Journal of Food Technology 3 (3): 397-403, 2005

Antinutritional Factors Content and Minerals Availability of Pearl Millet (*Pennisetum glaucum*) as Influenced by Domestic Processing Methods and Cultivar

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Abstract: In this study four pearl millet cultivars Ashana, Dahabaya, Dembi, GB-87-35, were investigated. Results showed that pearl millet contained 1.6-2.3% ash and 11.4-13.0% protein. Pearl millet cultivars were very rich in major mineral specially P. Trace minerals were also high for all cultivars specially Fe content. HCl-extractability of minerals (as an index of their bioavailability) varied between the cultivars and type of mineral as well as antnutritional factors contents. Phytic acid content ranged from 969.3 to 1101.0 mg/100 g and polyphenol ranged from 306.7 to 669.4 mg/100 g. Cooking was found to reduce antinutritional factors contents and caused a slight increase in mineral content while bioavailability was significantly ($p \leq 0.01$) improved for all minerals investigated. Germination for 2, 4 and 6-days significantly ($p \leq 0.01$) decreased major and trace (except Cu and Co) minerals contents. Bioavailability of all minerals was significantly ($p \leq 0.01$) improved throughout the germination time.

Key words: Millet, cultivar, antinutrient, availability, minerals, domestic process

INTRODUCTION

Pearl millet is a staple food for a large section of the population in Asian and African countries. Besides supplying calories and proteins in the diet, pearl millet is a good source of essential minerals. Like other cereals grains the abundance of antinutrients such as phytic acid and polyphenols inhibit proteolytic and amylolytic enzymes, limit protein and starch digestibility and make poor human bioavailability of minerals. Pearl millet is also versatile foodstuff. It is used mainly as cooked, whole, dehulled or ground flour; dough or a grain like rice. These are made into unleavened breads (roti), fermented foods (kisra and jalabies), thin and thick porridges (toh). Steam-cooked dishes (couscous); non-alcoholic beverage and snake[1]. In Sudan it is staple diet of the people in the Western- region (Darfur) where it is consumed as thick porridge (aseeda) or a thin porridge (nasha) or kisra from fermented or unfermented dough and also brewed into local beer (maressa) fermented millet malt and other alcoholic drinks. Among millets, pearl millet contains a higher protein content and better amino acid balance than sorghum. Large variations in protein content from 6 to 21% have been observed[2]. Protein content of 15.4, 14.8 and 16.3% was reported by Klopfenstein *et al*[2] for gray, yellow and brown pearl millet, respectively. Local Sudanese cultivars investigated by Elyas *et al*[3] gave a rage of 10.8-14.9% of protein comparable to those reported by Ali *et al.*[4](12.5-13.6%). Sigh *et al.*[5] stated that fibre content of pearl millet account for 1.5 to 1.7% for high protein inbred lines and 1.25 to 1.3% for normal protein varieties. Klopfenstein *et al.*[6] found that gray, yellow and brown pearl millet contained about 2.25, 2.01 and 2.56% crude fibre, respectively. Oshodi *et al.* [7] reported a value of 3.1% of crude fibre for a Nigerian cultivar. Phytic acid content of pearl millet represents more than 70% of the total phosphorus of the grain [8]. A value of 990 mg/100 g of phytic acid was reported by Khetarpaul and, while Kumar and Chauhan[9] reported a value of 825.7 mg/100 g. Elhag *et al.*[10] reported values of 943 and 1076 mg/100 g phytic acid for two Sudanese cultivars. Simwamba *et al.*[11] stated that both environmental and genetic conditions influence the phytate level of pearl millet. Polyphenols have been considered as antinutrient because they interact with food constituents and make them unavailable. Klopfenstein *et al.*[6] reported that the goitrogen is mainly found in the bran and that it produces goitre due to the inhibition of the normal conversion of thyroxine (T_4) to triiodothyronine (T_3), the more active form of the hormone. The high mineral content of pearl millet could be associated with the grain goiterogenicity. Polyphenols content of pearl millet ranging from 590- 1060 mg/ 100 g [31]. Khetarpaul and Chauhan[12] reported a value of

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761 mg/ 100 g. Elhag *et al.*[10] gave values of 304 and 444 mg/100 g of polyphenols for two of pearl millet cultivars. Polyphenols can form complexes with metal cations through their carboxylic and hydroxylic groups and thus interfere with the intestinal absorption of minerals. Numerous experiments in both humans and animals have shown that polyphenols strongly inhibit iron absorption. This action has been attributed to the galloyl and catechol groups of phenolic compounds [13]. Values for individual minerals in pearl millet vary widely [14] and are dependent to a large extent on the mineral composition of the soil. In this study we would like to investigate the effect of domestic processing methods on antinutritional factors and minerals availability of pearl millet cultivars.

MATERIALS AND METHODS

Four certified cultivars (Ashana, Dahabaya, Dembi and GB 87-35) of pearl millet (*Pennisetum glaucum*) were used in this study and were obtained from El Obeid Agricultural Research Station, Western Sudan. The seeds of each cultivar were cleaned from husks, damaged grains and foreign materials by hand and divided into two lots. One lot was kept in clean bottles at 4°C for sprouting (malting). The remaining seeds were milled into fine flour using Quadramat Junior mill and kept in nylon bags for further processing and chemical analysis. Unless otherwise stated all reagents used in this study were of reagent grade.

Processing methods

Cooking: Cooking of the samples was performed by suspending the flour of each cultivar in distilled water in the ratio of 1:10 (flour: water) and the slurry was shaken continuously to avoid lumps while boiling in a water bath for 20 min. The viscous mass was spread out thinly in Petri dishes and oven dried at 70°C. The dry flakes were milled into fine flour (0.4 mm) and kept in nylon bags at 4°C for further analysis [15].

Germination: Germination was performed according to the method of Bhise *et al.* [16] with a slight modification. The millet grains were soaked in distilled water for 10 h at room temperature (28±2). The water was discarded and steeped in 1 to 2 volumes 0f 0.2% formaldehyde solution for 40 min to retard mould growth during germination. The soaked grains were then washed with distilled water several times and steeped in water for 20 min to remove residual formaldehyde. The wet millet grains were then spread evenly (about 1 cm) on medical guaze saturated with distilled water in stainless trays and germinated for three different time intervals (2, 4 and 6 days). Distilled water was sprinkled on the grains every 12h to avoid drying. The germinated grains were cleaned from the shoot and root manually, then allowed to dry completely in good air circulating room and further cleaned from residual shoot and root. The grains were milled into fine flour (0.4 mm) and kept in clean tightly closed plastic bags.

Chemical analysis

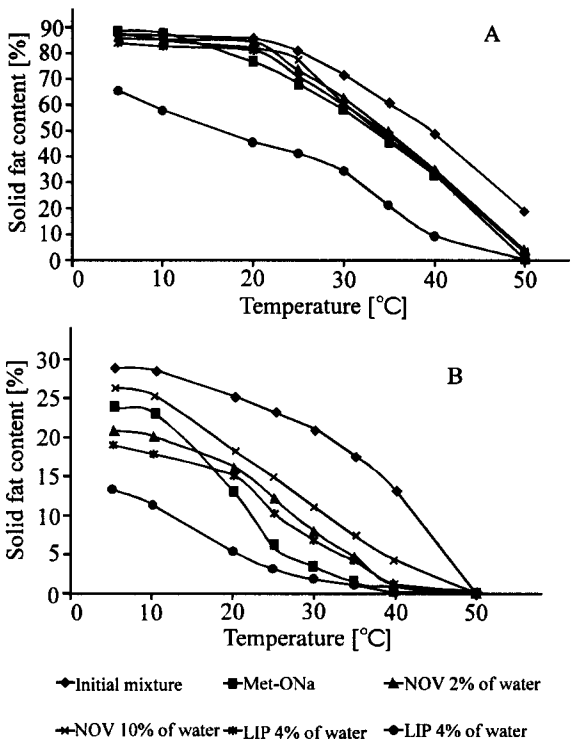
Phytic acid determination: Phytic acid content was determined by the method described by Wheeler and Ferrel, using two grams of a dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron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

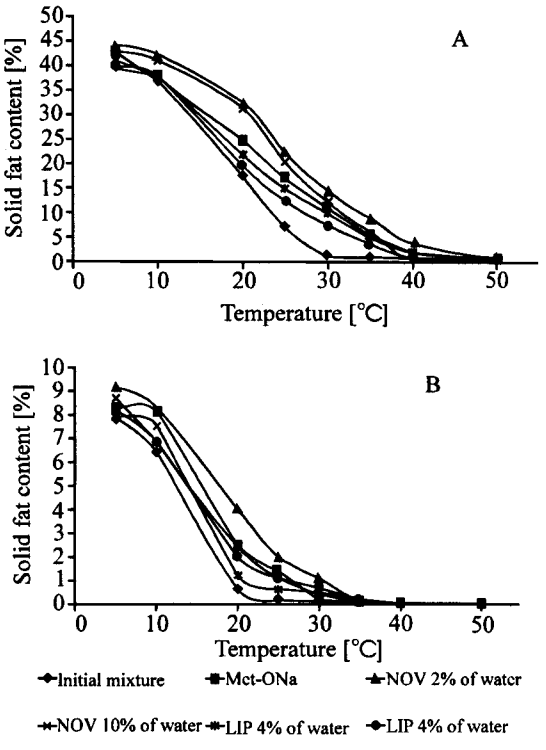
Total polyphenols determination: Total polyphenols were determined using Purssion blue spectrophotometric method [17]. About 60 mg of sample were shaken manually for 60 second with 3.0 mL of methanol in a test tube. The mixture was filtered and then the tube was quickly rinsed with additional 3.0 mL of methanol and the contents poured at once into the funnel. The filtrate was mixed with 50 mL of water and analysed within an hour. About 3.0 mL of 0.1M $FeCl_3$ in 0.1 N HCl was added to 1.0 mL of the filtrate followed immediately by timed addition of 3.0 mL of 0.008M $K_3Fe(CN)_2$. The absorbance was read at 720 nm using a spectrophotometer (Jeway 6306 uv/vis) after 10 min. A standard curve was prepared using tannic acid as a standard. Polyphenol content was calculate as follows:

$$\text{Polyphenol \% (Tannic equivalent)} = \frac{C \times 56 \times 100}{60}$$

Where:
C: Concentration corresponding to optical density.
56: Volume of extract.
60: Weight of sample (mg).

Mineral extraction: About 5 gm were weighed in a dry crucible. The crucible was placed in muffle furnace for 3 h at 250°C. The contents were cooled and transferred to 250 mL beaker, 12 mL of 5N HCl and 3 mL of conc. HNO_3 were added. The beaker was placed in a sand bath to boil for 10 min. thereafter about 100 mL of distilled water were added and allowed to boil for another 10 min and then cooled. The contents were filtered through Whatman No. 41 filter paper and the volume was complete to 250 mL with distilled water. The extract was stored in bottles for individual mineral analysis [18]. From the extract, the elements Ca, Cu, Co, Fe, Mg, Mn and Zn contents were determined using Perkin Elmer Atomic Absorption.





Phosphorus determination: Phosphorus was determined by the vanadate-molydate- yellow method [18]. 5 mL of the aliquot extracted above was transferred into 50 mL volumetric flask, 10 mL ammonium molybdate-vanadate reagent (22.5 g $(NH_4)_6 MO_7O_{24} 4H_2O$ in 400 mL H_2O) + (1.5 g ammonium vanadate in 300 mL boiling distilled water) + 250 mL Conc. HNO_3 in one litre volume) was added to it and mixed again. After 30 min, the density of the colour was read at 450 nm using Jenway 6305 UV/Vis Spectrophotometer. Astandard curve was prepared to determine phosphorus concentration.

Potassium and sodium determination: An aliquot of the above extract was used to determine the content of K and Na according to the AOAC [19] using Coring 400 Flame Photometer. The extract was taken into a conical flask and diluted with distilled water (if necessary). The standard solution of KCl and NaCl were prepared by dissolving 2.54, 3.33 g of KCl and NaCl, respectively each in 1000 mL of distilled water. 10 mL of the solution were taken and diluted to one litre to give 10 ppm concentration. The flame photometer was adjusted to zero degree using distilled water as a blank and to 100 degree using standard solution.

Mineral K or Na% = $(FR \times DF \times 100)/(10^6 \times S \times 10)$

Where :
FR: Flame photometer reading
DF: Dilution factor
S: Sample weight

Minerals extractability: To 2.0 g of each sample 20 mL of 0.03N HCL was added. The mixture was incubated at 37°C with continuous shaking for 3 h. The mixture was filtered through Whatman No.1 filter paper. The filtrate was dried at 70°C over night and analysed for mineral content as described above [20].

$$\text{Mineral extractability (\%)} = \frac{\text{Mineral extractibility in 0.03 HCL}}{\text{Total mineral}} \times 100$$

Statistical analysis: Each sample was analysed in triplicate. The figures were then averaged. Data were assessed by analysis of variance (ANOVA) [21] and by Duncan's multiple range test with a probability $P \leq 0.01$ [22].

RESULTS AND DISCUSSION

Effect of cooking on antinutritional factors and content and availability of minerals: As shown in Table 1, phytic acid content of the cultivars was ranged from 969.3 to 1101 mg/100 g while polyphenols were ranged from 306.65 to 669.39 mg/100 g. Values obtained in this study for phytic acid and polyphenols were higher those reported by Khaterpaul and Chauhan [23] but comparable with those reported by Elhag *et al.* [10]. Variations in phytic acid and polyphenols contents among different cultivars can be attributed to both genetic and environmental conditions [11]. Cooking of pearl millet slightly reduced phytic acid content with a maximum reduction rate of about 10% for Dembi cultivar. This result agrees with the finding of high for Ashana cultivar. Ashana cultivar had a higher value of Mg compared to other cultivars with high availability recorded for GB-87-35 cultivar. Higher amount of Ca was observed for GB-87-35 cultivar as well as its availability. All cultivars were very rich in P content with cultivar Dembi contained higher amount. However, the availability of P for all cultivars was found to be less than 50% with a maximum value of 39% reported for the same cultivar. Most of the major minerals content and availability were significantly ($P \leq 0.01$) increased due to cooking process. Khaterpaul and Chauhan [23] reported similar results showing an increase in availability percentage of Ca and P when pearl millet was subjected to autoclaving. They attributed this increase in content and availability to the decrease in phytic acid content. Trace minerals content and availability varied between the cultivars and was found to follow similar trend of major ones before and after cooking (Table 3). Our findings supported the data obtained by Abdalla *et al* [24].

De-Boland *et al.* (1975) who reported that the rate of destruction of phytate was slow during heat processing and he observed that about 30 min autoclaving reduced phytic acid content of cereals and oil seed products by less than 10%. The total polyphenols content of the cultivars (Table 1) was found to be reduced by about 6-7%. Abdalla *et al.* [24] reported that domestic cooking during preparation of aseeda removed only about 8% of the polyphenols while Kataria *et al.* [25] showed about 9% reduction in polyphenols as a result of ordinary cooking of unsoaked seeds of mung bean. Table 2 shows the effect of cooking on major minerals of peal millet cultivars. Na and K contents varied between the cultivars with maximum values obtained for Dahabaya cultivar. However, the available Na and K were observed to be

Table 1: Effect of cooking on antinutritional factors content (mg/100 g) of pearl millet cultivars

		Phytic acid		Polyphenols	
Cultivars	Treatment	Total	% reduction	Total	% reduction
Ashana	Uncooked	969.30(±0.00)^a	-	306.65(±2.92)	-
	Cooked	907.08(±0.00)^b	6.42	288.71(±0.00)^b	5.85
Dahabaya	Uncooked	991.11(±0.00)^a	-	445.88(±2.94)^a	-
	Cooked	921.83(±9.36)^b	7.00	421.33(±2.96)^b	5.51
Dembi	Uncooked	1101.04(±0.00)^a	-	669.39(±0.00)^a	-
	Cooked	988.85(±0.00)^b	10.19	625.65(±2.93)^b	6.53
GB-87-35	Uncooked	1068.19(±0.00)^a	-	473.00(±2.93)^a	-
	Cooked	988.45(±16.34)^b	7.46	438.59(±0.00)^b	7.27

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 cooking on major mineral content (mg/100 g) and availability (%) of pearl millet cultivars

		Na		K		Mg		Ca		P	
Cultivars	Treatment	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Ashana	Uncooked	16.25(±0.00)^b	73.26(±0.00)^b	434.26(±0.00)^a	71.45(±1.99)^a	93.00(±0.00)^b	56.29(±0.84)^b	52.78(±0.48)^b	32.45(±0.68)^b	1106.77(±1.76)^b	35.41(±0.32)^b
	Cooked	23.33(±0.55)^a	81.26(±1.93)^a	448.65(±0.42)^a	74.30(±0.54)^a	97.14(±0.29)^a	59.45(±0.57)^a	55.29(±0.15)^a	39.55(±0.84)^a	1120.58(±0.00)^a	36.47(±0.00)^a
Dahabaya	Uncooked	19.36(±0.28)^b	63.84(±1.61)^a	452.53(±1.37)^b	69.50(±0.61)^b	75.39(±1.36)^b	59.83(±2.12)^b	54.45(±0.72)^b	32.59(±0.26)^a	1147.59(±2.17)^b	36.14(±0.31)^b
	Cooked	24.49(±1.76)^a	70.30(±4.02)^a	467.45(±4.65)^a	73.34(±0.00)^a	79.35(±0.64)^a	64.27(±1.60)^a	56.69(±0.18)^a	36.66(±0.26)^b	1179.77(±3.27)^a	37.26(±0.00)^a
Dembi	Uncooked	15.21(±0.55)^b	63.12(±4.11)^b	370.47(±0.00)^b	63.26(±0.38)^b	84.51(±0.94)^b	52.73(±0.80)^b	49.08(±0.00)^b	27.73(±10.61)^b	1290.35(±6.46)^b	39.60(±0.00)^b
	Cooked	22.83(±0.55)^a	70.87(±1.02)^a	382.91(±4.43)^a	66.80(±0.73)^a	89.67(±0.00)^a	63.50(±0.64)^a	51.82(±0.17)^a	33.44(±0.49)^a	1322.44(±5.78)^a	41.99(±0.00)^a
GB-87-35	Uncooked	12.94(±0.68)^b	66.22(±1.63)^b	420.82(±2.22)^b	64.85(±0.34)^b	76.29(±0.84)^b	61.14(±1.78)^b	58.78(±0.32)^b	35.06(±0.31)^b	1205.80(±1.96)^b	31.05(±0.32)^b
	Cooked	20.80(±1.15)^a	72.55(±0.00)^a	435.22(±2.88)^a	68.43(±0.80)^a	79.56(±0.39)^a	67.40(±0.00)^a	60.99(±0.00)^a	40.76(±0.86)^a	1238.23(±5.2)^a	33.57(±0.25)^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 3: Effect of cooking on trace mineral content(mg/100 g) and availability (%) of pearl millet cultivars

		Fe		Zn		Mn		Cu		Co	
Cultivars	Treatment	Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Ashana	Uncooked	10.70(±0.09)^b	26.67(±0.08)^b	1.79(±0.01)^b	43.33(±0.23)^b	1.32(±0.00)^b	48.13(±0.51)^b	0.62(±0.00)^a	25.05(±0.56)^b	0.063(±0.00)^a	87.49(±2.13)^a
	Cooked	11.55(±0.12)^a	28.97(±0.24)^a	1.84(±0.01)^a	46.03(±0.13)^a	1.36(±0.00)^a	49.95(±0.19)^a	0.63(±0.01)^a	27.19(±0.24)^a	0.063(±0.00)^a	86.10(±7.94)^a
Dahabaya	Uncooked	7.50(±0.06)^b	23.99(±0.37)^b	1.26(±0.00)^a	44.92(±0.52)^b	1.65(±0.003)^b	46.23(±0.31)^b	0.53(±0.002)^b	21.99(±0.40)^b	0.061(±0.002)^a	87.42(±0.28)^a
	Cooked	8.04(±0.06)^a	27.14(±0.00)^a	1.30(±0.03)^a	47.11(±0.39)^a	1.69(±0.003)^a	48.93(±0.14)^a	0.53(±0.002)^a	24.02(±0.12)^a	0.062(±0.002)^a	87.04(±0.50)^a
Dembi	Uncooked	10.91(±0.03)^b	25.04(±0.17)^b	1.67(±0.02)^b	43.33(±0.68)^b	1.64(±0.003)^b	44.32(±0.18)^b	0.96(±0.01)^a	21.79(±0.84)^b	0.061(±0.00)^a	86.30(±0.57)^a
	Cooked	11.78(±0.02)^a	27.61(±0.29)^a	1.71(±0.01)^a	45.95(±0.21)^a	1.69(±0.01)^a	47.43(±0.34)^a	0.96(±0.01)^a	23.68(±0.58)^a	0.062(±0.00)^a	86.24(±1.04)^a
GB-87-35	Uncooked	11.16(±0.04)^b	26.51(±0.12)^b	1,35(±0.00)^b	42.22(±0.00)^b	0.76(±0.01)^b	45.34(±0.20)^b	0.87(±0.02)^a	21.32(±0.52)^b	0.103(±0.004)^a	87.35(±0.44)^a
	Cooked	12.21(±0.09)^a	27.98(±0.38)^a	1.39(±0.02)^a	44.77(±0.57)^a	0.78(±0.01)^a	48.52(±0.31)^a	0.90(±0.00)^a	21.38(±0.26)^a	0.104(±0.001)^a	87.12(±1.46)^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 4: Effect of germination on antinutritional factors content (mg/100 g) of pearl millet cultivars

Cultivars	Germination time (days)	Phytic acid		Polyphenols	
		Total	% reduction	Total	% reduction
Ashana	0	969.30(±0.00)^{a}	-	306.65(±2.92)^{a}	-
	2	639.10(±0.00)^{b}	34.07	213.76(±0.00)^{b}	30.29
	4	341.74(±0.00)^{c}	64.74	188.26(±1.48)^{c}	38.61
	6	178.22(±19.77)^{d}	81.61	171.87(±2.05)^{d}	43.95
Dahabaya	0	991.11(±0.00)^{a}	-	445.88(±2.94)^{a}	-
	2	752.72(±0.00)^{b}	24.05	340.73(±0.00)^{b}	23.58
	4	527.83(±0.00)^{c}	46.74	309.92(±2.61)^{c}	30.49
	6	228.56(±0.00)^{d}	76.94	279.86(±2.97)^{d}	36.80
Dembi	0	1101.04(±0.00)^{a}	-	669.39(±0.00)^{a}	-
	2	824.06(±4.68)^{b}	25.16	490.99(±0.00)^{b}	26.65
	4	703.00(±0.00)^{c}	36.15	468.08(±2.98)^{c}	30.07
	6	370.90(±0.00)^{d}	66.31	439.80(±2.57)^{d}	34.29
GB-87-35	0	1068.19(±0.00)^{a}	-	473.00(±2.93)^{a}	-
	2	784.57(±9.32)^{b}	26.55	354.10(±2.55)^{b}	25.13
	4	521.32(±0.00)^{c}	51.20	323.80(±0.00)^{c}	31.54
	6	342.34(±16.31)^{d}	67.95	303.45(±0.00)^{d}	35.85

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 germination on major mineral content (mg/100 g) and mineral availability (%) of pearl millet cultivars

Cultivars	Germnation days	Na		K		Mg		Ca		P	
		Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Ashana	0	16.25(±0.00)^{a}	73.29(±0.00)c	434.41(±3.32)a	71.45(±1.99)d	93.00(±0.00)a	56.29(±0.84)^{a}	52.78(±0.48)^{a}	32.45(±0.68)^{a}	1106.77(±1.76)^{b}	35.41(±0.32)^{d}
	2	14.36(±0.00)^{d}	75.53(±1.69)^{c}	373.65(±4.09)^{d}	76.64(±0.52)^{c}	88.97(±0.16)^{d}	66.32(±0.38)^{c}	40.32(±0.60)^{b}	52.59(±0.31)^{c}	1008.72(±0.00)^{d}	42.70(±0.00)^{c}
	4	14.50(±0.00)^{c}	80.10(±1.95)^{b}	407.28(±0.36)^{c}	88.63(±0.00)^{b}	91.66(±0.10)^{c}	73.78(±0.00)^{b}	37.81(±0.71)^{c}	61.84(±0.21)^{b}	1089.79(±0.00)^{c}	52.18(±0.00)^{b}
	6	15.73(±0.00)^{b}	87.59(±2.00)^{a}	424.07(±2.08)^{b}	98.66(±2.42)^{a}	92.40(±0.34)^{b}	83.67(±0.28)^{a}	36.38(±0.34)^{d}	68.50(±0.42)^{a}	1116.78(±2.78)^{a}	67.34(±0.18)^{a}
Dahabaya	0	19.36(±0.28)^{a}	63.84(±1.61)^{d}	452.53(±1.37)^{a}	69.50(±0.61)^{d}	75.39(±1.36)^{e}	50.83(±2.12)^{d}	54.45(±0.73)^{a}	32.59(±0.26)^{d}	1147.59(±2.17)^{c}	36.14(±0.31)^{d}
	2	16.59(±0.00)^{d}	72.89(±1.21)^{c}	394.49(±3.46)^{d}	79.40(±1.31)^{c}	71.19(±0.34)^{c}	71.39(±0.49)^{c}	44.83(±0.40)^{b}	54.04(±0.62)^{c}	1041.03(±1.50)^{d}	44.37(±0.15)^{c}
	4	17.90(±0.00)^{c}	80.73(±0.00)^{b}	424.42(±0.96)^{c}	89.91(±0.95)^{b}	73.05(±0.10)^{d}	77.61(±1.25)^{b}	42.72(±0.51)^{c}	66.59(±0.69)^{b}	1192.81(±0.00)^{b}	56.38(±0.00)^{b}
	6	18.45(±0.00)^{b}	93.11(±6.56)^{a}	444.80(±1.13)^{b}	101.75(±1.15)^{a}	74.37(±0.44)^{de}	87.63(±0.00)^{a}	41.55(±0.00)^{d}	73.40(±0.51)^{a}	1280.35(±2.21)^{a}	68.95(±0.00)^{a}
Dembi	0	15.21(±0.55)^{a}	63.12(±4.11)^{d}	370.47(±0.00)^{a}	63.26(±0.38)^{d}	84.51(±0.94)^{a}	52.73(±0.80)^{d}	49.08(±0.00)^{a}	27.73(±0.61)^{d}	1290.35(±6.46)^{b}	39.60(±0.00)^{d}
	2	13.44(±0.00)^{c}	73.46(±0.57)^{c}	324.29(±3.03)^{d}	78.64(±0.93)^{c}	79.20(±0.40)^{d}	69.47(±0.36)^{c}	39.29(±0.10)^{b}	43.93(±1.48)^{c}	1189.05(±2.08)^{d}	47.40(±0.17)^{c}
	4	14.12(±0.00)^{b}	78.94(±0.20)^{b}	343.52(±1.97)^{c}	89.21(±1.30)^{b}	81.14(±0.00)^{c}	76.32(±0.51)^{b}	37.74(±0.00)^{c}	54.39(±0.32)^{b}	1270.41(±0.00)^{c}	57.96(±0.20)^{b}
	6	14.54(±0.20)^{b}	87.77(±1.01)^{a}	358.13(±4.14)^{b}	99.29(±1.11)^{a}	87.07(±0.44)^{b}	85.91(±0.29)^{a}	36.06(±0.00)^{d}	60.12(±0.69)^{a}	1318.72(±0.00)^{a}	70.10(±0.00)^{a}
GB-87-35	0	12.94(±0.68)^{a}	66.22(±1.63)^{d}	420.82(±2.22)^{a}	64.85(±0.34)^{d}	76.29(±0.84)^{a}	61.14(±1.78)^{b}	58.78(±0.32)^{a}	35.06(±0.31)^{d}	1205.80(±1.96)^{c}	31.05(±0.32)^{d}
	2	11.41(±0.05)^{c}	72.18(±0.73)^{c}	365.01(±2.43)^{c}	71.34(±0.00)^{c}	71.79(±0.16)^{d}	76.30(±0.00)^{c}	46.44(±0.47)^{b}	53.07(±0.56)^{c}	1092.05(±0.00)^{d}	39.36(±0.42)^{c}
	4	12.18(±0.02)^{b}	80.00(±0.00)^{b}	398.84(±2.21)^{b}	80.58(±1.15)^{b}	73.46(±0.46)^{c}	81.01(±0.58)^{b}	44.60(±0.33)^{c}	60.06(±0.50)^{b}	1210.19(±2.18)^{b}	49.96(±0.00)^{b}
	6	12.56(±0.06)^{ab}	90.24(±3.11)^{a}	414.37(±6.58)^{a}	92.20(±0.94)^{a}	75.10(±0.27)^{b}	87.50(±0.50)^{a}	41.98(±0.09)^{d}	67.76(±0.49)^{a}	1310.03(±3.29)^{a}	69.28(±0.46)^{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 6: Effect of germination on trace mineral content (mg/100 g) and mineral availability (%) of pearl millet cultivars

Cultivars	Germination days	Fe		Zn		Mn		Cu		Co	
		Total	Available	Total	Available	Total	Available	Total	Available	Total	Available
Ashana	0	10.70(±0.09)^{a}	26.67(±0.08)^{a}	1.78(±0.01)^{a}	43.33(±0.23)^{a}	1.32(±0.00)^{a}	48.13(±0.51)^{a}	0.62(±0.004)^{a}	25.05(±0.56)^{a}	0.063(±0.00)^{a}	87.49(±2.13)^{a}
	2	12.81(±0.02)^{c}	36.73(±0.37)^{c}	2.09(±0.02)^{c}	54.76(±0.12)^{c}	1.57(±0.002)^{c}	56.35(±0.23)^{c}	0.56(±0.01)^{b}	47.60(±0.48)^{c}	0.055(±0.00)^{b}	89.62(±0.89)^{a}
	4	13.34(±0.00)^{b}	46.08(±0.16)^{b}	2.17(±0.02)^{b}	60.78(±0.10)^{b}	1.74(±0.00)^{b}	56.66(±0.35)^{b}	0.55(±0.001)^{b}	68.06(±0.65)^{b}	0.045(±0.00)^{c}	80.67(±1.31)^{c}
	6	13.87(±0.01)^{a}	63.18(±0.10)^{a}	2.33(±0.02)^{a}	77.98(±0.00)^{a}	1.98(±0.001)^{a}	69.77(±0.56)^{a}	0.56(±0.004)^{b}	87.66(±0.28)^{a}	0.058(±0.00)^{ab}	97.12(±1.42)^{a}
Dahabaya	0	7.50(±0.06)^{d}	23.99(±0.37)^{d}	1.26(±0.00)^{d}	44.92(±0.52)^{d}	1.65(±0.001)^{d}	46.23(±0.31)^{d}	0.53(±0.002)^{c}	21.99(±0.40)^{d}	0.060(±0.00)^{a}	87.42(±0.28)^{a}
	2	8.90(±0.00)^{c}	32.55(±0.12)^{c}	1.46(±0.05)^{c}	53.81(±0.86)^{c}	1.97(±0.00)^{c}	53.75(±0.10)^{c}	0.48(±0.004)^{b}	43.48(±0.40)^{c}	0.054(±0.00)^{b}	89.71(±0.00)^{a}
	4	9.27(±0.02)^{b}	43.59(±0.23)^{b}	1.58(±0.02)^{b}	62.27(±0.23)^{b}	2.22(±0.004)^{b}	59.69(±0.00)^{b}	0.47(±0.004)^{c}	63.69(±0.00)^{b}	0.043(±0.00)^{c}	80.50(±1.50)^{b}
	6	9.80(±0.02)^{a}	55.92(±0.33)^{a}	1.67(±0.02)^{a}	87.59(±0.28)^{a}	2.44(±0.01)^{a}	74.19(±0.24)^{a}	0.47(±0.004)^{c}	79.98(±0.67)^{a}	0.055(±0.00)^{ab}	99.02(±1.34)^{a}
Dembi	0	10.91(±0.03)^{d}	25.04(±0.17)^{d}	1.67(±0.02)^{d}	43.33(±0.68)^{d}	1.64(±0.003)^{d}	44.32(±0.18)^{d}	0.96(±0.01)^{a}	21.79(±0.84)^{d}	0.061(±0.01)^{a}	86.30(±0.57)^{a}
	2	13.10(±0.001)^{c}	33.94(±0.06)^{c}	1.92(±0.04)^{c}	51.92(±0.27)^{c}	1.93(±0.01)^{c}	51.13(±0.24)^{c}	0.86(±0.00)^{b}	44.49(±0.16)^{c}	0.054(±0.00)^{b}	88.53(±0.47)^{a}
	4	13.43(±0.03)^{b}	42.02(±0.00)^{b}	2.06(±0.06)^{b}	63.93(±0.12)^{b}	2.20(±0.002)^{b}	58.86(±0.20)^{b}	0.85(±0.00)^{c}	65.45(±0.12)^{b}	0.047(±0.00)^{c}	79.77(±0.49)^{b}
	6	14.36(±0.02)^{a}	61.77(±0.05)^{a}	2.19(±0.02)^{a}	85.39(±0.75)^{a}	1.36(±0.01)^{a}	67.48(±0.78)^{a}	0.87(±0.004)^{b}	79.95(±0.12)^{a}	0.057(±0.01)^{b}	93.04(±1.51)^{a}
GB-87-35	0	11.16(±0.04)^{d}	26.51(±0.12)^{d}	1.35(±0.00)^{d}	42.22(±0.00)^{d}	0.76(±0.01)^{d}	45.34(±0.20)^{d}	0.87(±0.02)^{a}	21.32(±0.52)^{d}	0.103(±0. 04)^{a}	87.35(±0.44)^{a}
	2	13.29(±0.04)^{c}	31.76(±0.00)^{c}	1.59(±0.02)^{c}	53.26(±0.00)^{c}	0.89(±0.01)^{c}	52.36(±0.47)^{c}	0.79(±0.003)^{b}	42.60(±0.43)^{c}	0.090(±0.01)^{b}	89.79(±0.00)^{a}
	4	13.93(±0.01)^{b}	40.45(±0.19)^{b}	1.65(±0.02)^{b}	64.27(±0.23)^{b}	0.99(±0.00)^{b}	60.53(±0.69)^{b}	0.78(±0.01)^{b}	62.55(±0.17)^{b}	0.080(±0.00)^{c}	82.04(±0.57)^{a}
	6	14.43(±0.02)^{a}	53.66(±0.09)^{a}	1.78(±0.03)^{a}	81.29(±0.31)^{a}	1.09(±0.001)^{a}	73.75(±0.00)^{a}	0.80(±0.01)^{b}	79.75(±0.40)^{a}	0.095(±0.00)^{b}	98.80(±0.73)^{a}

Values are means (±SD). Means not sharing a common superscript letter in a column are significantly different at o≤01 as assessed by Duncan's multiple range tests

Effect of germination on anti-nutritional factors: Germination of four pearl millet cultivars resulted in a significant (P≤0.01) decrease in phytic acid content (Table 4). It was found that as the germination time increased, the rate of reduction in phytic acid also increased significantly (P≤0.01). Germination of seeds for 2 days reduced phytate by a range from 24% for

Dahabaya to 34% for Ashana. In the fourth day of germination the level of reduction was increased in the ranged from 36.1% for Dembi to 64% for Ashsna. Moreover, germination for 6 days progressively reduced phytate content in the range from 66% Dembi to 81% for the cultivars Ashana. The results obtained agree with the results reported by Kumar and Chauhan[9] and Kheterpaul and Chauhan [26]. Reduction in phytate content is likely due to the fact that, phytase is responsible for the complete hydrolysis of phytate and several seeds or grains are known to contain the enzyme. Phytase activity reportedly increased during germination of several seeds

[32]. Disappearance of phytate during germination depends on phytase activity that was found to be increased after 48 h germination of bush beans[32]. Polyphenol content of the cultivars was also decreased after germination of the seeds. However, the rate of reduction is lower compared to that of phytate even after germination for 6 days. Khetarpaul and Chauhan[26] reported insignificant reduction of polyphenols in pearl millet grains after germination for 24 h and suggested that germination is not effective method in lowering polyphenol content. The presence of the enzyme polyphenolic oxidase may account for the loss of polyphenols during germination of food legumes[25].

Effect of germination on major and trace minerals: Table 5 shows the effect of germination pearl millet cultivars seeds on major minerals content and availability. It was found that for all cultivars major minerals content decreased with the germination time. However, the availability of all major minerals was significantly ($P \leq 0.01$) increased with the germination time and reached 99.29% for K in Dembi cultivar. Results revealed that germination enhances utilization of major minerals by making them available. The decrease in minerals content may be attributed to leaching effect during soaking and rinsing as suggested by Lorenz[27]. Malleshi and Desikachar[28] found a decrease of 23 and 16% in Ca and P contents, respectively for pearl millet germinated for 48 h and similarly for foxtail millet. They also reported a continuous and gradual decrease in Ca and P contents of finger millet after germination for different time intervals and they stated that the reduction could be due to metabolic and leaching losses and transfer of nutrient to the growing embryo. Saharan *et al.*[29] observed that sprouting of high-yielding cultivars of rice and faba bean caused significant losses in the total Ca and P due to leaching of nutrients. The improvement of minerals availability may be due to reduction in antinutrients as a result of germination. Kumar and Chauhan[9] investigated the HCI-extractability of P during germination of grain pearl millet and reported a significant enhancement of P extractability. They also observed that the longer the germination periods resulted in higher P extractability. Moreover, they reported that divalent cations may be present as mineral-phytate chelates in ungerminated grains. Degradation of phytic acid during germination probably releases complexed divalent metal ions and increases their extractability. The effect of germination on trace minerals content and availability of pearl millet cultivars is shown in Table 6. Trace minerals content for all cultivars slightly increased (except Cu and Co) with germination time while the availability of such minerals was increased significantly ($P \leq 0.01$) with a maximum value of 99.02 for Co. It was observed that Co was the most available trace mineral for all cultivars because after germination of the cultivars seeds for 6 days its availability exceeded 90% for all cultivars. Obizoba and Ath[30] reported that sprouting caused greater increase in minerals content of pearl millet. The increases were controlled by the sprouting time.

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