Potential of moringa leaf and baobab fruit food-to-food fortification of wholegrain maize porridge to improve iron and zinc bioaccessibility

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Abstract

Food-to-food fortification (FtFF) with moringa leaf (iron source) and/or baobab fruit (citric acid and ascorbic acid source) (each 13-15 g/100 g porridge dry basis) was studied to improve iron and zinc nutritive quality in African-type wholegrain maize-based porridges using *in vitro* dialysability assay. Moringa FtFF decreased percentage and total bioaccessible iron and zinc, by up to 84% and 45%, respectively. Moringa was very high in calcium, approximately 3% dry basis and calcium-iron-phytate complexes inhibit iron bioavailability. Baobab FtFF increased percentage and total bioaccessible iron and zinc, especially in porridges containing carrot+mango (β-carotene source) and conventionally fortified with $FeSO_4$, by up to 111% and 60%, respectively. The effects were similar to those when ascorbic and citric acids were added as mineral absorption enhancers. While moringa FtFF could be inhibitory to iron and zinc bioavailability in cereal-based porridges, baobab fruit FtFF could improve their bioavailability, especially in combination with conventional iron fortification.

Introduction

Mineral deficiencies of iron and zinc are prevalent in Africa, particularly among children and women of reproductive age (Gupta et al. 2020; Mantadakis et al. 2020). These deficiencies are in part due to monotonous consumption of cereal foods that are often low in bioavailable minerals and high in mineral bioavailability inhibitors, notably phytate and polyphenols (Gibson et al. 2018). The situation is exacerbated by the fact that subsistence farmers and poor communities do not generally consume commercially produced foods fortified with micronutrients (Bouis et al. 2018).

To help alleviate mineral deficiencies, at-risk communities are encouraged to diversify their diets by including vegetables rich in essential minerals and fruits rich in promoters of mineral bioavailability (WHO 2017). As part of this strategy, food-to-food fortification (FtFF) of staple starchy foods with micronutrient-dense fruits, vegetables and other plant food ingredients is increasingly recognized as a tool in the fight against mineral deficiencies in developing countries (Chadare et al. 2019; Kruger et al. 2020). Thus, FtFF of African cereal staple foods like porridges with locally available leafy vegetables and fruits could be valuable in helping to alleviate dietary mineral deficiencies. In this regard, moringa (*Moringa oleifera*) leaves are being widely studied (Glover-Amengor et al. 2017; Kumssa et al. 2017). Moringa leaves are both high in iron, calcium and zinc and in their bioavailability promoters such as organic acids and β-carotene (Moyo et al. 2011; Adetola et al. 2019). Moringa leaves have also been reported to have high iron bioaccessibility when compared to other commonly consumed green leafy vegetables (Amagloh et al. 2017).

Recently, our group reported that FtFF of wholegrain pearl millet porridge with moringa leaves, carrot and mango (sources of β-carotene) produced only a moderate increase in the percentage iron bioaccessibility when added at a level of 10% and actually decreased bioaccessibility when added at much higher levels (30%) (Van der Merwe et al. 2019). Similarly, FtFF of wholegrain pearl millet porridge with moringa leaves only (15%) was found to reduce the percentage iron and zinc bioaccessibility (Adetola et al. 2019). The potential negative effects of moringa leaves were attributed primarily to the formation of calcium-phytate-mineral complexes because of their very high calcium content (2-4 g/100 g, dry basis (db)). The high content of phenolics in pearl millet, of the order of 278-353 mg catechin equivalents/100 g (Adetola et al. 2019; Van der Merwe et al. 2019), possibly also played a role. In contrast, both studies showed that FtFF with baobab (*Adansonia digitata*) fruit pulp enhanced iron bioaccessibility. Baobab fruit is high in ascorbic and citric acids, promoters of iron absorption (Lönnerdal, 2000), but relatively low in iron (Tembo et al. 2017; Stadlmayr et al. 2020). As baobab widely available in tropical Africa, it could potentially serve to enhance iron bioavailability in other cereal staples.

Maize is by far the most widely cultivated cereal in sub-Saharan Africa where it plays a critical role in food security (Ekpa et al. 2018). Also, it contains substantially lower levels of polyphenols (Oboh et al. 2011) compared to pearl millet. Thus, the primary aim of this study was to determine the effects of FtFF of African-type wholegrain maize porridge with moringa leaves and baobab fruit on iron and zinc bioaccessibility. The study investigated both FtFF of a simple maize porridge and a complex porridge, which additionally was FtFF with locally available plant foods that are rich sources of provitamin A sources, namely carrot and mango. This was done because Vitamin A deficiency affects some 500 million women and children worldwide, with poor diet quality and diversity being the root causes (Nair et al. 2016). The study also compared the effects of these FtFF on iron and zinc bioaccessibility with conventional fortification using $FeSO_4$ and inclusion of ascorbic acid and citric acid.

Materials and methods

Materials

White maize (not genetically modified) (cultivar PHI 2369), kindly donated by Tongaat Hulett Starch, Germiston, South Africa, was milled to a coarse wholegrain flour using a hammer mill fitted with a 1 mm opening screen. Dried baobab fruit pulp was obtained from Nautica Organic Trading, Durban, South Africa. Moringa leaf powders were obtained from MorNutritional Products, Tooseng Village, South Africa and SupaNutri, Oudtshoorn, South Africa. Carrot powder and mango fruit powder were from Sunspray Food Ingredients, Johannesburg, South Africa. All ingredients were stored in light- and air-tight containers at 10^{o}C prior to analysis.

Porridge formulations and preparation

Two types of FtFF wholegrain maize-based porridges were formulated (Table 1):

Maize porridge FtFF with moringa or baobab (Experiment 1)

Five porridge formulations were prepared: maize flour alone (1), maize flour fortified with moringa leaf powder (2), maize flour fortified with baobab fruit pulp (3), maize flour fortified with ascorbic+citric acid (4) and maize flour fortified with $FeSO_4$ (5). Deionised water (100 ml) was added to 30 g porridge formulation (comprising maize, maize+moringa or maize+$FeSO_4$) and slurried to suspend the flour. The slurries were heated to boiling and maintained for 15 min with constant stirring. They were then cooled at room temperature (25^oC), frozen at -20^oC and then freeze-dried. The freeze-dried porridges were finely milled using hammer mill with a 250 μm opening screen and stored at -20^oC prior to analysis. Baobab fruit pulp or ascorbic+citric acid were added and thoroughly mixed into the cooked maize porridge flour, as baobab fruit is usually consumed raw, like most fruits.

Maize porridge containing carrot+mango as provitamin A source FtFF with moringa and/or baobab (Experiment 2)

Five porridge formulations were prepared: maize flour fortified with $FeSO_4$ and carrot+mango powder (1), maize flour fortified with $FeSO_4$ and ascorbic acid and carrot+mango powder (2), maize flour fortified with moringa leaf powder and carrot+mango powder (3), maize flour fortified with $FeSO_4$ and baobab fruit pulp and carrot+mango powder (4), and maize flour fortified with moringa leaf powder and baobab fruit pulp and carrot+mango powder (5). Corn starch was added so that all the formulations had a constant percentage of maize. A 100 g (db) serving of each of the five porridge formulations was calculated to provide approximately 200 μg retinol equivalents based on the carotenoid contents of carrot+mango alone, which if all was retained in the porridge would meet approximately 40% of the daily vitamin A requirement for women of 19-50 years (WHO and FAO 2004).

The five porridge formulations were similar in iron contents with one serving designed to meet approximately 20% of the daily iron requirement for women of 19-50 years (58.5 mg), either through fortification with moringa or with $FeSO_4$, calculated at low (5%) bioavailability, as from a predominantly plant-based diet (WHO and FAO 2004).

The formulations were prepared as described for Experiment 1. Carrot powder was added to the hot cooked porridge and cooked for another 5 min with stirring. The cooked porridge was cooled to 25^oC, after which mango powder was added and thoroughly mixed.

Analyses

The mineral, phytate, total phenolics, tannin and organic acid contents of the FtFF-porridge formulations were calculated from the contents of the ingredients and not the porridges as consumed.

Moisture contents

The moisture contents of the plant food ingredients were determined using the ISTA (2018) high constant temperature oven method.

Mineral contents

Acid digestion of the ingredient flours was performed by the US EPA method 3051A (US EPA 2007). Iron, zinc, calcium, magnesium and phosphorus contents of the digested ingredients were analysed by EPA method 200.7 (US EPA 1994) using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (Thermo Scientific model iCAP 6000, Bremen, Germany). Elements were quantified using wavelengths of 239.5 nm for Fe, 206.7 nm for Zn, 315.8 for Ca, 285.2 nm for Mg and 214.9 nm for P. Two independent samples of each foodstuff were analysed in duplicate.

Phytate content

Phytate content was measured using the extraction and indirect quantitative assay of Frühbeck et al. (1995). Dowex1-anion-exchange resin-AG 1 x 4 (4% Cross-linkage, chloride form, 100-200 mesh) in glass barrel Econo-columns (0.7x5 cm) was used to purify the sample extracts and to separate the inorganic and organic (phytate) phosphorus. The sodium phytate standard (P-8810, Sigma-Aldrich, Johannesburg, South Africa) and purified extracts were reacted with Wade reagent, after which absorbance was measured colorimetrically at 500 nm. Two independent samples of each foodstuff were analysed in duplicate.

Total phenolic contents

The Folin-Ciocalteu method according to Waterman and Mole (1994) was used to quantify total phenolic content (mainly free total phenolics) and reported in catechin equivalents (CE). Two independent samples of each foodstuff were analysed in duplicate.

Tannin contents

Condensed tannins were quantified by the modified Vanillin-HCl method of Price et al. (1978) and expressed in CE. Two independent samples of each foodstuff were analysed in duplicate.

Organic acid contents

Organic acids (ascorbic, citric and malic acids) were determined using the extraction and quantification assay procedure of Tembo et al. (2017), with slight modification. Samples (2 g) were extracted in 20 ml metaphosphoric acid (0.3 g/L) and centrifuged (3500 x *g*, at $4^{o}C$). The supernatants and the ascorbic acid (100468, Merck), citric acid and malic acid standards (251275 and 240176, respectively, Sigma-Aldrich) were then filtered through Millipore 0.45 µm PTFE filters, after which the organic acids were quantified by RP–HPLC using acidified (pH 2.6) 10 mM KH_2PO_4 as mobile phase and a Phenomenex-C18 column 250 x 4.6 mm (Torrance, CA). Simultaneous absorbance at 254 nm was used to detect ascorbic acid and at 210 nm to detect citric and malic acids.

In vitro mineral dialysability assay

The porridge formulations were subject to *in vitro* digestion to mimic the gastric and intestinal digestion in humans. The *in vitro* equilibrium dialysability assay procedure of Miller et al. (1981) was used. Digestive enzymes and bile salts used were pepsin (P-7000), pancreatin (P-1750), and bile extract (B-8631) (Sigma-Aldrich). Dialysis tubing Spectra/Por 7 (Ø = 20.4 mm) with a molecular weight cut-off of 10 kDa was used (G.I.C. Scientific, Johannesburg, South Africa). Mineral contents of the dialysates were measured using ICP-AES as described. Iron and zinc bioaccessibility was calculated as the percentage of the mineral in the dialysate relative to the total mineral content in the porridge sample. The total amount of bioaccessible minerals was then calculated by percentage mineral bioaccessibility

× total mineral content and was expressed as mg/100 g (dry basis (db)) porridge formulation. Two independent dialysability assay experiments were performed, with the intestinal step being each time performed in triplicate and each dialysate analysed in duplicate.

Contribution of iron and zinc contents to mineral requirements

The contribution of iron and zinc from 100 g porridge (db) (a 300–400 g serving size depending on its solids content) to the recommended nutrient intakes (RNI) for women of 19-50 years was calculated and expressed as a percentage of the RNI. Likewise, the percentage contribution that the bioaccessible iron and zinc in the different porridge formulations could potentially make to the daily iron and zinc absolute requirement (AR) of women of 19-50 years (WHO and FAO 2004; Sandstead 2015) was calculated.

Statistical analyses

Data were analysed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics software 25.0 (Armonk, NY). Tukey's Honest Significant Difference Post-hoc test was applied to determine significant differences between means at a confidence level of 95%. Fisher's Least Significant Difference Post-hoc test was applied for pair-wise comparison between the basic and the fortified porridge formulations at a confidence level of 95%.

Results and discussion

In the study, the plant foods were used in a dried, i.e. shelf-stable form rather than as fresh products in order to ensure consistency of nutrient composition throughout the study duration. However, in practical FtFF, the foods would be more affordable if fresh products were used. Also, the small particle size of the dried powders, the process of drying and the fine milling of the porridge formulations may have impacted on mineral bioaccessibility as the highly complex food matrix effects (Ferruzzi et al. 2020) would not be identical to those with fresh foods.

The mineral and antinutrient contents of the porridge formulations were calculated from contents of the plant foodstuffs rather than measured in the porridge formulations themselves. This was done because, as explained in the Introduction, the primary aim of the study was to determine the effects of moringa leaves and baobab fruit as FtF fortificants on iron and zinc bioaccessibility in African-type wholegrain maize porridge. It is recognised that the contents of the organic components such as the phenolics, phytate and organic acids but not the minerals would probably be different in the porridges to the sum of the components. This is because thermal processing can both alter the composition and also destroy these organic components in cereal foods (Taylor and Kruger 2019). However, simply knowing their contents in the final porridges would not substantially help to better understand the causes of the measured effects on mineral bioaccessibility reported in this present study. This is because these are the result of highly complex interactions between the antinutritional factors and promoters of bioaccessibility and the food matrix and its specific components (e.g. fibre and protein) that take place during food processing, preparation and assay (Ferruzzi et al. 2020).

Mineral contents

Wholegrain white maize was low in iron, only 2.0 mg/100 g (Table 2). This value was somewhat lower than the USDA FoodData Central value of 3.0 mg/100 g db (FDC 168920) (USDA 2019). The iron content of the wholegrain maize was 25–33% of that in wholegrain pearl millet (Adetola et al. 2019; Van der Merwe et al. 2019) and illustrates the problem of monotonous maize diets with respect to iron deficiency. FtFF of the maize porridge with moringa leaf powder substantially increased the iron content (Experiment 1). However, a 100 g db (300–400 g actual serving) of the moringa FtFF-maize porridge would only contribute approximately 11% of the iron RNI for women of 19–50 years at low (5%) iron bioavailability. According to CODEX, a food source is recognized as a good source of a mineral if one serving meets ≥15% of the RNI (Lewis 2019). With the porridges that had FtFF with carrot+mango powder (Experiment 2), fortification with moringa resulted in a serving contributing nearly 20% of the iron RNI for adult women at 5% bioavailability. As explained, the formulations were designed to provide approximately 20% of the iron RNI. Notably, the extent of increase in contribution to the iron RNI by moringa leaf FtFF would depend on the actual iron content in the moringa leaves. This can vary greatly, between 30 and >100 mg/100 g (db) as with the SupaNutri moringa, but typically around 50–60 mg/100 g (Van der Merwe et al. 2019). FtFF of the maize porridge (Experiment 1) with baobab fruit pulp had no effect on the iron content of the maize porridge as the iron contents of baobab and maize were similar.

The zinc content of the wholegrain maize of 1.9 mg/100 g (Table 2) was slightly lower than the USDA (2019) value of 2.5 mg/100 g. Although the zinc content of the MorNutri moringa (3.1 mg/100 g) was significantly higher ($p<0.05$) than that of maize, it was not high enough to substantially increase the zinc content of the FtFF-porridge (Experiment 1) at the 15:100 fortification ratio. FtFF with baobab also had no effect on the zinc content of the maize porridge alone as the zinc content of baobab, although only half that of maize, did not result in a significant dilution of the zinc content. The zinc contents of most of the porridge formulations in Experiment 2 were similar as were their percentage contributions to the zinc RNI (14-15%), except for the two moringa fortified-maize-based porridge formulations, which were slightly higher due to moringa's somewhat higher zinc content.

Antinutrient contents

The moringa leaf powder products had the highest phytate contents of all the ingredients, approximately 20% (MorNutri) and >100% (SupaNutri) higher than wholegrain maize (Table 3). Like other cereal grains, the wholegrain maize was also high in phytate (830 mg/100 g), which was in the range previously reported (710–1050 mg/100 g) (Hotz and Gibson 2001; Kruger et al. 2014). Although the phytate content of the MorNutri moringa was higher than in the maize, FtFF of maize porridge did not substantially increase the phytate content in Experiment 1. However, in Experiment 2 where a 1:1 blend of the two moringa powder products was used, the phytate content of the FtFF porridge was increased considerably, by approximately 36%. Baobab did not substantially affect the phytate contents of the FtFF porridges in either experiment as its phytate content was only half that of maize.

The total phenolic content of the wholegrain white maize (19 mg/100 g) was by far the lowest of the ingredients (Table 3) and much lower than wholegrain pearl millet (278 mg/100 g) (Adetola et al. 2019). The baobab fruit pulp and moringa leaf powders were very high in phenolics relative to the maize, 90- and 63–75-fold, respectively. Only the baobab fruit pulp contained condensed tannins, an apparently very high content of approximately 10 g CE/100 g. It should be noted that while this value is probably an overestimate, as the catechin standard exaggerates foodstuff true tannin content (Price et al. 1978), the true content was probably still high. Contamination of the pulp with seed fragments, which are high in tannins (approx. 23 g/100 g) (Osman 2004) could have contributed to its very high tannin content. The inclusion of either moringa or baobab increased the total phenolic content of the FtFF porridges greatly, with moringa+baobab FtFF by some 10–12-fold in Experiment 1 and rather less when they were included individually. Baobab FtFF greatly increased the apparent tannin contents of the porridges from undetectable levels to >1 g CE/100 g.

Organic acid contents

Baobab was highest ($p<0.05$) in ascorbic and citric acids (174 mg/100 g and 4440 mg/100 g, respectively) (Table 4). It was also the highest in malic acid, except for SupaNutri moringa. The values for ascorbic, citric and malic acids in baobab fruit were similar to those reported elsewhere, 162–499 mg/100 g (Bamalli et al. 2014), 2570–3300 mg/100 g and 210–2360 mg/100 g, respectively (Magaia et al. 2013; Tembo et al. 2017). Thus, in both Experiments the contents of ascorbic acid, citric acid and malic acid in the baobab FtFF porridges were increased substantially. The moringa powders, like baobab, were also high in citric acid (approximately 65% of baobab) and in malic acid but contained very little ascorbic acid (only 2 and 9 mg/100 g). These ascorbic acid values are in general agreement with the low content for dried moringa leaves of 17 mg/100 g reported by Gopalakrishnan et al. (2016). Hence, in both Experiments moringa FtFF of the porridges would only substantially increase the citric acid and malic acid contents. Mango powder was also notably high in ascorbic acid and high in citric acid. The ascorbic acid content was in the same range as the FoodData Central value of 220.6 mg/100 g (FDC 169910) (USDA 2019). However, the addition of mango to the porridge (Experiment 2) did not substantially increase the porridge ascorbic acid and citric acid contents because the proportion of mango was relatively low, only 3.5 g/100 g. Carrot powder contained negligible ascorbic acid, 3.5 mg/100 g, far lower than the FoodData Central value for fresh carrot of 50.4 mg/100 db (FDC 170393). The low ascorbic acid content of the carrot powder may have been a consequence of the drying process.

Mineral bioaccessibility

Iron bioaccessibility

Moringa leaf FtFF of wholegrain maize porridge (Experiment 1) at a 15:100 ratio greatly decreased percentage and total bioaccessible iron, by 84% and 52%, respectively (Table 5). This was despite the three-fold higher iron content (Table 2) and lower phytate:iron molar ratio of 11:1 compared to the maize porridge control (34:1) (Table 3). In contrast, conventional fortification of maize porridge with an equivalent amount of iron, as $FeSO_4$, decreased the percentage bioaccessible iron by 38%, but increased total bioaccessible iron by 104%. Similarly, in Experiment 2 moringa FtFF of the maize-based porridge (13.5 g/100 g) greatly decreased percentage and amount of bioaccessible iron, by 80% and 78% respectively, compared to the control maize-based porridge conventionally fortified with a similar amount of iron as $FeSO_4$. In contrast to this study, Van der Merwe et al. (2019) showed that moringa leaf FtFF of a pearl millet-based porridge at a ratio 10:100 increased iron bioaccessibility. However, when the fortification proportion was increased to 30:100, the percentage iron bioaccessibility was decreased. The different findings may be related to the 3–4-fold higher iron content of wholegrain pearl millet compared to wholegrain maize. This resulted in the much lower calcium×phytate:iron molar ratio at the lower level of moringa addition. Khoja et al. (2021) found that although moringa leaves had the highest amount of bioaccessible iron when compared to other plant foodstuffs such as baobab fruit pulp, fenugreek sprouts and seeds, it had the lowest percentage bioaccessible iron and substantially inhibited iron ($FeSO_4$) uptake by Caco-2 cells. These findings agree with research which has shown that green leafy vegetables that are high in phenolics and calcium such as spinach and amaranth limit iron absorption. Bonsmann et al. (2008) found that consumption of spinach together with wheat bread by childbearing women could potentially decrease iron absorption when compared to kale, which contained significantly less polyphenols and calcium. Similarly, Cercamondi et al. (2014) found that consumption by women of an amaranth leaf vegetable sauce together with a maize paste slightly increased fractional iron absorption but did not increase the amount of absorbed iron.

Given the above, the strong inhibitory effects of moringa were most likely driven by the very high calcium content (Table 2) in combination with its high phytate content (Table 3). Rousseau et al. (2020) reported that high contents of calcium in foods could increase the inhibitory effect of phytate on iron bioavailability by forming precipitates of iron-calcium-phytate complexes, which are less soluble than phytate complexes formed with either of the minerals. Similarly, Hallberg and Hulthén (2000) reported that food calcium contents more than 50 mg/100 g inhibited non-haem iron bioavailability in plant-based foods. Furthermore, the higher content of phenolics in the moringa fortified-porridges compared to the control porridges could also have contributed to the negative effect of moringa on iron bioaccessibility. Moringa leaves have been found to be particularly high in polyphenols such as chlorogenic acid (Van der Merwe et al. 2019), which can chelate iron (Andjelković et al.

2006). Also, moringa leaf is high in oxalate and dietary fibre (Amalraj and Pius 2015), both of which have been found to negatively impact iron bioavailability (Baye et al. 2015; Brigide et al. 2019).

Therefore, moringa FtFF of maize porridge (Experiment 1) may result in a substantial reduction in contribution of iron from one serving (100 g porridge, db) to the AR for adult women, as opposed to conventional fortification with $FeSO_4$, which could substantially improve it (Table 5). Similarly, in Experiment 2, moringa FtFF of the maize-based porridge containing provitamin A sources would likely greatly reduce contribution to the iron AR for an adult woman, whereas with conventional iron fortification using $FeSO_{4,}$ one porridge serving could meet the AR.

In Experiment 1, baobab FtFF of maize porridge at the 15:100 ratio slightly but significantly increased ($p<0.05$) iron bioaccessibility in terms of percentage and amount (Table 5), as was found with baobab FtFF of wholegrain pearl millet porridge (Adetola et al. 2019). Notably, baobab increased iron bioaccessibility, notwithstanding the fact that the baobab fortified-maize and baobab+$FeSO_4$ fortified-maize-based porridge (Experiment 2) formulations were high in total phenolics and contained tannins. The increase in the percentage bioaccessible iron with the maize-based porridge (Table 5, Experiment 1) was rather lower than with ascorbic+citric acid inclusion. This was probably due to tannins and other phenolics derived from baobab pulp (Table 3). In Experiment 2, baobab fortification of the $FeSO_4$ fortified-maize-based porridge, containing plant food provitamin A sources greatly increased both percentage and total bioaccessible iron, by approximately 107% and 111%, respectively, compared to the $FeSO_4$ fortified-maize-based control.

The inclusion of ascorbic acid in the $FeSO_4$ fortified-maize-based porridge, at approximately twice the level present in baobab fruit, however, resulted in a slightly lower increase in percentage and total bioaccessible iron (93% for both). The greater improvement in iron bioaccessibility with baobab fortification was very likely due to its high content of other organic acids including citric acid (Table 4). Citric acid addition alone to pearl millet porridge was found to increase both percentage and total bioaccessible iron (Adetola et al. 2019). Similarly, it has been found that citric acid addition in pressure cooked and uncooked rice substantially increased percentage iron bioaccessibility (Hemalatha et al. 2005). Ascorbic acid and citric acid are reported to overcome the effect of iron bioavailability inhibitors such as phytate and polyphenols in a food matrix by maintaining the iron in a soluble and absorbable form (Lönnerdal 2000; Hurrell and Egli 2010). Additionally, ascorbic acid enhances iron bioavailability, primarily by reducing insoluble Fe^{3+} to soluble Fe^{2+} (Hurrell and Egli 2010).

As seen, the positive effect of baobab fruit FtFF on iron bioaccessibility was far greater in the presence of added iron (as $FeSO_4$) (Experiment 2) than in its absence (Experiment 1) (Table 5). This is presumably because the organic acids in baobab fruit also enhanced the solubility of the added iron, thereby preventing the chelating effect of the phytate in the food matrix on the iron ions. This suggests that the potential of baobab fruit fortification or, for that matter, organic acid inclusion will only be maximised in the presence of sufficient potentially available iron in the food matrix. In support of this, baobab fortified-pearl millet porridge (Adetola et al. 2019) increased iron bioaccessibility by approximately 50% when compared to baobab fortified-maize porridge (Experiment 1) with the baobab fortified-pearl millet porridge containing more than four-times the amount of iron compared to the simple maize porridge.

The ascorbic acid:iron molar ratio of the maize+baobab and maize+citric+ascorbic acid porridges (3.6:1) (Table 4) was higher than 2:1, which has been reported to improve iron bioavailability from cereal-based foods (Troesch et al. 2011). However, a much higher ratio, 40:1 is considered to be optimal for improving iron bioavailability from cereal-based meals with high levels of iron bioavailability inhibitors (Teucher et al. 2004). Possibly, such a high ratio is only required in the absence of other organic acids such as citric acid.

In Experiment 2, the carrot and mango in the formulations may have also contributed to the greater improvement in iron bioaccessibility compared to Experiment 1 (Table 5). Carrot is very high in β-carotene, approx. 70751 µg/100 g (db) and mango contains a significant amount, 3869 µg/100 (db) (USDA, 2019). Beta-carotene has been shown to form a soluble complex with iron in the intestinal lumen, thus also preventing it from being chelated by phytate and polyphenols (Garcia-Casal et al. 1998).

Although, bioaccessible iron measured by the *in vitro* dialysability assay is only suggestive of the potential impacts in humans, the assay's prediction of the direction of effect is known to be more reliable (Fairweather-Tait et al. 2005). Hence, the dialysability data in this work and that on pearl millet porridges (Adetola et al. 2019; Van der Merwe et al. 2019), indicate that baobab fruit FtFF of cereal porridges can have substantial positive effect on iron bioavailability. In terms of contribution to the AR of adult women (Table 5), the enhancing effect of baobab FtFF of maize porridge alone (Experiment 1) is not substantial as the calculated increase was only from 46% to 50%. However, with the baobab FtFF porridge containing provitamin A sources and conventionally fortified with $FeSO_4$ (Experiment 2), one serving of the FtFF porridge could double the contribution to the AR.

Zinc bioaccessibility

In Experiment 1, none of the food fortificants improved zinc bioaccessibility (Table 5). In fact, moringa and conventional iron fortification of maize porridge substantially decreased the percentage bioaccessible zinc by 49% and 33%, respectively, and the amount of bioaccessible zinc by 45% and 31%, respectively. Because of the substantial negative effect of moringa and conventional iron fortification on zinc bioaccessibility, the moringa and iron fortified-simple maize porridges could potentially reduce the contribution of zinc to adult woman's AR. These findings agree with those of Hemalatha et al. (2009) where iron fortification of pressure-cooked rice-based grains with $FeSO_4$, decreased zinc bioaccessibility by approximately 27%.

The very high calcium content of moringa together with its high phytate content was also probably largely responsible for the reduction in zinc bioaccessibility. Sandström et al. (2001) reported that in the presence of phytate, calcium further inhibits zinc absorption due to co-precipitation of calcium with phytate and zinc. The calcium×phytate:zinc molar ratio of 318:1 in the maize porridge (Table 3) was above the critical value of 200:1 at which zinc bioavailability is impaired (Morris and Ellis 1989). *In vivo*, the co-occurrence of different minerals with similar chemical structures, such as iron, zinc and calcium, within a food matrix may affect mineral bioavailability as they compete for transport proteins and other uptake mechanisms (Sandström et al. 2001).

In contrast, in Experiment 2 baobab FtFF of the $FeSO_4$ fortified-maize-based porridge and baobab+moringa FtFF increased zinc bioaccessibility substantially, in terms of percentage by approximately 45% and 31%, respectively, and amount by 60% and 83%, respectively. This was notwithstanding the high phytate:zinc molar ratios (41–43:1) of the baobab and moringa+baobab FtFF porridges, which were more than 2-fold higher than the critical limit (10–15:1) (Hunt 2003). Also, this was notwithstanding the very high calcium×phytate:zinc molar ratio (509:1) of the moringa+baobab FtFF porridge. Baobab and moringa+baobab fortification of maize porridge containing plant-based provitamin A sources could potentially improve the contribution of a serving from 29% to around 50% of the zinc AR for adult women.

The positive effect of baobab fruit on zinc bioaccessibility could also be due to its high citric acid content (Table 3) in combination with β-carotene from the carrot and mango. Gabaza et al. (2018) also attributed an observed positive effect of baobab fruit on zinc bioaccessibility in a fermented wholegrain maize porridge to its high citric acid content. Similarly, Hemalatha et al. (2005) found that mango fruit FtFF of both uncooked and cooked rice grain substantially increased zinc bioaccessibility and attributed this to the mango's high citric acid content. Citric acid is reported to enhance zinc bioavailability by chelating it in a soluble and absorbable form (Gibson 2006). The role of β-carotene in zinc bioavailability has not been elucidated. However, some studies have shown that β-carotene fortification of cereal grains increased percentage zinc bioaccessibility (Gautam et al. 2010). Also, Van der Merwe et al. (2019) showed that baobab and/or moringa fortification of pearl millet-based porridge, containing approximately 40% plant β-carotene sources substantially increased zinc bioaccessibility. Similarly, Kruger (2020) found that FtFF of a maize-based porridge with orange-fleshed sweet potatoes rich in β-carotene increased percentage zinc uptake by Caco-2 cells. The β-carotene in these plant foods may have improved zinc solubility in the same way it does with iron (Garcia-Casal et al. 1998).

Conclusions

FtFF of wholegrain maize porridge with moringa leaf powder was found to have a strong inhibitory impact on iron bioaccessibility, despite moringa's high iron content. The potential exists for these interactions to negatively affect the porridge's contribution of iron to the AR for adult women. The adverse effect of moringa on iron bioaccessibility is likely due to a combination of its high calcium and phytate contents resulting in the formation of unavailable insoluble iron-calcium-phytate complexes. Zinc bioaccessibility from wholegrain maize porridge was not improved by moringa or baobab fruit FtFF alone. In fact, moringa FtFF is substantially inhibitory for zinc, as with iron bioaccessibility.

In contrast to moringa, baobab FtFF of maize porridge does appear to enhance iron bioaccessibility, despite baobab's low iron content. However, it is far more effective in the presence of sufficient potentially available iron, e.g. as $FeSO_4$. The positive effect of baobab is very likely due to its high contents of ascorbic and citric acids, which would enhance the solubility of the additional iron in the food matrix and maintain the iron in the Fe^{2+} form. Thus, baobab FtFF of conventionally iron fortified wholegrain maize porridge could potentially substantially increase its contribution to an adult woman's iron AR. Similarly, in the presence of the added plant provitamin A sources, both baobab and moringa+baobab potentially could significantly enhance zinc bioaccessibility from the porridge.

Baobab fruit FtFF of staple cereal foods containing sufficient potentially available iron, could be a practical strategy to complement existing nutritional interventions aimed at improving iron bioavailability in the diets of at-risk populations in Africa.

Disclosure statement

The authors report no potential conflicts of interest.

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Table 1: Maize-based porridge formulations (per 100 g db)

Porridge formulations	**Wholegrain white maize (g)**	**Moringa leaf (g)**	**Baobab fruit pulp (g)**	**Ascorbic acid (mg)**	**Citric acid (mg)**	**$FeSO_4$ (mg Fe)**	**Carrot+mango (1:1 ratio) (g)**	**Corn starch (g)[1]**
Experiment 1 (Maize porridge FtFF with moringa or baobab)								
Maize	100.0	0	0	0	0	0	0	0
Maize+moringa[2]	87.0	13.0	0	0	0	0	0	0
Maize+baobab	87.0	13.0	0	0	0	0	0	0
Maize+ascorbic+citric acid	100.0	0	0	23.3	595	0	0	0
Maize+$FeSO_4$	100.0	0	0	0	0	4.7	0	0
Experiment 2 (Maize porridge containing carrot+mango (as provitamin A source) FtFF with moringa and/or baobab)								
Maize+$FeSO_4$+carrot+mango	64.5	0	0	0	0	9.4	7.0	28.5
Maize+$FeSO_4$+ascorbic acid+carrot+mango	64.5	0	0	64.3	0	9.4	7.0	28.5
Maize+moringa[3]+carrot +mango	64.5	13.5	0	0	0	0	7.0	15.0
Maize+$FeSO_4$+baobab +carrot+mango	64.5	0	15.0	0	0	9.4	7.0	13.5
Maize+moringa[3]+baobab +carrot+mango	64.5	13.5	15.0	0	0	0	7.0	0

[1]Corn starch added to porridge formulations in Experiment 2 so that they had a constant percentage of maize, [2]MorNutri moringa,
[3]MorNutri:SupaNutri moringa (1:1 ratio)

Table 2: Mineral contents (mg/100 g db) of wholegrain white maize flour, baobab fruit pulp, moringa leaf powder, carrot powder and mango fruit powder and their maize-based FtFF porridge formulations (100 g db) and their estimated percentage contribution to the daily recommended nutrient intake (RNI) for adult women (19–50 years)

Porridge ingredients	Iron	Zinc	Calcium	Percentage contribution of iron to RNI (58.8 mg/day)[1]	Percentage contribution of zinc to RNI (9.8 mg/day)[1]
White maize	2.0[a] ±0.0	1.89[c] ±0.03	5[a] ±0	3.5	19.3
Moringa leaves (MorNutri)	34.2[c] ±1.2	3.08[e] ± 0.21	2335[d] ±110	NA[3]	NA
Moringa leaves (SupaNutri)	114.4[d] ±1.0	2.62[d] ± 0.03	3723[e] ±34	NA	NA
Baobab fruit pulp	1.7[a] ±0.1	0.95[b] ± 0.01	363[c] ±2	NA	NA
Carrot	5.3[b] ±0.1	2.78[d] ±0.01	167[b] ±2	NA	NA
Mango	1.9[a] ±0.1	0.35[a] ±0.01	61[a] ±2	NA	NA
Experiment 1 (Maize porridge FtFF with moringa[4] or baobab)					
Porridge formulations[2]					
Maize	2.0	1.89	5	3.5	19.3
Maize+moringa	6.2	2.05	309	10.6	20.9
Maize+baobab	2.0	1.77	52	3.4	18.1
Maize+ascorbic+citric acid	2.0	1.89	5	3.5	19.3
Maize+$FeSO_4$	6.8	1.89	5	12.2	19.3

Experiment 2 (Maize porridge containing carrot+mango (as provitamin A source) FtFF with moringa[5] and/or baobab)					
Porridge formulations[2]					
Maize+$FeSO_4$+carrot +mango	11.0	1.33	11	18.7	13.6
Maize+$FeSO_4$ +ascorbic acid+carrot+mango	11.0	1.33	11	18.7	13.6
Maize+moringa+carrot+mango	11.6	1.72	421	19.8	17.5
Maize+$FeSO_4$ +baobab+carrot+mango	11.3	1.47	66	19.1	15.0
Maize+moringa+baobab +carrot +mango	11.9	1.89	476	20.2	19.0

Values are the mean ±1 SD of two independent samples analysed in duplicate (n=4). Means with different superscripts letters in a column differ significantly ($p<0.05$),
[1]RNI for women aged 19-50 consuming a plant-based diet with the lowest level of mineral bioavailability, 5% for iron and 15% for zinc (WHO and FAO 2004),
[2]Mineral contents of all the porridge formulations were calculated from each of the ingredients in the formulations, [3]Not applicable, [4]MorNutri moringa,
[5]MorNutri:SupaNutri 1:1 ratio

Table 3: Phytate, total phenolics and tannin contents of wholegrain white maize flour, baobab fruit pulp, moringa leaf powder, carrot powder and mango fruit powder and their maize-based FtFF porridge formulations and their calculated phytate:mineral molar ratios

Porridge ingredients	Phytate (mg/100 g db)	Total phenolics (mg CE /100 g db)	Tannins (mg CE/100 g/100 g db)	Phytate:iron molar ratio	Calcium × phytate:iron molar ratio	Phytate:zinc molar ratio	Calcium × phytate:zinc molar ratio
White maize	830^{c} ±34	19^{a} ±1	0	34.3:1	4.5:1	43.4:1	6:1
Moringa leaves (MorNutri)	1004^{d} ±79	1194[1]	0	2.5:1	144.6:1	32.3:1	1882:1
Moringa leaves (SupaNutri)	1877^{e} ±67	1418^{d} ±14	0	1.4:1	128.9:1	70.9:1	6584:1
Baobab fruit pulp	414^{b} ±37	1707^{e} ±22	9860 ±220	20.1:1	181.7:1	43.0:1	389:1
Carrot	212^{a} ±17	241^{b} ±7	0	3.4:1	14.1:1	7.6:1	31:1
Mango	154^{a} ±11	396^{c} ±9	0	7.0:1	10.7:1	43.6:1	66:1
Experiment 1 (Maize porridge FtFF with moringa[3] or baobab) **Porridge formulations[2]**							
Maize	830	19	0	34.3:1	4.5:1	43.4:1	6:1
Maize+moringa	853	201	0	11.6:1	89.1:1	41.2:1	318:1
Maize+baobab	776	239	1286	32.7:1	42.4:1	43.4:1	56:1
Maize+ascorbic+citric acid[1]	830	19	0	34.3:1	4.5:1	43.4:1	6:1
Maize+$FeSO_4$[2]	830	19	0	9.8:1	1.3:1	43.4:1	6:1
Experiment 2 (Maize porridge containing carrot+mango (as provitamin A source) FtFF with moringa[4] and/or baobab) **Porridge formulations[2]**							
Maize+$FeSO_4$+carrot+mango	548	34	0	4.2:1	1.2:1	40.8:1	11:1
Maize+$FeSO_4$+ascorbic acid+carrot+mango	548	34	0	4.2:1	1.2:1	40.8:1	11:1
Maize+moringa+carrot+mango	743	211	0	5.4:1	56.8:1	42.9:1	450:1
Maize+$FeSO_4$+baobab+carrot+mango	610	291	1479	4.6:1	7.5:1	41.0:1	67:1
Maize+moringa+baobab+carrot+mango[7]	805	467	1480	5.7:1	68.0:1	42.9:1	509:1

Values are the mean ±1 SD of two independent samples analysed in duplicate (n=4). Means with different superscripts letters in a column differ significantly ($p<0.05$), [1]Single analysis only, [2]Phytate, total phenolics and tannin values of porridge formulations were calculated from the individual plant food ingredients, [3]MorNutri moringa, [4]MorNutri:SupaNutri 1:1 ratio

Table 4: Organic acid contents (mg/100 g db) of wholegrain white maize flour, baobab fruit pulp, moringa leaf powder, carrot powder and mango fruit powder and their FtFF maize-based porridge formulations

Porridge ingredients	Ascorbic acid	Citric acid	Malic acid	Ascorbic acid:iron molar ratio
White maize	Not detected	52[a] ± 8	73.2[a] ± 11.9	0.0:1
Moringa leaf powder (MorNutri)	9.1[a] ±0.9	2779[c] ± 208	780.2[d] ± 76.2	0.1:1
Moringa leaf powder (SupaNutri)	2.1[a] ±0.0	2968[c] ± 390	285.9[b] ± 3.0	0.0:1
Baobab fruit pulp	173.9[c] ±11.3	4440[d] ± 409	636.9[c] ± 29.3	31.6:1
Carrot	3.5[a] ±1.0	Not detected	Not detected	0.2:1
Mango	150.9[b] ± 5.5	1043[b] ± 98	Not detected	25.9:1
Experiment 1 (Maize porridge FtFF with moringa[2] or baobab)				
Porridge formulations[1]				
Maize	Not detected	52	73.2	0.0:1
Maize+moringa	1.2	408	165.2	0.6:1
Maize+baobab	22.7	624	146.5	3.6:1
Maize+ascorbic+citric acid	23.3	647	73.2	3.6:1
Maize+$FeSO_4$	0	52	73.2	0.0:1
Experiment 2 (Maize porridge containing carrot+mango (as provitamin A source) FtFF with moringa[3] and/or baobab)				
Porridge formulations[1]				
Maize+$FeSO_4$+carrot+mango	5.4	70	47.1	0.2:1
Maize+$FeSO_4$+ascorbic acid+carrot+mango	68.7	70	47.1	2.0:1
Maize+moringa+carrot+mango	6.1	458	119.2	0.2:1
Maize+$FeSO_4$+baobab+carrot+mango	31.5	736	142.7	0.9:1
Maize+moringa+baobab+carrot+mango	32.3	1125	214.8	0.9:1

Values are the mean ±1 SD of three independent samples analysed in triplicate (n=9). Means with different superscripts letters in a column differ significantly ($p<0.05$), [1]Organic acid contents of all the porridge formulations were calculated from each of the ingredients in the formulations, [2]MorNutri moringa, [3]MorNutri:SupaNutri 1:1 ratio

Table 5: Effects of food-to-food fortification of simple and complex (with a provitamin A source) wholegrain white maize porridge, with baobab fruit pulp and moringa leaf on iron and zinc bioaccessibility and the porridges' (100 g db) percentage contribution of bioaccessible iron and zinc to the absolute requirements (AR) of women of 19–50 years

Porridge formulations	Percentage bioaccessible iron	Amount of bioaccessible iron (mg/100 g db)	Percentage contribution of iron to AR (1.46 mg/day)[1]	Percentage bioaccessible zinc	Amount of bioaccessible zinc (mg/100 g db)	Percentage contribution of zinc to AR (1.75 mg/day)[2]
Experiment 1 (Maize porridge FtFF with moringa[4] or baobab)						
Maize (Control)	28.4[c] ±1.8	0.58[b] ±0.04	39.7	54.8[cd] ±4.1	1.03[d] ±0.08	59.3
Maize+moringa	4.4[a] ±0.6 (-84%)[3]	0.28[a] ±0.04 (-52%)	18.9 (-52%)	27.7[a] ±3.8 (-49%)	0.57[a] ±0.08 (-45%)	32.5 (-45%)
Maize+baobab	33.6[d] ±1.3 (19%)	0.67[bc] ±0.03 (16%)	46.2 (16%)	54.3[c] ±1.6	0.96[c] ±0.03 (-7%)	55.0 (-7%)
Maize+ascorbic+citric acid[1]	36.0[e] ±1.8 (27%)	0.74[c] ±0.04 (27%)	50.5 (27%)	58.2[d] ±4.1	1.10[d] ±0.08	63.0
Maize+$FeSO_4$[2]	17.5[b] ±3.1 (-38%)	1.19[d] ±0.22 (104%)	81.2 (104%)	37.3[b] ±2.6 (-33%)	0.71[b] ±0.05 (-31%)	40.4 (-31%)
Experiment 2 (Maize porridge containing carrot+mango (as provitamin A source) FtFF with moringa[5] and/or baobab)						
Maize+$FeSO_4$+carrot+mango (Control)	15.7[c] ±0.3	1.73[c] ±0.04	118.5	37.5[a] ±0.7	0.50[a] ±0.01	28.5
Maize+$FeSO_4$+ascorbic acid+carrot+mango	30.4[d] ±0.7 (93%)	3.34[d] ± 0.08 (93%)	228.9 (93%)	40.9[a] ± 1.6	0.54[a] ±0.02	31.1
Maize+moringa+carrot+mango	3.2[a] ±0.3 (-80%)	0.37[a] ±0.03 (-78%)	25.2 (-78%)	39.6[a] ±9.0	0.68[b] ±0.15 (36%)	38.9 (36%)
Maize+$FeSO_4$+baobab+carrot+mango	32.5[e] ±2.0 (107%)	3.66[e] ±0.22 (111%)	250.6 (111%)	54.3[b] ±1.1 (45%)	0.80[c] ±0.02 (60%)	45.7 (60%)
Maize+moringa+baobab+carrot+mango	4.71[b] ±0.31 (-70%)	0.56[b] ±0.04 (-68%)	38.4 (-68%)	49.1[b] ±6.6 (31%)	0.91[d] ±0.12 (83%)	52.1 (83%)

Values are the mean ±1 SD of two independent dialysability assay experiments with the intestinal step being each time performed in triplicate and each dialysate analysed in duplicate (n=12). Means with different superscripts letters in a column differ significantly ($p<0.05$)

[1]WHO and FAO (2004), [2]Sandstead (2015), [3]Percentage values in brackets are significantly different to the control maize porridge by pairwise comparison ($p<0.05$)

[4]MorNutri moringa, [5]MorNutri:SupaNutri 1:1 ratio