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# Comprehensive Analysis of Proximate, Antioxidant, Functional, and Anti-Nutritional Properties of Millet Flour: A Comparative Study of Brown Top Millet, Little Millet, and Foxtail Millet

MADHURI TIWARI[1], DEVINDER KAUR[1*] AND VINEETA PURANIK[1]
*[1*]Centre of Food Technology, University of Allahabad, Prayagraj. India*
**Corresponding E-mail: devi_sonu@yahoo.com*

***Abstract:*** This study presents a comprehensive evaluation of the proximate composition, antioxidant activity, functional properties, and anti-nutritional factors of flours derived from three millet varieties: Brown Top Millet (*Panicum ramosum*), Little Millet (*Panicum sumatrense*), and Foxtail Millet (*Setaria italica*). The proximate analysis revealed significant variations in the nutritional composition, with Foxtail Millet exhibiting the highest protein content (11.92 ± 0.11%) and moisture content (11.06 ± 0.35%), while Brown Top Millet showed the highest carbohydrate content (75.47 ± 0.00%) and ash content (5.38 ± 0.31%). Functional property assessment indicated that all millet flours had similar bulk density (0.10 ± 00 g/ml), with Little Millet demonstrating the highest oil absorption capacity (245.51 ± 0.50%) and Foxtail Millet exhibiting the highest swelling power (9.41 ± 2.45 g/g). Antioxidant analysis highlighted Foxtail Millet as having superior antioxidant activity, with DPPH radical scavenging activity of 85.38 ± 0.10%, total phenolic content of 77.15 ± 0.25 mg GAE/g, and total flavonoid content of 74.15 ± 0.06 mg/g. The study also identified antinutritional factors, with Little Millet showing the lowest phytic acid content (330 ± 0.10 mg/100g) but the highest tannin content (3.33 ± 0.67%). The findings demonstrate the unique nutritional and functional properties of each millet type, offering valuable insights for their application in food product development and potential health benefits.

***Keywords*:** Proximate analysis, Functional properties, Antioxidant activity, Antinutritional factors, Foxtail millet, Brown top millet, Little millet, Nutritional composition.

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## 1. Introdution

Millets are small, round-shaped cereal grains that come in various colors and sizes, depending on the specific variety. They belong to the Poaceae plant family, which also includes maize and sorghum. These plants naturally thrive in arid and semi-arid regions, such as those found in central Africa and Asia (Saleh *et al.,* 2013). India led millet production in Asia, yielding 11,066 metric tons, with China following closely. In Africa, Nigeria was the largest producer, with 5,163 metric tons, followed by Niger and Sudan (FAO STAT). Millets are often referred to as "Nutri Cereals" due to their rich nutritional profile. They are an excellent source of protein, dietary fiber, B-complex vitamins, and essential minerals such as iron, zinc, potassium, magnesium, and calcium. These grains are known to help manage conditions like diabetes and can aid in preventing other lifestyle-related disorders, including cardiovascular diseases (Dayakar *et al*., 2017). Brown top millet, one of the lesser-known and least explored millet varieties, has recently started gaining attention. India, particularly the arid plains of Karnataka and Andhra Pradesh, is the traditional home of brown top millet (*Panicum ramosum*). This millet thrives especially well in districts like Tumkur, Chitradurga, Chikkaballapura, and Bellari (Ashoka and Sunitha, 2020). Browntop millet is a small, green-tinted grain known for its exceptionally high fiber content, which is around 12%, making it the highest among millets. Due to its high fiber content, browntop millet may be beneficial in preventing common health conditions, including diabetes (Ashoka and Sunitha, 2020; Sarita and Singh, 2016). Foxtail millet (*Setaria italica*) is recognized as a significant millet variety globally, ranking as the sixth highest-yielding grain in terms of production (Saleh *et al*., 2013). Foxtail millet is among the world's oldest cultivated crops, with archaeological evidence of its earliest cultivation found in northern China, dating back approximately 7,400–7,935 years. Remains of this ancient grain have also been discovered in Europe, dating back around 4,000 years (Lu *et al*., 2009). Foxtail millet is rich in essential nutrients, including starch, protein, vitamins, and minerals (Table 1). Due to its coarse texture, about 79 % of foxtail millet is digestible, while the remaining portion is high in fiber and contains some anti-nutritional components. Like other millets, foxtail millet is a good source of crude fiber, which aids digestion and promotes bowel movements, acting as a natural laxative that supports a healthy digestive system (Bernard, 1996). These nutritional qualities have made foxtail millet a key ingredient in China for preparing noodles, nutritious gruels or soups, brewing alcoholic beverages, and making cereal porridges and pancakes (Krishna, 2013). Beyond its nutritional value, foxtail millet has been

found to offer several health benefits, including cancer prevention, as well as hypoglycemic and hypolipidemic effects (Yang *et al*., 2013; Zhang *et al*., 2015). Little millets, also known as saamai or kutki, are short-duration grains that can tolerate both drought conditions and waterlogging. Little millet is also referred to as "cool food" due to its cooling effect on the body when eaten during the summer (Pradeep and sreerama, 2018). Like other millets, little millet offers superior nutritional benefits compared to cereals, though its consumption is limited. It is also rich in nutraceuticals such as resistant starch, phytates, phenolics, sterols, lignans, and gamma-aminobutyric acid (Anurag *et al*., 2018).

This research aims to provide a thorough examination of the proximate, antioxidant, functional, and anti-nutritional properties of millet flours, specifically focusing on Brown Top Millet, Little Millet, and Foxtail Millet. By comparing these three varieties, we seek to highlight their unique nutritional profiles and functional benefits, as well as their potential drawbacks. Understanding these aspects will contribute valuable insights into their suitability for various dietary applications and their role in promoting health and well-being. This comprehensive analysis will not only enhance our knowledge of millet as a staple food but also support informed decisions regarding its utilization in both traditional and modern culinary practices.

## 2. Materials and Methods

### *2.1. Millet flours sample*

Three different types of millet flour were obtained from Sri Saraswathi Organics Pvt Ltd. and Sri SaraswathiSiridhnaya Mill, located on JogiMatti Road, opposite Nagara Katte, close to DIC, Chickpet, Chitradurga, Karnataka. The millet flours were brown top millet, little millet, and foxtail millet.

### *2.2. Determination of proximate composition of millets flour*

Using the standard analytical techniques described by AOAC (2016), the proximate composition of millet flours, comprising moisture, ash, protein, fat, and crude fibre, was ascertained. The sample was dried at 105°C until a consistent weight was reached in order to determine the moisture content. Weighing the residue left over after the sample was burned for eight hours at 650–700°C allowed us to quantify the amount of ash present. While crude fibre was examined in accordance with the AOAC (2005) protocol, crude fat was extracted using the Soxhlet method with petroleum ether. The Kjeldahl method was utilized to calculate crude protein by employing the factor N ×

6.25. Subtracting the total percentages of moisture, ash, crude fibre, fat, and protein from 100 yielded the carbohydrate content (%), in accordance with the AOAC (2005) recommendations. Carbohydrate (%) = 100 - (% moisture + % fat + % crude fibre + % protein + % ash) was the calculation that was applied.

## 2.3. Antioxidant Properties

### 2.3.1. Method of extraction of antioxidant

Following a 24-hour period at room temperature, a 1-gram sample of flour was dissolved in 10-milliliters of 50 % methanol. 978 x g centrifugation was used for 15 minutes to separate the mixture after incubation. Following the procedure outlined by (Moore *et al.*, 2006), the resultant supernatant was filtered through Whatman filter paper and refrigerated at 4°C for subsequent antioxidant analysis.

### 2.3.2. DPPH radical scavenging activity

Using the DPPH radical scavenging method, the antioxidant activity of millet flour extracts was evaluated in accordance with the protocol described by De Ancos *et al.* (2002). 90 µL of distilled water, 3.9 mL of a methanolic 0.1 mM DPPH solution, and a 10 µL aliquot of the acidified methanolic extract were mixed together. After giving this mixture a thorough vortex, it was kept in the dark for half an hour. The antioxidant activity was represented as the % suppression of the DPPH radical, and the absorbance was measured at 515 nm. The following formula was used to calculate the % inhibition.

$$\% \text{ inhibition of DPPH} = [\text{Abs control} - \text{Abs sample}/\text{Abs control}] \times 100$$

### 2.3.3. Total phenolic content

Singleton, Orthofer, and Lamuela-Raentos (1995) proposed a spectrophotometric method for measuring the total phenolic content in the millet flour extracts. Using this approach, a 50 ml volumetric flask was filled with 0.1 ml of acidified methanolic extract and 5 ml of distilled water. 2.5 ml of a 1:2 diluted Folin-Ciocalteu's reagent and 7.5 ml of a 15 % sodium carbonate solution were added to this combination. After the mixture was well combined, it was increased to a final volume of 50 millilitres. The blue hue that appeared after 30 minutes was measured at 760 nm. The phenolic content was reported as mg of gallic acid equivalents (GAE) per 100 g of the sample on a dry weight basis, and a calibration curve was created using a standard gallic acid solution.

### *2.3.4. Total flavonoid content*

The method outlined by (James *et al.*, 2008) was used to determine the total flavonoid content (TFC) of the millet flour extract. 150 µl of 10 % $AlCl_3$ was mixed with two millilitres of methanolic extract. After ten minutes, 1 millilitre of 1 M NaOH and 1.2 millilitres of distilled water were added. Following an additional incubation of 10 minutes, the absorbance was measured at 510 nm in comparison to a blank.

## *2.4. Functional Properties*

### *2.4.1. True density and Bulk density*

According to ASAE (2001), the liquid displacement method was used to assess the true density. The method outlined by Wang and Kinsella (1976) was used to calculate the samples' bulk densities. Using this procedure, a 50 ml graduated cylinder containing five grammes of the sample was tapped on a table until the volume of the sample remained constant. Next, the sample's final volume was noted. The following formulas were used to get the bulk density:

Bulk Density (g/ml) = Weight of Sample/ Volume of Sample

### *2.4.2. Water and oil absorption capacity*

The method of (Wani *et al.*, 2013) was used to calculate the samples' oil absorption capacity (OAC) and water absorption capacity (WAC). Using a glass rod, one gramme of the sample was combined with 10 millilitres of either water or refined vegetable oil in a 30-milliliter centrifuge tube. For half an hour, the tube was kept at room temperature. After centrifuging the mixture for 25 minutes at 1,917 x g, the clear supernatant was poured into a measuring cylinder. The tube was inverted to remove any last droplets. Weighing was done on the tube, sample, and absorbed water or oil. The oil/water absorption capacity was determined by calculating the amount of water or oil that was absorbed by 1 g of the sample.

$$\text{Water/Oil Absorption Capacity (\% )} = \frac{\text{(Final weight of tube + sample) - (Initial weight of tube + sample)} \times 100}{\text{weight of sample}}$$

### *2.4.3. Swelling Capacity*

The technique described by (Raghavendra *et al.*, 2004) was used to assess swelling capacity. A calibrated measuring container was filled to the designated

level with distilled water after five grams of the dry sample were added. The cylinder was then allowed to incubate for eighteen hours at room temperature. The wheat bran's final volume was measured following the incubation period.

SC was calculated using a formula

SC (mL/g)= volume occupied by sample / original sample weight

## 2.5. *Antinutritional Factors*

### *2.5.1. Determination of Tannin content*

With minor adjustments, the Schanderl (1976) approach was used to analyse the tannin content. A 0.5 g sample of millet flour was weighed and then placed in a 250 ml conical flask with 75 ml of distilled water. For thirty minutes, the flask was slowly boiled. Following a boil, the mixture was centrifuged for 20 minutes at 2000 rpm, collecting the supernatant and adjusting the volume to the mark in a 100 ml volumetric flask. After that, a meticulous 1 ml of the sample extract was transferred to a different flask that included 10 ml of sodium carbonate solution, and it was diluted with distilled water to make 100 ml.After giving the mixture a good shake and letting it sit for 30 minutes, the colour that appeared was assessed at 700 nm using a spectrophotometer. A standard curve was made using tannic acid, and the percentage of tannin in the sample was given as the outcome. In the event that the absorbance was higher than 0.7, a 1:4 sample dilution was used. Tannic acid in the dose range of 10-100 mg was used to create a standard graph. The sample's tannin content was determined using the standard graph and expressed as a percentage (%).

### *2.5.2. Determination of Phytate content*

The technique outlined in AOAC (2005) was used to ascertain the phytate concentration of millet flours. A 125 ml Erlenmeyer flask containing a flour sample was weighed, and 3 % TCA was employed for extraction. An $FeCl_3$ solution was combined with a 10-milliliter aliquot following the suspension's centrifugation. The heated mixture was then treated with a few drops of 3 % TCA. Following the addition of 3 millilitres of 1.5 M NaOH, the mixture was filtered using Whatman No. 2 filter paper. After dissolving the precipitate in 40 millilitres of hot 3.2 N $HNO_3$, the filter paper was rinsed several times with water, collecting the rinses in the same flask. After the flask cooled, water was added to dilute the solution to the appropriate volume, and the colour was measured at 480 nm in just one minute.

## 3. Statical Analysis

Data were presented as means of triplicate measurements from the nutritional, functional, antinutritional, and antioxidant activity studies. IBM SPSS Statistics 16 software was used for the analysis. One-way analysis of variance (ANOVA) with a significance threshold of $p < 0.05$ was used to determine statistical significance, and Duncan's test was used after.

## 4. Results and Discussion

### *4.1. Proximate Composition of flour from selected millets*

The proximate composition of the millet flours reveals significant differences across the three varieties (Table 1). Foxtail Millet exhibited the highest protein content (11.92 ± 0.11 %) and moisture content (11.06 ± 0.35 %), suggesting its potential as a protein-rich food source. The moisture content, while beneficial for maintaining freshness, may also make Foxtail Millet more susceptible to microbial growth, necessitating proper storage. The moisture (9.3 %), protein (11.6 %), fat (4.9 %), crude fiber, and carbohydrate (76 %) content of foxtail millet flour reported by Devisetti *et al*. (2014) are consistent with the findings of the present study. The proximate composition of Brown Top Millet flour observed in the present study is consistent with the values reported in the literature by Sirisha *et al*. (2022). Brown Top Millet had the highest carbohydrate content (75.47 ± 0.00 %) and ash content (5.38 ± 0.31 %), indicating its value as an energy-dense food with potentially higher mineral content. In contrast, Little Millet had the lowest ash content (1.24 ± 0.20%) but a relatively high carbohydrate content (73.73 ± 0.00 %), positioning it as a good energy source with lower mineral content. These variations highlight the potential of each millet type to fulfill different nutritional needs depending on dietary requirements.

**Table 1: Proximate Analysis of flour from selected millets**

| *Millets flour* | *Moisture (%)* | *Ash (%)* | *Protein (%)* | *Fat (%)* | *Crude Fibre (%)* | *Carbohydrate (%)* |
|---|---|---|---|---|---|---|
| Foxtail | 11.06±0.35$^{a}$ | 4.24±0.22$^{c}$ | 11.92±0.11$^{a}$ | 4.25±0.02$^{a}$ | 13.61±0.34$^{b}$ | 67.92±0.48$^{c}$ |
| Brown top | 8.80±0.90$^{b}$ | 5.38±0.31$^{b}$ | 8.6±0.20$^{c}$ | 1.7±0.08$^{c}$ | 8.1±0.30$^{c}$ | 75.47±00$^{a}$ |
| Little millet | 11.20±0.32$^{b}$ | 1.24±0.20$^{a}$ | 10.19±0.26$^{b}$ | 3.66±0.17$^{b}$ | 7.4±0.37$^{a}$ | 73.73±00$^{b}$ |

*Note:* All values represent the means ± standard deviations from three separate experiments. Different superscripts (a to c) within the same column denote significant differences ($p < .05$).

## 4.2. Functional Properties of flour from selected millets

Functional properties such as bulk density, true density, water absorption capacity (WAC), oil absorption capacity (OAC), and swelling power demonstrated significant variation among the millet flours (Table 2). All millet flours had a similar bulk density (0.10 ± 00 g/ml), suggesting consistent behavior in food formulations where bulk density is a critical factor. The higher swelling power of Foxtail Millet (9.41 ± 2.45 g/g) compared to the other millets may enhance its suitability in products requiring thickening or gelling properties. On the other hand, Little Millet showed the highest OAC (245.51 ± 0.50 %), which could improve its application in food products that require enhanced mouthfeel and flavor retention, such as baked goods and meat substitutes. The functional properties, including bulk density, water absorption, oil absorption, and swelling capacity, are consistent with the values reported by Abedin *et al*. (2022). The moderate WAC in all millet flours indicates their potential for use in products where moisture retention is important, such as in bakery and processed food items.

**Table 2: Functional Properties of flour from selected millets**

| *Millets Flour* | *Bulk density(g/ml)* | *True density(g/ml)* | *WAC(%)* | *OAC(%)* | *Swelling power(g/g)* |
|---|---|---|---|---|---|
| Foxtail | 0.10±00$^{a}$ | 1.41±0.15$^{c}$ | 118.75±0.22a | 129.42±0.42$^{b}$ | 9.41±2.45$^{a}$ |
| Brown top | 0.10±00$^{a}$ | 1.22±0.02$^{b}$ | 104.5±2.3$^{a}$ | 231.18±18$^{a}$ | 9.2±1.20$^{a}$ |
| Little millet | 0.10±00$^{a}$ | 1.33±0.04$^{a}$ | 107±2.04$^{b}$ | 245.51±0.50$^{c}$ | 8.73±1.80$^{a}$ |

*Note:* All values represent the means ± standard deviations from three separate experiments. Different superscripts (a to c) within the same column denote significant differences ($p < .05$).

## 4.3. Antioxidant Properties of flour from selected millets

The antioxidant properties of the millet flours, including DPPH radical scavenging activity, total phenolic content (TPC), and total flavonoid content (TFC), varied significantly (Table 3). Foxtail Millet exhibited the highest antioxidant activity, with DPPH scavenging activity of 85.38 ± 0.10%, TPC of 77.15 ± 0.25 mg GAE/g, and TFC of 74.15 ± 0.06 mg/g. The antioxidant properties, including DPPH, TPC, and TFC, of millet flours are consistent with the results reported by Devisetti *et al*. (2014) and Abedin *et al*. (2022). These results suggest that Foxtail Millet could be an excellent source of antioxidants, making it a potential ingredient for functional foods aimed at reducing oxidative stress and promoting overall health. In comparison, Brown Top Millet showed the lowest

antioxidant activities, indicating that it may be less effective in applications where high antioxidant content is desired. However, it may still provide other nutritional benefits, as seen in its proximate composition.

**Table 3: Antioxidant properties of flour from selected millets**

| *Millets flour* | *DPPH (%)* | *TPC (mg GAE/g)* | *TFC (mg/g)* |
|---|---|---|---|
| Foxtail | 85.38±0.10[a] | 77.15±0.25[a] | 74.15±0.06[a] |
| Brown top | 72.40±0.13[c] | 70.30±0.19[c] | 63.13±0.40[c] |
| Little millet | 77.64±0.09[b] | 71.49±0.42[b] | 68.86±0.65[b] |

*Note:* All values represent the means ± standard deviations from three separate experiments. Different superscripts (a to c) within the same column denote significant differences ($p < .05$).

## *4.4. Anti-nutritional factors of flour from selected millets*

The analysis of antinutritional factors revealed that Little Millet had the lowest phytic acid content (330 ± 0.10 mg/100g) but the highest tannin content (3.33 ± 0.67%)(Table 4). High tannin content can negatively affect the bioavailability of nutrients, particularly proteins and minerals, suggesting that Little Millet may require processing methods such as soaking, fermentation, or cooking to reduce tannin levels before consumption. Foxtail Millet had a higher phytic acid content (620 ± 0.14mg/100g), which can impair mineral absorption, though it showed moderate tannin levels. Brown Top Millet, with a moderate phytic acid content (490± 0.31 mg/100g) and lower tannin content, may present a balanced option among the millets, with fewer concerns related to antinutritional factors. According to the literature by Sirisha *et al*. (2022), the phytic acid content of Brown Top Millet flour is 368.33 mg/100 g, and the tannin content is 2.12 %. These values are consistent with the findings of the present study.The phytic acid content and tannin percentage of foxtail millet flour are similar to the values reported by Devisetti *et al*. (2014).

**Table 4: Antinutritional factors of flour from selected millets**

| *Millets flour* | *Phytic (mg/100g)* | *Tannin (tannic acid %)* |
|---|---|---|
| Foxtail | 620±0.14[a] | 2.64±0.36[a] |
| Brown top | 490±0.31[b] | 1.39±0.62[a] |
| Little millet | 330±0.10[b] | 3.33±0.67[a] |

*Note:* All values represent the means ± standard deviations from three separate experiments. Different superscripts (a, b) within the same column denote significant differences ($p < .05$).

## 5. Conclusion

The comprehensive analysis of the proximate, functional, anti-oxidant, and anti-nutritional properties of the three millet flours underscores the unique strengths and potential challenges of each type. Foxtail Millet stands out for its high protein and antioxidant content, making it an ideal candidate for health-focused food products. Brown Top Millet's high carbohydrate content and moderate functional properties suggest its suitability for energy-dense food formulations. Little Millet, with its high tannin content, may require careful processing but offers a good balance of energy and oil absorption properties, making it versatile in various culinary applications. Each millet type's distinct properties can be leveraged in food product development to meet specific nutritional and functional needs. However, the presence of antinutritional factors like tannins and phytic acid in these millets suggests the need for careful consideration in food formulation to optimize nutrient bioavailability. Overall, this study underscores the potential of millet flours as nutritious and functional ingredients, particularly in regions where they are staple foods, and supports their inclusion in diverse dietary applications to enhance nutritional quality.

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