

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(9): 1291-1295
www.biochemjournal.com
Received: 02-06-2024
Accepted: 06-07-2024

Vaibhav Jadhav
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

RB Kshirsagar
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

Vishal Raut
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

BS Agarkar
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

Kadam GR
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

Corresponding Author:
Vaibhav Jadhav
College of Food Technology,
Vasantrao Naik Marathwada
Krushi Vidyapeeth, Parbhani,
Maharashtra, India

Advanced physico-chemical and nutritional characterization of biofortified pearl millet varieties: A comparative evaluation of Dhanshakti and AHB-1200

Vaibhav Jadhav, RB Kshirsagar, Vishal Raut, BS Agarkar and Kadam GR

DOI: <https://doi.org/10.33545/26174693.2024.v8.i9Sp.2338>

Abstract

This research examines the Physico-Chemical and Nutritional compositions of the biofortified pearl millet varieties, Dhanshakti and AHB-1200, compared to a control variety. The results showed that Dhanshakti had superior grain dimensions and thousand kernel weight, while AHB-1200 exhibited higher bulk and true densities. In terms of nutritional composition, AHB-1200 had significantly increased fat content, while Dhanshakti had higher levels of protein (11.46%) and carbohydrates (74.15%), making it a potential functional food for addressing malnutrition. Mineral analysis indicated that Dhanshakti had elevated levels of iron (84.43 mg/kg) and calcium (918.2 mg/kg), which can help mitigate micronutrient deficiencies. However, both biofortified varieties also presented higher levels of anti-nutritional factors, such as phytic acid and polyphenols, which may affect nutrient absorption. Overall, the nutritional profiles of Dhanshakti and AHB-1200 demonstrate their potential to improve dietary quality. These findings highlight the importance of biofortified pearl millet varieties in enhancing food security and nutrition, particularly in regions susceptible to malnutrition. Further research is necessary to explore processing methods that could reduce the effects of anti-nutritional factors while maximizing nutritional benefits.

Keywords: Physico-chemical, nutritional characterization, biofortified pearl millet varieties

Introduction

Millets are a group of small-seeded cereal crops or grains belonging to the Gramineae family, cultivated globally for both food and fodder. The most widely grown species, in terms of global production, include pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet (*Panicum glaucum*), and finger millet (*Eleusine coracana*) (Crawford and Lee, 2003) ^[10]. A key characteristic of millets is their remarkable ability to tolerate and thrive under adverse conditions, such as continuous or intermittent drought, which sets them apart from other cereals like maize and sorghum (LCRI, 1997) ^[11]. Millets serve as a principal food source in arid and semi-arid regions worldwide. According to Ikwelle *et al.* (1993) ^[12], pearl millet is ranked as the most important cereal in southern Sudan and northern Guinea.

Pearl millet (*Pennisetum glaucum* L.) is a staple food for millions of people living in the arid and semi-arid regions of Asia and Africa, including countries like India and Nigeria. It is well-known for its ability to grow in harsh conditions, tolerating both ongoing and unpredictable droughts. Compared to crops like rice and wheat, pearl millet requires less irrigation and fewer inputs, making it suitable for dryland farming. It also contributes to reducing atmospheric carbon dioxide and helps maintain productive land, ensuring food security for smallholder farmers (Nambiar *et al.*, 2011) ^[1]. Nutritionally, pearl millet is packed with essential nutrients like proteins, minerals, vitamins, and antioxidants. It is naturally gluten-free and rich in polyphenols and other compounds that promote good health. Often called a "Smart food" (Tako *et al.*, 2015) ^[2], pearl millet offers several health benefits, such as lowering fat absorption in the intestines and releasing sugar slowly, which can reduce the risk of high blood pressure, heart disease, and diabetes (Varsha and Narayanan, 2017) ^[3].

Despite its impressive nutritional profile and resilience, pearl millet has faced limited popularity, largely due to the short shelf life of its milled flour (Goswami *et al.*, 2020) [4]. Within just 5–7 days of milling, the flour tends to develop undesirable off-flavors and a bitter taste, which discourages wider consumer acceptance and leads to post-harvest waste. This issue disproportionately affects women, who are traditionally responsible for food preparation. Over the past decade, there has been a noticeable decline in pearl millet consumption in urban areas and commercial markets (Rani *et al.*, 2018) [5], which has subsequently reduced incentives for smallholder farmers to cultivate this crop. While there have been attempts to address the problem of rancidity through post-harvest processing and pre-milling techniques, these methods often impair the nutritional quality of the flour, particularly in terms of its micro- and macro-nutrients (Goyal *et al.*, 2015) [6]. As a result, a deeper understanding of the biological mechanisms that cause rancidity in pearl millet is critical to reviving its role in dryland agriculture and promoting its widespread use.

Pearl millet grain contains a larger germ layer compared to most cereals, except for maize (Taylor, 2004), and is known for its relatively high lipid content (5–7%), which predominantly consists of unsaturated fatty acids (Sharma *et al.*, 2015) [8]. During the milling process, the bran and germ layers of the grain rupture, releasing endogenous lipases that initiate the hydrolysis of stored lipids, resulting in the formation of free fatty acids (FFAs). These lipases are more thermostable than lipoxygenases and exhibit greater enzymatic activity under low-moisture conditions. As such, preventing the initial release of FFAs is vital to controlling the oxidation of seed lipids and the subsequent development of rancidity. Although lipases are believed to be primarily responsible for the post-milling rancidity of pearl millet (Kumar *et al.*, 2021) [9], the detailed biochemical and molecular mechanisms driving this process remain poorly understood, and the specific lipase(s) involved have not yet been conclusively identified.

The present study aims to contribute to the understanding of pearl millet seeds, particularly to enhance post-harvest handling and storage techniques. By investigating the relevant physicochemical properties of biofortified pearl millet varieties, including Dhanshakti and AHB-1200, which are rich in iron and zinc, the research seeks to provide insights that can improve storage operations and equipment. These varieties hold promise for addressing nutrient deficiencies while ensuring better shelf life and processing outcomes for this highly nutritious yet underutilized crop.

Materials

Raw material

The Dhanshakti variety of biofortified pearl millet was freshly harvested and obtained from the Bajra Research Scheme, Mahatma Phule Krishi Vidyapeeth, Dhule, Maharashtra. The AHB-1200 variety was procured from the National Agriculture Research Project (NARP), Vasantrao Naik Marathwada Krishi Vidyapeeth, Sambhajinagar (Aurangabad), Maharashtra. The control (non-biofortified) variety is procured from the local market of Parbhani.

Chemicals and glassware

The chemicals, standard reagents, and indicators used in this research were of analytical grade. Necessary glassware was taken from departments including Department of Food

Engineering, Department of Food Chemistry and Nutrition, Department of Food Processing Technology, and Department of Food Microbiology and Safety, the College of Food Technology, VNMKV, Parbhani.

Processing and analytical equipment

The research study utilized various equipment and processing machinery, including a Vernier calliper, weighing balance, grinder, mechanical sieve shaker, hot air oven, Soxhlet apparatus, Micro Kjeldahl unit, muffle furnace, pH meter, spectrophotometer, and centrifuge. These were obtained from the Department of Food Engineering, Department of Food Chemistry, Department of Food Processing and Nutrition, Department of Food Microbiology and Safety, as well as the niche area laboratory and pilot plant at the College of Food Technology, VNMKV, Parbhani.

Methods

A. Physical properties

Physical properties of pearl millet grain, including length, width, thickness, bulk density, true density, thousand kernel weight, gain volume, porosity, and angle of repose, were assessed using standardized measurement methods. Each property was determined according to established protocols to ensure the accuracy and reliability of the results.

1. Length, width and thickness

The length, width, and thickness of pearl millet grains were measured using a digital micrometer, with the average results recorded in millimeters. This precise method ensured accurate assessment of the grain dimensions.

2. 1000 kernel weight

The thousand kernel weight of the food grains was determined by counting 1,000 randomly selected grains and weighing them using a digital balance. The results were recorded in grams.

3. Grain volume

The grain volume of pearl millet was measured by placing a known quantity of grains into a measuring cylinder. The resulting volume was recorded in milliliters.

4. Bulk density

Bulk density was determined by dividing the mass of a specific quantity of grains by its volume, measured using a constant-volume cylinder. The results were expressed in kg/m³.

$$\text{Bulk density } (\rho_b) = \frac{\text{Weight of grains}}{\text{Volume of grains}}$$

5. True density

The true density of the food grains and powders was determined using the toluene displacement method. A sample of 10 g was weighed and placed in a measuring cylinder previously filled with a reference solution of kerosene or toluene. The increase in liquid level after adding the grains was recorded, and the true density was expressed in kg/m³.

$$\text{True density } (\rho_t) = \frac{\text{Weight of grains}}{\text{Volume of grains}}$$

6. Angle of repose

The angle of repose was measured by evaluating the height and diameter of a naturally formed heap of food grains, following the method described by Singh *et al.* (2010) ^[13].

$$\theta = \tan^{-1} \left(\frac{2H}{D} \right)$$

7. Porosity

Porosity refers to the fraction of space in bulk grain that is not occupied by the grain itself. It was calculated as a percentage using the equation derived from true and bulk density, as outlined by Vanrnamkhasti *et al.* (2008) ^[14].

$$\text{Porosity } (\epsilon) = \left[\frac{\rho_t - \rho_b}{\rho_t} \right] \times 100$$

B. Chemical properties

All proximate constituents were analyzed in triplicate, and the average values were used for the final results.

1. Proximate Analysis.

The proximate composition of the pearl millet grain was analyzed to determine its nutritional profile. Moisture content was assessed using the dry oven method (AOAC, 2000) ^[15]. The moisture-free sample was then used to analyze other proximate constituents, including fat, protein, crude fiber, and ash content, following AOAC guidelines (2000) ^[15]. The carbohydrate content was calculated using the difference method (Wadikar *et al.*, 2021) ^[16].

2. Mineral content

To prepare the mineral solution, the ash sample was digested, and the mineral content, including iron, zinc, calcium, magnesium, sodium, potassium, and manganese, was determined following the standard procedures outlined by Ranganna (1986) ^[17]. All proximate constituents were analyzed in triplicate, and the mean values were recorded for accuracy.

3. Anti-nutritional factor

The anti-nutritional factors were measured using the method described by Davies and Reid (1979) ^[18]. This involved specific analytical procedures to quantify the levels of compounds that could potentially hinder nutrient absorption or utilization in the body.

Results and Discussion

A. Physical Properties

The physical properties of grains play a vital role in assessing their appropriateness for different post-harvest processing and storage techniques. In this study, we examined the physical characteristics of biofortified pearl millet varieties, specifically Dhanshakti and AHB-1200, and compared them to a control variety. The findings are summarized in Table 1.

The grain length varied among the different varieties, with the control showing the highest measurement at 3.49±0.007 mm, followed by Dhanshakti at 3.19±0.006 mm and AHB-1200 at 2.97±0.009 mm. In terms of grain width, Dhanshakti had a slightly higher value of 2.69±0.008 mm compared to the control (2.52±0.004 mm) and AHB-1200 (2.48±0.003 mm). The thickness showed minor differences, with the control at 2.11±0.006 mm, Dhanshakti at

2.08±0.005 mm, and AHB-1200 at 2.02±0.007 mm. The results for the Dhanshakti variety align closely with findings reported by Samtiya *et al.* (2023) ^[19].

The thousand kernel weight was highest in the AHB-1200 variety, recorded at 10.03±0.01 g, which was slightly more than Dhanshakti at 9.93±0.03 g and the control at 9.87±0.01 g. The grain volume also peaked in AHB-1200 at 6.53±0.011 ml, followed by the control at 6.23±0.08 ml and Dhanshakti at 6.12±0.07 ml. Regarding bulk density, the control variety had the highest measurement at 847±1.95 kg/m³, while Dhanshakti exhibited the lowest at 711±1.5 kg/m³, with AHB-1200 in between at 819±2.91 kg/m³. For true density, AHB-1200 was recorded at 1310±1.98 kg/m³, surpassing the control (1258±2.12 kg/m³) and Dhanshakti (1206±2.04 kg/m³). These results for AHB-1200 regarding bulk and true density align with findings by Samtiya *et al.* (2023) ^[19].

The porosity was highest in the AHB-1200 variety at 42.93±0.11%, followed by Dhanshakti at 39.83±0.16% and the control at 35.92±0.18%. Regarding the angle of repose, which affects grain flowability, the variations were slight: Dhanshakti measured at 27° 7'±0.08, compared to the control at 26° 1'±0.05 and AHB-1200 at 26° 9'±0.05. These findings align with those reported by Pawase *et al.* (2021) ^[20] and Kalange *et al.* (2021) ^[21]. Overall, this data on the physical characteristics of biofortified pearl millet varieties is crucial for informing processing and storage decisions.

Table 1: Physical properties of pearl millet grains

Properties	Variety		
	Control	Dhanshakti	AHB-1200
Length (mm)	3.49±0.007	3.19±0.006	2.97±0.009
Width (mm)	2.52±0.004	2.69±0.008	2.48±0.003
Thickness (mm)	2.11±0.006	2.08±0.005	2.02±0.007
Thousand kernel weight (g)	9.87±0.01	9.93±0.03	10.03±0.01
Grain volume (ml)	6.23±0.08	6.12±0.07	6.53±0.011
Bulk density (kg/m ³)	847±1.95	711±1.5	819±2.91
True density (kg/m ³)	1258±2.12	1206±2.04	1310±1.98
Porosity (%)	35.92±0.18	39.83±0.16	42.93±0.11
Angle repose (°)	26° 1'±0.05	27° 7'±0.08	26° 9'±0.05

*Each value represents the average of three determinations

B. Chemical properties

1. Proximate analysis

The proximate composition of pearl millet grains offers valuable nutritional insights, particularly for biofortified varieties like Dhanshakti and AHB-1200. As detailed in Table 2, the control variety exhibited the highest moisture content at 8.45±0.04%, while Dhanshakti and AHB-1200 had lower levels at 7.46±0.03% and 7.61±0.04%, respectively. These moisture differences can significantly affect shelf life and storage stability. In terms of water activity, the values were comparable across the varieties, with Dhanshakti and the control recording 0.61±0.01 and 0.62±0.01, respectively. AHB-1200 had a slightly higher value of 0.66±0.02, which may suggest a different spoilage risk profile.

In terms of protein content, Dhanshakti demonstrated the highest level at 11.46±0.03%, followed closely by AHB-1200 at 11.07±0.05%, and the control variety at 10.24±0.02%. Notably, AHB-1200 also had a significantly

higher fat content at $9.79 \pm 0.01\%$, compared to Dhanshakti's $5.18 \pm 0.02\%$ and the control's $5.39 \pm 0.03\%$. This substantial variation in fat content suggests that AHB-1200 may serve as an energy-dense option. On the other hand, the carbohydrate content was lowest in AHB-1200 at $67.05 \pm 0.6\%$, while Dhanshakti ($74.15 \pm 0.5\%$) and the control ($73.26 \pm 0.4\%$) offered higher carbohydrate values, highlighting their potential for energy contribution from carbs.

The crude fiber content was found to be highest in AHB-1200 at $2.41 \pm 0.03\%$, which may provide better digestive health benefits compared to Dhanshakti ($1.70 \pm 0.03\%$) and the control ($1.84 \pm 0.02\%$). Additionally, the ash content, reflecting the total mineral content, was significantly greater in AHB-1200 at $3.1 \pm 0.03\%$, compared to Dhanshakti ($1.72 \pm 0.01\%$) and the control ($1.43 \pm 0.02\%$). This further underscores the enhanced nutritional profile of the AHB-1200 variety. Comparable findings on proximate composition have also been reported by Kulthe *et al.* (2016) [22].

These findings highlight the enhanced nutritional profile of biofortified pearl millet varieties, especially AHB-1200, which exhibits higher levels of protein, fat, fiber, and ash content. This makes AHB-1200 a promising option for addressing malnutrition and improving overall dietary quality.

Table 2: Proximate composition of pearl millet grain

Parameters (%)	Variety		
	Control	Dhanshakti	AHB-1200
Moisture	8.45 ± 0.04	7.46 ± 0.03	7.61 ± 0.04
Water activity	0.62 ± 0.01	0.61 ± 0.01	0.66 ± 0.02
Protein	10.24 ± 0.02	11.46 ± 0.03	11.07 ± 0.05
Fat	5.39 ± 0.03	5.18 ± 0.02	9.79 ± 0.01
Carbohydrates	73.26 ± 0.4	74.15 ± 0.5	67.05 ± 0.6
Crude fibre	1.84 ± 0.02	1.70 ± 0.03	2.41 ± 0.03
Ash	1.43 ± 0.02	1.72 ± 0.01	3.1 ± 0.03

*Each value represents the average of three determinations

2. Mineral content

The mineral composition of pearl millet is a crucial factor in its ability to help alleviate micronutrient deficiencies. As shown in Table 3, the iron content was significantly greater in Dhanshakti (84.43 ± 1.45 mg/kg) compared to AHB-1200 (66.18 ± 1.06 mg/kg) and the control variety (58.46 ± 1.12 mg/kg). This higher iron content in Dhanshakti underscores its potential role in combating iron-deficiency anemia. Similarly, zinc levels were highest in Dhanshakti (51.94 ± 1.97 mg/kg), with AHB-1200 (51.17 ± 1.39 mg/kg) closely following, both surpassing the control variety (41.24 ± 1.22 mg/kg).

Calcium content varied significantly among the varieties, with Dhanshakti showing the highest level at 918.2 ± 5.4 mg/kg, followed by AHB-1200 at 691.9 ± 4.21 mg/kg, and the control at 577 ± 4.87 mg/kg. Similarly, Dhanshakti also had the highest magnesium content at 1718 ± 4.91 mg/kg, which is vital for bone health and various enzymatic functions. In contrast, AHB-1200 recorded 1428 ± 7 mg/kg, which is lower than the control's value of 1486 ± 6.5 mg/kg. Regarding sodium content, Dhanshakti again showed a higher level at 786.6 ± 3.8 mg/kg, surpassing AHB-1200, which had 569.4 ± 2.4 mg/kg, and the control at 592 ± 2.9 mg/kg. Potassium, essential for heart function and muscle health, was also highest in Dhanshakti at 5196.3 ± 11.2

mg/kg, followed by AHB-1200 at 4134 ± 10 mg/kg, and the control at 4054 ± 12.8 mg/kg.

The manganese content was relatively consistent among all varieties, with Dhanshakti exhibiting the highest level at 12.11 ± 0.57 mg/kg. AHB-1200 and the control showed comparable values at 10.09 ± 0.2 mg/kg and 10.12 ± 0.55 mg/kg, respectively. The mineral content in both Dhanshakti and AHB-1200 aligns with findings reported by Samtiya *et al.* (2023) [19].

These findings indicate that Dhanshakti excels over both AHB-1200 and the control in several mineral contents, especially iron, zinc, calcium, magnesium, sodium, and potassium. This highlights Dhanshakti's strong potential to help combat mineral deficiencies in human diets.

Table 3: Mineral content of pearl millet

Parameters	Variety (mg/Kg)		
	Control	Dhanshakti	AHB-1200
Iron	58.46 ± 1.12	84.43 ± 1.45	66.18 ± 1.06
Zinc	41.24 ± 1.22	51.94 ± 1.97	51.17 ± 1.39
Calcium	577 ± 4.87	918.2 ± 5.4	691.9 ± 4.21
Magnesium	1486 ± 6.5	1718 ± 4.91	1428 ± 7
Sodium	592 ± 2.9	786.6 ± 3.8	569.4 ± 2.4
Potassium	4054 ± 12.8	5196.3 ± 11.2	4134 ± 10
Manganese	10.12 ± 0.55	12.11 ± 0.57	10.09 ± 0.2

*Each value represents the average of three determinations

3. Antinutritional factors

The presence of antinutritional factors, such as phytic acid and polyphenols, can affect the bioavailability of essential nutrients in pearl millet. As shown in Table 4, AHB-1200 had the highest phytic acid content (567 ± 1.3 mg/100 g), followed closely by Dhanshakti (548 ± 1.4 mg/100 g) and the control variety (498 ± 1.5 mg/100 g). Phytic acid is known to bind minerals like iron, zinc, and calcium, potentially reducing their absorption in the human body. While biofortified varieties like Dhanshakti and AHB-1200 exhibit enhanced mineral content, their higher levels of phytic acid may impact mineral bioavailability.

The polyphenol content was similar across the varieties, with AHB-1200 showing the highest level at 440 ± 1.7 mg/100 g, followed closely by the control at 438 ± 1.1 mg/100 g, and Dhanshakti at 432 ± 1.6 mg/100 g. While polyphenols are known for their antioxidant properties, they can also interfere with the digestion of certain macronutrients and inhibit the absorption of proteins and minerals. The findings regarding antinutritional factors align with those reported by Kulthe *et al.* (2016) [22] and Samtiya *et al.* (2023) [19].

The biofortified varieties of pearl millet, particularly AHB-1200, show elevated levels of antinutritional factors such as phytic acid and polyphenols. However, these levels remain within a manageable range. Additionally, appropriate processing techniques can be employed to reduce the impact of these factors, thereby improving nutrient absorption and overall bioavailability of essential nutrients.

Table 4: Antinutritional factor content in biofortified pearl millet

Parameters	Variety (mg/100 g)		
	Control	Dhanshakti	AHB-1200
Phytic acid	498 ± 1.5	548 ± 1.4	567 ± 1.3
Polyphenol	438 ± 1.1	432 ± 1.6	440 ± 1.7

*Each value represents the average of three determinations

Conclusion

This study underscores the enhanced nutritional profile of biofortified pearl millet varieties, especially Dhanshakti and AHB-1200, when compared to the control. Both varieties demonstrated elevated levels of protein, minerals, and fiber, with AHB-1200 exhibiting higher fat and fiber content, indicating its nutritional richness. Dhanshakti stood out with the highest concentrations of essential minerals like iron, calcium, and magnesium, showcasing its potential to combat micronutrient deficiencies. However, the increased levels of antinutritional factors, such as phytic acid and polyphenols, could affect nutrient bioavailability. Overall, these biofortified pearl millet varieties present a promising approach to enhancing food security and addressing malnutrition.

Acknowledgement

The authors would like to express their heartfelt gratitude to Research guide, Dr. R.B. Kshirsagar, Associate Dean and Principal, College of Food Technology, VNMKV, Parbhani, for his unwavering encouragement and support throughout this research. We also extend our sincere thanks to Mr. Sachin Giri for his continuous guidance and assistance during the research process.

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