraction.

of the pH tested and low or no solubility was recorded between pH 4 and 5. This result was expected because the isoelectric point of soy protein is around pH 4.5. Among the fractions tested, the 11S-CCUR sample had the highest solubility from pH 2–4 and 8–10. Between pH 5 and 7 the solubility of 11S samples from both procedures was the same. Between pH 3 and 5 the 7S samples from both procedures had the same solubilities. At the other pH levels, the CCUR samples showed greater solubility than the modified Nagano samples. Although the IM sample also showed similar behavior at pH 4–5, it had the lowest solubility at all the other pH levels tested compared to the other fractions.

Viscosity. Viscosity is another very important functional property in many soy protein applications. In some industrial uses of soy protein, such as adhesives, the protein must be applied at a high solids content but still retain a relatively low viscosity. The viscosity of these suspensions was studied at different protein concentrations. Samples of modified Nagano fractions were prepared at concentrations of 5, 7, and 9%. The viscosities were tested at 25°C and pH 7 (Fig. 2). The intermediate fraction showed a significant increase in viscosity at 9% protein concentration. Results showed that the concentra-

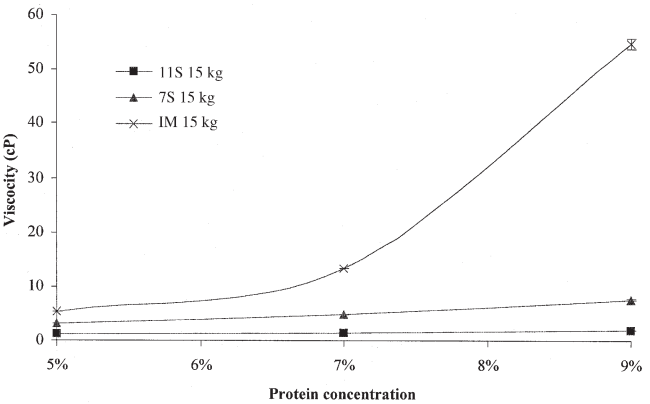

FIG. 2. Effect of protein concentration on viscosity of modified Nagano fractions. Each point represents an average of three data points. For abbreviations see Figure 1.

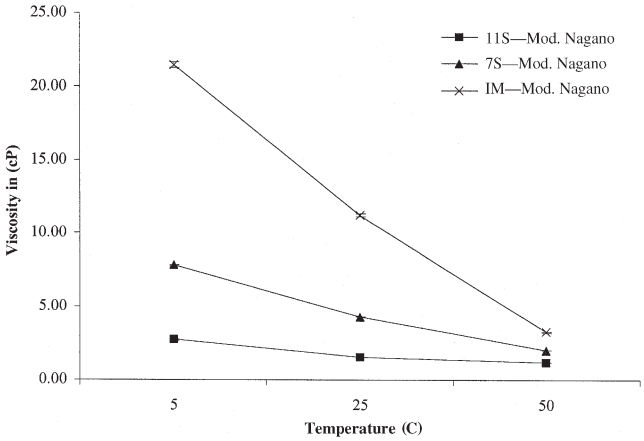

FIG. 3. Effect of temperature on viscosity of modified (Mod.) Nagano fractions. Each point represents an average of three data points. For abbreviations see Figure 1.

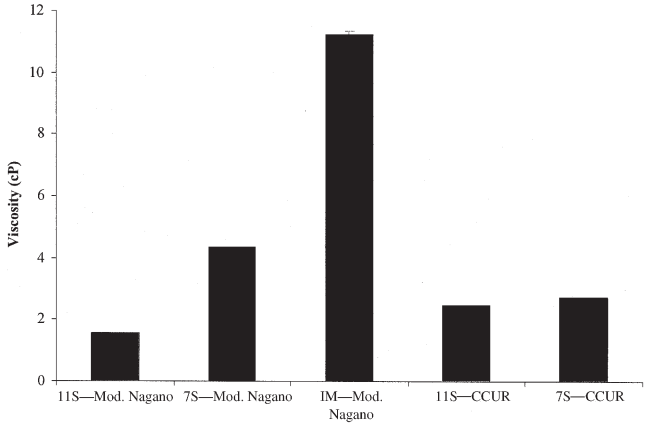

FIG. 5. Viscosity of modified (Mod.) Nagano fractions compared to the CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

tion had little effect on the viscosity of the 11S and 7S fractions. All the protein fractions had similar viscosities at 7% protein concentration; therefore, the effects of pH and temperature were studied at 7% protein concentration for the modified Nagano fractions. The change in viscosity with temperature showed that viscosity decreased with increasing temperature for all three samples (Fig. 3). IM had the highest viscosity, followed by 7S and 11S, and showed the largest drop in viscosity with increased temperature. The effect of pH on viscosity exhibited a trend similar to that observed for temperature (Fig. 4), with IM having the highest viscosity followed by 7S and 11S.

On the basis of the foregoing information, samples from the simplified pilot CCUR method (5) were compared with the three fractions from the modified Nagano process, at 7% protein, 25°C, and neutral pH. The results showed that IM had the highest viscosity (Fig. 5) followed by 7S-N, 7S-CCUR, and 11S-CCUR, with 11S-N being the least viscous.

Emulsification properties. (i) EC. EC is defined as the maximum amount of oil (in grams) emulsified by 1 g of protein before the oil-in-water emulsion inverts to a water-in-oil emulsion. The effect of pH on EC was studied using all fractions from the modified Nagano method at pH 2, 7, and 9.5 (Fig. 6). At pH 2, IM had the highest EC, followed by 7S-N and 11S-N. At the other two pH, IM had the lowest EC. In all three fractions, the lowest EC was recorded at pH 7. In the basic pH range, 7S-N had the highest EC. At pH 2 and 9.5, 11S-N showed no significant difference in its capacity to emulsify. When the fractions were compared separately with the respective solubility values at each pH, they followed a fairly linear relationship with the EC. But when all fractions were compared, the relationship was not so clear. Figure 7 compares the Nagano fractions with the CCUR fractions, measured at the natural pH of each fraction. The 7S-CCUR fraction showed the highest EC, and in both processing methods

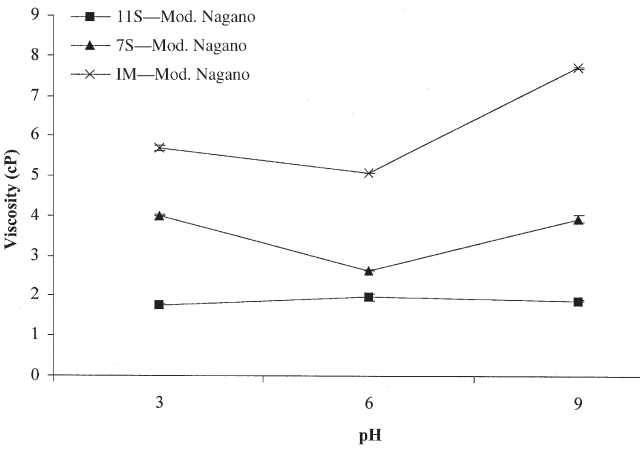

FIG. 4. Effect of pH on viscosity of modified (Mod.) Nagano fractions. Each point represents an average of three data points. For other abbreviations see Figure 1.

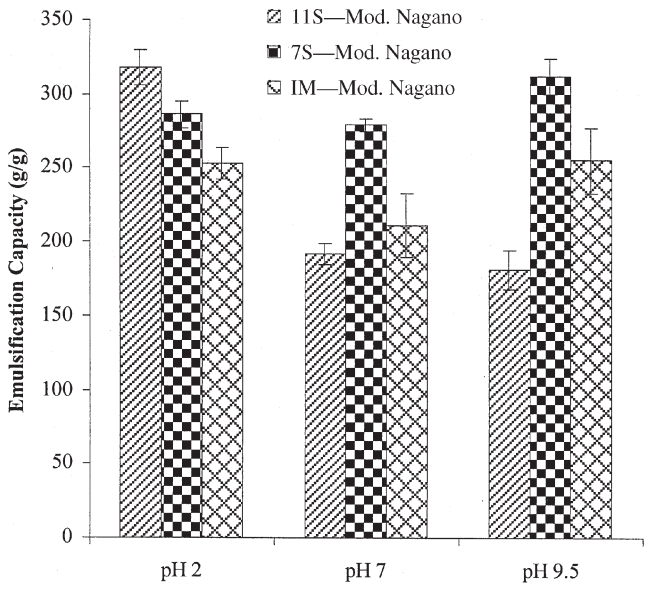

FIG. 6. Effect of pH on emulsification capacity of modified (Mod.) Nagano fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

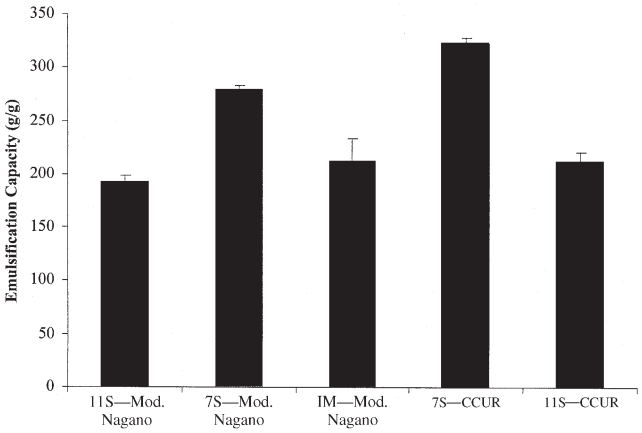

FIG. 7. Emulsification capacity of modified (Mod.) Nagano fractions compared to CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

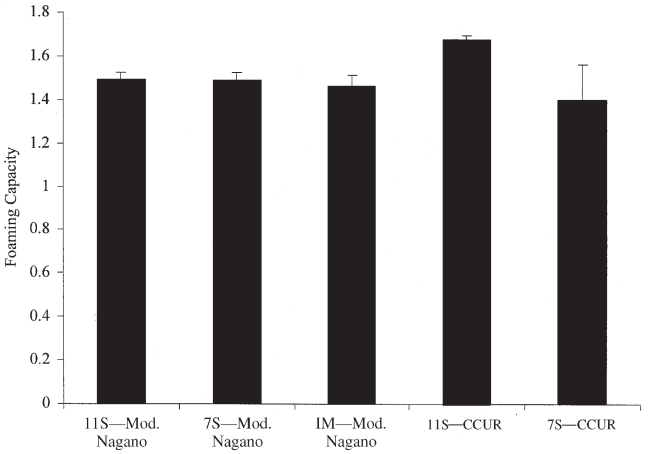

FIG. 9. Foaming capacity of modified (Mod.) Nagano fractions compared to CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

7S had a higher EC than 11S. In CCUR samples, the EC showed a linear relationship with solubility, but the Nagano fractions did not.

(ii) EA. For both food and nonfood applications, it is important to understand the EA as thoroughly as we understand the EC. The EA was measured by reading the absorbance at 500 nm of a protein–oil emulsion; the higher the absorbance, the greater the EA. Absorbance, recorded over time, was used in calculating the EAI. Figure 8 shows that the EAI of the IM fraction was greater than that of the 11S and 7S fractions in both processing methods.

Foaming properties. Three parameters were used to determine the foaming properties of the fractions. Foaming capacity is a measure of the efficiency of foam generation. K is used to estimate the potential of the foam to hold liquid and reduce drainage, thereby indicating foam stability. V_i is the rate of liquid incorporation into the foam and is used to estimate the density of the foam. The results in Figure 9 showed that there was no significant difference in foaming capacity between the Nagano fractions. However, 11S-CCUR had a slightly higher foaming capacity than 7S and the Nagano fractions. Figure 10 shows that both of the 11S fractions had significantly higher K values than the other fractions and that the 11S-CCUR fraction had a significantly higher K value than the 11S-N fraction. The higher K values of the 11S samples indicate that they had greater foam stability than the 7S or IM fractions. Figure 11 shows that the foam density was higher in the two 7S fractions than the 11S fractions. Among the 7S fractions, 7S-N showed a higher foam density than 7S-CCUR. Both 11S fractions had a lower foam density than IM, and 11S-N was higher than 11S-CCUR. Thus, our results show that foam stability (K values) and foam density (V_i values) were inversely related.

In this study the processing method influenced the functional properties of the fractions. The solubility curves for both the 7S and 11S fractions overlapped at moderate pH levels; however, at the more extreme pH levels, the fractions from the CCUR process had higher solubility than the fractions from the modified Nagano method. In the two-fraction process (CCUR), most other functionality properties seemed to be related to solubility. In the IM fraction of the modified

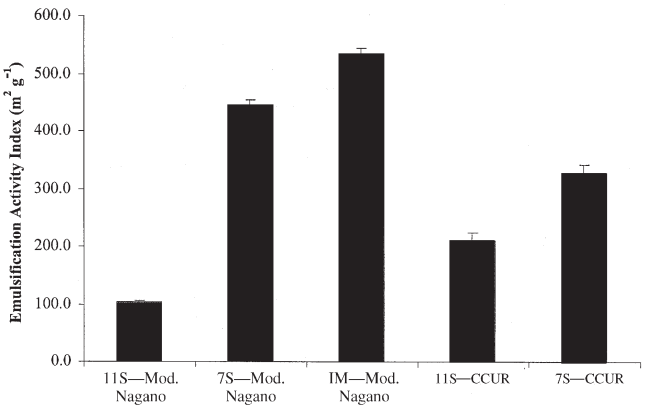

FIG. 8. Emulsification activity index of modified (Mod.) Nagano fractions compared to CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

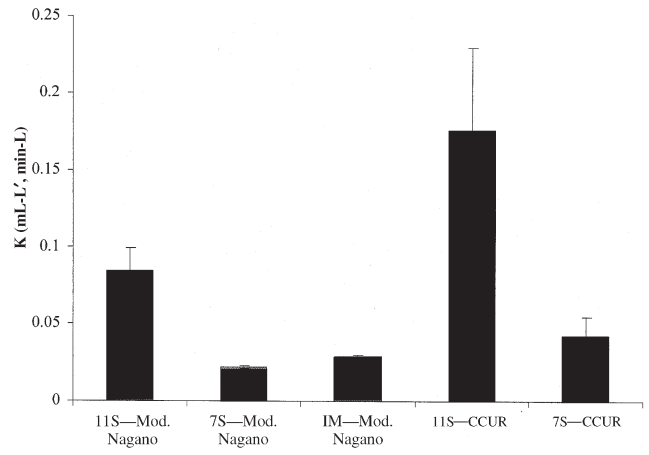

FIG. 10. Foaming stability of modified (Mod.) Nagano fractions compared to CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

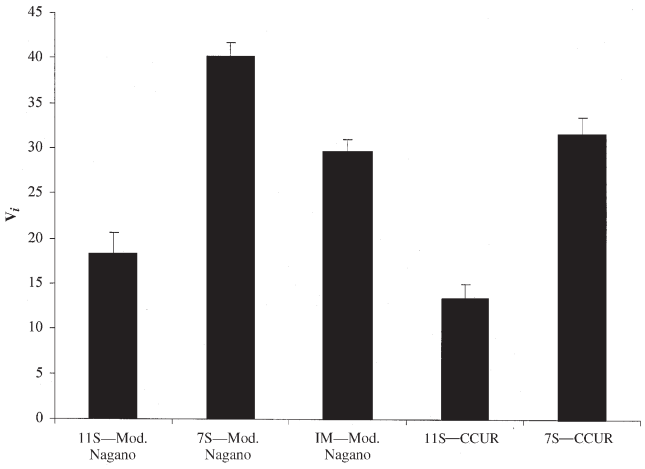

FIG. 11. Foaming density of modified (Mod.) Nagano fractions compared to CCUR fractions. Data represent an average of three replications; error bars are SD. For abbreviations see Figures 1 and 3.

Nagano method, it was difficult to relate solubility to other functional properties.

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REFERENCES

1. Myers, D.J., Past, Present and Potential Uses of Soy Proteins in Non-food Industrial Applications, in *Proceedings of the AOCS World Conference and Exhibition on Oilseed Technology and Utilization*, edited by T. Applewhite, AOCS Press, Champaign, 1993, pp. 278–285.
2. Myers, D.J., World Industrial Applications for Soy Protein and Potential for Increased Utilization, *Cereal Foods World 38*: 355–360 (1993).
3. Nagano, T., M. Hirotsuka, H. Mori, K. Kohyama, and K. Nishinari, Dynamic Viscoelastic Study on the Gelation of 7S Globulin from Soybeans, *J. Agric. Food Chem. 40*:941–944 (1992).
4. Wu, S.W., P.A. Murphy, L.A. Johnson, A.R. Fratzke, and M.A. Reuber, Pilot-Plant Fractionation of Soybean Glycinin and β-Conglycinin, *J. Am. Oil Chem. Soc. 76*:285–293 (1999).
5. Wu, S.W., P.A. Murphy, L.A. Johnson, M.A. Reuber, and A.R. Fratzke, Simplified Process for Soybean Glycinin and β-Conglycinin Fractionation, *J. Agric. Food Chem. 48*:2702–2708 (2000).
6. Sorgentini, D.A., J.R. Wagner, and M.C. Añón, Effects of Thermal Treatment of Soy Protein Isolate on the Characteristics and Structure–Function Relationship of Soluble and Insoluble Fractions, *Ibid. 43*:2471–2479 (1995).
7. Kinsella, J.E., Relationship Between Structure and Functional Properties of Food Proteins, in *Food Proteins*, edited by P.F. Fox and J.J. London, Elsevier Applied Science, London, 1982, pp. 51–103.
8. Hall, G.M., Basic Concept, in *Methods of Testing Protein Functionality*, edited by G.M. Hall, Blackie Academic & Professional, London, 1996, pp. 1–4.
9. Yamauchi, F., T. Yamagishi, and S. Iwabuchi, Molecular Understanding of Heat Induced Phenomena of Soybean Protein, *Food Rev. Int. 7*:283–322 (1991).
10. Hermansson, A.M., Physico-chemical Aspects of Soy Proteins Structure Formation, *J. Text. Studies 9*:33–58 (1978).
11. Vojdani, F., Solubility, in *Methods of Testing Protein Functionality*, edited by G.M. Hall, Blackie Academic & Professional, 1996, pp. 11–60.
12. Corn Refiners Association, Protein, in *Corn Refiners Association Standard Analytical Methods of the Member Companies,* Corn Refiners Association, Washington, DC, 1996, Method A-18.
13. Pearce, K.N., and J.E. Kinsella, Emulsifying Properties of Proteins: Evaluation of a Turbidimetric Technique, *J. Agric. Food Chem. 26*:716–723 (1978).

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