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Physico-chemical and Nutritional Composition of Ten Sorghum (*Sorghum bicolor* L.) Grain Varieties as Potential Feed for Livestock

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Abstract

Sorghum grain (*Sorghum bicolor* (L.) Moench) is gaining importance amongst livestock producers and animal nutritionists. Newly developed sorghum varieties should be evaluated for their suitability in small ruminant ration. The aim of this research was to determine the physico-chemical characteristics of ten sorghum varieties as potential feed for small ruminants. There were no significant differences ($P > 0.05$) in DM, CP and CF contents of the ten sorghum varieties. The sorghum black had higher GE, NDF and ADF contents and ATxArg had higher oil and mineral contents. Similarly, sorghum varieties labelled black, red, R17049 and FZ2CND were characterized by high tannins, phenolic and redness contents. Marcia, R17231, RTx436 contained high levels of yellowness and lightness. Fatty acid profile also varies amongst the ten varieties. The results of this study demonstrated that physiochemical and nutritional properties of sorghum varied by varieties.

Keywords: sorghum, tannin, polyphenols, redness, yellowness

1. Introduction

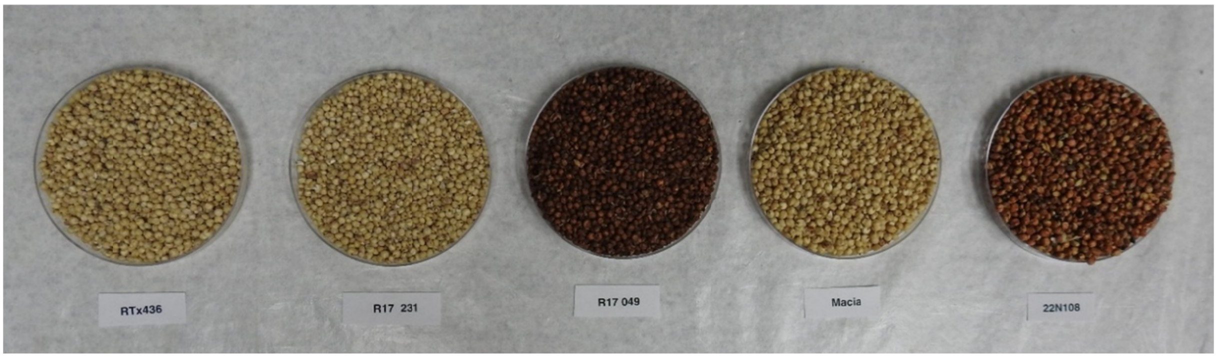
Corn is the most widely produced feed grain in the United States, accounting for more than 95 percent of total feed grain production (USDA, 2023). The demand for corn has increased significantly as corn-ethanol production continues to rise (Hodges et al., 2022). This demand has led to increased corn price and this trend is expected to remain into the future. Hence, there is urgent need to identify potential alternate energy source for livestock feed that require less water and can withstand high summer temperature.

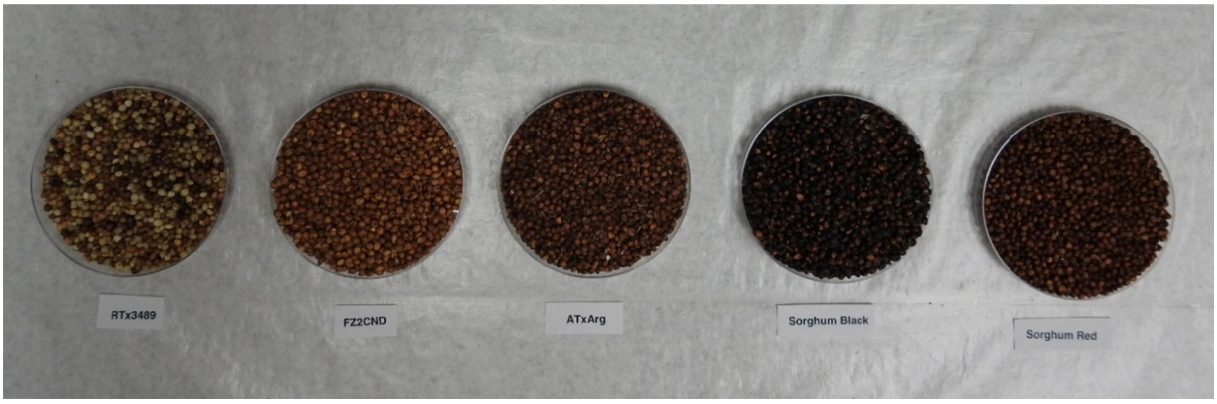
Sorghum (*Sorghum bicolor*, Moench) grain has been identified as a suitable replacement for corn (Mabelebele et al., 2015). It is drought tolerant and excellent feedstuff for poultry, pigs, and ruminants. Its utilization as a livestock feed is increasingly popular in regions characterized by environmental stresses, making it a suitable replacement for corn in animal feeding (Arroyo et al., 2016).

Sorghum seed varieties have been shown to vary in physical and chemical characteristics (Tasie & Gebreyes, 2020). Many studies have evaluated the inclusion of sorghum grains as an energy ingredient. It has been documented that sorghum grain color, texture, minerals, tannin, phenolics, and crude protein contents varies with sorghum varieties (Subramanian et al., 1990). These variations may lead to the poor feeding value of certain sorghum cultivars in animal ration. The nutritional content of sorghum varies depending on genotypes, seed color, and growing environmental conditions (Mohammed et al., 2015). There are currently few studies on the nutritional evaluation of sorghum varieties in small ruminant production. Therefore, the objective of the current study was to determine the physico-chemical characteristics of ten sorghum varieties as potential feed for small ruminants.

2. Materials and Methods

Ten sorghum varieties (ATxArg, Macia, FZ2CND, 22N108, RTx3489, R17049, R17231, RTx436, Sorghum Black and Sorghum Red) were used in this study. Seeds were supplied by S&W Seed Company Texas and the Department of Soil and Crop Sciences, Texas A&M University. Two pounds of each variety were cleaned to remove broken and foreign materials prior to laboratory analysis. Photos of labelled sorghum varieties are listed below:





2.1 Proximate Composition

Proximate analyses for dry matter, crude protein (N × 6.25), crude fiber and fat was carried out according to the standard methods of AOAC (2005). Ground samples of each variety were oven-dried and weighed. The sample, in a crucible, was ashed in a muffle furnace at 550 °C for 6 hours. The ash was acid digested by adding 1 mL 55% (v/v) HNO3. After cooling, calcium, magnesium, manganese, iron, sodium, potassium, aluminum and phosphorus concentrations were determined by AOAC method 6.1.2 (2005) using inductively coupled plasma spectroscopy. Neutral detergent fibre (NDF) and acid detergent fiber (ADF) were established according to the methods of Van Soest et al. (1991). The gross energy contents of the milled sample were determined with adiabatic bomb calorimetry (Gallenkamp, Autobomb, London, UK). For the acid detergent lignin (ADL) analysis, the samples were soaked in 12 M sulfuric acid for 3 h and thoroughly washed with boiling distilled water. Cellulose was calculated as ADF-ADL.

2.2 Color

The color of the grain was observed visually and Hunter L*a*b* representing L (lightness), a (redness) and b (yellowness) color values were measured with a Konica Minolta CR-400 c camera (Konica Minolta, Sakai, Osaka, Japan).

2.3 Extraction and Tannin Assay

Freeze-dried sorghum grain samples were extracted according to a procedure by Padda and Picha (2015) with some modifications. Samples were mixed with 10 mL of 80% acetone, heated in a water bath at 80 ºC for 10 min, followed by vortexing for 30 s and cooling to room temperature prior to centrifuging at 4500 g for 20 min. Supernatants were diluted as necessary for each measurement. Condensed tannins were determined using the Butanol-HCl method and expressed as leucocyanidin equivalent (% DM).

2.4 Phenolic Analysis

The Folin-Ciocalteu assay modified from Singleton and Rossi (1965) was used to quantify total phenolic content (TPC). A gallic acid standard curve was made from a 1 mg/mL gallic acid standard stock solution in methanol, followed by a serial dilution by a factor of two to obtain concentrations ranging from 1 mg/mL to 0.002 mg/mL. Then, 100 µL Folin-Ciocalteu reagent and 800 µL 5% sodium bicarbonate were added to the standard curve and to a 100 µL portion of supernatant. The standard curve and the samples were then heated at 40 °C for 30 min and cooled at room temperature for 10 min. Cooled samples were plated in triplicate in a 96-well plate, scanned at 765 nm, compared against the gallic acid standard curve, and reported as mg of gallic acid equivalents/g of feed.

2.5 Fatty Acid Analysis

A modified version of the microwave assisted extraction (MAE) method described by Bronkema et al. (2019) was used to extract FAs from samples using the CEM Mars 6 microwave digestion system, equipped with a 24-vessel rotor and Glasschem vessel set (CEM Corporation, Matthews, NC). This method was also described by Sergin et al. (2021). Briefly, 400 mg of lyophilized sample was added to a microwave vessel with 8 mL of 4:1 (v/v) solution of ethyl acetate: methanol and 0.1% butylated hydroxytoluene (BHT) as an antioxidant. FAs were extracted using the following microwave parameters: 55 °C for 15 min with initial ramp of 2 min at 400 W maximum power. Vessel contents were filtered using Whatman lipid free filters (Grade 597) (Weber Scientific; Hamilton, NJ) into a test tube containing 3.5 mL HPLC water. Samples were centrifuged at 2500 RPM for 6 min, and the top organic layer was transferred to a new tube and dried under nitrogen. Extracted oil was resuspended in 4:1 (v/v) dichloromethane: methanol with 0.1% BHT to bring each sample to 20 mg oil/ml. Dichloromethane was purchased from VWR Chemicals (Radnor, PA).

For the creation of fatty acid methyl esters (FAME), a modified methylation described by Agnew et al. (2016) was conducted. Two mg of suspended oil (100 mL) was aliquoted from each sample, dried under nitrogen, and resuspended in toluene with 20 µg of internal standard (methyl 12-tridecenoate, U-35M, Nu-Chek Prep, Elysian, MN). Two mL of 0.5 N anhydrous potassium methoxide was added and samples were heated at 50 °C for 10 min. Once cool, 3 mL of 5% methanolic HCl was added, and samples were heated at 80 °C for 10 min. Once cool, 2 mL of water and 2 mL hexane were added, samples were centrifuged (2500 RPM at room temperature for 5 min), and the upper organic phase was removed and dried to obtain FAMEs.

2.6 Statistical Analysis

Data for proximate analysis (dry matter, crude protein, crude fat, NDF, ADF, cellulose and lignin) of sorghum varieties was analysed using a general linear (proc GLM) model as follows:

$$Y = \mu + Sorg + \varepsilon \quad (1)$$

Where, Y = Measured response variable; Sorg = Sorghum variety effect; ε = Error term.

The Duncan Multiple Range Test (DMRT) was used for the post-hoc tests.

Data on physicochemical traits (total phenolics, tannin, calcium, potassium, sodium, phosphorus, and colour) as well as fatty acid content were subjected to principal component analysis (PCA) to study the relationship between characteristics of sorghum varieties. The PRINCOMP procedure of SAS was used to produce eigenvalue and eigenvector tables. Principal components were described in terms of proportion of explained variance and eigenvalue. Only principal components with eigenvalues above 1.0 were considered significant (O'Rourke and Hatcher, 2013). The PCA plots were mapped using the PROC SGPLOT procedure of SAS.

3. Results and Discussion

Proximate composition of freeze-dried sorghum varieties is presented in Table 1.

There were no differences (P > 0.05) in DM, CP and CF contents of the sorghum varieties. However, oil, NDF, ADF, cellulose, lignin and GE contents differed (P < 0.05) between sorghum varieties. The DM content ranged from 85.9 to 87.7%. This relatively high DM content of the sorghum varieties is appropriate for utilization indicating low minimal moisture to foster growth of moulds. Grains with below 85% moisture content are predisposed to mould and/or fungal infections (Hamito, 2010; Karlovsky, 2016). CP contents were not different (P > 0.05) between sorghum varieties. The CP content ranged from 9.1 to 12.9%. Kaijage et al. (2014) reported that Tanzanian sorghum had CP content similar to the varieties of the present study. The varieties tested in this study had higher CP contents than sorghum Sudan grass cultivars (5.4 to 7.8%), as well as Turkey sorghum varieties (6.9 to 7.7%) (Bean et al., 2013; Hassan et al., 2016).

The oil content in the studied varieties ranged from 2.9 to 3.9%, with ATxArg and RTx3489 having higher (P < 0.05) fat contents than the other varieties. This range was lower than the sorghum cultivars evaluated by Mabelebele et al. (2015). The discrepancy might be due to different agroecological zones. Sorghum black had higher (P < 0.05) NDF, ADF contents. Lignin contents were also higher (P < 0.05) in sorghum black and red. Our results are similar to the findings of Mabelebele et al. (2015). Varieties with higher tannin concentrations have higher ADF, NDF and lignin contents (Parnian et al., 2013). The contribution of polyphenols to the lignin fraction are usually responsible for the higher values of dietary fibre in sorghum tannin varieties. The gross energy (GE) content between varieties varies from 2600-3222 kca/kg. Sorghum black had a higher ($P < 0.05$) GE content. Mabelebele et al. (2015) observed no differences in GE content between varieties. Negative correlation between GE and tannin content of sorghum varieties had previously been reported (Talmadge et al., 1975). In our study, the variety with high tannin had higher GE. It has been documented that the colour of sorghum grain varies greatly due to pericarp colour and thickness, presence of testa, and endosperm texture and colour. Several studies reported that there is a relationship between sorghum grain colour and tannin content (Hahn & Rooney, 1985; Leeson & Summers, 2005). According to Ring et al. (1988), phenolic compounds, like tannin, change the pigmentation of the pericarp and testa of sorghum grain. In contrast, Waniska et al. (1996) suggest that seed colour is not a good parameter to predict tannin content. In light of our study, predicting tannin content in sorghum grain based on its lightness and yellowness seems impracticable because precision of tannin estimation depends on many intrinsic factors, outside those two colours.

Table 1. Proximate composition (% DM basis) and gross energy contents (kcal/kg) of ten sorghum varieties

Cultivar	DM	CP	CF	Fat	NDF	ADF	Cellulose	Lignin	GE
ATxArg	87.7	10.3	2.6	3.7^{a}	8.7^{c}	7.3^{c}	4.7^{e}	3.4^{b}	3056.5^{b}
Macia	86.4	10.3	2.2	3.0^{b}	10.8^{b}	6.0^{e}	6.1^{c}	0.8^{f}	2854.3^{c}
FZ2CND	85.9	10.9	1.9	3.0^{b}	10.3^{b}	8.4^{b}	6.1^{c}	2.9^{c}	2967.7^{c}
22N108	86.8	12.9	2.0	3.2^{b}	11.4^{b}	8.7^{b}	7.9^{a}	1.8^{e}	2646.6^{d}
RTx3489	86.5	11.7	1.9	3.9^{a}	8.0^{cd}	6.6^{cd}	5.4^{d}	2.0^{d}	2600.5^{d}
R17049	86.2	10.1	2.2	3.0^{b}	8.7^{c}	7.9^{b}	5.0^{de}	3.5^{ab}	3076.6^{b}
RTx436	86.3	10.5	1.7	2.9^{b}	9.0^{c}	5.5^{e}	4.0^{f}	1.9^{ed}	2790.8^{c}
R17231	86.2	9.1	2.1	3.0^{b}	7.2^{d}	6.9^{c}	5.0^{de}	2.2^{d}	2910.3^{c}
Sorghum black	86.2	10.9	2.4	3.1^{b}	15.1^{a}	9.8^{a}	6.6^{b}	3.6^{a}	3222.2^{a}
Sorghum red	86.8	10.2	1.9	3.2^{b}	9.3^{c}	8.6^{b}	5.4^{d}	3.7^{a}	3069.0^{b}
SEM	0.13	1.93	0.60	0.20	0.40	0.25	0.20	0.17	40.7
P-value	0.516	0.749	0.052	0.0179	<.001	<.001	<.001	<.001	0.0002

Note. DM: Dry matter; CP: Crude protein; CF: Crude fiber; NDF: Nitrogen detergent fiber; ADF: Acid detergent fiber; GE: Gross energy.

^{a}, ^{b}, ^{c}, ^{d}, ^{e}, ^{f}: different letters in columns indicate means of significant difference ($P < 0.05$).

The variables analysed using PCA included phenolic, tannin, calcium, potassium, sodium, phosphorus, lightness, redness and yellowness. The results of eigenvalues of the correlation matrix are presented in Table 2. There were 2 principal components (PC) extracted with eigenvalues > 1 and had a cumulative proportion of variance of 83.12%. These two PC adequately described the spread of the data on physicochemical properties of sorghum varieties.

Table 2. Analysis of the first 2 principal components for physicochemical traits of sorghum varieties

	Eigenvalue	Difference	Proportion
1	4.18956905	0.89842851	0.4655
2	3.29114054		0.3657

Table 3 shows the loadings of eigenvectors on the PC 1 and 2. Calcium, potassium, sodium, and phosphorus loaded higher than the other eigenvectors on PC 1. Thus, PC 1 described mineral contents of sorghum varieties. PC 2 is characterised by two physical parameters (lightness and yellowness) and chemical properties (phenolic and tannin contents). Lightness and yellowness had high and positive loadings on PC 2. While phenolic and tannin contents loaded strongly and negatively on the same PC, implying that negative scores on PC 2 indicate higher phenolics.

Table 3. Principal component loadings of physicochemical traits of sorghum varieties

Eigenvector	Prin1	Prin2
Phenolic	0.272150	-.424368
Tannin	0.237889	-.419632
Calcium	0.431211	0.254446
Potassium	0.420912	0.274211
Sodium	0.411654	0.266246
Phosphorus	0.421296	0.274326
Lightness	-.277670	0.415935
Redness	0.157450	-.141400
Yellowness	-.239569	0.405892

Figure 1 indicates that yellowness tends to correlate lightness values of sorghum seeds. Dykes et al. (2011) indicated that the yellow colour was consistent with higher levels of 3-deoxythocyanidins, a flavone found in parts of the sorghum seed. Similarly, tannin, phenolic contents correlate with redness in sorghum seeds of tested varieties. Awika (2017) noted that red sorghum has a higher tannin content compared to colorless sorghum (white).

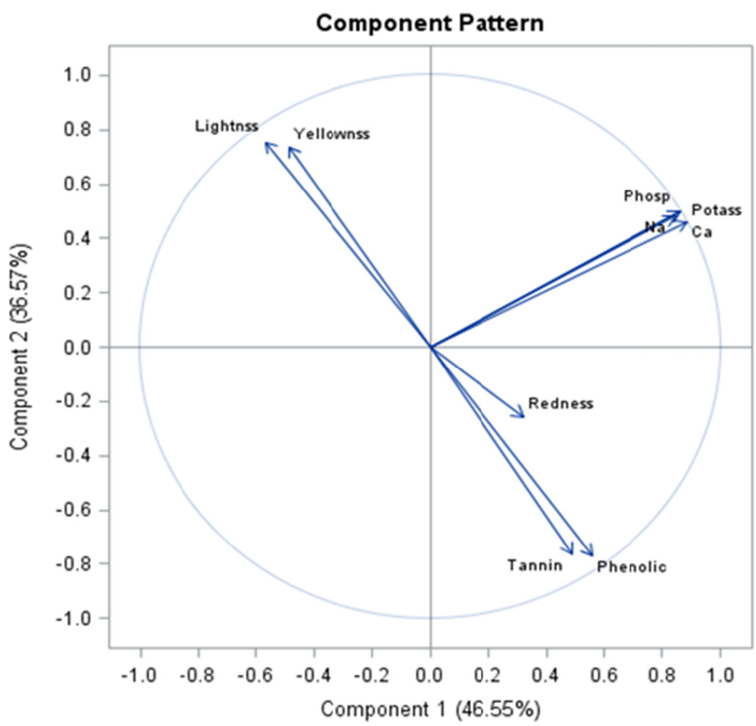


Figure 1. Component pattern of physicochemical traits of sorghum varieties

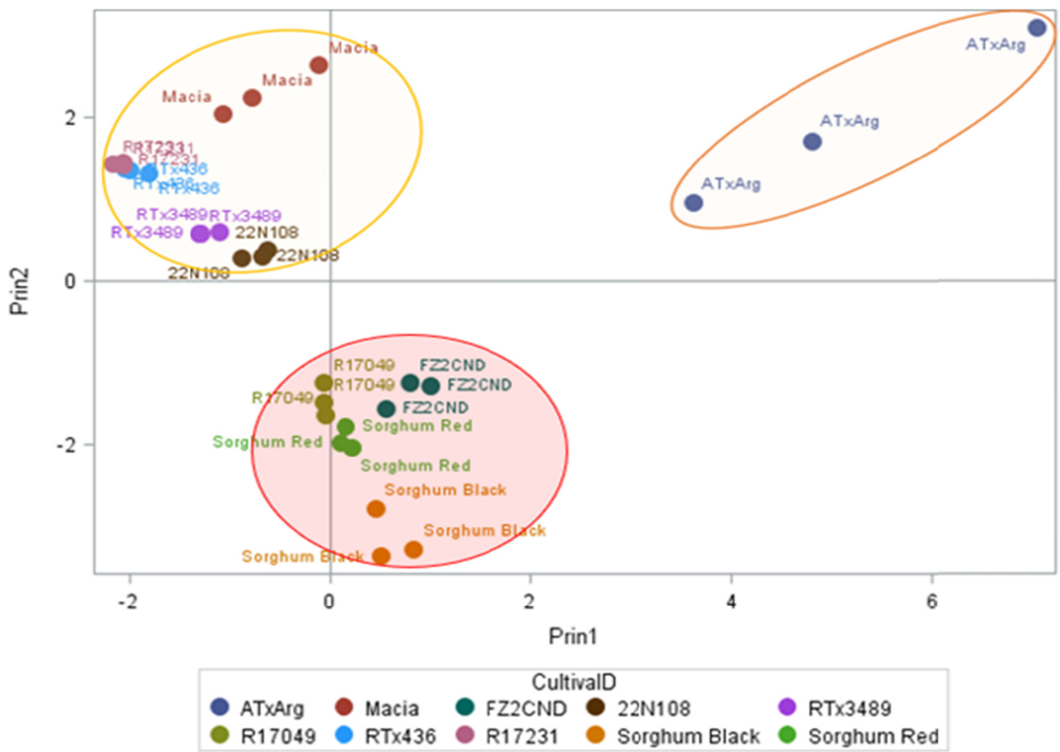


Figure 2. Plot of the first two PC scores of physicochemical traits

The author classified local red sorghum as tanned sorghum with a polyphenol content of more than 10 mg g^{-1}, while the colorless sorghum is classified as non-tanned. The redness of the sorghum grain testa is influenced by the presence of tannins (Earp et al., 2014; du Plessis, 2008). According to Figure 2, the sorghum varieties have different physicochemical traits. The variety ATxArg have higher contents of the minerals (calcium, phosphorus, sodium and potassium) than the other varieties. R17049, FZ2CND, sorghum red and sorghum black differed from the other varieties because they were characterised by high tannins, phenolics and redness contents. Lastly, Marcia, R17231, RTx436 contained high levels of yellowness and lightness.

The germ and aleurone layers are the main sources of fat with the germ layer yielding 80% and aleurone layer having 20% (Kulamarva et al., 2009). A total of 22 fatty acids were detected amongst the sorghum varieties investigated in this study and were analysed using PCA. The first 4 PC with eigenvalues > 1 were extracted as they described 88.2% of the variation of fatty acids, expressed as percent of total fat (Table 4). This indicated that the 22 detected fatty acids were classifiable into four main PC based on the correlation matrix.

Table 4. Analysis of the first 4 principal components in fatty acid analysis of sorghum varieties

	Eigenvalue	Difference	Proportion	Cumulative
1	9.99137726	5.49126751	0.4542	0.4542
2	4.50010975	1.83623132	0.2046	0.6587
3	2.66387843	0.40904868	0.1211	0.7798
4	2.25482975		0.1025	0.8823

The loadings of eigenvectors on PC 1, 2, 3 and 4 are presented in Table 5.

Fatty acids (eigenvectors) which had high loadings on PC1 included nervonic, γ-linolenic, linolenic and eicosapentaenoic, pentadecanoic, margaric, stearic, heneicosanoic, behenoic and tricosanoica acids). While vaccenic acid loaded negatively on PC 1. In PC 2, heptadecenoic, oleic, gondoic and erucic and arachidic acids had high loadings. While linoleic and myristic acid loaded poorly on PC2. Principal component 3 comprised of palmitic, arachidic, lignoceric which loaded highly. While heptadecenoic and linoleic acids loaded poorly. Lastly, PC 4 had palmitoleic and vaccenic acids with high loadings; adrenic acid loaded poorly.

Table 5. Principal component loadings of fatty acids of sorghum varieties

Eigenvector	Prin1	Prin2	Prin3	Prin4
Myristic	0.141525	-.296877	0.201830	0.209102
Pentadecanoic	0.273172	0.060948	-.086417	0.032236
Palmitic	-.059581	0.236328	0.376977	0.218650
Palmitoleic	-.177143	-.082678	0.002100	0.523615
Margaric	0.289349	-.004747	-.046066	-.073777
Heptadecenoic	0.126195	0.269474	-.270787	-.005894
Stearic	0.289920	0.038395	0.217414	0.008648
Oleic	0.066448	0.446943	0.048424	-.017521
Vaccenic	-.250051	-.091417	0.066047	0.314377
Linoleic	-.091402	-.361407	-.277932	-.135918
Linolenic	0.239431	-.134201	0.282436	-.099511
Arachidic	0.153912	0.297789	0.302247	0.004552
Gondoic	-.051662	0.392725	-.031500	0.012255
Gamma linolenic	0.293613	-.004755	-.192361	0.081977
EPA	0.293613	-.004755	-.192361	0.081977
Heneicosanoic	0.269783	-.102629	-.097424	0.288256
Behenoic	0.304743	0.018895	0.000867	0.090630
Erucic	-.081585	0.365565	-.232050	-.046540
Adrenic	0.044905	-.127182	0.123059	-.583372
Tricosanoic	0.282769	-.056711	-.113228	0.223003
Lignoceric	0.177287	-.084133	0.461073	-.046551
Nervonic	0.263830	-.025610	-.230214	-.029770

Note. The loadings of eigenvectors on PC 1, 2, 3 and 4.

Principal components 1 and 2 were plotted in Figure 4 because they had the highest cumulative proportion explaining that variation (65.87%). It demonstrates that the amounts of fatty acids between sorghum varieties is not similar. The variety FZ2ND had high contents of fatty acids of PC 1 (Figure 4), these include ω-3, 6 and 9 fatty acids which are critical for livestock feeding where they function as components for membranes and precursors for synthesis of prostaglandins and arachidonic acid (Tallima & Ridi, 2018). RTx3489 and Macia had high contents of fatty acids in PC 2. Notably in this group are ω-9 fatty acids (oleic, gondoic and erucic acids). Figure 4 also show that the variety 22N108 has high levels of linoleic and myristic acids.

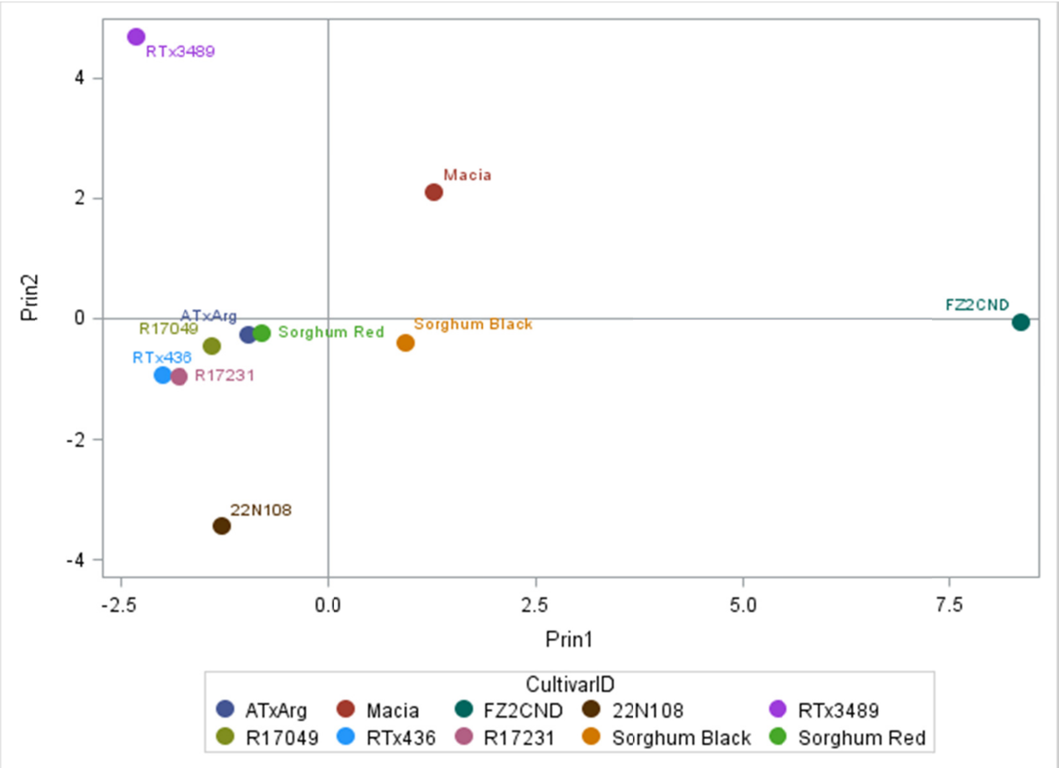


Figure 3. Component pattern of PC 1 and 2 of fatty acids in sorghum varieties

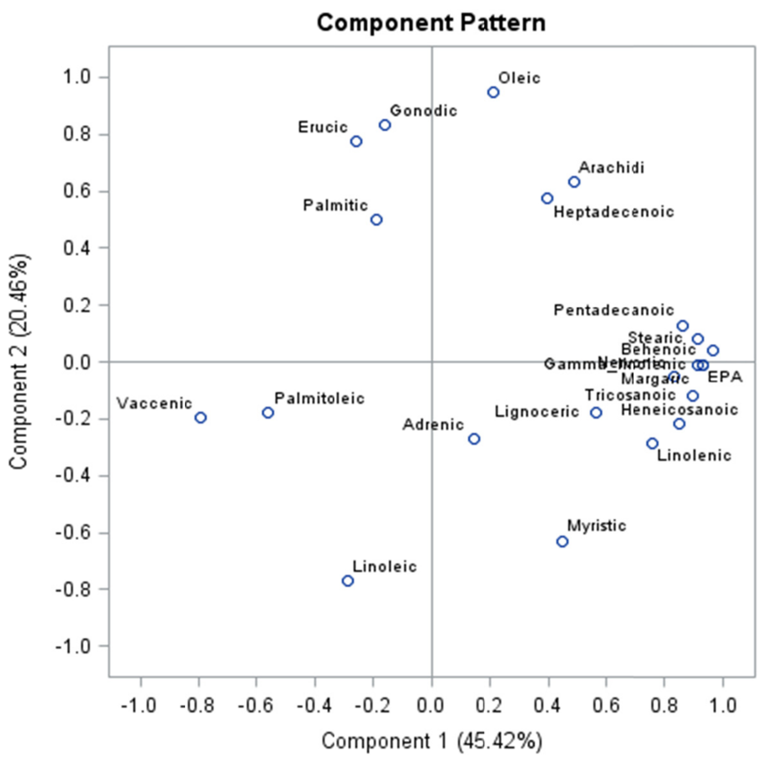


Figure 4. Plot of scores from of PC 1 and 2 of fatty acids of sorghum varieties

Table 5 indicates component patterns of PC 3 and 4 while Figure 5 shows a plot of the scores of sorghum varieties. The sorghum varieties Sorghum black and Macia have higher Components of lignoceric, palmitic and arachidic acids compared to other varieties. While R17231 and RTx436 had higher fatty acid contents of PC 4 (palmitoleic and vaccenic acids). Adrenic acid was higher in the variety R17049 compared to other varieties pattern of PC 1 and 2 of fatty acids in sorghum varieties.

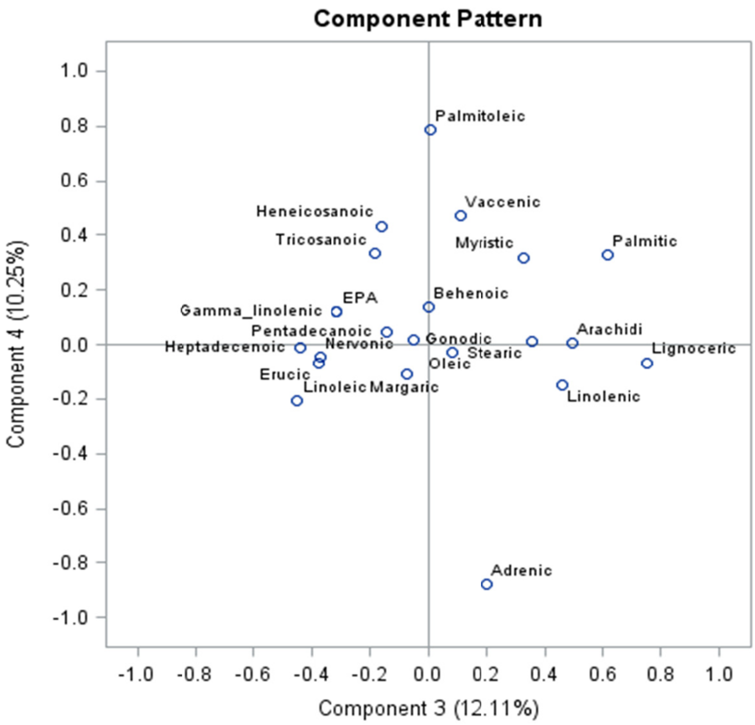


Figure 5. Plot of scores of sorghum varieties

4. Conclusion

There were variations in the nutritional profile and fatty acid contents of the ten varieties evaluated in the current study. The sorghum black had higher GE, NDF and ADF contents. ATxArg had higher fat and mineral contents. The variations could be attributed to factors like genotype and growing conditions. Similarly, sorghum varieties labelled black, red, R17049 and FZ2CND were characterised by high tannins, phenolic and redness contents. Marcia, R17231, RTx436 contained high levels of yellowness and lightness. Sorghum grain physiochemical and nutritional properties vary amongst different varieties. The choice of one variety over the other will depend on the production parameters in question. Physico-chemical characteristics of grains play a very important role in determining the quality of grains. This study represents a pilot study and further research will focus on the effects of different sorghum varieties on production parameters, volatile fatty acid (VFA), rumen microbiome and methane emissions in small ruminants.

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