During cooking, hard beans absorb less water and lose fewer solids and minerals than controls. This results from severe restriction of cell separation caused by at least two mechanisms contributing to the hard-to-cook defect: phytate depletion leading to Ca/Mg pectate formation and lignin deposition. Lack of further hydration during cooking suggests that starch gelatinization may be hindered in hard beans.

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Registry No. H_2O, 7732-18-5; Mg, 7439-95-4; Ca, 7440-70-2; K, 7440-09-7; starch, 9005-25-8.

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Mineral and Phytate Content and Solubility of Soybean Protein Isolates

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Laboratory preparations of sodium proteinate, obtained by aqueous extraction of defatted soybean flakes, precipitation at pH 4.5, and neutralization to pH 8, were fractionated by centrifugation and filtration into an easily sedimented insoluble fraction (4–7%), a gellike material (1–3%), and a soluble filtrate. The pH 8 soluble filtrate was then acidified to pH 4.5 and refractionated at pH 8 into a second insoluble (1–6%), a second gellike (1–3%), and a final soluble filtrate fraction (80–89%). These fractions plus a trypsin inhibitor concentrate were analyzed for calcium, magnesium, potassium, sodium, iron, manganese, copper, zinc, phosphorus, and phytic acid and compared to values for commercial soy proteinates. Mineral–phytate–protein interactions were examined by dialysis and gel filtration. Differences in binding by the various minerals were noted, but phytate content of the proteins did not correlate with mineral binding or protein insolubility. The calcium level of the first insoluble fraction was 4 times that of the unfractionated isolate.

The complex relationships among minerals, phytic acid, and plant proteins and their association with reduced mineral bioavailability have been studied extensively (Smith and Rackis, 1956; Saio et al., 1968; Okubo et al., 1975, 1976; O'Dell and de Boland, 1976; Erdman, 1979; Cheryan, 1980; Reddy and Salunkhe, 1981; Prattley and Stanley, 1982; Turnlund et. al., 1984). Neutralization of soy protein products reportedly decreases zinc bioavailability compared to acid-precipitated protein (unneutralized), 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) suggested that formation of protein–phytate–mineral complexes during processing of soybean protein isolates, rather than phytic acid content per se, may be an important factor in reduced bioavailability of minerals such as zinc. They reported that when soy protein is fed as the sole protein source in the diet, the need for supplemental zinc 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 occur when precipitation is carried out near pH 4.5, the isoelectric region for the major proteins. Phytic acid reacts with the proteins in water extracts of raw, defatted soybean flakes during acidification to pH 4.5, and the pH range of minimum solubility of the proteins is affected by both phytic acid and calcium concentrations (Saio et al., 1967). When the isoelectric protein isolate is subsequently neutralized (pH 6–8.5), soluble and insoluble protein–phytate complexes are formed (Smith and Rackis, 1956).

In a previous study, Honig et al. (1984) determined phytic acid contents of commercial and laboratory-prepared soybean protein isolates, including soluble and insoluble fractions of pH 8 sodium proteinates. In the present study we determined levels of major mineral elements as well as phytic acid levels in these same products before and after dialysis in order to determine relationships

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Table I. Mineral and Phytic Acid Content of Commercial Soybean Protein Samples[a]

component	defatted meal[b]	concentrate, Promosoy 100[c]	isolates			
			Promine R[c]	Edipro A	Supro 710	Supro HD90
			Units: Milligram/Gram			
P	7.1 ± 0.4	6.1		9.3 ± 0.35	7.9	8.35 ± 0.15
phytic acid				18.5 ± 0.50	16.2 ± 1.0	18.70 ± 2.10
Ca	2.23 ± 0.08	3.43	1.78	1.05 ± 0.07	1.21 ± 0.12	1.66 ± 0.11
K	25.2 ± 0.9			0.41 ± 0.07	0.82 ± 0.20	0.88 ± 0.11
Na	0.004 ± 0.001[d]			0.14 ± 0.10	9.63 ± 0.75	6.18 ± 0.72
			Units: Milligram/Kilogram			
Mg	3250 ± 100	3555	523	261 ± 29	362 ± 16	459 ± 39
Fe	138 ± 4	121	179	114 ± 11	143 ± 11	138 ± 24
Mn	38 ± 3	36	3	15 ± 0.5	10 ± 0.3	12 ± 1
Cu	20 ± 4	16	17	14 ± 0.2	18 ± 3.3	19 ± 0.9
Zn	52 ± 6	28	56	30 ± 2.6	29 ± 4.6	36 ± 2.2

[a] Mean ± standard deviation where results are from two or more determinations in our present study, otherwise quoted values. [b] Osborn, 1977. [c] Rackis, J. J., unpublished data. [d] Value obtained for sample where special precautions were taken to exclude dust contamination. Commercial meals contain 0.25 mg/g (Summers et al., 1983).

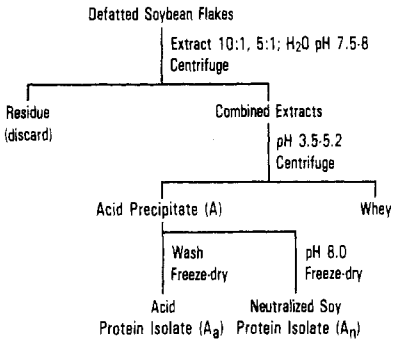

Figure 1. Preparation of soy protein isolate. Normal pH for preparation of acid precipitate (A) was 4.5; pH was varied in one experiment as described in text.

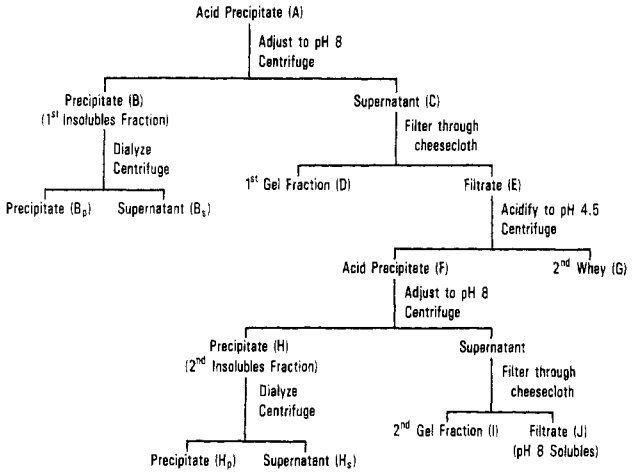

Figure 2. Fractionation of isoelectric protein isolates into pH 8 soluble and insoluble fractions.

between processing conditions and mineral content, phytic acid content, and solubility of soybean protein isolates neutralized to pH 8. Acid-precipitated isolates and their wheys, prepared at pH 3.5–5.2, and a trypsin inhibitor (TI) concentrate containing mainly acid-soluble whey proteins were also analyzed for minerals and phytic acid to see how levels in the neutralized pH 8 isolate might be affected by binding to acid-precipitated protein or whey proteins at acidic pH. Sodium proteinate was also fractionated by gel permeation chromatography on Sepharose 6B to determine whether minerals coeluted with phytic acid and 7S proteins as shown in previous work.

MATERIALS AND METHODS

Commercial soybean protein isolates, Edipro A, Supro 710, and Supro HD90 with minimum protein contents of 90%, were obtained from Ralston Purina Co., St. Louis, MO. Promine R isolate and Promosoy 100 protein concentrate were supplied by Central Soya Co., Chicago, IL. Defatted flakes prepared from certified Amsoy soybeans, 1976 crop, as described by Sessa et al. (1969), were used to prepare acid precipitated and neutralized soybean protein isolates (Figure 1). Soybeans and flakes had been stored at 4 °C. Commercial isolates are usually prepared by a process similar to that in Figure 1 except that the water is not distilled (we used deionized distilled water) and they are spray-dried instead of freeze-dried.

The laboratory-prepared acid precipitates (A, Figure 1), were further processed to pH 8 soluble and insoluble fractions (Figure 2). These were pH 4.5 isoelectric precipitates except when the effect of precipitation pH was studied. After the insoluble fraction (B) and gel fraction (D) were removed, the resultant filtrate (E) was reacidified and the steps repeated to remove the second insoluble fraction (H) and second gel fraction (I). Neutralization required nearly 1 h to attain constant pH. The first neutralization step followed shortly after the first precipitation, and the second neutralization took place after the reacidified precipitate had been standing in water overnight at 4 °C. To evaluate the extent of binding of various minerals and phytic acid, samples were dialyzed against at least 10 volumes of deionized distilled water for 2–7 days in Spectrapor (Fisher Scientific Co., Fair Lawn, NJ) cellulosic tubing of 6000–8000 MW cutoff. The trypsin inhibitor concentrate, obtained from E. C. Baker, was prepared as described by Baker and Rackis (1985).

Initial determinations of phytic acid were based on the ion-exchange HPLC method of Graf and Dintzis (1982) as described by Honig et al. (1984). Later determinations were based on the ion-exchange procedure of Harland and Oberleas (1977) and on modifications of that procedure by Ellis and Morris (1983). Following ion-exchange separation of phytic acid, 0.5-mL portions of the 0.7 N NaCl eluates were digested for 45 min in test tubes in a sandbath with 0.5 mL of 6 N H_2SO_4 and 2–3 drops of 65% $HClO_4$. Phosphorus was then determined colorimetrically (Chen et al., 1956) and phytic acid was calculated (% P × 3.55). Total P was determined as in Honig et al. (1984).

Calcium, magnesium, potassium, sodium, iron, manganese, copper, and zinc were determined by atomic absorption with a Techtron AA120 spectrometer (Varian Instrument Division, Palo Alto, CA). Dry ashing at 550 °C overnight was used earlier, but wet ashing and other atomic absorption conditions similar to those reported by

Table II. Effect of pH and Dialysis on Mineral and Phytic Acid Content of Commercial Protein Isolates[a]

component	Edipro A pH 4.5[b] undialyzed	Edipro A pH 4.5[b] dialyzed	Edipro A pH 8.0[b,c] undialyzed	Edipro A pH 8.0[b,c] dialyzed	Supro HD90, pH 6.3[b] undialyzed	Supro HD90, pH 6.3[b] dialyzed
			Units: Milligram/Gram			
P	9.3 ± 0.4		9.9 ± 0.5	7.7	8.4 ± 0.2	
phytic acid	18.5 ± 0.5	11.8 ± 0.6		17.1 ± 1.1	18.7 ± 2.1	14.9 ± 0.9
Ca	1.05 ± 0.07	0.51 ± 0.04	1.17	1.10	1.66 ± 0.11	1.97
K	0.41 ± 0.07	0.15 ± 0.01	1.45 ± 0.14	0.68 ± 0.05	0.88 ± 0.11	0.40 ± 0.02
Na	0.14 ± 0.1	0.37	16.1 ± 0.7	6.03 ± 0.45	6.18 ± 0.72	2.97 ± 0.01
			Units: Milligram/Kilogram			
Mg	261 ± 29	106 ± 4	265 ± 4	290	459 ± 39	578 ± 23
Fe	114 ± 11	142 ± 4	147 ± 2	123	138 ± 24	148
Mn	15 ± 0.5	7 ± 0.7	19 ± 1.1	13 ± 1.4	12 ± 1	14
Cu	14 ± 0.2	16 ± 0.8	7 ± 0.4	6	19 ± 0.9	23
Zn	30 ± 3	28 ± 2	32 ± 3	32 ± 5	36 ± 2	22

[a] Mean ± standard deviation, unless single determination. [b] pH that of a suspension of the product in distilled water. [c] Adjusted from pH 4.5 to 8.0 with NaOH and then freeze-dried.

Table III. Distribution of Minerals and Phytic Acid between Soluble and Insoluble pH 8 Protein Isolate Fractions[a]

fraction	pH 8 isolate (A_n)	1st insol (B)	1st gel fraction (D)	2nd insol (H)	2nd gel fraction (I)	filtrate (J)	2nd whey (G)
yield, %[b]	100	4–7	1–3	1–6	1–3	80–89	4–7
				Units: Milligram/Gram			
P	7.5	5.3 ± 0.16	6.3 ± 0.3	5.9 ± 0.4	6.4 ± 1.0	6.9 ± 0.7	9.1 ± 2.3
phytic acid	15.7	14.0 ± 0.8	17.7 ± 0.2	13.8 ± 0.1	15.5 ± 0.5	14.9 ± 0.4	12.5 ± 0.5
Ca	0.25	1.0 ± 0.34	0.43 ± 0.12	0.12 ± 0.03	0.14 ± 0.06	0.11 ± 0.04	2.9 ± 0.06
K	2.6	1.2 ± 0.20	2.4 ± 1.02	0.15 ± 0.05	0.29 + 0.07	0.22 ± 0.07	28 ± 0.9
Na	14.8	8.4 ± 2.2	11.8 ± 3.6	6.0 ± 1.3	11.7 ± 1.6	14.3 ± 1.8	206 ± 13.5
				Units: Milligram/Kilogram			
Mg	423	284 ± 73	438 ± 105	61 ± 30	98 ± 38	108 ± 41	6770 ± 940
Fe	98	212 ± 73	128 ± 14	133 ± 26	120 ± 20	116 ± 12	16 ± 2
Mn	4	4 ± 0.6	3 ± 0.8	4 ± 0.7	3 ± 0.5	3 ± 0.9	50 ± 4.8
Cu	17	17 ± 3.8	18 ± 3.1	20 ± 4.6	17 ± 3.5	19 ± 4.0	8 ± 1.1
Zn	15	19 ± 1.1	24 ± 3.3	12 ± 4.3	17 ± 2.9	11 ± 3.0	91 ± 0.7

[a] See Figures 1 and 2 for preparation and identification of freeze-dried fractions. Mean ± standard deviation for two or more preparations or determinations unless single determination. [b] Range of yields of each fraction from isolate A (Figure 2).

Table IV. Effect of Dialysis on Distribution of Minerals and Phytic Acid in Insoluble Fractions B and H[a]

fraction	precipitate B_p	supernatant B_s	precipitate H_p	supernatant H_s
yield, %[b]	1.1–5	0.6–3.3	0.3–4	0.2–1.6
		Units: Milligram/Gram		
P	3.6 ± 0.7	7.3 ± 1.2	5.7 ± 1.4	8.5 ± 1.4
phytic acid	5.7 ± 0.3	23.9 ± 1.1	8.5 ± 1.2	28.4 ± 0.5
Na	0.40 ± 0.23	4.8 ± 1.6	0.24 ± 0.02	4.9 ± 2.8
		Units: Milligram/Kilogram		
K	120 ± 40	810 ± 130	120 ± 70	770 ± 470
Ca	830 ± 200	600 ± 200	205 ± 23	670 ± 120
Mg	244 ± 107	166	47 ± 5	276 ± 83
Fe	232 ± 6	158 ± 26	180 ± 12	164 ± 35
Mn	5 ± 2.3	6 ± 1.9	7 ± 4.1	13 ± 5.7
Cu	20 ± 4.3	14 ± 1.1	16 ± 2.3	19 ± 2.3
Zn	20 ± 1.8	36 ± 4.8	17 ± 4.5	41 ± 6

[a] See Figure 2 for preparation and identification of freeze-dried fractions. Insoluble fractions B and H dialyzed 48 h against deionized distilled water and centrifuged. Mean ± standard deviation from two or more preparations. [b] Range of yields of each fraction from pH 8 protein isolate.

Garcia et al. (1972) were more suitable for our purposes. Values in Tables I–IV are from both methods. Values in Tables V and VI were obtained by the wet-ashing procedure. Samples of 0.05–2 g of different soybean protein preparations were digested with 15–20 mL of redistilled HNO_3 and 1–2 mL of 65% $HClO_4$. After digestion, HNO_3 and $HClO_4$ were fumed off and displaced by concentrated HCl (Garcia et al., 1972) to avoid interferences and hazards from $HClO_4$. Ashed samples in HCl were then brought to 25 mL with deionized, distilled water and further diluted 1:25 or 2:25 for high concentrations of K and Na and 1:10 or 2:10 for Ca and Mg determinations. The final dilutions of samples and standards for Ca and Mg determinations contained 0.5% La_2O_3. Reagent blanks were subjected to the same procedures to correct for background levels of minerals. Individual mineral element standards were 0.12–10 ppm dilutions of 1000 ppm standards (Fisher Scientific Co., Fair Lawn, NJ); 7–10% HCl was aspirated into the instrument between samples.

Gel permeation chromatography was performed on Sepharose 6B (2.5 × 90 cm) equilibrated with 0.05 M sodium borate buffer, pH 7.5 (Schnepf and Satterlee, 1985), containing 0.02% NaN_3; the same buffer with and without 1 N NaCl was used as eluent. The effluent was monitored

Table V. Effect of Precipitation pH on Mineral and Phytic Acid Concentrations of Protein Isolate and Whey[a]

sample	precipn pH	protein,[b] %	yield,[c] %	concn, mg/kg Ca	Mg	Fe	Mn	Cu	Zn	concn, mg/g K	P	phytic acid
isolate (A_a)[d]												
1	3.5		42.5	355	476	108	6	21	14	3.8	8.5	21.6
2[g]	4.5		56.4	279	396	106	9	17	16	1.1	6.7	15.3
3	5.2		56.9	485	730	111	7	17	24	2.9	4.4	8.4
pH 8 isolate[e]												
1st insoluble (B)												
1	3.5		1.0	1840	180	135	0	21	19	2.3	5.7	9.7
2[f]	4.5		3.2	1003	284	212	4	17	19	1.2	5.3	14.0
3	5.0		2.1	1600	406	114	0	14	18	2.6	3.7	10.7
filtrate (J)												
1	3.5		37.3	155	38	115	3	15	6	0.6	9.6	22.5
2[f]	4.5		44.6	106	108	116	3	19	11	0.2	6.9	14.9
3	5.0		43.6	120	98	112	2	16	11	0.3	6.5	15.8
whey[d]												
1[g]	3.5	42	57.5	2560	3110	51	30	9	64	32	3.1	4.5
2[g]	4.5	23	43.6	2496	5490	31	42	6.6	87	60	4.0	7.4
3	5.2	38.1	43.1	2260	5140	45	38	9.0	76	50	6.8	13.8
TI conc[h]	4.2	77		586	290	98	2	24	4	0.1	3.5	9.0

[a] Freeze-dried basis, single preparation unless otherwise noted. [b] Percent protein in freeze-dried fraction. Isolates assumed to be over 90%. [c] Percent of total aliquot of extract. [d] For preparation see Figure 1. [e] For preparation see Figure 2. [f] Mean of two or more preparations, except for protein yield. For deviations see Table III. [g] Mean of two preparations. [h] For preparation see Baker and Rackis (1985).

Table VI. Analyses of Eluted Fractions Obtained by Gel Filtration of pH 8 Soluble Isolate on Sepharose 6B[a]

fraction	protein mg	protein %[b]	concn, mg/kg Ca	Mg	K	Fe	Mn	Cu	Zn	concn, mg/g Na	P	phytic acid
I	820	40	127	51	248	68	1.4	4.5	31.1	35	1.3	0.7
II	200	36	101	61	240	50	7.6	3.9	13.2	36	0.9	0
III	650	47	100	83	199	56	3.0	3.8	9.4	30	0.7	0.2
IV	920	68	162	58	147	71	4.6	8.6	8.4	23	0.7	0.3
V	540	52	232	66	217	53	3.3	11.0	10.1	27	1.7	1.8
VI	390	27	370	335	233	29	8.5	12.8	7.6	46	8.2	21.2
VII	330	2.6	121	122	352	3.8	0.5	5.6	7.2	62	4.2	4.6
VIII	840	1.4	60	42	370	8		1.4	2.7	83	0.2	0

[a] See Figure 3 for origin of fractions; single determinations on freeze-dried samples. [b] Protein content of undialyzed, freeze-dried samples.

at 280 nm. Samples were 0.5–0.9 g of soybean proteinate (fraction A_n, Figure 1), dispersed in pH 7.5 borate buffer and filtered through glass wool. Column eluate fractions were analyzed for protein, phytic acid, and minerals.

Nondissociating gel electrophoresis was carried out by the procedure of Ornstein (1964) and Davis (1964) on 5% slab gels. Dissociating sodium dodecyl sulfate (SDS) electrophoresis was performed on 11% gels (Laemmli, 1970). Equipment from LKB Instruments Inc., Gaithersburg, MD, was used for both procedures. Protein was determined by the procedure of Lowry et al. (1951).

RESULTS AND DISCUSSION

Commercial Soybean Protein Products. Results of our analyses of commercial soybean isolates, unpublished data for another commercial isolate and a protein concentrate, and values from a collaborative study of mineral levels in defatted soybean meal (Osborn, 1977) are shown in Table I. The Na value of Edipro A and the Fe, P, and Cu levels of all isolates are similar to those of defatted meal. The high Na values for Supro 710 and Supro HD90 result from neutralization during commerical processing. Values for Ca, Mg, K, and Zn (except for Promine R) are lower in isolates than in meal, while Ca and Mg levels in the concentrate are higher than those in meal. These results for commercial isolates are generally comparable to those reported by O'Dell (1979) for soybean protein products and serve as a reference.

Commercial protein samples were dialyzed for at least 48 h against distilled deionized water in attempts to remove minerals and phytic acid. Dialysis of Edipro A at pH 4.5 (the product's pH as obtained) resulted in losses of Ca, Mg, K, Mn, and phytic acid (Table II); Fe, Cu, Zn, and Na values increased or showed little change. When this protein was adjusted to pH 8 and then dialyzed, there was a major decrease in added Na, a decrease in K compared with undialyzed pH 8 protein, and a small decrease in P; some of the apparent increase in K at pH 8 may be due to interference from the high level of Na. Dialysis of Supro HD90 at pH 6.3 decreased only K, Na, Zn, and phytic acid. These results indicate that all these minerals were bound to some extent at pH 6.3–8.0, although K and Na levels did decrease at pH 6.3.

Neutralized Laboratory-Prepared Protein Fractions. Mineral levels in laboratory preparations of pH 8 soluble and insoluble fractions (Figure 2) are compared in Table III with levels in pH 8 isolate A_n. The major differences noted were that the Ca level in first insoluble fraction B was 4 times that in unfractionated, unwashed, isolate A_n, while levels of Fe and Zn in B were also higher. This indicates that higher levels of Ca and possibly higher levels of Fe and Zn are associated with insolubility. On the other hand, levels of phytic acid, K, Na, and Mg were lower in B than in A_n and are inversely related to insolubility. In the first gel fraction D, phytic acid, Ca, and Zn were higher than in A_n. In the second insoluble fraction H, levels of phytic acid, P, and Na were lower than in A_n or soluble filtrate J. Levels of Ca, K, and Mg were all reduced in H–J compared to A_n while Zn was lower only in J. Whey fraction G was only 33% protein compared to over 90% in A_n or the other fractions. It consisted of acid-soluble proteins and minerals, especially NaCl formed by the neutralization and acidification steps. Levels of most minerals were higher in G than in the original isolate

or other fractions due to solubilization during reacidification. Phytic acid, Fe, and Cu, however, were at lower levels in G, indicating that they were not freely soluble at pH 4.5. If levels of Ca based on average yields in fractions B–J are added, a total of 0.29 mg/g is obtained; this cumulative value is comparable to 0.25 mg/g obtained for the unfractionated pH 8 isolate preparation (A_n) and is evidence of internal consistency of the data.

Yields of the final soluble filtrate fraction J varied from 80 to 89%, while yields of insoluble fractions B and H varied from 1 to 7% when prepared from a pH 4.5 protein precipitate. The various factors that affect these yields require further investigation. Yields of minerals and protein varied somewhat between individual preparations, especially fractions B and D. Some factors that may have affected these values were the precipitation pH, amount of Na^+ added during neutralization, and separations between fractions B and D. Levels of Ca were higher and protein yields lower for B if it was washed and washes added to fraction D. Levels of Ca, Mg, and Zn in laboratory-prepared isolates (Table III) were not as high as those in commercial samples (Table I), possibly because laboratory samples were prepared with deionized, distilled water while commercial samples are prepared with water that contains minerals.

Upon SDS gel electrophoresis, fractions B–J (Table III) exhibited no major differences in protein or subunit composition (data not shown).

Portions of fractions B–J except G (Table III) were also dialyzed against deionized distilled water for at least 48 h and analyzed for phytate and minerals. Conductance of suspensions of these fractions decreased significantly after dialysis, due mainly to losses of Na; little loss of other minerals occurred except for K, further indicating binding of all minerals at pH 8.

Precipitates (B_p and H_p) and supernatants (B_s and H_s) from dialysis of insoluble fractions B and H differ markedly in mineral and phytate values (Table IV). After dialysis, supernatants B_s and H_s contained 10–40% of the protein in fractions B and H. B_s had higher levels of Zn, K, P, and Na than did associated precipitate B_p; whereas levels of Ca, Fe, and Cu were higher in B_p. Levels of minerals were 1.5–20 times higher in H_s than in precipitate H_p, except for Fe and Cu. Phytic acid levels in fractions B_s and H_s were about 4 and 3 times greater than those of B_p and H_p, respectively, and 93% of P was in phytic acid in B_s and H_s, compared to 45 and 42% in B_p and H_p, respectively. Overall, there was a major loss of only K and Na in dialyzed fraction B as compared to undialyzed B (Table III); however, Ca, Mg, Zn, and K levels increased in dialyzed H. Possibly the larger amounts of phytic acid, Na, and K in B_s and H_s as compared to B_p and H_p associate with protein in a soluble complex that prevents dialysis and adsorbs minerals from outside the sample, while in the precipitate Ca and Fe complex directly to protein, causing aggregation. Most of our phytic acid determinations were made by the procedure of Harland and Oberleas (1977); however, results on the neutralized protein fractions were determined using the modification of Ellis and Morris (1983) as it gave more complete elution of phytic acid and better agreement between fractions and starting material.

Effects of Precipitation pH. We also investigated effects of minor pH variations during isolate precipitation on amount of insoluble protein and on levels of minerals in acid-precipitated isolates before and after neutralization. Aliquots of aqueous defatted meal extracts were adjusted to pH 3.5–5.2; resulting precipitates and wheys (Figure 1)

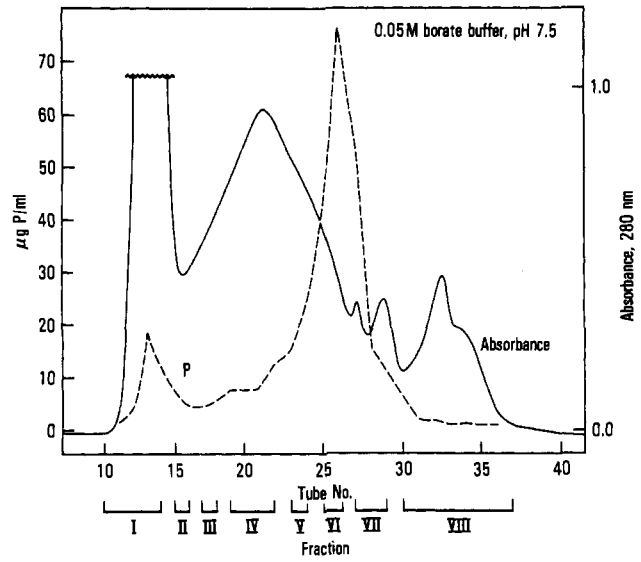

Figure 3. Chromatography of pH 8 solubles on Sepharose 6B using 0.05 M sodium borate buffer, pH 7.5.

and soluble and insoluble fractions obtained upon adjusting isolates to pH 8 (Figure 2) were analyzed for minerals and phytic acid. Levels of Zn were lowest in proteins isolated at pH 3.5 (Table V), levels of Zn in washed isolate and filtrate (J) were only 5–6 mg/kg, while P and phytic acid were highest in pH 3.5 precipitated proteins and lowest in the pH 5.2 isolate, indicating that Zn binding was at a minimum and phytic acid maximum at pH 3.5. Ca, Mg, K, Mn, and Zn were quite soluble (loosely bound) at precipitation pHs and were mainly in whey, where P and phytic acid levels were lower. Fe and Cu remained with protein at acidic pHs. Less Fe and Cu occurred in whey than in isolate, but on a mineral-to-protein basis, differences were small.

In a trypsin inhibitor (TI) concentrate (Table V), extracted at pH 4.2 and ultrafiltered at pH 2 to remove carbohydrates and some minerals, final levels of most minerals were comparable to those in protein isolates (Table V), although less phytate remained in the concentrate. Fe and Cu levels were also similar to those in whey on a mineral-to-protein basis. Comparison of mineral levels in the TI concentrate with those in pH 3.5 whey and isolate suggests that, between pH 4.2 and 2.0, relative strength of binding of minerals to protein is approximately in the order Cu > Fe > Ca > P > Mn > Mg > Zn > K. Amount of pH 8.0 insoluble protein was least at precipitation pH 3.5; a total of only 7% of isolate protein was in fractions B, D, H, and I (Table III), while 93% was in the soluble filtrate fraction (J). After precipitation at pH 4.5, only 83% of the protein was soluble at pH 8.0, whereas at pH 5.0, 87% of isolate protein was in J, indicating that differences in yield of insoluble fraction may be due to variations in acidification pH. It was shown by de Rham and Jost (1979) that mineral and phytic acid contents vary with precipitation pH; protein efficiency ratio (PER) also varied but was affected primarily by factors other than phytate.

Gel Filtration. A Sepharose 6B elution pattern for a neutralized soy protein isolate (fraction A_n, Figure 1) is shown in Figure 3. Protein, mineral, and phytic acid analyses of resulting fractions (Table VI) show most phytic acid (fractions V–VII) to be associated with the main P peak (Figure 3). Increased amounts of Mg, Ca, and Cu occurred in the same fractions. The highest levels of Ca and Cu occurred in fractions IV–VI, and of Mg in VI–VII. Higher Fe levels generally correlated with higher protein.

Zn, however, was most concentrated in fraction I, eluting at the void volume, in which aggregated protein and possibly nucleic acids (Obara and Kimura, 1967) led to turbidity and high 280- and 260-nm absorbance. The Zn to Cu ratio was 7:1 in this low-phytate, void-volume fraction. Absorbance after tube 28 (Figure 3) was due mainly to nonprotein constituents such as isoflavones and ribonucleic acids (Obara and Kimura, 1967). Phytate did not coelute with the major proteins, as expected for a phytate–mineral complex with those proteins, but rather eluted in a range associated with lower molecular weight proteins (as identified by gel chromatography and electrophoresis). Possibly, affinity of the phytate for protein was reduced by the Na level in the borate elution buffer. In earlier studies, the phytate content of 7S protein decreased after Sepharose 6B chromatography (Honig et al., 1984). With 1 N NaCl added to the elution buffer, both P and 7S protein eluted later (not shown), but P level was still maximum near the end of the main protein peak (7S area).

CONCLUSIONS

These studies demonstrate that Ca and Fe associate more with pH 8 insoluble than soluble soybean proteins. Some minerals, phytic acid, and protein may be soluble at pH 8 due to association, but our results do not clearly show how much protein is involved or reveal a specific composition for such a complex. All minerals bind to protein at pH 8. Fe and Cu binding varies little with pH (Tables II and V); other minerals associate more or less strongly with protein and are more readily removed by dialysis at pH 4.5. Gel filtration (Table VI) also showed that minerals associated with proteins at pH 8 and that most Ca and Mg coeluted with phytic acid. Only K and Na were at high levels in the last fraction, eluted after proteins and phytic acid. The ratio of Zn to Cu and Fe was much higher in soybean meal and in whey than in laboratory-prepared protein isolates, especially at pH 3.5 (Tables I and V). More pH 8 insoluble protein appeared to form when proteins were precipitated at pH 4.5 than at pH 3.5 or 5.0. Nash et al. (1971), however, reported increased protein denaturation as pH was lowered from 5.8 to 2.4; dialysis appeared to decrease protein solubility. Chen and Morr (1985) reported increased protein solubility in low-phytate soy protein at pH 3 but decreased solubility at pH 6 and equivalent solubility at pH 9 compared to high-phytate soy protein, which indicates that phytate may affect solubility near pH 6. Rodriguez et al. (1985) reported that ion exchange removes Ca and Mg from soy protein along with phytic acid but does not remove most of the iron. This is of interest in connection with our findings that phytic acid, but not Fe, dissociated from the major proteins upon gel chromatography near neutral pH. When Prattley and Stanley (1982) isolated protein bodies from soybean meal with glycerol and chromatographed the proteins in pH 6.8, 0.05 M Tris buffer, calcium and phytate were in the 7S peak. Under our conditions most minerals and phytic acid did not elute as a complex with 7S protein.

This study shows further evidence that each of the minerals associates with phytic acid and the various soy protein components to a different extent and indicates these associations are affected by water extraction, acidification, and addition of Na^+ or Cl^- ions.

Registry No. Ca, 7440-70-2; P, 7723-14-0; Zn, 7440-66-6; Cu, 7440-50-8; Mn, 7439-96-5; Fe, 7439-89-6; Na, 7440-23-5; K, 7440-09-7; Mg, 7439-95-4; phytic acid, 83-86-3; trypsin inhibitor, 9035-81-8.

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