Journal of Food Technology 3 (4): 516-522, 2005

Effect of Fermentation on Antinutritional Factors and Hcl Extractability of Minerals of Pearl Millet Cultivars

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Abstract: This study reports the effect of fermentation of pearl millet cultivars on the antinutritional factors and minerals content and availability. The flour of two pearl millet cultivars (Ashana and Dembi) was fermented for 14 h. The fermented flour was dried and milled. Phytic acid and polyphenols contents and hydrochloric acid (HCl) extractability of minerals of the fermented flours were determined at intervals of 2 h during fermentation. Phytic acid and polyphenols contents decreased significantly ($p \leq 0.01$) with increase in fermentation time with concomitant decrease in pH and increase in minerals content and extractability. When the flour was fermented for 14 h, Ashana had higher extractable Na and P, whereas Fe and Mn recorded high level in Dembi and Ashana, respectively. It was observed that cooking of the flour fermented for 14 h further increased minerals content and extractability for both cultivars. There was a good correlation between antinutritional factors reduction and increase in extractable minerals with increase in fermentation time.

Key words: Phytic acid, polyphenols, hydrochloric acid, extractability, minerals, pearl millet

INTRODUCTION

In developing countries, because of limited access to animal products (meat, fish, eggs) that provide high intakes of hemeiron and zinc, the main dietary sources of iron and zinc are cereals and legumes. In some countries in Sahelian Africa, millets, and particularly pearl millet (*Pennisetum glaucum*), can represent more than 75% of the total cereal production[1]. This cereal thus represents an important proportion of dietary intake for millions of people. Unfortunately, the iron and zinc in cereal based foods are poorly bioavailable due to factors that reduce their intestinal absorption, resulting in high rates of iron and zinc deficiency, especially in infants, children, and women of child-bearing age[2]. For instance, in Sudan where millet contributes to more than 20% of the available food energy, prevalence of iron deficiency anaemia is very serious in children. Furthermore, mineral absorption is also influenced by the level of mineral contents and by factors that enhance their absorption in the diet, as well as by the physiological status of the subjects, such as age, disease, or stores of mineral [3,4]. Over the years, many *in vivo*[5,6] or *in vitro*[7] studies have reported the negative effects of phytates, fibers, and tannins on iron or zinc bioavailability or *in vitro* availability. The overall conclusion of these studies shows that the effects of antinutritional factors on mineral availability are highly dependent on the food matrix. Moreover, results of different studies have differed considerably depending on the method used to estimate mineral bioavailability. Because of their cost and complexity, radio-isotopic measurements of mineral absorption in animals or humans are often impractical and consequently little used. The grain is traditionally processed either by germination or fermentation prior to consumption. Studies on biochemical changes in pearl millet due to these processes are limited. It was observed that the biological value and vitamin B content improved on fermentation of finger millet[8], while a decrease was observed in IVPD[9]. Rakhi and Khetarpau[10] reported that the HCl-extractabilities of calcium, iron, zinc, copper and manganese from the rice-defatted soy flour blend also improved. 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. Also Khetarpaul and Chauhan[11] reported that natural fermentation of precooked pearl millet flour, carried out at 20, 25 and 30°C for 72 h, brought about a significant increase in non-phytate, inorganic and HCl-extractable phosphorus. Further they reported that HCl-extractability of calcium, copper, iron, zinc and manganese was improved significantly. This study reports the effect of fermentation of pearl millet cultivars on the antinutritional factors and minerals content and availability.

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MATERIALS AND METHODS

Source of cereal grain: Two pearl millet cultivars (Ashana and Tembi) were obtained from ElObeid Agricultural Research Station, Western Sudan. All chemicals used in this study were of analytical grade.

Sample preparation: The cereal grains were cleaned manually to remove broken seeds, dust and other extraneous materials. The cleaned grains were milled and the dough was prepared in the ratio of 5% starter to flour. Then the slurry was allowed to ferment. Samples were withdrawn at zero time and at 2 h intervals up to 14 h of fermentation. The pH was measured after each withdrawal of sample using pH meter (pH 210-microprocesser pH meter, HANA instruments) and samples were dried at 60 °C in an air oven. The dried samples were flaky and reground to pass a 0.4 mm screen and stored at 4 °C in tightly closed containers.

Total minerals determination: Minerals were extracted from the samples by dry ashing method that described by Chapman and Pratt[12]. 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[13]. Calcium and magnisum were determined by titration method that described by Chapman and Pratt[12]. Sodium and potassium were determined by flame photometer (CORNIG EEL) according to AOAC[14] .

Hcl-extractability of minerals (bio availability): Minerals in the samples were extracted by the method described by Chauhan and Mahjan[15]. 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. Thereafter, extractability of each mineral was determined as a percentage of the individual total mineral.

Phytic acid determination: Phytic acid content was determined by the method described by Wheeler and Ferrel[16] 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.

Polyphenols determination: Total polyphenols were determined according to Purssion Blue spectrophotometric method[17] with a minor modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 mL methanol. The mixture was filtered. The filtrate was mixed with 50 mL of distilled water and analyzed within an hour. About 3.0 mL of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 mL of the filtrate followed immediately by timed addition of 3.0 mL of freshly prepared $K_3Fe(CN)_6$. The absorbance was monitor on a spectrophotometer (Pye Unicam SP6 – 550 UV) at 720 nm after 10 min from the addition of 3.0 mL of 0.1 M $FeCl_3$ and 3.0 mL of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared expressing the result as tannic acid equivalent i.e. amount of tannic acid (mg/100g) which gives a color intensity equivalent to that given by polyphenols after correction for blank.

Statistical analysis: Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) described by Snedecor and Cochran[18] and by Duncan'multiple range test[19] with a probability $p \leq 0.01$.

RESULTS AND DISCUSSION

Effect of fermentation on Phytate and polyphenols contents of the cultivars: Phytate content of untreated cultivars was 969.30 and 1101.04 mg/100g for Ashana and Dembi, respectively while total polyphenols was 306.65 to 669.39 mg/100g for the cultivars, respectively, (Table 1). Results of the present investigation were similar to those reported by Abdalla *et al.*,[20] and Kheterpaul and Chauhan[21] . Variations in phytic acid content among different genotypes can be attributed to both genetic and environmental conditions[22]. The protein content of the cultivars was 12.79 and 11.44% (data not shown). The cultivars contained appreciably high amount of protein, which was observed to be associated with phytate content as the protein content increased, phytate levels also increased[23]. For phosphorus, investigation showed values of 1101.77 and 1290.35 mg/100g for the cultivars, respectively. The percentage of phytic acid/total P was 88 and 85% for Ashana and Dembi cultivars, respectively. Chauhan *et al.*[16]. Stated that phytic acid represents more than 70% of total P in pearl millet. Results obtained in this study showed a linear relation between phytic acid and total P (correlation coefficient of 0.9805). Raboy *et al.*[24] concluded that, in various seeds, phytic acid positively correlates with total P, correlation coefficients being greater than 0.90. Factors that affect the total P content, such as soil, available P and fertilizers, can influence the phytic acid concentration[25]. phytic acid content decreased significantly ($p \leq 0.01$) within the first 2 h of fermentation. Thereafter, phytic acid content decreased progressively up to 14 h of fermentation with a concomitant decrease in the pH (Table 1). The phytic acid

Table 1:Effect of Fermentation on Phytic Acid and Polyphenols Contents (Mg/100g) of Pearl Millet Cultivars

	Cultivars					
	Ashana			Dembi		
Time(h)	pH	Phytic acid	Polyphenols	pH	Phytic acid	Polyphenols
Untreated	Untreated	969.30 (± 0.00)^{a}	306.65 (± 2.92)^{a}	Untreated	1101.04 (± 0.00)^{a}	669.39 (± 0.00)^{a}
0	6.16	921.70 (± 11.71)^{b}	285.00 (± 0.00)^{b}	6.18	1031.08 (± 0.00)^{b}	599.29 (± 2.87)^{b}
2	5.78	859.75 (± 0.00)^{c}	265.33 (± 2.92)^{c}	5.79	972.26 (± 0.00)^{c}	550.18 (± 1.42)^{c}
4	5.52	790.85 (± 0.00)^{d}	245.78 (± 2.53)^{d}	5.42	894.33 (± 9.19)^{d}	527.49 (± 0.00)^{d}
6	4.41	734.11 (± 9.28)^{e}	213.36 (± 0.62)^{h}	4.11	812.86 (± 0.00)^{e}	451.97 (± 0.00)^{i}
8	3.83	660.38 (± 0.00)^{f}	197.79 (± 0.00)^{i}	3.85	737.36 (± 9.12)^{f}	412.87 (± 0.87)^{j}
10	3.71	547.47 (± 29.34)^{g}	232.85 (± 0.87)^{e}	3.81	647.96 (± 0.00)^{g}	513.57 (± 0.00)^{e}
12	3.71	499.85 (± 15.62)^{h}	226.80 (± 0.00)^{f}	3.97	508.33 (± 0.00)^{h}	487.82 (± 2.89)^{f}
14	3.66	344.93 (± 0.00)^{i}	222.10 (± 0.73)^{g}	3.71	372.74 (± 9.22)^{i}	484.12 (± 0.00)^{g}
14, Cooked	3.66	309.24 (± 0.00)^{j}	215.03 (± 1.19)^{h}	3.71	328.55 (± 0.00)^{j}	471.37 (± 0.00)^{h}

Values are Means (± SD). Means not sharing a common superscript letter in a column are significantly different at P = 0.01 as assessed by Duncan's Multiple Range tests

Table 2:Effect of Fermentation on Major Mineral Content (Mg/100g) and Availability (%) of Pearl Millet Cultivar, Ashana

		Na		K		Mg		Ca		P	
Time (h)	pH	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Untreated	Untreated	16.25 (± 0.00)^{j}	73.29 (± 0.00)^{j}	434.41 (± 3.32)^{g}	71.54 (± 1.99)^{j}	93.00 (± 0.00)^{g}	56.29 (± 0.84)^{i}	52.78 (± 0.48)^{d}	32.45 (± 0.68)^{j}	1106.77 (± 1.76)^{j}	35.41 (± 0.32)^{j}
0	6.16	16.36^{i} (± 0.25)	76.69 (± 0.83)^{i}	437.42 (± 2.05)^{fg}	74.80^{i} (± 0.78)	93.08 (± 0.03)^{fg}	58.10 (± 0.20)^{h}	52.85 (± 0.03)^{d}	45.36 (± 0.08)	1115.41^{i} (± 0.49)	39.38 (± 0.17)^{i}
2	5.78	17.92 (± 0.68)^{h}	80.22 (± 0.00)^{h}	441.34 (± 0.00)^{f}	77.21 (± 0.00)^{h}	93.15^{f} (± 0.02)	60.23 (± 0.41)^{g}	52.89 (± 0. 5)^{cd}	49.16 (± 0.70)^{h}	1131.38 (± 3.40)^{h}	46.01 (± 0.00)^{h}
4	5.52	20.36 (± 0.64)^{g}	84.41 (± 0.84)^{g}	444.65 (± 0.77)^{e}	80.69 (± 0.47)^{g}	93.20 (± 0.01)^{f}	62.67 (± 0.00)^{f}	52.93 (± 0.7)^{cd}	51.23 (± 0.43)^{g}	1142.54 (± 0.00)^{g}	51.80 (± 0.31)^{g}
6	4.41	26.23 (± 1.18)^{f}	94.74^{f} (± 1.17)	453.46 (± 3.19)^{d}	93.71^{f} (± 0.79)	93.57 (± 0.00)^{e}	73.39 (± 0.20)^{e}	53.15 (± 0. 3)^{cb}	59.16 (± 0.66)^{f}	1179.89 (± 1.70)^{f}	64.95 (± 0.18)^{f}
8	3.83	29.67 (± 1.17)^{e}	99.90 (± 0.00)^{e}	463.38 (± 3.79)^{c}	99.55 (± 0.00)^{e}	93.62 (± 0.04)^{ed}	76.90 (± 0.24)^{d}	53.25 (± 0.6)^{ab}	61.19 (± 0.27)^{e}	1190.00 (± 0.00)^{e}	71.43 (± 0.40)^{e}
10	3.71	31.86 (± 0.23)^{d}	103.68 (± 0.66)^{d}	469.10 (± 3.17)^{b}	101.92 (± 0.54)^{d}	93.74 (± 0.02)^{cd}	78.87^{c} (± 0.00)	53.28^{b} (± 0. 5)^{a}	62.27 (± 0.00)^{d}	1195.35 (± 1.95)^{d}	73. 8 1 (± 0.00)^{d}
12	3.71	32.23 (± 0.47)^{c}	106.68 (± 0.00)^{c}	453.35 (± 2.92)^{d}	102.78 (± 0.47)^{c}	93.82 (± 0.04)	79.40 (± 0.23)^{c}	53.41 (± 0. 2)^{ab}	59.92 (± 0.24)^{c}	1202.65 (± 0.00)^{c}	75.11 (± 0.00)^{c}
14	3.66	33.09 (± 0.23)^{b}	110.73 (± 1.09)^{b}	480.32 (± 4.52)^{a}	108.84 (± 0.95)^{b}	94.03 (± 0.09)^{b}	80.71 (± 0.25)^{b}	53.41 (± 0. 6)^{ab}	63.02 (± 0.43)^{b}	1207.44 (± 1.55)^{b}	77.06 (± 0.17)^{b}
14, Cooked	3.66	35.25 (± 0.00)^{a}	116.48 (± 0.59)^{a}	483.65 (± 0.59)^{a}	111.69 (± 0.55)^{a}	94.53 (± 0.20)^{a}	81.46 (± 0.33)^{a}	53.52 (± 0.02)^{a}	64.78 (± 0.00)^{a}	1221.27 (± 0.00)^{a}	79.11 (± 0.17)^{a}

Values are Means (± SD). Means not sharing a common superscript letter in a column are significantly different at p= 0.01 as assessed by Duncan's Multiple Range tests

content of the various grains did not differ among cultivars at different levels of fermentation times. Generally, cereal has been regarded as the major source of dietary phytate[25-27]. The majority of ingested phytate is undergraded during transit through gastrointestinal tract[28]. Metal phytate complexes are highly insoluble over a wide pH range[28]. 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[28]. Phytic acid is a powerful inhibitor of iron-driven hydroxyl radical formation because it forms a catalytically inactive iron chelate[29]. Steeping and germination as well as fermentation decreased the phytic acid content of pearl millet cultivars. Gupta and Sehgal[30] have observed decrease in phytic acid contents of cereal grains used for preparing weaning foods as a result of soaking, fermentation and germination. Other researchers have reported decrease in the level of phytic acid during fermentation[10,11].Polyphenols content for the two cultivars followed a trend similar to that reported for phytate when the flour was fermented for up to 14 h (Table 1). It was observed that cooking of 14 h-fermented flour causes further reduction in both phytate and polyphenols content for the two cultivars.

Effect of fermetation on minerals content and extractability of the cultivars: The major minerals content and extractability of Ashana and Dembi cultivars are shown in Table 2 and 3, respectively. The data obtained showed that P and K were the major mineral constituents while Na and Ca were the least constituents in the untreated grain of the cultivar Ashana (Table 2). Values obtained in this study were in the range reported by Abdalla *et al.*[20]. HCl-extractability of major minerals of untreated grains revealed that Na and K were the most available minerals and Ca and P were the least available ones. Fermentation of the flour of the cultivar greatly affects both content and extractability of major minerals.

Table 3: Effect of Fermentation on Major Mineral Content (Mg/100g) and Availability (%) of Pearl Millet Cultivar, Dembi

		Na		K		Mg		Ca		P	
Time (h)	pH	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Untreated	Untreated	15.21 (± 0.55)^{h}	63.12 (± 4.11)^{g}	370.47 (± 0.00)^{g}	73.26 (± 0.38)^{i}	84.51 (± 0.94)^{c}	52.73 (± 0.94)^{c}	49.08 (± 0.00)^{h}	27.73 (± 0.61)^{i}	1290.35 (± 6.4)^{i}	39.60 (± 0.00)^{j}
0	6.18	15.36 (± 0.30)^{gh}	64.41 (± 1.78)^{g}	372.45 (± 1.00)^{g}	74.69 (± 0.72)^{h}	84.60^{cb} (± 0.01)	61.54 (± 0.45)^{h}	49.09^{h} (±0. 1)^{g}	38.95 (± 0.18)	1300.32 (± 0.0)^{h}	42.56 (± 0.15)^{i}
2	5.79	16.13 (± 0.00)^{g}	68.50 (± 0.00)^{f}	374.05 (± 1.34)^{g}	76.05 (± 0.37)^{h}	84.66 (± 0.04)^{cb}	63.20 (± 0.00)^{g}	49.12 (± 0.02)^{g}	42.27 (± 0.25)^{g}	1314.18 (± 1.6)^{g}	46.37 (± 0.08)^{h}
4	5.42	16.99 (± 0.27)^{f}	71.10 (± 0.63)^{f}	376.49 (± 4.29)^{f}	78.09 (± 0.00)^{g}	84.69 (± 0.02)^{cb}	64.51 (± 0.67)^{f}	49.19 (± 0.01)^{f}	44.36 (± 0.10)^{f}	1328.60 (± 1.9)^{f}	49.31 (± 0.00)^{g}
6	4.11	22.95 (± 0.30)^{e}	80.04 (± 1.22)^{e}	385.11 (± 2.48)^{e}	92.28 (± 0.56)^{f}	85.14 (± 0.02)^{ab}	74.12 (± 0.33)^{e}	49.34 (± 0.01)^{e}	52.49 (± 0.28)^{e}	1365.70 (± 1.7)^{e}	61.56 (± 0.15)^{f}
8	3.85	24.26 (± 0.56)^{d}	84.35 (± 0.80)^{d}	391.45 (± 0.00)^{d}	97.80 (± 0.94)^{e}	85.24 (± 0.04)^{a}	78.25 (± 0.27)^{d}	49.38 (± 0.00)^{d}	53.75 (± 0.00)^{c}	1378.90 (± 3 .9)^{d}	65.88 (± 0.15)^{e}
10	3.81	26.14 (± 0.31)^{c}	87.11 (± 0.00)^{c}	396.54 (± 0.00)^{c}	100.55 (± 0.00)^{d}	85.28 (± 0.03)^{a}	79.26 (± 0.16)^{c}	49.41 (± 0.03)^{c}	55.05 (± 0.00)^{c}	1382.66 (±4.1)^{cd}	67.67 (± 0.00)^{d}
12	3.79	27.54 (± 0.94)^{b}	89.15 (± 0.57)^{c}	393.20 (± 3.17)^{cd}	102.96 (± 1.87)^{c}	85.31 (± 0.02)^{a}	79.53 (± 0.38)^{bc}	49.46 (± 0.04)^{b}	53.71 (± 0.34)^{d}	1386.37 (± 1.7)^{c}	70.01 (± 0.00)^{c}
14	3.71	27.33 (± 0.30)^{b}	93.23 (± 1.14)^{b}	402.02 (± 2.60)^{b}	109.23 (± 0.65)^{b}	85.37 (± 0.00)^{a}	80.25 (± 0.57)^{b}	49.51 (± 0.01)^{a}	58.18 (± 0.09)^{b}	1394.00 (± 5.1)^{b}	72.85 (± 0.14)^{b}
14,cooked	3.71	29.18 ± 0.52)^{a}	96.06 (± 0.34)^{a}	407.09 (± 0.00)^{a}	111.06 (± 0.76)^{a}	85.40 (± 0.01)^{a}	81.63 (± 0.25)^{a}	49.53 (± 0.03)^{a}	59.90 (± 0.14)^{a}	1406.48 (± 1.4)^{a}	74.27 (± 0.00)^{a}

Values are Means (± SD). Means not sharing a common superscript letter in a column are significantly different at p≤ 0.01 as assessed by Duncan's Multiple Range tests

Major minerals content increased significantly (p≤ 0.01) when the flour was fermentation for 14 h with a higher increment observed for Na and P. The HCl extractability of the minerals was significantly (p≤ 0.01) increased with the fermentation time from 0 to 14 h for each mineral. For Na and K HCl-extractability exceeded 100% while for other minerals it was almost doubled. Further increment in major minerals content and extractability was observed when 14h-fermented flour was cooked (Table 2). Rakhi and Khetarpau[10] reported that the HCl-extractabilities of calcium, iron, zinc, copper and manganese from the rice-defatted soy flour blend also improved. 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. Also Khetarpaul and Chauhan[11] reported that natural fermentation of precooked pearl millet flour, carried out at 20, 25 and 30°C for 72 h, brought about a significant increase in non-phytate, inorganic and HCl-extractable phosphorus. Further they reported that HCl-extractability of calcium, copper, iron, zinc and manganese was improved significantly. The data obtained for Dembi cultivar (Table 3) showed that P and K were the major mineral constituents while Na and Ca were the least constituents in the untreated grain of the cultivar. Values obtained in this study were in the range reported by Abdalla *et al.*[20]. HCl-extractability of major minerals of untreated grains revealed that Na and K were the most available minerals and Ca and P were the least available ones. Fermentation of the cultivar flour greatly affects both content and extractability of major minerals. Major minerals content increased significantly (p≤0.01) when the flour was fermentation for 14h with a higher increment observed for P. The HCl extractability of the minerals was significantly (p≤0.01) increased with the fermentation time from 0 to 14 h for each mineral. For K, HCl extractability exceeded 100% while for P it was almost doubled. Further increment in major minerals content and extractability was observed when 14h-fermented flour was cooked (Table 3). Similar observations were reported by Rakhi and Khetarpau[10]and Khetarpaul and Chauhan[11] . For both cultivars the increment in 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[11]. The trace minerals content and extractability of Ashana and Dembi cultivars are shown in Table 4 and 5, respectively. The results obtained showed that Fe and Zn were the major mineral constituents while Co and Cu were the least constituents in the untreated grain of the cultivar Ashana (Table 4). Similar results were reported by Abdalla *et al.* [19]. HCl-extractability of trace minerals of untreated grains revealed that Co and Mn were the most available minerals and Fe and Cu were the least available ones. Fermentation of the cultivar flour greatly affects both content and extractability of trace minerals. Trace minerals content increased gradually with the fermentation time from 0 to 14 h for each mineral. The HCl extractability of the minerals was significantly (p≤ 0.01) increased when the flour of the cultivar was fermented for 14 h. For Fe and Cu, HCl extractability was almost doubled. Further increment in trace minerals content and extractability was observed when 14 h fermented flour was cooked (Table 4). Rakhi and Khetarpau[10] reported that the Hcl-extractabilities of

Table 4:Effect of Fermentation on Trace Mineral Content (Mg/100g) and Availability (%) of Pearl Millet Cultivar, Ashana

		Fe		Zn		Mn		Cu		Co	
Time (h)	pH	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Untreated	Untreated	10.70	26.67	1.78	43.33	1.32	48.13	0.62	25.05	0.063	87.49
		(± 0.09)^{h}	(± 0.08)^{i}	(± 0.01)^{g}	(± 0.23)^{i}	(± 0.00)^{g}	(± 0.51)^{i}	(± 0.004)^{h}	(± 0.56)^{j}	(± 0.003)^{d}	(± 2.13)^{d}
0	6.16	10.97	27.68	1.78	44.74	1.33	49.74	0.63	26.97	0.063	86.31
		(± 0.02)^{g}	(± 0.00)^{h}	(± 0.01)^{g}	(± 0.48)^{h}	(± 0.00)^{g}	(± 0.24)^{h}	(± 0.003)^{g}	(± 0.40)^{i}	(± 0.001)^{cd}	(± 0.19)^{d}
2	5.78	11.03	29.31	1.80	45.54	1.37	52.24	0.64	29.17	0.064	87.27
		(± 0.00)^{g}	(± 0.29)^{g}	(± 0.01)^{f}	(± 0.14)^{g}	(± 0.00)^{f}	(± 0.44)^{g}	(± 0.002)^{f}	(± 0.00)^{h}	(± 0.001)^{cd}	(± 0.67)^{d}
4	5.52	11.10	31.95	1.81	47.77	1.38	53.91	0.64	32.52	0.065	89.14
		(± 0.01)^{f}	(± 0.09)^{f}	(± 0.01)^{f}	(± 0.64)^{f}	(± 0.00)^{ef}	(± 0.31)^{f}	(± 0.002)^{f}	(± 0.44)^{g}	(± 0.001)^{c}	(± 0.00)^{c}
6	4.41	11.18	46.27	1.84	60.09	1.40	69.44	0.65	47.87	0.067	96.49
		(± 0.00)^{e}	(± 0.10)^{e}	(± 0.02)^{e}	(± 0.48)^{e}	(± 0.01)^{ed}	(± 0.20)^{e}	(± 0.002)^{e}	(± 0.33)^{f}	(± 0.00)^{b}	(± 0.58)^{b}
8	3.83	11.22	51.52	1.87	64.44	1.42	74.99	0.66	51.76	0.069	97.87
		(± 0. 1)^{ed}	(± 0.04)^{d}	(± 0.01)^{d}	(± 0.00)^{d}	(± 0.01)^{cd}	(± 0.39)^{d}	(± 0.002)^{d}	(± 0.00)^{e}	(± 0.00)^{a}	(± 0.51)^{a}
10	3.71	11.25	53.11	1.90	66.09	1.44	76.47	0.66	53.42	0.069	98.51
		(± 0. 0)^{ed}	(± 0.10)^{c}	(± 0.01)^{c}	(± 0.54)^{c}	(± 0.00)^{cb}	(± 0.27)^{c}	(± 0.001)^{cd}	(± 0.19)^{d}	(± 0.00)^{a}	(± 0.19)^{a}
12	3.71	11.30	55.92	1.93	68.59	1.45	77.00	0.67	54.60	0.069	98.45
		(± 0.0)^{c}	(± 0.00)^{b}	(± 0.02)^{a}	(± 0.29)^{b}	(± 0.00)^{cb}	(± 0.13)^{c}	(± 0.001)^{c}	(± 0.40)^{c}	(± 0.00)^{a}	(± 0.19)^{a}
14	3.66	11.39	51.13	1.91	68.54	1.48	78.11	0.67	57.32	0.070	98.88
		(± 0.01)^{b}	(± 0.13)^{b}	(± 0.00)^{cb}	(± 0.56)^{b}	(± 0.05)^{a}	(± 0.38)^{b}	(± 0.001)^{b}	(± 0.49)^{b}	(± 0.00)^{a}	(± 0.00)^{a}
14	3.66	11.58	57.75	1.93	70.39	1.46	79.78	0.68	59.35	0.070	99.04
Cooked		(± 0.00)^{a}	(± 0.24)^{a}	(± 0.02)^{ab}	(± 0.49)^{a}	(± 0.00)^{ab}	(± 0.16)^{a}	(± 0.003)^{a}	(± 0.41)^{a}	(± 0.00)^{a}	(± 0.16)^{a}

Values are Means (± SD). Means not sharing a common superscript letter in a column are significantly different at p=0.01 as assessed by Duncan's Multiple Range tests

Table 5:Effect of Fermentation on Trace Mineral Content (Mg/100g) and Availability (%) of Pearl Millet Cultivar, Dembi

		Fe		Zn		Mn		Cu		Co	
Time (h)	pH	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Untreated	Untreated	10.70	26.67	1.78	43.33	1.32	48.13	0.62	25.05	0.063	87.49
		(± 0.09)^{h}	(± 0.08)^{i}	(± 0.01)^{g}	(± 0.23)^{i}	(± 0.00)^{g}	(± 0.51)^{i}	(± 0.004)^{h}	(± 0.56)^{j}	(± 0.003)^{d}	(± 2.13)^{d}
0	6.18	10.97	27.13	1.68	44.87	1.65	46.33	0.96	23.43	0.062	86.73
		(± 0.02)^{g}	(± 0.35)^{i}	(± 0.01)^{e}	(± 0.17)^{h}	(± 0.00)^{h}	(± 0.25)^{i}	(± 0.002)^{g}	(± 0.32)^{i}	(± 0.00)^{d}	(± 0.66)^{d}
2	5.79	11.03	29.36	1.69	46.29	1.66	48.08	0.97	26.57	0.062	87.17
		(± 0.00)^{g}	(± 0.05)^{h}	(± 0.03)^{e}	(± 0.00)^{g}	(± 0.00)^{g}	(± 0.10)^{h}	(± 0.00)^{f}	(± 0.36)^{h}	(± 0.00)^{d}	(± 0.77)^{d}
4	5.42	11.10	32.36	1.70	48.74	1.68	51.17	0.98	29.50	0.063	88.15
		(± 0.01)^{f}	(± 0.08)^{g}	(± 0.03)^{e}	(± 0.12)^{f}	(± 0.00)^{f}	(± 0.22)^{g}	(± 0.01)^{e}	(± 0.38)^{g}	(± 0.00)^{d}	(± 0.25)^{c}
6	4.11	11.39	43.17	1.76	63.79	1.70	67.70	1.00	50.53	0.065	96.90
		(± 0.02)^{c}	(± 0.01)^{f}	(± 0. 2)^{d}	(± 0.59)^{e}	(± 0.01)^{e}	(± 0.19)^{f}	(± 0.01)^{d}	(± 0.38)^{f}	(± 0.00)^{c}	(± 1.14)^{b}
8	3.85	11.22	45.81	1.79	67.64	1.73	70.14	1.01	54.71	0.066	97.99
		(± 0.01)^{e}	(± 0.19)^{e}	(± 0. 1)^{cd}	(± 0.45)^{d}	(± 0.01)^{d}	(± 0.18)^{e}	(± 0.01)^{c}	(± 0.00)^{e}	(± 0.00)^{cb}	(± 0.41)^{a}
10	3.81	11.49	48.88	1.81	69.22	1.75	73.77	1.01	57.15	0.066	98.73
		(± 0.01)^{b}	(± 0.07)^{d}	(± 0. 2)^{cb}	(± 0.00)^{c}	(± 0.00)^{c}	(± 0.00)^{d}	(± 0.00)^{bc}	(± 0.32)^{d}	(± 0.00)^{cb}	(± 0.00)^{a}
12	3.79	11.30	50.00	1.82	70.40	1.76	75.29	1.02	58.58	0.067	98.32
		(± 0.00)^{d}	(± 0.06)^{c}	(± 0. 2)^{cb}	(± 0.34)^{b}	(± 0.00)^{b}	(± 0.37)^{c}	(± 0.00)^{b}	(± 0.00)^{c}	(± 0.00)^{ab}	(± 0.19)^{a}
14	3.71	11.59	53.62	1.84	70.76	1.76	77.30	1.02	59.62	0.067	98.85
		(± 0.00)^{a}	(± 0.08)^{b}	(± 0.35)^{b}	(± 0.35)^{b}	(± 0.00)^{a}	(± 0.21)^{b}	(± 0.00)^{b}	(± 0.36)^{b}	(± 0.00)^{ab}	(± 0.00)^{a}
14	3.71	11.58	56.38	1.85	73.77	1.77	79.87	1.03	61.72	0.068	98.86
Cooked		(± 0.00)^{a}	(± 0.00)^{a}	(± 0.02)^{a}	(± 0.00)^{a}	(± 0.00)^{a}	(± 0.00)^{a}	(± 0.00)^{a}	± 0.28)^{a}	(± 0.00)^{ab}	(± 0.17)^{a}

Values are means (± SD). Means not sharing a common superscript letter in a column are significantly different at p=0.01 as assessed by Duncan's Multiple Range tests

calcium, iron, zinc, copper and manganese from the rice-defatted soy flour blend also improved. 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. Also Khetarpaul and Chauhan[11] reported that natural fermentation of precooked pearl millet flour, carried out at 20, 25 and 30°C for 72 h, brought about a significant increase in non-phytate, inorganic and HCl-extractable phosphorus. Further they reported that HCl-extractability of calcium, copper, iron, zinc and manganese was improved significantly. The results obtained for Dembi cultivar (Table 5) showed that Fe and Zn were the major mineral constituents while Cu and Co were the least constituents in the untreated grain of the cultivar. Results obtained in this study were in the range reported by Abdalla *et al.*[19]. HCl-extractability of trace minerals of untreated grains revealed that Co and Mn were the most available minerals and Fe and Cu were the least available ones. Fermentation of the cultivar flour greatly affects both content and extractability of the minerals. Trace minerals content increased gradually with the fermentation time from 0 to 14 h. The HCl extractability of

the minerals was significantly ($p \leq 0.01$) increased with the fermentation time from 0 to 14 h for each mineral.

Co recorded higher Hcl extractability than the other minerals while for Fe and Cu it was almost doubled. Further increment in trace minerals content and extractability was observed when 14h-fermented flour was cooked (Table 5). The increment in 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[11].

CONCLUSIONS

Fermentation of the flour of 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 the cultivars. Results indicated that The availability of Fe, Ca and P increased significantly with the fermentation time and reached it maximum value after 14 h. Cooking of the fermented flour cause further increment in the availability of such minerals.

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