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Some Nutritional Attributes of Selected Newly Developed Lines of Sorghum (*Sorghum bicolor*) after Fermentation

B. H. Abdelseed[1], A. H. Abdalla[2], A. El-Gasim A. Yagoub[3], I. A. Mohamed Ahmed[4], and E. E. Babiker*[1]

ABSTRACT

Total energy, protein content and digestibility, antinutritional factors, and total and extractable minerals of normal sorghum (Type II) and four newly developed lines of sorghum (Eri-1, SHK-ABA-4, SHK-ABA-6 and SHK-ABA-10.) were studied before and after fermentation. Phytic acid and Tanin contents of raw flour of the normal sorghum were, respectively, 41.73 mg 100 g^{-1} and 170.54 mg 100 g^{-1}, while the same values for the four lines ranged from 16.07 to 38.64 mg 100 g^{-1} and from 31.90 to 184.25 mg 100 g^{-1}, respectively. Polyphenols content of raw flour of the normal sorghum was 604.56 mg 100 g^{-1}, exceeding the values found for the four lines in the range of 476.46 to 544.44 mg 100 g^{-1}. According to our results, fermentation of normal sorghum flour and that of the new lines significantly (P≤ 0.05) decreased the antinutritional factors i.e. phytate, tannins, and polyphenols. The total energy of raw flour of the normal sorghum was 369.87 Kcal 100 g^{-1} while it ranged from 367.23 to 372.57 Kcal 100 g^{-1} for the new lines. In all cases, this energy slightly decreased after fermentation. Protein digestibility of normal sorghum was 22.60% and, for the new lines, it ranged from 37.00 to 57.19%. After fermentation, protein digestibility and the total and extractable Ca, P, and Fe increased significantly (P≤ 0.05) for all genotypes studied.

Keywords: Antinutrients, Fermentation, Minerals, Protein, Sorghum lines.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a drought resistant indigenous crop of Africa and, as such, plays a significant role in the food security of the rural populations of southern and eastern Africa (Dendy, 1995). Cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low because of the presence of antinutritional factors such as phytate and polyphenols (Valencia *et al*., 1999). It was observed that sorghum grains had low starch and protein digestibilities due to the presence of certain antinutritional factors, which, also, contribute to poor sensory characteristics of processed sorghum grains (Hassan and El Tinay, 1995).

As with other foodstuffs, certain nutritional inhibitors and toxic substances are associated with sorghum grains (Hassan and El Tinay, 1995). These factors modify the nutritional value of the individual grains, and some of them have very serious consequences. The effects of phytic acid in human and animal nutrition are related to the interaction of phytic acid with proteins,

[1] Department of Food Science and Technology,Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.
*Corresponding author; e-mail: elfadilbabiker@yahoo.com
[2] Department of Crop Production, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.
[3] Faculty of Agriculture, University of Zalingei, P. O. Box: 6, Zalingei, Sudan.
[4]United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan.

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vitamins, and several minerals, thereby restricting their bio-extractability (Svanberg and Lorri, 1997). In view of the anti-nutritional effects of phytic acid, many attempts have been made to reduce phytate. Phytic acid is said to chelate mineral cations and proteins, forming insoluble precipitates, which lead to reduced bioavailability of trace mineral cations and reduced digestibility of proteins (Svanberg and Lorri, 1997).

A nutritional limitation to the use of sorghum is the poor digestibility of its protein when wet cooked. The factors affecting wet cooked sorghum protein digestibility may be categorized into two main groups: exogenous factors (grain organizational structure, polyphenols, phytic acid, starch, and non-starch polysaccharides) and endogenous factors (disulphide and non-disulphide crosslinking, kafirins hydrophobicity, and changes in protein secondary structure). All these factors have been shown to influence sorghum protein digestibility (Duodu *et al*., 2003).

Various simple technologies have been investigated to improve the protein digestibility of sorghum, including fermentation (Lorri and Svanberg, 1993; Hassan and El Tinay, 1995) and supplementation with protein-rich sources (Ibrahim *et al*., 2005). Of equal importance is the application of different processing technologies in order to increase the bioavailability of indigenous nutrients in grains such as protein, starch, and minerals. One such household-level technology that is widely practiced in many developing countries is the fermentation technique. The different ways by which the fermentation process can affect the nutritional quality of foods include improving the nutrient density and increasing the bioavailability of nutrients. The latter may be achieved by degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of promoters for absorption, and influencing the uptake of nutrients by the mucosa (Taylor and Taylor, 2002). Sorghum is generally consumed as fermented flat bread (*Kisra*), thick porridge (*Asseda*), thin fermented gruel (*Nasha*), boiled grain (*Balela*), and non-alcoholic beverages (*Sharboat, Abreh and Hulomor*). Most of these indigenous foods are produced by traditional processes (Hassan and El Tinay, 1995). Recently large efforts have been directed to improve the nutritional quality of cereal grains, particularly to improve the level of essential amino acids as well as protein digestibility. In the present study, we would like to investigate the nutritional value of newly developed sorghum lines compared to that of normal sorghum, before and after fermentation.

MATERIALS AND METHODS

Seeds of four newly developed (conventional breeding) sorghum lines, namely, Eri-1, SHK-ABA-4, SHK-ABA-6 and SHK-ABA-10, were obtained from the Department of Agronomy, Faculty of Agriculture, University of Khartoum, Sudan, and were grown at the University top farm. The seeds of the cultivars were small, slightly white in color, and with seeds testa. All chemicals used in this study were of analytical grade.

Flour Sample Preparation

The seeds of the cultivars were cleaned manually to remove broken seeds, dust and other extraneous materials. The cleaned grains were milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass a 0.4 mm mesh size screen and were stored at 4 ^{o}C before being used for further analysis.

Fermentation of Sorghum

Sorghum flour was fermented according to the traditional method (lactic acid fermentation) practised by the Sudanese housewives (El Tinay *et al.*, 1985). The final

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media had a pH of 4.9. Fermentation was carried out at 37°C for 24 hours. After a distinct incubation period, the samples were dried in a hot air oven (Heraeus UT 5042, Germany) at 60°C for 16 hours. Dried samples were ground to pass a 0.4 mm screen and were stored in polyethylene bags at 4°C prior to analysis.

Protein Content Determination

Raw and fermented flour of sorghum lines were analyzed for protein by adopting standard AOAC (1995) method.

Total Energy (Calorific Value) Determination

Energy was calculated as described by Osborne and Voogt (1978) using the Atwater factors: 1g of carbohydrates (C.) provides (4Kcalories), 1g of protein (P.) provides (4Kcalories) and 1g fat (f.) provides (9Kcalories).

Total Minerals Determination

Minerals were determined in the samples by the dry-ashing method described by Chapman and Pratt (1961). The amount of iron was determined using atomic absorption spectroscopy (Perkin–Elmer 2380). Ammonium vanadate was used to determine phosphorus, by the ammonium molybdate method of Chapman and Pratt (1982). Calcium was determined by a titration method described by Chapman and Pratt (1961).

HCl-extractable Minerals Determination

Hydrochloric acid extractability of minerals was performed according to the Chauhan and Mahjan (1988) method. A sample of about 1.0 g was extracted using 10 ml of 0.03 N HCl and shaking at 37°C for 3 hours. Thereafter, the extract was filtered and the clear filtrate obtained was dried at 100°C and placed in a muffle furnace at 550°C for 4 hours. Later, the samples were cooled and about 5 ml of 5N HCl were added and boiled gently for 10 minutes, followed by cooling and diluting to 100 ml with distilled water. Minerals were determined as described in the previous section.

$$\text{Mineral extractability\%} = \frac{\text{Mineral extractable in } 0.03\text{NHCl (mg } 100\text{ g}^{-1})}{\text{Total minerals (mg } 100\text{ g}^{-1})} \times 100$$

Phytic Acid Determination

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

Polyphenols Determination

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Butler, 1977) with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 minute in 3.0 ml methanol. The mixture was filtered. The filtrate was mixed with 50 ml distilled water and analyzed within an hour. About 3.0 ml of 0.1M $FeCl_3$ in 0.1M HCl were added to 1 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared $K_3Fe(CN)_6$. The absorbance was monitored on a spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm 10 minutes after the addition of 3.0 ml of 0.1M $FeCl_3$ and 3.0 ml of 0.008M $K_3Fe(CN)_6$. A standard curve was prepared, expressing the result as tannic acid equivalents; that is, the amount of tannic acid (mg 100 g^{-1})

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that gives a color intensity equivalent to that given by polyphenols after correction for blank.

Tannin Content Determination

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 of 1% (v⁄v) concentrated HCl in methanol for 20 minutes 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 minutes 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 100 g^{-1}) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then, tannin content was calculated and expressed in mg 100 g^{-1}.

Protein Digestibility Determination

Protein digestibility *(in vitro)* was assessed by employing pepsin according to the method of Akeson and Stahmann (1964). The nitrogen content of the sample and the undigested residue were determined by the microkjeldahl method (AOAC, 1995). The digested protein of the sample was calculated by subtracting residual protein from total protein of the sample.

$$\text{Protein digestibility}(\%) = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

Statistical Analysis

Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) as described by Snedecor and Cochran (1987) and by Duncan-multiple range test at a probability of $P \leq 0.05$.

RESULTS AND DISCUSSION

Changes in Antinutritional Factors Content of Raw and Fermented Flour

Phytate, polyphenols, and tannins contents of the raw and fermented flour of the normal sorghum and the newly developed lines are

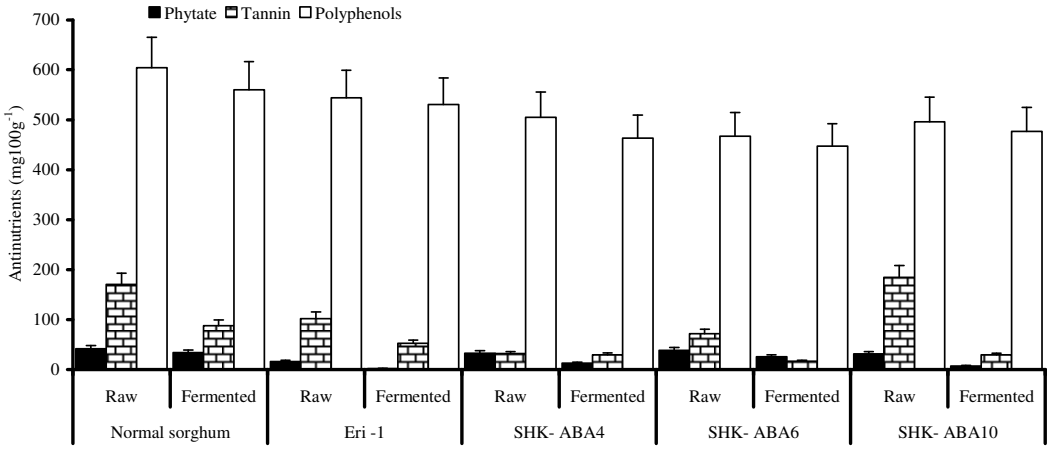

Figure 1. Antinutritional factors content (mg 100 g^{-1}) of raw and fermented flour of normal sorghum and newly developed lines. Error bars indicate the standard deviation of three independent samples.

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shown in Figure 1. Phytate content of raw seeds of normal sorghum (41.73 mg 100 g^{-1}) was significantly ($P \leq 0.05$) higher that of the new lines (16.07 to 38.64 mg 100 g^{-1}) and fermentation of flour significantly ($P \leq 0.05$) reduced phytate content in all cases (Figure 1). Polyphenols content of raw seeds of normal sorghum was 604.56 mg 100 g^{-1}, while, for the newly developed lines, it ranged from 467.46 to 544.44 mg 100 g^{-1}. Again, fermentation significantly ($P \leq 0.05$) reduced these values for all genotypes. Tannin content of the normal sorghum (170.54 mg 100 g^{-1}) was higher than that of the lines Eri1, SHK-ABA4 and SHK-ABA6, but, lower than that of the line SHKABA10. Similar to the case of phytate and polyphenols, fermentation significantly ($P \leq 0.05$) reduced tannin content of all the new lines, which were found to be lower than that of the normal sorghum. The results obtained showed that the development of new lines of sorghum by using conventional breeding significantly ($P \leq 0.05$) reduced the antinutrients content as reported by Dykes and Rooney (2006). Further reduction in antinutritional factors was observed after fermentation of both normal sorghum and the new lines as reported by many researchers (Ibrahim *et al*., 2005; Idris *et al*., 2005). The level of phytic acid in the seeds was reduced by more than 50% during the fermentation period. It has been suggested that the loss of phytic acid during fermentation might be due to the action of fermenting microorganisms that hydrolyze phytate into inositol and orthophosphate (Sandberg and Andlid, 2002). The low pH (4.9) of the fermented flour may have provided a favourable condition for phytase activity. Phytate-degrading enzymes have been detected in various bacterial genera, such as *Bacillus* (Kerovuo *et al*., 2000) and *Pseudomonas* (Richardson and Hadobas, 1997). Similar observations have been reported for tannins and polyphenols by other investigators. Abdel Rahman (2005) reported reduction in tannin contents during fermentation of millet cultivars. Hassan and El Tinay (1995) also found that natural fermentation of high and low tannin content of sorghum varieties decreased their contents by 63% and 61.4%, respectively. Also, El-Khalifa and El-Tinay (1994) achieved 92% decrease in tannin content of a high tannin sorghum cultivar through fermentation.

Changes in Total Energy, Crude Protein, and IVPD

The total energy of normal sorghum flour and that of the new lines before and after fermentation is shown in Figure 2. For the raw flour of the normal sorghum, this energy

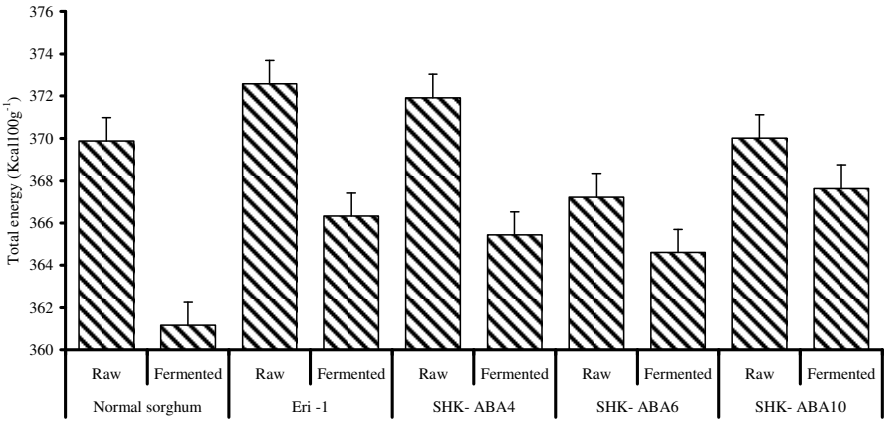

Figure 2. Total energy (Kcal 100 g^{-1}) of raw and fermented flour of normal sorghum and newly developed lines. Error bars indicate the standard deviation of three independent samples.

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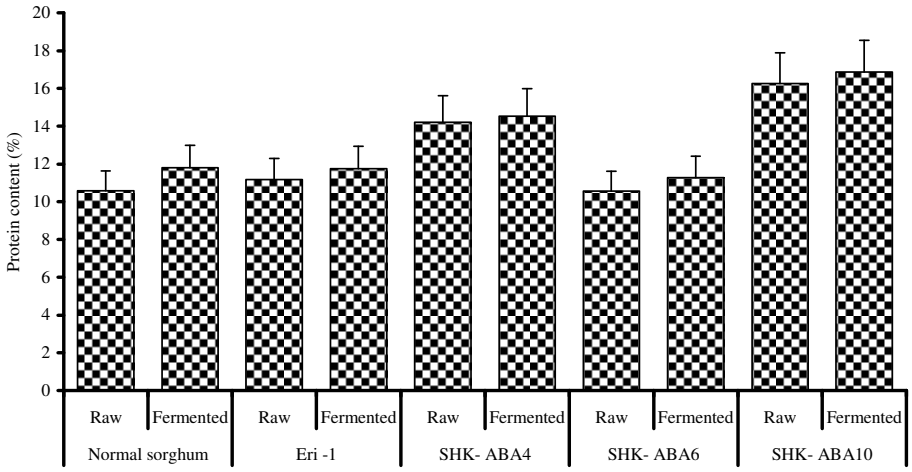

Figure 3. Protein content (%) of raw and fermented flour of normal sorghum and newly developed lines. Error bars indicate the standard deviation of three independent samples.

was 369.87 Kcal 100 g^{-1} while it ranged from 367.23 to 372.57 Kcal 100 g^{-1} for the newly developed lines. Fermentation of the flour reduced the total energy in all cases. The reduction in the total energy during fermentation is likely due to utilization of some nutrients by fermenting microorganisms. As shown in Figure 3, protein content of the normal sorghum was 10.58% and increased to 11.81% after fermentation. The newly developed lines had a higher protein content than the normal sorghum. In ranking the new lines, it was observed that line SHK-ABA10 (16.27%) had the highest protein content, followed by line SHK-ABA4 (14.20%). The other lines had a normal protein content higher than that of the normal sorghum. Fermentation was found to cause a marginal change in the protein content for all lines as well as the normal sorghum i.e. fermentation does not seem to be a viable method for raising the protein content. Similarly, Abdalla *et al.* (1998) reported a marginal and insignificant change in the protein content of pearl millet flour during fermentation. These findings indicate that yeast or natural fermentation of cereals improves the protein content only slightly, which can be attributed to the loss of dry matter, mainly carbohydrates.

The *in vitro* protein digestibility (IVPD) of raw and fermented normal sorghum and newly developed lines is shown in Figure 4. The IVPD of raw normal sorghum was 22.60% and increased significantly ($P \leq 0.05$) to 36.50% after fermentation. The IVPD of the new lines was found to be significantly ($P \leq 0.05$) higher than that of the normal sorghum and varied from 37.00 to 57.19%. Further improvement in IVPD of sorghum was observed after fermentation of the lines flour, which raised its value to a range between 44.01 to 70.38%. The results indicate that both breeding and fermentation cause a significant ($P \leq 0.05$) improvement in IVPD and an increase in protein availability for sorghum. The lactic acid fermentation process has been reported to improve the *in vitro* protein digestibility of non-tannin cereal grains and of high-tannin varieties (Lorri and Svanberg, 1993). In children meals, the protein digestibility was reported to increase after lactic acid fermentation of whole grain sorghum (non-tannin) flour that was prepared as Nasha, a traditional fermented Sudanese food for infants and young children. The highest increase in digestibility was observed in high-tannin sorghum gruels fermented with a natural starter culture (Hassan and El Tinay, 1995). *In vitro* studies have also shown that phytate protein complexes are formed by electrostatic interactions involving the terminal N-amino groups, the s-amino group

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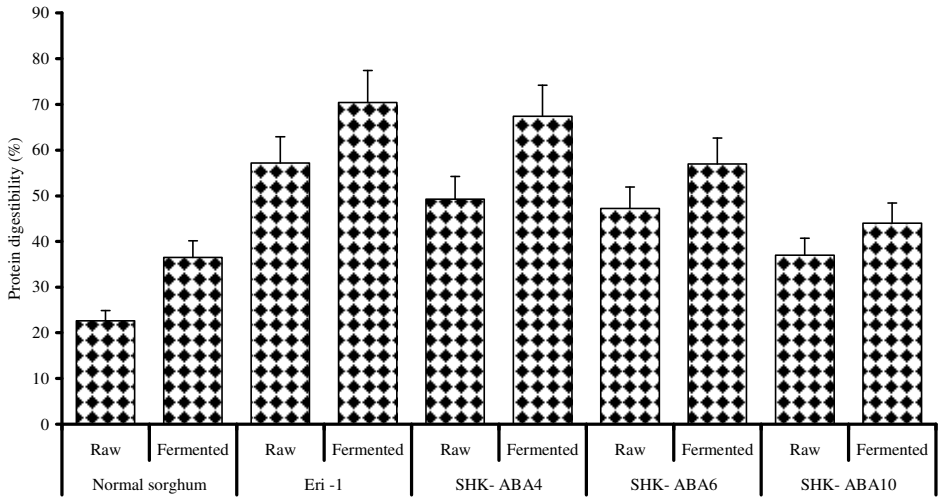

Figure 4. *In vitro* protein digestibility (%) of raw and fermented flour of normal sorghum and newly developed lines. Error bars indicate the standard deviation of three independent samples.

of lysine, the imidazole group of histidine, and the guanidyl groups of arginine (Taylor and Taylor, 2002). Many of these complexes are insoluble and are not biologically available for humans under normal conditions. Taylor and Taylor (2002) proposed that, during fermentation, insoluble proteins (prolamine and glutelin) undergo structural changes, which make them more accessible to pepsin attack, rather than being broken down into smaller sub-units. These changes are likely to have a marked effect on the digestibility of the seed protein and may be partly responsible for the increased protein digestibility observed in this study in fermented sorghum flour.

Changes in Total and Extractable Ca, P, and Fe

Calcium content of the raw flour of the normal sorghum was 19.34 mg 100 g^{-1}, of which about 6.02 mg 100 g^{-1} were HCl-extractable (Figure 5). Fermentation of the normal flour significantly ($P\leq 0.05$) increased both total and extractable Ca. The newly developed lines contained significantly ($P\leq 0.05$) higher amounts of Ca than the normal sorghum, with values ranging from 50.18 to 75.58 mg 100 g^{-1}. Aafter fermentation, further increases in Ca were observed that varied from 75.21 to 100.11 mg 100 g^{-1}. Fermentation also significantly ($P\leq 0.05$) increased the extractable Ca contents to a range of 37.60 to 67.00 mg 100 g^{-1}. The results obtained showed that both breeding and fermentation significantly ($P\leq 0.05$) increased the level of Ca of sorghum. Total P of the raw flour of the normal sorghum was 287.05 mg 100 g^{-1}, of which about 78.79 mg 100 g^{-1} was extractable (Figure 6). The total and extractable P were higher in the new lines, ranging from 233.13 to 388.75 mg 100 g^{-1} and from 23.33-99.60 mg 100 g^{-1}, respectively. As shown in Figure 6, in all cases, fermentation caused further increase in both total and extractable P.

Total Fe of the raw flour of normal sorghum was 4.61 mg 100 g^{-1}, of which 1.23 mg 100 g^{-1} was extractable (Figure 7). These values were higher for only some of the newly developed sorghum lines. However, in all cases, fermentation of the flour significantly ($P\leq 0.05$) increased both total and extractable Fe of sorghum. Similar trend for the major and trace minerals extractability was observed by Idris *et al.* (2005) in ground whole sorghum flour

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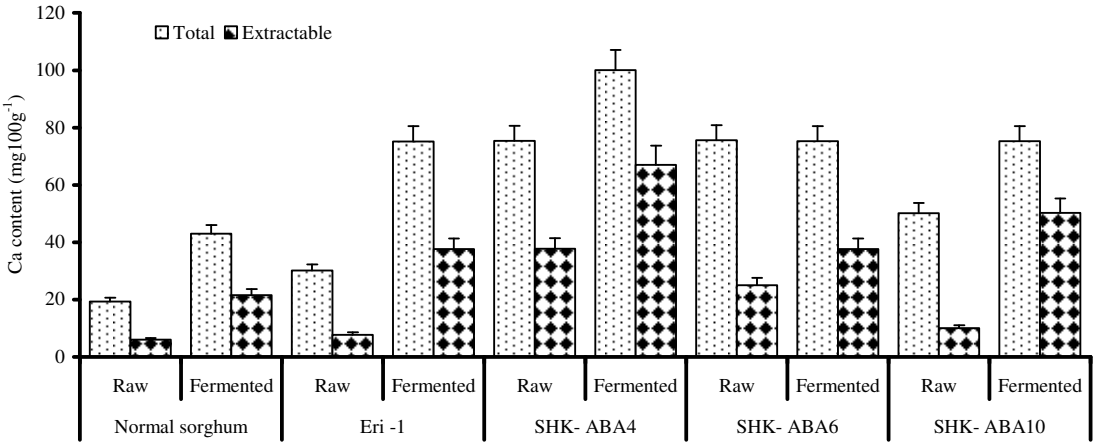

Figure 5. Total and extractable Ca (mg 100 g^{-1}) of raw and fermented flour of the normal sorghum and the newly developed lines. Error bars indicate the standard deviation of three independent samples.

incubated for different periods of time**.** Moreover, the results obtained indicated that the residual phytate might play an important role in reducing Fe 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. The increase in HCI extractable Fe may be attributed to reduction in phytate as well as tannins and polyphenols in the treated flour, which reduces the prevalence of iron deficiency. However, the residual tannin and phytate greatly lowered Fe extractability. Overall, results of our study indicated that fermentation of sorghum flour significantly ($P \leq 0.05$) increased the extractability of Ca,

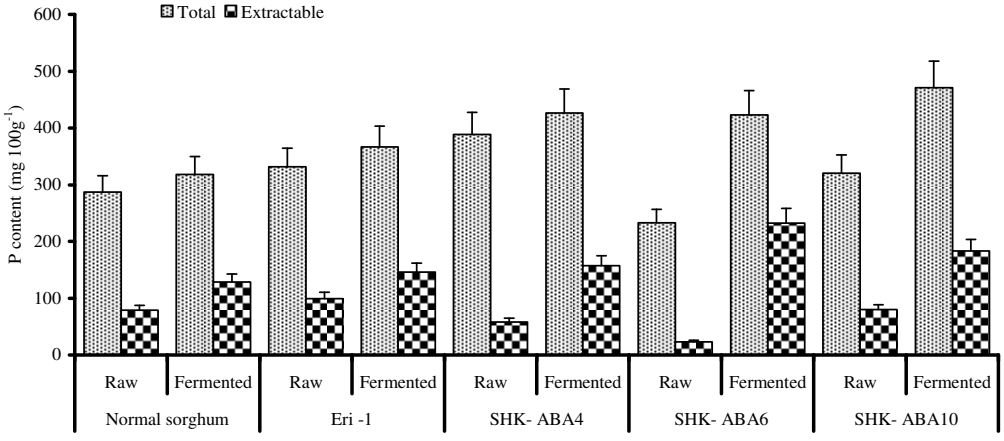

Figure 6. Total and extractable P (mg 100 g^{-1}) of raw and fermented flour of the normal sorghum and the newly developed lines. Error bars indicate the standard deviation of three independent samples.

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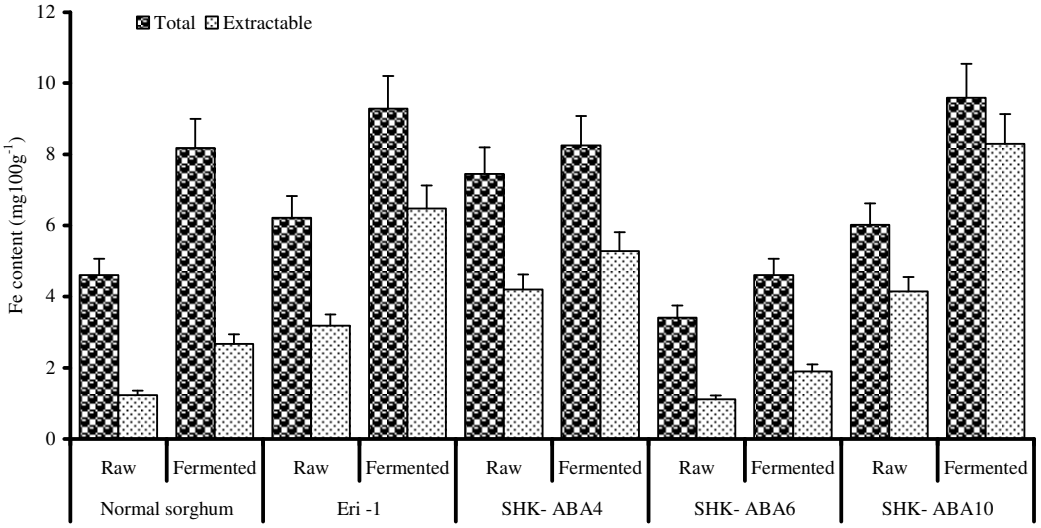

Figure 7. Total and extractable Fe (mg 100 g^{-1}) of raw and fermented flour of the normal sorghum and the newly developed lines. Error bars indicate the standard deviation of three independent samples.

P, and Fe and the degree of the increment depends on the sorghum line under investigation. Higher HCl extractability of minerals may be partly ascribed to the decrease in phytic acid, as a significant negative correlation between the phytic acid and HCl extractability of dietary essential minerals has been observed (Duhan *et al*., 2002). Similar results are reported by Idris *et al.* (2005) in sorghum cultivars. The increment in total and extractable P is likely because of the release and solubilisation of phytate phosphorus by the action of the enzyme phytase produced during fermentation of the flour, as reported by Khetarpaul and Chauhan (1990). The difference in P content and extractability between the lines may lie in the chemical, as well as quantitative, differences of phytase in the lines. Moreover, total Ca and Fe were also greatly improved, which was likely due to release and solubilisation of such minerals during fermentation. Rakhi and Khetarpau (1994) reported that the total and HCl extractabilities of calcium, iron, and phosphorus from flour blend of rice and defatted soy greatly improved when the seeds were germinated and malted.

CONCLUSIONS

Breeding as well as lactic acid fermentation of sorghum are potential methods for improving the protein content and digestibility, as well as HCl-extractability, of calcium, phosphorus, and iron. The availability of minerals from plant foods such as cereals is limited due to the presence of antinutrients. Therefore, consumption of fermented cereal may help to alleviate the prevalent mineral deficiencies caused by their limited bioavailability and may lead to a better mineral status of the population of developing countries.

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بعضی از خواص تغذیه ای ارقام انتخاب شده جدید سورگوم بعد از تخمیر

ب. ح. عبدالسد، ا. ح. عبدالله، ا. الجاسم. یعقوب، ای.ا.محمد احمد، ای. ای. بابیکر

چکیده

مقدار انرژی کل، پروتئین و هضم پذیری آن، عوامل ضد تغذیه‌ای و عناصر معدنی کل و قابل استخراج چهار رقم سورگم تازه اصلاح شده (Eri-1, SHK-ABA-4, SHK-ABA-6, SHK-ABA-10) قبل و بعد از تخمیر مورد مطالعه قرار گرفت. مقدار اسید فایتیک آرد خام قبل از تخمیر در محدوده ۱۶/۰۷ تا ۳۸/۶۴ و بعد از تخمیر ضمن کاهش معنی دار($P \leq 0.05$) در محدوده ۲/۲۷ تا ۳۵/۵۱ میلی گرم در صد گرم بود. مقدار تانن آرد خام قبل از تخمیر در محدوده ۳۱/۹۰ تا ۱۸۴/۲۵ و بعد از تخمیر ضمن کاهش معنی دار ($P \leq 0.05$) در محدوده ۱۶/۵۰ تا ۵۲/۲۵ میلی گرو بر صد گرم بود. در حالیکه مقدار پلی فنل های آرد خام قبل از تخمیر در محدوده ۴۷۶/۴۶ تا ۵۴۴/۴۴ بود، بعد از تخمیر ضمن کاهش معنی دار ($P \leq 0.05$) ۴۴۷/۳۸ تا ۵۳۰/۵۸ میلی گرم درصد گرم شد. مقدار انرژی کل آرد خام قبل از تخمیر درحدود، ۳۶۷/۲۳ تا ۳۷۲/۵ کیلو کالری برای صد گرم و بعد از تخمیر ضمن کاهش کمی در محدوده ۳۶۵/۴۴ تا ۳۷۰/۶۰ کیلو کالری برای صد گرم بود. هضم پذیری پروتئین آرد خام در دامنه ۳۷/۰۰ تا ۵۷/۱۹ درصد و بعد از تخمیر ضمن افزایش معنی دار($P \leq 0.05$) در دامنه ۴۴/۰۱ تا ۷۰/۳۸ درصد بود. تخمیر آرد ارقام سورگوم موجب افزایش معنی‌دار ($P \leq 0.05$) در مقدار عناصر کل وقابل استخراج کلسیم (Ca) فسفر (P) و آهن (Fe) شد.

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