Phytate Content of Taiwanese Diet Determined by ^{31}P Fourier Transform Nuclear Magnetic Resonance Spectroscopy

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Phytate and trace element concentrations of the total daily diet of upper social groups in Taiwan were determined by ^{31}P FT NMR spectroscopy and by instrumental neutron activation analysis, respectively. Results of 15 samples are 2.21 ± 0.71 g of phytate/kg of dry diet with the range 1.07–3.76 g/kg. The molar ratios of phytate to Zn and Ca were calculated, and bioavailabilities of these elements are discussed.

INTRODUCTION

Phytic acid (*myo*-inositol 1,2,3,5/4,6-hexakis[dihydrogen phosphate]) is a common constituent of most cereal grains and some vegetables and fruits (Oberleas, 1973; Cheryan, 1980). Typical phytic acid contents of some cereals and oil seeds are (grams per kilogram, dry basis) soft wheat, 11.3; rice, 8.9; soyabeans, 14; peanut meal, 17; sesame meal, 50. In general, phytates constitute about 1–2% by weight of many cereals and oilseeds, although 3–6% have been reported for some varieties. Also note that in general 60–90% of all phosphorus in these seeds is present as phytic phosphorus.

The physiological role of phytic acid has been controversial. Since it contains, for some important cereal and vegetables, up to 60–90% phosphorus, as phytic phosphorus, one may say that phytic acid is the main store of phosphorus. In the body, phosphorus becomes available during germination by sequential hydrolysis catalyzed by the enzyme phytase, which is also usually present in the seeds (O'Neill et al., 1980). On the other hand, since phytic acid is negatively charged at all pH values normally encountered in foods, it will easily make complexes with positively charged cations and/or proteins. Also, it is found that, owing to its very tight binding to proteins, phytate is one of the most effective chemical agents for decreasing the affinity of hemoglobin for oxygen by making complexes with deoxyhemoglobin (Benesch, 1968; Isbrandt, 1980). Although the precise nature and the extent of binding of phytic acid with cations and proteins and their physicochemical behavior and nutritional role are not known completely (Lee, 1988), the insolubility of these complexes is a major reason for their adverse nutritional effect in biological systems. Therefore, the mere presence of a mineral in a diet is no indication of its bioavailability, the percentage that can be absorbed and utilized by the body.

Chinese diets consist of large amounts of rice and soyabeans; both are very rich in phytate. Therefore, it is important to measure phytate and trace element content of a diet and to calculate their molar ratios to have some ideas on the bioavailabilities of these elements.

Many attempts have been made to determine the amount of phytate in diet. Ion exchange (Harland and Oberleas, 1986; Ellis and Morris, 1986), high-performance liquid chromatography (Lehrfeld, 1989), iron precipitation (Kikunaga, 1985), and ^{31}P NMR methods (O'Neill et al., 1980; Mazzola et al., 1986; Ersöz et al., 1990) have been used for this purpose. Among these, high-performance liquid chromatography and ^{31}P NMR methods can separate not only hexaphosphate but also other hydrolysis products.

Phosphorus nuclear magnetic resonance spectroscopy has been applied to a wide range of biochemical problems. Due to the five equatorial–one axial or five axial–one equatorial positions of phosphate groups in phytate (Figure 1), its NMR spectrum is fairly distinctive, consisting of four signals that bear a 1:2:2:1 relationship to one another at pH ranges 1–5 and 10–12. As seen from Figure 1, the 1,3 and 4,6 positions are equal, giving rise to a 1:2:2:1 signal ratios. During hydrolysis, the lower inositol phosphates give rise to additional signals, some of which are resolved from those of the hexaphosphate (Isbrant and Oertel, 1980; Wise et al., 1983). All of these extra peaks are to high field of the phytate C-2 phosphate and do not interfere with the phytate determinations. However, the appearance of extra peaks in the spectrum provides a method for observation of hydrolysis. In this work we used ^{31}P FT NMR spectroscopy to determine phytate content of 15 diet samples. The Zn, Ca, Fe, and Se contents of these samples were also determined by instrumental neutron activation analysis (Liu et al., 1991).

EXPERIMENTAL PROCEDURES

Collection of Diet Samples. The purpose of this work was to study the trace element and phytate intake of a healthy Taiwanese population. Here, only certain socioeconomic and educational groups were considered. They were five graduate students (THGS) from National Tsing Hua University, five male staff members (THFS), and five female members of faculty families (THFF). These subjects all live in Hsinchu city. Most of the food taken originates from the island, especially from the south, west, and costal plain. Therefore, the foods taken are not local specialties but rather representative of typical foods in Taiwan.

Information about the subjects is given in Table I. Samples were collected by applying a duplicate portion technique. In this technique, everything consumed by the subjects during the full 3-day period is collected in clean polyethylene containers (Mumcu et al., 1988; Liu et al., 1991).

Samples were homogenized with a Polytron homogenizer (Model PT-II, Inyon, Switzerland) for 5 min at 4000 rpm and then for 10 min at 25 000 rpm. After homogenization, they were freeze-dried and powdered in an all-Teflon cylindrical mill and sieved by a 0.9 × 0.8 mm sieve. Samples were weighed before and after homogenization and freeze-drying to determine their water content. Details of sample collection, criteria for selection of subjects, homogenization, possibly trace element contamination

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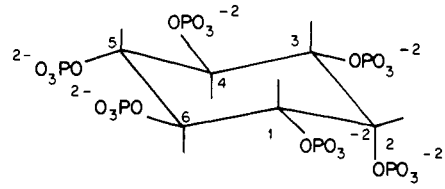

Figure 1. Structure of phytate in five-equatorial-one-axial position.

Table I. Information about the Subjects and Their Dry Weight of Daily Intakes

sample ID[a]	sex	body wt, kg	sample consumed in dry wt,[b] g/day
THGS-1	M	77	433
THGS-2	F	45	320
THGS-3	M	65	451
THGS-4	F	49	289
THGS-5	M	60	465
THFF-1	F	70	405
THFF-2	F	51	322
THFF-3	F	38	248
THFF-4	F	52	254
THFF-5	F	56	251
THFS-1	M	78	308
THFS-2	M	68	428
THFS-3	M	80	418
THFS-4	M	63	494
THFS-5	M	62	392
male av[c]		69 ± 8	424 ± 57
female av[c]		52 ± 10	298 ± 56
all av[c]		61 ± 13	365 ± 85

[a] THGS, Tsing Hua graduate students; THFF, Tsing Hua faculty families; THFS, Tsing Hua faculty staff. [b] Dry weights are the averages of 3-day collection. [c] One standard deviation from the arithmetic mean.

during homogenization and freeze-drying can be found elsewhere (Mumcu et al., 1988; Liu et al., 1991).

Determination of Trace Elements by Instrumental Neutron Activation Analysis. Aliquots of 250–300-mg diet samples in small cleaned polyethylene bags along with IAEA mixed human diet standard H-9 and animal muscle standard H-4 were irradiated in the Tsing Hua open-pool reactor (THOR) with a thermal neutron flux of 2.0×10^{12} n cm^{-2} s^{-1}. Both short (5 min) and long irradiations (30 h) were carried out. As a result, concentrations and daily intakes of Cl, Na, K, Ca, Mg, Al, Fe, Zn, Br, Mn, Rb, Se, Cr, Co, Cs, and Sc were determined. Complete elemental analyses and their results are presented elsewhere (Liu et al., 1991).

^{31}P FT NMR Studies. The following solutions were used during the extractions of phytate from the diet samples for NMR studies.

1. Phytate stock solution was prepared by dissolving 0.2 g of sodium phytate (Sigma P-5756, with 98% purity, 11% water) in 5 mL of 3% (w/v) trichloroacetic acid (CCl_3COOH).

2. CCl_3COOH was prepared by dissolving 3.0 g of CCl_3COOH in 100 mL of distilled water.

3. H_3PO_3 solution was Merck 30% H_3PO_3 solution diluted to 0.24% H_3PO_3.

4. D_2O had a minimum isotopic purity of 99.8%; Merck D_2O was used.

5. Standard phytate solutions, 0.1–0.5-mL phytate stock solutions which contain 0–2.0% phytate, were used to draw a calibration curve. The calibration curve was plotted by drawing peak area ratios of the phytate C-2 peak to that of H_3PO_3 vs percent (w/v) phytate.

Phytate Extraction. Diet samples (2.5 g) were stirred vigorously in 15 mL of 3% (w/v) CCl_3COOH solution. After 10 min of centrifuging at 3000 rpm, 0.1 g of Na_4EDTA was added to 5 mL of supernatant and the pH was adjusted to 4.5 ± 0.5 by adding 0.1 N NaOH. Excess quantities of EDTA were added to the extract before NMR analysis to complex multivalent cations. Chelation of sufficient concentrations of paramagnetic ions allows phytate phosphorus to give well-resolved NMR spectra. Finally, 0.5 mL of 0.24% H_3PO_3 as an internal standard and 0.5 mL of D_2O were added to lock the instrument. From this solution, 2.5 mL is pipetted for NMR measurements. The flow chart of extraction of phytate from diet samples is given in Figure 2.

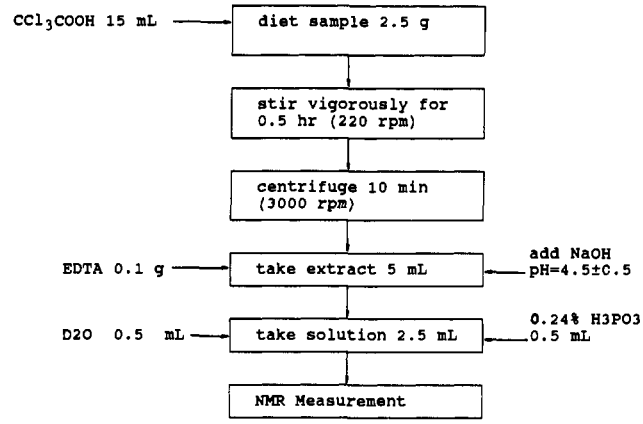

Figure 2. Steps in extraction of phytate from diet samples for ^{31}P FT NMR measurements.

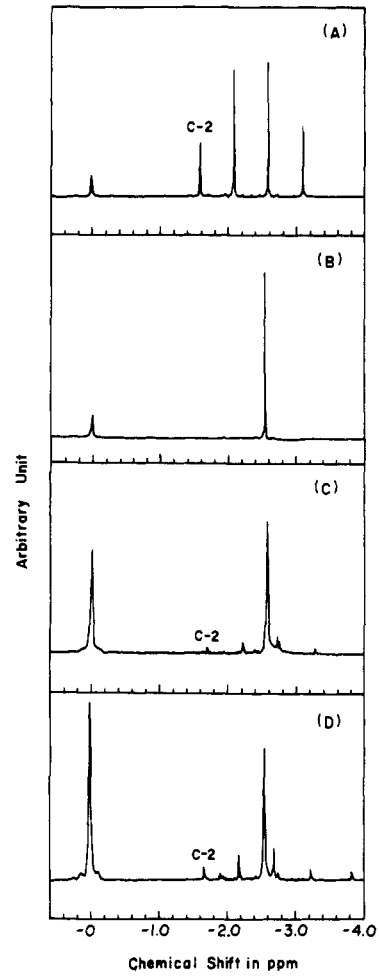

Figure 3. ^{31}P NMR spectrum of pure phytate (A), phosphate (B), U.S. diet TDD-1D (C), and Taiwanese diet THFF-3 (D). Vertical coordinates are in arbitrary units. The peak at 0 ppm is of the H_3PO_3 used as internal standard for the intensity calculation.

^{31}P FT NMR Spectroscopy. The ^{31}P NMR experiments were carried out by a Bruker MSL 300 FT NMR spectrometer at 121.49 MHz in the Fourier transform mode with deuterium lock. The ^{31}P chemical shifts were measured with reference to internal standard of H_3PO_3 solution; downfield shifts were positive. The spectrum width was 4000 Hz with 12-μs pulses applied with a repetition time of 2 s. The free induction decay was accumulated into 16K data points.

Table II. Phytate and Trace Element Content on Dry Weight Basis of Taiwanese Diet Samples

sample ID	phytate, g/kg	phytate, mmol/kg (×1000)	Zn, mg/kg	Zn, mol/kg (×1000)	Ca, g/kg	Ca, mol/kg (×1000)	molar ratios [Ph]/[Zn]	[Ph]/[Ca]	[Ph][Ca]/[Zn]
THGS-1	1.71 ± 0.20	2.59	18.0 ± 0.4	0.277	0.85 ± 0.05	21.2	9.4	0.122	0.20
THGS-2	3.11 ± 0.26	4.71	13.5 ± 0.4	0.208	0.88 ± 0.08	22.0	22.6	0.214	0.50
THGS-3	2.43 ± 0.24	3.69	19.3 ± 0.6	0.297	0.79 ± 0.02	19.8	12.4	0.186	0.25
THGS-4	1.55 ± 0.27	2.35	24.4 ± 0.8	0.375	1.00 ± 0.08	25.0	6.3	0.094	0.16
THGS-5	2.28 ± 0.26	3.45	12.7 ± 0.4	0.195	1.52 ± 0.04	38.0	17.7	0.091	0.67
THFF-1	1.29 ± 0.26	1.96	20.5 ± 0.9	0.315	1.47 ± 0.11	36.8	6.2	0.094	0.23
THFF-2	3.02 ± 0.12	4.57	17.7 ± 2.2	0.272	1.60 ± 0.09	40.0	16.8	0.114	0.67
THFF-3	1.95 ± 0.14	2.95	24.9 ± 1.4	0.383	1.36 ± 0.06	34.0	7.7	0.087	0.26
THFF-4	2.10 ± 0.15	3.17	19.8 ± 1.2	0.305	0.63 ± 0.02	15.8	10.4	0.200	0.16
THFF-5	1.87 ± 0.11	2.84	16.9 ± 1.3	0.260	0.86 ± 0.07	21.5	10.9	0.132	0.23
THFS-1	1.07 ± 0.37	1.62	22.6 ± 1.7	0.348	1.50 ± 0.10	37.5	4.65	0.043	0.17
THFS-2	1.74 ± 0.07	2.64	27.5 ± 2.2	0.423	1.48 ± 0.06	37.0	6.24	0.071	0.23
THFS-3	2.02 ± 0.22	3.06	24.1 ± 1.5	0.371	1.39 ± 0.04	34.8	8.25	0.088	0.29
THFS-4	2.27 ± 0.21	3.43	18.0 ± 1.1	0.277	2.07 ± 0.14	51.8	12.4	0.066	0.64
THFS-5	3.76 ± 0.56	5.70	21.8 ± 1.5	0.335	1.70 ± 0.03	42.5	17.0	0.134	0.72
male av	2.16 ± 0.78	3.27 ± 1.18	20.5 ± 4.5	0.315 ± 0.070	1.41 ± 0.42	35.3 ± 10.5	10.4 ± 1.7	0.092 ± 0.011	0.40 ± 0.24
fem av	2.13 ± 0.69	3.23 ± 1.05	19.7 ± 4.1	0.303 ± 0.063	1.11 ± 0.36	27.9 ± 9.1	10.7 ± 1.7	0.116 ± 0.012	0.32 ± 0.19
all av	2.14 ± 0.71	3.24 ± 1.06	20.1 ± 4.2	0.309 ± 0.064	1.27 ± 0.41	31.8 ± 10.3	10.5 ± 1.6	0.102 ± 0.010	0.36 ± 0.21
median	2.10	3.17	19.8	0.305	1.39	34.8	10.4	0.091	0.26

Table III. Comparison of Averages of Phytate Content of Diet Samples and Daily Dietary Intake in Different Countries

country	no. of samples	phytate, g/kg	zinc, mg/kg	daily phytate intake, g/person	daily Zn intake, mg/person	ref
Taiwan	15	2.14 ± 0.71	20 ± 4	0.78 ± 0.26	7.3 ± 1.5	this work
Turkey	10	1.55 ± 0.70	26 ± 6	0.79 ± 0.35	11.3 ± 4.4	Ersöz et al. (1990)
Italy	13	1.19 ± 0.52	22 ± 5	0.75 ± 0.35	13.9 ± 2.7	Carnovale et al. (1987)

The 121.49-MHz ^{31}P FT NMR spectra of pure phytate, standard U.S. Diet TDD-1D, and Taiwanese diet THFF-3, are shown in Figure 3, parts A, C, and D, respectively. The phosphate spectrum is also given for comparison in Figure 3B. As predicted by the symmetry of the molecule (Figure 1), the ^{31}P FT NMR spectrum of phytic acid consists of four peaks with relative intensities of 1:2:2:1. On the other hand, diet samples present extra peaks, as seen in Figure 3C,D; all are to high field of the C-2 peak. These can be used for the determination of hydrolysis products.

The percent phytate content of the diet sample is calculated from the equation (O'Neill et al., 1980)

$$\%\ (w/w)\ \text{phytate} = \frac{\text{area of C-2 peak}}{\text{area of } H_3PO_3} \frac{V}{S} C$$

where V is the volume (mL) of 0.24% H_3PO_3 + CCl_3COOH solution used in the extraction, C is the reciprocal of the slope obtained in the calibration curve

$$\left(\frac{\text{area } H_3PO_3}{\text{area C-2}}\right) (w/v)\ \%\ \text{of phytate}$$

and S is the weight (g) of the sample.

RESULTS AND DISCUSSION

Phytate, Zn, and Ca contents of the diet samples are given in Table II. The phytate results are the average of three measurements. We have also calculated moles per kilogram of these substances in the diet, which will be used later in zinc and calcium bioavailability discussions. As seen from the table, there is no difference in the phytate content of male and female diets. This is expected since similar foods are consumed by both males and females. Although there are extensive studies on the phytate content of individual food items (O'Neill et al., 1980), there are only a few works on the phytate content of the total diet (Ersöz et al., 1990; Carnovale et al., 1987). Table III presents average phytate and daily dietary intake results from Turkey (Ersöz et al., 1990) and Italy (Carnovale et al., 1987) compared to this work. There are significant differences in phytate content among the three diets, but daily phytate intakes are very similar. This is probably due to the differences in total diet intake and the types of food consumed in different countries.

Although the phytate content of the Italian diet is much less than that of the Taiwanese diet, the Taiwanese daily intake on a dry weight basis is small; as a result, daily phytate intakes in these countries are almost equal. The average daily Taiwanese dietary intake of zinc is 7.29 ± 1.53 mg/person. This is somewhat lower than the WHO and RDA daily estimates, which are 11 and 15 mg/person, respectively, as recommended by the National Academy of Science, U.S.A. (*Recommended Dietary Allowances*, 1989; WHO, 1983).

To evaluate zinc bioavailability, phytate/zinc molar ratios, [Ph]/[Zn], were calculated; they range from 6.2 to 22.6. It was indicated by many workers that [Ph]/[Zn] is more important for the bioavailability studies than zinc or phytate content separately (Ellis et al., 1982). Harland and Peterson (1978) found that [Ph]/[Zn] in the vegetarian diet of monks ranged from 10 to 24.5, while in normal diets the ratio was 6. They followed up their studies 10 years later and found that the monks' daily phytate intake was reduced from 4.57 to 0.972 g (Harland et al., 1988). They noticed that a 5-fold decrease in phytate intake also affected the zinc value of blood, which increased from 85.2 ± 4.6 μg/dL in 1977 to 104.6 ± 3.1 μg/dL in 1987. Morris and Ellis (1980) showed that zinc bioavailability in rats is unaffected if [Ph]/[Zn] is lower than 12. A ratio of 6 was calculated for typical diets by Oberleas and Harland (1981), but since that time further research has advised caution when the ratio is 10 or above. Although the average [Ph]/[Zn] for the Taiwanese diet is 10.5 ± 1.6, 6 of 15 samples have values higher than 12. As seen from Table III, the Taiwanese diet is high in phytate but low in zinc content. However, in diets in which threshold values of phytate/zinc molar ratio are not attained, an allowance must be made for other factors affecting the zinc status, such as the presence of dietary fiber, competing ions, or protein intake. On the other hand, Davies et al. (1985) proposed that the (phytate × Ca)/Zn molar ratio,

[Ph][Ca]/[Zn], is more accurate for predicting zinc bioavailability. The molar ratio above which human zinc deficiency might be observed was proposed to be 0.5. In this study we found that [Ph][Ca]/[Zn] values vary between 0.16 and 0.72, averaging 0.36 ± 0.21. In this case only 23% of the subjects have higher values of [Ph][Ca]/[Zn] than the proposed 0.5 value.

The [Ph][Ca]/[Zn] molar ratio calculation was reevaluated by Wing et al. (1990). They indicated that [Ph]-[Ca]/[Zn] molar relations give the impression that increasing the calcium or phytate concentrations by a certain factor will have the same effect on growth as decreasing the zinc concentration by the same factor. However, it was shown by Morris and Ellis (1980) that this is not always true. On the other hand, Wing et al. (1990) proposed that a mass action law related to the

$$\text{free Zn} + \text{free phytate} \rightarrow \text{Zn–phytate complex}$$

reaction should be used.

$$K = [\text{Zn–phytate complex}]/[\text{free Zn}][\text{free phytate}]$$

This equation predicts that free zinc available for absorption should not be expected to be proportional to total phytate and total zinc concentrations but rather to this product.

Zinc, even in the physiological range, decreases the bioavailability of copper. In the presence of dietary phytate, excess calcium lowers zinc absorption and utilization. Both of these effects become of critical importance when dietary levels of essential elements are limiting (O'Dell, 1989).

The above discussions indicate that there is no consensus on the degree to which phytate impacts the physiological accessibility of metal cations (Martin and Evans, 1986). Although the phytic acid contents of cereal grains have been shown to affect adversely the intestinal absorption of ions like zinc and iron (Oberleas, 1973; Ellis and Morris, 1986), others report that phytic acid poses no hindrance to iron uptake and increases Cu uptake (Lee et al., 1988). There are very few experiments in the literature which measure both trace elements and phytate in the daily duplicate diet (Ersöz et al., 1990; Carnovale et al., 1987; Lehrfeld, 1989). Since processing of foods, other ingredients added, and different methods of cooking may hydrolyze phytate and alter the capacity to bind minerals in the gastrointestinal tract, the studies on natural diet environment are very important.

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Registry No. Zn, 7440-66-6; Ca, 7440-70-2; phytic acid, 83-86-3.