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RESEARCH ARTICLE

Evaluation of nutritional profiles, phytochemical constituents and antioxidant activities in *Sorghum bicolor* [L.] Moench (red vs. white)

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ABSTRACT

Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most widely cultivated cereal grain globally. It is valued for its rich nutritional profile, gluten-free nature, and health-promoting properties, particularly its antioxidant potential. However, limited comparative data exist on the nutritional content, phytochemicals, and antioxidant activities between the red and white sorghum. This research aims to compare these characteristics between the two variants of sorghum, which is the red sorghum and white sorghum. Red and white sorghum were dehydrated at 60 °C for 1 hour. Proximate analysis was performed to determine the nutritional content, Folin-Ciocalteu assay for total phenolic content (TPC) and aluminum chloride colorimetric method for total flavonoid content (TFC). Antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH+) and 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS+) assays. The results were analyzed using one-way analysis of variance (ANOVA), and differences with $p < 0.05$ were considered statistically significant. Red sorghum exhibited significantly higher protein ($p = 0.001$), while white sorghum exhibited higher ash content ($p = 0.036$). Although red sorghum showed slightly higher TPC (235.03 ± 59.82 µg GAE/g) and TFC (6.53 ± 3.27 µg QE/g) than white sorghum (214.03 ± 74.08 µg GAE/g and 4.95 ± 2.8 µg QE/g, respectively), the differences were not statistically significant ($p > 0.05$). The DPPH assay showed that WS exhibited significantly higher radical scavenging activity with 87.4% inhibition (IC_{50} = 260.01 µg/ml) compared to RS at 77.4% inhibition (IC_{50} = 268.67 µg/ml). Conversely, the ABTS assay revealed stronger antioxidant activity in RS, which had a higher inhibition percentage (67.6%, IC_{50} = 326.00 µg/ml) than WS (49.0%, IC_{50} = 827.27 µg/ml), indicating cultivar-dependent differences in antioxidant efficacy. Thus, red and white sorghum each exhibit distinct strengths, with significant differences in protein, ash content and free radical inhibitions (DPPH and ABTS). However, their comparable TPC, TFC suggest equal potential for functional food applications.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is a versatile cereal crop of significant global importance. It ranks fifth in production among cereal crops. It belongs to a genus comprising approximately 25 species within the grass family and serves as a staple food in the semi-arid regions of Africa and Asia. Sorghum is cultivated in nearly 100 countries worldwide; between 2001 and 2020, global cultivation averaged 40.9 million hectares, led by India, Sudan and Nigeria (Deb et al., 2004; Khalifa & Eltahir, 2023). There are various types of sorghum such as grain sorghum, forage sorghum, sweet sorghum and broomcorn. Sorghum grain is widely consumed in Africa and Asia, where it is primarily processed into flour for making bread, porridge, and various traditional dishes, serving as a vital staple food (Davana et al., 2020; Khoddami et al., 2021; Ali et al., 2024). In contrast, other sorghum types have distinct uses; forage sorghum is cultivated for livestock feed, while broomcorn is grown for broom production (Stefaniak et al., 2012; Taylor & Taylor, 2023).

In recent years, sorghum has gained global attention as a highly nutritious, gluten-free ancient grain, making it especially beneficial for individuals with gluten intolerance or celiac disease (Hamaker & Bugusu, 2003; Kumar et al., 2023). Its gluten-free nature has led to its increasing use as a substitute for wheat in a variety of food products, such as pasta, bread, biscuits, and porridge (Chavez et al., 2018;

Pineli et al., 2015; Hegde et al., 2023). Sorghum-based foods may also help regulate glycemic response, contributing to a reduced risk of metabolic disorders like diabetes (Zhang & Hamaker, 2009; Camara et al., 2024). Its nutritional profile closely resembles that of wheat, with a chemical composition comparable to rice and maize (Singh et al., 2014; Mir et al., 2018; Widowati & Luna, 2022). Moreover, sorghum is a valuable source of phenolic compounds and other bioactive substances that have been linked to potential health benefits, including cancer prevention, cardiovascular protection, and the reduction of chronic inflammation and oxidative stress (Xu et al., 2015; El Mahdi et al., 2024).

The phytochemical composition of red and white sorghum shows notable differences that have implications for nutrition, food processing, and industrial applications. Red sorghum is typically richer in tannins, anthocyanins, and flavonoids (especially 3-deoxyanthocyanidins) than white sorghum, which contributes to its higher antioxidant capacity (Flores-Garcia et al., 2024). These compounds play significant roles in health promotion due to their anti-inflammatory and anticancer properties. Meanwhile, white sorghum is generally lower in phenolic content but has comparable starch and amylose levels, making it more suitable for certain food products with milder flavor profiles and less bitterness (Soeka et al., 2024). Studies have also shown that pre-processing of sorghum can influence the bioavailability and expression of these phytochemicals differently across red and white varieties (Hamaker et al., 1988; Liu et al., 2018; Putra, 2025). Moreover, while red sorghum often exhibits higher polyphenol and antioxidant levels, white sorghum may contain more accessible proteins and micronutrients, depending on variety and environment (Wulandari et al., 2024). White sorghum tends to have a milder flavor compared to red sorghum, making it more favorable for certain food applications (Shen et al., 2018; Punia et al., 2021).

Recent research has highlighted that the nutritional composition, phytochemical profiles and antioxidant content of sorghum vary significantly depending on the pericarp color. Although sorghum is not extensively cultivated in Malaysia, increasing interest has been driven by its adaptability to environments and its potential in functional food development. However, comparative data on macronutrient composition, phytochemicals and bioactivities of locally available red and white sorghum varieties remain scarce. Thus, this study explores the comparative characteristics of red and white sorghum varieties, focusing on the aforementioned characteristics. The outcome of this research provides a deeper understanding of the health benefits and functional properties of sorghum and ultimately contributes to its wider utilization as a nutritious and accessible food source.

2. MATERIALS AND METHODS

2.1. Sample preparation

Sorghum samples were obtained from local farmers in Bachok (5.9262 °N, 102.4608 °E). The parent seeds were verified and purchased from a local agricultural supplier in Selangor. The grains were carefully inspected for any signs of contamination, particularly fungal growth, and only clean, intact grains were selected. These selected samples were then stored in airtight containers until further analysis. To ensure proper documentation and future reference, the samples were submitted to the Herbarium of the Faculty of Earth Science, UMK and assigned voucher numbers WS-001 (white sorghum) and RS-001 (red sorghum).

2.2. Sample pre-treatment

Pre-treatment was conducted according to Luo et al. (2018) with modifications. Fifty (50) g of both white and red sorghum samples were weighed, dried using a dehydrator (Puncak Steel, Malaysia) at 60 °C for 1 hour until the moisture content was low, and then ground into a fine powder until further use.

2.3. Sample extraction

Extraction was performed following Hou et al. (2016), with modifications. A total of 50 g of ground samples were added to 200 ml of distilled water at solid-to-solvent ratio of 1:4 in a 250 ml reagent bottle. Distilled water was selected as food-safe solvent to extract polar bioactive compounds from sorghum. The mixture was sonicated at 40 kHz for 60 minutes at 50 °C and later was filtered using filter paper (8-11 µm). The extract was frozen at -20 °C overnight and freeze-dried (ScanVac, Denmark) the next day until a dried sample was obtained. The dried samples were kept at -20 °C until further use.

2.4. Macronutrient determination

All samples were evaluated for macronutrients composition based on the methods described by the Association of Official Analytical Chemists (AOAC) 1999 and Pearson 1981. The analysis includes the determination of carbohydrate, fat, protein, crude fibre, ash, and moisture content.

2.4.1. Moisture content

The moisture content of sorghum samples was analysed using a moisture analyser (A&D MX-50) following standardized procedures. The instrument was preheated to a drying temperature of 105 °C and approximately 5 g of finely ground sample was uniformly distributed on the sample pan. The analyser continuously heated the sample, recording the weight loss due to moisture evaporation until a stable weight

was achieved. All measurements were performed in triplicate to ensure accuracy. The percentages of moisture were calculated using the following formula:

Moisture content (%) = (W0-W1) / W1 X 100

where W0 is the weight of samples before drying (g) and W1 is the weight of samples after drying (g).

2.4.2. Ash content

A total of 3 g of sorghum sample was placed in a porcelain crucible and subjected to ignition in a muffle furnace at 550 °C for 5 hours until white ash formed. After ignition, the crucible was transferred to a desiccator, allowed to cool and then weighed. This procedure was repeated until consistent weights were obtained in two consecutive measurements. The ash content was determined using the following formula:

Ash content (%) = (W0-W1) / W1 X 100

Where W0 is the weight of samples before ignition (g) and W1 is the weight of samples after ignition (g).

2.4.3. Protein content

The Kjeldahl method was used to determine protein content. Approximately 1 g of sample was digested with 12 ml of concentrated sulphuric acid (H_2SO_4) and two catalyst tablets in a digestion block at 400 °C for 2 hours. After cooling, the clear digest was diluted with 80 ml of distilled water and 50 ml of 40% sodium hydrochloride (NaOH). Ammonia released during distillation was collected in 30 ml of boric acid with methyl red indicator and quantified by titration with hydrochloric acid (HCl). The nitrogen content was converted to protein using a 6.25 conversion factor using the following formula:

$$\text{Protein (\%)} = \frac{\text{(Vs-Vb) X N X14.01 X 100}}{\text{W X 100}} \text{ X F}$$

where; Vs is the amount of standardized acid used in titrating the sample (ml), Vb is the amount of standardized acid used to titrate the blank (ml), N is the normality of standard HCL, W is the weight of sample used (g) and F is the factor to convert nitrogen to protein.

2.4.4. Fat content

Approximately 5 g of the dried samples were weighed into a thimble, which was then placed into the automated Soxtec™ 2055 Fat Extraction System. Subsequently, 85 ml of petroleum ether was added to the samples and the mixture was heated at 40 - 60 °C to extract the fat. After extraction, the petroleum ether was evaporated from the extract and then dried in an oven. The extract was allowed to cool in a closed container with a desiccator before being weighed. The fat content was calculated using the following formula:

Fat content (%) = (W0-W1) / Ws X 100

where W0 is the weight of empty dish with sample (g), W1 is the weight of empty dish (g) and Ws is the weight of the sample (g).

2.4.5. Fiber content

One gram (1 g) of the defatted samples was digested with 1.25 % H_2SO_4 and 1.25 % NaOH using the Fibertec 8000 Auto-Fibre Analysis System (FOSS Brand) to determine the fiber content. The reagents were preheated internally within the automated system and water used to wash the samples. Finally, the sample was rinsed with distilled water. The fiber content was calculated using the following formula:

Fiber content (%) = (W0-W1) / Ws X 100

Where, W0 is the weight of empty dish with sample (g), W1 is the weight of empty dish (g) and Ws is the weight of the sample (g).

2.4.6. Carbohydrate activity

Carbohydrate content was calculated according to Pearson (1981) using the following formula:

Carbohydrate (%) = 100- [a+ b + c+ d + e]

Where a is the moisture content, b is the ash content, c is the protein content, d is the fat content and e is the fiber content.

2.5. Determination of phytochemicals

2.5.1. Total phenolic content

Total phenolic content (TPC) of the sorghum extract was measured using a modified Folin-Ciocalteu method based on Babeanu et al. (2018). Gallic acid standards (10 - 1000 µg/ml) were used, while extract concentration ranged from 500 to 3000 µg/ml. Each sample was mixed with 2 ml of 0.2 N Folin-Ciocalteu reagent and left at room temperature for 5 minutes, followed by 0.5 ml of 7.5% (w/v) sodium carbonate. After 60 minutes of incubation in the dark, absorbance was read at 765 nm using a UV-1900i spectrophotometer. TPC was reported as µg gallic acid equivalent per gram of extract (µg GAE/g).

2.5.2. Total flavonoid content

The total flavonoid content (TFC) of the sorghum was determined using a modified aluminium chloride method as described by Kumari et al. (2021). A one mg/ml quercetin stock solution was prepared by dissolving 10 mg of quercetin in 10 ml of ethanol. From this stock solution, various concentrations of quercetin (10 – 1000 µg/ml) were prepared

for the standard curve, while extract concentration ranged from 500 to 3000 µg/ml. For the assay, 0.25 ml of the sorghum extract was mixed with 1.25 ml of deionized water and 75 µL of 5% sodium nitrite solution. After 6 minutes, 150 µL of 10% aluminium chloride hexahydrate solution was added, followed by 0.5 ml of 1 M NaOH. The solution was vortexed and incubated at room temperature for 5 minutes. The absorbance was measured at 415 nm using a UV-1900 spectrophotometer (Shimadzu). The experiment was conducted in triplicates, with quercetin as the standard. The results were expressed as µg of quercetin equivalent (QE) per gram of extract (µg QE/ g extract).

2.6. Antioxidant assays

2.6.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of the sorghum extract was measured using a DPPH radical scavenging assay following the method of Shah and Modi (2015) with slight modifications. Ascorbic acid standard solution was prepared by diluting a 1 mg/ml stock solution to obtain various concentrations ranging from 0.2 to 1 µg/ml. For the assay, 1 ml of 0.1 mM DPPH solution was mixed with 1 ml of sorghum extract at different concentrations (100 to 500 µg/ml). The mixture was shaken vigorously and incubated for 30 minutes in the dark. After incubation, the absorbance was measured at 517 nm using a a UV-1900 spectrophotometer (Shimadzu). The experiment was performed in triplicates, and ascorbic acid was used as a positive control. The radical scavenging activity (RSA) of the DPPH radical compound was calculated using the following formula:

$$\text{Inhibition of DPPH (\%)} = (A0-A1)/A0 \times 100$$

where; A0 is the absorbance of the control reaction and A1 is the absorbance of sorghum extract. The results were expressed as IC_{50} value (µg/ml). Data were presented as percentage inhibition and IC_{50}.

2.6.2. 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) assay

The antioxidant activity of the sorghum extracts was evaluated based on the method of Shah and Modi (2015) with modification. The ascorbic acid standard solution was generated by diluting the 1 mg/ml stock solution to various concentrations ranging from 0.002 to 0.01 mg/ml. The ABTS reagent was prepared by dissolving 2.45 mM of potassium persulfate solution and 7 mM of ABTS in a ratio of 1:1 (v/v) and incubating the mixture for 12–16 hours at room temperature in the dark. Then, the ABTS reagent was diluted with ethanol until the absorbance reading is approximately 0.700 ± 0.002 at 734 nm before analysis. Next, 2 ml of the diluted ABTS solution and 1 ml of extracts with different concentrations (100 to 500 µg/ml) will be mixed and left for 20 minutes in the dark. The absorbance was measured at 734 nm using the spectrophotometer (UV-1900 Shimadzu). The experiment was performed in triplicates, and ascorbic acid with different concentrations was used as a positive control. The capability of the sample to scavenge the ABTS radical compound (RSA) was calculated using formula:

$$\text{Inhibition of ABTS (\%)} = (A0-A1)/A0 \times 100$$

Where; A0 is the absorbance of the control reaction and A1 is the absorbance of Sorghum extract. The results were expressed as IC_{50} value (µg/ml). Data were presented as percentage inhibition and IC_{50}.

2.7. Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) in GraphPad Prism (version 10.4). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Macronutrients composition

Red and white sorghum are both primarily composed of carbohydrates and proteins, though minor differences exist in their respective levels (Chhikara et al., 2019; Felicia & Deborah, 2021; Pontieri et al., 2022). In terms of protein content, sorghum provides approximately 9–13%, which is notably higher than many traditional cereals, enhancing its value as a plant-based protein source (Singh et al., 2024). In this study, two types of sorghum (red and white) were assessed for their macronutrient composition. The results show that red sorghum (RS) has a significantly higher protein content (28.5 ± 0.57%) compared to white sorghum (WS) (25.25 ± 0.55%), as presented in Table 1. The present result aligns with previous findings by Sazonova et al., (2022), where red sorghum varieties contain a higher protein content of 28.5 ± 0.57 % compared to white sorghum, which has a protein content of 25.25 ± 0.55 %. The result was further supported by Wulandari et al. (2019), who reported higher protein values in red sorghum (35.50 %). The elevated protein levels observed in red sorghum may be attributed to its distinct biochemical profile, including a higher concentration of essential amino acids, as suggested by Khan et al. (2023). This compositional advantage reinforces the nutritional superiority of red sorghum, particularly in protein-rich dietary applications and in the development of functional foods aimed at improving protein intake.

The ash content was significantly higher in WS (10.93 ± 0.44%) than in red sorghum (10.41 ± 0.31%), as shown in Table 1. The finding aligns with Nemukondeni et al. (2022), who reported that white sorghum generally contains higher levels of dry matter, possibly due to its elevated mineral content. Sorghum is rich in essential minerals, including iron, zinc, magnesium, and phosphorus, all of which play critical roles in maintaining metabolic functions (Pontieri et al., 2024). In particular, the potassium content was found to be significantly higher in white sorghum, further supporting its superior mineral profile. A study by Afify et al. (2012) showed that white sorghums investigated (Dorado, Shandaweel-6, and Giza-15) had ash content ranging from 1.43% to 1.45%, with the 'Dorado' variety had the highest at 1.45%, and this decreased after processing due to mineral loss. However, the ash values observed in both red and white sorghum varieties in this present study are higher than previously reported, suggesting a greater mineral content that may support various physiological functions and enhance the grain's antioxidant potential (Llopart et al., 2016).

Table 1: Macronutrient composition of red sorghum (RS) and white sorghum (WS). Values are mean ± standard deviation. Asterisk (*) indicates a statistically significant differences between RS and WS at $p < 0.05$.

Macronutrients (%)	WS	RS
Moisture	10.44 ± 0.16	10.45 ± 0.17
Ash	10.93 ± 0.44*	10.41 ± 0.31*
Fat	1.02 ± 1.02	0.94 ± 0.67
Fiber	8.23 ± 2.09	7.19 ± 3.25
Protein	25.25 ± 0.55*	28.5 ± 0.57*
Carbohydrate	42.95 ± 0.85	42.5 ± 0.99

There were no significant differences ($p > 0.05$) in carbohydrate, fiber, fat and moisture contents between the red and white sorghum samples (Table 1). Carbohydrates are one of the main components of Sorghum with starch serving as a primary form of energy. Previous study showed minor variations in carbohydrate content among the three sorghum varieties (white, red, and black) (Pontieri & Giudice, 2016), which aligns with the result in this present study. Although both sorghum varieties are rich in carbohydrates, their carbohydrate composition differs. Red sorghum generally contains higher levels of structural carbohydrates, such as non-starch polysaccharides including cellulose and hemicellulose, compared to white sorghum (Knudsen et al., 1988). These slowly digestible starch in red sorghum leads to a lower glycemic index, causes a slower and more gradual rise in blood glucose levels (Rajeshkumar et al., 2024). Additionally, red sorghum is noted for its fiber content that could interacts with phenolic compounds, forming barriers that slow down digestion and reduce the glycemic response (Baah et al., 2025). Meanwhile, white sorghum contains a higher proportion of non-structural carbohydrates and lower dietary fiber, resulting in higher digestible energy and making it more easily digestible than red sorghum (Pan et al., 2019). This suggests that red sorghum is more beneficial for managing blood sugar levels and supporting metabolic health due to its slower digestion and lower glycemic response, whereas white sorghum could be preferred for quicker energy release and easier digestibility.

The fat content in red and white sorghum varies with total oil content reported to range from 2.32% to 13%, depending on the variety and cultivation conditions (Hadbaoui et al., 2010). In the present study, the total fat content between red and white sorghum showed no significant difference (Table 1). This finding also aligns with Hadbaoui et al. (2010), who reported similar oil yields (6.2–6.5%) for both varieties with direct extraction, but observed higher oil content in red sorghum (13.0%) compared to white sorghum (11.2%) when using acid hydrolysis. Both red and white sorghum has been shown to share similar fatty acid profiles, predominantly oleic and linoleic acids, with unsaturated fatty acids comprising over 84% of their total fat content (Hadbaoui et al., 2010; Aremu et al., 2022). White sorghum tends to have a higher total saturated fat content, while red sorghum contains lower levels of unsaturated and polyunsaturated fats (Pontieri et al., 2022). The presence of beneficial fatty acids like oleic and linoleic acid in both varieties may promote heart health by reducing cholesterol levels (Tanwar et al., 2023). Although red and white sorghum was shown to have similar fat contents in this study, the extraction process can lead to nutrient losses, particularly in lipids, which may affect the overall nutritional profile (Galán et al., 2018).

Moisture content plays a crucial role in determining the physical and storage properties of sorghum grains. Research shows that moisture levels ranging from 10% to 30% dry basis significantly influence grain characteristics such as diameter, sphericity, and angle of repose (Adinoyi et al., 2017). In this study, red and white sorghum shows no significant difference (Table 1). Moisture content is a critical factor as it influences the geometric mean diameter and sphericity of sorghum grains; as moisture content increases, grain dimensions expand while bulk density decreases, thereby affecting storage efficiency and overall conservation strategies (Gely & Pagano, 2017). For safe storage, moisture content should ideally remain between 15% and 20% in sealed containers to prevent damage and deterioration (Weinberg et al., 2008). While higher moisture content may improve certain physical traits, it also increases the risk of spoilage during storage.

1.2. Total phenolic content

White sorghum exhibited a TPC of 214.03 ± 74.08 µg GAE/g, while red sorghum showed a higher TPC of 235.03 ± 59.82 µg GAE/g (Figure 1). However, the difference was not statistically significant ($p>0.05$). The TPC observed in red sorghum aligns with the findings of Espitia-Hernández et al. (2020), who reported that red sorghum typically contains greater levels of phenolic compounds, attributed to its darker pigmentation and higher tannin content. Similarly, earlier research observed that red sorghum had greater phenolic content compared to white sorghum, primarily because of its elevated tannins and anthocyanins (Dykes & Rooney, 2006; Dykes et al., 2011). Differences in reported TPC values across studies may result from variations in environmental factors, extraction techniques, and genetic differences in sorghum samples (Upadhyaya et al., 2013; Zhang et al., 2015; Xiong et al., 2019). The distinction in TPC between the two types is largely attributed to their pericarp structure. According to Espitia-Hernández et al. (2020), the higher tannin and anthocyanin levels in red sorghum give it stronger antioxidant properties. In contrast, white sorghum, with less pigmentation, has fewer flavonoids and anthocyanins, resulting in lower TPC, as confirmed by Khoddami et al. (2015), who found that darker sorghum varieties tend to have higher polyphenolic content.

The bioactive properties of sorghum are closely tied to its phenolic composition. On the other hand, white sorghum, despite its lower phenolic content, remains an important gluten-free dietary option, providing essential vitamins and minerals. Althwab et al. (2015) also supported these findings, showing that phenolic-rich sorghum varieties can significantly contribute to dietary antioxidants and help combat oxidative stress.

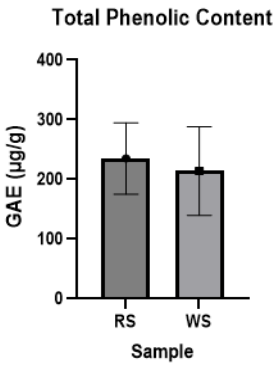

Figure 1: TPC of red sorghum (RS) and white sorghum (WS), expressed as GAE µg/g extract. Data are presented as mean ± standard deviation.

The comparable TPC levels, with no significant difference observed (p value > 0.05), may indicate genetic consistency across sorghum varieties, suggesting both are suitable for cultivation in diverse environments without compromising their bioactive content. This also implies that either variety can be used interchangeably in functional food formulations targeting antioxidant support, offering greater flexibility in sourcing and product development.

1.3. Total flavonoids content

The total flavonoid content in sorghum grains showed a higher mean value in red sorghum (6.53 ± 3.27 µg QE/g) compared to white sorghum (4.95 ± 2.8 µg QE/g). However, the difference was not statistically significant, with p value > 0.05 (Figure 2).

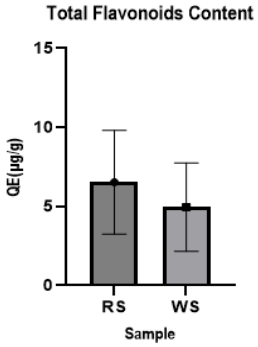

Figure 2: TFC of red sorghum (RS) and white sorghum (WS), expressed as QE µg/g extract. Data are presented as mean ± standard deviation.

According to Espitia-Hernández et al. (2020), red sorghum has more flavonoids because of its darker colour and higher anthocyanin content. Dykes et al. (2011) also found that red sorghum naturally has more flavonoids due to its outer layer and genetic makeup. Differences in flavonoid levels reported in other studies may be attributed to variation in environmental conditions, sorghum genotypes or the analytical methods used for flavonoid quantification. According to the previous research on flavonoid content in sorghum, a similar trend is observed. For example, Taleon et al. (2012) found that flavonoid concentrations in red sorghum reached as high as 0.255 µg QE/g. Additionally, Sharanagat et al. (2019) reported that the flavonoid levels in white sorghum were consistently lower, confirming that pigmentation is a key factor influencing flavonoid content. The high flavonoid levels in red sorghum are mostly due to its colour. Afandy et al. (2023) found that red sorghum has more anthocyanins and flavonoids, which help it act as a strong antioxidant. In contrast, white sorghum, which is lighter in colour, has fewer of these compounds. Khoddami et al. (2015) also observed that darker sorghum grains tend to have more flavonoids and other beneficial compounds. Similarly, the non-significant difference in TFC suggests both varieties offer comparable flavonoid content, supporting their interchangeable use in antioxidant-rich functional foods.

3.4. Antioxidants activities

3.4.1. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of sorghum varies between red and white cultivars. The DPPH assay showed that WS had a significantly higher percentage inhibition (87.4 ± 0.35%) compared to RS (77.4 ± 1.18%) with p = 0.0001, while ascorbic acid achieved 100 ± 0.00% inhibition (Table 2). The IC_{50} value of WS was slightly lower at 260.01 µg/ml compared to 268.67 µg/ml for RS, indicating higher antioxidant efficiency in WS. This suggests that although RS showed lower percentage inhibition, its antioxidant activity is comparable to WS. Ascorbic acid standard, with an IC_{50} of 4.63 µg/ml, remained the most potent antioxidant.

Table 2: Percentage inhibition (%) and IC_{50} values of red sorghum (RS) and white sorghum (WS) determined using the DPPH assay. Percentage inhibition is expressed as mean ± standard deviation at 500 µg/ml. Different letters indicate significant differences at $p < 0.05$.

	DPPH assay	
Sample	Percentage inhibition (%)	IC_{50} value (µg/ml)
Ascorbic acid	100 ± 0.00[a]	4.63
RS	77.4 ± 1.18[b]	268.67
WS	87.4 ± 0.35[c]	260.01

These results differ from previous studies by Ra et al. (2015), which reported significantly stronger antioxidant activity in red sorghum cultivars like the Korean variety *Hwanggeumchalsusu*, with an IC_{50} value as low as 10.9 µM. The enhanced activity in those cultivars was attributed to high levels of polyphenolic compounds like luteolinidin, whereas in the present study, the red showed higher TPC and TFC than white sorghum but the differences were not statistically significant (Figure 1 and 2). Additionally, the use of methanol extract in Ra et al. (2015) as compared to water extract in this present study may have influenced the results, as solvent type can affect the extraction efficiency of antioxidant compounds and thus impact the measured activity. These findings agree with previous literature emphasizing the critical role of polyphenols in antioxidant efficiency (Awika, 2003; Awika et al., 2003; Dykes et al., 2005; Dykes et al., 2011; Khoddami et al., 2015).

Moreover, the antioxidant potential and phenolic profiles are influenced by both genetic factors and environmental conditions, as demonstrated in studies involving sorghum from diverse geographical origins (Seo et al., 2023). Considerable variability in phenolic content and antioxidant activity has been observed, with certain genotypes exhibiting notably high levels of specific compounds such as taxifolin and ferulic acid (Bhukya et al., 2020). The study highlights that while white sorghum exhibits stronger antioxidant potential, its effectiveness is still moderate and comparable to red sorghum. The higher antioxidant activity observed in white sorghum suggests that it may have greater bioactive potential compared to red sorghum, although additional processing techniques such as fermentation or extraction optimization could further enhance these properties in both red and white sorghum.

3.4.2. 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) Assay

Sorghum is increasingly recognized for its nutritional and functional properties, particularly its antioxidant potential (Ragaee et al., 2006). The ABTS assay revealed that RS had a significantly higher percentage inhibition (67.6 ± 0.03%) compared to WS (49.0 ± 0.30%) with p = 0.001, while ascorbic acid showed complete inhibition at 100 ± 0.00% (Table 3). These results indicate stronger free radical scavenging activity in RS than in WS. The IC_{50} values further support this trend, with RS having a lower IC_{50} (326.00 µg/ml) than WS (827.27 µg/ml), suggesting greater antioxidant potency. As expected, ascorbic acid demonstrated the highest antioxidant efficiency with the lowest IC_{50} value of 1.54 µg/ml.

Table 3: Percentage inhibition (%) and IC_{50} values of red sorghum (RS) and white sorghum (WS) determined using the ABTS assay. Percentage inhibition is expressed as mean ± standard deviation at 500 µg/ml. Different letters indicate significant differences at $p < 0.05$.

	ABTS assay	
Sample	Percentage inhibition (%)	IC_{50} value (µg/ml)
Ascorbic acid	100 ± 0.00[a]	1.54
RS	67.6 ± 0.03[b]	326.00
WS	49.0 ± 0.30[c]	827.27

These findings are consistent with established literature, which associates antioxidant capacity with phenolic content and pigmentation in sorghum varieties. Espitia-Hernández et al. (2020) reported that darker sorghum types, such as black and red, exhibit higher antioxidant activity, while lighter-colored varieties, including white sorghum, tend to have reduced bioactive compound levels and thus lower antioxidant effectiveness. The comparatively greater antioxidant performance of red sorghum is likely due to its richer composition of polyphenolic compounds such as condensed tannins, flavones (e.g., luteolin and apigenin), and 3-deoxyanthocyanidins, which are primarily concentrated in the bran and pigmented outer layers. For example, red sorghum flour demonstrates a significantly lower IC_{50} value (98.85 ppm), indicating stronger radical scavenging activity than white sorghum flour and other processed forms (Indrianingsih et al., 2023; Hong et al., 2020; Jaćimović et al.,

2023). The antioxidant capacity of sorghum is closely linked to its total phenolic content, which has been associated with protective effects against oxidative stress and related chronic conditions, including cardiovascular diseases and cancer (Shen et al., 2018). In contrast, the limited phenolic profile of white sorghum accounts for its weaker radical scavenging ability. While differences in extraction methods, assay conditions, and genotypic variation may influence the absolute IC_{50} values across studies, the observed trend affirms the potential of pigmented sorghum varieties, particularly red sorghum, as promising sources of natural antioxidants for application in functional food formulations.

5. CONCLUSION

In conclusion, red and white sorghum exhibit distinct nutritional and functional attributes. Specifically, red sorghum exhibited significantly higher protein content and ABTS radical scavenging activity. Meanwhile, white sorghum exhibited higher ash content and DPPH radical scavenging activities. However, the comparable nutrients (fat, fiber and carbohydrate), total phenolic content (TPC) and total flavonoid content (TFC) between the two varieties highlight their equivalent potential for application in functional food formulations. Further research is required to explore the effects of different processing methods, optimized extraction methods, or combination approaches to enhance the functional properties of both sorghum varieties.

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