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Effect of Malt Pretreatment on Antinutritional Factors and HCl Extractability of Minerals of Sorghum Cultivars

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Abstract: Sorghum grains of cultivars Wad Ahmed and Tabat were germinated for 1, 2 and 4 days to obtain 1-, 2- and 4- day-old malts. About 1% of sorghum malt was added to sorghum flour. The mixtures were incubated at 30°C with shaking for 30, 60, 90 and 120 min. Phytic acid and tannin contents and minerals extractability (as an index for minerals bioavaliability) were assayed for all treatments. The results revealed that phytate and tannin contents were significantly (P = 0.05) reduced when sorghum flour was pretreated with malt. When a mixture containing 4-days-old malt was incubated for 120 min significantly ($P \leq 0.05$) reduced phytate and tannin contents by 39.9% and 26.7%, respectively for Wad Ahmed cultivar while for Tabat cultivar they were reduced by 36.6% and 23.8%, respectively. HCl extractability of both major and trace minerals was also significantly (P = 0.05) improved as a result of malt pretreatment especially when sorghum flour was mixed with 4-days malt and incubated for 120 min.

Key words: Malt, phytate, tannin, sorghum, minerals, extractability

Introduction

Malting of cereals is a processing procedure traditionally used in many African countries for the manufacture of alcoholic and soft drinks (Taylor, 1983). Malting is a process involving germination and drying of cereal seeds, the prime objective being to promote the development of hydrolytic enzymes that are not active in raw seeds (Dewar *et al*., 1997). 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 (Chavan and Kadam, 1989). 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 (Valencia *et al.,* 1999). An adequate mineral absorption is important especially for infants, children, elderly people and people in clinical situation (Bergman *et al.,* 1999). 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 (Vijayakumari *et al.,* 1998). In the Sudan sorghum comes first in volume of cereals produced and is the staple food for people in rural areas, particularly the low-income groups as food or sometimes processed to produce alcoholic or soft beverages or as feed for livestock (Brudevold and Southern, 1994). Many attempts to reduce phytate have been made. It is reported that phytate is reduced in malted oats by 99% (Larrson and Sandberg, 1995) and malted pea by 75% (Beal and Mehta, 1985). Marfo *et al.* (1990) found that 72 h fermentation significantly decreased phytate content in foodstuffs, (80-98% for rice, cassava, and cocoyam and 52-65% for sorghum, maize, soybean, cowpea, and yam). Other attempts to reduce the phytate content such as fertilization (Elsheikh *et al*., 2000) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier *et al*., 1996) have been tried. Moreover, vigorous efforts are directed towards coupling the beneficial effects of tannins in sorghum as a field crop with methods for overcoming the anti-nutritional effects of tannins in seeds by direct removal of seed testa, inactivation, or by extraction. Extractable tannin content was markedly reduced when grains were soaked in water and stored under a carbon dioxide atmosphere (Reichert *et al*., 1980). In feeding trials with rats (Yasaman *et al*., 1990) and chicks (Teeter *et al*., 1986), tannins reduced weight gain and feed conversion. Sorghum is rich in minerals 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 that are undesirable (El Sheikh *et al*., 2000). 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. In the present study we would like to evaluate the efficiency of malt pretreatment on antinutritional factors contents and HCl extractability of minerals (*in vitro* availability) of sorghum cultivars.

Materials and Methods

Source and germination of seeds: Two sorghum

Table 1: Percent reduction in Phytate* and tannin** content of sorghum flour incubated with malt of different age at different period of time

Cultivars	Incubation period (min)	1st day malt		2nd day malt		4th day malt	
		Phytate	Tannin	Phytate	Tannin	Phytate	Tannin
Wad Ahmed	0	0.0	0.0	0.0	0.0	0.0	0.0
	30	$3.9±0.15^{c}$	$2.4±0.93^{c}$	$6.4±0.10^{c}$	$3.8±0.19^{d}$	$9.9±2.10^{bc}$	6.6±0.59d
	60	$9.8±0.02^{b}$	$4.5±0.70^{b}$	$13.1±0.76^{bc}$	$8.0±0.23^{c}$	$17.8±4.01^{b}$	$13.5±0.92^{c}$
	90	$13.1±0.52^{b}$	$7.6±0.51^{ab}$	$22.2±1.80^{ab}$	$11.8±0.91^{b}$	$32.0±8.40^{a}$	$19.8±0.90^{b}$
	120	$17.8±1.81^{a}$	$9.74±0.51^{a}$	$28.9±1.77^{a}$	$16.7±0.82^{a}$	$39.9±6.60^{a}$	$26.7±1.66^{a}$
Tabat	0	0.0	0.0	0.0	0.0	0.0	0.0
	30	$3.8±0.97^{cd}$	$2.9±0.08^{b}$	$6.1±0.61^{d}$	$4.3±0.80^{c}$	$8.8±1.56^{d}$	$6.2±0.70^{d}$
	60	$7.0±0.75^{bc}$	$3.8±0.93^{b}$	$11.5±1.12^{c}$	$7.5±0.21^{c}$	$17.8±1.55^{c}$	$12.4±1.03^{bc}$
	90	$10.1±0.41^{ab}$	$7.6±0.20^{a}$	$16.4±0.60^{b}$	$13.8±0.64^{b}$	$27.6±1.08^{b}$	$18.6±1.59^{ab}$
	120	$15.1±0.53^{a}$	$9.4±0.97^{a}$	$24.0±0.81^{a}$	$18.1±0.96^{a}$	$36.6±0.70^{a}$	$23.8±2.19^{a}$

Values are means of three replicates ± SD. Means not sharing a common superscript letter in a column for each cultivar are significantly different at P ? 0.05 as assessed by Duncan's multiple range test. *Phytate content of Wad Ahmed and Tabat cultivars was 265 and 233 mg/100g respectively. **Tannin content of the cultivars was 0.96 and 0.35% respectively.

(*Sorghum biocolor*) cultivars, locally known as Wad Ahmed (high tannin) and Tabat (low tannin) were obtained from Pioneer Company, Khartoum, Sudan. The seeds were carefully cleaned and freed from broken and extraneous matter. Seeds were germinated according to the method described by Bhise *et al.* (1988) for different periods to obtain 1-, 2- and 4-days old malt. The germinated seeds were sun-dried and the root portions were manually removed. The seeds were milled into fine flour to pass a 0.4 mm sieve and kept at 4°C before use.

Addition and incubation of malt to sorghum flour: About 1% of 1-, 2- or 4-days-old malt was added to sorghum flour in triplicate. Samples were shaken for 30 min and then mixed with water 1:2 (w/v) and incubated at 30°C in a shaker for 30, 60, 90 and 120 min, thereafter dried at 65°C and ground to pass a 0.4 mm sieve and kept at 4°C before use.

Total minerals determination: Minerals were extracted from the samples by dry ashing method that described by Chapman and Pratt (1961). The amount of iron, zinc, manganese, cobalt and copper were determined using Atomic Absorption Spectroscopy (Perkin-Elmer 2380). Ammonium Vandate was used to determine phosphorus along with Ammonium Molybdate method of Chapman and Pratt (1982). Calcium and magnesium were determined by titration method that described by Chapman and Pratt (1961). Sodium and potassium were determined by flame photometer (CORNIG EEL) according to AOAC (1984).

HCl extractability of minerals (*in vitro* availability): Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). One gram of the sample was shaken with 10 ml of 0.03 M HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven dried at 100°C and then dry acid digested. The amount of the extractable minerals was determined by the methods described above.

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

Tannin determination: Quantitative estimation of tannins was carried out using the modified vanillin–HCl method by Price *et al.* (1978). A standard curve was prepared expressing the result as catechin equivalent i.e. amount of catechin (mg/100g) which gives a color intensity equivalent to that given by tannin after correction for blank.

Statistical analysis: Each determination was carried out on three separate samples and analyzed in triplicate, the figures were then averaged. Data was assessed by the analysis of variance (ANOVA) (Snedecor and Cochran, 1987). Means comparisons for treatments were made by using Duncan's multiple range test (Duncan, 1955). Significance was accepted at P = 0.05.

Results and Discussion

Effect of malt pretreatment on phytate and tannin contents: Table 1 shows the effect of malt pretreatment on phytate (percent reduction) during incubation of sorghum flour with 1% malt (1-, 2-, or 4-days old) for different period of time (30, 60, 90 or 120 min). Phytic acid content of untreated sorghum cultivars was 265 and 233 mg/100g for Wad Ahmed and Tabat cultivars, respectively. For each cultivar and for each malt age, percent reduction in phytate increased significantly (P = 0.05) with increasing time of incubation. When the flour was mixed with 1%, 1-day old malt, percent phytate reduction varied from 3.9 to 17.8% for Wad Ahmed cultivar while for Tabat varied from 3.8 to 15.1% depend on the incubation time. For 1%, 4-days old malt percent

Table 2: Total mg/100g) and available %) major and trace minerals of untreated sorghum cultivars

Minerals	Cultivars			
	WadAhmed		Tabat	
	Total	Available	Total	Available
Na	6.3±0.20	66.70±2.80	7.0±0.60	64.3±2.20
K	450.0±2.00	46.3±6.40	441.7±2.40	51.0±6.70
Ca	10.8±1.50	3.3±0.95	12.5±0.77	35.7±1.20
Mg	59.3±5.70	46.4±1.06	63.0±1.00	55.6±1.60
P	303.0±2.00	43.4±1.40	283.3±2.30	44.9±1.50
Fe	3.80±0.10	4.20±0.40	4.50±0.12	6.00±0.45
Zn	3.21±0.11	47.7±0.60	3.53± 0.18	53.8±5.70
Mn	3.90±0.30	40.8±2.36	3.50±0.10	46.2±4.70
Co	0.21±0.010	66.7±2.80	0.18±0.02	72.2±5.60
Cu	0.00	0.00	0.00	0.00

Values are means of three replicates ± SD.

reduction in phytate was significantly increased and was found to be varied from 9.9 to 39.9% and from 8.8 to 36.6% for Wad Ahmed and Tabat cultivars, respectively. Results showed that as the age of malt and incubation time increased, percent reduction in phytate was significantly increased. The results indicated that phytic acid reduction was significantly affected by addition of sorghum malt because the germination stage had a substantial effect on the reduction in phytate content due to the action of endogenous phytases obtained during germination that degrade the phytate into inorganic phosphorus and inositol and its intermediate forms. The rate of reduction depends upon the age as well as the amount of malt. Valverde *et al*. (1994) reported that germination of lentils greatly reduced phytate content compared to soaking or cooking. Similar results were also reported when sorghum flour was treated with malt and incubated for different time intervals (Elkhalil *et al*., 2001). Percent reduction in tannin content was similar to that obtained for phytate. However, the rate of reduction in tannin was less than that of phytate due the fact that phytate can be degraded by the malt enzymes but tannins only washed out.

Effect of malt pretreatment on minerals extractability (availability): The data obtained for the cultivars showed that among major minerals studied, K and P were the major constituents (450 and 303 mg/100g) followed by Mg (59.3 mg/100g) while Na and Ca were the least constituents (6.3 and 10.8 mg/100g) in the untreated grain of the cultivar Wad Ahmed. For Tabat cultivar, K and P were the major constituents (441.7 and 283.3 mg/100g) followed by Mg (63.0 mg/100g) while Na and Ca were also the least constituents (7.0 and 12.5 mg/100g) in the untreated grain (Table 2). For both cultivars HCl-extractability of major minerals of untreated grains revealed that Na and Mg were the most available minerals and Ca and P were the least available ones.

The data obtained for trace minerals studied showed that Mn and Fe were the major mineral constituents (3.90 and 3.80 mg/100g) followed by Zn (3.21 mg/100g) while Co was the least constituents (0.21 mg/100g) and no Cu was detected in the untreated grain of the cultivar Wad Ahmed. For Tabat, Fe and Zn were the major minerals constituent (4.50 and 3.53 mg/100g) followed by Mn and Co (3.50 and 0.18 mg/100g) and the grains were lacking Cu. For both cultivars HCl-extractability of trace minerals revealed that Co and Zn were the most available minerals and Fe was the least available one (Table 2). Malt pretreatment of the flour greatly affects extractability of major minerals (Table 3). Major minerals content increased significantly ($P = 0.05$) specially P (data not shown) when the flour of the cultivars was pretreated with malt. The increment in P content is likely due to the release of phytate P by the action of the enzyme phytase produced during germination of the grains and also due solubilization of phytate P during incubation as reported by Khetarpaul and Chauhan (1990). The HCl extractability of the minerals was significantly ($P=0.05$) increased with the age and incubation time. Addition of malt to the flour of the cultivars increased major minerals extractability with an increase in malt age and incubation time (Table 3). Sodium and phosphorus were the most available (87 and 76%) minerals when a mixture of a 4-days old malt and sorghum flour were incubated for 120 min for Wad Ahmed cultivar. However, for Tabat cultivar, Na and K were the most available minerals (84 and 81%) followed by Mg (76%) and finally P and Ca (72%). Results indicated that addition of sorghum malt to sorghum flour greatly improved the availability of minerals. The improvement in minerals availability is likely due the reduction in phytate and tannin content of the flour. Rakhi and Khetarpau (1995) reported that, the HCl-extractabilities of calcium, iron, zinc, copper and manganese from the rice-defatted soy flour blend greatly

Table 3: Effect of malt pretreatment and incubation for different period of time on major minerals availability (%) of sorghum flour of two cultivars

Minerals	Malt age (days)	Cultivar: Wad Ahmed — Incubation period (min.) 0	30	60	90	120	Tabat 0	30	60	90	120
Na	1	67±2.8^{d}	69±1.9^{cd}	71±2.3^{bc}	75±1.5^{ab}	76±1.8^{a}	64±0.0^{c}	69± 0.5^{bc}	71±2.1^{ab}	73±2.7^{ab}	73±2.4^{a}
	2	67±0.0^{c}	71±1.3^{b}	74±1.1^{b}	78±2.5^{a}	81±1.6^{a}	65±1.9^{c}	71±1.4^{b}	73±0.8^{b}	76±0.3^{a}	78± 2.3^{a}
	4	67±2.7^{e}	73±1.5^{d}	76±1.5^{c}	82±1.1^{b}	87±1.1^{a}	65±1.9^{e}	73±2.4^{d}	76±0.6^{c}	81±2.3^{b}	84±1.3^{a}
K	1	46±6.4^{c}	50±1.3^{bc}	53±2.4^{ab}	56±1.6^{ab}	59±1.8^{s}	51±0.7^{d}	55±0.8^{cd}	59±1.4^{bc}	63±2.7^{ab}	66±2.3^{a}
	2	47±1.6^{d}	52±0.7^{cd}	55±1.9^{bc}	58±1.7^{ab}	62±2.2^{a}	51±1.5^{d}	57±1.9^{c}	63±1.3^{b}	68±1.8^{ab}	73±2.0
	4	47±0.8^{c}	52±0.6^{c}	58±1.9^{b}	64±2.1^{a}	70±1.7^{a}	52±0.6^{c}	59±1.6^{c}	63±2.6^{b}	74±2.5^{a}	81±1.8^{a}Ca
	1	33±0.9^{c}	38±2.9^{b}	41±1.1^{b}	45±3.0^{a}	49±1.9^{a}	35±1.2^{e}	41±2.0^{d}	46±1.6^{c}	51±2.6^{b}	56±2.6^{a}
	2	34±1.6^{e}	40±2.4^{d}	45±1.4^{c}	52±2.9^{b}	57±3.1^{a}	36±1.4^{e}	42±1.7^{d}	49±1.6^{c}	55±2.9^{b}	61±1.9^{a}
	4	34±.6^{e}	43±1.7^{d}	51±1.0^{c}	59±1.8^{b}	67±2.1^{a}	36±1.1^{e}	45±2.3^{d}	54±1.0^{c}	63±1.9^{b}	72±0.7^{a}
Mg	1	46±0.6^{e}	41±0.9^{d}	52± 0.9^{c}	54±0.9^{b}	58±1.5^{a}	55±1.5^{e}	59±1.0^{d}	61±1.0^{c}	64±1.2^{b}	67±1.7^{a}
	2	46±.0^{e}	51±1.8^{d}	55±1.4^{c}	59±1.6^{b}	69±1.1^{a}	56±2.2^{e}	59±1.1^{d}	63±1.1^{c}	67±1.7^{b}	70±1.5^{a}
	4	47±1.1^{e}	52±2.1^{d}	58±1.9^{c}	63±1.8^{b}	69±1.9^{a}	56±2.1^{e}	61±1.9^{d}	66±1.5^{c}	71±1.3^{b}	76±2.9^{s}
P	1	43±1.4^{c}	46±3.0^{c}	51±2.5^{b}	55±0.9^{a}	58±1.8^{a}	45±1.4^{d}	47±2.7^{cd}	50±1.5^{bc}	52±0.7^{ab}	56±3.3^{a}
	2	44±1.2^{e}	48± 2.3^{d}	54±0.9^{c}	59±1.8^{b}	64±1.8^{a}	45±3.0^{e}	49±1.3^{d}	53±1.1^{c}	57±0.5^{b}	61±1.1^{a}
	4	44±2.3^{e}	51±0.8^{d}	60±1.9^{c}	68±1.6^{b}	76± 3.4^{a}	45±3.0^{e}	52±1.6^{d}	59±1.4^{c}	64±2.3^{b}	72±0.9^{a}

Values are mean of three replicates (± SD). Means not sharing a common superscript letter in a row for each cultivar are significantly different at P ≤ 0.05 as assessed by Duncan's multiple range test.

Table 4: Effect of malt pretreatment and incubation for different period of time on trace minerals availability (%) of sorghum flour of two cultivars

Minerals	Malt age (days)	Cultivar: Wad Ahmed — Incubation period (min.) 0	30	60	90	120	Tabat 0	30	60	90	120
Fe	1	4± 0.4^{c}	7±0.2^{d}	9±0.9^{c}	12±1.5^{b}	15±0.5^{a}	6±0.4^{e}	9±0.9^{d}	12±1.3^{c}	15±1.7^{b}	17±1.3^{a}
	2	4±1.7^{e}	7±0.5^{d}	11±0.8^{c}	14±1.5^{b}	18±1.7^{a}	6±0.5^{c}	10±0.9^{b}	12±1.8^{b}	16±1.7^{a}	19± 1.7^{a}
	4	4± 0.5^{e}	8±0.7^{d}	12±1.1^{c}	15±1.5^{b}	19±1.6^{a}	7±0.2^{d}	11±1.2^{c}	14±1.6^{b}	18±1.8^{a}	21±2.3^{a}
Zn	1	47±2.0^{d}	51±1.5^{c}	55± 2.4^{b}	58±1.7^{a}	62±1.9^{a}	54±3.8^{d}	61±1.2^{c}	64±1.1^{bc}	68±1.9^{ab}	72±1.3^{a}
	2	48±0.9^{e}	52±0.5^{d}	57±2.3^{c}	63±2.1^{b}	69±0.9^{a}	54±1.8^{d}	62±1.4^{c}	67±1.7^{bc}	70±1.8^{ab}	75±1.7^{a}
	4	48±1.5^{e}	55±1.8^{d}	62±1.1^{c}	68±1.9^{b}	75 ±1.4^{a}	54±2.1^{d}	64±2.3^{c}	70.±1.7^{b}	76±1.9^{b}	81±1.5^{a}
Mn	1	41±1.9^{d}	44±1.3^{d}	46±2.4^{c}	49±3.2^{ab}	51±2.6^{a}	46±2.5^{c}	49±2.3^{bc}	51±0.5^{ab}	53±2.4^{a}	55±1.9^{a}
	2	42±0.4^{e}	46±1.9^{d}	50±1.1^{c}	55±1.9^{b}	59±1.8^{a}	46±2.5^{e}	51±0.9^{d}	54 ±1.1^{c}	58±1.6^{b}	62±2.1^{a}
	4	41±0.9^{e}	48±1.5^{d}	56±1.8^{c}	61±1.0^{b}	70±2.2^{a}	46±2.1^{d}	55±2.4^{d}	61±1.6^{c}	67±1.7^{b}	74±2.2^{a}
Co	1	67±4.7^{a}	69±2.1^{a}	70±1.8^{a}	72±3.4^{a}	73±1.2^{a}	72±0.3^{c}	75±2.0^{bc}	77±1.8^{abc}	80±1.6^{ab}	81±1.7^{a}
	2	67±4.7^{c}	70±1.8^{bc}	74±2.4^{abc}	76±2.0^{ab}	79±1.7^{a}	73±0.5^{d}	75±0.8^{cd}	78±1.9^{bc}	82±2.2^{ab}	85±1.1^{a}
	4	67±2.2^{c}	73±3.4^{bc}	77±06^{b}	80±2.1^{ab}	85±1.8^{a}	73± 0.5^{c}	77±3.1^{c}	84±1.1^{b}	88±1.9^{ab}	93±3.1^{a}
Cu	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values are mean of three replicates ± SD. Means not sharing a common superscript letter in a row for each cultivar are significantly different at P ≤ 0.05 as assessed by Duncan's multiple range test.

improved. Further they reported that higher HCl-extractability of minerals may be partly ascribed to the decreased content of phytic acid, as a significant negative correlation between the phytic acid and HCl-extractability of dietary essential minerals was obtained. Malt pretreatment of the flour greatly affects and extractability of trace minerals (Table 4). Trace minerals content increased significantly (P = 0.05) when the flour of the cultivars pretreated with 1% malt (data not shown). It was observed that HCl extractability of trace minerals was significantly (P=0.05) increased with the age and incubation time. Addition of 1% malt to the flour of the cultivars increased trace minerals extractability with an increase in malt age and incubation time (Table 4). When a mixture of 1%, 4-days old malt and the flour of Wad Ahmed cultivar were incubated for 120 min, Co and Zn became the most available minerals (85 and 75%) and Fe was the least available (19%). For Tabat cultivar, also Co and Zn were the most available minerals (93 and 81%) followed by Mn (74%) and Fe was the least available mineral (21%). It is clear from the data obtained that the percent availability of Co and Zn are greater in Tabat compared to Wad Ahmed. Results indicated that addition of sorghum malt to sorghum flour greatly improved the availability of trace minerals. The improvement in the availability is likely due the reduction in phytate and tannin content of the flour as reported by Rakhi and Khetarpau (1995). Moreover, results revealed that as the age of the malt and the incubation time were increased, both major and trace minerals availability increased this basically due to the fact that more phytase will be available which solubilize phytate of the flour.

Conclusion: Utilization of sorghum malt to lower phytic acid and tannin contents and to improve the extractability of major and trace minerals is a promising and simple method. The rate of reduction of phytate and tannin contents with a concomitant increment of minerals availability depends on the age and incubation period of the malt.

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