



# Physical and Structural Characterization of Underutilized Climate-Resilient Seed Grains: Millets, Sorghum, and Amaranth

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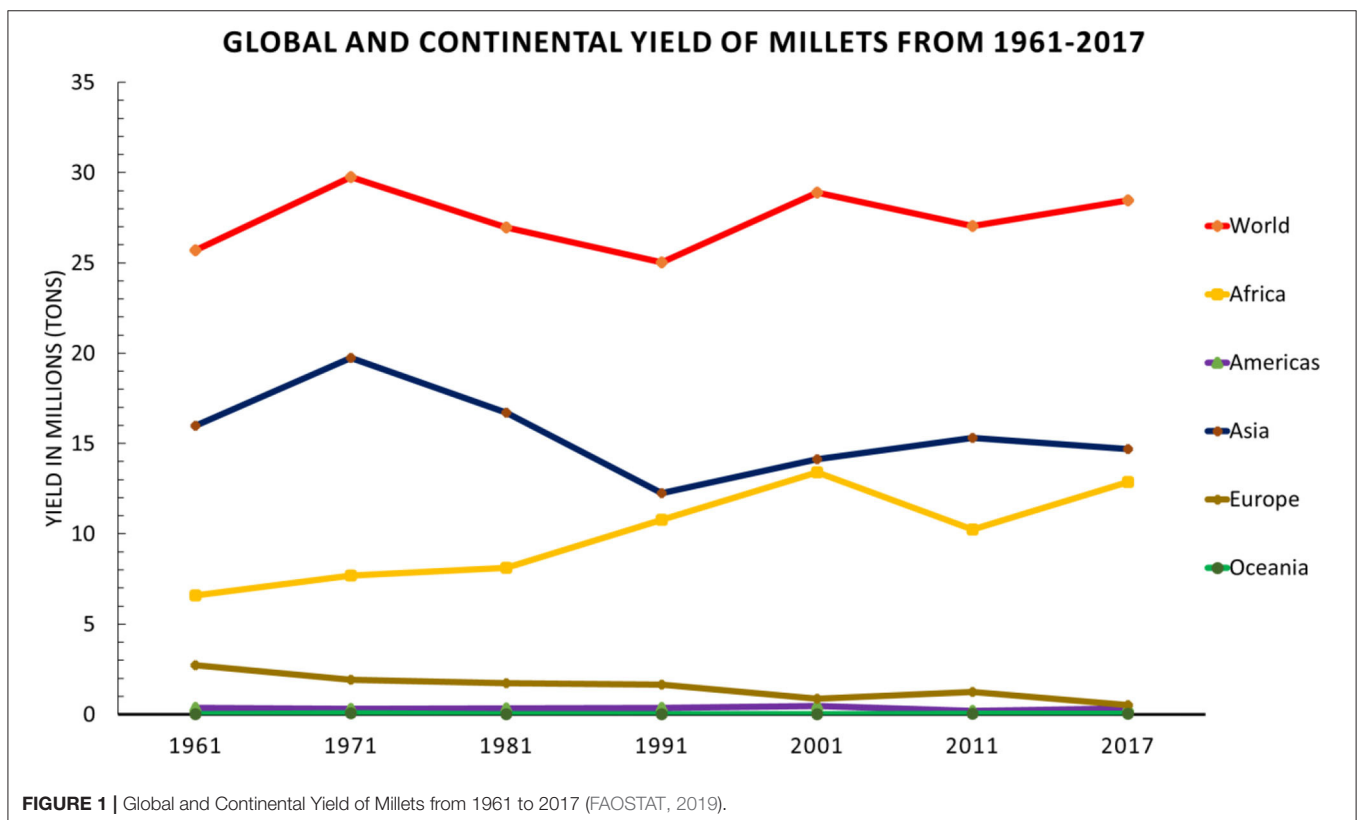
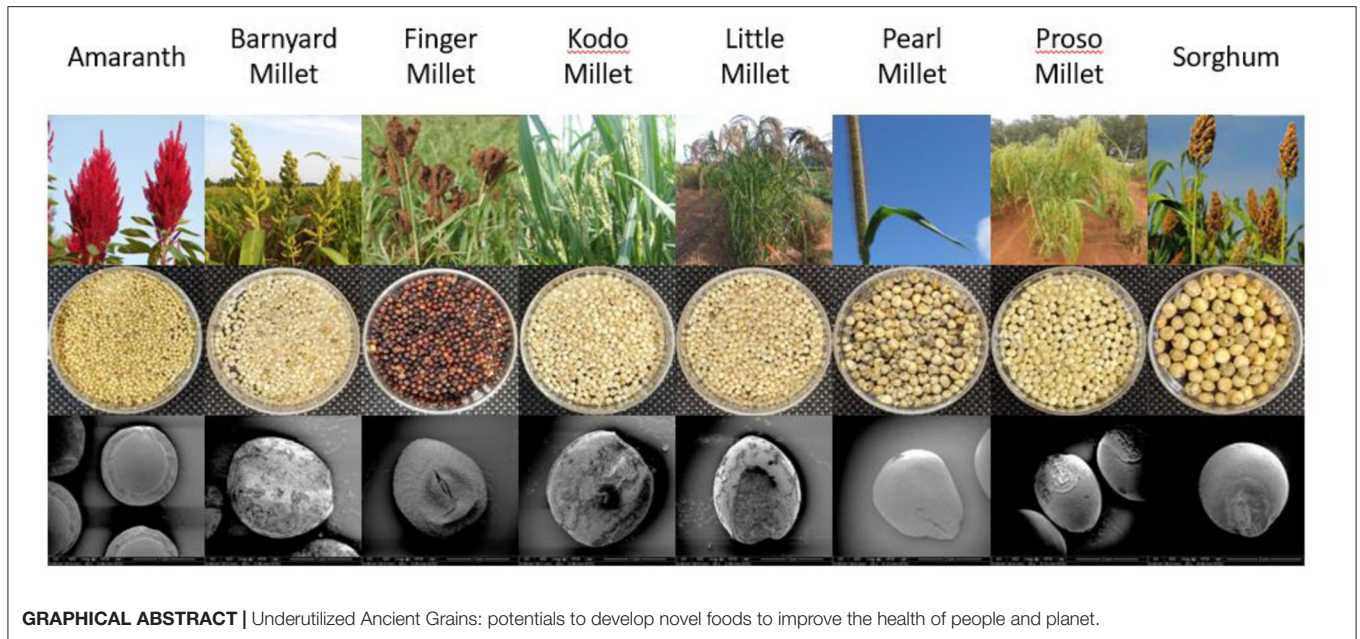
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While the world is facing food and nutritional challenges leading to the multifaceted burden of malnutrition (underweight and overweight), there is a need to sustainably diversify and explore underutilized crops. Climate-resilient crops, which have the potential to withstand climate crises, have drought resistance, and provide healthy foods with essential vitamins and minerals. Ancient seed grains like amaranth, millets, and sorghum are highly nutritious seed grains that are underutilized, and there is a need for comprehensive research into their properties. This study will specifically investigate amaranth alongside barnyard, finger, kodo, little, pearl, proso millets, and sorghum. Physical and structural properties of the ancient seed grains can provide useful data for storage and food processing. The angle of repose, porosity, and water activity of the grains varied from 19.3° to 23.9°, 3.6 to 17.4%, and 0.533 to 0.660 at 25.5°C, respectively. Additionally, Scanning Electron Microscopy (SEM) was used to observe the surface characteristics and overall shape of each grain. SEM images of the millets shows the impact of dehulling on the surface morphology of the grains (little, barnyard, proso, and kodo millets). This calls for research and development of novel food processing technologies to minimize loss and damage during processing of climate-resilient crops.

**Keywords:** millet, scanning electron microscope, food and nutrition security, sustainable diets, SDG #2, ancient grains, UN sustainable development goal

## INTRODUCTION

The bold and transformative 2030 Agenda for Sustainable Development was officially launched in September 2015 by 193 member states of the United Nations. The 2030 agenda calls on countries to begin efforts to achieve the 17 Sustainable Development Goals (SDGs) over the next 10 years (United Nations, 2020). SDG 2 focuses on Zero Hunger, to achieve food security, improved nutrition, and promote sustainable agriculture. Food and nutrition security are interconnected and in order to achieve them in the near future, there is a need to explore underutilized crops as food sources leading to diversification of healthy diets to feed 9 billion population in 2050. Millets are a group of small nutritious seed grains that are primarily grown in Africa and Asia. **Figure 1** shows production of millets by geographical region and year. The various millets



represent both cold and warm season crops that can be suited for dry and wet conditions, depending upon the species. Pearl millet, which originated in Africa, is suited for hot and dry climates with little rainfall but also tolerates hot and wet climates. By

contrast, proso millet is from a colder climate, originating from Central and Eastern Asia, so it performs better in cooler areas. Millets generally reach maturity faster than crops like maize or wheat. Foxtail and proso millet can mature in 60–90 days

while pearl and finger millet can mature in up to 100–110 days (Myers, 2018b). Millet uses vary from being processed into infant formula, fermented foods, flour, breads, beer, and snacks and can be prepared in a multitude of ways. Millets are a highly underutilized seed grain in the Western world, and in the US, they are often used as animal feed or cover crops to promote healthy soil (Myers, 2018b).

Amaranth is another ancient cereal grain that was first utilized by humans 6,000 years ago and was once an important crop to the Aztecs. Like millet, amaranth has drought resistance and includes many different varieties that are adaptable to a wide range of environments (Myers, 2018a). The leaves of the vegetable amaranth plant can be boiled and consumed like spinach, while the grain amaranth can be popped, ground into flour, or turned into many ready-to-eat products, such as millets (Robinson, 1986). In the US, amaranth has gained popularity in commercial products like sandwich bread, breakfast cereals, granola bars, and crackers (Myers, 2018a).

Millets and amaranth have the potential to help address climate change challenges (Saxena et al., 2018) and the need to find alternative crops to feed the growing population with nutritious food. To utilize and popularize these forgotten grains, novel value-added food product development, optimization, and research is required in this area. Ancient seed grains are gaining popularity among millennials and Gen Z for their low-calorie and low-fat contents. An ideal way to introduce consumers to an unfamiliar ingredient, like millet or amaranth, is to develop a familiar product with this new food ingredient. They also have potentials to be used in food fortification as natural alternatives due to rich micronutrient profile and create a new demand for millet and amaranth-based foods.

The health benefits of ancient seed grains are numerous, as there are several different varieties and each with its own strengths. All varieties of millets and amaranth are naturally free from gluten and have a lower caloric content than rice and wheat. Millets are also lower in carbohydrates than rice, which can help prevent the development of diabetes and obesity which has become a serious problem in our modern world. Pearl and proso millet are higher in protein than all other millets as well as rice. However, they both have a lower protein content than amaranth, (Valcárcel-Yamani and Caetano da Silva Lannes, 2012). Barnyard millet has a higher amount of fiber compared to rice, wheat, and other millets, which aids in digestion and nutrient retention. Finger millet contains a third of the daily recommended calcium intake, which is the most significant percentage of any essential mineral found in millets (Saleh et al., 2013). Other high percentages of essential minerals include iron in barnyard millet, zinc in foxtail and barnyard millets, niacin in proso millet, and riboflavin in barnyard millet.

Amaranth has a high protein content and contains almost twice as much protein as maize. Although the total protein content is not as high as a legume's, amaranth can be consumed as a source of "complete" protein because it has an ideal balance of the nine essential amino acids (Myers, 2018a). A more complete comparison between millets, amaranth, rice, and wheat can be seen in **Table 1**.

Hidden Hunger is a worldwide issue (deficiency due to vitamins and minerals): specifically, it is the double burden of malnutrition (stunting/underweight and obesity/overweight), which can affect any age, gender, or nationality. Children deficient in iron and zinc are shown to have stunted growth, impaired immune function, and irreversible neurological and psychomotor impairments (Mehta et al., 2017). Efforts to solve this crisis have recently included the biofortification of pearl millet in India, Govindaraj et al. (2019) have attempted to genetically improve cultivars of pearl millet to increase the densities of iron and zinc. There is a need to specifically design food processing equipment for ancient seed grains, develop new value-added food products, and expand the market needs for diversification of healthy diets.

The physical and structural properties of grains are critical and impact food processing parameters that are essential in the design of equipment for handling, harvesting, processing, and storing of grains (Baryeh, 2002). The shape of the grains is important in the design of sorting and sizing machines (Jain and Bal, 1997). Water activity, bulk density, true density, and porosity are important parameters to consider in the designing of storage structures for grains (Singh et al., 2010). The angle of repose is an important measure in the designing of storage, processing, and transporting structures (Bhadra et al., 2017). Scanning Electron Microscopy (SEM) is used to identify microstructural features of grains that would be otherwise hard to differentiate with human visual observations. The present study is investigating the physical and structural properties of barnyard millet, finger millet, kodo millet, little millet, pearl millet, proso millet, sorghum, and amaranth as an observational exercise to establish a physical characterization method. This information can help researchers and food industries explore the usage of the underutilized ancient seed grains in the development of new sustainable diets.

## MATERIALS AND METHODS

### Materials

#### Ancient Grains

The amaranth sample was provided by Dr. Rob Myers (Division of Plant Sciences, Columbia, MO), from his 2018 harvest of the Crimson Glow cultivar. The barnyard, little, kodo, and proso millets were obtained from Manna Foods (Southern Health Foods Pvt. Ltd, Chennai, India). The finger, pearl, and sorghum millets were supplied by Babco Foods International, LLC (Bridgewater, NJ 08807). All grain samples were stored at room temperature. The reasons for using commercial samples were based on the accessibility of raw materials and food availability to consumers/food start-up company interested to utilize ancient seed grains for new product development. Packaged food materials are subjected to food quality standards before entering commercial markets. Thus, for this study, we used commercially available ancient seed grains samples currently available for human consumption as an observational exercise to establish a physical characterization method. **Table 2** shows the whole grain image (photographic representation) used in this study along with information related to the geography of domestication, major producers of ancient seed grains.

**TABLE 1** | Nutritional Comparison of Millets, Amaranth, Rice, and Wheat (per 100 g) (Valcárcel-Yamani and Caetano da Silva Lannes, 2012; Saleh et al., 2013; Kumar et al., 2018; Nithyanantham et al., 2019).

Grain	Protein (g)	Carbohydrates (g)	Fat (g)	Calcium (mg)	Iron (mg)	Calorific value (kcal)
Amaranth	14.60	68.8–70.3	8.81	180.1–217.0	9.2–21.0	–
Barnyard Millet	6–13	55.0–56.88	3.53–3.9	18.33–22	15.47–19.7	300
Finger Millet	7.3–10	71.52–72.6	1.43–1.5	240–410	3.67–4.87	334–336
Kodo Millet	8.3–10	63.82–66.6	3.03–3.6	10–35	1.7–4.47	353
Little Millet	7.7–15	60.9	5.2	17.0–30	9.3	329
Pearl Millet	10.6–14	67.0–69.10	4.8	10–42	10.3–11.0	363
Proso Millet	10–13	67.09	3.09	14–23	1.0–3.4	352.5
Rice	3.61–6.8	75.33–90.39	0.87–2.7	0.05–10	0.47–2.03	341.2–396.8
Sorghum	10.4–10.82	70.7–72.97	3.1–3.23	25–35.23	4.01–6.57	329
Wheat	11.6–13.78	69.88–71.0	1.90–2.0	30–43.41	3.5–5.24	348–438

## Methods

### Angle of Repose

The angle of repose was calculated by filling a cylinder with grain that was then slowly lifted to create a conical mound (Akaaimo and Raji, 2006). The base diameter and the height of the cone were measured, and the following equation was used to calculate the angle of repose:

$$\phi = \tan^{-1} \frac{2H}{d}$$

where  $\phi$  is the angle of repose,  $H$  is the height, and  $d$  is the diameter. The measurements were replicated ( $n = 10$ ) times and the average value was reported. A plastic cylinder of 37 mm diameter was used. This value is an important property because it can impact the design of storage containers and processing equipment due to the differences in each grain.

### Bulk Density

The bulk density of grains was measured using two methods: the water displacement method and mass/volume relationship. The variability between both methods was compared. The water displacement method was done by filling a 25 ml graduated cylinder with 15 ml of water, 4.5 grams of each grain was added, and the volume of water displaced was measured. For the mass/volume method bulk density was measured using the procedure described by Mariotti et al. (2006). Four gram of each grain was poured into a 25 ml graduated cylinder gently tapped 10 times and the volume was noted. The ratio of the mass of the sample of grain to its total volume was then calculated ( $n = 5$ ). The results are expressed in  $\text{Kg}/\text{m}^3$ .

### True Density

The true density of the millets was measured using a Quantachrome Ultrapycnometer 1000 (Anton Paar, Graz, Austria). In total, 4.5 grams were weighed out and placed in the small cell for measurement. The equipment was operated using the multi-run setting with 5 runs and a standard deviation of 0.5%. The final three runs were averaged and reported by the equipment. This was repeated three times, each with a different sample of 4.5 grams to account for the variability in grain size and shape.

### Color

The color of each millet sample was measured using a Konica Minolta Chroma Meter CR-400/410 (Konica Minolta, Chiyoda, Tokyo, Japan). The device utilizes an  $L^*$ ,  $a^*$ ,  $b^*$  color space system to quantify the color of an object.  $L^*$  represents lightness and darkness,  $a^*$  represents redness when positive and greenness when negative, and  $b^*$  represents yellowness when positive and blueness when negative. The device was calibrated using a white sample provided by the manufacturer. For each sample, a container was filled, and the meter was placed on the sample. Five measurements were taken ( $n = 5$ ), with the average value reported.

### Porosity

The porosity of the millet grains was calculated using the true density values determined by the pycnometer and the experimental bulk density with the following equation.

$$\% \text{ porosity} = 1 - \frac{\text{bulk density}}{\text{true density}}$$

### Scanning Electron Microscopy

The internal and external structure of each millet type was determined using a Quanta 600F ESEM Scanning Electron Microscope located at the Electron Microscopy Core at the University of Missouri—Columbia. The grains were prepared by coating them with Platinum. Comparative analysis of multiple ancient seed grains at 5 KV power between 20 and 50X magnification.











### Shape

The shape of each grain was determined by evaluating the SEM images and using the data characterizations McDonough and Rooney (1989) determined to classify the different shapes of pearl millet they observed. These shape types include lanceolate, obovate, hexagonal, and globose.

### Water Activity







The water activity was measured by the Aqua Lab Cx-2 Water Activity Meter (Decagon Devices, Inc., Pullman, Washington 99163). The equipment determines the water activity of a sample by measuring the relative humidity of the sample when the

**TABLE 2** | Geography of domestication, major producers, and photographic representation of Ancient seed grains.

Grain	Scientific name	Geography of domestication	Year of domestication	Major producer(s)	Plant image	Whole grain image	Common name
Amaranth	<i>Amaranthus spp.</i>	Mexico & Central America (1)	8,000–7,000 BP (2, 3)	Latin America, USA, India, Africa, China, Eastern Europe, and Russia (1)			Spanish: Amaranto Hindi: Rajgira Kannada: Dhantina Soppu Tamil: Thandu Keerai Chinese: Hon-toi-moi Yoruba: Efo Tete Jamaican: Callaloo
Barnyard Millet	<i>Echinochloa esculenta</i>	Japan (4)	6,000 years ago (4, 5)	China, Korea, Japan, India (4)			Bengali: Shyama Hindi: Sanwa Kannada: Oodalu Oriya: Khira Punjabi: Swank Tamil: Kuthiraivolly Telugu: Udalu, Kodisama
Finger Millet	<i>Eleusine coracana</i>	East Africa (Ethiopia) (4)	5,000–4,000 years ago (4, 6)	Eastern and Southern Africa, India, Nepal, China (4)			Bengali: Marwa Gujarati: Nagli, Bavto Hindi: Ragi, Mandika Kannada: Ragi Oriya: Mandia Punjabi: Mandhuka, Mandhal Tamil: Keppai, Ragi Telugu: Ragi Chodi Hausa: Tamba Birom: Pwana Uganda: Bulo Swahili: Mandua Winbi
Kodo Millet	<i>Paspalum scrobiculatum</i>	India (4)	3,000 years ago (4, 7)	India (4)			Bengali: Kodo Gujarati: Kodra Hindi: Kodon Kannada: Harka Marathi: Kodra Oriya: Kodua Punjabi: Kodra Tamil: Varagu Telugu: Arikelu, Arika
Little Millet	<i>Panicum sumatrense</i>	India (4)	>6,400 BP (4, 8)	India, Nepal, Pakistan, Sri Lanka, Myanmar, Philippines (4)			Bengali: Sama Gujarati: Gajro, Kuri Hindi: Kutki, Shavan Kannada: Same, Save Marathi: Sava, Halvi, Vari Oriya: Suan Punjabi: Swank Tamil: Samai Telugu: Samalu

(Continued)

TABLE 2 | Continued

Grain	Scientific name	Geography of domestication	Year of domestication	Major producer(s)	Plant image	Whole grain image	Common name
Pearl Millet	<i>Pennisetum glaucum</i>	Northeast Mali (4)	4,500 BP (4, 9)	West and Central Africa, East and Southern Africa, India, Nigeria, USA (4)			Bengali: Bajra Gujarati: Bajri Hindi: Bajra Kannada: Sajje Marathi: Bajri Oriya: Bajra Punjabi: Bajra Tamil: Kambu Telugu: Sajja Marghi: Yadi Afrikaans: Bulrush Millet, Manna Sepedi: Leotsa Sesotho: Nyalothi isiNdebele: Inyouti Shangaan: Mhuga, Mhungu isiZulu: Unyaluthi, Unyawoti, Unyawothi
Proso Millet	<i>Panicum miliaceum</i>	Northern China (4)	10,300–8,700 BP (4, 10)	China, Kazakhstan, Afghanistan, India, Turkey, Romania, USA, Australia (4)			Bengali: Cheena Gujarati: Cheno Hindi: Chena; Barri Kannada: Baragu Marathi: Vari Oriya: China Bachari bagmu Punjabi: Cheena Tamil: Pani varagu Telugu: Variga
Sorghum	<i>Sorghum bicolor</i>	Northeast Africa (4, 11)	5,000 years ago (4, 12)	Nigeria, Burkina Faso, Niger, Mali, Sudan, Ethiopia, Cameroon, India, China, USA, South America, Australia (4)			Bengali: Jowar Gujarati: Jowari, Juar Hindi: Jowari, Juar Kannada: Jola Marathi: Jwari Oriya: Juara Punjabi: Jowar Tamil: Cholam Telugu: Jonna Yoruba: Okababa Hausa: Dawa, Jero Igbo: Soro South Africa: Mabele Thoro, Amazinba, Amabele East Africa: Mtama

BP-“Before Present” means before 1950—commonly used convention in radiocarbon dating.

**Data Sources:** FAO ICRISAT., 1996; ICAR- Indian Institute of Millets Research (IIMR), 2020; The United Sorghum Checkoff Program., 2020; (1) Arendt and Zannini, 2013; (2) Joshi et al., 2018; (3) Arreguez et al., 2013; (4) Taylor and Duodu, 2018; (5) Nasu and Momohara, 2016; (6) Hillu et al., 1979; (7) de Wet et al., 1983; (8) Fuller, 2011; (9) Manning et al., 2011; (10) Lu et al., 2009; (11) De Wet and Harlan, 1971; (12) Doggett, 1988; (13) Louw, 2020a; (14) Louw, 2020b. **Photo sources:** International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 2021, Indian Institute for Millet Research, Myers, 2018a, Amaranth: Pigweed Kin High in Protein., 2003, Food Engineering and Sustainable Technologies (FEAST Lab), University of Missouri.

sample water vapor pressure and air water vapor pressure reach equilibrium. The sample cups were filled less than half full and 5 measurements were made per sample. The average values were reported.

### Statistical Analysis

A one-way ANOVA was conducted for all experiments. The number of replicates varies for different experiments with a minimum of ( $n = 3$ ) for color, ( $n = 10$ ) for the angle of repose, and ( $n = 5$ ) for true density and water activity. All data

were analyzed using SAS<sup>TM</sup> software, Version 9.4 of the System for Windows 10.

## RESULTS AND DISCUSSION

### Angle of Repose

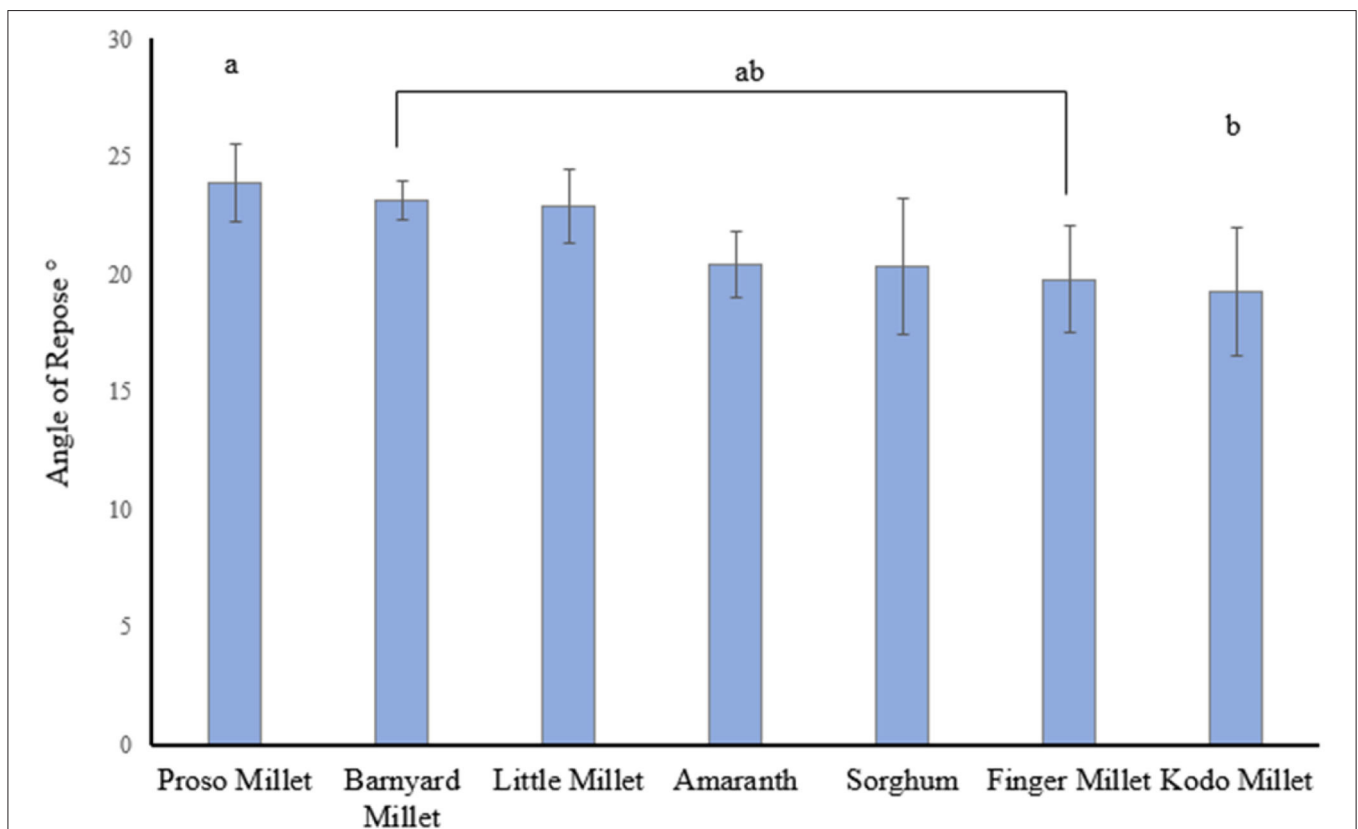
While designing food processing equipment and storage systems, the angle of repose is an important physical parameter to be estimated for each grain. An ancient grain with a high angle of repose will pile higher and require more energy to transport than

a grain with a lower angle of repose. The angle of repose varied from 19.3° to 23.9° with kodo millet having the smallest angle and proso millet with the largest angle. The angle of repose values is shown in **Figure 2**. The base measurements for kodo, sorghum, little, finger, barnyard, and proso were not significantly different from each other. The height measurements for little, barnyard, and amaranth were not significantly different from each other, while proso, little, barnyard, amaranth, finger, sorghum, and kodo were not significantly different from each other. The values for base, height, and angle are presented in **Table 3**.

Previous studies on the angle of repose use two main methods: a cylindrical method that was employed in this study and a “box”

method. This method uses a plywood box with two plates, one fixed and the other unfixed. The box is filled with the samples and then either gradually inclined until a slope is formed or the front panel is quickly removed to allow a slope to form. Rough rice (Varnamkhasti et al., 2008), popcorn kernels (Karababa, 2006), and pearl millet (Baryeh, 2002) used this “box” method. These values are reported to vary from 37° to 38°, 25.3° to 30.8°, and 34.4° to 49°, respectively.

Soybean (Davies and El-Okene, 2009) has been found using the same method this study uses, along with *Prosopis africana* seeds (Akaaimo and Raji, 2006). The reported values vary with respect to moisture content from 30° to 51° and 21.57° to 22.71°,



**FIGURE 2** | Angle of Repose for Ancient Grains. Mean values not connected by the same letters are significantly different (Tukey's HSD,  $P < 0.05$ ).

**TABLE 3** | Color, shape, and water activity of ancient grains.

Ancient Grain	Color			Shape	Water activity		Angle of repose		
	L*	a*	b*		Temp (°C)	Diameter (cm)	Height (cm)	Angle (°)	
Amaranth	58.08 <sup>d</sup> ± 0.91	5.86 <sup>b</sup> ± 0.18	24.71 <sup>a</sup> ± 0.82	Globose	0.583 <sup>b</sup> ± 0.003	25.2	10.44 <sup>a</sup> ± 0.32	1.94 <sup>ab</sup> ± 0.11	20.4 <sup>ab</sup> ± 1.55
Barnyard Millet	63.83 <sup>b</sup> ± 0.20	3.35 <sup>f</sup> ± 0.03	19.38 <sup>b</sup> ± 0.08	Globose	0.561 <sup>d</sup> ± 0.001	25.1	9.73 <sup>a</sup> ± 0.21	2.08 <sup>a</sup> ± 0.08	23.1 <sup>ab</sup> ± 0.97
Finger Millet	23.67 <sup>g</sup> ± 0.42	9.00 <sup>a</sup> ± 0.29	4.46 <sup>c</sup> ± 0.26	Obovate	0.533 <sup>f</sup> ± 0.003	25.5	10.26 <sup>a</sup> ± 0.4	1.84 <sup>ab</sup> ± 0.19	19.8 <sup>ab</sup> ± 2.56
Kodo Millet	59.12 <sup>d</sup> ± 0.52	4.68 <sup>d</sup> ± 0.05	18.79 <sup>d</sup> ± 0.17	Globose	0.563 <sup>d</sup> ± 0.002	25.3	10.12 <sup>a</sup> ± 0.67	1.76 <sup>b</sup> ± 0.18	19.3 <sup>b</sup> ± 3.04
Little Millet	57.69 <sup>c</sup> ± 0.45	3.24 <sup>e</sup> ± 0.03	19.57 <sup>e</sup> ± 0.16	Obovate	0.581 <sup>b</sup> ± 0.002	26.1	10.04 <sup>a</sup> ± 0.29	2.12 <sup>a</sup> ± 0.13	22.9 <sup>ab</sup> ± 1.76
Pearl Millet	52.86 <sup>e</sup> ± 0.08	2.48 <sup>g</sup> ± 0.02	16.40 <sup>f</sup> ± 0.03	Hexagonal	0.578 <sup>c</sup> ± 0.004	25.0	-	-	-
Proso Millet	72.64 <sup>a</sup> ± 0.19	2.22 <sup>e</sup> ± 0.04	30.93 <sup>g</sup> ± 0.09	Obovate	0.555 <sup>e</sup> ± 0.001	26.1	9.68 <sup>a</sup> ± 0.3	2.14 <sup>a</sup> ± 0.13	23.9 <sup>a</sup> ± 1.86
Sorghum	56.10 <sup>f</sup> ± 0.34	4.69 <sup>h</sup> ± 0.02	23.16 <sup>h</sup> ± 0.12	Globose	0.660 <sup>a</sup> ± 0.004	25.3	10.11 <sup>a</sup> ± 0.79	1.86 <sup>ab</sup> ± 0.21	20.3 <sup>ab</sup> ± 3.26

Mean values not followed by the same letter in the same column are significantly different (Tukey's HSD,  $P < 0.05$ ).

L\* = darkness to lightness, + = lighter, - = darker; a\* = greenness to redness + = redder, - = greener; b\* = blueness to yellowness + = yellower, - = bluer.

respectively. This study uses the cylindrical method as opposed to the box method because large amounts of grains are typically stored in cylindrical silos, so the values obtained would be the most applicable.

## Bulk Density

Bulk density's values of the grains ranged from 1,153 to 1,408  $\text{kg}/\text{m}^3$  when using the water displacement method and 728.16–828.72  $\text{Kg}/\text{m}^3$  with the mass/volume method. The values of bulk density for both methods followed the same trend for the grains with sorghum showing the least bulk density at 1,153  $\text{Kg}/\text{m}^3$  for the water displacement method and 728.16  $\text{Kg}/\text{m}^3$  for mass/volume method and kodo millet showing the highest bulk density at 1,408  $\text{Kg}/\text{m}^3$  for water displacement method and 828.72  $\text{Kg}/\text{m}^3$  for mass/volume method.

Sorghum, while the largest in appearance, had the lowest bulk density, and kodo millet had the highest bulk density. Previous studies done on the bulk density of minor millets include a study by Subramanian and Viswanathan (2003), which employed a mass/volume method. A container of known volume was filled to the top with grain which was weighed. The bulk density was calculated as a ratio of the mass of grains to the volume of the container. In the study by Subramanian and Viswanathan (2003), the millet with the greatest bulk density was barnyard millet, at 888.7  $\text{kg m}^{-3}$  and little millet had the lowest bulk density, at 748.1  $\text{kg m}^{-3}$ . Similarly, the bulk density of soybeans (Kibar and Öztürk, 2008) and popcorn kernels (Karababa, 2006) have been previously found with similar values. Both use a similar method of filling a 1,000 mL container full of the respective samples which were then weighed. A ratio of sample mass to container volume was calculated. The bulk density of soybeans varied from 719 and 766  $\text{kg} \cdot \text{m}^{-3}$ , while the popcorn kernels varied from 703 to 771  $\text{kg} \cdot \text{m}^{-3}$ . The distributions of bulk density are shown in **Figure 3**.

The observed difference in the values expressed in the water displacement method and the mass/volume method may be due to the presence of air spaces between and/or within the grains (Manger, 1966). Bulk density calculation using the mass/volume method includes the volume of the grains, open and closed pores, and the interparticle voids (Lorraine and Flint, 2002) resulting in a higher volume and lower density. In the water

displacement method, water is absorbed by the grains and it adheres to the pores of the grains and between the grains resulting in a lower volume of water displaced and a larger bulk density value.

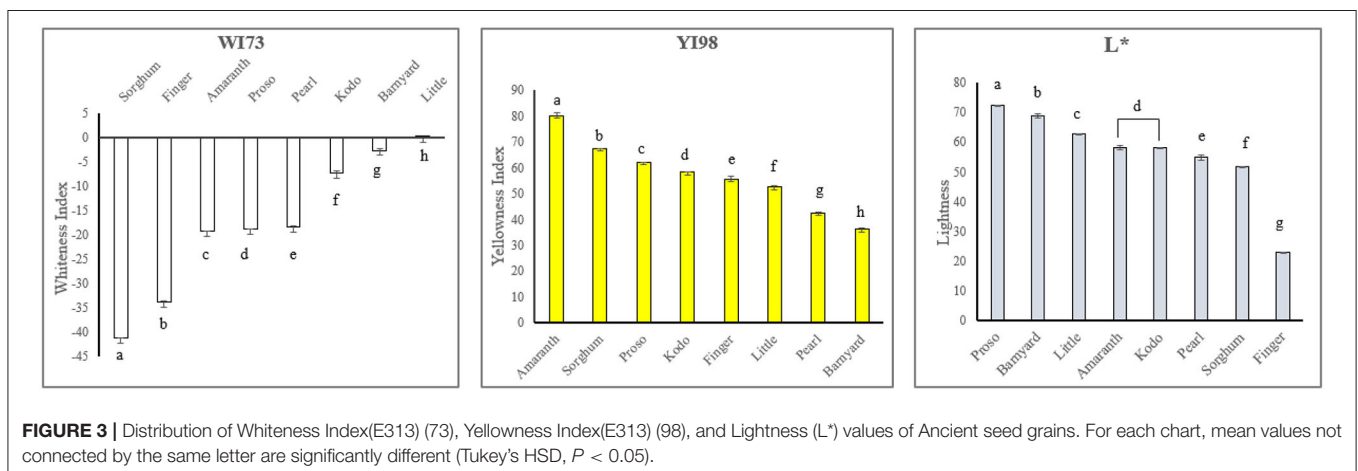
Two methods for bulk density measurements were analyzed in this study to observe which one would be more suitable for the ancient grains of smaller size profiles. The comparison results are presented in **Table 4**. In this study, we establish the water displacement method was not ideal for bulk density measurement of ancient grains of smaller size profile. The presence of pore spaces in the grains leads to the loading of gains on the water surface. As a result, the mass/volume method was determined to be more suitable for the bulk density measurement of ancient grains.

## True Density

The true density of the grains, as shown in **Table 4**, varied from 1390.17 to 1459.4  $\text{kg} \cdot \text{m}^{-3}$  with amaranth having the lowest true density and kodo have the largest. The masses of proso, barnyard, little, kodo, and amaranth could be grouped and were not significantly different from each other, while amaranth, little, kodo, and sorghum can be grouped and were also not significantly different from each other. The volume of each grain was significantly different from each other, except for sorghum and proso. The density of each grain was significantly different from each other.

Previous work finding the true density of soybean and rough rice has been done using the toluene displacement method. Kibar and Öztürk (2008) used the liquid displacement method outlined by Baryeh (2001) and Abalone et al. (2004), with toluene instead of water because the soybeans would absorb that substance less than it would water. Varnamkhasti et al. (2008) also found the true density of rough rice by using the toluene displacement method outlined by Mohsenin (1986).

The true density of soybeans varied from 905 to 983  $\text{kg} \cdot \text{m}^{-3}$ . The true density of rough rice of two different cultivars were 1269.1 and 1193.43  $\text{kg} \cdot \text{m}^{-3}$ . While the method of this study differed from these two studies, the results are comparable. The true density of amaranth is most similar to the true density of





the densest rough rice cultivar determined by Varnamkhasti et al. (2008).

## Color

Significant results of the color analysis show that the finger millet was the darkest, having the most redness and blueness of all of the grains. Oppositely, proso millet was the lightest, having the most yellowness and greenness of all of the grains. These values are shown in **Table 3**. The whiteness and yellowness indices were measured, WI(E313-73) and YI(E313-98), respectively. Deviation from whiteness can be perceived as yellowness, which is not always desirable in food. It is important to establish a base value for each grain in order to identify when one has spoiled, or in a comparison of grains like millets which can be very similar to the eye, it can be a way to differentiate between types. Barnyard millet had the greatest whiteness value, and proso millet had the highest yellowness value. The whiteness and yellowness of each grain were significantly different from each other, respectively. The  $L^*$  value of each grain was significantly different from each other except for amaranth and kodo. The  $a^*$  values for ancient grains were significantly different from each other except for proso and little. The  $b^*$  values for each grain were all significantly different from each other. The distributions of whiteness and yellowness indices Whiteness Index(E313-73) and Yellowness Index(E313-98) and  $L^*$  values are presented in **Figure 3**.

## Porosity

The porosity, as shown in **Table 4**, varied from 3.6 to 17.4%, for water displacement method, and 44.5–48.1% with mass/volume method. Kodo millet and sorghum having the least and most porosity, respectively from both methods. The porosities of two rough rice cultivars were found to be 56.98 and 60.4% (Varnamkhasti et al., 2008). The porosity of popcorn kernels decreased from 42.56 to 40.87% when the moisture content of the kernels increased from 8.85 to 17.12% (Karababa, 2006). The porosity of *Prosopis africana* seed varied from 21.57 to 22.71% at 11% mc (Akaaimo and Raji, 2006).

## Scanning Electron Microscopy

The scanning electron microscopy images shown in **Table 5** reveal the unique surface characteristics of each grain. Finger millet has a highly textured surface with numerous round protrusions over its entire surface compared to the rest of the grains. Sorghum and pearl millet appear to be relatively smooth. Barnyard millet, kodo millet, little millet, and proso millet all appear to have a rough surface. Amaranth is the only grain that has a distinct separation in its surface, with an outer ring around the grain itself. This is a unique structural feature present in amaranth grains. Wild-type rice is shown to have wax crystals on the outer surface, which gives it a similar appearance to finger millet (Bai et al., 2015). This is the first study to consolidate and report the structural morphology of the ancient grains: amaranth, millets, and sorghum using SEM micrographs.

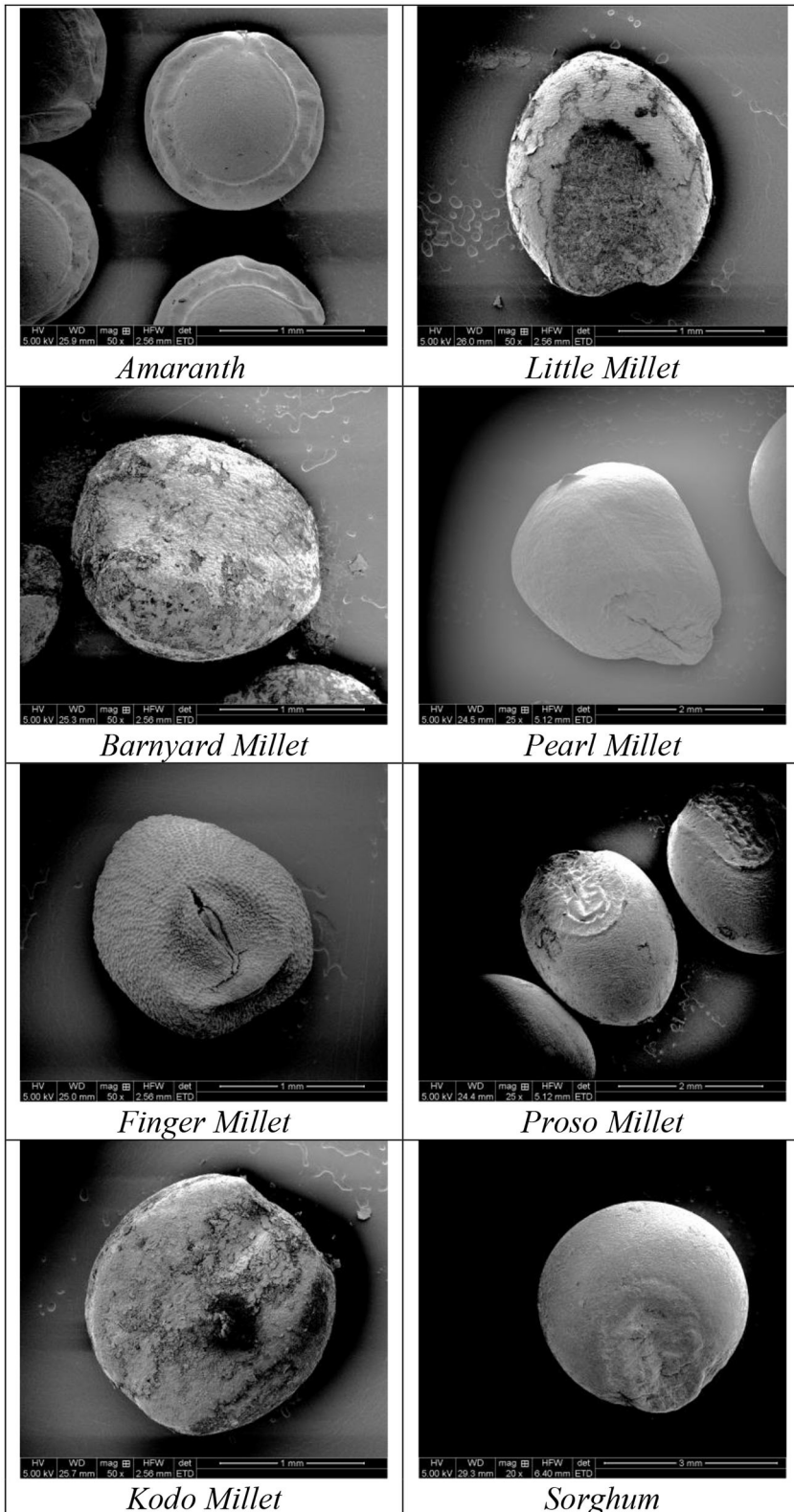
The surface features shown by the SEM photos can be explained due to the mechanical dehulling processes that the grains must undergo to be used as edible food and for further food processing. Little, barnyard, proso, and kodo millets must

**TABLE 4** | True density, bulk density, porosity, and angle of repose of ancient grains.

Grain	Water displacement method				Porosity			Mass/Volume method				
	Mass (g)	True density Volume (cm <sup>3</sup> )	Density ( $\frac{kg}{m^3}$ )	Mass (g)	Displacement (mL)	Bulk density	Density ( $\frac{kg}{m^3}$ )	Mass (g)	Volume (cm <sup>3</sup> )	Bulk Density ( $\frac{kg}{m^3}$ )	True Density ( $\frac{kg}{m^3}$ )	Porosity
Amaranth	4.502 ± 0.003	3.239 <sup>a</sup> ± 0.003	1390.17 <sup>f</sup> ± 0.001	4.504	3.4	3.4	1.325	4.006 ± 0.002	5.117 ± 0.030	783.10 <sup>b</sup> ± 13.571	1415.57 <sup>d</sup> ± 8.987	44.7%
Barnyard Millet	4.503 ± 0.001	3.112 <sup>e</sup> ± 0.003	1447.17 <sup>b</sup> ± 0.001	4.507	3.4	3.4	1.326	4.000 ± 0.000	5.017 ± 0.037	797.39 <sup>b</sup> ± 5.846	1462.85 <sup>b</sup> ± 3.744	45.5%
Finger Millet	4.507 ± 0.002	3.188 <sup>c</sup> ± 0.009	1413.73 <sup>d</sup> ± 0.004	4.501	3.4	3.4	1.324	4.000 ± 0.000	5.083 ± 0.069	787.03 <sup>b</sup> ± 10.610	1428.75 <sup>c</sup> ± 3.483	44.9%
Kodo Millet	4.502 ± 0.001	3.085 <sup>f</sup> ± 0.001	1459.40 <sup>a</sup> ± 0.001	4.504	3.2	3.2	1.408	4.000 ± 0.003	4.833 ± 0.075	828.72 <sup>a</sup> ± 12.030	1492.13 <sup>a</sup> ± 5.079	44.5%
Little Millet	4.502 ± 0.001	3.138 <sup>d</sup> ± 0.003	1494.60 <sup>c</sup> ± 0.002	4.502	3.4	3.4	1.324	4.000 ± 0.000	5.033 ± 0.047	794.77 <sup>b</sup> ± 7.395	1451.62 <sup>bc</sup> ± 10.519	45.2%
Proso Millet	4.503 ± 0.003	3.214 <sup>b</sup> ± 0.002	1400.87 <sup>e</sup> ± 0.000	4.508	3.4	3.4	1.326	4.005 ± 0.002	5.133 ± 0.094	780.49 <sup>b</sup> ± 14.390	1432.90 <sup>c</sup> ± 7.951	45.5%
Sorghum	4.499 ± 0.003	3.222 <sup>b</sup> ± 0.007	1396.47 <sup>e</sup> ± 0.002	4.498	3.9	3.9	1.153	4.000 ± 0.000	5.500 ± 0.191	728.16 <sup>c</sup> ± 25.400	1403.30 <sup>e</sup> ± 25.285	48.1%

Mean values not followed by the same letter in the same column are significantly different (Tukey's HSD,  $P < 0.05$ ).

**TABLE 5** | Scanning Electron Microscopic (SEM) images of ancient grains: Amaranth, Millets, and Sorghum.



be dehulled before consuming. While dehulling, the grains experience shearing forces which cause the rough, irregular surfaces. The scanning electron microscopic images of the millets (**Table 5**) shows the impact of dehulling on the surface morphology of the grains. This is an important observation, as surface morphology affects various physical characterization, including the angle of repose, coefficient of friction, and porosity. This is the first report that provides a microscopic comparison of the surface morphology of ancient seed grains, which would be helpful to advance research related to millets.

## Shape and Size

The ancient grains in this study were categorized in three out of the four shape types determined by McDonough and Rooney (1989). Amaranth, barnyard millet, kodo millet, and sorghum were globose in appearance, while finger millet, little millet, and proso millet were obovate. Pearl millet was the only grain with a hexagonal shape.

In increasing order, the size of the grains starts at the smallest with amaranth, followed by finger millet, little millet, barnyard millet, kodo millet, proso millet, pearl millet, and sorghum being the largest.

Baryeh (2002) observed lower bulk density with larger grains. Also showed that millet grains with a lower moisture content had a higher bulk density. Similar to Baryeh (2002), we also found that sorghum, having the larger grain size had lower bulk density using the water displacement method ( $1,153 \frac{\text{kg}}{\text{m}^3}$ ) compared to ancient seed grains with smaller size like amaranth, finger millet, little millet, and barnyard millet ( $1,324\text{--}1,326 \frac{\text{kg}}{\text{m}^3}$ ). While comparing the porosity data for sorghum (17.4%) and with other ancient seed grains (amaranth, finger, little, barnyard, kodo, and proso millets) the porosity range was between 3.6 and 8.5%. Similar trends were found for bulk density and porosity using mass/volume method. This could be explained by the fact that smaller grains could fill larger volumes by compactly packing themselves, filling interparticle voids, and minimizing porosity.

## Water Activity ( $a_w$ )

Water activity is an important food safety parameter. Water activity value ranges from 1.0 (pure water) to 0.0 (complete dry conditions). The water activity value provides an indicator of microorganisms that can grow by utilizing the water in the food samples (ancient seed grains). Higher the water activity, bacteria, yeast, and mold can grow resulting in food spoilage during storage. Food products with a water activity below 0.60 have extended shelf-life during storage.

Water activity varied from 0.533 at a temperature of 25.5°C to 0.660 at a temperature of 25.3°C, with finger millet having the lowest water activity and sorghum having the greatest. The remaining values are shown in **Table 3**. This study did not study the effect of water activity based on different moisture content. The water activity was measured for all samples upon opening the sealed packaging from the suppliers. Amaranth, barnyard, and pearl can be grouped and were not significantly different from each other in terms of water activity. Likewise, kodo, sorghum, amaranth, and barnyard can be grouped. The water activity of

amaranth and little, little and pearl, and kodo and barnyard were not significantly different from each other.

## CONCLUSIONS

The results of this study show that while the millets appear very similar to the human eye, they all vary with respect to the physical and structural properties. The potential for ancient seed grains to be grown in North America is far greater than what is currently being realized. They are commonly assumed to be useful only in developing countries, but field trials in Missouri and other locations show amaranth and millets to have good adaptation in temperate areas of North America (Myers, 2018b). Further research and breeding could help improve their potential even further, including improved yield and nutritional factors. Parts of the Midwestern and Great Plains areas of the U.S. are particularly viable areas to produce amaranth and other ancient seed grains. This, combined with the minimal water required to grow ancient seed grains like millet and amaranth compared to corn and wheat, could contribute to the growing effort to reduce freshwater consumption during climate crisis.

From the physical and structural characterization study, sorghum had a larger appearance, size, and the lowest bulk density, while kodo millet had the highest bulk density. Kodo millet had less porosity, while sorghum had high porosity from both density measurement methods. This is the first report that provides microstructural comparison clearly showing the impact of mechanical dehulling on the surface morphology of the ancient seed grains, which is invisible to human visual observation. Little, barnyard, proso, and kodo millets must be dehulled to make the seeds edible for human consumption. This study reports important microstructural observations that could directly impact physical characterization, including the angle of repose, coefficient of friction, porosity, and development of food processing equipment.

Color analysis shows that the finger millet was the darkest, having the most redness, and blueness of all the ancient seed grains. Barnyard millet had the greatest whiteness value (L), and proso millet had the highest yellowness value. The whiteness and yellowness of each ancient seed grain were significantly different from each other. This is an important food processing parameter to consider while developing new food products, ingredients from millets and would be of interest to food industries and plant-based food start-up companies.

This observational study establishes the methodology for physical characterizations of ancient grains. Further investigation on specific genetic traits, the effect of moisture content on the physio-chemical properties for different ancient seed grains is recommended. Since each ancient seed grain exhibits different physical and structural properties, further research, including chemical, functional properties, and nutrigenomics of the ancient seed grains for defined genetic lines, into environmental growth conditions is recommended to develop new products with enhanced health benefits. New value-added functional/nutraceutical food products can be formulated using single seed grains or the combination of amaranth as well as

barnyard, finger, kodo, little, pearl, proso millets, and sorghum to balance macro/micronutrient profiles.

To develop sustainable food processing technologies to utilize climate-resilient crops and improve agri-business, economic activity, and support for farming communities, a partnership between different stakeholders in food systems is critical. We conclude that ancient climate-resilient seed grains like millets, sorghum, and amaranth can lead to diversification of sustainable diets and have a positive impact to improve the health of people and the planet.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## AUTHOR CONTRIBUTIONS

SG contributed to the formal analysis, investigation, visualization, and writing (original draft, reviewing, and editing). RM was responsible for the resources and

the writing (review and editing). KK carried out the conceptualization, resource management, methodology, visualization, writing (review and editing), supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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