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RESEARCH ARTICLE

Impact of Traditional and Sustainable Processing Methods on the Mineral and Antinutrient Composition of Biofortified Pearl Millet (*Pennisetum glaucum*) Cultivars

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Abstract

The study aims to investigate the mineral and antinutritional properties of biofortified pearl millet [*Pennisetum glaucum* (L.) R.Br.] varieties. Pearl millet contains antinutrients such as phytate and phenols, which reduce the bioavailability of micronutrients like zinc and iron, despite its decent nutritional composition. Thus, it is necessary to increase bioavailability through processing, which also improves the grain's nutritional profile and can be utilised to mitigate micronutrient deficiencies, the main dietary issue facing the world today. The mean iron, zinc, calcium, phytate, phenol, and tannin contents were 6.60, 3.42, 28.43, 486.83, 346.00, and 171.09 mg/100 g. Dhanshakti has shown significantly ($P<0.05$) high iron content (8.66 mg/100 g), the lowest phytate ($P<0.01$) and phenol content ($P<0.001$) of 560.56 mg/100 g and 298.94 mg/100 g, respectively, compared to other varieties studied. RHB 234 has a low tannin content of 145.45 mg/100 g ($P<0.001$). After being soaked and germinated, grains have decreased minerals and phytate, while increases in phenols and tannins were observed. Therefore, traditional processing may reduce the quantity of both minerals and antinutrients, but it may also boost the bioavailability of the minerals because it reduces the amount of phytate, a key antinutrient that lowers the bioavailability of vital minerals like iron and zinc.

Keywords: Bioavailability, Germination, Iron, Millet, Soaking, Zinc

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R.Br.] is an important crop in the semi-arid and arid environments of South Asia and sub-Saharan Africa that are typically challenged by erratic rainfall, high mean temperature, low soil organic carbon content, and poor water-holding capacity. Pearl millet is highly valued for its nutrient-rich grain for human consumption**.** The crop forms the livelihood and nutritional security source for more than 90 million people in South Asia and sub-Saharan African regions. The demand for pearl millet is anticipated to increase in the future due to increasing human and livestock populations, expanded industrial uses, and growing healthy food consciousness in these regions. Being a self-sufficient crop, it is consumed primarily for food purposes in most developing countries (Deevi *et al.,* 2024).

"Biofortification" or "biological fortification" is the process of nutritional enhancement of food crops in which conventional plant breeding, modern biotechnology, and agronomic practices are used to develop and grow them for increasing the bioavailability of nutrients to the human population. Pearl millet has been grown by farmers for ages; replacing the regular varieties with biofortified crops can lessen micronutrient deficiencies in developing nations like

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India. Keeping this in view, various biofortified varieties of pearl millet have been released to fight iron and zinc deficiencies in India (Bansal and Kawatra 2020).

The scope of pearl millet for its utilisation in nutritional foods and nutraceuticals is very high. The importance of including this millet in the daily diet is well-studied and documented by various researchers. It contains anti-nutrient compounds like phytic acid and polyphenols, which reduce mineral bioavailability because of their chelating properties (Hemanth *et al.,* 2025). Biofortification of pearl millet is like a double-edged sword that cuts down the economic burden and simultaneously supplies the required nutrition to the poor, offering great scope for food security as well as nutritional security (Singhal *et al.,* 2022; Kumar *et al.,* 2022; Selladurai *et al.,* 2022).

In India, pearl millet is the principal source of dietary energy (360 kcal/kg) for rural populations in drier parts of the country. It is a rich source of protein, calcium, phosphorus and iron. Pearl millet comprises a fairly high amount of thiamine, riboflavin and niacin. In general, pearl millet grain has higher fat and protein content than sorghum. The energy level (784 cal/kg) is highest among the whole grain cereals (Gupta *et al.,* 2022).

According to Cyerin *et al.,* (2023), processing methods like soaking, sprouting, and cooking increase the millet grain's nutritional value. Mineral availability is improved by the sprouting method, and it also helps in lowering the anti-nutritional components. Studies in the past have emphasised the significance of sprouting because it increases the nutrient bioavailability in a variety of dietary grains, which has a significant impact on nutritional quality.

Materials and methods

Plant materials and preparation

The experimental materials used for the study were four biofortified varieties and one control variety (ABV-04, AHB-1200, RHB 234, Dhan Shakti and Pittaganti), which were collected from ICAR institutes, ICRISAT and State Agricultural Universities. In the laboratory, the samples were cleaned manually to remove broken grains, stones, chaff and dirt and were stored in fresh, clean Ziplock bags.

Soaking and germination

Cleaned raw grains were soaked in water in a ratio of 1:3 for 12 h at 30 ± 2°C. The soaked grains were stirred periodically to remove the gases accumulated around the grains, and the soaking water was changed after every 2 hr interval to prevent the growth of undesirable microbes. The grains were dried at 60 ± 2°C using a tray dryer to 13% moisture content.

After drying, pearl millet grains were washed in water and subjected to the germination process as described by Sara and Valerie (2016) method with some modifications such as using muslin cloth and drinking water for soaking, to avoid contamination.

Minerals analysis

Raw, soaked, and germinated grains were analysed separately for minerals (iron, zinc and calcium) by using ICP (Inductively Coupled Plasma-Optical Emission Spectrometry) (Murcia *et al*., 1999).

Antinutrient analysis

Determination of phytic acid: Phytic acid was estimated using Wheeler and Ferrel's method (1971).

Determination of phenols: The Folin-Ciocalteu method was used to measure the total amount of polyphenol content (Singleton *et al*., 1999).

Determination of tannins: Tannin content was estimated by using the FDR method (AOAC, 1980)

Statistical analysis

A factorial complete randomized block design was used to determine whether there were significant differences among the varieties and the effect of processing on the grain nutrients and antinutrients.

Results and Discussion

Effect of processing on the mineral composition of biofortified pearl millet

The mineral content of all varieties has decreased after soaking and germination (Table 1), which might be due to metabolic loss and transfer of nutrients to the growing embryo (Sade 2009).

Table 1: Effect of processing on minerals of biofortified pearl millet

Name of the varieties	Processed grains	Iron	Zinc mg/100 g	Calcium
Control (Pittaganti)	1	5.78	2.21	41.44
	2	5.23	1.76	34.15

	3	4.78	1.31	25.41
	4	3.18	0.96	25.04
	5	2.18	0.53	15.52
ABV-04	1	7.48	6.12	40.66
	2	7.05	4.30	33.07
	3	6.73	3.19	21.81
	4	6.65	2.50	22.46
	5	6.28	2.48	17.67
AHB 1200	1	8.42	3.23	42.60
	2	8.12	2.79	31.17
	3	7.82	2.55	24.79
	4	7.37	2.32	24.54
	5	6.69	1.90	14.57
Dhanshakti	1	8.66	4.77	43.64
	2	8.52	3.91	36.25
	3	8.07	2.85	30.59
	4	7.56	2.54	25.62
	5	7.32	2.07	17.51
RHB 234	1	8.30	4.10	43.22
	2	7.04	3.60	32.04
	3	6.36	2.14	25.41
	4	5.11	2.06	24.41
	5	4.41	1.86	17.26
	Mean	6.60	2.72	28.43
	Range	2.18-8.66	0.53-6.12	14.57-43.64
	SEM	0.47	0.19	2.42
	SED	0.66	0.28	3.42
	CD	12.29	12.62	14.74
	CV	1.32	0.56	6.87

1-Raw grains; 2-soaked grains; 3-24 hr sprouted grains; 4-48 hr sprouted grains; 5-72 hr sprouted grains.

Iron: The iron content of the raw, soaked, 24-hr, 48-hr and 72-hr germinated grains ranged from 5.78-8.66, 5.23-8.52, 4.78- 8.07, 3.18-7.56 and 2.18-7.32 mg/100 g, respectively (Table 1). A significant variation (P<0.05) in the iron content among the varieties studied was observed. Significantly high iron content was observed in raw grains of the Dhanshakti variety (8.66). The control variety Pittaganti (5.78) had the lowest iron content. The mean iron content across all varieties and processing methods was 6.58 mg/100 g, with a range from 2.18 mg/100 g to 8.66 mg/100 g. Dhanshakti has shown significantly (P<0.001) high iron content (8.52 mg/100 g) after soaking and germination (8.07 mg/100 g). Control variety Pittaganti (2.18) has the lowest iron content after 72 hr of germination. There is a significant variation (P<0.001) in the iron content of different varieties during different processing methods (soaking, 24 hr germination, 48 hr germination and 72 hr germination). Lestienne *et al.,* (2005) reported an iron reduction of 35% after soaking. There is a 63.7% reduction of iron in finger millet as recorded by Kumar *et al.,* (2021), after 96 hr germination. Iron losses are because of iron ions leaching into the soaking media (Saharan *et al*., 2001). But AbdelRahaman *et al.,* (2007) reported that the HCl extractability of iron increased significantly (P<0.01) from the beginning of germination and reached a maximum value at day 6. According to Suma and Urooj (2014) iron content significantly increased (P<0.05) upon germination.

Zinc: Zinc ranged between 1.33-6.12 mg/100 g. There is a significant variation (P<0.05) in the zinc content among the varieties studied. ABV 04 raw grains (6.12) have the highest zinc content among all the varieties. The lowest was observed in control variety grains (2.21). After processing, a significantly higher (P<0.001) zinc

content was observed in soaked grains of ABV-04, whereas the lowest was observed in the control after 72-hour germination. In the present study, there is a decreasing pattern of zinc from raw grains to processed grains. Bello (2017) observed that zinc concentrations of all the flour samples studied were higher than the control (2.63 mg/100 g) and decreased significantly as the steeping duration progressed, i.e., 4.82 (24 hr) to 4.08 (72 hr) mg/100 g during lime steeping and 5.32 (24 hr) to 4.83 (72 hr) mg/100g during ash steeping. In the study of Thorat *et al.,* (2017), the mean zinc content decreased after 12 hr of soaking and germination. There is a negative impact of germination on the zinc of finger millet according to Kumar *et al.,* (2021), as there was a 16.7% reduction of zinc after 96 hr germination. In contrast, the germination process increased the zinc level from 137.9 (Proagro-9444) to 345.9 (Dhanshakti) per cent compared to the ungerminated samples in the study of Iswarya and Narayanan (2016).

Calcium: There is a significant variation for calcium (P<0.001), but no significant variation (P>0.05) was observed during processing. However, the mean content of calcium has decreased during soaking and germination (Table 1). Higher calcium content was found in Dhanshakti raw grains (43.64 mg/100g), whereas ABV 04 grains (40.66mg/100g) had low calcium content. The calcium content of non-germinated finger millet grains reduced after soaking by 9.30%, followed by a linear increase with the increase in germination time, as revealed by Kumar *et al.,* (2021). In contrast to the present results, Bello *et al.,* (2017) reported that steeping in ash solution significantly (P<0.05) increased the calcium concentration from 62.15 mg/100 g (24 hr) to 63.46 mg/100 g (72 hr). Lime steeping increased from 54.28 mg/100g (24 hr) to 54.63 mg/100 g (48 hr) and later decreased to 53.02 mg/100 g at 72 hr, while the control had the lowest value (49.36 mg/100 g). The calcium content of raw pearl millet flour studied by Sharma *et al.,* (2023) was observed to increase during germination from 35.76 to 71.21 mg/100 g.

Effect of processing on the antinutritional composition of biofortified pearl millet

Phytic acid: Phytic acid ranged from 361.45- 647.84 mg/100 g, as mentioned in Table 2. The highest was observed in RHB 234, and the lowest was observed in Dhanshakti grains. Phytic has significantly reduced in all varieties (P<0.01) during different processing methods (P<0.001). The highest loss of phytate was observed in Dhanshakti with low phytic acid content (361.45 mg/100 g) (Table 2) after 72 hr germination. Four cultivars studied by Abdelrahaman *et al*., (2007) showed that the phytic acid content decreased significantly (P<0.01) within the first 2 days of germination. Thereafter, it decreased at a lower rate from day 4 to day 6 of germination. A decrease in the level of phytic acid during soaking may be attributed to leaching into the soaking water under the concentration gradient. A decrease in the level of phytic acid during germination can be due to phytase activity in the germinating grains. The inherent phytase activity in pearl millet grains hydrolyses phytic acid during germination and accounts for the reduction in phytic acid in sprouts. Phytase activity was observed during germination of wheat, barley, rye and oats, which hydrolyse phytate to phosphate and myoinositol phosphates (Sokrab *et al*., 2012).

Table 2: Effect of processing on antinutrients of biofortified pearl millet

Name of the varieties	Processed grains	Phytic acid	Total phenols mg/100 g	Tannins
Control (Pittaganti)	1	575.94	347.60	197.73
	2	502.78	351.72	194.42\
	3	499.04	361.07	209.14
	4	418.41	386.55	294.64
	5	378.10	412.73	301.91
ABV-04	1	565.99	317.14	172.88
	2	496.55	330.23	155.40
	3	488.65	349.84	190.82
	4	414.26	363.89	211.07
	5	373.52	388.14	247.67
AHB 1200	1	624.56	308.31	139.51
	2	563.20	309.81	127.52

	3	512.81	318.85	169.79
	4	432.15	348.10	224.43
	5	406.83	380.14	219.00
Dhanshakti	1	560.56	305.61	143.12
	2	481.90	306.14	114.73
	3	427.43	312.89	149.31
	4	412.59	336.68	182.51
	5	363.11	357.99	203.75
RHB 234	1	647.84	309.68	163.94
	2	576.50	310.30	117.80
	3	524.92	338.39	191.24
	4	503.73	350.26	194.49
	5	447.28	364.68	204.84
	Mean	487.95	487.95	342.67
	Range	363.11-647.84	305.61-412.73	114.73-301.91
	SEM	10.01	10.18	13.95
	SED	14.16	14.40	19.72
	CD	3.64	5.08	12.97
	CV	28.45	28.93	39.6239.62

1-Raw grains; 2-soaked grains; 3-24 hr sprouted grains; 4-48 hr sprouted grains; 5-72 hr sprouted grains.

Polyphenols: Total polyphenol content ranged from 346.00-416.06 mg/100 g. Polyphenols increased significantly ($P<0.001$) during soaking and germination. The highest phenol content was observed in RHB 234 grains, whereas the lowest was observed in raw grains of the Dhanshakti variety. Significantly ($P<0.001$), high polyphenol content was observed in ABV 04 (416.06 mg/100 g) followed by RHB 234 (414.68 mg/100 g) after 72 hr germination. Dhanshakti grains have shown significantly ($P<0.001$) lower content (306.14 mg/100 g) after soaking and 357.99 mg/100 g after 72 hr germination compared to other varieties. Sprouting significantly ($P<0.05$) elevated the polyphenol content from 120.34 to 123.86 and from 125.12 to 128.62 mg/100 g for Ugandi and Dembi yellow, respectively, in the study of Ahmed *et al*., (2010). According to Hithamani and Srinivasan 2014, Sprouting did not alter the polyphenol content, while roasting increased polyphenol content by 12%, which may be due to an increase in the tannin content. Bio-accessible polyphenol content in the native sample was 1.89 mg/g. There was a 20% increase in the bioavailable polyphenol after sprouting.

It was found that the total phenol content in non-germinated finger millet grains was 1.50%, which decreased to 0.49% after 24 hr of germination. However, prolonged germination time increased the phenol content, and it was found to be 1.13% after 96 h of germination (Kumar *et al.,* 2021). The polyphenol content for both maize genotypes studied by Sokrab *et al*., (2012) showed an increase with germination, and the increment exceeded 100% for the genotype at the end of the germination period. The increment in polyphenols could be a result of solubilization of condensed tannin when the seeds were soaked in water and migration of polyphenols to the outer layer as a result of germination, as indicated by the browning of the germinated seeds.

In barnyard millet, Sharma *et al*., (2016) found that the predicted values of TPC and TFC reached their highest values of 54.23 mg GAE/100 g dry weight basis and 42.95 mg RUE/g dry weight basis after soaking for 11.8 h followed by germination for 36.5 hr at 33□C. Since phenolic content is bound to non-starch polysaccharides in grain cell walls through associations such as ester and ether bonds, the increase in phenolic content during germination in barnyard millet could have resulted from the action of cell wall-degrading enzymes, which modified the cell wall structure of the grain and released the phenolic content.

Tannins: Tannins ranged from 171.09-255.24 TAE mg/100 g. Significantly ($P<0.001$) higher tannin content was observed in the control and lowest in Dhanshakti. Tannin content was significantly ($P<0.001$) increased during soaking and germination. Control has recorded significantly ($P<0.001$) high phenols after soaking

(209.14 mg/100 g) (Table 2) and after germination (72 hr) (255.24 mg/100 g). Dhanshakti has recorded a low phenol content of 124.47 mg/100 g after 72 hr of germination. An investigation of three sorghum cultivars carried out by Ahmed *et al*., (1996) showed that for all cultivars, tannin content was slightly increased when seeds were germinated for different periods. This could be a result of the solubilization of tannin when the seeds were soaked in water and the migration of tannin to the outer layer as a result of germination, as indicated by the browning of the germinated seeds.

Germination of pearl millet increased the tannin content significantly ($P \leqslant 0.05$) in both varieties (from 0.21% to 0.28% for MRB and 0.23% to 0.36% for K) studied by Pushparaj and Urooj (2011). According to Samtiya *et al.,* (2021), during germination, a part of the tannins may enter the endosperm along with the imbibed water and are likely to form complexes with reserve seed protein and hydrolytic enzymes, thus inactivating them. In the human body, tannins show multifunctional properties that attract researchers' interest. However, they negatively affect the nutritional value of food. Tannin combines with protein and forms a complex, lowering food efficiency, growth, and iron absorption. Other severe side effects that can appear from excessive tannin consumption are damage to the gastrointestinal tract and the elimination of specific tissues, proteins, and essential amino acids. Therefore, they should be reduced to a safer level before being consumed by humans (Samtiya *et al.,* 2021). Tannins are known to function as antinutrients, reducing the accessibility of vital nutrients by causing protein precipitation (Choudhary *et al.,* 2023).

Conclusion

The present study has demonstrated that there is a decrease in mineral content after soaking and germination. Phenolics and tannins in grains have increased during processing. Even though minerals have decreased after processing, the bioavailability of the reduced minerals is higher due to reduced phytic acid, which is a primary chelating antinutrient against the minerals.

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