

Comparative Analysis of Proximate Composition, Phenolic, and Flavonoid Content of Selected Cereal Flours from Haryana, India

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Abstract

Background: Cereal grains are a part of the Indian diet, with wheat being the most widely consumed. However, the increasing demand for nutrient-dense and functional foods has shifted consumer interest in millets and sorghum due to their superior nutritional and bioactive properties. **Objectives:** This study compared the proximate composition, total phenolic content (TPC), and total flavonoid content (TFC) of selected millet flours, sorghum, and wheat flour commonly available in India. **Materials and Methods:** Standard Association of Official Analytical Chemists methods were used for proximate analysis, whereas TPC and TFC were quantified using spectrophotometric assays. **Results:** The results showed significant differences among the cereals. Wheat flour exhibited the highest protein (13.68 ± 0.11 g/100 g) content but had the lowest TPC and TFC values, indicating limited phytochemical richness. In contrast, sorghum flour demonstrated significantly higher TPC (6.32 ± 0.16 mg GAE/g) and TFC (2.49 ± 0.35 mg QE/g) compared to pearl millet, finger millet, and wheat, highlighting its potential as a functional grain with strong antioxidant properties. Millet flours exhibited higher dietary fiber and ash content compared to wheat. The fiber content of finger millet was 4.78 ± 0.02 g/100 g, which is the highest among the other samples. **Conclusion:** Overall, the findings emphasize that while wheat flour remains valuable for its protein contribution, sorghum and millets are superior in terms of phytochemical composition and functional potential. These insights support the diversification of cereal consumption patterns in India, encouraging the inclusion of sorghum and millets in daily diets and industrial food applications for better nutritional security and health benefits.

Key words: Diet, dietary fiber, edible grain, functional food, nutrients

INTRODUCTION

Wheat is one of the most extensively consumed staple cereals worldwide. However, consumers' focus has shifted toward alternative cereals with better nutritional qualities and functional properties due to growing concerns about non-communicable diseases (NCDs), such as obesity, type 2 diabetes, and cardiovascular disorders. Millets and sorghum are commonly cultivated in India's semi-arid regions. These crops have rich nutrient profiles, low input needs, and resistance to climate stress.^[1]

The analysis of proximate composition provides valuable insights into the fundamental nutritional value of cereal grains. In addition, these insights impact the flour's functional properties

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in food applications, as well as its nutritional value, including its calorie content. Although wheat has a lower dietary fiber and micronutrient density than millets, it is still a good source of protein and carbohydrates.^[2] On the other hand, millets, finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), pearl millet (*Pennisetum glaucum*), and little millet (*Panicum sumatrense*), are known to contain higher amounts of dietary fiber, essential amino acids, and bioactive compounds.^[3] These qualities demonstrate their quality as promising candidates for the development of functional foods targeting metabolic health.

The phytochemical composition of millets and sorghum, especially phenolic compounds, is their most important characteristic.^[4] Phenolic acids, flavonoids, and tannins are examples of phenolic compounds that can scavenge free radicals, chelate pro-oxidant metals, and alter oxidative stress pathways.^[5] The regular consumption of foods high in phenolics has been associated with better glycemic control, gut health, and a lower risk of developing chronic diseases.^[6] While phenolics are present in wheat as well, they are less abundant than in millets and sorghum. The antioxidant activity of cereals is associated with their total phenolic content (TPC) and total flavonoid content (TFC). Studies indicate that pigmented cereals such as finger millet (rich in polyphenols) and sorghum (containing unique 3-deoxyanthocyanidins) exhibit stronger antioxidant capacities compared to non-pigmented cereals such as wheat.^[7,8] However, the variability in antioxidant activities is influenced by genetic background, growing conditions, and processing techniques.^[9]

Despite the growing recognition of their health benefits, millets and sorghum remain underutilized in food systems compared to wheat. Consumer preference, lack of awareness, and limited scientific documentation on their comparative nutritional and phytochemical attributes contribute to their marginalization.^[3] Thus, a systematic comparison of proximate composition and phenolic content of commonly available millet flours, sorghum, and wheat flour in India is critical to highlight their nutritional and functional superiority. Such evidence can support the promotion of these underutilized grains as sustainable alternatives in both household diets and industrial food formulations. The present study aims to compare the proximate composition, TPC, and TFC of selected millet flours, sorghum, and wheat flour available in India. Quantifying and comparing the proximate composition and phytochemical content of millets, sorghum, and wheat flours provides valuable insights for selecting cereal ingredients for nutraceutical formulations, designing functional foods, and implementing clinical nutritional interventions.

MATERIALS AND METHODS

Procurement of raw materials

Flour samples of pearl millet (*P. glaucum*), finger millet (*E. coracana*), sorghum (*Sorghum bicolor*), and wheat

(*Triticum aestivum*) were procured from local markets in Ambala, Haryana, India. The samples were obtained from a local producer commonly available for household consumption. All flours were stored in airtight containers at room temperature ($25 \pm 2^\circ\text{C}$) in a cool, dry place until further analysis.

Proximate composition analysis

The proximate composition (including moisture content, crude protein, crude fat, crude fiber, and total ash) of flours was determined using standard methods as described by the Association of Official Analytical Chemists (2005).^[10] The carbohydrate content was calculated by difference, i.e., by subtracting the sum of moisture, crude protein, crude fat, and total ash from 100%. The total energy (kcal/100 g) was estimated using conversion factors: 4 kcal/g for protein, 4 kcal/g for carbohydrates, and 9 kcal/g for fat.^[11]

TPC

The Folin–Ciocalteu (FC) method was used to analyze the TPC with some modifications. In brief, 1 g of the sample was extracted with 10 mL of 1% acidified methanol. The mixture was vortexed for 1 min and stirred on a shaker for 18 h with temperature control (4°C). After this centrifugation at 10,000 rpm for 15 min was carried out, the clear supernatant was collected, reacted with FC reagent, and incubated in the dark. The absorbance was then recorded at 760 nm. The phenolic concentration was calculated from a gallic acid calibration curve and expressed as mg GAE/g DM, following the procedure described by Grover *et al.* (2024).^[12]

TFC

Flavonoid content was analyzed using the aluminum chloride (AlCl_3) colorimetric assay with slight modifications. For this, 1 mL of the methanolic extract was added to a 10 mL volumetric flask that already contained 4 mL of distilled water. Then 0.3 mL of 5% sodium nitrite was added, followed by 3 mL of 10% AlCl_3 . After 1 min, 2 mL of 1 molar sodium hydroxide was added, and the solution volume was adjusted to 10 mL with distilled water. The resulting pink solution was thoroughly mixed, and its absorbance was read at 510 nm using a UV-Vis spectrophotometer. A quercetin standard curve (10–100 $\mu\text{g}/\text{mL}$) was prepared, and results were expressed as mg QE/g.^[12]

Statistical analysis

The proximate composition test was performed in triplicate ($n = 3$) to ensure the reliability of the measurements. The results were expressed as mean standard deviation (SD). The coefficient of variation was calculated to assess the relative variability of the data, using Equation 1.

$$CV_i (\%) = (Standard\ Deviation\ (SD)/Mean) \times 100 \quad (\text{Eq.1})$$

This approach allowed evaluation of both central tendency and precision in the analytical results. Statistical analyses were performed using Microsoft Excel 2019 (Microsoft Corp., USA).

RESULTS AND DISCUSSION

Proximate analysis

Table 1 presents the proximate composition of pearl millet, finger millet, sorghum, and wheat flour. The moisture content of flours impacts the storage stability, microbial safety, and processing parameters. Our study found that finger millet flour had the lowest moisture content ($8.11 \pm 0.02\%$), followed by pearl millet ($9.12 \pm 0.02\%$), wheat ($10.22 \pm 0.02\%$), and sorghum flour ($10.33 \pm 0.04\%$). The moisture values of all the tested flours were below the permissible level (14.5%), ensuring that all samples are microbiologically safe for storage, have reduced enzymatic activity, and can be processed.^[13] Such low moisture values are advantageous for the formulation of functional and therapeutic food ingredients, as they minimize oxidative and microbial degradation during storage.

The protein plays a crucial role in determining both the nutritional value and the functional properties of dough.^[14] Our study found that wheat flour had the highest protein content ($13.68 \pm 0.11\%$), followed by pearl millet ($10.87 \pm 0.02\%$), sorghum ($10.21 \pm 0.03\%$), and finger millet ($7.39 \pm 0.01\%$). These findings are consistent with those of Owheruo *et al.* (2019), they have reported that finger millet protein content was lower than that of pearl millet flour.^[15] Similarly, another study has demonstrated that, compared to sorghum, pearl millet flour has a higher protein content.^[16] Thus, our findings are consistent with previously published literature. However, for baked goods, gluten plays a crucial role, primarily as the protein in wheat; this protein helps retain gas and provides elasticity during bread manufacturing.^[17] However, the lack of gluten in millet flour limits its use in baked products. At the same time, they are generally used in baked products for their nutritional values; for example, they are a good source of essential amino acids such as lysine.^[18] Thus, incorporating millet into bakery products can improve protein quality and amino acid profile.

The pearl millet flour had the highest content of lipids ($6.05 \pm 0.03\%$), followed by sorghum ($2.76 \pm 0.01\%$), finger millet ($2.06 \pm 0.01\%$), and wheat ($1.49 \pm 0.03\%$). These findings are consistent with previous literature.^[15,19,20] Flours with a high fiber content are susceptible to lipid oxidation. This highlights that the pearl millet flour should be packed in oxygen-impermeable materials.^[21] From a nutraceutical perspective, millet lipids are rich in unsaturated fatty acids, tocopherols, and phytosterols, which confer potential cardioprotective and antioxidant benefits. Carbohydrates

were the primary macronutrient among all flours, with finger millet showing the highest level ($75.34 \pm 0.02\%$), followed by wheat ($73.18 \pm 0.10\%$), sorghum ($72.71 \pm 0.10\%$), and pearl millet ($69.93 \pm 0.04\%$). Carbohydrates are the primary component responsible for the significant calories in flour.

Crude fiber was substantially higher in millet flours compared to wheat flour. Finger millet exhibited the highest fiber content ($4.78 \pm 0.02\%$), followed by pearl millet ($2.40 \pm 0.02\%$), sorghum ($2.58 \pm 0.03\%$), and wheat ($0.62 \pm 0.02\%$). The high dietary fiber content of millets offers various health benefits, including improved gut health, better glycemic regulation, and increased satiety.^[3] However, a high fiber content may reduce consumer acceptability of bread in terms of its textural properties.^[22] At the same time, high fiber is advantageous for the development of porridge, cookies, and extruded snacks.^[23-25] Pearl millet has relatively lower carbohydrate and higher fiber content; therefore, it can be used for the development of low-glycemic index foods.^[26] Functional foods developed from pearl millet can be used to manage diabetes, as demonstrated in clinical studies.^[27] Ash content indicated the total mineral content, and our study found that the ash content of pearl, finger, sorghum, and wheat flour was $1.63 \pm 0.02\%$, $2.32 \pm 0.01\%$, $1.41 \pm 0.01\%$, and $0.81 \pm 0.02\%$, respectively. These findings align with previous studies, indicating that millets are rich sources of essential minerals, including iron, calcium, magnesium, and zinc, offering potential nutraceutical and pharmaceutical relevance for anemia prevention, bone health, and enzymatic regulation.^[28,29] Overall, these findings highlight the nutritional benefits of the millets and sorghum over wheat in terms of bioactive and mineral composition. Their inclusion in functional food formulations, clinical nutrition interventions, and therapeutic diets can contribute to nutritional security and reduce disease risk, aligning with the global shift toward plant-based, nutrient-dense functional ingredients.

Total flavonoid compounds and total phenolic compounds

Our findings found that TPC varies significantly among the different cereal flours [Table 2]. The sorghum flour exhibited the highest TPC value (6.32 ± 0.16 mg GAE/g), followed by pearl millet (4.62 ± 0.34 mg GAE/g), finger millet (3.49 ± 0.25 mg GAE/g), and wheat (0.84 ± 0.16 mg GAE/g). These differences can be attributed to the intrinsic genetic variability and environmental conditions.^[30] These findings align with a previous study, which states that the TPC of sorghum is higher than that of wheat.^[31] Phenolic compounds are responsible for the antioxidant potential and can help reduce oxidative stress and the risk of chronic diseases.^[32] The higher TPC of sorghum and pearl millet flour suggests that they can neutralize reactive oxygen species, which could be beneficial in mitigating oxidative stress-mediated pathophysiological processes, including diabetes, cardiovascular disorders, and neurodegenerative diseases.

Table 1: The proximate composition of pearl millet, finger millet, sorghum, and wheat flour

Parameter	Pearl millet flour		Finger millet flour		Sorghum flour		Wheat flour		F-value	P-value
	Mean±SD	Cvi	Mean±SD	Cvi	Mean±SD	Cvi	Mean±SD	Cvi		
Moisture (%)	9.12±0.02 ^b	0.24	8.11±0.02 ^c	0.21	10.33±0.04 ^a	0.40	10.22±0.02 ^a	0.17	987.4	<0.001
Crude protein (%)	10.87±0.02 ^b	0.19	7.39±0.01 ^d	0.19	10.21±0.03 ^c	0.28	13.68±0.11 ^a	0.84	1632.1	<0.001
Crude fat (%)	6.05±0.03 ^a	0.55	2.06±0.01 ^c	0.69	2.76±0.01 ^b	0.51	1.49±0.03 ^d	1.76	2231.8	<0.001
Crude fiber (%)	2.40±0.02 ^b	0.71	4.78±0.02 ^a	0.43	2.58±0.03 ^b	1.27	0.62±0.02 ^c	3.95	1349.6	<0.001
Ash (%)	1.63±0.02 ^b	1.33	2.32±0.01 ^a	0.54	1.41±0.01 ^c	1.00	0.81±0.02 ^d	2.55	1527.3	<0.001
Carbohydrate (%)	69.93±0.04 ^c	0.05	75.34±0.02 ^a	0.02	72.71±0.10 ^b	0.14	73.18±0.10 ^b	0.13	1023.9	<0.001
Energy (kcal)	377.65±0.24 ^a	0.06	349.47±0.18 ^d	0.05	356.53±0.33 ^c	0.09	360.87±0.23 ^b	0.06	873.5	<0.001

Values are mean±standard deviation (n=3). Different superscript letters within a row indicate significant differences among cereals according to Tukey's HSD test (P<0.05)

Table 2: Total phenolic content and total flavonoid content of cereal flour

Cereal	TPC (mg GAE/g)	CVI	TFC (mg QE/g)	Cvi
Sorghum	6.32±0.16 ^a	2.53	2.49±0.35 ^a	13.65
Pearl millet	4.62±0.34 ^b	7.36	1.23±0.17 ^b	13.82
Finger millet	3.49±0.25 ^c	7.16	0.98±0.18 ^b	18.37
Wheat	0.84±0.16 ^d	19.05	0.52±0.08 ^c	15.38
F-value	226.4		48.2	
P-value	<0.001		<0.001	

Values represent mean±standard deviation (n=3). Different superscript letters indicate significant differences between cereals according to Tukey's HSD test (P<0.05).

Pearl millet also exhibits a relatively high phenolic content compared to wheat, with the primary phenolic acids identified as ferulic, p-coumaric, and caffeic acids, which are known to exert antiglycation, antihypertensive, and anti-inflammatory effects.^[33] These bioactive compounds may contribute to improved endothelial function, modulation of glucose metabolism, and inhibition of lipid peroxidation, thereby extending the potential pharmacological relevance of millet-based foods.

A similar trend was observed for TFC. Sorghum flour had the highest TFC (2.49 ± 0.35 mg QE/g), followed by pearl millet (1.23 ± 0.17 mg QE/g), finger millet (0.98 ± 0.18 mg QE/g), and wheat (0.52 ± 0.08 mg QE/g). The high TFC in sorghum is attributed to the high levels of anthocyanins, flavan-3-ols, and flavones, especially in pigmented sorghum varieties.^[34] These flavonoids are known for modulating oxidative stress and inflammatory cascades by activating the Nrf2 pathway and inhibiting the NF-κB pathway, thereby emphasizing their therapeutic potential in the prevention of chronic diseases.

Finger millet also exhibits moderate flavonoid levels, while wheat again recorded the lowest TFC, validating the trend observed for phenolic compounds. These observed differences among cereals highlight the potential of millets, particularly

sorghum and pearl millet, as functional food ingredients with higher phenolic and flavonoid concentrations compared to wheat. This highlights that sorghum, pearl millet, and finger millets are promising candidates in the development of nutraceuticals, functional foods, and health-oriented dietary products aimed at combating oxidative stress-related conditions such as diabetes, cardiovascular diseases, and neurodegenerative disorders. However, millet utilization may be limited due to the presence of anti-nutritional factors such as tannins, phytates, and oxalates, which can affect mineral bioavailability and functional properties. Whereas the traditional processing methods, such as soaking, fermentation, and germination, have been shown to reduce these components and enhance the nutritional quality of millet-based foods.

Finger millet also exhibited moderate flavonoid levels, whereas wheat showed the lowest TFC, validating the trend observed for TPC. These variations among cereals emphasize the potential of millets, particularly sorghum and pearl millet, as functional food ingredients owing to their higher phenolic and flavonoid concentrations compared to wheat. This suggests that sorghum, pearl millet, and finger millet are promising candidates for the development of nutraceuticals, functional foods, and health-oriented dietary products aimed at mitigating oxidative stress-related conditions such as diabetes, cardiovascular diseases, and neurodegenerative disorders. However, the utilization of millets may be limited by the presence of anti-nutritional factors, such as tannins, phytates, and oxalates, which can reduce mineral bioavailability and impact functional properties. Traditional processing methods, including soaking, fermentation, and germination, have been demonstrated to reduce these compounds and thereby improve the nutritional quality of millet-based foods. Furthermore, incorporating processed millets into composite flours or ready-to-cook formulations may enhance consumer acceptance and broaden their application in modern diets. Future studies could explore optimized processing techniques to balance nutritional quality, sensory acceptability, and functional benefits of millet-based products.

Limitations of the study

While the present study provides valuable comparative data on the proximate composition, phenolic content, and flavonoid content of millets, sorghum, and wheat flour, certain limitations need to be acknowledged. First, the analysis was conducted on flour samples available from the local market of Ambala, India, which may not fully capture the variability across different cultivars, geographical regions, or seasonal harvests. Cereals are known to exhibit significant genetic and environmental diversity, which can influence their nutrient and phytochemical profiles; hence, the findings cannot be generalized to all varieties. Second, only TPC and TFC were estimated using spectrophotometric methods, which, while useful for comparative purposes, do not provide information on the specific phenolic compounds present or their individual bioactivities. Advanced analytical approaches, such as high-performance liquid chromatography, liquid chromatography-tandem mass spectrometry (LC-MS/MS), or metabolomics, would offer a more detailed characterization.

Another limitation lies in the exclusive use of *in vitro* estimations without assessing *in vivo* bioavailability and bioaccessibility. The biological relevance of phenolic compounds depends not only on their concentration but also on their release during digestion, metabolism, and interaction with gut microbiota. Furthermore, the study did not evaluate the impact of processing methods, such as fermentation, cooking, extrusion, or baking, which are known to alter the nutritional and phytochemical profiles of cereals significantly. Finally, the study was limited to proximate, phenolic, and flavonoid parameters, excluding other relevant phytochemicals, such as carotenoids, phytosterols, and dietary fibers, that may also contribute to health-promoting properties.

CONCLUSION AND FUTURE PROSPECTS

The comparative evaluation of millet, sorghum, and wheat flours demonstrates that millets and sorghum possess distinct nutritional and functional advantages over wheat. Millets, particularly finger millet and pearl millet, were found to be richer in dietary fiber, ash, and phenolic content, while sorghum exhibited notable flavonoid concentrations with potential antioxidant benefits. In contrast, wheat, although widely consumed, exhibits comparatively lower levels of bioactive compounds, highlighting the importance of diversifying cereal consumption to enhance dietary quality. These findings reinforce the potential role of millets and sorghum as functional food ingredients that can contribute to the prevention and management of lifestyle-related disorders due to their superior phenolic and flavonoid profiles.

By highlighting these differences, the study provides scientific evidence to support policy-level interventions and consumer awareness initiatives that promote the inclusion

of underutilized grains such as millets and sorghum into mainstream diets. The results also underline the importance of repositioning traditional cereals within modern food systems as sustainable, nutrient-rich, and health-promoting alternatives.

Future research should focus on expanding the scope of comparative studies to include multiple cultivars of millets, sorghum, and wheat grown across India's diverse agroclimatic zones, thereby generating more representative datasets. Advanced analytical techniques, such as LC-MS/MS and nuclear magnetic resonance-based metabolomics, should be employed for the detailed profiling of individual phenolic compounds and flavonoids, along with their antioxidant and anti-inflammatory bioactivities. Investigating the bioaccessibility and bioavailability of these phytochemicals through simulated digestion models and human intervention studies will provide a clearer understanding of their physiological relevance.

In addition, the impact of traditional and modern processing methods (e.g., fermentation, sprouting, extrusion, and baking) on the stability and transformation of phenolic compounds warrants systematic investigation. Given the current global emphasis on functional foods, future work should also explore the development of millet- and sorghum-based formulations with optimized sensory properties and validated health benefits. Integrating clinical and nutrigenomics studies will further elucidate the role of these cereals in modulating metabolic pathways linked to NCDs. On a broader scale, the findings can inform policy frameworks and nutrition guidelines, thereby supporting the mainstreaming of millets and sorghum in sustainable food systems and contributing to national and global food security initiatives.

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