Iron Bioavailability of Fortified Maize and Sorghum Porridges

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Abstract

In sub-Saharan Africa, iron deficiency anemia poses a significant public health problem, negatively affecting the health and development of millions of young women and children. One strategy to help reduce this problem is via fortification of commonly consumed food products made from grains such as maize or sorghum. Using the established Caco-2 cell bioassay for Fe bioavailability, the present study tested whether varying levels of iron and ascorbic acid added to flavored whole grain maize and sorghum porridges deliver bioavailable iron. The results demonstrated that the fortified iron in maize and sorghum porridge improved these foods as sources of dietary Fe, with the effect much stronger in maize than sorghum porridge. Consistent consumption of these foods could therefore improve the Fe status and alleviate the risk of iron deficiency anemia of those who consume them.

Keywords: Maize; Sorghum; Sorghum bicolor; Zea mays; Porridge; Iron; Bioavailability; Caco-2; In vitro digestion; Bioassay; Bio fortification

Introduction

Micronutrient malnutrition is a major health problem affecting billions of people worldwide. It is especially prevalent in Sub-Saharan Africa, with over a third of the region's population suffering from vitamin and mineral deficiencies [1]. Certain food staples in this region, such as cereal porridges, tend to be nutrient-poor and their regular consumption may give rise to micronutrient malnutrition, especially amongst vulnerable populations [2]. Micronutrient malnutrition can affect key development outcomes, such as poor physical and mental development in children, increased vulnerability to disease and illnesses, as well as general losses in societal productivity [1, 3].

In particular, iron deficiency anemia (IDA), a pernicious and widespread disorder, is often a direct result of insufficient intake of bioavailable iron (Fe). IDA is especially common amongst pregnant women, women of reproductive age, and children and often leads to losses in work productivity, low birth weight, increased child and maternal mortality, and stunted childhood development [3]. A primary cause of IDA is low intake of bioavailable Fe in the diet. Poor dietary Fe bioavailability is often due to non-diverse, monotonous diets based largely on staple food crops such as maize, rice, sorghum and wheat. Such foods are commonly high in inhibitors of Fe bioavailability such as phytate and polyphenolic compounds [4]. These risk factors are exacerbated by periods of life when daily iron requirements are higher, namely, during periods of growth, pregnancy, lactation and menstruation [4].

Iron fortification can be a cost-effective means to reduce the prevalence of IDA provided consideration is given to technological issues such as the Fe fortificant, food matrix, food preparation and flavorings that may impact Fe absorption; and the food is palatable and consumed in adequate quantities by consumers [4].

Given the above, the primary objective of the present study was to conduct an in vitro Caco-2 experiment of fortified whole grain maize and sorghum porridges intended for commercial use in Sub-Saharan Africa to confirm the products provide bioavailable Fe.

The study was designed to assess iron bioavailability at varying molar ratios of ascorbic acid (AA) to Fe (AA:Fe), considering impact of overages and cooking, at quantities presumed to have minimal, if any, effect on the organoleptic quality of the cereals.

Materials and Methods

Samples Source and Preparation

The porridges were made from three flavoured extruded cereals containing:

1. whole grain white maize (vanilla and chocolate)
2. whole grain yellow maize (vanilla and chocolate)
3. whole grain sorghum (vanilla and chocolate)

All cell culture, sample analyses and formulations were conducted in the Glahn Lab at the Robert Holley Center for Agriculture and Health, Ithaca, NY.
two flavours) contained 11% and 18.5% of Codex’s Nutrient Reference Values (NRV) for iron (as NaFeEDTA) and 15% of the NRV for zinc (as chloride), vitamin B12, and folic acid per serving size (50 g dry weight). Thus, amounts for each 50 g serving size were 1.5 mg and 2.6 mg iron, respectively, 1.7 mg zinc, 36 µg DFE folate, and 0.36 µg vitamin B12. As per the experimental protocol, AA was added at 15 mg, 20 mg and 30 mg per serving.

The above formulations resulted in AA:Fe molar ratios ranging from 1.19 to 4.08 depending on the cereal. Due to the presence of intrinsic Fe in each cereal, AA:Fe values were calculated for fortified Fe and fortified Fe plus intrinsic Fe.

All of the samples, except what is noted below, were tested on an ‘as prepared’ basis using cooking methods described on product labels, before conducting the in vitro bioavailability assay. Because cooking (adding boiling water) is known to oxidize AA, two samples of sorghum were tested on an ‘as is’ basis (dry cereal) using the same dose of AA:Fe (see Table 1) to determine if cooking causes a significant loss of AA and thus affects Fe bioavailability.

### Table 1: Iron concentration and molar ratios of AA:Fe in maize and sorghum cereals

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Cereal Fe* (µg/g)</th>
<th>Added Fe (% NRV)</th>
<th>Added AA (% NRV)</th>
<th>Molar Ratio (AA:Fe) with only added AA and Fe</th>
<th>Molar Ratio (AA:Fe) with Total Fe</th>
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</thead>
<tbody>
<tr>
<td>White maize</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>----</td>
<td>----</td>
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<td>11</td>
<td>0</td>
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<td>----</td>
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<td>11</td>
<td>15</td>
<td>3.06</td>
<td>1.19</td>
</tr>
<tr>
<td>White maize</td>
<td>49</td>
<td>11</td>
<td>20</td>
<td>4.08</td>
<td>1.58</td>
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<tr>
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<td>18.5</td>
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<td>2.43</td>
<td>1.25</td>
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<tr>
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<td>1.88</td>
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<tr>
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<td>11</td>
<td>0</td>
<td>----</td>
<td>----</td>
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<tr>
<td>Yellow maize</td>
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<td>11</td>
<td>15</td>
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<td>2.29</td>
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<td>----</td>
</tr>
<tr>
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<td>35</td>
<td>11</td>
<td>0</td>
<td>----</td>
<td>----</td>
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<td>15</td>
<td>3.06</td>
<td>1.44</td>
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<tr>
<td>Sorghum (uncooked)</td>
<td>35</td>
<td>11</td>
<td>15</td>
<td>3.06</td>
<td>1.44</td>
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<tr>
<td>Sorghum (uncooked)</td>
<td>35</td>
<td>18.5</td>
<td>20</td>
<td>2.43</td>
<td>1.46</td>
</tr>
</tbody>
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### Iron Content Analysis

Dried, ground food samples (0.5 g) were treated with 3.0 mL of 60:40 HNO3 and HClO4 mixture in a Pyrex glass tube and left overnight to destroy organic matter. The mixture was then heated to 120 °C for two hours and 0.25 mL of 40 µg/g Yttrium added as an internal standard to compensate for any drift during the subsequent inductively coupled plasma atomic emission spectrometer (ICP-AES) analysis. The temperature of the heating block was then raised to 145 °C for 2 h. If necessary, more nitric acid (1–2 mL) was added to destroy the brownish color of the organic matter. Then, the temperature of the heating block raised to 190 °C for ten minutes and turned off. The cooled samples in the tubes were then diluted to 20 mL, vortexed and transferred onto auto sampler tubes to analyze via ICP-AES. The model of the ICP used was aThermo iCAP 6500 series (Thermo Jarrell Ash Corp., Franklin, MA, USA). For the measurement of 58Fe isotopes in Caco-2 cells, cell isolates were treated as described above, and 58Fe was quantified using inductively coupled plasma mass spectrometry (Agilent Model 7500CS, Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA 95051).

### Iron Bioavailability Analysis

The in vitro digestion protocol was conducted as per an established, highly validated in vitro digestion model [6, 7]. Briefly, exactly 1 g of each sample was used for each sample digestion. To initiate the gastric phase of digestion, 10 mL of fresh
saline solution (0.9% sodium chloride) was added to each sample and mixed. The pH was then adjusted to 2.0 with 1.0mol/L HCl, and 0.5ml of the pepsin solution (containing 1 g pepsin per 50 mL; certified > 250 U per mg protein; Sigma #P7000) was added to each mixture. The mixtures were under gastric digestion for 1 h at 37 °C on a rocking platform (model RP-50, Laboratory Instrument, Rockville, MD) located in an incubator. After 1 h of gastric digestion, the pH of the sample mixture was raised to 5.5–6.0 with 1.0mol/L of NaHCO3 solution. 2.5 mL of the pancreatic bile extract solution was added to each mixture. The pancreatic bile extract solution contained 0.35 g pancreatin (Sigma #P1750) and 2.1 g bile extract (Sigma #B8631) in a total volume of 245 mL. The pH of the mixture was then adjusted to approximately 7.0, and the final volume of each mixture was adjusted to 15.0 mL by weight using a salt solution of 140 mmol/L of NaCl and 5.0 mmol/L of KCl at pH 6.7. At this point, the mixture was referred to as a "digest". The samples were then incubated for an additional two hours at 37 °C, at which point the digest were centrifuged, and supernatants and pellet fractions collected and transferred to tubes for analysis. Three independent replications of the in vitro digestion procedure were carried out for all of the food samples. For some samples, as noted in the specific results section, Fe bioavailability was assessed in both the presence and absence of AA. The AA was added to the digests at the start of the gastric digestion phase at a concentration of 10 µmol/L. This treatment has been shown to expose some additional differences between samples and thus provides further information on the matrix of the digest.

**Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis of Polyphenols**

For polyphenol extraction, 5 mL of methanol:water (50:50 v/v) was added to either 500 mg of maize or sorghum, and vortexed for one minute before incubating in a sonicating water bath for 20 minutes at room temperature. Samples were again vortexed and placed on a compact digital Rocker (Labnet International, Inc., Edison, NJ, USA) at room temperature for 60 minutes before centrifuging at 4,000 g for 15 minutes. Supernatants were filtered with a 0.2 µm Teflon™ syringe filter and stored at -20°C until chemical analysis.

Extracts and standards were analyzed by an Agilent 1220 Infinity Liquid Chromatography (LC; Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to an Advion expressL® compact mass spectrometer (CMS; Advion Inc., Ithaca, NY, USA). Two-µL samples were injected and passed through an Acquity™ UPLC BEH Shield RP18 1.7 µm 2.1 x 100 mm column (Waters, Milford, MA, USA) at 0.35 mL/minute. The mobile phase consisted of ultra-pure water with 0.10 % formic acid (solvent A, hereby referred to as "A") and acetonitrile with 0.10 % formic acid (solvent B). Polyphenols were eluted using linear gradients of 86.7 to 77.0% A in 0.50 minutes, 77.0 to 46.0% A in 5.50 minutes, 46.0 to 0% A in 0.50 minutes, held at 0% A for 3.50 minutes, 0 to 86.7% A in 0.50 minutes, and held at 86.7% A for 3.50 minutes for a total run time of 14 minutes. From the column, flow was directed into a variable wavelength UV detector set at 278 nm. Flow was then directed into the source of an Advion expressL® CMS, and electro spray ionization (ESI) mass spectrometry was performed in negative ionization mode using selected ion monitoring with a scan time of 50 milliseconds for the 18 polyphenol masses of interest. Capillary voltage and temperatures were 300°C and 100 volts, respectively. ESI source voltage and gas temperature were 2.6 kilovolts and 240°C respectively. Desolvation gas flow was 240 L/hour. Advion Mass Express™ software was used to control the LC and CMS instrumentation and data acquisition. Individual polyphenols were identified and confirmed by comparison of MS and LC retention times with authentic standards. Polyphenol standard curves for flavonoids were derived from integrated areas under UV absorption peaks from 8 replications. Standard curves for catechin and 3,4-dihydroxybenzoic acid were constructed from MS ion intensities using 8 replications.

**Phytate Analysis**

For phytate (phytic acid) determination, a 500 mg sample from each cereal was first extracted in 10 mL of 0.66 M hydrochloric acid under constant motion for 16 hours at room temperature. A 1 mL aliquot of total extract was collected using a wide bore Pipet tip, and then centrifuged (16,000 g) for 10 minutes to pellet debris. A 0.5 mL sample of supernatant was then neutralized with 0.5 mL 0.75 M sodium hydroxide and stored at -20°C until the day of analysis. A phytate/total phosphorus kit (K-PHYT; Megazyme International, Ireland) was used to measure liberated phosphorus by phytase and alkaline phosphatase. Phosphorous was quantified by colorimetric analysis as molybdenum blue with phosphorus standards read at a wavelength of 655 nm against the absorbance of a reagent blank. Total phytate concentrations were calculated with Mega-Calc™ by subtracting free phosphate concentrations in the extracts from the total amount of phosphorous that is exclusively released after enzymatic digestion.

Phytate levels in the cereals were as follows: white maize, 0.65 g/100g; yellow maize 0.60 g/100g; and sorghum 0.66 g/100g.

**Statistical Analysis**

Data were analyzed using the software package GraphPad Prism (GraphPad Software, San Diego, CA). Data were analyzed using analysis of variance incorporating normalization of variance, if needed, and Tukey’s post test to determine significant differences (p < 0.05) between groups. Unless noted otherwise, values are expressed as mean ± standard deviation (SD); n = 3 independent replications.

**Results and Discussion**

As expected for both the white and yellow maize, the presence of added Fe at 11% NRV and above clearly resulted in more Fe uptake from the porridge, which was further enhanced by the addition of AA. Such results are to be expected as it is widely known that AA can offset the inhibitors of Fe uptake present in maize [10].

For sorghum, similar effects were observed; however, the maximal effect appears to be slightly less. This also is not surprising as sorghum may possess substantially more inhibitors of Fe bioavailability such as polyphenolic compounds [11]. Maize on the other hand, has little to no polyphenols; however, the germ fraction has recently been identified as an inhibitor of Fe uptake from whole grain maize [12]. Phytic acid is suspected to be the Fe uptake inhibitor present in the germ fraction as it is the storage form of phosphorous and thus essential for growth and development of the maize plant. The same may also be true for sorghum, however to our knowledge this has not been reported. Use of NaFeEDTA as the source of Fe is likely to be the best choice for the form of fortified Fe as the EDTA helps to offset the potential inhibitory effects of these compounds and other ingredients of the food matrix [13]. The presumed mechanism for this benefit is that the EDTA complexes the Fe, maintaining solubility yet allows exchange of the Fe with the Fe transporter on the surface of the intestinal epithelial cell.

Of course, other considerations for this product, such as flavor, shelf life, etc. could influence the final selection of the AA: Fe fortification level. In addition, the level of intrinsic Fe also influences this molar ratio. As shown in Table 1, the ratio decreases substantially when the intrinsic Fe is accounted for and thus should be factored into the formulation if a targeted minimal AA: Fe is desired. It should be noted that the white maize cereal was relatively high in intrinsic Fe concentration [12]. Such levels can be monitored when maize cereal batches are procured, and if abnormally high then contamination of Fe is likely due to soil or dust. Such contamination can influence Fe bioavailability [14].

The flavoring does not appear to have any effect on the Fe bioavailability. It was noted during the phytate extraction and analyses that the extract was slightly cloudy for both maize and the sorghum. Normally these extracts are clear, as they were in the controls for the assay. Perhaps this was due to the flavoring.

Overall, the results of the present study indicate that levels of 11% and 18.5% NRV Fe, and the associated AA levels, in the fortified whole grain maize and sorghum cereals would be a significant source of dietary Fe.

Phytate to Fe ratios were high in the unfortified cereals ranging from 11-17 phytate relative to the intrinsic Fe which is common in maize and sorghum. Generally, any ratio above 10:1 phytate to Fe has the maximal inhibitory effect on iron absorption. The added ascorbate and Fe lowered these ratios to the range of approximately half of the above.

**Conclusion**

To summarize, the Fe and ascorbate fortification clearly improve the nutritional quality of the cereals. The Caco-2 cell Fe uptake is quite high in the fortified products. If consumed on a regular basis, it would be expected that the product formulated at the lowest level of added Fe will improve Fe nutrition and help contribute to alleviation of Fe deficiency.

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**Conflict of Interest and Funding Disclosure**

Conflicts of Interest: Lisa Fleige is an employee of PepsiCo, Inc, which manufactures cereal products under the brand name Quaker Oats®

**Ethical Approval**

N/A

**References**


