Table III. Ratio of ω3 to ω6 Polyunsaturated Fatty Acids[a] of the Dorsal Muscle Lipids of Some Cultured and Wild Freshwater Fishes

fish	cultured mean	cultured SD	wild mean	wild SD
carp	0.59	0.16	1.18**	0.13
rainbow trout	2.77	0.16	3.68**	0.62
eel	2.96	0.79	1.43**	0.12

[a] For the ratio of ω3 to ω6 polyunsaturated fatty acids, see Materials and Methods. Key: **, $P < 0.01$ compared to the cultured fish.

and rainbow trout > wild eel > wild carp > cultured carp. It may be inferred from these results that the beneficial effects of freshwater fishes on cardiovascular disease would be similar in order.

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Phosphorus-31 Nuclear Magnetic Resonance Spectroscopic Determination of Phytate in Foods

Eugene P. Mazzola,* Brian Q. Phillippy, Barbara F. Harland,[1] Ted H. Miller,[2] Jennifer M. Potemra,[2] and Eileen W. Katsimpiris[3]

A direct quantitative method for the determination of phytate in foods, using phosphorus-31 Fourier transform nuclear magnetic resonance spectroscopy, has been substantially modified to improve its convenience and accuracy and eliminate interference from paramagnetic ions. An ion chromatographic method for phytate has also been employed, and good agreement was obtained between the two procedures.

Phytate (*myo*-inositol, hexakis(dihydrogen phosphate); Figure 1) is a naturally occurring organic substance that binds nutrient mineral cations, making them unavailable for nourishing the body (Oberleas et al., , 1966). It is found in most fruits, vegetables, and grains. Thus, the measurement of phytate is especially important in vegetarian diets (Harland and Peterson, 1978), in diets selected for their high content of plant fiber, and in diets that may be marginally deficient in minerals. There is increasing need for a precise method of determining phytate as Americans change their diets to incorporate more foods of plant origin. When dietary phytate levels are high and dietary mineral intakes are low, mineral status in animals and humans may be compromised. A tool that may be employed for estimating mineral bioavailability in phytate-containing diets is the phytate:mineral molar ratio. For a description of the calculation of this ratio, as well as a comprehensive list of phytate-containing foods, see Oberleas (1983b) and Oberleas and Harland (1981).

Food and Drug Administration, Divisions of Chemistry and Physics (E.P.M., T.H.M., J.M.P., E.W.K.), Food Technology (B.Q.P.), and Nutrition (B.F.H.), Washington, D.C. 20204.
[1] Present address: Department of Human Nutrition and Food, Howard University, Washington, DC 20059.
[2] Summer Intern, American University, Washington, DC 20016.
[3] Summer Fellow, Society for Applied Spectroscopy, Baltimore-Washington Chapter.

A detailed account of the history of phytate methodology development may be found in a review by Oberleas (1983a). Heubner and Stradler (1914) developed the first method for quantifying phytate, based on the fact that, in the presence of excess ferric ion, phytate is insoluble in dilute acid. Various modifications in the acid extraction of foods and feeds, in the purification of phytate, and in the quantification of phytate have been used through the years.

Many attempts have been made to simplify and shorten the analysis of foods for phytate, but because analytical methods have been laborious and imprecise, understanding the role of phytate in the bioavailability of minerals from plant food has been delayed. Averill and King (1926), Young (1936), and Latta and Eskin (1980) modified the iron precipitation procedure, which shortened analytical time. Harland and Oberleas (1977) developed a method using step gradient elution with anion-exchange column chromatography. Ellis and Morris (1982) compared the anion-exchange method to an iron precipitation method. However, the ability of these methods to discriminate against lower inositol phosphates (pentaphosphates, tetraphosphates, etc.), which have been detected in certain processed foods (deLange et al., 1961; O'Neill et al., 1980; Phillippy and Johnston, 1985), has not been demonstrated.

This lack of specificity prompted O'Neill and co-workers (1980) to develop a ^{31}P Fourier transform nuclear magnetic resonance (^{31}P FT NMR) spectroscopic technique for a more precise analysis of foods for phytate. NMR is well suited for this task because the ^{31}P NMR spectrum of

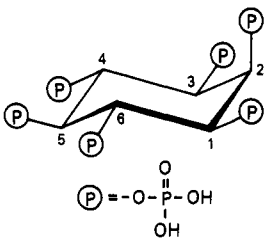

Figure 1. Structure of phytate at pH 4.5 (Isbrandt and Oertel, 1980).

phytate is fairly distinctive, consisting of four signals that bear a 1:2:2:1 relationship to one another at certain pH ranges.

Graf and Dintzis (1982a,b) and Camire and Clydesdale (1982) modified the anion-exchange purification and concentration procedure, using reversed-phase HPLC in the determinative step. Lee and Abendroth (1983) improved the HPLC procedure by using an ion-pairing technique, and Phillippy and Johnston (1985) recently introduced a method using ion chromatography, which is a form of HPLC utilizing ion-exchange resins.

Because of the heightened interest in phytate, development of a confirmatory procedure that would be specific for phytate was desirable. Two considerably different analytical methods were performed, which yielded comparable phytate values for some cereal foods. The first is an ion chromatographic method; the second contains several substantial modifications of the ^{31}P FT NMR determinative step of O'Neill et al. (1980). These are (1) use of the method of standard addition, rather than an internal standard, for elimination of food matrix effects (Willard et al., 1981), (2) use of sufficient concentrations of EDTA to remove NMR signal interference due to paramagnetic ions (Becker, 1980), and (3) elimination of sucrose as a viscosity-increasing relaxation agent (Becker, 1980).

EXPERIMENTAL SECTION

Materials. Sodium phytate was purchased from Sigma Chemical Co., St. Louis, MO. All reagents were used as received without further purification. Certified wheat bran was purchased from the American Association of Cereal Chemists, St. Paul, MN; soy isolate was purchased from the Ralston-Purina Co., St. Louis MO; texturized vegetable protein and corn bran were purchased from A. E. Staley Manufacturing Co., Decatur, IL; and wheat bread was purchased locally.

Phytate Determination by Ion Chromatography. Wheat bread was dried in an 85 °C oven and homogenized in a Waring blender; the other foods were extracted as received. Five grams of sample was placed in a 200-mL Nalgene bottle, and 100 mL of 1.2% HCl was added. For the soy isolate, the HCl was added in steps and stirred with a glass rod to form a paste, thereby preventing clumping. The mixtures were shaken 30 min in a mechanical shaker (Precision Scientific Co., Chicago, IL) and filtered through coarse filter paper. The extracts were assayed by ion chromatography according to Phillippy and Johnston (1985).

Phytate Determination by ^{31}P NMR Spectroscopy. Food extracts were obtained as described in the previous method. The extracts were vacuum filtered, six 10-mL aliquots were taken from the filtrate, 1.5 mL of 0.5 M Na_4EDTA was added to each aliquot, and phytate standard additions were made to five of the aliquots for each food. The equivalent quantities of sodium phytate used (58.8% purity by visible spectrophotometry) and the percentage of phytate in the original test samples to which they correspond were as follows: (wheat bran) 75 mg (15%), 60 mg (12%), 45 mg (9%), 30 mg (6%), 15 mg (3%); (isolated soy and texturized vegetable protein) 45 mg (9%), 35 mg (7%), 25 mg (5%), 15 mg (3%), 5 mg (1%); (corn bran and wheat bread) 35 mg (7%), 25 mg (5%), 15 mg (3%), 5 mg (1%), 2.5 mg (0.5%). Each solution was adjusted to pH 4.5 with sodium hydroxide, and the solution was diluted to 15 mL (0.05 M EDTA).

^{31}P FT NMR spectra, described by 8192 data points, were obtained at 32 MHz with proton noise decoupling on a Varian Associates FT-80A NMR spectrometer. Spectral widths of 4 kHz were used, which correspond to acquisition times of ca. 1 s. Pulse widths of 8 μs were employed, which correspond to tip angles of ca. 40° with 10-mm sample tubes. Volumes of 3 mL were used for detection of phosphorus NMR signals, and 3000 transients were accumulated for each solution. Spectra were plotted with 40-fold horizontal expansion, i.e., 100-Hz plot widths, and the intensity of axial phosphate signals was determined by standard electronic integration.

Unknown analyte concentrations were determined from correlations of signal intensity vs. phytate concentration by least-squares analysis. They correspond to negative x intercepts in plots of signal intensity (y axis) vs. concentration (x axis) (Willard et al., 1981).

RESULTS AND DISCUSSION

In the pH ranges 1–5 and 10–12, the ^{31}P NMR spectrum of phytate consists of four signals in a 1:2:2:1 relationship as predicted by the symmetry of the molecule (O'Neill et al., 1980; Isbrandt and Oertel, 1980; Figure 1). In accordance with O'Neill and co-workers (1980), the signal due to the 2-axial phosphate group was well-resolved with respect to both the orthophosphate and the other equatorial phytate resonances at pH 4.5. The signal appeared at lowest field and was selected for integration. In addition, integration of the other three phytate signals, for verification of the approximate 1:2:2:1 intensity ratios, served as a check against erroneous contribution to the axial phosphate integrals due to accidental chemical shift equivalence.

While the ^{31}P FT NMR report of O'Neill et al. (1980) addressed the question of lower inositol phosphate nonspecificity, it was not without disadvantages: (1) phytate values for foods that contain appreciable amounts of paramagnetic ions, e.g., iron and manganese, were lower than those determined by other methods; moreover, no phytate signal was observed for isolated soy, which is known to contain phytate; (2) lengthy pulse delay times had to be employed to ensure complete relaxation of the internal standard; (3) large quantities of sucrose were used to reduce the longitudinal relaxation time (T_1) of both phytate and the internal standard via increased viscosity (Becker, 1980). These highly concentrated sugar solutions were difficult to both prepare and maintain in solution. In addition, pH adjustment was both arduous and uncertain, and electrode fatigue was a serious liability.

The problem of long T_1 relaxation times for phytate, and especially for the internal standard, which resulted in the use of both lengthy pulse delay times and viscous sucrose solutions, was obviated by use of the standard addition method of analysis. Several advantages are realized by this technique: (1) phosphorus NMR spectra can be obtained more quickly under standard, rapid pulsing, noise-decoupled conditions without either the requirement of pulse delay times for nuclear Overhauser enhancement suppression (Martin et al., 1980) or the employment of unwieldy, thick sucrose solutions; (2) matrix effects, which are a potential source of error in food analysis, can be eliminated. The absence of interferences due to matrix

Table I. Comparison of Phytate Values Obtained by NMR and Ion Chromatography

	phytate, % w/w				
method	wheat bran	textrzd veg protein	isol soy	wheat bread	corn bran
^{31}P NMR[a]	3.3	1.5	1.1	0.6	0
ion chromatogr[b]	3.2	1.4	1.0	0.7	0

[a] Precision ca. ±10%. [b] Precision ca. ±5%.

effects can be demonstrated either by concentration–response relations that have correlation coefficients of essentially unity or, graphically, by straight-line plots.

The problem of paramagnetic species, which was likely responsible, at least in part, for the low phytate values reported by O'Neill et al. (1980) for certain foods, was resolved by competitive complexation with EDTA. Numerous foods such as wheat bran and soy are rich (from an NMR standpoint) in paramagnetic ions, principally iron, manganese, cobalt, and copper. The dietary concern about phytate stems, of course, from its complexation of such multivalent cations. Chelation by phytate with relatively small amounts of these paramagnetic species, moreover, caused complete disappearance of the phytate phosphorus NMR spectrum via extreme broadening of the resonance lines. Since EDTA–metal complexes are, in general, both kinetically and thermodynamically quite stable, it was felt that EDTA might preferentially bind the paramagnetic ions and thereby effectively separate them from the phytate. Excess quantities of EDTA were, therefore, added to food extracts before NMR analysis to sequester the majority of multivalent cations. Chelation of sufficient concentrations of paramagnetic ions in this manner provided sharp, well-resolved phytate phosphorus NMR spectra for all the foods examined including wheat bran, which is known to be rich in iron. Phytate NMR signals were also observed for isolated soy, where O'Neill and co-workers, in the absence of EDTA, saw none. In addition, EDTA affected the signal intensity of neither phytate standards in the absence of paramagnetic species nor phytate in bran samples in the presence of 10-fold excesses of EDTA (0.75 M).

The phytate content of several foods was determined, and the results were in good agreement with values obtained by ion chromatography (Table I). One-hour accumulation times were sufficient to permit spectra to be obtained that could be reproducibly integrated, i.e., with precisions ranging from ca. 5% for the smaller phytate standard additions (1–3%) to ca. 1% for the larger additions. Concentration–response plots for each of the foods were linear even at the higher phytate concentrations, indicating that food matrices were still intact. Correlation coefficients for the points that determined these lines were 0.99985 or greater. No evidence of phytate hydrolysis was observed under the experimental conditions used for extraction, separation, or analysis.

Lower inositol phosphates are currently being synthesized. Metal binding capacities will be determined as part of the assessment of their dietary significance. ^{31}P NMR spectra should be valuable both in the identification of these partial phytate hydrolysis products and in investigations of their metal-binding properties. Owing to the definitiveness of the phytate ^{31}P NMR spectrum, this method should be useful when the presence of lower inositol phosphates is suspected or as a substantially different confirmatory analytical technique.

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Registry No. Phytate, 83-86-3.

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