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# Bioavailability of iron, zinc, folic acid, and vitamin A from fortified maize

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**Several strategies appear suitable to improve iron and zinc bioavailability from fortified maize, and fortification per se will increase the intake of bioavailable iron and zinc. Corn masa flour or whole maize should be fortified with sodium iron ethylenediaminetetraacetate (NaFeEDTA), ferrous fumarate, or ferrous sulfate, and degermed corn flour should be fortified with ferrous sulfate or ferrous fumarate. The choice of zinc fortificant appears to have a limited impact on zinc bioavailability. Phytic acid is a major inhibitor of both iron and zinc absorption. Degermination at the mill will reduce phytic acid content, and degermed maize appears to be a suitable vehicle for iron and zinc fortification. Enzymatic phytate degradation may be a suitable home-based technique to enhance the bioavailability of iron and zinc from fortified maize. Bioavailability experiments with low phytic acid–containing maize varieties have suggested an improved zinc bioavailability compared to wild-type counterparts. The bioavailability of folic acid from maize porridge was reported to be slightly higher than from baked wheat bread. The bioavailability of vitamin A provided as encapsulated retinyl esters is generally high and is typically not strongly influenced by the food matrix, but has not been fully investigated in maize.**

**Keywords:** maize; bioavailability; iron; zinc; phytic acid

## Introduction

Effective fortification programs aim to improve the bioavailable nutrient intake of the whole population, with the intent of eliminating or preventing micronutrient deficiencies in the general population and the most vulnerable groups. Being widely consumed, maize flour is a suitable vehicle for mass fortification.[1] It serves as a major staple food in several African and Latin American countries[2] and may be fortified with one or more micronutrients, such iron, zinc, folic acid, and/or vitamin A. As for other mass fortification programs, fortification of centrally processed maize flour on a large scale may not reach population groups not having access to processed foods.

Bioavailability refers to the amount of ingested micronutrient absorbed and utilized in the body and can be influenced by the chemical form of the fortificant, the nutrient composition of the fortification premix, the dietary composition and food matrix, the overall dietary intake, and the physiological state of target individuals. A fortification program therefore needs to be adjusted to the consumption pattern and composition of the staple food in question, as well as the regional diet and individual factors in the target population. This article reviews the properties of added fortificants to maize flour and the dietary and individual factors affecting the nutrient bioavailability of added iron, zinc, folic acid, and vitamin A.

## Maize flour fortification

Major maize-consuming countries are located in Central America and Sub-Saharan Africa, and Egypt is also a major maize-consuming country. Large-scale produced maize flour is degermed and sold as flour or grits, and can be precooked.[3] In several countries, maize flour is usually consumed as gruel or porridge, and in certain settings, a fermentation,

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**Table 1.** Overview of countries fortifying maize flour under mandatory legislation (adopted from FFI country profiles)

| Country | Iron fortificant | Amount added Fe (ppm) | Zinc (ppm) | Folic acid (ppm) | Vit. A (IU/kg) | Vit. B12 (ppm) | Estimated maize flour intake g/day |
|---|---|---|---|---|---|---|---|
| Brazil | Ferrous fumarate | 42 | | 1.5 | | | 69 |
| Costa Rica | Unknown | 22 | | 1.3 | | | 15 |
| Kenya | NaFeEDTA | 5 | 30 | 0.5 | 0.2 | 0.002 | 219 |
| Mexico | Ferrous sulfate | 40 | 40 | 2 | | | 337 |
| Nigeria | Unknown | 16 | 20 | | 9 | | |
| South Africa | Electrolytic Fe | 35 | 15 | 2 | 2.1 | | 284 |
| Tanzania | NaFeEDTA | 10 | 30 | 1.5 | | 0.005 | 160 |
| Uganda | NaFeEDTA | 40 | 30 | 0.5 | 0.5 | 0.003 | 66 |
| Venezuela | Ferrous fumarate | 50 | | | 2.8 | | 160 |
| USA | Unknown | 28.6 | | 1.5 | | | |

soaking, or germination step is added. Considering that these traditional treatments of maize flour occur at the household level, effective fortification of maize flour can be challenging as centrally processed fortified flour is not always available. In Mexico and Central America, maize is often first cooked with lime (calcium hydroxide) or ash (potassium hydroxide) in the nixtamalization process, before the dough is baked into tortillas or is dried to yield masa flour.[3] The nixtamalization process typically uses whole maize; only the pericarp of the grain is removed, using an alkali process.[4] Maize flour is fortified in several countries and Table 1 provides an overview of countries currently adding one or more nutrients to maize flour under mandatory legislation.[2] Micronutrients that may be added to maize flour include iron, zinc, folic acid, vitamin A, and vitamin B12, as well as vitamins B1, B2, B3, B6, and vitamin D, if public health issues for those micronutrients have been found.[5] Fortification of corn masa flour with iron and other micronutrients has been extensively reviewed by Sustain in 1997,[6] and will be discussed in the nixtamalization section below.

### *Overview of existing recommendations for maize flour fortification*

In 2002, the Institute of Nutrition of Central America and Panama/Pan American Health Organization (INCAP/PAHO)[7] published guidelines for Latin America and the Caribbean, featuring recommended iron compounds for the fortification of wheat and maize flour. The comprehensive World Health Organization (WHO) guidelines published in 2006 on food fortification with micronutrients[1] included the following recommendation for maize flour iron fortification: the recommended iron compounds for high-extraction or nixtamalized maize flour (corn masa flour) are NaFeEDTA at 1× the recommended fortification level. Ferrous fumarate, ferrous sulfate, or encapsulated versions thereof have to be fortified at 2× the recommended fortification level. The recommendation differs for low-extraction maize flour, where the recommended iron compounds are ferrous sulfate, ferrous fumarate, or encapsulated versions to be fortified at 1× the recommended fortification level, whereas electrolytic iron is to be added at 2× the recommended fortification level. Furthermore, the recommendations suggest that each country should estimate the level of fortification that would provide the required iron that is lacking in the common diet. The recommended vitamin A fortificants for cereal flours are dry stabilized forms of retinyl acetate and retinyl palmitate, which have shown satisfactory stability[1] and should be used if vegetable oil is not already fortified and the population is considered at risk of vitamin A deficiency.

In 2008, the Second Technical Workshop on Wheat Flour Fortification, held in Stone Mountain, Georgia, USA, proposed the average levels of nutrients for iron,[8] vitamin A,[9] folic acid,[10] vitamin B12[11] (not part of this review), and zinc[12] to be added to fortified flour on the basis of extraction rate, fortificant, and estimated per capita flour consumption.[13] While a detailed evaluation for maize flour was not included in the different working groups of the technical workshop, a recommendation on fortification levels was given for wheat flour on the basis of the estimated per capita daily consumption, which could likely be generalized to maize fortification with limitations for iron and zinc, as there is much less experience in fortifying maize flours with iron, and the sensory stability may differ between the two staple foods.[14] However, similar considerations apply and previous recommendations for iron[7] are valid.

## Bioavailability of fortificants used for maize flour fortification

Micronutrients affect nutritional status only to the extent by which they are available for absorption through the gastrointestinal tract and for systemic utilization. For this reason, the bioavailability of a fortified micronutrient is a key determinant to successful food fortification and describes the fractional amount of micronutrient absorbed and utilized.

## Iron bioavailability from fortified maize

### *Iron absorption and bioavailability*

The mechanisms of iron absorption are reviewed elsewhere.[15] A major fraction of the iron consumed in the diet is nonheme iron, which typically has variable bioavailability, being influenced by a range of dietary enhancers (organic acids, ascorbic acid, and muscle protein) and inhibitors (phytic acid, polyphenols, and calcium).[16] Monotonous plant-based diets do not contain significant amounts of heme iron or absorption enhancers, and contribute therefore to low dietary iron bioavailability and a high prevalence of iron deficiency.[15]

The contribution of fortified and native nonheme iron to daily iron needs is primarily determined by its solubility in the gastric contents, the composition of the diet, the iron status and physiological condition (infection or inflammation) of the individual. Only iron that is soluble in the gastric juice can enter the common nonheme iron pool.[17] Iron compounds have therefore been classified according to their solubility in water and dilute acid. To compare the different iron compounds, the relative bioavailability is used as a critical measure to judge an iron fortification compound in relation to water-soluble ferrous sulfate, which has by definition a relative bioavailability of 100%.[18]

### *Iron fortificants used for maize flour fortification*

The most bioavailable iron fortificants are compounds with the highest solubility in the human proximal intestine. However, owing to their chemical and physical properties, water-soluble iron compounds tend to react with the food matrix, causing adverse changes during storage. To achieve an impact equivalent to that of highly bioavailable fortificants, larger amounts of lower bioavailability fortificants are used.

NaFeEDTA is an iron fortificant and is both water-soluble and iron absorption enhancing, which is related to its chelating properties.[19] In high-phytate cereal diets, NaFeEDTA was shown to have two- to three-fold higher absorption than ferrous sulfate,[20] whereas in meals prepared with low-extraction flours, it had similar bioavailability to ferrous sulfate.[21] According to the current guidelines for maize flour fortification,[13] NaFeEDTA is the first-choice fortificant for whole-grain maize flour and corn masa flour.

Ferrous sulfate is a water-soluble iron compound that is widely used with many fortification vehicles. Because it is highly water soluble, sensory changes can occur, including increased rancidity, color changes, and off-flavors during storage. It is the recommended compound for degermed maize flour and flours with a short shelf life. Ferrous fumarate, which is poorly soluble in water and tends to cause fewer sensory changes than ferrous sulfate, has also been used for maize flour fortification (for example, in Venezuela). Studies investigating the absorption of ferrous fumarate have reported a similar absorption to ferrous sulfate in adults, but a decreased relative bioavailability in preschool children.[22] However, this could not be confirmed in women, preschool children, or infants in a recently published study that found the relative bioavailability of ferrous fumarate to be close to that of ferrous sulfate.[23] The differences between the studies were mainly ascribed to the differences in the iron status of the participating subjects and the presence of ascorbic acid, both known to enhance differences in bioavailability between water-soluble and insoluble iron compounds.[24] In maize fortification, which does not foresee cofortification with ascorbic acid and which targets the general population, the differences in bioavailability between ferrous fumarate and ferrous sulfate may be small.[23] It cannot be fully ruled out, however, that ferrous sulfate may have higher bioavailability than ferrous fumarate in infants and young children that are iron deficient.[23]

The heterogeneous group of elemental iron powders is widely used in food fortification, as it does not cause sensory changes. Several reviews investigated the potential use of elemental iron powders;[25,26] however, only electrolytic iron has been recommended as a potentially effective fortificant, albeit with relatively low bioavailability.

A further potential class of iron fortification compounds are amino acid chelates, such as ferrous bisglycinate, which has been shown to have superior bioavailability over ferrous sulfate but inferior bioavailability over NaFeEDTA in cooked maize flour.[27] Ferrous bis-glycine chelate, however, was shown to induce organoleptic changes upon storage in maize flour.[28] In contrast, ferric trisglycinate caused limited organoleptic changes, but its bioavailability has not been as comprehensively characterized. In one study, ferric trisglycinate was added to a maize porridge given to young adults with a mean serum ferritin of 8 μg/L (therefore, on average, iron deficient)[29] and resulted in an iron absorption of only 2.3%.

### Dietary factors influencing iron absorption from fortified maize flour

Several dietary components are known to affect the iron bioavailability of fortified maize flour. The main iron absorption inhibitors in maize are phytic acid in the germ of the maize kernel[30] as well as calcium from lime used to treat maize flour in some forms of the nixtamalization process. The antinutritional effect of the phytic acid in maize flour is due to the complexing action of phytate on divalent and trivalent metal cations.[31] Calcium may compete with zinc, manganese, copper, and iron for absorption in the intestine.[32] The main factors enhancing iron absorption are the addition of ascorbic acid and/or EDTA and the degradation of phytic acid.[18]

### Phytic acid

At the pH in the stomach and intestine, phytic acid is mostly present as phytate (myo-inositol hexakisphosphate (IP6)), mainly in the form of a mixed salt of calcium/magnesium/potassium. IP6 is a strong chelator of divalent and trivalent metal cations, such as iron, zinc, calcium, manganese, and magnesium. Cereals, such as whole-maize flour, contain 1–2% phytic acid.[33] It is well known that absorption of native and fortification nonheme iron is strongly inhibited by IP6 in a dose-dependent manner, which is in direct relation to the molar ratio of phytic acid to iron.[34] Phytic acid also decreases the absorption of other essential nutrients, including zinc and calcium.[35] Mineral-phytate complexes have a very low solubility under the pH conditions of the upper gastrointestinal tract, where most minerals are absorbed.[36]

Phytic acid levels in whole maize are higher (7.2–22.2 mg/g dry weight, DW) than those in wheat (3.9–13.5 mg/g DW) or rice (0.6–10.8 mg/g DW), and are similar to the levels found in sorghum (5.7–33.5 mg/g DW) and wild rice (22.0 mg/g DW).[33] Phytic acid localization in maize differs from most other cereals as it is concentrated in the germ: in a whole maize kernel containing 8.9–9.6 mg phytic acid/g, the germ contained nearly 60% of the total phytic acid content (5.7–6.4 mg/g), while little phytic acid is present in the endosperm (0.4 mg/g) and hull (0.7–2.5 mg/g).[30] The milling (degermination) process is expected to remove a large portion of the phytic acid, a process that, in some countries such as South Africa and in North America and Europe, is applied to a large proportion of the maize consumed, notably in industrially processed maize, and is related to increases in shelf life. Whole-maize flour, in contrast, was reported to contain 9.8–21.3 mg phytic acid/g dry matter, while degermed maize is reported to contain negligible amounts of phytic acid.[37] Corn flakes, produced mostly with degermed cooked maize, contain 0.4–1.5 mg phytic acid/g dry matter.[38]

Genetically modified maize cultivars with low phytic acid content have been studied as potential vehicles for iron fortification. In a study by Mendoza *et al.*, the iron fortification compound was the greatest determinant of iron bioavailability, irrespective of the maize phytic acid content, likely because the phytic acid reduction was not sufficient to meaningfully affect iron bioavailability between the two maize cultivars.[39]

Different methods of processing wild-type maize, such as soaking, germination, and rinsing, have been investigated for reducing the whole-maize phytate content and phytate-to-mineral molar ratio.[40]

### *Nixtamalization*

The cooking of maize with calcium-containing lime is the first step in the production of several maize products (such as tortillas or masa) and shows several nutritional benefits, including improved niacin bioavailability.[41] Fortification with iron appears challenging: the acceptability to consumers and millers of iron fortification with water-soluble products (ferrous sulfate and ferrous fumarate) was recently investigated, and the authors reported that despite the objective difference in color between unfortified and fortified products, this difference did not result in lower hedonic scores in a consumer panel.[42,43] The same authors reported that fortification with elemental iron and ferric pyrophosphate resulted in no discoloration,[44] but the bioavailability of these compounds is known to be low, in particular in products high in phytic acid, such as corn masa flour, where they are generally not recommended.[7] The nixtamalization process does only marginally reduce (by approximately 20%) the phytic acid content in the maize kernels;[45] this proportion is not likely to enhance iron bioavailability significantly. Calcium content increases with the addition of lime, resulting in calcium contents of at least 100 mg/100 g of maize flour.[46] In single-meal studies in phytic acid–free wheat rolls, calcium inhibited iron absorption from 3.5 mg ferrous sulfate by 20–60% at calcium levels of 75–160 mg. This would suggest a relevant inhibition of calcium on iron bioavailability if corn tortillas are consumed alone and fortified with ferrous fumarate or ferrous sulfate. However, in a study in maize fortified with NaFeEDTA and 60 mg ascorbic acid, no significant effects of the addition of 200 mg calcium on iron bioavailability were detected,[47] suggesting another reason for the superiority of NaFeEDTA for fortification of corn masa flour. It has been suggested that the effect of calcium on iron bioavailability is overestimated from single-meal studies with multiple enhancers and inhibitors present, compared to multiple-meal studies where calcium will only have a small effect on iron absorption.[32,48]

### *Iron absorption enhancers*

**Ascorbic acid.** Ascorbic acid is a well-described enhancer of iron absorption, and an ascorbic acid:iron molar ratio of at least 2:1 is recommended, while in phytic acid–rich foods, a molar ratio of 4:1 is recommended,[20] as this has been shown to increase iron bioavailability by at least twofold in single-meal studies. A 40-mg ascorbic acid dose in an orange juice serving was reported to increase endogenous iron bioavailability from unfortified maize meals by threefold,[49] at an ascorbic acid to Fe ratio of 15–17:1, as the maize in this test meal was not fortified. The addition of ascorbic acid after processing at households can be an effective means of enhancing iron bioavailability from maize. Point-of-use fortification with ascorbic acid is practiced with micronutrient powders (MNP).[47]

**EDTA.** EDTA can be added as an absorption enhancer to iron-fortified foods, and EDTA:Fe molar ratios such as 0.3:1 have been reported to enhance iron bioavailability from wheat-based breakfast meals by approximately 20%. Increasing the EDTA:Fe molar ratio to 0.7:1 and 1:1 did not appear to enhance bioavailability further.[50] Walter *et al.* reported the enhancing effect of EDTA addition to maize tortillas fortified with ferrous fumarate, improving iron bioavailability by 70% when added at an EDTA: Fe ratio of 2:1.[51]

### *Nutritional effects of phytase during food processing*

Depending on the extraction rate of the flour during milling, the phytic acid and iron contents are significantly decreased. Furthermore, in fermented baked products prepared with low-extraction flours (therefore with a limited native phytic acid level), phytic acid content may be considerably reduced owing to phytase being active during fermentation, increasing iron absorption in bread rolls compared to wheat products processed differently.[52] However, even low amounts of phytic acid can significantly inhibit iron absorption.[53] It has been recommended that the ratio of phytic acid to iron should be reduced to at least 1:1 and, if possible, even below 0.5:1, to achieve a meaningful increase in iron absorption.[1] Removing 86–95% of the total phytic acid from a semi-synthetic test meal by acid washing was found to double iron absorption.[53] Complete dephytinization can be achieved by using native or added phytase during the manufacturing process. No investigation on the fermentation of low-extraction maize on iron absorption was found in the literature, and maize has a lower phytase activity compared to wheat or barley.[54] Hurrell *et al.*[35,55] examined the influence of phytic acid degradation on iron absorption from porridge made from various cereals in healthy adults. Dephytinized cereal porridges were prepared by adding phytase to an aqueous slurry adjusted to pH 5.0–5.5 and holding at 40 °C until all the phytate had been degraded. Dephytinization significantly enhanced iron absorption from maize from 1.8% to 8.9%.

### Point-of-use fortification with phytase

Especially in settings where people do not have access to processed foods and prepare maize-based meals at home from whole maize, the degradation of phytic acid needs to take place at a household level. Phytic acid degradation in a whole maize–based complementary food has been investigated by making use of naturally occurring phytase in whole-grain wheat or rye; 10% wheat or rye was added to 90% whole-grain maize before processing the complementary food.[56,57] Layrisse *et al.* showed that phytase addition improves iron bioavailability by a factor of two from a maize flour–based test meal when directly added, regardless of whether the fortification compound is ferrous sulfate or ferrous bisglycine chelate.[27] In this case, the enzyme remains active during consumption and degrades phytate during its stomach transit time. Similar findings were reported by Troesch *et al.*,[47] where the addition of phytase increased iron absorption from iron sulfate–fortified high-extraction maize (8:1 phytic acid:iron molar ratio) by twofold. Furthermore, the study examined the effect of phytase addition on iron bioavailability from maize with low amounts of added highly bioavailable iron (NaFeEDTA) in combination with ascorbic acid. The addition of phytase increased iron absorption from both NaFeEDTA, and NaFeEDTA plus ascorbic acid. The combined addition of phytase, ascorbic acid, and NaFeEDTA resulted in 7.4% iron absorption, compared with absorption of 1.5% from iron sulfate without ascorbic acid in the same meal. This approach can therefore be an effective means of increasing absorption of low amounts of highly bioavailable iron in maize-based meals and can be used for targeted household fortification.

## Zinc bioavailability from fortified maize

Zinc absorption takes place in the upper part of the intestine (duodenum and jejunum) via two distinct transport processes: a diffusion-mediated nonsaturable transport and a saturable carried-mediated component.[58] The absorptive capacity is not saturated at typical dietary zinc concentrations, and zinc balance is determined by the total daily absorbed zinc (TAZ) and by daily zinc losses, which occur primarily via the gastrointestinal tract (endogenous zinc losses, EZL) and to a lesser extent in the kidneys. Zinc balance is maintained by the regulation of EZL and TAZ, which are both strongly correlated by recent (or daily) zinc intakes.

While a range of food constituents have been described to potentially affect zinc bioavailability,[58] a recent review identified two leading determinants: recent zinc and phytic acid intake from the diet.[12,59] Accordingly, a mathematical model for zinc absorption in humans has been proposed as a function of dietary zinc and phytate intake, which explained 82% of the variability in the human studies included.[60] Recently, the same authors updated the predictive model for zinc bioavailability from foods, including the effect of calcium, iron, and protein, and the model improved to explain 88% of the biological variability.[61] Additional factors that may affect zinc bioavailability from maize in humans are therefore accompanying protein quantity and quality, dietary calcium content, and the presence of organic acids.[58] As opposed to iron bioavailability, however, dietary factors other than phytic acid most likely play a minor role in zinc nutrition.[58]

### Zinc fortification compounds

Zinc compounds commonly used in fortification, such as zinc oxide (water insoluble) and zinc sulfate (water soluble), are unlikely to differ in their bioavailability in zinc-fortified foods.[59] This was repeatedly shown in studies in both adults and children[59] and in zinc-fortified maize tortillas, where zinc bioavailability from zinc oxide and zinc sulfate was not significantly different.[62,63]

Zinc gluconate, mostly used in zinc supplements and MNP, was studied in Ghanaian infants in a maize-based complementary food and was reported to provide adequate bioavailable zinc to infants, when provided at relatively high dosages of 5 or 10 mg/meal. However, the bioavailability of zinc from zinc gluconate was not compared to other zinc fortification forms.[64] Zinc bioavailability from zinc methionine consumed with a higher amount of ascorbic acid (100 mg) and sodium iron EDTA was compared to the bioavailability of zinc sulfate consumed with half the amount of ascorbic acid (50 mg). No significant difference in zinc bioavailability was found between the two formulations, indicating that the effect of the form of zinc used for fortification on zinc bioavailability would be of minor magnitude.[65]

### EDTA

Hotz *et al.* investigated the effect of the addition of EDTA as a means to enhance zinc bioavailability from zinc oxide in maize tortillas served with a complete meal of beans and coffee.[62] The test meals had a phytic acid:zinc molar ratio of 17:1, while the addition of EDTA to the zinc in the tortilla resulted in an EDTA:zinc molar ratio of 0.5:1; this molar ratio was 0.2:1 in the complete meal, an amount that did not result in a significant increase in zinc absorption from zinc oxide in maize tortillas. In a further study on rice flour meals, where the phytic acid:zinc molar ratio was 1:1 and the EDTA:zinc molar ratio was 1.4:1, a significant increase in fractional zinc absorption from added zinc oxide was found,[66] suggesting that an EDTA:zinc molar ratio that is greater than one may enhance zinc absorption. In a recently completed study on maize porridge fortified with zinc sulfate with a phytic acid:zinc molar ratio of 9:1, the addition of EDTA at an EDTA:zinc molar ratio of 1:1 increased the bioavailability of the zinc, while this increase was somewhat lower in an identical maize porridge fortified with zinc oxide. The addition of EDTA:zinc at a higher molar ratio of 2:1, by contrast, decreased zinc bioavailability, indicating the possible adverse effect of a high EDTA level on zinc bioavailability.[67]

### Effect of calcium on zinc bioavailability in the presence of phytic acid

The evidence for an effect of calcium on zinc bioavailability in humans has been conflicting, with some studies indicating an enhancing effect of calcium[68,69] while other studies suggest an inhibitory effect.[70] Miller *et al.*, however, found an improved fit in his predictive model for zinc bioavailability for an algorithm including calcium, iron, and protein intakes. The effect of iron and calcium on zinc bioavailability is suggested to be most pronounced in the presence of phytic acid, as calcium and iron would preferentially bind to phytic acid, having therefore a bioavailability-enhancing effect on zinc.[61] This may be relevant in the case of corn masa flour, where phytic acid remains unchanged in the nixtamalization process, but calcium content is enhanced, therefore potentially increasing zinc (but not iron) bioavailabiltiy. However, more direct evidence from studies in nixtamalized corn with varying calcium and phytic acid content appears to be needed to substantiate this hypothesis further.

### Low phytic acid maize

Maize cultivars with reduced phytic acid content (LPA) have become available and have been investigated as potential vehicles for dietary zinc. In a short-term study of polenta meals, the LPA cultivar was reported to have a phytic acid:zinc molar ratio of 17:1, compared to 36:1 in the wild-type counterpart, and this resulted in a fractional zinc absorption of 30% and 17%, respectively.[71] This indicates the potential benefit of consuming low phytic acid maize cultivars. In contrast to this short-term study, a 10-week study of children in Guatemala investigated zinc absorption from LPA maize as part of the usual diet. While a reduction of 20–30% in phytic acid intake compared to the control groups was estimated, this decrease did not result in improved fractional zinc absorption, most likely because maize only partly contributed to the total phytic acid intake.[72] In summary, LPA maize has been shown to improve zinc bioavailability in controlled settings and would likely constitute a suitable vector for increasing dietary zinc supply and fortification, owing to its lower phytic acid level and favorable phytic acid:zinc molar ratio.

### Effect of enzymatic phytate degradation

Phytate degradation in cereals including maize can be achieved by the addition of exogenous microbial phytase, by adding a natural source of phytase, such as whole wheat or rye,[57] or by traditional methods through a combination of germination, soaking, and fermentation.[40] Much of the evidence relating to zinc bioavailability has been gathered on the enzymatic degradation of phytic acid. In a study of children with rickets in Malawi, soaking and fermentation did not affect phytic acid content and zinc absorption from maize, whereas the addition of phytase enzyme increased zinc bioavailability by twofold.[73] In a metabolic study of Malawian children recovering from tuberculosis and undergoing rapid weight gain, the microbial phytase treatment of a corn soy porridge, which reduced the phytic acid:zinc molar ratio from 30:1 to 7:1, increased fractional zinc absorption from 24% to 41%, but did not affect zinc absorption in healthy children.[74] In the same population, consuming a maize-based diet with an estimated phytic acid:zinc molar ratio of 23:1, EZL have been found to be abnormally high when compared to subjects in Western settings.[74] This was explained either by phytic acid directly binding to endogenous zinc or by tropical enteropathy affecting the zinc losses from the gut mucosa.[74] While these studies clearly indicate the potential for enhancing zinc bioavailability by phytic acid degradation, additional evidence is needed from studies of healthy subjects and of the extent of phytic acid removal required to obtain a nutritionally relevant effect on zinc bioavailability. The effect of direct phytic acid addition to maize test meals was recently compared to the complete dephytinization of maize meals and resulted in a similar twofold increase in zinc bioavailability compared to the non-dephytinized control meal with a phytic acid:zinc molar ratio of 9:1.[75]

## Folic acid bioavailability from fortified maize

Folic acid (pteroylmonoglutamic acid) is the oxidized monoglutamate form of the vitamin, which is used in supplements and food fortification.[76] Folic acid has been reported to undergo limited degradation when added to corn masa flour before baking, which was ascribed to microbial degradation.[77] The bioavailability of folic acid can be defined as the vitamin fraction available for utilization in normal physiological functions and for storage as measured by changes in folate status.[78] Natural folate and folic acid differ in bioavailability, with food folate bioavailability being lower than folic acid.[79] Factors thought to affect bioavailability include the stability of the vitamin before (i.e., during processing) and during ingestion, the food matrix, (micro)nutrient interactions, and folate analogs that may impair bioavailability by inhibiting the coenzymatic function and the interconversion of folate.[80]

Food fortification with folic acid has been shown to be an effective method in the primary prevention of neural tube defects and the improvement of the micronutrient status of populations over time. Unfortified whole maize contains 30 μg/100 g folate, while dehulled, degermed maize contains 10 μg/100 g folate.[81] Lower folate intake levels can be expected in populations where the diet consists of unfortifed wheat, maize, or rice in concomitance with a diet low in folate-rich vegetable and fruits. This may be the case in Central America and many Sub-Saharan African countries, where almost half of the daily energy intake comes from maize flour or maize meal.

There are reported differences in bioavailability from different food matrixes. For example, folate bioavailability from a single dose of 600 mL orange juice (840 μg or 1900 nmol) was only 30% compared with an oral supplement of folic acid.[82] There is increasing experience in folate fortification of maize flour[83] and products produced from maize. In 1975, Colman *et al.* published results from a pilot field trial on the effects of folic acid fortification of maize meal in South Africa. In this short-term study, apparent bioavailability as evaluated by the plasma response of folic acid from fortified maize meal and fortified rice was 50–60% of that of folic acid in aqueous solution, compared to 30–40% for fortified wheat bread. It was further estimated that, in spite of differences in response, an amount of 400 μg of folic acid could be added to the average daily intake of maize meal to provide 200 μg/day of absorbed folic acid.[84]

The rise in red-cell folate concentration induced by supplemental folic acid in tablet form was compared to folic acid–fortified maize or bread.[85] A maize meal providing 500 μg of added folic acid per day has yielded comparable erythrocyte folate values to those obtained with 300 μg/day of folic acid in tablet form,[86] or to ones receiving 900 μg/day of folic acid in bread,[85] indicating approximately 60% and 33% bioavailability of folic acid in the tablet form, respectively. Another small study compared changes in red-cell folate in Irish women taking either folic acid supplements or folic acid–fortified food[87] and found that an increased intake of folic acid either as supplements or as fortified foods was effective in increasing folate status.

## Vitamin A bioavailability from vitamin A–fortified maize

None or hardly any vitamin A is present in maize or its products, with the exception of yellow maize, which contains provitamin A carotenoids.[88] Commercial retinol preparations used for fortification are esterified with palmitic or acetic acid to improve stability. The choice of a vitamin A fortificant is largely governed by the characteristics of the food vehicle.[1] Since vitamin A is fat soluble, it is easily added to fat-based or oily foods. When the food vehicle is either dry (like maize) or a water-based liquid, an encapsulated form of the vitamin is needed. Encapsulated retinyl acetate and retinyl palmitate are the main forms of vitamin A that are available for use as fortificants in cereals such as maize.[1] β-Carotene has been used as a cereal fortificant; however, the conversion and bioavailability of provitamin A carotenoids is lower than that of retinol.[89]

The physical form of vitamin A slightly influences the extent of vitamin A absorption. For example, higher plasma values and lower fecal losses of vitamin A were observed in rats when vitamin A was given as alcohol in an aqueous dispersion or emulsion, rather than as ester in an oily preparation.[90] Comparable differences in fecal losses between aqueous versus oily preparations have been described for healthy infants[90] and adults.[91]

Several studies were conducted to assess the absorption of labeled retinyl esters. Physiological single doses (about 900–1000 μg retinyl equivalents (RE)) of oil-soluble retinyl esters to healthy children were almost completely absorbed (96–99%) and retained by about 80%.[92] No difference was observed between the absorption and retention of retinyl palmitate and acetate in these studies. Although physiological doses of vitamin A as retinyl esters were shown to be almost completely absorbed, bioavailability estimates of retinyl esters in a maize matrix are not available and future research in this area is warranted.

The bioavailability of β-carotene and other provitamin A carotenoids is substantially affected by the plant matrix in which they are embedded and by other meal components, such as added fat and soluble fiber.[93] In contrast, the absorption of retinyl esters is relatively unresponsive to other meal components, such as fat[94] or fiber content. Vitamin E enhanced the absorption of retinyl acetate when given as a single high dose,[95] but the effect of vitamin E as a fortificant in maize products on vitamin A absorption is not known. In a study of healthy women receiving white maize flour porridge fortified with β-carotene or retinyl palmitate before boiling, the postprandial response curves of retinyl palmitate (as a marker for total retinyl esters) in the triacylglycerol-rich lipoprotein fraction were measured after ingestion of porridge.[96] Half of the retinyl palmitate dose (286 μg) was required, compared to β-carotene (527 μg), to achieve the same formation of retinyl esters in triacylglycerol-rich lipoproteins, suggesting a high bioavailability.

## Conclusion

Efficacy-based bioavailability data, including the technical experiences of current maize flour fortification efforts, are necessary to provide guidance to governments and processors on how to implement effective maize flour fortification programs. In particular for iron, a similar evaluation to wheat flour is needed to determine useful and effective fortification levels and fortificants depending on the daily maize flour intake and the estimated iron need in the population. While degermed maize fortification with iron may be similar to wheat flour fortification with respect to product stability, corn masa flour fortification and whole maize fortification is likely more technically challenging.

Nondegermed maize has a high phytic acid content, which reduces the bioavailability of minerals such as iron and zinc. Preferred fortification compounds have been identified for NaFeEDTA for corn masa flour and nondegermed maize, and ferrous fumarate and ferrous sulfate for degermed maize. Zinc oxide and zinc sulfate can be used for zinc fortification, and no significant difference in bioavailability has been reported between the two compounds in fortified maize.

The reduction of phytic acid using endogenous or exogenous phytase can be an effective strategy for the improvement of bioavailability. Several human studies suggest that the direct addition of phytase at the point of consumption can enhance iron and zinc bioavailability from maize-based foods, such as porridge, while smaller effects on iron bioavailability have been reported. The use of low phytic acid maize cultivars has been reported to increase the bioavailability of iron and zinc and may be suitable maize vehicles for fortification.

Bioavailability from folic acid is typically higher than that of natural food folates. Fortification of maize meal provides bioavailable folate and can be seen as an effective and feasible means to increase folic acid status in a population. The main forms used for the vitamin A fortification of maize-based products are encapsulated retinyl acetate and retinyl palmitate. Studies of single oral doses of retinyl esters given to children demonstrate almost complete absorption. Although there are limited studies on vitamin A bioavailability from vitamin A–fortified maize, a high bioavailability is likely.

## Conflicts of interest

M.J.B. and B.H. are employees of DSM, a manufacturer of mineral and vitamin premixes. K.K. is the director of Sight & Life, a humanitarian nutrition think tank of DSM. D.M. declares no conflicts of interest.

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