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Evaluation of anti-nutrient and mineral content in different pearl millet varieties

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Abstract

Pearl millet is one of the most popular crops in India. It is nutritionally comparable and even superior to major cereals with respect to protein, fat, energy, vitamins & minerals. The present investigation was carried out to evaluate the mineral content and the anti-nutrients present in pearl millet varieties i.e., white grain variety (ICMV-221), hybrids varieties (HHB-226, HHB-223 and HHB-197) and HC-20 variety. Calcium content was highest in HHB-226 and lowest in ICMV-221. No significant differences in calcium content of HHB-197 and HHB-223 was witnessed. Significant ($P < 0.05$) differences in iron content of different varieties of pearl millet were observed. ICMV-221 contained significantly ($P < 0.05$) higher zinc content than all the other varieties of pearl millet however, no significant difference in zinc content of HC-20 and HHB-223 as well as HHB-197 and HHB-226 were noticed. The phytic acid and polyphenol content of all pearl millet varieties varied from 620.00 to 875.00 and 304.17 to 438.33 mg/100g.

Keywords: Pearl millet, mineral content, anti-nutrients, phytic acid and polyphenols

Introduction

Changing life style, changes in meal pattern and awareness about health as well as healthy foods has increased the demand for consumption of the high content of minerals present in cereals. In this category, more emphasize is on Pearl millet [*Pennisetum glaucum* (L.)] which is the fourth most important grain crop next to rice, wheat and corn. Moreover, pearl millet is nutritionally comparable and even superior to major cereals with respect to protein, fat, energy, vitamins & minerals (Hadimani *et al.*, 1995; Abadalla *et al.*, 1998) ^[9, 3]. It also has better mineral profile because of higher level of calcium but owing to certain inherent factors, the availability of these mineral to human system is low (Kumar and Kapoor, 1984) ^[11].

Materials and Methods

Mineral content

Mineral content of the sample were determined by wet digestion method. One g of flour was weighed and dispersed in a 150 ml conical flask. 25-30 ml diacid mixture ($\text{HNO}_3:\text{HClO}_4$) in ratio 5:1 was added in flask and kept for overnight. Then the contents were digested by heating until clear white precipitates settled down at the bottom. The crystals left were dissolved by adding double distilled water. Then the contents were filtered through Whatman # 42 filter paper. The filtrate was made to 50 ml volume by using double distilled water and used for the determination of trace minerals viz. iron, zinc, magnesium using Atomic Absorption Spectrophotometer in Central laboratory, Haryana Agricultural University, Hisar.

Calcium

Calcium was estimated using standard method of AOAC (1995) ^[5].

Reagents

1. Ammonium oxalate: Saturated solution
2. Methyl red indicator: Dissolve 0.5 g methyl red in 100 ml of alcohol
3. Dilute acetic acid
4. Dilute sulphuric acid
5. Dilute ammonium hydroxide

- 0.1 N Potassium permanganate (KMnO₄)
- 0.1N Potassium permanganate: Dilute 10 ml of 0.1N Potassium permanganate solution to 100 ml with water (1 ml = 0.2 mg of Ca). Prepare fresh solution before use.

Procedure

Twenty ml of the digested mineral solution was taken in a 250 ml beaker. 25 ml of water was added to it. 10 ml of saturated ammonium oxalate solution and 2 drops of methyl red indicator was added to the ash solution. Solution was made alkaline by adding dilute ammonia and then slightly acidic with a few drops of acetic acid until the colour was faint pink (5.0 pH). The solution was heated to boiling and was allowed to stand at room temperature for overnight. The solution was then filtered through Whatmann No. 42 paper and washed with water, till the filtrate was oxalate free. The point of filter paper was broken with platinum wire or glass rod. Then the precipitates were washed with hot dilute sulphuric acid from wash bottle into beaker in which the calcium was precipitated and then with hot water and titrated hot (temperature 70-80 °C) with 0.01N Potassium permanganate (KMnO₄) till permanent pink color.

$$\text{Ca (mg/100g)} = \frac{X \times 0.2 \times V}{V_1 \times W} \times 100$$

Where

X = Titre value

V = Total volume of ash solution

V₁ = Volume taken for estimation

W = Weight of sample

Total dietary fibre

The total dietary fibre was determined by slight modification of an enzymatic method using AOAC method (1995) [5].

Reagents:

- 78% ethanol (207 ml of water was taken into one liter volumetric flask and 95% ethanol was added to make one liter.
- Phosphate buffer: 0.08 M, pH 6.0-6.5. 1.4 g of Na₂HPO₄ and 8.4 g of NaH₂PO₄ anhydrous was dissolved in one liter of water. The pH was checked and adjusted with NaOH. The buffer made was stored in tightly capped container at room temperature.
- 0.275 N NaOH. Diluted 275 ml of 0.1N NaOH solution to one liter with water in a volumetric flask and stored in tightly capped container at room temperature.
- 0.325 M HCl. Diluted 325 ml of 1.0N HCl solution to one liter with water in a volumetric flask and stored in tightly capped container at room temperature.
- Enzymes – α-amylase, protease and amyloglucoside obtained from Sigma Aldrich Inc. Pvt. Ltd.

Procedure

Sample preparation: one g sample (S) was weighed and defatted it in Soxhlet apparatus.

Extraction of water soluble material: The sample was dispersed in a 50 ml of pH 6.0 phosphate buffer and was kept for 40 min. at 60 °C to ensure interaction of pectin with minimum degradation.

Starch and protein hydrolysis: The pH of solution was adjusted to 6.0-6.5 for optimum amylase and protease

activity. The suspension was then cooled to 20-30 °C. Then add 0.10 ml α-amylase was added to the beaker and mixed well. The beaker was covered with aluminum foil and placed in a boiling water bath. Beaker was agitated gently at 5 minutes interval, temperature of the beaker reached at 95 °C. Solutions were allowed to cool at room temperature. The pH of the solution was adjusted to 7.5±0.2 by adding 10 ml of 0.275 N NaOH to beaker. The pH was checked and adjusted with NaOH. Solution of protease in phosphate buffer (5 mg/ml) was prepared fresh. 0.1 ml of 5 mg protease solution was added to beaker. The pH of the solution was adjusted between 4.0 and 4.6 by the addition of 10 ml of 0.325 M HCL to beaker, pH was checked and adjusted with NaOH, 0.1 ml of amyloglucoside was added to beaker and covered it with aluminum foil. Then place in 60 °C water bath, with continuous agitation; incubated for 30 minutes after the internal temperature of the beaker reached 60 °C. Four volumes of 95% ethanol added to beaker. The solution was kept overnight at room temperature for complete precipitation.

Filtration: After complete precipitation, the bed of celite in crucible was wetted and redistributed by using 78% ethanol. Gentle suction was maintained and quantitatively transferred the precipitate and suspension from beaker to the crucible. The residue was washed with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol and two 10 ml portions of acetone. The crucible containing residues was dried overnight in a 105°C air oven. The crucible was cooled in desiccators and the weight as “residue + celite + crucible weight” (R) was recorded. The crucible was transferred to muffle furnace and heated to 525 °C 5 hours. After complete ashing, crucible was cooled in desiccators. The weight was recorded i.e. weight of “ash + celite + crucible weight” (A).

Calculations

$$\text{TDF (\%)} = \frac{R - A}{S} \times 100$$

Anti-nutrients

Total phenolic content

Reagents

- Gallic acid
- Methanol
- Sodium carbonate (20% w/v)
- Folin-ciocalteu reagent

Procedure

Extraction of sample: one g sample was extracted separately with distilled water and 80% ethanol by refluxing for 1hour and then centrifugation was carried out at 2000 rpm for 30 minutes. After filtration, the residue was re-extracted twice under same conditions. The solvent was removed to get extractives. These extract were filtered and concentrated by keeping it in oven.

The total phenolics were determined by Folin-Ciocalteu reagent method using Gallic acid as standard for which a calibration curve was obtained with solutions 0.08, 0.04, 0.02, 0.01 and 0.05 mg/ml of Gallic acid. A 1.0 ml of diluted extract (all fraction were diluted with ethanol to adjust the absorbance with the calibration limits), 1.0ml of 1mol/L Folin-ciocalteu reagent and 2.0 ml of Na₂CO₃ (20% w/v) were mixed and the volume was made to 10 ml. After 8 minutes, the mixture was centrifuged at 600 rpm for 10

minutes. Then the absorbance of supernatant solution was measured at 730 nm using Spectronic 20 (Elico Ltd. Hyderabad) spectrophotometer and against a blank prepared similarly but containing distilled water instead of extract. The concentration of phenolics thus obtained was multiplied by the dilution factor and the results were expressed as the equivalent to milligrams of Gallic acid per gram of extract (mg GAE/g).

Phytic acid

Phytic acid was determined by the method of Haug and Lantzsch (1983) [10].

Reagents

1. Phytate reference solution: Dissolved 30.54 mg sodium phytate (5.5% water, 97% purity and containing 12 Na/mole) in 100 ml 0.2 N HCl, which gave a solution containing 200 µg phytic acid per ml.
2. Ferric ammonium sulphate solution: Dissolved 0.2 g ferric ammonium sulphate in 100 ml 0.2 N HCl and made to 1000 ml with water.
3. Bipyridine solution: Dissolved 10 g 2-2 bipyridine and 10 ml thioglycolic acid in water and made to 1000 ml.

Extraction

One g finely ground sample was extracted with 25 ml 0.2 N HCl for three h with continuous shaking in a shaker. After proper shaking, it was filtered through Whatmann No.1 filter paper. The volume was made to 25 ml with 0.2 N HCl.

Estimation

0.5 ml of the sample extract was pipetted into a test tube. One ml ferric ammonium sulphate solution was added. The tube was heated in a boiling water bath for 30 min. The contents of the tube were mixed and centrifuged for 30 min. at 3000 rpm. One ml supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. The absorbance at 519 nm against distilled water was measured. For plotting a standard curve different concentrations i.e., 0.2-1.0 ml of standard sodium phytate solution containing 40-200 µg phytic acid were taken and made to 1.4 ml with water.

$$\text{Phytic acid (mg/100g)} = \frac{M \times V \times 100}{W \times V_1 \times 1000}$$

Where

M = concentration of extract obtained from graph

V = volume made of extract (ml)

W = weight of the sample

V₁ = volume of extract aliquot taken (ml)

0.5 OD corresponded to 120 µg phytic acid.

Results and discussion:

Calcium, iron, zinc and copper content of different varieties ranged from 61.17-65.67, 6.20-10.74, 5.75-7.13 and 3.83-5.89 mg/100 g, respectively (Table 1). Calcium content was highest in HHB-226 and lowest in ICMV-221. No significant differences in calcium content of HHB-197 and HHB-223 was witnessed. HC-20 contained highest iron content (10.74 mg/100g), followed by ICMV-221(9.72 mg/100g), HHB-197 (8.76 mg/100g), HHB-223 (6.83 mg/100g) and HHB-226 (6.20 mg/100g). Significant (P<0.05) differences in iron content of different varieties of pearl millet were observed. ICMV-221 contained significantly (P<0.05) higher zinc

content than all the other varieties of pearl millet however, no significant difference in zinc content of HC-20 and HHB-223 as well as HHB-197 and HHB-226 were noticed. HC-20 contained significantly (P<0.05) high copper content, whereas HHB-223 contained lowest copper content. Significant (P<0.05) varietal influence with respect to copper content was observed in all varieties (Table 1). The results indicate that HC-20 contained highest amount of iron and copper content, whereas ICMV-221 contained highest zinc content and HHB-226 contained highest zinc and calcium content, respectively. The total dietary fibre content of ICMV-221, HC-20, HHB-197, HHB-226 and HHB-223 were 19.66, 18.65, 17.55, 17.88 and 17.61g/100g, respectively (Table 1). ICMV-221 contained significantly (P<0.05) high total dietary fibre content than HHB-197, HHB-226 and HHB-223. However, no significant differences in total dietary fibre content of HHB-197, HHB-226 and HHB-223 was observed. Calcium content in ICMV-221, HC-20, HHB-197, HHB-226 and HHB-223 varieties of pearl millet was 61.17, 64.17, 62, 65.67 and 62.17 mg/100g, respectively (Table 1). Varietal influence in respect to calcium content was observed in all varieties. Malik (1999) [12] and Poonam (2002) [15] reported a lower range of calcium in pearl millet i.e. 19.9 to 22.5 and 15.9 mg/100g, respectively. Similarly, Abdalla *et al.* (2010) [1] observed lower values of calcium (16.08-16.09 mg/100g) content in pearl millet varieties. However, similar range of calcium content (46.0- 68.0 mg/100g and 61.42-66.16 mg/100g) in different pearl millet varieties has been observed by Anju (2005) and Chaudhary (2011) [7], respectively. The mean values of iron content in pearl millet cultivars ranged from 6.20 to 10.74 mg/100g (Table 1). Significantly (P<0.05) higher (10.74 mg/100g) and lower (6.20 mg/100g) values of iron content was found in HC-20 and HHB-226, respectively whereas, iron content of ICMV-221 and HHB-197 was 9.72 and 8.76 mg/100 g. The results of the present study are in close consent with the results of Anju (2005) as iron content of pearl millet varieties ranged from 9.62-14.60 mg/100g. Poonam (2002) [15] and Abdalla *et al.* (2009) [2] on the contrary, gave very low values of iron content in pearl millet varieties i.e. 2.13 and 2.4-2.5 mg/100g. Abdalla *et al.* (2010) [1] and Chaudhary (2011) [7] reported higher iron content in pearl millet varieties which ranged from 17.88-18.65 and 10.30-11.49 mg/100g, respectively. Nambiar *et al.* (2011) [13] also reported 8.0 mg/100g iron in pearl millet flour.

Zinc content of the pearl millet varieties ranged from 5.62 to 7.13 mg/100g. Highest (7.13 mg/100g) and lowest (5.62 mg/100g) values for zinc content was observed in ICMV-221 and HHB-226, respectively. No significant differences were observed in zinc content of HC-20 (6.27 mg/100 g) and HHB-223 (6.21 mg/100 g). These results are comparable to the reported value as 4.23-7.38 mg/100g and 4.12-5.20 mg/100g zinc content by Anju (2005) and Nithya *et al.* (2006) [14]. On the contrary, lower value of 1.80 mg/100g for zinc content has been reported by Sharma (1994) [16] and Kumar & Kapoor (1984) [11] whereas, Abdalla *et al.* (2010) [1] observed higher zinc content (6.7-7.29 mg/100g) of pearl millet varieties. Nambiar *et al.* (2011) [13] and Chaudhary (2011) [7] also reported lower value of zinc (3.1 mg/100g and 4.47-5.29 mg/100g, respectively) content in pearl millet flour than the present investigation. Copper content of all pearl millet varieties ranged from 3.83 to 5.89 mg/100g. Highest (5.89 mg/100g) and lowest (3.83 mg/100g) values for copper content was observed in HC-20 and HHB-223, respectively. The total dietary fibre content of ICMV-221, HC-20, HHB-197, HHB-226 and HHB-223 were 19.66, 18.65, 17.55, 17.88

and 17.61 g/100g, respectively. ICMV-221 contained higher total dietary fibre content than HHB-197, HHB-226 and

HHB-223. Godara (2013) [8] reported lower value for total dietary fibre (16.4 g/100g) content in pearl millet.

Table 1: Mineral content and TDF (total dietary fiber) in different varieties of pearl millet (per 100g on dry basis)

Varieties	Calcium (mg)	Iron (mg)	Zn (mg)	Cu (mg)	TDF (g)
ICMV-221	61.17±0.29	9.72±0.04	7.13±0.22	5.22±0.09	19.66±0.74
HC-20	64.17±0.29	10.74±0.04	6.27±0.02	5.89±0.04	18.65±0.23
HHB-197	62.00±0.50	8.76±0.06	5.75±0.03	4.83±0.02	17.55±0.29
HHB-226	65.67±0.58	6.20±0.07	5.62±0.03	4.10±0.04	17.88±0.08
HHB-223	62.17±0.29	6.83±0.10	6.21±0.03	3.83±0.15	17.61±0.09
CD at 5% level	0.75	0.19	0.18	0.15	0.68

Values are mean± S.D of three replicates.

The phytic acid and polyphenol content of all pearl millet varieties varied from 620.00 to 875.00 and 304.17 to 438.33 mg/100g (Table 2). HHB-223 contained highest phytic acid content followed by HHB-197, HC-20, HHB-226 and ICMV-221. Significant ($P < 0.05$) differences were observed in phytic acid content of these varieties. HC-20 contained significantly ($P < 0.05$) higher polyphenol content than ICMV-221, HHB-197, HHB-226 and HHB-223 (Table 2). Significant differences in polyphenol content in the pearl millet varieties were observed except HC-20 and HHB-197. Result indicates that HHB-223 contained highest phytic acid content and lowest polyphenol content. The phytic acid content of pearl millet ranged from 620 to 875 mg/100g (Table 1); it was lowest in ICMV-221 whereas, HHB-223 contained highest phytic acid content (875 mg/100 g). Malik (1999) [12] and Poonam (2002) [15] also reported similar range of phytic acid (620.3 to 724.5 and 710.15 mg/100g, respectively), whereas 603.33 to 678.33 mg/100g of phytic acid was also observed in twelve varieties of pearl millet by Anju (2005). Lowest phytic acid (620 mg/100g) content was found in white grain variety (ICMV-221) whereas HHB-223 contained highest phytic acid (875 mg/100 g). Polyphenol content of pearl millet varieties ranged from 304.17 to 438.33 mg/100g. Highest polyphenol content (438.33 mg/100g) was observed in HC-20 followed by HHB-197 (433.33 mg/100 g), ICMV-221 (405.67 mg/100 g), HHB-226 (365.67 mg/100 g) and HHB-223 (304.17 mg/100g). Variation in polyphenol content has been reported (608-788 mg/100g, 741.29 to 767.54 mg/100g and 502.78 to 658.30 mg/100g) by Sharma and Kapoor (1996) [16], Archana *et al.* (2000) [6] and Anju (2005), respectively.

Table 2: Phytic acid and polyphenol in different varieties of pearl millet (mg per 100g on DM basis)

Varieties	Phytic acid	Polyphenol
ICMV-221	620.00±5.00	405.67±4.51
HC-20	817.67±2.52	438.33±5.20
HHB-197	857.33±4.62	433.33±3.82
HHB-226	730.00±0.00	365.67±1.15
HHB-223	875.00±5.00	304.17±2.93
CD at 5% level	7.26	6.99

Values are mean± S.D of three replicates

Lowest polyphenol (304.17 mg/100g) content was observed in HHB-223 variety of pearl millet whereas highest polyphenol (438.33 mg/100 g) content was noticed in HC-20.

Conclusion

The study showed that calcium content was highest in HHB-226 and lowest in ICMV-221. HC-20 contained highest iron content followed by ICMV-221, HHB-197, HHB-223 and HHB-226. Highest zinc content was found in white grain

variety (ICMV-221) whereas lowest in HHB-226 variety of pearl millet. Lowest phytic acid content was found in white grain variety whereas HHB-223 contained highest phytic acid. However, lowest polyphenol content was in HHB-223 variety of pearl millet whereas highest polyphenol content was noticed in HC-20.

Thus, all above mentioned pearl millet varieties can be utilized for processing and to prepare iron, calcium rich healthy foods and also help in diversifying its use for achieving food and nutritional security.

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