J Amer Oil Chem Soc (2007) 84:369–376
DOI 10.1007/s11746-007-1050-8

ORIGINAL PAPER

Fate of Phytic Acid in Producing Soy Protein Ingredients

Nicolas A. Deak · Lawrence A. Johnson

Received: 28 November 2006 / Accepted: 22 February 2007 / Published online: 13 March 2007

Abstract Phytic acid (myo-inositol hexaphosphate) is present in soybeans and soy protein products at 1–2% dry matter. Phytate causes poor absorption of essential electrolytes and minerals, and binds to proteins and co-precipitates with isoelectric soy protein isolates. We determined how phytic acid partitioned during different procedures to prepare soy protein ingredients. Procedure and soybean variety significantly affected phytic acid content and recovery. High-sucrose/low-stachyose (HS/LS) soybeans contained significantly ($P < 0.05$) less phytate than did a typical variety of commodity soybeans (IA2020). In addition, phytate was more readily extracted from the commodity soybeans than from HS/LS soybeans. Among all procedures studied, ethanol-washed soy protein concentrate had the highest phytate contents and yields in the protein products for both soybean varieties (~80 mg/g and 99%, respectively). When protein extraction was carried out at room temperature the protein products had significantly lower phytate yields (60–78%) than when extraction was at 60 °C (80–99%). The protein products obtained from normal soybeans had significantly higher phytate contents than the same products made from HS/LS soybeans. When fractionating soy proteins, the glycinin-rich fraction contained significantly less phytate than the β-conglycinin fraction except for the fractionation procedure performed at room temperature instead of 4 °C.

N. A. Deak · L. A. Johnson (✉)
Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, 1041 Food Sciences Building, Ames, IA 50011-1061, USA
e-mail: ljohnson@iastate.edu

Keywords Phytate · Phytic acid · Soybeans · Soy protein · Soy protein concentrate · Soy protein isolate · Soy protein fractionation

Introduction

Phytic acid (myo-inositol 1,2,3,4,5,6, hexakis dihydrogen phosphate) is present in soybeans and soy protein products in concentrations between 1.0 and 1.5% [1]. At pH values normally encountered in food, phytate is highly negatively charged and complexes or binds to positively charged molecules such as metallic cations and proteins [2]. Phytate binds nutritionally important minerals, such as iron, zinc and calcium, and these mineral-phytate complexes are poorly absorbed [3].

The solubility profile of phytate is quite different in the presence of protein than in the absence of protein. Different mechanisms for the interaction of phytate with protein predominate at different pH regions, <pH 5, pH 5–7, and >pH 7 [2]. At low pH, most proteins have net positive charge while phytic acid is negatively charged, consequently protein-phytic acid interaction is the result of strong electrostatic interaction. In the intermediate pH region, both protein and phytic acid have net negative charges, however, some protein-phytate complexes still form [2]. In the high pH region, multivalent cations, such as calcium, are essential for protein-phytate complexing [4]. Saio et al. [5] found that a single protein molecule may bind many molecules of calcium and phytic acid. The behavior of phytate at alkaline pH is strongly influenced by salt linkages or alkaline-earth ion bridges [2]. This mechanism also explains why phytic acid is soluble in the presence of protein above pH 6, even though phytate salts by themselves are insoluble at alkaline pH [6].

Considerable research has focused on removing phytic acid from soy protein products. Addition of NaCl disrupts alkaline-earth ion bridges to produce phytate-reduced soy protein products [7]. Ford et al. [8] used low pH in combination with $CaCl_2$ to remove 90% of the phytate from soy protein concentrates (SPC). Omosaiye et al. [9] developed an ultrafiltration procedure to eliminate phytic acid from soy protein isolates (SPI) and full-fat SPCs. Kumagai et al. [10] removed phytate by using ion-exchange resins. Saito et al. [11] reported on a method for separating soybean glycinin and β-conglycinin by using phytase and suggested that phytate may affect protein solubility and related functional properties.

Very little is known about the fate of phytic acid during processing of soy protein ingredients. Honing et al. [12] studied the effectiveness of dialysis to remove phytate from several SPIs and soy protein fractions, and suggested that processing conditions affected the formation of phytate-protein complexes.

During previous research [13, 14], we observed that two different soybean varieties, IA2020, a normal commodity soybean line, and a line genetically modified to be high in sucrose and low in stachyose contents (HS/LS) fractionated differently. We hypothesized that phytic acid may play a role in fractionating soybean storage proteins since the myo-inositol metabolism was genetically modified in HS/LS soybeans [15]. The objective of our present study was to determine the fate of phytic acid when producing soy protein ingredients.

Experimental Procedures

Materials

Air-desolventized, hexane-defatted white flakes were prepared from a normal soybean variety, IA2020, and HS/LS soybeans (2 HS Soybeans, Low Stachyose, Lot-980B0001 OPTIMUM, Pioneer a DuPont Company, Johnston, IA, USA) by using a French Oil Mill Machinery Co. extractor-simulator (Piqua, OH, USA) in the pilot plant at the Center for Crops Utilization Research. The flakes were milled with a Krups grinder (Distrito Federat, Mexico) in small quantities (~10 g) to prevent overheating and retain the native protein state while obtaining flour with 100% of the material passing through a 50-mesh screen. The flours were stored in sealed containers at 4 °C until used.

Preparation of New Soy Protein Concentrates (NSPC)

NSPCs were prepared according to the Crank and Kerr patent [15] in which protein was extracted at pH 7.5 and the Johnson patent [16] in which protein was extracted at pH 8.5, and the extracts were neutralized and dried. About 100 g of defatted soy flour was extracted with de-ionized water at 10:1 water-to-flour ratio, the pH was adjusted to 7.5 or 8.5 with 2N NaOH, and the resulting slurry was stirred for 30 min at 60 °C. The slurry was centrifuged at 14,300×*g* for 30 min to obtain a protein extract and an insoluble fiber residue, which was re-extracted with additional de-ionized water at 5:1 water-to-insoluble-fiber ratio, the pH was adjusted as previously described and the resulting slurry was stirred for 30 min. After centrifuging at 14,300×*g* for 30 min, the resulting second protein extract was combined with the first extract, and the insoluble fiber was sampled and discarded. The combined extract was adjusted to pH 7.0 with 2N HCl and freeze-dried. The freeze-dried products were stored in sealed containers at 4 °C until used. These procedures were replicated three times for each flour type.

Preparation of Ethanol-washed Soy Protein Concentrate (EWSPC)

About 100 g of defatted soy flour was extracted with 60% ethanol/40% de-ionized water at 10:1 solvent-to-flour ratio and 40 °C, and the resulting slurry was stirred with a magnetic stirrer for 30 min in a covered container to avoid ethanol evaporation. After centrifuging at 14,300×*g* for 30 min, the protein concentrate was obtained as the residual solids and the extract (supernatant, primarily soluble sugars) was discarded. The resulting protein concentrate was air-desolventized at 25 °C for 24 h, freeze-dried and stored in sealed containers at 4 °C until used.

Preparation of SPI

About 150 g of defatted soy flour was extracted with de-ionized water at 10:1 water-to-flour ratio, the pH was adjusted to 8.5 with 2N NaOH, and the resulting slurry was stirred for 30 min at 60 °C. After centrifuging at 14,300×*g* for 30 min, the insoluble fiber-rich residue was discarded. The protein extract was adjusted to pH 4.5 with 2N HCl and centrifuged as described above. A protein curd was obtained as the precipitate and the supernatant (whey) was discarded. The curd was re-dissolved in de-ionized water, and sufficient 2N NaOH was added to achieve pH 7 with approximately 10% solids content. The resulting slurry was freeze-dried and stored in sealed containers at 4 °C until used.

Preparation of Fractionated Soy Proteins by Using a Modified Nagano (Wu) Procedure

The soy protein fractionation procedure utilized as the control in the present study has been reported by Wu et al.

[17] and was a modification of a procedure first reported by Nagano et al. [18]. About 100 g of defatted soy flour was extracted with de-ionized water at 15:1 water-to-flour ratio, the pH was adjusted to 8.5 with 2N NaOH, and the resulting slurry was stirred for 1 h. After centrifuging at 14,300×*g* and 15 °C for 30 min, the protein extract (1st extract) was decanted, and the amount of insoluble fiber-rich residue was determined and sampled for proximate composition. Sufficient $NaHSO_3$ was added to the resulting protein extract to achieve 10 mM SO_2 concentration and the pH was adjusted to 6.4 with 2N HCl. The slurry was stored at 4 °C for 12–16 h and then centrifuged at 7,500×*g* and 4 °C for 20 min. A glycinin-rich fraction was obtained as the precipitated curd, which was redissolved in de-ionized water, and the pH was adjusted to 7 with 2N NaOH. The fraction was sampled and stored in sealed containers at –80 °C until freeze-dried. To the supernatant (2nd protein extract), sufficient salt was added to obtain 250 mM NaCl, the pH was adjusted 5 with 2N HCl, and the resulting slurry was stirred for 1 h. The slurry was centrifuged at 14,000×*g* and 4 °C for 30 min. An intermediate mixture of glycinin and *β*-conglycinin was obtained as the precipitated curd; this fraction was treated as described above. The supernatant (3rd protein extract) was combined with de-ionized water in the ratio of 2 times the volume (3rd protein extract) and the pH adjusted to 4.8. The resulting slurry was centrifuged at 7,500×*g* and 4 °C for 20 min. A *β*-conglycinin-rich fraction was obtained as the precipitated curd. This fraction was treated as described above, and the amount of supernatant (whey) was determined and sampled for proximate composition. This procedure was replicated twice.

Preparation of Fractionated Soy Protein by Using a New Simplified (Deak) Procedure

We prepared fractionated soy protein using a new simplified procedure developed in our lab and known as the Deak procedure [13]. About 100 g of defatted soy flour was extracted with de-ionized water at 15:1 water-to-flour ratio, the pH was adjusted to 8.5 with 2N NaOH, and the resulting slurry was stirred for 1 h. After centrifuging at 14,300×*g* and 15 °C for 30 min, the protein extract (1st protein extract) was decanted and the amount of insoluble fiber residue was determined and sampled for proximate composition. Sufficient $NaHSO_3$ and $CaCl_2$ to obtain 5 mM concentrations each of SO_2 and Ca^{2+} were added to the protein extract and the pH adjusted to 6.4 with 2N HCl. The resulting slurry was stored at 4 °C for 12–16 h (identified as D4C) in one case, and stirred for 1 h at ~25 °C (DRT) in the other. In both cases, the fractionation procedure was continued by centrifuging the slurry at 14,000×*g* and 4 °C for 30 min. The glycinin-rich fraction was obtained as the precipitated curd. The curd was neutralized and treated as previously described. The supernatant (2nd protein extract) was adjusted to pH 4.8 with HCl, and the slurry was stirred for 1 h and then centrifuged at 14,000×*g* and 4 °C for 30 min. A *β*-conglycinin-rich fraction was obtained as the precipitated curd. This fraction was treated as described above, and the amount of supernatant (whey) was determined and sampled for proximate composition. Both procedures (D4C and DRT) were replicated twice.

Analyses and Mass Balances

Moisture content was determined by oven-drying for 3 h at 130 °C [19]. Quantitative HPLC analyses of phytate contents were done according to the method described by Kwanyuen et al. [20]. Mass balances for dry matter and phytate were determined.

Statistical Analyses

The data were analyzed by Analysis of Variance (ANOVA) and General Linear Model (GLM). Least Significant Differences (LSD) were calculated at $P < 0.05$ to compare treatment means using the SAS system (version 8.2, SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Phytic Acid Contents of Starting Soy Flours

The IA 2020 soy flour contained 25.6 ± 0.5 mg/g phytic acid and the HS/LS soy flour contained 22.1 ± 0.5 mg/g phytic acid on dry weight bases (these contents were statistically different; LSD = 1.8 mg/g, $P < 0.05$). We attributed this difference in phytic acid content (13.7%) to the HS/LS soybeans being modified in myo-inositol metabolism [15]. Unusually high amounts of galactinol (galactopyranosyl-myo-inositol), a precursor in biosynthesis of stachyose, were detected in this flour [20] probably allowing less myo-inositol to enter into phytic acid metabolism.

Phytic Acid in NSPCs

Phytate contents of the products and partitioning when using NSPC procedures are shown in Table 1. For the NSPC extracted at pH 7.5, there were significant differences in phytate contents between the two soybean varieties. IA2020 flour retained significantly less phytate in the spent flakes and, as a consequence, yielded significantly more in the NSPC, compared to NSPC prepared from HS/LS flour.

Table 1 Phytic acid contents and yields of solids and phytic acid in soy protein products ($n = 3$)

Procedure/product	IA 2020			HS/LS			LSD	
	Phytate (mg/g)	Solids yield (%)	Phytate yield (%)	Phytate (mg/g)	Solids yield (%)	Phytate yield (%)	Phytate (mg/g)	Phytate (%)
NSPC, pH 7.5								
Spent flour	22.6 ± 1.0	29.7 ± 0.5	26.2 ± 1.6	28.6 ± 1.0	32.8 ± 0.3	42.5 ± 1.8	2.2*	3.8*
Concentrate	18.0 ± 0.4	70.4 ± 0.3	49.5 ± 1.4	14.6 ± 1.2	67.4 ± 0.3	44.8 ± 3.7	2.0*	6.3
Total		100.1 ± 0.2	75.8 ± 1.3		100.2 ± 0.2	87.3 ± 3.8		6.5*
NSPC, pH 8.5								
Spent Flour	30.4 ± 1.7	28.9 ± 0.7	34.3 ± 2.8	28.7 ± 1.1	30.1 ± 0.7	39.2 ± 2.3	3.3	5.8
Concentrate	15.9 ± 0.3	71.5 ± 1.5	44.3 ± 1.6	14.1 ± 0.4	69.1 ± 1.0	44.1 ± 1.3	0.8*	3.3
Total		100.4 ± 0.9	78.6 ± 1.8		99.2 ± 1.0	83.4 ± 3.6		6.4
SPI								
Spent Flour	27.4 ± 1.2	36.5 ± 0.5	39.1 ± 1.2	28.6 ± 1.0	34.8 ± 0.8	45.1 ± 2.5	2.6	4.4*
Isolate	12.6 ± 0.7	40.7 ± 0.7	20.0 ± 1.0	12.2 ± 1.4	42.4 ± 0.4	23.4 ± 2.9	2.5	4.9
Whey	9.8 ± 0.4	24.4 ± 0.4	9.3 ± 0.5	10.8 ± 0.3	23.4 ± 0.4	11.5 ± 0.3	0.7*	0.8*
Total		101.6 ± 1.0	68.3 ± 0.7		100.6 ± 1.6	80.0 ± 4.9		7.9*
EWSPC								
Concentrate	27.7 ± 0.4	76.1 ± 0.7	82.1 ± 2.1	27.4 ± 0.5	78.4 ± 0.2	97.4 ± 2.1	1.1	4.8*
Extract	15.8 ± 0.3	24.8 ± 0.4	15.3 ± 0.4	3.1 ± 0.2	22.2 ± 0.7	2.1 ± 0.3	1.3*	0.8*
Total		100.8 ± 1.0	97.4 ± 1.8		100.7 ± 0.6	99.5 ± 1.9		3.9

*Denotes significant difference at $P < 0.05$. *HS/LS* high-sucrose/low-stachyose soybeans, *IA2020* a specific line of normal soybeans, *NSPC* new soy protein concentrate prepared by alkali extraction, neutralizing and drying, *pH 7.5* and *8.5* extraction pH for NSPC, *SPI* soy protein isolate, *EWSPC* ethanol-washed soy protein concentrate, *LSD* least significant difference

We attributed this difference (18.9%) to more protein-phytate complex formation at pH 7.5 for the IA2020 flour, since all other variables for both flours were the same. The phytate contents of the NSPCs prepared from both flours were about the same, but the IA2020 yielded NSPC with significantly higher phytate content. The total phytate recovery with this procedure was significantly different for both flours and less than 100%. In both cases, more phytate entered the procedure than was recovered after treatment. We attributed this difference to two mechanisms: (1) to hydrolysis by processing and (2) to phytase activity. There is conflicting evidence in the literature about endogenous phytase activity in soybeans. Although early research failed to show phytase activity in soybeans [2], Selle et al. [22] reported phytase activities ranging from 10 to 95 FTU/kg for 22 different soybean meal samples. We attributed our low phytate recovery to both mechanisms and the differences in phytate recovery between soybean varieties to higher phytase activity in IA2020 soybeans, since all procedure variables were the same.

For the NSPC extracted at pH 8.5, there were no significant differences between soybean varieties in phytate partitioning, but the NSPC obtained from IA2020 soy flour contained slightly more phytate (11.3%). This difference was consistent with differences in phytate contents of the starting flours. There were no differences in phytate contents and partitioning for NSPC extracted at pH 8.5 and NSPC extracted at pH 7.5 when using HS/LS soy flour. Significant differences occurred, however, when IA2020 flour was used. Significantly more (LSD = 3.1 mg/g) phytate remained in the spent flour when extracted at pH 8.5 and, as a consequence, this procedure produced NSPC with significantly (LSD = 1.0 mg/g) less phytate content. Phytate partitioning followed the same pattern. These differences may have been due to differences in the strength of the phytate-protein complex formation at these pHs. The protein of the NSPC extracted at pH 7.5 seemed to have higher affinity for phytate than did protein in NSPC extracted at pH 8.5. In addition, phytic acid is less soluble at higher pHs [2], which could account for the difference in phytate extractability. This phenomenon was not observed for HS/LS soybeans and we hypothesize that the phytate was complexed to protein differently in HS/LS flour.

Phytic Acid in EWSPC

EWSPC had the highest phytate contents among all products produced (Table 1). There were no differences in

phytate contents between EWSPCs prepared from IA2020 and HS/LS flours, however, significantly higher phytate yields were observed in the EWSPC prepared from HS/LS flour. The total phytate recovery in this procedure was the highest among all procedures tested and almost all of the initial phytic acid was accounted for. Phytase activity was probably inhibited by ethanol. The differences in phytate partitioning suggested that phytic acid was complexed in IA2020 differently than it was in HS/LS soy flour.

Phytic Acid in SPI

There were no significant differences in phytate contents between the soybean varieties for spent flour and SPI (Table 1); however, differences were observed in phytate partitioning. The phytate yields of the spent flour and whey fractions were significantly higher when using the HS/LS soy flour. Significant differences were also observed in total phytate recovery when producing SPI. The recovery of phytate when using HS/LS soy flour was 11.7% higher, suggesting that IA2020 flour had more phytase activity. Lower total recoveries for SPI also suggested that there may have been some acid hydrolysis of phytic acid. When comparing SPI to the NSPC extracted at pH 8.5, two major differences must be noted. One difference was that two extraction steps were used in the NSPC procedure, but the higher dilution did not extract significantly more phytate; a second difference was the acid precipitation during SPI production that would account for the reduced phytate recovery. Phytic acid is soluble at the pH of SPI precipitation [2], yet significant amounts of phytate were found in SPI. This phenomenon can be explained by the protein-phytate complex formation at pH <5.0 and, consequently, co-precipitation.

Phytic Acid Partitioning in Soy Protein Fractionation

Phytate contents and yields for three different soy protein fractionation procedures were determined using both normal (IA2020) and HS/LS flours (Table 2). There were significant differences among procedures and among soybean varieties for both phytic acid content and partitioning. All three procedures started the same way, using 15:1 water-to-flour ratio at pH 8.5 and room temperature. This extraction procedure was significantly more efficient in extracting phytate from IA2020 soy flour (77.2 ± 3.5% phytate extracted) than for HS/LS soy four (53.9 ± 2.1% phytate extracted). Consequently, not only did the IA 2020 soy flour contain more phytate, but the phytate was more easily extracted, yielding a 1st protein extract, which was the starting point for all fractionation procedures, with significantly higher phytic acid content. There were no significant differences among the phytate contents of the spent flours for all fractionation procedures when using HS/LS soybean variety. While the HS/LS spent flours had similar phytate contents to the other procedures (NSPC and SPI), the IA2020 flour gave spent flours with less phytate than did NSPC extracted at pH 8.5 and SPI. For IA2020, extraction temperature probably influenced the efficiency of phytate extraction, since the fractionation procedures extracted protein at room temperature while the other two procedures extracted protein at 60 °C.

Wu fractionation procedure. When using the Wu fractionation procedure, 50 and 40% of the phytate originally present in IA2020 and HS/LS flours were lost, respectively. We attributed this difference in phytate loss between soy varieties to differences in phytase activity in the soy flours. The Wu fractionation procedure also yielded the lowest amount of total phytate for both varieties. The Wu fractionation procedure produces three fractions: a glycinin-rich, a β-conglycinin-rich and an intermediate fraction (mixture of the two proteins). These products had the lowest phytate contents and yields among all products studied despite this procedure starting with the highest phytate content. The glycinin-rich fraction had the lowest phytate content among the three fractions, followed by the intermediate fraction, and then the β-conglycinin-rich fraction. This trend was observed for both soybean varieties.

The low phytate content of the glycinin-rich fraction was probably due to the pH at which this fraction was precipitated (pH 6.4). This observation is in accordance with data reported by Okubo et al. [4] where no specific binding occurred between phytic acid and glycinin in the pH range of 6.0 to 10.0. Furthermore, this fraction was precipitated in the intermediate pH range of phytate-protein complex formation [2]. In addition, the Wu fractionation procedure used sulfites as reducing agents, which alters protein structure [23], and, as a consequence, probably altered phytate binding specificity.

During precipitation of the intermediate fraction, NaCl was added to achieve 0.25 M concentration. The intermediate fraction was precipitated at pH 5.0, which is between the low and intermediate pH ranges for phytate binding to protein [2]. Phytic acid is not as tightly bound to the protein as it is at lower pHs [2]. In addition, DeRham et al. [7] reported that adding NaCl to protein extracts could disrupt the alkaline-earth ion bridges yielding proteins low in phytic acid. During the Wu fractionation procedure, this last mechanism is likely.

Higher phytate contents were found in the β-conglycinin-rich fraction than the two previously discussed fractions. This trend was observed for both soybean varieties and was significant (LSD = 0.9 mg/g and 1.1 mg/g for IA2020 and HS/LS soybeans, respectively, at

Table 2 Phytic acid contents and yields of solids and phytic acid in fractionated soy protein products ($n = 2$)

Procedure/product	IA 2020			HS/LS			LSD	
	Phytate (mg/g)	Solids yield (%)	Phytate yield (%)	Phytate (mg/g)	Solids yield (%)	Phytate yield (%)	Phytate (mg/g)	Phytate (%)
Wu								
Spent Flour	17.1 ± 0.1	27.5 ± 0.7	18.4 ± 0.4	31.1 ± 2.6	25.0 ± 0.2	35.1 ± 2.7	7.8*	8.2*
Glycinin	2.9 ± 0.1	11.1 ± 1.2	1.3 ± 0.2	1.2 ± 0.1	15.4 ± 0.6	0.8 ± 0.1	0.5*	0.5
Intermediate	3.2 ± 0.0	17.3 ± 0.4	2.2 ± 0.0	1.7 ± 0.2	8.8 ± 0.3	0.7 ± 0.1	0.7*	0.4*
β-Conglycinin	10.3 ± 0.2	10.7 ± 0.3	4.3 ± 0.1	7.7 ± 0.1	10.5 ± 0.2	3.7 ± 0.0	0.5*	0.2*
Whey	18.4 ± 0.3	36.4 ± 0.7	26.1 ± 0.1	11.2 ± 0.3	40.6 ± 0.9	20.6 ± 1.0	1.4*	3.1*
Total		103.0 ± 0.6	52.2 ± 0.2		100.3 ± 0.8	60.9 ± 3.5		7.4*
D4C								
Spent Flour	21.9 ± 0.9	30.2 ± 0.3	25.9 ± 0.8	30.1 ± 1.3	35.5 ± 0.3	48.5 ± 1.6	4.7*	5.4*
Glycinin	10.9 ± 0.4	15.5 ± 0.7	6.6 ± 0.5	7.7 ± 0.1	18.0 ± 0.0	6.3 ± 0.1	1.1*	1.5
β-Conglycinin	14.5 ± 0.1	23.1 ± 0.5	13.0 ± 0.4	10.1 ± 0.3	20.5 ± 0.2	9.3 ± 0.2	1.0*	1.3*
Whey	24.4 ± 3.4	31.2 ± 0.4	29.7 ± 3.7	10.7 ± 1.4	26.0 ± 0.5	12.5 ± 1.4	11.2*	12.2*
Total		100.0 ± 0.0	75.2 ± 3.1		100.0 ± 1.0	76.6 ± 2.9		9.3
DRT								
Spent Flour	22.3 ± 1.6	30.8 ± 0.4	26.8 ± 2.3	31.1 ± 2.6	37.8 ± 0.5	53.2 ± 5.1	5.8*	17.0*
Glycinin	18.9 ± 0.3	15.7 ± 1.6	11.6 ± 1.0	9.6 ± 0.1	14.3 ± 0.8	6.2 ± 0.3	1.1*	3.1*
β-Conglycinin	12.0 ± 0.1	23.3 ± 0.8	10.9 ± 0.5	8.1 ± 0.6	22.2 ± 0.6	8.1 ± 0.8	1.8*	2.8*
Whey	10.4 ± 0.5	30.1 ± 0.4	12.2 ± 0.8	9.1 ± 2.0	25.7 ± 0.7	10.6 ± 2.6	6.2	8.2
Total		99.9 ± 0.9	61.6 ± 2.6		100.1 ± 1.1	78.3 ± 2.0		9.9*

*Denotes significant difference at $P < 0.05$. *HS/LS* high-sucrose/low-stachyose soybeans, *IA2020* a specific line of normal soybeans, *Wu* fractions produced with the Wu procedure, *D4C* fractions produced with the Deak fractionation procedure with chilling, *DRT* fractions produced with the Deak fractionation procedure without chilling, *glycinin* glycinin-rich fraction, *β-conglycinin* β-conglycinin-rich fraction, *LSD* least significant difference

$P < 0.05$). When comparing the phytate contents of this fraction for the two soybean varieties, there was a big difference. The β-conglycinin-rich fraction produced from IA2020 soy flour had 25% more phytate; however, the difference in phytate yields was lower (13.9%). In both cases, these differences were significant (Table 2). The higher phytate contents of these fractions were probably due to this fraction being precipitated at pH 4.8 after twofold addition of de-ionized water diluted the NaCl concentration to 1/3 of that of when the intermediate fraction was precipitated. This pH was in the region where phytic acid is tightly bound to protein, since phytate has a negative net charge and protein has a net positive charge [2].

Deak soy protein fractionation procedure. In previous studies we developed a new soy protein fractionation procedure [24] and reported compositional and functional characteristics of the fractions obtained for IA2020 soy flour [13] and HS/LS soy flour [14]. The procedure was based on differences in calcium binding to glycinin and β-conglycinin in the presence of a reducing agent. The two soybean varieties fractionated differently when using this procedure, producing enriched protein fractions with higher purities when using IA2020 soy flour compared to HS/LS soy flour [13, 14].

Preferential calcium binding to glycinin and consequential precipitation of this fraction has been widely reported in the literature [25–27] and calcium binding to phytic acid and protein have already been discussed. Graf [28] reported that calcium binding to phytic acid is temperature, pH, and ionic strength dependent. He found that calcium affinity for phytate increased with increasing temperature and 2 mM concentration of free Ca^{2+} ions was critical for phytate precipitation. Cheryan [2] reported that an excess of Ca^{2+} ions displaced the phytate-protein complex. The different behaviors of the two soybean varieties in fractionation behavior in the presence of Ca^{2+} may have been due to differences in phytate contents. IA2020 soy flour had higher phytate content and the phytate extracted more readily than did the phytate of HS/LS soy flour. As a consequence, the amounts of phytate present in the extracts prepared from IA2020 soy flour were greater than those of the extracts from HS/LS soy flour.

In the Deak procedure, we added an excess of Ca^{2+} (5 mM), which worked better for IA2020 soy protein

extracts than for extracts prepared from HS/LS soybeans. We have also found that mM amounts of reducing agent were necessary to increase protein purities of the fractions [13]. For the IA2020 variety, the total phytate yield for the D4C procedure was similar to the previous procedures (NSPC and SPI) and significantly higher than the total recovery in the Wu procedure. This was probably due to the extract being held at 4 °C, which minimized enzyme activity. Thus, there were no significant differences in total phytate recovery between soybean varieties for the D4C procedure.

The phytate content of the glycinin-rich fraction produced by using this procedure was significantly higher than that of the same fraction produced by the Wu procedure. These increased content and yield of phytate were probably due to insolubilization of phytate by calcium and co-precipitation of the phytate salt with the glycinin-rich fraction. The lower phytic acid content of the fraction obtained from HS/LS soybeans indicated that the amount of free Ca^{2+} ions available to specifically bind to the protein was higher compared to the same procedure using IA2020 soy flour. This finding probably explains why HS/LS soy flour produced fractions with lower purities [14], because if there are more free Ca^{2+} ions available to bind to prote\in at constant pH, there will be higher co-precipitation of glycinin and β-conglycinin.

The β-conglycinin-rich fraction had higher phytic acid content than did the glycinin-rich fraction. These differences, however, were proportionally less significant than when using the Wu procedure, where the β-conglycinin-rich fraction yielded between three and four times as much phytate as did the glycinin-rich fraction. The β-conglycinin-rich fraction from the D4C procedure had higher phytate content than did the same fraction from the Wu procedure. Since this fraction was precipitated at pH 4.8, phytate was tightly bound to the protein and the amounts of calcium remaining in the system might account for the increased phytate.

For the Deak fractionation procedure at 25 °C, the only variable that changed was extraction temperature. This treatment involved 1 h stirring at room temperature that would explain the lower total phytate yields for the IA2020 flour due to endogenous phytase activity. The calcium affinity for phytate increases at higher temperature, which explains why more phytate was precipitated in the glycinin-rich fraction than did the same fraction of the D4C procedure. The phytate contents of the β-conglycinin-rich fractions were approximately the same for both procedures, probably because the calcium binding to β-conglycinin was not influenced by temperature or calcium concentration, since similar amounts were also bound to this fraction when using the Wu procedure.

Acknowledgment This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Iowa State University, Ames, IA, Project N. 6571, was supported in part by USDA NRI grant No. 2001-35503-10814, the Center for Crops Utilization Research, Hatch Act and State of Iowa funds.

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