

EFFECT OF GERMINATION ON MINERAL BIOAVAILABILITY OF SORGHUM-BASED COMPLEMENTARY FOODS

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ABSTRACT

Many people living in developing countries depend on diets based on cereal staples. Such diets lack diversity, which may result in micronutrient deficiencies. A complementary food made from cereals is often low in mineral content and contains significant quantities of mineral absorption inhibitors like phytic acid and condensed tannins. Anti-nutritional factors are plant constituents, which play an important role in humans, reducing the digestibility of nutrients and the absorption of minerals. Infant malnutrition due to nutritionally inadequate diets is one of the major concerns in Ethiopia. Children in rural Ethiopia are especially prone to micronutrient deficiencies as they eat from the family dish, which is predominantly plant-based. The main objective of this study is, therefore, to investigate the effect of germination on bio-availability of minerals of sorghum-based complementary foods. Two varieties of sorghum (*Sorghum bicolor* (L.) Moench) grains (varieties 76T1#23 and Meko) were collected, cleaned, soaked for 22 hours at room temperature ($22 \pm 2^{\circ}\text{C}$) and germinated for 36 and 48 hours at the soaking temperature. The germinated seeds were dried at 55°C for 24 hours and the ungerminated sorghum seeds were also dried at 55°C for 2 hours to facilitate milling. Both ungerminated and germinated sorghum grains were milled into a homogeneous fine powder. Germination of sorghum grains for 36 and 48 hours decreased phytic acid levels (mg/100g) significantly ($p < 0.05$) for variety 76T1#23 from 399.12 to 255.66 and 190.11, and from 464.94 to 293.18 and 203.76 for variety Meko, respectively. During germination of sorghum grains for 36 and 48 hours, molar ratio of phytate: iron was decreased significantly ($p < 0.05$) from 4.12 to 2.06 and 1.35 for variety 76T1#23, and from 5.49 to 2.35 and 1.58 for variety Meko, respectively. Similarly, germination of sorghum grains for 36 and 48 hours decreased significantly ($p < 0.05$) phytate:zinc molar ratio of sorghum flour from 21.18 to 12.76 and 9.31 for variety 76T1#23; and from 25.72 to 15.54 and 10.64 for variety Meko, respectively. In contrast, germination of sorghum grains for 36 and 48 hours increased significantly ($p < 0.05$) the contents of total phosphorus, non-phytate phosphorus, iron, zinc and calcium. Hence, germination appeared to be a promising food processing method to improve bioavailability of minerals and to decrease phytate levels, and therefore to decrease deficiencies of minerals in infants.

Key words: Germination, anti-nutritional factors, mineral bio-availability

INTRODUCTION

Good nutrition is essential for adequate growth and cognitive development of children and for protection against infection [1-4]. Many people living in developing countries depend on diets based on cereal staples. Such diets lack diversity, which may result in micronutrient deficiencies [5]. Minerals such as iron, zinc and calcium are essential micronutrients and play a vital role in growth, health and development of infants [6]. Zinc and iron are two of the micronutrients that are most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies. In children, mineral deficiency leads to poor growth, impaired immunity and increased morbidity from common infectious diseases and mortality [7]. Zinc and iron deficiency arises to a large extent from low contents of minerals in the diet and /or impaired bio-availability of minerals in the diet, largely attributable to the high phytic acid and tannin contents of the diets [7, 8]. Infant malnutrition due to nutritionally inadequate diets is one of the major concerns in Ethiopia. Children in rural Ethiopia are especially prone to micronutrient deficiencies as they eat from the family dish, which is predominantly plant-based [7].

A complementary food made from cereals is often low in mineral content and contains significant quantities of mineral absorption inhibitors, phytic acid and condensed tannins [9]. Phytic acid forms complex compounds with cations such as zinc, iron, magnesium and calcium at physiological pH of the plant. The mineral-phytic acid complex, phytate, is either insoluble or difficult to hydrolyze during digestion [10,11]. Phytic acid inhibits absorption of min by humans and other monogastric animals in a dose-dependent manner [7, 11, 12]. Phytic acid in cereal-based foods can be degraded by adding commercial exogenous phytases or by activating the native phytases by a combination of soaking and germination [12]. Condensed tannins are nutritionally important since they bind to and reduce the bio-availability of minerals and proteins. Tannin also inhibits the activity of some enzymes and, therefore, adversely influences protein digestibility and cellulose breakdown [13].

Sorghum (*Sorghum bicolor* (L.) Moench) is a critically important cereal crop in sub-Saharan Africa on account of its drought tolerance; hence, can consistently produce a crop under climatic conditions where other cereals fail. Sorghum is particularly adapted to drought prone areas: hot, semi-arid tropical environments [14]. Sorghum is the fifth most important cereal crop in the world next to wheat, rice, maize, and barley in terms of production. The annual production of sorghum is over 60 million tons on about 45 million hectares, of which Africa produces 40% [14-17]. It is believed that sorghum originated in Africa, more precisely in Ethiopia; between 5000 and 7000 years ago [16]. Although nutritionally sorghum is comparable with other grains, it is regarded as an inferior grain. In Ethiopia, sorghum flour is used to feed infants in the form of thick and thin porridge [16]. Since it is used without any treatment except cooking, the anti-nutritional factors affect the bio-availability of essential minerals.

Germination increases endogenous phytase activity which leads to phytate degradation. Germination also decreases the levels of phytic acid and condensed tannins present in cereals [18-22]. The main objective of this study was, therefore, to investigate the effect of germination on bioavailability of minerals of sorghum-based complementary foods.

MATERIALS AND METHODS

Sample collection

Two varieties of sorghum (*Sorghum bicolor* (L.) Moench) grains (varieties 76T1#23 and Meko), were collected for this study. Variety 76T1#23 was purchased from the Ethiopian Seeds Enterprise (ESE), Arsi Basic Seeds Storage and Preparation Center, Asella town, Arsi district, Ethiopia. The second variety, variety Meko, was obtained from the Agricultural Research Institute of Ethiopia (EARI), Melkassa Agricultural Research Center (MARC), Awash Melkassa town, Ethiopia.

Sorghum flour preparation

Sorghum grains collected were cleaned to remove stones, dust and light materials, glumes and stalks, and broken, undersized and immature grains. Cleaning was done by winnowing and hand sorting. The cleaned sorghum grains were divided into two portions. The first portion was not subjected to any treatment (served as control). The second portion was washed three times using deionized water and soaked in a volume of water 3 times the weight of seeds for 22 hours at room temperature ($22\pm 2^{\circ}\text{C}$). The steeping water was drained off and the soaked sorghum grains were washed twice using deionized water to protect the growth of microorganisms during germination. The soaked sorghum seeds were again divided into two sub-portions and the first sub-portion was germinated for 36 hours and the second sub-portion was germinated for 48 hours, at the soaking temperature and watered 2-3 times a day to enhance the germination process [22]. Germinated sorghum grains were washed with running deionized water and dried in a drying oven (Memmert, Germany) at 55°C for 24 hours. The ungerminated sorghum grains were also dried in a drying oven at 55°C for two hours to facilitate the milling process [23, 24].

The dried sorghum grains, both ungerminated and germinated, were separately milled to a fine homogeneous powder using a Cyclotec sample mill (Tecator, Hogans, Sweden) and then passed through a 1.18 mm aperture size laboratory test sieve (Endecotts Ltd., London, England). The milled samples were then packed in airtight polythene plastic bags and stored at room temperature until laboratory analysis.

Chemical analyses

Mineral determination: A fresh sample (ca. 2.5 g) was ashed at 550°C for six hours in a muffle furnace (Carbolite, Aston Lane, Hope, Sheffield, England, UK). When ashing was incomplete (when color of ash was not white), several drops of concentrated nitric acid were added and the samples were re-ashed for three more hours at 550°C . The ashed samples were dissolved in five milliliter 6N HCl and diluted to 50 ml with deionized water. The concentrations of iron, zinc and calcium

were determined in an aliquot using an atomic absorption spectrophotometer (Varian SpectrAA-20 Plus, Varian Australia Pty., Ltd., Australia). For the determination of calcium, lanthanum chloride (10% w/v) was added to both standards and samples to suppress interference from phosphorus [25]. The same digest was used to determine total phosphorus [26].

Phytic acid content determination: A fresh sample of sorghum flour (ca. 0.15g) was extracted with 10ml 2.4% HCl in a mechanical shaker for one hour at room temperature. The extract was centrifuged at 3000rpm for 30 minute (Dynac II centrifuge, Clay Adams, Bacton, Dickinson and Company, USA). One milliliter of Wade reagent (containing 0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% of sulfosalicylic acid in water) was added to threemilliliter of the sample solution (supernatant) and the mixture was mixed on a Vortex for five seconds. Phytic acid (analytical grade sodium phytate) was used as standard for phytic acid determination. The absorbance of both the sample and standard solutions were measured using deionized water as blank using UV-VIS spectrophotometer [27]. The calibration curve was constructed from a series of standard solution using SPSS version 15.

Condensed tannin determination: A fresh sample of sorghum flour (ca. 2.0g) was extracted with 10ml of 1% HCl in methanol for 24 hours at room temperature with a mechanical shaker and centrifuged at 1000rpm for five minutes (Dynac II centrifuge, Clay Adams, Bacton, Dickinson and Company, USA). One milliliter of supernatant was taken and mixed with fivemilliliter of Vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and four percent Vanillin in methanol). D-catechin was used as standard for condensed tannin determination (40mg of D-catechin was dissolved in 1000 ml of one percent HCl in methanol). The absorbance of the sample and standard solutions were measured using deionized water as blank UV-VIS spectrophotometer [28]. The calibration curve was constructed from a series of standard solution using SPSS Version 15. Concentration of tannin was read in mg of D-catechin per gm of sample.

Phytate phosphorus and Non-phytate phosphorus determination: Phytate phosphorus (mg/100 g) is equal to [phytic acid content in mg/100g x 0.2818]. Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus [10].

Data management and statistical analysis: Each determination was carried out in triplicate and results were reported as an averaged value (mean \pm standard deviation, mean \pm SD). Data were analyzed by the one-way analysis of variance (ANOVA) using SPSS Version 15.0. Differences between treatments were determined by the Fisher's Least Significance Difference (LSD) method. Statistical significance was set at $p < 0.05$.

RESULTS

Minerals content of sorghum flour

For varieties 76T1#23 and Meko, germination was found to increase significantly ($p < 0.05$) levels of minerals (iron, zinc and calcium). For both varieties, sorghum flour germinated for 48 hours contained highest minerals content while lowest values minerals were recorded for ungerminated sorghum flour (Table 1).

Total phosphorous, phytate phosphorous and non-phytate phosphorous contents

For both varieties, ungerminated sorghum flour contained lowest total phosphorous (TP) and non-phytate phosphorous (NPP) contents while sorghum flour germinated for 48 hours contained the highest value. As the content of non-phytate phosphorus increased, the phytate phosphorous (PP) content (mg/100g) was significantly ($p < 0.05$) decreased. In general, ungerminated sorghum flour sample contained highest phytate phosphorous whereas sorghum flour germinated for 48 hours contained the lowest value (Table 2).

Antinutritional factors

Ungerminated sorghum flour contained significantly ($p < 0.05$) highest phytic acid values whereas lowest values of phytic acid were recorded for sorghum flour germinated for 48 hours. The condensed tannin contents of sorghum flour investigated was below detection limit during laboratory determination (as mg of tannin per 100g of sorghum flour analyzed (Tables 3 and 4).

Bioavailability of minerals

Phytate:iron, phytate:zinc and calcium: phytate molar ratios

The results in Tables 3 and 4 indicated that germination decreased the phytate:iron and phytate:zinc molar ratios of sorghum flour, whereas, germination increased the calcium:phytate molar ratios.

DISCUSSION

The contents of zinc and calcium in ungerminated sorghum flour obtained in this study were comparable with values reported elsewhere whereas the values of iron were higher than values recorded in a previous study [7]. In the current study, germination increased iron, zinc, calcium and phosphorous contents of sorghum flour significantly ($P < 0.05$) (Tables 4 and 5), which were consistent with results of a previous study, which stated that the levels of certain minerals increased considerably in the case of germinated flours [23]. Similarly, a two-fold increase in iron level of sprouted hungry rice (acha) is observed [22]. The observed increase in minerals contents (iron, zinc, calcium and phosphorous) of sorghum flour during germination might be due to loss of water-soluble constituents during steeping and washing [10, 29]. During germination, the total phosphorous (TP) and non-phytate phosphorous (NPP) contents of the sorghum flour increased significantly while phytate phosphorous (PP) content was decreased by the same amount that non-phytate phosphorous increased, which is similar to results of a previous investigation [10].

Germination resulted in a considerable loss of phytic acid. As the period of germination was prolonged, a significant and successive reduction in phytic acid was witnessed; after 48 hours germination, a loss of up to 45% was observed [10]. These results are consistent with results obtained in the present investigation. Germination has been found to decrease the levels of anti-nutrients present in cereals and maximizes the levels of some of the utilizable nutrients, which is consistent with results of the present study [22]. The phytic acid content was reduced during germination due to an increase in the phytase activity, which degrades phytic acid in plant-based foods [10, 16, 22, 30], and which agrees with results of the present study.

Results obtained in the present study indicated that the two sorghum varieties investigated contain no condensed tannins since the seeds are without pigmented testa and red/brown pericarps. Sorghum varieties are divided into three types based on their genetics and chemical analyses. Of those, Type I sorghums ($b_1b_1B_2$, $B_1b_2b_2$, $b_1b_2b_2$) do not have a pigmented testa, and contain low levels of phenols and no tannins. The varieties of sorghum investigated in this study might be related to Type I sorghums with tannin-free contents, which are predominant types grown in the world [14]. In contrast, the red sorghum grain is classified as a high polyphenol sorghum variety containing a moderate amount of condensed polyphenols [17].

Phytate: zinc molar ratio is used to estimate the likely absorption of zinc from a diet. Diets with a phytate: zinc molar ratio greater than 15 have relatively low zinc bio-availability, those with phytate: zinc molar ratios between 5 and 15 have medium zinc bio-availability and those with a phytate: zinc molar ratio less than 5 have relatively good zinc bio-availability [7, 11].

Low values (phytate:zinc molar ratio <15) were found in sorghum flour germinated for 36 and 48 hours, indicative of favorable zinc bio-availability, whereas high values (>15) were found in ungerminated sorghum flour samples, indicative of low availability of zinc. In the present study, only sorghum flour sample of variety 76T1#23 germinated for 48 hours gave phytate: zinc molar ratios of less than 10.

The critical calcium: phytate molar ratio is 6:1. The calcium: phytate molar ratio >6 is indicative of unfavorable for calcium absorption [7]. All the sorghum flour samples analyzed in this study exhibited calcium: phytate molar ratios less than 6, which indicated that calcium is available for absorption from such diets.

The high calcium content of food may jeopardize bio-availability of iron and zinc. High calcium levels in foods can promote the phytate-induced decrease in zinc bio-availability when the $[\text{calcium}] \times [\text{phytate}] : [\text{zinc}]$ millimolar ratio exceeds 0.5 [7]. However, values observed in the sorghum flour samples analysed indicated that the possible contribution of calcium in the complementary foods in exacerbating the low bio-availability of zinc and iron due to phytate is probably minimal. When the phytate: iron molar ratio >0.15 , indicates low iron bioavailability [7]. All sorghum flour samples analyzed (both germinated and ungerminated) contain phytate: iron

molar ratios >0.15 . However, germination of sorghum grain resulted in a 3–4-fold reduction in the phytate: iron molar ratios.

CONCLUSIONS AND RECOMMENDATIONS

The use of germinated sorghum flour in the formulation of complementary foods provides gruels of low anti-nutrients content, therefore, bio-availability of nutrients. Utilization of simple utensils makes germination process suitable for low-income families living in rural areas in developing countries. Hence, germination is a promising food processing method for weaning food preparation, especially in developing countries.

Traditional food processing techniques used in Africa are deeply rooted in tradition and experience. For significant changes to be made in African food systems, researchers should not neglect existing traditional food technologies. The products of these technologies are vital to national survival and provide subsistence to majority of the people. It is recommended that animal feeding trials be carried out to evaluate the effects of germination on the nutritional qualities of complementary foods.

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Table 1: Effect of germination on minerals content of sorghum flour, mean \pm SD

Sample Code ^π	Variety 76T1#23			Variety Meko		
	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)
Ungerminated	8.21 \pm 0.69 ^a	1.86 \pm 0.87 ^a	17.09 \pm 0.83 ^a	7.19 \pm 0.29 ^a	1.78 \pm 0.51 ^a	20.99 \pm 0.63 ^a
Germinated 36	10.55 \pm 0.71 ^b	1.97 \pm 0.51 ^b	25.85 \pm 0.64 ^b	10.60 \pm 1.13 ^b	1.86 \pm 0.63 ^b	27.68 \pm 0.28 ^b
Germinated 48	11.99 \pm 0.91 ^b	2.01 \pm 0.65 ^b	25.93 \pm 0.45 ^b	10.98 \pm 0.89 ^b	1.89 \pm 0.71 ^b	29.62 \pm 0.47 ^b

Values within the same column with different superscript letters are significantly different from each other (at $p < 0.05$).

^π Ungerminated = 100% made from ungerminated sorghum flour (control); Germinated 36 = 100% made from sorghum flour germinated for 36 hours and Germinated 48 = 100% made from sorghum flour germinated for 48 hours.

Table 2: Effect of germination on total phosphorous, phytate phosphorous and non-phytate phosphorous contents of sorghum flour (mg/100 g), mean \pm SD

Sample Code ^π	Variety 76T1#23			Variety Meko		
	Total phosphorous	Phytate phosphorous	Non-phytate Phosphorous	Total phosphorous	Phytate phosphorous	Non-phytate Phosphorous
Ungerminated	208.42 \pm 1.54 ^a	112.47 \pm 0.59 ^a	95.95 \pm 1.23 ^a	183.04 \pm 2.3 ^a	131.02 \pm 0.94 ^a	52.02 \pm 0.87 ^a
Germinated 36	221.49 \pm 0.98 ^b	72.04 \pm 1.11 ^b	149.45 \pm 1.31 ^b	192.25 \pm 1.47 ^b	82.62 \pm 0.51 ^b	109.63 \pm 0.96 ^b
Germinated 48	223.26 \pm 1.13 ^b	53.57 \pm 0.91 ^c	169.69 \pm 1.18 ^c	193.56 \pm 1.73 ^b	57.42 \pm 0.63 ^c	136.14 \pm 1.14 ^c

Values within the same column with different superscript letters are significantly different from each other (at $p < 0.05$).

^π Ungerminated = 100% made from ungerminated sorghum flour (control); Germinated 36 = 100% made from sorghum flour germinated for 36 hours and Germinated 48 = 100% made from sorghum flour germinated for 48 hours.

Table 3: Effect of germination on phytate, phytate: iron, phytate: zinc, calcium:phytate, [calcium][phytate]:zinc contents of sorghum flour (variety 76T1#23)

Sample Code ^π	Condensed tannin	Phytate (mg/100g)	Phytate/iron* (molar ratio)	Phytate/zinc ^ψ (molar ratio)	Calcium/phytate ⁶ (molar ratio)	[phytate x Ca]/Zn ^ω (mol/Kg)
Ungerminated	ND	399.12 ± 3.7 ^a	4.12 ± 0.4 ^a	21.18 ± 0.2 ^a	0.71 ± 0.1 ^a	0.09
Germinated 36	ND	255.66 ± 2.3 ^b	2.06 ± 0.2 ^b	12.76 ± 0.1 ^b	1.67 ± 0.3 ^b	0.08
Germinated 48	ND	190.11 ± 1.2 ^c	1.35 ± 0.1 ^c	9.31 ± 0.6 ^c	2.25 ± 0.5 ^c	0.06

Values within the same column with different superscript letters are significantly different from each other (at p<0.05). ND= Not Detected

*= mg of Phytate/MW of Phytate: mg of iron/MW of iron

^ψ= mg of Phytate/MW of Phytate: mg of Zinc/MW of Zinc

⁶= mg of Calcium/MW of Calcium: mg of phytate/MW of phytate

^ω = (mol/kg Phytate) x (mol/kg Calcium)/(mol/kg Zinc)

^π Ungerminated =100% ungerminated sorghum flour (control); Germinated 36= 100% sorghum flour germinated for 36 hours and Germinated 48= 100% sorghum flour germinated for 48 hours

Table 4: Effect of germination on phytate, phytate: iron, phytate: zinc, calcium: phytate, [calcium][phytate]: zinc contents of sorghum flour (for variety Meko)

Sample Code ^π	Condensed tannin	Phytate (mg/100g)	Phytate/iron* (molar ratio)	Phytate/zinc ^ψ (molar ratio)	Calcium/phytate ⁶ (molar ratio)	[phytate x Ca]/Zn ^ω (mol/Kg)
Ungerminated	ND	464.94 ± 5.7 ^a	5.49 ± 0.6 ^a	25.72 ± 0.9 ^a	0.75 ± 0.52 ^a	0.14
Germinated 36	ND	293.18 ± 6.2 ^b	2.35 ± 0.5 ^b	15.54 ± 0.4 ^b	1.56 ± 0.34 ^b	0.11
Germinated 48	ND	203.76 ± 3.6 ^c	1.58 ± 0.4 ^c	10.64 ± 0.6 ^c	2.40 ± 0.84 ^c	0.08

Values within the same column with different superscript letters are significantly different from each other (at p<0.05). ND= Not Detected

*= mg of Phytate/MW of Phytate: mg of iron/MW of iron

^ψ= mg of Phytate/MW of Phytate: mg of Zinc/MW of Zinc

⁶= mg of Calcium/MW of Calcium: mg of phytate/MW of phytate

^ω = (mol/kg Phytate) x (mol/kg Calcium)/(mol/kg Zinc)

^π Ungerminated =100% made from ungerminated sorghum flour (control); Germinated 36= 100% made from sorghum flour germinated for 36 hours and Germinated 48= 100% made from sorghum flour germinated for 48 hours.

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