

A Method for Phytic Acid Determination in Wheat and Wheat Fractions¹

E. L. WHEELER and R. E. FERREL, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

ABSTRACT

To assure adequate iron and calcium supplementation in cereal foods and feeds, and to measure the effectiveness of processing designed to reduce their phytate content, a relatively rapid, accurate method is necessary for the determination of phytic acid in wheat, wheat milling by-products, and high-protein flours made from them. A modification of the McCance-Widdowson method for determining phytic acid is reported. The phytate is extracted with trichloroacetic acid and precipitated as the ferric salt. The iron content of the precipitate is determined colorimetrically and the phytate phosphorus content calculated from this value assuming a constant 4 Fe:6 P molecular ratio in the precipitate. The factors affecting this ratio were investigated and are discussed. Applicability of the method to other whole grain cereals is demonstrated.

A large part of the phosphorus in wheat and other cereal grains is present as phytin, the Ca-Mg salt of phytic acid. In wheat the phytic acid is largely distributed among those tissues that go into the by-products of flour milling and is present in large amounts in the high-protein flours (wheat protein concentrates) prepared for human consumption by remilling selected by-product fractions.

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Our concern with phytic acid is centered on two factors: the uncertain availability of its phosphorus to monogastric animals, recently reviewed by Taylor (1) and Nelson (2); and the degree of its interference with calcium and iron absorption (3,4) in human nutrition. Studies by deLange et al. (5) and preliminary investigations at this laboratory indicated that phytic acid content could be significantly decreased by certain processing treatments. Before these treatments could be thoroughly investigated, however, a relatively simple, reliable method for determining phytic acid was needed.

Literature methods have generally relied on the marked insolubility of the ferric salt for phytate determination. Many variations have been reported in methods for extracting, precipitating, and purifying the precipitate, and in direct and indirect methods for determining the phytate content of the precipitate. The quicker indirect colorimetric determination of the bound iron depends upon a constant composition in the precipitate, which has been variously reported to have an iron-to-phosphorus ratio of from 3:6 to 4:6.

After studying these various modifications and their effect on composition of the precipitate, we have developed a relatively simple method for determination of phytic acid applicable to wheat and wheat fractions as well as other cereals.

MATERIALS AND METHODS

A single lot of wheat protein concentrate (WPC) prepared in this laboratory by remilling the shorts from HRS wheat was used throughout this work. All chemicals used were C.P. or reagent grade. A Beckman DK-2 spectrophotometer was used for colorimetric iron measurements, but comparable results were obtained on a Coleman Universal Model 14 instrument. Phosphorus was determined by the method of Allen (6).

The barium phytate (Ba_4Phy) standard was prepared in the following way. Commercial phytin was dissolved in 3% trichloroacetic acid (TCA) and filtered. Ferric phytate (Fe_4Phy) was precipitated from the filtrate by adding concentrated $FeCl_3$ solution. The precipitate was washed with 3% TCA, slurried in water, and converted to sodium phytate and $Fe(OH)_3$ by addition of NaOH. The $Fe(OH)_3$ was filtered out, the sodium phytate solution adjusted to about pH 6 with HCl, and Ba_4Phy precipitated by addition of $BaCl_2$. The Ba_4Phy was redissolved in HCl and the double precipitation cycle repeated twice; the final Ba_4Phy was washed thoroughly with water followed by methanol, and dried in vacuum at 80°C. Found: Ba = 44.6%, C = 6.0%, P = 14.5%. Calculated: Ba = 45.9%, C = 6.0%, P = 15.5%. Inorganic P was present as 6.2% of the total phosphorus. Further purification altered neither the compositional analysis nor the amount of inorganic phosphorus present.

Proposed Method

1. Weigh a finely ground sample (40 mesh) estimated to contain 5 to 30 mg. phytate P into a 125-ml. Erlenmeyer flask.
2. Extract with 50 ml. 3% TCA for 30 min. with mechanical shaking or 45 min. with occasional swirling by hand.
3. Centrifuge the suspension and transfer a 10-ml. aliquot of the supernatant into a 40-ml. conical centrifuge tube.

4. Add 4 ml. FeCl_3 solution (made to contain 2 mg. ferric iron per ml. in 3% TCA) to the aliquot by blowing rapidly from the pipet.

5. Heat the tube and contents in a boiling-water bath for 45 min. If the supernatant is not clear after 30 min., add 1 or 2 drops of 3% sodium sulfate in 3% TCA and continue heating.

6. Centrifuge (10 to 15 min.) and carefully decant clear supernatant.

7. Wash precipitate twice by dispersing *well* in 20 to 25 ml. 3% TCA, heating in boiling-water bath 5 to 10 min., and centrifuging.

8. Repeat wash once with water.

9. Disperse the precipitate in a few ml. water and add 3 ml. 1.5N NaOH with mixing.

10. Bring volume to approximately 30 ml. with water and heat in boiling-water bath for 30 min.

11. Filter hot (quantitatively) through a moderately retentive paper (S & S 597, Whatman No. 2).

12. Wash precipitate with 60 to 70 ml. hot water and discard filtrate. If phosphorus determination is desired, it may be made on this filtrate.

13. Dissolve the precipitate from the paper with 40 ml. hot 3.2N HNO_3 into a 100-ml. volumetric flask.

14. Wash paper with several portions of water, collecting the washings in the same flask.

15. Cool flask and contents to room temperature and dilute to volume with water.

16. Transfer a 5-ml. aliquot to another 100-ml. volumetric flask and dilute to approximately 70 ml.

17. Add 20 ml. of 1.5M KSCN, dilute to volume, and read color immediately (within 1 min.) at 480 nm.

18. Run a reagent blank with each set of samples.

19. Calculate iron content from a $\text{Fe}(\text{NO}_3)_3$ standard run at the same time or read from a previously prepared standard curve.

20. Calculate the phytate phosphorus from the iron results assuming a 4:6 iron:phosphorus molecular ratio.

RESULTS AND DISCUSSION

Most recent methods are modifications of the technique of McCance and Widdowson (7), who rejected the earlier titrametric procedures because of unsatisfactory end point determination and analyzed for phosphorus in the Fe_4Phy precipitate. They extracted phytate with N/6 HCl, whereas other workers have used TCA solutions. We investigated the effectiveness of these two extractants on a series of commercially prepared WPCs from various sources. The McCance and Widdowson technique was followed in all other respects. Results are shown in Table I.

In every case more phytate phosphorus was found in TCA extracts, although differences were not uniform. In addition, the TCA extracts were much cleaner than those with HCl. When HCl extracts were neutralized to about pH 6.0, a large amount of floccular white precipitate formed, whereas in TCA extracts only traces were present. This was probably a protein-phytic acid complex, because if it was

TABLE I. COMPARISON OF HCl AND TCA AS PHYTIC ACID EXTRACTANTS^a

Sample	Total P mg./100 g.	Phytate Phosphorus			
		HCl Extract		TCA Extract	
		mg./100 g.	% Total P	mg./100 g.	% Total P
WPC-1	826	476	57.6	686	83.0
WPC-2	633	425	67.1	437	69.0
WPC-3	1073	505	47.1	696	64.9
WPC-4	886	587	66.3	650	73.4
WPC-5	486	331	68.1	347	71.4
WPC-6	1383	622	45.0	632	45.7
WPC-7	733	471	64.3	556	75.9
WPC-8	904	577	63.8	683	75.6

^aData adjusted to 14% m.b.

INOSITOL HEXAPHOSPHATE (Phytic acid)

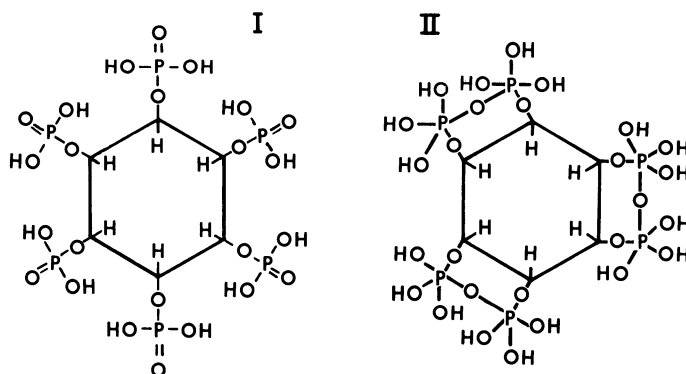


Fig. 1. Proposed structures for phytic acid.

TABLE II. EFFECT OF NEUTRALIZATION ON COMPOSITION OF FERRIC PHYTATE

Source	Treatment	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P ^a
WPC	neutralized	79.4	105.5	4.51:6
	not neutralized	72.0	105.5	4.08:6
Ba ₄ Phy	neutralized	58.3	77.0	4.55:6
	not neutralized	51.0	76.3	4.02:6

^aIn this and the following tables, phosphorus is taken as an absolute value in calculating Fe:P ratios. Thus any variation in both methods is assigned to iron values. For that reason values between 3.90:6 and 4.10:6 are considered as satisfying theoretical requirements.

removed only traces of phytic acid were found in the supernatant. This complex may account in part for the lower results with HCl. Extracting 30 min. with mechanical shaking or 45 min. with occasional hand swirling was as effective as longer extraction times, and 3% TCA was as good as the 5% used by deLange et al. (5).

Direct determination of phosphorus in the Fe_4Phy precipitate requires a time-consuming perchloric acid digestion. The procedure can be simplified by determining iron colorimetrically and calculating phytate phosphorus from the results. For this method to be accurate, the precipitate must always have a known and constant composition. Other workers have reported Fe:P ratios ranging from 3:6 to 4:6 (5,8,9).

Brown and co-workers (10) indicated that the most probably phytate salt form for a trivalent cation was Fe_4Phy , even though their studies suggested the Neuburg structure (II) with 18 titratable hydrogens to be most likely. The more recent NMR work of Johnson and Tate (11) indicated the Anderson structure (I) for wheat phytate was most probable, which also favors the Fe_4Phy composition (Fig. 1).

The McCance and Widdowson method (7) recommended neutralization of the extracts before precipitation of Fe_4Phy . Anderson (12) and Makower (13,14) claimed better results if precipitation was carried out in acid solution. Our investigations supported these latter claims, as shown in Table II. We also found that fast addition of FeCl_3 (as in blowing from a pipet) gave more consistent results than slower addition.

Neutralization of HCl extracts has the effect of increasing ionic concentration through the presence of NaCl. Anderson (12) found little effect with 0.25N NaCl at the iron concentrations we recommend. However, he also found precipitation more complete at more acid concentrations, which would cause much higher salt concentrations if neutralized. Accordingly, we studied the effect of various NaCl concentrations on Fe_4Phy formation from WPC extracts and the Ba_4Phy standard (Table III). There was a general trend with both materials for Fe:P ratios to become less than 4:6 as NaCl concentration increased. This was probably due to formation of mixed Na-Fe salts. Variations in composition of phytate salts with changes in composition of precipitating cations have been demonstrated by others (3). The

TABLE III. EFFECT OF NaCl ON COMPOSITION OF FERRIC PHYTATE

NaCl conc. in 3% TCA	Used in Extract Only			Used in Extract and Wash		
	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P
WPC Extracts						
0	77.2	112.3	4.10:6
2.5	74.4	112.9	3.95:6	70.0	112.3	3.74:6
3.7	76.6	114.2	4.08:6	73.1	112.0	3.92:6
10	71.3	114.2	3.75:6	64.0	112.9	3.40:6
Ba_4Phy Standard						
0	70.4	106.8	3.96:6	71.4	106.9	4.00:6
2.5	67.7	105.0	3.86:6	66.5	106.5	3.75:6
3.7	66.7	106.0	3.78:6	62.8	104.9	3.60:6
10	64.6	106.0	3.66:6	59.8	105.3	3.41:6

TABLE IV. EFFECT OF IRON CONCENTRATION ON COMPOSITION OF FERRIC PHYTATE

Fe:P Mole Ratio Used	Found		Fe:P
	$\mu\text{M Fe}$	$\mu\text{M P}$	
	WPC Extracts		
4:6	71.5	116.7	3.70:6
8:6	80.0	117.4	4.08:6
12:6	80.2	112.3	4.29:6
16:6	76.4	108.4	4.23:6
	Ba ₄ Phy Standard		
4:6	50.4	74.7	4.04:6
8:6	51.5	74.7	4.14:6
16:6	52.5	73.4	4.28:6

effect was most marked when the salt was present in both the extraction and washing steps, indicating that cationic exchange was taking place with the extremely insoluble Fe₄Phy. In fact, in the Ba₄Phy series there was a decreased recovery of phosphorus from 98 to 92% with increasing salt concentrations when present in the extraction and wash steps, suggesting an increasing solubility as sodium content of the mixed Na-Fe salt increased.

Anderson (12) found 100% recovery of phytate phosphorus with just theoretical amounts of iron (4 moles Fe to 6 moles P) but did not indicate the Fe:P ratio of the precipitates. Earley (9) found, under his conditions, that iron had to be at least 3.6 times theoretical for the precipitate to have a ratio of 4 Fe:6 P. In our studies with Ba₄Phy standard, quantitative recoveries of phosphorus and 4:6 Fe:P ratios were obtained with theoretical and twice theoretical amounts of iron (Table IV). Above these levels, additional iron was retained in the precipitate and Fe:P ratios were too high. With WPC extracts a theoretical amount of iron gave precipitates with low Fe:P ratios but near quantitative recovery of phosphorus, perhaps owing to formation of mixed phytate salts with the naturally occurring metal ions. A twofold excess of iron gave satisfactory ratios and quantitative phosphorus recoveries. The recommended twofold excess of iron, based on estimated phytate phosphorus, should allow for reasonable variation on this estimate and still yield satisfactory results.

Anderson (12) showed that Fe₂(SO₄)₃ and FeNH₄(SO₄)₂ gave improved recovery of phytic acid over a wider range of acidities than did FeCl₃, presumably owing to the sulfate ion. He also found that these salts precipitated the lower phosphate esters of inositol whereas FeCl₃ did not. In our investigation no significant difference was found, using the various salts, in either Fe:P ratios or phosphorus recoveries (Table V). Our data confirm the evidence that wheat phytate was present only as the hexaphosphate ester as found by Johnson and Tate (11).

TABLE V. EFFECT OF DIFFERENT FERRIC SALTS ON COMPOSITION OF FERRIC PHYTATE

Salt Used	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P	%P Recovered
FeCl ₃	74.1	113.0	3.94:6	100
FeNH ₄ (SO ₄) ₂	75.2	112.2	4.03:6	99
Fe ₂ (SO ₄) ₃	72.5	113.2	3.86:6	100

TABLE VI. EFFECT OF $\text{SO}_4^{=}$ ON COMPOSITION OF FERRIC PHYTATE

Additive ^a	Used in Extract Only			Used in Extract and Wash		
	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P
			WPC Extracts			
Na_2SO_4	78.3	110.0	4.27:6	86.9	112.8	4.62:6
H_2SO_4	75.0	103.5	4.35:6	66.6	106.5	3.75:6
			Ba_4Phy Standard			
Na_2SO_4	73.7	98.7	4.48:6	80.5	99.4	4.86:6
H_2SO_4	71.4	99.0	4.33:6	69.4	101.3	4.11:6

^aAll at 0.18M $\text{SO}_4^{=}$ in 3% TCA.

TABLE VII. EFFECT OF H_2SO_4 ON COMPOSITION OF FERRIC PHYTATE

Extractant	Wash	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P
3% TCA	3% TCA	74.5	111.5	4.01:6
3% TCA	0.1N H_2SO_4	80.5	112.5	4.29:6
0.5N H_2SO_4	0.1N H_2SO_4	77.0	107.0	4.31:6

TABLE VIII. EFFECT OF AMOUNT AND CONCENTRATION OF PHYTATE PHOSPHORUS ON FERRIC PHYTATE PRECIPITATION USING Ba_4Phy

Phytate Phosphorus Present		Found	
Total mg.	Conc. mg./ml.	Fe:P	% Recovered
0.64	0.064	3.66:6	92
0.62	0.10	3.80:6	95
3.2	0.11	4.03:6	97
3.2	0.16	4.05:6	98
1.6	0.20	4.11:6	99
2.2	0.30	4.08:6	97
3.1	0.31	4.03:6	99
4.7	0.31	4.17:6	96
6.2	0.31	4.20:6	95
4.6	0.46	4.22:6	97
6.4	0.64	4.26:6	97
9.3	0.93	4.22:6	84

TABLE IX. RECOVERY OF ADDED PHYTATE PHOSPHORUS

Sample	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P	% Recovery
WPC	37.5	56.2	4.00:6	...
WPC and Ba_4Phy (56.8 $\mu\text{M P}$ added)	78.0	112.5	4.16:6	99
Ba_4Phy (56.8 $\mu\text{M P}$)	38.2	56.8	4.04:6	100

TABLE X. APPLICATION OF PROPOSED METHOD TO VARIOUS WHOLE GRAIN CEREALS

Grain	$\mu\text{M Fe}$	$\mu\text{M P}$	Fe:P	Phytate P ^a mg./g.
Barley	80.5	121.2	3.99:6	1.88
Rice	56.6	87.1	3.85:6	1.35
Oats	82.0	121.2	4.05:6	1.88
Milo	122.0	180.2	4.05:6	2.79
Corn	92.0	136.8	4.03:6	2.12
Wheat	107.0	159.0	4.04:6	2.47

^aAs-is moisture basis.

Earley (9) carried out his extraction and precipitations in solutions containing 10% Na_2SO_4 . We have observed that traces of Na_2SO_4 added at the precipitation step aided coagulation of the Fe_4Phy , particularly at lower phytate concentrations. Accordingly we investigated the effect of SO_4^{2-} , present as Na_2SO_4 and H_2SO_4 , on precipitate compositions (Table VI). Na_2SO_4 caused extra iron to be incorporated in the precipitate. It was not clear whether it was bound or simply occluded. The effect was most marked when the salt was present in both the extraction and wash steps. Precipitates formed in the presence of H_2SO_4 also included more than the theoretical amount of iron, but this was apparently removed when the acid was also present in the wash steps. In the WPC extracts there was a reduced amount of phosphorus in the precipitates when H_2SO_4 was present. Since TCA was also present, it was not clear whether the H_2SO_4 led to poorer extraction or poorer precipitation. Further studies with H_2SO_4 (Table VII) on WPC indicated that sulfuric acid depressed the extraction of phytate (as did HCl). In addition, in the absence of TCA, removal of excess iron from the precipitate was poorer. Other work (data not given) showed that, even at concentrations of 0.003%, Na_2SO_4 present in precipitation and wash steps caused a high Fe:P ratio (4.2:6). However, a few drops of 3% Na_2SO_4 solution, added only at the precipitation step, aided coagulation without appreciably affecting results.

The effective range of the proposed method was studied using the Ba_4Phy standard. Data shown in Table VIII indicate it was applicable at phytate phosphorus concentrations between 0.10 and 0.65 mg. per ml. with a reasonable degree of accuracy. However, as the total amount of phytate phosphorus—and therefore precipitate—increased, it became more difficult to wash out occluded iron, and ratios tended to become greater than theoretical. Sample sizes and dilutions given in the proposed procedure yield amounts and concentrations in the applicable range.

In the WPC used in the studies, 88% of the total phosphorus was extracted by our method. Ninety percent of the extracted phosphorus was organic phosphorus and essentially all of this was determined as phytate phosphorus. Added phytate was recovered quantitatively (Table IX), suggesting that the phosphorus not extracted from WPC was present as phospholipids, phosphoproteins, or nucleic acids, all known to be present in wheat.

When the method was applied to whole wheat and other whole grain products it appeared to be equally applicable, as shown in Table X.

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