

A MODIFIED METHOD FOR PHYTATE ANALYSIS USING AN ION-EXCHANGE PROCEDURE: APPLICATION TO TEXTURED VEGETABLE PROTEINS

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ABSTRACT

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A modified method for phytate analysis has been developed which involves concentrating phytate on an anion-exchange resin, stripping the resin of contaminating inorganic phosphate with 0.05*M* NaCl, and eluting the phytate with 0.7*M* NaCl. The final eluate is then digested, inorganic phosphate is measured, and the hexaphosphate equivalent

is calculated from the hydrolyzed phosphate. The method is simple, reproducible, and rapid. The concentration of phytate in 10 soybean-based meat substitutes ranged from 0.12 to 1.63% when the products were analyzed by the ion-exchange method; all but three sources contained phytate in excess of 1%.

Textured vegetable proteins, primarily of soybean origin, are increasingly being used as human foods as a result of developments in food technology and an expanding need for sources of protein. The protein in these products is less expensive and can be prepared with a higher yield per unit of land than meat from animals or fowl. The use of plant food as protein frequently is promoted without considering the total nutrient adequacy of the substituted food, the effect the components of such foodstuffs may have on other dietary nutrients (1,2,3), or the effect other nutrients may have on the utilization of the protein in the substituted food (4,5).

Plant seeds such as the soybean contain the anion phytate, myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (6). Phytate has been shown to bind zinc and other minerals, and concomitantly, zinc and other minerals have been demonstrated to improve the utilization of protein (4). Because meat is currently a valuable source of available zinc and other trace elements, it is important to determine the phytate content of plant protein being used as meat substitutes and to assess the effect of this phytate on the biological availability of zinc and other minerals.

Many methods for measuring phytate involve the following basic steps: 1) extraction of the dried, finely ground food with hydrochloric acid or trichloroacetic acid, 2) precipitation of phytate with ferric ion, 3) centrifugation and washing of the precipitate, 4) wet or dry ashing of the precipitate, 5) colorimetric determination of the phosphorus in the ashed precipitate, and 6) calculation of phytate equivalents from the phosphorus analyses (7,8).

In many indirect methods for the analysis of phytate, iron is determined in the supernatant fluid from a measured volume of standardized iron solution following phytate precipitation (7). Iron may also be determined in the washed precipitate, provided the precipitate has been hydrolyzed. Hydrolysis is necessary in the latter instance because ferric phytate is not completely soluble in concentrated sulfuric or nitric acid.

This paper reports a modified method for the determination of phytate using

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an ion-exchange column prior to digestion and colorimetric measurement of the phosphate.

MATERIALS AND METHODS

Samples of dried material (5 g) were extracted for 2 hr at room temperature with 100 ml of 1.2% HCl or with 1.2% HCl containing 10% sodium sulfate if the extracts were to be precipitated with iron. The extracts were then vacuum-filtered. To avoid hydrolysis from mold or bacterial enzymes, the filtrate was refrigerated for no more than 1 week before use (9,10). The methods (9,10) were used as a basis for comparing the ion-exchange method.

In the ion-exchange method, glass barrel Econo-columns (Biorad Laboratories, Richmond, Calif., 0.7×15 cm) equipped with a 3-way Luer fit stopcock (Propper Manufacturing Co., W. Germany) were clamped vertically and packed with about 0.5 g of 200–400 mesh AGI-X8 chloride form anion-exchange resin (Biorad Laboratories, Richmond, Calif.). Before use, the columns were washed first with 0.7M NaCl to assure chloride saturation of the resin and then with distilled water until the eluate was salt-free. The sample extract was diluted before being placed on the column so that the total anion concentration was less than 0.05M (about 1:25). The sample container was rinsed and the column was washed with 15 ml of distilled water to elute most of the inorganic phosphate. The remaining inorganic phosphate was eluted with 15 ml of 0.05M NaCl.

The phytate was then eluted from the column with 15 ml of 0.7M NaCl and collected in a micro-Kjeldahl flask or digestion tube. To the digestion vessel was added 0.5 ml of concentrated H_2SO_4 , 3.0 ml of concentrated HNO_3 , and a few boiling chips. Digestion was continued until only the H_2SO_4 remained (25–30 min). The end point had been reached when a white cloud of vapors separated from and remained suspended above the H_2SO_4 in the digestion vessel for 5 min. (Digestion timing is critical because incompletely digested products will not react with color reagents and overdigestion will cause sublimation of the phosphorus

TABLE I
Comparison of Three Methods for Determining Phytate using
Sodium Phytate as a Standard Material (1 mg/ml)^a

Quantity Tested ml	Direct Digestion Phosphorus Assay Phytate mg	Iron Precipitation Phosphorus Assay		Ion-Exchange Phosphorus Assay	
		Phytate mg	Recovery %	Phytate mg	Recovery %
1	0.42 ± 0.0	0.43 ± 0.0	101.8	0.43 ± 0.0	100.4
2	0.81 ± 0.0	0.82 ± 0.0	101.2	0.81 ± 0.0	100.5
4	1.61 ± 0.0	1.74 ± 0.0	107.9	1.62 ± 0.0	100.4
8	3.35 ± 0.0	3.81 ± 0.2	113.8	3.28 ± 0.0	97.9
16	6.74 ± 0.0	7.20 ± 0.1	106.9	6.61 ± 0.0	98.1
Mean % recovered			106.3		99.5

^aMean ± standard error for six determinations.

TABLE II
Comparison of the Separation of Free Phosphate from Phytate
in Wheat Bran by the Ion-Exchange Column Method

Sample No.	Water Eluate		0.05M NaCl Eluate		0.7M NaCl Eluate	
	Before digest. mg P/g	After digest. mg P/g	Before digest. mg P/g	After digest. mg P/g	Before digest. mg P/g	After digest. mg P/g of bran
1	1.35	1.22	0.27	0.30	0.10	10.49
2	1.35	1.24	0.20	0.27	0.12	10.41
3	1.35	1.24	0.17	0.30	0.17	10.41
4	1.35	1.24	0.17	0.32	0.17	10.41
5	1.35	1.24	0.15	0.25	0.30	10.41
6	1.42	1.33	0.12	0.30	0.20	10.70
7	1.44	1.35	0.20	0.30	0.24	10.28
8	1.47	1.33	0.15	0.27	0.15	10.57
9	1.47	1.19	0.15	0.30	0.15	10.41
10	1.47	1.30	0.20	0.30	0.15	10.28
Mean \pm S.E.	1.40 \pm 0.02	1.27 \pm 0.02	0.18 \pm 0.01	0.29 \pm 0.01	0.18 \pm 0.02	10.44 \pm 0.04

TABLE III
Comparison of the Separation of Free Phosphate from Phytate in a Solution
of Potassium Acid Phosphate and Sodium Phytate by the Ion-Exchange Column Method

Sample No.	Water Eluate		0.05M NaCl Eluate		0.7M NaCl Eluate	
	Before digest. $\mu\text{g P}$	After digest. $\mu\text{g P}$	Before digest. $\mu\text{g P}$	After digest. $\mu\text{g P}$	Before digest. $\mu\text{g P}$	After digest. $\mu\text{g P}$
1	130	130	47	47	0	219.8
2	130	133	50	53	5	229.2
3	133	135	50	50	10	219.8
4	135	130	50	53	5	229.2
5	133	130	50	49	10	226.1
6	133	130	53	49	5	229.2
7	127	133	47	53	5	223.0
8	127	130	50	53	5	229.2
9	130	130	47	50	10	229.2
10	130	135	50	50	7	226.1
Mean \pm S.E.	131 \pm 0.5	132 \pm 0.8	49 \pm 0.5	51 \pm 0.6	6.0 \pm 1.1	226.1 \pm 1.2

(11.) About 10–15 ml of distilled water was added to the digestion vessels, the contents were mixed thoroughly, and the vessels were placed in a boiling water bath for 15 min to hydrolyze any pyrophosphates which may have formed during digestion. The digestate was quantitatively transferred to a volumetric flask and diluted to volume. An aliquot of the digested eluate was then taken for phosphorus determination by the Fiske-Subbarow method (12).

All data were analyzed statistically according to methods of Snedecor and Cochran (13).

RESULTS AND DISCUSSION

To determine recovery by the method (Table I), aliquots of a sodium phytate solution (inositol hexaphosphoric acid, corn type V, 97% pure, sodium salt; Sigma Chemical Co.) were analyzed to serve as standards. Identical aliquots were analyzed following iron precipitation and digestion and following elution from the column by 0.7M NaCl. As shown in Table I, there was a significant difference between methods ($P < 0.05$).

A test of the reproducibility of the ion-exchange method was made with both wheat bran (Table II) and a mixture of potassium phosphate and sodium phytate (Table III). Each of the three fractions from the column (water, 0.05M NaCl, and 0.7M NaCl) was tested colorimetrically for phosphate both before and after digestion, in an attempt to show the effectiveness of the NaCl molarities in isolating the phytate ion.

Table III shows the recovery of phosphorus and phytate when a solution containing 186.7 μg of inorganic phosphorus and 228.4 μg of phytate phosphorus was added as purified potassium acid phosphate and sodium phytate, respectively. With both the bran and the prepared phytate-phosphate mixture, most of the inorganic phosphate was in the water fraction. The 0.05M NaCl removed an additional quantity of inorganic phosphate. In spite of this, a small but detectable quantity of inorganic phosphate remained in most of the

TABLE IV
Comparison of Two Methods for Determining Phytate Content
(% dry wt) of Soybean-Based Foodstuffs^a

Foodstuff	Iron Precipitation Phosphorus Assay	Ion-Exchange Phosphorus Assay
TVP ^b pork	1.85 \pm 0.0	1.42 \pm 0.0
TVP bacon	1.37 \pm 0.0	0.95 \pm 0.0
TVP ham	1.78 \pm 0.0	1.26 \pm 0.0
TVP beef	1.68 \pm 0.0	1.36 \pm 0.0
TVP beef chunks	1.66 \pm 0.0	1.36 \pm 0.0
TVP bacon + vitamins	1.42 \pm 0.1	1.15 \pm 0.0
TVP unflavored + vitamins	1.82 \pm 0.0	1.63 \pm 0.0
Chicken analog	0.59 \pm 0.0	0.27 \pm 0.0
Ham analog	0.28 \pm 0.0	0.12 \pm 0.0
Textured soy concentrate	1.48 \pm 0.0	1.50 \pm 0.0

^aMean \pm standard error for six determinations.

^bTextured vegetable protein.

0.7M NaCl fractions. It was felt that this does not detract from the method for proximate analysis of phytate.

Table IV shows the analysis of ten vegetable protein products intended to extend or replace meat. These were analyzed by both the iron precipitation and the ion-exchange methods. There were no significant differences between the methods. The ion-exchange method gave a lower concentration in most samples and less variation in the phytate values between replicates than the iron precipitation method.

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