Phytate Content of Indian Foods and Intakes by Vegetarian Indians of Hisar Region, Haryana State

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The presence of phytate in the diet results in a reduction in the bioavailability of essential minerals. The Indian (vegetarian) diet is rich in cereals and legumes, foods that are known to be rich in phytate. As a result of questionnaires and interviews, the dietary habits of 298 rural Indians have been determined and samples of their dietary raw materials and processed foods obtained. Levels of phytate, Ca, and Zn in raw and processed foods and in total diets have been determined. Phytate intakes are 2–3 times higher than in Europe, and the sensitive [phytate][Ca]/[Zn] ratio suggests that the populations are on the borderline of human zinc deficiency. Further studies are needed in which the chemical nature of the "phytate" present after cooking and processing is determined.

INTRODUCTION

Phytate (inositol hexaphosphate) is widely distributed in foods of plant origin; it stores most of the phosphorus in cereals and legumes. Phytates have been shown to reduce the bioavailability of minerals (Davies and Nightingale, 1975; Nolan and Duffin, 1987) and to inhibit proteolytic (Singh and Krikorian, 1982; Serraino *et al.*, 1985) and amylolytic (Deshpande and Salunkhe, 1982) enzymes. While the exact nature and degree of binding of phytate to minerals and proteins are difficult to determine and their resultant nutritional roles are complex (Lee, 1988), it is clear that high levels of intake are associated with adverse nutritional effects in man. However, recent research suggests a positive role of phytates in terms of reducing cell proliferation and hence reducing the risk of colonic cancer (Graf and Eaton, 1990). Given the distribution of phytate within plant-based foods, it is clear that vegetarian populations will be particularly susceptible to phytate-related nutritional disorders; in particular, they are particularly susceptible to reduced zinc bioavailability due to a combination of lowered zinc intakes and the physicochemical binding effects of the phytate.

In rural India the reasons for populations adapting a vegetarian lifestyle are cultural and/or economic, rather than philosophical, religious, or health-related. This situation is thus rather different from the situation within Western, industrialized regions (Ellis *et al.*, 1982). Indian vegetarians, heavily dependent upon cereals and legumes, will thus consume high levels of phytate. The exact level (and nature of the phytate species, including partially hydrolyzed forms) will depend upon the individual plant-based foods consumed and their relative proportions in the diet; the methods of cooking will also play an important role, since Khokhar and Chauhan (1986) and Duhan *et al.* (1989) have shown soaking, fermenting, sprouting, autoclaving, and boiling to reduce the inositol hexaphosphate content of the consumed foods either from heat treatment or by allowing phytase to act on the phytic acid substrate. While there have been many studies on the phytate content of individual food items, investigations into the phytate contents of the total diet are much fewer. Studies have been carried out on daily dietary intakes in the United States (Ellis *et al.*, 1982) and Britain (Wise *et al.*, 1987), in Italy (Carnovale *et al.*, 1987), Sweden (Torelm and Bruce, 1982), and Turkey (Ersöz *et al.*, 1990), and, most recently, in Taiwan (Wang *et al.*, 1992). There is, however, no information available on the intakes of phytate in India or on its level in individual diets and its distribution in the components of different meals. The present study was carried out to obtain such data and included a comparison of the average daily intakes throughout the year for children, adolescents, adults, and older people and a comparison of landholding and landless status. Given concerns over the effect of phytate on zinc bioavailability, molar phytate/zinc and molar (phytate × calcium)/zinc ratios were also calculated.

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METHODS

Dietary Surveys and Food Consumption Record. Surveys based upon the Indian Council of Medical Research (ICMR, New Delhi) questionnaire model were carried out (Pushpamma *et al.*, 1983). Interviews (lasting at least 1 h) were conducted with 63 children (33 male, 30 female) aged 4–9 years, 64 adolescents (36 male, 28 female) aged 10–19 years, 121 adults (61 male, 60 female) aged 20–45 years, and 50 older adults (25 male, 25 female) aged 46 years and above. All of the participants regularly consumed two meals a day. The questionnaire covered 30 landholding families and 30 landless families in three rural villages, approximately 30 km from Hisar, Haryana State. The surveys were carried out in July, November/December, and March/April to take into account seasonal dietary changes. The results have been expressed as an average of these seasons to represent mean annual intakes. The questionnaire covered 20 areas including general family history and details, dietary habits, cooking practices, foods consumed, and diet-related disease states. Full details of the questionnaire are available from the senior author. Approximately 1 week after the survey was carried out in each village, the actual food consumption of each individual was recorded by weighing their food for 3 consecutive days. The average of these 3-day records was taken as

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the daily food intake for that season. Daily intakes for the year were estimated by taking the average of all seasons.

Dietary Samples. Meals were prepared by the families included in this study according to the above food consumption for data for all 298 subjects. The individual cooked foods corresponding to each day's total diet were collected in locally available screw-capped polythene containers, marked with the name of the subject and the date and time of collection. Dietary collections were carried out over 3 days; the individual food samples corresponding to each day's meals were transported to the laboratory on the day of collection and kept frozen. Liquid foods such as milk and milk products were measured and collected in labeled polythene bottles and treated as above.

Collection of Uncooked Foods. Uncooked foodstuffs (cereals, grain legumes, oil seeds, and vegetables) grown and consumed by landed families and those procured by landless families were collected as above from the homes of the individual subjects and from local village markets. The quantities obtained were comparable with the amounts required for the individual families within the program. Nine samples of each foodstuff (three villages, each sample in triplicate) were obtained and dried to constant weight at 40 °C in an air-flow oven. The dried samples were ground, thoroughly mixed, and stored in sealed polythene bottles until required for analysis.

Collection of Cooked Foods. Evaluation of the dietary survey information revealed that all subjects (including all of the different age groups) within the families included in this study shared a common meal pattern and that the foods consumed were similar across the seasons. Moreover, the sources of food and the means and methods employed for cooking were similar. As a result of these findings, 12 cereal-based, 7 legume-based, and 15 vegetable-based cooked foods that were consumed by the 298 subjects were collected from all of the families ($n = 60$). Samples were collected across three consecutive days and from the three different villages. The samples were kept frozen until required and then thawed and dried at 40 °C in an air-flow oven. They were then homogenized and ground in a locally manufactured mixer-cum-grinder containing a stainless steel blade. Subsequent analyses of these cooked foods for mineral composition and phytate content provided a data set from which, together with average daily food consumption information, phytate and mineral intakes were calculated.

Phytate Analysis. Dry, powdered samples were used for the phytate analyses. Following the earlier work of Haug and Lanzsch (1983), two methods of analysis were used for raw and cooked cereals and legumes. The procedure of Davies and Reid (1979) was used for grain legumes, their products, and vegetables, while that of Haug and Lanzsch (1983) was followed for cereals, cereal-based foods, and oilseeds.

In the former case, legume or vegetable powders (1.0 g) were extracted in triplicate, for 4 h with 0.5 N nitric acid (20 mL, constant agitation). After filtering, the filtrate (1 mL) was diluted with distilled water to a final volume of 1.4 mL, to which was added ferric ammonium sulfate (1.0 mL containing 50 μg of iron). The tubes were stoppered and placed in a boiling water bath (20 min) and then allowed to cool. Amyl alcohol (5 mL) was added to each tube followed by 0.1 mL of ammonium thiocyanate (100 g L^{-1}), the tubes were shaken thoroughly and centrifuged (3000 rpm), and the absorbance of the amyl alcohol layer was determined at 465 nm against amyl alcohol exactly 15 min after the addition of the ammonium thiocyanate using a Spectronic 21 instrument. A linear relationship was found over a range of phytate concentrations from 20 to 200 nmol.

For cereals and oilseeds triplicate samples of powder (1.0 g) were extracted with 0.2 N hydrochloric acid. One milliliter of extract was pipetted into a test tube fitted with a ground glass stopper together with 1 mL of ferric ammonium sulfate (0.2 g hydrated, in 100 mL of 2 N hydrochloric acid, diluted to 1 L with distilled water). The tube was heated in a boiling water bath for 30 min, cooled in ice for 5 min, and then allowed to reach ambient temperature. After centrifugation (3000 rpm), the supernatant (1 mL) was mixed with 2,2′-bipyridine solution (Haug and Lanzsch, 1983) and the absorbance measured at 519 nm against distilled water using a Spectronic 21 instrument. A linear relationship was observed between 3 and 30 μg of phytate P.

Table 1. Phytate Contents in Different Raw Foodstuffs Calculated on a Dry Matter Basis

foodstuff	phytate[a] (g kg^{-1})	moisture (g kg^{-1})
cereals		
pearl millet, *Pennisetum typhoideum*	8.1 ± 1.2	124
rice, *Oryza sativa*	2.9 ± 0.2	133
wheat, *Triticum sativum*	9.3 ± 1.2	128
legume grains		
chickpea (whole), *Cicer arietinum*	10.1 ± 1.2	98
chickpea (split), *C. arietinum*	9.1 ± 1.0	98
chickpea (white), *C. arietinum*	6.6 ± 0.3	95
black gram (split), *Phaseolus mungo* Roxb.	8.0 ± 0.5	109
green gram (split), *Phaseolus aureus* Roxb.	10.8 ± 0.6	101
lentil, *Lens esculenta*	10.1 ± 0.9	109
rajmah, *Phaseolus vulgaris*	11.3 ± 0.1	120
soybean, *Glycine max* Merr.	21.8 ± 1.2	81
green leafy vegetables		
bathua leaves, *Chenopodium album*	3.0 ± 0.3	896
mustard leaves, *Brassica campestris*	0.9 ± 0.1	898
other vegetables		
bottle gourd, *Lagenaria vulgaris*	0.5 ± 0.1	961
carrot, *Daucus carota*	6.3 ± 0.1	860
cauliflower, *Brassica oleracea*	0.8 ± 0.1	908
ladies finger, *Abelnoschers esculentus*	0.5 ± 0.1	896
potato, *Solanum tuberosum*	1.6 ± 0.1	747
tinda, *Citrullus vulgaris*	0.6 ± 0.03	935
miscellaneous		
jaggery	0	39
sugar	0	4
gingerly seeds, *Sesanum indicum*	0	5.3
SE (M)	0.8	
level of significance ($P < 0.05$)	2.4	

[a] Values are means ± SD of nine replicates.

The phytate contents were calculated per kilogram of the daily diet from the total phytate content *of the cooked food* constituting part of the daily meal and the total amount of these foods consumed daily. The calcium and zinc contents (measured below) were calculated in a similar manner.

Zinc Analysis. The powdered, dried samples were digested in a nitric acid/perchloric acid (5:1) mixture and analyzed for zinc using the atomic absorption spectrometric method of Lindsey and Norwell (1969). Zinc levels were measured with a Perkim-Elmer 2380 instrument, wavelength 213.9 nm, using appropriate Sigma standards and reference materials. Digestions were carried out in triplicate on hot plates (80 °C) for 4 h after the samples were kept overnight in the digestion medium or until the contents were clear and homogeneous. The samples were then dried, and each analysis was conducted in duplicate.

Calcium Analysis. Calcium levels were determined in the same manner as described for zinc using appropriate Sigma standards and reference materials. The analysis was carried out at 422.7 nm. Detailed information on the zinc and calcium contents of Indian plant foods, and on the intakes of these and other minerals, is presented elsewhere (Khokhar and Pushpanjali, 1994).

Statistical Treatment. Data were subjected to statistical analysis; descriptive variables, ANOVA variance, and *t*-test procedures were followed (Panse and Sukhatme, 1961).

RESULTS

The phytate and moisture contents of the raw foodstuffs included in this study are shown in Table 1. Wheat and pearl millet contained the highest levels among the cereals, with high levels also being found among grain legumes (especially soybean). Of the green

Table 2. Phytate Content of Cereal-Based Cooked Foods

	phytate[a] (g kg^{-1} of dry wt)
pear millet chapati (unleavened bread)	5.4 ± 0.6
missi chapati (wheat and chickpea)	6.3 ± 0.8
wheat chapati	6.5 ± 1.9
bajra Khichri (broken and dehusked pearl millet)	4.5 ± 1.0
porridge (wheat)	4.3 ± 2.5
halwa (cream of wheat + oil + sugar)	4.8 ± 1.1
rice pudding	2.3 ± 1.0
puri (refined wheat bread, fried)	6.2 ± 1.2
rice (boiled)	2.4 ± 0.6
sweet rice	2.1 ± 1.0
vermicilli	2.9 ± 0.7
wheat churma (wheat chapatis crushed with fat and sugar)	6.5 ± 1.4

[a] Values are means ± SD of nine replicates.

Table 3. Phytate Content of Legume-Based Cooked Foods

	phytate[a] (g kg^{-1} of dry wt)
chickpea flour curry (karhi)[b]	8.6 ± 0.9
green gram + kachri curry (dhal)	2.9 ± 0.7
white chickpea flour curry (karhi)	6.1 ± 0.7
black gram split curry (dhal)	3.0 ± 0.7
cowpea curry (dhal)	2.7 ± 0.5
khichri (rice + green gram (dehusked))	2.7 ± 0.8
green gram split curry (dhal)	2.8 ± 0.1

[a] Values are means ± SD of nine replicates. [b] Names in parentheses are the local Indian names of the recipes.

vegetables, bathua leaves showed the highest content, while carrots contained the greatest amount of phytate of the root vegetables examined. Vegetables contributed significantly lower amounts of phytate to the vegetarian Indian diet because of their lower overall phytate contents and the much greater intakes of phytate-rich cereals and grain legumes.

Wide variations were observed in the phytate contents of the prepared foods examined in this study. Ratios of phytate contents in uncooked and cooked samples varied between 1.3 (rice) and 5.2 (carrot). Such variation is likely to reflect the physical nature of the plant material and the conditions of cooking. Values for cereal-, legume-, and vegetable-based cooked foods are shown in Tables 2–4. For cereal-based foods, the phytate contents ranged from 6.5 to 2.1 g kg^{-1} (wheat chapati–sweet rice), with comparable ranges for legume-derived foods (8.6–2.7 g kg^{-1}, chickpea flour curry–khichri) and vegetable dishes (5.6–0.3 g kg^{-1} chholia curry–long melon). Two of the most widely, and heavily, consumed foods, chickpea curry and wheat chapatis, contain high levels of phytate (6.1 and 6.5 g kg^{-1}, respectively). The phytate, zinc, and calcium contents of the diets of Indian vegetarian children, adolescents, adults, and older people are shown in Table 5. Mean phytate values varied from 4.8 to 5.2 g kg^{-1} (children–adolescents), with no statistical differences being found between different age groups. This is ascribed to the similar dietary patterns of all family members, including children. Given this finding, it would be expected that differences in phytate intake among the different age groups would be a function of their total dietary intakes. This is shown to be the case (Table 6), with mean daily intakes of phytate for children varying from 0.72 to 1.16 g; corresponding figures for adolescents (1.35–1.78 g), adults (1.56–2.50 g), and the elderly (1.29–2.08 g) were observed.

Mean zinc contents of the diets of the different age groups varied between 18 and 21 mg kg^{-1}, while those of calcium varied between 827 and 1057 mg kg^{-1} (Table 5).

Average phytate daily intakes for adult and elderly vegetarian subjects showed males within each group to consume significantly ($p < 0.01$) higher amounts of phytate than females (Table 6). There were no significant ($p < 0.05$) differences between landholding and landless subjects for phytate intake, but in landholding families, elderly males consumed significantly ($p < 0.01$) more phytate than females. In landless families males had a higher consumption of phytate than females, in agreement with their higher cereal intake. The effect of landholding was also seen in adolescents and adults, where landless female adolescents and adults consumed significantly ($p < 0.01$) higher amounts of phytate. In both cases these differences may be ascribed to varying cereal consumption.

The phytate/zinc molar ratio for the four age groups show mean values between 23.9 and 25.7, while the (phytate × calcium)/zinc molar ratio varied between 0.5 and 0.7 mol kg^{-1} (Table 5).

DISCUSSION

In the consideration of the data on phytate contents and intakes presented in this paper, it is important to emphasize that the analytical method employed measures various partially hydrolyzed inositol phosphates as well as the hexaphosphate species. These hydrolysis products may be produced during food preparation and processing by the action of phytase (Nayini and Markakis, 1983), and their biological properties are known to vary. The data obtained in the present study may therefore overestimate the metal chelating and *in vivo* effects of phytate. Methods are available for selectively measuring phytic acid (hexaphosphate) (Lehrfeld, 1989; Ersž *et al.*, 1990), but these were unavailable to the present workers. Nevertheless, the information presented here on the phytate contents of Indian plant foods, and processed foodstuffs, and the intakes of Indian vegetarians offers a comparison with information produced by other groups.

The mean daily intake of phytate for Indian children aged 4–9 years was found to be 0.72–1.16 g, while that of adolescents (10–19 years) was 1.35–1.78 g. The phytate intakes of the latter were thus considerably higher than those reported (0.5–0.8 g) for male and female students at a higher education establishment in Scotland (Wise *et al.*, 1987). The mean daily phytate intakes of Indian adult (20–45 years) and elderly subjects (over 45 years of age) were 1.56–2.50 and 1.29–2.08 g, respectively. In comparison, the corresponding figures obtained for adults in Turkey (Ersöz *et al.*, 1990), Italy (Carnovale *et al.*, 1987), Taiwan (Wang *et al.*, 1992), and the United States (Harland and Peterson, 1978) were all closely grouped between 0.75 and 0.79 g. It is clear, therefore, that the Indian children examined in the current study exhibited mean daily phytate intakes comparable with those of adults from other parts of the world.

The zinc binding effect of phytate means that consideration must be taken of both dietary phytate and dietary zinc intakes. In a recent study on Taiwanese diets the average phytate/zinc molar ratio was 10.5 ± 1.6 (Wang *et al.*, 1992). Earlier, Oberleas and Harland (1981) had found molar ratios of around 6.0 for typical U.S. hospital diets. Ellis *et al.* (1982) recorded phytate/

Table 4. Phytate Content of Vegetable-Based Cooked Foods

	phytate[a] (g kg^{-1} of dry wt)		phytate[a] (g kg^{-1} of dry wt)
bottle gourd	0.5 ± 0.1	onion	2.3 ± 0.1
brinjal	5.5 ± 2.3	potato and carrot	2.3 ± 0.8
chholia (chickpea, immature pods)	5.6 ± 0.9	potato curry	1.8 ± 0.1
chkotre	0.4 ± 0.1	potato + radish pods	1.2 ± 0.2
carrot	1.2 ± 1.6	ridge ground	3.2 ± 0.8
long melon	0.3 ± 0.1	bathua raita (in yogurt)	1.5 ± 0.1
okra	0.4 ± 0.1	bottle gourd raita (in yogurt)	0.4 ± 0.1
mustard leaves, sag	0.9 ± 0.7		

[a] Values are means ± SD of nine replicates.

Table 5. Ca, Zn, and Phytate Concentrations and [Phytate]/[Zinc] and [Phytate][Calcium]/[Zinc] Ratios of the Diets of the Indian Population According to Age (Calculated on Dry Matter Basis)

age group	phytate (g kg^{-1})	phytate (mmol kg^{-1})	Zn (mg kg^{-1})	Zn (mmol kg^{-1})	Ca (mg kg^{-1})	Ca (mmol kg^{-1})	[Ph][a]/[Zn]	[Ph][Ca]/[Zn]
children (4–9 years)	4.8 ± 0.9 (4.5–5.0)	7.21	18 ± 4 (17–19)	0.28	1057 ± 355 (820–1250)	26.4	25.7	0.68
adolescents (10–19 years)	5.2 ± 0.7 (5.0–5.50)	7.83	20 ± 3 (19–23)	0.31	927 ± 239 (817–1031)	23.2	25.2	0.59
adults (20–45 years)	4.9 ± 0.7 (4.6–5.3)	7.42	20 ± 3 (19–23)	0.31	894 ± 305 (702–1086)	22.3	23.9	0.53
aged population (over 45 years)	5.1 ± 0.5 (4.8–5.4)	7.71	21 ± 3 (20–23)	0.32	827 ± 187 (791–886)	20.7	24.1	0.50
level of significance	NS[c]		NS		NS			

[a] [Ph] = concentration of phytate. [b] Figures in parentheses represent the range. [c] Not significant.

Table 6. Mean Daily Consumption of Phytate According to Sex and Landholding (LH) or Landless (LL) Status

group		phytate intake[a] (g day^{-1})	*t* value[b]	*t* value[c]
children				
LH	M	1.16 ± 0.43	0.02	0.58
	F	0.72 ± 0.32		
LL	M	1.07 ± 0.47	0.05	3.40**[d]
	F	1.12 ± 0.35		
adolescents				
LH	M	1.78 ± 0.58	0.41	0.16
	F	1.35 ± 0.04		
LL	M	1.74 ± 0.68	0.01	0.18
	F	1.38 ± 0.30		
adults				
LH	M	1.90 ± 0.50	2.90[d]	4.02[e]
	F	1.56 ± 0.47		
LL	M	2.50 ± 0.61	4.80[d]	1.90
	F	1.86 ± 0.68		
aged population				
LH	M	1.81 ± 0.33	5.90[d]	1.50
	F	1.29 ± 0.30		
LL	M	2.08 ± 0.52	4.70[d]	0.10
	F	1.30 ± 0.25		

[a] Values are presented as mean ± SD. [b] Comparison between male and female. [c] Comparison between landholders (LH) and landless (LL) (M/M or F/F). [d] Significant at 1% level. [e] Significant at 5% level.

zinc molar ratios of 3.3 ± 1.4, 4.5 ± 0.8, and 7.6 ± 0.7 for regular, ovo-lacto vegetarian, and soy meat substitute hospital diets. In the present study we have found mean phytate zinc molar ratios between 23.9 and 25.7 for diets consumed by the different age groups.

In diets high in phytate and low in zinc (such as that in the present study) consideration should be taken of the effect of other factors on zinc bioavailability. The (phytate × calcium)/zinc molar ratio has been suggested by Davies *et al.* (1985) to be a more accurate predictor of this variable. Because milk and milk products are taken in highly diluted forms, calcium intakes are known to be low in Indian vegetarian populations. The ratios obtained (0.50–0.68) are considered to be at the borderline, above which human zinc deficiency might be observed. Wang *et al.* (1992) have recently calculated the (phytate × calcium)/zinc ratio for Taiwanese diets to vary between 0.16 and 0.72, with a mean of 0.36 ± 0.21.

The major contributor to dietary phytate within Indian vegetarian populations was found to be wheat; this cereal is consumed by all age groups throughout the year, with only partial substitution by pearl millet in the colder months. Other staples, such as dhal, chickpea flour karhi, and vegetable curries, also contributed significantly to the total phytate intake. In general, landholding families did not have phytate intakes different from those of landless families, although some differences between individual age groups were noted. Within some age groups, landless females consumed considerably higher amounts of phytate when compared with their landholding equivalents.

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