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Effect of Germination, Fermentation and Cooking on Phytic Acid and Tannin Contents and Hcl-extractability of Minerals of Sorghum (*Sorghum biocolor*) Cultivars

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Abstract: Sorghum (*Sorghum biocolor*) also known as 'Dura' in Sudan is a source of carbohydrate, protein and mineral that is comparable to other common cereal grains. However, antinutrients like phytate and tannins reduce the nutrient bioavailability, which can be improved by suitable processing methods such as germination, fermentation and cooking. In our study, sorghum cultivars (Wad Ahmed and Tabat) were germinated for different periods (24, 48 and 96 h), fermented for different period of time up to 14 h (2 h interval) and then cooked. Major changes in phytate occurred during germination (96 h) and in tannin occurred after the germinated or fermented flour was cooked. The reduction in phytate content accompanied by increase in HCl-extractable minerals of more than 100%. The study revealed that germination or fermentation followed by cooking is a potential process for decreasing the antinutrient levels and enhancing availability of minerals.

Key words: Sorghum, germination, fermentation, cooking, HCl-extractability, minerals

INTRODUCTION

The production and distribution of plant foods is comparatively more economical because they exhibit better shelf life and can be, stored and processed with less expensive methods. Therefore, greater emphasis has been placed throughout the world on the increasing the production of plant foods, improving their nutritional quality and developing simple and economic methods for their storage and processing[1]. Among the plant foods, cereals are grown over 73.5% of the world-harvested area. Diets in developing countries are based mainly on cereals and legumes. Cereal grains contributed over 60% of the world food production and along with pulses and oilseeds form a major bulk of dietary protein, calories, vitamin and minerals to the world population in general and to the developing world in particular[1]. With increasing dependence upon cereal grains to provide both energy and protein requirements of human in developing countries, the need for raising the overall nutritional value of cereal grains has become increasingly important and much effort has been made to improve the amount and quality of cereal nutrients. The methods employed to improve the nutritional quality and organoleptic properties of cereal-based foods include genetic improvement, amino acid fortification, supplementation or complementation with protein rich sources and processing technologies which include milling, malting, fermentation or sprouting[1]. Cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low due to the presence of antinutritional factors such as phytate and polyphenols[3]. An adequate mineral absorption is important especially for infants children, elderly people and people in clinical situation[3]. It is evident that the nutritional importance of a given food/ feed stuff depends not only on nutrient composition of raw foodstuff but also on the amount utilized[4]. In the Sudan sorghum comes first in volume of cereals produced. It is the staple food for people of the semiarid areas of Africa, particularly the low-income groups [5]. It is also a principal source of alcoholic beverages in many countries[6] and feed for livestock[6]. Despite the documented potential, relative to other common cereals like maize and rice, sorghum has low nutritional value and inferior organoletpic qualities[7]. Sorghum is rich in mineral content but its nutritional quality is dictated mainly by its chemical composition; presence of considerable amounts of anti-nutritional factors such as tannin, phytic acid, polyphenol and trypsin inhibitors which are undesirable and efforts are directed to minimize their content in the seed[8]. Hence elimination or inactivation of such anti-nutritional compounds is absolutely necessary to improve the nutritional quality of sorghum and effectively utilize its full potential as human food, one way of solving this problem through food processing. In the present study we would like to evaluate the efficiency of processing methods such as cooking, germination and fermentation of sorghum flour on the reduction or elimination of anti-nutritional factors (phytate and tannin) and the improvement of

minerals availability.

MATERIALS AND METHODS

Two sorghum (*Sorghum biocolor*) cultivars, locally known as Wad Ahmed (high tannin) and Tabat (low tannin) were obtained from Pioneer Company, Khartoum, Sudan. About 4 kg of each variety was cleaned from damaged seeds, foreign materials and other extraneous grain or grits. For treated and treated grains about 1.0 kg of the seeds was milled to a fine powder. Unless otherwise stated all reagents used in this study were of reagent grade.

Germinated of the seeds: About 1.0 kg was germinated according to the method of Bhise *et al.*[9]. The sorghum grains of the cultivars were steeped overnight in distilled water. The wet grains were then soaked in 1-2 volumes of 0.2% formaldehyde solution for 40 min to prevent mould growth during germination. The soaked grains were then washed with distilled water several times and soaked in water for 20 min to remove remaining formaldehyde. The moistened sorghum was then spread out thinly on jute bags moistened with distilled water and germinated at room temperature (30±2°C). The grains were moistened two times a day to avoid drying. The samples were then taken to obtain 24, 48 and 96 hour germinated grains. The root and shoot portions removed manually and sun dried. The grains were milled into fine powder to pass a 0.4 mm screen and store at 4°C in polyethylene bags.

Sorghum fermentation: According to El Tinay *et al.* (1979) method about 400 g of the flour were mixed with water at ratio of 1:2 (w/v), then fermented by using a previously fermented starter. After thorough mixing, the samples were taken at 2 h intervals till the end of fermentation, which was terminated after 14 h at room temperature (30±2°C). The samples were dried at 70°C for 48 h, then ground to pass a 0.4 mm screen and store at 4°C.

Cooking of the flour: About 0.25 Kg of the flour before and after germination or fermentation were cooked in distilled water in a ratio of 1:2 (w/v) for 20 min in boiling water bath with stirring. Thereafter dried at 65°C in a hot air oven drier (Heraus UT 5042, Germany), then ground to pass a 0.4 mm screen and stored at 4°C in polyethylene bags.

Minerals analysis

Determination of total minerals: Total minerals were determined according to the method described by Champan and Partt[11]. Two grams of sample were weighed in a clean dry crucible. The crucible was placed in a muffle furnace for 4 h at 550°C. Samples were cooled and 10 mL of 3 N HCl was added, covered with watch glass and boiled gently for 10 min, then cooled. The contents were filtered through Whatman filter paper (No. 4) and the volume was made to 50 mL with distilled water. Minerals were determined by flamephotometry or atomic absorption spectrophotometer (Perkin-Elmer 2380).

Phosphorus determination: Phosphorus content was carried out according to the method of Chapman and Pratt [11] by using spectrophotometer (JENWAY 6305 UV/Vis.). A standard curve was prepared to determine P concentration in the sample.

Potassium and sodium determination: An aliquot of the above extract was used to determine the content of K and Na according to the AOAC[12] using Coring 400 Flame Photometer.

Determination of HCl-extractable minerals: Minerals extractability of untreated and treated sorghum was determined according to the method of Kumar and Chauhan[13]. About 1.0 gram of the sample was extracted in 10 mL 0.03 N HCl by shaking the content at 37°C for 3 h. The clear extract obtained after filtration through Whatman filter paper was oven dried at 100°C, then heated in a muffle furnace at 550°C for 3 h, after cooling, about 3 mL of 3 NHCl was added and the volume was made to 50 mL with distilled water. Mineral content of the extract was determined as described above.

Determination of tannin content: Quantitative estimation of tannins was carried out using the modified vanillin-HCl method according to Price *et al.*[14]. A standard curve was prepared using catechin.

Determination of phytic acid: Phytic acid content was determined according to the method of Wheeler and Ferrel[15] by using spectrophotometer (JENWAY 6305 UV/V).A standard curve of different (Fe $(NO_3)_2$) concentrations was plotted to calculate the ferric ion concentration. Phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 iron: phosphorus molar ratio.

Statistical analysis: All data were subjected to statistical analysis using Spss. VII.0, means were tested by analysis of variance (ANOVA). Value of probability of 5% was used to indicate significance according to Duncan multiple range test (DMRT) [16].

RESULTS AND DISCUSSION

Effect of germination, fermentation and/or cooking on phytate and tannin: Germination was more effective in decreasing phytate content (Table 1). It was observed that germination for 96 h reduced phytate by 90.9 and 88.3% for WadAhmed and Tabat cultivars, respectively while tannin content reduced by 73.6 and 34.3% for the cultivars, respectively. Fermentation of the cultivars flour was also found to be effective in reducing phytate and tannin for both cultivars (Table 2) but to a less extend compared to germination process. Cooking of the germinated or fermented flour (Table 3) significantly reduced both phytate and tannin contents. However, cooking of germinated flour was more effective in reducing tannin of the seed and was found to remove tannin completely from the cultivar Tabat (Table 3). Germination was observed to increase tannin content of sorghum seeds by bringing it to the surface but when the seeds were soaked prior germination and cooked after germination this process was observed to enhance leaching during soaking and evaporation during boiling [17]. Cooking of fermented flour was also found to be effective in reducing tannin content significantly compared to the amount of phtate reduced.

Effect of germination, fermentation and/or cooking on Minerals bioavailability: The grains of sorghum traditionally processed either by germination or fermentation prior to consumption. Germination was more effective in increasing the extractability of both major and trace elements especially when the rains were germinated for 96 h (Table 4). For both cultivars minerals extractability significantly ($p<0.05$) increased with time. For some minerals (Ca, Mn and Co) the extractability exceeded 100%. For both cultivars fermentation of the grains flour was also significantly ($p<0.05$) increased minerals extractability with time, which was observed to exceed 100% for Ca after 10 h fermentation (Table 5). Results indicated that fermentation was more effective in increasing the bioavailability of Ca compared to germination. Phytate complexes with essential elements such as Zn, Fe and Ca and reduces their bioavailability which can be enhanced by degradation of phytate [18]. Lopez *et al.*[19] observed that natural lactic acid fermentation decreased the phytic acid in corn meal due to phytase production by the microbes. Phytase activity was also found during germination of wheat, barley, rye and oats, which hydrolyse phytate to phosphate and myoinositol phosphates[20]. The increase in HCl extractable minerals may be attributed to reduction in phytate and presence of enhancers such as organic acids

Table 1: Effect of germination on phytate (mg/100g) and tannin (%) contents of sorghum cultivars

	Cultivars							
	Wad Ahmed				Tabat			
Germination time (h)	Phytate total	% Reduction	Tannin total	% Reduction	Phytate total	% Reduction	Tannin total	% Reduction
0	265.0 (+9.4)^{a}	-	0.96 (+0.02)^{a}	-	233.0 (+4.0)^{a}	-	0.35 (+0.00)^{a}	-
24	141.4 (+3.2)^{b}	46.7 (+1.2)^{c}	0.68 (+0.01)^{b}	29.2 (+2.1)^{c}	150.8 (+6.3)^{b}	35.3 (+2.7)^{c}	0.28 (+0.00)^{b}	20.0 (+0.00)^{b}
48	79.5 (+4.8)^{c}	70.0 (+1.8)^{b}	0.37 (+0.02)^{c}	61.4 (+1.0)^{b}	82.7 (+7.9)^{c}	64.5 (+3.4)^{b}	0.23 (+ 0.00)^{c}	34.3 (+0.00)^{a}
96	24.1 (+2.0)^{d}	90.9 (+1.8)^{a}	0.25 (+0.03)^{d}	73.6 (+0.64)^{a}	27.2 (+4.8)^{d}	88.3 (+2.1)^{a}	0.23 (+0.00)^{c}	34.3 (+ 0.00)^{a}

Values are means of three replicates (+ SD), Means not sharing a common superscript letter in a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

Table 2: Effect of fermentation on phytate (mg/100g) and tannin (%) contents of sorghum cultivars

	Cultivars									
	Wad Ahmed					Tabat				
Fermentation time (h)	pH	Phytate total	% Reduction	Tannin total	% Reduction	pH	Phytate total	% Reduction	Tannin total	% reduction
0	5.99	265.0 (+9.4)^{a}	-	0.96 (+0.03)^{b}	-	5.82	233.0 (+4.0)^{a}	-	0.35 (+0.02)$_{a}$	-
2	5.76	215.8 (+4.9)^{b}	18.6 (+1.9)^{g}	0.72 (+0.03)^{b}	25.0 (+2.1)^{d}	5.33	196.4 (+9.6)^{b}	15.5 (+1.4)^{e}	0.26 (+0.02)^{bc}	26.2 (+2.8)^{c}
4	5.22	186.4 (+7.9)^{c}	29.6 (+2.9)^{f}	0.71 (+0.03)^{b}	26.0 (+2.8)^{d}	5.00	169.6 (+6.3)^{c}	27.2 (+2.00)^{d}	0.25 (+0.02)^{bc}	28.1 (+5.0)^{de}
6	5.00	168.6 (+1.8)^{d}	36.4 (+0.69)^{e}	0.64 (+0.01)^{c}	33.3 (+2.0)^{c}	4.82	137.2 (+6.5)^{d}	42.7 (+0.67)^{c}	0.23 (+0.04)^{bcd}	33.3 (+7.8)^{cde}
8	4.50	130.9 (+0.0)^{f}	50.6 (+1.9)^{d}	0.62 (+0.02)^{c}	35.1 (+2.6)^{c}	4.00	118.3 (+4.7)^{e}	49.2 (+2.1)^{b}	0.22 (+0.03)^{cde}	38.1 (+4.6)^{bcd}
10	3.99	119.4 (+0.0)^{f}	54.9 (+0.0)^{c}	0.53 (+0.02)^{d}	44.8 (+1.0)^{b}	3.88	100.5 (+6.3)^{f}	56.9 (+2.7)^{a}	0.21 (+0.01)^{de}	39.5 (+4.4)^{bc}
12	3.77	106.8 (+6.3)^{g}	59.7 (+2.4)^{b}	0.40 (+0.04)^{e}	58.3 (+0.0)^{a}	3.53	97.4 (+6.3)^{f}	58.2 (+2.7)^{a}	0.18 (+0.02)^{ef}	48.6 (+4.9)^{ab}
14	3.60	85.9 (+7.9)^{h}	67.6 (+3.0)^{a}	0.37 (+0.01)^{e}	61.1 (+2.1)^{a}	3.33	91.1 (+5.4)^{f}	62.3 (+2.4)^{a}	0.16 (+0.01)^{f}	55.2 (+6.3)^{a}

Values are means of three replicates (+ SD). Means not sharing a common superscript letter in a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

Table 3: Effect of cooking, of flour before and after germination or fermentation on phytate (mg/100g) and tannin (%) contents of sorghum cultivars

	Cultivars							
	Wad Ahmed				Tabat			
Treatment	Phytate total	% Reduction	Tannin total	% Reduction	Phytate total	% Reduction	Tannin total	% Reduction
Untreated	265.0 (+9.61)^{a}	-	0.96 (+0.02)^{a}	-	233.0 (+10.54)^{a}	-	0.35 (+0.03)^{a}	-
Cooked	222.1 (+3.2)^{b}	16.2 (+1.7)^{c}	0.43 (+0.02)^{b}	55.2 (+2.1)^{c}	195.8 (+7.90)^{b}	16.0 (+3.37)^{c}	0.18 (+0.02)^{b}	48.6 (+5.70)^{c}
Germinated and cooked	107.9 (+7.95)^{d}	59.3 (+3.02)^{a}	0.18 (+0.01)^{d}	81.2 (+1.05)^{a}	88.0 (+6.3)^{d}	62.2 (+2.7)^{a}	0.00 (+0.00)^{d}	100.0 (+0.00)^{a}
Fermented and cooked	165.5 (+4.83)^{c}	37.6 (+1.78)^{b}	0.23 (+0.01)^{c}	75.7 (+1.21)^{b}	151.8 (+4.74)^{c}	36.0 (+1.68)^{b}	0.072 (+0.01)^{c}	78.1 (+1.57)^{b}

Values are means of three replicates (+ SD). Means not sharing a common superscript letter in a column are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

Table 4: Effect of germination on HCl-extractability (%) of minerals of sorghum cultivars

		Cultivars							
		Wad Ahmed				Tabat			
		Germination period (h.)							
Minerals		0	24	48	96	0	24	48	96
Na	Total	6.3 (+0.25)^{a}	6.3 (+0.25)^{a}	6.4 (+0.40)^{a}	6.4 (+0.40)^{a}	7.3 (+0.29)^{a}	7.0 (+0.00)^{a}	7.3 (+0.29)^{a}	7.3 (+0.17)^{a}
	Extract	66.7 (+4.56)^{b}	71.4 (+0.0)^{b}	75.5 (+0.02)^{b}	85.9 (+7.85)^{a}	66.2 (+4.00)^{c}	75.3 (+6.85)^{b}	85.0 (+0.29)^{b}	89.0 (+0.00)^{a}
K	Total	450.0 (+25.0)^{a}	450.0 (+0.0)^{a}	450.0 (+25.0)^{a}	450.0 (+0.00)^{a}	441.7 (+7.22)^{a}	441.7 (+28.8)^{a}	441.7 (+7.22)	441.7 (+14.4)^{a}
	Extract	46.3 (+6.64)^{d}	50.0 (+3.2)^{c}	64.8 (+3.23)^{b}	74.1 (+6.41)^{a}	50.9 (+0.00)^{c}	62.5 (+11.5)^{bc}	71.7 (+8.61)^{ab}	77.3 (+3.23)^{a}
Ca	Total	10.8 (+0.10)^{b}	12.5 (+0.15)^{a}	12.5 (+0.00)^{a}	12.5 (+0.00)^{a}	12.5 (+0.00)^{b}	14.4 (+0.35)^{a}	14.5 (+0.49)^{a}	14.7 (+0.20)^{a}
	Extract	33.3 (+1.09)^{d}	78.9 (+2.81)^{c}	88.7 (+0.11)^{b}	120.0 (+0.01)^{a}	35.8 (+1.00)^{a}	89.6 (+0.95)^{c}	97.2 (+0.00)^{b}	112.4 (+0.40)^{a}
Mg	Total	59.3 (+0.93)^{b}	59.3 (+0.93)^{b}	60.0 (2.0)^{b}	63.0 (+2.11)^{a}	63.0 (+0.00)^{b}	63.8 (+0.50)^{a}	63.8 (+0.00)^{a}	63.9 (+0.41)^{a}
	Extract	46.4 (+0.00)^{c}	64.8 (+7.19)^{b}	75.8 (+3.35)^{a}	81.8 (+5.71)^{a}	55.6 (+0.00)^{d}	74.0 (+0.46)^{c}	79.9 (+1.55)^{b}	85.4 (+4.70)^{a}
P	Total	303.4 (+6.35)^{a}	289.7 (+6.4)^{ab}	277.7 (+7.3)^{bc}	268.6 (+1.67)^{c}	283.4 (+8.06)^{a}	271.3 (+5.6)^{ab}	262.9 (+6.3)^{bc}	253.7 (+5.30)^{c}
	Extract	43.4 (+1.4)^{d}	61.0 (+2.61)^{c}	70.7 (+3.04)^{d}	92.4 (+2.35)^{a}	45.1 (+1.55)^{d}	62.7 (+1.99)^{c}	73.9 (+0.00)^{b}	94.2 (+4.38)^{a}
Fe	Total	3.8 (+0.64)^{a}	3.8 (+0.01)^{a}	3.9 (+0.00)^{a}	3.9 (+0.02)^{a}	4.5 (+0.00)+a	4.5 (+0.01)^{a}	4.5 (+0.17)^{a}	4.5 (+0.00)^{a}
	Extract	4.30 (+0.35)^{d}	7.2 (+0.66)^{c}	9.2 (+0.40)^{b}	13.3 (+0.92)^{a}	7.1 (+0.64)^{d}	13.7 (+0.81)^{c}	18.4 (+0.45)^{b}	23.3 (+0.00)^{a}
Zn	Total	3.21 (+0.03)^{a}	3.22 (+0.12)^{a}	3.25 (+0.03)^{a}	3.30 (+0.20)^{a}	3.50 (+0.20)^{b}	3.53 (+0.00)^{b}	3.55 (+0.02)^{ab}	3.60 (+0.80)^{a}
	Extract	47.2 (+3.03)^{d}	69.3 (+2.150^{c}	79.3 (+2.30)^{b}	84.5 (+0.90)^{a}	56.0 (+0.76)^{d}	71.4 (+2.90)^{c}	76.9 (+0.60)^{b}	80.3 (+0.17)^{a}
Mn	Total	3.90 (+0.10)^{a}	3.9 (+0.20)^{a}	3.9 (+0.20)^{a}	3.9 (+0.00)^{a}	3.50 (+0.11)^{a}	3.50 (+0.10)^{a}	3.50 (+0.00)^{a}	3.50 (+0.00)^{a}
	Extract	40.8 (+0.92)^{d}	74.1 (+1.91)^{c}	86.2 (+0.76)^{b}	103.5 (+1.40)^{a}	45.8 (+5.16)^{d}	76.5 (+2.19)^{c}	81.4 (+0.00)^{b}	96.7 (+1.67)^{a}
CO	Total	0.21 (+0.01)^{a}	0.21 (+0.02)^{a}	0.21 (+0.00)^{a}	0.21 (+0.02)^{a}	0.18 (+0.01)^{a}	0.18 (+0.02)^{a}	0.18 (+0.02)^{a}	0.19 (+0.01)^{a}
	Extract	66.7 (+0.00)^{c}	115.8 (+5.48)^{b}	138.1 (12.6)^{a}	147.8 (+4.75)^{a}	72.2 (+9.58)^{c}	111.1 (+5.53)^{b}	122.2 (+11.)^{ab}	131.6 (+0.00)^{a}
Cu	Total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Extract	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values are means of three replicates±SD. Means not sharing a common superscript letter in a row are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

Table 5: Effect of fermentation on HCl-extractability (%) of minerals of sorghum cultivars

	Cultivars							
	Wad Ahmed							
	Period of fermentation (h)							
Minerals	0	2	4	6	8	10	12	14
Na	66.7(+ 2.77)^{e}	69.8 (+ 0.69)^{d}	72.4 (+ 2.07)^{d}	75.6 (+ 1.00)^{c}	78.7 (+ 1.15)^{b}	81.0 (+ 1.40)^{b}	85.7 (+ 1.76)^{a}	87.3 (+ 2.06)^{a}
K	46.3 (+ 6.40)^{h}	52.4 (+ 0.98)^{g}	58.6 (+ 2.18)^{f}	64.9 (+ 0.98)^{e}	71.2 (+ 1.97)^{d}	77.1 (+ 2.15)^{c}	83.9 (+ 0.95)^{b}	89.5 (+ 0.96)^{a}
Ca	33.3 (+ 0.95)^{f}	37.5 (+ 1.66)^{ef}	38.3 (+ 1.91)^{ef}	40.3 (+ 0.70)^{e}	95.3 (+ 1.66)^{d}	104.5(+ 4.16)^{c}	114.7(+ 4.14)^{b}	124 (+ 3.37)^{a}
Mg	46.4 (+ 0.00)^{f}	47.4 (+ 1.9)^{f}	48.5 (+ 1.91)^{ef}	50.6 (+ 1.23)^{e}	59.3 (+ 2.23)^{d}	65.0 (+ 1.15)^{c}	71.4 (+ 1.04)^{b}	78.9 (+ 0.00)^{a}
P	43.4 (+ 1.40)^{e}	47.3 (+ 1.68)^{d}	50.1 (+ 1.31)^{d}	54.9 (+ 1.00)^{c}	57.0 (+ 1.96)^{c}	60.7 (+ 2.09)^{b}	63.9 (+ 2.75)^{ab}	66.6 (+ 2.06)^{a}
Fe	4.1 (+ 0.41)^{h}	5.9 (+ 0.69)^{g}	7.5 (+ 0.55)^{f}	8.9 (+ 0.46)^{e}	10.4 (+ 0.40)^{d}	12.4 (+ 0.57)^{c}	14.3 (+ 0.66)^{b}	15.8 (+ 1.39)^{a}
Zn	47.7 (+ 0.95)^{h}	53.2 (+ 1.11)^{g}	57.6 (+ 2.64)^{f}	61.4 (+ 1.40)^{e}	66.4 (+ 2.32)^{d}	70.1 (+ 1.63)^{c}	75.3 (+ 1.15)^{b}	78.8 (+ 1.80)^{a}
Mn	40.8 (+ 0.58)^{h}	49.2 (+ 1.07)^{g}	54.4 (+ 1.11)^{f}	58.9 (+ 1.83)^{e}	63.7 (+ 1.76)^{d}	67.9 (+ 0.95)^{c}	72.4 (+ 3.02)^{b}	79.4 (+ (1.42)^{a}
Co	66.7 (+ 0.00)^{g}	68.9 (+ 1.26)^{fg}	72.7 (+ 1.28)^{fg}	76.4 (+ 0.69)^{de}	79.9 (+ 1.84)^{cd}	83.3 (+ 0.73)^{bc}	87.7 (+ 0.73)^{ab}	90.5 (+ 0.70)^{a}
Cu	0.00	0.00	00.0	00.0	0.00	0.00	0.00	0.00
Na	64.3 (+ 2.77)^{f}	72.7 (+ 2.08)^{e}	76.1 (+ 1.47)^{d}	79.6 (+ 1.39)^{c}	82.5 (+ 2.26)^{c}	86.3 (+ 1.96)^{b}	88.5 (+ 2.20)^{b}	92.2 (+ 1.33)^{a}
K	51.0 (+ 5.65)^{h}	58.6 (+ 2.15)^{g}	64.2 (+ 1.28)^{f}	70.5 (+ 1.82)^{e}	77.1 (+ 1.43)^{d}	83.3 (+ 1.39)^{c}	90.3 (+ 1.21)^{b}	96.4 (+ 2.48)^{a}
Ca	35.7 (+ 1.22)^{f}	39.4 (+ 1.60)^{ef}	43.4 (+ 1.85)^{de}	47.6 (+ 1.35)	86.6 (+ 2.35)^{c}	105.4 (+ 4.48)^{b}	113.3 (+ 3.32)^{a}	116.5 (+ 10.5)^{a}
Mg	55.6 (+ 1.55)^{f}	56.7 (+ 1.51)^{f}	56.7 (+ 1.51)^{f}	60.0 (+ 2.46)^{e}	71.0 (+ 1.33)^{d}	76.4 (+ 0.83)^{c}	80.4 (+ 1.44)^{b}	84.6 (+ 0.85)^{a}
P	44.9 (+ 1.45)^{g}	48.7 (+ 1.10)^{f}	52.7 (+ 1.13)^{e}	56.7 (+ 1.96)^{d}	60.4 (+ 1.15)^{c}	63.3 (+ 1.79)^{b}	65.8 (+ 1.10)^{b}	69.5 (+ 2.05)^{a}

Table 5: Continue

	Cultivars							
	Tabat							
	Period of fermentation (h)							
Minerals	0	2	4	6	8	10	12	14
Fe	6.0 (+ 0.40)^{h}	7.7 (+ 0.47)^{g}	10.3 (+ 0.40)^{f}	12.5 (+ 0.61)^{e}	14.9 (+ 0.98)^{d}	17.5 (+ 0.66)^{c}	19.6 (+ 0.96)^{b}	22.0 (+ 1.88)^{a}
Zn	53.8 (+ 0.65)^{f}	59.7 (+ 1.25)^{e}	63.6 (+ 1.64)^{de}	67.6 (+ 1.14)^{d}	72.9 (+ 1.22)^{c}	76.6 (+ 0.98)^{bc}	79.3 (+ 1.23)^{b}	86.2 (+ 2.06)^{a}
Mn	46.2 (+ 2.45)^{h}	51.5 (+ 0.75)^{g}	57.3 (+ 2.21)^{f}	60.5 (+ 1.72)^{e}	64.2 (+ 0.96)^{d}	67.6 (+ 1.14)^{c}	73.9 (+ 1.57)^{b}	80.7 (+ 1.32)^{a}
Co	72.2 (+ 0.56)^{b}	75.5 (+ 0.50)^{ab}	78.8 (+ 2.05)^{ab}	82.5 (+ 1.27)^{ab}	84.6 (+ 0.55)^{ab}	89.9 (+ 1.43)^{ab}	93.3 (+ 2.25)^{a}	96.4 (+ 1.50)^{a}
Cu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values are means of three replicates±SD. Means not sharing a common superscript letter in a row are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

Table 6: Effect of cooking and/or fermentation or germination on total (mg/100g) and HCl-extractability (%) of minerals of sorghum cultivars

		Cultivars			
		Wad Ahmed		Tabat	
Minerals		Uncooked	Cooked	Uncooked	Cooked
Na	Total	6.3 (+0.25)	6.0 (+0.50)	7.0 (+0.00)	7.3 (+0.29)
	Extract	66.7 (+2.77)	63.9 (+9.64)	64.3 (+0.00)	89.9 (+0.00)
K	Total	450.0 (+25.0)	400.0 (+12.50)	441.7 (+14.43)	441.7 (+2.0)
	Extract	46.3 (+6.41)	43.8 (+0.00)	51.0 (+5.65)	79.8 (+0.50)
Ca	Total	10.8 (+0.15)	10.8 (+0.15)	12.5 (+0.00)	12.5 (+0.00)
	Extract	33.3 (+0.95)	30.3 (+2.22)	35.7 (+1.22)	79.3 (+0.17)
Mg	Total	59.3 (+0.00)	60.0 (+0.50)	63.0 (+1.00)	59.7 (+0.69)
	Extract	46.4 (+0.00)	41.7 (+1.65)	55.6 (+1.55)	76.0 (+0.05)
P	Total	303.0 (+25.0)	305.30 (+2.50)	283.0 (+2.31)	9.4 (+0.68)
	Extract	43.3 (+6.41)	40.3 (+0.00)	44.9 (+1.45)	31.9 (0.00)
Fe	Total	3.8 (+0.15)	3.8 (+0.68)	4.50 (+ 0.12)	283.3 (+1.87
	Extract	46.3 (+6.41)	43.8 (+0.00)	6.00 (+0.45)	56.6 (+1.83)
Zn	Total	3.21 (+25.0)	3.2 (+12.50)	3.53 (+ 0.88)	2.5 (+0.12)
	Extract	47.7 (+6.41)	49.3 (+0.00)	53.8 (+5.65)	66.7 (+0.00)
Mn	Total	3.90 (+25.0)	3.8 (+12.50)	3.5 (+0.01)	3.4 (+0.00)
	Extract	40.8 (+6.41)	40.2 (+0.00)	46.2 (+2.45)	79.4 (+2.95)
Co	Total	0.21 (+0.02)	6.21 (+0.02)	0.18 (+0.02)	0.18 (+0.00)
	Extract	66.7 (+8.25)	66.7 (+9.55)	72.2 (+5.55)	116.7 (+0.60)
Cu	Total	0.00	0.00	0.00	0.00
	Extract	0.00	0.00	0.00	0.00
Na	Total	6.2 (+0.29)	7.3 (+0.29)	6.3 (+0.25)	7.5 (+0.00)
	Extract	85.5 (+0.00)	89.9 (+0.00)	86.2 (+1.85)	92.0 (+0.00)
K	Total	450.0 (+2.00)	441.7 (+2.0)	450.0 (+0.00)	441.7 (+0.00)
	Extract	62.8 (+0.00)	79.8 (+0.50)	89.3 (+2.88)	89.9 (+0.00)
Ca	Total	10.8 (+0.00)	12.5 (+0.00)	10.7 (+0.00)	12.5 (+0.00)
	Extract	83.3 (+0.00)	79.3 (+0.17)	90.3 (+1.09)	98.4 (+0.00)
Mg	Total	51.2 (+1.15)	59.7 (+0.69)	59.3 (+0.00)	62.0 (+0.00)
	Extract	67.6 (+0.00)	76.0 (+0.05)	79.4 (+1.33)	84.7 (+0.00)
P	Total	6.5 (+0.00)	9.4 (+0.68)	303.4 (+6.34)	284.0 (+5.26)
	Extract	26.2 (+1.55)	31.9 (0.00)	63.7 (+1.76)	69.3 (+1.75)
Fe	Total	305.9 (+1.47	283.3 (+1.87	6.3 (+0.00)	11.1 (+0.00)
	Extract	50.6 (+0.00)	56.6 (+1.83)	30.7 (+1.09)	49.5 (0.00)
Zn	Total	2.9 (+0.00)	2.5 (+0.12)	3.0 (+0.00)	3.2 (+0.00)
	Extract	64.4 (+1.96)	66.7 (+0.00)	73.3 (+0.00)	76.1 (+4.79)
Mn	Total	3.5 (+0.00)	3.4 (+0.00)	3.7 (+0.00)	3.5 (+0.00)
	Extract	68.6 (+0.00)	79.4 (+2.95)	75.7 (+2.70)	81.7 (+1.50)
Co	Total	0.21 (+0.00)	0.18 (+0.00)	0.21 (+0.00)	0.18 (+0.00)
	Extract	100.0 (+0.00)	116.7 (+0.60)	104.8 (+4.75)	105.6 (+0.00)
Cu	Total	0.00	0.00	0.00	0.00
	Extract	0.00	0.00	0.00	0.00

Values are means of three replicates (+ SD). Means not sharing a common superscript letter in a row are significantly different at $p<0.05$ (as assessed by Duncan's multiple range test)

and ascorbic acid[21]. Sorghum cultivars WadAhmed and Tabat contained only about 10.8 and 12.5 mg/l00 g Ca, respectively of which only 33.3 and 35.7% are bioavailable in the raw grain for the cultivars, respectively. Processing improved its bioavailability up to >100%. For both cultivars either germination or fermentation was able to

increase Fe extractability more than 25%. This could be due to the fact that the residual polyphenols during fermentation of sorghum may increased due to microbial activity, which may hydrolyse the condensed tannins to lower molecular weight phenols as reported by Khetarpaul and Chauhan[22] for pearl millet. The galloyl (trihydroxybenzene) groups are mainly responsible for inhibiting the iron absorption[23] There is high prevalence of iron deficiency anaemia mainly due to the poor bioavailability of iron caused by the inhibitors such as phytates, tannins and fiber in plant foods[21]. Zn is a coenzyme for an estimated 200 enzymes, many of which affect protein synthesis and thus growth[24]. In sorghum, processing has resulted in the increase of Zn extractability when both cultivars grains were germinated or fermented (Table 4 and 5). Cooking of the flour before and after germination or fermentation (Table 6) has significantly ($p<0.05$) increased both major and trace minerals extractability for both cultivars. However, the rate of increment is low compared to germination or fermentation alone. Jood and Kapoor[25] reported that ordinary cooking of soaked seeds had a significant improvement in HCl-extractability of all minerals. Koksel *et al.*[26] reported that neither cooking method nor the dehulling process had a significant influence on the concentration of four of six elements (Fe, Cu,Zn, Mg) measured for barley. Valencia *et al.*[2] found that cooking quinoa samples did not affect the amount of soluble iron and only small reduction of phytate content occurred. Kumar *et al.*[13] investigated the effects of cooking on characteristics of legumes: green gram, cow pea and chickpea. They observed poor extractability rate of phytate phosphorus with water and HCl, which could be due to formation of insoluble complexes between phytate phosphorus and other components of legumes which reduced the rate of mineral bioavailability.

CONCLUSION

Major biochemical changes were observed when sorghum grains were germinated or fermented and then followed by cooking. Germination (96 h) was effective in improving minerals extractability. Fermentation was more effective in reducing pH and phytate and increasing the mineral bioavailability. The results indicate that a combination of germination followed by cooking is a potential process for developing a food product of improved nutritive value and digestibility from sorghum.

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