FUNCTIONAL PROPERTIES OF A NEW SOY PROTEIN ISOLATE¹

M. SHEMER,² H. L. CREININ,³ R. E. McDONALD, and W. E. IRWIN,⁴ Miles Laboratories, Marschall Division, Elkhart, IN 46514

ABSTRACT

Cereal Chem. 55(3): 383-391

A soybean protein fraction, SP 55, has been obtained by an aqueous fractionation from soy flour. The soy protein isolate exhibits unusual heat coagulation, viscosity, and emulsification properties. Its instantaneous thermosetting properties

occur between 85 and 90°C. It also displays an unusual viscosity versus pH relationship. SP 55, at as high as 50% solids in water, is a free-flowing viscous material at both isoelectric and neutral pHs.

Soy protein isolate is generally produced in the following manner (1): Defatted soy meal or flour is subjected to an aqueous alkaline system (pH 9) that extracts the soluble proteins. The aqueous extract is separated from the insoluble residue, and the soy isolate is obtained from the clarified supernatant by isoelectric precipitation (pH 4-5) of the protein. The isolate is washed with water and spraydried at the isoelectric point, or it may be neutralized to pH 7 and spray-dried. The process yields a soy protein isolate that contains greater than 90% protein on a dry basis.

Soy protein isolates are used in most edible applications for their functional properties. Firm, resilient, self-supporting gels of soy protein isolates can be obtained, usually at concentrations above 15% (2). These gels, however, usually lack the firm binding properties of egg albumen. Tough, chewy soy protein isolates held in a matrix of a more tender gel binder were described in the literature as a matrix that resembles meat (3). This article describes a soy protein isolate (SP 55) that exhibits unusual rheological and emulsifying properties and has thermosetting characteristics that resemble the coagulation properties of egg albumen.

MATERIALS AND METHODS

Sample Preparation

SP 55 was obtained by a two-step aqueous fractionation of defatted soy flour. The isolation scheme is shown in Fig. 1. A 10:1 weight ratio of 37–40° C tap water to soy flour was used. Sodium sulfite (0.1% of the weight of the soy flour) was added to the water prior to the addition of the soy flour as an antioxidant. Extraction was performed by stirring the mixture with a marine-type paddle for 30 min at pH 5.5 using phosphoric acid as the acidulant. The aqueous extract (supernatant) was separated from the insoluble residue by centrifugation at 5,000 rpm in a Lourdes Beta-Fuge, model A-2, using a continuous head (No. 1605). The feed to the centrifuge was maintained at a slow rate, usually 200–250

Presented at the 60th Annual Meeting, Kansas City, MO, Oct. 1975.

²Present address: Miluda Research and Development, D.N. Anschrit, Israel.

Present address: Ross Laboratories, Columbus, OH.

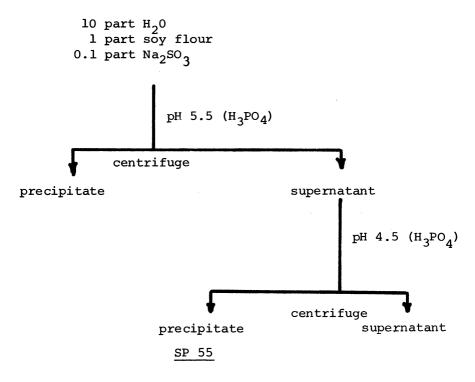
⁴Author to whom communications should be addressed.

^{© 1978} The American Association of Cereal Chemists

ml/min, so that solids (determined with a DeLaval Gyrotester) in the supernatant were less than 0.25% by volume. The supernatant from the first centrifugation was adjusted to pH 4.5 with phosphoric acid to precipitate the SP 55. The SP 55 was separated from the supernatant by centrifugation. When fresh SP 55 was desired, this material was used directly. Dry material was obtained by neutralizing the protein to pH 7 with 2% NaOH and either spray-drying or freeze-drying the protein.

Viscosity Measurements

All viscosities were measured with a Brookfield Syncro-Lectric Viscometer, model HAT. When viscosities of high concentrations of SP 55 were determined, fresh SP 55 was used; spindle speed was 20 rpm. When SP 55 and albumen were compared for viscosity at lower concentrations and different pHs, the proteins used were spray-dried. A known amount of material, either commercial egg albumen or SP 55, was dispersed in distilled water. The pH was adjusted to the



- 1. Use as is or
- 2. Neutralize to pH 7 and spray dry or freeze dry

Fig. 1. Isolation scheme for SP 55.

desired value for SP 55 (pH 5.5, 6.0, or 7.0) with either H₃PO₄ or NaOH. The egg albumen was used as is (pH 6.9). The solutions were made up to the desired weight and stored overnight in a refrigerator, and viscosities were determined the next day at 25°C at 100 rpm.

Shear Tests

Shear tests were performed with a Food Technology Corporation Texturepress (formerly known as the Allo-Kramer Shear Press). The apparatus was the Texturepress (model S2HE) with the Texturecorder (model E2) and standard cell (C-400). A 2,275 kg test ring was on the instrument.

Twenty-five grams of solution were poured into small aluminum cups 6 cm in diameter and 1.5 cm deep. The cups were placed in a steam kettle (100°C) and the proteins were gelled by steaming for 15 min. They were cooled to room temperature, removed from the cups, and shear was determined. Shear rate was at a setting of 8 (14.7 cm/min) on the Allo-Kramer.

Gel Strength and Emulsification Properties

We observed that the SP 55 appeared to have both emulsification and binding properties when it was incorporated into artificial sausage type emulsions. Therefore, a test was developed that determined the rupture point of an artificial emulsion that was similar in its major components and composition to that of a sausage emulsion. The relative emulsification capacity of various proteins was

TABLE I
Proximate Analysis of SP 55

Protein	93%
Ash	4%
Fat	0.1%
Carbohydrates	3%
pH	6.9
Protein dispersibility index	94%

TABLE II Vsicosity^a of SP 55 Solution Versus pH

рН	Solids (%)	Viscosity (cP)
4.39	50.0	200.000
	50.0	200,000
4.53	49.9	62,000
4.65	49.7	42,000
4.82	49.5	32,000
5.02	49.3	30,000
5.34	49.0	24,000
6.17	48.5	19,600

[&]quot;Spindle speed: 20 rpm; temperature: 22-25°C.

also observed on the final emulsion.

In this procedure, 875 ml of water, 250 g of commercial soy protein isolate, soy protein concentrate or SP 55, and 17.5 g of NaCl were mixed in a Hobart Food Cutter, model 84142. The pH was adjusted to pH 6.6, and 112 g of a hydrogenated vegetable oil (Wiley melting point $36.1-38.3^{\circ}$ C, iodine value 58-63) were added. The mixture was mixed for an additional 5 min. The mixture was placed in several 100-ml $(31.5 \times 164 \text{ mm})$ tubes. The tubes were placed in a 90° C water bath for 40 min and then cooled in an ice bath for one hour. The gels were removed from the tube, cut into 30-mm sections, allowed to warm to room temperature $(22-25^{\circ}\text{C})$, and tested for rupture point with an Instron Universal Tester, model 1102 TM. A 1,000-lb load cell was on the Instron, and the shear rate was 0.5 in./min. The probe used was a 0.25-in. stainless steel rod.

Prior to running the gel strength test, the relative degree of fat separation was noted, as either no, slight, moderate, or heavy degree of separation.

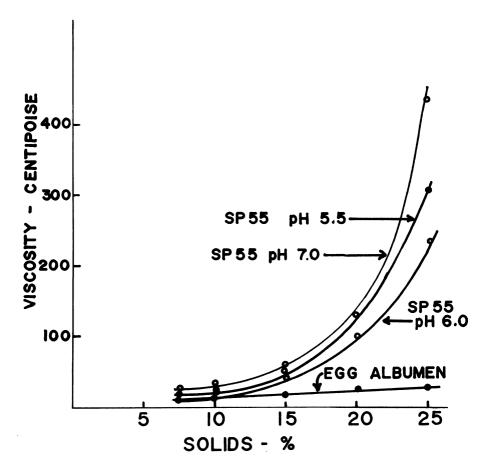


Fig. 2. Comparison of viscosity of egg albumen and SP 55 solutions (various pHs) at different solids content.

Analytic Methods

Protein (N \times 6.25), fat (by ether extraction), ash, and moisture were determined by AOAC methods (4). Carbohydrates were calculated by difference. Protein dispersibility index (PDI) was determined by AOCS method Ba 10-65 (5).

RESULTS AND DISCUSSION

Yield and Composition

The yield of SP 55 was approximately 10% of the starting amount of soy flour. The chemical composition of SP 55 is shown in Table I. Its proximate analysis is similar to that of most soy isolates containing 93% protein on a dry basis. SP 55 is highly soluble, having a PDI of 94%.

Viscosity Relationships

Some interesting properties of SP 55 are its appearance and rheological behavior. Isolated at pH 4.5, SP 55 is a free-flowing viscous material with solids content of about 40%. On standing, however, it exudes water until the solids content is about 50%. Under these conditions, it still remains a free-flowing viscous material. Most other soy isolates when isolated at pH 4.5-4.6 are cake-or curd-like in appearance and nature and do not flow readily.

Most soy protein isolates are free-flowing solutions at concentrations up to about 30% solids at neutral pHs. The viscosity of a solution of soy globulins increases as the pH is raised and vise versa (2). This phenomenon may represent the unfolded state of the protein molecules at elevated pHs and the more aggregate state of the protein near the isoelectric point pH 4.6 (6,7). SP 55, however, shows different properties (Table II). At high solids, SP 55 is a free-flowing material at pH 4.39. The fluidity or viscosity of the material is dramatically altered by pH adjustment. As the pH of the system was raised, the viscosity was reduced from 200,000 cP at pH 4.39 to slightly under 20,000 cP at pH 6.0. The solids concentration of the highest pH solution was 48.5% as a result of the addition of NaOH to adjust the pH.

The viscosity of SP 55 at different pHs and strengths was compared with that of commercial egg albumen (Fig. 2). Egg albumen solutions had low viscosities (from 7.5 to 25% solids), and the slight increase in viscosity in that range was linear as solids increased. The suspensions were yellow in appearance and were

TABLE III
Effect of Sodium Chloride on Viscosity of 50% SP 55 Solutions at pH 4.39

NaCl (%)	Viscosity (cP)	
	200,000	
0.1	36,000	
0.6	19,200	
1.0	13,280	
2.0	12,000	

relatively clear except for a slight amount of white precipitate that appeared at the bottom. The SP 55 suspensions were more opaque than were the egg albumen suspensions. Viscosities were slightly higher than those for the egg albumen at low solids, but increased rapidly as solids were increased above 20%.

The addition of NaCl to the 50% solutions of SP 55 at pH 4.39 (Table III) caused the viscosity of the SP 55 solutions to decrease rapidly, even at concentrations as low as 0.1%, suggesting that an increase in ionic strength was causing a dissociation of the SP 55 into smaller units.

When 50% solids isoelectric solutions of SP 55 were elevated in temperature and viscosity was measured (Table IV), a decrease in viscosity occurred between 22 and 53°C. When the temperature was greater than 80°C, however, the solution solidified into a hard gel. This thermosetting property resembles the heat coagulability properties of egg albumen.

TABLE IV
Viscosity of 50% SP 55 Solutions at Different Temperatures

Temperature (°C)	Viscosity (cP)	
22	200,000	
29	77,000	
37	41,600	
47	22,000	
53	17,600	

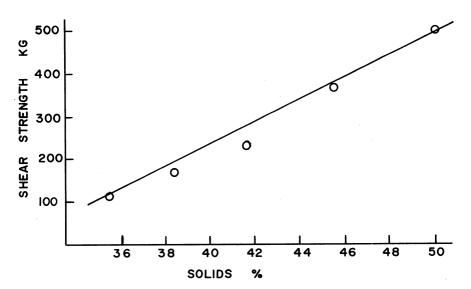


Fig. 3. Effect of solids content on shear strength of SP 55 gels.

Gel and Shear Characteristics

The gelling observation noted above was of interest since soy proteins are used in many applications as gelling agents.

The shear strength of gels made with SP 55 was dependent, as would be expected, on the solids content of the solutions (Fig. 3). The shear strength of the gels varied linearly, the solids content of the gels going from approximately 100 kg for the 38% solids gel to more than 500 kg for the 50% gel at pH 4.6. For comparitive purposes, a piece of hard Swiss cheese had a shear strength of only 5 kg on the Allo-Kramer.

Sodium chloride had a negligible effect on the shear strength of heat-coagulated SP 55 (Table V) compared with the effect it had on the viscosity (Fig. 2). Salt added to 43% solids SP 55 gels produced a slight increase in shear strength, going from approximately 300 kg at no added salt to approximately 330 kg at 1.2% NaCl. The combined observation of decreased solution viscosity and retained heat-set gel strength could be useful if SP 55 were incorporated into a product that was later heat set. The addition of a small amount of salt would facilitate the addition of other ingredients without getting thickening from the heat-coagulating protein.

The shear strength of SP 55 and egg albumen gels at lower concentrations and different pHs (SP 55 only) was compared (Table VI). When solids were below 15%, both egg albumen and SP 55 formed weak gels and did not register on the Allo-Kramer. At three different pHs (5.5, 6.0, and 7.0), the gel strength of SP 55

TABLE V
Effect of Sodium Chloride on Shear Strength of 43% Solids SP 55 Gels

NaCl (%)	Shear Strength (kg)
	297
0.2	336
0.6	342
1.2	331

TABLE VI
Comparison of Shear Strength of SP 55 and Egg Albumen Gels
at Lower Solids Concentrations and Different pHs^a

		SP 55 (pH)		
Solids (%)	5.5	6.0	7.0	Egg Albumen
15	8	10	15	11
20	32	24	35	33
25	87	75	74	87

[&]quot;Values expressed in kilograms.

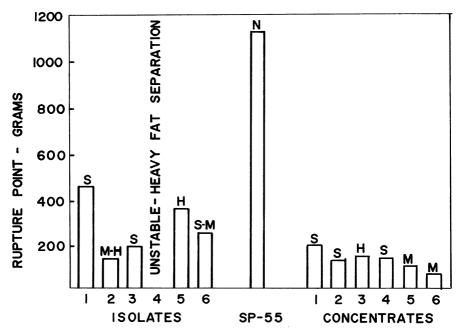


Fig. 4. Gel strength test of SP 55 soy concentrates and soy isolates. Notation above bars refers to amount of fat separation in sample. N, none; S, slight; M, moderate; H, heavy.

and egg albumen at solids content of 15, 20, and 25% had similar values, indicating that SP 55 has the potential for being a functional substitute as a firming agent for egg albumen.

Most foods that use soy ingredients are mixtures of water, fat, protein, and salt and are heated for both functional and microbiological purposes. Therefore, SP 55 was compared with other commercially available soy ingredients, concentrates, and isolates to see how it functions in a model system containing other commonly used food ingredients. Six isolates and six concentrates were compared with this test (Fig. 4). Generally, the isolates showed higher gel strengths than did the concentrates, probably due to their higher protein content. Gel strength of SP 55, however, was more than twice that of the best commercial soy protein isolate tested—greater than 1,100 g compared with 400 g for the isolate.

SP 55 was the only protein that, when included into the heat-set emulsion, did not show any fat separation. One of the other isolates tested separated to such a degree that measuring the gel strength using this test was not possible. These results indicate that SP 55 not only is an excellent gel-forming agent but also helps to form an emulsion that does not separate when heat set.

The chemical characterization of this protein is in progress and will be reported at a later date.

Acknowledgment

We thank Ivan Good for his skilled technical assistance.

Literature Cited

- SMITH, A. K., and CIRCLE, S. J. Soybeans: Chemistry and technology. Avi Publishing Co.: Westport, CT (1972).
- 2. CIRCLE, S. J., MEYER, E. W., and WHITNEY, R. W. Rheology of soy protein dispersions. Effect of heat and other factors on gelation. Cereal Chem. 41: 157 (1964).
- 3. FRANK, S. S., and CIRCLE, S. J. The use of isolated soybean protein for non-meat, simulated sausage products. Frankfurter and bologna types. Food Technol. 13: 307 (1959).
- 4. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (ed. 12). The Association: Washington, DC (1975).
- 5. AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (ed. 3). The Society: Champaign, IL (1975).
- 6. KELLEY, J. J., and PRESSEY, R. Studies with soybean protein and fiber formation. Cereal Chem. 43: 195 (1966).
- 7. ISHINO, K., and OKAMOTO, S. Molecular interaction in alkali denatured soybean proteins. Cereal Chem. 52: 9 (1975).

[Received January 7, 1977. Accepted November 15, 1977]