

Phytic Acid. I. Determination of Three Forms of Phosphorus in Flour, Dough, and Bread¹

U. TANGKONGCHITR, P. A. SEIB, and R. C. HOSENEY²

ABSTRACT

Cereal Chem. 58(3):226-228

An analytical scheme was devised to measure phytate phosphorus, inorganic phosphorus, and phosphorus not precipitated by ferric ion in flour, dough, and bread. Determination of the various types of phosphorus

made possible the calculation of mass balance for phosphorus. Spiking experiments were used to prove the validity of the scheme.

A group of respected nutritionists have proposed seven dietary goals (Select Committee 1977) for U.S. citizens. The only positive recommendation, as Paul LaChance (1980) of Rutgers University pointed out, is that Americans increase their consumption of complex carbohydrates by eating more fruits, vegetables, and whole-grain foods. Whole grains, however, contain phytic acid. When phytic acid is ingested at 2-8 g per day, which is 0.57-2.2 g of phytate phosphorus (Pp), it interferes with the bioavailability of divalent minerals, notably zinc, iron, calcium, and magnesium (Erdman and Forbes 1977, Hoff-Jorgensen et al 1946, McCance and Widdowson 1942, Nahapetian and Bassiri 1975). Because some American diets are deficient or marginal in those minerals, the National Academy of Sciences has proposed that cereal foods be enriched by adding zinc, iron, calcium, and magnesium (NAS/NRC 1974). Reduction of the phytic acid in whole grain foods appears prudent, especially because other sources of phytic acid, such as vegetable proteins (DeRham and Jost 1979), are being consumed in increasing amounts.

One report, however, indicates that humans might adapt to moderately high intakes of phytate. Walker et al (1948), studied three subjects eating ~1.02-1.22 g of phytic acid per day (0.29-0.35 g of Pp) in the form of brown bread and found that, after 4-8 weeks, the subjects showed positive calcium, magnesium, and iron balances.

Phytate analysis is a subject of current interest. Phytate in rice bran has been determined by high-performance liquid chromatography (Tangendjaja et al 1980). Latta and Eskin (1980) separated phytic acid in various plant materials by anion-exchange chromatography and rapidly quantitated it in the eluant, using the Wade reagent. Cosgrove (1980) has reviewed assay methods for inositol phosphates.

Phosphorus occurs in wheat in the form of several classes of compounds. Most of it is inorganic phosphorus (Pi) and phosphate esters of *myo*-inositol (O'Dell et al 1972, Nahapetian and Bassiri 1975). Increases in total phosphorus (Pt) of wheat are linearly correlated (coefficient = 0.98) with increases in Pp (Lolas et al 1975). The remainder is phospholipids with small amounts of phosphosugars in nucleic acids and phosphoproteins.

We wanted to follow Pp from flour to bread. To do so required an analytical scheme to measure the various types of phosphorus. We used an analytical scheme that accounted for phosphorus in one of four categories: Pi; phosphorus extractable (Pe) with a mixture of aqueous 1.2% HCl and 10% Na₂SO₄; Pp, the fraction of

¹Contribution 80-410-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, 66506.

²Graduate research assistant, professor, and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan 66506.

Pe precipitated by ferric ion; and supernatant phosphorus (Ps), the fraction of the extract not so precipitated. This article reports the development and justification of this scheme.

MATERIALS AND METHODS

Sodium Phytate Monohydrate

Sodium phytate (39.25% water), purchased from Sigma Chemical Co., St. Louis, MO, was purified by a slight modification of the procedure described by Johnson and Tate (1969). Sodium phytate (10 g) was dissolved in 300 ml of a mixture of water and methanol (3:1, v/v). The solution was allowed to stand at room temperature for two days, during which rhombic and needle-shaped crystals were deposited in the flask. The transparent crystals were recovered by filtration and dried to constant weight under vacuum at 110°C over phosphorus pentoxide. During drying, the transparent crystals became opaque. The opaque crystals, which were stored in a closed container over anhydrous calcium sulfate, gave the elemental analysis of the pure monohydrate salt of sodium phytate. Calculations for $C_6H_6O_{24}P_6Na_{12}H_2O$ predicted 7.65% C, 0.85% H, and 19.73% P. Actual findings were: C, 7.69%; H, 0.90%; and P, 19.03%.

Determination of Forms of Phosphorus

Figure 1 gives an outline of the mass-balance determination of phosphorus. All phosphorus determinations were done in duplicate. In this work, the values of Pe, Pp, and Ps for flour samples are reported in milligrams of phosphorus per 100 g of dry solid. Dry solids consisted of a whole wheat flour (WW) or doughs or bread freeze-dried before analysis.

Pt. Pt in the original dry sample was determined after digesting the solid sample (0.5 g of dried bread or dough) in a mixture of concentrated sulfuric acid (3 ml) and nitric acid (8 ml). The digest was made to volume (100 ml) with water, and a 2.0-ml aliquot was used to determine phosphorus colorimetrically.

Pi. The native Pi in the sample was determined by extracting a sample (5.000 g) with 100 ml of 12.3% aqueous trichloroacetic acid on a mechanical shaker for 12 hr at 25°C according to the procedure of Pons et al (1946). After centrifugation at $2,000 \times g$ for 30 min, an aliquot (5.0 ml) was diluted to an appropriate volume with distilled water. The solution was then analyzed directly for Pi.

Pe, Ps, and Pp. A sample of dried bread or dough (5.000 g) was extracted with 100 ml of 1.2% aqueous HCl containing 10% Na_2SO_4 (Earley 1944, Nahapetian and Bassiri 1975). The mixture was shaken at room temperature for 24 hr, centrifuged at $2,000 \times g$ for 40 min, and the supernatant decanted. An aliquot (5.0 ml) of the acid extract was used to determine Pe. The phytic acid in 10.0 ml of the acid extract was precipitated by adding 5 ml of aqueous 0.4% ferric chloride hexahydrate in 0.6% HCl containing 5% Na_2SO_4 . The mixture was heated in a boiling water bath for ~20 min to complete precipitation. The ferric phytate precipitate was collected as a solid pellet by centrifuging at $2,600 \times g$ for 30 min. The supernatant was decanted and saved for determination of Ps.

The yellowish pellet was dissolved in a mixture of concentrated H_2SO_4 (2 ml) and concentrated HNO_3 (3 ml), and transferred quantitatively to a micro-Kjeldahl flask for wet combustion. The digestion, which usually required ~20 min, was judged complete when white fumes filled the flask and hung over the liquid. Distilled water (10 ml) was slowly added to the acid digest while it was still warm, and the aqueous solution was boiled in a water bath 30 min. The solution was then transferred quantitatively and made to volume (100 ml) with distilled water. The Pp was determined colorimetrically as orthophosphate by the method of Lindberg and Ernster (1956) as modified by Nahapetian and Bassiri (1975). A standard curve was prepared with known concentrations (1.98×10^{-5} to 4.96×10^{-5} mg of phosphorus per 2.0 ml) of reagent-grade potassium dihydrogen phosphate (Fisher Scientific Company, Fairlawn, NJ).

To determine Pe, the 5.0-ml aliquot of the acid extract of flour or dough was wet-ashed as previously described. For Ps, the supernatant (~15 ml) was decanted into a micro-Kjeldahl flask,

evaporated to ~5 ml, and wet-ashed. Pe and Ps were quantitated colorimetrically as orthophosphate (Nahapetian and Bassiri 1975).

Spiking Experiments

Pt. WW (0.5000 g, containing natively 1.97 mg of Pt) was mixed with 7.1 mg of sodium phytate monohydrate, which contained 1.4 mg of Pp.

Pi. In a series of spiking experiments, 10, 15, 20, and 30 mg of Pi in the form of potassium dihydrogen phosphate was added to WW (5.000 g), which originally contained 1.13 mg of Pi.

Pp and Pe. A duplicate recovery experiment was done in which two levels of sodium phytate monohydrate, 36.3 and 64.7 mg, which are equivalent to 7.3 and 13.0 mg of Pp, were added to 5.000 g of WW, natively containing 17.0 mg of Pe and 16.5 mg of Pp.

RESULTS

The Pt in the WW spiked with 7.1 mg of sodium phytate monohydrate was determined to be 3.6 mg, which is 106% of the theoretical 3.4 mg.

The flours spiked with potassium dihydrogen phosphate (10, 15, 20, and 30 mg) contained 11.4, 15.7, 20.6, and 29.6 mg of Pi, respectively, which is equivalent to 95–102% recovery of added Pi.

In theory, the WW samples spiked with 36.3 and 64.7 mg of sodium phytate monohydrate should have contained 24.3 and 30.0 mg, respectively, of Pe and 23.8 and 29.5 mg, respectively, of Pp. After extraction with 100 ml of 1.2% HCl containing 10% Na_2SO_4 , the actual recoveries were: Pe, 23.8 and 30.2 mg (98.0 and 100.8% respectively), and Pp, 22.9 and 29.2 mg (97.0 and 99.0%, respectively).

DISCUSSION

The classical method (Oberleas 1973) of determining phytic acid involves extracting plant materials with a low-pH medium, precipitating ferric phytate, collecting and washing the precipitate,

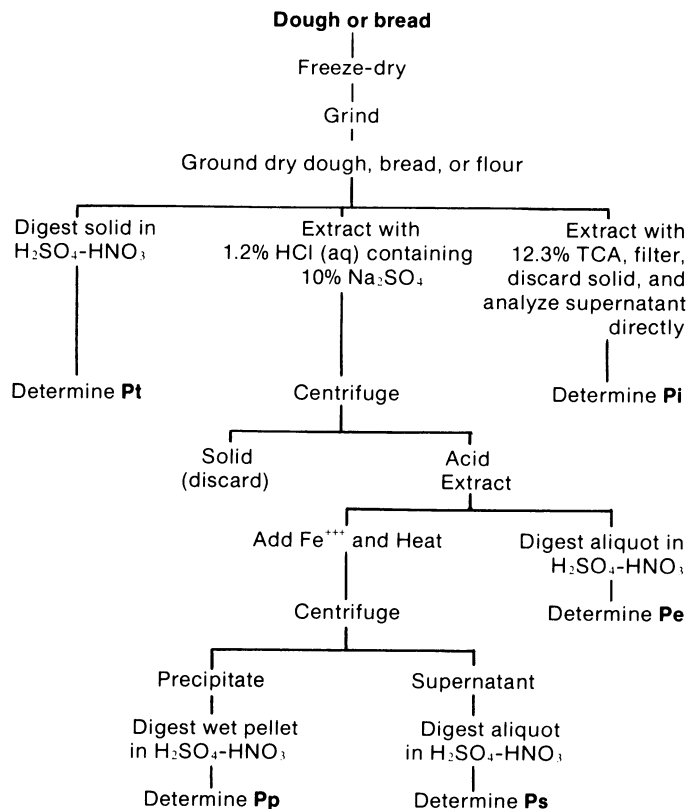


Fig. 1. Outline of mass-balance determination of various forms of phosphorus in flour, dough, and bread. Pt = total phosphorus, Pi = inorganic phosphorus, Pe = phosphorus extractable in 1.2% aqueous HCl containing 10% Na_2SO_4 , Pp = fraction of Pe precipitated by ferric ion, Ps = fraction of Pe not precipitated by ferric ion.

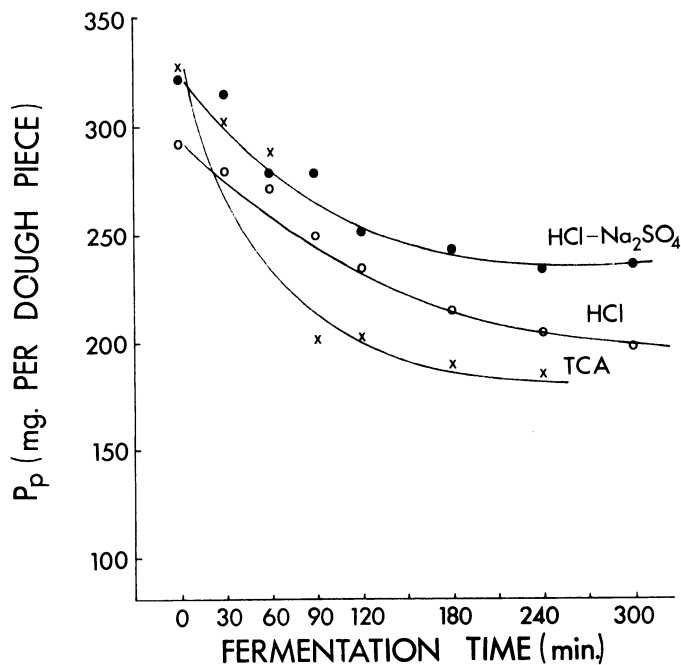


Fig. 2. Extraction of whole wheat dough by three methods. Pp = Phytate phosphorus, TCA = trichloroacetic acid.

and quantifying by determining iron (Earley 1944, de Lange et al 1961, Makower 1970, Wheeler and Ferrel 1971) or phosphorus (Nahapetian and Bassiri 1975, Wheeler and Ferrel 1971). We used the latter method because the molar ratio of iron to phosphorus in ferric phytate has been reported to be 4:6 (Earley 1944, Makower 1970), 3:6 (de Lange et al 1961), and sometimes higher than 4:6 (Ferrel 1978). The Fe-P ratio varies because phytate is partially hydrolyzed by phytase in some plant materials (Ferrel 1978, Minailovic et al 1965, Nagai and Funahashi 1962) to give *myo*-inositol pentaphosphates and tetraphosphates, both of which precipitate with ferric ion (Anderson 1963). Harland and Oberleas (1977) recently used ion-exchange chromatography in place of ferric ion precipitation to isolate phytate from extracts of vegetable proteins.

Quantifying ferric phytate by wet combustion followed by determining orthophosphate, however, also has a pitfall—the digestion step must be done carefully to avoid errors (Jones 1949).

Three extraction media have been reported by various investigators to solubilize phytic acid in plant materials, ie, 1.8% hydrochloric acid, 3% trichloroacetic acid, or a mixture of 1.2% hydrochloric acid with 10% sodium sulfate. We compared the three on a whole wheat dough at various fermentation periods (Fig. 2). The curves show that the best recovery of Pp with a single extraction step was achieved with the mixture of hydrochloric acid and sodium sulfate, which we used throughout the remainder of the work.

The analytical scheme described here serves two purposes. It allows one to verify the accuracy of the recovery of phosphorus in the various analytical steps because Pe must equal the sum of Pp and Ps. It also allows one to predict whether a mixture of phosphate esters of *myo*-inositol are accumulating in a dough during breadmaking. The difference between Pi and Ps would undoubtedly contain *myo*-inositol monophosphate and diphosphate esters. One can check for an accumulation of intermediary esters of *myo*-inositol by determining whether the loss of Pp equals the gain of Pi.

The validity of the analytical scheme for the various forms of phosphorus shown in Fig. 1 was checked by recovery experiments. When flour or dry ground dough was "spiked" with a known amount of sodium phytate monohydrate, the recovery of added Pt, Pe, and Pp was 97–106%. Spiking of flour and dough with potassium dihydrogen phosphate gave 95–102% recovery of Pi.

- ANDERSON, G. 1963. Effect of Fe/P ratio and acid concentration on the precipitation of ferric inositol hexaphosphate. *J. Sci. Food Agric.* 14:352.
- COSGROVE, D. J. 1980. Chromatography and analytical methods. Page 12 in: Cosgrove, D. J., ed. *Studies in Organic Chemistry*, Vol. 4. Inositol Phosphates, Their Chemistry, Biochemistry and Physiology. Am. Elsevier Scientific Pub. Co., Inc.: New York.
- DE LANGE, D. J., JOUBERT, C. P., and DU PREEZ, S. F. M. 1961. The determination of phytic acid and factors which influence its hydrolysis in bread. *Proc. Nutr. Soc. South Africa* 2:69.
- DE RHAM, O., and JOST, T. 1979. Phytate-protein interaction in soybean extracts and low-phytate soy protein products. *J. Food Sci.* 44:596.
- EARLEY, E. B. 1944. Determining phytin phosphorus. Stoichiometric relation of iron and phosphorus in ferric phytate. *Ind. Eng. Chem. Anal. Ed.* 16:389.
- ERDMAN, J. W., and FORBES, R. M. 1977. Mineral bioavailability from phytate-containing foods. *Food Prod. Dev.* 11(10):46.
- FERREL, R. E. 1978. Distribution of bean and wheat inositol phosphate esters during autolysis and germination. *J. Food Sci.* 43:563.
- HARLAND, B. F., and OBERLEAS, D. 1977. A modified method for phytate analysis using an ion-exchange procedure: Application to textured vegetable proteins. *Cereal Chem.* 54:827.
- HOFF-JORGENSEN, E., ANDERSON, O., and NIELSON, G. 1946. The effect of phytic acids on the absorption of calcium and phosphorus. *Biochem. J.* 40:555.
- JOHNSON, L. F., and TATE, M. E. 1969. Structure of phytic acids. *Can. J. Chem.* 47:63.
- JONES, W. M., Jr. 1949. *Inorganic Chemistry*. Blakiston Co.: Philadelphia, PA.
- LA CHANCE, P. A. 1980. Selling bakery products to benefit nutrition. Page 190 in: *Proc. 56th Annual Meeting. Am. Soc. Bakery Eng.*: Chicago, IL.
- LATTA, M., and ESKIN, M. 1980. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* 28:1315.
- LINDBERG, O., and ERNSTER, L. 1956. Determination of organic phosphorus compounds by phosphate analysis. Page 1 in: Glick, D., ed. *Methods of Biochemical Analysis*, Vol. 3. Interscience Publishers, Inc.: New York.
- LOLAS, G. M., PALAMIDIS, N., and MARKAKIS, P. 1975. The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. *Cereal Chem.* 53:867.
- MAKOWER, R. U. 1970. Extraction and determination of phytic acid in beans (*Phaseolus vulgaris*). *Cereal Chem.* 47:288.
- MCCANCE, R. A., and WIDDOWSON, E. M. 1942. Mineral metabolism on dephytinized bread. *J. Physiol.* 104:304.
- MINAILOVIC, M. L., ANTIC, M., and HADZIJEV, D. 1965. Chemical investigation of wheat. VIII. Dynamics of various forms of phosphorus in wheat during ontogenesis. The extent and mechanism of phytic acid decomposition in germinating wheat grain. *Plant Soil* 23:117.
- NAGAI, Y., and FUNAHASHI, S. 1962. Phytase (myoinositolhexaphosphate phosphohydrolase) from wheat bran. *Agric. Biol. Chem.* 26:794.
- NAHAPETIAN, A., and BASSIRI, A. 1975. Changes in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium and zinc in wheat during maturation. *J. Agric. Food Chem.* 23:1179.
- NAS/NRC. 1974. Proposed fortification policy for cereal-grain products. National Academy of Science, National Research Council: Washington, DC.
- OBERLEAS, D. 1973. Phytates. Page 363 in: *Toxicants Occurring Naturally in Foods*, 2nd ed. National Academy of Sciences: Washington, DC.
- O'DELL, B. L., DE BOLAND, A. R., and KOIRTYOHANN, S. R. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.* 20:718.
- PONS, W. A., and GUTHRIE, J. D. 1946. Determination of inorganic phosphorus in plant materials. *Ind. Eng. Chem., Anal. Ed.* 18:184.
- SELECT COMMITTEE ON NUTRITION AND HUMAN NEEDS, U.S. SENATE. 1977. *Dietary goals for the United States*. U.S. Government Printing Office: Washington, DC.
- TANGENDJAJA, B., BUCKLE, K. A., and WOITTON, M. 1980. Analysis of phytic acid by high-performance liquid chromatography. *J. Chromatogr.* 197:274.
- WALKER, A. R. P., FOX, R. W., and IRVING, J. T. 1948. Studies in human metabolism. *Biochem. J.* 42:452.
- WHEELER, E. L., and FERREL, R. N. 1971. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem.* 48:312.