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Phytate Removal from Soybean Proteins

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ABSTRACT

The ability of Ca++, Ba++ and Zn++ ions to complex with and precipitate phytate ions was investigated as a possible approach for producing phytate-reduced soy-protein isolates. The treatments involved adding divalent cation reagents to mildly alkaline soy-protein extracts, or using mildly alkaline solutions of the cation reagent as protein extractant. Treatments used up to 5% (w/v) divalent cation reagent in dilute NaOH at pH 8-9 at room temperature with stirring for 1 hr. The proteins that remained soluble, or that were extracted following the above treatments, were further fractionated by dialysis against distilled water and then adjusted to the isoelectric pH of 4.5 with HCl. All treatments generally precipitated 15-90% of the total extracted proteins. The resulting phytate-reduced fractions had their total P reduced by ca. 30-74%, but their yields were reduced to only 11-85% of the total extract proteins. On the basis of these findings and on other adverse factors, e.g., toxicity, possible off-flavor and reduced functionality from residual precipitant ions, the divalent cation-fractionation method is not recommended for producing phytate-reduced soy-protein isolates for food use.

INTRODUCTION

Phytate, the hexaphosphate salt of myo-inositol (1), is the principal storage form of phosphorus (P) in the soybean (2). Hydrogenated and monovalent salts of phytic acid are water soluble, whereas its divalent and trivalent metal salts are quite insoluble (3). For example, in the presence of magnesium ion, phytate precipitates at pH values of 5.2 or greater, and in the presence of the more active calcium ions, precipitation occurs at pH values of 4.2 or greater, where phytate exists as its tetrahydrogenated, octa-anion form (4). However, in the presence of soy protein, calcium-magnesium phytate remains soluble above pH 10, presumably because of complex formation with the proteins, but coprecipitates with the protein at acid pH values (5,6).

Coprecipitation and recovery of proteins and phytate during commercial preparation of soy-protein isolates results in a final product containing ca. 2-3% phytate (2,5). This relatively high concentration of phytate has led to concerns regarding the bioavailability of soy proteins, per se, as well as zinc, iron and other trace minerals in diets containing soy protein (1,2,6,7). A clear indication of the stability of these soy-protein–mineral–phytate complexes is demonstrated by the relative inability of various workers to remove more than 70-80% of the phytate from soy-protein extracts (2,6,8-11). A recent procedure using a sequential, 2-stage cation and anion exchange treatment, was developed to remove 95% or more of the phytate from soy-protein extracts (5).

McKinney et al. (12) reported on efforts to selectively precipitate and remove phytate from soy-protein extracts by adding $Ca(OH)_2$ and $Ba(OH)_2$ at concentrations of 0.1-1.0% and at pH 8.4 and 9.2. Because protein–mineral–phytate complexes are presumed to be associated mainly through ionic bonding, dissociating the above complexes should be possible by adding excess divalent cations. This paper describes several experiments conducted to investigate the possibility of using this approach for precipitating phytate from aqueous soy-protein extracts and for extracting soy proteins from defatted flakes to prepare soy-protein isolates with low phytate content.

MATERIALS AND METHODS

Low temperature, hexane-extracted soy flakes with high protein solubility were obtained from Ralston Purina Company (St. Louis, MO). The hollow fiber ultrafiltration (UF) unit was an Amicon Model DC-2 unit equipped with a H1P10-8 hollow fiber cartridge with a 10,000 MW cutoff. Dialysis tubing was Spectrapore No. 1, with a 32 mm flat diameter and a 6,000-8,000 MW cutoff, from Fisher Scientific, Fairlawn, NJ. All chemicals were purchased from Fisher Scientific except 2,4-diaminophenol dihydrochloride, which was purchased from Eastman Kodak Company, Rochester, NY. Demineralized, distilled water was prepared by passing glass-distilled water through a Barnstead Model BD-2 demineralizer.

All centrifugation treatments were performed at 25,860 × *g* for 20 min at 20 C and dialysis was against demineralized, distilled water for 18 hr at 0-5 C. Soy extracts and their fractions were freeze-dried before analysis.

Protein determination was by the micro-Kjeldahl procedure (13) following previously described modifications (5) and using a conversion factor of 5.70 (14). Total phosphorus (P) was determined by the method of Allen (15). The molybdenum blue complex was assayed at 640 nm in a Coleman Jr. II Spectrophotometer (5).

Details of the procedure used to prepare and treat soy-protein extracts with $CaCl_2$, $BaCl_2$ and Zn acetate are presented in Figures 1-3. In Trial 1, aliquots of soy-protein extract were treated with 5% (w/v) solutions of each of the above reagents and stirred for 1 hr at room temperature (20-25 C). The treated extracts were then centrifuged to remove the precipitate (cation-insoluble fraction) and the supernatants were concentrated 4:1 by hollow fiber UF and dialyzed against distilled water for 18 hr at 0-5 C. Dialyzed supernatants were centrifuged to remove the precipitate that had formed during dialysis (dialysis-insoluble fraction) and the resulting supernatant was then acidified to pH 4.5 with HCl and recentrifuged to recover the precipitate (dialysis-soluble fraction). In addition, an aliquot of the the untreated extract was concentrated 4:1 by hollow

fiber UF, dialyzed against distilled water and freeze-dried as above for treated fractions. A second procedure (Trial 2) was employed where defatted soy flakes were extracted for 1 hr with a dilute NaOH solution at pH 8-9 containing 5% (w/v) $BaCl_2$ (Figure 2). The solubilized protion was recovered by centrifugation, concentrated 4:1 by hollow fiber UF and dialyzed against distilled water as above. Following dialysis, the fraction was centrifuged to recover the dialysis insoluble fraction. Varying amounts of dry $BaCl_2$ were added to aliquots of alkaline soy-protein extract (Figure 3) to provide a final $BaCl_2$ concentration of 0-5% (w/v) in Trial 3. After centrifugation, the resulting supernatant fractions were dialyzed against distilled water as above, acidified to pH 4.5 and recentrifuged to recover the combined cation-soluble fraction.

RESULTS AND DISCUSSION

Because many of the resulting soy-protein fractions contained extremely low concentrations of phytate, we decided to use total P as an indicator of their phytate content. We felt this was a reasonable approach because only

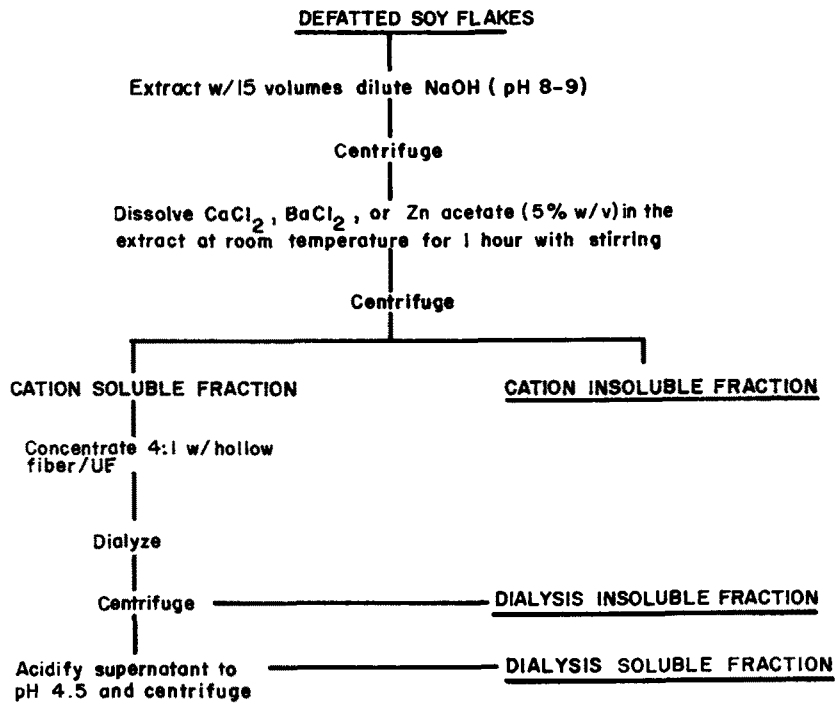

FIG. 1. Procedure for producing divalent cation-treated soy-protein extracts (Trial 1). All centrifugation steps were done at 25,860 × *g* for 20 min at 20 C. Dialysis was done for 18 hr at 0-5 C against 4 L distilled water.

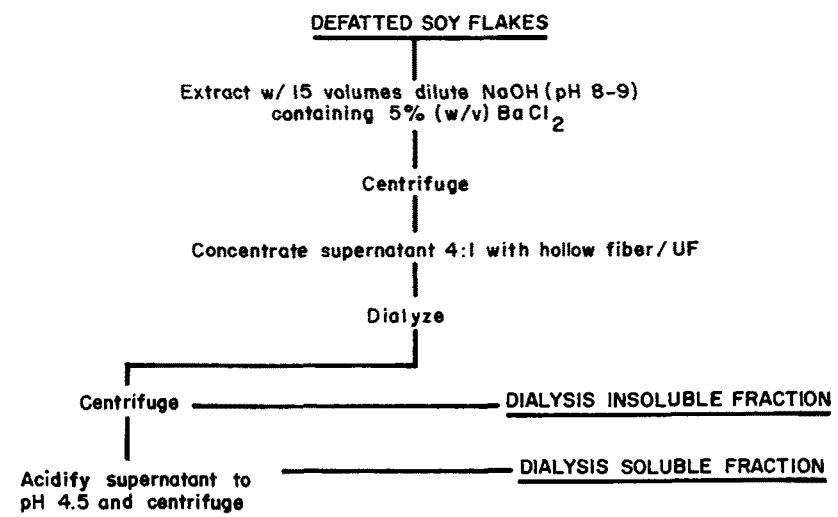

FIG. 2. Procedure for producing divalent cation-extracted soy-protein extracts (Trial 2). All centrifugation steps were done at 25,860 × *g* for 20 min at 20 C. Dialysis was done for 18 hr at 0-5 C against 4 L distilled water.

small quantities of these protein fractions were obtained, and also because phytate accounts for ca. 70% of the total P content of the soybean (2).

In Trial 1, $CaCl_2$, $BaCl_2$ and Zn acetate reagents were added in dry form to the alkaline soy extract to bring their final concentration to 5% (w/v). The length of the treatment was shortened from that of McKinney et al. (12) because the reaction was completed in less than 1 hr. McKinney et al. (12) used 1% $Ba(OH)_2$ and 0.1% $Ca(OH)_2$ solutions and allowed a total reaction time of 2-17 hr.

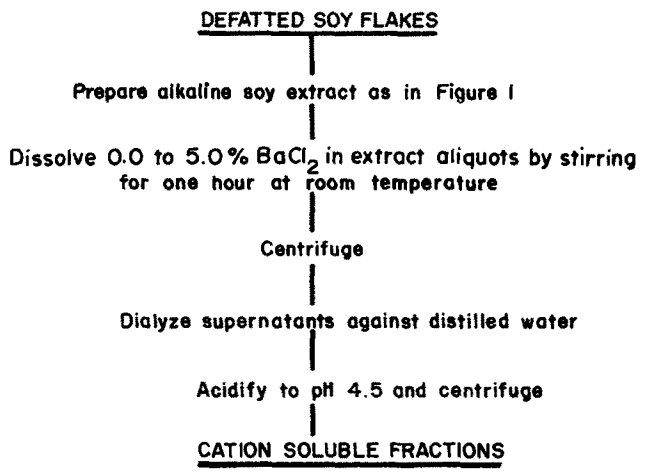

FIG. 3. Procedure for producing 0.0-5.0% $BaCl_2$-extracted soy proteins (Trial 3). The final fractions obtained are equivalent to a combination of dialysis-soluble and dialysis-insoluble fractions in Figures 1 and 2. All centrifugation steps were done at 25,860 × *g* for 20 min at 20 C. Dialysis was done for 18 hr at 0-5 C against 4 L distilled water.

As shown in Table I, the above treatments resulted in extensive precipitation of both protein and P. For example, $BaCl_2$ precipitated ca. 26% of the total protein and 89% of the P in the cation-insoluble fraction in Trial 1. $CaCl_2$ precipitated only ca. 14% of the protein and 64% of the P. Zn acetate caused an even more drastic protein precipitation (89%) and P precipitation (98.5%). Thus, these treatments are capable of precipitating P from soy-protein extracts, but only at the expense of unacceptable losses of the proteins as well. Even though the treatment results in low P content protein fractions, e.g., the dialysis-soluble fraction, their extremely low yields (1-10%) makes this process of little practical value. These findings were in general agreement with those of McKinney et al. (12) for P removal from soy proteins, e.g., 1% $Ba(OH)_2$ removed 89% and 0.1% $Ca(OH)_2$ removed 97% of the P from soy-protein extracts. However, McKinney et al. (12) did not mention problems with protein coprecipitation like those encountered in the present study. The protein precipitations experienced in this study are not caused by shifts in pH toward the protein's isoelectric point as we found that the pH of the reaction systems were 6.2, 6.9 and 7.2 for Zn acetate, $CaCl_2$ and $BaCl_2$.

Interactions between Group IIA cations (Ca++ and Ba++) and proteins are probably of the ionic type ("s" orbital interactions), whereas those involving Zn++ ions and proteins may result from complex ion formation with free amino groups on the proteins, e.g., N-terminal or ε-amino groups, through hybridization of the outer shell (4th shell) of Zn++ as either sp^3 or sp^3d^2 (16). This latter interaction would result in the formation of insoluble aggregates with enhanced stability, because the lone electron pairs of the amino group nitrogen could fill the hybridized outer shell of Zn++, thus making the resulting interactions somewhat covalent in character.

TABLE I

Fractionation of Soy Proteins with Divalent Cations

Extract/protein fraction[a]	Fraction yield (g/L)	Protein content (%)	Protein recovery (%)	Total P content (%)	Total P recovery (%)
Trial 1					
Untreated, contentrated, dialyzed extract	16.2	86.33	100	0.704	100
5% $BaCl_2$-treated extract					
Cation insoluble fraction	8.2	47.94	26.5	2.06	88.6
Dialysis insoluble fraction	10.9	88.72	65.1	0.194	11.0
Dialysis soluble fraction	1.6	80.54	8.4	0.0378	0.3
Total	20.7		100		99.9
5% $CaCl_2$-treated extract					
Cation insoluble fraction	4.3	43.94	14.4	2.53	63.5
Dialysis insoluble fraction	12.7	86.94	84.4	0.492	36.4
Dialysis soluble fraction	0.2	74.87	1.1	0.0682	0.1
Total	17.2		99.9		100
5% Zn Acetate-treated extract					
Cation insoluble fraction	24.6	70.28	88.8	0.957	98.5
Dialysis insoluble fraction	2.5	87.39	11.1	0.143	1.5
Dialysis soluble fraction	–	–	–	–	–
Total	27.1		99.9		100
Trial 2					
5% $BaCl_2$ extract					
Cation insoluble fraction	–	–	–	–	–
Dialysis insoluble fraction	7:6	98.76	89.1	0.180	97.9
Dialysis soluble fraction	1.0	83.54	10.9	0.0284	2.1
Total	8.6		100		100

[a]All fractions were freeze-dried before analysis. See Figures 1 and 2 for details of the procedures.

TABLE II

Effect of $BaCl_2$ On Yield and Composition of Cation Soluble Soy Protein Fraction

Extract fraction	Fraction yield (g/L)	Protein content (%)	Protein yield (g/L)	Total P content (%)	Total P yield (%)
Untreated	17.0	84.90	14.3	0.587	99.8
0.1% $BaCl_2$ treated	16.0	84.24	13.3	0.541	86.6
0.2% $BaCl_2$ treated	12.7	82.98	10.7	0.503	63.7
1.0% $BaCl_2$ treated	3.3	82.91	2.7	0.661	2.2
3.0% $BaCl_2$ treated	11.3	86.32	9.7	0.145	16.4
5.0% $BaCl_2$ treated	12.0	83.39	10.0	0.260	31.2

[a]All fractions were freeze-dried before analysis. See Figure 3 for details of procedure.

Trial 2 was conducted by extracting defatted soy flakes with a dilute NaOH (pH 8-9) solution containing 5% $BaCl_2$ (w/v) as in Figure 2. We speculated that the Ba++ ions might complex with and precipitate the phytate ions before they had an opportunity to dissolve and complex with the protein, thus providing more effective phytate removal. Ba++ ions were chosen as the phytate precipitant because it was shown above to be most effective for preferentially precipitating phytate in Trial 1. The results in Table I indicate that the alkaline Ba++ ion extraction resulted in dialysis insoluble and dialysis soluble fractions with a slightly higher protein and lower P content than was obtained by the $BaCl_2$ treatment in Trial 1. However, this latter approach resulted in much lower fraction yields (8.6 g/L) than those in Trial 1 (20.7 g/L).

Trial 3 was conducted to establish optimum $BaCl_2$ concentrations that would provide high-yield protein but low-yield P fractions. Results in Table II and Figure 4 show that both protein and P yields are minimal at 1% $BaCl_2$ treatment. Combining this information with the observation that trends in fraction yield and protein yield both closely follow total P content of these fractions as a function of $BaCl_2$ concentration, attempts to optimize this process for producing soy-protein isolates with high yield and low P content would probably meet with limited success.

Typical P content values for soy-protein isolates produced by the combination cation-anion exchange process were in the range of 0.094% with a protein content of 86.5% (5). By comparison, dialysis-insoluble fractions provided in the present study contained 0.180-0.492% P and 86.9-88.7% protein (Table I). Furthermore, whereas essentially complete recovery of the soy proteins is achieved by the ion-exchange process, only ca. 73-85% of the proteins are recovered in the cation soluble fraction (sum of dialysis-insoluble and dialysis-soluble fractions) from Ba++- and Ca++- treated soy extracts (Table I). In addition, the cation precipitation treatments require a number of time-consuming and rather tedious steps, e.g., centrifugation, dialysis and ultrafiltration, and also possess the serious problem of high residual precipitant ions, which might be undesirable from the standpoint of toxicity and functionality.

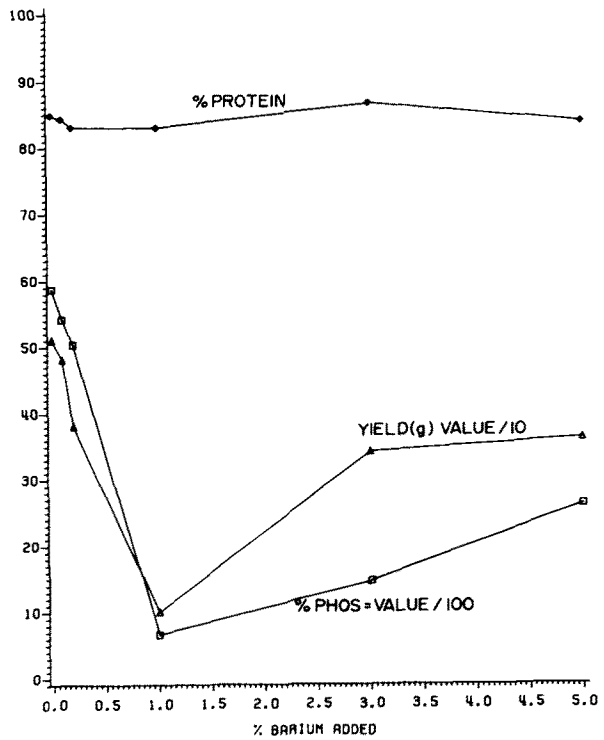

FIG. 4. Influence of $BaCl_2$ concentration on protein and P yields.

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