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Millets marvel: Unveiling the nutritional composition and antioxidant properties of Superfoods

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Abstract

The present study focusing on the comprehensive analysis of five varieties of millets (Foxtail, Proso, Barnyard, Kodo, and Little millets) concerning their protein content, antioxidant properties, and proximate composition. The objective is to extract proteins from millets using ammonium sulfate precipitation, quantify protein content using the Lowry method, assess antioxidant properties using the phosphomolybdate method, and conduct proximate analysis to determine their nutritional composition. Proteins are extracted via ammonium sulfate precipitation, selectively separating them based on solubility. The Lowry method, a colorimetric assay, measures protein content by reacting proteins with copper ions and calculating concentration from absorbance readings. Antioxidant properties are evaluated through the phosphomolybdate method, assessing millets' ability to scavenge free radicals and inhibit oxidative damage, yielding insights into their potential health benefits. Proximate analysis determines millets' moisture, protein, fat, ash, and carbohydrate content, offering a comprehensive understanding of their nutritional profile. These findings contribute valuable insights into millets' protein content, antioxidant properties, and proximate composition, promoting their utilization as a sustainable and nutritious food source, beneficial for both human health and agricultural practices.

Keywords: Millets, protein, antioxidant, nutritional composition, proximate analysis

Introduction

Millets, an important class of grains, are consumed by over 1/3rd of the world's population. They are annual, small-grained, warm-weather cereals belonging to the grass family. These traditional grains have been cultivated and consumed in the Indian subcontinent for over 5000 years ^[1]. Millets are highly nutritious and gluten-free, making them a popular alternative to wheat, rice, and other grains for individuals with celiac disease, gluten intolerance, or other dietary restrictions ^[2].

Various millet varieties include pearl millet, finger millet, foxtail millet, proso millet, barnyard millet, among others, each with unique characteristics and uses. Pearl millets, rich in protein, are predominantly grown in Africa and India. Finger millets, abundant in iron and calcium, serve as a staple food in many parts of Africa and South Asia, where they are used to prepare porridge, bread, and various traditional dishes ^[3]. Foxtail millets, a significant crop in China, are utilized to make noodles, dumplings, and other culinary delights ^[4]. Proso millets, widely cultivated in the United States and Europe primarily as birdseed, are gaining popularity as a human food ingredient ^[5]. Barnyard millets, though considered a minor crop in many regions, serve as a vital food source for tribal communities in India ^[6].

Millets boast high nutritional content, rich in protein, fibers, and micronutrients such as iron, magnesium, and zinc. They offer various health benefits and aid in managing health issues like diabetes mellitus and hyperlipidemia ^[7]. Despite comprising over 58% of global production, many Indians remain unaware of the health benefits and nutritional value of millets. Millets contain approximately 65% carbohydrates, 9% proteins, 3% fat, and 7% crude fiber, along with vitamins and minerals ^[8]. They serve as a good source of vitamin B, magnesium, antioxidants, manganese, phosphorus, and iron. While millets are rich in essential amino acids, they lack lysine and threonine but are relatively high in sulfur-containing amino acids like methionine and cysteine ^[9]. However, millets also contain certain anti-nutritional factors such as phytic acid, tannins, oxalates, protease inhibitors, and lecithin, which can hinder nutrient absorption ^[10].

Protein, an essential macronutrient crucial for human growth and repair, is found abundantly in millets. Antioxidants, molecules capable of inhibiting the oxidation of other molecules, play a vital role in preventing chronic diseases and improving overall health^[11]. Millets possess antioxidant properties due to the presence of various bioactive compounds like polyphenols, flavonoids, and tocopherols^[12]. Proximate analysis, a commonly used method, determines the nutritional composition of food products, including protein content, fat, carbohydrates, fiber, ash, and moisture^[13]. In this study, we compare the nutritional composition, protein content, and antioxidant properties of isolated proteins from five different types of millets.

Methodology

Collection of Sample

Millets were sourced from local markets to ensure the acquisition of high-quality millets suitable for extraction. Five distinct types of millets were selected for the study, namely Foxtail millet, Kodo Millet, Barnyard millet, Little millet, and Proso millet.

Isolation of Protein

Initially, 50 grams of each millet variety underwent washing and straining before being combined with extraction buffer, PVP, and PMSF. Following thorough blending, the mixture was subjected to filtration and centrifugation processes to eliminate fibers. The resulting supernatant containing proteins was then precipitated using 70% ammonium sulfate overnight. Subsequently, the precipitated proteins were centrifuged and resuspended in pH 7.0 phosphate buffer. The resuspended protein solution underwent dialysis against 50 mM phosphate buffer (pH 7.0) using a 12,000 Da MWCO dialysis bag. After three buffer changes, the crude protein extract was stored at -80 °C for further analysis, with total protein content estimated using the Lowry method.

Estimation of Protein by Lowry Method

Designated protein samples were diluted within the range of 20-100 µg and subjected to analysis. Sequential addition of K Na Tartrate-Na₂CO₃ solution, CuSO₄-K Na Tartrate-NaOH solution, and freshly prepared Folin reagent facilitated protein quantification through absorbance at 720 nm^[14]. The meticulously constructed standard curve of BSA was then employed to accurately estimate the protein concentration in the millet samples.

Estimation of Total Antioxidant Activity-Phosphomolybdenum Method

For the determination of total antioxidant activity in millet hydrolysate, 100 µl of sample (ranging from 125-500 µg) was incubated with 1 ml of Molybdate reagent (0.6 M H₂SO₄, 28mM disodium phosphate, 4mM ammonium molybdate) at 95°C for 90 minutes. After cooling, absorbance was measured at 695 using a multimode reader^[15]. Ascorbic acid standards (ranging from 25-125 µg) were employed for comparison. The total antioxidant capacity was then calculated as mg AAE/mg protein samples.

Data Analysis

Proteins isolated from different millet varieties underwent ammonium sulfate precipitation, followed by dialysis and resuspension in phosphate buffer for further analysis. The estimation of protein content in millet samples was

conducted using the Lowry method. Various concentrations of BSA solution (100, 200, 300, 400, 500 µg/ml) were prepared from the respective OD values obtained after the reaction with the Folin's-Ciocalteu reagent, with results indicated in Figure 2. The determination of antioxidant capacity utilized a standard curve of ascorbic acid, with results presented in Figure 4.

Proximate Analysis

Selected millet samples underwent proximate analysis, including parameters such as Protein, Fat, Carbohydrate, Moisture, and Ash, using AOAC methods.

Results

The antioxidant properties of millets, determined by the phosphomolybdate method, revealed significant variability among different types. Kodo millet exhibited the highest antioxidant property (1.41 mg.eq. ascorbic acid/g of protein), followed by little millet (0.14 mg.eq. ascorbic acid/g of protein), proso millet (0.3 mg.eq. ascorbic acid/g of protein), and barnyard millet (0.2 mg.eq. ascorbic acid/g of protein). Unfortunately, the antioxidant activity of foxtail millet couldn't be calculated due to precipitation during the reaction.

Protein analysis showed variations in protein content among millet varieties. Proso millet had the highest protein content (13.54%), followed by foxtail millet (12.15%), barnyard millet (11.2%), little millet (10.6%), and kodo millet (9.84%).

Fat analysis indicated that barnyard millet had the highest fat content (3.004%), followed by kodo millet (2.54%), foxtail millet (2.40%), little millet (1.53%), and proso millet (1.17%).

Ash content analysis revealed differences across millet types, with little millet exhibiting the highest ash content (1.342%), followed by proso millet (1.127%), foxtail millet (1.235%), barnyard millet (1.185%), and kodo millet (0.029%).

Discussion

The Lowry method serves as a reliable analytical tool for assessing millet protein content, widely employed in numerous studies demonstrating millet's protein content variability. Furthermore, millet's antioxidant activity, highlighted by its ability to counter free radicals, underscores its therapeutic potential against diseases like cancer and heart ailments. Various millet types exhibit distinct antioxidant capacities, with foxtail millet, kodo millet, and little millet emerging as prominent contenders.

Moisture content, a crucial parameter influencing millet quality, ranges between 7% and 14%, governed by factors like millet variety and storage conditions. Estimation of millet protein content via the Kjeldahl method underscores its nutritional significance, varying across millet varieties and ranging from 7.5% to 12.5%. Additionally, millets boast low-fat content, with variations across types such as foxtail, barnyard, proso, kodo, and little millets.

The ash content in millets, indicative of mineral richness, varies between 1% and 4%, with foxtail and barnyard millets showcasing notable levels. Finally, millets' carbohydrate content varies by type, with values ranging from 60.9 to 72.3 grams per 100 grams of raw millet, underlining their nutritional diversity and dietary significance.

Table 1: Amount of proteins in millet samples

Sample	OD (720 nm)		Average	A-B	$\mu\text{g/ml}$	Amount of protein ($\mu\text{g}/5\text{ g of millet}$)	Amount of protein ($\mu\text{g/g of millet}$)
	A	B					
Blank	0.07	0.072	0.071	-	-	-	-
Barnyard millet	0.328	0.322	0.325	0.254	149.3169	29863.37	5972.675 $\pm$ 0.003464
Foxtail millet	0.308	0.311	0.3095	0.2385	140.1992	39255.78	7851.156 $\pm$ 0.001732
Kodo millet	0.317	0.32	0.3185	0.2475	145.4933	29098.67	5819.733 $\pm$ 0.001732
Little millet	0.54	0.532	0.536	0.465	273.4345	54686.9	10937.38 $\pm$ 0.004619
Proso millet	0.61	0.58	0.595	0.524	308.1404	61628.08	12325.62 $\pm$ 0.017321

Table 2: Antioxidant activity of millet protein samples

Sample	mg.eq. ascorbic acid/ μg of protein
Barnyard millet	0.2 $\pm$ 0.011
Foxtail millet	-
Kodo millet	1.41 $\pm$ 0.008
Little millet	0.14 $\pm$ 0.0015
Proso millet	0.3 $\pm$ 0.003

Table 3: Percentage of moisture in different millets

Sample	Weight of empty dish (g)	Weight of sample (g) w_w	Weight of empty dish + sample (g)	Weight of empty dish + dried sample (g)	Weight of dried sample (g) w_d	Moisture content (%) ($w_w w_d$) *100 w_w
Barnyard	48.083	5.0213	53.1043	52.532	4.449	11.397 $\pm$ 0.5%
Foxtail	30.4066	5.0048	35.4114	34.790g	4.383	12.41 $\pm$ 0.5%
Kodo	53.0446	5.0345	58.0791	57.496	4.451	11.58 $\pm$ 0.5%
Little	50.7730	5.0222	55.7952	55.160	4.387	12.6 $\pm$ 0.5%
Proso	42.7917	5.0222	47.8139	47.160	4.368	13.02 $\pm$ 0.5%

Table 4: Percentage of protein in different millets

Sample	Weight of the Sample	Burette Reading (ml)		Volume of 0.1 N HCL consumed (ml)	Percentage of Nitrogen (%)	Percentage of Protein (%)
		Initial	Final			
Blank	-	0	0.4	0.4	-	-
Barnyard	0.5123	0	7	7	1.803	11.2 $\pm$ 0.3%
Foxtail	0.5256	0	7.7	7.7	1.944	12.15 $\pm$ 0.3%
Kodo	0.5155	0	6.2	6.2	1.575	9.84 $\pm$ 0.3%
Little	0.5337	0	6.9	6.9	1.705	10.65 $\pm$ 0.3%
Proso	0.5233	0	8.5	8.5	2.167	13.54 $\pm$ 0.3%

Table 5: Percentage of fat in different millets

Sample	Weight of dried sample (w_1) (g)	Weight of empty dish (w_2) (g)	Weight of dish + fat in sample (w_3) (g)	Weight of fat in sample (w_5) (g) [w_3-w_2]	Percentage of fat (%) [w_5/w_1] *100
Barnyard	2.017	49.0641	49.1247	0.606	3.0040 $\pm$ 0.4%
Foxtail	2.012	44.6306	44.6790	0.0484	2.40 $\pm$ 0.4%
Kodo	2.059	125.2665	125.3190	0.0525	2.54 $\pm$ 0.4%
Little	2.013	57.5845	57.6154	0.0309	1.53 $\pm$ 0.4%
Proso	2.005	125.2665	125.2900	0.0235	1.17 $\pm$ 0.4%

Table 6: Percentage of ash in different millets

Sample	Weight of crucible (g)	Weight of crucible + sample (g)	Weight of sample (g)	Weight of crucible + ash (g)	Weight of ash (g)	Ash content (%)
Barnyard	14.8979g	15.9184	1.0205	14.9100	0.0121	1.185 $\pm$ 0.3%
Foxtail	60.2294g	61.2571	1.0277g	60.2421	0.0127	1.235 $\pm$ 0.3%
Kodo	31.6154g	32.6485	1.0331g	31.6157	0.0003	0.029 $\pm$ 0.3%
Little	68.2388g	69.2447	1.0059g	68.2523	0.0135	1.342 $\pm$ 0.3%
Proso	14.8306g	15.8332	1.0026g	14.8419	0.0113	1.127 $\pm$ 0.3%

Table 7: Percentage of total carbohydrates in different millets

Sample	Moisture	Ash	Protein	Fat	Total carbohydrates (%) [moisture +fat+ ash +protein-100]
Barnyard	11.397	1.185	11.2	3.00	73.214 $\pm$ 0.4%
Foxtail	12.41	1.235	12.15	2.40	71.805 $\pm$ 0.4%
Kodo	11.58	0.029	9.84	2.54	76.011 $\pm$ 0.4%
Little	12.6	1.342	10.65	1.53	73.878 $\pm$ 0.4%
Proso	13.02	1.127	13.54	1.17	71.143 $\pm$ 0.4%

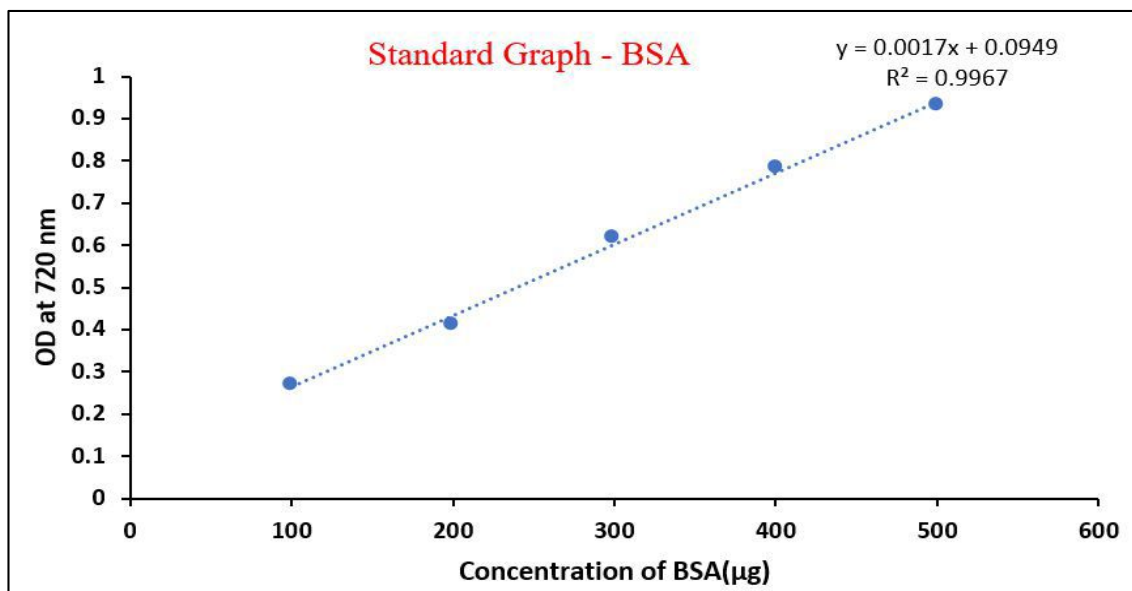


Fig 1: Standard Curve of BSA

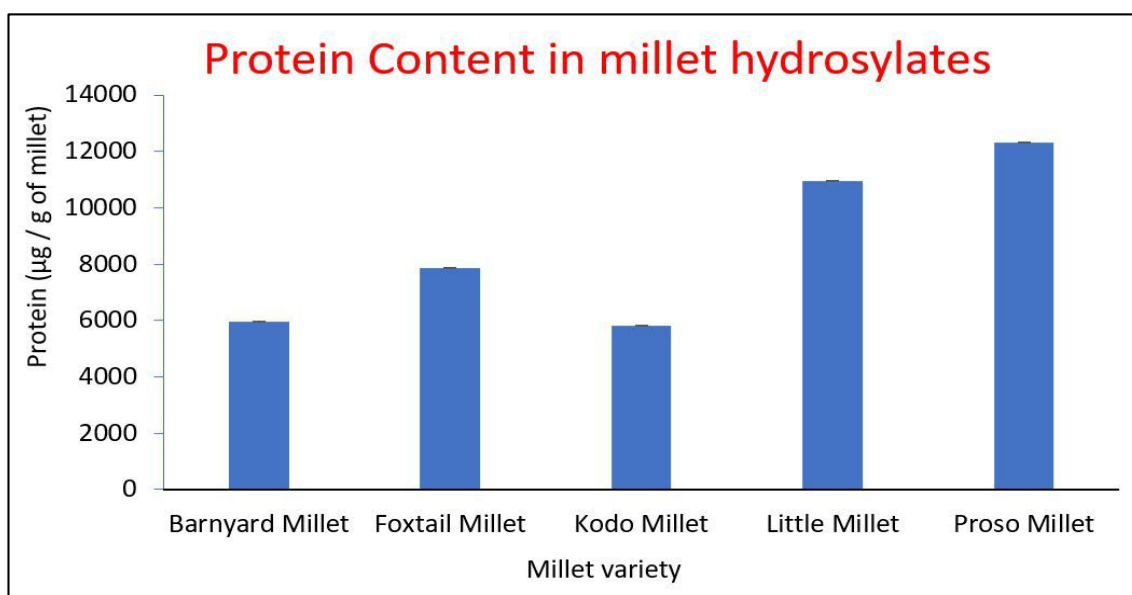


Fig 2: Graphical representation of proteins in millet samples

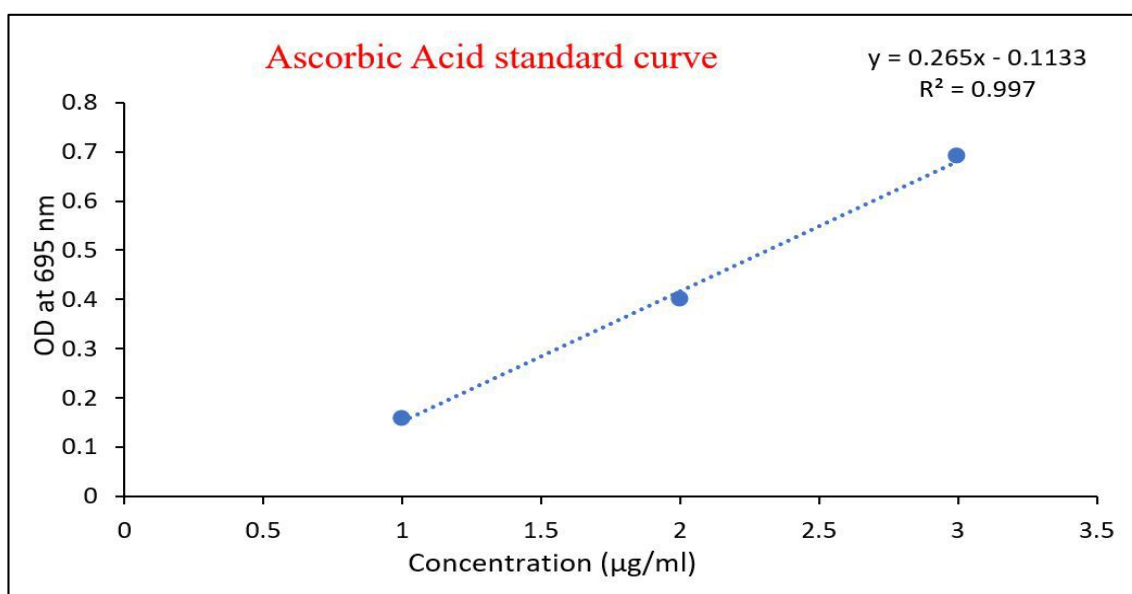


Fig 3: Standard graph of ascorbic acid

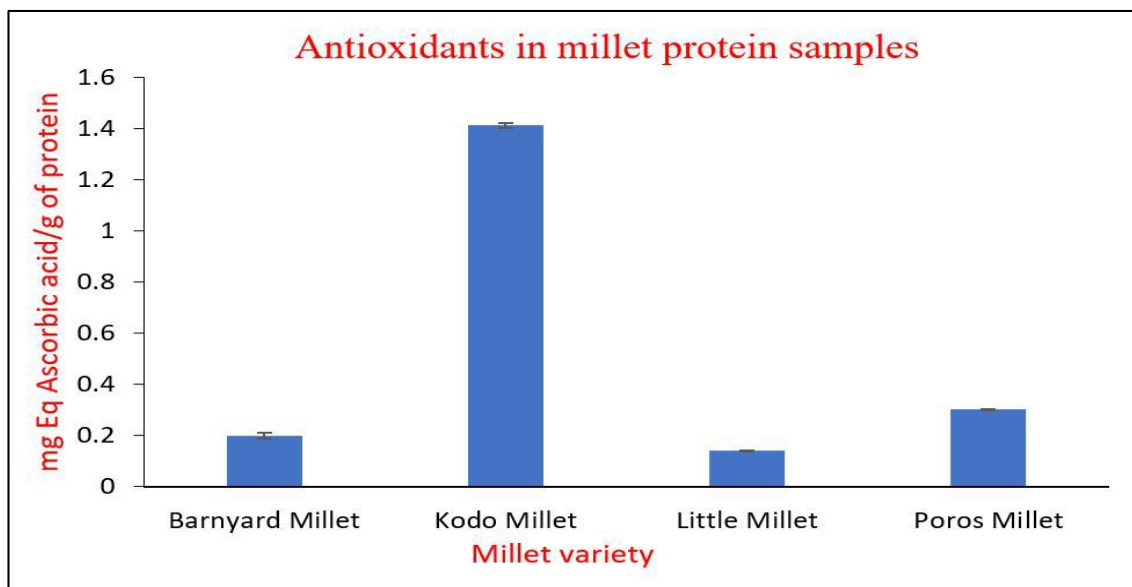


Fig 4: Graphical representation of the antioxidant activity of millet protein samples

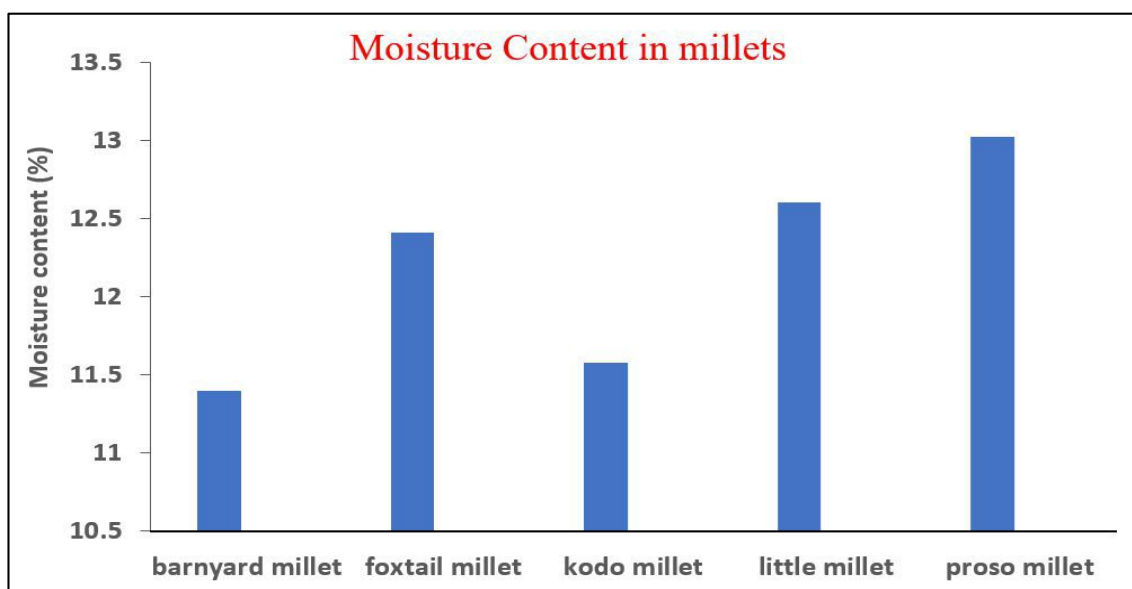


Fig 5: Graphical representation of moisture content in millet samples

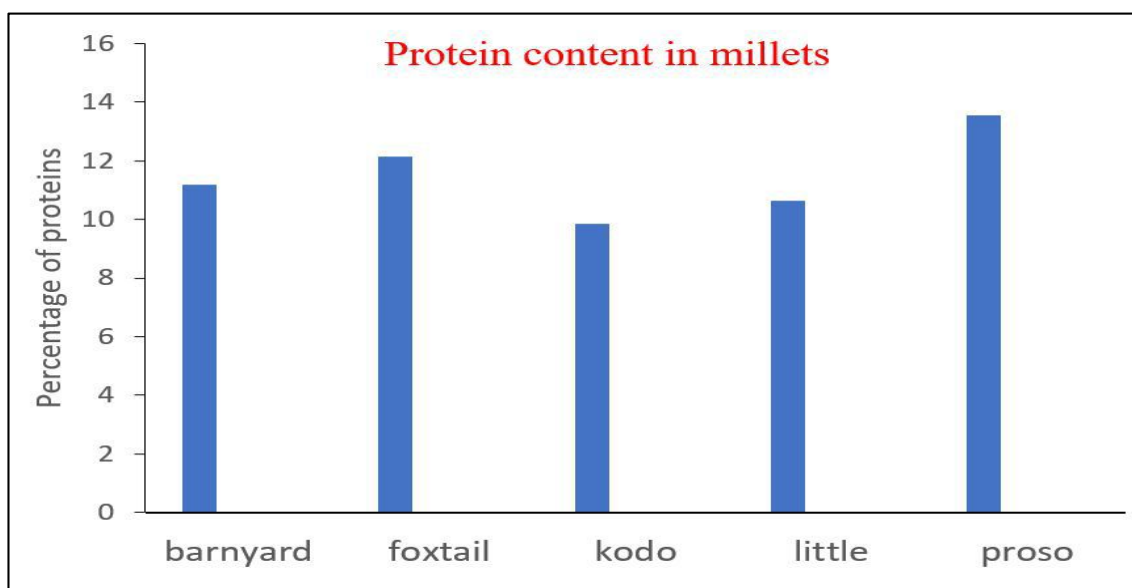
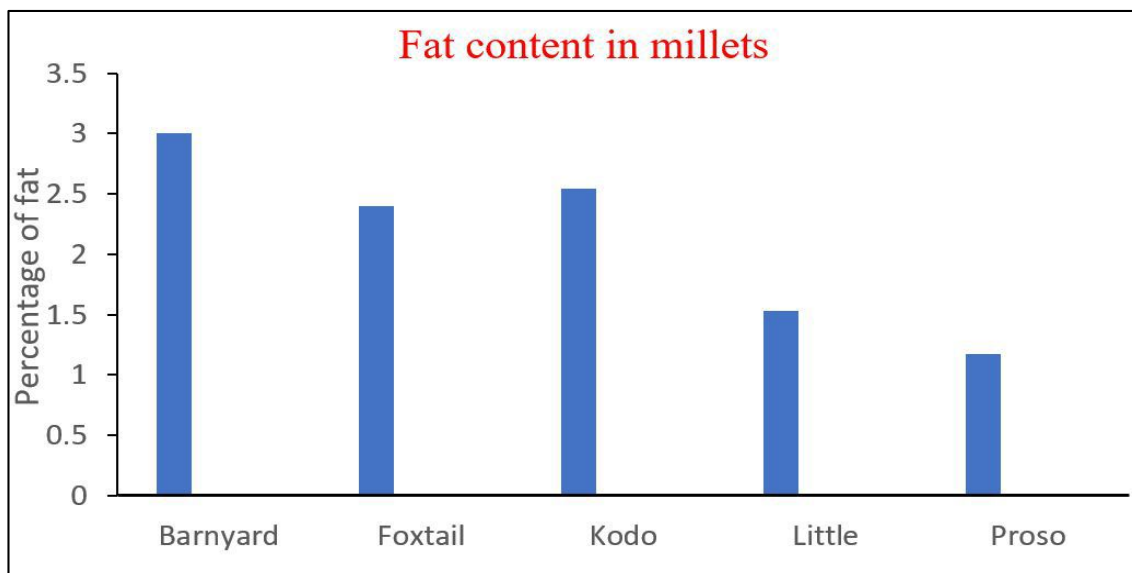
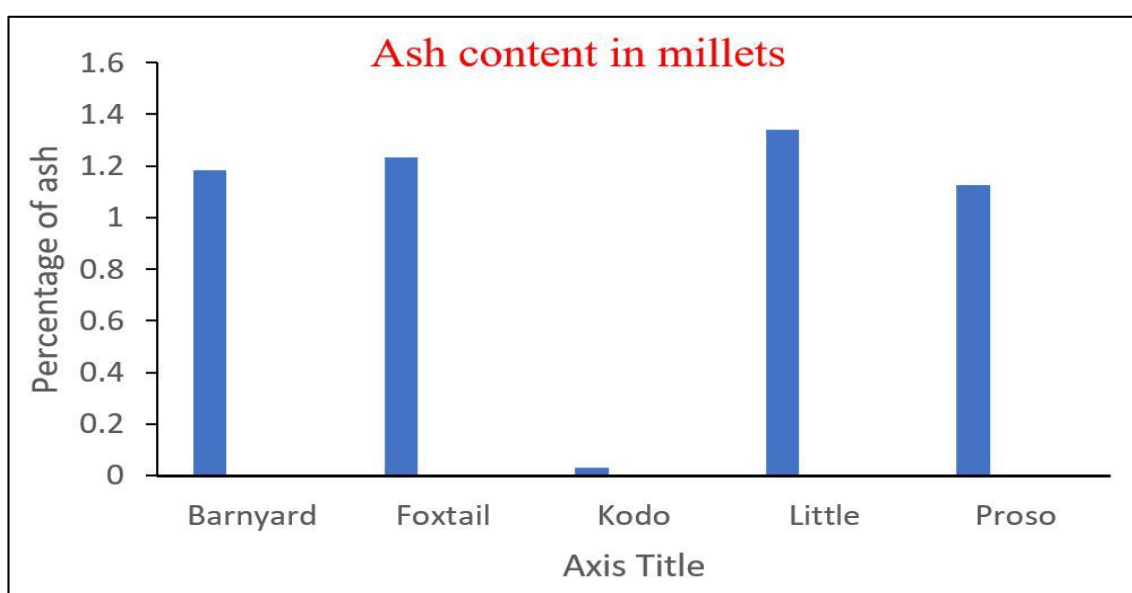
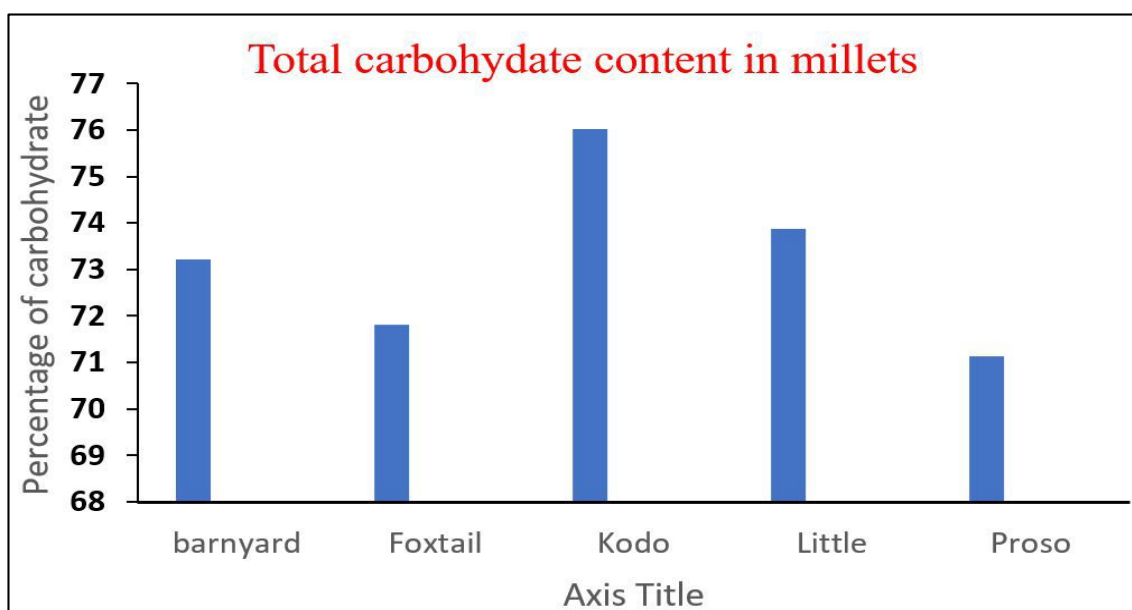


Fig 6: Graphical representation of protein in millet samples

**Fig 7:** Graphical representation of fat content in millets**Fig 8:** Graphical representation of ash content in millet samples**Fig 9:** Graphical representation of carbohydrate content in millets

Conclusion

The results of our study highlight the nutritional diversity and health-promoting properties of different millet varieties. Kodo millet demonstrated the highest antioxidant activity, followed by little millet, proso millet, and barnyard millet. However, the antioxidant activity of foxtail millet was not determined due to precipitation issues during the analysis.

Proso millet emerged as the millet variety with the highest protein content, while barnyard millet had the highest fat content. Little millet exhibited the highest ash content among the millet varieties studied.

The variability in nutritional composition among millet types underscores the importance of considering millets as valuable components of a balanced diet. Their low-fat content, coupled with significant protein, antioxidant, and mineral content, positions millets as excellent alternatives to traditional cereal grains.

Overall, millets offer a promising dietary solution with potential health benefits, making them worthy of further exploration and incorporation into food systems and dietary recommendations.

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