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Physico-chemical properties and nutritional variance of different genotypes of Indian major millet (Jowar)

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Abstract
Sorghum is one of the most important major millet crops in India. While determining the quality of grains Physico-chemical properties play a crucial role as it determines the overall acceptability of grains and their utilization in different value-added products. The present research was intended to evaluate the three sorghum genotypes *viz.* IS-23891 (white), GP-1539 (Red), and GP-2017-5 (Yellow) for their nutritional variance and physicochemical properties. Results revealed that the highest value of 1000 kernel weight (54.75 gm), 1000 kernel volume (50.18 ml), true density (1.25 g/ml), carbohydrates (73.54 gm) angle of repose(35º23´), and water absorption capacity (35%) was recorded for white coloured genotype IS-23891. Red coloured genotype GP-1539 gave the highest value of protein (11.57%) and fat (3.3%) and reducing sugar (0.35%) as well as it is rich in minerals such as Fe (5.73 ppm), Zinc (1.90 ppm), fibres and anthocyanins. Genotype GP-2017-5 is a rich source of Calcium (27.86 ppm) and crude fibres (3.2%). The higher value of Phosphorous (490 ppm) was found in genotype IS-23891 followed by GP-1539 (416.78 ppm) and GP-2017-5 (390.21 ppm). The lowest value of iron (3.27 ppm) and zinc (1.35 ppm) was found in GP-2017-5. Total sugar content of genotypes GP-1539, GP-2017-5 and IS-23891 was (1.85%), (1.35%), and (1.65%), respectively. The TPC showed obvious variations among different varieties ranging from 157.63 to 289.20 mg/100 g grain (Total, DW). Among all the sorghum genotypes, GP-1539 had the highest TPC, followed by GP-2017-5 while IS-23891 had the lowest. The grain color is one of the reasons that results in the TPC differences among these varieties, however the variation in brewing sorghum grains may come from other reasons, such as the strain and growing environment.

Keywords: Sorghum, genotypes, physico-chemical properties, reducing sugars, mineral content, anthocyanins, phytochemicals

Introduction

Sorghum is the principal staple food of Maharashtra, and also an important food of Andhra Pradesh, Tamil Nadu, Madhya Pradesh and Karnataka. Maharashtra ranks first in sorghum production with 1.81 million tons in 2019-20 and contributes almost 38.23 per cent share to all of India's production (DES-GOI, 2020). Sorghum is a popular type of Millet in India to make Rotis and other bread. It is locally known as Jowar. Organic jowar is a rich source of iron, protein, and fibre and, due to the presence of policosanols which aids in lowering cholesterol levels. Sorghum has a low glycemic index and also increases satiety. India is known for producing numerous traditional coarse cereal crops such as Sorghum, Pearl Millet, Finger Millet, and Foxtail Millet which come under the major millet category (White Paper on Millets, N.D.). Millets are small, round-shaped coarse grains, an indigenous crop to India with an impressive nutrient profile. They are famed as "poor man's food grain" due to their affordability. Millet grains have one in thing common - ample amounts of nutrition. Millets are stapled foods and are important sources of nutrients. Millet grains are rich sources of fibre, vitamins, minerals, and phytochemicals. Millets are cultivated in about 12 million ha. With an annual production of 13.7 (MT) and contribute 10% to the country's food grain basket (MAFW, GOI, 2020-21). It contains nearly all classes of phenolic compounds with simple phenolic acids, flavonoids & tannins being the dominant groups.

Most of the grain produced in African and Asian subcontinents is utilised for human consumption as sorghum is a staple food in these countries. Though sorghum is known for its nutritional quality, the main reason behind decreasing its consumption is the easy availability of rice and wheat through the public distribution system and easy methods of processing and cooking fine cereals such as rice (Ratnavathi CV, 2014) [21]. There has been a 44% decline within the area of sorghum cultivated in India from 1972-73 to 2004-05 i.e., 16.1 to 9.0 million ha. (Rao *et al.*, 2010) [20].

The overall concern for the development of functional foods has generated the need to study and use new food ingredients involved in maintaining and improving health. Sorghum is one among the most important grains considering the cultivated land areas and global production. Sorghum is a gluten-free grain and also rich source of nutrients and phytochemicals. In this perspective, sorghum has a huge potential for its exploitation in different functional food and development of healthy food products (Istrati *et al*., 2019) [11]. The phenolic compounds in sorghum are mainly composed of phenolic acids, 3-deoxyanthocyanidins, and condensed tannins.

Recently sorghum grain has been incorporated into other foods and it has been used to develop functional foods and beverages. Rather than this, the phenolic compounds, 3-deoxyanthocyanidins, and condensed tannins can be isolated and these isolated compounds can be used as encouraging natural multifunctional additives in broad food applications (Xiong *et al*., 2019) [28]. Coloured (black, purple, blue, red, etc.) cereal grains, rich in anthocyanins, have recently achieved a lot of attention in the food industry. Anthocyanins have demonstrated antioxidant potential in both *in vitro* and *in vivo* studies, and due to the presence of the anthocyanins rich food by means of these cereal grains reduces the risks of chronic diseases. As such, coloured grains are used to make promising new products and functional foods (Francavilla & Joye, 2020) [8]. The colour and stability of these anthocyanin are influenced by pH, light, temperature, and structure. In acidic conditions, anthocyanins appear as red but turn blue when the pH increases (Khoo *et al*., 2017) [12]. As compare to other cereals available epidemiological evidence advises that sorghum consumption reduces the risk of certain types of cancer in human due to the high concentration of phytochemicals in sorghum may be partly responsible (Awika & Rooney, 2004). Consumer demand for gluten-free cereal products is rising steadily with the increase in celiac disease and other allergic reactions to gluten from wheat-, rye- or barley-based foods (Bogue & Sorenson, 2008) [4]. Sorghum foods have a low glycemic index and glycemic load than wheat/rice and are suggested to prevent and manage/control diabetes (Banhur D and Ganapathy K. N. Sorghum as a Small Grains a Big Gain. Pdf, n.d.). The anthocyanins found in sorghum is generally 3-deoxyathocyanins which were a rare class of pigments and these anthocyanins are stable against light, heat and change in pH (Devi *et al*., 2012) [7].

Materials and Methods

Materials

Sorghum [sorghum bicolor (L.) Moench] genotypes i.e., IS-23891 (chalky white), GP-1539 (Red), and GP-2017-5 were procured from the Seed Technology Research and Breeder Seed Production Unit V.N.M.K.V. University Parbhani, Maharashtra. All the chemicals and glassware used in this research work were of analytical grade (AR) and were used from the PG laboratory in the Department of Food Chemistry and Nutrition. Equipment required in the present investigation was available at the College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Procedures recommended by the Association of Official Analytical Chemists (AOAC, 1990) [1] were used for this research work.

Methods

Physical Characteristics of Sorghum Genotypes

Physico-chemical characteristics are an important parameter which determines overall consumer acceptability, and therefore the study of these characteristics of the grains becomes a basic step in any research.

The sorghum grains are subjected to measure physical characteristics such as thousand kernel weight, thousand kernel volume, angle of repose, bulk density, true density and water absorption capacity. These physical characteristics as mentioned above determine the quality of the sorghum grain. Methods used to determine these physical characteristics are given below. All the measurements in this research work were done in triplicates.

1000 Kernel Weight

For determination of 1000 kernel weight neat, clean and sorted 1000 grains weight was measured by precise electronic weighing balance and the average weight was calculated.

1000 Kernel Volume

The volume of 1000 grains was measured directly by millilitre water displacement when grains were poured into a measuring cylinder containing water.

Bulk Density

The bulk density (BD) of grain was measured according to the standard method given by (Rybak-Chmielewska, 2003) [23].

It was calculated by using the formula: - $\rho = M/V$

True Density

True density is nothing but the quotient of mass per unit volume of a sample, without considering pores in the material, i.e. (true volume).

It was calculated by using the formula: - $\rho = W/V$

The angle of Repose

Angle made by free vertical fall of grain to the base of heap formed.

Formula: - $Q = \tan^{-1}(2h/d)$

Where, h- the height of cone (cm)

and d- diameter of cone(cm).

Water Absorption Capacity (WAC)

It is defined as the maximum amount of water that 1 g of material will imbibe and retain under low-speed centrifugation. To estimate water absorption capacity, five-gram flour was weighed in a 50 ml centrifuge tube, 30 ml water was added and stirred with a glass rod for 5 min, then allows to stand for 30 min, at ambient conditions, then centrifuged at 4500 rpm for 25 min. The volume of free liquid was measured and the retained volume was expressed as ml of water absorbed per gram of sample on a dry basis.

Chemical Characteristics of Sorghum Genotypes;

Analysis of chemical characteristics of sorghum genotypes was done by means of moisture content, fat, protein, ash, crude fibre and total carbohydrates, reducing and non-reducing sugars. All the analyses were done in triplicates and the results were expressed as the average value wherever applicable.

Moisture Content
Moisture content was determined using the (A.O.A.C., 1990) [1] method. The following formula was used to measure the moisture content.

$$\% \text{ Moisture} = \frac{\text{Initial weight - Final weight}}{\text{Weight of sample}} \times 100$$

Fat Content:
Fat content was determined using the (A.O.A.C., 1990) [1] method. Soxhlet apparatus was used to determine the crude fat content of samples.

$$\% \text{ Crude fat} = \frac{\text{Final weight of flask - Empty weight of the flask}}{\text{Weight of sample}} \times 100$$

Protein Content.
Protein content was determined using the (A.O.A.C., 1990) [1] method. Kjeldahl apparatus was used to determine the crude protein content of samples. Micro- Kjeldahl steam distillation unit was used for distillation purposes.

Per cent nitrogen and per cent protein was calculated by using the following formula.

$$\% \text{ Nitrogen} = \frac{\text{CBR X Normality of } H_2So_4 \text{ X Moles of nitrogen X D.F.}}{\text{Weight of sample}} \times 100$$

Where,
CBR= Corrected Burette Reading
Normality of Acid (H_2So_4) = 0.01
Moles of Nitrogen = 0.014
D.F. = Dilution Factor
Total protein = % Nitrogen X Protein factor [for cereals 5.7 & for Sorghum 6.25]

Ash content
Ash content was determined using the (A.O.A.C., 1990) [1] method. A muffle furnace was used to determine the ash content of the samples. The percent ash was calculated using the following formula.

$$\% \text{ Ash} = \frac{\text{Weight of crucible with ash - weight of empty crucible}}{\text{Total weight of the sample}} \times 100$$

Total Carbohydrate
The modified phenol sulphuric acid method was used to determine the total carbohydrate content of the samples. The standard graph was plotted using standard glucose solution and a comparison was done with the standard curve obtained using a graph. The graph was plotted as absorbance on the Y axis and concentration on the X axis.

Total Sugars
The sugar percentage was calculated by the method suggested by Nelson-Somogyi. This was calculated by the addition of reducing and non-reducing sugar.

Reducing and Non-reducing Sugar
The reducing and non-reducing sugar percentage was calculated by the method suggested by Nelson-Somogyi.

The reducing sugar was determined using a formula;

$$\text{Reducing sugar in sample (\%)} = \frac{\text{Sugar value from graph}}{\text{Aliquot sample used}} \times \frac{\text{The total volume of alcohol-free extract}}{\text{Weight of Sample}} \times \frac{1}{100}$$

Crude fibre
The crude fibre content was determined using the (A.O.A.C., 1990) [1] method.

$$\% \text{ Crude fibre} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W} \times 100$$

Where,
W = weight of the sample
W_1 = weight of ashing dish
W_2 = weight of sample before ignition
W_3 = weight of sample after ignition

Determination of minerals.
The mineral content of food was determined by the method given by (Ranganna, 1986).

Determination of Total Phenolic Content
Total phenolic content of sorghum were determined using Folin-Ciocalteu's method. 5 ml of Folin-Ciocalteu reagent was added in 1ml soarrghum seed extract sample in tube. Then, 4 ml of 7.5% sodium carbonate was added. After 1 hr of incubation at ambient temperature absorbance was read at 765nm against blank. The obtained results were taken as mg gallic acid equivalent per gram of fresh sample (mg GAE/g). The formula for calculating the total phenolic contents present in sorghum extract samples as given below.

$$C = c\ V/m$$

Where, C
= total poly phenolic content present p(mg GAE/g)
= gallic acid concentration obtained from calibration curve (mg/ml)
V = Extract volume (ml)
m = Extract mass (g)

Statistical analysis
The results of various physical and chemical measurements of grain quality were analysed. The Analysis of variance was calculated using the standard ANOVA procedure. The analysis of variance revealed significance at a $p < 0.05$ level. The standard error (SE) and critical difference (CD) at the 5% level were mentioned where required.

Results and Discussion
The physical appearance of the grains is presented in Table 1.

Table 1: The physical appearance of sorghum genotypes

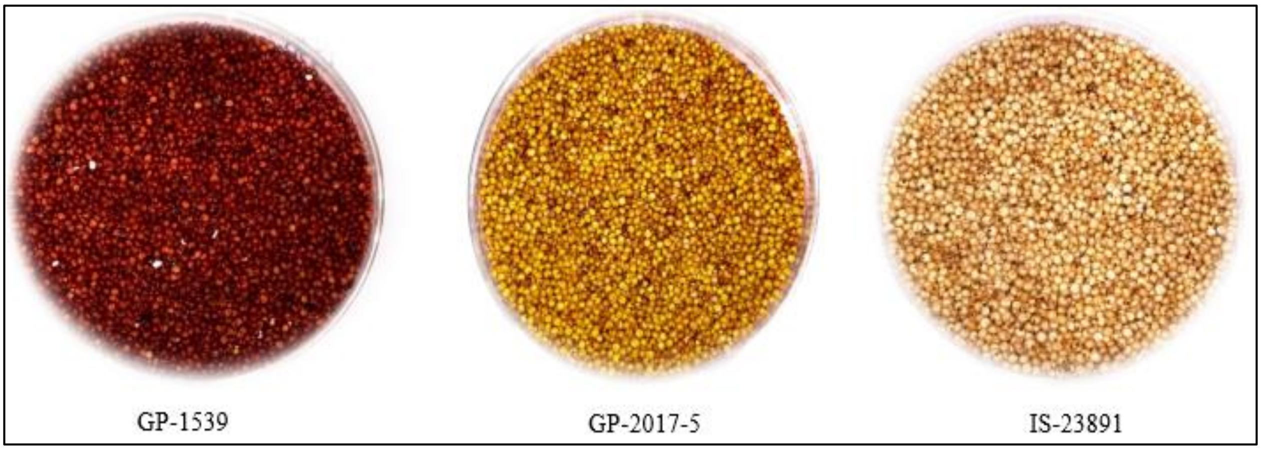
Sr. No.	Genotypes	The physical appearance of sorghum genotypes				
		VKC	Hunter Colour Meter			Shape
			L	A	B	
1.	GP-1539	Red	34.91	15.62	15.18	Bold
2.	GP-2017-5	Yellow	54.56	6.91	39.32	Round
3.	IS-23891	White	64.13	2.38	15.18	Round

Where,
VKC: Visual Kernel Colour
Hunter LAB test: L: lightness, A: redness, B: green colour value

The visual kernel colour of genotypes GP-1539, GP-2017-5 and IS-23891 were red, yellow and white respectively. There were significant differences in the Hunter LAB values among all the genotypes mentioned. The Hunter LAB values ranged from 64.13 (lightest) to 34.91 (darkest). The genotypes GP-1539 and GP-2017-5 had pigmented testa, except for genotype IS-23891. The genotype GP-1539 which was red gave the highest value for redness (15.62) due to the red pigmented testa, however, IS-23891 gave the lowest value for the same as it was white in colour as well as the absence of pigmented testa. Concerning the green colour value, the genotype GP-2.17-5 which was yellow gave the highest value and genotypes GP-1539 and IS-23891 gave the same values for green colour. The shape of the genotypes GP-1539, GP-2017-5 and IS-23891 was bold, round and round respectively.

Anthocyanins are water-soluble flavonoids and are responsible for red, violet, and blue colours in fruits, vegetables, and grains (Francavilla & Joye, 2020) [8]. The predominant anthocyanins found in sorghums were 3-deoxyanthocyanidins including the unique leuteolinidin and apigeninidin analogues. The results obtained are in line with the results obtained by (Mabelebele *et al*., 2015) [15].

Physical appearance of different sorghum genotypes



Kernel size and weight are key elements for differences in physical properties. Thus, knowing grain quality, flour quality and end-use application are necessary for hybrid selection (Liu *et al*., 2012) [13]. The physical properties of the above-mentioned genotypes are given in Table 2.

Table 2: Physical properties of different sorghum genotypes

Genotypes	Physical properties of sorghum grain					
	Thousand kernel weight (g)	Thousand kernels Volume (ml)	Bulk density (g/ml)	True density (g/ml)	The angle of repose (degrees)	WAC (%)
GP-1539	28.78	23.50	0.72	1.25	32º16´	35%
GP-2017-5	38.04	29.93	0.66	1.12	33º19´	32%
IS-23891	54.75	50.18	0.55	1.03	35º23´	30%
SE±	0.0194	0.1052	0.0091	0.0107	0.0138	0.3688
CD at 5%	0.0589	0.3193	0.0276	0.0326	0.0418	1.118

* Each value is a mean of three determinations

Physical properties of sorghum were determined such as thousand kernel weight, thousand kernel volume, bulk density, true density, angle of repose, and water absorption capacity which aid in sorghum identification and its utilization for the specific application. It was found that the highest thousand kernel weight (54.75g) and thousand kernel volume was (50.18ml) for sorghum genotype IS-23891. However, the lowest thousand kernel weight and volume were observed for genotype GP-1539 which was (28.78g) and (23.50ml) respectively. The result obtained was in close resemblance to the result reported by (Gürsoy & Güzel, 2010) [10]. Deviation observed may be because of genotypic differences, meteorological differences, cultivation methods etc.

The highest bulk density was found for genotype GP-1539 (0.72g/ml) and the lowest bulk density was observed for genotype IS-23891 (0.55g/ml). Bulk density is measured as a mass per unit volume or a degree of compactness whereas, true density can be defined as the "Quotient of mass per unit volume of a sample, without considering pores in the material, i.e. (True volume)." The true density recorded for the sorghum genotypes GP-1539, GP-2017-5, and IS-23891 were 1.25, 1.12, and 1.03 respectively. The result obtained for sorghum genotypes in the present study was in line with the findings of (Vannalli *et al*., 2008) [25]. In this determination, the true volume is considered as the amount of toluene solution displaced after adding a specific amount of sorghum grains. The angle of repose for genotype IS-23891 was 35º23´ which is the highest one whereas, the angle of repose recorded for GP-1539 and GP-2017-5 were 32º16´ and 33º19´ respectively. The result obtained for the angle of repose for sorghum was nearer to the results obtained. The highest water absorption capacity was found for genotype GP-1539 (35%) and the lowest was observed in IS-23891. The water absorption capacity of sorghum genotype GP-2017-5 was 32%. The values obtained for physicochemical properties were found to be identical to values reported by (Murty *et al*., 1982) [22].

Chemical composition of different sorghum genotypes

The proximate composition of GP-1539, GP-2017-5 and IS-23891 was determined using moisture, fat, protein, carbohydrates, crude fibres and Ash content. The data about the chemical composition is given in Table 3.

Table 3: Proximate composition of different sorghum genotypes

Genotypes	Chemical properties of sorghum grain					
	Moisture (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Crude fibres (%)	Ash (%)
GP-1539	7.2	3.3	11.57	72.36	3.2	1.9
GP-2017-5	9.4	2.78	10.37	71.20	3	1.57
IS-23891	8.6	3.11	9.57	73.53	2.8	1.39

* Each value is a mean of three determinations

Moisture content was ranged from (7.2 to 9.4%), fat (3.3 to 3.11%), protein (9.5 to 11.5%), carbohydrate (71.2 to 73.5%), crude fibres (2.8 to 3%), and ash content (1.3 to 1.9%). It is evident from the table that the highest moisture content was found for genotype GP-2017-5, followed by (8.6%) and (7.2%) for genotypes IS-23891 and GP-1539 respectively. No significant difference in the values of fat content was observed among the genotypes.

The obtained values for protein content in the present investigation are 11.57% for GP-1539 which was the highest protein content among selected genotypes whereas, the protein content of genotype GP-2017-5 was 10.37%. The genotype IS-23891 gave a lower level of crude protein content which was 9.57%. In addition to genetic factors, environmental effects may be a cause for the wide variation in protein content (Geleta *et al*., 2005) [9]. The obtained values for protein content are in line with the values reported by (Chavan *et al*., 2016) [5].

The carbohydrate content was found at 72.36 g/100g and comparable with the ranges of 71.20% to 73.53%. The values recorded for the carbohydrate content of the genotypes GP-2017-5, GP-1539, and IS-23891 were 71.20%, 72.36%, and 73.53% respectively. The results obtained were similar to the findings of (U.D. Chavan *et al*., 2015). The mean value for crude fibre content for genotype IS-23891 (2.8%), GP-1539 (3%), and GP-2017-5 (3.2%). The results obtained are in line with the results given by (Longvah *et al*., 2017) [14]. From the results obtained it is evident that there is no significant difference in crude fibre content was observed.

Based on recorded values for ash content it was found that the highest value of ash was recorded for GP-1539, followed by (1.39%) and (1.57%) for genotypes IS-23891 and GP-2017-5 respectively. The obtained values for ash content reported in the present study were in line with the findings of (Prabhakar *et al*., 2017) [18].

Total Sugars

Determination of total soluble sugar plays a crucial role in the preparation of value-added products from sorghum which determines the taste and acceptability of the product.

The data pertaining to the total sugar content is presented in Table 4.

Table 4: Total Sugars

Genotype	Total Sugar (%)	Reducing sugar (%)
GP-1539	1.85	0.35
GP-2017-5	1.35	0.21
IS-23891	1.65	0.26

It is evident from the table that total soluble sugars in the sorghum genotype ranged from 1.35% to 1.85%. Based on the results obtained it is found that all the genotypes studied were significantly different. Total soluble sugars are mostly responsible for the good taste of roti prepared from sorghum (S.V. Nirmal *et al*., 2017) [24].

From Table-4 it is seen that the highest total soluble sugars were found in genotype GP-1539 which was 1.85%, followed by 1.65% and 1.35% in sorghum genotypes IS-23891 and GP-2017-5 respectively. Yellow pericarp containing sorghum i.e., GP-2017-5 gave a lower amount of total soluble sugar as compared to the other two genotypes. Similar results are reported by (S.V. Nirmal *et al*., 2017) [24].

Mineral composition of different sorghum genotypes.

Sorghum grains are a good source of mineral which plays a crucial role in the human diet. Therefore, the utilization of sorghum by means of different value-added products in daily diet will aid in the supply of essential minerals to the human body. Data pertaining to the commonly found minerals in the sorghum accessions are presented in Table 5.

Table 5: Mineral composition of sorghum genotypes

Genotype	Mineral composition of grains (mg/100gm or ppm)			
	Calcium	Phosphorous	Iron	Zinc
GP-1539	24.71	416.78	8.57	1.90
GP-2017-5	27.86	390.21	5.27	1.35
IS-23891	18.71	490.55	4.33	1.70

*Each value is a mean of three determinations

Concerning the phosphorous content Genotype IS-23891, recorded the highest amount of phosphorous which is (490.55ppm). Likewise, the values of phosphorous content observed for genotypes GP-2017-5 and GP-1539 were (390.21 ppm) and (416.78 ppm). From the results obtained it is seen that the genotype GP-2017-5 gave a significant lower phosphorous content.

From the values presented in table-4, it can be concluded that the calcium content ranged from 18.71 to 24.53 (ppm). A higher level of calcium content was found in the genotype GP-2017-5 which is (27.86 ppm). The genotype -IS23891 gave a higher level of calcium which is (18.71 ppm) whereas, the calcium content exhibited by genotype IS-23891 is (24.71 ppm).

Iron plays a crucial role in the body's functioning so determining the iron content of grains is essential.

The genotype GP-1539 recorded a higher level of iron content (8.57 ppm) however, the iron content of the genotype GP-2017-5 and IS-23891 showed a significant difference in iron values which is (5.27 ppm) and (4.33 ppm) respectively. The lowest iron content was found in genotype IS-23891.

The zinc content of different sorghum genotypes as mentioned above ranged from (1.35 ppm) to (1.90 ppm). The genotype GP-1539 gave higher-level zinc which is (1.90 ppm). The genotype GP-2017-5 gave a lower zinc content which is (1.35 ppm). Likewise, zinc content was found in genotype IS-23891 which is (1.70 ppm). The values obtained for mineral content were in close resemblance with the values reported by (Chavan *et al*., 2016) [5]. Zinc and ferrous are the essential minerals found in sorghum which play a crucial role and surge the phytochemical composition of sorghum. Besides minerals, sorghum is a rich source of fibre, vitamins, and phytochemicals and is hence used in the enrichment of food products.

Table 6: Total Phenolic Content of Sorghum Genotypes

Genotypes	Total Phenolic Content (TPC) (mg/100gm)	Total Anthocyanin Content (mg/100gm)
IS-23891	157.63	9.71
GP-1539	289.20	53.30
GP-2017-5	220.40	27.74
S.E±	0.2433	0.2224
CD at 5%	0.7379	0.6747

Total phenolic content and total anthocyanin content are summarized in Table 6. On the whole, genotype GP-1539 showed higher phenolic compounds than other varieties, while the white grain sorghum, IS-23891 showed the lowest contents of all.

The TPC showed obvious variations among different varieties ranging from 157.63 to 289.20 mg/100 g grain (Total, DW). Among all the sorghum genotypes, GP-1539 had the highest TPC, followed by GP-2017-5 while IS-23891 had the lowest. The grain color is one of the reasons that results in the TPC differences among these varieties, however the variation in brewing sorghum grains may come from other reasons, such as the strain and growing environment. However, the results conducted by Wu G and co-investigators showed that there were no significant differences between free (soluble) and bound (insoluble) total phenolics in three red pericarp sorghum genotypes (WU *et al*., 2017) [27].

Conclusion

Thus, by taking into consideration the data obtained in the present investigation it can be concluded that the sorghum grains are packed with dense nutrients. Sorghum grain is gluten-free, high in resistant starch and a rich source of nutrients and most importantly contains a diverse range of bioactive phenolic compounds. Phenolic compounds found in sorghum are rare class of phenolic pigments found in cereal grains. Hence, the study of these physicochemical characteristics is necessary for determining processing parameters and utilization of sorghum in different value-added products.

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