Phytate-Mineral-Protein Composition of Soybeans: Gel Filtration Studies of Soybean Meal Extracts

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Defatted soybean flakes were extracted, dialyzed, and chromatographed with Tris-HCl buffer (pH 8, 0.03 M) on Sepharose 6B to investigate associations of essential minerals with specific proteins or phytic acid. Eight fractions (I-VIII) were analyzed for protein and phosphorus compositions, phytic acid, Ca, Mg, K, Na, Fe, Mn, Cu, and Zn. Protein composition was also characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and ultracentrifugation. Elution of agglutinin was affected by interaction with other extract components and Sepharose. Concentrations of Fe and Ca were highest in fraction IV, which was richest in glycinin and β-conglycinin. The content of Mn and agglutinin in the dialyzed, gel-filtered extract was in a molar ratio of 1.2, close to that reported in isolated soybean agglutinin. However, 25% of the Mn and 49% of the phytic acid were in fraction VII, where the molar ratio of Mn to agglutinin was 6. Fraction VII also had the highest levels of Mg, P, and Cu per milliliter of eluent. Fraction VIII corresponded to agglutinin. Phytic acid was apparently involved in some protein-mineral associations but not in others.

INTRODUCTION

The formation of soybean protein-phytate-mineral complexes during processing is associated with reduced bioavailability of minerals such as Ca, Zn, Mg, and Fe (Rackis and Anderson, 1977; Erdman, 1979; Cheryan, 1980; Prattley et al., 1982). Protein-phytate complexes are also implicated in reduced protein digestibility (de Rham and Jost, 1979; Ritter et al., 1987), changes in solubility and related functionality (Okubo et al., 1976; Chen and Morr, 1985), and changes in molecular weight and subunit composition of soy proteins (Brooks and Morr, 1985). Yoshida (1988, 1989) and Yoshida et al. (1986) reported on forms of associations of Fe, Mn, Cu, and Zn with soybean proteins during gel filtration in pH 7.4, 0.01 M Tris-HCl buffer. Lee and Johnson (1989) showed that Mn was more available from soybeans than from casein and Mn-soy protein interaction affected the manganese superoxide dismutase activity of rats. Levels of phytate P, soluble Fe, and Zn in fractions of defatted soybean meal extracts obtained by chromatography on Bio-Gel P-4 were also reported by Ellis and Morris (1981).

In previous studies we investigated effects of soy protein-phytic acid-mineral interactions on protein solubility and mineral binding after acidification and neutralization of a pH 8 buffer extract of defatted meal (Honig and Wolf, 1987; Honig et al., 1987). In this study we examined the protein-phytic acid-mineral composition of dialyzed pH 8 buffer extracts of soybean flakes by gel filtration without intermediate acidification and readjustment to 8. Gel filtration chromatographic fractions were characterized by mineral and protein analyses, ultracentrifugal analyses, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to better define mineral or phytic acid interactions with specific proteins. The minerals studied affect the functionality and nutritional value of soybean proteins. Possible effects of these associations on chromatographic behavior and on dimerization or higher aggregation of specific protein components, as indicated by gel filtration elution times and ultracentrifugal behavior, were also examined.

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MATERIALS AND METHODS

Preparation of Protein Extracts. Amsoy 71 soybeans, 1976 crop, were flaked and defatted. The resulting meal was then extracted with Tris-HCl buffer (0.03 M, pH 8) containing 0.01 M 2-mercaptoethanol in a 1:12 meal to buffer ratio and then reextracted 1:8 [instead of 1:20 as in Thanh and Shibasaki (1976)]. Sodium azide (0.02%) was added to the buffer as a preservative. The extract was centrifuged at 5000*g* and 5 °C and then filtered through cheesecloth. A portion of the combined extracts was dialyzed in dialysis tubing of 10-14-kDa exclusion limits (Serva Biochemicals, Westbury, NY) against 16 volumes of extraction buffer for 24 h at 5 °C with one buffer change and centrifuged at 18000*g*. The resulting supernatant was fraction A and the precipitate fraction B. Portions of A and B were freeze-dried for mineral and phytate analyses. Portions of the dialysis buffer were also freeze-dried (fraction C) and analyzed to determine the amount of minerals dialyzing out. Deionized water was used in all procedures.

Gel Filtration. Portions (20-25 mL) of fraction A containing 400-500 mg of protein, were applied to a 2.6 × 90 cm Sepharose 6B column equilibrated with extraction buffer and then eluted with the same buffer at 5 or 24 °C. The effluent was monitored at 260 and 280 nm. The column was standardized with Blue Dextran, catalase, ovalbumin (Pharmacia Fine Chemicals, Piscataway, NJ), soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), and DNP-aspartic acid (Mann Biochemicals, New York, NY), applied in 2 mL of buffer, to indicate molecular size of protein eluents. The effluent was pooled into nine fractions (on the basis of 260- and 280-nm absorbance and P analyses) and freeze-dried. The first eight fractions were then analyzed for minerals, phytic acid, and protein composition. Fraction 9, containing only low levels of protein and P, was not further analyzed. In one gel filtration experiment, the fractions were subjected to ultracentrifugal analysis before freeze-drying and further analyses.

Electrophoresis. Extract and gel filtration fractions were examined by SDS-PAGE (Laemmli, 1970) on 12% gels as modified by Fling and Gregerson (1986). Proteins were stained with Coomassie Blue R250 (Sigma) (Fling and Gregerson, 1986) and glycoproteins with periodic acid-Schiff (PAS) reagent (Schiff reagent, Sigma) (Konat et al., 1984). Levels of individual proteins in the fractions were determined from corresponding SDS-PAGE bands by means of an Ultroscan XL densitometer (Pharamcia-LKB, Bromma, Sweden).

Ultracentrifugal Analysis. Gel filtration fractions were concentrated by pervaporation from dialysis tubing (Kober, 1917),

Table I. Distribution of Protein, Phytic Acid, and Minerals in Dialyzed Soybean Extracts

		mg/50 g of meal								
fraction[a]	protein,[b] g	Ca	Mg	K	phytic acid	P	Fe	Mn	Cu	Zn
A	14.79	19.9	41.1	37.5	247	116.2	1.76	0.47	0.40	0.82
B	1.05	10.6	14.7	9.1	109	35.1	0.24	0.31	0.03	0.16
C		6.4	39.0	661.4	53	46.8	0.32	0.08	0.06	0.47

	mg/g[c]								
fraction[a]	Ca	Mg	K	phytic acid	P	Fe	Mn	Cu	Zn
A	1.3	2.8	2.5	16.7	7.9	0.12	0.03	0.03	0.06
B	10.1	14.1	8.7	103.8	33.4	0.23	0.30	0.03	0.30

	%[d]								
fraction[a]	protein	Ca	Mg	K	phytic acid	P	Fe	Mn	Cu	Zn
A	93.4	53.9	43.4	5.3	60.4	58.7	75.9	56	81.6	56.6
B	6.6	28.7	15.5	1.3	26.7	17.7	10.3	34.5	6.1	11.0
C		17.3	41.1	93.4	13.0	23.6	13.8	9.5	12.2	32.4

[a] A, soluble retentate; B, insoluble retentate; C, dialyzate (low molecular weight, unaggregated, nonprotein components). [b] Yield from total dialyzed extract of 50 g of meal. [c] Milligrams per gram of protein in fraction. [d] Percent of total protein or mineral component.

equilibrated against pH 7.6, 0.5 ionic strength buffer [standard buffer of Wolf and Briggs (1959)] containing 0.01 M 2-mercaptoethanol, and analyzed in a Beckman Model E ultracentrifuge at 48 000 rpm at room temperature. After analysis in standard buffer, samples were equilibrated against the 0.03 M Tris-HCl extraction buffer and reanalyzed in the ultracentrifuge, then dialyzed against distilled water, and freeze-dried. Compositions were calculated by enlarging ultracentrifuge patterns and measuring peak areas using a Sigma-Scan (Jandel Scientific, Sausalito, CA) computer program. Areas were corrected for radial dilution. Compositions are expressed as percentages of total areas under schlieren sedimentation patterns (Pickels, 1952).

Analytical. Phytic acid was determined according to the procedure of Ellis and Morris (1983) as adapted by Honig and Wolf (1987). Minerals were determined by atomic adsorption spectrometry (Garcia et al., 1972) as adapted by Honig and Wolf (1987). P was determined according to the procedure of Chen et al. (1956) as in Honig et al. (1984). Protein concentration was determined according to the procedure of Lowry et al. (1951) or the bicinchoninic acid (BCA) procedure of Smith et al. (1985) using BCA reagent (Pierce Chemical Co., Rockford, IL). Trypsin inhibitor activity (TIA) was determiend according to the procedure of Hamerstrand et al. (1981).

RESULTS AND DISCUSSION

Mineral Composition of Dialyzed Extract and Dialysate. Mineral analyses of the Amsoy 71 soybean meal extract fractions, from dialysis and centrifugation, are shown in Table I. Yields of protein, minerals, and phytate in these fractions represent distribution between soluble (A) and insoluble (B) dialyzed extract retentate and the dialyzate (C). Loss of minerals to the dialysis buffer varied from 93% for K to only 9.5% for Mn. Insoluble fraction B contained only 7% of the protein found in fraction A but was relatively rich in phytic acid (104 mg/g of protein compared to 17 mg/g in A). Fraction B also contained higher levels of other minerals except for Cu and Na (most Na was from sodium azide in the buffer and is not shown). Insoluble mineral–phytate complexes may explain high levels of phytate and certain minerals in fraction B. Protein compositions of fractions A and B were similar by SDS–PAGE, however, indicating nonspecific aggregation in fraction B.

Gel Filtration. Figure 1 presents the Sepharose 6B elution pattern of dialyzed extract (fraction A) chromatographed at 5 °C; similar results were obtained for other gel filtrations at 5 or 24 °C. Results are similar to those of Brooks and Morr (1985), although they used 0.5 ionic strength standard phosphate buffer (Wolf and Briggs, 1959) with 0.001 M dithioerythritol as the reducing agent

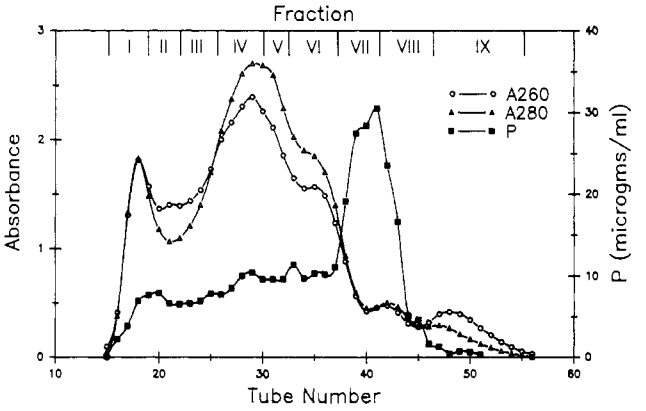

Figure 1. Sepharose 6B gel filtration of dialyzed Amsoy soybean meal components soluble in pH 8, 0.03 M Tris-HCl; column effluent was monitored at 260 and 280 nm and for P content. On the basis of these analyses, eluted material was divided into fractions I–IX, as indicated. V_e standards: Blue Dextran (2000 kDa), 176mL (V_o); catalase (232 kDa), 286 mL; ovalbumin and KTI (43 and 21.5 kDa, respectively), 352 mL; DNP-aspartic acid (0.3 kDa), 445 mL ($\sim V_t$). Volume per tube was 11 mL.

for Sepharose 6B gel filtration of a defatted soybean meal extract. Fraction I was highly turbid, indicating aggregation of protein and nonprotein material such as nucleotides and polar lipids. High 260 and 280 nm absorbance ratios in fractions I–III and VII–IX suggest ribonucleic acid containing components (Obara and Kimura, 1967), while Ellis and Morris (1983) report methanol–chloroform extractable phospholipid and Fe in the gel filtration void volume fraction of their soybean meal extract. In contrast to gel filtration of soybean isolates (Honig and Wolf, 1987) the elution volume of the P peak was 450 mL instead of 360 mL, while 26% of the extract protein eluted in fraction VI or later compared to only 7% of the isolate protein. Fraction VIII elutes after the total liquid volume of the column, V_t (445 mL as estimated by elution of DNP–aspartic acid), indicating that its components interact with the column.

SDS–PAGE. Sepharose 6B fractions were analyzed by SDS–PAGE with protein and glycoprotein stains (Figure 2). Fraction components were identified by comparison to standard proteins, identifications in the literature (Iwabuchi and Yamauchi, 1987; Hirano et al., 1987; Sathe et al., 1987; Sato et al., 1986), PAS staining, associated minerals, and ultracentrifugal behavior. Fraction I shows several bands corresponding to lipoxygenase, the α subunit of β-conglycinin, γ-conglycinin subunit,

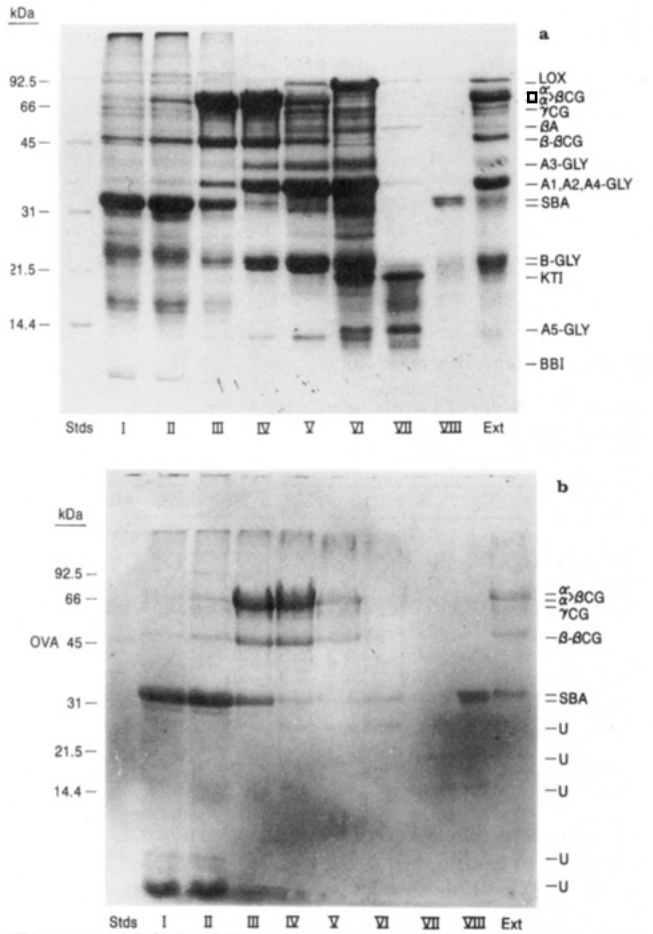

Figure 2. SDS–PAGE patterns for gel filtration protein fractions stained with (a) Coomassie Blue and (b) glycoprotein stain. (Stds) Bio-Rad low molecular weight standards (phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean Kunitz TI, and lysozyme, 92.5 to 14.4 kDa); (I–VIII) fractions I–VIII, respectively; (Ext) soybean extract control. Major soy proteins indicated in Ext, identified in other studies (Iwabuchi and Yamauchi, 1987; Hirano et al., 1987; Sathe et al., 1987), are BBI, Bowman–Birk inhibitor; A5-GLY, acidic glycinin polypeptide; KTI, Kunitz inhibitor; B-GLY, basic glycinin polypeptides; SBA, agglutinin subunits; A3-GLY, A1,A2,A4-GLY, acidic glycinin polypeptides; β-βCG, β subunit, β-conglycinin; βA, β-amylase; γCG, γ-conglycinin subunit; α′-, α-βCG, α′ and α subunits of β-conglycinin; LOX, lipoxygenase.

β-amylase, the β subunit of β-conglycinin, glycinin polypeptides, and a strong band of M_r 32 000 equivalent to an agglutinin subunit plus minor unidentified bands. Fraction II is similar to fraction I but contains more β-conglycinin. Both fractions I and II show much streaking, indicative of aggregation, as also suggested by the turbidity of fraction I. In fraction III β-conglycinin and agglutinin bands are strongest, while acidic and basic polypeptides of glycinin are present in lower concentrations. Fraction IV is similar to III, except for a decreased amount of agglutinin and increased levels of acidic and basic glycinin polypeptides. Minor agglutinin bands can be identified in fractions IV–VI. Fractions V and VI also have major bands corresponding to acidic and basic glycinin polypeptides. Fraction VI has strong bands corresponding to lipoxygenase, β-amylase, and Kunitz trypsin inhibitor (KTI). These are also among the bands in fraction VII and along with agglutinin are some of the main components identified by Iwabuchi and Yamauchi (1987) in soybean whey proteins. A minor band attributed to Bowman–Birk proteinase inhibitor appears in fraction VII. Fraction VII also shows much streaking attributable to modified protein. In fraction VIII a pair of soybean agglutinin subunits are the major components. The fraction V pattern is most similar to that of the starting extract.

SDS–PAGE gels of these same samples stained for glycoproteins are shown in Figure 2b. Bands correspond mainly to protein bands in Figure 2a. Major glycoproteins that have been identified in soybeans include β- and γ-conglycinin (with about 5% carbohydrate) and agglutinin (6%) (Nielsen, 1985). A glycoprotein band at M_r 62 000, especially in fractions III–IV, may correspond to a dimer of agglutinin subunits reported by Lotan et al. (1975) to form in soybean agglutinin samples on storage. Unidentified glycoproteins may be modified forms of these proteins or minor components. However, in fractions I and II, intense bands of M_r <8000 do not correspond to any bands in Figure 2a. They may be aggregates of nonprotein glycosides, such as saponins, glycolipids, or nucleotides with minor proteins or may be proteins that stain poorly with Coomassie Blue. Glycoproteins were minimally visible at about 10 μg with the PAS stain. Samples containing 250–400 μg of protein were applied per fraction for PAS stain compared to 125–150 μg for Coomassie Blue stain, permitting minor agglutinin subunit bands near M_r 32 000 in fractions IV–VII to be seen with the glycoprotein stain.

Table II. Distribution of Major Proteins in Gel Filtration Fractions Based on Densitometry[a]

fraction	protein, mg	% [b] LOX	BCG	GCG	BA	GLY	SBA	TI	U
I	18.9	0.2	7.4	1.9	2.7	4.6	18.6		64.7
II	18.9	0.06	13.9	2.4	1.8	4.8	11.5	1.1	64.5
III	45.4	0.22	33.5	3.2	1.2	19.4	10.8	0.2	31.6
IV	134.1	0.92	34.3	2.9	0.7	47.0	1.6		12.6
V	83.8	2.04	10.6	6.3	2.8	54.7	3.2	0.4	20.0
VI	76.3	5.82	1.9	2.5	3.7	30.5	3.4	8.5	43.0
VII	27.1	0.71	0.6	0.4	7.4	15.2	4.1	18.8	52.7
VIII	4.2	0.01	1.6	0.8	0.9	1.8	37.8	3.7	53.5
extract		2.9	22.2	2.8	2.1	43.8	5.2	3.5	17.5

[a] LOX, lipoxygenase; BCG, β-conglycinins; GCG, γ-conglycinin; BA, β-amylase; GLY, glycinin polypeptides; SBA, soybean agglutinins; TI, Kunitz and Bowman–Birk trypsin inhibitors; U, unidentified. [b] Percent of total protein in fraction.

Table III. Distribution of Glycoproteins in Gel Filtration Fractions Based on Densitometry

component[b]	fraction,[a] % I	II	III	IV	V	VI	VII	VIII
BCG	10	20	76	83	80	20		
GCG	1	3	3	3	4	5		
SBA	54	54	15	9	9	45	15	72
UGP	35	23	6	4	7	25	85	28

[a] Percent of glycoprotein in each fraction. [b] BCG, β-conglycinin; GCG, γ-conglycinin; SBA, soybean agglutinin; UGP, unidentified glycoprotein.

Protein Distribution. The distribution of major proteins in each gel filtration fraction was estimated from densitometry (Table II). For fraction I, a protein level of 0.4 mg/mL of eluent, as determined by BCA assay, does not parallel 280-nm absorbance (Figure 1) due to increased turbidity and nonprotein absorbance indicated by high 260-nm absorbance in this fraction. In fraction I, soybean agglutinin was the major component identified by SDS–PAGE (although 65% of the absorbance was due to unidentified proteins). The highest level of agglutinin at 0.1 mg/mL eluted in fraction III. The highest level of protein, including 1.4 mg/mL glycinin and 1 mg/mL β-conglycinin, eluted in fraction IV. Maximum elution of γ-conglycinin at 0.16 mg/mL was in fraction V. Most lipoxygenase (80%) eluted in fractions V and VI, while 72% of

Table IV. Composition of Tris Buffer Extract and Gel Filtration Fractions from Ultracentrifugal Analysis[a]

	Tris buffer, %						standard buffer, %				
fraction	2S	7S	9S	11S	15S	>15S	2S	7S	11S	15S	>15S
extract	20.2	17.7	23.8	30.4	7.9		22.8	36.3	32.0	8.8	
I	10.8	6.3	3.2	3.9	4.3	71.4	6.1	9.3			84.6
II	14.6	5.2	16.0	5.5	5.8	52.8	5.0	15.1	4.9	5.2	69.9
III	8.3	4.6	52.9	34.2			7.5	56.2	14.6	14.3	7.4
IV	6.3	12.2	40.5	33.5	7.5		2.6	46.3	40.1	7.9	3.1
V	15.3	41.5		32.3	10.9		12.1	25.8	56.9	5.2	
VI	60.7	32.0		7.3			58.1	26.0	15.9		
VII	96.5	3.5					95.4	4.6			
VIII	81.1	18.9					81.0	19.0			

[a] In Tris-HCl extraction buffer or after equilibration with standard buffer of Wolf and Briggs (1959).

the β-amylase eluted in fractions V–VII and 93% of the TI in fractions VI and VII. TI activity levels determined on each fraction were similar to levels indicated by densitometry. Most agglutinin (51%), 25% of the β-conglycinin, and only 7% of the glycinin eluted in fractions I–III ahead of the catalase standard. About 8% of the agglutinin appeared in fraction VIII. The distribution of protein components from an extract of Raiden soybean flakes (unpublished data) was similar except for some quantitative differences and a lack of A5 glycinin polypeptide.

The distribution of the glycoproteins, based on densitometry of the PAS stained gel, is presented in Table III. Agglutinin accounts for more than 50% of the glycoprotein in fractions I and II and over 70% in fraction VIII (not including the unidentified and presumably nonprotein glycoside band at M_r <8000 which accounts for 45% of the staining in fraction I, 53% in fraction II, and 2% in fraction III).

Ultracentrifugal Analyses. Composition of the Amsoy extract and its gel filtration fractions in 0.03 M Tris-HCl buffer, as compared to composition in standard buffer (Wolf and Briggs, 1959), was determined by ultracentrifugal analysis (Table IV). As expected, aggregated material (>15S) is maximum in fraction I and nearly as great in fraction II. Subsequent fractions show decreasing average component size until fraction VII shows 96% 2S composition. Fraction VIII, however, still contains 19% 7S protein, which may be agglutinin retarded on the column by interaction with the Sepharose 6B (Pusztai et al., 1988). This is less than the 38% agglutinin estimated by densitometry of the PAGE bands but some may elute as subunits and therefore sediment as part of the 2S fraction. Fractions II–V contain major amounts of 9S when analyzed in Tris-HCl and correspondingly higher levels of 7S in standard buffer. This differentiates the trimeric 7S β-conglycinin, which dimerizes to 9S protein in low ionic strength buffer (Iibuchi and Imahori, 1978), from other 7S and 6S proteins such as agglutinin, γ-conglycinin, β-amylase, and lipoxygenase. However, associations other than dimerization have apparently also occurred among proteins in fractions I–III since the proportion of proteins sedimenting as 2S plus 7S and 9S is greater than that of 11S proteins. Associations of agglutinin with other soybean glycosides and proteins have been reported (Bond et al., 1985; Einhoff et al., 1986). Differences in the content of 11S protein in fractions V and VI, as determined by ultracentrifugal analyses in Tris-HCl as compared to analyses in standard buffer, suggest partial dissociation of 11S into half-molecules in Tris-HCl buffer (Wolf and Briggs, 1958).

Mineral and Phytate Compositions. Mineral levels per gram of protein are shown in Table V, on the basis of the protein distribution presented in Table II. Levels of minerals per gram of protein were generally higher overall and especially in fractions I–III (except for Fe, K, and Zn)

Table V. Mineral Levels per Gram of Protein in Sepharose 6B Fractions

	mg/g					PAP,[b]	μg/g			
fraction	Ca	Mg	K	P	PA[a]	%	Fe	Mn	Cu	Zn
I	0.8	0.3	0.31	12	6.7	16	131	31	43	55
II	0.4	0.2	0.25	7.9	4.8	17	138	16	22	40
III	1.0	0.4	0.17	6.3	10.4	47	125	68	14	14
IV	1.0	0.3	0.02	3.0	4.3	40	124	6	3	8
V	1.0	0.9	0.07	6.4	10.8	47	104	22	32	37
VI	1.0	1.8	0.05	8.6	15.6	51	103	25	49	44
VII	4.5	13.5	0.42	37.4	120.3	91	335	109	135	66
VIII	3.5	9.0	90.2	9.7	2.1	6	338	53	57	65
total	1.2	1.8	1.0	8.3	16.3	56	136	29	31	29

[a] PA, phytic acid. [b] Percent phytic acid phosphorus (PAP)/total phosphorus.

compared to levels in corresponding fractions from gel filtration of undialyzed, pH 8 protein isolates (Honig and Wolf, 1987). This is consistent with findings of higher levels of minerals per gram of whey protein compared to levels in isolate protein (Honig and Wolf, 1987). Fraction IV had maximum concentrations of Fe and Ca per milliliter (not shown) corresponding to maximum levels of protein and the major protein components, β-conglycinin and glycinin, in this fraction. The level of other minerals per gram of protein is lower in fraction IV compared to other fractions. Okubo et al. (1976) found no binding of phytic acid to glycinin at pH 8, suggesting minerals are not primarily associated through phytic acid with the major proteins, especially glycinin after gel filtration. Khan et al. (1990) also found little association of Zn with the major 7S and 11S proteins. Levels of Mn were maximum in fraction III (70 ng/mL of effluent), which had 26% of the total Mn in the eight fractions while VII had 25%. Zn levels in the column effluent were highest in fraction V, while Mg, Cu, P, and phytic acid levels were highest in fraction VII with 54, 30, 31, and 49%, respectively, of their total content eluting in this fraction. By comparison, 86% of phytic acid eluted in fractions VI and VII during similar gel filtration of a soy protein isolate (Honig and Wolf, 1987). Phytic acid P as a percent of total P ranged from about 6–15% in fractions I, II, and VIII to 91% in fraction VII.

Discussion. These gel filtration results give some indication of soybean protein–phytate–mineral relationships, but in connection with previous results (Honig and Wolf, 1987) and results of others (Prattley et al., 1982; Brooks and Morr, 1985; Yoshida 1988, 1989), they also indicate that these relationships are readily affected by treatment prior to gel filtration and by conditions of the gel filtration.

As indicated by gel filtration, SDS–PAGE, and sedimentation analysis, 61% of the agglutinin elutes with aggregated or larger proteins; 25% elutes where 120-kDa proteins should elute and 13% elutes with smaller proteins or is held back on the column according to elution of

standard proteins. Elution of Mn shows some correspondence to elution of agglutinin. The ratio of moles of Mn/120 kDa mol of agglutinin in the total gel filtration fractions was calculated at 1.2, within the range of 1.0–1.7 reported by Jaffe et al. (1977) and Novakova et al. (1981), who also found 4 mol of Ca associated with 1 mol of native soybean agglutinin. The ratio of Mn to agglutinin in the fractions ranged from 0.3 in fractions II and VIII to 1.6 in fraction VI and 5.8 in fraction VII. Other manganoproteins are present in soybeans and other legumes (Yoshida, 1988), but fraction VII also contains bands indicative of modified agglutinin polypeptides. Although all Mn and some Ca in aggregate fractions I–III can be accounted for by direct association with agglutinin, phytic acid is also associated with some of the manganoprotein and appears to be involved in the aggregation of agglutinin or other manganoprotein polypeptides (Yoshida, 1988). The phytic acid and other minerals in these fractions may also be involved in the aggregation of β-conglycinin (Brooks and Morr, 1985). However, while SDS–PAGE and sedimentation analysis suggest the presence of modified agglutinin polypeptides, especially in fraction VII, they also show elution of some agglutinin later than would be expected for a 120-kDa protein or its 32-kDa subunit, indicating interaction with the column. On gel filtration of purified soybean agglutinin under the conditions used for our extract, little aggregation occurred (as indicated by failure to elute early) but more than 60% eluted in fraction VIII or later (unpublished results). We are studying this further. Some soybean Fe–protein may be aggregated with phospholipid (Ellis and Morris, 1981). This is consistent with our finding that most of the P in fractions I and II is not from phytic acid. However, more of the Fe appears to be bound directly to protein rather than through phytic acid, in agreement with Yoshida (1989). At 1 atom of Fe/mol of lipoxygenase (Nielsen, 1985), 10% of the Fe in the extract and 40% of that in fraction VI was associated with lipoxygenase. A larger percentage may also be directly associated with ferritin (Szcekan and Joshi, 1987; Laulhere et al., 1988). At 450–540 kDa/mol, ferritin would be expected to elute in fraction III or IV but would require futher isolation to be identified by SDS–PAGE in our fractions.

This study provides additional information to indicate associations of soybean proteins, particularly agglutinin, with phytic acid and minerals or other soybean components. Further study is needed to learn more of the factors affecting these associations and their relationship to soybean nutrition and functionality.

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Registry No. Ca, 7440-70-2; Mg, 7439-95-4; K, 7440-09-7; Na, 7440-23-5; Fe, 7439-89-6; Mn, 7439-96-5; Cu, 7440-50-8; Zn, 7440-66-6; phytic acid, 83-86-3; lipoxygenase, 9029-60-1; β-amylase, 9000-91-3; Kunitz trypsin inhibitor, 9088-41-9; Bowman–Birk trypsin inhibitor, 37330-34-0.