Phytic Acid and Phosphorus Content of Various Soybean Protein Fractions[1]

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ABSTRACT

Phytate-protein complexes formed during the manufacture of soy protein isolates reportedly affect mineral bioavailability in foods; therefore, we have estimated the amounts of such complexes by determining the phytic acid (PA) content by anion-exchange high-performance liquid chromatography (HPLC) before and after dialysis. Protein fractions analyzed include: 1) commercial soybean protein isolates, 2) laboratory-prepared isolates, 3) the two major components of the isolates, the 7S and 11S globulins, 4) the pH 8 insoluble material formed on neutralization of the isoelectric (pH 4.5) form of the isolate, and 5) the pH 8 soluble fraction of the isolate. After dialysis, the PA content of the various soybean protein isolates was 0.95–1.75%. The 11S globulins were essentially devoid of PA, whereas the 7S globulins contained 0.34%. PA values for the dialyzed pH 8 insoluble and soluble protein fractions were 0.22–1.41% and 0.36–1.29%, respectively. Yields of the pH 8 insoluble fractions were 10.2–28.6% of the total protein isolate. These data indicate that certain protein components of the soybean protein isolate are strongly complexed with phytic acid, especially at pH 8. However, variability in the nature and yield of the insoluble proteins and in the amount of phytic acid bound indicates that factors other than the pH and protein type are involved in the formation of phytate-protein complexes. Processing conditions appear to be important, because extensive dialysis of the commercial samples at pH 8 failed to remove appreciable amounts of phytic acid.

The phytic acid (PA) content of various cereals, food legumes, and some oilseeds is 0.6–5% (Rackis and Anderson 1977). Several studies have shown that PA forms complexes with di- and trivalent cations and proteins and is associated with reduced mineral bioavailability (Smith and Rackis 1956; Saio et al 1968; Okubo et al 1975, 1976; O'Dell and de Boland 1976; de Rham and Jost 1979; Erdman 1979; Cheryan 1980; Prattley and Stanley 1982). Although de Boland et al (1975) found a 1.52% PA content in a defatted soybean meal and a soybean protein isolate, Lease (1967) reported that in tests involving defatted soybean meal and various soybean protein isolates, only the isolates required supplemental zinc for chick feeding. Several recent studies have shown that neutralization of soy protein products decreased zinc bioavailability, compared to acid-precipitated protein, soy flour, or egg white, when fed to rats (Erdman et al 1980, Prattley et al 1982, Ketelson et al 1984). Rackis and Anderson (1977) suggest that the formation of protein-phytate-mineral complexes during the processing of soybean protein isolates, rather than PA content per se, may be the main factor in reduced bioavailability of such materials as zinc and that the need for supplemental zinc, in particular, may vary from 0 to 100 ppm, depending on the soybean protein product used and the conditions of manufacture.

In the preparation of soybean protein isolates, maximum yields of precipitated protein occur at approximately pH 4.5, the isoelectric region for the major proteins. Phytic acid reacts with the proteins in the water extracts of raw soybean flakes during acidification to pH 4.5. When the isoelectric protein isolate is neutralized (pH 6–8.5), soluble and insoluble protein-phytate complexes are formed (Smith and Rackis 1956).

In our study, we determined the PA content of commercial and laboratory-prepared soybean protein isolates. Both isoelectric and neutralized isolates were analyzed. We also determined the phytic acid content of the pH 8 soluble and insoluble fractions of neutralized soybean protein isolates and did analyses before and after extensive dialysis, to obtain information on the nature of the interaction between phytic acid and proteins as a function of pH.

Soybean protein isolates consist of four components as characterized by ultracentrifugation, with sedimentation constants of 2, 7, 11, and 15S (Wolf 1972). The major components, the 7S and 11S globulins, were prepared, purified, and analyzed for phytic acid content.

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

MATERIALS AND METHODS

Commercial soybean protein isolates, Edipro A, Supro 710, and Supro HD90, were obtained from Ralston Purina Co., St. Louis, MO. Isoelectric and neutralized soybean protein isolates were prepared in the laboratory by standard procedures (Smith and Circle 1972) from raw, defatted, dehulled soybean flakes (Fig. 1). The flakes were prepared from certified Amsoy 71 soybeans (1976 and 1982 crops), as described by Sessa et al (1969).

The laboratory-prepared isoelectric soybean protein isolate was further processed to prepare pH 8 soluble and insoluble fractions (Fig. 2). Centrifugations were done at 10,000 rpm (16,000 rcf) for 20 min. Adjustments to pH 4.5 were made with 2*N* HCl and to pH 8 with 2*N* NaOH for 40–60 min with constant stirring until the pH remained steady. On dialysis, part of the pH 8 insoluble protein remained as a precipitate (fractions D and J) and the rest was dispersed (fractions C and I). In one preparation, these fractions were all analyzed for PA. After centrifugation at pH 8, part of the precipitate was gellike and tended to decant off with the supernatant; it was filtered off from the supernatant with cheesecloth to give gel fraction K and filtrate L, which were analyzed separately for PA. Samples were usually freeze-dried before analysis.

The 7S and 11S globulins were isolated by procedures reported by Thanh and Shibasaki (1976); the general scheme is shown in Fig. 3. The 7S globulins were purified by chromatography on Sepharose 6B, equilibrated with 0.1*N* potassium phosphate buffer, pH 7.6, in the presence of 0.01*M* β-mercaptoethanol and 0.02% sodium azide.

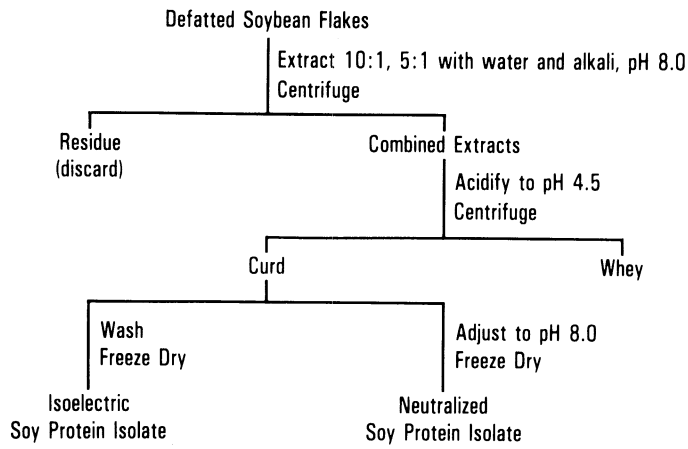

Fig. 1. Preparation of soy protein isolates (Smith and Circle 1972).

An acid-sensitive fraction was prepared according to the procedure reported by Anderson (1974). Samples were dialyzed against 10 volumes of deionized, distilled water with 4–8 changes for 2–7 days in Spectrapor cellulosic tubing of 6,000–8,000 mol wt cutoff (Fisher Scientific Co., Fair Lawn, NJ). No major decrease in phytic acid content was found after 48 hr.

Phytic acid was determined by the ion-exchange HPLC procedure of Graf and Dintzis (1982). Samples weighing 0.15–1 g were extracted with 20 ml of 0.5*N* HCl, and the filtered extracts were eluted through an AG 1X-8 ion-exchange column. Effluents eluted with 2*N* HCl were analyzed for phytic acid. Determination of phosphorus (P) was by the procedure of Chen et al (1956). Samples were digested for 1 hr with 0.5 ml of 6*N* H_2SO_4, followed by the addition of three drops of 65% $HClO_4$, and then were heated to a clear solution. For colorimetry, 3 ml of reagent (6*N* H_2SO_4:H_2O:2½% ammonium molybdate:10% ascorbic acid 1:2:1:1) and 3 ml of an appropriate dilution of the hydrolyzed sample were heated 1 hr at 37°C and read at 820 nm in a Beckman colorimeter. Sodium phytate (Sigma Chemical, St. Louis, MO), which analyzed as 61% PA and 17.2% P, was used as a PA standard. Na_2HPO_4 served as the P standard.

AG 1X-8 ion-exchange resin, 200–400 mesh, was supplied by Bio-Rad, Richmond, CA. Ammonium molybdate and L-ascorbic acid were ACS-certified grade (Fisher Scientific Co., Fair Lawn, NJ).

RESULTS AND DISCUSSION

The PA contents of some commercial soybean protein isolates before and after dialysis are shown in Table I. Values for the undialyzed samples are near the range of 1.9–2.5%, reported by Hartman (1979) as typical for commerical soybean protein isolates. However, the Edipro A sample at pH 4.5 lost 39% and Supro HD90 at pH 6.3 lost 30% of its PA when dialyzed for 48 hr against water. When these two protein isolates were brought to pH 8 before dialysis, PA losses were only 10 and 13%, respectively. The sample of Supro 710 at pH 6.9 or when brought to pH 8 showed little or no loss of PA on dialysis. These results indicate that when the protein isolates are at pH 8, more PA remains bound. Differences in production conditions, however, may also be a factor in the amount of PA bound. Okubo et al (1975) removed most of the phytate from a soybean protein isolate by dialysis against water at pH 5.5, but less was removed at higher pH values. The P content was similar for the three isolates (0.79–0.89%); based on 28.2% P in PA, this would correspond to 2.8–3.15% PA. Because actual PA values are lower (Table I), nonPA P compounds, such as phosphatides and nucleic acids, apparently are also present.

Yields and PA contents of the pH 8 insoluble (fractions A and G) and soluble (fraction H) proteins, prepared as shown in Fig. 2, were variable. The results for eight repetitions of the partial fractionation into A, G, and H are shown in Table II. Yields of fraction A were 2–19% of the total isolate; the PA content after dialysis averaged 0.80% but ranged from 0.54 to 1.2%. Similarly, the yields of fraction G were 1–19%; the PA content after dialysis averaged 1.03% but varied from 0.76 to 1.41%. In one of the fractionations, A and G were analyzed for PA before dialysis and contained 1.09 and 1.31% PA, respectively. The yields of soluble fraction H were 71–97% of the isolate, with an average value of 1.23% PA obtained before dialysis and 1.06% PA after dialysis. Fraction H usually had a PA content of at least 0.74% except in one fractionation where supernatant B was held at pH 8 overnight instead of acidifying to pH 4.5 immediately, as was done for the other fractions. This sample of fraction H contained only 0.36% PA. Otherwise, these results showed no consistent differences in PA content between the insoluble (A and G) and soluble (H) fractions. The PA contents, however, were all lower than values for unfractionated, undialyzed pH 8 protein isolate, indicating that some PA may be lost during fractionation. No effect of age of the soybeans or of rate of acidification was noted. Complexing of PA with proteins does not appear to be the only reason for formation of insoluble protein fractions. Other factors, such as protein aggregation and reversible and irreversible denaturation of proteins, must contribute to variability in recovery of insoluble protein and in the extent of PA binding to protein. Nash and Wolf (1967) also found wide variability in the amount of insoluble protein in commercial and laboratory-prepared soybean protein isolates at pH 7.6 in the presence and absence of 2-mercaptoethanol.

One preparation of isoelectric isolate was carried through the full fractionation (fractions A–L) shown in Fig. 2. The results from this extended fractionation are shown in Table III. Fractions C and I had values of 0.86 and 0.65% P and 0.84 and 0.57% PA, respectively, compared to 0.57% P and 0.97% PA for fraction L. Fractions D and J contained only 0.41 and 0.4% P and 0.33 and 0.22% PA, respectively. On the other hand, the gel fraction, K, had contents of 0.97% P and 1.41% PA, which were higher than values for the other fractions. Total yield of the insolubles in this

TABLE I
Phosphorus and Phytic Acid Content of Commercial Soybean Protein Isolates Before and After Dialysis

Isolate	Original pH	Phosphorus Content (%)	Phytic Acid Content: Undialyzed (%)	Phytic Acid Content: Dialyzed[a] (%)	Phytic Acid Content: Dialyzed pH 8[b] (%)
Edipro A	4.5	0.89	1.79	1.10	1.60
Supro HD90	6.3	0.84	2.00	1.40	1.75
Supro 710	6.9	0.79	1.61	1.65	1.55

[a] Dialyzed for 48 hr against water.
[b] Adjusted to pH 8 before dialysis for 48 hr against water.

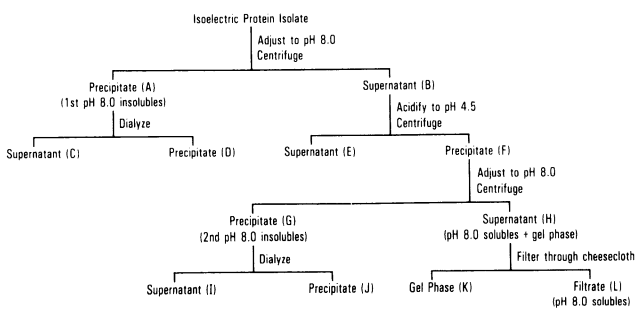

Fig. 2. Fractionation of isoelectric protein isolates into pH 8 soluble and insoluble fractions.

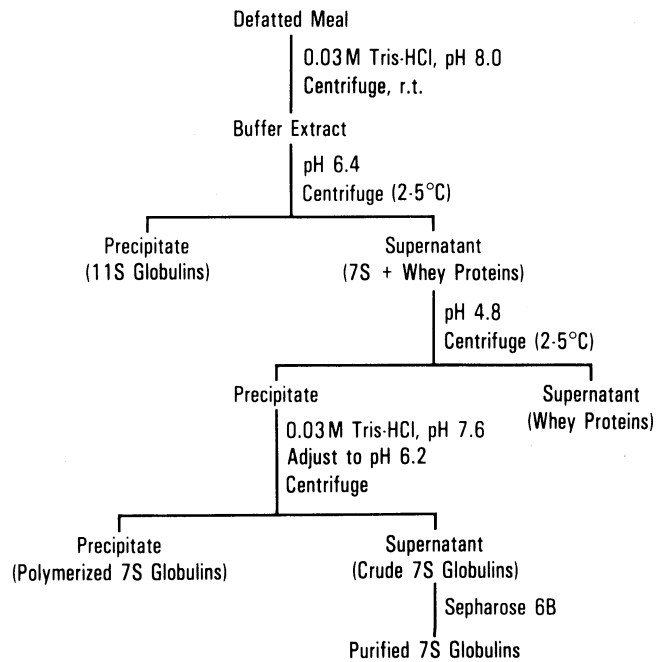

Fig. 3. Isolation of 7S and 11S globulins, according to the procedure of Thanh and Shibasaki (1976).

TABLE II
Protein Yield and Phytic Acid Content of pH 8 Insoluble and Soluble Fractions of Soybean Protein Isolate (Partial Fractionation)[a]

Fraction	Protein Yield[b]				% Phytic Acid			
	% of Meal		% of Total Isolate		Undialyzed		Dialyzed	
	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range
A-first insoluble	2.1	0.5-6.4	7.6	2.0-18.9	1.09	1.09[c]	0.80	0.54-1.21[d]
G-second insoluble	2.0	0.2-4.8	7.6	1.0-19.2	1.31	1.31[c]	1.03	0.76-1.41[e]
H-solubles	21.5	19.1-25.0	84.7	71.4-97.0	1.23	1.17-1.36[f]	1.06	0.36-1.58[e]

[a] See Fig. 2.
[b] Ranges represent data for eight preparations.
[c] One preparation.
[d] Five preparations.
[e] Six preparations.
[f] Three preparations.

TABLE III
Protein Yields and Phytic Acid Content of pH 8 Soluble and Insoluble Fractions of Soybean Protein Isolate (Extended Fractionation)[a]

Fraction	Protein Yield		Phosphorus (%)	Phytic Acid (%)
	% of Meal	% of Total Isolate		
C-supernatant	1.6	6.1	0.86	0.84
I-supernatant	1.0	3.9	0.65	0.57
D-precipitate	1.3	5.0	0.41	0.33
J-precipitate	0.3	1.3	0.40	0.22
K-gel phase	0.3	1.1	0.97	1.41
L-filtrate	21.6	82.6	0.57	0.97

[a] See Fig. 2.

TABLE IV
Phosphorus and Phytic Acid Content (%) of Laboratory Prepared Protein Isolates[a] and Acid-Sensitive Fraction[b]

Fraction	Phosphorus	Phytic Acid
pH 4.5 protein isolate, undialyzed	0.71	1.30
pH 8 isolate, undialyzed	0.75	1.60
pH 8 isolate, dialyzed	0.55	0.95
Acid-sensitive, undialyzed	0.22	0.28

[a] See Fig. 1 for preparation.
[b] See Anderson (1974) for preparation.

TABLE V
Phosphorus and Phytic Acid Contents (%) of 11S and 7S and Whey Fractions of Soybean Proteins[a,b]

Major Protein	Phosphorus	Phytic Acid
11S	0.06	0.01
7S-soluble	0.75	0.34
7S-polymerized	0.81	0.74
7S-purified[c]	0.18	0.06
2S-pH 4.8 whey	0.03	0.01

[a] See Fig. 3.
[b] Dialyzed against water.
[c] Chromatographed on Sepharose 6B; 0.1 μ sodium phosphate pH 7.6.

preparation was 17.4%. Characteristics of the different insoluble fractions, especially the gellike character of fraction K, may help explain the finding of Saio et al (1969) that coagulability of soybean protein for making tofu (a gellike product) was related to calcium content and PA/N ratio and that hardness of the tofu was inversely related to calcium content and P/N ratio.

For comparison, the P and PA contents of laboratory preparations of unfractionated pH 4.5 isoelectric protein isolate, pH 8 sodium proteinate, and acid-sensitive fraction are shown in Table IV. The undialyzed isoelectric protein isolate contained 1.3% PA after three washings with water. The pH 8 proteinate not washed before neutralization contained 1.6% PA before dialysis, compared to the 1.52% PA value reported by de Boland et al (1975). During dialysis for 48 hr, 40% of the PA was removed.

The acid-sensitive fraction is the part of water-extractable soybean protein that is insoluble in the presence of 1 M NaCl at pH 4.5 (Anderson 1974). Before dialysis, this fraction had P and PA contents of only 0.22 and 0.28%, respectively. This low value was expected, because Okubo et al (1976) reported that direct binding of PA to soybean glycinin is at a maximum at pH 2 and at a minimum at pH 4.8. Thus, the pH of 4.5 used in this study would be expected to result in low levels of PA binding to soybean protein.

Apparently, protein-phytate-mineral complexes are formed above pH 5.5 and hinder removal of phytate by dialysis or ultrafiltration (Okubo et al 1975, Cheryan 1980).

The 7S and 11S globulins were isolated by the procedure of Thanh and Shibasaki (1976), as shown in Fig. 3. Ultracentrifuge patterns of the major proteins of soybeans, their solubilities, and other properties have been reported by Wolf (1972). These fractions were examined for P and PA content to determine whether these values were affected by the type of protein or isolation procedure. Results are shown in Table V. The crude 11S had a very low P content and almost no PA. Because this fraction was acidified to only pH 6.4 during the isolation procedure (Fig. 3), the low PA content is consistent with the results of Okubo et al (1976), who found no binding of PA to 11S soybean protein above pH 4.8. In contrast, the soluble crude 7S fraction contained 0.75% P and 0.34% PA, and the insoluble polymerized 7S contained 0.81% P but twice as much bound PA. Both of these fractions were precipitated at pH 4.8, brought back to pH 7.6, and then reacidified to pH 6.2. Gel filtration on Sepharose 6B of the crude, soluble 7S protein removed a cloudy material in the void volume, which may have been lipids or nucleoproteins (Obara and Kimura 1967), and also increased the proportion of 7S in the major protein peak from 63 to 87%, as indicated by densitometry of the polyacrylamide gel electrophoresis pattern. The resulting purified 7S fraction contained only 0.18% P and 0.06% PA. The pH 4.8 whey, which was mainly 2S protein, contained very little P or PA after dialysis or ultrafiltration. The possibility that a protein-phytate-mineral complex was removed from the soluble 7S by Sepharose 6B chromatography requires further investigation of other column fractions. Such soluble complexes were reported by Prattley and Stanley (1982).

Our results for phytic acid content of the major proteins differ quantitatively from the results of Brooks and Morr (1984) but agree in regard to the differences between the phytate contents of the 7S and 11S proteins. Brooks and Morr prepared the proteins from Bragg soybeans, using a modification of the Thanh and Shibasaki (1976) procedure. Their 7S protein was high (1.4%) in phytic acid and their 11S protein was low (0.07%) in agreement with our observations (Table V). Brooks and Morr used a different variation of the Thanh and Shibasaki procedure than we did and did not dialyze their preparations, which may explain why their phytic acid values were higher than ours.

In conclusion, as a result of repeated precipitation at pH 4.5 with HCl and resolubilization to pH 8 with NaOH, 3–29% of the soybean protein isolates became insoluble. The P and PA contents of the soluble and insoluble fractions varied and did not correlate directly with solubility. Because much of the PA was not readily

dialyzable, it was probably complexed with protein. Such complexes are reported by Okubo et al (1975) and Prattley and Stanley (1982), but the nature of the complexes and their effect on solubility would seem to be quite variable. PA appeared to be associated to the greatest extent with the soft, gellike insoluble protein obtained in the extended fractionation shown in Fig. 2. Little PA was associated with 11S precipitated at pH 6.4; a larger amount of PA was associated with the 7S precipitated at pH 4.8, but apparently some phytic acid-7S complex could be separated from uncomplexed PA by resolubilization at pH 7.8 and precipitation at pH 6.2 or by column chromatography.

The PA content did not account for all of the P in any of the samples in which total P was analyzed, either before or after dialysis. This implies the presence of other nondialyzable phosphorus compounds. Koshiyama and Iguchi (1965) reported the presence of a ribonucleoprotein in soybean protein isolates. Phosphatides also occur in isolates (Nash et al 1967). The nature of other nondialyzable P compounds and other components associated with insoluble protein fractions requires further investigation.

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