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ORIGINAL ARTICLE

Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*)

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Abstract The effect of germination on the level of phytase activity and the contents of phytates and phosphorus of five Nigeria grown cereal grains was studied. The cereals screened were rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). Phytase activity was high (0.21–0.67 U g^{-1}) in all samples. Phytate content ranged between 5.6 and 6.2 mg g^{-1} while total phosphorus content ranged between 3.3 and 4.3 mg g^{-1}. During germination, the level of phytase activity increased and reached its maximal value after seven (16-fold), six (5-fold), five (7-fold), seven (3-fold) and eight (6-fold) days of germination for rice, maize, millet, sorghum and wheat respectively. After this initial increase, phytase activity declined slightly ($P<0.05$). The increase in phytase activity during germination was accompanied by a significant reduction in phytate ($P<0.05$) and a small but significant increase in total phosphorus.

Keywords Cereal grains · Phytase activity · Phytate · Phosphorus · Germination

Introduction

Cereals and legumes form a significant portion of the food supply for humans and other animals, as they are a major source of carbohydrates, proteins, lipids and minerals (Katina et al. 2005). The presence of antinutrients such as phytate has been a major limiting factor to the extensive utilization of these plant resources (Al-Numair et al. 2009). Phytate, myo-inositol (1,2,3,4,5,6) hexakisphosphate, is present in high concentrations in many food items derived from plants. It is the major storage form of phosphorus in mature grains and legumes (Kumar et al. 2010). Its hydrolysis is catalysed by the enzyme phytase (myoinositol hexaphosphate phosphohydrolase) to inositol and orthophosphate. About 60–90% of total phosphorus in plant feedstuffs is bound as phytate phosphorus (Wu et al. 2009). Phosphorus, in this form, is not utilised by human beings, birds or agastric animals because they lack sufficient endogenous intestinal phytase which releases orthophosphate from the phytate molecule in the gastrointestinal tract (Holm et al. 2002). The unabsorbed phytate is excreted and contributes to phosphate pollution in water bodies downstream of agriculturally intensive areas (Sharpley 1999). Furthermore, phytate works in a broad pH-region as a highly negatively charged ion and therefore its presence in the diet has a negative impact on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} (Wu et al. 2009). The need to maintain sufficient dietary phosphorus levels while reducing phosphorus excretion in poultry manure has led to an increase in the application of phytase to poultry feed (Hatten et al. 2001). The phytases (*myo*-inositol hexakisphosphate phosphohydrolases) are a subfamily of the high-molecular-weight histidine acid phosphatases. They catalyse the phosphate monoester hydrolysis of phytate, which results in the stepwise formation of *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates, as well as the liberation of inorganic phosphate (Sathe and Reddy 2002). The use of phytase reduces phosphorus excretion in poultry

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manure by allowing the birds to utilize more of the phytate phosphorus (Sharpley 1999). The use of phytase may also free cations and proteases bound in phytate phosphorus complexes and improve many production parameters and body structure characteristics in broilers and laying hens, such as body weight, bone ash content, feed consumption, egg weight, and egg shell quality (Hatten et al. 2001). The phytases used for this purpose are usually of microbial origin and the technology involved in the extraction, purification and thermo-stabilization of the enzymes is still beyond the reach of developing countries especially in Africa. High native phytase activities are present in cereals and cereal by-products (Steiner et al. 2007). It appears that phytase activity usually increases on germination (Sung et al. 2005) and germination has been used to induce phytase activity in cereals (Senna et al. 2006). The current study was conducted to assess the effect of germination on phytase activities and the contents of phytate and total phosphorus in samples of different Nigerian grown cereal grains. This information would help to determine the extent of germination that is likely to result in high yield of phytase enzyme.

Materials and methods

Samples The cereals screened are: rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). Rice and maize were cultivated in Mid-Western Nigeria while soghun, millet and wheat were cultivated in Northern Nigeria. They were harvested in 2008 and stored for 10–12 months at 25–30°C and 55–65% Relative Humidity prior to analyses. During analyses grains had moisture contents of 9.0 to 13.0%.

Germination of cereal grains For surface sterilization, seeds were soaked in 0.5% NaOCl for 3 min and then in 0.75% H_2O_2 for 2 min. After soaking, seeds were thoroughly rinsed with sterile water. The pretreated seeds were strained through two layers of gauze before spreading on plastic trays overlaid with wet tissue papers. The seeds were then allowed to germinate in disinfected dark cupboards at 24°–28°C for 0–10 days. The seeds were rinsed once a day with sterile water during the period of germination. After harvesting, the rootlets and sprouts were separated when vegetative growth was observed and only grain matter used. Seeds to be used for the determination of Phytate and total phosphorus were oven dried at 60°C for about 18 h, allowed to cool and then milled to a particle size of <0.5 mm.

Enzyme preparation The preparation of the crude enzyme was basically as described by Senna et al. (2006). Fresh samples of ungerminated and germinated seeds were homogenized in 0.1 M sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 12,000×g for 5 min. The pellets were discarded and supernatants were used for enzyme assays, described as total homogenate. All procedures were carried out at 4°C.

Assay of the enzyme activity Enzyme activity was determined at 40°C. The assay mixture consisted of 350 µL of 0.1 M sodium acetate buffer, pH 5.0 and 100 µL of 2 mM sodium phytate. This mixture was pre-incubated for 10 min at 40°C and the enzymatic reactions were started by adding 100 µL of the crude enzyme to the pre-incubated assay mixtures. After incubation at 40°C for 30 min, the liberated phosphate was measured by using the ammonium molybdate method (Heinonen and Lahti 1981). Added to the assay mixture were 1.5 mL of a freshly prepared solution of acetone/5 N H_2SO_4/10 mM ammonium molybdate (2:1:1 v/v) and 100 µL of 1.0 M citric acid. Any cloudiness was removed by centrifugation prior to measurement of the absorbance at 355 nm. To calculate the enzyme activity, a calibration curve was produced over the range of 5–600 nmol of phosphate (ϵ=8.7 $cm^2 \mu mol^{-1}$). Activity (units) was expressed as 1 µmol of phosphate liberated per minute. Blanks were run by adding the ammonium molybdate solution prior to adding the enzyme to the assay mixture.

Phytate extraction and determination Phytate extraction and determination was essentially according to the method of Wheeler and Ferrel (1971) with slight modifications. Instead of extraction using 3% TCA, 1.2 M HCl containing 10% Na_2SO_4 was used because of excessive foaming with TCA (Azeke et al. 2005).

Total phosphorus determination The method of Jacobs (1999) was applied for the determination of total phosphorus. This involved digestion of seeds (5 g) with 30 ml conc. HNO_3 and 20 ml conc. H_2SO_4.

Statistical analysis All assays were carried out in triplicates. Mean values with standard deviations were computed. Data were subjected to analysis of variance and read at 0.05 confidence level. Means that differed significantly were shown by the Tukey-Kramer test using the GraphPad InStat version 2.04a (GraphPad Software Inc, San Diego, California, USA, www.graphpad.com)

Results and discussion

Distribution of phytase activity, phytate and total phosphorus in cereal grains The phytase activities, phytate and

Table 1 The phytase activity, total phytate and total phosphorus contents of selected Nigerian grown cereal grains

	Rice	Maize	Millet	Sorghum	Wheat
Phytase (U/g)	0.41±0.01a	0.23±0.02b	0.37±0.04c	0.21±0.06b	0.67±0.01d
Phytate (mg/g)	5.8±0.61a	5.9±0.66a	5.7±0.28a	6.2±1.01b	5.6±0.61c
Phophorus (mg/g)	4.3±0.62a	3.9±0.65b	3.6±0.73c	4.0±0.96d	3.3±0.17e

Results are mean±SD of three independent determinations. Values in a row with different letters are significantly different ($P<0.05$).

total phosphorus contents of some Nigerian grown cereal grains are shown in Table 1. With the exception of maize and sorghum significant differences ($P<0.05$) were observed in the phytase activities (0.21–0.67 U g^{-1}) of the cereal grains screened. Steiner et al. (2007) reported a range of 0.4–6.0 U g^{-1} for oats, barley, triticale, rye and wheat. They reported 2.8 U g^{-1} for wheat, which is higher than that presented in this study (0.67 U g^{-1}), while Cossa et al. (2000) reported 0.520–1.4 U g^{-1} for wheat, which is in agreement with the present study.

In contrast to the findings of the present study, literature data show in general a larger variation in phytase activities in cereals. For example, as reported by Cossa et al. (2000), phytase activities in wheat were 0.520–1.4 U g^{-1}. One factor responsible for the large variations in literature values is the differences in analytical procedures used for the determination of phytase activity. As previously shown by Greiner and Egli (2003), phytase activities in cereals based on extraction methods are considerably lower than those obtained by means of direct incubation methods (Steiner et al. 2007). According to Greiner and Egli (2003) the incomplete extraction of plant phytases might be attributed to proteolytic degradation and a partial association of the enzymes with membrane structures. Differences in the composition of the cell walls, interaction of phytases with morphological grain fractions and differences in the location of the enzymes within the grains are considered to be additional factors responsible for the incomplete and different extraction rates of plant phytases (Steiner et al. 2007). Generally, high native phytase activities are present in cereals and cereal by-products, whereas lower activities have been reported for legume seeds (Steiner et al. 2007).

The phytate contents of the screened cereal grains ranged from 5.58 to 6.23 mg g^{-1}. Non-significant differences were found in the phytate contents of rice, maize and millet ($P>0.05$) while the phytate contents of sorghum and wheat were significantly different ($P<0.05$). These values agree with already published data for different cereals (Wu et al. 2009; Kumar et al. 2010). Nour et al. (2010), however, reported a much lower value (1.99–3.11 mg g^{-1}) for sorghum. Phytate is formed during maturation of plant seeds and so differences in phytate contents of cereal grains may be due to differences in degree of maturation during harvest, genetics, environmental fluctuations, location, irrigation conditions, soil type, year, and fertiliser application (Wu et al. 2009). The daily intake of phytate for humans on vegetarian diets, on an average, is 2,000–2,600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150–1,400 mg (Reddy 2002). Usually legume based food (cooked) items contain higher amounts phytate than do cereal-based food items.

The total phosphorus content observed in this study was between 3.33 mg g^{-1} (for wheat) and 4.34 mg g^{-1} (for rice). This again is in agreement with reported values for some cereals. For example, Nour et al. (2010) reported a range of 4.41–4.58 mg g^{-1} for three sorghum cultivars. Larsson et al.

Table 2 Phytase activity (U g^{-1} dry matter) during germination of cereal grains

Days of Germination	Rice	Maize	Millet	Sorghum	Wheat
0	0.41±0.01a	0.23±0.02a	0.37±0.04a	0.21±0.06a	0.67±0.01a
1	0.48±0.14a	0.24±0.02a	0.39±0.07a	0.19±0.04a	0.71±0.03a
2	0.61±0.09b	0.46±0.09b	0.53±0.09b	0.32±0.07b	0.92±0.32b
3	1.0±0.40c	0.61±0.07c	0.90±0.08c	0.43±0.09c	1.0±0.03b
4	2.2±0.39d	0.91±0.09d	2.0±0.78d	0.49±0.03cd	1.4±0.11c
5	3.2±0.45e	0.98±0.07d	2.6±0.37e	0.53±0.09d	2.0±0.21d
6	5.8±0.85f	1.3±0.09e	2.1±0.11d	0.57±0.06d	3.0±0.45e
7	6.9±0.82h	1.2±0.08f	1.9±0.10f	0.59±0.09d	3.7±0.12f
8	4.2±0.571	1.0±0.10d	1.2±0.10g	0.42±0.08c	3.9±0.23g
9	3.4±0.23j	1.0±0.07d	1.2±0.03h	0.40±0.04c	3.9±0.11g
10	3.0±0.13e	0.97±0.08d	1.1±0.10ch	0.36±0.04cb	3.8±0.09f

Results are mean±SD of three independent determinations. Values in a column with different letters are significantly different ($P<0.05$).

Table 3 Phytate content (mg g^{-1} of dry matter) during germination of cereal grains

Days of germination	Rice	Maize	Millet	Sorghum	Wheat
0	5.8±0.61a	5.9±0.66a	5.7±0.28a	6.2±1.01a	5.6±0.61a
1	5.6±0.67ab	5.9±0.88a	5.6±0.16a	6.0±0.46a	5.4±0.09a
2	5.5±0.29b	5.2±0.23b	5.2±0.39b	5.6±0.88b	5.1±0.10b
3	4.9±0.47c	4.8±0.51c	4.8±0.27c	4.9±0.22c	4.6±0.11c
4	3.0±0.55c	3.1±0.62d	4.0±0.77d	4.1±0.43d	4.2±0.05d
5	2.5±0.56d	2.7±0.22e	3.6±0.67e	3.3±0.37e	3.7±0.03e
6	1.2±0.29e	2.4±0.31f	2.8±0.52f	2.3±0.29f	3.1±0.91f
7	1.1±0.18ef	1.9±0.27g	2.2±0.20g	2.0±0.21g	2.1±0.03g
8	1.0±0.15f	0.93±0.22h	1.1±0.05h	1.7±0.09g	1.0±0.02h
9	1.1±0.23f	0.88±0.07h	0.89±0.09h	1.4±0.11h	0.88±0.01hi
10	0.96±0.27f	0.72±0.16h	0.85±0.11h	1.2±0.07i	0.79±0.02i

Results are mean±SD of three independent determinations. Values in a column with different letters are significantly different ($P<0.05$).

(1997) reported a range of 1.3–4.0 mg g^{-1} for white wheat and whole wheat while Barrier-Guillot et al. (1996) reported a range of 2.25–4.24 mg g^{-1} for 56 different wheat cultivars. This narrow range of phosphorus content shows that not only the phosphorus status of soil or fertilizer determines phosphorus contents of grains but also root uptake of phosphorus (Harrison and Dighton 1991). It has been found that about 60–90% of total phosphorus is bound as phytate phosphorus in cereals and legumes (Wu et al. 2009).

Effect of germination on phytase activity Germination resulted in significant increases ($P<0.05$) in phytase activity of all samples screened (Table 2). This increase may be as a result of *de novo* synthesis of the enzyme during germination (Sung et al. 2005). For rice and sorghum, the level of phytase was highest on the 7th day of germination (16- and 3-fold respectively), while for maize, millet and wheat, phytase activity was highest on the 6th (5-fold), 5th (7-fold) and 8th (6-fold) dayof germination respectively. After this increase, phytase activity declined slightly ($P<0.05$) probably due to the degradation of the enzyme by active protease (Houde et al. 1990) or due to inhibition by accumulating phosphate (Sung et al. 2005). A similar trend was reported for germinating rice (Kikunaga et al. 1991), lentil (Greiner 2002), barley (Sung et al. 2005) and soybean (Prazeres et al. 2004). While a maximum of 16-fold increase in phytase activity was observed for rice grains in this study, Kikunaga et al. (1991) reported a maximum of 7-fold increase also after germinating rice grains for 10 days.

Sung et al. (2005) reported that germination temperature affects intrinsic phytase acitivity. They observed that germinating barley at 25°C resulted in higher phytase activity than at 20°C or 15°C. Germination in this study was carried out at the prevailing room temperature of 25–32°C. A 24 h time lag was observed in all the samples before any significant increase in phytase activity was recorded. A similar time lag was also reported by Konietzny et al. (1995) for germinating spelt, Greiner (2002) for lupine seeds and Sung et al. (2005) for barley. It is likely that during the germination the expression of phytase delayed relatively even when other proteins are being synthesized. Many roles have been postulated for plant phytase during seed germination and seedling growth. The current belief is that such enzymes contribute to the

Table 4 Total phosphorus content (mg g^{-1} of dry matter) of germinating cereal grains

Days of germination	Rice	Maize	Millet	Sorghum	Wheat
0	4.3±0.62a	3.9±0.65a	3.6±0.73a	4.0±0.96a	3.3±0.17a
1	4.3±0.08a	3.9±0.22a	3.6±0.69a	4.0±0.11a	3.2±0.11a
2	4.4±0.17a	4.0±0.11ab	3.6±0.67a	4.0±0.09a	3.3±0.08a
3	4.4±0.53a	4.2±0.08b	3.6±0.10ab	4.2±0.12b	3.4±0.03b
4	4.8±0.64b	4.2±0.55b	3.7±0.05b	4.2±0.53b	3.4±0.09b
5	4.9±0.15b	4.3±0.61b	3.9±0.19c	4.3±0.56b	3.4±0.06b
6	4.9±0.29b	4.2±0.23b	3.9±0.20c	4.3±0.23b	3.5±0.21bc
7	4.8±0.43b	4.2±0.44b	3.9±0.89c	4.4±0.90c	3.5±0.12c
8	4.9±0.96b	4.3±0.21bc	4.0±0.56c	4.4±0.19c	3.5±0.07c
9	4.9±0.68bc	4.3±0.25c	4.2±0.27d	4.5±0.67d	3.6±0.05cd
10	5.0±0.44c	4.4±0.33c	4.3±0.19d	4.5±0.24d	3.6±0.11d

Results are mean±SD of three independent determinations

Values in a column with different letters are significantly different ($P<0.05$)

mobilization of phosphate from macromolecular organic phosphates (Duff et al. 1994). Due to high native phytase activities, germinated und ungerminated cereals have the potential to contribute substantially to the gastrointestinal hydrolysis of phytate in non-ruminant animals.

Effect of germination on phytate contents Germination for 10 days resulted in a significant reduction ($P<0.05$) in the phytate contents of all cereal grains screened (Table 3). The reductions were 81–88% for all samples. Non-significant changes ($P>0.05$) in phytate contents were observed within the first 24 h of germination, which also coincided with the initial lag phase observed for phytase activity (Table 2). The maximum reduction in phytate content occurred in rice at the 7th day of germination and in maize, millet and wheat at the 8th day while in sorghum at the 10th day. (Table 3). This also coincides with the reduced phytase activity observed for the same period. During the germination of cereals, phytate is degraded by intrinsic phytase enzyme (Kumar et al. 2010).

Naturally, there are dissimilarities in the capacities of various plant and microbial species to dephosphorylate phytate, due to differences in their intrinsic phytate-degrading activities (Egli et al. 2002) and the properties of the enzymes, such as protein stability and pH, as well as temperature optimum for phytate degradation (Konietzny and Greiner 2002). The degree of illumination during germination has been found to be an important factor in the reduction of phytates, with germinating under blue or red light being more effective than germinating in the dark (Khattak et al. 2007). Plant seeds utilise phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value. Many researchers such as Egli et al. (2002) have reported little intrinsic phytate-degrading activity in non-germinated cereal grains.

Effect of germination on phosphorus contents It appears that the effect of sprouting on the phosphorus contents of cereal grains was not as pronounced as on phytase and phytate. After germinating for 10 days, phosphorus contents of screened cereal grains increased by 14.1% for rice, 12.6% for maize, 19.7% for millet, 12.5% for sorghum and 8.4% for wheat (Table 4). The increase in total P resulted from changes in dry matter due to sprouting process. Nour et al. (2010) reported an increase of 19.1–35.6% for three varieties of sorghum after sprouting for 3 days while Hahm et al. (2009) reported a 6% increase in phosphorus after germinating sesame seeds for 3 days. They attributed the increase to phosphate translocation, which plays a significant role in grain metabolism during germination. Three days germination of seed samples in the present study resulted 1.6–7.4% increase in phosphorus, which agrees with these previous reports. Differences in cultivars could also account for some differences in phosphorus values in the present report when compared to literature values. Al-Numair et al. (2009) reported phosphorus increases of 6, 8 and 10% respectively for three cultivars of the same white beans (*Phaseolus vulgaris*) after germinating for 4 days. They also reported phosphorus increases of 1 and 8% for two cultivars of faba beans (*Vicia faba*) after germinating for same period. This is a possibility since increase in phytase activity during germination can result in increased phosphorus availability. During germination, phytase sequesters orthophosphate groups from the inositol ring of phytic acid to produce free inorganic phosphorus, along with a chain of intermediate myo-inositol phosphates (inositol pentaphosphate to inositol monophosphate) (Debnath et al. 2005). By releasing bound phosphorus in feed ingredients of vegetable origin, phytase makes more phosphorus available for bone growth and protects the environment against phosphorus pollution (Baruah et al. 2007).

Conclusion

Significant differences ($P<0.05$) were observed in the phytase activities of the different cereal grains screened and germination for 10 days resulted in significant increases in activity. Increase in phytase activity was accompanied by a significant decrease in the content of the antinutrient, phytate and slight increase phosphorus. The increase in phytase activity and the accompanying decrease in phytate content are expected to improve phosphorus availability and utilization, which would reduce phosphorus pollution. It is recommended that different cultivars of samples of these cereal grains and from different farmlands be screened for same parameters in order to find cultivars with higher phytase activity.

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