

Disodium Ethylenediaminetetraacetic Acid (EDTA) as an Enhancer of Iron and Zinc Bioaccessibility from Select Cereals and Pulses

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ABSTRACT

Introduction: In view of the widespread deficiencies of trace minerals like iron and zinc, strategies to enhance their bioavailability from plant foods is of interest. This study was therefore undertaken to explore ethylenediaminetetraacetic acid (EDTA), a known metal chelator, for a possible beneficial influence on the bioaccessibility of iron and zinc from food grains. **Methods:** EDTA was added at molar ratios ranging from 1:0.25 to 1:2 relative to the inherent iron and zinc to raw and heat-processed food grains. Bioaccessibility of iron and zinc was determined by an *in vitro* simulated gastrointestinal digestion procedure. **Results:** EDTA significantly enhanced iron bioaccessibility from all the food grains (from one-fold to thirteen-fold increase), in both raw and heat processed grains. The beneficial influence of EDTA on iron bioaccessibility was more prominent in cereals than in pulses. EDTA showed influence on zinc bioaccessibility to a lesser magnitude (from one-fold to five-fold increase). The impairment on zinc bioaccessibility by heat processing from the food grains was efficiently countered by EDTA. EDTA added at a level equimolar (1:1) to the inherent iron and zinc significantly enhanced the bioaccessibility of the iron and zinc among the food grains. **Conclusion:** EDTA could be an effective co-fortificant to enhance the bioaccessibility of iron and zinc from food grains, possibly in the form of a sprinkle, to combat mineral deficiencies.

Key words: Bioaccessibility, cereals, EDTA, iron, zinc

INTRODUCTION

Micronutrient deficiencies, such as that of iron and zinc, are widespread across the world and are more prevalent in the developing countries. One of the many factors responsible for these deficiencies is the people's dependence exclusively on plant based foods with poor bioavailability of micronutrients. The bioavailability of trace elements such as iron and zinc has been shown to be inhibited by the presence of antinutritional factors like phytates, tannins, and dietary fibre, while

on the other hand it is enhanced by organic acids (Woods, 2005; Gillooly *et al.*, 1983; Lönnerdal, 2000), spices like onion and garlic and β -carotene (Gautam, Platel & Srinivasan 2010; 2011). The antinutritional factors inhibit the bioavailability of metal ions by forming complexes with them, thus rendering them unavailable for absorption (Prasad, 1998).

Iron and zinc are essential for various vital functions in the human body, and their deficiencies have profound physiological and socio-economic consequences.

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Although staple cereals, millets, and pulses are good sources of iron and zinc, the presence of inherent antinutritional factors limits their bioavailability. One of the strategies to combat the deficiency of these vital trace elements would therefore be to improve their bioavailability from plant foods.

Ethylenediaminetetraacetic acid (EDTA), a known metal chelator, is often used as a food additive to preserve food, and as a co-fortificant to stabilise iron and improve its bioavailability. The Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (1999) permitted the use of CaNa_2EDTA and Na_2EDTA up to 2.5 mg/kg body weight/day, with the maximum acceptable daily intake set at 150 mg/person/day. Recent studies show that EDTA, when used as a co-fortificant along with iron at levels equimolar to the inherent iron content of food grains, it significantly enhances the bioaccessibility of both iron and zinc from food grains (Tripathi & Platel, 2011). However, the EDTA was added at only one concentration level as a co-fortificant. This study aimed at exploring EDTA as a possible enhancer to improve the bioaccessibility of iron and zinc from food grains. EDTA was added exogenously at varying levels to raw and heat processed food grains and the bioaccessibility of iron and zinc was studied.

METHODS

Cereals, namely, rice (*Oryza sativa*), wheat (*Triticum aestivum*), finger millet (*Eleusine coracana*), and sorghum (*Sorghum vulgare*), and pulses, namely, red gram (*Cajanus cajan*), and black gram (*Phaseolus mungo*), both decorticated, were procured locally and finely ground using hammer mill (mesh size: 32) comminuting mill (Cadmill Machinery Co Pvt Ltd, Ahmedabad, India). Pepsin, pancreatin, and bile extract,

were procured from Sigma Chemical Co, St Louis, MO, USA and were all of porcine origin. Na_2EDTA and other chemicals used were of analytical grade. Triple distilled water was used throughout the experiment, and the glassware used was acid washed and rinsed with triple distilled water.

Total iron and zinc content of the cereals was analysed using the dry ashing technique. Finely ground samples were ashed in a muffle furnace at 550 °C for 6 h. The ash was dissolved in concentrated hydrochloric acid (AOAC, 2000). An atomic absorption spectrometer (Shimadzu AAF-6701) was used to analyse total iron and zinc content, and the calibration of the measurements was done using commercial standards. All the measurements were carried out using standard flame operating conditions recommended by the manufacturer.

An *in vitro* method described by Luten *et al.* (1996) involving simulated gastrointestinal digestion and equilibrium dialysis was used to assess bioaccessible iron and zinc. The dialysable portion of iron and zinc representative of the bioaccessible fraction was analysed by atomic absorption spectrometry. The percentage bioaccessibility of iron and zinc was calculated as follows:

$$\text{Bioaccessibility (\%)} = 100 * Y/Z,$$

where Y is the element content of the bioaccessible fraction (mg mineral elemental/ 100 g grain), and Z is the total iron or zinc content (mg mineral element/100 g grain).

Na_2EDTA of particle size 150×10^6 M was added to the food grains at molar ratios of the inherent iron or zinc to EDTA, ranging from 1:0.25 to 1:2. For each of the grains examined, the molar ratios of inherent iron to EDTA were calculated individually and EDTA was added accordingly. In order to study the effect of EDTA on zinc bioaccessibility, molar ratios of inherent zinc to EDTA were used.

In order to test whether the effect of EDTA on mineral bioaccessibility would be retained even when the food grains were subjected to heat processing, the selected food grains were subject to three heat processing techniques employed at home, namely, pressure cooking, open pan boiling, and microwave cooking, in the presence of EDTA. Ten gram of the food grains was pressure cooked in 60 ml of triple distilled water for 10 min (15 psi). For open pan boiling, 10 g of cereal was cooked in 120 ml of triple distilled water without lid for 10 to 15 min.

For microwave (SAMSUNG Trio, Combi - CE1031LAT) cooking, 10 g of the food grains were cooked in 120 ml of triple distilled water with 450 W for 7 min. The cooked samples were further analysed for mineral bioaccessibility as described above.

The samples were determined in four replicates and the average values were considered. Statistical analysis was done using OriginPro (version 8) statistical

software. Results were analysed by one-way analysis of variance (ANOVA), and the significance level was calculated using the Tukey-Kramer comparison test. Results were considered significant at $P < 0.05$.

RESULTS

Effect of Na_2EDTA on iron bioaccessibility from food grains

The effect of EDTA added at varying levels ranging from 1:0.25 to 1:2 relative to the inherent iron on the bioaccessibility of iron from raw and heat processed cereals is presented in Figure 1. Presence of EDTA brought about a highly significant increase in the percentage of iron bioaccessibility from all the cereals examined, with the increase being dose-dependent. The bioaccessibility of iron from rice (Figure 1a), which was 16% in the control grain, was enhanced to 22% by EDTA at the molar ratio of 1:0.25 (iron:EDTA). The addition of EDTA at a molar ratio of 1:2 (iron:EDTA) brought about an enormous increase of

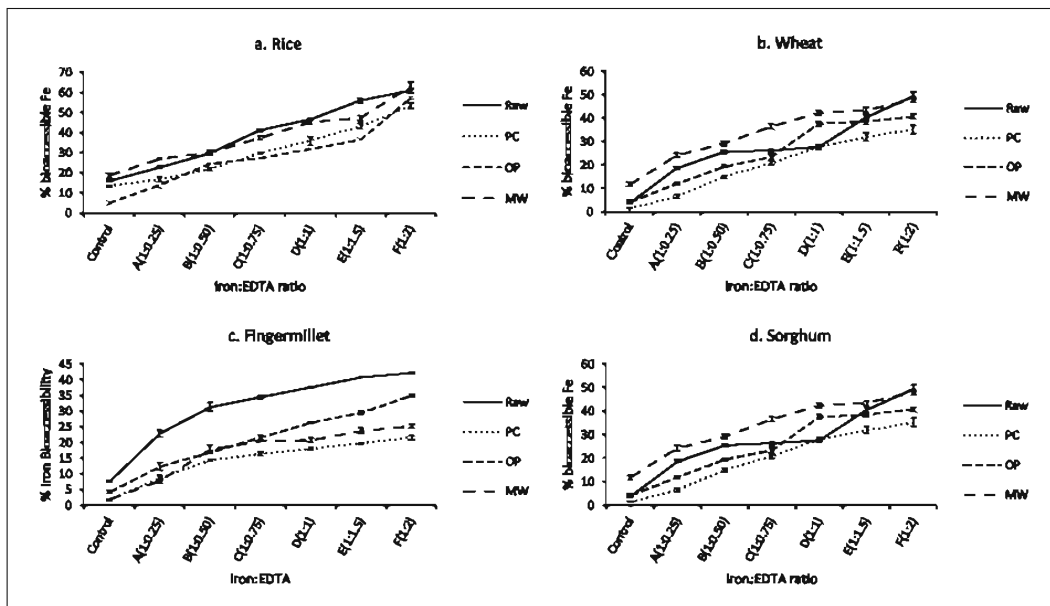


Figure 1. Effect of varying levels of EDTA on iron bioaccessibility from raw and heat processed cereals

Values are Mean \pm standard error of mean (SEM) of four replicate analyses

60% in iron bioaccessibility, which was 60%. Thus, there was an almost four-fold increase in iron bioaccessibility from rice with the addition of EDTA at the highest level examined here, whilst the increase was 50% at the lowest level. This increase in iron bioaccessibility was retained even when the rice was heat treated in the presence of varying levels of EDTA. The differences in the heat treatment methods such as pressure-cooking, open pan boiling, or microwave cooking did not make a difference to the beneficial effect of EDTA on iron's bioaccessibility from rice. The trend observed in the heat processed grain was similar to that seen in the raw grain. Bioaccessibility of iron from the pressure-cooked, open pan boiled, and microwave cooked rice was 13.4%, 5.11%, and 18.7%, respectively in the absence of EDTA. The addition of EDTA at a molar ratio of 1: 2 relative to iron enhanced bioaccessibility to 53%, 57%, and 63%, respectively. Although open pan boiling reduced the bioaccessibility of iron from rice, the addition of EDTA countered this negative effect. Thus, even small amounts of EDTA had significant beneficial effects on iron bioaccessibility from rice, both in the raw and cooked forms.

Similar significant increases in iron bioaccessibility were observed by the addition of EDTA to wheat, with the increases being dose-dependent (Figure 1b). The iron bioaccessibility of the control wheat was around 4%, and this increased to 18% when EDTA was added at a molar ratio of 1: 0.25 (iron: EDTA), and at the highest level of EDTA, it was 49%. The beneficial effect of EDTA was similar at various levels of heat processed wheat. Pressure cooking of wheat significantly decreased the bioaccessibility of iron, but this was effectively countered by the addition of EDTA.

The addition of EDTA to finger millet at varying levels brought about significant increases in iron bioaccessibility (Figure 1c). There was a three-fold increase

in bioaccessible iron when EDTA was added at the lowest level (1: 0.25), whilst the increase was more than five-fold at the highest level (1:2). This positive effect of EDTA was even more prominent when the grains were heat processed in the presence of this chelator. Additionally, EDTA effectively countered the negative effect of heat treatment on iron bioaccessibility. The percentage of bioaccessible iron in pressure-cooked, open pan boiled, and microwave cooked finger millet were 1.8%, 4.2%, and 2%, respectively and increased to 8.7%, 12.3%, and 7.7%, respectively, with addition of EDTA at the lowest level.

Heat processing of the finger millet by all the three methods reduced iron bioaccessibility in the absence of EDTA. However, the addition of EDTA to the grains at the lowest level increased iron bioaccessibility back to that of the raw grain, thus countering the negative effect of heat processing. The extent of the increase in iron bioaccessibility from the heat processed finger millet as a result of the addition of EDTA was greater than from the raw grain. Despite this, the percentage of bioaccessible iron in raw finger millet remained higher than that of the heat processed grains because of the initial significant decrease in iron bioaccessibility due to heat processing.

There was an enormous increase in iron bioaccessibility from sorghum as a result of the addition of EDTA from around 4.5% to 7% with the highest level of EDTA (Figure 1d). As in the case of the other cereals, EDTA brought about a dose-dependent increase in iron bioaccessibility. This beneficial effect of EDTA was retained even in heat processed sorghum. Whilst microwave cooking reduced iron bioaccessibility, addition of the metal chelator countered this negative effect.

The influence of EDTA on iron bioaccessibility from pulses is presented in Figure 2. As found in the case of cereals, the positive effect of EDTA on iron bioaccessibility in the pulses was

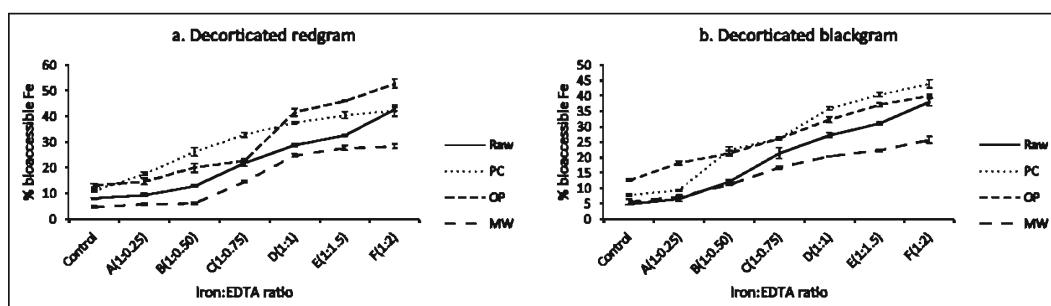


Figure 2. Effect of varying levels of EDTA on iron bioaccessibility from raw and heat processed pulses

Values are mean \pm SEM of four replicate analyses

dose-dependent. There was a small one-fold increase in iron bioaccessibility from decorticated red gram with the addition of EDTA at a molar ratio of 1:0.25, whilst at the highest level of EDTA added, the increase was five-fold (Figure 2a). Heat treatment, especially by pressure cooking and open pan boiling, brought about a marginal increase in iron bioaccessibility from the decorticated red gram, which was further enhanced by the presence of EDTA. The increase in iron bioaccessibility from cooked red gram as a result of addition of EDTA was similar to that observed in the raw grain.

The effect of EDTA on iron bioaccessibility from black gram was even more pronounced, with an eight-fold increase at the highest level of EDTA (Figure 2b). This beneficial effect of EDTA was retained even when the grain was heat treated, although the extent of increase in iron bioaccessibility was slightly lower. Among the three methods of heat treatment, the beneficial influence of EDTA on iron bioaccessibility was more prominent in open-pan boiled black gram.

Effect of Na₂EDTA on zinc bioaccessibility from food grains

Zinc bioaccessibility was also enhanced by the presence of EDTA in the cereals studied as shown in Table 1. This beneficial effect of EDTA on zinc bioaccessibility from these grains, however, was less

prominent compared to its effect on iron bioaccessibility. EDTA enhanced the bioaccessibility of zinc from rice from 22% to around 42% at the highest level, with the increase being less than slightly two-fold. A similar increase in zinc bioaccessibility was seen in the case of rice heat treated in the presence of EDTA. The effect of EDTA was highest in the open pan boiled rice, where EDTA at a molar ratio of 1: 2 (zinc: EDTA) brought about a three-fold increase in zinc bioaccessibility, whilst it was around two-fold in the pressure-cooked and microwave-cooked rice. Open pan boiling caused a marginal decrease in zinc bioaccessibility from rice, which was negated by the EDTA.

Zinc bioaccessibility from wheat was significantly enhanced by the added EDTA at all levels as shown in Table 1. EDTA enhanced zinc bioaccessibility from 25% at the molar ratio of 1: 0.25 to the inherent zinc, to 58% at the highest level of 1: 2 (zinc: EDTA). The percentage of zinc bioaccessibility in the absence of EDTA was 12%. Heat processing of wheat significantly decreased zinc bioaccessibility, and was seen in all the three methods of heat processing. Although the extent of this undesirable effect of heat processing on zinc bioaccessibility was countered by the addition of EDTA, heat processed wheat continued to have lower amounts of bioaccessible zinc at all the levels of EDTA. The positive effect of EDTA on

Table 1. Effect of EDTA on the bioavailability of zinc from raw and heat processed cereals

Grain	Percentage bioavailability of zinc Molar ratio of zinc:EDTA							
	0	1:0.25	1:0.5	1:0.75	1:1	1:1.5	1:2	
Rice	Raw	22.60 ±0.47	25.62±0.62	28.41±0.24*	29.23±0.18*	31.03±0.26*	38.00±0.60*	1.66±0.45*
	PC	19.22±0.54	22.93±0.60*	25.36±0.47*	27.02±0.51*	31.08±0.27*	34.70±1.15*	0.69±1.36*
	OPB	17.83±1.38	26.37±0.60*	31.28±0.49*	35.32±0.85*	40.12±0.50*	43.42±1.36*	51.28±0.47*
	MW	22.80±1.00	25.60±0.44*	28.05±0.41*	30.10±0.40*	32.60±0.15*	36.52±0.50*	39.66±1.18*
Wheat	Raw	12.08±0.78	25.11±1.25*	34.73±0.90*	45.22±0.77*	51.43±1.29*	55.31±0.86*	58.20±0.64*
	PC	3.85±0.17	3.86±0.12	6.04±0.33*	7.28±0.55*	10.46±0.37*	14.91±0.98*	24.70±1.80*
	OPB	7.06±0.51	8.20±0.35	9.08±0.31*	9.53±0.25*	10.33±0.38*	11.95±0.55*	15.00±0.72*
	MW	3.90±0.67	11.51±0.74*	12.85±0.85*	15.72±0.18*	18.30±1.16*	22.60±0.76*	28.30±0.52*

PC – pressure cooked; MW – microwave cooked; OPB – open pan boiled

Values are mean ± SEM of four replicate analyses

*Significantly ($p < 0.05$) higher than control (no added EDTA)

Table 2. Effect of EDTA on zinc bioavailability from raw and heat processed millets

Grain	Percentage bioavailability of zinc Molar ratio of zinc:EDTA							
	0	1:0.25	1:0.5	1:0.75	1:1	1:1.5	1:2	
Sorghum	Raw	5.05±1.45	11.63±0.72*	15.50±0.71*	34.17±2.05*	34.96±0.13*	43.64±0.08*	4.73±0.13*
	PC	4.85±0.54	6.14±0.47*	7.24±0.50*	8.83±0.36*	11.02±0.64*	15.64±0.79*	6.63±0.75*
	OPB	9.71±1.38	13.86±0.50*	15.47±0.49*	18.08±0.51*	18.52±0.61*	21.92±0.25*	5.31±0.58*
	MW	6.98±1.02	8.38±0.16*	9.30±0.41*	10.16±0.14*	11.46±0.29*	13.56±0.66*	5.25±0.50*
Finger millet	Raw	4.68±0.10	5.45±0.25*	6.37±0.11*	7.55±0.14*	8.54±0.10*	9.34±0.29*	1.97±0.61*
	PC	4.98±0.14	7.22±0.30*	9.03±0.05*	9.80±0.38*	11.11±0.07*	11.78±0.04*	5.38±1.06*
	OPB	4.45±0.16	6.37±0.19*	8.37±0.21*	9.96±0.53*	13.70±0.91*	14.58±0.79*	7.75±0.27*
	MW	11.84±0.10	15.77±0.33*	17.44±0.19*	20.22±0.36*	21.58±0.25*	24.32±0.65*	1.88±1.25*

PC – pressure cooked; MW – microwave cooked; OPB – open pan boiled

Values are mean ± SEM of four replicate analyses

*Significantly ($p < 0.05$) higher than control (no added EDTA)

zinc bioaccessibility was somewhat lower in open pan boiled wheat compared to the other two heat treatment methods.

EDTA enhanced bioaccessibility of zinc from finger millet from 4.6% to nearly 12% at the highest level as shown in Table 2. A similar trend was observed in heat processed finger millet, with the highest level of EDTA bringing about a three to four-fold increase in zinc bioaccessibility. Microwave cooking enhanced the bioaccessibility of zinc to a significant level, which was further enhanced by the addition of EDTA.

The beneficial effect of EDTA on the bioaccessibility of zinc was most prominent in sorghum, especially the raw grains, as compared to the other three cereals as shown in Table 2. At the lowest level added, EDTA enhanced zinc bioaccessibility was two-fold, whilst it was nine-fold at the highest level. This desirable effect was retained even when sorghum was cooked in the presence of EDTA, although to a lesser extent with the increase in zinc bioaccessibility being six, three, and two-fold in the pressure cooked, open pan boiled, and microwave cooked sorghum, respectively.

Table 3. Effect of EDTA on zinc bioavailability from raw and heat processed pulses

Grain	Percentage bioavailability of zinc Molar ratio of zinc:EDTA							
	0	1:0.25	1:0.5	1:0.75	1:1	1:1.5	1:2	
Red gram (D)	Raw	35.30±1.85	41.55±0.46*	43.00±0.38*	43.76±0.38*	44.51±0.69*	46.15±1.35*	9.48±3.35*
	PC	40.42±0.37	41.44±0.11	41.97±0.38	45.26±0.59	47.92±0.45*	48.76±0.21*	1.04±0.59*
	OPB	28.48±0.37	31.16±0.43	32.87±0.25*	33.61±0.15*	35.75±0.36*	37.10±0.04*	8.04±0.34*
	MW	23.77±1.69	25.41±0.81	25.71±1.38	26.21±0.95*	28.60±1.15*	30.03±1.03*	7.58±0.92*
Black gram (D)	Raw	24.54±1.64	27.78±0.29*	29.50±0.62*	31.37±0.54*	33.60±0.55*	44.10±0.81*	7.64±0.62*
	PC	23.34±0.63	25.37±0.88	27.62±0.62*	29.00±0.59*	33.62±0.38*	34.63±0.80*	7.34±0.22*
	OPB	26.02±0.47	31.34±0.94*	32.52±0.93*	33.57±0.20*	34.63±0.25*	35.89±0.43*	8.98±0.26*
	MW	16.93±0.48	19.30±0.44	21.58±0.39*	23.84±0.48*	26.51±0.63*	27.42±0.48*	0.31±0.42*

D - decorticated; PC - pressure cooked; MW - microwave cooked; OPB - open pan boiled

Values are mean±SEM of four replicate analyses

*Significantly ($p < 0.05$) higher than control (no added EDTA)

The effect of EDTA on zinc bioaccessibility from pulses is shown in Table 3. Although EDTA enhanced zinc bioaccessibility from both the pulses examined, the extent of the increase was relatively low compared to its effect on iron bioaccessibility. The extent of the increase in zinc bioaccessibility from raw red gram (decorticated) was only around 40% with the highest level of EDTA. The increase in zinc bioaccessibility with increasing levels of EDTA was only marginal, with very little difference between the levels. The same trend was observed in cooked red gram with all the methods of heat processing. In decorticated black gram however, the beneficial effect was higher compared to that in red gram. EDTA added at molar ratios of 1:2 (zinc:) increased zinc bioaccessibility from the raw grain by around two-fold. Similarly, the increase in zinc bioaccessibility from pressure-cooked, open pan boiled, and microwave cooked black gram was 1.6, 1.5, and 1.8 times, respectively, at the highest level of EDTA. Thus, the increase in zinc bioaccessibility from black gram as a result of EDTA addition was higher than that from red gram.

DISCUSSION

Enhancing iron and zinc bioavailability from grains is of utmost importance in view of their limited accessibility from plant-based foods. Metal chelators, such as EDTA warranted further exploration in this study. In this study, EDTA used at varying levels (1:0.25 to 1:2, relative to the inherent iron/zinc) had a significantly positive influence on iron and zinc bioaccessibility from commonly consumed cereals and pulses. This beneficial effect was seen both in raw as well as heat processed grains. Although the magnitude of the positive influence of EDTA varied with grain samples depending on their inherent iron or zinc concentrations, the increase in iron bioaccessibility brought about by EDTA was nevertheless significant. In general, the beneficial influence of EDTA on iron bioaccessibility from cereals was greater than that from pulses. Among the food grains examined, the positive effect was highest in wheat, with the increase in iron bioaccessibility ranging from around five to thirteen-fold with varying levels of EDTA. This positive influence of EDTA on iron bioaccessibility was seen even in heat

processed grains. Different forms of heat processing such as pressure-cooking, open pan boiling, and microwave cooking did not make any appreciable difference to this effect.

The magnitude of the beneficial effect of EDTA, however, was lower as far as zinc bioaccessibility was concerned, both in cereals and pulses. Zinc bioaccessibility was rarely enhanced by more than five-fold, with the only exception being sorghum, where it was nine-fold. Despite smaller increases, the zinc bioaccessibility was certainly higher with the addition of EDTA in most of the grains examined. Whilst heat processing had a decreasing effect on zinc bioaccessibility from the food grains in general, this was efficiently countered by EDTA. Earlier studies by Hemalatha, Platel & Srinivasan (2007) reveal that zinc bioaccessibility from heat processed cereals and pulses was lower than from their raw counterparts. In such a scenario, the addition of EDTA beneficially countered the compromised bioaccessibility due to heat treatment.

The actual amounts of EDTA added to the food grains in this study were well within the upper limit specified by the JECFA (1999), which is 150 mg per day. Considering all the grains, the average amounts of EDTA added at the lowest (1:0.25), and highest (1:2) levels to the grains were 4.4 mg/100g, and 35 mg/100g, respectively. In a majority of the grains examined, EDTA added at an equimolar (1:1) level to the inherent iron and zinc, which was around 20 mg/100g, enhanced iron and zinc bioaccessibility to an extent almost similar to the effect seen at the highest level (1:2). Thus, this amount of EDTA can be used to significantly enhance iron and zinc bioaccessibility. When added at this level, even if one consumed about 400 g of the staple cereals and 60 g of pulses, the normal blend in Indian meals, per day, the intake of EDTA would still be less than 100 mg/day.

Na₂EDTA is a hexadentate chelator

which is water soluble, odourless, white crystalline powder. It binds to transition metal ions like iron and zinc stoichiometrically, rendering them anionic as the part of the metal-EDTA complex. EDTA has the ability of forming complexes with alkali metal ions (Lanigan & Yamarik, 2002).

This is significant because the absorption of metal ions occurs in the alkaline environment of the duodenum. Ferric iron has been shown to have the highest stability constant to bind to EDTA, followed by copper, zinc, ferrous iron, calcium, magnesium, and sodium (Lanigan & Yamarik, 2002; Bothwell & MacPhail, 2004). Since plant foods contain iron in the ferric form, EDTA would be the ideal candidate to enhance iron bioaccessibility from vegetarian foods. The binding of ferric iron to EDTA is favoured in the low pH environment of the stomach. In the duodenum, the iron is dissociated from the complex and released for absorption, and EDTA is available to bind to other nutritionally important metals such as copper and zinc. Most of these metals are subsequently dissociated and released for absorption. Almost 95% of EDTA is excreted in faeces, while about 5% of metal/EDTA complexes are absorbed intact (Bothwell & MacPhail, 2004).

The role of EDTA has thus far been that of a co-fortificant (i.e., it has been used with iron fortification salts, as a stabiliser, and enhancer of iron absorption). This study demonstrated that EDTA could independently function as an enhancer of iron and zinc bioaccessibility from food grains.

Organic acids are known enhancers of mineral absorption by virtue of their ability to reduce ferric iron to the ferrous state. However, the amount in which these acids are required in order to exert their enhancing effect is relatively high. Amounts as high as 1 g each for citric, malic, and tartaric acids, and 15 mg for ascorbic acid were reported to be

efficacious in enhancing iron absorption from basic meals weighing around 100 g to 200 g (Gillooly *et al.*, 1983). The levels of EDTA used in our study were reasonably low, and therefore more feasible for use as an enhancer of mineral bioaccessibility.

EDTA could be used as a sprinkle to enhance the bioaccessibility of minerals from the meals consumed, similar to the micronutrient sprinkles recommended by the WHO (2011) for home fortification. A previous study showed that EDTA, used as a co-fortificant along with iron and zinc at an equimolar level to the inherent iron, did not alter the organoleptic properties of the products prepared from fortified millet flours (Tripathi *et al.*, 2011).

CONCLUSIONS

This study, carried out with individual food grains, should be extended to conventional composite meals consisting of both cereals and pulses to verify the effect of EDTA. Thus, EDTA merits exploitation to maximise the bioaccessibility of iron and zinc from commonly consumed food grains which are usually rich in antinutritional factors. Since EDTA is an approved food additive, it could probably be used in midday meals and in supplementary feeding programs, to address micronutrient deficiencies.

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