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# PHYTATE:ZINC AND PHYTATE X CALCIUM:ZINC MOLAR RATIOS IN SELECTED DIETS OF ETHIOPIANS

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**Abstract.** The phytate:zinc and phytate x calcium:zinc molar ratios of selected Ethiopian diets were determined. The effect of these molar ratios on zinc solubility (index of bioavailability) from the selected diets (porridge, gruel, sour dough bread, yeasted bread, *kitta*, and *injera*) was estimated using the *in vitro* method. The mean phytate:zinc and phytate x calcium:zinc molar ratios of the unfermented diets were grater than 10 and 0.5 M/kg, respectively, while *injera* and sourdough bread exhibited ratios less than the suggested critical values of 10 and 0.5 M/kg, respectively. Fermented gruel had phytate:zinc and phytate x calcium:zinc molar ratios slightly greater than the critical values. Diets with low phytate:zinc and phytate x calcium:zinc molar ratios exhibited relatively higher bioavailability of zinc. Thus, the molar ratio phytate:zinc and phytate x calcium:zinc predict the adequacy of zinc nutriture in a given diet.

## INTRODUCTION

The nutritional value of a diet in terms of macrominerals and trace minerals is dependent on the amount of mineral that is bioavailable for physiological processes in the organism much more than its content in the diet. Several dietary factors have been implicated in changes in the absorption of trace minerals, for example, zinc [1]. Erdman [2] observed that phytic acid found in cereals and legumes is thought to be a major contributor to the reduced availability of zinc from these foods.

The prediction of mineral bioavailability from phytate-containing foods is complicated by the complex interactions between minerals and phytic acid contained in foods. The formation of zinc-calcium-phytate complexes or other zinc-phytate complexes in the upper gastrointestinal tract of monogastric animals is believed to be a major mechanism by which phytate reduces dietary zinc bioavailability [3].

The phytate:zinc molar ratio has been suggested as an indicator of zinc bioavailability [4-5]. However, the phytate:zinc molar ratio does not indicate that high dietary calcium accentuates the effects of phytate on zinc bioavailability [6]. In contrast, it has been suggested that the dietary phytate x calcium:zinc molar ratio may provide a more useful assessment of zinc bioavailability than the phytate:zinc molar ratio alone [7-9].

Some of the cereal-based Ethiopian diets include *injera* (fermented pancake-like sour bread) *kitta* (unleavened flat bread), porridge, gruel (thin porridge, 10% dry matter), sourdough bread (*diffo dabbo*) and yeasted-bread. There is only scant information on the phytate:zinc molar ratios of few selected Ethiopian diets [10]. Information on the phytate:zinc and phytate x calcium:zinc molar ratios of most Ethiopian diets is, however, lacking. The objective of this investigation was to determine the phytate:zinc and phytate x calcium:zinc molar ratios as indexes of the effect of phytate on bioavailability of zinc in selected Ethiopian diets.

## EXPERIMENTAL

*Materials.* Brown and white varieties of tef (*Eragrostis tef*) were procured from the local market in Bishoftu, Shoa, Ethiopia in a single lot. *Faffa* (weaning food) and soy-fortified wheat flour (locally known as *Dubbie* flour) were procured from a store owned by Faffa Foods Factory, Addis Ababa, Ethiopia. Whole wheat flour and white flour were obtained from the Department of Milling and Baking Technology, Central Food Technological Research Institute (CFTRI), Mysore, India. The tef grains were cleaned of dust and other foreign materials and were ground in an electric grinder (Milone, M/S Rajkot, India) using 0.5 mm sieve size.

*Preparation of meals.* Procedures for preparation of *kitta*, porridge, gruels and natural lactic acid fermentation to produce *injera*, fermented gruel and sourdough bread (*Diffo dabbo*) were described previously [10-11]. Yeast-raised bread was prepared following the straight dough procedures described in AACC [12].

*In vitro zinc bioavailability.* All glassware were soaked for 24 h in a 10% (v/v) solution of nitric acid and rinsed three times with double distilled water before use. The *in vitro* digestion method developed by Narasinga Rao and Prabhavati [13] for estimating iron availability in food mixtures was used with minor modifications. Zinc solubility from the test diets at pH 7.5 was considered as an index of availability.

In brief, in a 125-mL Erlenmeyer flask 2.50 g dry food, 12 mL 0.5% pepsin (P-700, Sigma Chemical Co.) in 0.01 M HCl and approximately 30 mL 0.01 M HCl were combined, adjusted to pH 2.0 with 1.2 M HCl, and incubated in shaking incubator (100 rpm) at 37 °C for 90 min. Following incubation, each sample was immediately transferred to 50 mL centrifuge tubes and centrifuged for 20 min at 1800 X g or 4500 rpm and the supernatant filtered. The pH of the filtrate was adjusted to 7.5 by slowly adding 2 M NaOH, dropwise while magnetically stirring. The flasks were then incubated followed by centrifugation as described earlier. Zinc solubility of the food digestate was measured by filtering a 10 mL aliquot using ashless filter paper in a Buchner funnel under vacuum and wet digested with acid mixtures [10]. Samples of test foods and cereal flours were also subjected to a wet digest procedure. The wet digestion solutions were analysed for zinc, using an atomic absorption spectrophotometry (Model 3110; Perkin-Elmer, Norwalk, CT, USA). Calcium was determined by the titration method [14]. Phytic acid (*myo*-inositol hexaphosphate) contents of the cereal flours and their food products were determined using a spectrophotometer method [15]. Calcium, zinc and phytate were expressed as moles/kg of diet calculated on a dry weight basis [8]. The results were subjected to analysis of variance. Significant differences between means were determined by using Student's *t* test at the 5% level of probability [16].

## RESULTS AND DISCUSSION

Table 1 summarises the composition of raw cereal flours. The zinc content of the cereal flours ranged from 0.22 to 0.36 mM/kg. The data show that zinc contents of both brown tef and white tef flour are significantly greater than that of the other flours while white flour had the least zinc content. The content of calcium also varied from 7.24 to 51.41 mM/Kg. The weaning food, *Faffa*, and brown and white tef flours did not differ significantly in their content of calcium. The high calcium content of the *Faffa* flour might be a result of the addition of skim milk powder to the flour.

White flour had a lower mineral content than the other cereal flours and also the lowest phytic acid. The phytic acid contents of *Faffa*, *dubbie* and white flours did differ significantly from that of tef and whole wheat flours (Table 1). The phytic acid content of white flour is about three times less than that of the tef and whole wheat flours, probably due to removal of the bran during wheat milling.

Table 1. Comparisons of zinc solubility and two molar ratios for cereal flours.*

| Sample type | Soluble Zn % | Ca mM/kg | Zn mM/kg | (P)/(z) | Phyrate mM | (P)(C)/(Z) M |
|---|---|---|---|---|---|---|
| Brown tef flour | 8.03±0.13 | 51.41±0.32 | 0.36±0.07 | 30.03±2.51 | 10.76±0.80 | 1.54±0.05 |
| White tef flour | 9.14±0.50 | 49.32±0.13 | 0.35±0.02 | 29.07±1.17 | 10.27±0.71 | 1.43±0.07 |
| *Faffa* | 6.68±0.27 | 48.73±0.31 | 0.33±0.01 | 23.23±1.41 | 9.38±0.31 | 1.37±0.08 |
| *Dubbie* | 4.71±0.17 | 10.34±0.12 | 0.22±0.02 | 18.01±2.71 | 7.84±0.16 | 0.37±0.05 |
| Whole wheat flour | 6.23±0.11 | 10.73±0.86 | 0.32±0.01 | 33.46±4.54 | 10.66±0.81 | 0.36±0.02 |
| White flour | 4.23±0.14 | 7.24±0.14 | 0.22±0.01 | 14.20±0.39 | 3.15±0.14 | 0.10±0.01 |

* Mean ± S.D. of four determinations. (P)/(Z) = phytate:zinc; (P)(C)/(Z) = phytate x calcium: zinc.

The zinc solubility profiles following *in vitro* treatments of cereal flours are given in Table 1. The solubility of zinc after the *in vitro* digestion ranged from 4.23 to 9.14%. Zinc in tef flours was more soluble than from the other flours. Similarly, the phytate:zinc and phytate x calcium:zinc molar ratios (M/kg) varied in the range between 14 and 30 and 0.10 to 1.54, respectively.

Table 2 indicates soluble zinc, phytic acid and phytate:zinc and phytate x calcium:zinc molar ratios of porridge, gruel and *kitta* diets prepared from the cereal flours. The heat treatment during the preparation of the food products had a minor effect on the phytic acid content. Only small differences were observed between the cereals flours and foods prepared from these cereal flours in phytic acid content. Under these conditions clearly, little or no phytic acid is destroyed and thus the phytic acid contents of the uncooked flours shown in Table 1 would approximate to the foods that are normally eaten.

Digestion in HCl-pepsin and adjustment of the acid digest to pH 7.5 did not improve zinc solubility of the prepared foods over those values reported for the cereal flours (Table 1). Considering the solubility criterion bioavailability, it appears that lower zinc solubility from these foods lends support a reduction of zinc bioavailability by phytate due to formation of zinc-phytate complex at pH 7.5.

The lower solubilities of zinc following digestion of the pH 7.5 may also partially be attributed to the formation of insoluble calcium and zinc hydroxides as the pH values of the digests were adjusted to 7.5 with NaOH. Insoluble calcium and zinc hydroxide formation occurs at pH values greater than 6 [17].

Cooking also had no effect on the phytate:zinc and phytate x calcium:zinc molar ratios (Table 2). The prepared meals had values in the range 18-31 and 0.36-1.61 M/kg compared to 18-30 and 0.37-1.54 M/kg for the cereal flours. These results indicate that processing of the flours into porridge, gruel or *kitta* had little effect on the relative proportions of zinc, calcium and phytic acid.

Table 2. Comparisons of zinc solubility and two molar ratios for selected food products*.

| Flour type | Food type | Soluble Zn % | Phytate mM | (P)/(Z) | (P)(C)/(Z) M |
|---|---|---|---|---|---|
| Brown tef | *Kitta* | 9.61±0.13 | 10.76±0.42 | 30.03±3.12 | 1.54±0.15 |
| | Porridge | 6.64±0.12 | 10.99±0.56 | 30.67±9.60 | 1.58±0.12 |
| | Gruel | 8.03±0.13 | 11.22±0.15 | 31.32±1.74 | 1.61±0.30 |
| White tef | *Kitta* | 9.34±0.04 | 10.12±0.17 | 28.67±4.21 | 1.41±0.05 |
| | Porridge | 8.89±0.19 | 10.10±0.23 | 28.61±0.67 | 1.41±0.08 |
| | Gruel | 9.14±0.50 | 10.37±0.63 | 29.38±2.23 | 1.45±0.09 |
| *Faffa* | Porridge | 7.08±0.73 | 9.14±0.13 | 22.31±2.10 | 1.33±0.11 |
| | Gruel | 8.84±0.44 | 9.07±0.15 | 22.13±1.22 | 1.32±0.21 |
| *Dubbie* | Porridge | 4.91±0.77 | 7.99±0.35 | 17.99±0.93 | 0.37±0.07 |
| | Gruel | 5.91±0.31 | 7.76±0.24 | 17.76±1.82 | 0.36±0.08 |

* Mean ± S.D. of four determinations. (P)/(Z) = phytate:zinc; (P)(C)/(Z) = phytate x calcium:zinc.

Table 3 indicates the composition of the fermented products of tef, *dubbie*, whole wheat and white flours. Natural lactic acid fermentation of tef to produce gruel or *injera* significantly increased the solubility of zinc at pH 7.5. In contrast, the phytate:zinc, phytic acid content and phytate x calcium:zinc molar ratios decreased significantly compared to the unfermented gruel or unleavened flat bread *kitta*. Zinc solubility increased by an average of 46% in *injera* while the phytic acid, phytate:zinc and phytate x calcium:zinc molar ratios decreased by an average of 72 and 79%, respectively, in brown and white tef *injera* as compared to *kitta*. Such significant reductions in phytic acid, phytate:zinc and phytate x calcium:zinc molar ratios may be attributed to the action of the organisms, yeasts and *Lactobacillus*, present in the fermentation medium as compared to that in the raw cereal flours. Phytate destruction by natural lactic acid fermentation has been reported previously for tef [10].

Table 3. Comparisons of zinc solubility and two molar ratios for selected fermented food products.*

| Flour type | Food type | Soluble Zn % | Phytate mM | (P)/(Z) | (P)(C)/(Z) M |
|---|---|---|---|---|---|
| Brown tef | *Injera* | 54.19±0.27 | 2.96±0.19 | 8.27±0.21 | 0.43±0.08 |
| | Gruel | 50.37±0.37 | 5.93±0.14 | 16.56±0.41 | 0.85±0.12 |
| White tef | *Injera* | 55.38±1.15 | 2.10±0.12 | 5.94±0.41 | 0.29±0.02 |
| | Gruel | 54.78±0.18 | 5.35±0.16 | 15.15±0.69 | 0.75±0.13 |
| Dubbie | Sourdough | 27.37±0.47 | 0.93±0.03 | 4.26±0.21 | 0.04±0.01 |
| | Yeasted | 13.01±0.79 | 5.08±0.08 | 33.18±0.29 | 0.24±0.02 |
| Whole wheat | Sourdough | 26.28±0.13 | 1.63±0.42 | 5.01±0.27 | 0.05±0.01 |
| | Yeasted | 12.18±0.95 | 7.99±0.14 | 25.09±0.83 | 0.27±0.09 |
| White flour | Sourdough | 48.32±0.28 | - | - | - |
| | Yeasted | 22.67±0.82 | 1.16±0.65 | 5.21±0.38 | 0.38±0.02 |

* Mean ± S.D. of four determinations. (P)/(Z) = phytate:zinc; (P)(C)/(Z) = phytate x calcium:zinc.

The reduced solubility of zinc in *kitta* product as compared to *injera* and fermented gruel diets may be the result of the formation of stable calcium-phytate-zinc complexes in the *kitta* diet. These complexes would be resistant to digestion in the digestion tract and consequently inefficient absorption of zinc would occur.

The composition of yeast-raised and sourdough (*diffo dabbo*) breads is given in Table 3. A short leavening time (225 min) for *dubbie* flour yeasted-bread reduced the phytate content from 7.64 to 5.08 mM while prolonged leavening (24 h) of sourdough bread preparation reduced it to 0.93 mM. Sourdough bread produced from white flour contained no phytate. Whole wheat flour contained 10.7 mM phytic acid which was reduced to 1.6 mM after 24 h of leavening and baking the sourdough bread while in yeasted-bread the phytic acid content was reduced by 27%. Phytate destruction by yeast and sourdough fermentations has also been reported previously for bread [18-19].

The percentage of zinc soluble from breads varied according to the phytic acid contents of the breads (Table 3). The amount of zinc soluble from the breads increased when the fermentation time was increased. The solubility of zinc in sourdough bread prepared from white flour was the highest (48%) and least (12%) in yeast-raised bread produced from *dubbie* flours. The absence of phytic acid in white flour sourdough bread increased the white flour was the highest (48%) and least (12%) in yeast-raised bread produced from *dubbie* flours. The absence of phytic acid in white flour sourdough bread increased the solubility of zinc by 1.8-fold compared with whole wheat flour sourdough bread containing 0.93 mM of phytic acid and four-fold compared with yeasted-bread fermented for only 225 min. Navert *et al.* [20] observed that substantial reductions in phytic acid content of bread have been associated with greater zinc absorption in humans. The molar ratios, phytate:zinc and phytate x calcium:zinc obtained were thus ranged from 0 to 5.01 and 0 to 0.05 in sourdough breads and 5.21 to 33.2 and 0.24 to 0.38 M/kg, respectively, in yeasted breads (Table 3).

Since the effectiveness with which phytate can reduce zinc availability in diets depends upon the absolute levels of both zinc and phytic acid, it has been suggested that the dietary contents of phytic acid and zinc expressed as molar ratio, phytate:zinc could be a satisfactory means of predicting whether a phytate-containing diet may give rise to poor availability of zinc [21]. A molar ratio ≥ 10 has been established as the dividing point between dietary conditions providing adequate zinc homeostasis and those resulting in inadequate zinc nutriture [22]. Harland and Petersen [23] estimated the phytate:zinc molar ratio of a normal diet to be about 6.

With zinc solubility considered as bioavailability, our results for fermented gruel, *injera* and sourdough breads is consistent with observations that low phytate:zinc molar ratios in various foods are associated with increased zinc absorption by rats. Stuart *et al.* [24] reported that rats were able to absorb a significantly higher amount of zinc from the fermented sorghum food than from the cooked maize or sorghum gruels presumably due to the lower phytate:zinc molar ratios in this product.

With the phytate:zinc molar ratio as an index (suggested critical value 10 from animal studies) of zinc nutriture [21], foods which may make a significant contribution towards these critical ratios include unleavened flat bread *kitta*, unfermented gruel and unfermented porridge. The results in Table 2 suggest that long-term consumption of such diets might develop zinc deficiency. Bindra *et al.* [7] similarly listed foods with phytate:zinc molar ratios of more than 10 as wholemeal chapatti, tanok and soy bean flour.

*In vitro* studies have shown that calcium increases the precipitation of insoluble zinc-phytate complexes at intestinal pH. The formation of such a complex *in vivo* in the upper gastrointestinal tract of single-stomached animal is thought to be the mechanism by which phytate reduces dietary zinc bioavailability [25]. In the present study, tef flour and the weaning food *Faffa* contained high level of calcium compared to the other cereal flours. However, food products prepared from these flours contain calcium to phytate molar ratios significantly lower than the critical value (Ca/phytate molar ratio = 18) to impair zinc solubilization [26].

Bindra *et al.* [7], Ellis and Kelsay [8], and Fordyce *et al.* [9] have reported that the phytate x calcium:zinc molar ratio is a better index of zinc utilisation. Phytate x calcium:zinc molar ratios exceeding 0.5 M/kg may adversely affect zinc balance in humans and for the rat, molar ratios above 3.5 M/kg dry diet would predict poor zinc status. Perhaps the human is less able than the rat to digest and absorb zinc from insoluble complexes in the gastrointestinal tract.

If the phytate x calcium:zinc molar ratio is used as the index of the effect of phytate on dietary zinc bioavailability, the data (Table 2) indicate that zinc bioavailability may be likely to be impaired by these diets while the level of dietary phytate present in fermented diets (Table 3) would be likely to have little effect on the dietary zinc bioavailability. The results also suggest that diets with relatively high phytate x calcium:zinc molar ratios are characterized by low zinc solubility in contrast to diets with relatively low phytate x calcium:zinc molar ratios which exhibited relatively higher zinc solubility. The large discrepancy between phytate:zinc and phytate x calcium:zinc molar ratios as index of the phytate effect on bioavailability of zinc in yeasted-breads appears to be a result of the low dietary calcium content of the diets.

Thus, consumption of cereals with higher phytic acid contents, with higher phytate: zinc or phytate x calcium:zinc molar ratios, or without animal protein in the meal will likely result in poorer zinc availability. Establishing a nutrient database containing phytate, calcium and zinc analysis values for all foods will allow estimation of the phytate:zinc and phytate x calcium:zinc molar ratios in a diet with sufficient precision to predict the probable risk of zinc deficiency.

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