SOLUBILITY AND ULTRACENTRIFUGAL STUDIES ON SOYBEAN GLOBULINS[1]

A. M. NASH AND W. J. WOLF

ABSTRACT

Solubilities of laboratory and commercial preparations of soybean globulins were measured in 0.5 ionic strength, pH 7.6, potassium phosphate-sodium chloride buffer. Measurements with and without 0.01*M* 2-mercaptoethanol in the buffer gave an estimate of insoluble disulfide polymers. Compositions of the soluble proteins were determined by ultracentrifugation.

Five laboratory samples of soybean globulins prepared by isoelectric precipitation and freeze-drying had solubilities of 37–73% in buffer and 66–78% in buffer containing 2-mercaptoethanol. Five commercial globulin samples in the isoelectric form had solubilities of 6–59% in buffer and 10–66% when 2-mercaptoethanol was present. Seven commercial soybean proteinate samples had solubilities ranging from 6–81% in buffer as compared to 13–83% in buffer containing 2-mercaptoethanol.

Solubilities of all samples were increased by 2-mercaptoethanol, but wide variations occurred. Ultracentrifugal analysis also showed large variations in the compositions of different samples. Some commercial preparations closely resembled laboratory samples in solubilities and ultracentrifugal compositions. Other commercial samples had low protein solubilities and appeared extensively modified by the isolation process. Samples of lecithinated proteinate, pepsin-hydrolyzed protein, and alkali-modified protein were included for comparison.

In the United States, food-grade soybean proteins have been available commercially since about 1948 (1). Research on commercial preparations of food-grade soybean proteins includes nutritional studies (2,3), food formulations (1,4), and rheological studies (5–7). Little information is available, however, about the solubility properties of these products from different manufacturers.

Soybean proteins are readily extracted from defatted meal by water at pH 6.6 but are only slightly soluble at this pH after isolation by acid precipitation (8). Only about 60% of laboratory-prepared soybean globulins are soluble in buffer of pH 7.6 and 0.5 ionic strength. When the buffer contains 0.01*M* mercaptoethanol or sodium sulfite, an additional 20% of the proteins dissolves, presumably by reduction of disulfide-linked polymers of the 7S and 11S ultracentrifugal fractions (9). Kelley and Pressey (10) confirmed these results and also investigated the effect of alkali and urea on the solubility and ultracentrifugal properties of soybean globulins. Further studies in our laboratory indicate that the fraction of the globulins not dissolved in buffer containing reducing agents is insolubilized during acid precipitation (11). This insoluble fraction appears to be derived principally from the 2S and 7S fractions present in the initial aqueous extract of the meal. Here we report additional studies on the solubility and ultracentrifugal composition of laboratory-prepared soy-

[1] Manuscript received July 25, 1966. Contribution from the Northern Regional Research Laboratory, Peoria, Ill. 61604. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture. Presented at the 51st annual meeting, New York City, April 1966.

bean globulins and a number of commercially available soybean proteins of food grade.

Materials and Methods

Protein Preparations. Laboratory-prepared, isoelectric protein samples A, C, D, and E (Table I) were obtained from dehulled, undenatured hexane-defatted soybean meal by water extraction and hydrochloric acid precipitation at pH 4.5. The curd was separated by centrifugation and then freeze-dried. Sample B was prepared in the same manner from a commercial sample of hexane-defatted meal having a nitrogen dispersibility (12) of 73%, except that the initial aqueous extract was made at pH 7.6 by addition of dilute sodium hydroxide.

Commercial soybean protein samples used in this study varied in age: samples 0.1–0.5 years old represent current production. Since samples F, G, H, K, L, and M, 3–10 years old, are no longer available commercially, laboratory-stored samples were used. Sample H, received as a frozen curd, was freeze-dried; the curd had been prepared like that of sample G. The commercial samples were used as obtained, on the assumption that possible differences in mesh sizes would not affect the solubility measurements significantly under the conditions used.

Protein Solubility. Protein samples of 500 mg. (dry basis[2]) were dispersed in about 20 ml. of potassium phosphate-sodium chloride buffer (0.0325*M* K_2HPO_4, 0.0026*M* KH_2PO_4, 0.4*M* NaCl), pH 7.6, ionic strength 0.5 with or without 0.01*M* 2-mercaptoethanol. The dispersions were equilibrated by dialysis with stirring at 4°C. against buffer for 48 hr. or longer, adjusted to 25 ml., and clarified by centrifuging (20,000 × *g*) for 10 min. at 25°C. Solubilities are expressed as percentages of the total nitrogen (as determined by Kjeldahl analysis) remaining soluble after dialysis and centrifugation. Samples S and T, which were pepsin hydrolysates of soybean proteins, were not dialyzed against buffer because losses through the dialysis tubing would have been large.

Ultracentrifugal Analysis. Protein solutions from the solubility experiments were analyzed at room temperature in a Spinco[3] Model E ultracentrifuge (30-mm. double-sector cell) at 47,660 r.p.m. A separate analysis in a synthetic boundary cell gave the total protein concentration in area units. Sedimentation patterns were measured and calculated according to Pickels (13). Ultracentrifugal compositions (Table I) for the soluble portions of the samples were calculated by dividing the area of each component by the total area (obtained with the synthetic boundary cell) and multiplying by the percent solubility. Each component is expressed as the percentage of the total (soluble plus insoluble) protein to permit direct comparison of preparations with differing solubilities. By this method of calculation, the sum of the percentages of the soluble components should equal the percentage solubility; in general, the two values agreed within ± 7% of the total protein. Exceptions were samples

[2] Moisture was determined at 105°C. for 2 hr. *in vacuo*.

[3] Reference to specific equipment does not constitute endorsement by the U.S. Department of Agriculture.

TABLE I

SOLUBILITIES AND ULTRACENTRIFUGAL COMPOSITIONS OF SOYBEAN GLOBULINS

Sample	Preparation[a]	Sample Age	Buffer without 2-Mercaptoethanol						Buffer with 2-Mercaptoethanol					
			Protein Solubility	Ultracentrifugal Composition					Protein Solubility	Ultracentrifugal Composition				
				2S	7S	11S	15S	>15S		2S	7S	11S	15S	>15S
		years	%	%	%	%	%	%	%	%	%	%	%	%
A	Isoelectric	4	37.2	9	5	13	5	4	66.4	10	14	24	7	8
B	Isoelectric	4	56.5	7	7	15	7	14	67.3	7	14	24	7	8
C	Isoelectric	2	59.8	8	10	20	6	9	71.8	8	23	29	5	4
D	Isoelectric	0	69.4	9	11	26	12	15	77.8	9	24	33	6	7
E	Isoelectric	0	72.7	8	11	31	13	11	76.7	9	28	36	3	2
F	Isoelectric (I)	3	6.0	4	....	....	....	....	9.9	7	1	1	1	1
G	Isoelectric (I)	3	10.7	3	1	4	1	1	21.0	6	2	10	2	2
H	Isoelectric (I)	4	18.4	3	2	7	2	2	29.8	4	3	13	4	4
I	Isoelectric (II)	0.5	20.3	4	5	5	2	3	46.8	7	11	17	5	6
J	Isoelectric (III)	0.5	58.8	5	9	16	9	16	65.9	5	14	26	8	8
K	Sodium proteinate (IV)	8	6.1	4	1	....	....	....	13.2	9	2	1	....	....
L	Sodium proteinate (I)	3	11.7	6	2	2	....	....	22.0	10	4	4	1	....
M	Sodium proteinate (IV)	10	14.0	8	2	3	1	1	23.3	11	2	6	2	2
N	Sodium proteinate (IV)	0.5	33.3	18[b]	3[c]	....	3	6	83.0	26[b]	....	....	5	39
O	Sodium proteinate (II)	0.5	36.6	6	7	10	3	8	63.1	11	15	19	5	9
P	Sodium proteinate (III)	0.5	54.6	6	11	19	7	8	65.4	8	17	26	4	5
Q	Sodium proteinate (V)	0.1	81.0	36	....	3	....	37	82.3	37	....	4	....	37
R	Lecithinated sodium proteinate (II)	0.5	25.3	5	5	5	2	4	49.3	10	11	14	4	9
S	Pepsin hydrolyzed (IV)	0.5	93.8	24	7	....	....	....	97.3	26	8	....	....	....
T	Pepsin hydrolyzed (IV)	0.5	102.4	36	....	....	....	....	105.1	37	....	....	....	....

[a] Similar Roman numerals in parentheses indicate common sources of commercial samples. Ultracentrifuge patterns for selected samples are shown in Figs. 1–3.
[b] Sedimented as a 2S peak with a poorly resolved shoulder of 3S–5S.
[c] Sedimented as poorly resolved material in the region of the usual 7S and 11S fractions.

N, S, and T in which large portions of material failed to sediment away from the meniscus.

Results

Laboratory-Prepared Globulins. Previous studies (9–11), showing that soybean globulins are not completely soluble in buffer after isoelectric precipitation, were confirmed with five different samples (A to E in Table I). Samples A to C had been stored at laboratory temperatures for 2–4 years, whereas D and E were freshly prepared. Solubilities of the globulins in buffer without 2-mercaptoethanol ranged from 37 to 73%; the two freshly prepared samples had the highest solubilities. In all five samples the amount of soluble 2S component was nearly constant, but decreased amounts of faster-sedimenting components accounted for the lower solubility of the older samples (A to C). In buffer containing 0.01*M* 2-mercaptoethanol the solubilities of all samples were higher and the solubility range was narrower (66–78%). Each sample showed marked increases in 7S and 11S fractions but no significant changes in 2S fraction with the mercaptan. In contrast, the amounts of 15S and >15S materials remained nearly constant in the old samples but decreased in the fresh samples. These decreases in 15S and >15S fractions of samples D and E account for a large portion of the gain in 7S and 11S fractions. Differences in solubility in buffer and the effect of 2-mercaptoethanol on the solubility and ultracentrifugal composition of the laboratory globulin preparations are illustrated in Fig. 1 for samples A and E.

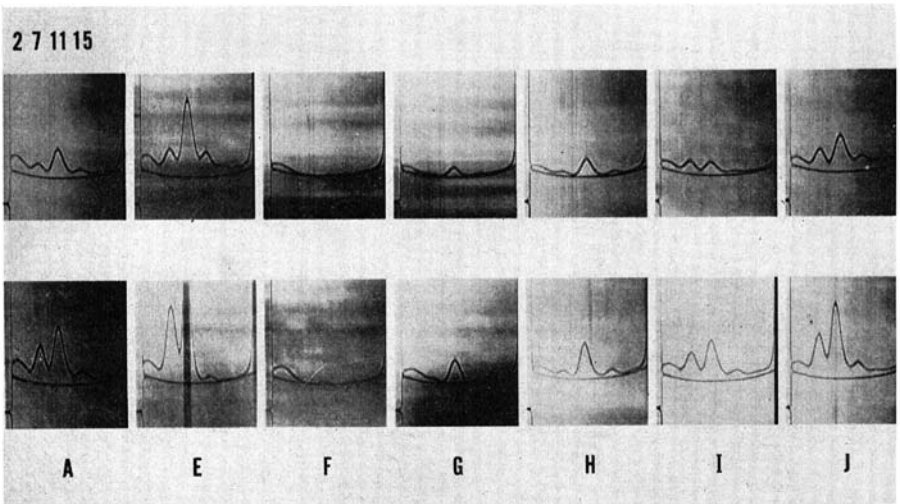

Fig. 1. Ultracentrifuge patterns for proteins soluble in buffer (upper) and buffer plus 0.01*M* 2-mercaptoethanol (lower) of samples A, E, F, G, H, I, and J (Table I). Patterns were taken 48 min. after the run was started, with a bar angle of 70°, and are directly comparable. Differences in areas reflect differences in solubilities. The major peaks are identified in Svedberg units for sample A as across top of upper figure.

Commercially Available Globulins. Commercial food-grade isolates of soybean globulins are available in several forms (14). Those most comparable

to our laboratory-prepared samples are isoelectric preparations dried at the pH at which they are precipitated (~ pH 4.5). Solubilities in buffer without 2-mercaptoethanol for commercial isoelectric samples F to J ranged from 6 to 59%. As noted with the laboratory-prepared samples, the mercaptan increased the solubility of all the commercial isoelectric proteins. The order of solubilities remained unchanged from that without mercaptan. Sample J was most comparable to our preparations, and sample F was most dissimilar. Figure 1 shows ultracentrifuge patterns for samples F to J with and without 2-mercaptoethanol.

Soybean proteins isolated commercially are also available as proteinates, usually prepared by neutralizing acid-precipitated globulins to pH 6.5–7.5 followed by drying. In buffer not containing the mercaptan the range of solubilities of proteinate samples K to Q was 6–81%. Samples K, L, and M had low solubilities; most of the soluble protein sedimented as 2S fraction. Sample Q had the highest solubility but contained unusually large amounts of 2S–3S protein plus a broad peak of 19S. Sample N also contained 2S–3S protein in excess of the 2S content of laboratory preparations, but, unlike Q, it contained only small amounts of fast-sedimenting proteins (Table I).

With 2-mercaptoethanol the solubilities of all proteinates were higher, but the solubility increases varied widely. Sample Q, with a solubility of 81% without 2-mercaptoethanol, increased but slightly in solubility. In contrast, samples K, L, and M with solubilities of 6–14% without the mercaptan showed greater solubility increases. The largest increase in solubility occurred with

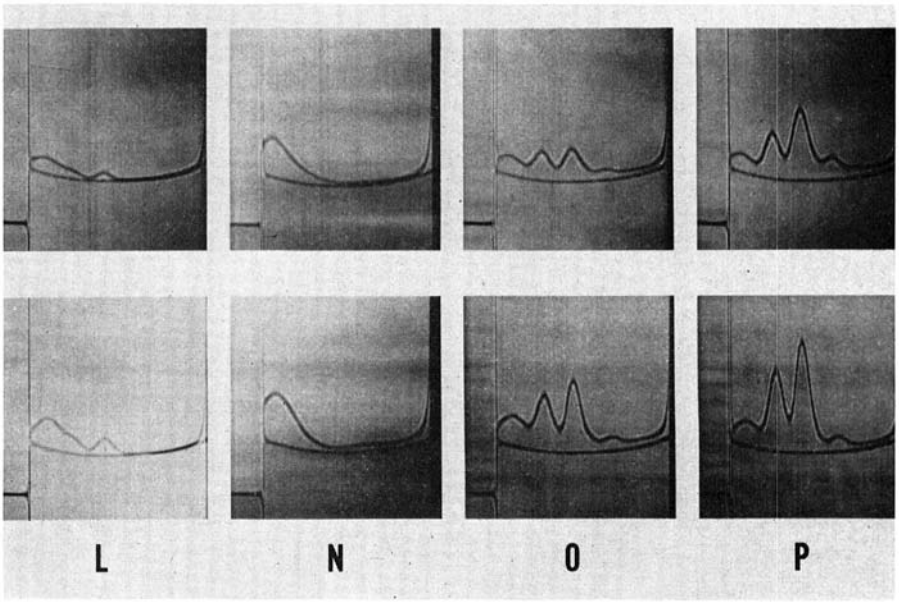

Fig. 2. Ultracentrifuge patterns for proteins soluble in buffer (upper) and buffer plus 0.01*M* 2-mercaptoethanol (lower) of samples L, N, O, and P (Table I). Conditions were identical to those described in Fig. 1.

sample N because of increased amounts of 2S–3S fraction and of two ill-defined peaks of 15S–17S and 35S. The ultracentrifuge pattern for sample N resembled the pattern for sample Q more closely than those of the other proteinates. The solubilizing effect of the mercaptan on sample N contrasted markedly to that on our laboratory samples, in which solubility increases were accompanied by large increases in 7S and 11S components (Fig. 1). The effect of 2-mercaptoethanol on the ultracentrifuge patterns of four proteinate samples is shown in Fig. 2.

Also available are several forms of soybean proteins that have been modified to give functional properties desired in foods. Sample R, a sodium soybean proteinate containing about 25% of added soybean lecithin, came from the supplier of samples I and O. Without reducing agent, sample R was less soluble than proteinate sample O but similar in solubility to isoelectric protein sample I. This relationship was also true with reducing agent. The ultracentrifuge pattern for sample R in 0.01*M* 2-mercaptoethanol is shown in Fig. 3.

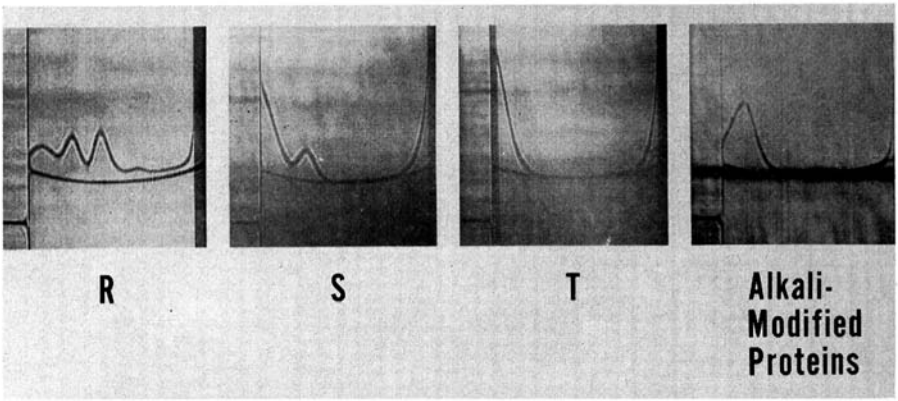

Fig. 3. Ultracentrifuge patterns for protein soluble in buffer plus 0.01*M* 2-mercaptoethanol of samples R, S, T, and an alkali-modified, industrial-grade, soybean protein.

Samples S and T were preparations that had been hydrolyzed with pepsin to make them soluble over a wide range of pH, particularly near the isoelectric point of soybean globulins. These pepsin hydrolysates are used as aerating agents in the food industry (15). Sample S was almost completely soluble in buffer, and only a small increase in solubility was noted with the mercaptan. Only 7–8% of the sample sedimented as a discrete boundary of about 7S (Fig. 3). Because the rest of the sample sedimented so slowly that only an incomplete boundary was observed, accurate calculation of component areas was impossible. The slowly sedimenting material presumably consisted of amino acids and polypeptides formed by peptic hydrolysis of soybean globulins. Sample T, completely soluble in buffer, was distinctly different from sample S; T contained none of the 7S fraction observed in S (Fig. 3).

Although not intended for food use, soybean globulins are also available

in alkali-modified forms for industrial uses as paper coating adhesives (16). Two such commercial samples were examined. The two had solubilities of 17 and 21% in buffer and 32 and 44% in buffer plus 2-mercaptoethanol.[4] In buffer, both samples sedimented as diffuse peaks with $s_{20,w}$ values of 2.6S–2.9S. With mercaptan, faster-sedimenting material was solubilized; the ultracentrifuge pattern showed a skewed peak of 3.9S and protein (about 5% of the pattern area) in the 7S–11S region. The ultracentrifuge pattern for the more soluble sample in the presence of mercaptan is shown in Fig. 3. Kelley and Pressey (10) have reported similar solubility and sedimentation data for alkali-treated soybean proteins.

Discussion

Previous studies showed that proteins extracted from defatted meal with water or salt solutions are soluble in pH 7.6, 0.5 ionic strength buffer (17, 18). Insolubility of isolated soybean proteins in this buffer is a measure of modification or denaturation during isolation. By this criterion, soybean proteins appear sensitive to modification, particularly when precipitated with acid at pH 4.5–5.0 (11). Denaturation of soybean proteins can be either reversible or irreversible. Reversible denaturation can occur by formation of buffer-insoluble disulfide polymers which are readily depolymerized and solubilized by 0.01*M* 2-mercaptoethanol (9). The amounts of the two denatured forms can be estimated by measuring solubilities of soybean protein samples in buffer with and without 2-mercaptoethanol. The difference between the two solubility measurements represents the amount of reversibly denatured protein. The protein insoluble in buffer containing the mercaptan presumably is irreversibly denatured.

Water extracts of laboratory-prepared soybean meal contain buffer-soluble disulfide polymers of the 7S and 11S components (19) which are converted to buffer-insoluble polymers (reversible denaturation) during isoelectric precipitation and drying and possibly during subsequent storage of the dry proteins. In samples A to C, which were 2–4 years old, the disulfide polymers were largely insoluble in buffer but were depolymerized to soluble monomers by 2-mercaptoethanol. In contrast, the fresh samples, D and E, had high solubilities in buffer which increased only moderately with the mercaptan even though the increases in pattern areas of the 7S and 11S components were similar to those of A to C. As indicated by the sedimentation patterns for E in Fig. 1 and also by the data in Table I, most of the disulfide polymers in the fresh preparations were buffer-soluble and occurred in the 15S and >15S range. Apparently buffer-soluble disulfide polymers are converted to buffer-insoluble polymers on aging.

Soybean proteins are isolated commercially as follows (5): Defatted meal is extracted with warm, dilute alkali and the insoluble residue is separated. The globulins are then precipitated by adding acid to the extract to pH 4.5–5.0, the whey is removed, and the curd washed with water. Finally, the washed curd is spray-dried, or converted into proteinate and then spray-dried. The

[4] Protein solubilities were measured with 375 mg. of proteins instead of 500 mg. as described under "Materials and Methods."

factors most likely to cause modification of the globulins are pH and temperature during extraction and the amount of heat-treatment during drying.

Surprising features of the commercial protein samples were the large variations in solubility and in ultracentrifugal composition. Of the isoelectric preparations, samples F to H had low solubilities even in the presence of mercaptoethanol. This property indicated extensive irreversible modification of the proteins during isolation. Similarity of sample J to our laboratory samples in both solubility and ultracentrifugal properties indicated milder contitions during its isolation. The properties of sample I indicated modification during isolation but to a smaller extent than that of samples F to H. Sample H, which we secured as a frozen curd and then freeze-dried, had a low solubility. Apparently modification of sample H was caused by conditions used in extraction and precipitation of the protein.

Variations similar to those observed among the commercial isoelectric samples were noted among the proteinates, although the age of samples K and M may also be a factor. A common feature of most of the commercial isoelectric and proteinate samples is a lower content of buffer-soluble 2S fraction than in the laboratory samples. Many of the commercial samples also showed an increased amount of 2S fraction soluble in buffer containing 2-mercaptoethanol, a result in contrast to findings on laboratory samples.

Irreversible denaturation in the commercial proteins involved mainly the components sedimenting faster than the 2S fraction. In samples with extremely low solubilities (F and K) the major soluble fraction was 2S protein. In samples of intermediate solubilities (G and H with mercaptan) the 11S fraction was in much higher concentration than the 7S fraction. This observation suggests that the 11S fraction is more stable than the 7S fraction. Differences in stability between these fractions occurred in alcohol denaturation studies (11,20).

A large source of variability among soybean globulin preparations is the amount of buffer-insoluble disulfide polymers. In laboratory preparations these polymers varied from 4% (sample E) to 29% (sample A); in the commercial isoelectric samples the amount of polymers varied from 4% (sample F) to 26% (sample I) and ranged from 1% (sample Q) to 50% (sample N) for the commercial proteinates.

Use of our solubility measurements to determine soybean protein modification has obvious limitations with such samples as N and Q. Although samples N and Q had high solubilities in buffer with 2-mercaptoethanol, they appeared modified when examined ultracentrifugally. The sedimentation properties of samples N and Q resembled those reported by Watanabe and Nakayama (21) for water extracts of soybean meal heated at pH 7.0 between 80° and 100°C. Modification of samples N and Q presumably results from heat-treatment during isolation.

An additional example of modification of soybean proteins is that obtained by peptic hydrolysis. These modified proteins are used as aerating agents in foods. In one procedure used to prepare such aerating agents, the pH is adjusted from 2.5 to 3.5 following peptic hydrolysis, and sodium chloride is added. The salt solubilizes unhydrolyzed or partially hydrolyzed

protein which acts as a foam stabilizer when the hydrolysate is aerated (15). Our studies on sample S revealed material of the 7S range (Fig. 3) which presumably acts as the foam stabilizer. However, sample T, also a pepsin hydrolysate, did not contain the high-molecular-weight material. Literature supplied by the manufacturer indicated that the two samples are representative of products designed for different applications: sample S is used in candy manufacture, whereas sample T is put into chiffon mixes and cake mixes.

Our limited studies on alkali-modified soybean proteins (Fig. 3) indicate that such preparations differ markedly from our laboratory-prepared proteins (Fig. 1, samples A and E) and from commercial food-grade proteins such as samples J, O, P, and R (Figs. 1–3). The alkali treatment hydrolyzes the proteins partially and destroys some of the sulfur-containing amino acids; such proteins are not intended for food uses (1,22).

Our results emphasize the wide variations among different commercial proteins and suggest the need to evaluate samples from several sources to determine suitability of soybean proteins for a given food application. In certain instances solubility measurements may be useful in such evaluations. For example, protein solubilities in buffer with and without 2-mercaptoethanol were used to predict quality of bakery products containing supplements of soybean proteins (23).

Acknowledgment

We are indebted to Bonita R. Heaton for performing the micro-Kjeldahl analyses.

Literature Cited

1. Circle, S. J. [with Johnson, D. W.] Edible isolated soybean protein. *In* Processed plant protein foodstuffs, ed. by A. M. Altschul; chap. 15, pp. 399–418. Academic Press: New York (1958).
2. Rackis, J. J., Smith, A. K., Nash, A. M., Robbins, D. J., and Booth, A. N. Feeding studies on soybeans. Growth and pancreatic hypertrophy in rats fed soybean meal fractions. Cereal Chem. **40:** 531–538 (1963).
3. Longenecker, J. B., Martin, W. H., and Sarett, H. P. Improvement in the protein efficiency of soybean concentrates and isolates by heat treatment. J. Agr. Food Chem. **12:** 411–412 (1964).
4. 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:** 307–313 (1959).
5. 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:** 157–172 (1964).
6. Circle, S. J., Whitney, R. W., Catsimpoolas, N., and Meyer, E. W. Rheology of soy protein dispersions. Further studies on factors affecting heat gelation. Abstr. 50th AACC annual meeting, Kansas City, Mo. April 1965.
7. Corey, H. Some physiochemical behaviors of soy protein. Abstr., 50th AACC annual meeting, Kansas City, Mo., April 1965.
8. Circle, S. J. Proteins and other nitrogenous constituents. *In* Soybeans and soybean products, ed. by K. S. Markley; vol. I, chap. 8, pp. 275–370. Interscience: New York (1950).
9. Wolf, W. J., Eldridge, A. C., and Babcock, G. E. Physical properties of alcohol-extracted soybean proteins. Cereal Chem. **40:** 504–514 (1963).
10. Kelley, J. J., and Pressey, R. Studies with soybean protein and fiber formation. Cereal Chem. **43:** 195–206 (1966).

11. WOLF, W. J., SLY, DAYLE ANN, and BABCOCK, G. E. Denaturation of soybean globulins by aqueous isopropanol. Cereal Chem. **41:** 328–339 (1964).
12. SMITH, A. K., BELTER, P. A., and JOHNSEN, V. L. Peptization of soybean meal protein. Effect of method of dispersion and age of beans. J. Am. Oil Chemists' Soc. **29:** 309–312 (1952).
13. PICKELS, E. G. Ultracentrifugation. Methods Med. Res. **5:** 107–133 (1952).
14. ZIEMBA, J. V. Let soy proteins work wonders for you. Food Eng. **38**(5): 82–84, 87–90, 93 (1966).
15. BURNETT, R. S. Soybean protein food products. *In* Soybeans and soybean products, ed. by K. S. Markley; vol. II, chap. 23, pp. 949–1002. Interscience: New York (1951).
16. BAIN, W. M., CIRCLE, S. J., and OLSON, R. A. Isolated soy proteins for paper coating from a manufacturer's viewpoint. *In* Synthetic and protein adhesives for paper coating, ed. by L. H. Silvernail and W. M. Bain; chap. IX, pp. 206–241. TAPPI Monograph Series No. 22, Technical Association of the Pulp and Paper Industry: New York (1961).
17. NAISMITH, W. E. F. Ultracentrifuge studies on soya bean protein. Biochim. Biophys. Acta **16:** 203–210 (1955).
18. WOLF, W. J., and BRIGGS, D. R. Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean proteins. Arch. Biochem. Biophys. **63:** 40–49 (1956).
19. WOLF, W. J. Physical and chemical studies on soybean proteins. Ph.D. thesis. University of Minnesota (1956).
20. ROBERTS, R. C., and BRIGGS, D. R. Characteristics of the various soybean globulin components with respect to denaturation by ethanol. Cereal Chem. **40:** 450–458 (1963).
21. WATANABE, T., and NAKAYAMA, O. Study of water-extracted protein of soybean. Nippon Nogeikagaku Kaishi **36:** 890–895 (1962).
22. SMITH, A. K. Vegetable protein isolates. *In* Processed plant protein foodstuffs, ed. by A. M. Altschul; chap. 10, pp. 249–276. Academic Press: New York (1958).
23. KURAMOTO, S. U.S. Patent 3,252,807 (May 24, 1966).