Overestimation of Phytic Acid in Foods by the AOAC Anion-Exchange Method

Jacob Lehrfeld*,† and Eugene R. Morris‡

National Center for Agricultural Utilization Research, Food Physical Chemistry, Agricultural Research Service, U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, and Beltsville Human Nutrition Research Center, Vitamin and Mineral Nutrition Laboratory, BARC-East, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

The currently used AOAC method may overestimate the phytic acid (inositol hexaphosphate) content of processed foods. These foods generally contain the lower phosphates (inositol penta-, tetra-, and triphosphate) in addition to phytic acid. These lower phosphates are included in the calculation of phytic acid determined by the AOAC anion-exchange method. The HPLC method quantitates phytic acid and the other inositol phosphates as separate entities. Research has indicated that phytic acid, and to a lesser degree other inositol phosphates, may reduce mineral bioavailability of foods. Consequently, a method that measures the individual inositol phosphates will more accurately predict the potential negative effect on mineral bioavailability. Phytic acid content of a human diet, an oat breakfast cereal, a wheat bran sample, and a sample of sodium phytate was determined according to the AOAC method and an HPLC method. The values obtained were in good agreement when the sample contained only phytic acid. However, the values diverged when samples contained appreciable amounts of inositol penta-, tetra-, and triphosphate.

INTRODUCTION

Phytic acid (PA), a common component in all cereal grains, has been shown to decrease the bioavailability of some trace elements and minerals (Morris, 1986) and to interfere with the activity of some digestive enzymes (Reddy et al., 1989; Thompson et al., 1987). The currently generally accepted AOAC method for measuring the phytic acid content in foods and feeds is based on the step gradient anion-exchange method developed by Harland and Oberleas (1986). In their procedure the phytic acid is extracted from a food or feed matrix with 2.4% HCl and washed through an anion-exchange column. Most of the associated impurities are washed off the column while the phytic acid is retained on the column. Phytic acid is then eluted with a 0.7 M NaCl solution and acid digested to inorganic phosphate. The implicit assumptions are that (1) all of the phytic acid is held on the column and (2) only phytic acid is eluted from the anion-exchange column by the 0.7 M NaCl solution. The phytic acid (the assumed sole source of phosphorus) in the eluent is subsequently converted into inorganic phosphate. On the basis of this assumption, the phytic acid content of the sample is calculated to be 28.2% × inorganic phosphate value. These assumptions are valid for most grains and legumes in their native state because they contain essentially only phytic acid. However, diets and foods or feeds containing processed grains or legumes can contain appreciable quantities of partially dephosphorylated isomers of phytic acid (Phillippy et al., 1988) such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3), and possibly the di- and monophosphates (IP2 and IP1). These phosphates are also held on the anion-exchange resin and eluted with 0.7 M NaCl. Therefore, these phosphates will be included in the calculation of phytic acid. Of all the possible phosphates, only IP6 and IP5 appear to interfere appreciably with the bioavailability of trace elements and minerals (Tao et al., 1986). Consequently, the inclusion of the other phosphates in the calculation of PA, as is done in the AOAC method, will tend to overestimate the negative effect that a particular food or diet will have on the bioavailability of trace elements and minerals. Recently reported methods permit the rapid identification and quantification of phytic acid and its partially dephosphorylated isomers IP3, IP4, and IP5 by HPLC (Lehrfeld, 1989; Sandberg and Ahderinne, 1986). Neither of these methods detects nor quantitates inositol mono- or diphosphates (IP1 or IP2). Philippy and Bland (1988) described a gradient technique using postcolumn detection to detect the lower phosphates. The latter method also resolves the enantiomers.

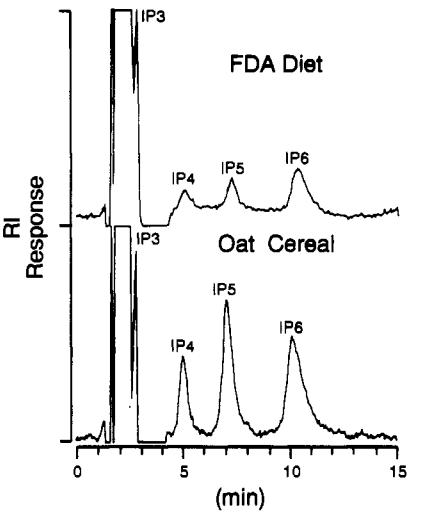

Figure 1. Reversed-phase HPLC of an FDA survey diet and an oat breakfast cereal by ion-pair chromatography. Samples were injected onto a Hamilton PRP-1 column (4.1 × 150 mm, 5 μm). Eluting solvent was 0.015 M formic acid and 0.4% TBNOH in 52% methanol. The pH was adjusted to 4.3 with sulfuric acid. The column temperature was 40 °C, and the elution rate was 0.9 mL/min. IP3, inositol triphosphate; IP4, inositol tetraphosphate; IP5, inositol pentaphosphate; IP6, phytic acid.

† National Center for Agricultural Utilization Research.
‡ Beltsville Human Nutrition Research Center.

Table I. Phytic Acid Analysis by AOAC and HPLC

sample	phytic acid,[a] mg/mL AOAC[b]	phytic acid,[a] mg/mL HPLC[c]	IP6, % HPLC	IP5, % HPLC	IP4, % HPLC	IP3, % HPLC
wheat bran	0.28	0.27 (3.6)[d]	92.4	6.5	0.9	0.2
wheat bran + spike	0.55	0.56 (0.12)	94.6	5.4		
oat cereal	0.058	0.019 (5.2)	42.6	35.8	17.5	4.1
oat cereal + spike	0.30	0.28 (0.7)	83.2	9.8	3.0	3.9
FDA diet	0.046	0.011 (3.6)	38.6	20.0	13.7	27.7
FDA diet + spike	0.28	0.25 (0.8)	86.6	7.9	1.1	4.4
sodium phytate (spike)	0.26	0.25 (2.4)	99.1	0.86	0.04	0.0

[a] mg/mL of phytic acid (IP6) in eluent from anion-exchange column (aliquots from the same eluent were used for both the AOAC and HPLC analyses). [b] IP2 and IP1 may be an additional source of phosphorus. [c] Average of two determinations. [d] Values in parentheses are coefficients of variation.

In this study the phytic acid content of seven samples was measured by the AOAC method (986.11, final action 1988) and by HPLC. The results were compared. Reasons for divergence and convergence of data are discussed.

MATERIALS AND METHODS

Sample Preparation. The diet (an FDA composite survey diet), a cereal (a whole grain toasted oat breakfast cereal), and a wheat bran sample and the corresponding samples spiked with sodium phytate were treated as described by Harland and Oberleas (1986). They were shaken with 2.4% HCl (10 mL of 2.4% HCl/g of sample) for 3 h at room temperature. The extracts were diluted and washed onto an AG 1-X4 column. The retained inositol phosphates were eluted from the column with 0.7 M NaCl.

Sample Analysis: Phosphorus. An aliquot of the eluent (0.7 M NaCl eluent described above) was digested with sulfuric and nitric acids, diluted, and treated with a molybdate solution followed by 1-amino-2-hydroxynaphthalene-4-sulfonic acid reagent, and the absorbance was read at 640 nm. Phosphorus was calculated from a standard calibration curve; phytate = 28.2% × P (Harland and Oberleas 1986).

Sample Analysis: HPLC. An aliquot (8 mL) of the eluent (0.7 M NaCl eluent described above) was washed through a 10-mL AG 50W-X8 200–400-mesh (H^+) column to exchange H^+ for Na^+, and the resulting solution was concentrated and used for HPLC analysis (Lehrfeld, 1989). A number of adjustments had to be made in the chromatographic conditions because of the reduced sample concentration. Size of the injection was raised to 100 μL rather than the 20 μL previously used, a phytic acid hydrolysate (3 mg/L) was added to the eluting solvent to reduce the effect from nonspecific adsorption, and the sample was reconstituted with 0.1–0.5% (v/v) tetrabutylammonium hydroxide (TBNOH) rather than 1% TBNOH to minimize the appearance of ghost peaks.

RESULTS AND DISCUSSION

Inositol phosphates found in grains and legumes usually contain over 92% phytic acid. We found raw wheat bran to contain 92.4% IP6, which is in good agreement with the 90.3% value found by Sandberg and Ahderinne (1986). However, during conversion of these grains or legumes into food products endogenous and exogenous phytases or processing conditions can cause extensive hydrolysis of phytic acid into IP5, IP4, IP3, IP2, and IP1. For example, the endogenous phytase in Scout 66 hard red winter wheat bran is so active that when a sample was suspended in pH 4.8 acetate buffer, the PA content was reduced from 4 to 0.2% within 1.5 h. Processing can also have a significant effect on the degree of hydrolysis. For example, in a minimally processed oat bran cereal, 76% of the phytate was phytic acid. This is in contrast to a toasted oat cereal in which the phytic acid content was only 42.6% of the phytate. An example of the isomer profile of a survey diet and a breakfast cereal can be seen in Figure 1. Of the total phytate, only 42.6% of the toasted oat cereal and 38.6% of the survey diet were phytic acid (Table I).

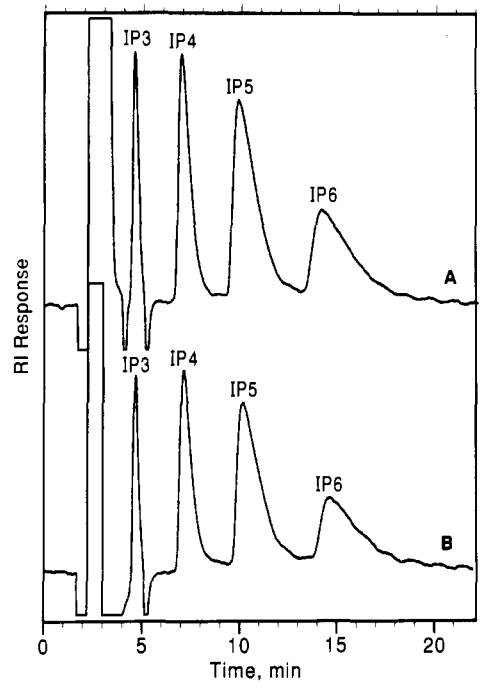

Figure 2. Reversed-phase HPLC of a phytic acid hydrolysate by ion-pair chromatography. Samples were injected onto a Hamilton PRP-1 column (4.1 × 150 mm, 5 μm). Eluting solvent was 0.015 M formic acid and 0.4% TBNOH in 51.8% methanol. The pH was adjusted to 4.3 with sulfuric acid. The column temperature was 40 °C, and the elution rate was 0.9 mL/min. IP3, inositol triphosphate; IP4, inositol tetraphosphate; IP5, inositol pentaphosphate; IP6, phytic acid. (A) Phytic acid hydrolysate; (B) phytic acid hydrolysate that has been absorbed onto an AG1-X4 column and then eluted with 0.7 M NaCl.

Isomers below IP5 do not seem to have a negative effect on bioavailability of minerals (Tao et al., 1986). Consequently, the HPLC method that can quantitate IP5 and IP6 in mixtures containing all of the isomers would more accurately assess the negative potential of the food on the bioavailability of minerals and trace elements. The AOAC method combines all of the isomers held by and eluted from the AG1-X4 column and reports them as phytic acid; thus, such values, in some circumstances, can be misleadingly high. Figure 2A is the HPLC profile of a phytic acid hydrolysate. Figure 2B is the HPLC profile of an aliquot of the same sample after absorption onto an AG 1-X4 column, desorption with 0.7 M NaCl, and exchange of H^+ for Na^+ (AG 50W-X8 column). The profiles and normalized ratios are almost identical, indicating that IP3, IP4, IP5, and phytic acid contained in the original extract will be in the solution to be analyzed for phosphorus and in the same approximate ratio. Whether IP2 and IP1 were also present is unknown. Similar results were obtained when a silica-based quaternary amine (SAX) column eluted with 2 N HCl (Lehrfeld, 1989) was substituted for the AG

1-X4 column eluted with 0.7 N NaCl. However, the ionic strength of the initial wash was reduced to 0.01 N HCl to prevent a substantial loss of IP3. To demonstrate the different values one can obtain from the same sample, we took a diet sample and a cereal sample, both of which contained appreciable amounts of partially hydrolyzed phytic acid, a sample of wheat bran that contained predominantly IP6 and a small amount of IP5, and a sample of commercial recrystallized sodium phytate and analyzed them for phytic acid by the AOAC method and the HPLC method. To determine if the food matrix interfered with the isolation or the analysis for phytic acid, the same samples were spiked with sodium phytate and reanalyzed by the AOAC method and HPLC. A summary of these results is in Table I.

A comparison of the AOAC and HPLC values for phytic acid (Table I) indicates that with the cereal and diet samples, which contain appreciable amounts of hydrolyzed phytic acid isomers, the AOAC method overestimates the phytic acid content by factors of 3 and 4.2, respectively. Because the concentrations of phytic acid were so low, the coefficient of variation with HPLC quantification could be as high as 20% (Lehrfeld, 1989). Even if one considers the extreme value and increases the concentration found by HPLC by 20%, the values found by AOAC will still be overestimated by factors of 2.5 and 3.5, respectively. The AOAC and HPLC values for samples containing over 83% IP6 are in reasonable agreement (wheat bran, spiked wheat bran, spiked cereal, spiked diet, and sodium phytate).

Another advantage of the HPLC method is the ability to quantitate inositol phosphates in the presence of nucleotides. Phillippy (Phillippy et al., 1988) pointed out the possible interference by nucleotides in the analysis of phytic acid. Diets containing meat, fish, mushrooms, yeast extracts, and sprouted grain products would be expected to contain naturally occurring nucleotides. In addition, the 5′-nucleotides are often added to many processed food products as flavor enhancers. Rossomando (1988), using similar column and solvent conditions (C-18 column and 10% methanol containing TBNOH buffered at pH 4.4), found that the monophosphate nucleotides eluted within 3 min and some triphosphates eluted within 5 min. With the 52% methanol used in the present assay the adenosine diphosphate peak precedes the IP3 peak by 0.7 min. The common mononucleotides would be expected to elute even earlier. Nevertheless, should a nucleotide elution peak coincide with an inositol phosphate peak, both can still be resolved by placing a UV detector in series with the RI detector and monitoring the effluent at 254 nm. The UV detector will quantitate only the nucleotide because the IPs are transparent in this region. The difference between the two calculated concentration values will be the concentration of inositol phosphate.

In summary, samples containing predominantly IP6 give the same value for phytic acid by AOAC and HPLC. However, when samples contain an appreciable amount of other inositol phosphates, the values from the two methods diverge because AOAC measures the phosphorus content of everything retained and subsequently eluted from the AG1-X4 column. Figure 2 demonstrates that if other inositol phosphates are extracted from the sample being analyzed, then they will be found in the eluent from the AG1-X4 column. Thus, the determination of phytic acid based on the phosphorus content of this eluent will overestimate the IP6 content. Consequently, the phytic acid content of processed foods (which often contain partially phosphorylated inositols formed by hydrolysis of phytic acid) should be quantified by HPLC.

ACKNOWLEDGMENT

We gratefully acknowledge the excellent technical assistance of Mary Hallengren and David Hill.

LITERATURE CITED

AOAC. *Official methods of analysis of the AOAC*; Association of Official Analytical Chemists: Arlington, VA, 1990; Method 986.11, final action 1988.

Harland, B. F.; Oberleas, D. Anion exchange method for determination of phytate in foods: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 667–670.

Lehrfeld, J. High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. *Cereal Chem.* **1989**, *66*, 510–515.

Morris, E. R. Phytate and dietary mineral bioavailability. In *Phytic Acid: Chemistry and Applications*; Graf, E., Ed.; Pilatus Press: Minneapolis, MN, 1986; pp 57–76.

Phillippy, B. Q.; Bland, J. M. Gradient ion chromatography of inositol phosphates. *Anal. Biochem.* **1988**, *175*, 162–166.

Phillippy, B. Q.; Johnston, M. R.; Tao, S. H.; Fox, M. R. S. Inositol phosphates in processed foods. *J. Food Sci.* **1988**, *53*, 496–499.

Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. Nutritional consequences of phytates. In *Phytates in Cereals and Legumes*; CRC Press: Boca Raton, FL, 1989; pp 81–110.

Rossomando, E. F. Enzyme assays and kinetic studies with HPLC. *Biochromatography* **1988**, *3*, 7–15.

Sandberg, A. S.; Ahderinne, R. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.* **1986**, *51*, 547–550.

Tao, S. H.; Fox, M. R. S.; Phillipy, B. Q.; Fry, B. E., Jr.; Johnson, M. R. Effect of inositol phosphates on mineral utilization. *Fed. Proc.* **1986**, *45*, 819.

Thompson, L. U.; Button, C. L.; Jenkins, D. J. Phytic acid and calcium affect the in vitro rate of navy bean starch digestion and blood glucose response in humans. *Am. J. Clin. Nutr.* **1987**, *46*, 467–473.

Received for review May 26, 1992. Revised manuscript received August 4, 1992. Accepted August 20, 1992.

Registry No. IP5, 25663-09-6; IP4, 27121-72-8; IP3, 27121-73-9; PA, 83-86-3.