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Effect of processing methods on antinutritional factors, protein digestibility and minerals extractability of winter sorghum cultivars

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ABSTRACT

The aim of this study was to investigate the effect of fermentation and/or cooking on the antinutritional factors (polyphenols, tannins and phytates), protein digestibility, total and extractable minerals of two Sudanese winter season cultivars (locally named as Abu Ragaba and Abu Kunjara) and one summer season cultivar (Wad Ahmed) as control. High tannin and phytate characterized Abu Kunjara, while high polyphenol characterized Wad Ahmed. The results obtained showed that the antinutritional factors of all cultivars were significantly ($P \leq 0.05$) decreased after fermentation and cooking. The reduction in antinutritional factors was concomitant with a significant ($P \leq 0.05$) increase in protein digestibility after processing of the samples. Total minerals contents (Ca, P, Fe and Mg) were fluctuated after fermentation and cooking. HCl-extractability of Ca, P, Fe and Mg was significantly ($P \leq 0.05$) increased after fermentation and cooking of the samples. However, HCl-extractability of such minerals of the raw samples was decreased after cooking for all cultivars. The increment in minerals extractability is attributed to the reduction in antinutritional factors.

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INTRODUCTION

The demand for cereals as food, feed and industrial raw materials is increasing due to population explosion in developing countries and short falls in cereals production in several developed countries. It is well established that the majority of the people in the developing countries depend mainly on cereal grains as their staple food due to limited income and high prices of animal foods (Sokrab *et al.*, 2012). Sorghum (*Sorghum bicolor* L. Moench) can grow under very harsh conditions such as infertile soils and excessive heat, conditions that are unsuitable for maize or wheat production. Sorghum, together with millet, represents the major form of energy and protein for the African population (Belton & Taylor, 2004). In the Sudan, sorghum comes first in volume of cereal production, covering more than 60% of the total cultivated cereal area, and with an annual production of about 4.0 million tons (FAO, 1997). Most local sorghum cultivars (e.g. Wad Ahmed, Gadamalhamam, Dabar, etc.) are grown in the summer season (June–August) in irrigated or rain-fed sectors. However, two unique sorghum cultivars, locally known as Abu Ragaba and Abu Kunjara, are grown by transplanting seedlings (30- to 35-days old) to the field at early October (which is the time for harvesting the summer season sorghum) and harvested at late January to early February. Therefore, they considered as winter season crop. These winter sorghum cultivars are cultivated in the humid soils of valleys of West and South Darfur states. Plant growth depends on the water conserved in the wet soils until harvesting (Mohamed Nour *et al.*, 2010). Sorghum, like legume and oil seed meal, has some limitations due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytate and tannins. These compounds are known to interfere with protein and carbohydrate digestion and mineral bioavailability. Reduction or elimination of these undesirable components is essential for improving the nutritional quality of sorghum and effectively utilizing their full potential as human food. Efforts, however, are directed to improve the nutritional value of the sorghum grains. Various simple processing

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methods such as soaking, sprouting, cooking and fermentation have been found to improve the nutritional value of plant grains (Mohamed Nour *et al.*, 2010; Yagoub & Abdalla, 2007). It is well known that fermentation decreased the values of antinutritional factors as well as increased the *in vitro* protein and starch digestibilities and thus improve the nutritional quality of summer sorghum flour (Elkhalifa *et al.*, 2004; Idris *et al.*, 2005; Abdelseed *et al.*, 2011; Mohamed *et al.*, 2011). Cooking on the other hand has been reported to reduce the *in vitro* protein digestibility of sorghum flour, whereas combining fermentation with cooking had significantly improved protein digestibility over wet cooking alone (Taylor & Taylor, 2002). Although much research has been done on the effect of fermentation and/or cooking on the nutritional quality of summer sorghum cultivars, research on the effect of such processing methods on nutritional values of Sudanese winter sorghum cultivars is scarce. Therefore, the broad objective of this study is to derive new physicochemical and nutritional information on the effect of domestic processing methods on the quality of Sudanese winter sorghum cultivars that can contribute to better future utilization of winter sorghum cultivars. In this context, the effects of fermentation and/or cooking on antinutrients, *in vitro* protein digestibility and minerals content and extractability were studied.

MATERIALS AND METHODS

Materials:

Three cultivars of Sudanese sorghum locally known as Wad Ahmed (summer season), and winter season Abu Ragaba (white grains) and Abu Kunjara (reddish brown grains) were obtained from Darfur Agricultural Research Station. About 2.0 Kg of each cultivar was cleaned from damaged seeds, foreign objects and other extraneous grain or girts, then milled into flour (0.4 mm screen). The flour was divided into four portions; one portion of each cultivar (250 g) was kept in clean polyethylene bags and stored at 4 °C as raw samples for subsequent analysis. Other portions were then fermented and/or cooked. All chemicals and reagents used were of analytical grade.

Preparation Of Fermented Samples:

Fermentation was carried out according to the method of El Tinay *et al.* (1985). About 500 g of the flour (two portions) of each cultivar were mixed with water at ratio of 1:2 (w/v), then fermented by using previously fermented starter (the starter formed 5 % of the dough). The fermentation was taken place at room temperature (28-32 °C) for 14 h (traditional fermentation). The pH of the fermented samples was measure at the end of fermentation period using a glass electrode pH meter (PUSL Munchem 2, KARL-KOLB, Germany). The samples were then dried and were reground to pass a 0.4 mm screen. The fermented flour was then divided into two portions one was stored at 4 °C until used for subsequent analysis and other portion was then cooked.

Preparation Of Cooked Samples:

Cooking of the samples was performed according to the method described by Arbab & El Tinay (1997). Cooked samples were prepared by suspending 250 g of the flour of each raw and fermented samples in distilled water in the ratio of 1:10 flour to water (w/v) and stirring to avoid lumps while boiling in water bath for 20 min. The viscous mass of cooked samples was spread out in thin layer and then dried. The dry flakes were milled into fine flour to pass a 0.4 mm screen and kept in polyethylene bags at 4 °C for further analysis.

Determination Of Total Polyphenols:

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price & Butler, 1977) with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 mL methanol. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was mixed with 50 mL distilled water and analyzed within an hour. About 3.0 mL of 0.1 M FeCl₃ in 0.1 M HCl was added to 1.0 mL filtrate, followed immediately by timed addition of 3.0 mL freshly prepared K₃Fe(CN)₆. After incubation for 10 min, the absorbance was monitored at 720 nm using a spectrophotometer (Pye Unicam SP6-550 UV, London, UK). A standard curve was obtained, expressing the result as tannic acid equivalents, that is, the amount of tannic acid (mg per 100 g) that gives a colour intensity equivalent to that given by polyphenols after correction for blank.

Determination of Tannin:

Quantitative estimation of tannin, as catechin equivalent, was carried out using the modified vanillin-HCl method of Price *et al.* (1978). The reagent was prepared daily.

Determination Phytic acid:

Phytic acid content of the samples was determined by the method described by Wheeler & Ferrel (1971) using 2.0 g dried sample. A standard curve was prepared expressing the results as Fe (NO₃)₃ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

In Vitro Protein Digestibility

In vitro protein digestibility of the samples was measured according to the method of Monjula & John (1991). A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 mL of 0.1 M HCl at 37°C for 3 h. The reaction was stopped by the addition of 15 mL of 10% trichloroacetic acid (TCA). The mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro- Kjeldahl's method. Digestibility was obtained by using the following equation:

$$\text{Protein digestibility} = \frac{\text{N in supernatant} - \text{N in blank}}{\text{N in sample}}$$

Determination Of Total Minerals:

Minerals were determined from the samples by the dry ashing method that described by Chapman & Pratt (1982). About 2.0 g of samples was acid-digested with diacid mixture (HNO₃/HClO₄, 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 made to 50 mL with double-distilled water and was used for determination of total minerals. Calcium was determined by a titration method. Phosphorus was determined spectrophotometrically by using molybdovanadate method. Iron and Magnesium were determined by Perkin-Elmer model 2380 atomic absorption spectrophotometer (Norwalk, CT, USA).

Determination of HCl extractable minerals:

Minerals in the samples were extracted by the method described by El Maki *et al.* (2007). One gram of the sample was extracted using 10 mL of 0.03 M HCl with shaking at 37 °C for 3 h. Then, the extract was filtered and the clear supernatant was dried at 100°C, incinerated at 550°C for 4 h. Thereafter, the samples were cooled and 5 mL of HCl were added and heated gently on a sand bath for 10 min. After cooling, samples were diluted to 100 mL using distilled water. The amount of the extractable phosphorus, calcium, iron and magnesium in the digested sample were determined in the same manner as described above for total minerals. Extractability of each element was calculated as a percentage of the total amount of the element.

$$\text{Mineral extractability \%} = \frac{\text{Mineral extractable in 0.03 N HCl}}{\text{Total minerals (mg/100g)}} \times 100$$

Statistical Analysis:

Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of variance (ANOVA) (Snedecor and Cochran, 1987). Duncan's multiple range test was used to separate means. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect Of Processing Methods On The Antinutritional Factors Content And In Vitro Protein Digestibility Of Sorghum Cultivars:

Table (1) shows the polyphenols, tannins and phytate content, and *in vitro* protein digestibility of raw and processed flour of three sorghum cultivars. Polyphenols contents of unprocessed sorghum flours were 554.27, 494.3 and 449.87 mg/100g, for Wad Ahmed, Abu Kunjara and Abu Ragaba, respectively. These values were within the range (270-2450 mg/100g) reported by Dlamini *et al.* (2007) and higher than the values (306.65 and 445.84 mg/100g) reported by Abdelrahman *et al.* (2005). There was a significant difference ($P \leq 0.05$) in polyphenols content between the two winter cultivars and Wad Ahmed (control), and between winter cultivars themselves. Wad Ahmed had the highest content followed by Abu Kunjara and then Abu Ragaba. This result suggested that sorghum cultivars (Wad Ahmed and Abu Kunjara) are high polyphenols genotypes, whereas, Abu Ragaba is low polyphenols genotype. The variations in polyphenols content may likely be due to genetic factors, environmental conditions, location, irrigation conditions, type of soil and degree of ripeness. Total polyphenols content of fermented flours of Wad Ahmed, Abu Kunjara and Abu Ragaba cultivars were 492.07, 470.70 and 392.77 mg/100g, respectively (Table 1). The result indicates that fermentation significantly ($P \leq 0.05$) reduced total polyphenols contents by 12.03, 5.60 and 13.50 % for Wad Ahmed, Abu Kunjara and Abu Ragaba, respectively. These values were less than the value (18% reduction) reported by Mohammed *et al.* (2011). Reduction in polyphenols after fermentation might be as a result of the activity of polyphenoloxidase or fermentation microflora (Reddy & Pierson, 1994). There was a significant difference ($P \leq 0.05$) between the

three cultivars in the percent of total polyphenols reduction. Abu Kunjara had the lowest reduction percent compare to the control and Abu Ragaba. The total polyphenols content of the raw-cooked sorghum flour was reduced by 29.13, 11.47 and 3.90 % for Wad Ahmed, Abu Kunjara and Abu Ragaba cultivars, respectively (Table 1). The result indicates that cooking significantly ($P \leq 0.05$) reduces the total polyphenols of sorghum. This is in fair agreement with Alonso *et al.* (2000) who reported a significant reduction ($P \leq 0.05$) of polyphenols due to thermal processing methods. The total polyphenols content of fermented cooked sorghum flour was reduced by 21.00, 17.07 and 14.93 % for Wad Ahmed, Abu Kunjara and Abu Ragaba, respectively (Table 1). The result indicates that combining fermentation with cooking significantly ($P \leq 0.05$) reduced the total polyphenols content. The reduction in polyphenols after cooking might be due to the fact that phenols react with protein during cooking forming poorly extractable protein-phenolic complexes (Osman *et al.*, 2010). Tannins contents, on the other hand, of raw sorghum flours were 158.03, 203.43 and 254.50 mg/100g, for Wad Ahmed, Abu Ragaba and Abu Kunjara respectively (Table 1). There was a significant difference ($P \leq 0.05$) in tannins content between the two winter cultivars and Wad Ahmed (control) and between winter cultivars themselves. Wad Ahmed had the lowest tannins content followed by Abu Ragaba and Abu Kunjara. This result suggested that Abu Kunjara is a high tannins genotype. This is likely due to the reddish brown color of Abu Kunjara grains which indicated that tannin is associated with the seed coat colour as reported previously (Rooney *et al.* 1984). Tannins content of fermented flour of Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars was 107.80, 90.20 and 126.83 mg/100g, respectively. The result indicated that fermentation significantly ($P \leq 0.05$) reduced tannins content by 32.40, 56.03 and 50.37% for the three cultivars, respectively. Similarly reduction of tannins content during fermentation was reported by many investigators (Idris *et al.*, 2005; Khattab & Arntfield, 2009). The reduction in tannins content after fermentation might be due to the effect of microorganisms and/or activation of tannins degradation (Reddy & Pierson, 1994). There was a significant difference ($P \leq 0.05$) between the three cultivars in the percent of tannins reduction during fermentation. Abu Ragaba had the highest percent reduction followed by Abu Kunjara then Wad Ahmed. Cooking of raw sorghum flour of Wad Ahmed, Abu Ragaba and Abu Kunjara significantly ($P \leq 0.05$) reduced tannins content by 13.77, 48.2 and 35.23%, respectively (Table 1). This result is lower than that reported by Idris *et al.* (2005) for two sorghum cultivars (55.2 and 48.6%), while Abdelhaleem *et al.* (2008) reported a reduction in tannins content 28.3 and 31.8% for two sorghum cultivars. The reduction in tannin after cooking may be as a result of heat degradation of tannin molecule as well as changes in chemical reactivity or the formation of insoluble complexes as explained by Alonso *et al.* (2000). There was a significant difference ($P \leq 0.05$) between the three cultivars in tannins content reduction during cooking. Abu Ragaba had the highest reduction followed by Abu Kunjara then Wad Ahmed. Cooking of fermented flour significantly ($P \leq 0.05$) reduced tannin content by 82.07, 74.67 and 63.10% for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively (Table 1). The result obtained match with those obtained by Idris *et al.* (2005) who found a reduction in tannins content of 75.7 and 78.1% of two sorghum cultivars. The phytate contents of sorghum cultivars Wad Ahmed, Abu Ragaba and Abu Kunjara were 280.57, 330.03 and 394.20 mg/100g, respectively (Table 1). The three cultivars show highly significant difference ($P \leq 0.05$) in phytate content between them. Abu Kunjara had the highest phytate content followed by Abu Ragaba and then Wad Ahmed. Fermentation of the three sorghum cultivars Wad Ahmed, Abu Ragaba and Abu Kunjara significantly ($P \leq 0.05$) reduced phytate content by 51.97, 53.57 and 52.90%, respectively. A similar trend of phytate reduction during fermentation was reported by Idris *et al.* (2005) who found 67.6 and 62.3% reduction in phytate of two sorghum cultivars for 14h fermentation. The loss in phytate during fermentation could be due to the action of either native phytase or that of fermenting microorganisms and thus hydrolysis of phytate into inositol and orthophosphate (Sandberg & Andlid, 2002). Cooking of the raw sorghum flour of Wad Ahmed, Abu Ragaba and Abu Kunjara slightly reduced phytate content by 16.47, 16.77 and 17.83%, respectively (Table 1). These results were in line with 16.2 and 16.0% reduction in phytate content of two sorghum cultivars investigated by Idris *et al.* (2005). Cooking cause a little reduction in phytate of sorghum. This is in agreement with the fact stated by Maga (1982) who found that phytate is fairly stable to heat. The observed reduction in phytate content of sorghum flours during cooking may be due to limited activation of phytase enzyme during cooking before denaturation by heat, or partly due to the heat labile nature of phytic acid and the formation of insoluble complexes between phytate and other components such as phytate-protein and phytate-protein-mineral complexes (Udensi *et al.*, 2007). Cooking of fermented flour significantly ($P \leq 0.05$) reduced phytate content by 38.53, 36.03 and 35.50% for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively (Table 1). These results match those obtained by Idris *et al.* (2005) who found a reduction in phytate content by 37.6 and 36.0% for fermented-cooked flour of two sorghum cultivars. The results were higher than those reported by Abdelhaleem *et al.* (2008) who found a reduction of 9.6% for fermented-cooked sorghum. Reduction obtained after fermentation and cooking may be due to acumulative effect.

Since the *in vivo* techniques are time consuming as well as expensive and the results from *in vitro* studies are equally reliable, *in vitro* methods have been successfully used in assessing the protein digestibility of foods. As shown in Table (1) the *in vitro* protein digestibility of Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars was 38.46, 40.03 and 32.33%, respectively. The results were lower than the results stated by Awadelkareem *et*

al. (2009) who reported 49.25 and 55.85% *in vitro* protein digestibility of African and Indian sorghum cultivars, respectively. Also the results obtained were lower than the results stated by Ibrahim *et al.* (2005) who analyzed two Sudanese sorghum cultivars high tannin and low tannin and recorded 47.9 and 53.0%, respectively. The *in vitro* protein digestibility of the cultivars showed a significant difference ($P \leq 0.05$) between them. Abu Ragaba (low tannin) had the higher digestibility followed by Wad Ahmed then Abu Kunjara. Fermentation significantly ($P \leq 0.05$) improved the *in vitro* protein digestibility of the three cultivars (Table 1), Wad Ahmed, Abu Ragaba and Abu Kunjara gave protein digestibility of 45.87, 47.17 and 38.93%, respectively. The result is agreed with Elkhalfifa *et al.* (2004) who found that fermented sorghum flour had higher *in vitro* protein digestibility than unfermented one. The reason for the increase in *in vitro* protein digestibility is attributed to enzymatic breakdown of complex storage proteins into simpler soluble products after fermentation (Yousif and El Tinay, 2001). Cooking of the raw sorghum flour of all cultivars significantly ($P \leq 0.05$) decreased the *in vitro* protein digestibility of all cultivars (Table 1). Abu Kunjara (high tannin) had the lowest *in vitro* protein digestibility (20.93%) followed by Wad Ahmed (25.63%) then Abu Ragaba (low tannin) (28.41%). These findings are agreed with Duodu *et al.* (2002) who reported that cooking significantly decrease *in vitro* protein digestibility of sorghum and maize cultivars. The result obtained also agreed with Belton and Taylor (2004) and Arabab and El Tinay (1997) who reported that sorghum protein digestibility decrease significantly after wet cooking. It is well documented that condensed polyphenols have a negative impact on digestibility of high-tannin sorghum (Hahn *et al.*, 1984), but the results indicated that tannins are not mainly responsible factors for lowering *in vitro* protein digestibility and may be many factors else had a role in this process. Duodu *et al.* (2003) reported that the low protein digestibility of cooked sorghum may be resulted from changes in proteins themselves during cooking. The formation of enzyme resistant disulphide bonded oligomers may cause low digestibility of proteins. Cooking of fermented flour significantly ($P \leq 0.05$) improved the *in vitro* protein digestibility over cooking alone for all cultivars (Table 1). Abu Ragaba (low tannin) had the highest digestibility followed by Wad Ahmed then Abu Kunjara (high tannin). The results match with those obtained by Yousif and El Tinay (2001), and Taylor and Taylor (2002) who reported that cooking reduced digestibility of sorghum while combining fermentation with cooking had significantly improved protein digestibility over wet cooking alone. The *in vitro* protein digestibility of the cultivars showed a significant difference ($P \leq 0.05$) between them for all treatments. Abu Ragaba (low tannin) had the highest *in vitro* protein digestibility for all treatments followed by Wad Ahmed and then Abu Kunjara (high tannin).

Effect Of Processing Methods On Minerals Content And Extractability Of Sorghum Cultivars:

As shown in Table (2) the total calcium content was significantly different ($P \leq 0.05$) between winter sorghum (Abu Ragaba and Abu Kunjara) and summer season sorghum (Wad Ahmed) which was found to be 16.90, 17.82 and 12.54 mg/100g for Abu Ragaba, Abu Kunjara and Wad Ahmed, respectively. Out of this amount about 20.60, 27.77 and 33.87% was found to be extractable for the cultivars Abu Kunjara, Abu Ragaba and Wad Ahmed, respectively. The values for total calcium are higher than that reported by Idris *et al.* (2005) while extractability for the investigated cultivars lower than that reported by Idris *et al.* (2005), who found 10.8 and 12.5 mg/100g total calcium for two sorghum cultivars while the extractability was 33.3 and 35.7%. The values of total calcium content are match with those reported by Khalil *et al.* (1984) who reported a 16.0 and 18.0 mg/100g for reddish-white and white sorghum. The results obtained showed low extractability of calcium of all cultivars. This could be attributed to the fact that legends form insoluble chelates with minerals. Fermentation significantly ($P \leq 0.05$) decreased the total calcium content of the three cultivars but increased the extractability (Table 3). The value of extractability after fermentation was 36.8, 30.6 and 25.23% for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. These results are in line with those of Idris *et al.* (2005) who reported that fermentation significantly increased mineral extractability. The increment in extractability is due to reduction in phytate by fermentation, thereby converting bound forms of minerals to free forms which are responsible for increased availability of minerals (Chompreeda and Field, 1984). Cooking of the raw sorghum flour of all cultivars significantly ($P \leq 0.05$) decrease calcium extractability (Table 2). These results agreed with those reported by Idris *et al.* (2005). The reduction in extractability may be due to complexation of minerals with other food constituents. However, total calcium content increased for Abu Ragaba and Wad Ahmed and decreased for Abu Kunjara as a result of cooking. This may be due to higher phytate content that occurred after cooking for Abu Kunjara (Table 1). Cooking of the fermented samples significantly ($P \leq 0.05$) increased calcium extractability (Table 2). The increment may be due to the high reduction in phytate content followed cooking of fermented flour. There was a significant difference ($P \leq 0.05$) among Abu Ragaba and Abu Kunjara for all treatments in regard to calcium extractability in which Abu Ragaba is superior to Abu Kunjara cultivar. The total phosphorus content of Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars was 282.31, 280.13 and 366.66 mg/100g, respectively (Table 2). The values were less than the ranges (396-407 mg/100m) and (410.28-424.45 mg/100g) reported by Khalil *et al.* (1984) and Mohamed Nour *et al.*, (2011), respectively. Out of this amount about 21.33, 24.30 and 15.97% was found to be extractable for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. These values were less than the values (43.4 and 44.9%) reported by Idris *et al.* (2005)

and more than the range (16-19.59%) reported by Mohamed Nour *et al.*, (2011) for winter sorghum cultivars. There was a significant difference ($P \leq 0.05$) between winter sorghum (Abu Ragaba and Abu Kunjara) and Wad Ahmed, and between the two winter cultivars in extractable phosphorus. The results obtained showed low phosphorus extractability for all cultivars. This may be due to the formation of insoluble chelates. Fermentation significantly ($P \leq 0.05$) decreased the total phosphorus content of the three cultivars, but increased the extractability (Table 2). The values of extractability after fermentation were 25.4, 27.87 and 19.10 % for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. These results are agreed with those previously reported (Idris *et al.*, 2005). The increment in extractability may be due to the reduction in phytate content (Table 1). Cooking of the raw sorghum flour of all cultivars significantly ($P \leq 0.05$) decreased phosphorus extractability (Table 2). The reduction in extractability may be due to complexation of minerals with other food constituents. Cooking of the fermented samples significantly ($P \leq 0.05$) increased extractability of phosphorus (Table 2). The increment may be due to reduction in phytate content followed cooking of fermented flour as shown in Table (1). Phosphorus extractability showed a significant difference ($P \leq 0.05$) among cultivars for all treatments. Abu Ragaba had the higher extractability followed by Wad Ahmed and then Abu Kunjara. As shown in Table (2) the total iron content (Fe) showed a significant difference ($P \leq 0.05$) between Wad Ahmed and Abu Ragaba and Abu Kunjara which was found to be 6.78, 9.08 and 9.23 mg/100g for the cultivars, respectively. The values are higher than the ranges (4.0-5.5 mg/100g) and (3.41-7.45 mg/100g) reported by Abdelrahman *et al.* (2005) and Abdelseed *et al.*, (2011), respectively. Out of this amount about 7.10, 7.80 and 4.55% were found to be available of the cultivars, Wad Ahmed and Abu Ragaba and Abu Kunjara, respectively. The values are higher than (4.2 and 6.0%) reported by Idris *et al.* (2005) for two sorghum cultivars and within the range (2.58-10.69%) reported by Mohamed Nour *et al.*, (2010). The results obtained showed that the extractability of iron of Abu Kunjara is lower than Wad Ahmed, while Abu Ragaba had the highest. Fermentation significantly ($P \leq 0.05$) increased both total iron content and extractability of the three cultivars (Table 2). This result was agreed with that reported previously by Idris *et al.* (2005). The increment in total iron and extractability may be due to decrease in levels of anti-nutritional factors which affect the absorption of minerals. Cooking of the raw sorghum flour of all cultivars significantly ($P \leq 0.05$) decreased iron extractability (Table 2). However, cooking of the fermented samples significantly ($P \leq 0.05$) increased extractability of iron. The values of extractability after cooking of fermented samples are 17.10, 19.83 and 12.20% for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively. This increment may be due to reduction of anti-nutritional factors. There was significant difference ($P \leq 0.05$) among the three cultivars in iron extractability for all treatments. Abu Ragaba had the highest extractability followed by Wad Ahmed and then Abu Kunjara. The total magnesium content of Wad Ahmed, Abu Ragaba and Abu Kunjara cultivars was 58.86, 52.32 and 26.64 mg/100g, respectively (Table 2). The values are in line with Khalil *et al.* (1984) who reported 46.4 and 55.6 mg/100g for white and reddish sorghum cultivars, respectively. The values are less than (59.3 and 63.0 mg/100g) reported by Idris *et al.* (2005) for two sorghum cultivars. Out of this amount about 28.15, 21.90 and 13.60% was found to be extractable for Wad Ahmed, Abu Ragaba and Abu Kunjara, respectively (Table 2). The values are less than (46.4 and 55.6%) reported by Idris *et al.* (2005). The results obtained showed that the extractability of magnesium is very low for all cultivars, however, Abu Ragaba is higher than Abu Kunjara and both cultivars are lower than Wad Ahmed. Low magnesium extractability may be due to the fact that magnesium like divalent cations may present as mineral-phytate chelates in raw seeds (Mamiro *et al.*, 2001). Fermentation significantly ($P \leq 0.05$) increased the total magnesium content of Abu Ragaba and Abu Kunjara and decreased its content in Wad Ahmed (Table 2). However, the extractability of magnesium in all cultivars was significantly ($P \leq 0.05$) increased. On the other hand, cooking of the raw sorghum flour significantly ($P \leq 0.05$) decreased magnesium extractability of the three cultivars (Table 2). Whereas, cooking of the fermented samples significantly ($P \leq 0.05$) increased magnesium extractability of all cultivars. This increment may be due to reduction in anti-nutritional factors. There was a significant difference ($P \leq 0.05$) among the three cultivars in magnesium extractability for all treatments. Abu Ragaba had higher extractability compared to Abu Kunjara and both cultivars are lower than Wad Ahmed.

Table 1: Antinutritional factors content (mg/100g) and *in vitro* protein digestibility (IVPD) of raw and processed winter sorghum cultivars.

Sorghum cultivar	Treatment	Polyphenols		Tannin		Phytate		IVPD
		Total	Reduction (%)	Total	Reduction (%)	Total	Reduction (%)	
Wad Ahmed	Raw	554.27 ^a (±1.75)	0.00 ⁱ	158.03 ^d (±3.25)	0.00 ^j	280.57 ^d (±1.22)	0.00 ^e	43.46 ^d (±0.15)
	Fermented	492.07 ^b (±1.16)	12.03 ^f	107.80 ^e (±1.11)	32.40 ^h	136.03 ^l (±0.60)	51.9 ^{7a}	50.87 ^b (±0.33)
	Cooked	392.83 ^b (±0.68)	29.13 ^a	136.27 ^e (±3.25)	13.77 ⁱ	234.37 ^e (±1.05)	16.47 ^d	30.63 ^h (±0.40)
	Fermented/cooked	437.50 ^c (±1.21)	21.0 ^b	28.37 ^f (±1.31)	82.07 ^a	172.43 ^j (±3.04)	38.53 ^b	37.63 ^f (±0.35)
Abu Ragaba (Winter white)	Raw	449.87 ^d (±1.46)	0.00 ⁱ	203.43 ^b (±2.29)	0.00 ^j	330.03 ^b (±0.78)	0.00 ^e	45.03 ^c (±0.45)
	Fermented	392.77 ^h	13.50 ^e	90.20 ⁱ	56.03 ^d	164.60 ^k	53.57 ^a	52.17 ^a

		(±5.88)		(±1.10)		(±0.50)		(±0.57)
	Cooked	432.67 ^f (±1.89)	2.90 ^h	104.40 ^h (±1.21)	48.20 ^f	272.17 ^e (±1.93)	16.77 ^d	33.41 ^g (±0.26)
	Fermented/cooked	379.07 ^f (±1.82)	14.93 ^d	51.13 ^k (±1.63)	74.67 ^b	209.23 ^h (±1.00)	36.03 ^{bc}	40.70 ^e (±0.26)
Kunjara (Winter red)	Raw	494.30 ^b (±0.62)	0.00 ⁱ	254.50 ^a (±1.64)	0.00 ^j	394.20 ^a (±3.99)	0.00 ^e	37.33 ^f (±0.55)
	Fermented	470.70 ^c (±1.61)	5.60 ^e	126.83 ^f (±1.59)	50.37 ^e	187.30 ⁱ (±1.25)	52.90 ^b	43.93 ^d (±0.60)
	Cooked	437.50 ^e (±3.38)	11.47 ^f	164.90 ^c (±1.21)	35.23 ^e	322.97 ^e (±2.24)	17.83 ^d	25.93 ⁱ (±1.01)
	Fermented/cooked	413.77 ^e (±1.27)	17.07 ^c	94.80 ⁱ (±1.10)	63.10 ^c	256.53 ^f (±1.27)	35.50 ^e	33.57 ^g (±0.45)

Values are means of triplicates samples (±S.D). Values bearing different superscript letters within columns are significantly different (P≤0.05).

Table 2: Total (mg/100g) and extractable (%) Calcium (Ca), phosphorous (P), Iron (Fe) and Magnesium (Mg) of raw and processed winter sorghum cultivars.

Sorghum cultivar	Treatment	Ca		P		Fe		Mg	
		Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable
Wad Ahmed	Raw	12.54 ^{def} (±0.55)	33.87 ^{cd} (±1.15)	282.31 ^{de} (±1.09)	21.33 ^e (±0.35)	6.78 ^e (±0.74)	7.10 ^{ef} (±0.40)	58.86 ^{bc} (±6.54)	16.42 ^{bc} (±0.13)
	Fermented	9.90 ^g (±1.10)	36.80 ^e (±1.91)	264.55 ^f (±24.75)	25.40 ^c (±0.70)	10.01 ^{cd} (±1.05)	10.55 ^d (±1.35)	50.60 ^c (±2.20)	15.33 ^{bcd} (±1.81)
	Cooked	20.17 ^a (±0.55)	32.17 ^d (±1.05)	224.54 ^g (±1.09)	18.33 ^f (±1.05)	7.03 ^e (±0.11)	6.70 ^{ef} (±0.30)	55.59 ^{bc} (±3.27)	13.72 ^{cde} (±1.97)
	Fermented/cooked	11.45 ^{efg} (±0.55)	41.60 ^b (±3.40)	293.76 ^d (±7.08)	29.13 ^b (±0.35)	12.46 ^{abcd} (±1.18)	17.10 ^b (±1.10)	58.86 ^{bc} (±0.00)	21.53 ^{ab} (±5.15)
Abu Ragaba (Winter white)	Raw	16.90 ^b (±0.55)	27.77 ^{ef} (±1.05)	280.13 ^{def} (±8.72)	24.30 ^{cd} (±1.30)	9.08 ^{de} (±1.72)	7.80 ^e (±0.20)	52.32 ^c (±0.00)	11.45 ^{def} (±1.64)
	Fermented	13.75 ^{cde} (±0.55)	30.60 ^{def} (±0.20)	228.80 ^g (±11.00)	27.87 ^b (±2.60)	11.28 ^{bcd} (±3.69)	11.20 ^{cd} (±0.10)	56.10 ^{bc} (±3.30)	18.15 ^{bc} (±1.65)
	Cooked	17.28 ^b (±4.32)	25.40 ^f (±5.40)	318.06 ^c (±3.78)	22.60 ^{de} (±0.60)	10.69 ^{cd} (±0.81)	6.85 ^{ef} (±0.05)	48.60 ^c (±16.20)	9.78 ^{ef} (±3.31)
	Fermented/cooked	14.04 ^{cd} (±0.00)	45.60 ^a (±0.60)	270.54 ^{ef} (±5.94)	29.63 ^b (±1.25)	14.06 ^{ab} (±2.46)	19.83 ^a (±0.75)	64.80 ^{ab} (±6.48)	22.94 ^a (±4.22)
Abu Kunjara (Winter red)	Raw	17.82 ^b (±0.54)	20.60 ^g (±1.20)	366.66 ^a (±3.78)	15.97 ^g (±0.12)	9.23 ^{de} (±3.51)	4.55 ^g (±0.55)	26.64 ^d (±1.25)	3.63 ^g (±0.13)
	Fermented	12.54 ^{def} (±0.55)	25.23 ^f (±2.75)	351.01 ^b (±1.14)	19.10 ^f (±1.55)	12.70 ^{abc} (±0.16)	6.45 ^f (±0.15)	50.50 ^c (±1.67)	8.21 ^f (±0.27)
	Cooked	10.26 ^{fg} (±0.54)	19.50 ^g (±0.50)	183.06 ^h (±7.02)	15.07 ^g (±0.85)	10.66 ^{cd} (±0.03)	4.30 ^g (±0.30)	25.56 ^d (±2.25)	2.54 ^g (±0.19)
	Fermented/cooked	15.81 ^{bc} (±0.55)	32.53 ^d (±1.10)	381.50 ^a (±4.75)	32.23 ^a (±1.45)	15.68 ^a (±0.25)	12.20 ^c (±0.20)	71.94 ^a (±2.18)	25.22 ^a (±2.57)
Lsd _{0.05}	2.309 [*]	3.729 ^{**}	15.38 ^{**}	2.033 [*]	3.025 [*]	1.015 [*]	9.579 ^{**}	4.199 ^{**}	
SE	0.7912	1.277	5.268	0.6967	1.036	0.3479	3.282	1.439	

Values are means of triplicate samples (±SD). Values bearing different superscript letters within columns are significantly different (P ≤ 0.05).

Conclusion:

Fermentation of the grains flour resulted in reduction in antinutritional factors (polyphenols, tannin and phytate), an improvement of the *in vitro* protein digestibility; in addition it appears to be most beneficial in increasing HCl-extractability of minerals. Combination of cooking with fermentation resulted in a reduction in antinutritional factors with a concomitant improvement in HCl-extractability of minerals and *in vitro* protein digestibility.

REFERENCES

- Abdelhaleem, W.H., A.H. El Tinay, A.I. Mustafa, E.E. Babiker, 2008. Effect of Fermentation, Malt-Pretreatment and Cooking on Antinutritional Factors and Protein Digestibility of Sorghum Cultivars, Pak J Nutr., 7(2): 335-341.
- Abdelrahman, S.M., H.B. El Makki, W.H. Idris, E.E. Babiker, A.H. El Tinay, 2005. Proximate composition, anti-nutritional factors and mineral content and availability of selected legumes and cereals grown in Sudan. J Food Technol., 3: 511-515.
- Abdelseed, B.H., H.A. Abdelwahab, A.Y. Abu ElGasim, I A. Mohamed Ahmed, E.E. Babiker, 2011. Some nutritional attributes of selected newly developed lines of sorghum after fermentation. J Agric Sci Technol., 13: 399-409.

- Alonso, A., A. Aguirre, F. Marzo, 2000. Effect of extrusion and traditional processing methods on anti-nutrients and *in vitro* digestibility of protein and starch in faba bean and kidney beans. *Food Chem.*, 68: 159-165.
- Arbab, M.E., A.H. EL Tinay, 1997. Effect of cooking with sodium bisulphate or ascorbic acid on the *in vitro* protein digestibility of two sorghum cultivars. *Food Chem.*, 59: 339-343.
- Awadelkareem, A. M., G. Muralikrishna, A.H. EL Tinay, A.I. Mustafa, 2009 Characterization of Tannin and Study of *in vitro* Protein Digestibility and Mineral Profile of Sudanese and Indian Sorghum Cultivars. *Pak J Nutr.*, 8: 469-476.
- Belton, P.S., J.R.N. Taylor, 2004. Sorghum and millets: protein sources for Africa. *Trends Food Sci Technol.*, 15: 94-98.
- Chapman, H.D., F.P. Pratt, 1982. *Methods of analysis soils plant and water*. University of California, Agriculture Division, USA, pp. 169.
- Chompreeda, P.T., M.L. Fields, 1984. Effect of heat and fermentation on the extractability of minerals from soybean meal and corn meal blends. *J Food Sci.*, 49: 566- 568
- Dlamini, N.R., J.R.N. Taylor, L.W. Rooney, 2007. The effect of sorghum and processing on the antioxidant properties of Africa sorghum-based foods. *Food Chem.*, 105: 1412-1419.
- Duodu, K.G., A. Nunest, I. Delgadillot, M.L. Parkert, E.N.C. Millst, P.S. Beltont, J.R.N. Taylor, 2002. Effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *J Cereal Sci.*, 35: 161-174.
- Duodu, K.G., J.R.N. Taylor, P.S. Belton, B.B. Hamaker, 2003. Factors affecting sorghum protein digestibility Mini Review, *J Cereal Sci.*, 38: 117-131.
- El Tinay, A.H., Z.M. El Mehdi, A. El Soubki, 1985. Supplementation of fermented sorghum Kisra bread with legume protein isolates. *J Agric Food Chem.*, 21: 679-687.
- Elkhalifa, A.O., B. Schiffler, R. Bernhardt, 2004. Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. *Nahrung/ Food*, 48: 91-94.
- El Maki, H.B., S.M. AbdelRahaman, W.H. Idris, A.B. Hassan, E. E. Babiker, A. H. El Tinay, 2007. Content of antinutritional factors and HCl-extractability of minerals from white bean (*Phaseolus vulgaris*) cultivars: influence of soaking and/or cooking. *Food Chem.*, 100: 362-368.
- FAO., 1997. *Production yearbook*. Food and Agricultural Organization of the United Nation. Rome, Italy., 51: 59-79.
- Hahn, D.H., L.W. Rooney, C.F. Earp, 1984. Tannins and polyphenols of sorghum. *Cereal Food World*, 29: 776-779.
- Ibrahim, F.S., E.E. Babiker, N.E. Yousif, A.H. ELTinay, 2005. Effect of fermentation on biochemical and sensory characteristics of sorghum flour supplemented with whey protein. *Food Chem.*, 92: 285-292.
- Idris, W.H., S.M. AbdelRahman, H.B. ELMaki, E.E. Babiker, A.H. EL Tinay, 2005. Effect of germination, fermentation and cooking on phytic acid and tannin contents and HCl-extractability of minerals of sorghum (*Sorghum bicolor*) cultivars. *J Food Technol.*, 3(3): 410-416.
- Khalil, J.K., W.N. Sawaya, W.J. Safi, H.M. Al Mohammed, 1984. Chemical composition and nutritional quality of sorghum flour and bread. *Plant Foods Hum Nutr.*, 34: 141-150.
- Khatab, R.Y., S.D. Arntfield, 2009. Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT - Food Sci Technol.*, 42: 1113-1118.
- Maga, J.A., 1982. Phytate: its chemistry, occurrence, food interaction nutritional significance and methods of analysis. *J Agric Food Chem.*, 30: 1-9.
- Mamiro, P.R.S., J. Vancamp, S.M. Mwikya, A. Huyghebaert, 2001. *In vitro* extractability of calcium, iron and zinc in finger millet and kidney beans during processing. *J Food Sci.*, 66: 1271-1275.
- Mohamed Nour, A.A., I.A. Mohamed Ahmed, E.E. Babiker, A.E.A. Yagoub, 2010. Investigations on winter season Sudanese sorghum cultivars: effect of sprouting on the nutritional value. *Inter J Food Sci Technol.*, 45: 884-890.
- Mohammed, N.A., I.A. Mohammed Ahmed, E.E. Babiker, 2011. Nutritional evaluation of sorghum flour (*Sorghum bicolor* L. Moench) during processing of injera. *Inter J Biol Life Sci.*, 7: 1.
- Monjula, S., E. John, 1991. Biochemical changes and *in vitro* protein digestibility of the endosperm of germinating *Dolichos lablab*. *J Sci Food Agric.*, 55: 529-539.
- Osman, N.M., I.A. Mohamed Ahmed, E.E. Babiker, 2010. Fermentation and cooking of sicklepod (*Cassia obtusifolia*) leaves: changes in chemical and amino acid composition, antinutrients and protein fractions and digestibility. *Inter J Food Sci Technol.*, 45: 124-132.
- Price, M.L., L.G. Bulter, 1977. Rapid visual estimation and spectrophotometer determination of tannin content of sorghum grain. *J Agric Food Chem.*, 25: 1268-1273.
- Price, M.L., S. Van Scoyoc, L.G. Butler, 1978. A critical evaluation of the vanillin reactions as an assay for tannin in sorghum grain. *J Agric Food Chem.*, 26: 1214-1218.

Reddy, N.R., M.D. Pierson, 1994. Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Res Inter.*, 27: 281-290.

Rooney, L.W., D.H. Hahn, C.F. Earp, 1984. Tannins and phenols of sorghum. *Cereal Foods World*, 29: 776-779.

Sandberg, A.S., T. Andlid, 2002. Phytogetic and microbial phytases in human nutrition. *Inter J Food Sci Technol.*, 37: 823-833.

Snedecor, G.W., W.G. Cochran, 1987. *Statistical Methods*, 17th (Edn). The Iowa State University Press, Ames, IA, USA, pp: 221-222.

Sokrab, A.M., I.A. Mohamed Ahmed, E.E. Babiker, 2012. Effect of malting and fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *Inter J Food Sci Technol.*, 47: 1037-1043.

Taylor, J., J.R.N. Taylor, 2002. Alleviation of the adverse effects of cooking on protein digestibility in sorghum through fermentation in traditional African porridges. *Inter J Food Sci Technol.*, 37: 129-138.

Udensi, E.A., F.C. Ekwu, J.N. Isinguzo, 2007. Antinutrient factors of vegetable cowpea (*Sesquipedalis*) seeds during thermal processing. *Pak J Nutr.*, 6: 194-197.

Wheeler, E.L., R.E. Ferrel, 1971. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem.*, 48: 312.

Yagoub, A.A., A.A. Abdalla, 2007. Effect of domestic processing methods on chemical, *in vitro* digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranea*) seed. *Res J Agric Biol Sci.*, 3: 24-34.

Yousif, N.E., A.H. El Tinay, 2001. Effect of fermentation on sorghum protein fractions and *in vitro* protein digestibility. *Plant Food Human Nutr.*, 56: 175-183.