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Phosphorus and Phytate Content of Soybean Protein Components

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This study was conducted to determine the P and phytate contents of the major soy protein fractions prepared from defatted Bragg soybeans and commercial defatted soy flakes. The 11S, 7S, and soy whey precipitate fractions from defatted Bragg soybeans and commercial defatted soy flakes contained 0.08%, 0.63–0.68%, and 10.49–15.20% P (dry basis), respectively. These same fractions from commercial defatted soy flakes contained 0.07%, 1.40%, and 45.37% phytate (dry basis), respectively. It was concluded that most of the P of 11S protein is non-phytate, whereas phytate accounts for a major portion of the P content of 7S and soy whey precipitate fractions.

Phytate, which is the hexaphosphate salt of myoinositol (Erdman, 1979), is the principal storage form of P in the soybean (Okubo et al., 1975), accounting for about 70% of its total P content. Phytate exists in the soybean in association with various mineral ions and is soluble in mildly alkaline soy extracts but becomes strongly associated with the proteins in the extract, especially at alkaline pH in the presence of divalent cations. Phytate coprecipitates with the proteins in the soy extract under acid conditions, such as those used to produce commercial soy protein isolates, resulting in final phytate concentrations of 2–3% (Brooks and Morr, 1982; Hartman, 1979). The relatively high concentration of phytate in commercial soy protein isolates may affect the bioavailability of the proteins, per se, as well as that of zinc, iron, and other trace minerals in the diet (Erdman, 1979; Cheryan, 1980; Hartman, 1979; Maddaiah et al., 1964; Okubo et al., 1975). Phytate has the potential for adversely affecting the solubility and related functional properties of soy proteins in commercial food product applications. And, finally, phytate, because of its strongly anionic nature and tendency to bind to soy proteins, may interfere with their fractionation and characterization in terms of determining molecular weight, subunit content and size, electrophoretic mobility, and other important physicochemical properties.

Thanh and Shibasaki (1976a) developed an effective procedure for fractionating the major storage soybean proteins, i.e., mainly 7S and 11S components (Damodaran and Kinsella, 1982; Gayler and Sykes, 1981; German et al., 1982; Meinke et al., 1981; Utsumi et al., 1981). However, little if any attention has been given to the possible interference of phytate ions in any of this work.

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The present study was conducted to modify the fractionation procedure of Thanh and Shibasaki (1976a) and to use it to determine the P and phytate contents of the resulting soy protein fractions.

MATERIALS AND METHODS

Soybean Protein Sources. Soybeans (var. Bragg) of the 1982 crop, from the Clemson University Agronomy Department, were finely ground in a Waring blender and defatted with hexane by Soxhlet extraction for 16 h. Commercial defatted soy flakes with high protein solubility were obtained from Ralston Purina Co. (St. Louis, MO).

Protein Fractionation. The major soy protein fractions, i.e., 7S, 11S, soy whey, and soy whey precipitate, were prepared from 10 g of defatted Bragg soybeans and 40 g of commercial defatted soy flakes according to a modified Thanh and Sibasaki (1976a) procedure outlined in Figure 1. Defatted soybeans or commercial defatted soy flakes were extracted with 20 parts (w/v) of dilute Tris-HCl buffer and centrifuged to provide whole soy extract. After completion of the indicated steps for preparing the 7S globulin fraction, the pH was adjusted to 7.8, and it was freeze-dried without dialysis as by Thanh and Shibaski (1976a). The final resolubilized and centrifuged 11S globulin fraction was directly freeze-dried without the overnight refrigerated storage treatment of Thanh and Shibasaki (1976a). All centrifugation treatments were at higher forces of 23700*g* to obtain more complete separation of sediment and supernatant fractions.

Analytical Methods. Protein was determined by the Bio-Rad Coomassie blue dye-binding method (Bio-Rad Laboratories, 1976), with bovine plasma γ-globulin as the reference protein. Total P was determined by the method of Allen (1940), as modified by Brooks and Morr (1982). The method involves perchloric acid digestion of protein samples and spectrophotometric assay of the phospho-

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Table I. Chemical Composition of Soy Protein Fractions[a]

protein fraction	apparent protein content, %	P content, % mean	P content, % SE	phytate content, % mean	phytate content, % SE	P to protein ratio	phytate to protein ratio	phytate to P ratio
defatted Bragg soybeans								
whole soy extract	53.35	0.52[a]	0.001	n.a.		0.0097		
7S component	90.91	0.63[a]	0.013	n.a.		0.0069		
11S component	83.71	0.08[a]	0.001	n.a.		0.0010		
soy whey	14.97	0.29[a]	0.005	n.a.		0.0193		
soy whey precipitate	5.24	10.49[a]	0.085	n.a.		2.0019		
commercial defatted soy flakes								
whole soy extract	78.95	0.60[b]	0.002	1.41[c]	0.010	0.0076	0.0179	2.35
7S component	112.60	0.68[b]	0.005	1.40[c]	0.012	0.0060	0.0124	2.05
11S component	100.80	0.08[b]	0.001	0.07[cd]	0.001	0.0008	0.0007	0.875
soy whey	16.88	0.25[b]	0.003	0.28[cd]	0.008	0.0148	0.0166	1.12
soy whey precipitate	4.49	15.20[b]	0.365	45.37[cd]	0.240	3.3853	10.1046	2.985

[a] Those means within each group followed by the same letter are different ($p < 0.05$). n.a. = not assayed due to insufficient sample. SE = standard error for $N = 3$.

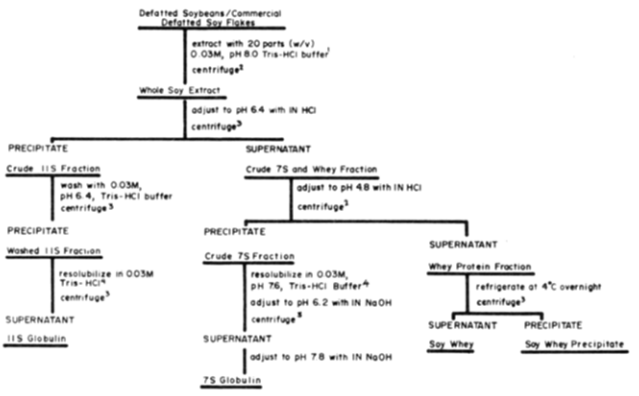

Figure 1. Procedure for fractionating soy protein components. [1]0.03M Tris-HCl–0.001 M dithioerythritol (DTE), pH 8.0, at room temperature for 1 h. [2]23700 *g* for 20 min at 20 °C. [3]23700 *g* for 20 min at 2 °C. [4]Add 1 N NaOH with stirring at pH 7.6 until protein dissolves.

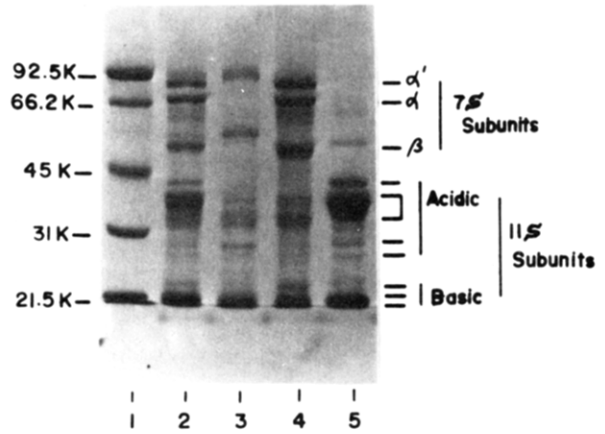

Figure 2. SDS-PAGE of (well 1) Bio-Rad low molecular weight standards (phosphorylase B, 92.5K; bovine serum albumin, 66.2K; ovalbumine, 45K; carbonic anhydrase, 31K; soy trypsin inhibitor, 21.5K), (well 2) whole soy extract, (well 3) soy whey, (well 4) 7S soy globulin, and (well 5) 11S soy globulin.

rus–molybdate complex at 640 nm. Phytate was extracted from protein samples with 1.2% HCl solution and determined by the procedure of Brooks and Morr (1982). Phytate P values were converted to phytate by assuming that it contains 28.2% P.

Gel Electrophoresis. Sodium dodecyl sulfate (SDS)–acrylamide gel electrophoresis was performed in 9% gels using a Bio-Rad Model 220 vertical unit (Bio-Rad Laboratories, Richmond, CA) by the method of Laemmli (1970) with slight modification. From 70 to 100 μg of freeze-dried protein was dissolved in buffer and added to each well. Low molecular weight SDS gel electrophoresis protein standards were purchased from Bio-Rad Laboratories (Richmond, CA). The SDS concentration was increased to 0.2% (w/v) in all solutions, and 0.01 M dithioerythritol (DTE) was used in place of mercaptoethanol. The 1.5 mm thick gels were run at a constant current of 30 mA. They were stained overnight in a solution containing 0.25% (w/v) Coomassie blue G-250 and 8.95% acetic acid and 45.4% methanol (v/v). Gels were destained in a solution containing 7% acetic acid and 5% methanol (v/v) in water.

RESULTS AND DISCUSSION

Comparison of SDS gel electrophoresis patterns of the soy protein fractions' subunits in Figure 2 with those of Gayler and Sykes (1981) confirmed that the modified fractionation procedure provides reasonable separation of the major soy protein components. In contrast to the ultracentrifugal sedimentation data of Thanh and Shibasaki (1976a), these SDS gel electrophoresis patterns clearly indicate minor contamination of the 7S and 11S components. Such contamination would be expected on the basis of pH–solubility properties of these proteins (Thanh and Shibasaki, 1976a). The soy whey precipitate fraction was not examined by SDS gel electrophoresis because it was only partially soluble in the dissociating buffer.

Initial experiments with defatted Bragg soybean protein fractions revealed substantial amounts of P (Table I). The fractionation procedure was therefore scaled up to process 40 g of commerical defatted soy flakes in order to obtain sufficient quantities of the protein fractions to analyze for phytate content.

Apparent protein content values determined by the Coomassie blue dye-binding procedure for the protein fractions are given in Table I. This protein determination method was chosen because it best accommodated the requirements for small sample size and was not subject to interference by Tris buffer. Since soy protein fractions are heterogeneous and contain a number of different subunits (Figure 2), it is impossible to select a reference protein that will duplicate their response to the Coomassie blue reagent. It was therefore necessary to select bovine plasma γ-globulin as the reference protein, as recommended by Bio-Rad Laboratories (1976). Protein values obtained by this procedure are highly reproducible, exhibiting standard errors (SE) of 0.002 and 0.006% among four replicate determinations for 7S and 11S soy protein components, respectively. Although these apparent protein content values are not absolute values, they are, nevertheless, useful for comparing relative protein content values among the

different soy protein components. As would be expected, the most highly purified protein fractions, i.e., 7S and 11S components, contained the highest apparent protein contents. Protein fractions prepared from commerical defatted soy flakes were more readily solubilized during the protein determination, and this factor may account for the slightly higher apparent protein content values for these protein fractions compared to defatted Bragg soybean protein fractions. Additional work is in order to study the protein content and distribution among the different protein fractions by micro-Kjeldahl, biuret, or other protein determination methods.

P content values of the freeze-dried protein fractions ranged from 0.08 to 15.20% (Table I). The 11S component contained the lowest P content of all fractions, whereas the 7S component contained a similar P content as whole soy extract and soy whey. Comparison of P/protein ratios for these protein fractions (Table I) confirms that the 11S component contains the lowest P content and that the soy whey precipitate contains the highest P/protein ratios of 2 to 3.4. A similar relationship was observed for phytate/protein ratios of these fractions, where once again the 11S component contained the lowest phytate content ratio of 0.007 compared to the highest value of about 10 for soy whey precipitate. Examination of the P/phytate ratios (Table I) reveals that all are below the theoretical value of 3.546, assuming that phytate ion contains 28.2% P. Thus, most of the P contained by 11S component is nonphytate, whereas phytate accounts for a major portion of the total P of soy whey precipitate. The P and phytate content values for the above protein fractions from defatted Bragg and commercial defatted soy flakes were separately grouped and statistically analyzed (Table I). P content values were statistically different ($P < 0.05$) for the different fractions within each group, i.e., Bragg and commercial groups. Phytate values were also statistically different for the protein fractions from commercial flakes, except for whole soy extract and 7S component, which were not significantly different ($P < 0.05$).

Smith and Rackis (1957) and Hartman (1979) suggested that removal of phytate from soy proteins might alter their basic physicochemical and functional properties in food products. Studies to date (Thanh and Shibasaki, 1976b, 1978; Damodaran and Kinsella, 1982; German et al., 1982) have ignored this potentially important matter and its impact upon the nomenclature of soy proteins and their subunits. In view of the present findings, it appears that the 11S soy protein component does not bind adequate amounts of phytate ion to make this a major concern with respect to its physicochemical and functional properties. However, further consideration of this matter is required for 7S and the other soy protein fractions, since they bind substantially more phytate ion, which could readily alter their physicochemical and functional properties. Although the soy whey fractions appear interesting in the present study with respect to their high phytate contents, they are not commercially important at this time.

Registry No. Phytate, 83-86-3; phosphorus, 7723-14-0.

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COMMUNICATIONS

Thiamin Odor and Bis(2-methyl-3-furyl) Disulfide

A potent odor fraction, previously isolated as a single packed column GLC peak from the products of the UV irradiation of thiamin hydrochloride (and shown to be predominantly 2,3-(methylenedithio)-2-methyltetrahydrofuran), was resolved further into eight fractions by separation on a Carbowax 20-M Pyrex glass capillary GLC column. Odor threshold determinations of these fractions showed that the most potent was one containing bis(2-methyl-3-furyl) disulfide, a minor component of the packed column peak. This compound (a known flavor compound) was found to be an extremely potent odorant with an odor threshold of 2 parts in 10^{14} parts of water.

The degradation products of thiamin have been studied for many years and earlier studies have been reviewed [cf. Dwivedi and Arnold (1973) and van der Linde et al. (1979)]. Some of us, after studying a number of different ways of degrading thiamin, had isolated a particular packed column gas liquid chromatography (GLC) peak (called the "thiamin odor compound" or the "thiamin odor peak") that had shown the highest odor potency (lowest