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Nutritional and Anti-nutritional Evaluation of Pearl Millet (*Pennisetum glaucan*) Influenced by Germination and Popping

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Abstract

Pearl mill*et al*so known as "bajra" is negligible, low demanded by people for food. It has high level of calcium, iron, zinc, lipids, and amino acids, antioxidants but also contain some anti-nutrients like tannin, phytic acid, trypsin inhibitor etc. Processing methods are required to improve the nutritional, sensory value and availability of macro and micronutrients and reduce the anti-nutrients. Effect of germination and popping on pearl millet were studied and compare with WRPMF (whole raw pearl millet flour). On germination protein content 24% increased significantly and decreased during popping of pearl millet insignificantly. The crude fiber content of germinated ranged from 11-12% was higher and 17-18% higher in popping of pearl millet significantly. A significant effect on tannin, phytic acid, oxalic acid and trypsin inhibitor activity was fond during germination and popping. By 48 hours of germination 28% tannin and 42% phytic acid were decreased in pearl millet. Result indicate that the processing (germination and popping) improve the quality of pearl millet and make it as important food.

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Introduction

Nutritional wellness is a prime aspect of healthy and maximum development of human genetic potential. Malnutrition is considered as a big hindrance in national development. For the sake of overcoming the intense problem of food insecurity and malnutrition, the quality of diet should be given significant weightage. Increase in yields of the food and its diversification should be encouraged at both household and national level. Especially, for improving the household food security, cultivation of traditional food crops suitable for the area shall prove to be a successful approach. Mill*et al*so has potential for using as human food and beverages. India is considered as hub of minor crops like millets. The nutritive value of millet is similar to cereals but their utilization is limited due to the presence of the anti-nutrients, poor digestibility of protein and low palatability. Commercial processing of these locally grown grains into valuable foods and beverages can be an important driver for economic development in developing countries.

Pearl millet (Pennisetum glaucum L.), also known as "bajra" is negligible, low demanded by people for food. It has high level of calcium, iron, zinc, lipids, and amino acids (Sade, 2009) such as lysine, tryptophan, threonine and fatty acid like omega-9, omega-6 and omega-3 fatty acid ratio. The phytochemicals like tannins, phytates (Onyango *et al*., 2013) act as antioxidant properties. It

has low glycemic index, small amount of flavonoids are present and it is gluten free millet. Pearl millet contains phospholipids which are useful in brain functions, behavioural disorder and stress. It may have therapeutic effects in some health problems like anaemia, constipation, diarrhoea, diabetes, CVD, celiac diseases, cancer and it is referred to as anti-inflammatory and it also acts as probiotic food (Vanisha *et al*., 2011).

The low bioavailability of minerals (such as iron, zinc etc.) in cereal-based food products is a remarkable problem for children in developing countries. Thompson, (1993) described that millet contains tannins (0.61), phytates (0.48%), polyphenols, trypsin inhibitors and dietary fiber which considered as "anti-nutrients" acting on mineral bioavailability. because of their metal chelating and enzyme inhibitor activities which termed as neutraceuticals.

These anti-nutrients in food can be reduced by food processing techniques such as decortications, malting, germination, fermentation, roasting, popping etc. which may also improve mineral content and bioavailability (Sade, 2009). Several studies indicated that the decrease levels of anti-nutrients during soaking, germination and popping might be responsible for the improved starch digestibility due to the swelling and rupturing of starch granules as well as the activation of amylase and phosphorylase (Kaur & Kapoor, 1990).

Germination improved the in vitro protein and starch digestibility in pearl millet. It also led to the reduction of anti-nutrients such as phytic acid, tannins, and polyphenols, which form complexes with protein (Hassan *et al*., 2006). Krishnan *et al*., (2012) founded that the in vitro extractability and bio-accessibility of minerals (such as calcium, iron and zinc) were increased and anti-nutritional factor (such as phytic acid, oxalic acid) was decreased in pearl millet germination process. Pearl millet has higher beta-amylase activity and higher free alpha-amino nitrogen in comparison to sorghum after malting (Pelembe *et al*., 2004).

Popping is one of the processing techniques which uses sand as heat transfer media with HTST (high-temperature short time) method resulting starch gelatinization and the endosperm bursts open giving highly desirable flavor and aroma. It is used as ready-to-eat food (Shobana *et al*., 2013) at commercial scale thus promoting utilization of millet grains (Saleh *et al*., 2013). Popping reduced anti-nutritional factors results increased in vitro protein digestibility reported by Sankara & Deosthale, 1983).

Materials and Methods

Preparation of processed forms

Whole raw pearl millet flour (WRPMF)

Also, pearl millet seeds were cleaned, free from dirt and foreign matter. Then, they were sundried and ground in a mixer into fine flour and stored in another container.

Germinated pearl millet flour (GPMF)

One portion of pearl millet seeds was soaked overnight. Next day, after draining of water, seeds were wrapped in a muslin cloth and hung in a humid atmosphere. This germination process was conducted for 48 hours and seeds were sun dried properly and grounded into powder form and kept for analysis.

Popped pearl millet flour (PPMF)

Another portion of pearl millet seeds were popped and cooled down. After grounded into powdered form, this powder was kept for analysis.

Analysis of chemical composition, various versions of samples were prepared.

Proximate Analysis

The samples were analysed for moisture according to A.O.A.C. (1980) method. The ash, fat, crude fiber, protein, total carbohydrate and iron examined which given by Raghuramulu *et al*., (2003). Mineral content like calcium and phosphorous analysed according to Sharma (2007).

Antinutrient Analysis

Tannins (Price *et al*., 1978)

Tannin content of sample is estimated using modified Vanillin-HCL in methanol. The vanillin reagent reacts with any phenol that has a phloroglucinol nucleus and produces a colored product, which is measured at 500 nm in a spectrophotometer.

1 gm of sample was extracted with 10 ml of 1% HCL in methanol for 24 hours at room temperature then

centrifuged at 5000rpm. Mixture of equal volumes of 8% HCL in methanol with 2% vanillin in methanol was used to prepare Vanillin HCL reagent. 1 ml of supernatant was mixed with 5 ml of vanillin HCL reagent into a test tube. Catechin standard was carried out with each run of sample. The absorbance was read using a colorimeter at 500 nm after 20 minutes incubation at room temperature. A standard curve was prepared expressing the result as catechin equivalents, i.e. amount of catechin (mg per ml) which gives color intensity equivalent to that given by tannin after correcting for blank.

$$\text{Tannins (mg/100)} = \frac{\text{Optical density of test X conc. of standard X 1000}}{\text{Optical density of standard X volume of extract X weight of sample}}$$

Phytic acid (Davies and Reid, 1979)

This method is based on the determination of pink color complex precipitate as ferric ions complexed with phytate at pH1-2 can't react with thiocyanate ion and the phytate phosphorus content calculated from this value assuming a constant 4 Fe: 6P molecular ratios in the precipitate.

Grounded 1 g of sample and extracted with 3% TCA by shaking, filtered and made up to suitable volume with water. To 1.4 ml of the filtrate, added 1 ml of ferric ammonium sulphate solution (21.6 mg in 100 ml water), mixed and placed in a boiling water bath for 20 min. Cooled the contents and added 5 ml of isoamyl alcohol and mixed. To this, the color 0.1 ml ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 min. The alcoholic layer was separated and the color intensity was read at 465 nm against amyl alcohol blank after 15 min. Standard $Fe(NO_3)_3$ was run along with the sample.

Calculation: Graph of standard was plotted and results were expressed as mg phytic acid/100 g dry wt.

Oxalic acid (Raghuramulu *et al*., 1983)

Oxalic acid is precipitated as oxalate and is titrated with standard $KMnO_4$.

0.5 – 1.0 g of sample was extracted with 10 times volume of distilled water. Extracted sample was centrifuged at 10,000 rpm for 20 minutes. 10 ml of supernatant was taken in conical flask and titrated against 0.01 N $KMnO_4$ till the solution turned faint pink color along with blank.

Calculation

$$\text{Oxalic acid (mg/100ml)} = \frac{S - B}{X} \text{ X } 0.45 \text{ X } 100$$

S = volume (ml) $KMnO_4$ used for sample titration; B = volume (ml) $KMnO_4$ used for a blank titration; X = volume (ml) of a aliquot of sample.

Trypsin inhibitor (Kakade *et al*., 1969)

Trypsin enzymatic activity is assayed using casein as substrate. Inhibition of this activity is measured in the extract.

0.2 to 1.0 ml aliquot, trypsin solution (0.05 mg/ml in 0.001M HCl) were pipette into separate triplicate set of test tubes and final volume adjusted to 1 ml and 2 ml with phosphotate buffer (0.1 M, Ph 7.6) for aliquot and trypsin solution respectively. 1 ml of trypsin solution, added to aliquot tubes and all the tubes kept in water bath at 37^o C. Added 6 ml of 5% TCA in one of the triplicate tubes of aliquot and trypsin solution, marked as blank. In others, added 2 ml of 2% casein solution and then kept at 37^o C for exactly 2o minutes. Then, added 6 ml of 5% TCA and measured absorbance at 280 nm after 1 hours against blank using a UV visible Elico spectrophotometer.

Calculation

Absorbance was plotted against the volume of extract. One trypsin unit (TU) is defines as an increase of 0.01 absorbance units at 20 minutes per 10 ml of the reaction mixture. Trypsin inhibitor is defined as the number of trypsin units inhibited (TIU).

Result and Discussion

Changes in nutrients during germination and popping

Proximate analysis of all versions of pearl millet was done and result shown in Fig. 1. It was found that the moisture content for WRPMF, GPMF and PPMF were 12.4±0.01, 15±0.05, 11.28±0.15 (g/100g) respectively. Significant difference was found in moisture content of

GPMF and PPMF when compared with WRPMF. It showed that during popping moisture content was decreased significantly but vice-versa was seen during germination.

Total ash content for WRPMF, GPMF and PPMF were 2.3±0.10, 2.2±0.10 and 2.5±0.05. After processing, no significant difference was found in ash content for all versions (GPMF and PPMF) when compared with WRPMF.

Fat content for all versions were WRPMF; 5.0±0.10, GPMF; 5.2±0.15, PPMF; 4.5±0.20 (g/100g). It was found that fat content in germination increased while decreased during popping significantly.

Pearl millet has reasonable crude fiber helps to make it low energy, mark able protein food stuff rich in fiber. For WRPMF, GPMF and PPMF, the crude fiber contents were 18.1±0.50, 20.2±1.0, 14.9±1.5 (g/100g). The crude fiber content increased significantly in germinated sample and decreased significantly in popped sample.

Millets are good source of protein and in the same line protein content was 11.1±1.5 g/100g in WRPMF, 13.8±0.96 g/100g in GPMF, 11.0±0.05 g/100g in PPMF. It was seen that significant increase was found in protein content of germinated samples and decrease in popping process.

Table.1 Nutrients analysis of pearl millet (g/100g)

	Moisture	Total ash	Crude fiber	Fat	Protein	Carbohydrate
WRPMF	12.4±0.01	2.3±0.10	18.1±0.50	5.0±0.10	11.1±1.5	67.2±1.9
GPMF	15±0.05	2.2±0.10	20.2±1.0	5.2±0.15	13.8±0.96	64.4±1.51
PPMF	11.28±0.15	2.5±0.05	14.9±1.5	4.5±0.20	11.0±0.05	70.1±1.0

Table.2 Anti-nutrients analysis of pearl millet

versions	Tannin (mg/100g)	Phytic acid (mg/100g)	Oxalic acid (mg/100g)	trypsin inhibitor activity (U/g)
WRPMF	217.2±1.03	858.6±2.6	21.2±1.05	7899.9±10.6
GPMF	148.9±2.04	497.4±1.54	15.1±3.9	5877.5±11.2
PPMF	108.5±2.1	689.76±1.80	17.2±2.1	7457.4±9.5

Fig.1 Effect of processing on nutrients of pearl millet (g/100g). WRPMF- whole raw pearl millet flour, GPMF- germinated pear millet flour, PPMF- popped pearl millet flour

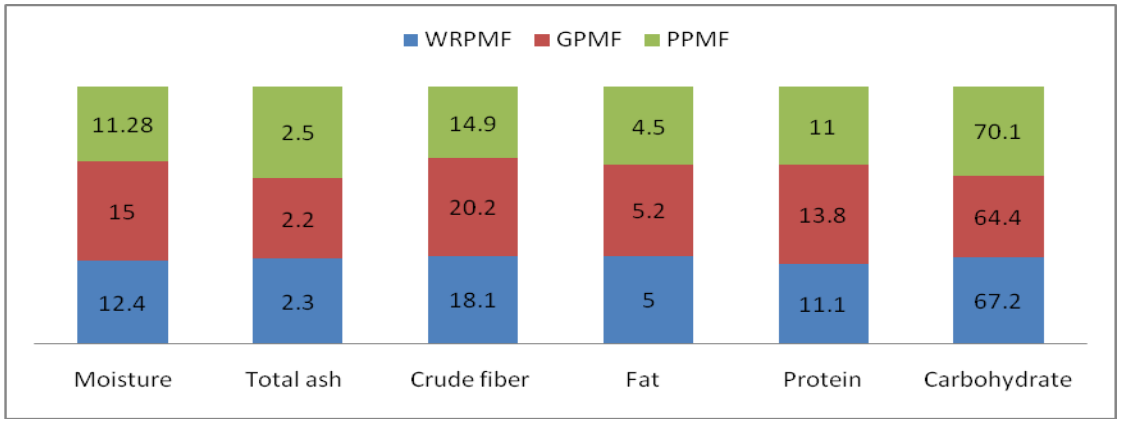


Fig.2 Effect of processing on anti-nutrients of pearl millet (mg/100g). WRPMF- whole raw pearl millet flour, GPMF- germinated pear millet flour, PPMF- popped pearl millet flour

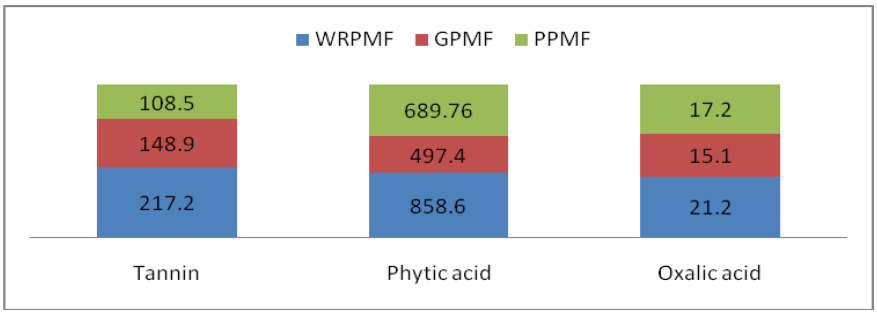
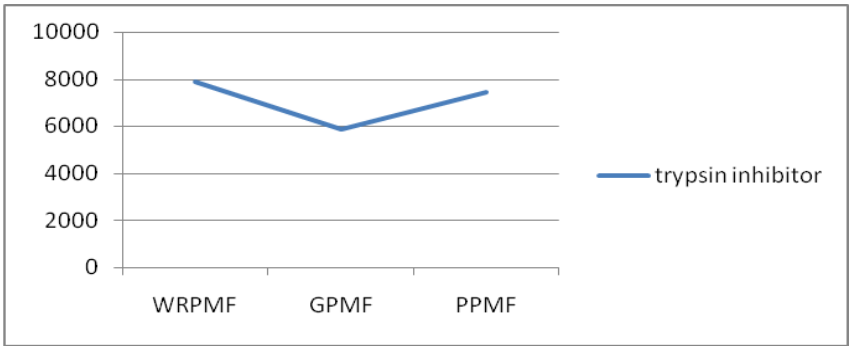


Fig.3 Effect on trypsin inhibitor activity (U/g) after germination (48 hours) and popping of pearl millet



Carbohydrate content for all samples (WRPMF, GPMF and PPMF) was 67.2±1.9, 64.4±1.51 and 70.1±1.0 (g/100g). It registered a significant decrease in germinated and an equal significant increase in popped forms respectively.

Mineral analysis

The calcium content of WRPMF, GPMF and PPMF were found as 41±0.57, 49±2.9 and 32±1.34 (mg/100g) respectively. Mineral analysis of processed forms of all versions revealed that calcium content increased significantly and decreased insignificantly during germination and popping respectively. Iron content for WRPMF, GPMF and PPMF were 8.0±1.2, 8.9±0.5, 9.8±1.5 mg/100g respectively. Significant different was found only in PPMF iron content. Phosphorous contents of all versions i.e. WRPMF, GPMF and PPMF were as 298±1.0, 281±1.3 and 301±2.1 (mg/100g). Phosphorus content decreased significantly during germination and increase insignificantly during popping of different versions respectively.

Changes in anti-nutrient during germination and popping

Fig. 2 shows the anti-nutrients like tannin, phytic acid and oxalic acid content of pearl millet versions. Tannin contents of WRPMF, GPMF and PPMF were found as 217.2±1.03, 148.9±2.04, 108.5±2.1 mg/100g respectively. Phytic acid content for WRPMF were 858.6±2.6 mg/100g, in germination it were 497.4±1.54 mg/100g (GPMF) while in popping it were 689.76±1.80 mg/100g (PPMF). Whereas, oxalic acid content for all version were WRPMF; 21.2±1.05, GPMF; 15.1±3.9, PPMF; 17.2±2.1 (mg/100g). While, in context of trypsin inhibitor activity resulted of processing in decreasing trends, WRPMF, GPMF and PPMF were stood at 7899.9±10.6, 5877.5±11.2 and 7457.4±9.5 U/g, indicating a decrease in the level of trypsin inhibitor activity (Fig. 3).

Conclusion

Germination and popping is simple, less expensive and household food processing methods. Germination of pearl millet resulted in improving the level of nutrients like moisture, protein, fat, minerals (calcium, iron) and lowered the level of anti-nutrients namely tannin, phytic acid, oxalic acid and trypsin inhibitor activity. Popping of pearl millet also increased the level of ash, carbohydrate, dietary fiber content and minerals such as iron significantly and reduced the anti-nutrients like tannin, phytic acid etc, and give pleasing texture to the product. Popping of pearl millet helps to make ready-to-eat food as snacks. Emphasis is given on the effect of germination and popping on nutritional quality of pearl millet. Processing and utilization of pearl millet as food

product development has beneficial aspect with regard to nutrition as improve the bioavailability of micronutrients and enhancing the nutrients and can be an alternative to cereals.

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