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Quality of protein and minerals as influenced by antinutrients of grains of sorghum cultivars grown under different levels of micronutrients

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Abstract

The seeds of two cultivars of Sudanese sorghum *(Sorghum bicolor)*, namely Tabat and Tetron, were grown under different levels of micronutrients (0, 2, 4 and 8 gm/5 kg soil) for two consecutive seasons. Antinutrients (tannin and phytate) content and *in vitro* available protein and minerals of the flour of the harvested seeds were examined. The results showed that both tannin and phytate were reduced with micronutrients level. Fertilization of Tabat seeds with a maximum dose of 8 gm/5kg soil, reduced tannin content by 73 and 50% and that of Tetron reduced by 50 and 83% during first and second growing seasons, respectively. Moreover, Phytate content of Tabat was reduced by 29 and 27% while that of Tetron was reduced by 20 and 17% during the growing seasons, respectively. The reduction in antinutrients was accompanied by a significant ($P \leq 0.05$) increase in protein digestibility and minerals extractability for both cultivars and during both seasons.

Introduction

Sorghum is a staple food in many African countries and it contains a reasonable amount of protein (7.5– 10.8%), ash (1.2–1.8%), oil (3.4–3.5%), fibre (2.3–2.7%) and carbohydrate (71.4–80.7%) with a dry matter ranging from 89.2% to 95.3% (AbdelRahaman *et al*., 2005). Attempts to reduce antinutrients effects have been tried by different means including conventional breeding (Abdelseed *et al*., 2011) and soaking (Elmaki *et al*., 1999) and malting (Idris *et al*., 2006) of sorghum grains, fermentation of sicklepod leaves (Osman *et al*., 2010) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier *et al*., 1996). Moreover, vigorous efforts are directed towards coupling the beneficial effects of tannins in sorghum as a field crop with methods for overcoming the antinutritional effects of tannins in seeds by direct removal of seed testa, inactivation or by extraction.

Extractable tannin content was markedly reduced when sorghum grains were soaked in water and germinated for different periods of time (Elmaki *et al*., 1999). Most of sorghum cultivars grown in Sudan contained only 9 to 11% protein content and most of the amino acids are limited (Elbashir *et al.,* 2008), which is critical for determining the nutritional value of foods. It is evident that the nutritional importance of a given food/feed stuff depends not only on nutrient composition or content, but also on the amount utilized by consumers (Vijayakumari *et al*., 1998). Soil pH influences solubility, concentration, ionic form and mobility of elements and consequently acquisition of such elements by plants (Fageria *et al*., 1997). The deficiency of essential micronutrients induces abnormal plant tissue pigmentation, size, and shape, which causes low leaf photosynthetic rates, and leads to various undesirable outcome, such as grain yield and composition (Masoni *et al*., 1996).

Today, although the production of energy and protein appears to be adequate to feed the developed world, agriculture systems in many developing countries still do not provide enough nutrients to meet human needs (Welch and Graham, 2004). Different approaches have been tried to improve the nutritional value of sorghum grain such as fermentation and malt pretreatment (Abdelhaleem *et al*., 2008) and supplementation with legumes (Asma *et al*., 2006), cluster bean (ELbashir *et al*., 2008) and pigeon pea (Abdallah *et al*., 2010) as well as conventional breeding (Abdelseed *et al*., 2011). Moreover, previous research focused only on the effect of micronutrients fertilization on total

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yield. However, in a previous study (Osman *et al.,* 2014) we observed that micronutrients fertilization improved the nutritional quality of sorghum cultivar (Gadambalia) seeds grown for two consecutive seasons. Therefore, the objective of this study was to investigate the *in vitro* available protein and minerals as influenced by antinutrients of flour of common sorghum cultivars (Tabat and Tetron) seeds grown for two consecutive seasons under different level of micronutrients fertilization.

Materials and Methods

Materials

Grains of two sorghum (*Sorghum biocolor* L. Monech) cultivars namely; Tabat and Tetron were obtained from the Department of Agronomy, Faculty of Agriculture, University of Khartoum, Shambat, Sudan. Micronutrients blend (MB) was obtained as a mixture of 14% water soluble Mo + 0.3% water soluble Mn + 0.3% water soluble B + 1.2% FeS + 0.02% Cu_2SO_4 + 0.02% $ZnSO_4$ + 0.004% $(NH_4)6[Mo_7O_{24}].4H_2O$, and macronutrients fertilizers (MF) (N-P_2O_5-K_2O) were donated by the Arid Land Research Center, Tottori University, Japan. Unless otherwise stated all the reagents used in this study were of analytical grade.

Plant experiment

After adjustment of the growing conditions (soil type, pH and temperature), the final experiments were carried out (2010 and 2011) at the Experimental Station of the Faculty of Agriculture, University of Khartoum, Shambat (latitude 15˚40′N and longitude 32˚32′E). The soil was sandy clay (82% sand and 18% clay) with pH of 7.2. The grains of the cultivar were seeded in pots with three grains per pot and after germination, were reduced to one plant per pot. Four doses (0, 2, 4 and 8 gm/5kg soil) of MB were applied to each pot. Beside micronutrients, all treatments received MF (N-P_2O_5-K_2O) at a fixed dose of 6 gm/5kg soil. After addition of fertilizer and germination of the seeds, the pH of the soil dropped to 5.7 and temperature between 20 and 25°C. To avoid water loss, the pots were watered under controlled conditions (weight difference). Each experiment was arranged in a factorial design with four replicates. At the end of each season, the grains were collected, sun dried, cleaned from dirt and broken grains and then ground to pass a 0.15 mm screen and stored at 4°C.

Determination of Tannin

Quantitative estimation of tannins was carried out using the modified vanillin–HCl method (Price *et al*., 1978). A 200 mg sample was extracted using 10 ml 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30 °C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg ml^{-1}) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

$$\text{Catechin equivalent (CE)\%} = \frac{\text{C x volume extracted (10 ml)}}{\text{sample weight (gm)}} \text{ X100}$$

Where C, concentration obtained from the standard curve (mg ml^{-1}).

Phytic acid determination

Phytic acid content was determined by the method described by Wheeler and Ferrel (1971) by using 2.0 gm dried sample. A standard curve was prepared and results were expressed as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

In vitro *protein digestibility (IVPD) determination*

IVPD was carried out using single enzyme (pepsin) digestion according to the method of Maliwal (1983). The digestibility was calculated using the following equation:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant} - \text{N in pepsin}}{\text{N in sample}} \text{ X100}$$

Minerals were extracted from the samples by the dry ashing method described by Chapman and Pratt (1982). About 2.0 gm of sample was acid-digested with diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was brought to 50 ml with double-distilled water and was used for determination of total calcium, phosphorus and iron. Calcium was determined by a titration method. Iron was determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). Phosphorus was determined by spectrophotometric method using molybdovanadate and the absorbance was measured at 730 nm.

*Extractable minerals (*in vitro *bioavailability) determination*

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988).

About 1.0 gm of the sample was shaken with 10 ml of 0.03 M HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven-dried at 100°C and then acid digested. The amount of extractable minerals was determined by the dry ashing method described above. Extractability (%) was determined as follows:

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractable in 0:03NHCl (mg/100gm)}}{\text{Total minerals (mg/100g)}} \text{ X100}$$

Each determination was carried out on three separate samples and analyzed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (Snedecor and Cochran, 1987). Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \leq 0.05$.

Results and Discussion

Changes in antinutrients content

Figure 1 shows tannin and phytate contents (mg per 100 gm) of sorghum cultivars seeds grown under different levels of micronutrient fertilizer and a constant level of macronutrients for two consecutive growing seasons. Results showed that tannin content of both Tabat and Tetron cultivars (Figure 1a) was gradually decreased with increase in fertilizer dose during both growing seasons. Tannin level of the cultivar Tabat significantly ($P \leq 0.05$) reduced from 0.11 to 0.03 mg/100gm when the seeds were fertilized with 8 g/5kg soil micronutrients during the first growing season, while during the second season it decreased to 0.05 mg/100gm (50% reduction). For Tetron cultivars, fertilization of the seeds with 8 g/5kg soil micronutrients significantly ($P \leq 0.05$) decreased tannin content from 0.12 mg/100gm to 0.06 and 0.02 mg/100gm during the first and second growing seasons, respectively. Although Tabat cultivar was slightly low in tannin content but the level of reduction in tannin was more or less similar to that of Tetron cultivar (Figure 1a). It is clear that there is a varietal variation with respect to tannin reduction which indicated that there is a seasonal response to micronutrients. Results indicated that micronutrients fertilization of sorghum seeds was very efficient in reducing tannin content. The loss in tannin during fertilization can be attributed to solubilisation by enzymes because the prime objective of fertilization is to promote the development of hydrolytic enzymes that are not active during seeds development. The reduction in tannin content after fertilization may be due to the action of the hydrolytic enzymes which

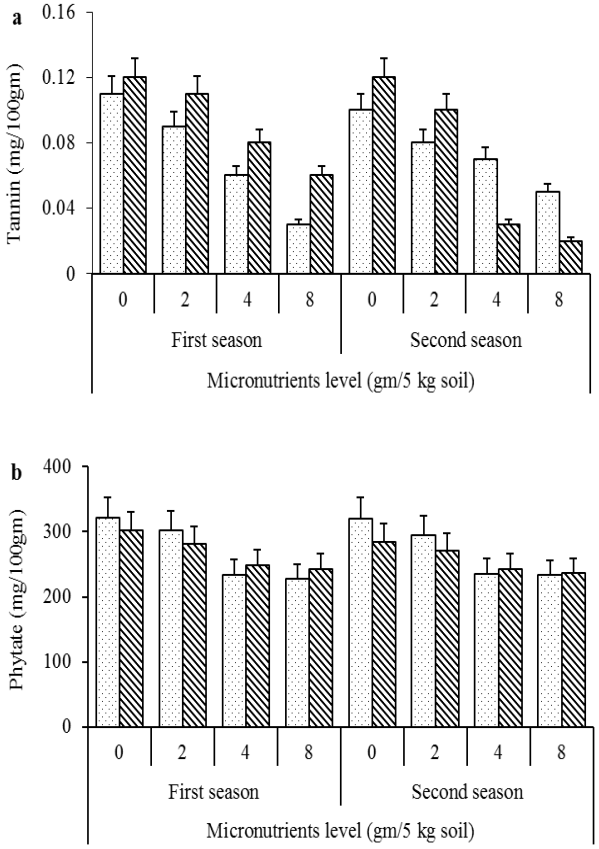

Figure 1. Antinutrients content (a, tannin; b, phytate) of flour of commonly consumed sorghum cultivars (dotted bars, Tabat; sliding lines bars, Tetron) grown under different level of micronutrients fertilization

break down and solubilize tannins as well as increase the chemical reactivity or the formation of insoluble complexes as explained by Osman *et al.* (2014). Moreover, they reported that there were seasonal variations in tannin content when the cultivar received micronutrients. Such variations could be attributed to genetics, physiological/biochemical mechanisms, responses to climate variations and responses to agronomic management practices. Genetic variations in plant acquisition of nutrients have been reported (Duncan and Carrow, 1999). Figure 1b shows the effect of micronutrients fertilization on phytate level of the seeds of Tabat and Tetron cultivars grown for two consecutive seasons. Phytate content of the seeds grown without micronutrients fertilizer was 321.1 and 301 mg/100gm for Tabat and Tetron seeds, respectively during the first season. However during the second season it slightly dropped for Tabat (320.6 mg/100g) while that of Tetron it was significantly ($P \leq 0.05$) dropped to 284 mg/100gm. Fertilization with micronutrients significantly ($P \leq 0.05$) dropped phytate level with micronutrient dose for both cultivars and during both seasons. The maximum percentage of phytate reduction for Tabat cultivar was 29 and 27% and that of Tetron was 20 and 17% during the

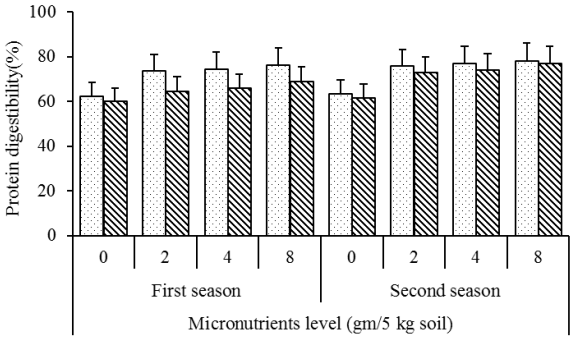

Figure 2. *In vitro* protein digestibility (IVPD) of flour of commonly consumed sorghum cultivars (dotted bars, Tabat; sliding lines bars, Tetron) grown under different level of micronutrients fertilization

first and second growing seasons, respectively. The apparent decrease in phytate content may be a result of the formation of insoluble complexes between phytate and other components such phytate–mineral complexes and accordingly the amount of free phytate was reduced. Moreover, the absorbed micronutrients may activate the phytase enzyme which hydrolyzes phytate (Sandberg and Andlid, 2002). It has been reported that micronutrients provided suitable pH for both tannase and phytatse activity (pH 5-6) and most of microelements such as Mn had no adverse effect on phytase activity (Seong *et al.,* 1996) and Zn on tanaase activity (Abdel-Naby *et al*., 1999).

Changes in protein and minerals in vitro *availability*

Figure 2 shows the effect of micronutrients fertilization on protein digestibility of sorghum cultivars seeds grown for two consecutive seasons. The *in vitro* protein digestibility (IVPD) of unfertilized seeds harvested during the first and second seasons was 62.25 and 63.21%, respectively for Tabat cultivar while that of Tetron was 60.1 and 61.6% for the seasons, respectively. The IVPD of sorghum seeds significantly ($P \leq 0.05$) increased with micronutrients level and reached maximum values of 76.21 and 68.70% for harvested seeds of Tabat and Tetron, respectively when fertilized with 8 g/5 kg soil during the first growing season. However, during the second growing season further and significant ($P \leq 0.05$) increment in IVPD was observed for both cultivars and reached 78.21 and 76.85% for the cultivars, respectively. The increase in protein digestibility by enzymatic breakdown of the harvested grains can be attributed to the reduction in antinutrients, which reported by many researchers (Fageer *et al.*, 2004; Abdalla *et al.*, 2010; Amro *et al*., 2005 and Abdelhaleem *et al*., 2008) to have an adverse effect on IVPD. Figure 3 shows the

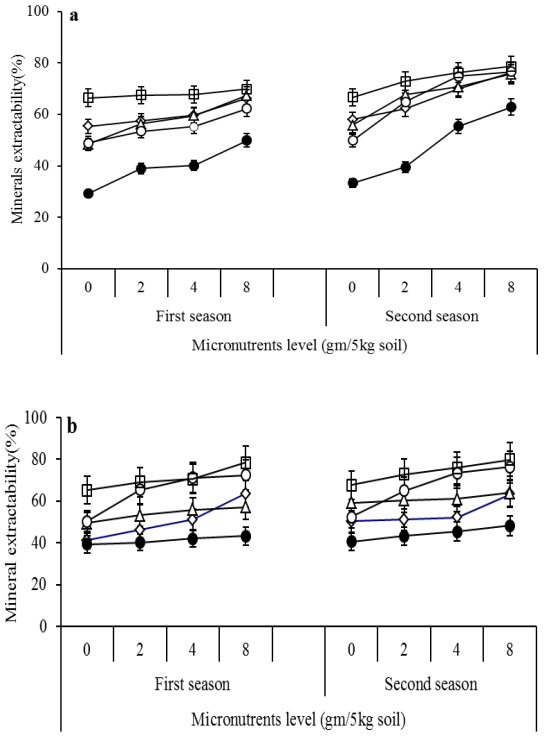

Figure 3. In vitro availability of some major minerals (◊, Ca; •, P; Δ, K; ○, Mg; □, Na) of flour of commonly consumed sorghum cultivars (a, Tabat; b, Tetron) grown under different level of micronutrients fertilization. Error bar indicates standard deviation

extractability of some major elements (Ca, P, K, Mg and Na) of sorghum seeds grown for two consecutive seasons under different levels of micronutrients fertilization. For Tabat cultivar, the extractability of all minerals increased significantly ($P \leq 0.05$) with micronutrients level (Figure 3a). The extractability of Ca, P, K, Mg and Na of unfertilized seeds was 55.33, 29.18, 48.31, 48.94 and 66.42%, respectively during the first growing season. However, during the second season it slightly increased to 58.0, 33.33, 55.73, 49.87 and 66.67% for the minerals, respectively. When the grains were fertilized with 8 g/5kg soil, the extractability of such minerals significantly ($P \leq 0.05$) increased to 66.23, 49.9, 67.26, 62.3 and 69.78% during the first growing season, while during the second season it significantly ($P \leq 0.05$) increased to 76.16, 62.79, 75.59, 76.58 and 78.67% for the minerals, respectively. A similar trend was observed for Tetron cultivar (Figure 3b). The extractability of some trace elements (Fe, Zn, Cu, and Mn) of sorghum seeds grown for two consecutive seasons under different levels of micronutrients fertilization is shown in Figure 4. For Tabat cultivar, the extractability of trace minerals increased significantly ($P \leq 0.05$) with micronutrients level (Figure 4a). The extractability of Fe, Zn, Cu, and Mn of unfertilized seeds was 15.29, 52.02, 34.44 and 43.86%, respectively during the first

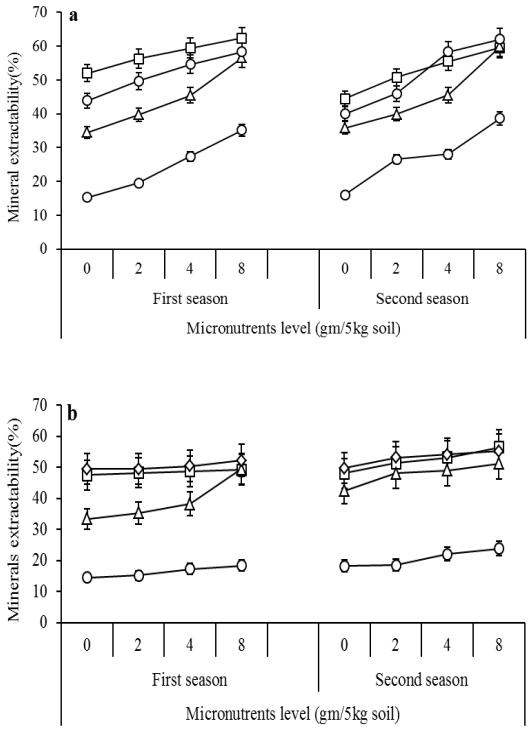

Figure 4. In vitro availability of some trace minerals (○, Fe; □, Zn; Δ, Cu; ◊, Mn) of flour of commonly consumed sorghum cultivars (a, Tabat; b, Tetron) grown under different level of micronutrients fertilization. Error bar indicates standard deviation

growing season, while during the second growing season it was 16.03, 44.44, 35.82 and 40.0% for the trace minerals, respectively. When the grains were fertilized with 8 g/5kg soil, the extractability of such minerals significantly ($P \leq 0.05$) increased to 35.17, 62.32, 56.51 and 58.28% during the first growing season, while during the second growing season it also significantly ($P \leq 0.05$) increased to 38.62, 59.51, 59.88 and 62.04% for the trace minerals, respectively. Changes in trace minerals extractability of Tetron cultivar (Figure 4b) are similar to those obtained for Tabat cultivar. The increase in HCI extractable major and trace minerals may be attributed to reduction in phytate as well as tannins in the treated seeds which reduces the prevalence of minerals deficiency. However, the residual tannin and phytate greatly lowered trace minerals extractability. It was observed that phytic acid, in the processed pigeon pea had a negative correlation with extractability of iron, which underlines the role of phytic acid in lowering the extractability of divalent cations in plant foods (Duhan *et al.,* 2002). Idris *et al*. (2005) observed that malting reduced the inhibitors such as phytates and tannins in sorghum meals. Overall, results indicated that treatment of seeds of sorghum with micronutrients significantly ($P \leq 0.05$) increased the extractability of both major and trace minerals and the degree of increment depends on micronutrients level. Higher extractability of minerals may be partly ascribed to the decreased content in phytic acid, as a significant negative correlation between the phytic acid and extractability of dietary essential minerals was observed (Duhan *et al.*, 2002). Many researchers reported the effect of antinutrients on minerals extractability of millet (Abdelrahaman *et al.*, 2007), sorghum (Idris *et al*., 2005 and 2007), corn (Fageer *et al*., 2004), white bean (Elmaki *et al.*, 2007) and faba bean (Elmaki *et al.,* 2005). In general, the results obtained showed that the cultivars highly respond to micronutrients application especially during the second growing season. However, the degree of response varied between the cultivars due to genetic variations in plant acquisition of micronutrients (Duncan and Carrow, 1999). It has been reported that the application of micronutrient-enriched NPK fertilizers provides a double benefit: increasing grain yield and improving the nutritional quality of the harvested grains, since micronutrient-enriched NPK fertilizers also increase the concentration of micronutrients in grain (Malakouti, 2008).

Conclusion

Micronutrients fertilization improved the availability of protein and dietary minerals. Utilization of such fertilizer is a promising and simple. The rate of increment of protein and minerals availability depends on the dose of the fertilizer as well as the season.

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