Evaluation of polyphenols and antioxidant contents of various sorghum varieties

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Abstract

Sorghum (Sorghum bicolor L. Moench) is a rich source of various bioactive compounds including tannins, phenolic acids and flavonoids at varying concentrations depending on their genotypes. Relative to other cereals or fruits, these compounds possess high antioxidant activity and offer health benefits. In the present study, three sorghum grain varieties and their brans namely IS8096-strawbrown, IS9350-light red, IS34060-white were analyzed for their antioxidant activity using 2,2'-azinobis (3-ethyl-benzothiazoline-6 sulfonicacid) (ABTS),2,2-dipheny l-1- picrylhydrazyl (DPPH) and ferric reducing ability of plasma assay (FRAP). All samples were evaluated for total phenols, flavonoids, tannin and antioxidant activities.

The results of bran extracts showed higher bioactive compounds compared to that flour extracts. The bran of straw brown IS8096 showed high levels of phenols, flavonoids, FRAP, taninns of 110.10±0.69 mg GAE/g,63.52±0.42mgQE/g,75.71±0.87mg/g, 1.90±0.48 mg/gm and IC_{50} value of DPPH and ABTS activities of 85.96±36 µg/ml, 25.91 ± 0.58µg/ml respectively. Hence these results can be used for selection of specific sorghum variety for breeding and also in food, nutraceuticals and other applications.

Keywords: Sorghum bicolor, antioxidant activity, phenols, flavonoids, tannins.

Introduction

Sorghum is fifth most major consumed cereal crop in many parts of the world mainly in Africa and Asia[1]. In India, sorghum yield was 7.7 million metric tons and ranked third for commodity value[2]. From past few decades, sorghum has drawn more interest because of its potential use as food with nutritional aspects such as gluten free, high levels of diverse phenolic compounds and agronomical qualities like heat tolerant and drought resistant crop[3]. Sorghum grain is an important source of ingredients for use in functional foods and other applications as it is a rich source of phenolic acids, condensed tannins (flavan-3,4-diols and/or flavan-3-ols), flavonoids (flavanones, flavan-3-ol derivatives and flavonols) and carotenoids[4,5].The pericarp of sorghum grains differs in color, thickness, presence of a pigmented testa and affects the phenolic composition as most sorghum polyphenlos are present in the outer layers of the grain[6].

Though sorghum bran is an industrial by-product and often removed in feed processing, food and biofuel production, it is an important component in sorghum and has a huge influence on the grain appearance and phenolic profile.

Previous studies showed the presences of various bioactive compounds in sorghum involved in the anti-inflammatory, diabetes, antioxidant, obesity and anti-proliferative activities[7-9]. Epidemiological studies have shown reduced incidence of gastrointestinal cancer due to consumption of sorghum[10]. However, data on antioxidant activities and polyphenols of specialty Sorghums and their applications is difficult to find. Such information is also critical if sorghum is to become a competitive source of the phytonutrients. In view of the food as medicine, if health maintenance or treatment is feasible through daily meals, the development of an antioxidant-rich sorghum variety can contribute to increase the nutritional value of sorghum food products as well as to promote health. The aim of the present work was to screen various polyphenols and antioxidant activities in bran and flour of three different sorghum varieties and further the results were evaluated for total phenolics, flavonoids, tannins and antioxidants activities. These results provide the basic data for selecting different sorghum varieties for different applications such as breeding of sorghum varieties with high level polyphenols, food formulations and value added health products.

Material and Methods

Samples: Samples with distinct sorghum genotypes were collected with grain color variation, namely IS8096-strawbrown, IS9350-light red and IS34060-white from Indian Council of Agriculture Research (ICAR), Indian Institute of Millets Research (IIMR). Then samples were dried at room temperature for 2 days and decorticated using a dehuller (Venture Tech India Private Limited) for bran and flour separation. Then samples were stored at -20 °C for further analysis.

Extraction of polyphenols by maceration: First, samples were defatted with hexane and dried for 12h in fume hood. Further maceration was carried out with 1% acidified methanol as solvent. The extraction procedure involved addition of 20 ml (v/v) of solvent to1g of sample and placed in orbital shaker (Bio-Technics, India) for 2 h.

Figure 1: Color gradient grains of selected sorghum varieties

Then samples were kept at -20°C for overnight to enhance the diffusion of polyphenols from cellular matrix and centrifuged for 10 min at 2790 g (Sorvall SS-34 rotor), this process was repeated twice for residue by addition of 10 ml solvent. The collected supernatants were dried by rotary flash evaporator (HS-2005S-N model) at 40˚C and extracts were kept at -20°C for further analysis. All experiments were carried out in triplicate.

Total phenolic content determination: The phenolic content of extracts was evaluated by modified Folin-Ciocalteau assay as described by Choiet et al[11] by using gallic acid standard (20–100 µg/ml). The extracts (0.1ml) were mixed with 1 ml of Folin–Ciocalteau reagent (0.2 N) and 2 ml (75 g/l) of sodium carbonate was added. The reaction absorbance was determined using an UV-2450 Spectrophotometer (Shimadzu) at 765 nm after incubation for 30 min in dark at room temperature. The results of phenolic content were expressed in milligram per gram gallic acid equivalents (mg /g GAE), (db).

Quantification of total flavonoids*:* The flavonoid content was estimated using the method of Chang et al[12]with some modifications. The extracts (0.5ml) were mixed with 100 µl of 1M potassium acetate, 100 µl of 10% $AlCl_3$ and 2.8 ml of distilled water. The reaction mixture was incubated at room temperature in dark for 30 min and absorbance of the reaction was determined at 415 nm by spectrophotometer (Shimadzu UV-2450). Catechin standard (20–100 mg/l) was used and the results were expressed as mg Quercetin per gram extract (mg QE/g) equivalents, dry basis (db).

Determination of tannin content: Quantitative estimation of the tannins was carried out using the method described by Makkar et al[13] with some modifications. Briefly, sample (0.25 ml) was added to vanillin reagent of 1.5 ml (4% w/v in methanol) and then concentrated HC1 (37 %) of 0.75 ml was added. Then the reaction was incubated in dark for 30 min at room temperature and absorbance was read by using spectrophotometer (Shimadzu UV-2450) at 500 nm. Catechin standard (20-100µg/ml) was used and tannins were expressed as milligrams catechin equivalent per gram extract (mg CE/g).

Determination of Antioxidant activity

Scavenging activity *of* DPPH radical: The DPPH % inhibition was quantified as described by Van Hung et al[14] with some modifications. 150 µl of the extracts were mixed with 2850 µl, of the DPPH working solution (0.2 mM DPPH- methanol solution). After a reaction time of 30 min in the dark at room temperature, the absorbance was then determined at 517 nm by UV-2450 Spectrophotometer (Shimadzu). Trolox (6-hydroxy-2, 5, 7, 8 –tetra methyl chroman-2 carboxilyc acid) standard (20–250 mg/l) was used and the percentage inhibition of DPPH was calculated using the following expression.

$$\%\,\text{inhibition} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control}= absorbance of control and A_{Sample} = absorbance of sample at time (t)=30 minutes.

Antioxidant capacity by *ABTS assay*: The ABTS radical scavenging activity (ABTS) was carried out as described by Chavez et al[15]. Briefly in 10 ml of distilled water, 7.4 mM ABTS salt and 2.6 mM of potassium persulfate were mixed and reacted for 12 h in the dark at room temperature. The ABTS working solution was prepared by diluted 95% methanol to obtain an initial absorbance of 0.7 ± 0.02 at 734 nm in a spectrophotometer UV-2450 (Shimadzu). Trolox standard (20–250mg/l) was used. Varying concentrations of samples (10 µl) were mixed with 990 µl of ABTS working solution and incubated at room temperature in dark. Absorbance was read after 30 min at 734 nm against methanol as blank and the percentage of inhibition was calculated.

$$\%\,\text{inhibition} = [(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$$

where A_{Control}= absorbance of control and A_{Sample} = absorbance of sample at time (t)=30 minutes.

Ferric reducing ability of plasma assay (FRAP)*:* The ferric reducing ability of plasma (FRAP) assay was determined based on a previous report with slight modification[16]. The FRAP reagent of 10 mM TPTZ (dissolved in 40 mM HCl), 20 mM ferric chloride and 0.1 mol/l acetate buffer (pH 3.6) were prepared at a ratio 1:1:10 (v/v/v). 100 µl of samples was allowed to react with 4.9 ml of FRAP solution at 37°C 10 min in the dark. Then absorbance at 593 nm was measured by UV-2450 Spectrophotometer (Shimadzu). The results of *FRAP assay* were expressed as mg $FeSO_4$/g equivalent of grain dry basis (db).

Statistical analysis: All experimental analysis was performed in triplicate and Analysis of Variance (ANOVA) was performed using GRAPH prism 8.0 software to determine the significance of differences between different groups. Tukey multiple range tests were applied

Results and Discussion

Evaluation of Total Phenolic Content: The content of total phenolics in flour and bran extracts were estimated by the Folin–Ciocalteu assay and expressed as gallic acid equivalents (GAE). The total phenolic contents of the samples in the present study were significantly different ($P \leq 0.05$) between the sorghum extracts and ranged from 31.65±0.07 mg GAE/g to 110.10±0.69 mg GAE/g (Table 1).

Among all the extracts, the bran of straw brown IS8096 showed high levels of phenols of 110.1±0.69 mg GAE/g whereas white IS34060 flour showed the lowest phenolic content of 31.65±0.07 mg GAE/g. These results are higher than those observed by Suganya Devi et al[17] with the highest TPC of 0.93 mg/g in bran extracts and Alfieri et al[18] with total phenolic compounds ranging from 0.60 to 20.73 g GAE/kg in a sub-set of 121 different sorghum genotypes. Hence the present results showed that bran samples have the highest total phenolic contents than the flour samples.

Quantification of Total Favonoid content: In our study, significant difference ($p \leq 0.05$) in total flavonoid content was observed between extracts of bran and flour which ranged from 15.20±0.12 to 63.52±0.42 mg QE/g (Table 1). Moreover, it was observed that the bran extracts have higher level of total flavonoids than flour extracts and IS8096 straw brown bran showed higher level of flavonoid at 63.52±0.42 mg QE/g followed by IS9350-light red bran with 44.69±0.32 mg QE/g and the white IS34060 flour showed lowest flavonoid content of 15.20±0.12 mg QE/g. The present results were higher than the previous studies by Wu et al[19] who showed the total flavonoid contents ranged from 7.56 ± 0.15 to 0.47 ± 0.09 mg CE/g, sample dry basis in six genotypes of sorghum.

Evaluation of tannins: Tannin contents of sorghum bran and flour extracts were determined by the modified vanillin-HCl method and the obtained results are presented in table 1. The statistical analysis revealed significant differences ($p \leq 0.05$) in tannin contents among the tested varieties.

Table 1
Levels of Phenol, flavonoids and tannins among different Sorghums bran and flour

Activity	Sample type	Sorghum variety		
		IS8096-strawbrown	IS9350-light red	IS34060-white
Phenols (mg/GAEgm)	Bran	110.10 ± 0.69^{cB}	76.08 ± 0.43^{bB}	51.65 ± 0.19^{aB}
	Flour	72.70 ± 0.08^{bA}	42.66 ± 0.04^{bA}	31.65 ± 0.07^{aA}
Flavonoids (mg/QEgm)	Bran	63.52 ± 0.42^{cB}	44.69 ± 0.32^{bB}	36.69 ± 0.17^{aB}
	Flour	46.69 ± 0.26^{cA}	35.54 ± 0.14^{bA}	15.20 ± 0.12^{aA}
Tannins (mg/CEgm)	Bran	1.90 ± 0.48^{bB}	1.73 ± 0.29^{aB}	nd
	Flour	0.33 ± 0.06^{bA}	0.22 ± 0.05^{aA}	nd

$^{a-c}$ Values with different superscripts in the same row for same sample are significantly different ($P \leq 0.05$).
$^{A-B}$ Values with different superscripts in the same column for same sorghum variety for different samples are significantly different ($P \leq 0.05$).
Abbreviations: nd-Not detected. Data are expressed as values are mean of triplicates ± standard deviation (n=3).

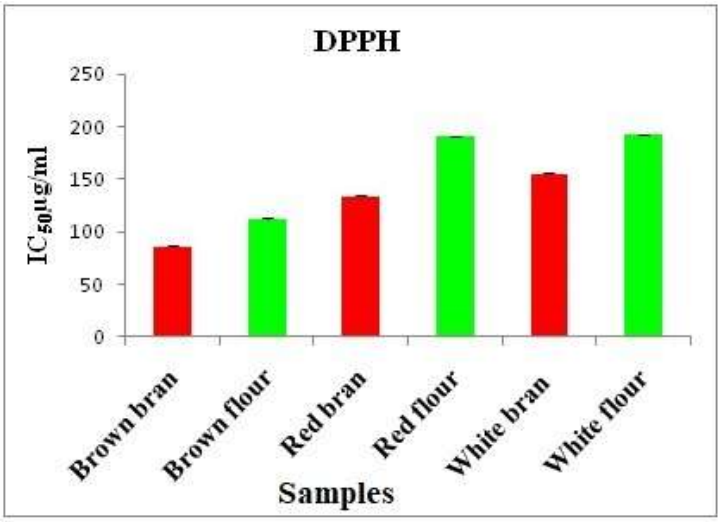

Figure 2: IC_{50} values of crude extracts in DPPH with lower IC_{50} value by IS8096brown bran showed higher antioxidant activity. Data are expressed as values are mean of triplicates ± standard deviation (n=3)

As shown in table 1, the bran of IS8096 straw brown showed high amount of tannins of 1.90±0.48 mg/CE gm and no tannins were observed in IS34060 white variety bran and flour respectively. The present results in our study are lower than the previous studies carried out by Dykes et al[20] showed tannin content which ranged from 11.9-15.5 mg catechins equivalents (CE)/g for different sorghum genotypes.

DPPH, ABTS and FRAP assay for antioxidant activity: In the present work, IS8096-strawbrown bran showed lower IC_{50} value of DPPH and ABTS activities of 85.96±0.36µg/ml, 25.91±0.28 µg/ml indicating higher inhibition activity and also showed higher antioxidant activity in FRAP assay of 75.71±0.87 mg/$FeSO_4$ (Figure 2-4). Previous studies by Hou et al[21] showed the highest value of 77.01 µmol Fe/g for FRAP by ultrasonic assistant extraction technology. Hence the present results showed that the sorghum bran samples have high antioxidant activity than flour samples, indicating that in sorghum bran, the phenolics are mainly concentrated showing consistently high antioxidant activity *in vitro*.[20]

Conclusion

In conclusion, this study showed that the bioactive compounds in sorghum bran were significantly higher than flour samples and their concentrations depend on the pericarp color and variety. The bran fractions, especially from straw brown IS8096 bran showed high levels of phenols, flavonoids and antioxidant activities.

Therefore, the obtained information can be beneficial in choosing specific sorghum variety for breeding with maximum polyphenols and antioxidant activities and these bioactive compounds can act as functional ingredient due to their nutritive and medicinal properties and can replace the usage of synthetic antioxidant in food formulations and value-added health products.

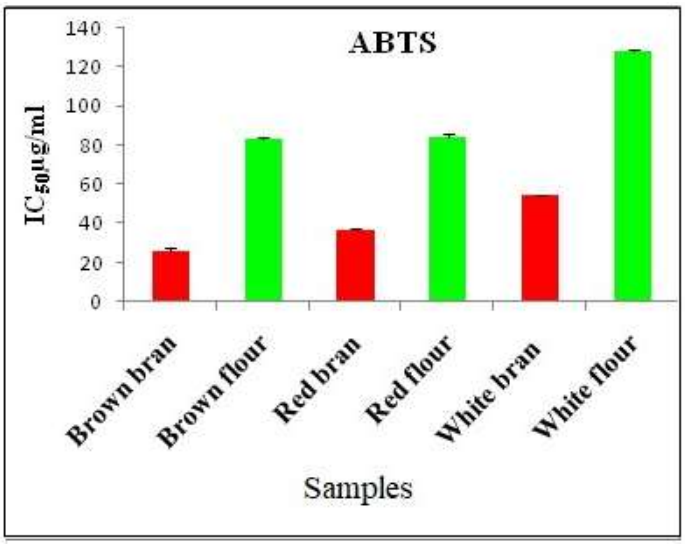

Figure 3: IC_{50} values of crude extracts in ABTS with lower IC_{50} value by IS8096 brown bran showed higher antioxidant activity. Data are expressed as values are mean of triplicates ± standard deviation (n=3).

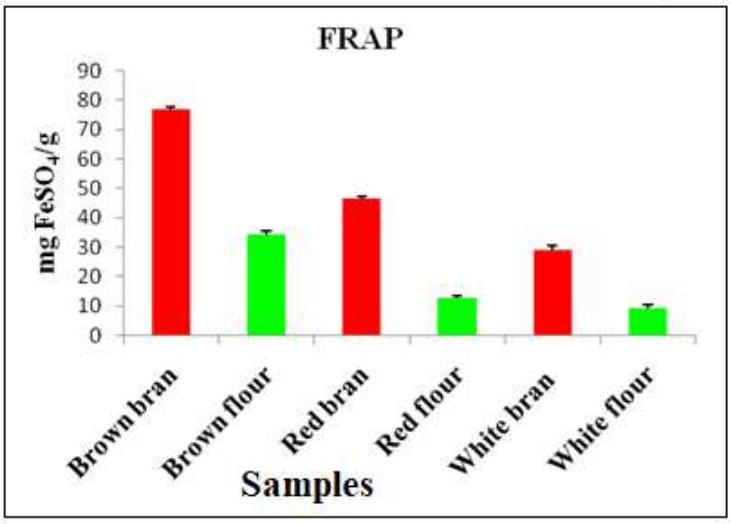

Figure 4: Levels of antioxidant activity among sorghum flour and their barns by FRAP assay Data are expressed as values are mean of triplicates ± standard deviation (n=3).

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