Agriculture Association of Textile Chemical and Critical Reviews Journal (2024) 64-69

AATCC Review

22 February 2024: Received
29 March 2024: Revised
24 April 2024: Accepted
09 June 2024: Available Online

www.aatcc.peerjournals.net

Research Article | Open Access

Characterization of Physicochemical, Functional and Antioxidant Properties of Proso Millet (*Panicum Miliaceum*)

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ABSTRACT

Proso millet (Panicum miliaceum) is an underutilized minor millet traditionally consumed as bird feed but nowadays, because of an increase in awareness about the health benefits of millet, the demand for millet products is increasing in the market. Millets are gluten-free, have a low glycemic index, and possess many functional and nutraceutical properties. The physicochemical and functional properties of millets are quite different from the cereals as millets are gluten-free which can be a challenge to develop new products with millets substituting the cereals in the formulations. The millet flour properties such as physical, functional and nutrient components affect the product's processing steps and quality. Hence, the present study was conducted to analyze the physicochemical and functional properties of proso millet grain and its flour. The study found that the proso millet grains were small, oval in shape, and yellow in colour. The L^*, a^* and b^* values were 76.25, 5.20, and 27.83 respectively. The water absorption capacity, oil absorption capacity, and swelling capacity of the proso millet flour were 1.15 g/g, 1.16 g/g, and 4.51 ml/g with a good amount of protein (12.10%) and crude fiber (5.55%). Tannin content was 228.24 mg tannic acid equivalent per 100 g, total phenolic content was 25.17 mg gallic acid equivalent per 100 g and 45.17 per cent DPPH inhibition activity. It can be concluded that proso millet can be a nutritious gluten-free functional ingredient with good physical properties and nutrients. Proso millet has the potential properties and it can be incorporated into different food product formulations to make them more nutritious.

Keywords: *Antioxidants, functional properties, proso millet, tannins, phenols, gluten-free flour, nutritional composition.*

1. INTRODUCTION

Millets are the most ancient minor cereal crops also regarded as the poor man's cereal and nutria-cereals. The term millet was derived from the French word "*mille*" which signifies thousands of grains [1]. Millet is the world's sixth most important cereal grain and is a major source of energy and protein for millions of people in India, Africa, and China, especially for the people living in arid and semi-arid regions [2]. Millets are a good source of energy, protein, fatty acids, vitamins, minerals, dietary fiber, and polyphenols but still in India, the consumption of some nutritious minor millets like kodo millet (*Paspalum scrobiculate* L.), foxtail millet (*Setaria italic* (L.) P. Beauvois), little millet (*Panicum sumatrense* Roth ex Roem. &Schult.) and barnyard millet (*Echinochloa esculenta*) is very low. Millets are more nutritious than other cereal grains as they are non-glutinous non-acid-forming and easily digestible [3]

Proso millet (*Panicum miliaceum*) is one of the underutilized minor millets known for its highest protein content among minor millets. During different processing, millets behave differently from cereal grains as millets are gluten-free and high in fiber and fat as compared to cereals. Therefore, it is important to assess the physical, functional, and chemical properties of millets for the development of novel food products as they relate to the processing behaviour of foods and food quality.

Hence, the present study was carried out to find out the physical, functional, and chemical properties of proso millet flour.

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DOI: https://doi.org/10.21276/AATCCReview.2024.12.02.64

2. MATERIALS AND METHODS

2.1 Research Location: The present study was conducted at the Department of Food Science and Nutrition, College of Community Science, Dharwad, Karnataka, India in the year 2022.

2.2 Procurement of sample: A local variety of organic dehulled proso millet was procured from the Green Organics of Dharwad, Karnataka.

2.3 Physio-chemical and functional properties of proso millet grain

2.4 Physical characteristics of proso millet

Physical properties define the quality of the product which affects the quality parameters of the final product.

Weight: The weight of the flour was measured using an electronic weighing balance with a sensitivity of 0.01 g.

Volume: To determine the volume of the sample ten grams of the sample was poured into a 25 ml graduated measuring cylinder. The cylinder was tapped ten times from a height of 8 to 10 cm. Volume was noted in ml.

Bulk density: The bulk density of the sample was analyzed by the method adopted by [4]. The bulk density (g/ml) was calculated as the ratio of sample weight to the sample volume by using the following formula.
Bulk Density= Weight of the flour (g)/ Volume of the flour (ml)

Colour: The colour assessment was carried out using a Konica Minolta spectrophotometer of model CM 2600/2500d. The three chromatic components were assessed in terms of the L^*, and b^* values were analyzed. The larger the L^* value indicates more lightness, the larger a^* value indicates more redness and the larger b^* value signifies more yellowness [5].

2.5 Functional properties of proso millet grains

Functional properties include hydration capacity, hydration index, swelling capacity, and swelling index which were analyzed using standard AACC (2000) methods [6].

Swelling capacity: 1000 grains were soaked in distilled water for 12 hours. The water was drained. The adhering water to the grains was removed by gently pressing with blotting paper. The increase in volume after soaking was noted as ml/1000 grains.
Swelling capacity = Volume of grains after soaking-Volume of grains before soaking

Swelling index: The swelling index was calculated by using the formula:
Swelling index = (Swelling capacity)/(the volume of 1000 grains) x 100

Hydration capacity: Thousand grains were soaked in distilled water for 12 hours. The water was drained. The adhering water to the grain was removed by gently pressing with blotting paper. The increase in weight after soaking was noted as g/1000 grains.
Hydration capacity = Weight of grains after soaking-Weight of grains before soaking

Hydration index: The hydration index was calculated using the formula:
Hydration index = (Hydration capacity)/(Weight of 1000 grains) x 100

2.6 Functional properties of Proso millet flour

Water Absorption Capacity: It was analyzed by the method of [7]. Five grams of sample was weighed and added to a pre-weighed centrifuge tube (W1). To this, 30 ml of water was added and stirred with a glass rod for five min. The content was allowed to stand for 30 minutes and then centrifuged at 11,000 rpm for 25 minutes. The free liquid was poured off. The inner side of the tube was wiped with tissue paper and the weight of the centrifuge tube was noted again (W2). The water absorption capacity was calculated using the following formula.
Water absorption capacity (%)= (W2-W1)/Weight of the sample x 100

Oil Absorption Capacity: Oil absorption capacity was determined by the method given by [8]. One gram of the sample was mixed with 10 ml of oil for 30 seconds in a mixer. The sample was allowed to stand at room temperature (27°C) for 30 minutes then it was centrifuged at 5000 rpm for 30 minutes and the volume of the supernatant was noted in a 10 ml graduated cylinder.
Oil absorption capacity (%) = Volume of oil absorbed/Weight of the sample x 100

Swelling power: Swelling power indicates the hydration capacity of starch. It was determined by adding five grams of dehydrated samples in 100 ml of distilled water. It was kept overnight at room temperature (20-30°C). Then swelling power was calculated by the following formula given by [9].
Swelling power (ml/g) = Change in volume (ml)/Weight of sample (g)

2.7 Proximates composition of proso millet flour

The proximate composition includes macronutrients, micronutrients, phytochemicals, and antioxidants.

Moisture estimation: Moisture of the sample was determined according to the procedure of [10] and was expressed in a g/100g sample.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g)} - \text{final weight (g)}}{\text{weight of the sample (g)}} \text{ x } 100$$

Protein estimation: The protein content of the dried sample was estimated as per cent total nitrogen by the method of [10]. The nitrogen content was calculated using the following formula:

$$\text{Nitrogen content (\%)} = \frac{\text{1.4 x Normality of the acid x titrant value}}{\text{Sample weight (g)}} \text{ x } 100$$

The protein content (%) was estimated by multiplying the nitrogen content with the nitrogen-to-protein conversion factor i.e. 6.25.

$$\text{Protein content (\%)} = 6.25 \text{ x \% Nitrogen}$$

Fat estimation: The fat content of the sample was determined by using the solvent extraction method[10].

$$\text{Fat content (\%)} = \frac{\text{The final weight of beaker (g)} - \text{Initial weight of beaker (g)}}{\text{Weight of the sample (g)}} \text{ x } 100$$

Crude fiber estimation: The crude fiber content of the sample was estimated by gravimetric method [10]. The loss in weight of the sample after ashing was the crude fiber content of the sample.

$$\text{Crude fiber (\%)} = \frac{\text{Wt. after oven drying} - \text{Wt. after ashing in a muffle furnace}}{\text{Weight of the sample (g)}} \text{ x } 100$$

Ash estimation: Total ash was estimated according to the procedure given by [10].

$$\text{Ash content (\%)} = \frac{\text{crucible wt. with sample} - \text{crucible wt. after ashing}}{\text{Weight of the sample (g)}} \text{ x } 100$$

Carbohydrate estimation: The carbohydrate content was calculated by different methods i.e. deducing the sum of the value of moisture, protein, fat, ash, and crude fiber from 100 [10].
Total carbohydrate (g/100g) = 100 - [Moisture + Fat + Protein + Ash]
Available carbohydrate (g/100g) = 100 - [Moisture + Fat + Protein + Ash + Crude fiber]

Total calorific energy: The calorific value was derived by multiplying carbohydrates, protein, and fat contents of buckwheat cookies with water constants viz., 4, 4, and 9 respectively, and expressed as per 100 g basis [10].
Total calorific energy (Kcal) = [(4×Carbohydrate%) + (9×Fat %) + (4×Protein%)]

Dietary fiber estimation: The soluble, insoluble, and total dietary fiber fractions were analyzed by enzymatic-gravimetric method. It was estimated by using amyloglucosidase [10].

2.8 Mineral estimation

Macro mineral: The calcium content was estimated by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute acid against standard potassium permanganate [10].

$$\text{Calcium (mg/100g)} = \frac{\text{Titre value x 0.2004 x volume}}{\text{Weight of the sample x aliquot}} \text{X } 100$$

Micro-minerals (mg/100g): The trace elements (iron, zinc, copper, and manganese) were estimated by wet digestion using the triacid mixture. A known aliquot of the test sample was diluted and micro minerals in the test sample (Cu, Mn, Zn, and Fe) were determined using an atomic absorption spectrophotometer [10]. Calibration of measurements was performed using commercial standards.

2.9 Estimation of antioxidant components

Tannins: Tannins were estimated calorimetrically using Folin-Denis reagent (FDR) based on the measurement of the blue colour formed by the reduction of phosphotungstomolybdic acid present in Folin-Denis reagent in an alkaline solution. Tannic acid was used as the standard and results were expressed as tannic acid equivalent [11].

Total phenolic content (TPC): Total phenol estimation was carried out with the Folin-Ciocalteau reagent. Gallic acid was used as the standard and results were expressed as gallic acid equivalent [12].

Total antioxidant capacity (TAC): The total antioxidant capacity was analyzed by using DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging activity which is based on electron transfer that produces a violet solution in methanol. The spectrophotometric method with DPPH was applied to perform an antioxidant activity [10].

Statistical analysis: The statistical analysis of the data was carried out using IBM SPSS software version 22 and MS Excel 2019. The statistical results were expressed in terms of mean values and standard deviation.

3. RESULTS AND DISCUSSION

3.1 Physical Properties of Proso Millet Grains

The physical properties of millet grains are the properties that provide an idea about their characteristics and quality. These properties affect the quality of the value-added products. It is essential to assess these properties as are the parameters of grain quality.

The physical properties of dehusked proso millet grains are presented in Table 1. The three dimensions of the grains were measured it was found that the average length, breadth, and thickness of proso millet grains were 2.14, 1.84, and 1.84 mm respectively. The length and breadth ratio of the grain was 1.20 giving it an oval shape. The weight and volume of the thousand grains were 4.50g and 3.43 ml respectively with a bulk density of 1.31 g/ml. The colour analysis of proso millet grains found that the grains had lightness, redness, and yellowness with the L^*, a^*, and b^* values of 76.25, 5.20, and 27.83 respectively. The C^* value was found 28.16 which depicts the chroma or intensity of the colour of the grain. The hue angle value (H°) was 79.42.

The present study reported the length of the proso millet grains was 2.17 mm, the breadth was 1.84 mm, the thickness was 1.84 mm and the length and breadth ratio was 1.20 which provided an oval shape to the proso millet grains (Table 1). The study by [13] reported similar results where the length of proso millet was 2.18 mm, the breadth 1.86 mm and the length/breadth ratio was 1.75. The results of the present study conformed with Shidenur, 2021 who reported a length of 2.3 mm, breadth of 1.85 mm, and length/breadth ratio of 1.25. The findings of [14] reported 2.36 mm length, 1.85 mm breadth,1.41 mm thickness, 1.27 for L: B ratio, and 0.83 sphericity of proso millet.

The present study reported that the average weight of 1000 grains of proso millet was 4.50 g, the volume was 3.43 ml and the bulk density was 1.31 g/ml (Table 1). The same results with a weight of 4.85 g, a volume of 3.36 ml, and a bulk density of 1.34 g/ml per 1000 grains of proso millet were reported by [15]. Similarly, the results of the present study followed the results reported by [16], [14], [17], and [13].

In the present investigation, the proso millet grains had lightness, redness, and yellowness with the L^*, a^* and b^* values of 76.25, 5.20, and 27.83 respectively (Table 1). The C* value was found 28.16 which depicts the chroma or intensity of the colour of the grain. These quantitative colour values allow them to evaluate the quality of their grain products. The results of the present study are by [16] reported L^* was 76.09, a^* was 5.18, and b^* was 28.73. Similar results were also reported by [18]. The yellow colour of the proso millet may be due to the presence of carotenoid pigments [19].

3.2 Functional Properties of Proso Millet Grains

The functional properties of grains define their capacity to interact with other ingredients with the change in their physical properties. Functional properties are important parameters for product development as these properties facilitate processing. Table 2 shows the functional properties of proso millet grains. The swelling capacity was 4.0 ml/1000 grains with a swelling index of 116.0 percent. Similarly, the hydration capacity was 5.13 with a hydration index of 114.0 percent.

The present study reported water absorption capacity of proso millet was 0.70 g/g, and the oil absorption capacity of 0.67 g/g (Table 2) which followed the findings of [20] and [13]. The millets with a higher percentage of fiber have higher water absorption and oil absorption capacity [21] because of the property of fiber to hold water and oil. The study [22] reported that the removal of husk leads to the loss of hydrophilic polysaccharides which decreases the water absorption capacity. Similarly, dehulling decreases the oil absorption capacity because of the reduced surface availability of hydrophobic amino acids [23].

In the present study, the swelling capacity was 4.00 ml/1000 grains, the swelling index was 116 per cent, the hydration capacity was 5.13 g/1000 grains, and the hydration index of 114 per cent and was found on par with the findings of [15] and [16]. As reported by [24] the swelling capacity and swelling index depend on the starch content which relaxes the crystalline structure and the groups of amylose and amylopectin associated with water molecules through hydrogen bonding.

3.3 Physico-chemical and Functional Properties of Proso Millet Flour

3.3.1 Physical and Functional Properties of Proso Millet Flour

The functional properties of raw ingredients of any product affect the processing parameters and the sensory qualities of the final product. Hence, it is important to find out these properties to meet the requirements of processing. Table 3 shows the physical and functional properties of the proso millet flour. The physical properties found that the volume of proso millet flour was 14.52 ml. The bulk density of proso millet flour was 0.69 g/ml. The colour analysis found that the proso millet flour had an L^* value of 90.34, a^* value of 71.93, and a b^* value of 16.93. The chroma and Hue values of proso millet were 16.93 and 71.04. The water absorption, oil absorption capacity, and swelling capacity of proso millet flour were 1.15 g/g, 1.16 g/g, and 4.51 ml/g respectively.

The present study reported that the weight, volume, and bulk density of proso millet flour were 10.00, 14.52 ml, and 0.69 g/ml (Table 3). The volume of 5.05g of proso millet flour was 7.50 ml and the bulk density was 0.67 g/ml reported by [15]. The proso millet flour had L^*, a^*, b^*, and chroma values of 90.34, 0.93, 16.93, and 16.93 respectively. the same study reported 86.45, 2.13, and 21.43 for L^*, a^*, and b^* values. Similar results were also reported by [13].

The water absorption capacity, oil absorption capacity, and swelling capacity of proso millet flour were found 1.15 g/g, 1.16g/g, and 4.51 ml/g. Whereas, [25] reported the WAC and OAC of polished grain flour were 130.9 g/100g and 67.8 ml/100 ml. The results of [15] reported WAC, OAC and swelling power 70.07 g/100g, 65.65g/100g and 6.64 g/100g. The variation in the WAC and OAC in the proso millet flour was because of the dehulling process carried out before milling. Proso millet starch has a higher water-binding capacity than wheat starch [26].

3.3.2 Chemical Properties of Proso Millet Flour

The chemical composition of proso millet flour such as proximate composition, total dietary fiber (soluble and insoluble), and antioxidants were analyzed and details are presented below. The proximates of proso millet flour are presented in Table 4. The proso millet contains 8.72 per cent moisture, 12.10 per cent protein, 1.2 per cent crude fat, 5.55 per cent crude fiber, 1.64 per cent ash, 76.33 per cent total carbohydrate, 70.78 per cent available carbohydrate 364 Kcal energy per 100 g of flour. The present study reported that the proso millet contained 8.72 per cent moisture, 12.10 per cent protein, 1.22 per cent crude fat, 5.5 per cent crude fiber, 1.64 per cent ash, 76.33 per cent carbohydrate, 70.78 per cent available carbohydrate, and 364 Kcal energy (Table 4). According to Nutritive Value of Indian Foods, 2007 [27], proso millet contains 70 per cent carbohydrates, 12.5 g of protein, 1.1 g of fat, 341 Kcal of energy, 2.2 g of crude fiber and 1.9 g of mineral. The results were found on par with the investigation of [20] who reported the moisture, fat, fiber, ash, and total carbohydrate were 11.75 g, 3.78 g, 4.87 g, 2.44 g, and 64.84 g respectively. [28], [15], [29], [16] and [25] also reported on-par results. Proso millet has higher protein content (12.10%) as compared to other minor millets such as kodo (8.3 g), browntop (8.89 g), little millet (7.7 g), and barnyard (6.2 g). Whereas, it has less crude fiber (2.2 g) as compared to other minor millets such as kodo (9.0 g), browntop (8.20 g), little millet (7.6g), and barnyard (9.8 g). Proso millet is good for developing products for growing children as it will meet their protein requirement and fiber helps to maintain gut health.

The dietary fiber components of proso millet flour are presented in Table 5. The total dietary fiber in the proso millet was 7.66 per cent out of which 4.72 per cent was insoluble and 2.95 per cent was soluble dietary fiber. In the present investigation, the proso millet flour had 2.95 per cent soluble dietary fiber and 4.72 per cent insoluble dietary fiber which both constitute total dietary fiber which was 7.66% (Table 5). [30] reported that 2.5% soluble and 19.5% insoluble dietary fiber is present in the millet and after decortication, the soluble fiber content increased which decreases the glycemic index of food.

3.3.3 Mineral Profile of Proso Millet Flour

The mineral composition of proso millet flour is given in Table 7. In proso millet flour, the calcium, iron, zinc, copper, and manganese contents were 29.93, 2.28, 1.75, 0.45 mg, and 1.13 mg/100 g of flour. The present study investigates the mineral content of proso millet flour which has 29.93 mg of calcium, 2.28 mg of iron, 1.75 mg of zinc, 0.45 mg of copper, and 1.13 mg of manganese per 100 g of flour (Table 6). According to [27], the calcium, iron, copper, manganese, and zinc content was 14 mg, 0.8 mg, 1.60 mg, 0.60 mg, and 1.4 mg respectively. [31] reported a total ash content of 1.53g which comprises of 3.84 mg iron, 20.91 mg of calcium, and 2.93 mg of zinc which was found on par with the results of the present study. The study of [20] reported 14.76 mg of calcium, 1.54 mg of iron, and 1.27 mg of manganese. The variation in the mineral content might be because of the varietal differences.

3.3.4 Antioxidant Properties of Proso Millet Flour

Many nutraceutical compounds like tannins, phenols, and flavonoids contribute to the total antioxidant properties. Table 7 shows the antioxidant properties of proso millet flour. The tannin content of proso millet flour was 28.24 mg tannic acid equivalent/100g, the total phenolic content was 25.17 mg gallic acid equivalent/100g and the antioxidant activity was 45.17 per cent DPPH inhibition activity. The tannin, total phenols, and antioxidant capacity of proso millet account for its antioxidant activity. The present study reported the tannin content of 28.24 mg TAE/100g, 25.17 mg GAE/100g, and 45.17% DPPH inhibition activity in proso millet flour (Table 7). [25] reported similar total phenol content and tannins and [31] reported similar DPPH inhibition activity in proso millet and reported a strong positive correlation between the phenolic content of broomcorn millet and antioxidant activity.

3. Conclusion

The good light-yellow colour, water absorption, oil absorption capacity, and high protein and antioxidant properties of proso millet flour make it suitable for bakery products and ready-to-eat products. The study concluded that proso millet flour has good physical, functional, and chemical properties which can be used to develop healthy convenience products such as ready to eat millet-based products that can be easily accepted by the common people.

4. Future scope of the study

- Impact of different processing on the physicochemical, functional, and sensory properties of proso millet.
- Development of proso millet flour incorporated food products.
- *In vitro* starch and protein digestibility along with amino acid profiling.
- Shelf life extension of proso millet flour.

Conflict of interest

The authors of the present paper confirm no conflicts of interest related to this work.

Acknowledgement

The authors are thankful to the University of Agricultural Sciences, Dharwad for providing the facilities to conduct the study and the encouragement by the faculty to accomplish the present work.

Table 1. Physical properties of Proso millet grains

Parameters		Proso millet grains
Length (mm)		2.14±0.04
Breadth (mm)		1.84±0.01
Thickness (mm)		1.84±0.01
Length-Breadth ratio		1.20±0.05
Shape		Oval
Thousand-grain weight (g)		4.50±0.15
Thousand-grain volume (ml)		3.43±0.06
Bulk density (g/ml)		1.31±0.06
Colour Values	L^* (lightness)	76.25±0.25
	a^* (Redness)	5.20±0.05
	b^* (yellowness)	27.83±0.29
	C^* (Chroma)	28.16±0.27
	H° (Hue)	79.42±0.12

Note: Values are expressed as mean ± standard deviation of three replications.

Table 2. Functional properties of Proso millet grains

Functional property	Proso millet grains
Swelling capacity (ml/1000 grains)	4.00±0.10
Swelling index (%)	116.00±0.01
Hydration capacity (g/1000 grains)	5.13±0.03
Hydration index (%)	114.00±0.02

Note: Values are expressed as mean ± standard deviation of three replications.

Table 3. Physical and functional properties of Proso millet flour

Physical properties		Proso millet flour
Weight (g)		10.00±0.002
Volume (ml)		14.52±0.03
Bulk density (g/ml)		0.69±0.001
Colour Values	L* (lightness)	90.34±0.02
	a* (Redness)	0.93±0.01
	b* (yellowness)	16.93±0.01
	C* (Chroma)	16.93±0.02
	H° (Hue)	86.83±0.010
Functional properties		**Proso millet flour**
Water Absorption Capacity (g/g)		1.15±0.01
Oil Absorption Capacity (g/g)		1.16±0.01
Swelling capacity (ml/g)		4.51±0.01

Note: Values are expressed as mean ± standard deviation of three replications.

Table 4. Proximate composition of Proso millet flour

Proximate principles (%)	Proso millet flour
Moisture	8.72±0.07
Protein	12.10±0.30
Crude Fat	1.22±0.03
Crude fiber	5.55±0.19
Ash	1.64±0.05
Total carbohydrate	76.33±0.31
Available carbohydrate	70.78±0.50
Energy (Kcal)	364±0.00

Note: Values are expressed as mean ± standard deviation of three replications.

Table 5. Dietary fiber components of Proso millet flour

Type of dietary fiber (%)	Proso millet flour
Soluble Dietary Fiber (SDF)	2.95±0.01
Insoluble Dietary Fiber (IDF)	4.72±0.12
Total Dietary Fiber (TDF)	7.66±0.13

Note: Values are expressed as mean ± standard deviation of three replications.

Table 6. Mineral profile of proso millet flour

Minerals (mg/100g)	Proso millet flour
Calcium (Ca)	29.93±0.24
Iron (Fe)	2.28±0.03
Zinc (Zn)	1.75±0.02
Copper (Cu)	0.45±0.02
Manganese (Mn)	1.13±0.04

Note: Values are expressed as mean ± standard deviation of three replications.

Table 7. Antioxidant properties of Proso millet flour

Antioxidants	Proso millet flour
Tannins (mg TAE/100 g)	28.24±0.42
Total Phenolic Content (mg GAE/100 g)	25.17±0.76
Antioxidant Capacity (% DPPH inhibition activity)	45.17±5.01

Note: Values are expressed as mean ± standard deviation of three replications. TAE-Tannic acid equivalent, GAE-Gallic acid equivalent. DPPH- 2,2-Diphenyl-picryl-hyrazyl-hydrate.

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