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Bioaccessibility of iron and zinc in selected complementary foods fortified with micronutrient powders in Kenya

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Abstract

Fortification with Micronutrient Powders (MNPs) is recommended as a strategy for increasing the micronutrient content in complementary foods. However, plant-based diets commonly consumed in developing countries are rich in phytates and tannins, which decrease the micronutrient bioavailability. The present work analysed the relationship between the antinutrient content, and also iron and zinc bioaccessibility, in home-made MNP-fortified complementary feeding porridges refined with white rice, maize, white sorghum, finger millet, pearl millet, Irish potato, and banana samples, which were obtained from the local market and milled into flour. Porridges were prepared using the flour, cooled to 50°C, fortified with MNPs, and subjected to *in vitro* digestion. Total and bioaccessible zinc and iron were quantified using atomic absorption spectrometry. Tannins and phytates were analysed using Folin-Denis and high-performance liquid chromatography methods, respectively. Porridges were classified as having poor bioavailability if their phytate-zinc and phytate-iron molar ratios were > 15 and > 0.4, respectively. Only pearl millet and soybeans showed tannin levels higher than the recommended values. The lowest phytate level was observed in refined white rice (0.11 ± 0.04 g/100 g), and the highest was in pearl millet (2.83 ± 0.10 g/100 g). Zinc bioaccessibility ranged from 7.31% (finger millet) to 26.05% (corn-soy blend). Only pearl millet porridge was classified as having poor zinc bioavailability. Iron bioaccessibility ranged from 20.73% (refined white rice) to 0.62% (pearl millet). Refined white rice and Irish potato were the only foods with the phytate-iron ratio within the recommended range. Iron bioaccessibility decreased significantly with an increase in both tannin (r = -0.31, p = 0.045) and phytate (r = -0.39, p = 0.01) contents. Zinc bioaccessibility showed a significant positive relationship with tannin levels (r = 0.41, p = 0.008), but an insignificant inverse relationship with phytate levels (r = -0.072, p = 0.700). Iron bioaccessibility was adversely affected by phytate and tannin levels. To improve iron and zinc bioavailability in complementary foods, strategies for lowering the phytate and tannin contents at the household level are recommended.

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Introduction

Plant-based complementary foods are widely consumed in developing countries. However, these foods contain antinutritional factors, especially phytates and polyphenolic compounds (such as tannins). These antinutrients decrease the bioavailability of micronutrients (Platel and Srinivasan, 2016), thus contributing to widespread iron and zinc deficiencies observed among young children aged 6 - 23 months in these regions (Bailey *et al.*, 2015). Phytic acid (primarily myo-inositol-6-phosphate) is an antinutrient, abundant in unrefined cereals, legumes, tubers, and oil seeds, and the most important inhibitor of zinc and iron absorption (Gupta *et al.*, 2015). Phytates form complexes with zinc, calcium, and iron, thus forming insoluble salts, and resulting in poor bioavailability (Baye *et al.*, 2013). Tannins are abundant in plant-based foods such as sorghum and millet (Pushparaj and Urooj, 2014), as well as in legumes such as soybeans (Jiao *et al.*, 2012).

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Micronutrient Powders (MNPs) are single-dose sachets of micronutrients in a powder form that are added to home-made complementary foods just before consumption to improve the micronutrient level (Zlotkin *et al.,* 2005). MNPs have been recommended as one of the strategies for increasing the micronutrient level in complementary foods, and World Health Organization (WHO, 2011) recommends the use of MNPs as a public health intervention. However, the information on the influence of antinutrients on zinc and iron bioavailability in home-made MNP-fortified complementary foods is scarce. Sandberg (2005) reported that *in vitro* methods can serve as a screening, ranking, or categorising tool for choosing fortification channels for many food formulations, especially because they are less expensive, faster, and offer better control over many experimental variables than *in vivo* methods. *In vitro* assays simulate digestion in the human gastrointestinal tract by subjecting food samples to stepwise digestive processes, thus ensuring that digestive fluids, pH, temperature, and time are maintained appropriately (Alminger *et al.,* 2014). *In vitro* bioaccessibility refers to the solubilisation of nutrients from the food matrix under enteric conditions, thus making them potentially absorbable in the gut (Barba *et al.,* 2017). The solubilisation process involves digestion and release of nutrients from the food matrix. Bioaccessibility is a crucial factor that determines the bioavailability of nutrients, and has been used to predict the bioavailability of minerals *in vivo*. The absorption of minerals in the gut is favoured by higher solubility (Zhu *et al.,* 2006) as it increases with increasing concentration of dissolved minerals. However, Cominelli *et al.* (2020) reported that phytates are highly negatively charged in the small intestine (pH 6 - 7), which enhances its ability to form complexes with iron and zinc, thereby resulting in the formation of insoluble salts and the loss of intestinal solubility (bioaccessibility). The information on the extent to which tannins may bind to iron and zinc in different foods is limited.

Notably, however, the precision of *in vitro* methods is lower than that of *in vivo* methods due to the physiological processes of digestion and absorption, which are influenced by a combination of factors such as age, genotype, nutritional status, amount of gastric and intestinal secretion, and gut microflora (Cardoso *et al.*, 2015). Manary *et al.* (2000) demonstrated the modulating effect of physiological needs on the estimation of the bioavailability of minerals in the diet. They reported that the *in vivo* bioavailability of zinc fortificants in cereal porridges fed to young Malawian children was in the range of 20 - 40%. Higher bioavailability values were observed among children consuming low-phytate diets but with high physiological needs, and a lower range of values was observed among normal healthy children.

Minerals such as zinc and iron play an important role in human health, deficiencies of which lead to immediate and long-term impacts. Micronutrient fortification of foods is a high-impact nutrition intervention aimed at curbing such deficiencies. A cheap and efficient way to estimate the bioavailability of minerals in fortified foods is *in vitro* assays. Therefore, the primary objective of the present work was to shed light on the *in vitro* bioaccessibility of zinc and iron in home-made complementary porridges fortified with MNPs. Comparisons were also made between conventional fortification and MNP fortification of corn-soy blend (CSB), which is the standard protocol in the management of moderately malnourished children. This information would be useful for MNP fortification programmes targeting locally consumed complementary foods.

Materials and methods

Food samples

Maize (*Zea mays* var. amylacea), soybean (*Glycine max* (L.) Merr.), rice (*Oryza sativa* L.), CSB, finger millet (*Eleusine coracana* Gaertn.), bulrush millet (*Pennisetum typhoideum*), white sorghum (*Sorghum bicolor* (L.) Moench.), cassava (*Manihot esculenta* Crantz), potato (*Solanum tuberosum*), and banana (*Musa* spp.) were purchased from the local market in Nairobi, Kenya. The samples were sorted to remove the extraneous matter, finely ground into flour, sieved through a fine mesh, and packaged in plastic bags. As a reference product, CSB (80:20; East African Nutraceuticals Ltd, Kenya) conventionally fortified with the same level of micronutrients as MNPs was also analysed. The CSB was pre-processed by extrusion cooking. The MNPs (Hexagon Nutrition Pvt Ltd, India) contained vitamin A (1250 IU), vitamin C (30 mg), vitamin B_1 (0.5 mg), vitamin B_2 (0.5 mg), vitamin B_3 (6 mg), vitamin B_9 (160 mcg), vitamin B_{12} (0.9 mcg), iron-ferrous fumarate 60% (12.5 mg), and zinc gluconate (5 mg).

Porridge preparation

Porridges were prepared by mixing 50 g of flour with 350 mL of distilled water in an aluminium cooking pot. The mixture was heated to boiling (92°C) with constant stirring for 10 min, cooled to 50°C, fortified with MNPs [vitamin A (1250 IU), vitamin C (30 mg), vitamin B_1 (0.5 mg), vitamin B_2 (0.5 mg), vitamin B_3 (6 mg), vitamin B_9 (160 mcg), vitamin B_{12} (0.9 mcg), iron-ferrous fumarate 60% (12.5 mg), and zinc gluconate (5 mg)], and then homogenised. Potato purée with the same consistency as the porridges was prepared. A summary of the study protocol is presented in Figure 1.

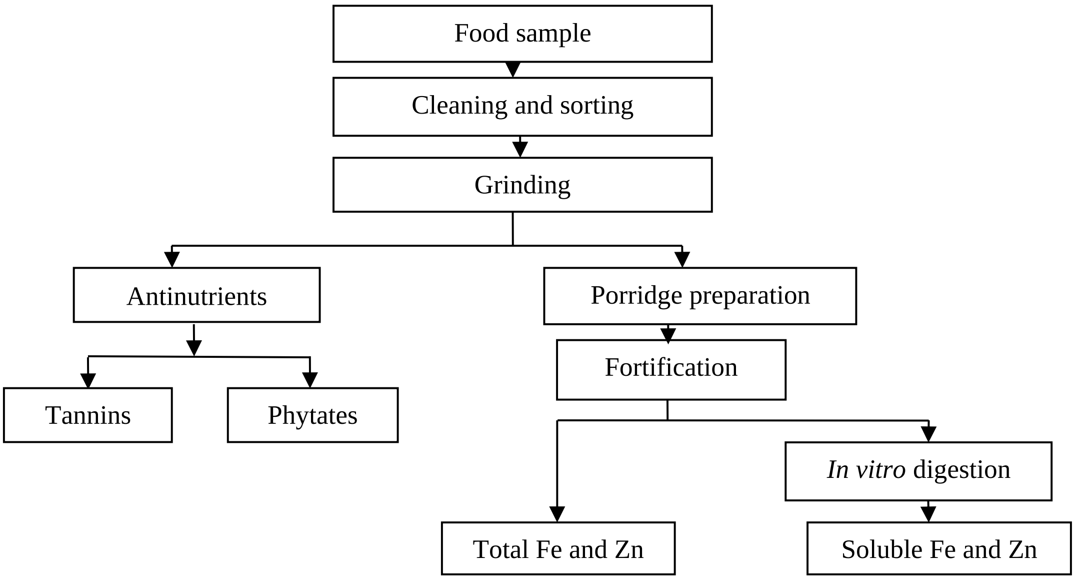

Figure 1. Flow chart illustrating the study protocol.

Determination of tannin content by colorimetric analysis

The tannin content was determined using the Folin-Denis spectrophotometric method (Pearson, 1976). The absorbance of the solution was read at 760 nm using a UV-vis spectrophotometer (Shimadzu-190). Standards with the tannic acid concentration of 5 - 25 µg/mL (Hopkin William, England) were prepared. A calibration curve was plotted, and the concentration of the unknown samples was expressed as the equivalent percentage of tannic acid.

Determination of phytate content by HPLC

The phytate content was determined using high-performance liquid chromatography (HPLC) (Camire and Clydesdale, 1982). The level of phytates was quantified using an HPLC apparatus (Model C-R7A plus, Shimadzu Corp., Kyoto, Japan) fitted with a 50377 RP-18 (5 µm) column cartridge at an oven temperature of 30°C, and a refractive index detector (RID) (Model RID-6A, Shimadzu Corporation, Kyoto, Japan) for identification. The mobile phase used was 0.005 N sodium acetate in distilled water at a flow rate of 0.5 µL per minute. Standard solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL of sodium phytate ($C_6H_6(OPO_3Na_2)_6 + H_2O$) in distilled water were prepared, and the phytate content was determined using Eq. 1:

$$\text{Phytate content (mg/g)} = (y / b) \times (\text{dilution factor / weight of sample}) \quad \text{(Eq. 1)}$$

where, y = peak area, and b = concentration.

Determination of total zinc and iron

The total zinc and iron concentrations in the porridge samples were determined using method 970.12 of the Association of Official Analytical Chemists (AOAC). Wet ashing was carried out using a nitric acid and hydrochloric acid mixture (HNO_3/HCI, 5:1 v/v). The samples (2 g each) were digested in triplicate on a hot plate using the concentrated hydrochloric acid (0.15 mL) and nitric (0.75 mL) mixture until the production of red fumes was stopped, and a clear solution was obtained. The samples were cooled and then diluted to 25 mL. The concentrations of zinc and iron in the digest were quantified using an atomic absorption spectrophotometer (AAS) (Shimadzu Corporation, Kyoto, Japan, AA-6200).

Determination of in vitro bioaccessibility of zinc and iron

In vitro enzymatic digestion was performed according to Skibniewska *et al*. (2002). Briefly, 5 g of porridge sample was mixed with 50 mL of deionised water, and the pH adjusted to 2.0 using 1 M HCl (Suprapure, Merck). Peptic digestion of the samples using a 0.5% pepsin (Sigma) solution was carried out for 2 h with constant shaking while being incubated at 37°C. After the pepsin digestion process, the samples were treated with 6% $NaHCO_3$ aqueous solution (Extrapure, Merck) to increase the pH to 6.8 - 7.0, and then, 0.4% pancreatin-bile (Sigma) solution (10 mL/40 mL of homogenate) was added, and the mixture was shaken in a thermostatic shaker at 37°C for 4 h. The digest was centrifuged at 2,500 rpm for 10 min, and the supernatant was digested by wet ashing. The concentrations of zinc and iron in the digest were quantified using an atomic absorption spectrophotometer (AAS) (Shimadzu Corporation, Kyoto, Japan, AA-6200).

Quality control of the analytical methods

The analytical samples were spiked with known levels of zinc and iron. The recovery of zinc and iron from the spiked samples was 99.2 and 98.7%, respectively. The final concentrations were adjusted for recovery. The R^2 values for the calibration concentration curve for zinc and iron were 0.998 and 0.996, respectively. All samples were analysed in triplicate. To avoid contamination, the containers were soaked in hot water containing detergent for about 2 h, cleaned and rinsed thoroughly with tap water, and then with deionised water. The glassware and plastic bottles were soaked overnight in 50% nitric acid and 2% nitric acid, respectively. They were rinsed twice with cold deionised water, and further rinsed with hot deionised water, and then immediately dried in an oven. All reagents used were of analytical grade. Standard zinc and iron solutions and enzymes were prepared immediately before use.

Data analysis

Data were analysed using SPSS version 20. The tannin and phytate contents in the different food samples were expressed as means and standard deviation, and compared using *t*-tests. The tannin level in processed cereal-based foods for infant and child feeding should be below 0.3 g/100 g (dry weight, dw) (Codex Stan 173-1989), whereas the phytate level of ≤ 25 mg/100 g (dw) is recommended (Onomi *et al*., 2004). The food samples were classified based on whether they met the recommended levels. The total and *in vitro* bioaccessible fractions of zinc and iron in the fortified complementary porridges were determined using laboratory analysis, which was expressed as mg/100 g (dw) of the porridges. Phytate/mineral molar ratios were computed, and the porridges were classified based on the recommended levels. Phytate/zinc molar ratios > 15 were associated with poor zinc bioavailability (Gibson *et al.*, 2006). Phytate/iron molar ratios < 1 and preferably < 0.4 significantly enhanced iron bioavailability (Hurrell, 2004). Pearson's correlation was used to establish the relationship between antinutrient content, phytate/mineral ratios, and *in vitro* bioaccessibility levels of zinc and iron.

Results

Antinutrient contents

Wide variations in the levels of tannins and phytates between the analysed samples were observed. Soybean flour showed the highest level of tannins, 0.37 ± 0.01 g/100 g (expressed as the equivalent percentage of tannic acid). The tannin contents of the cereal flours ranged from 0.05 to 0.31 g/100 g, with pearl millet showing the highest level (0.31 ± 0.01 g/100 g) and refined white rice (0.05 ± 0.01 g/100 g) the lowest, as shown in Table 1. The tannin levels of tubers and plantains were similar to those of refined white rice. Tubers (Irish potato and cassava) and banana contained a significantly lower tannin content when compared with the cereals, except for refined white rice ($p < 0.05$). Among the unrefined cereals, maize showed a significantly lower level ($p < 0.05$) of tannins.

In general, high levels of phytates were observed in the majority of the analysed flours (Table 1). The phytate contents of the cereal flours ranged from 0.11 ± 0.04 to 2.83 ± 0.10 g/100 g (expressed as inositol hexaphosphate). Among the cereals, refined white rice showed the lowest level of phytates (0.11 ± 0.04 g/100 g), whereas pearl millet the highest (2.83 ± 0.10 g/100 g). Among tubers and plantains, potato showed the lowest phytate content (0.05 ± 0.03 g/100 g), followed by banana (0.57 ± 0.07 g/100 g) and cassava (0.74 ± 0.11 g/100 g). No significant difference in the phytate levels was observed between

Table 1. Phytate and tannin contents of selected local complementary foods.

Product	Tannin content^{dw} (g/100 g)	Phytate content^{dw} (g/100 g)
White sorghum	0.20 ± 0.02^{e}	0.73 ± 0.07^{a}
Pearl millet	0.31 ± 0.01^{f}	2.83 ± 0.10^{e}
Finger millet	0.13 ± 0.02^{ad}	0.74 ± 0.09^{a}
Maize	0.09 ± 0.01^{bd}	0.94 ± 0.16^{ad}
White refined rice	0.05 ± 0.01	0.11 ± 0.04^{b}
Cassava	0.06 ± 0.01^{bc}	0.74 ± 0.1^{a}
Irish potato	0.05 ± 0.01^{c}	0.05 ± 0.03^{b}
Banana	0.05 ± 0.01^{c}	0.57 ± 0.07^{ac}
Soybean	0. 37 ± 0.01^{f}	0.66 ± 0.10^{ac}
CSB	0.14 ± 0.02^{a}	0.69 ± 0.12^{ac}

^{dw}dry weight. Values are means of three replicates ± standard deviation (SD). Means followed by different lowercase superscripts in the same column are significantly different (Tukey's test; $p < 0.05$).

Irish potato and refined white rice ($p > 0.05$). Maize, finger millet, white sorghum, cassava, banana, soybean, and CSB showed moderate phytate levels which were not significantly different ($p > 0.05$).

Total and in vitro bioaccessible zinc and iron

The total zinc content of the fortified porridges ranged from 14.08 to 21.97 mg/100 g (Table 2). Finger millet and banana porridges showed the highest total zinc content of 21.97 ± 1.72 and 20.42 ± 1.19 mg/100 g, respectively, which were significantly different from the other analysed porridges ($p < 0.05$). *In vitro* bioaccessibility is a measure of the amount of a nutrient available for absorption determined by mimicking the digestion process in the gut. Zinc bioaccessibility of the porridges ranged from 7.31 to 26.05% (Table 2). CSB fortified with MNPs showed the highest fraction of bioaccessible zinc (26.05%). Significantly lower bioaccessible zinc fractions were observed in pearl millet (9.98%) and finger millet (7.31%) when compared with other porridges ($p < 0.05$). In addition, zinc bioaccessibility in the conventionally fortified CSB (24.07%) was not significantly different ($p > 0.05$) from that of the MNP-fortified CSB (26.05%).

Table 2. Total and bioaccessible (BA) zinc in fortified local complementary foods^{dw}.

Product	Total zinc (mg/100 g) (mean ± SD)	BA zinc (mg/100 g) (mean ± SD)	% BA zinc	PA/zinc molar ratios
White sorghum	15.26 ± 1.3^{a}	2.15 ± 0.21^{d}	14.08^{ad}	4.72 ± 0.34^{a}
Pearl millet	16.04 ± 1.15^{a}	1.60 ± 0.18^{a}	9.98^{c}	17.40 ± 0.69^{b}
Finger millet	21.97 ± 1.72^{b}	1.60 ± 0.09^{a}	7.31^{c}	3.30 ± 0.13^{c}
Maize	17.40 ± 1.27^{a}	3.39 ± 0.57^{b}	19.45^{ad}	5.32 ± 0.49^{a}
White refined rice	15.72 ± 0.80^{a}	1.76 ± 0.29^{a}	11.18^{ad}	0.72 ± 0.26^{d}
Cassava	14.08 ± 1.02^{a}	3.29 ± 0.26^{b}	23.55^{abd}	5.19 ± 0.46^{a}
Irish potato	17.54 ± 1.41^{a}	2.48 ± 0.38^{b}	14.11^{ad}	0.31 ± 0.18^{d}
Banana	20.42 ± 1.19^{b}	3.21 ± 0.30^{b}	15.78^{ad}	2.74 ± 0.41^{c}
CSB	16.83 ± 1.35^{a}	4.34 ± 0.66^{b}	26.05^{ab}	4.87 ± 0.65^{a}
CSB$^{\#}$	14.19 ± 1.13^{a}	3.39 ± 0.46^{b}	24.07^{ab}	4.83 ± 0.96^{a}

^{dw}dry weight, PA: phytic acid, $^{\#}$conventionally fortified. Values are means of three replicates ± standard deviation (SD). Means followed by different lowercase superscripts in the same column are significantly different (Tukey's test; $p < 0.05$).

Unlike the zinc content, the total iron contents varied widely between the studied porridges. Among the iron-fortified porridges, the total iron content was highest in finger millet (53.35 ± 0.67 mg/100 g) and banana (34.71 ± 1.38 mg/100 g). Pearl millet, potato, and soybean showed moderate levels of the total iron content, 24.67 ± 1.05, 27.05 ± 1.91, and 2.34 ± 1.39 mg/100 g, respectively, and no significant difference was observed between the porridges ($p > 0.05$). The total iron contents in the iron-fortified porridges prepared from sorghum, cassava, maize, and CSB ranged from 18.54 to 20.30 mg/100 g. The iron-fortified white rice porridge showed the lowest total iron content (14.40 ± 1.50 mg/100 g). No significant difference was observed in the total iron contents between white sorghum, maize, cassava, soybean, and CSB ($p > 0.05$). Iron bioaccessibility in the porridges ranged from 0.56 to 20.73% (Table 3). The bioaccessible iron fraction was low in the fortified porridges prepared from sorghum (1.24%), millet (0.62%), Irish potato (1.93%), CSB products (ranging from 0.98 to 1.20%), and maize (0.56%). Finger millet (3.27%), cassava (4.28%), banana (2.65%), and soybean (3.16%) showed moderate levels of iron bioaccessibility. Refined rice porridge showed the highest iron bioaccessibility (20.73%) although it contained the lowest level of total iron. On the contrary, finger millet and banana, which contained higher levels of total iron when compared with other porridges, showed moderate levels of iron bioaccessibility. No significant difference was observed in the proportion of accessible iron between white sorghum, pearl millet, maize, Irish potato, and CSB ($p > 0.05$) despite having significantly different levels of total iron.

Table 3. Total and bioaccessible (BA) iron in fortified local complementary foods[dw].

Product	Total iron (mg/100 g) (mean ± SD)	BA iron (mg/100 g) (mean ± SD)	% BA iron	PA/Fe molar ratios
White sorghum	20.22 ± 1.08[a]	0.25 ± 0.02[ab]	1.24[ac]	3.07 ± 0.45[a]
Pearl millet	24.67 ± 1.05[b]	0.15 ± 0.02[b]	0.62[ac]	9.70 ± 0.10[d]
Finger millet	53.35 ± 0.67[c]	1.74 ± 0.09[dc]	3.27[bc]	1.17 ± 0.12[bc]
Maize	18.54 ± 1.85[a]	0.10 ± 0.01[b]	0.56[a]	4.29 ± 0.28[a]
White refined rice	14.40 ± 1.50[d]	2.98 ± 0.22[c]	20.73[d]	0.67 ± 0.20[c]
Cassava	18.75 ± 0.78[a]	0.80 ± 0.03[d]	4.28[b]	3.35 ± 0.39[a]
Irish potato	27.05 ± 1.91[b]	0.52 ± 0.03[ad]	1.93[ac]	0.16 ± 0.09[c]
Banana	34.71 ± 1.38[e]	0.92 ± 0.02[d]	2.65[bc]	1.39 ± 0.23[ce]
Soybean	22.34 ± 1.39[ab]	0.71 ± 0.01[d]	3.16[bc]	2.52 ± 0.54[ab]
CSB	19.24 ± 1.46[a]	0.19 ± 0.04[ab]	0.98[a]	3.64 ± 0.24[a]
CSB[#]	20.30 ± 2.01[a]	0.24 ± 0.01[a]	1.20[a]	2.87 ± 0.34[ab]

[dw]dry weight, PA: phytic acid, [#]conventionally fortified. Values are means of three replicates ± standard deviation (SD). Means followed by different lowercase superscripts in the same column are significantly different (Tukey's test; $p < 0.05$).

Phytate/mineral molar ratios

As reported in a previous study, a phytate/zinc molar ratio of > 15 is associated with poor bioavailability of zinc (Gibson *et al.*, 2006). Among the analysed porridges, only pearl millet showed a phytate/zinc molar ratio > 15. Similarly, phytate/iron molar ratios < 1, preferably < 0.4, significantly enhance iron bioavailability (Hurrell, 2004). Among all the fortified porridges, only refined white rice and Irish potato porridges showed phytate/iron molar ratios < 1, thus leading to a better iron bioavailability. The molar ratio of Irish potato puree was < 0.4. Significantly lower phytate/zinc molar ratios were observed in refined white rice and Irish potato when compared with the other porridges. Similarly, lower phytate/mineral ratios were observed for iron in both refined white rice and Irish potato.

Association between antinutrient content and mineral bioaccessibility

Zinc bioaccessibility significantly increased with increasing tannin content of the flours ($r = 0.405$, *p* = 0.008), but showed a non-significant inverse relationship with the phytate content (*r* = -0.072, *p* = 0.700). A significant inverse association between iron bioaccessibility and tannin (*r* = -0.311, *p* = 0.045) and phytate (*r* = -0.385, *p* = 0.012) levels was observed. Similarly, bioaccessibility of both iron and zinc decreased with increasing phytate/mineral ratios although the associations were not significant (zinc: *r* = -0.126, *p* = 0.4; and iron: *r* = -0.082, *p* = 0.6; Table 4).

Table 4. Association between antinutrient content, and bioaccessible (BA%) zinc and iron levels (mg/100 g).

	Fe	BA-Fe	Zn	BA-Zn	Tannin	PA	PA:Fe	PA:Zn
Fe (Corr)	1	-.130	.621**	-.404**	-.017	-.006	-.661**	-.403**
Sig.		.400	.001	.008	.900	.900	.001	.008
BA-Fe(Corr)		1	.040	-.136	-.311*	-.385*	-.082	-.158
Sig.			.800	.400	.045	.012	.600	.300
Zn (Corr)			1	-.076	-.104	-.057	-.540**	-.874**
Sig.				.600	.500	.700	.001	.001
BA-Zn (Corr)				1	.405**	-.072	.323*	-.126
Sig.					.008	.700	.037	.400
Tannin(Corr)					1	.569**	.041	.128
Sig.						.001	.800	.400
PA (Corr)						1	.147	.264
Sig.							.400	.100
PA:Fe (Corr)							1	.594**
Sig.								.001
PA:Zn (Corr)								1

*Pearson correlation (Corr) is significant (Sig) at the 0.05 level (2-tailed), **Pearson correlation (Corr) is significant (Sig) at the 0.01 level (2-tailed), -: inverse relationship, and PA: phytic acid.

Discussion

In developing countries, monotonous diets are consumed as they are rich in starchy cereals and tubers. Plant-based foods contain high levels of antinutrients which inhibit the absorption of nutrients such as iron and zinc in the human gut, and in turn leads to many iron and zinc deficiencies. The present work showed that refined white rice and starchy tubers (Irish potato) contained lower levels of phytates and tannins when compared with unrefined cereals, and are potentially better complementary foods considering their antinutrient profiles. As observed in the present work, plantains, roots, and tubers contain relatively lower levels of phytates when compared with wholegrain cereals (Phillippy *et al.*, 2003; Honfo *et al.*, 2007), which is consistent with the findings of Trinidad *et al.* (2009), Gibson *et al.* (2010), Eleazu *et al.* (2013), and Anbuselvi and Balamurugan (2014). The refining of cereals removes the outer layer, which is rich in antinutrients, thus enhancing the bioaccessibility of the nutrients. However, refining lowers the total iron and zinc contents of cereals since they are found at higher concentrations in the outer layers. The relationship between antinutrients and mineral content in refined cereals and legumes has been previously reported by Oghbaei and Prakash (2016).

There is a considerable amount of scientific evidence indicating that antinutrients lower the bioavailability of iron and zinc in foods. As reported by Gupta *et al.* (2015), phytates lower the mineral bioavailability even at low concentrations. On the other hand, tannin levels higher than 10% have been reported to affect the bioavailability of minerals (Ghavidel and Prakash, 2007; Fernandes *et al.*, 2010). Our findings are in agreement with the findings reported in the literature. The *in vitro* zinc bioaccessibility of the analysed products ranged from 7.31 to 26.05%, which falls within the moderate range of bioaccessibility (5 to 30%) based the classification proposed by Gibson *et al.* (2006). These findings show that tannin levels did not lower the bioaccessibility of zinc, and if anything, increased with increasing levels of tannins.

Phytate/zinc molar ratios have been used to estimate zinc bioavailability, and a ratio > 15 is associated with poor zinc bioavailability (Gibson *et al.,* 2006; Miller *et al.,* 2007). The present work showed a similar trend in which *in vitro* bioaccessibility of zinc decreased with increasing phytate/zinc ratios although the association was not significant. In the present work, the phytate/zinc ratios of all the fortified products, except pearl millet, were less than 15. Overall, in all analysed products except pearl millet, the levels of antinutrients did not affect zinc bioaccessibility.

Unlike zinc bioaccessibility, both tannins and phytates significantly affect iron bioaccessibility at relatively lower levels. Our findings are consistent with those of Afsana *et al.* (2004) who reported that tannin levels of 1 - 2% did not affect zinc bioavailability although iron bioavailability was significantly reduced. Iron bioaccessibility of all the products ranged from 0.56 to 4.28% except for refined white rice, which showed a high level of 20.73%. Similar bioaccessible levels have been reported in previous studies (Kapsokefalou *et al.,* 2005; Haro-Vicente and Martínez-Graciá, 2006).

A phytate/iron molar ratio > 1 is an indication of poor iron bioavailability (Mitchikpe *et al.,* 2008; Hurrell and Egli, 2010). Only refined white rice and Irish potato porridges showed phytate/iron molar ratios < 1, thus indicating better iron bioavailability, and the best among them was Irish potato purée, with a molar ratio of < 0.4. Despite the low phytate/iron ratio observed in potato, its iron bioaccessibility was far below that of refined white rice. This could have been due to the presence of other antinutrients such as oxalates, which also affect iron bioavailability but were not investigated.

The findings of the present work showed that the total *in vitro* bioaccessible iron content was highest in refined white rice (2.98 mg/100 g). However, refining is not recommended for reducing antinutrients due to the associated loss of nutrients and bioactive compounds. Unlike zinc, bioaccessible iron levels in the analysed foods were suboptimal for complementary feeding despite fortification with MNPs. The limitation of the present work was that the *in vitro bio*accessibility model assessed the gut solubility of iron and zinc only. The influence of other factors on mineral bioavailability was not investigated, and therefore, *in vivo* models are required to validate the present findings. The native content of iron and zinc in the food products was also not analysed.

Furthermore, our study compared MNP and conventional fortification with respect to zinc and iron bioaccessibility. No significant difference in zinc bioaccessibility was observed between conventional and MNP food fortification, as was the case with CSB. This implied that antinutrient-mineral interactions occurred even when micronutrients were added at the point of consumption. A major limitation in the comparison of iron bioaccessibility between the two types of fortification was that phytate/iron ratios for the samples analysed were significantly different, and hence, comparison was not possible.

Conclusion

Majority of the plant-based local complementary foods contain high levels of antinutrients which affect zinc and iron bioavailability. This might be a major contributing factor to the high number of micronutrient deficiencies reported among children aged 6 - 23 months in Kenya. Most of the analysed foods contained tannin levels within the permitted range, but phytates were present at higher levels than the permitted range. The present findings indicated that iron bioaccessibility was more adversely affected by antinutrients when compared with zinc in MNP-fortified porridges. All the fortified products, except for Irish potato and refined white rice, showed a higher phytate/iron ratio than the permitted range, thus affecting iron bioaccessibility. MNP food fortification did not significantly differ from conventional industrial fortification in terms of zinc bioaccessibility. Further research using *in vivo* models is recommended to validate the present findings. Assessment of iron and zinc bioavailability in different diet combinations is also recommended.

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References

Afsana, K., Shiga, K., Ishizuka, S. and Hara, H. 2004. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. Bioscience, Biotechnology and Biochemistry 68(3): 584-592.

Alminger, M., Aura, A., Bohn, T., Dufour, C., El, S. N., Gomes, A., ... and Santos, C. 2014. *In vitro* models for studying secondary plant metabolite digestion and bio-accessibility. Comprehensive Reviews in Food Science and Food Safety 13: 413-436.

Anbuselvi, S. and Balamurugan, T. 2014. Phyto-chemical and anti-nutrient constituents of cassava and sweet potato. World Journal of Pharmacy and Pharmaceutical Sciences 3(3): 1440-1449.

Bailey, R. L., West, K. P. and Black, R. E. 2015. The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism 66: 22-33.

Barba, F., Mariutti, L. R., Bragagnolo, N., Mercadante, A., Barbosa-Cánovas, G. and Orlien, V. 2017. Bio-accessibility of bioactive compounds from fruits and vegetables after thermal and non-thermal processing. Trends in Food Science and Technology 67*:* 195-206.

Baye, K., Mouquet-Rivier, C., Icard-Verniere, C., Rochette, I. and Guyot, J. P. 2013. Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sour dough used to make *injera*. Food Chemistry 138: 430-436.

Camire, A. L. and Clydesdale, F. M. 1982. Analysis of phytic acid in foods by HPLC. Journal of Food Science 47(2): 575-578.

Cardoso, C., Afonso, C., Lourenço, H., Costa, S. and Nunes, M. 2015. Bio-accessibility assessment methodologies and their consequences for the risk-benefit evaluation of food. Trends in Food Science and Technology 41*:* 5-23.

Cominelli, E., Pilu, R. and Sparvoli, F. 2020. Phytic acid and transporters: What can we learn from low phytic acid mutants. Plants 9 (1): 69.

Eleazu, C. O., Okafor, P. N., Amajor, J., Awa, E., Ikpeama, A. I. and Eleazu, K. C. 2013. Chemical composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (*M. paradisiacae*) flour. African Journal of Biotechnology 10(74): 16937-16947.

Fernandes, A. C., Nishida, W. and Da Costa Proença, R. P. 2010. Influence of soaking on the nutritional quality of common beans (*Phaseolus vulgaris* L.) cooked with or without the soaking water: A review. International Journal of Food Science and Technology 45: 2209-2218.

Ghavidel, R. A. and Prakash, J. 2007. The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds. LWT - Food Science and Technology 40: 1292-1299.

Gibson, R. S., Bailey, K. B., Gibbs, M. and Ferguson, E. L. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food and Nutrition Bulletin 31: S134-S146.

Gibson, R. S., Perlas, L. and Hotz, C. 2006. Improving the bioavailability of nutrients in plant foods at the household level. The Proceedings of the Nutrition Society 65(2): 160-168.

Gupta, R. K., Gangoliya, S. S. and Singh, N. K. 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. Journal of Food Science and Technology 52: 676-684.

Haro-Vicente, J. F. and Martínez-Graciá, C. 2006. Optimization of *in vitro* measurement of available iron from different fortificants in citric fruit juices. Food Chemistry 98(4): 639-648.

Honfo, F. G., Kayodé, A., Coulibaly, O. and Tenkouano, A. 2007. Relative contribution of banana and plantain products to the nutritional requirements for iron, zinc and vitamin A of infants and mothers in Cameroon. Fruits 62*:* 267-277.

Hurrell, R. and Egli, I. 2010. Iron bioavailability and dietary reference values. The American Journal of Clinical Nutrition 91(5): 1461S-1467S.

Hurrell, R. F. 2004. Phytic acid degradation as a means of improving iron absorption. International Journal for Vitamin and Nutrition Research 74(6): 445-452.

Jiao, Z., Si, X., Zhang, Z., Li, G. and Cai, Z. 2012. Compositional study of different soybean (*Glycine max* L.) varieties by ^{1}H NMR spectroscopy, chromatographic and spectrometric techniques. Food Chemistry 135(1): 285-291.

Kapsokefalou, M., Alexandropoulou, I., Komaitis, M. and Politis, I. 2005. *In vitro* evaluation of iron solubility and dialyzability of various iron fortificants and of iron-fortified milk products targeted for infants and toddlers. International Journal of Food Sciences and Nutrition 56(4): 293-302.

Manary, M. J., Hotz, C., Krebs, N. F., Gibson, R. S., Westcott, J. E., Arnold, T., ... and Hambidge, K. M. 2000. Dietary phytate reduction improves zinc absorption in Malawian children recovering from tuberculosis but not in well children. The Journal of Nutrition 130(12): 2959-2964.

Miller, L. V., Krebs, N. F. and Hambidge, K. M. 2007. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. The Journal of Nutrition 137(1): 135-141.

Mitchikpe, E. C. S., Dossa, R. A. M., Ategbo, E. A. D., van Raaij, J. M. A., Hulshof, P. J. M. and Kok, F. J. 2008. The supply of bio-available iron and zinc may be affected by phytate in Beninese children. Journal of Food Composition and Analysis 21(1): 17-25.

Oghbaei, M. and Prakash, J. 2016. Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. Cogent Food and Agriculture 2(1): 1136015.

Onomi, S., Okazaki, Y. and Katayama, T. 2004. Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet. Bioscience, Biotechnology and Biochemistry 68: 1379-1381.

Pearson, D. 1976. The chemical analysis of food. 17th ed. Scotland: Churchill Livingstone.

Phillippy, B. Q., Bland, J. M. and Evens, T. J. 2003. Ion chromatography of phytate in roots and tubers. Journal of Agricultural and Food Chemistry 51(2): 350-353.

Platel, K. and Srinivasan, K. 2016. Bioavailability of micronutrients from plant foods: An update. Critical Reviews in Food Science and Nutrition 56(10): 1608-1619.

Pushparaj, F. S. and Urooj, A. 2014. Antioxidant activity in two pearl millet (*Pennisetum typhoideum*) cultivars as influenced by processing. Antioxidant 3*:* 55-66.

Sandberg, A. S. 2005. Methods and options *in vitro* dialyzability; benefits and limitations. International Journal for Vitamin and Nutrition Research 75(6): 395-404.

Skibniewska, K. A., Kozirok, W., Fornal, L. and Markiewicz, K. 2002. *In vitro* availability of minerals from oat products. Journal of the Science of Food and Agriculture 82(14): 1676-1681.

Trinidad, T. P., Mallillin, A. C., Sagum, R. S., Briones, D. P., Encabo, R. R. and Juliano, B. O. 2009. Iron absorption from brown rice/brown rice-based meal and milled rice/milled rice-based meal. International Journal of Food Sciences and Nutrition 60(8): 688-693.

World Health Organization (WHO). 2011. Use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6 - 23 months of age. Geneva: WHO.

Zhu, L., Glahn, P. R., Yeung, C. K. and Dennis, M. 2006. Iron uptake by Caco-2 cells from NaFeEDTA and $FeSO_4$: Effects of ascorbic acid, pH, and a Fe(II) chelating agent. Journal of Agricultural and Food Chemistry 54(20): 7924-7928.

Zlotkin, S., Schauer, C., Christofides, A., Sharieff, W., Tondeur, M. C. and Hyder, S. M. Z. 2005. Micronutrient sprinkles to control childhood anaemia. PLOS Medicine 2(1): e1.