Physical and functional properties of ancient grains and flours and their potential contribution to sustainable food processing

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\textbf{ABSTRACT}

Sustainable agricultural practices supporting food diversification and value addition of underutilized crops can support global food security and nutrition. Rediscovered ancient seed grains and flours (barnyard millet, finger millet, little millet, sorghum) are emerging as sustainable alternatives due to high nutritional content and climate resilience. The physical and functional properties varied significantly ($p < 0.05$) among seed grains and flours in this observational study. All the tested grains showed good antioxidant activity due to a good phenolic content. Understanding these properties is essential for food processing operations. This study indicates that millet and sorghum could be used successfully in food products formulation.

\textbf{INTRODUCTION}

Hunger, malnutrition, and food insecurity are ongoing global issues that affect people in developed and developing countries,\textsuperscript{[1]} resulting in preventable health inequities among affected communities.\textsuperscript{[2,3]} The State of Food Security and Nutrition (SOFI) 2020\textsuperscript{[1]} report shows that the prevalence of severe food insecurity increased from 2014 to 2019 in all world regions except for the United States and Europe. However, the United States also deals with food insecurity issues, especially in vulnerable communities of color, indigenous tribes, food deserts with limited access to affordable and healthy foods.\textsuperscript{[4,5]} The United Nations Sustainable Development Goal to “End hunger, achieve food security and improved...
nutrition and promote sustainable agriculture”-SDG 2[6] is a call to action to meet this challenge and pledges “to leave no one behind.” To achieve this, the United Nations proposed an integrated approach to change the current global agriculture and food systems.[7,8]

Global climate change, declining agrobiodiversity, land constraints, water scarcity, and environmental degradation are significantly challenging to the world’s agricultural production.[9] The current agrarian production trajectory is not sustainable, and there needs to be a more sustainable approach to agriculture to cope with the aforementioned challenges.[1] Sustainable agriculture aims to address present food demands while preserving resources for future generations.[10] This could be done by combining traditional farming techniques with modern technologies[11] to increase food production, protect the environment, and sustain the agricultural system’s viability.[7,8,12]

Agricultural diversification is essential to making agricultural food systems more sustainable[9,13] and attaining food and nutritional security.[14] Approximately 30,000 edible plant species are known, but only 30 of these known plants feed the world, and only a few are cultivated on a large scale.[15] Global agricultural production currently focuses on cultivating few high-yielding staple grains – maize, wheat, rice, barley, and lesser extent, sorghum.[16] This has resulted in a reduction in the biodiversity of agricultural cropping systems worldwide and a decline in the cultivation of traditional crops.[17] Key strategies to securing a sustainable food system and nutrition security involve popularizing the production of underutilized crops,[14,18] encouraging crop diversification,[19] and prioritizing the dietary quality of food.[20]

Globally, countries stand to benefit from producing underutilized grains.[21] In some regions in Africa, like sub-Saharan Africa, where water is scarce, climate change is of great concern.[17] Crops will need to survive on very fragile soils that will undergo severe fluctuations in temperatures and rainfall.[22] Since underutilized grains adapt well to marginal and precarious environments, they can play a significant role in reducing the vulnerability of farming systems to climate change, maintain high yields, and producing crops with diverse quality attributes.[17,23,24] Because most of these grains are grown in rural communities, they are an accessible means of adaptation for indigenous farmers,[22] thereby providing income opportunities for these small farmers.[25] Growing underutilized grains increases women’s employment opportunities, thereby enhancing their social status.[26] Along with their adaptation to adverse ecological conditions, some underutilized grains have significant levels of important micronutrients[17] and have the potential for reversing the trend of micronutrient deficiencies (hidden hunger) in both developing and developed countries.[21,25]

Although agricultural grasses (Poaceae family) that contain edible seeds can be grouped under cereals, there are still no clear classifications. Millets and sorghum have been commonly named pseudo cereals, seed grains, and underutilized grains. Millets and sorghums are well adapted to harsh weather conditions and can be grown in low agricultural potential environments.[27] According to FAOSTAT,[16] the United States, India, Nigeria, Mexico, and China are the largest producers of millets and sorghum globally. Millets and sorghum are underutilized seed grains in both the developed and developing world. In Africa and Asia, millet and sorghum grains are essential staple food crops for millions of people. In most developed countries, sorghum is primarily used as animal feed.[28] These underutilized crops have been shown to adapt to a wide range of climate conditions and may be nutrient-dense and offer better growth prospects in marginal production areas.[14,17,24]

Nutrient composition for millets and sorghum are similar to other cereals (mainly carbohydrates, proteins, fat, crude fiber, minerals, vitamins) and are a rich source of energy (Tables 1 and 2). Both millets and sorghum have very low lipids content. The protein content in millets is usually variable and depends on the variety, cultivars, growing conditions. Millets and sorghum are rich in essential vitamins and minerals like potassium, calcium, magnesium, iron, and zinc.[31] Especially finger millet, which has three times more calcium than milk.[32] Millets and sorghum are both gluten-free, making them suitable for people with celiac disease.[21,33] Singh et al.,[34] and Mal et al.,[35] reported that millets are a rich source of phytochemicals and micronutrients. Xiang et al.,[36] identified ten phenolic compounds for finger millets and reported that darker-colored finger millet varieties had higher phenolic contents and
antioxidants. These nutritional factors make millets a suitable functional food that can potentially prevent or delay the occurrence of cardiovascular diseases. [29] Sorghum contains higher polyphenols, flavonoids, and extractable phenolic acids than other major cereals. [37–40] Phenolics in sorghum have been reported to have unique functional and bioactive properties in foods. [41,42]

The amino acid composition of millets and sorghum is an important nutritional trait to consider when assessing protein quality. [43] Amino acids play a major role in the synthesis of proteins and as intermediates in metabolism. [44] Humans can synthesize all amino acids necessary except for the nine essential amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine. [44] Essential amino acids must be taken through the diet. A comparative amino acid profile for selected millets and sorghum is presented in Figure 1. In general, millets and sorghum have higher levels of leucine, isoleucine, arginine, phenylalanine than other essential amino acids. Lysine is an important building block for synthesizing proteins in humans, and as with most grains, millets and sorghum are deficient in lysine. [45]

**Contribution to sustainable food processing**

The physical and functional characteristics of grains and flour describe their processing and storage properties. The grains’ size, density, and porosity are essential parameters to know during equipment design and agricultural processes like sorting, mixing, storing, and transporting grains. [46,47] Color is one of the most relevant characteristics related to grain quality as it affects the color of the resulting food and has implications on consumer preference. [48] Functional properties of the flour, like solubility, water holding capacity, and dispersibility, reflects the interaction between the flour’s composition and molecular structure. [49] The moisture content and water activity of flour indicate product quality and shelf stability. [50] 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, thiobarbituric acid reactive}

### Table 1. Average nutritional composition of selected millets and sorghum grains (g/100 g).

| Grain        | Protein | Fat  | Ash  | Carbohydrate | Crude Fiber | Energy (kcal) | References |
|--------------|---------|------|------|--------------|-------------|---------------|------------|
| Barnyard     | 11.0    | 3.9  | 4.5  | 55.0         | 13.6        | 300           | [29]       |
| Finger       | 7.7     | 1.5  | 2.6  | 72.6         | 3.6         | 336           | [29]       |
| Little       | 9.7     | 5.2  | 5.4  | 60.9         | 7.6         | 329           | [29]       |
| Maize        | 12.1    | 4.6  | 1.8  | 66.2         | 2.3         | 358           | [29,30]    |
| Rice         | 7.5     | 2.4  | 4.7  | 78.2         | 10.2        | 362           | [29,30]    |
| Sorghum      | 10.4    | 3.1  | 1.6  | 70.7         | 2.0         | 329           | [29]       |
| Wheat        | 14.4    | 2.3  | 1.8  | 71.2         | 2.9         | 348           | [29,30]    |

Data sources: [29,30]

### Table 2. Mineral and vitamins composition of millets and sorghum (mg/100 g).

| Minerals     | Barnyard | Finger | Little | Sorghum | Maize | Rice | Wheat |
|--------------|----------|--------|--------|---------|-------|------|-------|
| Ca           | 22       | 344    | 17     | 13      | 10    | 10   | 41    |
| P            | 280      | 283    | 220    | 289     | 89    | 160  | 306   |
| K            | -        | 408    | 126    | 363     | 270   | 130  | 363   |
| Na           | -        | 11     | 7.9    | 2       | 37    | 6    | 3     |
| Mg           | 82       | 137    | 61     | 165     | 0.163 | 32   | 120   |
| Fe           | 18.6     | 3.9    | 9.3    | 3.4     | 2.3   | 0.5  | 3.9   |
| Cu           | 0.60     | 0.47   | 0.05   | 1.7     | 0.22  | 0.25 | 0.9   |
| Mn           | 0.96     | 5.49   | 0.68   | 1.6     | 0.163 | 1.1  | 13.3  |
| Zn           | 3        | 2.3    | 3.7    | 1.7     | 0.46  | 1.2  | 1     |
| Thiamin (Vitamin B1) | 0.33 | 0.42 | 0.30 | 0.33 | 0.155 | 0.41 | 0.41 |
| Riboflavin (Vitamin B2) | 0.10 | 0.19 | 0.09 | 0.1 | 0.055 | 0.0049 | 5.46 |
| Niacin (Vitamin B3) | 4.2 | 1.1 | 3.2 | 3.7 | 1.77 | 1.62 | 5.5 |

Data source: [30]
substances (TBARS) and total phenolic content are among the most frequently used method for determining the antioxidant properties of plant extracts.\(^51\) As highlighted by other authors,\(^7,52,53\) it is essential to carefully consider the effects of unit operations such as milling, kneading, and baking stages on the final quality of the products made using these underutilized grains. Therefore, understanding the physical and functional attributes of the grains is critical to selecting the best grain for transformation into value-added products. The aim of this study is to assess differences in physical, functional, and antioxidant properties between barnyard millet (Echinochloa utilis), finger millet (Eleusine coracana), little millet (Panicum sumatrense), and white sorghum (Sorghum bicolor), in order to develop food products with improved nutritional value.

**MATERIALS AND METHODS**

**Materials**

This observational study was conducted to establish methodologies for the analysis of millets and sorghum. Considering the food availability and accessibility, currently available commercial samples for human consumption were used in the study. White sorghum and finger millet were obtained from Babco Foods International LLC (New Jersey, USA). Barnyard and little millets were obtained from Manna foods (Chennai, India). Millet and sorghum grains were milled using a Butterfly Matchless 750-watt mixer grinder to get flour.

**Chemicals, solvents, reagents**

Acetic acid, gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Acros Organics (Morris Plains, NJ). Folin-Ciocalteu, Thiobarbituric acid (TBA), Malondialdehyde tetra butylammonium salt (MDA) were obtained from Sigma (St. Louis, MO). Ethanol was purchased from Thermo Fisher Scientific (Hampton, NH). Sodium bicarbonate was purchased from Duda Energy LLC (Decatur, AL).

**Physical properties**

*Grain size analysis:* Grain size analysis was estimated using the National Institutes of Health (NIH) inspired open-source image analysis software ImageJ. ImageJ is an open-source program for image processing and analysis.\(^54\) It can be used to calculate the area, pixel values, distance, and angle of user-defined selections. ImageJ supports standard image processing functions such as contrasting.
smoothing, sharpening, and filtering. More details on ImageJ can be found in the ImageJ user guide.\textsuperscript{[55]} The area of the major and minor axis were measured, and the diameter of the grains was calculated using Eq (1).

\[
\text{Diameter } D = 2\sqrt{\frac{A}{\pi}} \quad \text{Eq(1)}
\]

Where A is the area of the grain.

Density Measurements (Bulk, Tapped, True): Bulk density ($\rho_b$) was determined according to the method described by Zungur Bastioğlu et al.\textsuperscript{[56]} Approximately 4 g of grains was measured into a 10 mL graduated cylinder. The bulk density was calculated by dividing the sample weight by the sample volume. For the tapped density ($\rho_t$), the cylinder was mechanically tapped using a shaker at 1000 rpm for 20 minutes.\textsuperscript{[57]} The bulk density of flour was measured using 2 g of flour and followed the same procedure used to measure grains. The experiment was replicated six times, and the average value was recorded.

\[
\text{Bulk density (kg/m}^3\text{)} = \frac{\text{Weight of grains (kg)}}{\text{Volume of grains (m}^3\text{)}} \quad \text{Eq(2)}
\]

The true density of the grains was determined using a gas pycnometer (Quantachrome Ultrapycnometer 1000 Anton Paar, Graz, Austria). Four grams of grains was placed in the sample cell and known quantity of helium under pressure was allowed to flow into the sample cell containing the material. True grain density was expressed as the ratio of the weight of grains in the sample cell to the volume measured by the pycnometer. The equipment was set to multi-run three times, and the average value was displayed. This process was repeated six times with a different grain sample. Before use, the pycnometer was calibrated according to the manufacturer’s recommendation.

Porosity: Porosity is defined as the ratio of void spaces inside the grain to the apparent volume of the grains.\textsuperscript{[58]} The porosity of grains was calculated from the bulk density and true density values using the Equation described by Jain & Bal.\textsuperscript{[59]}

\[
\text{Porosity(\%)} = \frac{(\rho_t - \rho_b)}{\rho_t} \times 100 \quad \text{Eq(3)}
\]

where $\rho_b =$ bulk density and $\rho_t =$ true density

Carr compressibility index and Hausner ratio

The flow characteristics of the grain and flour samples was measured by calculating the Carr Index\textsuperscript{[60]} and Hausner ratio\textsuperscript{[61]} from the bulk density ($\rho_b$) and tapped density ($\rho_t$) values. Compressibility index (CI) and Hausner ratio (HR) were calculated using equations (3) and (4).

\[
\text{Carr Index CI} = \frac{\rho_t - \rho_b}{\rho_t} \times 100 \quad \text{Eq(4)}
\]

\[
\text{Hausner Ratio (HR)} = \frac{\rho_t}{\rho_b} \quad \text{Eq(5)}
\]

Angle of repose: The angle of repose of the grains was determined using the vertical cylinder method described by Akaaimo & Raji.\textsuperscript{[62]} A cylinder open at both ends was placed on a horizontal surface and filled with grains, then the cylinder was carefully lifted to create a heap. The base and diameter of the heap were measured, and angle of repose was calculated using Equation (6). The angle of repose of flour was determined using a modified version of the fixed funnel method described by Beakawi Al-Hashemi & Baghabra Al-Amoudi.\textsuperscript{[63]} A funnel with a wide opening was attached to a stand at a distance 10 cm above a horizontal table surface. Two grams of grains was poured through the funnel to create a heap, and the height and diameter measured using Equation (6).
Angle of repose (°) = \tan^{-1}\left(\frac{2H}{D}\right) \hspace{1cm} \text{Eq}(6)

Where H = height of the heap, D = Diameter of the heap

**Color**

Color measurements of grains and flour was done using a Chroma Meter (Konica Minolta CR-410, Chiyoda, Tokyo, Japan) to get the L*, a*, and b* color parameters. The L*, a* and b* values stand for lightness, greenness/redness, and blueness/yellowness, respectively. The chromameter was calibrated against the values on a white sample provided by the manufacturer. Six measurements were taken for each of the grain and flour samples, and the average value was reported. Color difference (ΔE), chroma, and hue angle (θ°) were calculated using Equation (7), (8), and (9).

\[ ΔE = \sqrt{(L_1^* - L^*) + (a_1^* - a^*) + (b_1^* - b^*)} \hspace{1cm} \text{Eq}(7) \]

\[ \text{Chroma} (C*) = \sqrt{(a^2) + (b^2)} \hspace{1cm} \text{Eq}(8) \]

\[ \text{Hue angle (h*}) = \tan^{-1}\left(\frac{b^*}{a^*}\right) \hspace{1cm} \text{Eq}(9) \]

**Water solubility:** The flour’s solubility was determined by the method described by Singh and Singh.\(^\text{[64]}\) Two grams of flour sample was placed in a 50 mL centrifuge tube. Distilled water (30 mL) was added, and the mixture placed in a shaking water bath for 30 minutes at 37°C. The mixture was centrifuged at 5000 rpm for 5 minutes and the supernatant was decanted and dried in a 225°C oven for two hours. Solubility was calculated using Equation (10).

\[ \text{Water solubility} (\%) = \frac{\text{weight of dried supernatant}}{\text{weight of dried sample}} \times 100 \hspace{1cm} \text{Eq}(10) \]

**Water holding capacity:** Water holding capacity was determined using the method of Dayakar Rao et al.,\(^\text{[65]}\) with slight modifications. Flour sample (1.5 g) was placed in a pre-weighed centrifuge tube to which 5 mL of distilled water were added. The mixture was vigorously vortexed for 15 seconds and centrifuged at 5000 rpm for 10 minutes. The supernatant was poured into aluminum dishes and dried for 2 hours at 130°C. Water holding capacity (WHC) according to the weight of samples was calculated by using Equation (11).

\[ \text{Water holding capacity} (g/g) = \frac{\text{weight of wet sample} - \text{weight of dried precipitate}}{\text{weight of dry sample}} \hspace{1cm} \text{Eq}(11) \]

**Water activity**

The water activity (aw) was measured with a water activity meter (Cx-2, Decagon Devices, Inc., Pullman, Washington, 99,163) with a 0.001 sensitivity at room temperature.

**Moisture content**

Moisture content of the flour was measured with a halogen moisture analyzer (HE53, Mettler Toledo, Columbus, Ohio 43,240). The procedure was repeated four times for all flour samples.
**Dispersibility**

Dispersibility was measured using the method described by Jinapong et al., [57] One gram of grain flour was added to 10 mL distilled water and poured into a 50 ml beaker. The sample was stirred vigorously with a spatula for 15 seconds. The wet mixture was poured through a sieve (212 µm). The sieved sample was transferred onto a pre-weighed aluminum pan and dried in an oven at 105°C until it is completely dry. The dispersibility was calculated according to Equation (12).

\[
\% \text{ Dispersibility} = \frac{(10 + W) \times \%TS}{W \times \left(\frac{100 - MC}{100}\right)}
\]

Eq(12)

Where MC = moisture content of the flour, % TS = percentage of dry matter in the wet flour mixture after sieving, and W = weight(g) of the flour sample.

**Antioxidant properties**

*Preparation of extracts for the determination of total phenolic content:* The extracts were prepared according to the method described by Maliak & Singh. [66] Flour samples (0.5 gram) was weighed into a 15 mL centrifuge tube and 5 mL 80% ethanol was added to it as a solvent. The mixture was centrifuged at 5000 rpm for 20 minutes and the supernatant saved. To the sediment, 2.5 mL of 80% ethanol was added, and the mixture was centrifuged for 20 minutes at 5000 rpm. The supernatant was recovered and added to the supernatant from the previous step. The supernatant was covered with aluminum foil and allowed to stand for 24 hours. After 24 hours, 5 mL of distilled water was added to the residue to obtain sample extract.

*Preparation of extract for DPPH and TBARS*

The extracts were prepared according to the method described by Maliak & Singh. [66] Half (0.5) gram of flour was weighed into a 15 mL centrifuge tube, and 5 mL of ethanol was added to it as a solvent. The sample was mixed and left to stand for 24 hours. After 24 hours the mixture was centrifuged at 5000 rpm for 20 minutes. The supernatant was recovered and used for analyses.

**Total phenolic content**

The evaluation of total phenol content of the flour samples was done using a modified version of the Folin-Ciocalteu method described by Maliak & Singh. [66] Sample extract (2 mL) was added to scintillation vials and made up to 3 mL with distilled water. 0.5 mL of Folin-Ciocalteu reagent was added to each vial. After 3 minutes 20% sodium bicarbonate was added to each vial. The mixture was placed in boiling water for one minute then allowed to cool to room temperature. The absorbance of the mixture was then read at 650 nm using distilled water as blank. Gallic acid was used as a reference and the results were expressed in milligrams of gallic acid (GAE) per gram of the sample extract.

**DPPH radical scavenging activity**

Antioxidant activity was determined using the method described by Horvat et al., [67] To each flour sample, 0.2 mL was taken and added to a mixture of 1 mL 0.5 mMol/L 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanol solution and 2 mL ethanol. The mixture was incubated in a dark place for 30 minutes. Absorbance was measured at 517 nm. All experiments were repeated four times (n = 4). The percentage of inhibition of free radical DPPH was calculated against blank using Eq (13). For the blank, the sample was replaced with ethanol in the mixture. The DPPH scavenging activity was measured using Eq (13).

\[
\text{DPPH} \% \text{ scavenging activity} (\% \text{Inhibition}) = 1 - \left(\frac{A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100
\]

Eq(13)
where $A_{\text{sample}} = \text{Absorbance of the samples at time} = 30 \text{ minutes}$ and $A_{\text{blank}} = \text{Absorbance of the blank at time} = 0$.

*Thiobarbituric acid reactive substances (TBARS) test:* Lipid oxidation using TBARS method was measured as described by Zed & Ullah.\cite{LB45} A 4.0 mM standard solution of thiobarbituric acid (TBA) was prepared in acetic acid. The sample extract (1 mL) was mixed with 1 mL TBA reagent. The mixture was heated in a boiling water bath at 95°C for 1 hour. The mixture was cooled to room temperature, and the absorbance was measured using UV-visible spectrophotometer at 532 nm. Malondialdehyde MDA was used as a reference and different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM MDA were prepared. The TBARS was calculated using Eq (14).

$$TBARS \ (\mu M/g) = \frac{(Ac \times V)}{W} \quad \text{Eq(14)}$$

where $Ac$ is the concentration determined from the calibration curve and $W$ is the weight of the sample taken while $V$ is volume in mL of the total extract prepared.

**Statistical analysis**

The generated data were subjected to one way-analysis of variance (ANOVA) and the Tukey HSD (honest significance difference) test was used to compare means. Data was analyzed using JMP 14.0 software (SAS Institute Inc, Cary, NC). Significance was accepted at 95% confidence interval ($p < .05$). Results were expressed as mean ± standard deviation.

**RESULTS AND DISCUSSION**

**Grain size**

Grains were spread out on a plain sheet of white paper with a 1 cm scale placed at the bottom. The scale was used to obtain dimensions in mm since images give dimensions in pixels. Grains were arranged without touching or overlapping each other to simplify the image processing process, as shown in Figure 2. Images were captured with a Samsung Galaxy S8 camera with resolutions at about 1440 × 2960 pixels. The images were then converted to 8-bit binary images. The calibration of the scale was done with the known scale that was placed in the image. Then a part of the image was selected for analysis.

The diameter of the grains is given in Figure 3. The diameter ranged between 0.39 to 3.05 mm ± 0.41 for barnyard millets, 1.51 to 3.07 mm ± 0.21 for finger millet, 0.29 to 2.95 mm ± 0.31 for little millet and 3.58 to 5.48 mm ± 0.34 for sorghum. Ramashia et al.\cite{BS66} obtained similar diameters for the similar length, width and thickness of finger millet cultivars. Similar results have also been reported by Liu et al.\cite{GZ74} results for sorghum with kernel diameter ranging from 1.93 to 2.55 mm. Jain & Bal\cite{MH99} observed similar trends for three pearl millet cultivars with lengths ranging from 1.70 to 3.12 mm, 1.83 to 2.95 mm, and 2.0 to 3.36 mm. Variability among different cultivars, varieties of millets, and sorghum grown at different geographical and climatic conditions needs to be considered. Considering the prevalent use of cell phones in Africa & Asia, this low cost and simple methodology could be developed further for quality control in the field, small holder farmers, small & medium scale processing plants.

**Density**

The analysis showed that bulk density values ranged from 784.3 to 800 kg/m$^3$ for barnyard millet, 784.3 to 800 kg/m$^3$ for little millet, 769.2 to 800 kg/m$^3$ for finger millet, and 689.7 to 769.2 kg/m$^3$ for Sorghum (Table 3 and 5). Low bulk density value of finger millets could imply that of all the grains...
finger millet would occupy the least space during storage. Goswami et al.,[71] reported similar bulk density (684.99 to 777.50 kg/m$^3$) results for finger millet grains. Jain & Bal[59] reported results for the bulk density of three pearl millet cultivars ranging from 830.0 to 866.1 kg/m$^3$.

The result shows that the bulk density of the flours was highest with finger millets (696.74 ± 29.72 kg/m$^3$) and lowest on little millets flour (553.59 ± 31.44 kg/m$^3$). Similar results were reported by Dharmaraj et al.,[72] with bulk densities ranging from 0.77– 0.83 g/ml for decorticated finger millet whole meal before and after hydrothermal treatment. Akinola et al.,[73] reported a 1.27 g/ml bulk density for the same material.

Figure 2. (a) Image capture of sorghum grains. (b) 8-bit image after applying band pass filter. (c) binary image of grains after thresholding. (d) outline of grains after analysis.

Figure 3. Size distribution of grains. (A) Barnyard millet (B) Finger millet (C) Little Millet (D) Sorghum.
density value for pearl millet flour. Flours with higher bulk densities indicate their suitability for use in food preparations, while lower bulk density flours are more suitable for weaning food formulation preparation.\[69\] Since Little millets had the least bulk density, it could be beneficial in formulating complementary foods.\[74\]

Bulk density is an essential parameter in determining the separation, cleaning, sorting, packaging, and transportation requirement of particulate or powdery foods.\[75\] Tapped density is the bulk density obtained after mechanically tapping a container and was used to calculate porosity. To obtain consistently reproducible results, a mechanical shaker at a fixed rate and time was used to determine tapped density in this study. True density was determined using the gas pycnometer instead of the water displacement method due to the small size of the grains that could cause some of the grains to float in water.

**Porosity**

Porosity values was calculated based on the relationship between bulk and true densities. The mean porosity results varied from 45.49 ± 0.29% for barnyard millet, 44.92 ± 0.71% for finger millet, 45.24 ± 0.89% for little millet and 20.02 ± 1.07% for sorghum. Similar results were reported for maize, green wheat and pearl millet at vary moisture content.\[58,59,76\] Ramashia et al.\[69\] reported lower porosity results for finger millet cultivars ranging from 24.31 ± 2.10 to 32.41 ± 5.40%.

**Carr compressibility index (CI) and Hausner ratio (HR)**

Carr’s compressibility index (CI) and the Hausner ratio (HR) of the grains were used to determine the flow properties of the grains and flours. CI and HR results presented in Table 5 were interpreted as shown in Table 3. The CI and HR values for all the grains and flour indicated excellent flowability with no significant difference between the grains. This is in accordance with results from Meera et al.\[77\] for several brown rice varieties.

**Angle of repose**

Angle of repose is the angle formed between the slope of the pile and a horizontal plane when the pile is stationary.\[78\] The angle of repose values for millet and sorghum grains and flour are shown in Table 5 and was interpreted according to the classifications shown in Table 4. There was a significant difference (p > .05) between the angle of repose of the grains with values ranging from 14.5 ° for finger millet and 21.2° for little millet. The low angle of repose values could be due to the fact that raw grains are round and easily slide on each other, resulting in a lower angle of repose. Materials with low angle of repose flow easily and can be transported using very little energy.\[80\] A similar angle of repose results was obtained by Bhadra et al.\[81\] for sorghum grains. There was no significant difference in the angle of repose for the flours. The vertical cylinder and funnel methods were employed to measure the

| Flow Character                  | Hausner Ratio | CI%  |
|--------------------------------|---------------|------|
| Excellent/very free flow        | 1–1.1         | ≤10  |
| Good/free flow                  | 1.12–1.18     | 11–15|
| Fair                            | 1.19–1.25     | 16–20|
| Passable                        | 1.26–1.34     | 21–25|
| Poor/cohesive                   | 1.35–1.45     | 26–31|
| Very poor/very cohesive         | 1.46–1.59     | 32–37|
| Very, very poor/approx. non-flow| >1.60         | >38  |
angle of repose of grains and flour due to the limited quantity of sample available. Other methods for determining the angle of repose of granular material like the tilting box and revolving drum method would require a larger amount of sample.

**Water activity and moisture content**

The grains’ water activity varied significantly, with sorghum having the highest mean value and finger millet with the lowest mean value. There was no significant difference in the water activity of the flour samples. The water activity of Sorghum and millet flours ranged between was 0.37 ± 0.00 to 0.41 ± 0.01, indicating high microbial stability. The moisture content of barnyard flour varied significantly at 7.7 ± 0.07% to 8.5 ± 0.19% for little millet. Similar results were obtained by Liu et al., with mean moisture content ranging from 9.20 to 14.77% for different sorghum varieties. Ramashia et al. reported moisture content values varying from 7.88 ± 1.92 to 9.38 ± 3.08% for finger millet flour varieties. Moisture content and water activity of flour are important physical properties of grains and flour and good indicators of storage stability.

**Water solubility**

Water solubility is a critical quality parameter that affects the acceptance of a product by consumers. The solubility index of flour is its ability to dissolve in water. There was a significant difference (p < .05) between the solubility values of the flours. Solubility index values ranged from 4.99 ± 2.18% for little millet to 1.42 ± 0.15% for barnyard millet (Table 5). According to these results, all the samples can be considered as non-soluble. The difference in water solubility could be because of the high starch content and low protein content and fat in finger millet. These results are similar to that of Gull, Prasad, and Kumar, as they reported water solubility index of 4.13% for pearl millet flour.

**Water holding capacity**

Water holding capacity measures the amount of water absorbed by starch and is used as an index of gelatinization. The flours’ water holding capacity ranged from 1.08 ± 0.06 to 1.43 ± 0.09 g/g, with sorghum flour having the highest value and barnyard flour with the lowest value. The water holding capacity was found to be similar to the results obtained from a previous study. The high-water absorption observed in sorghum and finger millet could be due to their high starch content since starch and proteins contain hydrophilic parts that enhance water uptake.

**Dispersibility**

Dispersibility is a measure of the rehydration ability of flour or starch. The higher the dispersibility, the better the sample reconstitutes in water. Finger millet had the least mean dispersibility value of 28.78 ± 4.97%, while little millet had the highest mean value of 5.53 ± 5.84%. Lower dispersibility values observed for the flours imply that the flour samples may clump during rehydration.

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**Table 4. Classification of flowability of powder based on repose angle**

| Description                  | Repose Angle |
|------------------------------|--------------|
| Very free flowing            | <30°         |
| Free flowing                 | 30–38°       |
| Fair to passable flow        | 38–45°       |
| Cohesive                     | 45–55°       |
| Very cohesive (non-flowing)  | >55°         |

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Color

Table 6 shows the results of the color measurements of grain and flour samples as recorded in terms of the L* (Lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma), and H* (Hue angle) values. Color values varied significantly (p < .05) among the grains. Barnyard millet grains had the highest L* value of 65.79 to 65.55 ± 0.35 and finger millet grains the least at 23.94 to 24.24 ± 0.16. These results are like Ramashia et al., [69] with L* values ranging from 19.23 ± 0.42 for black to 52.97 ± 1.76 for milky cream grain cultivars of finger millets. Siwela et al. [89] had similar results that ranged from 45.9 ± 0.9 for finger millet grain. A similar L* value trend was noted for the flour samples. After milling the lightness (L*) values for all the flours increased. Aboubacar & Hamaker [90] had similar results for sorghum flour. Color is an important grain quality parameter that affects the resulting product and could influence consumer acceptance. [69]

Total phenolic content

Phenolic compounds are the major contributors to the antioxidant activity of cereals. [91] The total phenolic contents were determined using the Folin Ciocalteu (FC) method and results (Figure 4) are expressed in terms of the gallic acid equivalent (GAE) in mg/g of the extract. The FC method has been

| Grain properties | Barnyard millet | Finger millet | Little millet | Sorghum |
|------------------|----------------|--------------|---------------|---------|
| Bulk Density Kg/m³ | 797.4 ± 5.9 | 787.0 ± 10.6 | 794.8 ± 7.4 | 728.2 ± 25.4 |
| True Density Kg/m³ | 1462.9 ± 3.7 | 1428.8 ± 3.5 | 1451.6 ± 10.5 | 1403.3 ± 25.3 |
| CI% | 3.7 ± 2.4 | 3.2 ± 2.9 | 5.0 ± 1.5 | 5.7 ± 3.0 |
| Haussner | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.1 ± 0.0 | 1.1 ± 0.0 |
| Porosity % | 45.5 ± 0.3 | 44.9 ± 0.7 | 45.2 ± 0.9 | 48.1 ± 2.7 |
| Angle of Repose (°) | 21.2 ± 1.0 | 14.5 ± 3.9 | 21.2 ± 1.2 | 20.0 ± 1.1 |
| Water Activity | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 |

| Flour properties | Barnyard millet | Finger millet | Little millet | Sorghum |
|------------------|----------------|--------------|---------------|---------|
| Bulk Density Kg/m³ | 570.6 ± 40.7 | 696.7 ± 29.7 | 553.6 ± 31.4 | 580.4 ± 30.8 |
| CI% | 11.1 ± 1.7 | 9.7 ± 4.7 | 7.9 ± 2.5 | 11.9 ± 2.4 |
| Haussner | 1.1 ± 0.0 | 1.1 ± 0.1 | 1.1 ± 0.0 | 1.1 ± 0.0 |
| Angle of Repose (°) | 30.8 ± 2.8 | 31.1 ± 3.5 | 30.8 ± 2.7 | 29.5 ± 4.4 |
| Water solubility % | 1.4 ± 0.2 | 2.6 ± 0.7 | 5.0 ± 2.2 | 2.8 ± 0.5 |
| Water holding capacity (g/g) | 1.1 ± 0.1 | 1.4 ± 0.1 | 1.1 ± 0.1 | 1.4 ± 0.1 |
| Moisture Content (%) * | 7.7 ± 0.1 | 8.3 ± 0.2 | 8.5 ± 0.0 | 7.8 ± 0.1 |
| Water Activity | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.4 ± 0.0 |
| Dispersibility % | 33.8 ± 6.5 | 28.8 ± 5.0 | 45.5 ± 5.8 | 30.3 ± 5.0 |

The mean ± standard deviation, n = 6. Values followed by the same letters in the same row indicate no significant difference by the Tukey HSD test at 5% level of significance (p < 0.05). *n = 4

Table 6. Color characteristics of Sorghum and Little, barnyard and finger millets.

| Grain Color | L | a* | b* | C | h* | Δ e |
|-------------|---|----|----|---|----|-----|
| Barnyard | 65.6 ± 0.4 | 3.9 ± 0.0 | 21.1 ± 0.1 | 21.5 ± 0.1 | 79.7 ± 0.0 | - |
| Finger | 24.1 ± 0.2 | 8.8 ± 0.1 | 3.2 ± 0.1 | 9.4 ± 0.2 | 20.1 ± 0.3 | - |
| Little | 57.7 ± 0.6 | 3.9 ± 0.1 | 20.0 ± 0.2 | 20.3 ± 0.2 | 79.1 ± 0.0 | - |
| Sorghum | 54.38 ± 0.54 | 5.18 ± 0.1 | 22.9 ± 0.3 | 23.5 ± 0.3 | 77.3 ± 0.2 | - |

| Flour Color | Barnyard | 78.3 ± 0.8 | 1.7 ± 0.0 | 17.3 ± 0.2 | 17.3 ± 0.2 | 84.5 ± 0.0 | 23.5 ± 0.6 |
|-------------|----------|-------------|-----------|-------------|-------------|-----------|-------------|
| Finger | 55.1 ± 0.9 | 4.6 ± 0.1 | 8.2 ± 0.2 | 9.4 ± 0.2 | 61.0 ± 0.1 | 53.9 ± 4.8 |
| Little | 78.0 ± 0.5 | 1.8 ± 0.0 | 16.3 ± 0.1 | 16.4 ± 0.2 | 83.7 ± 0.8 | 23.1 ± 0.3 |
| Sorghum | 74.0 ± 0.6 | 2.2 ± 0.0 | 18.4 ± 0.2 | 18.6 ± 0.2 | 83.3 ± 0.0 | 27.6 ± 0.4 |

Mean ± standard deviation, n = 6. Values followed by the same letters in the same column indicate no significant difference by the Tukey HSD test at 5% level of significance (p < 0.05).
validated as the standard method for determining of total polyphenols in plant extracts. The TPC of the four grains are shown in Figure 3. The highest TPC was found among the grains in finger millet (0.042 mg GAE/g) and the lowest in little millet (0.012 mg GAE/g). These results correspond to that of Siwela et al. that reported TPC ranging from 0.05–1.84 mg GAE/100 mg for finger millets varieties. Awika et al. reported TPC of 0.8 mg GAE/g in white sorghum compared with 9.8–22.5 mg GAE/g in brown and black sorghum varieties. The phenolic compounds content in millet and sorghum grains would vary depending on the morphological fraction, variety, climate and cultivation methods used.

**DPPH**

The DPPH assay method is based on the reduction of DPPH, a stable free radical with purple color absorbed at 517 nm. Antioxidants neutralizes DPPH free radicals resulting in a discoloration that indicates the antioxidant efficacy. As seen in Figure 5 the DPPH radical scavenging activity ranged from 51.97% for little millet to 63.99% for finger millet. However, there was no significant difference in the DPPH activities of the grains. Pradeep & Guha reported % inhibition of DPPH values ranging from 90.2 to 95.5% for little millets undergoing different processing methods. Sreeramulu et al. reported DPPH scavenging activity of 1.73 (mg Trolox equivalent/g) for finger millet. Shejawale et al. reported DPPH activity ranging from 65.16% to 82.22% for six foxtail millet varieties at different processing conditions.

**TBARS**

Thiobarbituric acid reactive substance (TBARS) is a widely known method for detecting lipid oxidation. The TBARS assay measures the pink pigment formed through the reaction of thiobarbituric acid (TBA) and malondialdehyde (MDA) in the presence of heat. The spectrophotometric method for TBA analysis was used in this study because it is a simpler and cost-effective alternative to HPLC procedures. Absorbance was measured at 532 nm. The results for the TBARS test is presented in Figure 6. The TBARS concentration was highest in Barnyard millet at 0.6803 μM/g and lowest in sorghum at 0.3257 μM/g. Similar result were reported by Baublis et al. for wheat flour.

![Figure 4](image-url) (A) Standard calibration curve of gallic acid (b) Total phenol content of barnyard, finger, little millets and sorghum in Gallic acid equivalent (GAE) in mg/g of the extract. Mean values not followed by the same letter are significantly different (Tukey’s HSD, p < .05). Data expressed in Mean ± SD of n = 4 replicates.
CONCLUSION

This study showed significant variations in the physical properties of the grains. Barnyard and little millet grains were found to have comparable properties, while finger millet and sorghum grains differed significantly from the rest of the grains. The mean values obtained for the diameter of barnyard millet, finger millet, little millet and sorghum were 1.60, 1.99, 1.90 and 4.54 mm, respectively. There was no significant difference in the flow properties of the grains and flour. Bulk density, true density, porosity, color attributes, solubility, water holding capacity, and dispersibility varied significantly between the flours. Little millet had the highest solubility of the raw grains at 4.99%. Results indicated the presence of antioxidant activity in all the grains. The highest DPPH antioxidant activity was found in sorghum and little millet. Total phenolic content and TBARS were highest in barnyard millet and finger millet. Physical and functional properties of all the millets and sorghum indicate a potential application into food products. The methodologies described in this study can be adapted...
by small farmers and farmers in developing countries as low-cost alternatives for grain quality determination. Sorghum and millets are important in terms of their agronomic and nutritional advantages and can play a significant role in global food security. For people living in less developed countries or in countries ravaged by drought conditions and climate change, millet and sorghum may be a nutritious and valuable alternative to provide sustainable food and economic security. Support from the local governments and non-government organizations (NGOs) can help create awareness among all stakeholders involved to promote more cultivation and consumption of the grains. The effect of unit operations like sieving, grading, milling, kneading, and baking on final product quality is significant to sustainable innovations of underutilized grains. Therefore, further studies on the effects of moisture content and processing techniques on the grains’ physical, rheological, and nutritional properties are essential to design innovative products made using these underutilized grains.

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Highlights

(1) Image] was used to determine grain diameter efficiently.
(2) There were variations in the physical and functional properties of the grains and flour
(3) All grains indicated antioxidant activities

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