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Changes of Total Phenolics, Tannins, Phytate and Antioxidant Activity of Two Sorghum Cultivars as Affected by Processing

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ABSTRACT

Sorghum (*Sorghum bicolor (L.) Moench*) is a rich source of bioactive compounds such as polyphenols, tannins, and phytate. Polyphenols have been recognized as the most abundant source of anti-oxidants in our diet. The quantity and quality of polyphenols, tannins and phytate in foods are affected by processing due to their highly reactive nature, which may affect their anti-oxidant activity and the nutritional value of foods. The aim of the present study was to investigate how some domestic processing methods such as soaking (in different solutions), germination, fermentation using *(Saccharomyces cerevisia*) and wet cooking influence phenolic compounds, tannins, phytate content and free radical scavenging activity of two sorghum cultivars {high tannin sorghum (Assuit 14) and low tannin sorghum (Giza 15)} grown in southern Egypt. The results indicated that soaking in (distilled water, KOH 2%, NH_4OH 30%, and NaOH 2%), and germination had significant reduction of total phenolic, tannin and, phytic acid content and antioxidant activity. While fermentation process of Giza 15 increased the total phenolic content and the antioxidant activity of the fermented flour, while a significant decrease of tannin and phytic acid was found. On the other hand, wet cooking treatment for 2h of Giza 15 grains showed an increase of antioxidant activity (58%), and a decrease in phytic acid, phenolic compounds and tannins contents. Our results illustrated that sorghum Assiut 14 had higher content of phenolics, tannins, phytate as well as antioxidant activity by DPPH than Giza 15. For (IC_{50}) Assuit 14 had 7.544 while Giza15 had 22.147. All processing methods affect on phenolics, tannin and,phytic acid content and activities of antioxidant components of sorghum differentially, suggesting that treating the seed before use is able to change the bioactive compounds content of sorghum and the antioxidant activity as related.

Keywords: Processing, Phenolic compounds, phytic acid , Tannin, Antioxidant activity, Sorghum grains.

INTRODUCTION

The plants kingdom is considered a major food source for humans in the worldwide especially cereal. After wheat, rice, maize and barley sorghum ranks fifth of important cereals concerning world production. Also it is very important source of food for peoples in the semi and tropics of Asia and Africa (Awika *et al*., 2003a; FAO 2005 and Liu *et al.*, 2012). sorghum is found in Upper Egypt. Sorghum contains valuable amount of protein (7.5–10.8%), oil (3.4–3.5%), ash (1.2– 1.8%), fiber (2.3–2.7%) and carbohydrates (71.4–80.7 %) with a dry matter ranged from 89.2 to 95.3%, depending on type of cultivar (Rahaman *et al.*,2005).

It has been suggested that antioxidants may contribute to the health benefits of cereal-based foods by reducing the incidence of aging-related chronic diseases including heart diseases and some types of cancer (Miller *et al.*, 2000).

As other cereals, sorghum also is an important source of bioactive compounds such as 3-deoxyanthocyanidins, tannins, vitamin E, carotenoids, and other antioxidants (Awika and Rooney 2004). These compounds reduce the damage caused by free radicals and thus promote benefits to human health (Valko *et al.*, 2007).

Antioxidant compounds of sorghum such as phenolic compounds, tannins and phytate provide protection against a variety of cancers mediated through antioxidation properties (Oboh 2006 ; Dykes and Rooney 2007; Goufo and Trindade 2014).

Tannins are known for their effects as antioxidants at the same time tannins are related with reducing protein digestibility (Duodu *et al*., 2002) that could be related to their ability for enzymes inhibition(Scalbert *et al*., 2000). However, like other cereals, sorghum grains need to be processed before human consumption, which may modify their chemical composition, and functional and nutritional value. Some processing employed to improve the organoleptic, nutritional and antioxidant properties of sorghum include soaking, cooking, germination and fermentation. Soaking, germination and pressure-cooking proved to be effective household strategies to reduce the levels of polyphenols and tannins in grains (Shweta *et al.,* 2010) that led to improvement for both product color and digestibility. The negative effect of sorghum tannins could be reduced by some procedures like alkali treatment. (Beta *et al.*1999). Some reports exist on antioxidant activity of fully processed products like cookies and bread containing sorghum bran, as well as extrusion cooked products (Awika *et al.*, 2003b).

The aim of our study was to study the effect of phenolics, tannins and phytic acid on the antioxidant activity of tow sorghum varieties and to determine the effect of different domestic process on these active compounds and on the antioxidant

MATERIALS AND METHODS

Materials

The samples of sorghum cultivars (*Sorghum bicolor (*L.*)Moench*); low tannin (Giza 15) and high tannin (Assiut 14) samples were obtained from Agriculture Research Center at Shandawel, Sohag, Egypt, Season 2013.

All chemicals used in this study obtained from Alpha Chemicals Company and Sigma – Aldrich Company.

Technological Methods

Sorghum grains soaking:

Sorghum seeds soaked by using distilled water (1:5 w/v) for 20h (Afify et al.,2011), and were soaked in various alkali solutions (2% KOH for 20h, 30% NH4OH for 20h and 2% NaOH for 10 h) the soaked seeds were maintained at 4°C. At the end of soaking period, seeds were washed with distilled water to remove residual soaking solution (Mulimani and Supriya 1994). After that,

the seeds were dried and milled using a blender home mill (Moulinex blenders) to get fine flour that was kept at – 4º C until analysis.

Germination of sorghum grains:

Sorghum seeds were germinated according to the method of (Elkhalifa and Bernhardt 2010). The sorghum grains (50 g) steeped in distilled water for 20h with two changes of water during the day. The wet sorghum is placed inside Petri dishes with cotton saturated with distilled water for 12h. The germinated grains were dried and milled in a blender home mill (Moulinex blenders) to obtain fine flour and kept at – 4º C until analysis.

Fermentation of sorghum flour:

Sorghum flour was fermented according to the traditional method practiced described by (El Tinay *et al.*, 1979 ; Chinedu *et al.*,2010). The flour was mixed with distilled water (45% w/v) in a plastic bucket covered with a lid, kneaded into dough, and allowed to ferment using yeast (*Saccharomyce scerevisiae*) and incubated at 40°C for 12h. The fermented dough was dried and ground to flour then stored in polyethylene bags at – 4º C prior to analysis.

Cooking of sorghum grains:

According to (Duodu *et al.*,2002) 50 g of sorghum seeds were boiled in distilled water (1: 5 w/v), for 2h with stirring that followed by submerring with distilled water, the cooked seeds were dried and milled to get fine flour then kept at – 4º C for further analysis.

Analytical Methods

Gross Chemical Composition:

Moisture, crude fat, crude protein, fiber ash and minerals (Magnesium, calcium, iron, and zanic) contents were determined according to (AOAC 2005). Total carbohydrate content of grains was calculated by difference.

Determination of phenolics content (PCs):

The total phenolic content in sorghum seeds or sorghum flour were extracted by using 0.3% acidified methanol water 60:40 in ultrasonic (Digital Ultrasonic Cleaner Bath, Item Code 100405-230, Model : 405) and were determined using the Folin-Ciocalteau method with slight modification (Taga *et al.*,1984). Using Folin-Ciocalteu method. Absorbance was measured at 750 nm against a blank. Gallic acid was applied to construct the standard curve (Spectrophotometer,SP-2000UV).

Tannin assay:

The content of tannin of sorghum before and after treatments were assayed by the vanillin–HCl method (Price *et al.*,1978) with modification. The results were prepared as catechin equivalents, i.e. amount of catechin (60mgcatechin / 100mL absolute methanol) which gives a colour intensity equivalent to that given by tannins after correcting for blank, the absorption was measured at 500 nm by spectrophotometer. Then tannin content (mg CE/100g) was calculated according to the equation:

$$\text{Catechin equivalent(Tannin)mg/100g} = \frac{\text{C} \times \text{volume extracted}}{\text{Dry sample weight (g)}} \times 100$$

Where C, concentration obtained from the calibration curve (mg/ mL).

Determination of phytate:

Phytate content was determined according to (AOAC 1990) with some modification by (Sorour *et al.*, 2003). Dowex® 50WX 8 hydrogen form hydrogen form, 200-400 mesh, and phytic acid was determined from the standard curve according to the equation:

Phytic acid (mg / 100g dw) = Phytate P × 3.446.

Determination of antioxidant activity :

The method described by (Hatano *et al.,*1988) with some modifications used for the determination of the antioxidant activity stable DPPH radical. The decrease in absorbance was measured at 517 nm against a blank without extract with a spectrophotometer. From a calibration curve obtained with different amounts of extract the $IC_{50}$was calculated. The IC_{50} was that concentration of an antioxidant which was required to quench 50% of the initial DPPH radicals under the experimental conditions given.

Statistical analysis:

Data analysis was performed using the SAS software (version 9.1, SAS Institute). Mean separation of data was carried out using least significant difference Duncan's test at 5% and probability levels.

RESULTS AND DISCUSSION

Chemical composition:

The results in Table (1) show the chemical composition of the studied sorghum grains cultivars. The content of crude protein, fat, fiber, ash and carbohydrates vary depending on the type of sorghum grains cultivar. Crude protein content ranged between (12.5-12.7%) while crude fat content was 3.7% for both varieties, ash content was (1.55 and 1.85%), moisture (7.55 and 6.86%), crude fiber (1.54 and 2.54%) and carbohydrates (80.51 and 76.43%) for Giza 15 and Assuit14 cultivar, respectively. Sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of the poorest people living in Africa, Asia and the semi-arid tropics worldwide (Klopfesntein and Hoseney 1995).

Table 1. Gross chemical composition of sorghum grains (on dry weight basis).

Sorghum Cultivars	Crude Protein %	Crud fat %	Ash %	Moisture %	fibers % Crude	Carbohydrate %	Minerals mg/100g						
							P	Ca	Mg	Na	K	Fe	Zn
Giza 15	12.7	3.7	1.55	7.55	1.54	80.51	314.5	197.8	123	1535.9	235.2	27	8.3
Assuit 14	12.5	3.7	1.85	6.86	2.54	76.43	414.3	229.4	138.1	1214.9	299.4	20	6.8

***Means of triplicates.**

Data of minerals average values revealed that sodium was the predominate element present in all grains. phosphorus, potassium, calcium, magnesium, ferric (iron) and zinc were present by sensible amount in all grains under investigation. Processed sorghum seeds or flour were found to be important sources of calories and proteins to

the vast majority of the population as well as for poultry and livestock (FAO 1997).

Total phenolics content.

The results presented in Tables (2 & 3) show the affect of different processing on phenolics content of sorghum cultivars. The phenolics contents were 178.28 and 825.36 mg gallic acid equivalent (GAE)/100g raw sample of Giza 15 (low sorghum tannin) and Assuit 14 (high tannin sorghum), respectively. The results of phenolic content are in the same trend with that of (Dykes 2008) who reported that total phenol contents of low and high sorghum tannin varied from 180 to 600 mg and from 800 to 2000 mg (GAE/100g),respectively. All soaking, germination and wet cooking procedures showed significant decreases for total phenolics content. The high reduction levels of phenolics content in sorghum(Giza15) grains were showed after soaking in water (43.4%) and in NH_4OH 30. However,these losses were more in sorghum (Assiut 14)grains varied from 61.0 to 73.0% after soaking in NaOH 2% and NH_4OH 30%,respectively. The reduction of phenolic compounds after soaking in different solution may be to loss with soaking solution. The results approved with (Afify *et al.*,2012),who found that the loss of total phenols ranged between 21.97% and 28.30 in sorghum after treatments and who reported that this loss was expected as the effect of soaking in the removing of the phenolic compounds. The reduction in phenolics as a result of alkali soaking might also be due to abstraction of hydrogen atoms and rearrangements of the structures, which affecting mainly the phenolic groups. (Kennedy *et al.,*1984; Cilliers and Singleton 1990). They also reported that, a minor part of the phenolic degradation after alkali soaking might have been caused by the possible opening of the C-ring and rearrangements to other products. Germination of Giza 15 and Assuit 14 for 38hreduced the phenolics content by 23.9 and 48.8% respectively. The decrease in phenolics content after germination could be the water-soluble compounds, that are found in the pericarp and testa leached out in the water (Awika and Rooney2004; Beta *et al.,*1999 ; Waniska 2000), or a new insoluble complexes with proteins could be formed. (Riedl and Hagerman 2001).Wet cooking for 2h led to total phenols losses of 32.3 and 32.2% in Giza 15 and Assuit 14respectively, of their initial values of sorghum grains. Total phenolic content significantly decreased during cooking (Table 3). The reactive hydroxyl groups of phenolic compounds may have reacted, or created insoluble complexes with food components such as protein or minerals, or even more polymerised into condensed phenolics that led to a decrease of measured phenolic hydroxylic groups. (Barroga *et al.,*1985). On the other hand, fermentation of Giza 15 flour for 12h increased phenolic compounds by 31.8% , but Assuit 14 was loss 3.8%. (Katina *et al.,*2007) explained the increase on total phenolic content after fermentation by the fact that metabolic activity of microbes during fermentation process could change the active compounds level, or could breakdown the structural of cereal cell walls that led to liberation and/or synthesis of numerous bioactive compounds during fermentation, enzymes such as amylases, xylanases and proteases derived from the grain and microbes contribute to the modification of grain composition (Katina *et al.,* 2007; Loponen *et al.,* 2004).

Table 2. Effect of soaking on phenolics content in sorghum grains.

Sorghum Cultivars	Phenolic content (mg GAE/100g dw).								
	Control T0	Soaking in water T1	% Reduction	Soaking in KOH 2% T2	% Reduction	Soaking in NH4OH 30 % T3	% Reduction	Soaking in NaOH 2%T4	% Reduction
Giza 15	178.28^{b}	100.89^{f}	43.41	171.01^{c}	4.08	102.20^{f}	42.70	168.51^{c}	5.50
Assuit 14	825.36^{a}	266.86^{g}	67.70	308.20^{f}	62.66	222.92^{h}	73.00	321.5^{e}	61.0

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

Table 3. Effect of germination, fermentation and wet cooking on phenolics content in sorghum.

Sorghum Cultivars	Phenolic content (mg GAE/100g dw).						
	Control T0	Germination for 38h T5	% Reduction	Fermentation for 12h T6	% Reduction	Wet cooking for 2h T7	% Reduction
Giza 15	178.28^{b}	135.7^{d}	23.9	234.89^{a}	31.8(+)	120.67^{e}	32.3
Assuit 14	825.36^{a}	426.07^{d}	48.4	793.796^{b}	3.8	486.08^{c}	41

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

Tannin content:

Tables (4&5) showed the effect of different process on sorghum tannins content. The tannin contents in Assuit 14(high tannin sorghum) had 1302.588 mg catechin equivalent (CE)/100g sample while Giza 15(non tannin sorghum) had 3.5793 mg catechin equivalent (CE)/100g. Sorghums with a pigmented testa have tannins (Awika and Rooney 2004 ;Hahn *et al.,*1984). Soaking in distilled water, KOH 2%, NH4OH 30%, NaOH 2%, germination, fermentation and cooked processes showed significant decrease in tannin content ($P \leq 0.05$). Soaking in all alkali solutions was effective in removing considerable amount of tannins in both high and low tannin sorghum varieties tested in this study that arrived to not detected amount for both sorghum varieties. The tannin content lost during sorghum soaking in distilled water, KOH 2%, NH4OH 30% and NaOH 2% was 100 and 97.7% ,100 and 100%;100 and 99.6% ; 100 and 100% for Giza 15 and Assiut 14 grains, respectively. The reduction of tannins after soaking in different solution may be due to removing of tannins by washing in the soaking solution. These results are in accordance with recorded by (Mulimani and Supriya 1994 ; Afify *et al.*,2012) who reported that the reduction of tannin by soaking may be due to the effect of soaking in the tannin removal. (Babar *et al.,*1988) observed that overnight soaking of jack bean seeds in 2% $NaHCO_3$ removed considerable amounts of tannic acid .

Table 4. Effect of soaking on tannins content in sorghum.

Sorghum Cultivars	Tannins content (mg catechin equivalent (CE)/100g)								
	Control T0	Soaking in water T1	% Reduction	Soaking in KOH 2 % T2	% Reduction	Soaking in NH4OH 30 % T3	% Reduction	Soaking in NaOH 2% T4	% Reduction
Giza 15	3.5793[a]	ND[b]		ND[b]		ND[b]		ND[b]	
Assuit 14	1302.588[a]	29.542[e]	97.7	ND[f]		5.194[f]	99	ND[f]	

ND= Not Detected

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

Also after fermentation, tannins were decreased in Giza 15 flour by 100%, and in Assuit 14 flour by 48.6%,wherase during grains germination for 38h. The reduction was 100% and 27.8%.respectively for both varieties. The wet cooking process also caused a significant reduction in tannin content for both varieties this reduction were 100% for Giza 15 and 91.9% for Assuit 14 of their initial values of sorghum grains. After fermentation, tannins were decreased may be as a result of microbial activity during fermentation like tannase enzyme and metabolic for microbial fermentation. These results approved with (Abdelhaleem *et al.,*2008 and Rahman and Osman 2011).These results agree with that of (Abdelhaleem *et al.,*2008) Tannin content reduced by 68.s0% and 74.5%, respectively for both (high and low tannin) cultivars using natural fermentation. While the reduction of tannins after cooking may be a result of complexes tanins with sorghum grain components such as protein. Prolamins (proteins with high proline content) bind strongly with sorghum tannins. (Emmambux and Taylor2003). In the aqueous media polymerization process of tannins occurs between some pigmentes such as anthocyanins and with tennin molecule. (Remy *et al.,*2000), vanillin-HCl method could not be able to detect such as those complexes.

Table 5. Effect of germination, fermentation and wet cooking on tannins contents in sorghum

Sorghum Cultivars	Tannins content (mg catechin equivalent (CE)/100g)						
	Control T0	Germination for 38h T5	% Reduction	Fermentation for 12h T6	% Reduction	Wet Cooking for 2h T7	% Reduction
Giza 15	3.5793[a]	ND[b]		ND[b]		ND[b]	
Assuit 14	1302.588[a]	940.288[b]	27.8	669.176[c]	48.6	105.83[d]	91.9

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

Phytic acid

The effect of soaking in water, KOH 2%, NH4OH 30%, NaOH 2%, germination, fermentation and cooking on Phytic acid content for Giza 15 and Assuit 14 sorghum is shown in Tables(6 & 7). Phytic acid content in raw sorghum Giza 15 and Assuit 14 were 160.88 and 215.5 mg/100, respectively. The high tannin sorghum (Assiut 14)occurred high reduction ability during all processing compared to low tannin sorghum(Giza 15). The ascending order of reduction being: water soaking (6.7; 16.5%); soaking in KOH 2% (7.8; 18.5%); soaking in NaOH 2 % (11.7; 18.8%); Soaking in NH4OH 30% (11.7; 23.6%); wet cooking (12.7; 25.9%); germination (13.6 ; 26.4%)and 36 h fermentation (15.6 ; 28%).

Table 6. Effect of soaking on phytate in sorghum.

Sorghum Cultivars	Phytate content (mg/ 100g)								
	Control T0	Soaking in water T1	% Reduction	Soaking in KOH 2 % T2	% Reduction	Soaking in NH4OH 30 % T3	% Reduction	Soaking in NaOH 2% T4	% Reduction
Giza 15	160.88[a]	150.11[b]	6.7	148.33[b]	7.8	142.123[c]	11.7	141.99[c]	11.7
Assuit 14	215.486[a]	179.97[b]	16.5	175.59[c]	18.5	164.552[d]	23.6	174.974[c]	18.8

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

Table 7. Effect of germination, fermentation and wet cooking on phytate in sorghum

Sorghum Cultivars	Phytate content (mg/ 100g)						
	Control T0	Germination for 38h T5	% Reduction	Fermentation for 12h T6	% Reduction	Wet Cooking for 2h T7	% Reduction
Giza 15	160.88[a]	138.94[dc]	13.6	135.72[d]	15.6	140.46[c]	12.7
Assuit 14	215.49[a]	158.59[e]	26.4	154.91[f]	28	159.63[e]	25.9

Values in the same row bearing different sidescripts are significantly ($p \leq 0.05$) different(as assessed by Duncan's multiple range test).

The reduction in phytic acid caused by soaking may be due to water solubilization of some phytic acid salts. Germination of sorghum resulted in a substantial reduction of phytic acid content. This substantial reduction of phytic acid content in germinated sorghum seeds may be due to the activity of the enzyme phytase, which hydrolyses phytic acid to inorganic phosphate and inositol (Eskin and Wiebe 1983). The reduction due to fermentation observed in this study was higher than other treatments. Enzymatic hydrolysis of phytic acid by endogenous phytase of sorghum and/or by phytase which was produced by the microorganism, may

account for most of the reduction of phytic acid during fermentation. The low pH of fermented product and temperature of fermentation may also provide favorable conditions for phytase activity. The reduction due to wet cooking may be caused by the limited activation of phytase enzyme during cooking and before its denaturation by heat. These observations indicate that enzymatic methods (malting and fermentation) for phytic acid reduction are more effective than physical methods (soaking and heating).

Antioxidant activity:

Results presented in Tables (8 & 9) show the examined processing effect on sorghum antioxidant activity. High tannin sorghums had higher antioxidant activity than non-tannin sorghum grains ($p<0.005$) assayed by DPPH). The DPPH radical scavenging activity of Giza15 was 22.147 % and of Assuit14 was 7.544 % for raw sorghum grains. All processing used in this study caused a significant decrease($p<0.005$) in the antioxidant activity for both sorghum varieties expect the fermentation and wet cooking of Giza 15 caused an increase of the DPPH radical scavenging activity of the sample. The DPPH scavenging activity in sorghum was decreased during soaking period. It was decreased of the original value after soaking for 20 h in distilled water, Giza15 (83%) Assuit 14 (434%), during soaking in KOH 2% for 20h Giza 15 was 47.5%. Assuit 14 was 446%, during soaking in NH_4OH 30% for 20h Giza 15 was 90.5%. Assuit 14 was 403%, during soaking inNaOH 2% for 10h Giza 15 was 135%. Assuit 14 was 327%, during germination for 12h Giza 15 was 21%. Assuit 14 was 122%, This decreased in antioxidants activity after soaking in different solution may be cause of loss some antioxidants components they found in the raw sorghum grain like that phenolic compounds, tannins, phytate, caused by leaching in soaking solution. The results approved with (Afify *et al.*,2012). The variance of the antioxidant activities of sorghum varieties could be related to the difference in their content of phenolics and to the differences of phenolics. In fact, several studies of structurally related phenolic compounds have appered differences in their antioxidant activity (Rice-Evans 1999 ; Bors 2001 ; Ramadan *et al.,*2012) they reported that, the decrease in antioxidants activity after germination may be as a cause of activation of some enzymes and other metabolic processes during germination who crak the antioxdants compounds, which leads to loss of antioxidants activity in sorghum grains. After fermentation for 12h Giza 15 and after wet cooking for 2h antioxidant activity were increase by 47% and 58%, respectively. While the decrease of antioxidant activity for these process of Assuit 14 was 5.4% and 6.4% ,respectively. Fermentation with *Saccharomyces cerevisiae* had a positive influence on DPPH inhibitory effect in Giza15 sorghum grains, this increased may be as a result of the increase of total phenolic compounds that was appeared in this study for Giza 15 variety by fermentation process or for the production of other compounds from yeast during fermentation such as ascorbic acid, carotenoids and tocopherols, These results approved with(Đorđević *et al.*,2010). On the other hand the increase of antioxidants activity after Giza 15 cooking (58%) may be result of the thermal processing known to alter the antioxidant profile and generate more antioxidants that contribute in antioxidant activity. Increase in antioxidant activity due to thermal processing, caused by the formation of Maillard browning pigments which enhanced the antioxidant activity approved with (Sharma *et al*.,2012).The decrease of antioxidant activity after Assiut 14 cooking may be cause of some antioxidants components had been lost in boiling water such as polyphenol compounds. These results are in the same line with that of (de Morais Cardoso *et al.*,2014).

Table 8. Effect of soaking on antioxidant activity in sorghum.

Sorghum Cultivars	50% DPPH radical scavenging activity (EC 50 mg)								
	Control T0	Soaking in water T1	% Reduction	Soaking in KOH 2 % T2	% Reduction	Soaking in NH4OH 30 % T3	% Reduction	Soaking in NaOH 2 % T4	% Reduction
Giza 15	22.15^{f}	40.55^{c}	83	32.66^{d}	47.5	42.19^{b}	90.5	52.04^{a}	135
Assuit 14	7.544^{h}	40.334^{b}	434	41.233^{a}	446	37.95^{c}	403	32.232^{d}	327

Values in the same row bearing different side scripts are significantly ($p\leq 0.05$) different(as assessed by Duncan's multiple range test).

Table 9. Effect of germination, fermentation and wet cooking on antioxidant activity in sorghum.

Sorghum Cultivars	50% DPPH radical scavenging activity (EC 50 mg)						
	Control T0	Germination for 38h T5	% Reduction	Fermentation for 12h T6	% Reduction	Wet Cooking for 2h T7	% Reduction
Giza 15	22.15^{f}	26.79^{e}	21	11.745^{g}	47 (+)	9.305^{h}	58 (+)
Assuit 14	7.544^{h}	16.74^{e}	122	7.95^{g}	5.4	8.026^{f}	6.4

Values in the same row bearing different side scripts are significantly ($p\leq 0.05$) different(as assessed by Duncan's multiple range test).

Relationship between antioxidant activities and phenolics content, tannin and phytic acid content.

phenolic acids, flavonoids and condensed tannins are the formsof phenolics in sorghum (Serna-Saldivar and Rooney1995). The pigmented sorghum which have (proanthocyanidins) condensed tannins(Waniska and Rooney2000). In this study a clear relation observed among phenolic, tannin and phytic acid content and the antioxidant activity, however the sorghum variety(Assiut14) with high content of total phenolic content, tannin content and phytic acid content showed higher antioxidant activity by DPPH by 65% than sorghum variety (Giza15)low in these active

compounds. It suggested that some groups of phenols were strong antioxidants, like condensed tannins. This relation between these active compounds and antioxidant capacity indicated that they could used as indicators for in sorghum as antioxidant activity. High antioxidant activity of black pericarp sorghums were correlated with the high pigment content(Awika *et al.,*2004). Tannins in sorghum are of condensed type, sorghums containing tannins have higher antioxidant capacity than, most non-tannin sorghums because it is able to bind the free radicals Awika *et al.,*2004). Different genotypes of sorghum with a wide range of phenol profiles have been known as strong free radical scavengers. (Dykes 2008).

(Hagerman *et al.*,1998) found that The efficiency of sorghum tannin was 15 to 30 times higher than simple phenol concerning peroxyl radical quenching. The antioxidant activity of the colorless phenols may be detrmined by DPPH assay because their reaction with DPPH is slow. Hence it is important to consider color of sorghum as an indicator of antioxidant activity. It is obvious also that in this study, in spite of the clear reduction of phenolic compounds, tannin and phytic acid content after some treatments (T6 and T7) the sorghum grain showed higher antioxidant activity than control. In fact, total phenol content of the examined sorghum varieties related with their DPPH antioxidant activity in some mean, which indicated that reducing possibility was not the only mechanism for the antioxidant activity of sorghum seeds, that is why samples with low concentration of total phenol, tannin or phytic acid may have remarkable activity as antioxidant. Different results of the antioxidant activity could be measured by different methods rely on different mechanisms (Sun and Ho 2005).This information may be helpful to encourage the utilization of sorghum grain in functional foods related industries.

CONCLUSION

All the studied process were able to change the content of total phenolics ,tannin ,phytate and the antioxidant activity and these process had positive or negative effect. Taking together the results suggest the antioxidant activity of these tow sorghum varieties is affected by their content of phenolic compounds, phytic acid, however the sorghum (Assiut14) variety with high content of total phenolic content, tannin content and phytic acid content showed higher antioxidant activity by DPPH than sorghum (Giza15) variety low in these active compounds.

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التغيرات التى تحدث للمركبات الفينولية والتانينات والنشاط المضاد للأكسدة لصنفين من الذرة الرفيعة نتيجة لبعض المعاملات التصنيعية

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يعتبر محصول الذرة الرفيعة من المحاصيل الغنية بالمواد الفعالة حيويا مثل الفينولات والتانينات والفيتات حيث تعتبر الفينولات من اكثر المصادر لمضادات الاكسدة فى الاغذية ، نظرا لطبيعة هذه المركبات القابلة للتفاعل والتغير السريع فانها تتأثر بشدة بالمعاملات التصنيعية وبالتالى يتأثر نشاطها المضاد للأكسدة لذلك يهدف هذا البحث لدراسة تأثير بعض المعاملات التصنيعية مثل النقع فى المحاليل المختلفة وكذلك معاملات الانبات والتخمر والطبخ العادى على محتوى المواد الفينولية والتانينات والفيتات بالاضافة الى النشاط المضاد للأكسدة فى بذور صنفين من الذرة الرفيعة (اسيوط ١٤ مرتفع التانينات , جيزة ١٥ منخفض التانينات) النامية جنوب مصر. واظهرت النتائج ان جميع معاملات النقع فى (الماء المقطر , هيدروكسيد البوتاسيو ٢% هيدروكسيد الصوديوم ٢% وهيدروكسيد الامونيوم ٣٠%) بالاضافة للانبات قد أدت الى خفض محتوى كل من التانينات والفيتات والبولى فينولات وبالتبعية النشاط المضاد للأكسدة لكلا الصنفين محل الدراسة على الجانب الآخر ادت معاملة التخمر بالخميرة الى زيادة محتوى الفينولات والنشاط المضاد للأكسدة فى دقيق الصنف جيزة ١٥ وفى نفس الوقت سببت نقص محتوى كل من الفيتات والتانينات ومن ناحية أخرى تبين أن معاملة الطبخ لبذور الصنف (جيزة ١٥) قد أدت الى نقص محتوى كل من الفيتات والتانينات والفينولات ولكنها سببت زيادة النشاط المضاد للأكسدة بحوالى ٥٨% . اكدت النتائج ان الصنف اسيوط ١٤ كان افضل فى المحتوى من هذه المواد الفعالة وكذلك اعلى فى النشاط المضاد للأكسدة من الصنف جيزة ١٥ وبشكل عام فان ان جميع المعاملات التصنيعية المستخدمة فى الدراسة كان لها تأثير بشكل ما على اصناف الذرة الرفيعة محل الدراسة لكن هذا التأثير قد اختلف باختلاف الاصناف وباختلاف محتواها من المواد الفعالة المدروسة.