

Effect of Germination on Biofortified Pearl Millet Cultivars' Nutrient Content

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Abstract – Iron deficiency anaemia is one of the micronutrient nutrient deficiency diseases wide spread globally. Biofortification of staple foods play a critical role in alleviating micronutrient malnutrition. Biofortified pearl millet (BPM) offers a novel, yet practical solution to combat anaemia in millet-consuming communities of India. *Aim:* To compare the nutritional value of BPM and to examine the effect of germination on nutrient content of the same. *Methods:* Nutrients (moisture, ash, protein, fat, calcium, iron, zinc); anti-nutrients (phytate & tannin) content were estimated per standard AOAC procedures; carbohydrate (difference method) and energy (factorial method) were calculated. *Results:* A significant difference ($p < 0.05$) was observed in moisture, protein, carbohydrate, ash, dietary fibre, iron, phytate (Proagro-9444; DHANSHAKTI and ICMH-1201), fat (Proagro-9444 and DHANSHAKTI), acid insoluble ash, zinc and calcium (DHANSHAKTI and ICMH-1201) in the selected cultivars. Germination significantly altered the moisture (DHANSHAKTI), protein, acid insoluble ash, calcium, iron (Proagro-9444; DHANSHAKTI and ICMH-1201), fat (DHANSHAKTI and ICMH-1201), ash (Proagro-9444 and DHANSHAKTI) and zinc (Proagro-9444) content of the cultivars. *Conclusion:* On germination, phytate and tannin content decreased but calcium, iron and zinc content improved significantly.

Keywords – Biofortification, Germination, Nutrient content, Pearl millet.

I. INTRODUCTION

Increasing awareness and knowledge among health care providers and correction of iron deficiency anemia during adolescence will go a long way in improving the health of future parents [1]. Mineral deficiencies adversely affect the health of more than three billion people worldwide [2]. Children and women of reproductive age are most at risk, with global anemia prevalence estimates 42 and 30 per cent in pregnant women and non-pregnant women aged 15–49 years. Africa and Asia account for more than 85 per cent of the absolute anemia burden in high-risk groups and India is the worst hit [3]. Iron deficiency is the principal cause of anemia that leads to morbidity and mortality of mother and child at childbirth, and impairs cognitive skills and physical activity [4], but Zn deficiency leads to immune system dysfunctions and high susceptibility to infectious diseases, retardation of mental development, altered reproductive biology, gastrointestinal problems and stunted growth of children [5-6].

Nutritional deficiency problems have enormous socioeconomic impacts at the individual, community and national levels [7-8]. India annually loses about four million disability-adjusted life years (DALYs) due to iron

deficiency and another 2.8 million (DALYs) due to Zn deficiency [9]. Pharmaceutical supplementation, industrial food fortification and agricultural approaches of dietary diversification and biofortification have been suggested to address these problems. Biofortification of staple crops such as millets is most cost-effective, sustainable, consumer acceptable, pro-rural and pro-poor intervention [10]. Biofortification is a process of enhancing the nutritional quality by applying by advanced plant breeding techniques.

Millet is one of the oldest cultivated foods and has been a staple in Southeast Asia and India since early times, but was replaced by rice later [11]. Pearl millet is one of the four most important cereals (rice, maize, sorghum and millets) grown in the semi-arid and dry lands of Africa and Southeast Asia [12]. It is the staple diet for rural households in the world's poorest countries, its stover is a valuable livestock feed in India and Africa [13]. Pearl millet and other millet grains survive in arid hence; its production and importance are growing concurrently with global warming. It accounts for >50% of total cereal grain consumption in communities in India, especially in Maharashtra, Gujarat, and Rajasthan, where the rural poor heavily depend on this crop. Pearl millet is also widely used in Northern Karnataka [14]. Pearl millet biofortification is on improving Fe density as an associated trait. Depending on the genotypic composition of the trials, moderate to high correlations between Fe and Zn densities have been observed.

Pearl millet (*Pennisetum glaucum*) is an excellent dietary source of calcium, iron, manganese, and methionine- an amino acid lacking in the diets of hundreds of millions of the poor who live on starchy foods such as cassava, plantain, polished rice, and maize meal. Millet use is diverse and can be used in porridge, soups, sprouts, bread and stuffing's, fermented beverages, and baby foods [15], rich in iron and zinc, contains a high amount of antioxidants and these nutrients along with the antioxidants may be beneficial for the overall health and wellbeing.

Germination and malting of cereals is a way not only to produce fermentable extract for the brewing and distilling industries but can also be a way to produce ingredients enriched with health promoting compounds. Malt extracts have also been shown to be good substrates for the growth and application of probiotic bacteria [16].

II. MATERIALS AND METHODS

II.1. Grain Procurement and Processing

Three pearl millet cultivars namely Proagro-9444; DHANSHAKTI and ICMH-1201 developed by International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India, were the base materials for this study and were procured within a month of harvest. Of the three samples, Proagro 9444 was considered as control, and the rest two were taken as biofortified cultivars.

II.2. Processing of Pearl Millet

II.2.1. Cleaning

The harvested threshed millet (Proagro-9444; DHANSHAKTI and ICMH-1201) cultivars were cleaned off from glumes and few damaged grains. Then the three millets cultivars were labeled, separately packed in muslin cloth bag which were named, covered using a high-density polyethylene bags and stored at $10 \pm 2^\circ\text{C}$ for future use. The total pearl millet grains were divided into two portions, one-half was used for germination and the other half was used as such (ungerminated).

II.2.2. Germination

A known weight of the three selected whole pearl millet grains were washed twice and then soaked in just sufficient quantity of purified water (aqua guard) for eight hours at room temperature ($32 \pm 2^\circ\text{C}$). Later the water was drained, the grains were then allowed to sprout after spreading on trays lined with wet muslin cloth and kept for 72 hours at room temperature ($32 \pm 2^\circ\text{C}$), with spraying of water as and when required to enable sprouting as indicated by [17-18] who have also established that prolonged germination time helps in better mineral bioavailability. All three cultivars were germinated for 72 hours and then the germinated millets were sun dried after covering with a clean, washed, white muslin cloth for nutrient and anti-nutrient analysis.

II.2.3 Drying

The ungerminated cultivars were also spread on clean trays and to prevent contamination they were covered with clean, washed, white muslin cloth then sun dried to reduce the moisture content to less than 13% which is the maximum permissible limit for human consumption as recommended by [19-20].

II.2.4. Estimation of Quality Parameters

The ungerminated and germinated whole pearl millet grains were analyzed for moisture (oven dry method), ash (dry ashing method), crude fiber (fibra plus), total dietary fiber (enzymatic-gravimetric method), as per [21]. The crude protein [22], fat (Soxhlet method with petroleum ether [23], total carbohydrate (difference method as per [24]), and energy (factorial method) were also quantified. Minerals (calcium, iron, zinc) contents were determined by atomic absorption spectrophotometry (AAS) using an SP 191 PyeUni cam spectrophotometer using di-acid digestion method [21]. Vitamin-C and vitamin A (carotene) were carried out as per [25] method using a spectrophotometer. Phytic acid and tannin estimation was done by [26]. The heavy metal lead content was assessed

by the method of [21]. All the estimations were ($n= 6$) tests were done as per standard procedure.

II.3. Statistical Analysis

Results of the nutrient and anti-nutrients analysis were subjected to statistical analysis of t-test using SPSS version no 20 for comparing the cultivars nutritional value and the effect of processing on grain composition.

III. RESULTS AND DISCUSSION

The results of nutrient analysis were presented in Table-1 to 4.

III.1. Nutrient Content of Pearl Millet Cultivars

III.1.1 Moisture Content

Moisture is an important determinant of food quality and affects the physical, chemical aspects of food which relates to the freshness and stability for the storage for a long period of time, preservation and resistance to deterioration. It is necessary to calculate the content of other food constituents (i.e., total solids) on a uniform basis (i.e., dry weight basis).

The moisture content of pearl millet cultivars differed significantly at 5 % level and ranged from 6.82 ± 0.06 (DHANSHAKTI-germinated) to 9.39 ± 0.05 percent (ICMH-1201- ungerminated). Germinated as well ungerminated pearl millet cultivar's moisture content was well below the 13 percent quoted by [19] for whole grains. Both the ungerminated and germinated ICMH-1201; contained more moisture compared to other two (DHANSHAKTI and Proagro-9444) cultivars.

There was a significant reduction in the moisture content of three cultivars after germination and drying compared to their respective ungerminated cultivars. The highest reduction was noted in Proagro 9444 (14.2 percent) followed by ICMH with 12.03 percent and the least 9.5 percent) was in DHANSHAKTI .

As per [17] and [27] had established that germination enhances moisture content; however, the present finding is quite contrary to the above report, because in the present study after 72 hours of germination the grains were subjected to covered sun drying.

III.1.2. Protein Content

Protein is best measured as the sum of individual amino acid residues. Ungerminated pearl millet cultivars had 9.78 ± 0.42 (Proagro-9444) to 11.80 ± 0.09 g /100 g (DHANSHAKTI) of protein.

It is quite clear from the results that the protein content showed an upward revision on germination in the three cultivars studied. Among the germinated cultivars, DHANSHAKTI had the highest (14.83 ± 1.13 g/100 g) and Proagro9444 had the least (13.71 ± 0.03 g/100 g). The percentage increase of protein in germinated cultivars was from 25.6 (DHANSHAKTI) to 40.1 (Proagro-9444) percent.

Table – I

Nutrient Content of Pearl millet Cultivars				
Nutrients	Cultivars	DHANSHAKTI	ICMH-1201	Proagro-9444
	Samples	Whole	Whole	Whole
Process		Mean ± SD		
Moisture (g /100 g)	Ungerminated	7.54 ^{a,b} ± 0.07	9.39 ^a ± 0.05	8.23 ^a ± 0.08
	Germinated & Dried	6.82 ^b ± 0.06	8.26 ± 0.06	7.06 ± 0.04
Fat (g /100 g)	Ungerminated	1.03 ^{a,b} ± 0.06	1.41 ± 0.11	1.21 ^{a,b} ± 0.03
	Germinated & Dried	2.33 ^b ± 0.73	2.45 ^b ± 0.05	1.64 ± 0.09
Ash (Acid soluble) (mg /100 g)	Ungerminated	5.78 ± 0.18	6.26 ^a ± 0.26	2.46 ^{a,b} ± 0.16
	Germinated & Dried	5.97 ^b ± 0.12	6.88 ± 0.78	2.98 ^b ± 0.14
Acid Insoluble Ash (AIA) (mg /100 g)	Ungerminated	0.02 ^{a,b} ± 0.01	0.04 ^{a,b} ± 0.02	0.03 ± 0.05
	Germinated & Dried	0.01 ^b ± 0.06	0.02 ^{a,b} ± 0.01	0.02 ^b ± 0.04

*All data reported on a dry basis and represent the mean of six determinations. SD – Standard deviation
Values with similar super scripts are significantly different ($p < 0.005$).

Earlier researchers have opined that soluble proteins increases due to the action of proteolytic enzymes that hydrolyses protein-polyphenol complexes in the seed during germination [28], protein content of pearl millet varies due to the varietal difference and the amount of nitrogen available in the soil [29], In cereals and legumes, on germination, due to increased protease enzyme activity there is break down of peptide bonds in proteins (protein hydrolysis) hence, the protein content increases [30-32]. The above reports help to substantiate the increase in protein content of the germinated cultivars of the present study.

III.1.3. Fat Content

The fat content of the three ungerminated cultivars was significantly different ($p < 0.05$) and it ranged from 1.03 ± 0.06 g / 100 g (DHANSHAKTI) to 1.41 ± 0.11 g/ 100 g (ICMH-1201). Earlier studies by [33-37] had indicated a fat content of 4.36 to 7.11 percent, 4.1 to 5.5 percent, 4.6 percent, 6 to 8 percent, 4.36 to 7.11 percent, but the present study finding is much lower than the above values which could be due to the varietal difference.

The fat content of germinated pearl millet ranged from 1.64 ± 0.09 (Proagro-9444) to 2.45 ± 0.05 g/ 100 g (ICMH 1201).The process of germination had facilitated a significant increase ($p < 0.05$) in the fat content. The percentage increase in the fat content was between 35.5 (Proagro-9444) to 126.2 (DHANSHAKTI). The fat content of ICMH-1201 germinated sample's was significantly higher at five percent level, than the other

two germinated cultivar's fat content. Lipids are present in a free and bound form in pearl millets (extractable with water-saturated butanol) [38], and there is enhanced activity of lipase during germination [39]. Both these factors could have helped to increase the fat content in the germinated samples. But ICMH-1201 had a moderate increase in fat content (73.75 percent).

III.1.4. Carbohydrate Content

Carbohydrates are the sugars, starches, and fibers found in fruits, grains, vegetables and milk products. The total carbohydrate content of the three cultivars varied significantly at 5 percent level. While the ICMH-1201 and Proagro-9444 had more or less the same quantity of carbohydrate before (74.25 ± 0.31 ; 72.13 ± 0.17) and after (72.82 ± 0.46 ; 69.31 ± 0.26) germination, but DHANSHAKTI contained a higher amount of carbohydrate than ICMH-1201 and Proagro-9444. The germinated cultivar's carbohydrate was significantly ($p < 0.005$) lower than their corresponding ungerminated cultivars. The percentage decrease ranged from 1.9 (DHANSHAKTI) to 3.9 (Proagro-9444). The decrease in total carbohydrate could be attributed to their consumption, as a source of energy during the germination process [40 and 17]. As per [41-42] reported that starch in the endosperm gets degraded slowly during the course of germination and, with the degradation of starch, the total soluble sugar (reducing and non-reducing sugar) content were elevated significantly during the period of germination. The present finding is in line with the above reports.

3.1.5. Energy Content

The ungerminated cultivars calorific value ranged from 369 to 379 Kcal. After germination, a 3.5 (ICMH 1201), 6.6 (DHANSHAKTI) and 5.3 (Proagro 9444) percent increase in energy content was observed and it was from 383 (ICMH 1201) to 403 (DHANSHAKTI) kcal. The increase in energy content could be attributed to the higher protein and fat content of the germinated cultivars (refer discussion under section 3.1.2 and 3.1.3 for protein and fat).

3.1.6. Dietary Fiber Content

Dietary fibre of the cultivars differed significantly at 0.05 percent level ranged from 7.52 ± 0.88 g/100g (DHANSHAKTI-germination) to 10.96 ± 0.16 g/100g (ICMH-1201- ungerminated). ICMH-1201 recorded the highest dietary fiber content in both germinated (8.29 ± 0.48 g/100g) and ungerminated (10.96 ± 0.16 g/100g) samples.

Table – II
Nutrient content of Pearl millet cultivars

Nutrients	Cultivars	DHANSHA	ICMH-	Proagro-
		KTI	1201	9444
Samples		Whole	Whole	Whole
Process		Mean ± SD		
Carbohydrate (g / 100 g)	Un germinated	74.25 ^a	72.35 ^a	72.13 ^{a, b}
		±	±	±
	Germinated & Dried	0.31	0.26	0.17
		±	±	±
Dietary fiber (g / 100 g)	Un germinated	10.93 ^a	10.96 ^a	10.42 ^a
		±	±	±
	Germinated & Dried	0.12	0.16	0.03
		±	±	±
Protein (g / 100 g)	Un germinated	11.80 ^{a, b}	10.58 ^{a, b}	9.78 ^{a, b}
		±	±	±
	Germinated & Dried	0.09	0.09	0.42
		±	±	±
		14.83 ^b	14.06 ^b	13.71 ^b
		±	±	±
		1.13	0.07	0.03
		±	±	±

*All data reported on a dry basis and represent the mean of six determinations. SD – Standard deviation
Values with similar super scripts are significantly different (p < 0.005)

It is quite clear that the dietary fiber of germinated cultivars was lower compared to ungerminated cultivars. The decrease in dietary fiber ranged from 23.1 (Proagro-9444) to 31.1 (DHANSHAKTI) percent. The reduction in dietary fibre could be justified because soaking process decreases the fiber content due to reduction in water-soluble fiber content of seed namely β -glucan [43] and the germination increases the activity of β -amylase enzyme which hydrolyzes cell wall of carbohydrates during germination [44] and according to [45] during the first stage of seed germination process, β -glucanase enzymes degrades the endosperm cell walls and α amylase degrades starch.

3.1.7 Ash (Acid Soluble) Content

The ash content of pearl millet cultivars (ungerminated) ranged from 2.46 ± 0.16 mg/100g (pro agro9444) to 6.26 ± 0.26 mg/100g (ICMH-1201). In the germinated pearl millet cultivars; the ash content was between 2.98 ± 0.14 mg/100g (pro agro9444) to 6.88 ± 0.78 mg/100g (ICMH 1201). Compared to ungerminated cultivars, all the germinated cultivar's ash content registered a considerable increase. The percentage increase was highest in Proagro-9444 (21.1 percent), and the least in DHANSHAKTI (3.2 percent). It could be noticed that, whether ungerminated or germinated, the Proagro-9444 cultivar had the least ash, but ICMH-1201 had the highest ash. There was a significant increase (p<0.05 percent level) in the ash content on germination.

3.1.8. Acid insoluble Ash (AIA) Content

The Acid Insoluble Ash (AIA) content is the proportion of a sample that is not hydrolyzed by sulphuric acid and not subsequently volatilized upon incineration of Acid-insoluble residue.

The AIA content of ungerminated and germinated pearl millet cultivars ranged from 0.01 ± 0.06 mg/100g (DHANSHAKTI-germinated) to 0.04 ± 0.02 mg/100g (ICMH-1201-ungerminated). There was a noticeable

decrease in the AIA content at five percent level in the pearl millet cultivars on germination. The percentage decrease was higher (50 percent) in DHANSHAKTI and ICMH-1201, but lower (33.3 percent) in Proagro-9444. Prior to soaking and germination, the millets were washed thoroughly in running tap water and that could have removed any dust / dirt adhering to the millets' seed coat, hence the reduction in AIA in germinated and dried samples.

III.2. Mineral Composition of Pearl Millet Cultivars

III.2.1 Calcium Content

Calcium content in ungerminated pearl millet cultivars varied from 39.84 ± 0.24 mg/100g (Proagro 9444) to 41.81 ± 1.05 mg/100g (DHANSHAKTI) which is almost the same 42mg/100g, as reported by [46] but much lower than the 72 mg/100 g calcium as revealed by [47].

DHANSHAKTI, ICMH-1201 and Proagro-9444's calcium content improved by 66.2, 50.4 and 57.2 percent and this increase was significant at 5 % level.

As per [30] reported that germination and fermentation would increase the mineral content due to an increase in fitase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein and enzyme hence minerals become free, therefore increasing the availability of minerals.

Table – III
Minerals content of Pearl millet cultivars

Nutrients	Cultivars	DHANSHAKTI	ICMH-	Proagro-
		1201	1201	9444
Samples		Whole	Whole	Whole
Process		Mean ± SD (mg/ 100 g)		
Calcium	Un germinated	41.81 ^{a, b}	40.58 ^{a, b}	39.84
		±	±	±
	Germinated & Dried	1.05	0.46	0.24
		±	±	±
Iron	Un germinated	9.68 ^a	8.86 ^{a, b}	4.6 ^{a, b}
		±	±	±
	Germinated & Dried	15.32	13.68 ^b	10.08 ^b
		±	±	±
Zinc	Un germinated	3.59 ^a	3.31 ^a	2.97 ^b
		±	±	±
	Germinated & Dried	16.01	8.71	7.06 ^b
		±	±	±
		0.81	0.24	0.38
		±	±	±

*All data reported on a dry basis and represent the mean of six determinations. SD – Standard deviation
Values with similar super scripts are significantly different (p < 0.005)

III.2.2 Iron Content

The iron content of ungerminated pearl millet was 9.68 ± 0.11 (DHANSHAKTI), 8.86 ± 0.07 (ICMH 1201) and 4.6 ± 0.12 (Proagro-9444) mg/100g. As per [47], reported a iron content of 50 mg/kg for ICMH-1201 which is lower compared to the present finding. As per [48], evaluated the mineral content of 225 accessions of pearl millet, and reported a mean iron content of 42.9 with a range of 19.7 to

86.4 µg/g. The iron value for DHANSHAKTI and ICMH-1201 of the present study are higher, while that of Proagro-9444 was near the above mean value.

The germinated DHANSHAKTI, ICMH 1201 and Proagro 9444, had 15.32 ± 0.84 ; 13.68 ± 0.08 and 10.08 ± 0.20 mg/100g of iron respectively. All three pearl millet cultivars Fe content increased after germination. ICMH-1201 showed an increase of 54.4 percent. While DHANSHAKTI's iron increment was nearly three times (145.2 percent), Proagro's was twice (119.1 percent) that of ICMH-1201.

III.2.3 Zinc Content

The zinc content was 3.59 mg/100g (DHANSHAKTI), which is 35.9 mg/kg. The germination process increased the zinc level from 137.9 (Proagro-9444) to 345.9 (DHANSHAKTI) percent compared to the ungerminated samples. The ungerminated cultivars contained 3.59 ± 0.10 (DHANSHAKTI), 3.31 ± 0.19 (ICMH-1201), 2.97 ± 0.35 (Proagro9444) mg/100g of zinc. According to [47] zinc content of ICMH-1201 was 31 mg/kg and [48] from their analysis of 225 accession of pearl millet, reported the mean value of 40.3 (range: 13.5–82.4) µg/g. The present result of 3.31 ± 0.19 mg/100g (ICMH 1201-ungerminated) corroborates well with that of the former report and lower than that of the latter's mean value.

Germinated cultivars logged a remarkable increase in zinc content that varied between 137.9 (Proagro 9444) to 345.9 (DHANSHAKTI) percent.

Of the three germinated cultivars, DHANSHAKTI contained the highest zinc content (16.01 ± 0.81 mg/100g), whereas Proagro-9444 had the least (7.06 ± 0.38 mg/100g) Zn. From the fore going discussions, it is clear that there is a significant enhancement in the mineral content of pearl millet after germination. In pearl millet, a greater concentration of minerals is located in the covering layers and germ than the endosperm portion of the kernel [49]. For the present analysis pearl millet was used without removing the outer covering and this account for the high iron and zinc content of the selected cultivars.

III.3. Anti-nutrients Factors of Pearl Millet Cultivars

Unprocessed pearl millet cultivars contained 0.56 ± 0.07 (DHANSHAKTI) to 0.58 ± 0.06 mg/100g (Proagro9444) phytate content, and the processed (germination and drying) had 0.23 ± 0.08 to 0.40 ± 0.07 mg/100g of phytate. The percentage of reduction in phytate content was between 28.5 (DHANSHAKTI) to 46.5 percent (Proagro-9444).

Tannin content of ungerminated pearl millet varied from 0.42 ± 0.01 (ICMH 1201) to 0.59 ± 0.07 (Proagro-9444) mg/100g. On germination, there was a significant ($p < 0.005$) decrease in tannin content which ranged from 0.22 ± 0.08 (DHANSHAKTI) to 0.34 ± 0.28 (ICMH-1201) mg/100g. On the whole, sprouting (germination) of pearl millet significantly decreased the anti-nutrients (phytate and tannin) content.

Table – IV
Phytate and Tannin content of Pearl millet cultivars

Anti-Nutrients	Cultivars	DHANSHAKTI	ICMH-1201	Proagro-9444
		Whole	Whole	Whole
Process		Mean \pm SD (mg/100g)		
Phytate	Ungerminated	$0.56^{a,b}$	$0.58^{a,b}$	$0.58^{a,b}$
		\pm	\pm	\pm
		0.07	0.09	0.06
	Germinated & Dried	0.40^b	0.40^b	0.23
		\pm	\pm	\pm
		0.07	0.03	0.08
Tannin	Ungerminated	0.54^b	0.42	0.59^b
		\pm	\pm	\pm
		0.11	0.01	0.07
	Germinated & Dried	0.22^b	0.34	0.28^b
		\pm	\pm	\pm
		0.08	0.28	0.17

**All data reported on a dry basis and represent the mean of six determinations.

SD – Standard deviation

Values with similar super scripts are significantly different ($p < 0.005$)

IV. CONCLUSION

Germination helps to decrease the phytate and tannin content in biofortified pearl millet. It also helps to significantly improve the fat, ash and protein content. The process of germination enhances the dializability of minerals, hence an increase in calcium, iron and zinc content. On the whole, DHANSHAKTI could be rated as superior cultivar in terms of mineral content.

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