

The physical and chemical characteristics of several accessions of sorghum cultivated on drylands in East Nusa Tenggara, Indonesia

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Abstract. Mukkun L, Lalel HJD, Kleden YL. 2021. The physical and chemical characteristics of several accessions of sorghum cultivated on drylands in East Nusa Tenggara, Indonesia. *Biodiversitas* 22: 2520-2531. Many sorghums' accessions are widely cultivated in East Nusa Tenggara (ENT) Province, but no information is available on their physical and chemical properties. This study aimed to analyze the physical and chemical properties of several sorghum accessions cultivated in the dryland of ENT province. Seven sorghum accessions were obtained from farmers, namely Numbu, Kawali, Okin, red local, brown local, black local, and white local. Physical properties of the grain were carried out visually, including color, shape, and seed husks. Proximate content, total polyphenols, anthocyanins, and tannins were analyzed to determine each accession's chemical properties. Quantitative data were analyzed by ANOVA followed by Duncan Multiple Region Test (DMRT) at $P \leq 0.5$. Meanwhile, qualitative data were tabulated and presented in tables and figures. The results showed that all sorghums have almost the same nutritional content as other staple food to develop into a food substitute for rice to support food security. Furthermore, local black sorghum contains high polyphenol compounds with high antioxidant activity, indicating that it has excellent potential to be developed into functional foods. The high anthocyanin content in black sorghum can also be developed into a safe and environmentally friendly food coloring.

Keywords: antioxidant activity, bioactive compounds, dryland, polyphenols, sorghum

Abbreviations: DPPH: 1,1-diphenyl-2-picrylhydrazyl; CD 50: the amount of sorghum flour methanol extract that can capture 50% of DPPH free radicals

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the food crops for dry climate areas with high adaptability to drought, which can produce on marginal land and is relatively resistant to pests and diseases (Susila 2014; Kondombo et al. 2016). Furthermore, due to the plants' ability to form ratoons, it can be harvested several times by making use of moisture from the rest of the rainy season and that remains in the soil after the rainy season (Stefoska-Needham et al. 2015). Based on previous studies, sorghum has the potential for food and health. However, research on sorghum as a source of food and health is mainly carried out by foreign researchers, while in Indonesia, this research is still limited.

The nutritional content of sorghum varies depending on genotypes, seed color, and growing environmental conditions (Mohammed et al. 2011). The Mandau variety contains 12% of protein, 3% of fat, and 76% of carbohydrates, while the Keris variety contains lower protein, namely 6.38%, 3.6% of fat, and only 25.20% of carbohydrates (Subagio and Aqil 2014). Besides, sorghum is a good source of minerals, especially calcium, zinc, phosphorus, and magnesium (Martino et al. 2012). Similarly, its dietary fiber is a very useful element to health, because it can prevent heart disease, obesity, hypertension, and diabetes (Moraes et al. 2015; Girard and

Awika 2018). The phenolic compound acts as antioxidants, therefore, it can prevent cancer and tumors (Sehitoglu et al. 2014; Xie et al. 2019), as well as inhibit the development of various diseases caused by viruses such as HIV (Human Immunodeficiency Virus) (Lin et al. 2013). A recent report stated that polyphenol compounds could inhibit the development of the coronavirus; therefore, research on using plant-derived polyphenol compounds for preventive and medicinal purposes would be beneficial (Chojnacka et al. 2020). The presence of tannins in colored sorghum is reported to inhibit protein absorption and digestibility, but its ability as an antioxidant and anti-cancer needs to be taken into account (de MoraesCardoso et al. 2017; Lee et al. 2020). Polyphenolic compounds can also inhibit the activity of digestive enzymes, especially amylase and trypsin then reduce the digestibility of starch and preventing a drastic increase in blood sugar (Moraes et al. 2015).

To increase sorghum production, the Agency for Agricultural Research and Development has released 15 varieties of sorghum with various superior characters until 2014 (Gunawan 2017). The National Atomic Energy Agency (BATAN) has also produced at least three sorghum varieties, namely Pahat, Samurai 1, and Samurai 2 varieties (Dudato et al. 2020). Local sorghum is also the most crucial genetic source for developing high-yielding varieties with desired characteristics. Santoso et al. (2004) has identified as many as 39 sorghum accessions

originating from various regions in Indonesia.

The local sorghum in East Nusa Tenggara is very diverse with 31 accessions, and these types have characteristics such as different colors, shapes, and sizes of seeds. These accessions have been classified based on differences in color, namely black, red, brown, and white sorghum (Mukkun et al. 2018). However, no study has been conducted on its physical and chemical properties; therefore, it has not been widely used as a staple or functional food.

This study aimed to characterize the physical and chemical properties of local sorghum in East Nusa Tenggara. The results would be very beneficial for mapping as a complementary food for rice to improve food security in the community and transform sorghum into a functional food ingredient. Furthermore, this research can also increase the economic value and preserve sorghum germplasm, which is almost extinct.

MATERIALS AND METHODS

Plant materials

Three accession of sorghum (Numbu, Kawali, Okin) were obtained from a farmer group in Kawalelo Village,

East Flores District, and 4 accessions (Local Red, Local Black, Local Brown, Local White) were collected from Hamba Praing Village, East Sumba District, East Nusa Tenggara Province (Figure 1). Both areas are classified as semi-arid and characterized by low rainfall, short rainy season, and long dry season. Low amount of rainfall (<1000 mm) with a short rainy season (only 4-5 months) in a year also requires farmers to cultivate drought-resistant crops such as sorghum (BPS-NTT, 2021). Monthly rainfall data during the growing season from January to July 2019 in East Flores and East Sumba ranged from 72 to 334 mm and 4.2 to 297.7 mm respectively (BMKG, 2020).

Kawali and Numbu (white grain) varieties were released in 2001 as national variety, while Okin (light pink) is a crossed breed sorghum between Numbu and Kawali. The red, black, brown, and white sorghum are local varieties that have long been cultivated by the farmers (Figure 2). Planting was carried out in early January and harvested from April to May 2019. Numbu and Kawali were harvested at about 110 days after planting, while local varieties have a longer harvesting date, 4 to 5 months after planting. The samples in the form of panicles were harvested from farmers' gardens, then threshed manually, and sun-dried for 3 days to allow a water content level of 10-12%.

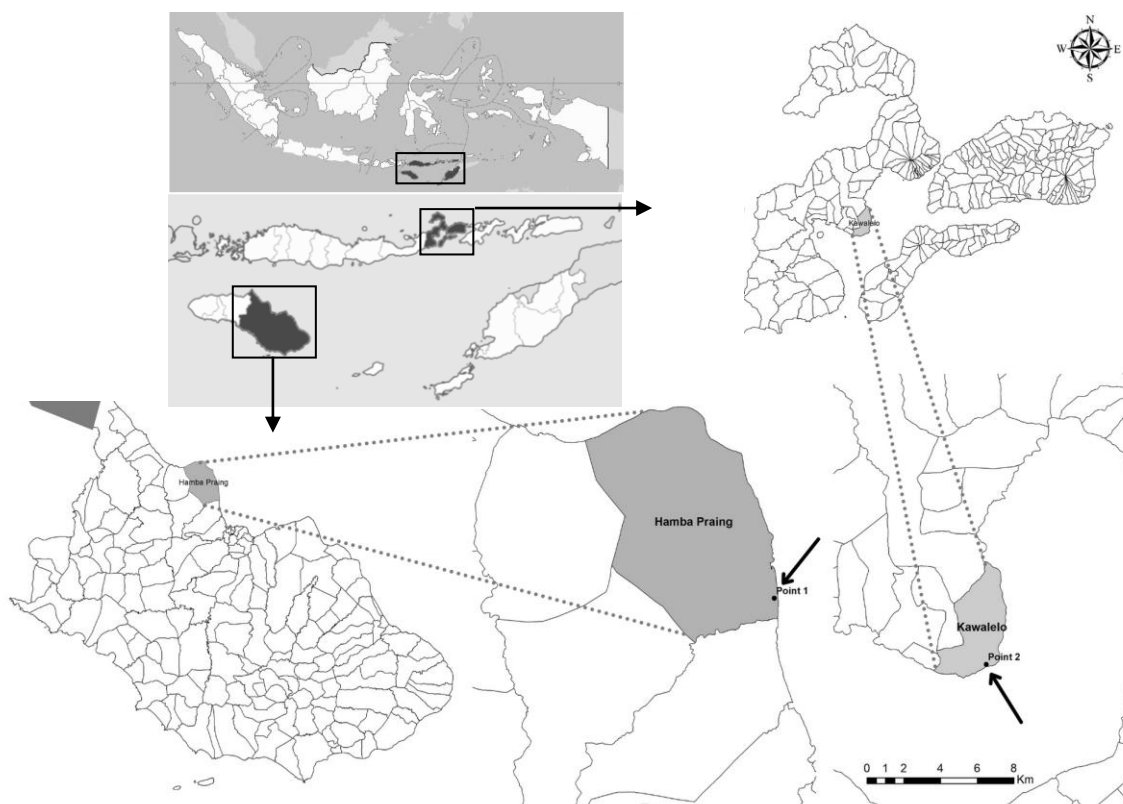


Figure 1. Sorghum sampling locations in East Nusa Tenggara, Indonesia. Point 1. Hamba Praing Village, East Sumba District (9°33'41''S, 120°13'59''E), Point 2 Kawalelo Village, East Flores District (8°26'54.0''S, 122°53'00.5''E)



Figure 2. Sorghum grains as research materials. A. Numbu, B. Kawali, C. Okin, D. Black Local, E. Red Local, F. Brown Local, and G. White Local

Sample preparation

Panicles of each variety/accession were harvested from farmers' gardens, then threshed manually, and sun-dried for three days to allow a water content level of 10-12%. Each genotype's whole grains were manually cleaned to remove foreign particles and then grounded using a mortar, followed by powder utilizing a milling machine.

Physical analysis

Sorghum's physical properties are essential to observe because they are closely related to the grains' chemical compositions. The physical attributes of sorghum grains differ in color, shape, and presence of a pigmented testa and affect the chemical content, such as phenolic composition. The color, shape, and panicle compactness and shape were observed according to the method described by ICRISAT (2008). The size of the grains is measured by weighing 1000 grains at 12% moisture content and expressed in grams (Kusumawati et al. 2013). Plant height measurements were carried out by measuring ten sorghum plants randomly for each variety and accession from the stem base to the panicle's tip (IBPGR and ICRISAT 1993). Bulk Density was measured using the method described by Li et al. (2016). Sorghum seeds were placed in a measuring cup and compacted until the volume reaches 100 mL. All sorghum seeds from the measuring cup were removed and weighed. The bulk density was expressed as g mL^{-1} . All physical analysis attributes were conducted in triplicate.

Chemical analysis

Water content

The water content of sorghum grains was analyzed using the thermogravimetric method (AOAC, 2011).

Approximately 1 g sample was put in a weighted Petri dish, and then heated in an oven at 105 °C until the grain weight was constant (24 h). The difference between the initial weight of the grain and after drying was calculated as dry basis moisture content (% DM).

Protein content

The protein content was analyzed using the Micro Kjeldahl method (AOAC 2011). A sample of 0.2 g was placed in a 30 mL Kjeldahl flask, and then 1.9 ± 0.1 g of K_2SO_4 , 40 ± 10 mg of HgO , and 2.0 ± 0.1 mL of H_2SO_4 were added as well as a boil for 1-1.5 h until the solution is clear. After cooling, 8-10 mL of $\text{NaOH-Na}_2\text{S}_2\text{O}_3$ were added, and then distilled. The distillation product is then titrated by 0.02 M HCl until a gray color occurs.

Fat content

The fat content was determined based on the Soxhlet extraction method (AOAC 2011). The measuring flask was dried in the oven. Furthermore, sorghum flour was weighed as much as five g, wrapped in filter paper, and covered with fat-free cotton. The filter paper containing the sample was placed in a Soxhlet extraction device coupled with a condenser. The hexane solvent was placed in the flask and then refluxed for at least 5 h. The measuring flask contained the extracted fat and was then heated to evaporate the solvent mixed with the sample fat. Percentage of fat was calculated using the following formula:

$$\% \text{ Fat} = \frac{\text{Weight (extraction + residue)} - \text{weight (extraction cup)}}{\text{Weight sample}} \times 100$$

Ash content

The determination of the ash content was based on the furnace method (AOAC 2011). A total of 3.0-5.0 g sample was placed in a weighted plate and then placed in an ashing furnace until the weight was constant. The crude fiber content was analyzed using a 1 g sample placed in a 300 mL Erlenmeyer flask, and then H₂SO₄ 0.3 N was added under back cooling for 30 min. After that, 50 mL of 1.5 N NaOH were added and filtered again for 30 min. The liquid in the Erlenmeyer flask was filtered using a filter paper with a known weight. Furthermore, it was washed successively with 50 mL of hot water and 25 mL of acetone. The residue, along with the filter paper, was dried until its weight was constant and then weighed.

Carbohydrates

Carbohydrates content was determined by difference (AOAC, 2011) using formula:

$$\text{Carbohydrate (\%)} = 100 - (\text{water content} + \text{protein} + \text{ash} + \text{fat})$$

Starch extraction and analysis

Extraction of starch from sorghum grains was conducted using hot water techniques (Izuagie et al. 2012; Chavan et al. 2016). About 100 g of grains of each type of sorghum was ground to the powder form and put in a separate beaker glass contained 300 mL of distilled water. All beakers were kept in a water bath at 40°C for about 24 hours. The soaked flour was then blended and filtered through a muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 minutes. The sediment was collected and suspended in excess 0.02% NaOH and then kept for 4 hours. The supernatant was discarded to remove any residual proteins and phenolic compounds. The NaOH treatment was repeated until getting colorless supernatant. The sediment was again suspended in distilled water and neutralized to pH 7. The slurry was filtered and washed using distilled water. After dried at 60°C, the starch was ground to powder and kept in sealed plastic bags for further analyses.

Amylose levels analysis

The amylose levels of the materials were measured using the International Rice Research Institute (IRRI) method (Li et al. 2016). 100 mg of flour was placed in a test tube, then 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added. The mixture was heated again for about 10 min in boiling water until a gel forms and transferred to a 100 mL volumetric flask, and shaken until the mark was right with the water. The 5 mL solution was pipetted and placed in a 100 mL measuring flask. Subsequently, 1 mL of 1 N acetic acid and 2 mL of iodine solution were added, precisely until the mark, shaken, and let stand for 20 min. Furthermore, the color intensity was measured using a spectrophotometer at a wavelength of 625 nm. The amylose level in the sample was calculated using a standard amylose curve. A range of amylose standard (potato amylose from Sigma) at a concentration of 25, 50, 75, 100, and 150 mg.l⁻¹ was prepared. The absorbances were measured at a wavelength of 625 nm. The standard

curve was $Y = 0.0028 X + 0.047$ ($R^2 = 0.99$).

Amylopectin levels analysis

Amylose and amylopectin were separated by mixing 1 M KOH with flour at 2 °C until dissolved (Li et al. 2016). 2 mL of the solution were passed over a CL-2B sepharose column and eluted with water. The fraction was collected every 40 min (proving starch with Lugol and observed with a spectrophotometer at a wavelength of 500 nm). The fraction from the first peak contains amylopectin, dried, and weighed. Calculations can also be carried out by subtracting the total starch from amylose.

Total polyphenols analysis

Total polyphenols were calculated using Folin-Ciocalteu Method (AOAC 2011). A total of 0.1 mL of liquid extract was added with 75 mL of distilled water, 10 mL of Folin-Ciocalteu, and 10 mL of Na₂CO₃. The solution was then adjusted to 100 mL with distilled water, mixed uniformly, and left at room temperature for 30 minutes. The absorbance readings were carried out at 760 nm using a spectrophotometer. Negative control was carried out by replacing 0.1 mL of sample solution with 0.1 mL of distilled water. The standard for phenols used tannic acid. Tannic acid (Sigma) was used as phenolic standard at the range of 0.02 to 0.1 mg.L⁻¹. The standard curve was $Y = 0.1574X$ ($R^2 = 0.995$).

Total anthocyanins analysis

A total anthocyanin s was measured following method described by Gusti and Wroistad (2000) using spectrophotometer at wavelength of 520 nm and 700 nm. Two separated sample dilutions were prepared at pH 4.5 to form the colored oxonium and colorless hemiketal complexes, respectively. Total anthocyanins were calculated using the following formula:

$\text{Anthocyanins (mg.l}^{-1}\text{)} = (A \times \text{MW} \times \text{DF} \times 1000) / (x \times 1)$, where A is absorbance resulted from $(A_{\text{max}} - A_{700})_{\text{pH}1} - (A_{\text{max}} - A_{700})_{\text{pH}4.5}$; MW is molecular weight; DF is dilution factors; and is molecular absorptivity.

Tannin analysis

The tannin content of the samples was determined according to the modified Vanillin-HCl methanol method as described by Mohammed et al., (2011). The Vanillin-HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% Vanillin in methanol. The solutions of the reagent were mixed just before use. About 0.2g of the ground sample was placed in a small conical flask. Then 10 mL of 1% concentrated HCl in methanol was added. The conical flask was capped and continuously shaken for 20 minutes, and the content was then centrifuged at 2500 rpm for 5 minutes. About 1.0 mL of the supernatant was pipetted into a test tube containing 5mL of Vanillin-HCl reagent. Absorbance at 450 nm was read on a spectrophotometer (corning, 259) after 20 minutes of incubation at 30°C; a blank sample was carried out with each sample run. A standard curve was prepared to express the result as catechin equivalent, i.e., catechin (mg per mL), which gives color intensity equivalent to that

given by tannin after correcting for blank. Tannin content was expressed as catechin equivalent as follows:

$$\text{Tannin (\%)} = \frac{C \times 10 \times 100}{200}$$

Where:

C : Concentration corresponding to the optical density

10 : Volume of the extract (mL).

200 : Sample weight (mg).

Antioxidant activity

Antioxidant activity was measured by testing the ability to scavenge free radicals using the method described by Dykes et al. (2014) with some modification. Extract solutions were prepared by dissolving the extract at concentrations of 25, 50, 100, 200, 400, 800, and 1000 ppm in chloroform: methanol (2:1). A total of 4 mL of an extract solution in chloroform: methanol (2:1) were mixed with 1 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl) 0.2 mM solution in methanol. The mixture was reacted for 30 min before its absorbance was measured at a wavelength of 517 nm. The loss of violet color from DPPH indicates an increase in the ability of DPPH radical scavenging. All chemical analyses were performed in triplicate.

Data analysis

Data of bulk density and the grain's chemical properties were statistically analyzed using Analysis of Variance (ANOVA) followed by Duncan Multiple Region Test (DMRT) at the level of 5% to determine the differences between sorghum varieties or accessions. Meanwhile, physical properties data are presented in tabular form. The Pearson correlation coefficients for the relationship between different properties were calculated using IBM SPSS Statistic 25.

RESULTS AND DISCUSSIONS

Physical characters

Grain characteristics

Sorghums cultivated by farmers in East Nusa Tenggara are highly diverse in seed characteristics, both in color, shape, and panicle compactness and shape (Table 1 and Figure 3). Kawali dan Numbu is hybrid sorghum released as sorghum for consumption, characterized by white grain color. Their panicles are compact, and the seed shape is flat round with an average weight per 1000 seeds of 29 and 34 g. Meanwhile, local sorghums are more varied in color, namely white, black, red, and brown. The shape of the seed varies from ellipse, narrow ellipse to flat round. However, all of them have a loose panicle shape. The local sorghum seed weight ranges from 30 to 38.5 g per 1000 seeds, which is relatively higher than that of the hybrid sorghum, namely Kawali. Morphologically, local sorghum has a taller stem compared to hybrid sorghum (Table 1). The Numbu and Kawali varieties are classified as short sorghum with stem heights of 90 and 120 cm. Meanwhile, the five local accessions were classified as tall sorghum

with stem heights ranging from 249 to 280 cm. Based on stem height, sorghum is classified into very short (<76 cm), short (76-150 cm), medium (151-225 cm), tall (226-300 cm), and very tall (>300 cm) sorghum (IBPGR and ICRISAT, 1993; Martiwi et al. 2020).

Although hybrid varieties have a higher yield potential (1 to 4 tone/ha) than local varieties (<1 tone/ha) (Sinha and Kumaravadivel 2016), however, due to their more drought resistance, lower input, disease resistance, long stems which are useful as livestock feed, local varieties are therefore still grown by farmers (Mishra et al. 2008; Tamiru et al. 2015). Their extreme photoperiod sensitivity gives farmers the flexibility to adjust planting dates to take advantage of early rains while still getting a modest crop when rains are delayed, with little variation in time of flowering and maturity. Cross-pollination among varieties of sorghum can occur because of their uncovered panicles and produce new types of sorghum which ultimately increase the diversity of sorghum (Pabendon et al. 2012; Zubair 2016).

Figure 3 shows the appearance of sorghum accessions, both local sorghum and national varieties before harvest. Inflorescence compactness and shape of sorghum Numbu and Kawali are compact and elliptic, while local accessions vary from very loose to semi-loose compactness with elliptic, erect, and dropping primary branches (Table 1). Inflorescence compactness and shape is one of the morphological traits used as key characters in identifying and selecting traits for breeding purposes. Previous studies on sorghum morphological variability conducted in other countries also reported high variation in types of inflorescence, and it was found that inflorescence compactness and shape was a predominant character in the grouping of sorghum landraces from Ethiopia (Verma et al. 2017). Martiwi et al. (2020) also reported that there were seven variabilities in inflorescence compactness and shape of 29 accessions from Java Island.

Many biological, climatic, and physical factors affected the on-farm genetic diversity of sorghum. These were farm size, yield, altitude, rainfall, temperature, and various edaphic factors. On-farm genetic diversity was affected considerably through the individual and interaction effects of these factors. Biological factors such as the number of varieties planted, farm size, and the presence of farmer groups were reported to increase the diversity of sorghum (Sinha and Kumaravadivel 2016). Rainfall and temperature are the main climatic factors that determine sorghum production. Sorghum is best adapted to areas with an average annual rainfall of between 450 and 750 mm (Yibrah et al. 2018). Although sorghum is classified as a drought-tolerant plant, to be able to produce optimally requires an adequate supply of water in the flowering phase (6.5-7.2 mm/day) (Sharma et al. 2019). Water-stress responses in sorghum can be physiological, morphological, and phenological. The level of tolerance to water stress is different for each sorghum genotype or variety and allow for farmers to select varieties that best suit local farming conditions and hence making sorghum suitable to a range of conditions (Hadebe et al. 2017).



Figure 3. Physical performance of sorghum accession before harvest. A. Numbu, B. Kawali, C. Okin, D. Black Local, E. Red Local, F. Brown Local, and G. White Local

Soil physicochemical properties such as pH, nutrient availability, texture significantly affect the diversity of sorghum. The pH is related to the availability of nutrients in the soil, such as nitrogen and phosphorus. Sorghum requires a neutral to alkaline soil pH of 5.5 to 7.6 (Mekbib et al. 2009). Altitude also has been reported to impact the diversity of sorghum. The lowest diversity occurred in the highland and lowland, whereas the highest was in the intermediate.

According to Awika, (2017), the grain color is caused by the presence of polyphenol compounds, especially tannins. Colored sorghum has a higher tannin content compared to colorless sorghum (white). The results of the analysis of total polyphenols (Table 5) showed that local colored sorghum is classified as tanned sorghum with a polyphenol content of more than 10 mg g^{-1} , while the colorless sorghum is classified as non-tanned. Tannins are anti-nutritional compounds; therefore, processing technology is needed to reduce tannin compounds, especially for colored sorghum. Cooking and fermentation are processes that can reduce tannin compounds and increase the palatability of sorghum (Chhikara et al. 2019). The shape of the grain also varied, including flat, round ellipses, and narrow ellipses. Furthermore, the plants also have unique husk properties, starting from being very short because the husk only covers the base of the seeds, but there are types of seeds where the entire seed is covered by

the husk even longer than the seeds, and the seeds with long husks will produce lower yields than those with short husks.

Bulk density

The bulk density is a description of the dry matter density in terms of weight compared to the volume of foodstuff. The higher the bulk density value, the denser the dry matter, and the lower the bulk density, the less dense a food or other material. Table 2 shows a high diversity of bulk density of sorghum accessions originating from East Sumba and East Flores District, ranging from $0.58\text{--}0.83 \text{ g mL}^{-1}$. Accession White Local has the highest bulk density of about 0.82 g mL^{-1} . The seeds with a high dry matter density provide a high flour yield as well (Mishra et al. 2015). In other cereal products such as rice, it was reported that the bulk density ranges from $0.77\text{--}0.90 \text{ g mL}^{-1}$ (Taylor et al., 2014). Black local sorghum was the accession with the lowest bulk seed density (0.58 g mL^{-1}), while local white had the highest density, although the difference was not significant. In addition to the influence of genetic factors, the environment also significantly influences the growth and development of seeds. The low bulk density of seeds generally affects the subsequently processed products' low yield (weight) (Li et al. 2016; Xiong et al. 2019).

Table 1. Physical characteristics of sorghum from East Nusa Tenggara, Indonesia

Accession/ variety	Grain color	Grain origin	Weight 1000 grain (g)	Grain shape	Panicle compactness and shape	Mean plant length (cm)
Numbu	White	East Flores	34.00	Flat round	Compact elliptic	95
Kawali	White	East Flores	29.00	Flat round	Compact elliptic	125
Okin	White	East Flores	33.00	Flat round	Semi compact elliptic	100
Black local	Black	East Sumba	30.06	Narrow ellipses	Very loose drooping primary branches	265
Red local	Red	East Sumba	33.89	Flat round	Semi-loose drooping primary branches	280
Brown local	Brown	East Sumba	36.50	Ellips	Very loose, erect primary branches	249
White Local	White	East Sumba	38.50	Flat round	Semi-loose, drooping primary branches	268

Table 2. Bulk density of different type of sorghum

Type/variety	Replication (mg/L)			Average (mg/L)
	1	2	3	
Numbu	0.78	0.78	0.80	0,79 ^a
Kawali	0.58	0.63	0.70	0,64 ^a
Okin	0.78	0.80	0.80	0,79 ^a
Red Local	0.50	0.63	0.61	0,60 ^a
Black Local	0.57	0.60	0.62	0,58 ^a
Brown Local	0.60	0.60	0.65	0,62 ^a
White Local	0.83	0.83	0.83	0,83 ^a

Note: Means followed by the same letter in a column are not significantly different ($P \leq 0.05$)

Chemical compositions

The chemical composition and nutrition value of whole sorghums (Table 3) are dominated by carbohydrates followed by protein, fat, minerals, and crude fiber. The nutritional value of sorghum is similar to other important food such as rice, corn, and wheat.

Protein

The protein content ranges from 9.03 to 12.73%, which showed that sorghum could be a protein source to meet the vegetable needs of protein. The black local sorghum type had the highest protein content at 12.73%, followed by Okin, and the lowest for the brown local accession, although it did not differ significantly between sorghum accessions. This result is lower than the results of the study conducted by de MoraisCardoso et al. (2017) on different sorghum varieties, which attained 17.8%. The protein content of accessions varied from 7.99 to 17.80%, showing the potential for improving the nutritive value of sorghum (Geleta et al. 2005). Sorghum proteins are classified as prolamins and not prolamins. About 79% of the total protein sorghum is prolamin, while the rests are albumins, globulins, and glutelin (de MoraisCardoso et al. 2017). Sorghum proteins are rich in glutamic acid and nonpolar amino acids (proline, leucine, and alanine) and have lysine as the main limiting amino acid. In addition, they may be deficient in 5 other essential amino acids (methionine, cysteine, isoleucine, valine, and threonine) (Moraes et al. 2012).

Fat

The fat content is also significantly influenced by sorghum's genotype, in which the colored sorghum has a

higher fat content than the white. Brown sorghum contains the highest fat (3.28%), followed by red sorghum (2.6%) and brown (1.98%). According to de MoraisCardoso et al. (2017), the sorghum's fat content ranges from 1.24-3.07 g/100g, and 83-88% are unsaturated fat. The major fatty acids of sorghum are linoleic (45.6-51.1%), oleic (32.2-42.0%), palmitic (12.4-16.0%), and linolenic acids (1.4-2.8%) (Afify et al. 2012).

Minerals

Table 3 also shows that black sorghum contains the highest total minerals (2.87%), while the lowest is in Kawali (0.81%) with white seed coat color; therefore, colored sorghum is a good source of minerals. The plant is a good source of the mineral, especially Ca, Fe, and Zn (Martino et al. 2012; Stefoska-Needham et al. 2015). According to Stefoska-Needham et al. (2015) sorghum is rich in iron, which is 5.4 mg per 100 g compared to rice, which contains low iron (1.8 mg per 100 g); therefore, black sorghum is very suitable for overcoming iron deficiency problems sufficiently high prevalence in humans.

Carbohydrate

Carbohydrate is the main component of sorghum. The carbohydrate content of some sorghum also varies between 70.24% in black sorghum, and 77.52% in Kawali sorghum. This result is almost the same as the research conducted by Moench et al. (2011), namely 60 to 80%.

Starch, amylose, and amylopectin content

Starch is the main carbohydrate in sorghum and is the largest source of energy. Table 3 shows that sorghum varieties and accessions' starch content were significantly different ($P \leq 0.05$). Local white sorghum contains the highest starch (99.76%), followed by local brown (99.46%), local red (98.25%), Kawali (96.89%), local black (96.43%), and the lowest was found in the Numbu variety (86.45%). The high starch content in local white sorghum indicates that sorghum can be developed into a starch source both for consumption and as an industrial raw material. Sorghum starch is a good type of starch because it contains a higher proportion of resistant starch (RS) and slowly digestible starch (SDS) components. The higher proportion of RS and SDS in sorghum can help consumers experience satiety for a longer time, potentially cutting the frequency of snacking and caloric intake, which in turn contributes to a low glycemic index and reduced risk of

developing chronic health conditions, such as type 2 diabetes and overweight (Teferra and Awika, 2019). The ability of sorghum-based diets to reduce hyperglycemia makes sorghum one of only a few “ancient grain” crops with demonstrated health benefits that consumers are seeking (Taylor and Duodu 2015).

The sample's amylose content ranged from 77.90% to 92.71%, while amylopectin ranged from 7.29% to 22.10% (Table 4). Amylose is a polysaccharide made up of straight-chain glucose units with 1,4-D-glucopyranoside bonds. Meanwhile, amylopectin is made up of the same units, but there are also branching with 1,6-D-glucopyranoside bonds. The composition of amylose and amylopectin determines the rheological characteristics, such as gelatinization, retrogradation, and gelling (de MoraesCardoso et al. 2017). The higher the amylose content, the drier the rice (hardened when cool and less sticky). The results showed that only 2 out of 7 accessions were classified as sorghum rice with moderate amylose content, namely Kawali and brown local sorghum seeds with an amylose content of 21.42% and 22.10%, respectively. Similarly, sorghum rice from five other sorghum accessions was classified as rice with low amylose content or less than 20%. Meanwhile, local white sorghum was classified as waxy sorghum because of its low amylose content (7.29%).

Total polyphenol content

There were significant differences in the total polyphenol content, anthocyanins, and antioxidant activity of the sorghum tested (Table 5). The highest polyphenol content was recorded in local black sorghum (23.07 mg g⁻¹), followed by Kawali (18.49 mg g⁻¹), red local (17.01 mg g⁻¹), and brown local (15.17 mg g⁻¹). Meanwhile, local white sorghum contains the lowest polyphenols, namely 2.52 mg g⁻¹. This result agrees with the results of Wu et al. (2017) who found higher total polyphenols in black and brown sorghum when compared to other varieties. The high amount of polyphenol compounds in black sorghum and other colored sorghum is due to its anthocyanin content. Anthocyanins are the primary pigments responsible for black, purple, blue, pink, red, and brown

color sorghum. The high amount of polyphenol compounds in black sorghum and other colored sorghum is due to its anthocyanin content. Anthocyanins are the primary pigments responsible for black, purple, blue, pink, red, and brown color sorghum (Vanamala et al. 2018). Xiong et al. (2020) have identified 119 phenolic compounds in black and brown sorghum where flavonoids, including anthocyanins, are the main compounds. Sorghum varieties with colored testa and thick pericarp contain the highest phenolic compounds compared to colorless. Phenolic compounds are concentrated in testa and pericarp, consisting of 3 groups, namely, phenolic acids, flavonoids, and condensed tannins (Stefoska-Needham et al. 2015). The presence of phenolic compounds in colored sorghum has become an interesting issue recently, especially for preventing and treating chronic diseases. Recently, several research groups reported the anticancer activity for polyphenol-rich sorghum. Treatment of colon and breast cancer cells with sweet sorghum extracts led to a suppression of cell proliferation (Stefoska-Needham et al. 2015; Xie et al. 2019). Recently, growing evidence supports the use of phenolic extracts as human health-promoting anti-inflammatory and antioxidant agents (Vanamala et al. 2018). Phenolic compounds in sorghum have health benefits for humans because they can act as antioxidants by scavenging free radicals.

Table 4. Starch, amylose, and amylopectin content of different sorghum accessions

Sorghum varieties	Starch content (%)	Amylose content (%)	Amylopectin content (%)
Numbu	86.45a	16.38d	83.62d
Kawali	96.89d	21.42e	78.58b
Okin	88.79b	14.30b	85.70f
Black Local	96.43c	14.49c	85.51e
Red Local	98.25e	22.10e	77.90a
Brown Local	99.46f	16.51d	83.49c
White Local	99.76g	7.29a	92.71g

Note: Means followed by the same letter in a column are not significantly different ($P \leq 0.05$)

Table 3. Nutritional content of different sorghum varieties

Sorghum variety/accession	Nutritional content					
	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Mineral (%)	Carbohydrate (%)
Numbu	8.83 ^c	9.93 ^a	1.81 ^c	1.22 ^b	0.97 ^b	76.73 ^d
Kawali	8.97 ^d	9.29 ^a	1.30 ^a	2.02 ^c	0.81 ^a	77.52 ^d
Okin	8.84 ^c	10.47 ^a	1.85 ^c	1.05 ^a	1.24 ^c	75.74 ^{cd}
Black Local	8.65 ^b	12.73 ^a	2.66 ^d	2.02 ^c	2.87 ^g	70.24 ^a
Red Local	9.91 ^f	10.16 ^a	1.98 ^c	3.10 ^e	1.76 ^f	73.81 ^b
Brown Local	9.06 ^e	9.03 ^a	3.28 ^e	2.97 ^d	1.58 ^e	72.91 ^b
White Local	8.55 ^a	10.32 ^a	1.56 ^b	3.25 ^f	1.45 ^d	74.64 ^{bc}

Note: Means followed by the same letter in a column are not significantly different ($P \leq 0.05$)

Table 5. Polyphenol and anthocyanin content of different type of sorghum

Types	Total polyphenols (mg g ⁻¹)	Anthocyanins (mg g ⁻¹)	Tannin (mg g ⁻¹)	CD50 (g)
Numbu	7.49 ^b	0.95	0.95 ^b	475.67 ^b
Kawali	18.49 ^f	0.84	0.84 ^a	5.19 ^b
Okin	13.10 ^c	0.12	1.10 ^c	476.70 ^b
Black Local	23.07 ^g	3.24	0.85 ^a	4.05 ^a
Red Local	17.01 ^e	1.10	2.59 ^d	6.22 ^a
Brown Local	15.17 ^d	1.49	5.49 ^e	5.67 ^a
White Local	2.52 ^a	nd	1.23 ^c	750.13 ^b

Note: nd: not detected. Means followed by the same letter in a column are not significantly different ($P \leq 0.05$)

Table 6. Correlation between physicochemical properties of sorghum accessions

	Polyphenol	CD 50	Anthocyanin	Tannin
CD 50	0.92 ^{**}	-	-	-
Anthocyanin	0.65 [*]	0.60 [*]	-	-
Tannin	0.84 ^{**}	0.39	0.033	-
Amylose	0.62 [*]	0.77 [*]	0.03	0.17
Amylopectin	0.64 [*]	0.77 [*]	0.03	0.17
Carbohydrate	0.44	0.38	0.64 [*]	0.32
Bulk Density	0.86 ^{**}	0.97 ^{**}	0.39	0.41 [*]

Note: * $P \leq 0.05$, ** $P \leq 0.01$

Anthocyanins content

Anthocyanins are plant-specific flavonoid compounds that accumulate in plant cells' vacuoles and display red, purple, and blue colors found in leaves, petals, seeds, and plant tissue. This study showed that the highest anthocyanins were found in local black sorghum (3.24 mg. g⁻¹), although it was not significantly different from other varieties (Table 5). Meanwhile, local white sorghum does not contain anthocyanins or is undetectable. Compared to commercial sources of anthocyanins such as fruits and vegetables, the anthocyanin content of black sorghum was much higher than the main sources of anthocyanin such as strawberries, red grape, blackberry, and red cabbage, with an average anthocyanin content of 0.15-0.35, 0.3-7.5, 1.15, and 0.3-0.9 mg g⁻¹, respectively (Sehitoglu et al. 2014). The sorghum has an additional advantage in terms of storage stability relative to fruits and vegetables. Furthermore, due to the low moisture content of the seeds (10-14%), anthocyanins in sorghum are more stable than fruits with a moisture content of more than 80% (Dykes et al. 2013). The most dominant anthocyanins on black sorghum are 3-deoxyanthocyanidins, more stable than the anthocyanins in fruits and vegetables currently used as a source of anthocyanins for commercial purposes (Dykes et al. 2013; Zhu 2018). Therefore, black sorghum is very competitive as a source of natural anthocyanins and food coloring, which can benefit health. Red and brown sorghum in this study also contain anthocyanins which are pretty high compared to white sorghum (Kawali, Numbu, and Okin). Therefore, apart from black sorghum, red and brown sorghum are also potential sources of anthocyanins. Previous studies also found that red and brown sorghum contains higher anthocyanins than colorless ones (Zhu 2018). Regular consumption of anthocyanin-rich food

products may help to prevent various chronic disorders and to improve health conditions. This is because the anthocyanins in sorghum have unique properties; for example, 3-deoxyanthocyanidins are strong phase II enzyme inducers, a property not reported for their anthocyanin analogs. The unique polyphenols found in sorghum do indeed exert important bioactive properties not related to their direct antioxidant activity that may provide superior health benefits. Such indirect effect on endogenous detoxifying enzymes is highly relevant because it indicates these compounds can exert properties with essential consequences on oxidative stress and cancer prevention at low levels of intake (Sehitoglu et al. 2014; Awika 2017).

Tannins content

Tannins are phenolic compounds that often act as a defense mechanism against pathogens and predators (de MoraesCardoso et al. 2017). The tannin content of several varieties/accessions of sorghum analyzed (Table 5) was significantly different at the $P \leq 0.05$ level, ranging from 0.84 for Kawali to 5.49% for local brown. Dykes et al. (2014) classified sorghum into two groups, namely sorghum tannins containing tannins greater than 10 mg/g, while those containing fewer tannins were classified as low-tannin sorghum. Based on this classification, all of the varieties/accessions of sorghum tested were classified as low-tannin sorghum. Processing methods such as dehulling and decortication reduce the tannin content of sorghum. Decortication for 6-8 min to 70-81% extraction rates decrease in tannins by 79-92% (Dykes et al. 2014; Acquisgrana et al. 2020). Taylor and Duodu (2015) also reported that spontaneous fermentation of sorghum for 36 h was found to decrease tannins by 27%. Tannins are often

considered to have a negative impact on the absorption of nutrients from the food consumed because they can form tannin-protein or tannin-mineral complexes. Therefore, the nutrient digestibility of protein and some minerals is reduced. However, tannins and other polyphenol groups discovered in food are lately considered to have positive values because they act as antioxidants, which can inhibit various oxidation processes that are detrimental to human health (Moraes et al. 2015; de MoraisCardoso et al. 2017).

Antioxidant activity

In Table 5, the antioxidant activity was significantly higher in the colored local sorghum (local black, brown, and red) than the white one (Numbu, Okin, and local white). Black sorghum had the highest antioxidant activity than other sorghum with the lowest CD50 value of 4.05 mg, but not significantly different from the local red and brown. These results prove that polyphenol content has a positive correlation with antioxidant activity in the form of capturing free radicals from DPPH, which is displayed in CD50, namely the amount of sorghum flour methanol extract that can capture 50% of DPPH free radicals. Black, red, and brown sorghum with high antioxidant abilities can be used as raw materials of various functional food products that can be used by people classified as susceptible to various degenerative diseases. Tannins are the main polyphenol compounds possessed by various types of cereals and legumes such as sorghum, namely oligomeric compounds of flavan-3-ol and flavan-3,4-diol (Dykes et al. 2014; Vanamala et al. 2018). The colored sorghum had three to ten times more antioxidant activity compared to the colorless type (Table 5). Therefore, colored sorghum has the potential to be processed into functional food. According to Rao et al. (2018), sorghum's antioxidant activity can be maintained during processing, namely 57-78% when roasting and 70-100% when extrusion. The black sorghum with low tannins but high anthocyanins have high antioxidant activity, such as red and brown sorghum (Table 5). Anthocyanins were reported to contribute to fruits' high antioxidant activity (Barros et al. 2013). Black sorghum contains total anthocyanins (3.24 mg g⁻¹), which were higher than colorless sorghum; even the anthocyanin was not detected on local white sorghum (Table 5).

The correlation test results (Table 6) showed a highly significant correlation between total polyphenol content and antioxidant activity ($r = 0.92$; $P \leq 0.01$). This means that phenol compounds contribute 92% to antioxidant activity. Furthermore, black sorghum also contains the highest anthocyanins (3.24 mg g⁻¹), and therefore the high phenolic compounds and antioxidant activity are caused by the high levels of anthocyanins and tannins in red and brown sorghum. This result is in line with Dykes et al. (2013) who reported condensed tannins and dyes such as anthocyanins increased phenol and antioxidant activity in colored sorghum. Furthermore, the close correlation ($r = 0.60$; $P \leq 0.05$) between antioxidant activity and anthocyanin content showed that 60% of the antioxidant activity was contributed by anthocyanins (Table 6). Condensed tannins, also known as proanthocyanidins or

procyanidins, are made up of polymerized flavanol units and contribute to food astringency. This compound is common in sorghum, which has colored peel, red finger millet, and barley (Kaufman et al. 2013). Bulk density, amylose, and amylopectin also significantly correlated with antioxidant activity with correlation coefficients of 0.97 and 0.77, respectively. Red and brown sorghum contained relatively high tannins and anthocyanins and had a positive correlation with total phenol, with r of 0.84 at $P \leq 0.01$ and 0.65 at $P \leq 0.05$, respectively (Table 6). Anthocyanins and tannins are part of flavonoids beneficial to health as an anti-viral, anti-fungal, anti-bacterial, anti-toxic, anti-allergic anti-inflammatory (Stefoska-Needham et al. 2015; Sehitoglu et al. 2014). The high level of bioactive compounds in sorghum, which positively affects health as an antioxidant and inhibitor of pathogenic growth, means that sorghum is classified as a functional food ingredient (Przybylska-Balcerek et al. 2019).

Furthermore, black sorghum also contains the highest anthocyanins (3.24 mg g⁻¹), and therefore the high phenolic compounds and antioxidant activity are caused by the high levels of anthocyanins and tannins in red and brown sorghum. This result is in line with Dykes et al. (2013) who reported that condensed tannins and dyes such as anthocyanins increased phenol and antioxidant activity in colored sorghum. Furthermore, the close correlation ($r = 0.60$; $P \leq 0.05$) between antioxidant activity and anthocyanin content showed that 60% of the antioxidant activity was contributed by anthocyanins (Table 6). Condensed tannins, also known as proanthocyanidins or procyanidins, are made up of polymerized units of flavanol and contribute to food astringency. This compound is common in sorghum, which has colored peel, red finger millet, and barley (Kaufman et al. 2013). Bulk density, amylose and amylopectin also significantly correlated with antioxidant activity with correlation coefficients of 0.97 and 0.77, respectively. Red and brown sorghum contained quite high tannins and anthocyanins and had a positive correlation with total phenol, with r of 0.84 at $P \leq 0.01$ and 0.65 at $P \leq 0.05$, respectively (Table 6). Anthocyanins and tannins are part of flavonoids that are beneficial to health as anti-viral, anti-fungal, anti-bacterial, anti-toxic, anti-allergic, and anti-inflammatory (Stefoska-Needham et al. 2015; Sehitoglu et al. 2014). The high level of bioactive compounds in sorghum, which has a positive effect on health as an antioxidant and inhibitor of pathogenic growth, means that sorghum is classified as a functional food ingredient (Przybylska-Balcerek et al. 2019).

The bioactive content, such as polyphenols in sorghum, is greatly influenced by physical properties and the presence of other bioactive compounds. Bulk density has a positive correlation with polyphenol content ($r = 0.86$; $P \leq 0.01$) and antioxidant activity ($r = 0.97$; $P \leq 0.05$). Other compounds such as tannins, anthocyanins, amylose, amylopectin, and carbohydrates were also significantly correlated with polyphenol content.

The results showed that sorghum in the dry area of East Nusa Tenggara had a high diversity of both physical and chemical properties. All sorghum samples (the white and colored sorghum) have almost the same nutritional content

as staple foods such as rice and corn, and therefore can be developed into a rice substitute to promote food security. The presence of color in sorghum indicates the presence of bioactive compounds such as tannins, which have a positive impact on health. Moreover, black, red, and brown local sorghum contain high polyphenol compounds, with high antioxidant activity indicating that the plant has the potential to be developed into functional foods. The high anthocyanin content in black sorghum (three times higher than in fruits) can also be developed into a safe and environmentally friendly food coloring.

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