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Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties

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ABSTRACT

Objective: To investigate the changes in total phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. **Methods:** The changes in total phenols, total flavonoids, tannins, phenolic acids compounds, flavonoid components, vitamin E, β-carotene and antioxidant activity during soaking of sorghum grains were determined. **Results:** Total phenols, total flavonoids, tannins, vitamin E, β-carotene and antioxidant activity in raw sorghum were ranged from 109.21 to 116.70, 45.91 to 54.69, 1.39 to 21.79 mg/100 g, 1.74 to 5.25, 0.54 to 1.19 mg/kg and 21.72% to 27.69% and 25.29% to 31.97%, respectively. The above measured compounds were significantly decreased after soaking. *p*-Hydroxybenzoic acid, vanillic acid, syringic acid and cinnamic acid represent the major phenolic acids in Dorado variety. While ferulic acid, *p*-coumaric acid, gallic acid and caffeic acid represent the major phenolic acids in Shandaweel-6. On the other hand, protocatechuic acid represents the major phenolic acids in Giza-15. Regarding flavonoids components, Dorado was the highest variety in kampferol and naringenin while Shandaweel-6 was the highest variety in luteolin, apigenin, hypersoid, quercetin and christen. Finally, Giza-15 was the highest variety in catechin. Phenolic acids, flavonoid compounds and antioxidant activities were decreased after soaking. **Conclusions:** Sorghum varieties have moderate quantities from total phenols, total flavonoids, tannins, phenolic acids compounds, flavonoid components, vitamin E, β-carotene and antioxidant activity which decreased after soaking.

1. Introduction

Sorghum [*Sorghum bicolor* (*S. bicolor*) L. Moench] is a crop that is widely grown all over the world for food and feed. It is one of the main staples for the world's poorest and most insecure people in many parts of the developing world[1,2].

Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins. Sorghums phenolic acids are located in the pericarp, testa, aleurone layer, and endosperm[3]. The most abundant phenolic acids in sorghum are ferulic acid, *p*-coumaric acid and vanillic acid, which are predominant in bran layer of grains[4]. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa[5]. Most sorghum does not contain condensed tannins, but all contain phenolic acids[6]. Compared to other cereal crops, sorghum has unique chemical component of tannin including type II sorghum (tannins present in pigmented testa) and type III sorghum (tannins present in pigmented testa and pericarp), while non-tannin sorghum is classified as type I[7]. The tannins in sorghums have the highest levels of antioxidants compared to cereals[8]. The evidence of possible benefits of tannins in the diet has led to research that focuses on sorghum tannins and health[9,10].

Free radicals may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells[11]. This oxidative stress may be related to many disorders such as cancer, atherosclerosis, diabetes and liver cirrhosis[12].

Epidemiological studies have suggested that increased consumption of whole grains, fruits and vegetables is associated with reduced risks of chronic diseases[13-15]. This association may be attributed to the natural antioxidants

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from plant foods such as vitamin C, tocopherol, carotenoids, polyphenolics and flavonoids which prevent free radical damage by modulating the effects of reactive oxidants. Also, some plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. It can be also used in stabilizing food against oxidative deterioration[16-18].

Sorghum phenolic compounds have been shown to have antioxidant activity. These phenolic compounds possess structural features favorable for radical scavenging and/or metal chelation, which would enable them to be effective antioxidants. A potential therefore exists to use sorghum bran as a cheap source of natural antioxidants to prevent the development of oxidative rancidity in edible oils and other lipid food systems[5]. Some varieties of sorghum are recognized as important sources of dietary antioxidants because of the phenolic compounds found in the grain[19,20]. Many cereal and grain legumes are soaked before further processing. During soaking, water enters the kernel by molecular absorption, capillary absorption and hydration. Soaking gives a volume increase in the grain[21]. Soaking, fermentation and germination are three biological processes of significant impact on phytate and phenolic compounds. Several studies demonstrated that germination and fermentation affect condensed phenolic compounds[22].

The objective of this study was to increase the efficient use of three white sorghum by studying the biochemical changes of total phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity after soaking.

2. Materials and methods

2.1. Samples and chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzotiazoline-6-sulphonic acid) (ABTS), sodium carbonate (Na_2CO_3), butylated hydroxytoluene (BHT), gallic acid, catechin, β-carotene and α-tocopherol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA), Folin-Ciocalteu reagent was purchased from LOBA Chemie, India. All other chemicals used were of analytical reagent grade.

Three white sorghum varieties (*S. bicolor* L. Moench), named Dorado, Shandaweel-6 and Giza-15 grown during the 2007 season, were used. Dorado and Giza-15 varieties were obtained from Central Administration for Seed Certification (CASC), Ministry of Agriculture and Land Reclamation (MOALR), Giza, Egypt. Shandaweel-6 variety was obtained from the Crops Research Institute, Agricultural Research Center (ARC).

2.2. Soaking of sorghum grains

Sorghum grains were soaked in distilled water for 20 h with a ratio of 1:5 w/v and the soaked water was changed twice. At the end of soaking period, the soaked water was discarded. The grains were rinsed twice with distilled water and the grains were dried in oven at (45±5 ℃). The grains were milled in a laboratory mill to obtain fine flour and kept at −20 ℃ until analysis.

2.3. Biochemical analysis

2.3.1. Determination of total phenols

Total phenols were determined colorimetrically as described by Matkowshi and Piotrowska[23]. Sample (1 g) was mixed with 10 mL 80% methanol in a dark bottle and shaking for 2 h. The color was developed by Folin-Ciocalteu reagent and sodium carbonate. A volume of 0.250 mL was mixed with 0.250 mL Folin-Ciocalteau reagent and 0.50 mL of 10% sodium carbonate (Na_2CO_3) and the volume was completed to 5 mL with distilled water. After incubation in dark at room temperature for 30 min, the absorbance of the reaction mixture was measured at 725 nm against blank. Gallic acid was used as a standard.

2.3.2. Determination of total flavonoids

Total flavonoids were determined according to the methods of Nabavi *et al*[24]. Sample (1 g) was mixed with 10 mL 80% methanol and shaking for 2 h. Total flavonoids extract (0.4 mL) was added to 4 mL of H_2O. Then 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. The color was measured at 510 nm against a blank reagent. Catechin was served as standard compound.

2.3.3. Determination of tannins

Tannins were determined as described by Price *et al*[25] followed with minor modification by Osman[26]. Sample (1.0 g) was mixed with 10 mL of 1% methanol/HCl solution in a in a dark bottle and shaking for 20 min at room temperature. Then the mixture was filtrated. The tannins in the supernatant were estimated by using 1 mL of supernatant and 5 mL of vanillin/HCl mixture (by mixing equal volumes of 2% vanillin in methanol and 8% methanol/HCl) in a test tube and kept for 20 min at room temperature. The formed color was determined at 500 nm. Catechin was used to prepare the standard curve.

2.3.4. Fractionation of phenolic acids and flavonoid compounds using HPLC

The phenolic acids and flavonoid compounds of the samples were extracted according to the method described by Goupy *et al*[27] and Mattila *et al*[28] by using HPLC instrument (Hewlett Packard, series 1050, country) composed of column C18 hypersil BDS with particle size 5 μm. The separation was carried out with methanol and acetonitrile as a mobile phase, flow with 1 mL/min. Quantification was carried out for a calibration based on the standards phenolic acid and flavonoid.

2.3.5. Determination of vitamin E as α-tocopherol using HPLC

The vitamin E was quantified according to the method described by Pykaa and Sliwiok[29] by using HPLC instrument (Hewlett Packard, series 1050, country) composed

of column C18 hypersil BDS with particle size 5 μm. Samples (10 g) were extracted by using hexane and saponified with 25% methanolic KOH. The extracted α-tocopherol was filtrated through 0.20 μm millipore membrane filter and set up to a known volume. Three milliliters were collected in a vial for subsequent HPLC separation. Separation was carried out with methanol as a mobile phase, flow with 1 mL/min. Quantification was carried out for a calibration based on the standard α-tocopherol.

2.3.6. Determination of β-carotene

β-carotene was determined using the method outlined by Santra *et al*[30]. Samples (2 g) were added to water-saturated *n*-butanol and mixed by handshaking, and kept in the dark for 16-18 h for extraction of β-carotene. Samples were centrifuged at 10 000 g for 10 min. The absorbance of collected supernatant was measured at 440 nm on spectrophotometer. β-carotene was used to prepare the standard curve.

2.3.7. Determination of antioxidant activity

2.3.7.1. Radical scavenging activity by using DPPH method

The antioxidant activity of plant methanol extracts was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to Brand-Williams *et al*[31]. Samples (1 g) were extracted with 80% methanol (10 mL) for 2 h. Briefly, 2.4 mg of DPPH in 100 mL methanol was prepared and 3.9 mL of this solution was added to 0.1 mL of methanolic extract. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 515 nm. The radical scavenging percentage was calculated using the following equation:

$$\% \text{ Radical scavenging} = (1 - A_f/A_o) \times 100$$

Where, A_o is the initial absorbance and A_f is the final absorbance.

The antioxidant capacity was expressed as BHT equivalent antioxidant capacity (BHT EAC μmole/g dwt).

2.3.7.2. Radical scavenging activity by using ABTS method

The ABTS assay was carried out according to Re *et al*[32]. After preparing the ABTS radical (7 mM, ABTS was dissolved in 10 mL deionized water), ammonium persulfate solution was prepared (2.45 mM - 10 mL). ABTS radical cation was produced by reacting 10 mL of ABTS stock solution with 10 mL of ammonium persulfate and then mixed, homogenized and kept in an amber flask for at least 16 h and protected from light). For the sample, an aliquot of 200 μL of the radical formed was pipetted and diluted in 10 mL 96° ethanol of analysis grade. The absorbance was measured at 734 nm. An aliquot of 980 μL of the diluted radical was pipetted and transferred to cuvette, and 20 μL of the sample was added, homogenizing and agitating for a few seconds. The calculation of the radical scavenging percentage was made using the above formula. The antioxidant capacity was expressed as BHT equivalent antioxidant capacity (BHT EAC μmole/g dwt).

2.4. Statistical analysis

For the analytical data, mean values and standard deviation were reported. The data obtained were subjected to one-way analysis of variance (ANOVA) and least significant difference (LSD) was at $P<0.05$.

3. Results

Table 1 exhibited the changes in total phenols, total flavonoids and tannins during soaking of sorghum grains. The results showed that total phenols, total flavonoids and tannins content in raw sorghum ranged from 109.21 to 116.70, 45.91 to 54.69 and 1.39 to 21.79 mg/100 g, respectively. Shandaweel-6 was the highest variety in total phenols and total flavonoids content. Meanwhile, Giza-15 was the highest variety in tannins content.

In general, after soaking, the total phenols, total flavonoids and tannins content of sorghum was decreased. Data revealed that the losses were between 21.97% to 28.30%, 21.97% to 28.30% and 7.19% to 30.38% for total phenols, total flavonoids and tannins content, respectively.

Table 2 presented the changes in phenolic acids composition before and after soaking. HPLC analysis showed that sorghum phenolic acids consists of different compounds *i.e.* ferulic acid, protocatechuic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, gallic acid, caffeic acid, syringic acid and cinnamic acid, with different concentrations.

Dorado was the highest variety in *p*-hydroxybenzoic acid, vanillic acid, syringic acid and cinnamic acid while Shandaweel-6 was the highest variety in ferulic acid, *p*-coumaric acid, gallic acid and caffeic acid. Giza-15 was the highest variety in protocatechuic acid. After soaking, phenolic acids composition was significantly ($P<0.05$)

Table 1
Changes in total phenols, total flavonoids and tannins (mg/100 g dwt) during soaking of sorghum grains (mean±SD).

Treatments	Varieties	Tannins	Total flavonoids	Total phenols
Raw	Dorado	1.99 ± 0.26^{c}	45.91 ± 2.93^{b}	110.52 ± 2.01^{b}
	Shandaweel-6	1.39 ± 0.20^{c}	58.85 ± 1.64^{a}	116.70 ± 2.51^{a}
	Giza-15	21.79 ± 2.07^{a}	54.69 ± 0.96^{a}	109.21 ± 2.97^{b}
Soaking	Dorado	1.72 ± 0.19^{c}	33.92 ± 3.14^{d}	71.42 ± 2.61^{c}
	Shandaweel-6	1.29 ± 0.22^{c}	45.92 ± 3.29^{b}	70.00 ± 1.99^{c}
	Giza-15	15.17 ± 0.48^{b}	39.21 ± 2.86^{c}	61.24 ± 2.03^{d}
LSD		1.5803	4.7592	4.0922

Numbers in the same column followed by the same letter are not significantly different ($P<0.05$).

Table 2
Effect of soaking of sorghum grains on phenolic acids composition (μg/g dwt) (mean±SD).

Treatments	Varieties	Cinnamic acid	Syringic acid	Caffeic acid	Gallic acid	Vanillic acid	*p*-Hydroxybenzoic acid	*p*-Coumaric acid	Protocatechuic acid	Ferulic acid
Raw	Dorado	15.02±3.75^{a}	17.48±2.51^{a}	14.67±2.51^{ab}	14.84±2.00^{a}	23.43±2.52^{a}	16.39±0.10^{a}	51.29±3.08^{b}	156.31±2.11^{bc}	163.91±4.44^{b}
	Shandaweel-6	12.81±0.11^{abc}	15.71±2.84^{a}	20.83±3.05^{a}	21.51±3.62^{a}	22.19±3.05^{a}	13.41±2.63^{b}	71.88±4.39^{a}	150.28±4.95^{cd}	173.46±3.10^{a}
	Giza-15	9.76±0.87^{bcd}	16.06±1.60^{a}	13.55±4.40^{b}	18.96±4.01^{a}	15.45±2.28^{c}	6.13±1.21^{c}	41.88±4.03^{c}	178.22±3.00^{a}	120.47±3.00^{c}
Soaking	Dorado	13.33±2.09^{ab}	16.29±1.11^{a}	12.63±4.19^{b}	12.69±4.02^{a}	20.92±4.32^{ab}	11.51±2.11^{b}	20.37±3.51^{d}	152.03±3.05^{bc}	106.29±4.00^{d}
	Shandaweel-6	9.26±2.14^{cd}	14.89±2.71^{a}	12.74±2.52^{b}	16.59±1.97^{a}	15.86±2.60^{bc}	7.36±1.50^{c}	21.14±2.20^{d}	145.49±3.20^{d}	110.87±3.33^{d}
	Giza-15	6.17±1.40^{d}	15.86±2.30^{a}	10.42±0.67^{b}	15.61±2.89^{a}	14.56±2.20^{c}	4.76±0.20^{c}	9.34±1.12^{e}	156.66±3.77^{b}	98.51±3.28^{e}
LSD		3.6554	4.0276	5.5805	5.3059	5.1711	2.7728	5.7839	5.8617	6.1503

Numbers in the same column or raw followed by the same letter are not significantly different (P<0.05).

Table 3
Effect of soaking of sorghum grains on flavonoids composition (μg/g dwt) (mean±SD).

Treatments	Varieties	Naringenin	Christin	Catechin	Quercetin	Hypersoid	Kampferol	Apigenin	Luteolin
Raw	Dorado	28.62±2.99^{a}	3.58±0.55^{a}	5.58±1.03^{a}	22.34±2.02^{b}	34.62±4.00^{c}	36.44±2.00^{a}	61.58±2.97^{a}	167.26±4.15^{c}
	Shandaweel-6	26.77±3.10^{ab}	3.86±0.57^{a}	5.67±1.11^{a}	29.43±3.97^{a}	50.41±3.29^{a}	32.80±2.52^{ab}	65.58±4.25^{a}	210.70±2.00^{a}
	Giza-15	22.85±4.59^{bc}	3.34±0.78^{a}	6.13±0.99^{a}	25.73±3.00^{ab}	30.16±3.42^{cd}	17.88±2.74^{d}	25.74±3.05^{d}	112.56±3.74^{e}
Soaking	Dorado	20.57±0.50^{bcd}	3.15±0.76^{a}	5.29±0.73^{a}	22.87±2.88^{b}	25.16±3.10^{de}	30.94±2.25^{b}	33.77±3.30^{c}	121.27±3.56^{d}
	Shandaweel-6	20.64±1.95^{bcd}	2.75±0.53^{a}	5.43±0.87^{a}	27.50±1.80^{ab}	42.34±4.19^{b}	24.60±2.89^{c}	43.98±3.00^{b}	186.11±3.20^{b}
	Giza-15	18.97±4.79^{bcde}	2.60±0.77^{a}	4.45±1.00^{a}	22.81±3.20^{b}	22.76±2.90^{e}	14.78±1.16^{d}	19.46±3.00^{e}	66.74±2.01^{f}
LSD		5.9288	1.1915	1.7150	5.1156	6.2536	3.9286	5.3841	5.7294

Numbers in the same column or raw followed by the same letter are not significantly different (P<0.05).

decreased and this is due to the losses of total phenols in water as mentioned before.

Table 3 showed the effect of soaking of sorghum grains on flavonoids composition. HPLC analysis showed significant differences in flavonoids between sorghum varieties with different compounds *i.e.* luteolin, apigenin, kampferol, hypersoid, quercetin, catechin, christin and naringenin. Shandaweel-6 had the highest amount of luteolin, apigenin, hypersoid, quercetin and christen while Dorado had the highest amount of kampferol and naringenin and Giza-15 had the highest amount of catechin. There was a significant (P<0.05) decrease in flavonoids composition after soaking.

Table 4 presented the changes in vitamin E and β-carotene contents during soaking. It could be noticed that vitamin E content in raw sorghum ranged from 1.74 to 5.25 mg/kg. Vitamin E amount was significantly higher in Dorado than other varieties. After soaking vitamin E content was significantly reduced and ranged from 1.50 to 4.25 mg/kg. From the same table, β-carotene content ranged from 0.54 to 1.19 mg/kg in raw sorghum. Giza-15 contains the higher amount of β-carotene than other varieties. After soaking β-carotene content was significantly reduced and ranged from 0.40 to 1.04 mg/kg.

Table 5 exhibited the changes in antioxidant activity (AO) and antioxidant capacity (AC) during soaking. Data revealed that DPPH and ABTS scavenging activity in raw sorghum varied from 21.72% to 27.69% and 25.29% to 31.97%, respectively. Shandaweel-6 recorded the highest AO and AC due to its highest content of total phenols and flavonoids contents. Also, AC in raw sorghum varied from 12.82 to 16.39 μmole/g and 13.82 to 17.52 μmole/g, respectively. After soaking DPPH and ABTS scavenging and antioxidant capacity were significantly decreased.

Table 4
Effect of soaking of sorghum grains on vitamin E and β-carotene (mg/kg) (mean±SD).

Treatments	Varieties	β-carotene	Vitamin E*
Raw	Dorado	0.62±0.01^{b}	5.25±0.18^{a}
	Shandaweel-6	0.54±0.01^{bc}	4.42±0.10^{b}
	Giza-15	1.19±0.03^{a}	1.74±0.02^{d}
Soaking	Dorado	0.47±0.03^{bc}	4.25±0.21^{b}
	Shandaweel-6	0.40±0.03^{c}	2.35±0.10^{c}
	Giza-15	1.04±0.29^{a}	1.50±0.14^{e}
LSD		0.2151	0.2522

* Vitamin E as β-tocopherol.
Numbers in the same column or raw followed by the same letter are not significantly different (P<0.05).

Table 5
Effect of soaking of sorghum grains on AO and AC (mean±SD).

Treatments	Varieties	AC_{ABTS} (μmole/g)	AC_{DPPH} (μmole/g)	AO_{ABTS} (%)	AO_{DPPH} (%)
Raw	Dorado	15.29±0.22^{b}	14.33±0.84^{b}	28.00±0.42^{b}	24.59±1.34^{b}
	Shandaweel-6	17.52±1.55^{a}	16.39±1.10^{a}	31.97±2.82^{a}	27.69±1.88^{a}
	Giza-15	13.82±1.25^{bc}	12.82±0.63^{bc}	25.29±2.28^{bc}	21.72±1.07^{c}
Soaking	Dorado	12.36±1.15^{cd}	11.66±0.36^{cd}	23.21±2.15^{c}	20.28±0.63^{cd}
	Shandaweel-6	15.23±0.69^{b}	12.99±1.48^{bc}	28.49±1.29^{b}	22.47±2.57^{bc}
	Giza-15	11.81±0.82^{d}	10.70±0.10^{d}	22.17±1.54^{c}	18.59±0.18^{d}
LSD		1.8462	1.5699	3.4082	2.6648

NumberS in the same column or raw followed by the same letter are not significantly different (P<0.05).

4. Discussion

Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins. The antioxidative phytochemicals in grains, vegetables and fruits have received increased attention recently for their potential role in prevention of human diseases as well as in food quality improvement[3,33].

The present findings were in agreement with Glennie[34] who reported that concentrations of total phenols of white sorghum grains ranged from 80 to 100 mg/100 g and with Yang[35] who reported that total phenols content of non-tannin sorghums ranged from 90-1 820 mg gallic acid equivalent (GAE)/100 g sample. Previous studies found that sorghum tannins content ranged from 10 to 2056, 20 to 190 g and 0 to 1310 mg/100 g tannin as catechin equivalents[36-38]. In addition, our findings are somewhat close with the findings of Youssef[6] who found that Giza-15 contains 18 mg tannin /100 g.

This reduction of total phenols, total flavonoids and tannins after soaking may be attributing to leaching of phenols into the soaking medium. The results approved with Nwosu[39] who reported that this reduction was expected as soaking helped in the removal of the soluble anti-nutrients like tannins. The lower level of total phenols and total flavonoids after soaking may be due to the release of phenolic compounds into soaking water. This can be the result of longer soaking duration leading to more phenolics diffuse outside[40].

These research results were in agreement with Awika and Rooney[19] who reported that sorghum grains contain 100-500 μg/g dwt ferulic acid. Also, gallic acid ranged from 12.9-46.0 μg/g dwt, whereas cinnamic acid ranged form 2.0 to 19.7 μg/g dwt[20]. And the results were partially in agreement with Hahn *et al*[41] who mentioned that *p*-hydroxybenzoic acid, protocatechuic acid and vanillic acid ranged from 15 to 36, 24 to 141, 8 to 50 μg/g dwt, respectively. The reduction of phenolic acids compositions after soaking is due to the losses of total phenols in water as mentioned before.

Due to the biological benefits attributed to these compounds, there is a need to determine their presence and levels in sorghum[19,42]. Several phenolic compounds have been isolated from sorghum like naringenin (a flavanone) which was found in sorghum[43]. Also, luteolin, apigenin, kampferol, catechin, and naringenin were found in sorghum[19,42]. The reduction of flavonoids composition after soaking is due to the losses of total phenols and flavonoids after soaking and diffusion in soaking water.

It is well known that free radicals cause autoxidation of unsaturated lipids in food. On the other hand, antioxidants are believed to intercept the free radical chain during oxidation and to donate hydrogen from the tocopherol hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid[44]. Previous studies found that grains contain low to moderate levels of tocopherol and the α-tocopherol content in raw sorghum was 1.4 mg/kg[45,46].

Yellow endosperm sorghums contain carotenoids, including β-carotene which is considered the most important precursor of vitamin A, since one molecule can potentially be transformed into two molecules of retinol. Vitamin A deficiency affects approximately 250 million people in semiarid regions of Africa and Asia, where sorghum (*S. bicolor* Moench) is a major staple crop, even though β-carotene content in this population was low and would not be sufficient to cover daily requirement of vitamin A[47,48]. Our present results approved with Reddy *et al*[49] who reported that β-carotene in raw sorghum ranged from 0.56 to 1.13 mg/kg. Also, Salas Fernandez *et al*[48] found that yellow endosperm sorghum had β-carotene ranged from 0.22 to 3.23 mg/kg, even though that, β-carotene content would not be sufficient to cover daily requirements of vitamin A[50].

In general, cooking time, soaking and fermentation period were reported to have pronounced effects on the vitamin levels and anti-nutritional factors present in natural foods[51]. Phenolics are considered as a major group of compounds that contribute to the antioxidant activities of grains and thus may contribute significantly to the health benefits associated with whole food consumption[52]. Sorghum and barley are two important food grains reported to contain significant quantities of phenolic compounds[53]. Sorghum, as other cereal grains, fruits and vegetables has phytochemical compounds, which have been evaluated for antioxidant properties[54].

Radical scavenging is the main mechanism by which antioxidants act in foods. Several methods have been developed including DPPH and ABTS radical scavenging methods. The DPPH radical, is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts[55].

It was found that non-pigmented sorghum showed relatively radical scavenging activity ranged from 7% to 67%[56]. While the antioxidant activity (as ABTS) for whole grain was (19 μmole/g)[57], the same range for whole grain (10.8-22.6 μmole/g) of sorghums was reported[58]. It was also reported by Dicko *et al* that antioxidant activities ranged from 16 to 80 μmol/g[59]. It was found that, the antioxidant capacity measured by ABTS assay of non-tannin sorghum grains ranged from 9.7-78.9 μmol TE/g sample[35], which was close to (8-75 μmol TE/g sample) as reported[60]. Also, it was reported that, white sorghum had the antioxidant activity of 14 μmol/g (as ABTS)[61].

This reduction of antioxidant activity and antioxidant capacity after soaking due to the leaching occurred in total phenols, flavonoids, vitamin E and β-carotene contents in soaking water.

Sorghum varieties contain various phytochemicals which have gained increased interest due to their antioxidant activity and other potential health benefits. Consequently, sorghum could serve as an important source of phytoceuticals. Sorghum varieties have moderate quantities from total phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity. Besides that, after soaking sorghum still have phenols and antioxidant

components. The demand for natural antioxidants for use in foods has increased recently because of debates about the long-term safety of synthetic antioxidants such as BHT.

Conflict of interest statement

We declare that we have no conflict of interest.

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