Determination of Phytate in Turkish Diet by Phosphorus-31 Fourier Transform Nuclear Magnetic Resonance Spectroscopy

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The phytate content of daily diet samples from upper middle class families in Turkey was determined by phosphorus-31 Fourier transform nuclear magnetic resonance spectroscopy (^{31}P FT NMR). Samples, collected by a duplicate-portion technique, were homogenized and freeze-dried. Diet samples were stirred for 0.5 h in aqueous 3% (w/v) trichloroacetic acid, EDTA was added, and the pH was adjusted to 4.5 ± 0.5 before analysis. The average phytate content of 10 diet samples was found to be 1.55 ± 0.70 g/kg.

Phytate, *myo*-inositol 1,2,3,4,5,6-hexakis(dihydrogen phosphate), is the highest analogue of a series of widespread naturally occurring inositol phosphates (Oberleas, 1971) found especially in plant seeds and in many roots and tubers (Figure 1). Due to its strong chelating power, phytate acts as a carrier or storage site for trace minerals during plant growth (Reddy et al., 1982). Phytic acid is nutritionally important since mineral deficiencies can be induced by phytate in humans and monogastric animals whose diets consist predominantly of whole grains and legumes which have high phytate concentrations (Miller et al., 1980).

Processes such as cooking, fermentation, germination, etc. cause hydrolysis of phytate (O'Neill et al., 1980). Several established methods of phytate determination were evaluated in the past. Of these, iron analysis methods (Garcia-Villanova et al., 1982) and some chromatographic methods (Harland and Oberleas, 1985) were used the most. These methods, however, have the disadvantages that they require the measurement of the other phosphate compounds and are too time-consuming.

Many attempts were made to simplify and shorten the analysis of foods for phytate. In a recent study, O'Neill and co-workers (1980) developed a ^{31}P Fourier transform nuclear magnetic resonance (^{31}P FT NMR) spectroscopic technique for a more precise analysis for the phytate content of foods.

In the present study the ^{31}P NMR spectroscopic technique was further improved and used to determine the phytate concentration of daily diet samples.

EXPERIMENTAL SECTION

Sample Collection. Parameters considered for the purpose of selection of diets to be collected were socioeconomic, cultural, age, and health levels of the subjects, geographic region of the subjects, and season of the year. Daily diet samples were collected from families of members of the Chemistry Department of the Middle East Technical University (METU). One healthy individual, between 20 and 60 years old, from each chosen family participated in the study. Rice and wheat samples were purchased from the local market.

A duplicate-portion technique was used as the sample collection method. Samples were collected for three consecutive days from each subject. During meal time, the servings were duplicated and samples were collected in an equal amount as consumed by applying same eating system. Everything consumed was collected, excluding inedible parts, skin of fruits, seeds, bones, etc. Details of the sample collections and preparations are given by Mumcu et al. (1988) and Mumcu and Aras (1988).

Homogenization and Freeze-Drying. The collected diet samples were homogenized for 2 min at 1500 rpm and then for 5 min at 3000 rpm in a Robot Coupe Model R8 homogenizer with a plastic container and special titanium blades. After homogenization, diet samples were freeze-dried with a Labconco Model 75050 freeze-dryer.

Preparation of Samples for Analysis. (*a*) *Solutions.* The phytate stock solution was prepared by dissolving 0.25 g of sodium phytate in 5 mL of 3% (w/v) trichloroacetic acid. The sodium salt of phytic acid with a 98% purity, containing ca. 11% water (Sigma, lot no. P-5756), was used without further purification. Trichloroacetic acid solution was prepared by dissolving 3.0 g of trichloroacetic acid in 100 mL of distilled water. Phosphorus acid solution was prepared by dissolving 0.25 g of H_3PO_3 in 100 mL of distilled water. Liquid deuterium oxide, minimum isotopic purity of 99.7 atom % D was obtained from Merck and Co., Inc.

The standard calibration solutions of 0–2.0% phytate were prepared by pipeting different volumes of the phytate stock solution (from 0.1 to 0.5 mL) into NMR test tubes. The calibration curve was drawn by plotting the peak area ratio versus percent phytate.

(*b*) *Diet Samples.* A 2.5-g diet sample was stirred vigorously for 0.5 h in an aqueous solution of 10 mL of 3% (w/v) trichloroacetic acid to extract the phytate. Since this solution is unstable, it was prepared daily as required. After the extract solution was vacuum filtered through filter paper, about 0.1 g of ethylenediaminetetraacetic acid (EDTA) was added and the acidity was adjusted to pH 4.5 ± 0.5 by addition of 0.1 N NaOH. Excess quantities of EDTA were added to food extracts before NMR analysis in order to chelate sufficient concentrations of potentially interfering paramagnetic ions and allow phytate to give a well-resolved phosphorus NMR spectrum. Two milliliters of extract was pipeted into the NMR tube containing 1.0 mL of 0.25% H_3PO_3, which was added as an internal standard. At the end 0.4 mL of D_2O was added in order to lock the instrument.

(*c*) *Rice and Wheat Samples.* For the analysis of uncooked rice and wheat samples, the same procedure was applied. These were ground and sieved only, but not homogenized or freeze-dried.

^{31}P FT NMR Spectroscopy. The ^{31}P NMR experiments were performed on a Bruker AC 80 NMR spectrometer at 32.438 MHz in the Fourier transform mode. Spectrometer settings were as follows: spectral width, 4000 Hz; number of data points/FID, 16K; pulse width, 8 μs, which corresponds to tip angles of ca. 40°; number of acquisitions, 2000; interpulse delay, 2 s; 10-mm NMR tubes were used for ^{31}P NMR analysis. The ^{31}P chemical shifts were measured with reference to an internal standard of phosphorus acid solution at pH 2.3.

The calibration and phytate phosphate peak areas were calculated from the triangulated signals resulting from peak extrap-

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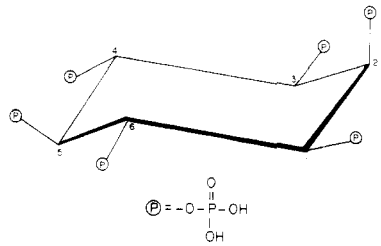
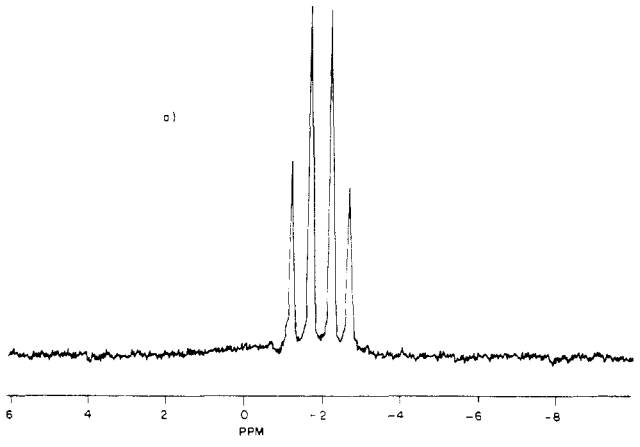


Figure 1. Structure of phytate.



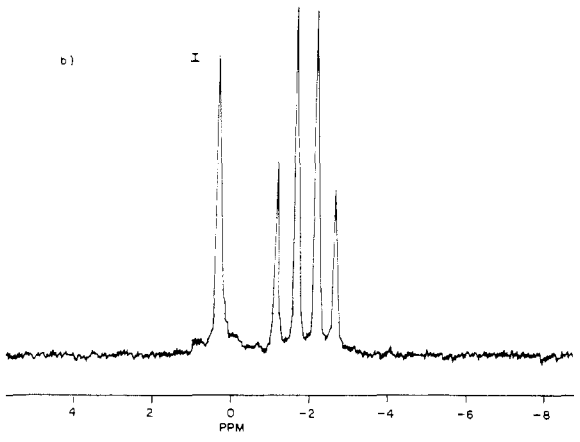


Figure 2. ^{31}P NMR spectrum of phytate (a) and uncooked rice (b) (I = internal standard).

olation of these spectra. The phytate content of the sample is calculated from the following equation (O'Neill et al., 1980)

$$\% \text{ phytate} = \frac{\text{phytate peak area}}{\text{standard peak area}} \times \frac{V}{S} \times C$$

where V is the volume of 0.25% H_3PO_3/trichloroacetic acid solution used in the extraction (mL), C is the reciprocal of the slope, and S is the sample weight.

Nuclear Overhauser enhancement (NOE) arises from proton noise decoupling of the ^{31}P signal. While it may be eliminated by the addition of paramagnetics (Glonek, 1976), the procedure was ruled out on the grounds of irreproducibility. For a given value of T_1 and pulse angle, the residual NOE for any repetition time in a gated experiment can be estimated. In this study the inverse gated-decoupling experiment was used to eliminate the NOE effect with a suitable D_1 time.

RESULTS AND DISCUSSION

Pure phytate gives a ^{31}P NMR spectrum with four resonances with the intensity ratios 1:2:2:1 due to the symmetry of the molecule (Figure 2a). As already mentioned, processes such as cooking, fermentation, germination, etc. cause hydrolysis of phytate (O'Neill et al., 1980). It was reported that phytate exists in aqueous solutions as the 1-ax/5-eq conformer at high pH. ^{13}C NMR measurements of phytate show that a 1:2:2:1 intensity pattern is associated with the 1-ax/5-eq conformation. This is found over a wide pH range (0.5–8). However, at high pH, a ring conformational change occurs. As one might expect the high-pH conformer is of the 5-ax/1-eq form, which gives a 4:1:1 pattern. As a result of this, phytate phosphorus gives a single broad peak instead of four peaks, and the C-2 phosphorus peak is lost (Isbrandt and Oertel, 1980).

Phytate phosphate NMR peaks of 1:2:2:1 ratio are seen in the spectra that we obtained from our stock solutions and from extracts of uncooked rice and wheat samples (Figure 2). On the other hand, in cooked diet samples only a single broad phosphorus peak is obtained (Figure 3).

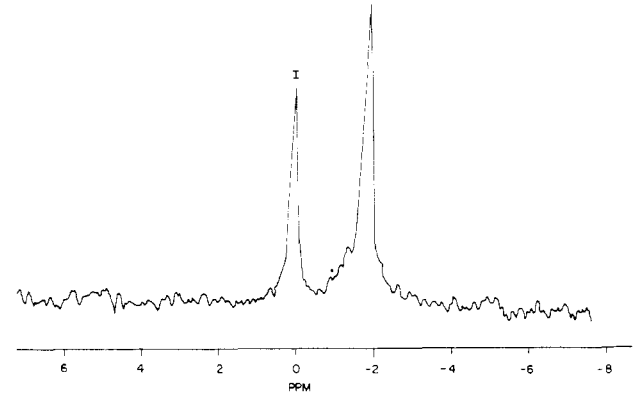


Figure 3. ^{31}P NMR spectrum of a cooked diet sample (I = internal standard).

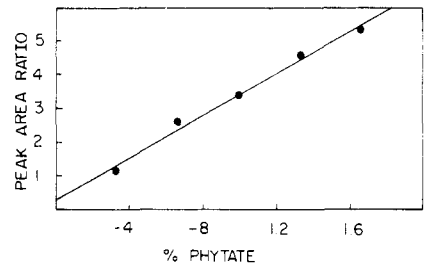


Figure 4. Experimental calibration curve by ^{31}P NMR.

Table I. Phytate Content (g/kg) of Wheat and Rice

sample	this work	lit. value[a]
rice	1.51 ± 0.07	1.4–22.0
wheat	7.50 ± 0.05	6.0–13.5

[a] Reddy et al., 1982.

In earlier reports, O'Neill et al. (1980) and Mazzola et al. (1986) presented plots of peak area ratio of phytate C-2 phosphorus to standard. In our experimental calibration curve, however, the peak area ratio is calculated as the ratio of the value of the integral over all four phytate peaks of the samples to the integral over the standard peak. Figure 4 shows the experimental calibration curve by NMR with this total peak area calculation.

The results for phytate contents of wheat and rice samples are in agreement with literature values (Table I).

We have also determined the phytate content of NIST Reference Material 8431a, human mixed diet, by the same technique. The experimental result of three determinations is 2.10 ± 0.10 g/kg, compared to 2.10 g/kg given by NIST. The agreement is very good.

The results from METU samples obtained by the ^{31}P

Table II. Phytate Content (g/kg) of METU Diet Samples (Based on Dry Weight)

sample	phytate content
METU-1	0.97 ± 0.28
METU-2	1.31 ± 0.55
METU-3	1.34 ± 0.11
METU-4	2.17 ± 0.34
METU-5	0.50 ± 0.20
METU-6	1.79 ± 0.08
METU-7	2.84 ± 0.11
METU-8	1.60 ± 0.21
METU-9	2.32 ± 0.47
METU-10	0.68 ± 0.15
average	1.55 ± 0.70
median	1.47

NMR technique are given in Table II. The average phytate content of 10 samples is found to be 1.55 ± 0.70 g/kg. Unfortunately, in the literature there are not many results on cooked total diet samples. Therefore it is difficult to compare our results with others. The phytate content of the samples can be correlated to the foods from which the samples came. In general, when the consumption of wheat and wheat products was high, the phytate content was also high. When the diet samples of METU-5 and METU-9 are examined, we see that the amount of consumed bread and rice pilaf is higher in METU-9 (18 slices of bread, 3 portions of pilaf) than METU-5 (8 slices of bread, 2 portions of rice pilaf), so the phytate content of METU-9 is also high.

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