SOY PROTEIN SOLUBILITY: THE EFFECT OF EXPERIMENTAL CONDITIONS ON THE SOLUBILITY OF SOY PROTEIN ISOLATES[1]

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ABSTRACT

For heterogeneous amorphous proteins, there cannot be a thermodynamically defined solubility at a given temperature and pressure. Instead, the solubilities of these proteins are dependent upon experimental conditions. In order to compare data from the different solubility methods now in use, we have made a systematic study of the effects of experimental conditions upon the solubilities of a freeze-dried, acid-precipitated soy curd, of three commercial soy isolates, and of a commercial sodium caseinate. Significant effects (as much as a 30% change in the solubility) that varied from isolate to isolate were caused by increasing the blending speed from mild shaking to high-speed blending at 11,000 rpm, or by changing from low-force centrifugation to centrifugation at 200,000 $\times$ g for 3 hr. Protein solubilities, however, were not affected by initial protein concentration or by equilibration times longer than 100 min. Increasing temperature from 25° to 62°C raised the solubilities of some of the isolates significantly (8–14%). The Biuret reaction was used to determine "protein solubility" instead of "nitrogen solubility" as determined by Kjeldahl.

Solubility is an important physicochemical and functional property of soy proteins. Loss of solubility has been widely used as an indication of denaturation. High solubility is normally a desired functional property. Yet, at a given temperature and pressure, there cannot be a unique thermodynamically defined solubility for heterogeneous amorphous proteins such as soy proteins. Variations in experimental conditions for determining protein solubility such as blending, centrifuging, equilibration time, and initial protein concentration will yield different solubility values for the same sample. Therefore, it is impossible to compare solubility data measured under different conditions without knowing the quantitative effect of these conditions. Even though the Nitrogen Solubility Index (NSI) and the Protein Dispersibility Index (PDI) methods have been adopted as the official methods of the American Oil Chemists' Society (1), many investigators find it more convenient to use other methods (2–14).

Paulsen *et al.* (2) and Hermansson (3) have examined solubility and dispersibility methods for nonsoy proteins. In this study, we examine the effects of a wide range of experimental conditions on the solubility of soy isolates.

MATERIALS AND METHODS

Protein Isolates

The following five laboratory and commercially prepared protein isolates were studied.

A. Laboratory-prepared curd. One part of commercial defatted soy flakes was extracted with ten parts of dilute NaOH. After removal of spent flakes, the total extract was adjusted to pH 4.5 with dilute HCl to precipitate the curd. The curd was washed three times with H_2O, resuspended at pH 7.0, and freeze-dried.

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B. Edi-Pro N, a general purpose commercial soy protein isolate, manufactured by Ralston Purina Company.

C. A commercially available sodium caseinate.

D. A commercially available soy isolate not manufactured by Ralston Purina Company.

E. Supro 710, a commercial soy protein isolate recently developed for use in nondairy coffee whiteners, manufactured by Ralston Purina Company.

All the isolates were in the proteinate form. The pH of 0.9% w/w slurries in distilled water ranged from 6.5 to 7.0. All the samples were stored at room temperature in closed containers. No noticeable changes in solubility during the span of this study were observed.

Protein Quantitation

Protein quantitation was carried out by the Biuret method reported by Layne (15). Sample concentrations were kept low to ensure complete dissolution of all the protein in the Biuret reagent. Throughout this paper, protein solubility will be expressed as:

$$\text{Solubility} = \frac{(10^2)\,(A')}{(A)}$$

where A and A′ are the dilution-corrected Biuret absorbances of the sample before and after removal of the insoluble proteins, respectively. Since the Biuret reaction is specific for the peptide bond[2] (16), the above definition gives the "protein solubility." This Biuret "protein solubility" differs from the "nitrogen solubility" obtained using Kjeldahl data. For the soy isolates studied here, we have estimated the "nitrogen solubility" to be 5 ± 2% higher than the "protein solubility."

General Experimental Conditions

Except for those studies where protein concentration was the independent variable, 0.9% w/w slurries in distilled H_2O at pH 7.0 were used. In the concentration studies, the protein-to-H_2O ratio was varied from 0.4 to 9.5%. To ensure complete wetting of the sample, a small portion of H_2O was first added to the sample, and the mixture was stirred with a spatula until a uniform paste was formed. The remainder of the H_2O was then added in increments, with stirring, to form a uniform slurry. Except for blending experiments, the slurry was equilibrated for 2 hr at 25° C in a shaker bath. Blending experiments were carried out in a Sorvall Omni-Mixer calibrated according to AOCS procedures (1). Centrifuging experiments were carried out on 15-ml samples at various *g*-forces using International Model K, Beckman Model J-21B, and Beckman Model L5-65 centrifuges.

Precision and Reproducibility of Data

Statistical analysis on data taken under similar conditions but on different samples and on different days gave coefficients of variation that ranged from 1.2 to 2.0%.

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[2]We have experimentally verified this claim for seven purified globular proteins and have determined the molar mean residue extinction coefficient to be 34.2 ± 0.8.

RESULTS AND DISCUSSION

Blending Conditions

In Fig. 1, the solubility (expressed as the percentage of protein remaining in solution after 10 min of blending followed by 20 min of centrifuging at 42,000 × *g*) is plotted against the blending speed. The experimental conditions cover a range from gentle shaking for 2 hr (0 rpm) to blending at 11,000 rpm. Figure 1 clearly shows that blending speed has an appreciable effect on protein solubility. Further, this effect varies greatly among the isolates studied. The solubility of isolate B increases linearly with increasing blending speeds for a total rise of 19%, while that of isolate C decreases slightly. Isolates D and E show an induction period where increasing blending speeds up to 3000 rpm cause little change in the solubility. After this induction period, the solubility of isolate D jumps sharply by 13% and then settles down to a steady linear rise for an overall change of 31%, whereas that of isolate E shows only a linear rise of 12%. Since no effort was made to maintain constant temperature, rising temperatures will also affect isolate solubilities. A profile showing the final sample temperature after blending is given. Specific temperature effects are discussed later.

Centrifuging Conditions

The effect of centrifugation conditions upon solubility is displayed in Fig. 2, where the solubility is plotted against the logarithm of the average centrifugal force in *g* times the duration of centrifugation in sec [log (f × t)]. The

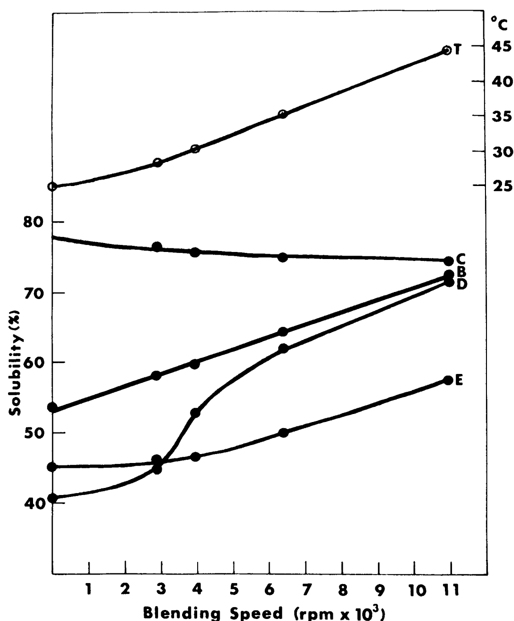

Fig. 1. The effect of blending speed on protein solubility: B, C, D, and E = isolates; T = temperature of slurry after 10 min of blending at the indicated speed.

experimental points cover a range from centrifuging at 460 × g for 10 min to centrifuging at $2 \times 10^5 \times g$ for 3 hr. Under the former and latter conditions, all particles with sedimentation coefficients greater than 6.1×10^4 S and 45 S, respectively, will be removed from solution. As in the blending studies, the effect of centrifugation conditions upon protein solubility is both appreciable and varied. The solubility of isolate E decreased smoothly from 48 to 38% as log (f × t) increased from 5.5 to 9.5. The absence of discrete changes in the slope of the curve is indicative of the presence of aggregates that have a random distribution of sedimentation coefficients. The solubilities of isolates B, C, and D do not exhibit reductions until very high centrifugation conditions are attained. The reductions are 10, 9, and 3%, respectively. This indicates the absence of significant amounts of very fast sedimenting particles in these isolates. With the exception of an added inflection in the low centrifugation [log (f × t)] region, the solubility curve for isolate A behaves like those of isolates B, C, and D. The inflection indicates the presence in isolate A of fast sedimenting particles that are absent from isolates B, C, and D.

Equilibration Time

Figure 3 gives the solubility as a function of equilibration time in the shaker bath. All samples except isolate B reach maximum solubility plateaus after 45 min of equilibration. This is in agreement with published data on meal protein extraction (17). The solubility of isolate B reached a plateau after 100 min of equilibration. A 2-hr equilibration time was selected for the balance of this work to assure complete equilibration.

Concentration

Figure 4 shows the solubility of the protein isolates as a function of the weight percentage of the total protein in the initial slurry. Because of difficulties in wetting the sample, we were unable to get reliable data for high concentrations of

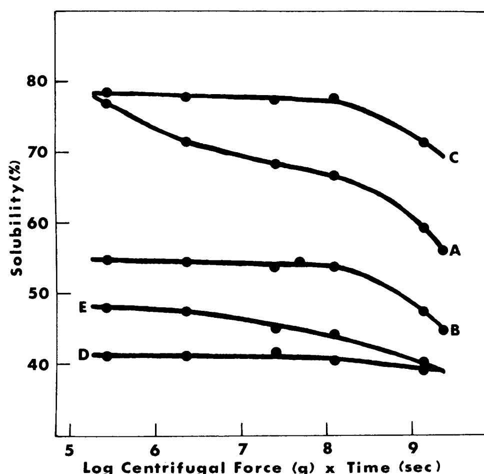

Fig. 2. The effect of centrifugation on protein solubility: A, B, C, D, and E = isolates.

isolate C. The data (Fig. 4) show that there is no dependence of the solubility on initial protein concentration in the range of concentrations studied. The measured solubilities are 69.5, 53.6, 79.7, 40.2, and 46.1%, respectively, for isolates A through E. Thus, it appears that all five isolates have one protein fraction that is soluble and another that is insoluble at all protein concentrations. Cogan *et al.* (17) and Smith *et al.* (6) observed a 3% decrease in meal extractability for a 2–3% increase of initial meal protein concentration. However, major differences in experimental conditions preclude a meaningful comparison of our data with theirs.

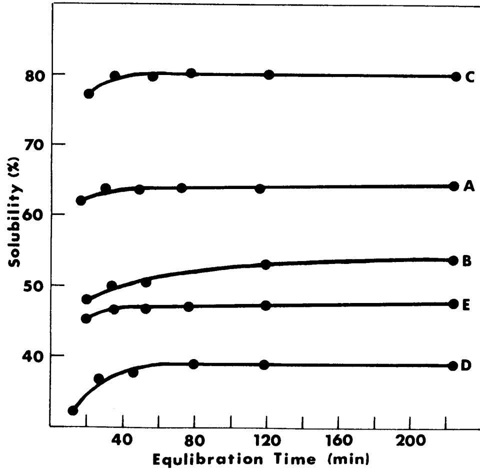

Fig. 3. The effect of equilibration time on protein solubility: A, B, C, D, and E = isolates.

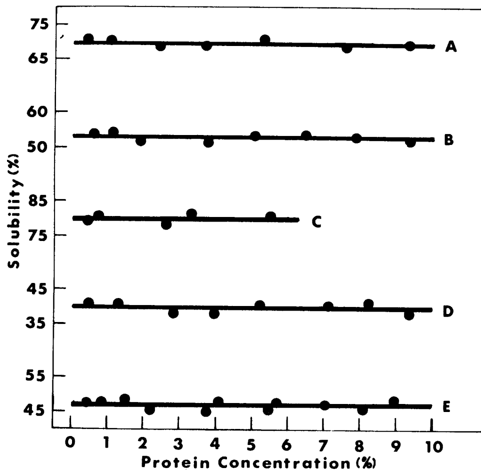

Fig. 4. The effect of protein concentration on protein solubility: A, B, C, D, and E = isolates.

Temperature

Figure 5 gives the solubility of the isolates as a function of equilibration temperature. The samples were equilibrated for 2 hr in a shaker bath at the desired temperature and then centrifuged at room temperature. Again, for the 25° to 62° C range studied, the effects are both appreciable and varied. The effects range from no change for isolate C to a 14% increase in solubility for isolate D. The effect on isolate E is a gentle linear increase in solubility of 8%. Isolates A, B, and D show similar behavior, with overall increases in solubility of 11, 7, and 14%, respectively. Most of the solubility increases take place between 30° and 40° C. After 40° C, the rate of increase in the solubility decreases such that the solubilities of isolates B, C, and D reach a plateau. With the exception of isolate D, which shows a 4.0% solubility increase, negligible solubility increases are caused by raising the temperature from 25° C (our conditions) to 30° C (NSI and PDI conditions).

SUMMARY AND CONCLUSIONS

As a result of this study, we have selected the following standardized experimental conditions: gentle shaking (120 shakes/min) of 0.9% w/w slurries for 2 hr at 25° C, centrifugation at 42,000 × *g* for 20 min, and the use of "protein solubility" as determined by Biuret analysis. This method generally gave us clear and foamless solutions suitable for intrinsic viscosity, uv absorption, and optical rotation work.

From the data presented in Figs. 1–5, the protein solubility data measured under different experimental conditions can now be compared. As an example, we have compared the solubilities from the above standard procedure with NSI solubilities. The use of gentler blending conditions, higher centrifugation conditions, lower equilibration temperatures, and Biuret "protein solubility" instead of Kjeldahl "nitrogen solubility" by the above method provides values generally lower than those by NSI. In Table I, the measured quantitative effects

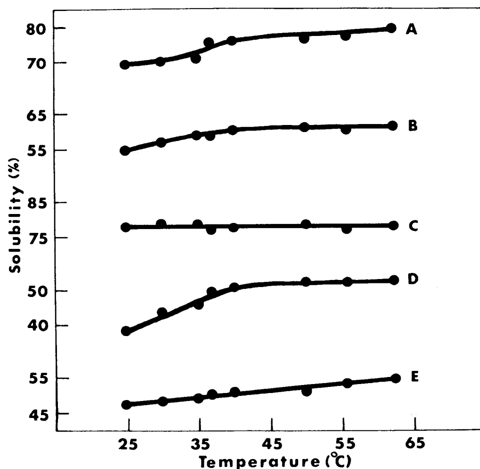

Fig. 5. The effect of temperature on protein solubility: A, B, C, D, and E = isolates.

TABLE I
Comparison of Solubility Data: Developed Method *vs.* NSI

	Effect on Solubility due to Variations in Experimental Conditions						
Isolate	Developed method %	Blending %	Centrifugation %	Temperature %	Kjeldahl *vs.* Biuret %	Estimated NSI %	Measured NSI %
A	69	0	10	1	5	85	85
B	55	1	2	1	5	64	67
D	40	0	1	4	5	50	(45)[a]
E	47	0	3	0	5	55	55

[a]Measurement was made on a 1-yr-old sample.

of each of these variations on the solubilities of isolates A, B, D, and E are presented along with the solubilities measured by our developed method. These separate effects when added to the solubility values derived from our developed method give us a predicted NSI solubility. These predicted NSI values compare favorably with the measured NSIs. (The nonsoy isolate, isolate C, was not included because we do not have a reliable estimate of the difference between Biuret "protein solubility" and Kjeldahl "nitrogen solubility.")

The solubilities of the five isolates studied showed appreciably different blending, centrifuging, and temperature dependence. It is therefore not possible to generalize our findings. The measurements made in this study must be repeated for each new isolate individually before meaningful comparison of solubility data obtained under different experimental conditions can be made. This problem can be eliminated by the acceptance of standard methods by all researchers. However, the presently available standard methods, NSI and PDI, do not meet the needs of many research problems, as providing clear solutions for optical measurements. Therefore, we favor the adoption of an additional method, such as the one developed in this study. This method would be suitable to problems requiring clear solutions. Solubility data should then be determined by this new method as well as by NSI. Such a procedure should result in more meaningful comparisons of soy protein solubility data.

Acknowledgment

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Literature Cited

1. AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.). Methods Ba 11-65 and Ba 10-65. The Society: Chicago (1970).
2. PAULSEN, T. M., HOLT, K. E., and ANDERSON, R. E. Determination of water-dispersible protein in soybean oil meals and flours. J. Amer. Oil Chem. Soc. 37: 165 (1960).
3. HERMANSSON, A. M. Functional properties of proteins for foods—solubility. AULs Halvarsskrift 2: 1 (1973).
4. SMITH, A. K., CIRCLE, S. J., and BROTHER, G. H. Peptization of soybean proteins. The effect of neutral salts on the quantity of nitrogenous constituents extracted from oil-free meal. J. Amer. Chem. Soc. 60: 1316 (1938).
5. MATTIL, K. F. Composition, nutritional, and functional properties, and quality criteria of soy protein concentrates and soy protein isolates. J. Amer. Oil Chem. Soc. 51: 81A (1974).

6. SMITH, A. K., RACKIS, J. J., ISNARDI, P., CARTTER, J. L., and KROBER, O. A. Nitrogen solubility index, isolated protein yield, and whey nitrogen content of several soybean strains. Cereal Chem. 43: 261 (1966).
7. BELTER, P. A., and SMITH, A. K. Protein denaturation in soybean meal during processing. J. Amer. Oil Chem. Soc. 29: 170 (1952).
8. FUKUSHIMA, D. Studies on soybean proteins. I. Water dispersibility of protein of defatted soybean flour as a criterion for degree of denaturation. Bull. Agr. Chem. Soc. Jap. 23: 7 (1959).
9. BETSCHART, A. A., SAUNDERS, R. M., BICKOFF, E. M., and KOHLER, G. O. Solubility profiles of protein concentrates (Abstr. No. 138). Cereal Sci. Today 18: 276 (1973).
10. 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 (1956).
11. WOLF, W. J., and TAMURA, T. Heat denaturation of soybean 11S protein. Cereal Chem. 46: 331 (1969).
12. ANDERSON, R. L., WOLF, W. J., and GLOVER, D. Extraction of soybean meal proteins with salt solutions at pH 4.5. J. Agr. Food Chem. 21: 251 (1973).
13. NASH, A. M., and WOLF, W. J. Solubility and ultracentrifugal studies on soybean globulins. Cereal Chem. 44: 183 (1967).
14. WOLF, W. J., RACKIS, J. J., SMITH, A. K., SASAME, H. A., and BABCOCK, G. E. Behavior of the 11S protein of soybeans in acid solutions. I. Effects of pH, ionic strength and time on ultracentrifugal and optical rotatory properties. J. Amer. Chem. Soc. 80: 5730 (1958).
15. LAYNE, E. Spectrophotometric and turbidimetric methods for measuring proteins, p. 450. In: Methods in enzymology, Vol. III, ed. by S. P. Colowick and N. O. Kaplan. Academic Press: New York (1957).
16. POMERANZ, Y. Evaluation of factors affecting the determination of nitrogen in soya products by the biuret and orange-G dye-binding methods. J. Food Sci. 30: 307 (1965).
17. COGAN, U., YARON, A., BERK, F., and MIZRAKI, S. Soybean protein: effect of processing conditions on yields and purity. J. Amer. Oil Chem. Soc. 44: 321 (1967).

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