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Effect of Soaking on Nutritional Value of Sorghum (*Sorghum bicolor L)*

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Abstract: ***This study was carried out to investigate the effect of soaking of sorghum (white Feterita) on chemical composition, total minerals and its availability, phytic acid, in vitro protein digestibility, phytate phosphorus and non phytate phosphorus. sample was purchased from local market in Khartoum. Soaked samples were prepared by soaking in distilled water and 4% citric acid at room temperature then all samples were sun dried, milled and kept in poly ethylene bags at 4^0Cfor evaluating. All data were subjected to statistical analysis using (SPSS). The results showed that moisture, lipid and crude fiber contents were decreased significantly (P≤0.05) compared with the control, while ash, crude protein and available carbohydrates contents were increased significantly (P≤0.05) compared with the control. Mineral content significantly (P≤0.05) not affected by Soaking in distilled water and 4% citric acid except magnesium content which was increased. Minerals availability significantly (P≤0.05) increased by soaking in 4% citric acid except potassium availability which was decreased, on the other hand soaking in distilled water significantly (P≤0.05) decreased the availability of minerals except iron which was increased. The anti-nutritional factor phytic acid was also affected by soaking showing significant (P≤0.05) reduction from 278.6(mg/100g) in the control to 250.07 in the sample soaked in distilled water. Phytate phosphorous decreased significantly(P≤0.05). In conclusion, soaking of feterita enhanced their nutritive value, using 4%citric acid resulted in higher nutritive value compared with distilled water.***

Keywords: sorghum, proximate composition, mineral content, protein digestibility, phytate

1. Introduction

Cereals are the major stable foods. They are important part of dietary and a large variety grown for edible purpose, also contribute nutrient intake of human being (when consumed as whole grains) significant source of energy, dietary fiber, vitamins, minerals and photochemical, higher in outer part of grain [1]. In Sudan Sorghum (cereal) called dura (Feteria) is grass species cultivated for grain industry as it contained anti-nutritive compounds, which lead to some negative effects in human digestive system[2] . Cereals are rich in minerals, but availability of these minerals is usually low due to the present of anti-nutritional factors which were synthesize chemicals compounds when consumed by animals or human may result in reduction in the nutritive value, such as phytic acid, lectines, protease inhibitors, tannins, polyphenoles and α-amylase inhibitors[3], also dietary fiber known to bind with nutritional significant mineral and thus reduce availability of minerals [4], these factors have negatively affected the nutritive value; inhibit protein and carbohydrates digestibility and pathological change in intestinal and liver tissue [3]. Significantly influence the functional and nutritional properties of foods, these lead to interference in the absorption of the minerals from small intestinal and adversely affect various metabolic processes. Phytic acid concern in foods and animals feed industries, because the phosphorus in this form is unavailable to monogastric animals due to a lack of endogenous phytase enzymes specific for the degradation of phytic acid [5]. Soaking is one of the methods used to improve the nutrition value of raw material in the manufacturing of food products [2]. Soaking process save energy cost by shortening cooking times and offers an additional advantage of rendering grain nutritionally superior by removing certain anti-nutritional factors such as Phytic acid[6]. The decrease of anti-nutrients content during soaking may be attributed to the leaching out into soaking water under the influence of the concentration gradient [7]. Thus the objective of this research is to study the effect of soaking in distilled water and 4%citric acid on nutritional characters, minerals availability and anti-nutritional factor (phytic acid) of the grain

2. Materials and Methods

Materials

Food materials

Sorghum (Feterita) season 2015 was purchased from the local market of Khartoum, cleaned and sorted by removing broken kernels and extraneous matter, part of the grains milled separately and passed through 60 mesh sieve then kept at 4°C for subsequent chemical analysis. The remaining part was used for soaking in 4% citric acid and distilled water for 15hs at room temperature and then the grains were sun dried for 24hs, milled and kept at 4°C for analysis.
Methods

Approximate analysis

Moisture, crude fiber, crude fat, crude protein, and ash were carried out according to the official standard method[8]. The total carbohydrate of the samples was calculated by subtracting the value of protein, oil, fiber, ash, and moisture content from 100.

Determination of total minerals

Total minerals which include K, Ca, Mg, Fe, Zn and P were determined according to the method of Chapman and Pratt [9] as described below:

Two grams of sample were weighed in clean dry crucible the crucible was placed in a muffle furnace for 4 hours at 550°C, sample were cooled and 10 ml of 3NHCl was added, covered watch glass and boiled gently for 10 minutes, then cooled. The contents were filtered through what man filter paper No 4 and the volumes was made to 50 ml with distilled water, and were taken for mineral determination either by flame photometry for potassium.(K) determination or by atomic absorption spectrophotometer (Perkin-Elmer 2380).

Determination of minerals availability
One gram of sample was extracted in 10ml (0.03N) HCL by shaking the content at 37°C for 3h. the clear extract obtained after filtration through what man filter paper No4 was oven dried 100°C then placed in a muffle furnace at 550°C for 3h, cooled and 3ml of (0.03N) Hcl were added and the volume was made to 50ml with distilled water and were taken for mineral determination [10].

$$Mineral\ extractability = \frac{\text{extracted mineral in 0.03N) Hcl}}{Total\ minerals\ (mg/100g)} \times 100$$

Determination of Phytic acid
The Phytic acid content was determined according to the method of Wheeler and Ferrel[11] .

Phytate phosphorus
Phytate phosphorus determined according to the formula as follow:

$$phytate\ phosphorus\ mg/100 = \frac{A \times 28.18}{100}$$

Where:
A: Phytic acid content.

Non phytate phosphorus
Non phytate phosphorus was calculated by difference between the total phosphorus and phytatephosphorus content.

Determination of *in vitro* protein digestibility
In vitro protein digestibility was determined for samples by using pepsin enzyme the method described by Maliwal[12] was used as modified by Monjula and Jon [13].

Two g of the sample containing 16mg nitrogen taken in triplicates and hydrolyzed with one mg pepsin in 15ml of (0.1N) HCl at 37°C for 2hours; the reaction was terminated by addition of 15ml of 10% (W/V) trichloroacetic acid (TCA). The mixture was filtered quantitatively through what man filter paper NO1. The TCA soluble fraction was assayed for nitrogen using the micro – kjeldhyl method. Digestibility was calculated using the following equation:

$$Protein\ digestbility = \frac{\text{N in Supernatant} - \text{N in blank}}{N\ in\ sample} \times 100$$

Where
N in blank = N in pepsin enzyme.

Statistical analysis
All data were subjected to statistical analysis using *SPSS Vito* means tested by analysis of variance Factorial design. Value of probability of 5% was used to indicate significance according to DMRT [14].

3. Results and Discussions

Proximate composition of Feterita as affect by soaking in distilled water and 4%citric
Results of proximate composition of white Feterita as affected by soaking in distilled water and 4%citric acid are shown in Table1.

The results obtained showed that different treatments significantly (P≤0.05) decreased moisture, lipid and fiber content and significantly (P≤0.05) increased ash, protein and carbohydrate contents of Feterita, while no significant differences (P≤0.05) were observed between soaking in distilled water and 4%citric acid on moisture, ash, lipid, fiber and carbohydrate contents except protein content.

Results indicated that the values of the proximate composition of raw Feterita was differ from the values obtained by other author[15] for moisture, ash, lipid, fiber, protein and carbohydrates contents of Sorghum cultivars. The result showed that moisture contents after soaking in distilled water and 4%citric acid were significantly (P≤0.05) decreased to 8.52% and 8.46%, respectively. These results were higher than the range 8.38-8.76% and 6.07 -6.56% for raw and soaked Sorghum cultivars [16].

Ash contents of Feterita soaked in distilled water and 4%citric acid were significantly (P≤0.05) increased from 1.60% to 2.35% and 2.47%, respectively. The results were higher than the range1.43%- 1.45% and 1.26-1.42% for the raw and soaked Sorghum cultivars, respectively[16].

Fat content of Feterita soaked in distilled water and 4%citric acid significantly (P≤0.05) decrease from 3.34% to 2.43% and 2.42%, respectively. This results was lower than the ranges 3.58-3.91% and 3.53- 4.10% for raw and soaked Sorghum cultivars [16], and 12.45% and 8.51% for Sorghum soaked in distilled water and lime, respectively[17]. Soaking process can decrease the fat content of Sorghum grain due to absorption of water which lead to activation of the enzyme and digest food reserve substance.

Fiber content was significantly (P≤0.05) decreased due to soaking in both distilled water and 4%citric acid from 2.39% to 1.54% and 1.39%, respectively. This results were in agreement with the values obtained for soaked Sorghum[16] and higher than the value reported for raw Feterita[18] and lower than the values of 7.86% and 6.71% for soaked Sorghum in distilled water and lime, respectively[17]. Soaking process could decrease fiber content as Sorghum contained soluble and insoluble fiber in water so water-soluble fiber content, which is called β-glucan, may be reduced.

Protein content significantly (P≤0.05) increased by soaking in both distilled water and 4%citric acid from 10.24% to 10.39% and 10.28% respectively. This result is in agreement with author who mentioned that protein content of soaked

Sorghum cultivars range from 9.82%-12.30% [16], and lower than14.4%- 14.37% for Sorghum cultivars [19].

Carbohydrate contents of Feterita as affected by soaking in distilled water and 4%citric acid were significantly (P≤0.05) increased from 72.09% to74.90% and 74.81%, respectively. This result was found to be lower close to the range of 71.48- 73.86% for soaked Sorghum cultivars[16] , and higher than the value of 57.33% and 66.53% for Sorghum soaked in distilled water and lime, respectively [17]. The variation in the carbohydrate contents is probably due to the increases and decreases that took place in the other components as a consequence of the processing variables. Since carbohydrate values are obtained by difference it means its values depend on factors responsible for the values of other components.

Table 1: Proximate composition(g/100g) of Feterita as affected by soaking in distilled water and 4% citric acid

Treatment	Moisture	Ash	Lipids	Fiber	Protein	Carbohydrates
A	10.34^{a} ±0.07	1.60^{b} ±0.06	3.34^{a} ±0.11	2.39^{a} ±0.09	10.24^{b} ±0.01	72.09^{b} ±0.19
B	8.52^{b} ±0.32	2.35^{a} ±0.12	2.43^{b} ±0.08	1.54^{b} ±0.16	10.32^{a} ±0.06	74.90^{a} ±0.23
C	8.46^{b} ±0.14	2.47^{a} ±0.15	2.42^{b} ±0.09	1.39^{b} ±0.22	10.28^{b} ±0.1	74.81^{a} ±0.49
$Lsd_{0.05}$	0.4202^{*}	0.2921^{*}	0.291^{*}	0.1879^{*}	0.1879^{*}	0.5992^{*}
SE±	0.1414	0.09832	0.100	0.06325	0.06325	0.2017

Mean (±SD) values bearing different superscript(s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Feterita whole seeds
B ≡ Feterita soaked in distilled water
C ≡ Feterita soaked in 4% citric acid

Effect of soaking in distilled water and 4%citric acid on total and available minerals of Faterita

The total (mg/100g) and available (%) of minerals of Feterita as affected by soaking in distilled water and 4% citric acid are shown in Table 2.

Calcium content significantly (P≤0.05) not affected by soaking in distilled water and 4%citric acid their values were 220.20 mg/100g, 216.26 mg/100g and 218.68mg/100g. These results were higher than the range 22.91-33.90 mg/100g and 18.90-26.74 mg/100g for Calcium content of raw and soaked Sorghum cultivars, respectively [16]. Available Calcium significantly (P≤0.05) not affected by soaking in distilled water while soaking in 4%citric acid significantly increased it from 32.21% to35.84.

Magnesium content of Feterita significantly (P≤0.05) increased by soaking in distilled water and 4%citric acid from 1.88 mg/100g for control to 2.20 mg/100g and 2.54 mg/100g, respectively. This result was lower than the range of 120.10-137.14 mg/100g and 108.13-126.71 mg/100g for Magnesium content of raw and soaked Sorghum cultivars[16]. Availability of Magnesium was significantly (P≤0.05) decreased by soaking in distilled water (from 50.46% to 49.96%), and increased by soaking in 4%citric acid(from 50.46 to 57.31%).

Total and available Potassium content of Feterita were significantly (P≤0.05) decreased due to the soaking in both distilled water and 4% citric acid from 418.24 mg/100g to 411.16mg/100g and 407.40mg/100g, respectively. These results were higher than the range 230.20-264.53mg/100g and 163.92-248.43mg/100g for Potassium content of raw and soaked Sorghum cultivars, respectively[16]. Out of this amount about 50.06% found to be available which decreased to 45.13% and 44.13% after soaked in distilled water and 4%citric acid, respectively. These results agreed with author who mentioned that available Potassium of Sorghum cultivars varied from 46.3-51.0% [20]. Generally, reduction after soaked may attributed to the leaching of minerals in to the soaking medium.

Iron content significantly(P≤0.05) was not affected by soaking in distilled water and 4% citric acid while availability significantly (P≤0.05) increased . This result disagree with the values of 7.65mg/100g and 3.98-5.19mg/100g of Iron content for raw and soaked Sorghum cultivars respectively[16], and 3.80-4.50 mg/100g for different Sorghum cultivars[20] . The percentage of available iron was more or less similar to the percentage obtained by some author [20], and lower than 8.02- 13.16% and 14.62- 20.75% for raw and soaked Sorghum cultivars, respectively[21].

Zinc content was 0.235mg/100g significantly (P≤0.05) was not affected by soaking in distilled water and 4%citric acid. This result lower than 3.21-3.53mg/100g for Zinc content of Sorghum cultivars[20], and 3.44-5.02 mg/100g and 3.44-3.78mg/100g of raw and soaked Sorghum cultivars, respectively[16]. The available Zinc was significantly (P≤0.05) increased by soaking in 4%citric acid, while soaked in distilled water significantly (P≤0.05) decreased its availability. These results were not compatible with the percentage of 7.35- 9.73% and 9.09- 10.23% for raw and soaked Sorghum cultivars, respectively[21]. and in agreement with the percentage of 47.7-53.8% for different Sorghum cultivars[20].

Table 2: Total and available of minerals of Feterita as affected by soaking in distilled water and 4% citric acid

Treatments	Ca		Mg		K		Fe		Zn	
	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)	Total (mg/100g)	Available (%)
A	220.21^{a} ±2.21	32.21^{b} ±0.09	1.88^{b} ±0.44	50.46^{b} ±0.36	418.24^{a} ±1.76	50.06^{a} ±0.30	3.42^{a} ±1.21	5.66^{c} ±0.25	0.235^{a} ±0.09	50.52^{b} ±0.73
B	216.26^{a} ±0.98	31.25^{b} ±1.19	2.20^{a} ±0.05	49.96^{c} ±0.59	411.16^{ab} ±1.01	45.13^{b} ±1.69	2.60^{a} ±0.21	6.76^{b} ±0.05	0.246^{a} ±0.02	47.46^{c} ±1.58
C	218.68^{a} ±2.47	35.84^{a} ±0.87	2.54^{a} ±0.27	57.31^{a} ±3.62	407.40^{b} ±1.54	44.54^{b} ±2.57	3.17^{a} ±0.17	9.34^{a} ±0.48	0.221^{a} ±0.01	54.79^{a} ±1.66
$Lsd_{0.05}$	6.757^{**}	1.861^{*}	0.4912^{*}	2.792^{*}	9.02^{*}	2.51^{*}	0.7652^{ns}	1.763^{*}	0.05425^{ns}	2.752^{*}
SE±	2.274	0.6264	0.1653	0.9397	3.036	0.8448	0.256	0.5933	0.01826	0.9263

Mean (±SD) values bearing different superscript(s) in the same columns and rows are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Feterita whole seeds
B ≡ Feterita soaked in distilled water
C ≡ Feterita soaked in 4% citric acid

3 Effect of soaking in distilled water and 4%citric acid on phytic acid contents and *in vitro* protein digestibility of Feterita

Results of phytic acid (mg/100g) of Feterita as affected by soaking in distilled water and 4%citric acid presented in Table 3.
Soaking in distilled water significantly (P≤0.05) decreased Phytic acid content, but 4%citric acid shows no significant (P≤0.5) decreased compared to the control.

Phytic acid content for Feterita whole grain found to be 278.61mg/100g. This result was in agreement with that reported by Idris *et al.*[22] who found that Phytic acid content of Sorghum cultivars was 265mg/100g. Soaking in distilled water and 4%citric acid significantly (P≤0.05) decreased Phytic acid content to 250.07 mg/100g and 266.60 mg/100g, respectively. These results obtained were disagree with authors who mentioned that Phytic acid content of raw and soaked Sorghum cultivars ranged from 556.52-606.07 mg/100g and from 409.71-425.86 mg/100g, respectively[21], and higher than the values of 64.72 mg/100g and 50.19 mg/100g of soaked Sorghum in distilled water and lime respectively[17]. The reduction in Phytate content caused by soaking may be due to water solubilization of some Phytate salt.

Protein digestibility content of Feterita was 64.11%. This value was higher than the range 32.33– 40.03% for Sorghum cultivars [19]. Soaking in distilled water significantly (P≤0.05) increase the protein digestibility content from 64.11% to 65.45%, but 4% citric acid soaked significantly (P≤0.05) decrease it to 62.51%.

Table 3: Phytic acid (mg/100g) and *In vitro* protein digestibility (%) of Feterita as affected by soaking in distilled water and 4% citric acid

Treatment	Phytic acid (mg/100g)	IVPD (%)
A	278.61^{a} ±0.52	64.11^{b} ±1.05
B	250.07^{b} ±1.01	65.45^{a} ±1.06
C	266.60^{a} ±2.41	62.51^{c} ±0.86
$Lsd_{0.05}$	12.45^{**}	1.625^{*}
SE±	4.191	0.5468

Mean values (±SD) bearing different superscript(s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Feterita whole seeds
B ≡ Feterita soaked in distilled water
C ≡ Feterita soaked in 4% citric acid

Effect of soaking in distilled water and 4%citric acid of Feterita on total phosphorus, phytate phosphorus and non-phytate phosphorus

The total Phosphorus, Phytate and non-Phytate phosphorus content of Feterita as affected by soaking in distilled water and 4% citric acid listed in Table 4 values expressed as dry matters basis (mg/100g).

The data shows that soaking in 4%citric acid significantly (P≤0.05) decreased Phosphorus content from183.77 mg/100g to182.45 mg/100g while no significant (P≤0.05) difference was observed after soaking in distilled water. These results were in disagreement with the range of 275.75-358.65 mg/100g of Phosphorus contents of soaked Sorghum[16]. Reduction after soaking may be attributing to leaching of Phosphorus in to the soaking media.

Distilled water and 4% citric acid soaking of Feterita show significant (P≤0.05) decreased in Phytate phosphorus content from 80.24 mg/100g to 71.02 mg/100g and 76.78 mg/100g, respectively. These results found to be lower than the range 159.79 174.01 mg/100g and 117.63-122.43 mg/100g for raw and soaked Sorghum cultivars [21].

Soaking in distilled water and 4%citric acid significantly (P≤0.05) increased Non- phytate phosphorus of Feterita from 103.50 mg/100g to 113.80 mg/100g and 105.70 mg/100g, respectively. The reduction and improvement of

Phytate phosphorus and non- phytate phosphorus during soaking may be due to the reduction of phytic acid content.

Table 4: Total Phosphorus, Phytate and non-phytate phosphorous (mg/100g) of Feterita as affected by soaking in distilled water and 4%citric acid

Treatment	Total Phosphorous (mg/100g)	Phytate phosphorous (mg/100g)	Non-phytate phosphorous (mg/100g)
A	183.77^{a} ±2.98	80.24^{a} ±0.15	103.50^{b} ±3.01
B	185.83^{a} ±0.70	72.02^{c} ±0.29	113.80^{a} ±0.87
C	182.45^{b} ±1.17	76.78^{b} ±0.70	105.70^{b} ±0.60
$Lsd_{0.05}$	3.028^{*}	1.321^{**}	4.488^{**}
SE±	1.019	0.4446	1.51

Mean values (±SD.) bearing different superscript(s) in the same column are significantly different (P≤0.05) according to DMRT.

Key:
A ≡ Feterita whole seeds
B ≡ Feterita soaked in distilled water
C ≡ Feterita soaked in 4% citric acid

4. Conclusion

1) Soaking in distilled water and 4% citric acid increased the content of magnesium but calcium, iron and zinc were not affected and potassium decreased.
2) Availability of minerals increased by soaking and the rate of increment is higher in soaking in 4% citric acid except the availability of potassium which were decreased by soaking.
3) Reduction in phytic acid content leads to increase the available minerals and *in vitro* protein digestibility.
4) Soaking may be useful for production of infant and young children foods with high protein digestibility and mineral availability.

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