

# Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes

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**Abstract** Two corn genotypes, Var-113 (high phytate) and TL-98B-6225-9×TL617 (low phytate) were fermented for 14 days. The fermented flour was dried and milled. Phytic acid and polyphenols contents and hydrochloric acid (HCl) extractability of minerals from the fermented flours were determined at intervals of 2 days during fermentation period. Phytic acid and polyphenols decreased significantly ( $P \leq 0.05$ ) with an increase in fermentation period, with a concomitant increase in HCl extractable minerals. For both genotypes the major and trace minerals content was increased with fermentation period. When the grains flour was fermented for 14 days, TL-98B-6225-9×TL617 genotype had higher extractable calcium (94.73 %) while Var-113 had higher extractable phosphorus (76.55 %), whereas iron recorded high extractability levels (84.93 %) in TL-98B-6225-9×TL617 and manganese recorded high extractability levels (81.07 %) in Var-113. There was good correlation between phytate and polyphenols levels reduction and

the increment in extractable minerals with fermentation period.

**Keywords** Corn · Genotype · Fermentation · Antinutrients · Minerals · HCl-extractability

## Introduction

The increment in maize production resulted from additional land area planted, genetic improvement and more efficient technological field practices as well as from the introduction of new more highly productive varieties. Most of the production in the developing countries is for human consumption while that in the developed world it is mainly for industrial use and animal feed (FAO 1992). 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 the high prices of animal foods. A comparison of available data for wheat, corn and rice puts corn as the second most important cereal grain after wheat and before rice in terms of yield per hectare (FAO 1992). Because of the great importance of maize as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value, mainly with respect to protein, many efforts have been made to improve the biological utilization of the nutrients it contains.

Maize in Sudan is usually consumed after processing such as cooking to prepare thick porridge (*asida*), or thin one (*nasha*) and *muttala*, a fermented maize bread, 20 cm thick and hard crusts on the top and on the bottom surfaces. In between the two crusts the bread is very white and has a spongy texture (Dirar 1993).

Like other cereals, the nutritive value of corn is inadequate due to its deficiency in essential amino acids (lysine and tryptophan), and the presence of antinutritional factors

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such as phytate, tannins and polyphenols (Fageer et al. 2004). These antinutritional factors chelate dietary minerals in the gastrointestinal tract reducing their bioaccessibility and bioavailability as reported for millet (AbdelRahaman et al. 2007), sorghum (Idris et al. 2005) and corn (Sokrab et al. 2011). Minerals are involved in activation of intracellular and extracellular enzymes, in regulation of critical pH level in body fluids necessary for control of metabolic reactions and in osmotic balance between the cell and the environment. A deficiency of any one of the essential minerals can result in severe metabolic disorders and compromise the health of the body. The presence of phytate in the human diet has a negative effect on mineral uptake. Minerals of concern in this regard include zinc, iron, calcium, magnesium, manganese and copper (Konietzny and Greiner 2003). More than 60 % of the phosphorus in corn meal is in the form of phytate phosphorus, which is poorly available for absorption and utilization in the gastrointestinal tract. The inhibitory effect of phytate on trace mineral absorption can also be predicted in vitro by the molar ratio of phytate to such mineral. Domestic processing techniques have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing. Reductions of such antinutritional factors by processing methods such as gamma irradiation, dehulling, soaking, sprouting, cooking, malting and fermentation have been documented by many researchers (Abdelhaleem et al. 2008; Bains et al. 2011; Ertas and Turker 2012; Gupta and Nagar 2012; Idris et al. 2005; Obizoba and Atii 1994; Osman et al. 2012) and still progressing. It has been reported that germination of various pearl millet cultivars increased significantly the HCl-extractable parts of both major and trace minerals, and also reduced significantly ( $P \leq 0.01$ ) the phytic acid and polyphenols contents of millet cultivars (AbdelRahaman et al. 2007). In Sudan, research into mineral content and extractability in corn as an important food has not been fully documented. Therefore, in this study we would like to evaluate the effect of fermentation on the antinutritional factor content and hydrochloric acid (HCl) extractability of minerals in high and low phytate corn genotypes.

## Material and methods

### Materials

Two corn genotypes Var-113 (high phytate) and TL98B-6225-9×TL617 (low phytate) were obtained from the Department of Agronomy, University of Khartoum. The samples were carefully cleaned and freed from foreign materials and part of the grains was ground to pass a 0.4-mm screen and kept in polyethylene bags at 4 °C for further

analysis. All chemical used in this study were commercially available and of reagent grade.

### Fermentation process

Corn flour of the genotypes was fermented according to the traditional method (lactic acid fermentation) practiced by the Sudanese housewives (El Tinay et al. 1985). Initially, however, a natural fermentation was performed by the original microorganisms present in the corn flour. Approximately, 95 g flour was mixed with 200 ml of sterile deionized water and then 5 g starter obtained from previously fermented dough were added and mixed well with a glass rod. The slurry was allowed to ferment at 30 °C (temperature used in a traditional Sudanese kitchen) for 14 h. Samples were withdrawn at zero time and at 2 h intervals throughout the fermentation period. The pH was measured after each withdrawal of sample using a pH meter (model 315i, WTW, Weilheim, Germany). Then samples were dried in a hot air oven (Heraeus UT 5042, Germany) at 60 °C for 16 h. Dried samples were ground in a mortar and pestle to pass through 0.4 mm screen and stored at 4 °C in tightly closed containers until used for determination of phytic acid, polyphenols and HCl extractability of minerals.

### Phytic acid determination

Phytic acid content of the samples was determined by the method described by Wheeler and Ferrel (1971). Briefly, 2 g of corn flour was extracted with 50 ml of 3 % trichloroacetic acid (TCA) for 3 h with shaking and precipitated as the ferric salt. The iron content of the precipitate was determined colorimetrically (Hach DR3 spectrophotometer, Loveland, Colorado, USA). The phytate content calculated from this value assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. The iron content in the unknown samples was read from the previously prepared standard curve (different solution of ferric nitrate having varied concentration of  $\text{Fe}^{+++}$ ). Phytic acid content was determined by multiplying the phytate phosphorus content by a constant factor of 3.55.

### Polyphenols determination

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Butler 1977) with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3 ml methanol. The mixture was filtered. The filtrate was mixed with 50 ml distilled water and analyzed within 1 h. About 3 ml of 0.1 M  $\text{FeCl}_3$  in 0.1 M HCl was added to 1 ml filtrate, followed immediately by timed addition of 3 ml freshly prepared  $\text{K}_3\text{Fe}(\text{CN})_6$ . The absorbance was monitored on a

spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm after 10 min from the addition of 3 ml of 0.1 M FeCl<sub>3</sub> and 3 ml of 0.008 M K<sub>3</sub>Fe(CN)<sub>6</sub>. A standard curve was obtained, expressing the result as tannic acid equivalents; that is, the amount of tannic acid (mg/100 g) that gives a color intensity equivalent to that given by polyphenols after correction for blank.

#### Minerals composition

Minerals were determined from the samples by the dry ashing method that described by Chapman and Pratt (1982). Briefly, 2 g of corn flour were weighed in dry crucible and placed in a muffle furnace for 3 h at 550 °C. After cooling, samples were transferred to 250 ml beaker and then 12 ml of 5 N HCl and 3 ml of concentrated HNO<sub>3</sub> were added. The beaker was placed in a sand bath to boil for 10 min. Thereafter, 100 ml distilled water were added and allowed to boil for another 10 min. The contents were filtered through Whatman ashless filter paper No. 41. The filtrate was made up to 250 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. All other minerals were determined by atomic absorption spectrophotometer (Perkin-Elmer 2380, Norwalk, Connecticut, USA).

#### HCl extractability of minerals (in vitro bioavailability)

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). About 1 g 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 acid-digested. The amount of the extractable minerals was determined by the methods

described above. HCl extractability of minerals (%) was determined as follows:

HCl extractability of minerals (%)

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

#### Statistical analysis

Three separate batches, for a particular treatment, were taken and analyzed separately and the results were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor and Cochran 1987) and by Duncan's multiple range test with a probability  $P \leq 0.05$  using SAS/STAT software.

## Results and discussion

#### Effect of fermentation on antinutritional factors content and phytate/phosphorus ratio of corn genotypes

Table 1 shows the effect of fermentation on antinutritional factors content and phytate/phosphorus ratio of corn genotypes. Phytate content of untreated genotypes was 1047.00 and 87.16 mg/100 g for Var-113 and TL-98B-6225-9×TL617, respectively while polyphenols content was 460.50 and 363.70 mg/100 g for the genotypes, respectively. Variations in phytate and polyphenols contents between the two genotypes can be attributed to both genetic and environmental conditions. The value of phytate for Var-113 was much higher than those reported for white and yellow corn (Marfo et al. 1990) but much lower than that of TL-98B-6225-9×TL617. Khan et al. (1991) reported very high levels

**Table 1** Effect of fermentation on anti-nutritional factors content (mg/100 g) and phytate/P ratio of two corn genotypes (Var-113 and TL-98B-6225-9×TL617)

Fermentation time (h)	Var-113					TL-98B-6225-9×TL617				
	Phytic acid	% reduction	Phytate/P	Polyphenols	% reduction	Phytic acid	% reduction	Phytate/P	Polyphenols	% reduction
0	1047.00 <sup>a</sup>	–	11.34	460.50 <sup>a</sup>	–	87.16 <sup>a</sup>	–	0.49	363.70 <sup>a</sup>	–
2	1047.00 <sup>a</sup>	0.00	8.63	159.40 <sup>b</sup>	65.38	38.71 <sup>b</sup>	55.59	0.21	217.60 <sup>b</sup>	40.17
4	968.30 <sup>b</sup>	7.52	4.60	155.10 <sup>c</sup>	66.32	24.70 <sup>c</sup>	71.66	0.12	205.30 <sup>c</sup>	43.55
6	819.40 <sup>c</sup>	21.74	3.89	121.50 <sup>d</sup>	73.62	13.20 <sup>d</sup>	84.85	0.07	182.90 <sup>d</sup>	49.71
8	303.50 <sup>d</sup>	71.01	1.40	98.17 <sup>e</sup>	78.68	9.60 <sup>e</sup>	88.98	0.05	175.10 <sup>e</sup>	51.86
10	223.10 <sup>e</sup>	78.69	1.01	95.95 <sup>f</sup>	79.16	3.40 <sup>f</sup>	96.10	0.02	172.90 <sup>f</sup>	52.46
12	187.50 <sup>f</sup>	82.09	0.80	91.50 <sup>g</sup>	80.13	1.54 <sup>g</sup>	98.23	0.01	171.90 <sup>g</sup>	52.73
14	155.00 <sup>g</sup>	85.20	0.69	91.40 <sup>g</sup>	80.14	0.31 <sup>h</sup>	99.64	0.001	151.70 <sup>h</sup>	58.29

Values are means of three replicates. Means not sharing a common superscript letter in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test

(715–760 mg/100 g) of phytate for maize products when compared with that of genotype TL-98B-6225-9×TL617 genotype but much lower than that of Var-113 genotype. Deshmukh et al. (1995) reported phytate phosphorus content ranging from 132 to 234 mg/100 g of maize varieties. Compared to TL-98B-6225-9×TL617 genotype (14.47 %), Var-113 had low protein content (11.70 %) but higher value of phytate. It has been reported that appreciably high amount of protein was observed to be associated with phytate content and it was found that as the protein content increased, phytate levels also increased (Reddy and Pierson 1994). This observation is a departure from an otherwise good correlation between protein content and phytate level. The explanation for this deviation is not clear, but may lie in chemical (as well as quantitative) differences between the protein and phytate of the genotypes. As shown in Table 1 the ratio of phytic acid/total phosphorus for corn genotypes was 11.34 and 0.49 with an average value of 5.91. AbdelRahaman et al. (2007) stated that phytic acid represents more than 70 % of total phosphorus in pearl millet. Results obtained in this study showed a linear relation between phytic acid and total phosphorus. Raboy et al. (1991) concluded that, in various seeds, phytic acid positively correlates with total phosphorus, correlation coefficients for some cultivars being greater than 0.90. Factors that affect the total phosphorus content, such as soil, available phosphorus and fertilizers, can influence the phytic acid concentration. Polyphenols content was also varied between the two genotypes and this variation is likely to be due to the fact that the amounts of such factor depend on variety, stage of maturity, edible portion and storage conditions.

As shown in Table 1 and for the two genotypes, polyphenols content decreased significantly ( $P \leq 0.05$ ) within the first 2 days of fermentation but phytic acid significantly ( $P \leq 0.05$ ) decreased within the first 4 days of fermentation. Thereafter, they decreased at a higher rate from day 6 to day 14 of fermentation and the reduction exceeded 85 % for phytate and 58 % for polyphenols at the end of fermentation process for both genotypes. The results showed that fermentation as a source of degrading enzymes had significantly ( $P \leq 0.05$ ) reduced phytic acid and polyphenols contents of the genotypes grains with time. Generally, cereal has been regarded as the major source of dietary phytate. The majority of ingested phytate is undergraded during transit through the gastrointestinal tract (Graf and Easton 1990). During digesta movement in the human body, dietary phytate-mineral complexes may dissociate and may form other complexes through the gastrointestinal tract. In the upper part of the small intestine, which is characterized by maximum mineral absorption, the insoluble complexes are highly unlikely to provide absorbable essential elements. Thereby, the chemical interactions of phytate in the upper gastrointestinal tract are of particular concern since the site and

degree of phytate degradation can affect the nutritional value of a phytate-rich diet (Kumar et al. 2010). One phytate molecule can bind up to six divalent cations, and the metal could possibly bridge at least two phytate molecules, depending on the redox state (Graf and Easton 1990). Phytic acid is a powerful inhibitor of iron-driven hydroxyl radical formation because it forms a catalytically inactive iron chelate. Abdelseed et al. (2011) have observed decrease in phytic acid contents of sorghum grains as a result of fermentation. The decrease in the level of phytic acid during fermentation may be attributed to the action of the enzyme phytase released during fermentation. Other researchers have reported a decrease in the level of phytic acid during fermentation due to phytase activity in the fermented flour (Kerovuo et al. 2000; AbdelRahaman et al. 2005; Idris et al. 2005). The polyphenols content for both genotypes was also significantly ( $P \leq 0.05$ ) decreased with fermentation of genotypes flour and the reduction exceeded 58 % for both genotypes. Many researchers reported that fermentation greatly decreased polyphenols contents (AbdelRahaman et al. 2005; Idrir et al. 2005). Moreover, fermentation of malted samples caused further reduction in polyphenols as reported for sorghum (Abdelhaleem et al. 2008). The reduction in polyphenols content after fermentation is due to the action of tannase enzyme which is released by the microorganisms during fermentation (Barthamuef et al. 1994)

#### Effect of fermentation on total and extractable minerals of corn genotypes

The effect of fermentation on major minerals content and HCl extractability of corn genotypes grains is shown in Table 2. The major mineral content varied between the genotypes. The data obtained for unfermented flour indicated that phosphorus and potassium were the major mineral constituents of the genotypes grains. For genotype Var-113, total phosphorus was 92.33 mg/100 g and out of this amount about 33 % was extractable while that of genotype TL-98B-6225-9×TL617 was 178.70 mg/100 g and out of this amount about 27.40 % was extractable. For both genotypes calcium of unfermented flour represents the third mineral but the amount extractable was higher compared to all minerals. The variation in mineral content between the genotypes may be due to genetic as well as environmental factors. As shown in Table 2 the major minerals content of fermented samples was increased significantly ( $P \leq 0.05$ ) with the fermentation period as well as the HCl extractability of such minerals. The extractability of minerals of nutritional importance (calcium and phosphorus) significantly ( $P \leq 0.05$ ) increased with the fermentation period, with maximum values obtained at day 14. Genotype TL-98B-6225-9×TL617 gave higher extractable calcium (94.73 %) compared to that of genotype Var-113 (82.53 %) after 14 days of fermentation.

**Table 2** Effect of fermentation on major minerals content (mg/100 g) and HCl-extractability (%) of two corn genotype (Var. 113 and TL-98B-6225-9×TL617)

Genotype	Fermentation time (h)	pH	Na		K		Mg		Ca		P		
			Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	
Var-113	0	5.94	13.28 <sup>g</sup>	38.00 <sup>h</sup>	206.17 <sup>e</sup>	57.1 <sup>h</sup>	51.67 <sup>g</sup>	67.87 <sup>h</sup>	74.50 <sup>h</sup>	69.80 <sup>g</sup>	92.83 <sup>h</sup>	33.00 <sup>h</sup>	
	2	5.83	14.32 <sup>f</sup>	54.57 <sup>g</sup>	219.70 <sup>de</sup>	60.33 <sup>g</sup>	52.03 <sup>g</sup>	68.27 <sup>g</sup>	78.23 <sup>g</sup>	70.57 <sup>f</sup>	121.30 <sup>g</sup>	35.15 <sup>g</sup>	
	4	5.48	14.49 <sup>f</sup>	65.33 <sup>f</sup>	241.90 <sup>cde</sup>	68.30 <sup>f</sup>	55.11 <sup>f</sup>	74.13 <sup>f</sup>	84.00 <sup>f</sup>	74.13 <sup>e</sup>	160.40 <sup>f</sup>	43.46 <sup>ef</sup>	
	6	4.93	16.22 <sup>e</sup>	72.61 <sup>e</sup>	251.10 <sup>bcd</sup>	76.30 <sup>e</sup>	56.63 <sup>e</sup>	75.73 <sup>e</sup>	87.27 <sup>e</sup>	76.13 <sup>d</sup>	210.70 <sup>e</sup>	47.11 <sup>de</sup>	
	8	4.53	17.97 <sup>d</sup>	78.22 <sup>d</sup>	266.10 <sup>abc</sup>	81.82 <sup>d</sup>	59.19 <sup>d</sup>	79.36 <sup>d</sup>	90.40 <sup>d</sup>	79.10 <sup>c</sup>	218.00 <sup>d</sup>	54.87 <sup>d</sup>	
	10	4.23	20.30 <sup>c</sup>	82.07 <sup>c</sup>	286.80 <sup>ab</sup>	93.20 <sup>c</sup>	62.63 <sup>c</sup>	82.42 <sup>c</sup>	93.19 <sup>c</sup>	81.34 <sup>b</sup>	222.10 <sup>c</sup>	62.12 <sup>c</sup>	
	12	4.09	22.28 <sup>b</sup>	88.70 <sup>b</sup>	299.60 <sup>a</sup>	99.33 <sup>b</sup>	67.33 <sup>b</sup>	84.48 <sup>b</sup>	95.23 <sup>b</sup>	82.08 <sup>a</sup>	233.50 <sup>b</sup>	66.05 <sup>b</sup>	
	14	3.92	23.07 <sup>a</sup>	96.80 <sup>a</sup>	305.30 <sup>a</sup>	103.40 <sup>a</sup>	68.33 <sup>a</sup>	87.23 <sup>a</sup>	96.76 <sup>a</sup>	82.53 <sup>a</sup>	225.20 <sup>b</sup>	76.55 <sup>a</sup>	
	TL-98B-6225-9×TL617	0	5.85	10.80 <sup>h</sup>	39.77 <sup>h</sup>	125.00 <sup>cde</sup>	37.50 <sup>h</sup>	43.33 <sup>h</sup>	62.90 <sup>h</sup>	69.67 <sup>f</sup>	79.10 <sup>h</sup>	178.70 <sup>g</sup>	27.40 <sup>h</sup>
		2	5.76	12.12 <sup>g</sup>	45.03 <sup>g</sup>	123.40 <sup>e</sup>	46.05 <sup>g</sup>	45.27 <sup>g</sup>	68.14 <sup>g</sup>	72.33 <sup>e</sup>	81.17 <sup>g</sup>	185.10 <sup>f</sup>	30.63 <sup>g</sup>
		4	5.19	14.47 <sup>f</sup>	52.28 <sup>f</sup>	131.60 <sup>de</sup>	57.29 <sup>f</sup>	47.15 <sup>f</sup>	72.88 <sup>f</sup>	74.63 <sup>d</sup>	83.42 <sup>f</sup>	199.10 <sup>e</sup>	34.42 <sup>f</sup>
		6	4.76	16.21 <sup>e</sup>	58.51 <sup>e</sup>	150.80 <sup>bcd</sup>	62.09 <sup>e</sup>	49.50 <sup>e</sup>	81.14 <sup>e</sup>	76.05 <sup>c</sup>	86.30 <sup>e</sup>	200.90 <sup>d</sup>	38.26 <sup>e</sup>
		8	4.35	20.13 <sup>d</sup>	62.25 <sup>d</sup>	162.20 <sup>abc</sup>	67.54 <sup>d</sup>	50.81 <sup>d</sup>	84.23 <sup>d</sup>	77.30 <sup>c</sup>	92.40 <sup>d</sup>	200.90 <sup>d</sup>	44.36 <sup>d</sup>
		10	4.21	21.68 <sup>c</sup>	68.90 <sup>c</sup>	164.10 <sup>abc</sup>	72.33 <sup>c</sup>	52.07 <sup>c</sup>	86.30 <sup>c</sup>	78.93 <sup>b</sup>	93.15 <sup>c</sup>	204.30 <sup>c</sup>	52.10 <sup>c</sup>
12		4.06	23.42 <sup>b</sup>	76.66 <sup>b</sup>	175.80 <sup>ab</sup>	80.50 <sup>b</sup>	53.87 <sup>b</sup>	87.84 <sup>b</sup>	81.10 <sup>a</sup>	94.30 <sup>b</sup>	206.10 <sup>b</sup>	61.76 <sup>b</sup>	
14		3.85	27.20 <sup>a</sup>	83.53 <sup>a</sup>	180.30 <sup>a</sup>	88.13 <sup>a</sup>	56.32 <sup>a</sup>	91.72 <sup>a</sup>	82.07 <sup>a</sup>	94.73 <sup>a</sup>	213.30 <sup>a</sup>	66.10 <sup>a</sup>	

Values are means of three replicates. Means not sharing a common superscript letter in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test

**Table 3** Effect of fermentation on trace minerals content (mg/100 g) and HCl-extractability (%) of two corn genotype (Var. 113 and TL-98B-6225-9×TL617)

Genotype	Fermentation time (h)	pH	Fe		Zn		Mn		Cu		Co		
			Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	Total	Extractable	
Var-113	0	5.94	1.95 <sup>c</sup>	32.30 <sup>h</sup>	1.25 <sup>f</sup>	50.60 <sup>g</sup>	0.68 <sup>f</sup>	68.40 <sup>g</sup>	0.12 <sup>e</sup>	41.60 <sup>h</sup>	0.70 <sup>a</sup>	86.30 <sup>g</sup>	
	2	5.83	2.20 <sup>d</sup>	34.21 <sup>g</sup>	1.40 <sup>e</sup>	54.14 <sup>f</sup>	0.71 <sup>ef</sup>	69.35 <sup>f</sup>	0.37 <sup>d</sup>	43.23 <sup>g</sup>	0.68 <sup>a</sup>	86.41 <sup>g</sup>	
	4	5.48	2.31 <sup>d</sup>	35.33 <sup>f</sup>	1.54 <sup>d</sup>	54.14 <sup>f</sup>	0.73 <sup>def</sup>	70.25 <sup>e</sup>	0.61 <sup>c</sup>	46.25 <sup>f</sup>	0.70 <sup>a</sup>	87.07 <sup>f</sup>	
	6	4.93	2.44 <sup>d</sup>	39.85 <sup>e</sup>	1.61 <sup>d</sup>	56.43 <sup>e</sup>	0.75 <sup>cde</sup>	73.10 <sup>d</sup>	0.63 <sup>c</sup>	47.10 <sup>e</sup>	0.73 <sup>a</sup>	89.54 <sup>e</sup>	
	8	4.53	3.33 <sup>c</sup>	44.30 <sup>d</sup>	1.74 <sup>c</sup>	61.17 <sup>d</sup>	0.77 <sup>cd</sup>	76.69 <sup>c</sup>	0.66 <sup>bc</sup>	50.27 <sup>d</sup>	0.76 <sup>a</sup>	91.17 <sup>d</sup>	
	10	4.23	3.72 <sup>b</sup>	48.00 <sup>c</sup>	1.83 <sup>b</sup>	63.33 <sup>c</sup>	0.82 <sup>bc</sup>	76.53 <sup>c</sup>	0.71 <sup>b</sup>	57.23 <sup>c</sup>	0.81 <sup>a</sup>	96.27 <sup>c</sup>	
	12	4.09	3.91 <sup>b</sup>	55.30 <sup>b</sup>	1.91 <sup>a</sup>	64.07 <sup>b</sup>	0.84 <sup>ab</sup>	78.23 <sup>b</sup>	0.77 <sup>a</sup>	59.62 <sup>b</sup>	0.82 <sup>a</sup>	96.93 <sup>b</sup>	
	14	3.92	4.34 <sup>a</sup>	60.18 <sup>a</sup>	1.96 <sup>a</sup>	66.34 <sup>a</sup>	0.87 <sup>a</sup>	81.07 <sup>a</sup>	0.79 <sup>a</sup>	62.09 <sup>a</sup>	0.88 <sup>a</sup>	98.57 <sup>a</sup>	
	TL-98B-6225-9×TL617)	0	5.85	4.57 <sup>d</sup>	21.00 <sup>h</sup>	1.19 <sup>g</sup>	47.90 <sup>g</sup>	0.64 <sup>f</sup>	33.30 <sup>h</sup>	0.18 <sup>f</sup>	49.10 <sup>h</sup>	0.50 <sup>f</sup>	52.00 <sup>h</sup>
		2	5.76	4.78 <sup>cd</sup>	38.10 <sup>g</sup>	1.42 <sup>f</sup>	51.47 <sup>f</sup>	0.71 <sup>ef</sup>	34.77 <sup>g</sup>	0.54 <sup>e</sup>	53.33 <sup>g</sup>	0.63 <sup>e</sup>	54.90 <sup>g</sup>
		4	5.19	4.99 <sup>c</sup>	49.41 <sup>f</sup>	1.54 <sup>e</sup>	53.57 <sup>e</sup>	0.77 <sup>de</sup>	37.77 <sup>f</sup>	0.62 <sup>d</sup>	61.97 <sup>f</sup>	0.71 <sup>de</sup>	63.10 <sup>f</sup>
		6	4.76	5.21 <sup>b</sup>	61.62 <sup>e</sup>	1.67 <sup>d</sup>	53.37 <sup>e</sup>	0.79 <sup>de</sup>	44.52 <sup>e</sup>	0.66 <sup>d</sup>	67.87 <sup>e</sup>	0.76 <sup>cd</sup>	66.67 <sup>e</sup>
		8	4.35	5.34 <sup>b</sup>	68.39 <sup>d</sup>	1.75 <sup>cd</sup>	65.37 <sup>d</sup>	0.83 <sup>cd</sup>	55.59 <sup>d</sup>	0.69 <sup>d</sup>	73.50 <sup>d</sup>	0.78 <sup>cd</sup>	70.97 <sup>d</sup>
		10	4.21	5.61 <sup>a</sup>	75.99 <sup>c</sup>	1.79 <sup>bc</sup>	68.94 <sup>c</sup>	0.87 <sup>bc</sup>	63.27 <sup>c</sup>	0.90 <sup>c</sup>	75.47 <sup>c</sup>	0.82 <sup>bc</sup>	74.22 <sup>c</sup>
12		4.06	5.71 <sup>a</sup>	78.73 <sup>b</sup>	1.87 <sup>ab</sup>	72.62 <sup>b</sup>	0.93 <sup>ab</sup>	71.07 <sup>b</sup>	1.07 <sup>b</sup>	79.00 <sup>b</sup>	0.87 <sup>b</sup>	76.14 <sup>b</sup>	
14		3.85	5.72 <sup>a</sup>	84.93 <sup>a</sup>	1.91 <sup>a</sup>	79.16 <sup>a</sup>	0.97 <sup>a</sup>	73.22 <sup>a</sup>	1.21 <sup>a</sup>	81.93 <sup>a</sup>	0.97 <sup>a</sup>	80.34 <sup>a</sup>	

Values are means of three replicates. Means not sharing a common superscript letter in a column are significantly different at  $P \leq 0.05$  as assessed by Duncan's multiple range test



In a similar study, Abdelseed et al. (2011) observed an increase in the HCl extractability of calcium of sorghum lines after fermentation. Moreover, Eltayeb et al. (2008) reported that malting of millet followed by fermentation greatly improved the HCl extractability of calcium due to reduction in antinutrients level.

Another important mineral considered in this study, having a significant role in nutrition, is phosphorus. The phosphorus content for both genotypes significantly ( $P \leq 0.05$ ) increased with the fermentation time. The increment in phosphorus content may be due to hydrolysis of phytate by the enzyme phytase, which is released after fermentation. Phosphorus extractability increased significantly ( $P \leq 0.05$ ) with increase in fermentation period (Table 2) for both genotypes. Despite it contains higher level of phytate, genotype Var-113 gave higher extractable phosphorus (76.55 %) compared to that of TL-98B-6225-9×TL617 (66.10 %) after 14 days of fermentation. Variation in extractable phosphorus at different periods of fermentation was observed by Eltayeb et al. (2008) for millet cultivars. For both genotypes, all other minerals studied (sodium, potassium and magnesium) gave an HCl extractability trend similar to that obtained for calcium and phosphorus. According to FAO (1992), the corn germ is relatively rich in minerals, with an average value of 11 % as compared with less than 1 % in the endosperm. The germ provides about 78 % of the whole kernel minerals. The most abundant mineral is phosphorus, found as phytate of potassium and magnesium.

Table 3 shows the effect of fermentation on total and HCl extractable trace minerals of corn genotypes. The genotypes varied in total and extractable trace minerals. Iron content was 1.95 mg/100 g for Var-113 and out of this amount about 32.30 % was extractable while that of TL-98B-6225-9×TL617 was 4.57 mg/100 g and about 21 % was extractable. For both genotypes the trace minerals content and the HCl extractability increased significantly ( $P \leq 0.05$ ) with the fermentation period. The genotypes were significantly ( $P \leq 0.05$ ) differing in HCl extractability at various levels of fermentation. The HCl extractability of iron increased significantly ( $P \leq 0.05$ ) from the beginning of the fermentation and reached a maximum value at day 14. Genotype TL-98B-6225-9×TL617 gave higher HCl extractability of iron (84.93 %) while that of Var-113 was 60.18 %. The difference in iron extractability between the two genotypes is due to differences in level of phytate between them. In a similar study, Abdelseed et al. (2011) observed an increase in iron extractability of sorghum lines after fermentation. Moreover, Eltayeb et al. (2008) reported that malting of millet followed by fermentation greatly improved the HCl extractability of iron. Total and extractable zinc, manganese, copper and cobalt increased significantly ( $P \leq 0.05$ ) when the grains flour was fermented for 14 days. Similarly, Afoakwa et al. (2011) reported that fermentation significantly increased the copper

content of the Ghanaian cocoa beans. The increment in HCl extractability of both major and trace minerals of corn genotypes is likely to be due to the reduction of antinutrients (phytate and polyphenols) as a result of fermentation during which phytase and tannase enzymes released during fermentation which hydrolyzed phytate and polyphenols, respectively. The mechanism of the release of minerals might be through the dephosphorylation of phytate in which the removal of phosphate groups from the inositol ring decreases the mineral binding strength of phytate, and thus, results in increased bioavailability of essential dietary minerals (Sandberg et al. 1999).

## Conclusion

Fermentation is a powerful method in improving total and extractable major and trace minerals of corn genotypes by reducing the antinutritional factors (phytate and polyphenols) by the action of the enzymes released during fermentation.

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