Assessment of Home-Based Processing Methods To Reduce the Phytate Content and Phytate/Zinc Molar Ratio of White Maize (*Zea mays*)

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Various methods of processing maize suitable for household use in rural Malawi, Central Africa, were investigated for their ability to reduce its phytate content and phytate/zinc molar ratio. These methods included fermentation, germination, and soaking. Penta- and hexainositol phosphates were measured by HPLC, and zinc was measured by atomic absorption spectrophotometry. Natural lactic fermentation of maize flour slurries resulted in 88% phytate retention compared to unprocessed, unrefined maize flour porridges, whereas lower phytate retention was observed when a starter culture (61%) or germinated flour (71%) was also used. Fermentation of cooked maize flour porridges with germinated flour added resulted in 54−85% retention of phytate compared to controls. Soaking maize flour or pounded maize and decanting excess water resulted in 43 and 49% retention of phytate, respectively. The latter soaking procedures were simple and effective and were suitable for household use in rural Malawian communities.

Keywords: *Fermentation; germination; inositol phosphates; maize; phytate; Zea mays; zinc*

INTRODUCTION

In developing countries where cereals such as maize are the staple of the diet, the consumption of adequate amounts of bioavailable dietary zinc is compromised by the coexistence of a zinc absorption inhibitor, phytate. Phytate (*myo*-inositol hexaphosphate) is a strong chelator of zinc, particularly under physiological conditions (*1*), and is a potent inhibitor of zinc absorption. Other minerals of nutritional importance that are chelated by phytate are calcium, copper, iron, and manganese (*2*). The phytate/zinc molar ratio of the diet is a useful predictor of zinc bioavailability (*3*), and high ratios have been negatively associated with growth in children (*4*, *5*). Reduced phytate content of cereal-based meals has resulted in increased absorption of zinc (*6*, *7*). Therefore, at the population level, strategies to reduce dietary phytate content may lead to improved zinc status.

In Malawi, Central Africa, 62−89% of the dietary energy intake of children and women (*5*, *8*), and up to 75% of the energy from complementary diets of weanlings (*9*), are usually derived from maize (*Zea mays* L. Groench.), and these populations are considered to be at risk of zinc deficiency. More than 90% of Malawians live in rural areas (*10*), and access to centrally processed foods is limited. As a result, home-based strategies to reduce phytate content of maize may be the most feasible methods to improve zinc bioavailability in rural Malawi.

Several methods of decreasing the phytate content of cereals with relatively low technological requirements exist (*11*) and may be adapted for household use. These include methods to stimulate the activity of phytase enzymes, which hydrolyze phytate to remove inorganic phosphate from the *myo*-inositol phosphate ring, and thus reduce its mineral binding capacity (*11*). Endogenous cereal phytase enzymes, and/or exogenous phytases associated with microbiological organisms, may be activated by soaking, germination, or fermentation (*12*−*14*). Nonenzymatic methods to remove phytate from food substrates have also been used (*15*, *16*). The objective of the present study was to compare the ability of various, low-technology processing methods to reduce the phytate content of maize, which would be suitable for household use in populations of rural Malawi and possibly in other regions where unfermented maize is the major staple.

MATERIALS AND METHODS

All grains and flours used in this study were imported from Malawi, Central Africa, and were stored under refrigeration until use [white maize (*Zea mays* L. (Gram.)); bulrush millet (*Pennisetum glaucum*); sorghum (*Sorghum bicolor* (L.) Moench (Gram.))]. Various methods of fermentation and soaking of maize flour, maize porridges, or pounded maize were investigated for their ability to reduce phytate content. The same batch of maize flour was used for all control and processed samples to reduce variability in results due to differences in the baseline phytate and nutrient content of the maize flour. Specific details for each method are described below.

Fermentation, with and without Use of Germinated Flour. Unrefined white maize flour was diluted in tap water to prepare either slurries or cooked porridges (10% dry matter) for fermentation. All incubations were carried out in a water bath, at 25 °C, and all samples were heated on a hot plate to 90 °C for 10−13 min either before or after fermentation, as indicated. Lactic fermentation was followed by monitoring the change in pH. A pH of 3.8 was used as an endpoint as most enteropathic bacteria cannot survive at this level of acidity (*17*, *18*). Samples were dried in a 90 °C oven for up to 48 h prior to preparation for phytate analysis. As a control for all

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fermented samples, the phytate content of a maize flour porridge (10% dry matter) dried immediately after cooking was analyzed.

Preparation of Germinated Flours. Germinated white maize, bulrush millet, and sorghum grains were prepared by soaking the grains in excess water for 12 h, then draining the excess water, and incubating them in a plastic bag at 23−25 °C for 72 h. The germinated grains, including the vegetative portion, were then dried (23−25 °C, 2−3 days) and ground into flour in an electric mill.

Soaking. Maize flour was mixed with tap water at the dilution levels indicated and incubated at 25 °C. Raw, unsoaked maize flour was measured in triplicate for phytate content and used as a control. Soaked flour samples used for phytate and mineral analyses were oven-dried at 90 °C for ∼24 h, whereas samples used in the analysis of riboflavin and thiamin were freeze-dried (model 77530, Labconco, Kansas City, MO) to minimize loss due to heat lability. Whole maize was mechanically pounded to separate the germ and husk from the endosperm. Soaking of pounded maize was carried out in Malawi at ambient temperature (∼26−30 °C). Samples of pounded maize obtained from different local markets ($n = 4$) were analyzed separately for phytate content, and the mean of these samples was used as the control value.

Specific processing methods for all fermented and soaked samples prepared are described below:

(a) Natural Lactic Fermentation. Maize flour slurries of 25, 30, 46, and 55% dry matter by weight were prepared in duplicate. The slurries were incubated at 25 °C until the pH dropped to <3.8 (48−86 h). The 46 and 55% slurries were diluted with 0.7 and 1 part water, respectively, before cooking.

(b) Natural Lactic Fermentation with the Addition of Germinated Flour. Maize flour slurries of 25% dry matter by weight were prepared in triplicate. Germinated maize flour was added to the slurries (5% of the dry weight). The slurries were incubated at 25 °C until the pH dropped below 3.8 (∼48 h) and then cooked.

(c) Fermentation with the Addition of Starter Culture. Accelerated fermentation was carried out following the method of Svanberg et al. (*19*). A 25% maize flour slurry was incubated at 25 °C until the pH dropped below 5.3 (15 h). A portion of starter culture was added, equivalent to 5% of the slurry weight, and the incubation continued until the pH dropped below 3.8 (17 h). The starter culture used was a traditional Malawian fermented beverage produced by cooking a maize porridge (10% dry weight), cooling it to room temperature, adding germinated flour (10% of dry weight of flour), and fermenting it for 36 h. The pH of the fermented beverage was 3.6−3.8 after 36 h of fermentation.

(d) Fermentation of Maize Flour Porridges with the Addition of Germinated Flour. Maize flour porridges were cooked (10% dry matter) and cooled to <40 °C. Germinated maize, millet, or sorghum flours were then added to the porridges, equivalent to 5% weight of the dry matter content. The porridges were then incubated at 25 °C until the pH dropped below 3.8, ∼48−60 h. All samples were prepared in triplicate. Porridges prepared in the same way but without incubation were also compared to controls.

(e) Soaking of Maize Flour. Aqueous extractions of phytate from raw, unrefined maize flour were carried out to determine the amount of soluble phytate. One part flour (10 g) was mixed in 4 parts water and incubated for 1 h and then centrifuged. The supernatant was removed (30 mL), an equivalent volume of water was added back to the pellet, the mixture was rehomogenized, and the incubation and extraction processes were repeated. The pellet was then dried and analyzed for phytate content.

Change in phytate content was also determined in soaked maize flour samples after excess water had been decanted from the soaked slurry, rather than using centrifugation. One part flour was mixed in either 2 ($n = 1$), 3 ($n = 1$), or 4 ($n = 2$) parts water by weight and incubated for 12 h. Excess water was decanted (65% of water added). An equal amount of water was added back to the residue, and this slurry was incubated for a further 12 h ($n = 2$). For the 1:4 mixture, samples were also taken after the first extraction step ($n = 2$). The residual flour was dried and analyzed for phytate content.

The effect of soaking time on the change in phytate content during soaking of maize flour was also determined. One part flour was mixed in 4 parts water and incubated at 25 °C for 1, 3, 12, 16, or 24 h. All samples were prepared in duplicate.

(f) Soaking of Pounded Maize. One part pounded maize was soaked in 4 parts water for 1 h, and excess water was decanted (∼80% of the total water added). The soaked, pounded maize was dried on a mat before milling into flour. The dried flour was analyzed for phytate content.

HPLC Analysis of Phytate. Inositol penta- and hexaphosphates (IP5 + IP6) were determined according to a modified method of Lehrfeld (*20*). Ground samples were dried in a 90 °C oven until reaching constant weight. Approximately 5 g of dried sample was extracted with 5 mL of 0.67 M HCl by vortexing and sonicating in a bath for 20 min. Following centrifugation, 2.5 mL of the supernatant was removed and diluted in 22.5 mL of Millipore water. Concentration of inositol phosphates was performed using strong anion exchange columns (WAT023620, Sep-Pak Vac 1 cm^3 Waters Accell Plus QMA, Waters, Milford, MA). Columns were conditioned with 3 mL of 0.067 M HCl. Samples were then percolated through the column, and the column was washed with 2 mL of 0.067 M HCl. Inositol phosphates were eluted off the columns using 4 mL of 2 M HCl. All elutions were performed on a vacuum manifold at a flow rate of <1 mL/min. Eluants were evaporated to dryness, and the residue was reconstituted with 1 mL of Millipore water before injection into the separations module. The mobile phase used was a 40:60 solution of 0.04 M phosphoric acid and HPLC grade MeOH and contained 2% tetrabutylammonium hydroxide (40% solution in H_2O) and 4.5×10^{-6} M inositol hexaphosphophosphoric acid. The pH was adjusted to 4.0 with 4.5 M sulfuric acid. The mobile phase was degassed by vacuum filtration through a 0.45 μm filter. Standard dilutions were prepared from inositol hexaphosphoric acid dodecasodium salt (P-8810, Sigma Chemical Co., St. Louis, MO) and contained 3.2, 1.6, 0.8, and 0.4 μmol/mL inositol hexaphosphoric acid. Samples were injected into a Waters 2690 separations module with a flow rate of 0.45 mL/min, separated using a Hypersil H3ODS, 25 cm, 4.6 mm i.d., column with 3 μm pore size (H3ODS-250A, HiCHROM, Berkshire, U.K.) at 45 °C, and detected on a Waters differential refractometer (model 410).

This method was found to have an inter-run coefficient of variation of 10%, based on the analysis of eight replicates of five different samples of unrefined maize flour, processed by various methods. Standard reference materials are not available to determine the accuracy of phytate analysis; however, good interlaboratory comparison between results was found using similar methods of analyses. Samples of treated and untreated maize and soy flour blends analyzed in our laboratory were 573, 178, and 77 mg of IP5 + IP6/100 g compared to 660, 170, and 70 mg of IP5 + IP6/100 g in another laboratory (J. Lehrfeld, personal communication, 1997). Also, in our laboratory, a sample of wheat bran was analyzed to contain 3139 ± 102 mg of IP5 + IP6/100 g, compared with 3348 mg/100 g of dry weight (range: 3125−3571 mg/100 g), analyzed in another laboratory (D. Oberleas, personal communication, 1999). Thus, the accuracy of the method used in the present study was adequate.

Nutrient Analysis. As with cooking, some loss of nutrients may occur when soaking water is discarded. Therefore, the content of select nutrients in soaked flours and soaked pounded maize was also examined and compared to controls. The nutrients selected (zinc, calcium, iron, riboflavin, and niacin) are those for which maize is a major source and which are likely to be limiting in young childrens' diets in rural Malawi or other developing countries (*9, 21*). Methods used for dry ashing and atomic absorption spectrophotometry for zinc, iron, and calcium have been described previously (*22*). Mineral content was determined from one 5 g aliquot of dried sample using a Perkin-Elmer flame atomic absorption spectrophotometer (model 2280). Accuracy of the method was determined using a National Bureau of Standards, Standard Reference

Table 1. Effect of Processing Methods on the Phytate Content of Maize-Based Porridges (10% Dry Matter)[a]

processing method	*n*	IP6 + IP5 (mg/100 g of dry wt)	% retention phytate
control (maize porridge,10% dry matter)	3	661± 59	
(a) natural lactic fermentation	4	582 ± 51 (range: 540−647)	88 (82−98)
(b) natural lactic fermentation with germinated maize flour	3	471 ± 48	71
(c) fermentation with starter culture	1	406	61
(d) fermentation of cooked porridges with germinated grain flour added			
(i) germinated maize flour	3	358 ± 114	54
(ii) germinated millet flour	2	377 ± 80	57
(iii) germinated sorghum flour	3	565 ± 25	85
unfermented porridge with germinated millet flour	3	534 ± 44	81

[a] Mean ± SD.

Table 2. Effect of Soaking Unrefined White Maize Flour[a] on the Content of Phytate, Zinc, Niacin, and Riboflavin[b]

	maize flour (control) amount	soaked maize flour amount	soaked maize flour % retention
phytate (mg of IP6 + IP5/100 g)	710 ± 82 (2)[c]	306 ± 73 (2)	43
zinc (mg/100 g)	2.4 ± 0.1 (2)	1.7 ± 0.3 (2)	71
phytate/zinc molar ratio	28.9	17.7	61
niacin (mg/100 g)	0.06 (1)	0.03 (1)	50
riboflavin (mg/100 g)	1.64 (1)	1.38 (1)	84

[a] One part flour in four parts water for 12 h at 25 °C. [b] Mean ± SD. [c] Sample size for each analysis shown in parentheses.

Material, rice flour (1568a: certified values of 19.4 ± 0.5 μg of zinc/g and 7.40 ± 0.9 μg of iron/g versus analyzed values of 20.3 ± 0.8 μg of zinc/g and 7.70 ± 0.3 μg of iron/g). The calcium recovered from the Standard Reference Material, *Citrus* leaves (1572) and rice flour, were equivalent to 99 ± 1 and 90 ± 4%, respectively, of the certified percentage weight of calcium in the reference material. Riboflavin and niacin were also measured in a soaked maize flour sample based on standardized methods (*23*, *24*).

RESULTS

Retention of Phytate. The phytate and mineral contents analyzed in the unrefined raw maize flour samples here were found to compare well to previously published values for unrefined Malawian white maize (*22*, *25*); in the present study phytate, zinc, and calcium were 697−748 mg of IP5 + IP6/100 g, 2.4−2.5 mg of zinc/100 g, and 4−5 mg of calcium/100 g of dry weight compared to 599 mg of IP5 + IP6/100 g, 2.5 mg of zinc/100 g, and trace amounts of calcium. No analyzed value for the iron content of Malawian white maize is available for comparison. However, the iron content of unrefined yellow corn in the U.S. Department of Agriculture food composition tables is 3.5 mg of iron/100 g of dry weight (*26*), a somewhat lower content compared to 4.1 mg of iron/100 g in the white maize analyzed here.

The results for phytate retention in cooked, fermented samples are summarized in Table 1, and Tables 2 and 3 compare the results for changes in phytate content and select vitamin and mineral content of soaked flours and soaked pounded maize, respectively.

(a) Natural lactic fermentation of unrefined maize flour resulted in small decreases in phytate content; fermentation of 25, 30, 45, or 55% flour slurries for up to 86 h reduced phytate to 82, 82, 98, and 91% of controls.

Table 3. Effect of Soaking Pounded Maize[a] on the Content of Phytate, Zinc, Iron, and Calcium[a]

	pounded maize (control), *n* = 4, amount	soaked pounded maize, *n* = 4 amount	soaked pounded maize, *n* = 4 % retention
phytate (mg of IP6 + IP5/100 g)	697 ± 87	342 ± 58	49%
zinc (mg/100 g)	2.4 ± 0.4	2.2 ± 1.0	92%
phytate/zinc molar ratio	28.8	15.4	53%
iron (mg/100 g)	4.1 ± 1.1	4.4 ± 0.4	107%
calcium (mg/100 g)	4 ± 1	7 ± 1	175%

[a] Mean ± SD. [b] One part pounded maize in 4 parts water for 1 h at 25 °C.

(b) Fermentation of 25% raw maize flour slurries with the addition of 5% dry matter germinated maize flour resulted in phytate retention of 71% of controls, slightly less than when fermented without the addition of germinated flour.

(c) Following the addition of a starter culture to presoaked flour slurries, phytate was reduced to 61% of controls, somewhat less than with the addition of germinated flours.

(d) Fermentation of cooked porridges with the addition of germinated flour produced somewhat greater reductions in phytate content than when uncooked flours were fermented. With the addition of germinated flours of millet, maize, and sorghum to the slurries, phytate content was reduced to 54, 57, and 85%, respectively, of the level in controls.

(e) Aqueous extraction of flour using centrifugation resulted in the nearly complete removal of phytate, leaving only 3% of the phytate content of control samples (20 ± 16 mg of IP6 + IP5/100 g; *n* = 2). In samples that were extracted twice but from which excess water had been removed by decanting, increasing the proportion of soaking water to flour (1 part flour in 2, 3, or 4 parts water) resulted in greater losses of phytate (45, 31, and 24% of controls, respectively), as shown in Figure 1. When a single extraction was used in the 1:4 mixtures, a similar amount of phytate reduction was observed, as compared to the double extraction (26 versus 24% phytate retention, respectively).

The greatest loss of phytate in soaked maize flour samples occurred within the first hour of soaking (Figure 2); 43% of phytate remained after 1 h of soaking (soaked for 1 h, 306 ± 82, versus control, 710 ± 82), with little change up to 24 h.

(f) Soaking 1 part pounded maize in 4 parts water for 1 h and decanting excess water produced flour with phytate concentrations equivalent to 49% of controls, similar to the results found for soaking flour.

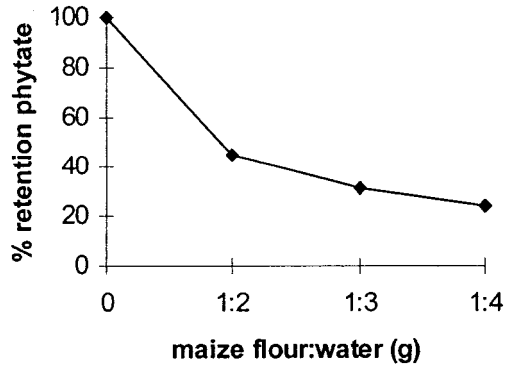

Figure 1. Effect of increasing proportion of soaking water in reducing phytate content (mg of IP6 + IP5/100 g of dry weight) of maize flour. One part maize flour was soaked in 2, 3, or 4 parts water, and extractions were made twice, at 12 h intervals, by decanting excess water from the residue.

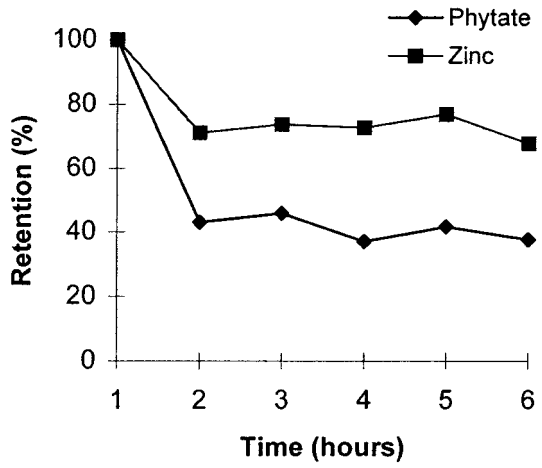

Figure 2. Effect of soaking time on the percent retention of phytate (mg of IP6 + IP5/100 g of dry weight) and zinc (mg of zinc/100 g of dry weight) from maize flour. One part maize flour was soaked in 4 parts water for 1 h, and excess soaking water was decanted from the residue.

Nutrient Loss. The zinc, niacin, and riboflavin contents were compared between soaked and unsoaked (control) maize flour (Table 2). Results suggest that some loss of these nutrients also occurs with soaking. When pounded maize was soaked for 1 h, only a slight reduction in zinc content occurred, and iron and calcium contents were retained and, in fact, showed a positive retention (Table 3).

DISCUSSION

The extent to which the different fermentation techniques used in this study reduced the phytate content of maize-based porridges was variable; between 54 and 88% of the phytate content of unprocessed, cooked maize porridges was retained after fermentation. In the present study, natural lactic fermentation of maize flour slurries did not substantially reduce phytate content (82−98% retained). A comparison of previous studies of phytate reduction in maize flour following natural lactic fermentation reveals variable results. Lopez et al. (*12*) measured the phytate content of natural lactic fermented maize flour slurries, incubated at 30 °C, at 24 h intervals, for up to 120 h. Phytate content was reduced to 72, 39, 44, 35, and 34% of controls at each interval. Natural lactic fermentation was also used to ferment maize flour dough, following traditional Nigerian practices (*13*), after which the phytate content dropped to 42% of controls after 48−72 h at ambient temperature. However, Koepf (*27*) did not observe phytate reduction when maize flour slurries were fermented for up to 72 h.

The addition of germinated maize flour (5% of dry weight) to the fermenting system reduced the phytate content of maize porridges to 71% of controls, somewhat lower than that achieved by natural lactic fermentation alone. Svanberg et al. (*19*) also observed greater reduction in phytate content of maize flour slurries when germinated sorghum flour (5% of dry weight) was added; fermentation without and with germinated flour added reduced phytate content to 41 and 30% of unfermented controls, respectively. The process of germination generally increases phytase activity in grains (*27*−*29*); therefore, the addition of germinated flours increases the amount of endogenous phytase activity present in the fermenting system. However, the level of activity achieved is very dependent on the grain types used (*27*). Amylase activity also increases during germination (*28*, *30*), hydrolyzing amylose and amylopectins to dextrins, maltose, and glucose, which are preferred substrates for lactic acid bacteria and may thus stimulate microbial growth and associated microbial phytase activity.

Svanberg et al. (*19*) successfully used an accelerated fermentation process to reduce the phytate content of maize flour slurries. Flours were first soaked for 24−48 h at ambient temperature before the addition of a starter culture and subsequent fermentation for 16−24 h at 30 °C; phytate content was reduced to 3% of controls. A similar process was used in the present study, except higher incubation temperatures during the soaking period were used, commensurate with the higher ambient temperature in Malawi, and the starter culture was prepared using a traditional Malawian lactic fermented beverage of maize porridge and germinated millet flour. However, phytate content was reduced only to 61% of controls in the present study.

When cooked maize flour porridges were fermented (*19*), phytate was reduced to 58% of controls when fermented with a starter culture and to 49% when both starter culture and germinated flour were added. In the present study, comparable results were observed when germinated millet or maize flour was added to cooked porridges prior to fermentation; phytate was reduced to 54−57% of controls, although the addition of germinated sorghum flour was less effective. Use of germinated flours with a higher endogenous phytase activity, such as rye and wheat, might be highly effective in reducing phytate content in fermented porridges; after 2 days of germination, phytase activities of rye and wheat were 6081 and 3090 PU/g compared to 32, 124, and 191 PU/g in millet, Malawi white maize, and sorghum, respectively (*27*). Unfortunately, wheat and rye cereals are not indigenous to Malawi.

The precise reasons for apparent differences in the ability of natural lactic fermentation to reduce the phytate content of maize are difficult to determine. Analytical methods that measure phytate phosphorus may overestimate the amount of phytate with mineral-binding capacity by including non-phytate phosphorus and the lower inositol phosphates derived from enzymatic hydrolysis in the analysis (*31*, *32*); only the higher inositol phosphates, hexa- and pentainositol phosphates (IP5 and IP6), are believed to form insoluble chelates with zinc (*32*) and have the most potent inhibitory effect

on non-heme iron absorption (*33*); hence, only these forms were quantified in the present analysis. Apart from methodological and analytical differences among the studies, a multitude of variables are expected to affect the outcome. The ability of a lactic acid fermenting system to reduce the phytate content of the substrate is dependent on various conditions of the fermenting system. The types of fermenting organisms depend on which types are present on the cereal seed coat, which in turn are dependent on environmental conditions during growth, harvest, and storage of the cereal (*34*). All fermenting organisms do not possess high levels of phytase activity. In the leavening of wheat flour dough, no phytase activity was associated with the fermenting organisms (*35*). Other factors, such as the incubation temperature and solids/water ratio, affect the succession of fermenting organisms and their metabolic activity (*34*). Ultimately, these conditions will influence the synthesis and activity of phytases by the microbial flora, as well as by the host material. Thus, the occurrence of lactic fermentation, as indicated by the gradual acidification of the slurry or dough, does not necessarily result in a predictable amount of phytase activity or inositol phytate hydrolysis. The precise mechanisms of the fermentation process that influence microbial phytase activity and subsequent hydrolysis of phytate are not yet understood and require further investigation (*36*).

In light of the many factors that can affect the amount of phytate hydrolyzed during lactic fermentation, and the highly variable results observed in the present study, as well as within and between previous studies, the effectiveness of fermentation used at the household level in reducing the phytate content of maize-based foods may be difficult to quantify. This was also noted in a study by Amoa et al. (*37*), where samples of fermented maize flour doughs used in the production of Ghanaian kenkey were collected from various local sites where the dough was being centrally produced. Across these samples, the authors observed a reduction in phytate phosphorus ranging from 15 to 78% of controls. It should be noted that fermentation methods with other cereal substrates, such as the leavening of wheat flour dough in bread-making, have successfully been used to reduce the phytate content (*35*) due to the higher activity of endogenous phytase in wheat (915−1581 PU/g), as compared to maize (0−46 PU/g; *38*). Some techniques, such as including germinated flours in the fermenting system, may improve phytate reduction to some degree; however, significant variability has also been observed with these methods in the present study. Nonetheless, fermentation techniques may still be advocated, due to the antimicrobial properties of lactic acid bacteria (*17*, *18*) and their potential to reduce diarrheal infection in children (*39*).

Most phytate in corn is in the form of a water soluble salt, such as sodium, potassium, or magnesium salts; de Boland et al. (*16*) observed that 86% of the phytate in corn germ could be removed by aqueous extraction (4 g/100 mL, pH 6.1). Also, 88% of the phytate phosphorus in corn is reported to be contained in the germ (*40*); therefore, the majority of phytate in maize could potentially be removed by aqueous extraction from the germ. High rates of extraction of water-soluble phytate (>99% extractable) were also observed in bean flour (*Phaseolus vulgaris* L.) using similar methods (*41*). In the present study, an aqueous extraction method using centrifugation to remove the soaking water reduced phytate to <3% of unsoaked flour. Although centrifugation is not a feasible method for use in rural homes, these results confirm that most phytate in white maize is indeed water soluble and extractable and that the amount of phytate removed by soaking is partly dependent on the efficiency of removal of the soaking water.

Using a less thorough method of removing water from the soaked flour residue resulted in a less efficient extraction of phytate, as expected. The results of these experiments indicate that although the removal of phytate may be improved by increasing the proportion of soaking water to flour (Figure 1), there may be no advantage to using a double extraction rather than a single extraction. Also, a soaking period of no more than 1 h could be used to achieve maximal removal of phytate by this method (Figure 2). It is likely that passive diffusion of phytate into the soaking water was the predominant mechanism of phytate reduction, rather than hydrolysis of IP6 and IP5 to lower inositol phosphates through activation of endogenous phytase enzyme during soaking. Although the lower inositol phosphates were not measured in the present study, the proportion of IP5 to IP5 + IP6 increased only from 3% in controls to 10% after 1 h of soaking, suggesting that little enzymatic hydrolysis occurred. Another method of soaking, using maize that had been pounded to separate the husk and the germ from the endosperm, also successfully reduced phytate content to 49% of controls. These levels of phytate reduction may be easily achievable at the household level in rural communities, as the methods are simple, require minimal technology, and involve no additional costs and little extra preparation time.

The effect of soaking maize flour resulted in some loss of zinc, although loss of zinc was minimal after soaking the pounded maize. Iron and calcium contents of the soaked pounded maize were also not reduced following soaking. The reasons for the differential results of mineral loss between soaked flour and soaked pounded maize are not clear but are probably related to differences in the solubility behaviors of zinc and iron in solution and in the presence of phytate (*42*). Nonetheless, a greater proportion of phytate was removed from the maize flour than zinc, and a reduced phytate/zinc molar ratio was observed in both the soaked maize flour and soaked pounded maize.

Some decrease in the content of niacin and riboflavin was observed from flour as well. However, as only one control and one processed sample were analyzed for vitamin content, these results must be regarded as preliminary; further analysis is needed to quantify the degree of loss by the soaking processes used here. The losses of water soluble vitamins would not be expected to exceed losses that normally occur with cooking in water; losses of niacin and riboflavin in boiled flour are estimated to be 10% (*43*).

The aim of this study was to identify a simple and reliable process to effectively reduce the phytate content of maize flour, which would be suitable for use in rural Malawian households. Although several previous studies have demonstrated that different methods of lactic acid fermentation of maize flour slurries could reduce phytate content by >50%, the amount of phytate reduction observed using these methods is inconsistent and may be dependent on a number of variables inherent to the fermenting system that could neither be detected

nor controlled at the household level. Methods of soaking milled or pounded maize to remove phytate by diffusion may be more effective at the household level as the dependent factors are nonbiological and therefore easier to control. The effect of soaking on the removal of other water soluble vitamins should be further quantified to determine changes in the nutritive quality of the processed products. The cultural acceptability of using such soaking methods at the household level should also be investigated.

ABBREVIATIONS USED

IP5 + IP6, inositol penta- and hexaphosphates; HPLC, high-pressure liquid chromatography; PU, phytase units.

SAFETY

Standard safety procedures for the handling of concentrated acids and tetrabutylammonium hydroxide are noted; otherwise, no special precautionary measures are required for the materials and laboratory methods presented here.

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