

Effect of Malt Pretreatment Followed by Fermentation on Antinutritional Factors and HCl Extractability of Minerals of Pearl Millet Cultivars

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Abstract: Millet grains of cultivars Ashana and Dembi were germinated for 6 days to obtain 6 day-old millet malt. Millet malt was added at a concentration of 5% to the flour. The mixtures were fermented for 14 h. Phytic acid, polyphenols and minerals content and HCl extractability were assayed every 2 h for all treatments. The results revealed that phytate and polyphenols contents were significantly reduced with fermentation time with a concomitant decrease in the pH of the media. Further decrease was observed when the 14 h fermented sample was cooked. For both cultivars major minerals content and extractability were significantly ($p \leq 0.05$) improved as a result of malt pretreatment and further improvement was observed after cooking of 14 h fermented sample. Trace minerals of both cultivars were slightly increased in content and availability compared to major ones. Results revealed that addition of malt followed by fermentation greatly increased the availability (extractability) of minerals and in some cases it exceeded 100%.

Key words: Malt, phytate, minerals, polyphenols, millet, fermentation

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. 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 thin plates (kisra) from fermented or unfermented dough, and also brewed into local beer (maressa). 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^[1]. Phytic acid content pearl millet represents more than 70% of the total phosphorus of the grain^[2]. A value of 990 mg/100g of phytic acid was reported by Khetarpaul and Chauhan^[3], while Kumar and Chauhan^[4] gave a value of 825.7mg/100g. Elhag *et al.*^[5] reported values of 943 and 1076 mg/100g phytic acid for two Sudanese cultivars. Polyphenols have been considered as antinutrient because they interact with food constituents and make them unavailable. The grain is comparable to some other cereal grains in term of nutrient content^[6]. However, it contains some antinutrient factors (phytate and polyphenols) that affect the nutrient absorption by human body system^[7]. Reduction of these

antinutrient factors by malting have long been documented by other researchers^[3,8] but such information is still need more investigation. Khetarpaul and Chauhan^[3] 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 with a corresponding decrease in phytate phosphorus. HCl-extractability of calcium, copper, iron, zinc and manganese was improved significantly and the improvement was most pronounced at 30°C. In this study we would like to evaluate the effect of malt pretreatment followed by fermentation on antinutritional factors content and hydrochloric acid extractability of minerals of pearl millet cultivars.

MATERIALS AND METHODS

Source and germination of seeds: Seeds of millet cultivars Ashana and Dembi were obtained from ElObed Agricultural Corporation, Western Sudan. The seeds were carefully cleaned and freed from broken and extraneous matter. The seeds were germinated for 6 days to obtain 6 day old malt. After germination, the unmalted and malted grains were milled into fine flour to pass a 0.4 mm mesh size screen.

Addition and incubation of malt to millet flour: Six days-old millet malt was added to millet flour at 5% concentration in triplicate. The slurry was allowed to ferment using 5% starter. 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-microprocessor pH meter, HANA instruments). Thereafter the 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^[9]. 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^[10]. Calcium and Magnisum were determined by titration method that described by Chapman and Pratt^[9]. Sodium and potassium were determined by flame photometer (CORNIG EEL) according to AOAC^[11].

Hcl-extractability of Minerals (in vitro availability): Minerals in the samples were extracted by the method described by Chauhan and Mahjan^[12]. 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, vegetables 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^[13] using two grams of a dried sample. A standard curve was prepared expressing the results as Fe (NO₃)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^[14] 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 FeCl₃ 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 K₃Fe(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 FeCl₃ and 3.0 mL of 0.008 M K₃Fe(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 determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the Aanalysis of Variance (ANOVA)^[15]. Duncan's multiple range test was used to separate means. Significance was accepted at (p≤0.01)^[16].

RESULTS AND DISCUSSION

Effect of malt pretreatment of millet flour on phytate and polyphenol contents: Table 1 shows the effect of malt pretreatment on phytate and polyphenol contents during incubation of millet flour with millet malt at 5% concentration before and after fermentation. Phytic acid content of untreated millet flour was 969.30 and 1101.04 mg/100 g for cultivars Ashana and Dembi, respectively while polyphenol content was 306.65 and 669.39 mg/100g for the cultivars, respectively. Fermentation of the flour pretreated with 5% malt significantly (p≤0.01) reduced both phytate and polyphenols content with the fermentation time from 0 h to 14 h. It was observed that fermentation of malt pretreated flour for 14 h reduced more than 50% of the total phytate for both cultivars. However,

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

| | | Cultivars | | | | |
|-------------------|-----------|------------------------------|------------------------------|-----------|-------------------------------|------------------------------|
| | | Ashana | | Dembi | | |
| Ferment. 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 | 6.18 | 881.16 (± 0.00) ^b | 215.39 (± 1.00) ^b | 6.23 | 976.33 (± 9.26) ^b | 468.79 (± 2.83) ^f |
| 2 | 5.48 | 810.59 (± 0.00) ^c | 197.23 (± 2.90) ^c | 5.67 | 905.80 (± 0.07) ^c | 430.26 (± 0.00) ^j |
| 4 | 4.93 | 726.82 (± 9.06) ^d | 204.32 (± 3.05) ^b | 5.18 | 808.74 (± 4.58) ^d | 435.42 (± 0.00) ⁱ |
| 6 | 3.84 | 655.94 (± 0.00) ^e | 233.09 (± 2.96) ^f | 4.11 | 689.37 (± 0.30) ^e | 456.91 (± 2.92) ^h |
| 8 | 3.78 | 555.77 (± 9.25) ^f | 247.92 (± 0.00) ^d | 3.84 | 576.33 (± 0.50) ^f | 459.85 (± 0.00) ^g |
| 10 | 3.72 | 430.30 (± 0.00) ^g | 251.39 (± 0.00) ^e | 3.72 | 448.02 (± 9.13) ^g | 475.60 (± 1.44) ^e |
| 12 | 3.65 | 375.55 (± 9.16) ^h | 260.33 (± 0.00) ^b | 3.69 | 366.53 (± 9.07) ^h | 480.11 (± 1.64) ^d |
| 14 | 3.52 | 286.77 (± 0.00) ⁱ | 259.19 (± 1.89) ^b | 3.33 | 264.50 (± 9.16) ⁱ | 525.30 (± 1.75) ^b |
| 14, Cooked | 3.52 | 245.00 (± 3.36) ^j | 239.81 (± 2.84) ^e | 3.33 | 232.61 (± 0.00) ^j | 488.33 (± 1.23) ^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 Malt Pretreatment Followed by Fermentation on Total (Mg/100g) and Available (%) Major Minerals of Pearl Millet Cultivar, Ashana

| Ferment time (h) | pH | Na | | K | | Mg | | Ca | | P | |
|------------------|-----------|---------------------------------|--------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|----------------------------------|--------------------------------|
| | | 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) ^j | 84.51 (± 0.94) ^c | 59.73 (± 0.94) ^c | 49.08 (± 0.00) ^h | 27.73 (± 0.61) ⁱ | 1290.35 (± 6.46) ^j | 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 (± 0.01) ^{cb} | 61.54 (± 0.45) ^h | 49.09 (± 0.01) ^{gh} | 38.95 (± 0.18) ^h | 1300.32 (± 0.00) ^h | 42.56 (± 0.15) ^j |
| 2 | 5.48 | 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 (± 1.57) ^g | 46.37 (± 0.08) ^h |
| 4 | 4.93 | 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 (± 1.94) ^f | 49.31 (± 0.00) ^g |
| 6 | 3.84 | 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 (± 1.70) ^e | 61.56 (± 0.15) ^f |
| 8 | 3.78 | 24.26 (± 0.56) ^d | 84.35 (± 0.80) ^d | 391.45 (± 0.00) ^d | 102.96 (± 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 (± 0.00) ^d | 65.88 (± 0.15) ^e |
| 10 | 3.72 | 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 (± 1.2) ^d | 67.67 (± 0.00) ^d |
| 12 | 3.65 | 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 (± 1.69) ^c | 70.01 (± 0.00) ^c |
| 14 | 3.52 | 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 (± 0.00) ^b | 72.85 (± 0.14) ^b |
| 14, | 3.52 | 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 (± 1.44) ^a | 74.27 (± 0.00) ^a |

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

Table 3: Effect of Malt Pretreatment Followed by Fermentation on Total (Mg/100g) and Available (%) Major Minerals of Pearl Millet Cultivar, Dembi

| Ferment time (h) | pH | Na | | K | | Mg | | Ca | | P | |
|------------------|-----------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|--------------------------------|----------------------------------|--------------------------------|
| | | Total | Available | Total | Available | Total | Available | Total | Available | Total | Available |
| Untreated | Untreated | 15.21 (± 0.55) ^j | 63.12 (± 4.11) ^h | 370.47 (± 0.00) ^g | 73.26 (± 0.38) ^j | 84.51 (± 0.94) ^b | 52.73 (± 0.80) ^j | 49.08 (± 0.00) ^g | 27.73 (± 0.61) ⁱ | 1290.35 (± 6.46) ^g | 39.60 (± 0.00) ^j |
| 0 | 6.23 | 15.71 (± 0.35) ^j | 66.25 (± 0.61) ^g | 373.18 (± 1.69) ^g | 74.92 (± 0.55) ⁱ | 84.61 (± 0.02) ^b | 62.23 (± 0.10) ^j | 49.10 (± 0.01) ^{gf} | 45.99 (± 0.55) ^j | 1309.54 (± 1.37) ^f | 47.56 (± 0.26) ^j |
| 2 | 5.67 | 16.82 (± 0.15) ^h | 68.44 (± 2.11) ^g | 377.14 (± 0.00) ^f | 77.02 (± 0.56) ^h | 84.63 (± 0.02) ^b | 63.90 (± 0.04) ^h | 49.18 (± 0.03) ^{ef} | 47.74 (± 0.00) ^h | 1327.13 (± 1.96) ^e | 52.67 (± 0.15) ^h |
| 4 | 5.18 | 18.68 (± 0.67) ^g | 71.88 (± 0.51) ^f | 378.74 (± 3.56) ^e | 79.58 (± 0.64) ^g | 84.66 (± 0.03) ^b | 65.38 (± 0.09) ^g | 49.17 (± 0.02) ^e | 49.01 (± 0.23) ^g | 1337.94 (± 0.00) ^e | 56.75 (± 0.13) ^g |
| 6 | 4.11 | 26.06 (± 0.51) ^f | 86.98 (± 0.90) ^e | 390.05 (± 2.13) ^d | 96.63 (± 0.93) ^f | 85.25 (± 0.03) ^a | 77.52 (± 0.15) ^f | 49.39 (± 0.00) ^d | 56.23 (± 0.30) ^f | 1374.38 (± 3.10) ^d | 69.32 (± 0.00) ^f |
| 8 | 3.84 | 29.50 (± 0.00) ^e | 91.06 (± 0.00) ^d | 397.70 (± 1.23) ^c | 103.40 (± 0.00) ^e | 85.28 (± 0.00) ^a | 81.12 (± 0.14) ^e | 49.41 (± 0.02) ^d | 58.18 (± 0.00) ^e | 1383.45 (± 0.00) ^c | 74.97 (± 0.00) ^e |
| 10 | 3.72 | 31.52 (± 0.12) ^d | 94.87 (± 0.56) ^c | 403.31 (± 0.81) ^b | 110.04 (± 1.31) ^d | 85.30 (± 0.05) ^a | 83.18 (± 0.19) ^d | 49.41 (± 0.02) ^d | 59.63 (± 0.05) ^d | 1393.66 (± 3.34) ^c | 77.41 (± 0.00) ^d |
| 12 | 3.69 | 32.43 (± 0.11) ^c | 96.25 (± 0.00) ^c | 397.86 (± 2.76) ^c | 112.76 (± 0.43) ^c | 85.32 (± 0.06) ^a | 83.23 (± 0.08) ^c | 49.47 (± 0.04) ^c | 60.93 (± 0.19) ^c | 1416.58 (± 1.92) ^b | 80.13 (± 0.15) ^c |
| 14 | 3.33 | 33.28 (± 0.21) ^b | 101.93 (± 0.42) ^b | 405.31 (± 3.12) ^a | 118.03 (± 1.88) ^b | 85.40 (± 0.02) ^a | 84.70 (± 0.44) ^b | 49.53 (± 0.03) ^b | 65.62 (± 0.00) ^b | 1424.84 (± 0.00) ^b | 82.04 (± 0.00) ^b |
| 14, | 3.33 | 35.42 (± 0.00) ^a | 105.24 (± 1.62) ^a | 406.98 (± 2.17) ^a | 121.44 (± 0.00) ^a | 85.32 (± 0.24) ^a | 85.78 (± 0.62) ^a | 49.61 (± 0.02) ^a | 66.88 (± 0.18) ^a | 1441.19 (± 2.07) ^a | 84.18 (± 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

the rate of polyphenol reduction for both cultivars was lower than that of phytate. Valverde *et al.*^[17] reported that germination of lentils greatly reduced phytate content compared to soaking or cooking. The results indicate that phytic acid reduction is significantly affected by addition of millet malt possibly due to the presence of phytase present in the malt. The rate of reduction depends upon the age as well as the amount of millet malt. The pH of the mixture was dropped with fermentation time and reached 3.52 and 3.33 for Ashana and Dembi cultivars, respectively. Cooking of malted and 14 h fermented flour caused further reduction in phytate and polyphenol contents (Table 1). Similar results were reported when sorghum flour was treated with malt and incubated for

different time intervals^[17]. Results indicated that malt pretreatment, followed fermentation and cooking (Table 1) was observed to reduce phytate and polyphenol contents. The results indicated that the enzymes obtained during germination of the seeds are active even during certain period of cooking. The present finding disagreed with the Fretzdorff and Weiper^[19] finding in which they observed that, there was no reduction of phytate content when the whole rye or its flour were cooked at 100°C.

Effect of malt pretreatment followed by fermentation 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.

Table 4: Effect of Malt Pretreatment Followed by Fermentation on Total (Mg/100g) and Available (%) Trace Minerals of Pearl Millet Cultivar, Ashana

| Ferment. time (h) | pH | Fe | | Zn | | Mn | | Cu | | Co | |
|-------------------|-----------|-----------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|-----------------------|-------------------------|
| | | 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 | 6.18 | (± 0.09) ^j | (± 0.08) ^j | (± 0.01) ^e | (± 0.23) ⁱ | (± 0.00) ^g | (± 0.51) ⁱ | (± 0.04) ^j | (± 0.56) ^j | (± 0.03) ^d | (± 2.13) ^e |
| | | 11.04 | 29.38 | 1.80 | 45.19 | 1.33 | 50.55 | 0.63 | 27.72 | 0.064 | 87.33 |
| 2 | 5.48 | (± 0.01) ^h | (± 0.00) ^j | (± 0.01) ^d | (± 0.13) ^h | (± 0.00) ^g | (± 0.18) ^j | (± 0.01) ^h | (± 0.28) ^h | (± 0.0) nd | (± 0.19) ^e |
| | | 11.11 | 31.82 | 1.82 | 47.98 | 1.38 | 54.31 | 0.64 | 30.93 | 0.064 | 88.61 |
| 4 | 4.93 | (± 0.01) ^g | (± 0.06) ^h | (± 0.02) ^d | (± 0.00) ^g | (± 0.00) ^f | (± 0.20) ^h | (± 0.02) ^g | (± 0.00) ^g | (± 0.00) ^f | (± 0.76) ^e |
| | | 11.15 | 35.15 | 1.82 | 49.72 | 1.39 | 57.76 | 0.65 | 34.56 | 0.065 | 90.36 |
| 6 | 3.84 | (± 0.00) ^g | (± 0.08) ^g | (± 0.02) ^d | (± 0.15) ^f | (± 0.00) ^f | (± 0.00) ^g | (± 0.01) ^f | (± 0.34) ^f | (± 0.00) ^f | (± 0.75) ^d |
| | | 11.19 | 52.87 | 1.89 | 66.52 | 1.44 | 72.41 | 0.66 | 55.77 | 0.068 | 98.61 |
| 8 | 3.78 | (± 0.01) ^g | (± 0.02) ^f | (± 0.02) ^f | (± 0.46) ^e | (± 0.01) ^e | (± 0.27) ^f | (± 0.03) ^e | (± 0.58) ^e | (± 0.00) ^b | (± 0.19) ^e |
| | | 11.23 | 57.75 | 1.91 | 70.11 | 1.47 | 77.61 | 0.67 | 60.41 | 0.069 | 99.25 |
| 10 | 3.72 | (± 0.00) ^g | (± 0.00) ^e | (± 0.02) ^b | (± 0.88) ^d | (± 0.04) ^d | (± 0.35) ^e | (± 0.02) ^d | (± 0.28) ^d | (± 0.00) ^b | (± 0.40) ^{bc} |
| | | 11.28 | 60.03 | 1.94 | 73.88 | 1.48 | 79.73 | 0.67 | 63.35 | 0.069 | 99.95 |
| 12 | 3.65 | (± 0.00) ^d | (± 0.33) ^d | (± 0.01) ^{ab} | (± 0.55) ^e | (± 0.04) ^e | (± 0.00) ^d | (± 0.03) ^g | (± 0.49) ^e | (± 0.0) ^{ab} | (± 0.33) ^{abc} |
| | | 11.34 | 62.92 | 1.94 | 77.70 | 1.49 | 81.09 | 0.68 | 64.58 | 0.070 | 100.43 |
| 14 | 3.52 | (± 0.00) ^e | (± 0.18) ^e | (± 0.02) ^a | (± 0.00) ^b | (± 0.02) ^b | (± 0.53) ^e | (± 0.00) ^{bc} | (± 0.28) ^b | (± 0.0) ^{ab} | (± 0.40) ^{bc} |
| | | 11.51 | 66.18 | 1.92 | 77.26 | 1.49 | 82.33 | 0.68 | 64.69 | 0.071 | 101.12 |
| 14, Cooked | 3.52 | (± 0.02) ^b | (± 0.03) ^b | (± 0.02) ^{ab} | (± 0.29) ^b | (± 0.02) ^{ab} | (± 0.24) ^b | (± 0.02) ^b | (± 0.09) ^b | (± 0.00) ^a | (± 0.00) ^a |
| | | 11.75 | 67.65 | 1.94 | 79.14 | 1.50 | 83.73 | 0.68 | 65.87 | 0.071 | 101.39 |
| | | (± 0.00) ^a | (± 0.08) ^a | (± 0.00) ^a | (± 0.14) ^a | (± 0.00) ^a | (± 0.27) ^a | (± 0.02) ^a | (± 0.00) ^a | (± 0.00) ^a | (± 0.40) ^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 Malt Pretreatment Followed by Fermentation on Total (Mg/100g) and Available (%) Trace Minerals of Pearl Millet Cultivar, Dembi

| Ferment. time (h) | pH | Fe | | Zn | | Mn | | Cu | | Co | |
|-------------------|-----------|------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|-------------------------|-----------------------|------------------------|-----------------------|
| | | Total | Available | Total | Available | Total | Available | Total | Available | Total | Available |
| Untreated | Untreated | 10.91 | 25.04 | 1.67 | 43.33 | 1.64 | 44.32 | 0.96 | 21.79 | 0.061 | 86.30 |
| 0 | 6.23 | (± 0.03) ^j | (± 0.17) ^j | (± 0.02) ^h | (± 0.68) ^j | (± 0.00) ^j | (± 0.18) ^j | (± 0.01) ^h | (± 0.84) ^j | (± 0.002) ^d | (± 0.57) ^f |
| | | 11.26 | 28.57 | 1.69 | 45.43 | 1.65 | 48.16 | 0.96 | 25.28 | 0.062 | 87.38 |
| 2 | 5.67 | (± 0.004) ^j | (± 0.00) ^j | (± 0.01) ^{hg} | (± 0.24) ^j | (± 0.00) ^j | (± 0.14) ^j | (± 0.00) ^h | (± 0.26) ^j | (± 0.00) ^d | (± 0.25) ^e |
| | | 11.30 | 30.79 | 1.71 | 47.25 | 1.67 | 51.48 | 0.97 | 28.63 | 0.062 | 88.14 |
| 4 | 5.18 | (± 0.01) ^h | (± 0.08) ^h | (± 0.02) ^g | (± 0.16) ^h | (± 0.002) ^h | (± 0.25) ^h | (± 0.01) ^g | (± 0.16) ^h | (± 0.00) ^{cd} | (± 0.50) ^e |
| | | 11.37 | 34.08 | 1.73 | 50.00 | 1.67 | 54.26 | 0.98 | 31.35 | 0.063 | 88.96 |
| 6 | 4.11 | (± 0.00) ^g | (± 0.02) ^g | (± 0.01) ^f | (± 0.00) ^g | (± 0.00) ^g | (± 0.24) ^g | (± 0.002) ^f | (± 0.32) ^g | (± 0.00) ^f | (± 0.41) ^d |
| | | 11.47 | 48.06 | 1.79 | 68.12 | 1.71 | 70.49 | 1.01 | 54.64 | 0.067 | 97.88 |
| 8 | 3.84 | (± 0.01) ^f | (± 0.05) ^f | (± 0.02) ^e | (± 0.24) ^f | (± 0.00) ^f | (± 0.28) ^f | (± 0.001) ^e | (± 0.27) ^f | (± 0.00) ^b | (± 0.75) ^f |
| | | 11.53 | 54.01 | 1.82 | 72.76 | 1.73 | 74.56 | 1.02 | 58.44 | 0.067 | 99.40 |
| 10 | 3.72 | (± 0.01) ^e | (± 0.00) ^e | (± 0.02) ^e | (± 0.10) ^e | (± 0.00) ^e | (± 0.34) ^e | (± 0.002) ^d | (± 0.16) ^e | (± 0.00) ^b | (± 0.62) ^b |
| | | 11.59 | 65.42 | 1.83 | 74.53 | 1.74 | 76.51 | 1.02 | 64.36 | 0.068 | 100.33 |
| 12 | 3.69 | (± 0.01) ^d | (± 0.01) ^d | (± 0.01) ^{cd} | (± 0.39) ^d | (± 0.004) ^d | (± 0.26) ^d | (± 0.002) ^{cd} | (± 0.42) ^d | (± 0.00) ^b | (± 0.00) ^a |
| | | 11.62 | 57.69 | 1.85 | 76.23 | 1.76 | 78.54 | 1.02 | 66.36 | 0.068 | 100.80 |
| 14 | 3.33 | (± 0.00) ^e | (± 0.05) ^e | (± 0.02) ^{bc} | (± 0.00) ^e | (± 0.00) ^e | (± 0.20) ^e | (± 0.003) ^e | (± 0.21) ^e | (± 0.00) ^{ab} | (± 0.35) ^a |
| | | 11.67 | 60.99 | 1.86 | 78.48 | 1.77 | 80.47 | 1.03 | 68.48 | 0.070 | 100.92 |
| 14, Cooked | 3.33 | (± 0.01) ^b | (± 0.09) ^b | (± 0.02) ^{ab} | (± 0.59) ^b | (± 0.59) ^b | (± 0.42) ^b | (± 0.002) ^b | (± 0.26) ^b | (± 0.00) ^a | (± 0.25) ^a |
| | | 11.91 | 63.72 | 1.87 | 81.68 | 1.79 | 81.64 | 1.04 | 70.40 | 0.070 | 101.14 |
| | | (± 0.00) ^a | (± 0.08) ^a | (± 0.02) ^a | (± 0.38) ^a | (± 0.004) ^a | (± 0.32) ^a | (± 0.01) ^a | (± 0.18) ^a | (± 0.00) ^a | (± 0.28) ^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

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. Malt pretreatment of the flour followed by fermentation greatly affects both content and extractability of major minerals. Major minerals content increased significantly (p ≤ 0.01) when the pretreated flour was fermentation for 14 h with a higher increment observed for Na and P. 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 phytate P during fermentation as reported by Khetarpaul and Chauhan^[5]. 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 other minerals it was almost doubled after 14 h fermentation (Table 2). Further increment in major minerals content and extractability was observed when 14 h fermented pretreated flour was cooked (Table 2). Rakhi and Khetarpau^[21] 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^[21] 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 with a corresponding decrease in phytate 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. 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. Malt pretreatment followed by fermentation greatly affects both content and extractability of major minerals of the cultivar. Major minerals content increased significantly ($p \leq 0.01$) when pretreated flour was fermented for 14 h with a higher increment observed for P. The increment of P content is likely due to solubilization of phytate P due to addition of malt (phytase) and fermentation. 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. For Na and K, extractability exceeded 100% while for Ca and P it was almost doubled after 14h fermentation. 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^[19] and Khetarpaul and Chauhan^[21]. 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^[3]. The trace minerals content and extractability of Ashana and Dembi cultivars are shown in Tables 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.*^[21]. HCl-extractability of trace minerals of untreated grains revealed that Co was the most available mineral and Fe was the least available one. Malt pretreatment followed by fermentation greatly affects both content and extractability of trace minerals of the cultivar. 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 ($p \leq 0.01$)

increased when the pretreated flour was fermented for 14 h. For Fe and Cu, extractability was almost doubled after 14h fermentation while that of Co exceeded 100%. Further increment in trace minerals content and extractability was observed when pretreated and 14h-fermented flour was cooked (Table 4). Rakhi and Khetarpau^[19] 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^[3] 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 with a corresponding decrease in phytate 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. HCl-extractability of trace minerals of untreated grains revealed that Co was the most available mineral and Cu was the least available one. Addition of malt followed by fermentation greatly affects both content and extractability of the minerals. Trace minerals content increased gradually with the fermentation time from 0 to 14h. 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 ($> 100\%$) while for other minerals it was almost doubled after 14 h fermentation. 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^[3].

CONCLUSIONS

Utilization of millet malt to lower phytic acid and polyphenol contents and to improve the extractability of major and trace minerals is a promising and simple method. The rate of reduction of phytate and polyphenol contents with a concomitant increment of minerals availability depends on the age, incubation period and concentration of the malt. The addition of malt to millet flour followed by fermentation and cooking, could be a complete process for preparing fermented malt food products.

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