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Appraisal of total phenolics, total flavonoids and antioxidants activity from bran and flour in various sorghum varieties

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Running title: Antioxidants of Sorghum

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ABSTRACT

Sorghum (Sorghum bicolor L. Moench) is an abundant source of bioactive components like flavonoids and phenolic acids at variable concentrations based on their genotypes. Especially the bran of sorghum grain has higher levels of them which are often removed as byproducts. These components own higher levels of antioxidant capacity and bid health aids. In the present work, varieties of sorghum grains namely IS15317- creamy white, IS7845-light red, and IS714-brown along with their brans were investigated for total phenols, and flavonoids. Antioxidant capability by FRAP,DPPH and ABTS were evaluated. Results showed higher levels of activities in bran samples than in flours. The brown IS714 bran showed maximum amounts of phenols, flavonoids, and FRAP with92.15±0.47 mg GAE/g,78.32±0.65 mg QE/g, 67.13±0.16mM $FeSO_4$ equivalent/g, and IC50 value by DPPHwith33.98±0.51µg/mL, and ABTS with31.78±0.66µg/mL. This information could provide data for assortment of sorghum varieties into food and industrial applications.

1. Introduction

Sorghum (Sorghum bicolor L. Moench),a versatile, drought resistant, fifth utmost produced, major cereal food crop and imperative staple food[1,2].In India, its production is 4.00 million tons in 3.75 million hectares which yielded 1.07 million

metric tons per hectare with 4.5million tons of production in the world [3].It ranked third in India for commodity value [4]. Sorghum is very economical to produce due to its capability to acclimatize to diverse stress environment.

In recent years a great attentiveness towards sorghum have been increased due to its advantageous food qualities like high nutrients, insignificant gluten content least digestibility potential low cholesterol and anti- carcinogenic properties. Most of phenolics are present in the outer layers of sorghum grains (bran) [5]. The extent of phenolic compounds, color, appearance, and nutritional quality in each variety differ by its genotype and the environment [6,7]. Diverse bioactive compounds like flavonoids, and phenolic acids are abundant in sorghum grains[8-12].

Consumers' preference for natural ingredients and their safety concerns over synthetic antioxidants have drawn growing interest for development of natural and novel antioxidants [13-15]. In vitro and in vivo studies showed sorghum phenolics are implicated in inhibition of the inflammation, diabetes, obesity, cancer, cardiovascular disease, hypertension, and oxidative stress,[16-25]. Thereby, they can be effective bioactive components having various applications in nutraceuticals, functional foods, pharmaceutical and cosmetics industries [26,27]. As levels of phytochemicals in each sorghum variety vary, it is difficult to choose the specific varieties for specific applications and also as the extraction process plays vital role to obtain higher yield of bioactive compounds. Therefore, various extraction methods and solvents may have a considerable effect on the amounts of the phenolics being extracted. Hence, extraction process that could be applied must be in such a way that it should be cost-effective with higher yield efficiency. Therefore, it is required to understand and screen-specific Sorghum

variety by profiling the different bioactive compounds in both bran and grain, as bran is mostly removed as waste.

The purpose of this study was to report on a comparative analysis of two extraction processes, namely maceration and soxhlet using 1% acidified methanol as solvent, from bran and flour in three sorghum grain varieties. Results were estimated by using spectroscopic analysis for their total phenols, flavonoids, and antioxidant capacity. Hence, this data on sorghum genotypes can assist in an assortment of specific varieties as functional grains and also their brans can be used as natural sources of the bioactive components as functional food additives in food industry with the maximum health aids.

2.Materials and Methods

2.1. Sample collection

Sorghum grains with varying colors viz., creamy white, light red, and brown namely IS15317, IS7845, and IS714 were collected from ICAR-Indian Institute of Millets Research (Fig.1). The collected grain samples were further dried for 3 days at room temperature, dehuller was used for decortication of samples to obtain bran and the flour. Further samples were kept at -20 °C until analyzed.

(a) IS 15317- Creamy White (b) IS7845 Light Red (c) IS 714 Brown

Fig. 1 Selected Sorghum grain varieties with different color gradient

2.2.Phenolics extraction Process

Before extraction of phenolics, the samples were initially defatted by using hexane twice and then dried in fume hood for overnight. Secondly, maceration and the soxhlet were carried by 1% acidified methanol [28,29]. For maceration (MAE), 30 mL (v/v)solvent and 1g sample were mixed, then kept in the orbital shaker at lower speed100 rpm for duration of 2 h. This procedure was repeated and further samples stored at -20°C for overnight. Then samples were placed at room temperature and centrifuged for 10 min at 2790 g. Procedure was twice repeated and residue obtained by solvent addition. Soxhlet extraction (SE) was done in the soxhlet apparatus. 5gsample thimble was kept at 40˚C using 250 mL of solvent. After extraction processes, using rotary evaporator, extracts were then evaporated at 40˚Cand were placed at -20°C for further study.

2.3 Evaluation of total phenolic contents (TPC)

Samples were analyzed for total phenolic contents using an improved Folin-Ciocalteu assay [2]. Extracts(0.5 mL) and 1mLof 0.2 N Folin-Ciocalteu reagent(1:10 v/v distilled water) were mixed, then 2mL sodium carbonate (7.5% w/v) was added. Further incubated in dark (30 min)at 22°C.Absorbance was determined by spectrophotometer at 725 nm. By using Gallic acid standard (20-100 µg/ml,$R^2 = 0.9983$). TPC was quantified as mg /g GAE.

2.4.Total flavonoids (TFs) quantification

Determination of total flavonoids was done by modified Aluminum Chloride method as reported [30]. Experiment was done by taking extract (0.5 mL)with aluminum chloride10 %(0.1mL), potassium

acetate 1M (0.1mL), and distilled water (2.8mL).Further incubated at room temperature for 30 min in dark. Absorbance had determined by spectrophotometer at 415 nm. Quercetin as the standard (20-100 µg/ml,$R^2 = 0.9986$) was used and results were expressed as mg QE/g.

2.5. Estimation of Antioxidant ability

2.5.1. Antioxidant ability by DPPH

DPPH was done with minor modifications [31]. Different concentrations (30-150 µg/mL) were prepared to1mg/mL of samples by 1mL methanol and then mixed along by 1mL of DPPH-methanol (0.2mM)and further incubated at 37°Cin dark for 30 min. Absorbance was taken in spectrophotometer at 517nm. Ascorbic acid (1mg/mL)standard (2.5-12.5µg/mL, $R^2 = 0.9903$) and methanol blank were used and values of IC_{50} were then calculated by formula.

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

Where, $A_{Control}$= absorbance of control

A_{Sample} = absorbance of sample

2.5.2. ABTS assay for Antioxidant ability

Antioxidant capability of extracts were estimated by Trolox-Equivalent Antioxidant Capacity method(TEAC) as reported[6]. Stock solution of ABTS was prepared with ABTS (8 mM) and potassium persulfate (3 mM) (1:1 ratio) and kept in dark for12 h. Freshly prepared ABTS (1 mL with 95% methanol) working solution was read at 734 nm until 1.1 ± 0.02absorbance. Further 0.1 mL sample with different concentrations were added to 2.9 mL working solution of ABTS and incubated at room temperature in dark for 30 min. Using spectrophotometer, absorbance was then read at 734 nm. Trolox (50 to 1000 µM) was taken as standard($R^2 = 0.9931$) and IC_{50} values were evaluated by formula.

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

Where, $A_{Control}$= control absorbance

A_{Sample}= sample absorbance

2.5.3. Antioxidant capacity byFRAP

FRAP analysis was done by minor modifications [31]. 20 µL extract was mixedto180µL FRAP reagent [300mM acetate buffer (3.6 pH), 20mM $FeCl_3.6H_2O$ solution and 10mM TPTZ in 40 mM HCl mixed in ratio 10:1:1] freshly prepared and incubated for a time period of 30 min in dark. Further absorbance was read at 593 nm by microplate reader. $FeSO_4$ was used as standard (0.2-1mM, $R^2 = 0.9984$) . Results were expressed as mM $FeSO_4$ equivalent /g.

2.6. Statistical Analysis

Analysis was carried out in triplicates and the calculation was analyzed using ANOVA, Data were represented as values mean of triplicates ± standard deviation (n=3).

3. RESULTS AND DISCUSSION

3.1. Evaluation TPC by FC assay

In sorghum, phenols are largely concerted in grain pericarp [32].From Table 1, present results showed higher TPC in bran samples with IS714 brown bran of 114.65±0.38 mg GAE/g by Soxhlet, compared to flour samples with significant variations in TPC at $p \leq 0.05$. Maximum TPC was observed by soxhlet extraction ranged from 9.52±0.07 to 114.65±0.38 mg GAE/g, whereas lower TPC was observed by maceration extraction which ranged from 4.26 ±0.16 to92.15±0.47 mg GAE/g as shown in figure 2. Earlier studies carried out by Rao, Santhakumar, Chinkwo, Wu, Johnson & Blanchard, (2018), investigated TPC content in black pericarp variety with 11.50±1.81 mg GAE /g, followed by 3.58±1.63 mg GAE /gin brown pericarp,[21]whereas Khoddami, Truong, Liu, Roberts & Selle, (2015) observed 3.38 ± 0.20 mg GAE /g in red pericarp [13]. Thereby, the present results were comparatively higher than previous results

as the variation in the content of phenolic compounds mainly depended on the variety, color, extraction process and environmental conditions present.

Table 1: Levels of TPC and TFC in bran and flour samples of different Sorghum grains.

Activity	Sample Type	Sorghum Variety					
		IS 15317- Creamy white		IS 7845 - Light Red		IS 714 - Brown	
		SE	MAE	SE	MAE	SE	MAE
Phenols (mg/GAE gm)	Barn	18.63 ± 0.18^{bB}	11.07 ± 0.08^{aB}	65.28 ± 0.41^{dB}	41.68 ± 0.14^{cB}	114.65 ± 0.38^{Fb}	92.15 ± 0.47^{eB}
	Flour	9.52 ± 0.07^{bA}	4.26 ± 0.02^{aA}	32.97 ± 0.35^{cA}	30.55 ± 0.11^{cA}	81.47 ± 0.22^{eA}	64.21 ± 0.35^{dA}
Flavonoids (mg/QE gm)	Bran	11.62 ± 0.11^{bB}	8.27 ± 0.07^{aB}	42.81 ± 0.36^{dB}	34.07 ± 0.24^{cB}	93.16 ± 0.49^{fB}	78.32 ± 0.65^{eB}
	Flour	7.96 ± 0.05^{bA}	2.39 ± 0.02^{aA}	31.54 ± 0.17^{dA}	28.03 ± 0.19^{cA}	72.04 ± 0.26^{fA}	56.34 ± 0.39^{eA}

a-f Values of distant superscripts differ significantly ($P \leq 0.05$)in same row. A– B Values of distant superscripts differ significantly ($P \leq 0.05$)in same column.

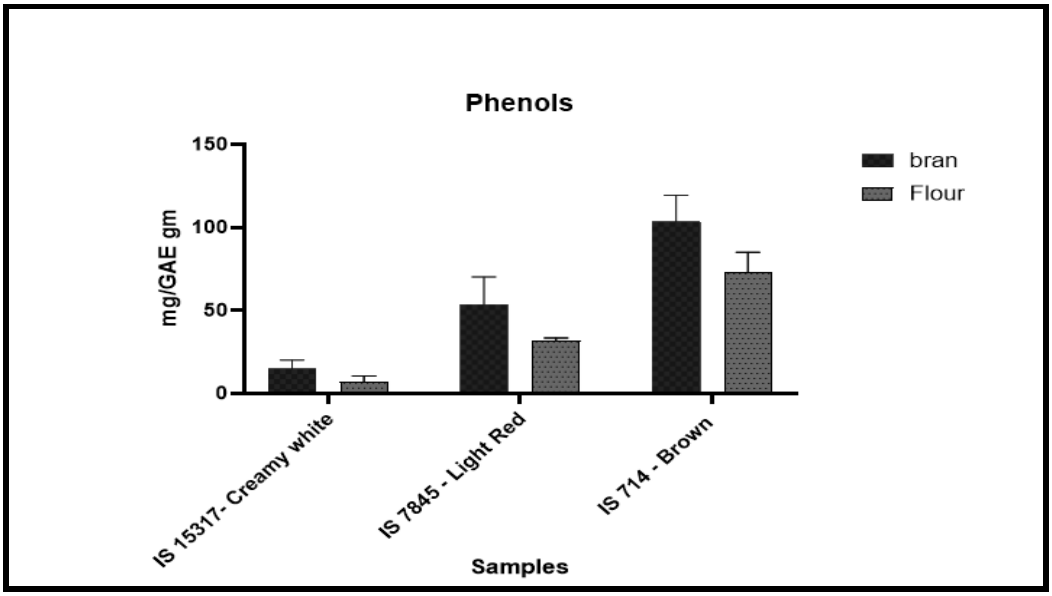

Fig 2: Graph showing the difference in phenol content of bran and flour among samples

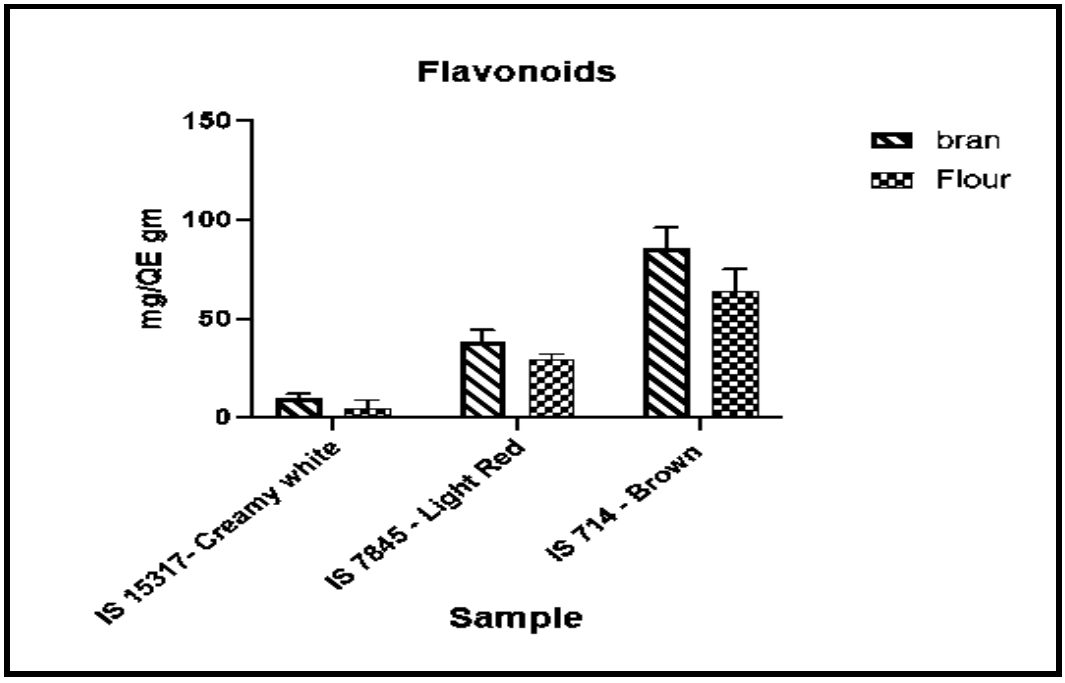

Fig 3: Graph showing the difference in flavonoids of bran and flour among samples

3.2. Estimation of Total Flavonoid Contents by aluminum chloride

Daily consumption of foods with high-level flavonoid can minimize risk of certain cancers, like colon, breast, and pancreatic cancers. The flavonoids levels in sorghum are highly concentrate in grain's exterior layers [33]. In our study, maximum levels of total flavonoid contents (Table1**)** were observed by the soxhlet extraction process ranged from 7.96±0.05 mg QE/g to 93.16±0.49mg QE/g compared to maceration extraction process that showed 2.39±0.02 mg QE/g to 78.32±0.65 mg QE/g which shown considerable variation in TFC ($p \leq 0.05$).Of all samples, IS 714 brown bran showed the highest total flavonoid content as shown in figure 3. Previous studies carried out by Moraes et al., (2015) described that TFC was 6.59 mg CE/g in

sorghum SC 21 brown variety[17] and Shuyu Shen et al., (2018)also reported TFC varied from 11.72 ± 1.69 to 61.10 ± 5.46 mg RE/100 gin eight sorghum grains[23]. The obtained results in this work were higher than previous studies, which may be owing to variance in extraction process, temperature conditions and genotypes.

3.3. Antioxidant ability by DPPH, ABTS and FRAP assay

Dietary consumption of phenolics having rich in antioxidants may contribute in averting numerous diseases associated with oxidative stress such as cardiovascular, type 2 diabetes and cancers[25]. The present work evaluated antioxidant activity by DPPH, ABTS and FRAP assay and found significant variances among the samples ($p \leq 0.05$). Results in Table 2 showed that, of all the samples the lowest IC_{50} value for DPPH and ABTS was observed in the brown IS 714brans with 33.98±0.51µg/ml and 32.98±0.09 µg/ml respectively in the soxhlet extraction process while lowest antioxidant activity was observed in IS 15317 with 767.34±0.38µg/ml and 462.08±0.05µg/ml by DPPH and ABTS using maceration. These results were higher than earlier reports by Zhu, Shi, Yao, Hao & Ren, (2017) with the lowest IC_{50} value 115.77 ± 9.01 µg/ml for DPPH in Jinza No.15sorghum variety [34].

Table 2 Antioxidant activities of different types of sorghum extracts.

Antioxidants Capacity	Type of Extraction	Sample Type	Sorghum Variety		
			IS 15317 Creamy white	IS 7845 Light Red	IS 714- Brown
DPPH (IC50 µg/mL)	MAE	Bran	578.05 ± 1.54^{cA}	306.92 ± 1.19^{bA}	143.99 ± 1.44^{aA}
		Flour	767.34 ± 0.38^{cB}	458.38 ± 2.16^{bB}	257.47 ± 1.49^{aB}
	SE	Bran	308.17 ± 1.37^{cA}	126.78 ± 1.04^{bA}	33.98 ± 0.51^{aA}

		Flour	367.21±1.20^{cB}	169.49±0.83^{bB}	81.21±0.61^{aB}
ABTS (IC50 µg/mL)	MAE	Bran	372.84±1.43^{cA}	154.62±1.04^{bA}	74.66±0.54^{aA}
		Flour	462.08±0.05^{cB}	225.48±1.08^{bB}	144.26±0.70^{aB}
	SE	Bran	192.83±1.07^{cA}	71.94±0.86^{bA}	32.98±0.09^{aA}
		Flour	286.09±1.47^{cB}	120.05±0.94^{bB}	82.76±0.49^{aB}
FRAP (mM FeSO4 Eq/gm)	MAE	Bran	04.13±0.04^{aB}	19.06±0.68^{bB}	31.78±0.66^{cB}
		Flour	02.21±0.02^{aA}	09.08±0.08^{bA}	19.06±0.18^{cA}
	SE	Bran	07.81±0.03^{aB}	20.19±0.10^{bB}	67.13±0.16^{cB}
		Flour	03.29±0.02^{aA}	12.33±0.06^{bA}	28.19±0.11^{cA}

a-c Values of distant superscripts differ significantly ($P \leq 0.05$)in same row.

A− B Values of distant superscripts in differ significantly ($P \leq 0.05$)in same column.

In FRAP assay, brown IS 714branshown the highest antioxidant capacity at 67.13±0.16mM $FeSO_4$Eq/gm by soxhlet extraction process while lowest activity was observed in flour sample of IS 15317 with 2.21 ±0.02mM FeSO4 Eq/gm by maceration. Previously Shiwangni Rao et al., (2018) showed the range of antioxidant activity by FRAP assay from 20.92 ± 2.69 mg/g TE to 2.31 ± 1.55 mg/g TE [21]. Therefore, present results are higher to those of earlier studies and these results imply that sorghum bran has high antioxidant capacity comparatively than sorghum flour and provide valuable comprehensive knowledge on the antioxidant potential of sorghum.

In conclusion, the present work showed that TPC, TFC, and antioxidant

levels were higher in the bran samples than in flour samples. The soxhlet extraction process results were good when compared to maceration by 1% acidified methanol solvent. Further of all samples analyzed the IS 714 brown bran had shown higher levels of TPC, TFC, and antioxidant activity while the lowest values were observed in IS 15317 creamy white flour.

Hence these findings can be a valuable source in choosing a specialty sorghum grain variety for plant breeding with higher phenolics and antioxidant ability. Therefore, these compounds maybe utilized as functional ingredient showing to their high nutritive properties and also medicinal value with value-added healthy products that are beneficial for human consumption and their health.

Conflict of interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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