Effects of reducing phytate content in sorghum through genetic modification and fermentation on *in vitro* iron availability in wholegrain porridges

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Abstract

Improved iron availability from sorghum porridges will benefit many malnourished communities in rural Africa where there is a high prevalence of iron deficiency. This research compared the efficacy of reducing sorghum phytate content by genetic modification (GM) and natural lactic acid fermentation on *in vitro* iron availability in porridges. GM low phytate, non-tannin (38% phytate reduction) and tannin (36% phytate reduction) sorghums and their null controls were processed into thick unfermented and fermented porridges. The inhibitory effect of the tannins seemed to prevent any increase in *in vitro* iron availability, regardless of the level of phytate reduction. Only the additive effect of GM in combination with fermentation in reducing the phytate content appeared to cause a substantial increase in *in vitro* iron availability in the GM fermented porridge (30%) made from the non-tannin line, compared to the GM unfermented porridge (8.9%) or the fermented porridge (17.6%) of the control sorghum. This could probably be of nutritional significance.

Keywords: Genetically modified, phytate, iron, dialysability, sorghum, traditional African porridges

1 Introduction

Iron deficiency is highly prevalent in the developing world (WHO, 2006), with up to 80% of pregnant women in developing countries suffering from iron deficiency (Murray-Kolb & Beard, 2009). Iron Deficiency Anemia (IDA), the most severe form of iron deficiency, affects as many as four billion people worldwide (WHO, 2006). In worst cases, the cause of iron deficiency may be inadequate dietary intake, but inhibitors of absorption, mostly found in plant foods, contribute significantly to these deficiencies (Rosado, 2003).

In most developing countries with a high prevalence of iron deficiency and IDA, the socio-economic status of those affected limits their consumption of red meat, which is one of the best sources of iron (Hunt, 2003). In these countries cereals, legumes and vegetables often constitute the main food sources.

Sorghum is one of the important crops used as a staple in Africa. In 2007 Africa produced 1.76×10^7 MT (FAO, 2007b) of sorghum, which provided on average 632 kJ/capita/day (FAO, 2007a). This was estimated to constitute on average approximately 25% of the daily energy intake of adults.

Sorghum, like other grains, contains non-heme iron, which has a very low bioavailability compared to that of heme iron in meat (Zimmerman, Chaouki & Hurrell, 2005). Also, sorghum is commonly consumed as wholegrain. While the bran of sorghum contains the most iron (Mahgoub & Elhag, 1998), it also contains phytate and sometimes tannins, depending on the cultivar, which further decrease the availability of non-heme iron (Hunt, 2003).

The research reported here is part of the Africa Biofortified Sorghum (ABS) project. The project seeks to develop a more nutritious and easily digestible sorghum through genetic modification containing increased levels of essential amino acids, especially lysine, increased levels of Vitamin A and more available iron and zinc, the latter, through phytate reduction (ABS, 2010).

The objective of this research was to compare the efficacy of reducing sorghum phytate content by genetic modification and that by fermentation on the *in vitro* iron availability in traditional African porridges.

2 Materials and methods

2.1 Samples

The two genetically modified low phytate (GMLP) sorghum lines were grown at Johnston, Iowa, in summer, confined field trails (ex Pioneer Hi-Bred, Iowa, USA). Both lines were genetically modified with kafirin suppression, lysine ketoglutarate reductase and myo-inositol kinase suppression. Two lines: parent P898012, Type II tannin sorghum (grown 2008) and Macia, a white tan-plant sorghum (grown 2009). For the tannin P898012 line, three independent GMLP and three independent null control samples were analysed. For the non-tannin Macia line, three independent GMLP and two independent null control samples were analysed. The relevant modification for this study is the suppression of myo-inositol kinase, which decreases the phytic acid synthetic capacity of the plant during seed development (Mendoza, 2002). The aim was to reduce the phytate contents of the GMLP non-tannin and tannin sorghums with approximately 40-50%.

2.2 Preparation of wholegrain flour and sorghum porridges

2.2.1 Flour

The samples were separately milled using a laboratory hammer mill (Falling Number 3100, Huddinge, Sweden) fitted with a 500 µm opening screen to give wholegrain flour. This was stored at 10^{o}C prior to food preparation.

2.2.2 Thick unfermented porridge (Ugali-type)

Distilled water (170 g) was added to 20 g flour. The mixture was heated to boiling and maintained with constant stirring for 5 min. The mixture was then left to cool at room temperature, after which it was frozen at -20°C.

2.2.3 Fermented uncooked flour

Macia sorghum flour (40 g) was mixed with 80 ml distilled water and incubated at 25°C for 48 h or until a pH lower than 4 was reached and this was used as a starter culture.

Fermented flour samples were prepared by mixing 20 g wholemeal flour, with 50 ml distilled water and 2 g starter culture. Incubation followed at 25°C for 36 h or until a pH below 4 was reached, after which it was frozen at -20°C.

2.2.4 Thick fermented porridge (Ting-type)

Fermented flour samples were prepared as described above (2.2.3). The fermented flour was then mixed thoroughly and cooked as described above (2.2.2).

All samples were freeze dried and then crushed to a particle size that passed through a 500 µm open sieve before analysis.

2.3 Analyses

2.3.1 Phytate content

This was determined through anion exchange chromatography, as described by Frubeck, Alonso, Marzo, & Santidrian (1995). Columns and resin used: Glass barrel Econo-columns, 0.7 x 15 cm (BioRad, Johannesburg, South Africa), Dowex 1; anion-exchange resin-AG 1 x 4, 4% cross-linkage, chloride form, 100-200 mesh (Sigma, Johannesburg, South Africa)

2.3.2 Tannin content

This was done by the Vanillin HCl assay of Price, Van Scoyoc, & Butler (1978). Reagent blanks that corrected for the colour of the extracts from the flour were included. Tannin content was expressed as mg Catechin Equivalents (CE) per 100 g sample (db). All sorghums were analysed for tannins and only non-tannin sorghums with no measurable tannin content was used in this study (results not displayed).

2.3.3 In vitro iron availability

This was determined according to the dialysis method of Luten, Crews, Flynn, Van Dael, Kastenmayer, Hurrell, Deelstra, Shen, Fairweather-Tait, Hickson, Farre, Schlemmer, & Frøhlich, (1996), with minor alterations. Due to potential precipitation of minerals, the tubing contents were acidified with 0.002 ml 65% nitric acid/ ml dialysate when decanted to keep minerals soluble. The mineral content of the flour and

dialysate were analysed through Iron Coupled Plasma Optical Emission Spectrometry (ICP-OES). The availability of the iron was calculated as the percentage of iron dialysable, compared to the total iron content. Enzymes used: Pepsin (P-7000), pancreatin (P-1750), bile extract (B-8631) (Sigma, Johannesburg, South Africa). Dialysis tubing used: Spectra/Por 7 (Ø = 20.4 mm) with a molecular mass cut-off (MMCO) of 10000 Da (Labretoria, Pretoria, South Africa).

2.3.4 Mineral analysis

Nitric-perchloric acid digestion on the wholemeal flour samples was done according to Zasoski & Barau (1977). The iron, zinc, calcium and phosphorus content of the flour samples were measured. Only the iron content of the dialysate was measured. The samples were analysed for these minerals by ICP-OES. Each element was measured in triplicate at an appropriate emission wavelength, chosen for high sensitivity and lack of spectral interferences.

2.3.5 Statistical analysis

All data were analysed with one way and/or multi-factor ANOVA, as appropriate.

3 Results and Discussion

3.1 Phytate content

The GMLP non-tannin and tannin sorghum flours had on average 38% and 36% reductions in phytate content, respectively, compared to their null controls (Table 1). The phytate content of the wholemeal sorghum flours varied between 772 and 1762 mg/100 g (Table 1). The phytate content of the GMLP sorghums was within the range

Table 1. Phytate contents (mg/100 g) of wholegrain flour and porridges as affected by sorghum type, genetic modification and fermentation.

Sample*	Whole grain flour	Thick unfermented porridge	Fermented flour	Thick fermented porridge	LS mean
Non-tannin control	1236^{cB} (69)	1165^{cB} (80)	395^{bB} (24)	212^{aB} (40)	740^{V}
Non-tannin GMLP	$772^{c,A}$ (48) [38%]	794^{cA} (57) [32%]	213^{bA} (26) [46%]	127^{aA} (58) [40%]	436^{U}
LS mean	979^{V}	943^{V}	310^{U}	168^{U}	
Tannin control	1762^{cB} (65)	1705^{cB} (667)	1467^{bB} (67)	1241^{aB} (123)	1544^{V}
Tannin GMLP	1121^{cA} (40) [36%]	1128^{cA} (64) [34%]	851^{bA} (48) [42%]	667^{aA} (89) [46%]	942^{U}
LS mean	1442^{V}	1417^{V}	1159^{U}	954^{U}	

[ ] Values in block parentheses are the % reduction in phytate of the GMLP sorghum relative to the value in its control.

Abc Values with different superscripts in the same row differ significantly ($p < 0.001$).
ABC Values with different superscripts in the same column differ significantly ($p < 0.001$).
UVW Values with different superscripts in the same column or row differ significantly according to multi-way analysis of variance ($p < 0.05$).
* Means and 1 standard deviation of two/three independent samples analysed in triplicate.

found for sorghum (300-2000 mg/100 g) (Lakshmi and Sumathi, 1997; Mahgoub & Elhag, 1998; Aldeyeye, Arogundade, Akintayo & Aisida, 2000; Oatway, Vasanthan & Helm, 2001).

Fermentation of the sorghum flours reduced the phytate content in the tannin and non-tannin sorghums by 17-24% and 68-72% compared to their respective flours. This reduction in sorghum phytate content due to fermentation has been well documented (Kayode, Nout, Bakker & Van Boekel, 2006; Oatway *et al.,* 2001; Feil, 2001, Matuschek, Towo & Svanberg, 2001). During fermentation, lactic acid bacteria ferment carbohydrates into various organic acids such as lactic acid, citric acid and acetic acid, which cause a reduction in pH to levels at which the enzyme phytase (endogenous or from lactic acid bacteria) can dephosphorylate phytate more effectively (Feil, 2001; Marfo, Simpson, Idowu & Oke, 1990).

With either the tannin and non-tannin sorghum, processing into thick unfermented porridge had no effect on phytate content. Heat treatment during the thick unfermented porridge preparation would have caused phytase to be denatured, before it could dephosphorylate and decrease the phytate content, as was also found by Mahgoub & Elhag (1998). However, extreme heat processing (e.g. autoclaving for 2-4 hours) can dephosphorylate phytate (Maga, 1982). Some studies have recorded some reduction in phytate content after traditional porridge preparation, but the processing methods included longer heat application than in this study (Marfo *et al.,* 1990) and preceding fermentation (Mahgoub & Elhag, 1998). Cooking of the fermented flours into a thick porridge further reduced the phytate contents of the tannin and non-tannin sorghum, by 15-22% and 40-46% of the phytate content in the

fermented flour. Marfo *et al.* (1990) and Mahgoub & Elhag, (1998) also found, that applying heat to fermented flour decreased the phytate content. This suggests that fermentation as a pre-treatment makes the phytate more susceptible to dephosphorylation during subsequent heat treatment.

After fermentation and porridge preparation, the GMLP sorghums still had phytate contents (tannin 34-46% and non-tannin 32-40%) lower compared with their controls. Thus the phytate reduction by genetic modification was still significant ($p<0.001$) even after the additional phytate reduction by fermentation.

3.2 Mineral content

The iron content of the sorghums varied between 4.7 and 8.4 mg/100 g flour (Table 2). The GMLP non-tannin sorghum had a much higher (approximately 2 fold) iron content compared to its control. As the grains were all grown under the same conditions and processed in the same manner, contamination can be ruled out as the reason for the high iron contents of the GMLP non-tannin sorghum compared to its control. It may have been possible that the genetic modification had an effect on the iron acquisition and storage in the plant. The modified grains were smaller than the controls and it is possible that the plant stored the same amount of iron in each grain, and subsequently this resulted in the higher concentration of iron.

The phosphorus contents varied between 314.4 and 351.0 mg/100 g in all the sorghums, but there was no significant difference ($p\geq0.005$) in phosphorus content between the GMLP sorghums and their controls. This finding is important because sorghum, like most grains have low phosphorus availability (Spencer, Allee, &

Table 2. Mineral (Fe, Ca, P) contents (mg/100 g flour, db) of tannin and non-tannin, genetically modified low phytate (GMLP) and sorghums and their null controls.

Sample*	Fe	Ca	P
Non-tannin control	5.4^{B} (0.3)	12.8^{A} (0.6)	314.4^{A} (4.1)
Non-tannin GMLP	8.4^{C} (0.2)	11.8^{A} (1.8)	320.0^{A} (8.9)
Tannin control	5.8^{B} (0.2)	24.1^{B} (1.1)	335.1^{B} (4.0)
Tannin GMLP	4.7^{A} (0.8)	19.9^{B} (4.4)	351.0^{B} (8.4)

ABC Values with different superscripts in the same column differ significantly ($p < 0.05$).

* Means and 1 standard deviation of two/three independent samples analysed in triplicate.

Sauber, 2000b). While phytate chelates other divalent minerals it also decreases the availability of phosphorus. The phosphorus bound within the phytate is not available for absorption (Spencer *et al.,* 2000b). If the phytate content is lowered without lowering the total phosphorus content as in these grains, it is probable that the amount of available phosphorus will increase (Spencer Allee, & Sauber, 2000a).

The calcium content of the tannin sorghums was significantly ($p<0.005$) higher than the non-tannin sorghums. It has been found that calcium inhibits iron absorption (House, 1999). Hallberg & Hulthén, (2000), however, found that only a calcium content higher than 50 mg/ 100g would inhibit non-heme iron availability. The calcium contents of these grains (11.8-24.1 mg/100g), however, are much lower and would probably not have any effect on the iron availability.

3.3 In vitro iron availability

In vitro iron availability, as measured by the dialysability assay in the sorghum flours varied between 5.8 and 13.3% (Table 3).

In both sorghum lines the thick unfermented porridge showed no significant ($p\geq0.05$) difference compared to its flour. Some researchers have also observed that heat processing had no effect on iron availability (Kayodé *et al.,* 2007; Hemalatha, Platel & Srinivasan, 2007b), while others have found a slight increase (Kayodé *et al.,* 2007; Hemalatha *et al.,* 2007b) or a reduction (Kayodé *et al.,* 2007). The fact that heat processing had no effect on the iron availability in this study may be due to its effect on the phytate (see 3.1) and tannin content. There was a reduction in the measurable tannin content (Table 4) after cooking the flour into unfermented porridge. However,

Table 3. In vitro available iron (%, db) of wholegrain flour and porridges as affected by sorghum type, genetic modification and fermentation.

Sample*	Whole grain flour	Thick unfermented porridge	Fermented flour	Thick fermented porridge	LS Mean
Non-tannin control	12.8^{aA} (1.3) [0.7]	10.6^{aA} (4.2) [0.6]	15.3^{bA} (4.2) [0.8]	17.6^{bA} (4.3) [1.0]	14.0^{U}
Non-tannin GMLP	13.3^{aA} (1.6) [1.1]	8.9^{aA} (2.2) [0.7]	28.7^{bB} (5.6) [2.4]	30.0^{bB} (5.4) [2.5]	20.2^{V}
LS Mean	13.1^{U}	10.0^{U}	22.2^{V}	24.5^{V}	
Tannin control	5.8^{aA} (0.8) [0.3]	5.8^{aA} (0.6) [0.3]	9.7^{bA} (1.0) [0.6]	6.5^{aA} (0.6) [0.4]	7.3^{U}
Tannin GMLP	6.5^{aA} (0.8) [0.3]	6.3^{aA} (1.7) [0.3]	12.9^{bB} (1.6) [0.6]	8.1^{aB} (1.8) [0.4]	8.5^{U}
LS mean	6.1^{U}	6.6^{UV}	11.3^{V}	7.3^{W}	

[] Values in block parentheses are the amount of available iron (mg/100 g, db).

abc Values with different superscripts in the same row differ significantly ($p < 0.05$).

ABC Values with different superscripts in the same column differ significantly ($p < 0.05$).
UVW Values with different superscripts in the same column or row differ significantly according to multi-way analysis of variance ($p < 0.05$).
* Means and 1 standard deviation of two/three independent samples analysed in triplicate.

Table 4. Tannin contents (mg CE/100 mg) of wholegrain flour and porridges as affected by genetic modification and fermentation.

Sample*	Whole grain flour	Thick unfermented porridge	Fermented flour	Thick fermented porridge	LS mean
Tannin control	1.83^{cB} (0.12)	0.45^{abB} (0.01)	0.53^{bB} (0.03)	0.33^{aB} (0.14)	0.54^{U}
Tannin GMLP	1.46^{cA} (0.10)	0.32^{bA} (0.14)	0.30^{bA} (0.03)	0.17^{aA} (0.09)	0.59^{U}
LS Mean	1.64^{V}	0.39^{U}	0.42^{U}	0.25^{U}	

Abc Values with different superscripts in the same row differ significantly ($p < 0.01$).
ABC Values with different superscripts in the same column differ significantly ($p < 0.01$).
UVW Values with different superscripts in the same column or row differ significantly according to multi-way analysis of variance ($p < 0.05$).
* Non-tannin sorghums were analysed for tannins and only sorghums with no measurable tannins were used (results not shown). Means and 1 standard deviation of two independent samples analysed in triplicate.

there was no increase in iron availability in the tannin sorghum (Table 3). A reduction in measurable tannin content after heat treatment may be due to the reaction of phenolic hydroxyl groups with food components such as protein and minerals, like iron, to form insoluble complexes (Matuschek *et al.,* 2001). Thus it is possible that the tannin content did not actually decrease and that the tannins may have been able to bind even more iron, than before the cooking. Kayodé *et al. (*2007) proposed this as a possible reason for the reduction of soluble iron observed after cooking tannin sorghum. The results for iron availability after thick unfermented porridge processing rather corresponds with the phytate contents (Table 1), which also showed no significant ($p \geq 0.001$) difference compared to the flour.

The fermented flours had significantly ($p < 0.05$) increased iron availability in both lines. The iron availability of the non-tannin and tannin fermented flours increasing by 16-54% and 40-50% respectively, compared to their wholemeal flours. The increased iron availability after fermentation in all the sorghums corresponded with the significant ($p < 0.001$) reduction in phytate content (Table 1). Many rural communities in Africa cultivate and consume tannin sorghums due its high resistance to pests (Matuschek *et al.,* 2001). It has been documented by other researchers that due to the high inhibitory affect of tannins on iron availability, any decrease in phytate content through phytase addition/processing is irrelevant when trying to increase iron availability in sorghum (Matuschek et al., 2001; Hurrell, Reddy, Juillerat & Cook, 2003; Towo, Matuschek & Svanberg, 2006). However, these researchers found that a reduction in both the phytate and tannin content led to increased iron availability. Matuschek *et al. (*2001) found that incubating sorghum with phytase decreased the

total phenolic content by 24% and suggested that the tannins may form complexes with the enzyme making them less assayable. While the formation of these complexes will decrease the measurable tannin content, tannins bound with the phytase will also not inhibit iron availability, possibly resulting in the increased iron availability observed in this study after fermentation. However, cooking of the fermented flours, as mentioned above, could have caused the tannins to bind more iron. This is probably the reason for the observed decrease in iron availability after the cooking of the tannin fermented flour. These results correspond with the above mentioned research, where a decrease of phytate content in tannin sorghums did not increase iron availability, without a decrease in tannin content.

Genetic phytate reduction did not significantly ($p<0.05$) increase iron availability in the non-tannin sorghum flour and thick unfermented porridge. This is probably due to the fact that the phytate content (Table 1) was not reduced enough. This is supported by the high phytate:iron molar ratios which ranged between 7.8 and 19.4. Different critical values above which iron availability is seriously impaired, has been published. These values include >1 (Hurrel, 2003) and >10-14 (Kayodé, Linnemann, Hounhouigan, Nout, & Van Boekel, 2006). While there was less phytate in the GMLP non-tannin sorghum, it is possible, that there was still enough phytate to bind most iron. The additive effect of the genetic phytate reduction and fermentation, in reducing the phytate content resulted in significantly ($p<0.05$) increased iron availability in the non-tannin sorghum. These samples also had significantly lower phytate:iron molar ratios which varied between 1.3 and 2.1. Comparing the porridges, the food consumed by target populations, the non-tannin GMLP fermented porridge had

significantly more available iron compared to the unfermented porridge. This suggest that porridges made from fermented non-tannin GMLP sorghum may supply 2-3 times the amount of available iron compared to a fermented porridge made from normal sorghum and unfermented porridge from the GMLP sorghum.

No published research could be found on the effect of reducing phytate content in sorghum through genetic modification on food iron availability. However, these findings can be compared to the work of Mendoza, Viteri, Lönnerdal, Young, Raboy & Brown (1998) on maize. They investigated the effect of GMLP maize, made into tortillas on iron availability. They conducted a human bioavailability study and found that tortillas made from GMLP maize, with a 65% phytate reduction, provided 33% more bioavailable iron compared to tortillas made from the same, but unmodified maize.

4 Conclusions

The reduction in phytate content in the sorghum lines by genetic modification (36-38%) is insufficient to bring about an improvement in iron availability in unfermented sorghum porridge. Also, the inhibitory effect of the tannins seems to prevent any increase in *in vitro* iron availability, regardless of the level of phytate reduction, in the tannin GMLP line. However, the additive effect of genetic modification in combination with fermentation in reducing the phytate content, appears to cause a substantial increase (at least 2 fold) in *in vitro* iron availability in non-tannin sorghum. This is probably of nutritional significance to communities in Africa, who consume sorghum as a staple.

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References

ABS. 2010. ABS Project Aim. http://biosorghum.org/abs_project.php. Accessed: 01 March 2011.

Aldeyeye, E.I., Arogundade, L.A., Akintayo, E.T., Aisida, O.A. & Alao, P.A. 2000. Calcium, zinc, and phytate interrelationships in some foods of major consumption in Nigeria. *Food Chemistry,* 71, 435-441.

Feil, B. 2001. Phytic acid. *Journal of New Seeds,* 3, 1-35.

FAO. 2007a. Food supply. http://faostat.fao.org/site/609/default.aspx#ancor Accessed: 01 March, 2011.

FAO, 2007b. Production. http://faostat.fao.org/site/567/default.aspx#ancor. Accessed: 01 March, 2011.

Frubeck, G., Alonso, R., Marzo, F. & Santidrian, S. 1995. A modified method for the indirect quantitative analysis of phytate in foodstuffs. *Analytical Biochemistry,* 225, 206-212.

Hallberg, L. & Hulthén, L. 2000. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron. *American Journal of Clinical Nutrition*, 71, 1147-1160.

Hemalatha, S., Platel, K. & Srinivasan, K. 2007b. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *Journal of Trace Elements in Medicine and Biology,* 21, 1-7.

House, W.A. 1999. Trace element bioavailability as exemplified by iron and zinc. *Field Crops Research***,** 60. 115-141.

Hunt, J.R. 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *American Journal of Clinical Nutrition,* 78, 633S-639S.

Hurrel, R.F. 2003. Influence of vegetable protein sources on trace element and mineral bioavailbility. *The Journal of Nutrition,* 133, 2973S-2977S.

Hurrell, R.F., Reddy, M.B., Juillerat, M. & Cook, J.D. 2003. Degradation of phytic acid in cereal porridges improves iron absorption by human studies. *American Journal of Clinical Nutrition,* 77, 1213-1219.

Kayodé, A.A.P., Linnemann, A.R., Hounhouigan, J.D., Nout, M.J.R. And Van Boekel M.A.J.S. 2006. Genetic and Environmental Impact on Iron, Zinc, and Phytate in Food Sorghum Grown in Benin. *Journal of Agricultural and Food chemistry,* 54, 256-262.

Kayodé, A.A.P., Linnemann, A.R., Nout, M.J.R. And Van Boekel M.A.J.S. 2007. Impact of sorghum processing on phytate, phenolic compounds and *in vitro* solubility of iron and zinc in thick porridges. *Journal of the Science of Food and Agriculture,* 87, 832-838.

Kayodé, A.A.P., Nout, M.J.R., Bakker, E.J. & Van Boekel M.A.J.S. 2006. Evaluation of the simultaneous effects of processing parameters on the iron and zinc solubility of infant sorghum porridge by response surface methodology. *Journal of Agricultural and Food Chemistry,* 54, 4253-4259.

Lakshmi, R.M. & Sumathi, S. 1997. Binding of iron, calcium and zinc by fibre of sorghum and ragi. *Food Chemistry,* 60, 213-217.

Luten, J., Crews, H., Flynn, A., Van Dael, P., Kastenmayer, P, Hurrell, R., Deelstra, H., Shen, L., Fairweather-Tait, S., Hickson, K., Farre, R., Schlemmer, U. & Frøhlich, W. 1996. Interlaboratory trial on the determination of the *in vitro* iron dialysability from food. *Journal of the Science of Food and Agriculture,* 72, 415-424.

Maga. J.A. 1982. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *Journal of Agricultural and Food Chemistry.* 30, 1-9.

Mahgoub, S.E.O. & Elhag, S.A. 1998. Effect of milling, soaking, malting, heat-treatment and fermentation of phytate level of four Sudanese sorghum cultivars. *Food Chemistry,* 61, 77-80.

Marfo, E.K., Simpson, B.K., Idowu, J.S. & Oke, O.L. 1990. Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea, and soybean. *Journal of Agricultural and Food Chemistry,* 38, 1580-1585.

Matuschek, E., Towo, E. & Svanberg, U. 2001. Oxidation of polyphenols in phytate-reduced high-tannin cereals: effect on different phenolic groups and on *in vitro* accessible iron. *Journal of Agricultural and Food Chemistry,* 49, 5630-5638.

Mendoza, C. 2002. Effect of genetically modified low phytic acid plants on mineral absorption. *International Journal of Food Science and Technology*, 37, 759–767.

Mendoza, C., Viteri, F.E., Lönnerdal, B., Young, K.A., Raboy, V & Brown K.H. 1998. Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *American Journal of Clinical Nutrition,* 68, 1123-1127.

Murray-Kolb, L.E. & Beard, J.L. 2009. Iron deficiency and child maternal health. *American Journal of Clinical Nutrition,* 89, 1S-5S.

Oatway, L., Vasanthan, T. & Helm, J.H. 2001. Phytic acid. *Food Reviews International,* 17, 419-431.

Price, M. L., Van Scoyoc, S. & Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry,* 26, 1214-1218.

Rosado, J.L. 2003. Zinc and copper: proposed fortification levels and recommended zinc compounds. *Journal of Nutrition,* 133, *2985S-2989S.*

Spencer, J.D., Allee, G.L. & Sauber, T.E. 2000a. Growing-finishing performance and carcass characteristics of pigs fed normal and genetically modified low-phytate corn. *Journal of Animal Science,* 78, 1529-1536.

Spencer, J.D., Allee, G.L. & Sauber, T.E. 2000b. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *Journal of Animal Science,* 78, 675-681.

Towo, E., Matuschek E. & Svanberg, U. 2006. Fermentation and enzyme treatment of tannin sorghum gruels: effects on phenolic compounds, phytate and *in vitro* accessible iron. *Food Chemistry,* 94, 369-376.

World Health Organization (WHO). 2006. Global database on anaemia. Geneva, Switzerland: WHO.

Zasoski, R.J. & Burau, R.G. 1977. A rapid nitric-perchloric acid digestion method for multi-element tissue analysis. *Communications in Soil Science and Plant Analysis,* 8, 425-436.

Zimmermann, M.B., Chaouki, N. & Hurrell, R.F. 2005. Iron deficiency due to consumption of a habitual diet low in bioavailable iron: a longitudinal cohort study in Moroccan children. *American Journal of Clinical Nutrition,* 81, 115-121.