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Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India

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Abstract

Several cereals and pulses commonly consumed in India were screened for zinc and iron contents and their bioaccessibility in the same was determined by equilibrium dialysis employing an *in vitro* simulated digestion procedure. Zinc content of cereals ranged from 1.08 mg/100 g in rice to 2.24 mg/100 g in sorghum. Zinc content of pulses was between 2.03 mg/100 g (whole chickpea) and 2.68 mg/100 g (decorticated chickpea). Iron content of cereals ranged from 1.32 mg% in rice to 6.51 mg% in sorghum, while that of pulses ranged from 3.85 mg% in decorticated green gram to 6.46 mg% in black gram. Dialyzability of zinc from pulses (27–56%) was generally higher than that from cereals (5.5–21.4%). Dialyzabilities of iron were almost similar from both cereals and pulses examined and were 4.13–8.05% in cereals and 1.77–10.2 % in pulses. A significant negative correlation between inherent phytate content and zinc dialyzability value was inferred in the case of pulses. Phytic acid content of the cereals had a significant negative influence on iron dialyzability. Inherent calcium had a negative influence on zinc dialyzability in cereals. Tannin did not have any significant influence on zinc or iron dialyzabilities from cereals and pulses. While both insoluble and soluble fractions of the dietary fibre generally interfered with zinc dialyzability, the insoluble fraction alone had this effect on iron dialyzability. The lower collective negative influence of the inherent factors on zinc dialyzability from pulses is consistent with their higher concentrations in these grains, relative to cereals. The negative correlation of inherent phytic acid with zinc and iron dialyzabilities was supported by enhanced dialyzabilities of these minerals upon partial removal of phytate from the grains by treatment with fungal phytase.

Keywords: Zinc; Iron; Cereals; Pulses; Bioaccessibility; Inherent factors

1. Introduction

Zinc is the fourth important micronutrient after vitamin A, iron and iodine, and is now receiving increasing global attention. Deficiency of this element, although not completely assessed, is believed to be as widespread as that of iron and is a cause for concern, especially in the developing countries (Prasad, 2003). Although the cause of suboptimal zinc status in some cases, may be inadequate dietary intake of zinc, inhibitors of zinc absorption are probably the most common causative factors. Although animal foods are rich sources of zinc in our diet, this micronutrient is derived mainly through food grains by a majority of the population in developing countries. Staple foods in developing countries include cereals and legumes, which are the main sources of zinc for most of the population, but even if net zinc intake appears adequate, compromised zinc status is common (Gibson, Yeudall, Drost, Mtitimuni, & Cullinan, 1998). In general, the average zinc intakes of vegetarians are lower than those of their omnivorous counterparts (Gibson, 1994).

Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes. Despite large scale intervention programmes, iron-deficiency anaemia remains the most widely prevalent nutritional problem in the world. Although many factors are responsible for the onset of iron deficiency, the most likely cause of this nutritional problem

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in developing countries is the poor bioavailability of dietary iron (Gibson, 1994; Sandberg, 2002).

Bioavailability of micronutrients, particularly zinc and iron, is low from plant foods (Gibson, 1994; Sandberg, 2002). Bioavailability of iron is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Among inhibitors, phytic acid, tannins, dietary fibre and calcium are the most potent, while organic acids are known to promote iron absorption (Gibson, 1994; Sandberg, 2002). Although not exhaustively evidenced, it is possible that the bioavailability of zinc from food grains is similarly influenced by such diverse factors coexistent in them. Cereals and pulses are known to contain high concentrations of one or more of the above inhibitors of iron absorption.

Information on the bioavailability of zinc from the food grains commonly consumed in India is limited. Such information would be useful for computing the recommended dietary allowances, as well as for evolving a dietary strategy to improve micronutrient intake. In this investigation, several food grains commonly consumed in India were screened for their zinc contents and bioaccessibility by measuring the *in vitro* dialyzability. Iron content and its bioaccessibility in these food grains were also determined in order to make a comparison. Various factors inherent in these grains, which may have an influence on the bioaccessibility values of zinc and iron, namely, phytic acid, tannin, calcium and dietary fibre, were also quantified Dialyzability values of zinc and iron of the food grains studied have been correlated with concentrations of the above inherent factors. A supplementary study was also conducted on selected grains, wherein the effect of removal of inherent phytate from the grains rich in it, on zinc and iron dialyzability, was examined.

2. Materials and methods

2.1. Materials

Cereals – rice (*Oryza sativa*), finger millet (*Eleusine coracana*), sorghum (*Sorghum vulgare*), wheat (*Triticum aestivum*) and maize (*Zea* mays), and pulses – chickpea (*Cicer arietinum*) – whole and decorticated, green gram (*Phaseolus aureus*) – whole and decorticated, decorticated black gram (*Phaseolus mungo*), decorticated red gram (*Cajanus cajan*), cowpea (*Vigna catjang*) and French bean (dry; *Phaseolus vulgaris*) were procured locally, cleaned and used as such. Pepsin, pancreatin and bile extract, all of porcine origin and phytase (from *Aspergillus ficuum)* were from Sigma Chemical Co., St. Louis, USA. All other chemicals used were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed (2% nitric acid in water) glassware was used throughout the study.

2.2. Total zinc, iron and calcium

Grain samples were finely ground and ashed in a muffle furnace at 550 °C for 10 h and the ash was dissolved in conc. HCl (Sp.Gr.1.18). Zinc and iron contents were determined by atomic absorption spectrometry (Shimadzu AAF-6701). In the case of calcium, lanthanum chloride was added to the mineral solution (final concentration 1%) to avoid interference from phosphate. Calibration of measurements was performed using commercial standards. All measurements were carried out using standard flame operating conditions, as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.

2.3. Bioaccessibility of zinc and iron

Bioaccessibilities of zinc and iron from various food grain samples were determined by an *in vitro* method described by Luten et al. (1996), involving simulated gastrointestinal digestion with suitable modifications. All food grain samples were finely ground in a stainless steel blender. The ground samples were subjected to simulated gastric digestion by incubation with pepsin (pH 2.0) at 37 °C for 2 h. Titratable acidity was measured in an aliquot of the gastric digest, by adjusting the pH to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin–bile extract mixture (1 l of 0.1 M sodium bicarbonate containing 4 g pancreatin + 25 g bile extract). Titratable acidity was defined as the amount of 0.2 M sodium hydroxide required to attain a pH of 7.5.

To simulate intestinal digestion, segments of dialysis tubing (Molecular mass cut off 10 kDa) containing 25 ml aliquots of sodium bicarbonate solution, being equivalent in moles to the sodium hydroxide needed to neutralize the gastric digest (titratable acidity) determined as above, were placed in Erlenmeyer flasks containing the gastric digest and incubated at 37 °C with shaking for 30 min or longer until the pH of the digest reached 5.0. Five ml of the pancreatin–bile extract mixture were then added and incubation was continued for 2 h or longer until the pH of the digest reached 7.0. At the end of simulated gastro-intestinal digestion, zinc and iron present in the dialyzate were analyzed by atomic absorption spectrometry. The dialyzable portion of the total minerals present in the sample (expressed as percent) represented the bioaccessible minerals.

Bioaccessibility (%) was calculated as follows: bioaccessibility (%) $= 100 \times Y/Z$, where, Y is the element content of the bioaccessible fraction (mg mineral element/100 g grain), and Z is the total zinc or iron content (mg mineral element/100 g grain).

2.4. Phytate, tannin and dietary fibre

Phytate in food grains was determined as phytin-phosphorus by the method of Thompson and Erdman (1982). Phytic acid values were computed by multiplying phytin phosphorus value by 3.55. Dietary fibre (soluble and insoluble) was estimated by an enzymatic – gravimetric method, as described by Asp, Johansson, Hallmer, and Siljestrom (1983). Tannin was estimated by the modified vanillin assay of Price, Scoyoc, and Butler (1978), using catechin as the standard.

2.5. Statistical analysis

All determinations were done in five replicates and the average values are reported. Statistical analysis of analytical data was done according to Snedecor and Cochran (1967). The data were also analyzed statistically by multiple regression tests, to infer the extent of modulation of zinc and iron bioaccessibilities by inherent phytic acid, tannin, calcium and dietary fibre, and by Pearson moment correlation, to infer the relationship between molar ratios of particular factors using the statistical software – SPSS programme.

2.6. Bioaccessibilities of zinc and iron from selected food grains after partial removal of phytate

Ground samples of decorticated green gram and black gram were pre-treated with commercial phytase to bring about hydrolysis of inherent phytate. The enzyme was added at a level of 2.2 U/g sample, suspended in 2 M glycine-HCl buffer, pH 2.5, and incubated for 60 min at 37 °C. At the end of phytase reaction, phytate content and bioaccessibilities of zinc and iron from the above samples were determined as described above.

3. Results and discussion

3.1. Zinc and iron contents in cereals and pulses

Zinc and iron contents of the food grains studied are presented in Table 1. As can be seen in the table, the inherent zinc concentration in cereals was lower, nearly half of that in pulses, except in the case of sorghum, whose zinc concentration was comparable to that of pulses. Thus, all the pulses examined here, with an uniformly higher amount of zinc compared to cereals, are a better source of this micronutrient. Zinc content of food grains observed in this study corresponded with the values reported by the Indian Council of Medical Research (ICMR) (Gopalan, Ramasastri, & Balasubramanian, 1999), where the zinc content of cereals ranged from 1.2 to 2.8 mg/100 g, and that of pulses ranged from 1.2 to 3.0 mg/100 g. The iron concentration of rice and wheat was about 30% higher than that of zinc while, in finger millet and maize, it was double and in sorghum, almost three times the zinc content. In all the pulses examined, iron concentration was similarly higher than that of zinc. Whole chickpea, red gram, black gram, and French bean had iron contents that were more than double their zinc concentrations. The iron concentrations of these cereals, as reported by the ICMR, range between 0.7 and 4.9 mg/100 g, while that in pulses is between 2.7 and 8.6 mg/100 g (Gopalan et al., 1999).

3.2. Bioaccessibility of zinc and iron from cereals and pulses

The bioaccessibility values of zinc present in various cereals and pulses, as determined by the *in vitro* digestibility procedure, are also presented in Table 1. Pulses in general had higher amounts of bioaccessible zinc than had cereals. Bioaccessibility of zinc from cereals ranged from 5.5% (sorghum) to 21.4% (rice), and that of pulses ranged from 27% (whole green gram) to 56.5% (decorticated chickpea) of the element present in them. Thus, bioaccessibility of zinc in pulses was several-fold higher than that in cereals. Among the cereals, the lowest availability of zinc from sorghum, although it had the highest concentration of this element (2.24 mg%) and the highest availability of this mineral in the case of rice with the lowest concentration of zinc (1.08 mg%), indicated that zinc bioaccessibility is not necessarily dependent on its concentration in the food grain. Bioaccessibility of iron was almost similar in the five cereals

Table 1
Zinc and iron bioaccessibility from cereals and pulses

Food grain	Zinc content (mg/100 g)	Iron content (mg/100 g)	Zn/Fe molar ratio	Zinc bioaccessibility (%)	Iron bioaccessibility (%)
Cereals					
Rice	1.08 ± 0.02	1.32 ± 0.03	0.70	21.4 ± 0.86	8.05 ± 0.36
Wheat	1.62 ± 0.05	3.89 ± 0.10	0.36	8.93 ± 0.44	5.06 ± 0.38
Finger millet	1.73 ± 0.04	2.13 ± 0.05	0.70	8.31 ± 0.50	6.61 ± 0.46
Sorghum	2.24 ± 0.07	6.51 ± 0.17	0.29	5.51 ± 0.32	4.13 ± 0.33
Maize	1.48 ± 0.05	3.21 ± 0.12	0.39	7.82 ± 0.68	7.83 ± 0.63
Pulses					
Chickpea					
Whole	2.03 ± 0.09	4.95 ± 0.12	0.35	44.9 ± 3.59	6.89 ± 0.54
Decorticated	2.68 ± 0.06	5.05 ± 0.16	0.46	56.5 ± 3.76	4.82 ± 0.36
Green gram					
Whole	2.40 ± 0.07	4.55 ± 0.13	0.46	27.0 ± 1.89	2.25 ± 0.11
Decorticated	2.19 ± 0.03	3.85 ± 0.08	0.49	40.8 ± 3.67	7.49 ± 0.45
Red gram	2.35 ± 0.02	4.93 ± 0.05	0.42	45.7 ± 3.20	3.06 ± 0.18
Black gram	2.30 ± 0.02	6.46 ± 0.10	0.31	33.4 ± 2.15	2.76 ± 0.19
Cow pea	2.57 ± 0.04	4.79 ± 0.08	0.45	53.0 ± 4.76	1.77 ± 0.09
French bean	2.18 ± 0.05	5.94 ± 0.12	0.32	52.5 ± 4.20	10.2 ± 0.77

Values are means ± SEM of five independent determinations.

examined and ranged from 4.13% in sorghum to 8.05% in rice, while that in pulses ranged from 1.77% in cowpea to 10.2% in French bean. Unlike the zinc bioaccessibility result, there was no striking difference between cereals and pulses in iron bioaccessibility. Percent bioaccessible iron values of this study in whole and decorticated chickpea (6.89 and 4.82, respectively) are less than that reported by Chitra, Singh, and Rao (1997) (25%), who measured only soluble ionic iron rather than total soluble iron. The bioaccessibility of zinc from chickpea, observed in this study (44.9% and 56.5% for whole and decorticated forms, respectively), is comparable to the 54.7% reported by Jood and Kapoor (1997).

Among pulses, whole grains of chickpea and green gram had lower availabilities of zinc than had their decorticated counterparts, indicating that the seed coat might consist of components that inhibit the bioavailability of minerals. In general, the bioaccessibility of zinc from all the food grains studied was higher than that of iron, this difference being more prominent in the case of pulses. The Zn:Fe molar ratio in cereals ranged from 0.29 to 0.7 and from 0.32 to 0.49 in pulses (Table 1). The Zn:Fe molar ratio was <1 in all the food grains examined. Thus, despite the lower molar concentrations of zinc relative to that of iron, zinc bioaccessibility was always higher than that of iron, in all the food grains examined.

In the case of iron, which is present in both divalent and trivalent forms, the former is known to be readily absorbed (Garrow, James, & Ralph, 2000). Unlike iron, all of the zinc present in plant foods exists in only one valency state (divalent), which could explain the higher bioaccessibility of this element than that of iron. The amount and/or quality of protein may influence trace element bioaccessibility (O'Dell, 1984). High protein diets, especially those based on animal protein, are reported to enhance the bioavailability of trace minerals, probably by formation of soluble amino acid complexes, which facilitate absorption of the former (Snedeker & Greger, 1983). This may explain the higher bioaccessibility of zinc in pulses than in cereals, the former being richer sources of proteins.

Minerals that are similar in chemical configuration are likely to compete with each other at the site of absorption, thus interfering with their bioavailability (Gibson, 1994). It is not known whether zinc bioavailability from food grains is influenced by minerals having similar chemical configurations. When zinc bioaccessibility from food grains in this study was viewed in relation to the inherent Zn:Fe molar ratio, although there was a trend towards increased *in vitro* availability of zinc from cereals with increased Zn:Fe molar ratios of the grains, such a trend was not discernible in the case of pulses. This could be due to the presence of other dominating factors, such as phytate, which overshadow the effect of Zn:Fe molar ratio alone in pulses. This implies that iron probably does not affect zinc absorption at the molar ratios inherent in the food grains. Iron in pharmacological supplements; however, has been reported to decrease zinc absorption in humans, especially when given in a water solution rather than with the diet (Troost et al., 2003). When iron bioaccessibility from food grains was viewed in relation to the inherent Zn:Fe molar ratio, it was found that iron dialyzability was independent of zinc concentration. Thus, the Zn:Fe molar ratio is probably not a major determinant of the *in vitro* availability of these minerals at the concentrations normally present in the food grains.

3.3. Concentrations of phytic acid, calcium, tannin and dietary fibre in food grains

Differences in the bioaccessibilities of zinc were observed between cereals and pulses, despite similar amounts of zinc inherent in them, while the same was not true in the case of iron bioaccessibility. It was also observed that zinc bioaccessibility from all the food grains tested was generally higher than that of iron. Such differences in the mineral bioaccessibility values could probably be attributed to various inherent factors associated with the grains. Several components present in the diet form soluble and insoluble complexes with trace elements under gastro-intestinal conditions affecting their bioavailability. Thus, it would be relevant to view the mineral bioaccessibility in relation to the concentrations of various modulating factors inherent in the grain. In this context, the concentrations of various factors, such as phytic acid, tannin, calcium and dietary fibre, inherent in these grains, which are likely to influence the bioaccessibility values of zinc and iron, were determined. The bioaccessibility values of zinc and iron of the food grains studied were correlated with concentrations of the above inherent factors.

Concentrations of phytic acid, tannin and calcium in various food grains examined for zinc bioaccessibility are presented in Table 2. Wheat, among the cereals, contained the highest amount of phytic acid, and rice the lowest, while, among the pulses examined, decorticated green gram had the highest content of phytic acid. Decorticated chickpea and green gram had higher phytic acid concentrations than had their whole grain counterparts. This is because phytic acid is mainly located in the cotyledon, with the hull containing less than 0.1% of the seed phytate (Cornovale, Lugaro, & Lombardi-Boccia, 1988). In general, calcium content of cereals was lower than that of pulses, except for finger millet. Among pulses, French bean and whole chickpea had especially high amounts of calcium (158 and 141 mg%, respectively). Among the food grains tested, finger millet had the highest amount of tannin, with similar high amounts being present in French bean and cowpea. Rice, on the other hand, had the lowest amount of tannin.

Total dietary fibre content was lowest in the case of rice, i.e. 3.65% (Table 3). Whole grains had higher dietary fibre contents than had their respective decorticated forms (in the case of chickpea, 27.3% vs. 8.76% and green gram 22.3% vs. 8.37%). The amounts of fibre in these whole legumes were similar to those of other whole legumes – French bean and cowpea (25.8% and 22.5%, respectively),

Table 2
Phytic acid, calcium and tannin contents of cereals and pulses

Food grain	Phytic acid	Phytate/Zn molar ratio	Calcium	[Phytate] × [Ca]/[Zn] molar ratio	Tannin
Rice	160 ± 5.30	14.1	7.26 ± 0.32	14.7	5.00 ± 0.21
Wheat	612 ± 9.40	37.2	36.9 ± 1.60	37.5	212 ± 4.86
Finger millet	417 ± 6.55	23.3	325 ± 9.23	23.9	2117 ± 22.8
Sorghum	295 ± 3.25	13.2	14.3 ± 0.42	13.0	68.3 ± 2.10
Maize	414 ± 7.26	27.4	7.19 ± 0.25	27.7	19.2 ± 0.48
Chickpea					
Whole	263 ± 5.20	12.9	141 ± 4.25	12.9	147 ± 6.24
Decorticated	324 ± 4.98	12.0	54.1 ± 1.24	12.0	58.3 ± 2.75
Green gram					
Whole	553 ± 8.12	22.7	90.3 ± 3.20	22.8	575 ± 11.2
Decorticated	630 ± 10.1	27.9	49.2 ± 2.15	28.5	43.3 ± 1.66
Red gram	388 ± 6.32	16.4	48.0 ± 1.86	16.4	65.0 ± 2.30
Black gram	539 ± 8.45	23.4	67.9 ± 2.33	23.2	55.8 ± 1.85
Cowpea	379 ± 4.92	14.2	87.9 ± 3.24	14.6	2208 ± 12.3
French beans	370 ± 7.50	16.5	158 ± 6.12	16.8	3075 ± 15.1

Values (mg/100 g) are means ± SEM of five independent determinations.

studied here. This higher fibre in the whole legumes is contributed essentially by the insoluble fraction present in the husk portion. Insoluble fibre content of decorticated pulses and of cereals, except rice, were similar. The soluble dietary fibre fraction was especially higher in the case of French bean (4.55%).

3.4. Correlation between bioaccessibility of zinc and iron and various inherent factors

When zinc bioaccessibility values of the food grains tested were viewed with respect to the phytate content of the grain, a negative correlation between dialyzability of zinc and phytate content was generally evident in both cereals and pulses. The multiple regression analysis, carried out to explain the influence of phytic acid on zinc bioaccessibility (Table 4), indicated that this negative effect of phytic acid was highly significant (1%) in the case of pulses, while in cereals, it was not statistically significant. However, when the negative influence of phytic acid on zinc bioaccessibility was viewed in terms of the phytate: zinc molar ratio (Table 2) present in the grain rather than the absolute amounts of phytate independent of zinc concentration, a negative correlation of zinc bioaccessibility was apparent with a phytate to zinc molar ratio range from 14.7 in rice to 23.9 in finger millet, while a further increase in this ratio, even up to 37.5 in wheat, had no corresponding negative effect. A similar trend was observed in pulses up to a phytate:zinc molar ratio of 22.8 (in whole green gram), beyond which the negative trend was not seen. However, such a negative correlation was not statistically significant.

In the case of iron, phytic acid content of the cereal grains produced a proportionate negative influence on its bioaccessibility, with the exception of sorghum. This negative effect

Table 3
Dietary fibre contents of cereals and pulses

Food grain	Total	Insoluble	Soluble
Cereals			
Rice	3.65 ± 0.08	2.80 ± 0.07	0.85 ± 0.02
Wheat	10.9 ± 0.29	8.30 ± 0.25	2.60 ± 0.07
Finger millet	13.1 ± 0.32	12.0 ± 0.46	1.10 ± 0.04
Sorghum	10.2 ± 0.41	9.40 ± 0.30	0.80 ± 0.03
Maize	11.5 ± 0.25	8.83 ± 0.26	2.70 ± 0.08
Pulses			
Chickpea – Whole	27.3 ± 1.01	24.4 ± 1.20	2.97 ± 0.15
Chickpea – Decorticated	8.76 ± 0.26	7.90 ± 0.23	0.86 ± 0.03
Green gram – Whole	22.3 ± 0.72	21.0 ± 0.52	1.38 ± 0.03
Green gram – Decorticated	8.37 ± 0.32	6.90 ± 0.26	1.47 ± 0.06
Red gram	12.6 ± 0.30	10.2 ± 0.15	2.50 ± 0.04
Black gram	16.5 ± 0.45	15.2 ± 0.28	1.30 ± 0.02
Cowpea	22.5 ± 0.80	20.7 ± 0.40	1.75 ± 0.03
French bean	25.8 ± 0.62	21.3 ± 0.42	4.55 ± 0.09

Values (g/100 g) are means ± SEM of five independent determinations.

Table 4
Estimated regression function of the influence of various inherent factors in relation to zinc bioaccessibility from food grains

Inherent factors	Regression co-efficient			
	Cereals		Pulses	
Constant	27.95	(1.473)	78.29	(16.46)
Iron	−1.631*	(0.532)	−0.766	(2.468)
Calcium	−0.040	(0.035)	0.083	(0117)
Phytic acid	−0.005	(0.005)	−0.047**	(0.017)
Soluble dietary fibre	−2.326*	(0.885)	0.455	(3.068)
Insoluble dietary fibre	−0.878	(0.494)	−1.249*	(0.569)
Tannin	0.007	(0.005)	0.006	(0.003)
	$R^2 = 0.955^{**}$		0.466^{*}	

The values in the parenthese indicate standard errors of the co-efficient *Significance at 5% level. **Significance at 1% level.

Regression model for zinc bioaccessibilty from cereals: $R^2 = 0.955^{**}$, $y = 27.95 - 1.631x_1 - 0.040x_2 - 0.005x_3 - 2.326x_4 - 0.878x_5 + 0.007x_6$.

Regression model for zinc bioaccessibilty from pulses: $R^2 = 0.466^{*}$, $y = 78.29 - 0.766x_1 + 0.083x_2 - 0.047x_3 + 0.455x_4 - 1.249x_5 + 0.006x_6$.

Where, y = zinc bioaccessibility, x_1 = iron, x_2 = calcium, x_3 = phytic acid, x_4 = soluble dietary fibre, x_5 = insoluble dietary fibre and x_6 = Tannin.

of inherent phytate on iron bioaccessibility from cereals was statistically significant (5%), as revealed by multiple regression analysis. However, a similar negative influence of phytate on iron bioaccessibility from pulses was not evident (Table 5).

Phytic acid is the principal storage form of phosphorus in cereals, legumes and oil seeds. It is the most potent inhibitor of zinc absorption, but also has a negative impact on non-haem iron absorption, especially in foods of plant origin (Sandberg & Svanberg, 1991), forming insoluble chelates with iron and zinc in the intestine, that are unavailable for absorption (Sandstrom, 1989; Wise, 1983). The inhibitory effect of phytate on the absorption of zinc, and to a lesser extent non-haem iron, has been reported to be dose-dependent (Hallberg, Brune, & Rossander-Hulten, 1989). Several investigators have tested the phytate to zinc molar ratio to predict the bioavailability of zinc in phytate-containing diets, using animal models. Such experiments showed that phytate:zinc molar ratios <12 had little effect on the bioavailability of zinc in rats. The inhibitory effect of phytate content of foods on zinc bioavailability has been reported by Oberleas and Harland (1981) and Turnland, King, Keyes, Gong, and Michel (1984), and molar ratios of phytate to zinc of more than 12 have been implicated in interference with zinc bioavailability in humans. Higher values, such as 15 (Sandberg, Anderson, Carlsson, & Sandstrom, 1986) and even 20 (Ferguson, Gibson, Thompson, & Ounpuu, 1989), of this critical ratio have been associated with clinical zinc deficiency in humans. In our study, although the zinc bioavailability value of the food grain did not have a correlation when viewed in relation to the phytate:zinc molar ratios, nevertheless, a significant negative correlation was inferred when viewed in terms of absolute concentration of the inherent phytate, especially in pulses. Only the hexa- and penta-phosphate esters of inositol appear to significantly inhibit the bioavailability of iron and zinc (Brune, Rossander-Hulten, Gleerup, & Sandberg, 1992; Lonnerdal et al., 1989). The method employed in our study, however, did not differentiate the various phosphates of inositol.

Table 5
Estimated regression function of the influence of various inherent factors in relation to iron bioaccessibility in food grains

Inherent factors	Regression co-efficient			
	Cereals		Pulses	
Constant	8.287	(1.015)	−0.095	(5.869)
Zinc	−0.776	(0.858)	0.651	(1.861)
Calcium	0.009	(0.017)	0.0918*	(0.022)
Phytic acid	−0.006*	(0.002)	0.0037	(0.003)
Soluble dietary fibre	1.430*	(0.481)	0.470	(0.618)
Insoluble dietary fibre	−0.104	(0.175)	−0.427*	(0.104)
Tannin	−0.0004	(0.003)	−0.0006	(0.001)
	$R^2 = 0.792^*$		0.753^{**}	

The values in the parenthese indicate standard errors of the co-efficient. *Significance at 5% level. **Significance at 1% level.

Regression model for iron bioaccessibility from cereals: $R^2 = 0.792^*$, $y = 8.287 - 0.776x_1 + 0.009x_2 - 0.006x_3 + 1.430\,x_4 - 0.104x_5 - 0.0004x_6$.

Regression model for iron bioaccessibility from pulses: $R^2 = 0.753^{**}$, $y = -0.095 + 0.651x_1 + 0.0918x_2 + 0.0037x_3 + 0.470\,x_4 - 0.427x_5 - 0.0006x_6$.

Where, y = iron bioaccessibility, x_1 = zinc, x_2 = calcium, x_3 = phytic acid, x_4 = soluble dietary fibre, x_5 = insoluble dietary fibre and x_6 = Tannin.

The negative impact of calcium content in food on the availability of iron is well recognized, and this may also be true of zinc bioavailability (Davies, Carswell, & Mills, 1985). When zinc bioaccessibility of the grains examined here was viewed in relation to calcium concentration, a decreasing trend was evident in cereals with increase in calcium concentration, which was, however, not statistically significant, as indicated by multiple regression analysis (Table 4). It appears unlikely that the calcium levels commonly found in mixed human diets will promote a phytate-induced decrease in zinc availability (Forbes, Parker, & Erdman, 1984). Some lacto-ovo vegetarians, however, who consume diets which are relatively high in both phytate and calcium, but low in zinc, may be at a risk of suboptimal zinc nutriture. Under such conditions, the [phytate] × [calcium]/[zinc] molar ratio may provide a more useful assessment of zinc bioavailability than phytate:zinc molar ratio alone (Davies, Carswell, & Mills, 1984). In view of the potentiation of the inhibitory effect of phytate by calcium, researchers have advocated the need to view the effect of calcium in terms of the [phytate] × [calcium]/[zinc] molar ratio (Bindra, Gibson, & Thompson, 1986; Ferguson et al., 1989).

When zinc bioaccessibility of various food grains was viewed in relation to calcium concentration in terms of molar ratio: [phytate] × [calcium]/[zinc] (Table 2), generally, in both cereals and pulses, as this ratio increased, there was a slight decrease in zinc bioaccessibility in the food grain. Very limited data exist on the critical [phytate] × [calcium]/[zinc] molar ratio inhibiting the bioavailability of zinc in human diets; however, a ratio between 150 and 200 has been suggested from a retrospective calculation of the data from Cossack and Prasad (1983). None of the food grains studied by us had [phytate] × [calcium]/[zinc] molar ratios exceeding this critical value. In fact, this ratio, in all the grains studied here, was <65, except finger millet which had a ratio of 190. Such an inverse relationship of calcium was, however, not evident for iron bioaccessibility values when the same were viewed in relation to calcium inherent in the grain, as well as the [phytate] × [calcium]/[iron] molar ratio. On the other hand, calcium seems to have a significant positive influence on iron bioaccessibility in the case of pulses. The positive influence of calcium on iron bioaccessibility could be due to its forming a ligand with phytate, thus sparing iron and allowing its free accessibility (Anderson, 2000).

The effect of dietary phytate on the bioavailability of zinc depends on the amounts of calcium in the diet relative to phytate. Maximum precipitation of phytate, and therefore maximum chelation of zinc in solution, occurs at Ca:phytate ratios >6:1, the percentage chelated decreasing with decreasing molar ratios (Wise, 1983). The critical Ca:phytate molar ratio for maximum dietary zinc precipitation for humans is unknown, although it has been suggested that zinc absorption is improved at a Ca:phytate molar ratio of 4.7 compared with a ratio of 8.0 when phytate:Zn molar ratios were >20 (Navert, Sandstrom, & Cederblad, 1985).

When zinc and iron bioaccessibility values in various food grains were viewed in relation to their tannin contents, it appeared that tannin did not have any significant influence on zinc and iron bioaccessibility in either cereals or pulses, accounting for only 0.7% and 0.6% of influence on zinc bioaccessibility (Table 4), and 0.04% and 0.06% on iron bioacessibility (Table 5). The negative role of tannin on iron absorbability from grains is equivocal. Tannin is reported to be a potent inhibitor of iron absorption (Hamdaoui, Doghri, & Tritar, 1995). Several studies have suggested that tannins exert a marked inhibitory effect on Fe absorption (Brune, Rossander-Hulten, & Hallberg, 1989; Gilooly et al., 1983) and this effect has been observed to be dose-related (Siegenberg et al., 1991; Tutanwiroon et al., 1991). On the other hand, Lombardi-Boccia, De Santis, Di Lullo, and Carnovale (1995) did not find any improvement in iron dialyzability from *Phaseolus vulgaris* beans after removal of the hulls, which contain most of the seed tannin. So also in our study, tannin at the levels present in the grains did not exert any statistically significant negative effect on the bioaccessibility of either of the minerals.

When the bioaccessibility of zinc from various cereals and pulses was viewed in relation to the dietary fibre content – total, insoluble and soluble (Table 3), it was evident that, in general, there was a negative influence of total dietary fibre on zinc bioaccessibility in cereals, (Table 4). This trend, however, was not consistent in the case of pulses. When the individual dietary fibre fractions of the food grains were related to zinc bioaccessibility value in cereals, both soluble and insoluble fractions negatively influenced zinc bioaccessibility, the effect being statistically significant (5% level) only for the soluble fibre fraction (Table 4). In the case of pulses, which generally had a high concentration of insoluble fibre, this fraction had a significant (5% level) negative influence on zinc bioaccessibility (Table 4). Similar to the effect on zinc bioaccessibility, insoluble dietary fibre negatively influenced iron bioaccessibility in cereals and pulses, the effect being statistically significant only in the latter (Table 5). On the other hand, the soluble dietary fibre fraction had a positive influence on iron accessibility from cereals and pulses, as indicated by the multiple regression analysis (Table 5), the effect being significant (5% level) in the case of cereals.

Thus, while both insoluble and soluble fractions of the dietary fibre in food grains generally interfered with zinc bioaccessibility, the insoluble fraction alone had this effect on iron bioaccessibility. The soluble fraction, on the other hand, had the opposite effect on bioaccessibility of iron, and more so in the case of cereals. It is speculated that the amount and type of dietary fibre matters in their influence on trace element bioavailability (Gibson, 1994). In general, the soluble fibre pectin, found predominantly in fruits, does not affect the absorption of zinc. By contrast, insoluble cereal and vegetable fibres (cellulose, hemicelluloses and lignin) are said to inhibit the bioavailability of zinc (Schwartz, Apgar, & Wien, 1986). The effects of dietary fibre on the bioavailability of trace elements are speculated to be confounded by the presence of other minerals and proteins in the food, as well as by the presence of phytate or oxalic acid (Kelsay, Prather, Clark, & Canary, 1988).

3.5. Relative effects of all inherent factors on zinc and iron bioaccessibility

In the case of zinc bioaccessibility from cereals, the multiple regression analysis, fitted to explain the collective influence of various inherent factors listed in Table 4, indicated that the inherent factors explained a 95.5% (R^2) variation in the bioaccessibility of zinc due to these factors, which is highly significant (1%). In the case of pulses, these inherent factors explained 46.6% of the variation, which was found to be significant at the 5% level (Table 4). Thus, the lower collective negative influence of the various inherent factors on zinc bioaccessibility from pulses is consistent with the higher zinc bioaccessibility value of these grains, relative to cereals. In the case of iron bioaccessibility from cereals, the collective influence of the inherent factors examined amounted to 79.2% (R^2), which was significant at the 5% level (Table 5). The extent of collective influence of the various inherent factors on iron bioaccessibility from pulses was essentially similar ($R^2 = 75.3\%$), which is statistically highly significant at the 1% level (Table 5). This explains the observed similar iron bioaccessibility values in both cereals and pulses.

3.6. Influence of reduction of phytate by treatment with phytase on mineral bioaccessibility

Among the various factors, phytate seems to have the maximum negative influence on the bioaccessibility of zinc, especially in pulses. This led us to examine whether removal of phytate from pulses would beneficially influence zinc bioaccessibility. Decorticated green gram and black gram, which had relatively higher phytate contents, were pre-treated with fungal phytase, which resulted in 36% and 24% reductions in phytate in green gram and black gram, respectively. Fig. 1 presents the zinc and iron bioaccessibility values in phytase-treated legumes. Reduction in phytate brought about a marginal increase (16%) in the bioaccessibility of zinc from black gram, while it did not seem to have any influence on the same from green gram. On the other hand, iron bioaccessibility significantly improved as a result of reduction in phytate content, the increases being 221% for black gram and 74.5% for green gram. Thus, partial removal of phytate from the food grains enhanced the bioaccessibility of minerals, especially of iron, supporting our inference of a negative correlation of inherent phytic acid with zinc and iron bioaccessibility.

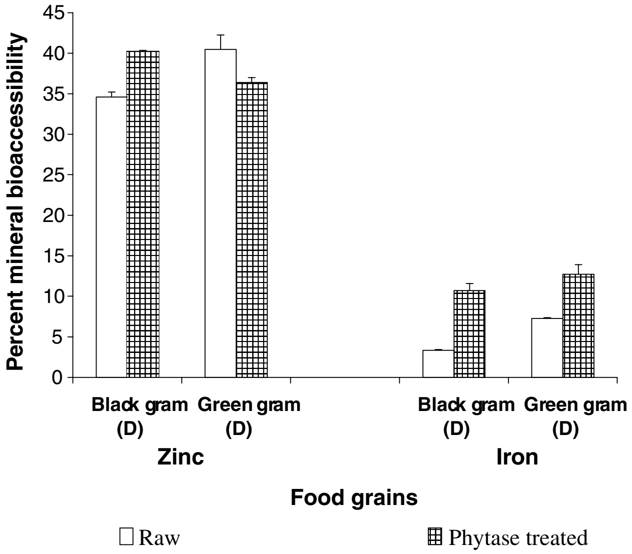

Fig. 1. Zinc and iron bioaccessibilities from phytase-treated grains D: decorticated grain.

Thus, removal of phytate from food grains will prove to be advantageous in terms of increase in the bioaccessibility of iron and zinc. There are several methods available to reduce the phytate content of various foods: leavening of bread (Navert et al., 1985), fermentation (Gibson et al., 1998), germination and milling (Gibson et al., 1998; Svanberg & Sandberg, 1988), treatment of foods with food grade phytase or the addition of phytase to the diet (Sandberg, Rossander-Hulten, & Turk, 1996).

4. Conclusion

Among the various cereals and pulses consumed in India, the bioaccessibility of zinc in pulses was generally higher than that in cereals, whereas bioaccessibilities of iron were similar in both cereals and pulses examined. Among the various inherent factors, phytate, tannin, calcium and dietary fibre, phytate, calcium and fibre (both soluble and insoluble) had a negative influence on the bioaccessibility of zinc. The lower collective negative influence of the various inherent factors on zinc bioaccessibility from pulses is consistent with the higher values of these in these grains, relative to cereals. Iron bioaccessibility was negatively influenced by inherent phytate and insoluble fibre. The negative correlation of inherent phytic acid with zinc and iron bioaccessibility was supported by the enhanced bioaccessibility of these minerals observed upon partial removal of phytate from the food grains by treatment with fungal phytase. The exceptionally higher bioaccessibility of zinc in rice could be attributed to the low levels of inhibitors of its absorption through interference with solubilization of the minerals.

The *in vitro* method employed here for the estimation of zinc and iron bioaccessibility is based on simulation of gastro-intestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that dialyzes through a membrane. Such *in vitro* methods are rapid, simple and inexpensive. The dialyzability of a mineral gives a fair estimate of its availability for absorption *in vivo* (i.e., bioaccessibility). Such relative estimates of the mineral bioaccessibility, in terms of *in vitro* zinc and iron dialyzability obtained in the present investigation for different food grains, are still valid and suffice to make a comparison among the various food grains consumed, as well as to form dietary strategies to derive maximum mineral availability.

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