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Reducing Phytic Acid from Sudanese Sorghum Bicolor (F.G) Using Simple Technique Methods

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Abstract: *An Experiment was conducted to reduce phytic acid from Sudanese Sorghum bicolor local name Feterita Gadarif (F.G) using a simple technique method. Approximate Analysis was done to determine the nutrition value of the grains before and after processing methods, to measure the change of nutrient content after processing treatments. The treatments of processing were divided into five methods of process, in addition of control (unprocessed cereal) such as dehulling, germination, soaking, vitamin C and storing methods respectively to reduce phytic acid content of the grains. The treatments shown significant decrease (p>0.05) in phytic acid contents of the processed sorghum as compared with unprocessed one (control). The highest loss of phytate registered in germination method (98%) followed by vitamin C treatment (86.2%), soaking (78.6%), storing (59.6) and dehulling method respectively. There were a significant increase (p>0.05) in metabolic energy. Ca, Fe and P of cereal nutrients content, obtained after processing operation by method of germination and some slight decrease of protein content of all processed grains*

Keywords: *phytic acid, determination, processing methods, germination*

1. Introduction

Food crops such as cereals, legumes and oilseeds are grown in over 90% of the world. Foods derived from these crops provide major source nutrients to mankind; one of these crops is sorghum bicolor, which is used for both animals and humans. Sorghum originated in northern Africa, which is used largely in world and over 300 million people depend on it. The cereals are rich in minerals but, the bio-availability of these minerals is usually low because of the presence of anti nutrition factors such as phytic acid (Valencia et al 1999), is an important constituent of sorghum. When Phytic acid or Phytate is discovered in salt form in 1903 Edward et al (2012), Phytic acid has been termed as anti-nutrient, due to its ability to bind minerals protein and starches, either directly or indirectly and thus alter their soluble functionality and absorption (Nelson, 1967). The effect on nutrition due to phytic acid in human and animals is related to the interaction of phytic acid with proteins, vitamins, and several minerals, thereby restricting their bio-extractability (Svanberg and Lorri. 1997). In the view of anti nutritional effects of phytic acid, many attempts have been made to reduce phytate

Therefore, the present study was conducted to use simple processing methods such as milling, soaking, germination, vitamin C and storing, to reduce phytic acid compound from sorghum and to shed light on phytic acid as a controversial component.

2. Materials and Methods

Sudanese Sorghum, Sorghum bicolor (Fatareta Gadarif) was purchased from Gadarif State local market of about 20 kg, for the experimental purpose which was cleaned out of damaged seeds and foreign objects. Then subject is processed to five treatments using a simple technique to reduce phytic acid, such as milling, soaking, germination, vitamin C and storing in addition to control (UN processed sorghum).

Chemical composition of sorghum were analyzed before and after processing treatment to determine nutrients content and phytic acid in the grain

2.1. Chemical Composition of Unprocessed Sorghum (F G)

The seeds were cleaned manually to remove broken seeds, dust, and other extraneous materials. The cleaned grains were milled into fine flour with hammer mill (Gibbons Electric, Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis. The seeds were chemically analyzed according to procedure of AOAC (1980) .The sorghum protein content were determined by adopting standard AOAC(1995) method. Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by dry-ashing methods, described by Chapman and Pratt (1961). The amount of iron was determined using atomic absorption spectroscopy (Perkin-Elmer 2380). Ammonium vanadate method of Chapman and Pratt (1982). Calcium was determined by a titration method described by Chapman and Pratt (1961), hydrochloric acid extractability of minerals was performed according to the Chapman and Mahjan (1988).

2.2. Phytic Acid determination

Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0g dried sample. A standard curve was prepared expressing the results as Fe (No) 3 equivalents, phytate phosphorus were calculated from the standard curve assuming a 4:6iron to phosphorus molar ratio

2.3. Processing Treatment

2.3.1. Dehuling 5.0 kg of cleaned cereals, moistened by adding water before hulling, to softening the surface of the grain and facilitating detachment of the pressure inside the machine. The commercial machine combines two stages, they are dehulling and milling. The grain was passed through the machine capacity of around 200to 275 kg/h for hulling, yield about 78%. The hulling grains were milled into fine flour with hammer mill (Gibbons Electric, Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis. The seed flour was chemically analyzed according to procedure of AOAC (1980). The sorghum protein content was determined by adopting standard AOAC (1995) method. Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by the dry-ashing methods, described by Chapman and Pratt (1961). Phytic acid was determined by the method, described by Wheeler and Ferrel (1971) using 2.0 g dried sample.

2.3.2. Soaked 5kg 0f the whole cleaned seeds was put in a pot filled with tap water, The grains were removed from the water after 12 hours sun –dried, then milled into a fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis. The processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing of sorghum protein content was determined by adopting standard AOAC(1995) method. Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by dry-ashing methods, described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0g dried sample. Then 2ml of the mill dried sample was weighted to determine phytic acid.

2.3.3. Germination According to the method of Koua Kou et al (2008). 3kg of the whole cleaned seeds was immersed in water overnight. The grains were spread on trays lined with cloth. It was kept wet by frequent spraying water. After 96 hours, the germinated grains were removed from the trays, sun –dried, then milled into fine flour with hammer mill (Gibbons Electric, Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis. The processed seed flour was chemically analyzed according to procedure of AOAC (1980). The processing sorghum protein content was determined by adopting standard AOAC (1995) method. Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971), using 2.0g dried sample. Then 2ml of the dried milled sample was weighted to determined phytic acid.

2.3.4. Vitamin C added 1kg of the whole cleaned seeds was milled in a laboratory mill to obtain fine flour, 150mg of ascorbic acid was mixed with the sample, then the processed seed flour were chemically analyzed according to procedure of AOAC (1980). The processing sorghum protein

content was determined by adopting standard AOAC (1995) method. Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971), using 2.0g dried sample. Then 2ml of the milled dried sample were weighted to determined phytic acid.

2.3.5. Storing Sorghum cereal (fetareta gadarif) were stored for 12 month ,2kg of the seeds then milled into fine flour with hammer mill (Gibbons Electric ,Essex K) to pass 0.4mm mesh size screen and were stored at 4C before being used for their analysis , the processed seed flour were chemically analyzed according to procedure of AOAC (1980) .The processing sorghum protein content were determined by adopting standard AOAC(1995) method . Energy was calculated as described by Osbonrne and Voogt (1978). Minerals were determined in the sample by the dry-ashing methods described by Chapman and Pratt (1961). Phytic acid was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample...Then 2ml of the milled dried sample were weighted to determined phytic acid.

2.4. Statistical Analysis

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

3. RESULTS

3.1. Results of the proximate analysis of minerals and calculated energy of the sorghum (F. G) are present in table (1) and (2). The result indicated that sorghum (F. G) had a high percentage of crude protein, Ether Extract and metabolizable energy.

Table1. *Chemical composition of sorghum (F.G) %*

Chemical profile	Sorghum(F.G)
Dry matter	93.83
Crude protein	14.3
Ether Extract	4.58
Crud fiber	2.69
Nitrogen free extract	7.31
Metabolizable energy (Kcal/kg)	383
Methionin	8.5mg/100g
Thiamin	0.38mg/100g
Lysine	117.6mg/100g
Niacin	3.8mg/100g

Analyzed values are means of duplicate sample. ME is calculated value by the equation of Carpenter and Clegg (1966).-NFE is calculated value.

Table2. *Minerals content of sorghum bicolor (F.G) mg/100g*

Chemical profile	Sorghum(F.G)
Ca	12.8
P	356
Mg	103
Fe	4.8
Mn	122

Analyzed values are means of duplicate sample.

ME is calculated value by the equation of Carpenter and Clegg (1966).

NFE is calculated value.

3.2. Chemical composition of sorghum before and after processing were shown in table (3), the result shown significant ($p>0.05$) change in some nutrient value of processed grains in energy, Ca, P, and Fe, and slight change in protein content.

Table3. *Effect of treatments on sorghum chemical composition before and after processing*

Treatments	CP	Energy (kcal/kg)	Ca	P	Fe	Phytate
Raw sorghum	145.30	3100.50b*	0.260a	0.38a	4.80a	889.20a
Dehulling	143.00	3101.00b	0.252	0.30b	3.26b	445.06b
Germination	143.4	3113.33a	0.265a	0.28b	3.26b	87.90g
Soaking	145.00	3100.67b	0.250	0.28b	4.83a	189.70e
Vitamin C	145.10	3100.00b	0.250	0.29b	4.90a	122.40f
Storing	143.00	398.20c	0.245	0.28b	3.30b	360.00c
Overall mean	144.13	2652.28	0.254	0.30b	4.06a	336.94d
Standard Error (SE±)	0.71	2.85	0.01	0.01	0.25	1.52
$LSD_{0.05}$	ns	8.70	Ns	0.03	0.77	4.68

$LSD_{0.05}$: least significant difference at 0.05 level of significance (for mean separations and comparisons)

* Different small letters represent significant differences between treatments means in each column.

Mean squares (from analysis of variance (ANOVA) table) for effect of different treatments on sorghum chemical composition before and after processing

Source of variation	Degree of freedom (d. f)	CP	Energy	Ca	P	Fe	Phytate
Treatments	5	3.6253	465.83	0.00016	0.0048	2.2189	293.0241
Experimental Error	12	1.4961	24.339	0.000047	0.00022	0.1956	6.9239
F-calculated value		2.4230	19.14	3.53	21.45	11.58	42.753
Level of Significance		Ns	***	*	***	***	***
Coefficient of variation (C.V. %)		8.50	1.86	2.70	4.94	10.89	7.81

N. s: not significant at 0.05 level of significance

3.1. Determination of Phytic Acid

Results of determination of phytic acid in raw sorghum and processed sorghum (F.G) are present in table (4). The result indicated a significant decrease ($p>0.05$) of phytic acid of all the processed treatments.

Table4. *Effect of treatments on sorghum phytate mg/ 100g before and after processing*

Treatments	Phytic acid content	Loss%
Raw sorghum	889.20a	0
Dehuling	445.20b	49.9
Germination	87.90g	90.1
Soaking	189.70e	78.6
Vitamin C	128.40f	86.2
Storing	360.00c	59.6

$LSD_{0.05}$: least significant difference at 0.05 level of significance (for mean separations and comparisons)

* Different small letters represent significant differences between treatments means in each column.

4. DISCUSSION

4.1. Chemical Composition

The moisture content of sorghum bicolor (F.G) was in the range obtained by AbdElnour (2001) who indicated that moisture content of Feterita and Dabar was 9.6-8.75 respectively. The crude protein content of sorghum bicolor

(F.G) (Table 3) was in the range observed by Hulse et al (1980) who found that the protein content of sorghum bicolor ranged between 8-16 % ,but lower than Elsayed (1999) analyzed the protein content of Tabat and Fetarita was 6.46-9.11 , but higher than the value reported by Dillon (2007) , and lower than that obtained by Mayada (2009).The metabolizable energy of sorghum (F.G) was in the range detected by Idris (2004) who found the carbohydrates content for sorghum was 80.7%.Minerals content was in range optimum by Hulse et al (1980) and Idris (2004)

4.2. Phytic Acid in Unprocessed Grains

The phytic acid content of sorghum (F.G) (Table4) are close to those reviewed by Marfo et al (1990) who reported that phytic acid content of red sorghum was 886mg /100g. Greiner (2006) and Konietzny and Koyode (2006) whom found that sorghum phytate ranged from 590 to 1180 and from 400 to 3500 mg/100g dwt. The result was high than the range obtained by Eammambux *et al* (2009) ,and high than the range reported by Makokha *et al* (2002) , also high than the found reported by Sathe (2002), these can be explained by that phytic acid content varied , stage of maturity , climatic , condition s type of soil , amount of available phosphorous and milling fraction of the grains

4.3. Removal of Phytate

Table (4) shown the removal of phytate after processed treatments , the highest loss of phytate ($p>0.05$) obtained by germination method (90.1 %) flowed by vitamin C method (86.2%),soaking method (78.6%),storing method (59.6%) and dehulling method (49.9%) respectively. In fact phytic acid in flour can be hydrolyzed by the enzyme phytase, and the optimum condition for phytase activity are PH range from 5.0 to 5.5 and temperature rang 50 to 55c.Germination method in sorghum grains reduced phytate up t0 90.9 after 96h Wisal (2004). The obtained was on line with Abdelrahman *et al* (2007) who reported that germination increase part of both major and trace minerals and also reduced significantly the phytic acid , germination is more effective way to remove phytic acid , and germination in 80-90£ removed 92% of phytate and releases vitamins and make grains and seeds more digestible . Vitamin C method reduced (P.A) 86.2%, vita C is strong enhancer of plant iron can overcome the inhibitors in plant foods. One study found that various doses of phytate reduced iron absorption by 10 to 50 %.But adding 50 mg of vitamin C counteracted the phytate and adding 150 mg of vitamin C increased iron absorption to almost 30%, similarly, in the presence of a large dose of tannic acid , 100 mg of vitamin C increased iron absorption from 2-8% .Snedecor *et al* (1987) The results of soaked sorghum was in range of result obtained by Motz *et al* ,(2001) who reported that soaking of maize for 1 h at room temperature already led to be reduction of phytic acid by 51%, but lower than the report obtained by Mahgoub and Elhage (1998) whom reported that soaking of sorghum flour at room temperature for 24 h reducing phytic acid level by 16-21%.The reduced of phytic acid using storing method was present in table (4) the result was in the same line with Sathe (2002) ,who reported that the decreased of phytic acid during storage from 0 to 65% in cereal according to temperature and humidity and from 2.5 to 76% in legume, and the reduction depend on the type of seeds , storage condition, and the age of the seeds

The processed of sorghum bicolor (F.G) by using five technical methods changed the nutrient values of the seeds after processing is shown in Table (3). The high content of crude protein metabolizable energy and fat obtained by germinated seeds , the result on line with many workers , they observed increase in proteins during germination of cereals , this increase could be attributed to a synthesis of enzymatic proteins by germination seeds . (W H O, 1998). Marero *et al* (1988) also reported that the increases in protein might be due to the fact some amino acids are produced in excess of the requirement during protein synthesis and these tend to accumulate in free amino acids pool. In the same line Koua kou *et al* (2008) indicated that the seeds of cereals during their germination develop a strong enzymatic activity. The results also in line with Ocheme and Chinma , (2007) who find that germination significantly increases the protein dry mater and ash content , while fat content and energy values of the flour sample showed a decrease , these protein increase not exceeding 14% of the starting protein content . This was found to be attributed to loss of dry weight through respiration during germination, in the same line Beal and Mehta (1985) reported that germination reducing up to 75% of phytate and increase phytase activity, but different from Martinez et al (1980) who reported that germination decreased the content of lysine and tryptophan and vitamin such as C, B, A and E

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