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ORIGINAL ARTICLE

Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains

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Objective and design: Food grains such as green gram, chickpea and finger millet are often subjected to traditional processing involving germination and fermentation. This study was designed to assess the effect of germination of these grains on the bioaccessibility of zinc and iron. The effect of fermentation of a cereal–pulse combination as encountered in the preparation of breakfast dishes – *idli*, *dosa* and *dhokla* – on the same was also evaluated. Bioaccessibility measurement was made employing an *in vitro* simulated digestion method.
Result: Zinc bioaccessibility was significantly decreased by germination (48 h) of finger millet (38%) and green gram (44%), while iron bioaccessibility was increased by 62% (green gram), 39% (chickpea) and 20% (finger millet), concomitant with a reduction in tannin content. A fermented batter of rice + black gram – 2:1 (*idli*) and 3:1 (*dosa*) – had higher bioaccessibility values for zinc (71 and 50%, respectively), while iron bioaccessibility values were increased in these cases of fermentation to an even greater extent, namely 277 and 127%, respectively. Zinc and iron bioaccessibility was not improved by fermentation of the combination of chickpea, green gram, black gram and rice (1:1:0.5:0.5; *dhokla*). A fermentation of cereal–legume combinations of *idli* and *dosa* batter significantly reduced both phytate and tannin, while in the case of *dhokla* batter there was a continued significant presence of phytate associated with additional legumes – chickpea and green gram.
Conclusion: Germination of food grains improved the bioaccessibility of iron but not that of zinc. Fermentation of a batter of cereal–pulse combination in the preparation of *idli* and *dosa* enhanced the bioaccessibility of both zinc and iron, but not that of the combination used for the preparation of *dhokla*.
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Introduction

Deficiency of micronutrients such as vitamin A, iron, iodine and zinc is a major public problem in developing countries, including India. Deficiency of zinc, 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 animal foods are rich sources of zinc, this micronutrient has to be derived mainly through food grains by a majority of the population in developing countries. Cereals are the primary sources of zinc in most vegetarian diets, secondary sources being legumes (Gibson, 1994). Bioavailability of minerals, especially iron, is low from plant foods (Gibson, 1994; Sandberg, 2002).

Bioavailability of trace minerals is known to be influenced by various dietary components that include both inhibitors and enhancers of their absorption, as well as by various food processing methods (Gibson, 1994; Sandberg, 2002). Food processing by heat generally alters the bioavailability of nutrients – both macro and micro. The digestibility and hence absorption of micronutrients such as iron is improved upon heat processing, which results in a softening of the food matrix and the release of protein-bound iron, thus facilitating its absorption (Lombardi-Boccia *et al.*, 1995). Bioaccessibility of zinc from food grains, however, was observed to be lower upon heat processing (Hemalatha *et al.*, 2006). Other domestic food processing methods such as germination and fermentation are known to improve

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mineral bioavailability by reducing the inhibitors of their absorption, such as phytic acid present in the grains (Gibson and Hotz, 2001; Kaur and Kawatra, 2002; Duhan *et al*., 2004). Besides reducing such factors, fermentation could also improve mineral bioavailability by virtue of the formation of organic acids, which form soluble ligands with the minerals, thereby preventing the formation of insoluble complexes with phytate. Germination and fermentation are common domestic processing methods widely used in Indian traditional cuisine. Combinations of cereals and pulses are fermented overnight and then cooked to make products – *idli*, *dosa* and *dhokla* – which are the primary breakfast items in most parts of India. Similarly, legumes such as chickpea, green gram, etc. are germinated and consumed in either the cooked or raw form. Information on the influence of germination and fermentation of cereals and legumes on the bioavailability of zinc is limited. The present investigation was therefore carried out to examine the effect of the germination and fermentation of a few cereals and pulses and their combinations, as commonly encountered in Indian cuisine, on the bioaccessibility of zinc.

Materials and methods

Materials

Cereals – rice (*Oryza sativa*) and finger millet (*Eleusine coracana*) – and pulses – whole and decorticated chickpea (*Cicer arietinum*), whole and decorticated green gram (*Phaseolus aureus*) and decorticated black gram (*Phaseolus mungo*) – were procured locally, cleaned and used for germination and fermentation studies. Porcine pancreatin, pepsin and bile extract were from M/s Sigma Chemical Co., St Louis, USA. All other chemicals used here were of analytical grade. Triple distilled water was employed during the entire study. Acid-washed glassware was used throughout the study.

Total zinc and iron

Finely ground grain samples were ashed in a muffle furnace at 550°C for 5 h and dissolved in concentrated HCl. Zinc and iron content were determined by atomic absorption spectrometry (Shimadzu AAF-6701). Calibration of measurements was performed using commercial standards. All measurements were carried out under standard flame operating conditions as recommended by the manufacturer. The reproducibility values were within 2.0% for both zinc and iron.

Bioaccessibility of zinc and iron

Bioaccessibility of zinc and iron in various food grain samples was determined by an *in vitro* method described by Luten *et al*. (1996) involving simulated gastrointestinal digestion with suitable modifications. The samples were finely ground in a stainless steel wearing blender and then subjected to 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 0.1 M sodium bicarbonate containing 4 g pancreatin + 25 g bile extract). The 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 cutoff: 10 kDa) containing 25 ml sodium bicarbonate solution, being equivalent in moles to the NaOH 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. Pancreatin–bile extract mixture (5 ml) was 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, which represents bio-available trace elements, were analyzed by atomic absorption spectrometry.

Estimation of phytate and tannin content

Phytate in food grains was determined as phytin-phosphorous by the method of Thompson and Erdman (1982). Phytate values were computed by multiplying the phytin phosphorous value by 3.55. Tannin was estimated by the modified vanillin assay of Price *et al*. (1978), using catechin as the standard.

Germination of cereals and legumes

Finger millet, chickpea and green gram were soaked for 16 h in triple distilled water (1:2.5 w/v) and the soaked grains were drained of water and allowed to germinate in a BOD incubator at 25°C for 24 and 48 h.

Fermentation of cereals and legumes

Different combinations of cereals and pulses, viz., rice + black gram (2:1) as in the preparation of *idli*, rice + black gram (3:1) as in the preparation of *dosa* and chickpea + green gram (decorticated) + black gram (decorticated) + rice (2:2:1:1) as in the preparation of *dhokla,* were soaked in water (grain:water = 1:2.5 w/v) for 10 h. The soaked grains were ground with the entire water to a fine batter. The batter was allowed to ferment for 14 h at laboratory temperature without the addition of any exogenous starter culture. A portion of the batter was steam cooked for 10 min in the case of *idli* and *dhokla*, while a portion of the batter for *dosa* was pan-fried (in a non-stick pan without oil).

Statistical analysis

Determinations of zinc and iron bioavailability in these variations of food samples, as well as all other chemical analyses, were carried out in five replicates. Statistical analysis of data was performed employing Student's *t*-test according to Snedecor and Cochran (1976).

Results

Effect of germination on bioaccessibility of zinc and iron

The influence of germination of various food grains on the bioaccessibility of zinc and iron is presented in Table 1. While zinc bioaccessibility was not affected by germination of chickpea for 24 and 48 h, the same was significantly decreased in the case of finger millet (by 30 and 38% respectively) and green gram (by 38 and 44%, respectively). On the other hand, germination of these grains for 24 and 48 h significantly increased the bioaccessibility of iron by 38 and 62% (green gram), and 37 and 39% (chickpea), respectively. A 20% increase in iron bioaccessibility from finger millet was seen at the end of 48 h germination. Thus, germination of food grains had contrasting effects on the bioaccessibility of zinc and iron.

Phytate content was not affected by germination of the test grains in our study, while tannin was reduced significantly, both at 24 and 48 h (Table 2). The reduction was 43 and 74% in the case of green gram, 44 and 50% in the case of finger millet and 47 and 52% in the case of chickpea, respectively after 24 and 48 h germination.

Effect of soaking on bioaccessibility of zinc and iron

Soaking of the legumes – green gram and chickpea – for 16 h generally did not seem to have a beneficial influence on either zinc or iron bioaccessibility (Table 3). In the case of green gram, zinc bioaccessibility was even reduced by 37% (Table 3).

Effect of fermentation on bioaccessibility of zinc and iron

Table 4 presents the influence of fermentation on bioaccessibility of zinc and iron from combinations of cereals and legumes. After fermentation, batters of rice + black gram – 2:1 (*idli*) and 3:1 (*dosa*) – showed significantly higher bioaccessibility values for zinc. The increases in zinc bioaccessibility value were as high as 71% in *idli* and 50% in *dosa*, respectively. Such an increased bioaccessibility of zinc, however, was not evident in the case of fermented *dhokla* batter; instead there was a slight decrease (10%). Since these fermented batters are heat-processed in the preparation of respective dishes, bioaccessibility of these minerals was also determined in the final products. In the case of *idli*, the zinc bioaccessibility value, although higher (27%) than that of unprocessed grains, was lower (26%) than that of the uncooked fermented batter. In the case of *dosa*, the zinc bioaccessibility value similarly decreased upon heat processing of the fermented batter (46% lower), and this value was

Table 3 Zinc and iron bioaccessibility in soaked legumes

Food grain	*Bioaccessible zinc (%)*		*Bioaccessible iron (%)*	
	Unprocessed	*Soaked for 16 h*	*Unprocessed*	*Soaked for 16 h*
Green gram	13.1 ± 5.16	8.19 ± 0.96**	6.12 ± 3.82	6.48 ± 0.67
Chickpea	21.4 ± 3.78	17.6 ± 5.01	4.08 ± 1.36	3.57 ± 1.03

Values are mean ± s.d. of five replicates.
**Significant decrease.

Table 1 Influence of germination on bioaccessibility of zinc and iron from finger millet, green gram and chickpea

Food grain	*Bioaccessible zinc (%)*			*Bioaccessible iron (%)*		
	Native	*Germinated – 24 h*	*Germinated – 48 h*	*Native*	*Germinated – 24 h*	*Germinated – 48 h*
Finger millet	3.94 ± 0.60	2.77 ± 0.96**	2.44 ± 0.29**	24.6 ± 0.85	26.3 ± 1.56	29.5 ± 0.63*
Green gram	37.47 ± 10.7	23.24 ± 5.54**	21.01 ± 3.58**	5.05 ± 0.22	6.95 ± 1.12*	8.18 ± 0.20*
Chickpea	45.09 ± 3.94	40.47 ± 8.35	40.13 ± 3.53	6.39 ± 0.69	8.78 ± 0.89*	8.87 ± 0.58*

Values are mean ± s.d. of five replicates.
*Significant increase; **significant decrease.

Table 2 Influence of germination on tannin and phytate content of finger millet, green gram and chickpea

Food grain	*Tannin*			*Phytate*		
	Native	*Germinated – 24 h*	*Germinated – 48 h*	*Native*	*Germinated – 24 h*	*Germinated – 48 h*
Green gram	450.0 ± 10.7	258.7 ± 8.18**	117.4 ± 4.02**	219.0 ± 13.4	208.9 ± 7.87	189.5 ± 18.6
Finger millet	973.9 ± 23.0	547.2 ± 14.4**	482.6 ± 4.83**	215.4 ± 12.6	203.9 ± 10.7	204.6 ± 7.33
Chickpea	215.2 ± 6.17	115.0 ± 5.12**	104.3 ± 3.18**	180.8 ± 6.28	170.0 ± 5.48	164.2 ± 11.7

Values expressed as mg per 100 g are mean ± s.d. of five replicates.
**Significant decrease compared to unprocessed sample.

Table 4 Influence of fermentation on bioaccessibility of zinc and iron from food grains

Food grain	*Bioaccessible zinc (%)*			*Bioaccessible iron (%)*		
	Raw grains	*Fermented batter – raw*	*Fermented batter - cooked*	*Raw grains*	*Fermented batter – raw*	*Fermented batter - cooked*
Idli						
Rice:black gram (D) (2:1)	31.7 ± 3.18	54.3 ± 13.2*	40.3 ± 5.59*	6.50 ± 1.41	24.5 ± 0.69*	23.1 ± 3.56*
Dosa						
Rice:black gram (D) (3:1)	29.7 ± 4.25	44.6 ± 8.18*	24.3 ± 3.04	6.90 ± 1.50	15.7 ± 4.20*	30.1 ± 6.93*
Dhokla						
Chickpea:green gram (D):black gram (D):rice (2:2:1:1)	54.0 ± 6.51	48.3 ± 7.58	37.1 ± 13.7**	12.0 ± 1.27	13.4 ± 1.23	13.3 ± 2.33

Values are mean ± s.d. of five replicates.
D: Decorticated.
*Significant increase compared to raw sample; **significant decrease compared to raw sample.

18% lower than that of the unprocessed grains. In the case of *dhokla*, the zinc bioaccessibility value decreased by 23% upon heat processing of the fermented batter, this final value being 31% lower than the unprocessed grain combination.

Fermentation of the combination of rice and black gram at both the proportions examined enormously improved the bioaccessibility of iron. Bioaccessible iron in the fermented *idli* batter was 276% higher and that in the *dosa* batter was 127% higher compared to the untreated grains used here. Heat treatment of the batter further increased the bioaccessibility of iron by 92% in the case of *dosa*, while that in the *idli* batter did not change. Thus, fermentation and heat processing as in the preparation of *dosa* resulted in a net increase of 335% in the bioaccessibility of iron from the rice–black gram combination. Fermentation of a combination of chickpea, green gram, black gram and rice as in the preparation of *dhokla*, however, did not improve the bioaccessible iron content of the native grains. Heat processing of the fermented batter of *dhokla* also did not alter the bioaccessible iron content; thus, the bioaccessible iron value of *dhokla* remained the same as that of the native grain combination used.

A reduction in the pH by 1.5 units as a result of fermentation of the batter of *idli*, *dosa* and *dhokla* was observed in the present study.

Discussion

Many food items are subjected to simple household processing before they are consumed. It is possible that some of these processes may influence the availability of zinc and iron. However, no systematic study on the effect of such processes on the availability of zinc/iron in foods appears to have been carried out. This paper reports the results of a study of the effect of soaking, germination and fermentation on zinc and iron availability from selected cereals and legumes consumed in India. Among the various legumes consumed in India, green gram and chickpea are most commonly germinated prior to use in the preparation of specific traditional dishes, especially in southern India. Germinated and malted finger millet is employed in the preparation of weaning/geriatric foods and as a beverage. Hence, these grains were screened in this study for evaluation of the possible influence of germination on bioaccessibility of zinc and iron. *idli*, *dosa* and *dhokla* are the common cereal and legume based fermented foods consumed in India. Food grains used in the preparation of these breakfast items are rice + black gram (*idli* and *dosa*) and rice + black gram + chickpea + green gram (*dhokla*) in varying proportions. These cereal–legume combinations were therefore studied here for the possible effect of fermentation on the bioaccessibility of zinc and iron.

Typical food processing methods such as germination and malting have been found to enhance iron absorption due to elevated vitamin C content or reduced tannin or phytic acid content, or both (Tontisirin *et al.*, 2002). These processes are known to activate phytases, which in turn hydrolyze phytate, rendering iron and zinc more available. During germination, endogenous phytase activity in cereals and legumes increases as a result of *de novo* synthesis and/or activation, resulting in reductions in inositol penta- and hexa-phosphates depending on the species and variety (Lorenz, 1980; Bartnick and Szafranska, 1987; Chavan and Kadam, 1989; Reddy *et al.*, 1989). Reddy *et al.* (1989) have reported reductions in phytate ranging from 36% for sprouted soya beans to 53% for germinated lentils. In addition, germination also reduces the content of polyphenols and tannins in some legumes (Camacho *et al.*, 1992).

Studies *in vitro* on iron bioavailability have shown a two-fold increase in germination and a five- to 10-fold increase in malting of minor millets (De Maeyer *et al.*, 1989). Germination of pigeon pea had a beneficial effect on the extractability of zinc and copper as reported by Duhan *et al.* (2004). Zinc and copper are generally present in association with phytic acid in plant foods, which may be responsible for their poor extractability. A decrease in the concentration of phytic acid by food processing such as soaking and germination may possibly release these metallic ions in free form. Germination significantly improved zinc absorption from cooked beans (Kannan *et al*., 2001).

Chopra and Sankhala (2004) have recently examined the influence of soaking and sprouting of horse gram (*Dolichos biflorus*) and moth bean (*Phaseolus aconitifolius*), which are rich in phytate and tannin, on *in vitro* iron availability. Soaking these legumes for 8 and 16 h reduced the levels of tannins and phytates. Degradation of phytates and tannins was more pronounced after 24 h of germination. *In vitro* iron availability significantly increased after soaking as well as after germination of these legumes (Chopra and Sankhala, 2004).

Phytate and tannin are reported to be potent inhibitors of iron bioavailability (Sandberg and Svanberg, 1991; Hamdaoui *et al*., 1995). In the absence of any decrease in phytate content of the grains, the reduction in tannin content during germination of the test grains in our study could be a factor that contributed to the increase in the bioaccessibility of iron. In addition, it is also possible that the vitamin C content of these grains increased during germination, and higher vitamin C contents could be an additional factor contributing to improvement in iron bioaccessibility. Unlike iron, a relationship between tannin content of foods and zinc bioaccessibility has not been established so far. The inhibitory effect of phytate on zinc bioavailability in foods has been reported by Oberleas and Harland (1981) and Turnland *et al*. (1984). The absence of any increase in zinc bioaccessibility upon germination of the tested food grains in our study is consistent with a lack of significant reduction of phytate content of these grains.

A two-fold increase in *in vitro* iron absorption has been observed in legumes and cereals germinated for different periods (Prabhavathi and Rao, 1979). Increases in iron ionizability could be observed during germination of green gram or wheat as early as 24 h, whereas such an effect was seen only after extended periods (48 and 72 h) in the case of chickpea. In the case of legumes, an increase was observed only with the whole grain, whereas no such changes were observed with decorticated legumes (Prabhavathi and Rao, 1979). In our study, improved iron bioacessibility was observed in chickpea germinated even for 24 h.

Thus, our observations on the improved bioaccessibility of iron upon germination of grains are in concurrence with several such observations reported in the literature. The information on improved bioaccessibility of iron from finger millet upon germination reported in this study is novel. While several studies have been reported on the influence of germination of food grains on iron bioaccessibility, ours is the first to report the same on zinc bioaccessibility.

Soaking of grains is the first step in the process of germination. It is possible that soaking brings about alterations in the factors that influence mineral availability. Soaking is claimed to reduce the phytic acid of most legumes because their phytic acid is stored in a relatively water-soluble form such as sodium or potassium phytate (Chang *et al*., 1977). Soaking may also remove polyphenols from certain beans (Ene-Obong and Obizoba, 1996). Soaking maize flour or pounded maize is reported to have resulted in 57 and 51% loss of phytate. The same authors have envisaged a reduction in phytate content and phytate:zinc molar ratio during fermentation of maize flour batter (Hotz and Gibson, 2001).

Our observation of reduced zinc bioaccessibility from germinated green gram could probably be attributed mainly to the effect of soaking itself (which is the first step in the germination process) rather than that of germination. Neither soaking nor germination had any beneficial influence on the bioaccessibility of zinc from chickpea. While germination brought about a significant increase in bioaccessible iron from green gram and chickpea, the same was not observed at the stage of soaking. Hence, the observed effect on iron bioaccessiblity could be attributed to changes occurring during germination. The absence of an increase in zinc and iron bioaccessibility as a result of soaking of food grains as observed by us could probably be because, in our study, the water in which the legumes were soaked was not discarded, but blended along with the grains for further determination of mineral bioaccessibility. Thus, the processed grain samples used for the determination of mineral bioaccessibility were not devoid of the soluble phytates that would have leached out of grain during soaking.

Microbial fermentation can enhance iron and zinc bioavailability via hydrolysis of phytate by microbial phytase derived from naturally occurring microflora on the surface of cereal grains (Sandberg, 1991). The beneficial effect of fermentation on mineral bioavailability could also be attributed to the formation of organic acids during this process, which form soluble ligands with iron and zinc (Tontisirin *et al*., 2002). In fact, we did evidence a reduction in the pH by 1.5 units as a result of fermentation of the batter of *idli*, *dosa* and *dhokla* in our study. This reduction in pH is certainly due to the synthesis of organic acids during fermentation. In Latin American countries, household food processing methods such as fermentation and germination are used in formulating infant foods (Devadas, 1998). *In vitro* measurements of soluble iron have been reported in cereal porridges prepared with soaking and fermenting flour slurries (Svanberg *et al*., 1993). Greater femoral zinc in rats fed with diets containing fermented soybean meal as compared to regular soybean meal has been reported, which probably results from increased zinc solubility in the small intestine (Hirabayashi *et al*., 1998).

Besides improving organoleptic properties, these processes activate phytases, which in turn hydrolyze phytate, improving iron and zinc bioavailability (Svanberg *et al*., 1993). Reductions in phytate content have been reported during lactic fermentation in the case of maize flour (Amoa and Muller, 1976) and leavening of wheat flour dough (Tangkongchitr *et al*., 1982). An increase in *in vitro* iron

Table 5 Influence of fermentation on tannin and phytate content of cereal-pulse combinations

Food grain	*Tannin*		*Phytate*	
	Raw grains	*Fermented batter – raw*	*Raw grains*	*Fermented*
Idli				
Rice:black gram (D) (2:1)	13.0±1.43	Traces**	85.9±4.81	Traces**
Dosa				
Rice:black gram (D) (3:1)	9.50±0.78	Traces**	65.4±4.02	32.7±5.70**
Dhokla				
Chickpea:green gram (D):black gram (D):rice (2:2:1:1)	182.6±5.39	Traces**	193.3±7.07	139.1±10.1**

Values are mean±s.d. of five replicates.
D: Decorticated.
**Significant decrease compared to raw sample.

bioavailability from iron-fortified dairy products (acidified milk and yoghurt) has been evidenced following milk fermentation or acidification. Lactic acidification and fermentation also increased zinc availability *in vitro* (Drago and Valencia, 2002). In our investigation, natural fermentation of cereal–legume combinations significantly reduced both phytate and tannin (Table 5) from the test grains. Phytate was completely removed during fermentation of the *idli* batter, while the reduction was 50 and 28% during fermentation of *dosa a*nd *dhokla* batter, respectively. Only traces of tannin were detectable in the fermented batters of *idli*, *dosa* and *dhokla*. The absence of any positive influence of fermentation on mineral bioaccessibility in the case of *dhokla* batter could be attributed to the continued presence of significant amounts of phytate. The additional legumes – chickpea and green gram – present in *dhokla* apart from rice and black gram (constituent grains for *idli* and *dosa*) have contributed to this significant phytate content.

In one of the earliest studies on the influence of food processing on *in vitro* iron availability, Prabhavathi and Rao (1979) have reported that fermentation of rice and black gram mixture and subsequent cooking (as in the preparation of *idli*) did not result in any change in the ionizable iron content. Our present observation on fermented food grains differs from this report. Most of these earlier studies reporting on the influence of fermentation on mineral bioavailability involved the use of externally added starter cultures for fermentation, while our study reports the influence of fermentation by cultures of lactic acid bacteria naturally associated with the grain. While several studies have been reported on the influence of lactic fermentation (using starter cultures) of grain flours on mineral bioaccessibility, information on the influence of natural fermentation, as encountered at the household level on zinc bioaccessibility, is limited.

The *in vitro* method employed here for the estimation of mineral availability is based on the simulation of gastrointestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that dialyses through a membrane. The dialysability of a mineral gives a fair estimate of its availability for absorption *in vivo*. Such *in vitro* methods are rapid, simple and inexpensive and offer the possibility of optimally controlling the assay conditions, which can lead to high accuracy. It is also ensured that the *in vitro* method employed in the present investigation gave reproducible mineral bioaccessibility values. Although the values from *in vitro* methods are relative rather than absolute estimates of mineral absorbability, being not subjected to the physiological factors that can affect bioavailability, such relative estimates on the comparative zinc/iron bioavailability values for food grains that were subjected to germination and fermentation are still valid and suffice to form a strategy to derive maximum mineral availability.

Concluding remarks

The present study has revealed that while germination of green gram, chickpea and finger millet for 24 and 48 h significantly enhanced the bioaccessibility of iron, bioaccessibility of zinc was not beneficially affected. In the absence of any decrease in phytate content of the grains, reduction in tannin content during germination of the test grains could have contributed to the increase in the bioaccessibility of iron. Fermentation of the batter of the cereal–pulse combination as in the preparation of *idli* and *dosa* significantly enhanced the bioaccessibility of both zinc and iron, the extent of increase in the case of iron being still better. However, such a beneficial influence of fermentation on zinc and iron bioaccessibility was not observed in the case of cereal–pulse combination used for the preparation of *dhokla*. Significant reductions of both phytate and tannin during fermentation of cereal–legume combinations of the *idli* and *dosa* batter must have contributed to the observed increase in mineral bioaccessibility. The absence of any positive influence of fermentation on mineral bioaccessibility in the case of *dhokla* batter could be attributed to the continued presence of significant amounts of phytate or any other protein that binds these micronutrients contributed by additional legumes – chickpea and green gram – present in *dhokla*. Thus, soaking, germination and fermentation of cereals and legumes (in which phytate is in the cotyledons) offers a practical household method to reduce inhibitors of mineral absorption, especially phytic acid and tannin, thereby contributing to enhanced zinc and iron absorption. In fact, such processing of cereals and legumes as part of the daily culinary is in wide practice across the Indian subcontinent.

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References

Amoa B, Muller HG (1976). Studies on kenkey with particular reference to calcium and phytic acid. *Cereal Chem* **53**, 365–375.

Bartnick M, Szafranska I (1987). Change in phytate content and phytase activity during the germination of some cereals. *J Cereal Sci* **5**, 23–28.

Camacho L, Sierra C, Campos R, Guzman E, Marcus D (1992). Nutritional changes caused by germination of legumes commonly eaten in Chile. *Arch Latin Americanos Nutricion* **42**, 283–290.

Chang R, Schwimmer S, Burr HK (1977). Phytate: removal from whole dry beans by enzymatic hydrolysis and diffusion. *J Food Sci* **42**, 1098–1101.

Chavan JK, Kadam SS (1989). Nutritional improvement of cereals by fermentation. *Crit Rev Food Sci Nutr* **28**, 349–400.

Chopra S, Sankhala A (2004). Effect of soaking and sprouting on tannin, phytate and *in vitro* iron in under-utilized legumes – horse gram (*Dolichos biflorus*) and moth bean (*Phaseolus aconitifolius*). *J Food Sci Tech* **41**, 547–550.

De Maeyer BM, Dallman P, Gurncy JM, Hallberg L, Sood SK, Srikantia SG (1989). *Preventing and Controlling Iron Deficiency Anaemia through Primary Health Care – A Guide for Health Administrators and Programme Managers*. WHO: Geneva.

Devadas RP (1998). Local strategies to support child nutrition. *Nutr Res* **18**, 233–239.

Drago SR, Valencia ME (2002). Effect of fermentation on iron, zinc and calcium availability from iron fortified dairy products. *J Food Sci* **67**, 3130–3134.

Duhan A, Khetrapaul N, Bishnoi S (2004). HCl – extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *J Food Comp Anal* **17**, 597–604.

Ene-Obong HN, Obizoba IC (1996). Effect of domestic processing on the cooking time, nutrients, antinutrients and *in vivo* protein digestibility of the African yam bean. *Plant Foods Hum Nutr* **49**, 43–52.

Gibson RS (1994). Content and bioavailability of trace elements in vegetarian diets. *Am J Clin Nutr* **59** (Suppl), 1223S–1232S.

Gibson RS, Hotz C (2001). Dietary diversification/modification strategies to enhance micronutrient content and bioavailability of diets in developing countries. *Br J Nutr* **85** (Suppl 2), S159–S166.

Hamdaoui M, Doghri T, Tritar B (1995). Effect of different concentrations of ascorbic acid, and tea mixture on non-haem iron absorption from a typical Tunisian meal fed to healthy rats. *Ann Nutr Metab* **39**, 310–316.

Hemalatha S, Platel K, Srinivasan K (2006). Influence of heat processing on the bioaccessibility of zinc from cereals and pulses consumed in India. *J Trace Elements Med Biol* **20**, in press.

Hirabayashi M, Matsui T, Yano H (1998). Fermentation of soybean meal with *Aspergillus usamii* improves zinc availability in rats. *BiolTrace Element Res* **61**, 227–234.

Hotz C, Gibson RS (2001). Assessment of home-based processing methods to reduce the phytate content and phytate : zinc molar ratio of white maize. *J Agric Food Chem* **49**, 692–698.

Kaur M, Kawatra BL (2002). Effect of domestic processing on zinc availability from rice bean (*Vigna umbellata*) diets. *Plant Food Hum Nutr* **57**, 307–318.

Lombardi-Boccia G, De Santis N, Di Lullo G, Carnovale E (1995). Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.). *Food Chem* **53**, 191–195.

Lorenz K (1980). Cereal sprouts: composition, nutritive value, food applications. *CRC Crit Rev Food Sci Nutr* **13**, 353–385.

Luten J, Crews H, Flynn A, Dael PV, Kastenmayer P, Hurrel R *et al.* (1996). Inter-laboratory trial on the determination of the *in vitro* iron dialyzability from food. *J Sci Food Agric* **72**, 415–424.

Kannan S, Nielson SS, Patricia A, Rodriguez-Burger AP, Mason AC (2001). Iron and zinc bioavailability in rats fed intrinsically labeled bean and bean–rice infant weaning food products. *J Agric Food Chem* **49**, 5063–5069.

Oberleas D, Harland BF (1981). Phytate content of foods: effect on dietary zinc bioavailability. *J Am Dietet Assoc* **79**, 433–436.

Prabhavathi T, Rao BSN (1979). Effects of domestic preparation of cereals and legumes on ionizable iron. *J Sci Food Agric* **30**, 597–602.

Prasad AS (2003). Zinc deficiency in humans: effect on cell mediated immunity. In: *Nutrition Goals for Asia – Vision 2020* 1st edn, Nutrition Foundation of India: New Delhi. pp 349–358.

Price ML, Scoyoc SV, Butler LG (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J Agric Food Chem* **26**, 1214–1228.

Reddy NR, Pierson MD, Sathe SK, Salunke DK (1989). *Phytates in Cereals and Legumes*. CRC Press: Boca Raton. pp. 68–72.

Sandberg AS (1991). The effect of food processing on phytate hydrolysis and availability of iron and zinc. In: Friedman M (ed). *Nutritional and Toxicological Consequences of Food Processing*. Plenum Press: New York. pp. 499–508.

Sandberg AS (2002). Bioavailability of minerals in legumes. *Br J Nutr* **88** (Supplement 3), S281–S285.

Sandberg AS, Svanberg U (1991). Phytate hydrolysis by phytase in cereals; effects on *in vitro* estimation of iron availability. *J Food Sci* **56**, 1330–1333.

Snedecor GW, Cochran WG (1976). *Statistical Methods* 6th edn. Iowa State University Press: Ames. 298pp.

Svanberg U, Lorri W, Sandberg AS (1993). Lactic fermentation of non-tannin and high tannin cereals: effects on *in vitro* estimation of iron availability and phytate hydrolysis. *J Food Sci* **58**, 408–412.

Tangkongchitr U, Seib PA, Hoseney RC (1982). Two barriers to the loss of phytate during bread making. *Cereal Chem* **59**, 216–221.

Thompson DB, Erdman JW (1982). Phytic acid determination in soy beans. *J Food Sci* **47**, 513–517.

Tontisirin K, Mantel G, Battacharjee L (2002). Food based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proc Nutr Soc* **61**, 243–250.

Turnland JR, King JC, Keyes WR, Gong B, Michel MC (1984). A stable isotope study of zinc absorption in young men: effects of phytate and cellulose. *Am J Clin Nutr* **40**, 1071–1077.