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Fermentation and Lactic Acid Addition Enhance Iron Bioavailability of Maize

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Maize is one of the most important cereal crops for human consumption, yet it is of concern due to its low iron bioavailability. The objective of this study was to determine the effects of processing on iron bioavailability in common maize products and elucidate better processing techniques for enhancing iron bioavailability. Maize products were processed to represent different processing techniques: heating (porridge), fermentation (ogi), nixtamalization (tortillas), and decortication (arepas). Iron and phytate contents were evaluated. Iron bioavailability was assessed using the Caco-2 cell model. Phytate content of maize products was significantly reduced by decortication (25.6%, $p = 0.003$) and nixtamalization (15%, $p = 0.03$), and iron content was reduced by decortication (29.1%, $p = 0.002$). The relative bioavailability (RBA, compared to 100% bioavailability of porridge with $FeSO_4$) of ogi was significantly higher than that of other products when fortified with $FeSO_4$ ($p < 0.001$) or reduced iron ($p < 0.001$). Addition of lactic acid (6 mg/g of maize) significantly increased iron solubility and increased bioavailability by about 2-fold ($p < 0.01$), especially in tortillas. The consumer panel results showed that lactic acid addition does not significantly affect the organoleptic characteristics of tortillas and arepas ($p = 0.166$ and 0.831, respectively). The results suggest that fermentation, or the addition of small amounts of lactic acid to unfermented maize products, may significantly improve iron bioavailability. Lactic acid addition may be more feasible than the addition of highly bioavailable but expensive fortificants. This approach may be a novel means to increase the iron bioavailability of maize products to reduce the incidence of iron deficiency anemia.

KEYWORDS: Fermentation; nixtamalization; Caco-2 cells; iron bioavailability

INTRODUCTION

Iron deficiency is the most prevalent nutritional deficiency worldwide, affecting over 2 billion people (*1*). Maize ranks as the largest cereal crop produced globally, and the third most important grain for human consumption, with 116 million tons consumed as food in 2003 (*2*). Due to the low iron bioavailability of maize, research is mainly focused on fortification strategies appropriate to maize products (*3*). Little attention, however, has been paid to food matrix effects in maize, except with phytate content. Because of the complexity and variation in maize processing, we can expect changes in the food matrix other than changes in phytate that will affect iron bioavailability.

Fortification is a common practice for increasing iron content of maize products. However, the highly bioavailable iron fortificant, ferrous sulfate ($FeSO_4$), is highly unstable and induces oxidative rancidity; on the other hand, elemental iron is very stable but has low bioavailability. Sodium iron EDTA has been proposed as the most effective fortificant, but its use is minimal due to its high cost (*4*). The fortification of maize with iron has been minimally successful at combating iron deficiency because

of the high level of phytate in maize, which significantly inhibits absorption of both intrinsic and added iron (*5*).

Traditional maize processing and its industrial adaptations are highly diverse and differentiated by cultural preferences. In general, traditional maize processing can be broadly categorized by four main processing techniques: heating, nixtamalization, decortication, and fermentation (*6*). Many of the contradictory results with maize iron fortification studies may be due to the use of products developed through different processing methods, thereby not accounting for strong food matrix effects.

A great level of interest is being given to defining best practices for maize product fortification (*4*); however, only a few studies have focused on the specific effects of processing on iron content and solubility (*7*, *8*), and none to our knowledge have taken a systematic approach comparing the different processes described above across the same starting material, especially including a bioavailability assessment.

The hypothesis of this study is that different processing techniques will affect the iron bioavailability of maize products, and, in particular, fermentation or lactic acid addition may beneficially affect iron bioavailability. The objective of this study is to evaluate the effect of the primary traditional processing techniques for maize, heating, nixtamalization,

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fermentation, and decortication, on phytate and iron content, iron solubility, and iron bioavailability. It is anticipated that by understanding the effects of processing, appropriate techniques can be transferred from one traditional process to another to improve iron bioavailability.

EXPERIMENTAL METHODS

Maize (cv. Northrup King 60-B6, 2004 harvest) was obtained from the Iowa Grain Quality Laboratory and cleaned by manual sorting to remove extraneous material and broken seeds. All chemicals were obtained from Sigma Aldrich (St. Louis, MO) unless otherwise noted. Mass balance technique, conducted in duplicate, was used to determine iron and phytate losses. Deionized water was used throughout the study.

Porridge Preparation (Heat). Maize (200 g) was ground in a coffee grinder to pass a 30-mesh screen (maize meal). The maize meal was mixed with 300 mL of H_2O and heated in a glass pot with stirring until the maize became stiff (porridge).

Arepa Preparation (Decortication). Maize (200 g) was tempered to raise the moisture content to 50%. Bran and germ were separated by using a blunt blade blender and mixed with water, and the floating fraction was collected (bran + germ). The endosperm fraction was run repeatedly through a KitchenAid grinder until smooth (arepa starch). Water was added to make the starch material malleable. Cakes of 50 g (1 cm thick) were formed and dry-fried in a Teflon-coated pan at 200 °C for 5 min per side (arepa).

Tortilla Preparation (Nixtamalization). Calcium hydroxide (400 mL of a 1% solution) was mixed with 200 g of maize in a glass pot. The maize mixture was cooked at 80 °C for 30 min and then left to steep at room temperature for 12 h. The steep liquid was drained, and the maize was rinsed three times with H_2O. The steep and rinse waters were retained for analysis (nejayote). The maize was run through the KitchenAid grinder repeatedly until smooth (masa). Water was added to make the masa malleable. Balls (25 g) were formed, pressed flat in a tortilla press, and dry-fried in a Teflon-coated pan at 200 °C for 2 min per side (tortilla).

Ogi Preparation (Fermentation). Maize (200 g) was steeped in 300 mL of H_2O at 25 °C for 24 h. The maize and steep water were blended in a Waring blender until smooth and then fermented spontaneously over 24 h at 25 °C (ogi, uncooked). The fermented maize was then boiled in a glass pot until stiff (ogi, cooked).

All maize samples were lyophilized, ground in a coffee grinder, passed through a 30-mesh sieve, and stored frozen at −20 °C for further use.

Iron Content. Total iron content in maize products and fractions was measured by a wet ashing method, followed by a colorimetric assay (*9*, *10*). Briefly, 1 g of sample was wet-ashed with nitric acid in a microwave at 250 W until the resulting liquid was clear. The sample was diluted to 10 mL, and the iron concentration was measured colorimetrically using ferrozine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt] as a chromogen in a microplate assay.

Phytate Content. Phytate was measured using anion exchange chromatography (*11*). Samples (1 g) were subjected to extraction with 2.4% HCl, centrifugation, and filtration. Supernatant was mixed with 0.75 mol/L NaOH and a 0.1 mol/L Na_2EDTA solution and run on an AG1-X4, 100−200 mesh, chloride form ion-exchange resin (Bio-Rad, Hercules, CA) in a 1.5 × 30 cm column. Phytate was eluted with a 0.7 mol/L NaCl solution, heat digested with H_2SO_4 and HNO_3, and then reconstituted with H_2O. Phosphorus content was measured using ammonium molybdate and 4-amino-3-hydroxy-1-naphthalenedisulfonic acid in 0.15 mol/L Na_2SO_3 and 0.92 mol/L $NaHSO_3$, and inositol hexaphosphate equivalents were calculated to estimate phytate concentration.

In Vitro Digestion. After preparation of the products with maize, freeze-dried products were fortified with 50 ppm of iron by adding $FeSO_4$ or reduced iron (RedFe, American Ingredients, Kansas City, MO) prior to digestion. Samples were prepared for bioavailability assessment as described earlier (*12*). Fortified samples were weighed to deliver 200 μg of total iron (∼3.5 g), mixed with water, and adjusted to pH 2.0 using 5 M HCl. Pepsin solution [porcine pepsin A (1:60000)

in 0.1 mol/L HCl] was added and incubated at 37 °C with shaking for 1 h. Following the pH adjustment to 6.0, pancreatin and bile solution were added [porcine pancreatin (4×USP) and bile extract in sodium bicarbonate solution], and incubation was continued for 15 min. Samples were heat-treated (4 min at 100 °C) to inactivate proteolytic enzyme activity (*13*) and centrifuged, and the supernatant was used for soluble iron and bioavailability experiments.

Lactic Acid Addition. To determine the effect of lactic acid addition on iron solubility and bioavailability, 6 mg/g lactic acid was added to arepa, tortilla, and porridge prior to in vitro digestion. This acidification level was chosen on the basis of reported values of maize products fermented over 24 h (*14*).

Cell Culture for Iron Bioavailability. All reagents for cell culture work were from Sigma Aldrich or Gibco BRL (Grand Island, NY) unless otherwise mentioned.

The following experiments were conducted in Caco-2 cells at passages 31−36 using the previously described method (*12*). Cells were grown in a culture flask with Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, 1% v/v nonessential amino acids, and 1% v/v antibiotic−antimycotic solution. Cells were maintained at 37 °C in an incubator with 5% CO_2. Trypsinized cells were seeded to collagenized (type 1 rat tail collagen) 12-well cell culture plates (Corning Costar) at a density of 5×10^4 cells/cm^2 for iron bioavailability experiments. The plates were maintained at 37 °C and 5% CO_2. Iron bioavailability experiments were conducted 15 days postseeding after a rinse with Earle's Balanced Salt Solution. Serum-free medium was prepared with DMEM with 1% v/v nonessential amino acids, 1% v/v antibiotic−antimycotic solution, 10 mmol/L piperazine-N,N′-bis[2-ethanesulfonic acid] (PIPES), hydrocortisone (4 mg/L), insulin (5 mg/L), selenium (5 μg/L), triiodothyronine (34 μg/L), and epidermal growth factor (20 μg/L), as modified from Glahn et al. (*15*). Serum-free medium (0.5 mL) and 0.5 mL of the supernatant of each digest were added to the cell culture well and incubated for 2 h. A subsequent 0.5 mL of serum-free medium was added after the 2 h incubation, followed by further incubation for 22 h. After 24 h total of incubation, the samples were removed by aspiration from the cell culture wells, and the cells were rinsed with 1 mL of Earle's Balanced Salt Solution. The cells were lysed in H_2O by sonication. Total lysate protein was determined according to the Bradford Coomassie assay (Pierce Laboratories, Rockford, IL). Ferritin in the lysates was determined by using a radioimmunoassay kit (Fer-Iron II, Ramco Laboratories, Stafford, TX) and a Cobra-II gamma counter with SpectraWorks software (Packard BioSciences, Meriden, CT). After ferritin concentration is normalized to cell protein concentration, the values are expressed as relative bioavailability (RBA) as compared to porridge fortified with $FeSO_4$.

Iron Solubility. Supernatant from the in vitro digest was mixed 1:1 v/v with 20% w/v trichloroacetic acid in 6 mol/L HCl, incubated for 20 h at 65 °C, and then subjected to centrifugation at 5000g for 10 min (*8*). Iron in the supernatant was measured using the colorimetric method described under Iron Content.

Sensory Analysis. Because lactic acid addition may change the organoleptic characteristics of food, we conducted a sensory analysis on lactic acid added products. Differences between untreated and lactic acid treated samples were assessed by using the triangle test (*16*). The protocol was approved for exemption by the human subjects review committee at Iowa State University. The products were prepared using commercial mixes that best emulated the processed products prepared in the above sections, either with or without food grade lactic acid (6 mg of powdered lactic acid; Purac, Lincolnshire, IL). All products were prepared as per the manufacturers' directions. Arepas (Harina PAN, Refinadora de Maiz Venezolana, C.A., Aragua, Venezuela) were shaped into cakes, 10 cm in diameter and 1 cm thick, and fried in a dry pan until crisp and browned both sides. Masa for tortillas (MASECA, Azteca Milling, City of Commerce, CA) was prepared, pressed in a tortilla press, and fried on both sides in a dry pan until the tortilla puffed with steam. Porridge (Bob's Red Mill Cornmeal, Coarse Ground, Milwaukie, OR) was prepared by boiling cornmeal with H_2O. Untrained panelists (44 total, 4 male and 40 female, ages 18−63) tested each of the three food products in a random sequence, receiving three samples (two
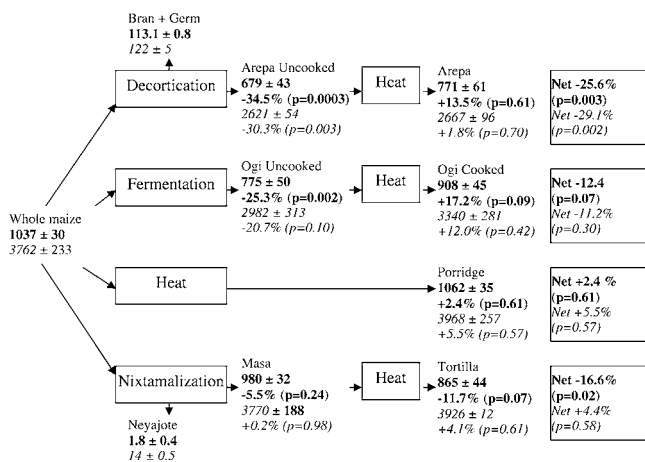
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Figure 1. Phytate and iron content of maize during processing. Phytate in bold expressed as milligrams of phytate per 200 g, dwb, starting sample weight (mean ± SE); iron in italics, expressed as micrograms of Fe per 200 g, dwb, starting sample weight (mean ± SE). Differences were determined between samples using Student's *t* test, *n* = 4–8.

Table 1. Phytate to Iron Molar Ratios for Maize Products[a]

	tortilla	arepa	ogi	porridge
no fortification	17.1 ± 1.3	20.7 ± 3.0	21.9 ± 2.6	30.0 ± 6.9
with fortification	4.9 ± 0.3	5.1 ± 0.6	5.6 ± 0.4	6.8 ± 0.7

[a] Mean ± SD, *n* = 4–8.

identical, one different treatment). Ballots were scored for panelists' ability to identify the product that was different.

Statistical Analysis. Differences in iron and phytate content between processing steps and between with and without lactic acid treatment in the same food product were determined using Student's *t* test. Differences in RBA and iron solubility among the processed products (grouped by fortification) were determined using ANOVA with Tukey's multiple-comparison test. To assess the interactive effect of processing and fortification, two-way ANOVA was used. Binomial probability distribution analysis was used for sensory analysis triangle test scores. All mean differences were deemed to be significant at $p \leq 0.05$. Analyses were performed using GraphPad software (GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego, CA), except for two-way ANOVA, which was performed with SAS (SAS 9.0 for Windows, Cary, NC).

RESULTS AND DISCUSSION

Changes in Iron Content during Processing. Iron content was found to not significantly change during processing except in arepa, where iron decreased 30.3% during decortication ($p = 0.003$), with a net decrease of 29.1% in the final product ($p = 0.002$) (**Figure 1**). It was not surprising to see the decrease in iron content with the physical removal of bran and germ, as iron content is high in the germ and, to a lesser extent, in the bran layer. Defatted germ is reported to contain 36.7 mg/100 g (*17*) and 2.79 mg/100 g for crude maize bran compared to 2.38 mg/100 g for whole maize (*18*). Because of a lack of waste streams, changes in iron content for porridge and ogi preparation were not significant. Because of the small weight of the waste stream in tortilla processing (neyajote), there was a negligible impact on iron content. Iron losses in steep water are low during nixtamilization because iron solubility is low due to the high pH associated with alkali treatment. However, in most traditional tortilla processes, the bran and germ layer are removed poststeeping, which should correspond to iron losses similar to decortication.

Changes in Phytate Content during Processing. The total phytate content of maize products was significantly affected by fermentation in ogi preparation (−25.3%, $p = 0.002$) and decortication in arepa preparation (−34.5%, $p = 0.01$) (**Figure 1**); however, only arepa and tortilla showed a significant net decrease of phytate compared to the starting material (−25.6%, $p = 0.003$, and −16.6%, $p = 0.02$, respectively). Decortication would physically decrease the phytate content due to bran and

germ removal, whereas fermentation would induce phytase activity. Fermentation, soaking, and germination are known to induce both endogenous phytases in seeds and exogenous phytases from microbial sources (*19*). Because endogenous phytase activity is low in maize [9 mIU/g as compared to 440 mIU/g for triticale (*20*)], phytate reduction in ogi is likely due to the microflora growth during fermentation.

More successful reductions in maize phytate have been seen from the addition of exogenous phytase from other sources, including the use of bacterial phytase (99.2%) (*21*), wheat phytase (88%), and germinated sorghum (72%) (*22*). Phytate was shown to be reduced 42% in fermented maize gruel and 60 and 98% in fermented maize gruel with 10 or 50 mg of wheat phytase, respectively (*8*). A 60% reduction in maize phytate after 96 h of germination at 32 °C was shown (*23*), but this level of reduction may have a minimal impact in improving iron bioavailability (*21*, *24*). Whereas germination has been implicated in reducing phytate content in maize porridges (*25*), the changes in starch functionality may limit its practical application in other maize products (arepa and tortilla).

Heating primarily reduced the phytate content of tortillas (11.7%, $p < 0.07$) but did not affect other maize products. The differences might be due to the higher heat achieved in dry heating in the tortilla preparation. The reduction of phytic acid content varies in the nixtamilization process on the basis of the conditions, such as alkali concentration, steeping time, and temperature (*7*).

It has been suggested that phytate has to be reduced by >90% to see a 2-fold improvement in iron bioavailability (*21*). The phytate to iron molar ratio in all of the unfortified and fortified products is high, ranging from 17 to 30 for unfortified products and from 4.9 to 6.8 in fortified products (**Table 1**). It has been shown that the phytate to iron molar ratio should be <1, and preferably 0.4, to observe a significant increase in bioavailability (*26*). None of our products achieved this low ratio.

Iron Solubility and Bioavailability. To compare the iron bioavailability of all maize products, results were normalized to porridge with $FeSO_4$ values and referred to as RBA. The bioavailability of unfortified maize products was low as compared to fortified maize products, ranging from 37 ± 13 to 46 ± 16% RBA (**Figure 2**). As iron content could not logically be matched between fortified and unfortified products, these data should be interpreted with caution; however, they serve to show the net improvement in RBA with fortification.

As anticipated, on average, the bioavailability of fortified products is about 2-fold higher than that of their unfortified counterparts except ogi with $FeSO_4$, which was 6.6-fold higher (RBA of 37 ± 13 versus 245 ± 16%). When $FeSO_4$-fortified products were compared, the RBA of ogi was 2.5-fold higher than those of the other products (RBA of 96 ± 6, 92 ± 11, and 100 ± 4% for tortillas, arepas, and porridge, respectively) ($p < 0.001$). When products were fortified with RedFe, the RBA values of ogi (142 ± 6%) and porridge (111 ± 6%) were higher than those of tortilla (79 ± 6%) and arepa (84 ± 6% ($p < 0.001$ and $p < 0.01$ for ogi and porridge, respectively).
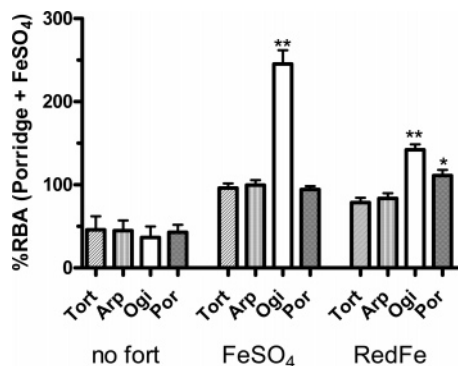
Figure 2. Relative bioavailability [RBA (ferritin per milligram of cell protein) normalized to porridge with FeSO₄] of maize products without fortification (no fort) or fortified with ferrous sulfate (FeSO₄) or reduced iron (RedFe). Tort, tortilla; Arp, arepa; Por, porridge. Columns with stars are significantly different within similarly fortified products as determined by ANOVA with Tukey's multiple-comparison test: *, $p < 0.05$; **, $p < 0.001$; $n = 9–14$.

The increase in iron bioavailability in ogi cannot be solely attributed to phytate reduction, as phytate levels were similar across all products, but can be attributed to increased iron solubility due to the acidic food matrix developed during lactic acid fermentation. Increased iron solubility was reported in various other lactic acid fermented products (*8, 27–31*), with the assumption that high-soluble-iron foods have higher bioavailability.

When the bioavailability data were analyzed with two-way ANOVA, both fortificant ($p < 0.0001$) and product ($p = 0.0158$) had a significant effect on iron bioavailability. A significant interaction between fortificant and product was also observed ($p = 0.008$). The effect of fortificant was not surprising, as it is well-known that FeSO₄ has high bioavailability as compared to RedFe (*32, 33*); however, the strong effect of product indicates a processing and food matrix effect on bioavailability. Our results suggest that choosing optimal fortification and processing techniques is important to achieve significant improvement in iron bioavailability. The iron bioavailability of ogi is higher than that of other maize products, suggesting that manipulation of the food matrix is an important strategy to enhance iron bioavailability in other products. Given the lactic acid production during the fermentation in ogi, it is feasible to add lactic acid to other products without further changing the processing conditions.

Overall, addition of lactic acid to unfermented products increased bioavailability on average 1.7-fold (**Figure 3**). In the unfortified products, tortilla had an increase of 2.1-fold ($p < 0.01$) and porridge and increase of 1.8-fold ($p < 0.0001$) with lactic acid addition. In FeSO₄-fortified products, RBA increased 2.0-fold in tortillas ($p < 0.001$), whereas in products with RedFe, the RBA increased 2.1-fold in tortillas ($p < 0.0001$) and 1.7-fold in arepas ($p < 0.0001$). Regardless of fortification, the RBA of tortilla was improved with lactic acid addition. Given the low iron solubility of tortillas as compared to other products (**Table 2**), much of this improvement in bioavailability is likely attributed to the increase of iron solubility accompanying the acidification; however, it cannot be solely attributed to iron solubility. The low solubility of iron in all of the tortilla products is likely due to the high pH attributed to the alkali treatment during processing. As expected, the pH of the tortillas (pH 7.8) was higher than those of arepa and porridge (both pH 6.1) and ogi (pH 4.0). The addition of lactic acid to arepa and porridge reduced the pH to 3.9−4.1 and 4.6 with tortillas (data not shown). As shown in **Table 2**, lactic acid addition significantly
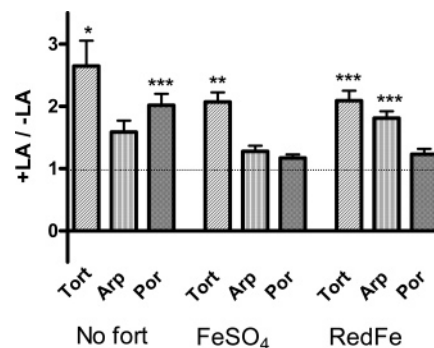

Figure 3. Relative bioavailability ratios [RBA (ferritin per milligram of cell protein) normalized to porridge with FeSO₄] of products with or without added lactic acid (+LA/–LA). Dotted line represents no effect of LA. Tort, tortilla; Arp, arepa; Por, porridge; RedFe, reduced iron. Student's *t* test was used to compare RBA of +LA and –LA products: *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$; $n = 5–14$.

Table 2. Percent Soluble Iron in Processed Maize Products Following in Vitro Digestion

	tortilla	arepa	ogi	porridge
no fortification	0.0 ± 0.9 a	6.7 ± 2.6 ab	14.9 ± 2.4 b	7.1 ± 4.3 b
+ lactic acid	18.4 ± 0.5 a**	26.8 ± 2.0 b**		24.7 ± 0.5 b*
+ FeSO₄	0.0 ± 0.2 a	30.6 ± 0.6 c	21.2 ± 1.5 b	28.4 ± 1.5 c
+ FeSO₄ + lactic acid	3.9 ± 0.1 a**	27.4 ± 2.2 b		25.6 ± 1.3 b
+ reduced Fe	0.0 ± 0.2 a	14.6 ± 0.6 b	13.8 ± 2.0 b	14.3 ± 0.4 b
+ reduced Fe + lactic acid	6.5 ± 0.9 a**	13.6 ± 1.5 b		12.7 ± 0.6 b

[a] Percent soluble iron is expressed relative to total iron in the sample. Mean ± SD, $n = 4–8$. Difference among processing treatments was determined by ANOVA with Tukey's multiple comparison. Means in the same row with the same letter are not significantly different. Lactic acid effect for each fortificant and treatment was determined individually by Student's *t* test: *, $p < 0.01$; **, $p < 0.001$.

improved iron solubility in tortillas ($p < 0.001$), regardless of fortification, but not in the other products, suggesting the importance of an acidic environment on iron solubility and bioavailability.

Lactic acid has been shown to enhance iron bioavailability through an enhancing effect on ferric iron transport across the intestinal epithelium (*34*), suggesting its effectiveness is greater with ferric than ferrous iron. Our results, showing greater effectiveness of lactic acid in arepa fortified with RedFe as compared to FeSO₄, support the previous study. The effect of lactic acid on improving bioavailability in maize food products is most likely from two different effects: pH reduction preventing the formation of insoluble oxides, most evidently observed in high-pH foods (tortillas) and, to a lesser extent, enhancement of absorption of ferric iron because the enhancement was less with moderate-pH foods (arepa and porridge). Given the very high phytate level in the porridge, acidification may have been insufficient to overcome the phytate inhibition in porridge.

Effect of Lactic Acid on Organoleptic Characteristics. Untrained panelists were capable of detecting a difference in organoleptic characteristics between lactic acid and typical porridges ($p < 0.0001$); however, they were not capable of detecting a difference in tortillas ($p = 0.166$) and arepas ($p = 0.831$). These results are summarized in **Table 3**.

Consumer testing suggested that the levels of lactic acid we used to improve iron bioavailability may not affect product quality except for porridge. The study was, however, limited, in that the consumer population is not a typical maize foods consuming population. It is possible that a maize-consuming

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Table 3. Number of Respondents Able To Correctly Differentiate Lactic Acid Added Products from Typical Products Using Triangle Testing[a]

product		p value
arepa	14/44	0.831
tortilla	19/44	0.166
porridge	32/43	<0.0001

[a] Binomial probability distribution.

population may be more sensitive to changes in formulation. On the other hand, the foods in this study were presented without any of the traditional seasonings and accompaniments that may act to mask off-flavors.

At the household level, the addition of lactic acid culture or acid steeps may be a useful technique in certain product formulations. In addition, given the availability of powdered lactic acid, flours could be supplied with added lactic acid. This may be a cost-effective means to increase the bioavailability of low-bioavailable fortificants such as RedFe rather than including high-cost, high-bioavailable fortificants. However, long-term storage studies would be necessary to evaluate the impact on nutrient stability and organoleptic acceptability of lactic acid enhanced maize products.

This study supports the premise that fortification alone is not sufficient to improve iron status in populations; rather, an effort to improve the bioavailability of traditional foods using appropriate technology is necessary for successful fortification strategies.

Conclusion. The effects of processing on iron bioavailability in maize are significant, especially with fermentation increasing bioavailability. The enhancing effect of fermentation may be attributed mostly to acidification from natural lactic acid production rather than phytate reduction. Lactic acid addition greatly improved iron bioavailability of low-bioavailable maize products, especially tortillas, without affecting organoleptic quality. The role of lactic acid and fermentation may have a beneficial impact on current iron fortification schemes, increasing iron solubility and bioavailability. Hence, the addition of lactic acid may be a novel means to increase the iron bioavailability of maize products.

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