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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Characterization of Tannin and Study of *in vitro* Protein Digestibility and Mineral Profile of Sudanese and Indian Sorghum Cultivars

Amir Mahgoub Awadelkareem[1], G. Muralikrishna[2], A.H. EL Tinay[3] and A.I. Mustafa[3]
[1]Department of Food Science and Technology, Faculty of Agriculture,
University of AL Zaim AL-Azhari, Khartoum, Sudan
[2]Department of Biochemistry and Nutrition, CFTRI, Mysore 570 013, Karnataka, India
[3]Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Sudan

Abstract: The study was conducted to investigate chemical composition, mineral profile, tannin content, and effect of cooking on *in vitro* protein digestibility , and separation and identification of free and bound phenolic acids of Sudanese sorghum cultivar (namely feterita) and Indian sorghum cultivar (namely CSH5). Chemical composition of the two sorghum cultivars was determined. Sudanese cultivar showed significantly ($p \leq 0.05$) high moisture, ash, protein, and fat while Indian cultivar was significantly higher ($P \leq 0.05$) in fiber and carbohydrate contents. Cupper, calcium, iron, phosphorus, potassium, and sodium were determined for the two cultivars. Results revealed that, Sudanese cultivar was significantly higher ($P \leq 0.05$) in cupper, calcium, iron, and sodium while Indian cultivar was significantly higher ($P \leq 0.05$) in phosphorus and potassium content. Tannin content in Sudanese cultivar was significantly ($P \leq 0.05$) higher compared to Indian cultivar. Effect of cooking on *in vitro* protein digestibility revealed that cooking significantly ($P \leq 0.05$) reduced the *in vitro* protein digestibility of the two sorghum cultivars. The phenolic acids (PAs) as free and bound form content were separated and identified using high performance liquid chromatography (HPLC) for the two sorghum cultivars. Syringic, p-coummaric, ferulic acid were detected as free form of phenolic acids of Indian cultivar while gallic , protocatechuic, gentisic, caffeic, p-coummaric , and ferulic acids were detected in free form of Sudanese cultivar. Gallic, protocatechuic , gentisic, and p-coummaric were not detected in free form in Indian cultivar while syringic acid was not detected in Sudanese cultivar in free form. Indian cultivar contained high caffeic and ferulic acid in free form compared to Sudanese cultivar. Syringic , caffeic, p- coummaric and ferulic acids were detected in bound form in Indian cultivar while gallic, protocatechuic, caffeic, p-coummaric and ferulic acid were detected in bound form in Sudanese cultivar. Gallic, protocatechuic and gentisic acids were not detected in free and bound form in Indian cultivar while p-coummaric acid was only detected in bound form in Indian cultivar. Syringic, caffeic, p-coummaric and ferulic acids content in bound form were high in Indian cultivar than Sudanese cultivar. Generally phenolic acids of the two cultivars exist mostly in bound form.

Key words: Sorghum, phenolic acid and *In vitro* protein digestibility

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Monech) is the fifth most important cereal crop in the world after wheat, rice, corn and barley .Sorghum out-performs other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed and alcohol and industrial products. The United States is the largest producer and exporter of sorghum, accounting for 20% of world production and almost 80% of world sorghum exports in 2001-2002 (USDA-FAS, 2003). World sorghum production was 57 million metric tons (FAOSTAT data, 2005). Its protein content is higher than that of corn although its nutritional protein quality is lower (Dowling *et al*., 2002). Sorghum grain quality is affected by factors such as genotype, climate, soil type and fertilization, among others, which can affect the chemical composition and nutrient value (Ebadi *et al*., 2005). The crude protein (CP) content of sorghum grains is highly variable (5.44-12.9%). The storage proteins in sorghum grain are mostly kafirins, which are prolamins that are soluble in aqueous alcohol in the presence of a reducing agent. According to Watterson *et al*. (1993), kafirins are the most abundant storage proteins in sorghum grain. They are of low nutritional quality, very hetero-geneous (Sastry *et al*., 1968), deficient in lysine, threonine and treptophan, and rich in leucine, proline and glutamic acid (Duodo *et al*., 2003). Recent studies have focused on increasing the digestibility of proteins such as kafirins (Dowling *et al*., 2002) and perhaps on reducing kafirins concentration without affecting the agronomic characteristic. Taylor *et al*. (2007) studied the binding of different kafirin species with sorghum Cts using chemical assay and by sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE),

reversed-phase high-performance liquid chromatography (RP-HPLC), and free zone capillary electrophoresis (EZE). The results showed that δ-kafirin bond have more CTs than the other kaffirin species. Tannins bind to proteins, carbohydrates and minerals and thus reduce digestibility of these nutrients. To reduce these negative effects, decortication, fermentation, germination and chemical treatments (i.e. HCl, formaldehyde and alkali) are used (Beta *et al*., 2000 and Dicko *et al*., 2005). Nutritional constraint to use of sorghum as food is the poor digestibility of sorghum protein on cooking. Digestibility may be used as indicator of protein availability. Its essentially a measure of the susceptibility of protein to proteolysis. A protein with high digestibility is potentially of better nutritional value than one of low digestibility because it would provide more amino acids for absorption on proteolysis. All sorghums contain phenolic acid, which are located in the pericarp, testa, aleurone layer and endosperm (Hahn *et al*., 1984; McDonough *et al*., 1986). Phenolic acids consist of two classes: hedroxybenzoic and hydroxycinamic acids. Hydroxybenzoic acids are directly derived from benzia acids and include gallic, p-hyroxyberoic, vanillic, syringic, and protocatechuic acids, among others. The hydroxycinamic acids have a C6- C3 structure and include coumaric, caffeic, ferulic and sinapic acids. Hahn *et al*. (1983) identified free and bound phenolic acids in sorghum. Free and bound phenolic acids are extracted in methanol and in boiling 2M HCL, respectively. Free phenolic acids are found in the outer layers of the kernel (pericarp, testa, and aleurone), whereas the bound phenolic acids are associated with the cell walls (Hahn *et al*., 1984). According to Hahn *et al*. (1983), the phenolic acids in sorghum are present mostly in bound form with ferulic acid being dominant (24-47%). In addition ,gallic acid is found only in bound form (12.9-46ug/g, dry wt), whereas cinnamic acid is found only in free form (2.0-10.7ug/g, dry wt) with exception of one variety (SCO719, red pericarp with pigmented testa), which is also reported to contain cinnamic acids in bound form only (19.7ug/g,dry wt) (Hahn *et al*.,1984). The objectives of the present work is to examine the chemical composition, mineral composition, tannin content, and effect of cooking on *in vitro* protein digestibility of African and Indian sorghum cultivars and isolation and identification of bound and free phenolic acids by chromatographic way.

MATERIALS AND METHODS

Sudanese sorghum cultivar (*feterita*) was obtained from the local market, Khartoum north, Sudan while the Indian cultivar (CSH5) was provided by department of grain science and technology , CFTRI , Mysore, India. The seeds were cleaned and freed from foreign material and broken kernels. The clean seeds were milled in Barabeder Quadrumat Junior Mill (Regulation No 1) into flour to pass a 0.4 mm screen. The flour was stored in polyethylene bags at 4?C for further analysis. Unless otherwise stated, all reagents used in this study are of lab-grade

Cooking: Cooking of samples was done according to method followed by El Tinay *et al*. (1979).

Proximate analysis: The determination of moisture, crude fibre, crude fat and ash were carried out according to AOAC (1984) methods.

Determination of total minerals: Minerals were extracted from the samples by dry ashing method that was described by Chapman and Pratt (1982). The amount of iron, Ca and Cu were determined using atomic absorption spectroscopy (Perkin-Elmer 2380). Ammonium vandate was used to determine phosphorous along with ammonium molybdate method of Chapmann and Pratt (1982). Sodium and potassium contents were determined by flame photometer (CORNIGEEL) according to AOAC (1984).

Determination of tannin content: Quantitative estimation of tannins was carried out using the modified vanillin – HCl method according to Price *et al*. (1978).

Determination of *in vitro* protein digestibility: *In vitro* protein digestibility was carried out according to Saunder *et al*. (1973) method.

Isolation and characterization of phenolic acids

Isolation and characterization of free phenolic acids: Free phenolic acids were isolated according to the method of Ayumi *et al*. (1999). Two grams of flours were extracted with 70% ethanol (4 x 50 mL., 1 h each); the supernatants were obtained by centrifugation and concentrated, and the pH was adjusted in the range of 2 - 3 with 4 M HCl. Phenolic acids were separated by ethyl acetate phase separation (5 x 50 mL), and the pooled fractions were treated with anhydrous disodium sulfate to remove moisture, filtered and evaporated to dryness. Phenolic acids taken in methanol were estimated colorimetrically by using Folin-Cioclateu method with gallic acid as the reference standard (Kaluza *et al*. 1980) as well as by HPLC (model LC-10A, Shimadzu), on a reversed phase Shimpak C_{18} column (4.6 x 250 mm), using a diode array detector (operating at 280 nm). A solvent system consisting of water/acetic acid/methanol (isocratic, 80:5:15) was used as mobile phase at a flow rate of 1 mL/min (Wulf and Nagel, 1967). Standfards such as caffeic, coumaric, ferulic, gallic, gentisic, protocatechuic, syringic, and vanillic acid were used for identification and quantification of phenolic acids present in the flour samples. Quantification of phenolic acids present in the sample was carried out by

measuring the area under respective peaks and plotting against a standard graph prepared (2-10 µg) for each individual phenolic acid using the above-mentioned standards.

Isolation and characterization of bound phenolic acids: Bound phenolic acids were extracted according to the method of Erk-Nordkvist *et al.* (1984). Sorghum flour of two cultivar (2 g each) were extracted with 70% ethanol (4 x 50 mL) and hexane (4 x 50 mL) to remove free phenolic acids and fat, respectively. The dried samples were extracted with 1 M sodium hydroxide (2 x 100 mL, 2 h each) containing 0.5% sodium borohydride under nitrogen atmosphere, and the clear supernatants were collected followed by centrifugation. The combined supernatants were acidified with 4 M HCl to pH 1.5, and the phenolic acids were processed and analyzed by colorimetry as well as by HPLC as mentioned in the case of free phenolic acids.

Statistical analysis: Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the Analysis of Variance (ANOVA) (Snedecor and Cochran, 1987). Duncan Multiple Range Test (DMRT, 1955) was used to separate means. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition of sorghum: Table 1 shows the results of the proximate composition of Sudanese (feterita) and Indian (CSH5) sorghum cultivars. Data are expressed on dry matter basis (per 100 gm material). The moisture content of Sudanese and Indian cultivars was assessed as 7.49 and 6.77% respectively. These values are comparable to the range of 5.7 to 10% reported by Yousif and Magboul (1972), but significantly lower than the range of 8.89 to 9.88 stated by Arbab (1995) may be due to climatic or location differences. Results show that Sudanese and Indian sorghum cultivars contain ash 2.29 and 1.54% respectively. The value are within the range of 1.5 to 2.6%, 1.4-1.8%, 1.5 - 3.9% reported by Awad El Kareem (2002); Abdel Rahman (2002); Hassan (1995), respectively. The crude protein content of two sorghum cultivars Sudanese and Indian is given in Table 1. Results, however, showed values of 14.0 and 10.02% respectively. The protein content of Sudanese cultivar is significantly higher than Indian cultivars. The values are within the range of 8.61 to 18.21% reported by Sastry *et al.* (1968). The protein content of Sudanese cultivar (feterita) is higher than the value stated by Awad El Kareem (2002) who reported the protein content of feterita was 13.13.The crude fibre analysis for the two sorghum cultivars Sudanese (feterita) and Indian (CSH5) showed the values of 1.65 and 1.72% respectively. The fibre content of Indian variety was significantly higher than Sudanese cultivar. Results obtained were found to be within the range of 1.2 to 1.9% and 1.4 to 2% reported by El Tinay *et al.* (1979) and Abdel Rahman (2002) respectively. However, the crude fibre content of the two cultivars(1.65 and 1.72%) were significantly lower ($P \leq 0.05$) than those reported by Hassan (1995) who stated the fiber content of sorghum cultivars ranged between 1.8% and 2.17%. The fat content is 3.12% (for Sudanese) and 2.84% (for Indian). The fat content of Sudanese cultivar is significantly ($P \leq 0.05$) higher than Indian cultivar. The fat content of Sudanese cultivar was in the range reported by Awad El Kareem (2002) who stated the fat content of two Sudanese sorghum cultivar (Dabar and Fetarita) ranged between 3.1 and 3.8%, while the fat content of Indian cultivar within the range reported by Shepherd *et al.* (1970) who stated that the fat content of sorghum cultivars ranged from 1.5 to 2.5%.The results of carbohydrates content are viewed in Table 1 as 71.46 and 77.11% for Sudanese and Indian cultivars, respectively. The carbohydrates content of Indian cultivar was significantly ($P \leq 0.05$) higher than Sudanese cultivar. The results obtained were in the range reported by Osman (2004) who recorded carbohydrates content of three Sudanese local cultivars (Tabat, mugud and feterita) to be ranging between 71.33 and 78.78%.

Mineral content: The mineral content of Sudanese and Indian sorghum cultivars are showen in Table 2. Total sodium content of Sudanese and Indian sorghum cultivars was 6.18 and 5.83 mg/100g respectively. Sodium content of Sudanese cultivar agrees with the result stated by Badi (2004) who reported that sodium content of two sorghum cultivars ranged from 6.3 to 7.0 mg/100g and both of the cultivars showed lower results than the results recorded by Khalil *et al.* (1984). Total potassium for both Sudanese and Indian were 225.23 and 367.51 mg/100g, respectively. Indian cultivar contains much amount of potassium compared to Sudanese. Potassium content of Sudanese and Indian is lower than 441.7 and 450 mg/100g reported by Badi (2004) and 430-458 mg/100g reported by Khalil *et al.* (1984), while potassium content of Indian cultivar within the range of 363-901 mg/100g stated by Deosthale and Belvady (1978). Total calcium content of Sudanese and Indian cultivars were 2.43 and 3.33 mg/100g respectively. The results obtained for both cultivars were less than results recorded by Badi (2004) and Khalil *et al.* (1984) who recorded 10.8 and 18 mg/100g, respectively. Total iron contents for Sudanese and Indian cultivars were 15.54 and 11.32 mg/100g respectively. Results obtained from two cultivars were higher than results reported by Badi (2004) who reported iron content of the Sudanese sorghum cultivars (Wad Ahmed and Tabat) as 3.8 and 4.5 mg/100g, respectively, while the results were in agreement with results stated by

Table 1: Proximate composition of African and Asian sorghum cultivars

	Parameter					
Cultivars	Moistures	Ash	Protein	Fat	Fibre	Carbohydrates
African	7.49[a]0.02	2.29[a]±0.01	14.00[a]±0.00	3.12[a]±0.01	1.65±0.05	71.46[a]±0.04
Asian	6.77[b]±0.05	1.54[b]±0.04	10.02[b]±0.08	2.84[b]±0.07	1.72±0.10	77.11[b]±0.29
± LSD	0.08	0.07	0.13	0.12	0.19	0.47

Values are means (±SD) of 3 replicates per treatment

[ab]Means with different superscripts in the same row were significantly different (P≤0.05).

Table 2: Minerals content (mg/100g) of Sudanese and Indian sorghum cultivars

	Parameter					
Cultivars	Cu	Ca	Fe	P	Na	K
Sudanese	0.41[a]±0.10	2.43[b]±0.10	15.54[a]±0.10	263.30[b]±1.00	6.18[a]±0.01	225.23[b]±0.0
Indian	0.32[b]±0.01	3.33[a]±0.09	11.32[b]±0.11	314.15[a]±2.05	5.83[b]±0.08	367.51[a]±1.54
± LSD	0.16	0.21	0.23	3.66	0.13	2.47

Values are means (±SD) of 3 replicates per treatment.

ab Means with different superscripts in the same row were significantly different (P≤0.05).

Table 3: Tannins and *Invitro* protein digestibility (IVPD) of Sudanese and Indian sorghum cultivars

	Parameters		
		(IVPD)	
	Tannin	Uncooked	Cooked
Sudanese	1.19[a]±0.01	49.25[b]±0.06	26.11[b]
Indian	0.08[b]±0.00	55.85[a]±0.40	33.11[a]
± LSD	0.01	0.65	0.24

Values are means (±SD) of 3 replicates per treatment.

ab Means with different superscripts in the same column were significantly different (P≤0.05).

Deosthale and Belvady (1978) who reported the iron content of sorghum cultivars to be ranging from 4.70 to 14.05 mg/100g. Total phosphorus content of two sorghum cultivars (Sudanese and Indian) were 263.30 and 314.15 mg/100g respectively, which are less than 407 and 396 mg/100g reported by Khalil *et al.* (1984) and 388 to 756 mg/100g stated by Deosthale and Belvady (1978). Phosphorus content of Indian cultivar is significantly (P≤ 0.05) higher than Sudanese cultivar. Total copper content for two sorghum cultivars Sudanese and Indian were 0.41 and 0.32 mg/100g, respectively. Results obtained were in agreement with Deosthale and Belvady (1978) who reported the copper content of sorghum cultivars to range from 0.39 to 1.58 mg/100g.

***In vitro* protein digestibility of sorghum:** Table 3 showed the *in vitro* protein digestibility of Sudanese and Indian cultivars as 49.25 and 55.85% for uncooked sample, while *in vitro* protein digestibility was 26.11 and 33.11% for Sudanese and Indian cultivar, respectively. Indian cultivar had significantly (P≤ 0.05) higher *in vitro* protein digestibility for cooked and uncooked compared to Sudanese. These results obtained agree with Chibber *et al.* (1980) who reported that, uncooked and cooked high tannin sorghum varieties both shown to have low *in vitro* protein digestibility. The lowest *in vitro* protein digestibilities obtained in case of Sudanese cultivar positively correlated to its tannin content, this finding agrees with Chavan *et al.* (1979) who observed significant lowering in *in vitro* protein digestibility (IVPD) in a high tannin cultivar. The IVPD of high tannin grain was found to be improved to the level of low tannin grains upon dehulling the grain. The minimum level of tannin requires to show the growth depressing effects in rats was 0.64 to 0.84% sorghum tannin (Fuller *et al.*, 1996). The tannin content of white and yellow grain cultivar grown in India for human consumption is below this level. Hence, the tannins present in Indian cultivars may not pose a significant problem of protein digestibility. Many efforts should be done to improve *in vitro* protein digestibility including sodium hydroxide, potassium hydroxide and sodium carbonate treatments which significantly increased *in vitro* protein digestibility from 48 to 69%, 69.1 and 72% respectively (Chavan *et al.*, 1979). *In vitro* protein digestibility of both cooked cultivars (Sudanese and Indian) is significantly decreased, these findings agree with Duodo *et al.* (2002) who reported that cooking significantly decrease *in vitro* protein digestibility of sorghum and maize cultivar. Results obtained also agreed with Mertz *et al.* (1984) and Maclean *et al.* (1981) who reported effect of cooking on protein digestibility at the three level of organizations, cooking caused a significant reduction in protein digestibility for both sorghum varieties (high and low tannins). Results obtained from the two cultivars Sudanese (high tannin sorghum) and Indian (low tannin sorghum) indicate that tannin content is not the only responsible factor for lowering *in vitro* protein digestibility and may be many other factors had a role in this process. An old hypothesize that cooling cooked porridge leads to formation of resistant starch which may form complexes with kafirin (major fraction) proteins that are less susceptible to enzyme attack

Table 4: Phenolic acid (%) in Indian and Sudanese sorghum cultivar (identified and quantified by HPLC)

		Phenolic acid						
Type		Gallic	Proto-catechuic	Gentisic	Syringic	Caffeic	Beta Coumaric	Ferulic
FPA	Indian	ND	ND	ND	13.40	69.30	ND	16.00
	Sudanese	30.00	6.80	17.30	ND	38.80	3.90	3.00
BPA	Indian	ND	ND	ND	1.90	1.70	7.00	65.00
	Sudanese	8.50	11.80	ND	1.40	1.10	6.70	45.00

FPA = Free phenolic acid. BPA = Bound phenolic acid. ND = Not detected

(Bach Knudsen and Munk, 1985). The results obtained for Sudanese (high tannin) and Indian cultivars (low tannin content) were significantly ($P \leq 0.05$) lower than the result stated by Ibrahim (2004) who analyzed two Sudanese sorghum cultivars [Wad Ahmed (high tannin) and Dabar (low tannin)] and recorded 47.9 and 53%, respectively. Fermentation of sorghum cultivar (high and low tannin) significantly decreased *in vitro* protein digestibility by 63% (low tannin) and increased (IVDP) by 17.5% of high tannin sorghum cultivar (Romo Parad *et al*., 1985). Ibrahim (2004) stated that supplementation of high tannin (Wad Ahmed) and low tannin (Dabar) sorghum cultivar with 5 and 10% whey protein significantly increased protein digestibility. For Sudanese cultivar (Feterita) may be supplementation with highly rich protein concentrate or isolate of legumes increased *in vitro* protein digestibility. *In vitro* protein digestibility of Sudanese cooked samples was higher than the value reported by Arbab and El Tinay (1997) who reported the *in vitro* protein digestibility of cooked sorghum (12 and 18%) will treatment with reducing agent. The results obtained for Sudanese and Indian were higher than the results stated by Arbab and El Tinay (1997) for uncooked samples. It is interesting that the use of a reducing agent during cooking doesn't appear to completely reverse the effect of lowered sorghum protein digestibility on cooking. Cooking sorghum flour with a reducing agent did improve protein digestibility but not to the level of uncooked sorghum flour (Oria *et al*., 1995). Similar results have been reported from SDS-PAGE analysis of pepsin indigestible residues of sorghum protein body preparation (Duodo *et al*., 2002). During cooking, more of such disulphide cross-linked protein oligomers and polymers are formed. When sorghum is cooked, enzymatically resistant protein polymers are formed through disulphide bonding of the β-kafirin and δ-kafirin (Oria *et al*., 1995). Finally, the causes of the poor digestibility of sorghum proteins appear to be multi-factorial. Depending on the nature of the sorghum used (whole grain, endosperm protein body preparation, high tannin grain or condensed – tannin – free grain), different factors may contribute with some being more important than others. The probable causes of reduced protein digestibility indicate that a number of currently used processing technologies may be applied to improve sorghum protein digestibility. These processing technologies including dry cooking "popping", extrusion, malting, fermentation and grain refinement (Duodu *et al*., 2001; Hamaker *et al*., 1994; Elkhalifa and Chandrashekar, 1999; Taylor and Taylor, 2002 and Duodo *et al*., 2002).

Tannin content of sorghum cultivars: The tannin content of Sudanese and Indian cultivar showed in table 3, as 1.19 and 0.08% as catechin equivalent respectively. Sudanese cultivar had significantly ($P \leq 0.0$) higher tannin content compared to Indian cultivar. Results obtained for two cultivars agree with Jambunthan and Mertz (1973) who reported that tannin content of high tannin sorghum is 2.69% and for low tannin sorghum is 0.5%. Result findings agreed with Radhakrishnan and Sivaprasad (1980) who reported arrange of 0.01 to 2.056% tannin as catchin equivalents in the sorghum grown in India. The tannin content of cultivars commonly grown and consumed in India ranged from 0.43 to 0.64% tannic acid equivalents. For Sudanese sample (high tannin content) several investigators have implicated the high tannin character of certain sorghum genotypes to their ability to resist bird damage. It is observed that bird resistance is offered only during the milk stage of grain development, where the tannin content is higher. As the grain matures, the tannin content is reduced and the seeds become palatable (Price *et al*., 1967). The usefulness and practicability of high tannin cultivars to save the yield from bird damage in the field and subsequent processing of grain to remove tannins before consumption needs to be evaluated. Results obtained were comparable to result stated by Chavan *et al*. (1979) who analyzed two low tannin sorghum cultivars and two high tannin sorghum and recorded the tannin content for low tannin cultivars range from 0.40 to 0.46% (catechin equivalent) and for higher tannin sorghum ranged from 3.44 to 3.60% (as catechin equivalent).

Phenolic acid content: The phenolic acids (PAs) (free and bound) content separated and identified by high performance liquid chromatography (HPLC) of the two cultivars are shown in Table 4. Free phenolic acids of Indian cultivar detected were 13.40% syringic acid, 69.30% Coumaric and 16.0% ferulic acid while free

phenolic acids of Sudanese cultivar were callic acid (30%), 6.8% protocatechuic acid, 17.30 gentisic acid, 38.5% caffeic acid, 3.90 p-comaric acid and 3.0% ferulic acid. Gallic, protocatechuic, gentisic, and p-coumaric were not detected as free phenolic acids for Indian cultivar while syringic acid was not detected in Sudanese cultivars. Indian cultivar contained high caffeic (69.30%) and ferulic (16.00%) compared to Sudanese which contained 38.80 and 3.00% respectively. Syringic acid content of Indian cultivar was 13.40% .The bound phenolic acid figures of Indian cultivar were 1.90, 1.70, 7.00 and 65.0% as syringic, caffeic, P-coumaric and ferulic acids, respectively. Gallic, protocatechuic and gentisic acids were not detected in free or bound form while p-coumaric was detected in bound form of Indian cultivar. Bound phenolic acids values of Sudanese cultivar were 8.5, 11.80, 1.40, 1.10, 6.70 and 45.0%, as gallic, protocatechuic, syringic, caffeic, p-coumaric and ferulic acids, respectively. Syringic, caffeic, p-coumaric and ferulic acids content in bound form of Indian cultivar were higher than Sudanese cultivar. The results obtained from two cultivars are comparable with Hahn *et al*. (1983) and Adam and Liu (2002) who reported that, the phenolic acids exist mostly in bound form (esterifies to cell wall polymers), with ferulic acid as being the most abundant bound phenolic acid in sorghum and other cereals. Findings obtained agreed with Hahn *et al*. (1984); Waniska *et al*. (1989) who reported that, several other PAs have been identified in sorghum including syringic (15C), protocatechuic (16C), caffeic (17C), p-coumaric (20C) as more abundant. The PAs like other phenols are though to help in plant defense against pests and pathogens. The PAs show good anti-oxidant activity (Subba Rao and Muralikrishna, 2002), and thus may contribute significantly to the health benefits associated with whole grain consumption. From these findings in spite of Sudanese cultivar demonstrated as high tannin sorghum cultivar and Indian as low tannin cultivar, phenolic acid content of syringic, cuffeic, p-coumanic and ferulic for Indian cultivar were higher than Sudanese. These observations agreed with Waniska *et al*. (1989) who reported that, in sorghum the level of PAs do not correlate with the presence of level of other phenol (anthocyanin or tannin). Waniska *et al*. (1989) observed increased level of free PAs in certain sorghums with pigmented testa (containing tannin) compared to the ones without pigmented testa. In general sorghums have level of PAs comparable to those of other cereals (Andersen *et al*., 2001; Adam and Liu, 2002; Hahn, 1983, 1984) significant varietals differences are observed in phenolic acid composition and ratios of bound and free forms of these compounds in sorghum. Adam and Liu (2002) found a strong correlation between anti-oxidant activity and levels of bound ferulic acid in wheat, corn, rice and oats.

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