

CHANGES IN TOTAL PHENOLICS AND DPPH[•] SCAVENGING ACTIVITY DURING DOMESTIC PROCESSING IN SOME CEREAL GRAINS

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Abstract

The aim of this work to investigate the changes in total phenolic content and free radical scavenging abilities against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) during soaking and germination of three cereal grains; wheat (Sids 1), corn (H310 White) and sorghum (Giza 15) grown in Sohag Governorate, Egypt. Total phenolic compounds in wheat, sorghum and corn were 381.4, 288.5 and 204 mg/100g, respectively. Soaking and germination processes showed significant decrease in total phenolic compounds and antioxidant activity. Losses of total phenols during soaking for 12 hour were 15.18, 14.9 and 5.96 % of its initial values in raw materials, in wheat, sorghum and corn, respectively. Germination process for 48 hour led to decrement of total phenols ranged from 39.3 - 43.95 % in investigated cereal grains of its initial values in raw materials. Sorghum had higher antioxidant activity than wheat and corn, it was 37.28, 34.44, 33.05% in raw materials, respectively. DPPH radical scavenging activity decreased during soaking and germination processes. Losses was 6.85, 13.49 and 16.84% during soaking while in Germination DPPH activity decreased to 30.79, 21.6, 20.12% of its initial values after germination for 48 hour in wheat, corn and sorghum, respectively. Gross chemical composition for the whole grains was studied.

Keywords: total phenolic, wheat, DPPH assay, soaking, germination

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1. INTRODUCTION

Grains in particular, are a major source of antioxidants in our daily diets. The main antioxidative components in grain are classified as phenolic compounds such as anthocyanins, tannins, and ferulic acid, and other substances (Tome' *et al.*, 2004). Whole grain products are recommended for healthy diets as being recognized sources of dietary fiber and antioxidant substances (Ragaee *et al.*, 2006).

Consumption of foods containing rich antioxidant activity substances, such as grains, vegetables, and fruits, may prevent many diseases and promote good health (Willet, 1994 and Temple, 2000).

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).

The process of cereal seeds germination has been used for centuries for the purpose of softening the kernel structure, improving its

nutritional value, and reducing anti-nutritional effects. In fact, the germination process is also one of methods used to improve the functionality of oat seed protein (Kaukovirta-Norja, *et al.*, 2004). In general soaking period was reported to have pronounced effects on the vitamin levels and anti-nutritional factors present in natural foods (Fadahunsi, 2009).

Radical scavenging is the main mechanism by which antioxidants act in foods. Several methods have been developed including 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (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 (Kumar *et al.*, 2011).

The aim of this work to investigate the changes in total phenolics content and free radical scavenging abilities against the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH assay)

during soaking and germination of three cereal grains cultivars.

2. MATERIAL AND METHODS

Materials:

Grains: Three cereal crops, including wheat (*Triticum aestivum* L.) Sids 1, corn (*Zea mays* L.) Hybrid 310 and sorghum (*Sorghum bicolor* L.) Giza 15 collected from Sohag Governorate, Egypt.

Chemicals: DPPH (2, 2-diphenyl-1-picrylhydrazyl), 6-hydroxy-2, 5, 7, Folin-Ciocalteu reagent, acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, ethanol, hexane, and ethyl acetate were HPLC grade.

Technological processes:

Soaking: Grains samples were soaked in water (1:5, w/v) for 12 hr at room temperature, water was changed every 6 hr (Abdel-Gawad, 1993).

Germination: Soaked grains samples were germinated in betry dishes coating with moistened filter paper at room temperature for 12, 24, 36 and 48 hr (Youssef *et al.*, 1987).

Milling: All soaked and germinated grains were dried then conditioned by rising its moisture content up to 14 %, then left for 24 hr as tempering time. Milling was run in a Buhler experimental mill (type 212) by progressively receiving the whole flour (Sorour, M. A 1997).

Analytical methods:

The moisture content was measured in an air oven at 70°C, crude fat and ash content were determined according to AOAC (2000).

The nitrogen content was analyzed using the standard Kjeldahl procedure (AOAC, 2000). Protein content was determined by multiplying the nitrogen content by 6.25 and 5.7 food commodities nitrogen/protein factors according to Merrill and Watt (1973), Mosse (1990), Chang (1998). Total carbohydrate content of grains has been calculated by difference as the following formula:

$$100 - \% \text{ of } [protein + fat + ash] \text{ in grains}$$

It should be clear that carbohydrate estimated in this fashion includes fibre, as well as some components that are not strictly speaking

carbohydrate, e.g. organic acids (Merrill and Watt, 1973).

Potassium, calcium, iron, and zanic were determined using Perkin Elmer Atomic Absorption Spectro-photometer 2380. Phosphorus content was determined by Specter-photometer according to AOAC (1980).

Extraction of total antioxidants:

Ten grams of dry sample was ground fine using a coffee grinder then weighed and transferred into a test tube (25 x 150 mm). For extraction; 40 mL of methanol was added in a test tube and vortexes to mix with the sample well triplicate. The test tubes were capped and placed in a 60°C water bath for 20 min. The tubes were vortexed twice during the incubation. Then, the solvent layer from each tube was separated by centrifugation at 2000 rpms for 15 min.

The solvent supernatant was transferred to clean, previously weighed and labeled test tubes. The residue was mixed with 20 mL of the same solvent again and vortexed. The solvent supernatant was combined with the previous one. The tube with supernatant was then placed in a vacuum centrifuge evaporator to remove solvent. The dried extract in the tube was weighed to measure the extraction yield of the samples. All samples were kept at -20°C prior to testing (Oufnac, 2006).

Determination of total phenolic compound content:

The total phenolic content of whole flour extract was determined using Folin-Ciocalteu reagent (Velioglu *et al.*, 1998). Folin-Ciocalteu reagent was diluted 10 times with deionized water. Dried grains sample extract (0.02 g) was re-dissolved in 1 ml methanol and 0.1 mL of this sample extract solution was mixed with 0.75 ml diluted Folin-Ciocalteu reagent. The reaction solution was left at room temperature for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g/L) was added. The mixture was incubated at room temperature for 90 min and filtered through a 0.45 µm syringe filter. The absorbance of the solution was determined at 750 nm using a UV-Visible SpectraMax Plus384 Spectro-

photometer. The test for each extract was triplicated. The averaged absorbance was used in calculation. The total phenolics content was expressed as μg Gallic acid Equivalent / gram dry grain sample using gallic acid standard curve.

DPPH radical scavenging determination:

The whole flour extract solution for the DPPH test was prepared by re dissolving 0.2 g of each dried extract in 10 mL methanol. The concentration of DPPH solution was 0.025 g in 1000 mL of methanol. 2 mL of the DPPH solution was mixed with 100 μL of the sample extract/methanol solution and transferred to a cuvette. The reaction solution was monitored at 517 nm, after an incubation period of 30 min at room temperature, using a Spectro-photometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

$$\text{Inhibition\%} = \frac{(\text{Abst}=0 \text{ min} - \text{Abst}=30 \text{ min}) / \text{Abst}=0 \text{ min} \times 100}$$

Where $\text{Abst}=0 \text{ min}$ was the absorbance of DPPH at zero time and $\text{Abst}=30 \text{ min}$ was the absorbance of DPPH after 30 min of incubation.

Statistical analysis:

All soaking and germination processes were performed in triplicate. The data were expressed as mean \pm standard deviation. The analyses were processed using Excel 2003 software.

3. RESULTS AND DISCUSSION

Chemical composition:

The results in Table (1) showed the chemical composition and minerals of studied cereal grains cultivars. The data revealed that corn grain has the highest level of fat content, while wheat grain has highest protein content. The levels of protein, fat, ash and carbohydrates vary depending on the type of grain cultivar. Data of the average values of minerals revealed that potassium and phosphorus were the predominate elements present in all grains under investigation. The chemical composition

of the studied grains has the same trend with that showed in the food composition table for Egypt (FCTE, 2006).

Effect of soaking and germination on phenolic compounds content:

The results presented in Table (2) and Fig. (1) showed the effect of soaking in phenolic contents in wheat, corn and sorghum. Wheat has higher phenolic content than corn and sorghum grains. Phenolic contents of wheat, corn and sorghum raw samples were 381.4, 288.5, and 204.0 mg (GAE)/100g, respectively. The phenolic contents has decreased to 84.82% of its initial value in the wheat grains after soaking for 12 hr, while it was 94.04 and 85.10% of their initial values of corn and sorghum grains. These results lower than those obtained by Glennie (1983) who reported that concentration of total phenolics of white sorghum ranged from 80 to 100 mg/100 g. Ysang (2009) reported that the total phenols content of non-tanin sorghums ranged from 90-1820 mg gallic acid equivalent (GAE)/100 g sample. Afify *et al.* (2012) found that the losses of total phenols ranged between 21.97% and 28.30 in sorghum after soaking.

Several possible reasons have been suggested for reductions in polyphenol concentrations upon soaking.

Losses may result simply from leaching into the soak water (Deshpande *et al.*, 1982; Igbedioh *et al.*, 1995): Losses may also be attributed to decreases in extractability, as lower molecular weight phenolic compounds polymerize, thus becoming insoluble in water (Deshpande *et al.*, 1982).

Other investigators (Bravo, 1998) have attributed the losses to binding of polyphenols with other organic substances such as carbohydrate or protein. Alternatively, during the period of soaking, the enzyme polyphenol oxidase may be activated, resulting in degradation and consequent losses of polyphenols (Jood *et al.*, 1987; Jood *et al.*, 1998 and Saxena *et al.*, 2003). This reduction of total phenols, after soaking may be attributing to leaching of phenols into the soaking medium (Afify *et al.*, 2012).

Table (1) Gross chemical composition of wheat, corn and sorghum (on dry weight basis).

Cultivars	Protein %	Fat %	Carbohy- -drate %	Ash %	Minerals mg/100g				
					Fe	P	Ca	K	Zn
Wheat Sids1	12.73	0.57	85.13	1.57	8.04	307.00	86.24	127.40	1.18
Sorghum Giza15	12.29	1.43	85.76	0.52	9.92	118.50	56.84	96.84	4.45
Corn H310	9.56	1.99	86.68	1.77	6.66	273.67	68.60	154.84	2.94

The results approved with Nwosu (2010) showed that this reduction was expected as soaking helped in the removal of the soluble antinutrients like tannins. Akillioglu (2010) reported that the result of longer soaking duration leading to more phenolics diffuse outside.

The effect of germination on phenolic contents of wheat, corn and sorghum are shown in Table (2) and Fig. 1. Phenolic content in wheat was decreased gradually during germination period. The total phenolics content in wheat grains was decreased to 76.14, 69.66, and 58.91% of its initial value of control after 24, 36, and 48 hr of germination, respectively. In corn grains, the

phenolic content decreased after 24, 36, and 48 hr of germination to 85.09, 65.96, and 56.05% of its control value, respectively. While in sorghum the phenolic content decreased to 72, 70.43, and 60.07% of its value in the raw grains after the same germination periods, respectively. Decreases in the polyphenol contents during germination have been reported by several authors for pulses (Mc-Grath *et al.*, 1982; Rao and Deosthale, 1982 and Osuntogun *et al.*, 1989). These decreases may be attributed to increased the activity of polyphenol oxidase and other catabolic enzymes as observed by (Kruger, 1976) for wheat.

Table 2. Effect of soaking and germination process on phenolic content *mg (GAE)/100g* in wheat, corn and sorghum (Mean \pm SD)

Samples	Control	Soaking	Germination			
		12 hr	12 hr	24 hr	36 hr	48 hr
Wheat	381.4 \pm 6.9	323.5 \pm 10.61	338.6 \pm 11.27	290.4 \pm 3.66	265.7 \pm 5.51	224.7 \pm 9.77
Corn	288.5 \pm 7.07	271.3 \pm 4.24	290.6 \pm 3.09	245.5 \pm 9.52	190.3 \pm 5.1	161.7 \pm 6.58
Sorghum	204 \pm 11.31	173.6 \pm 2.55	159.4 \pm 2.79	149.6 \pm 6.93	143.5 \pm 5.46	130.7 \pm 8.78

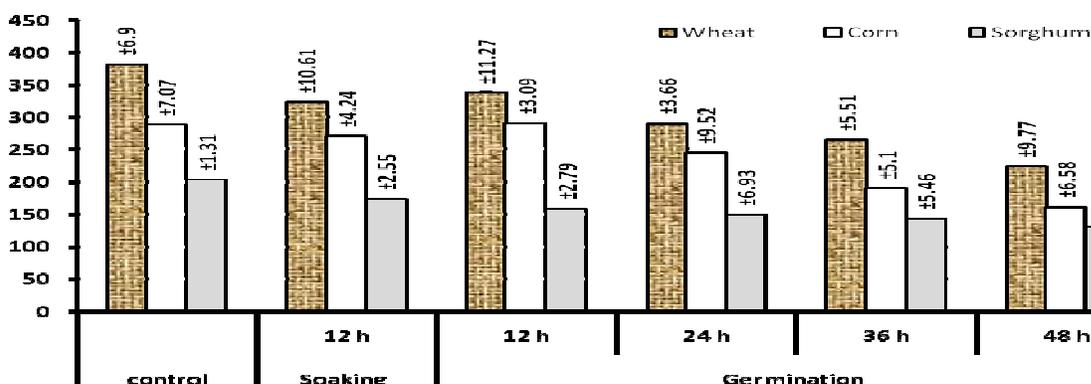


Fig. 1. Effect of soaking and germination time in phenolic content *mg (GAE)/100g* in Wheat, Corn and Sorghum

Table 3. Effect of soaking and germination process in % DPPH scavenging activity in wheat, corn and orghum

Samples	Control	Soaking		Germination		
		12 hr	12 hr	24 hr	36 hr	48 hr
Wheat	34.44±0.8	32.08±3.3	31.89±3.54	30.74±3.8	30.63±1.88	27.0±1.34
Corn	33.05±1.94	28.59±1.41	30.11±3.83	28.16±4.4	26.86±2.45	26.4±1.83
Sorghum	37.28±0.89	31.00±3.58	31.09±1.11	28.41±1.63	27.56±1.31	25.8±1.5

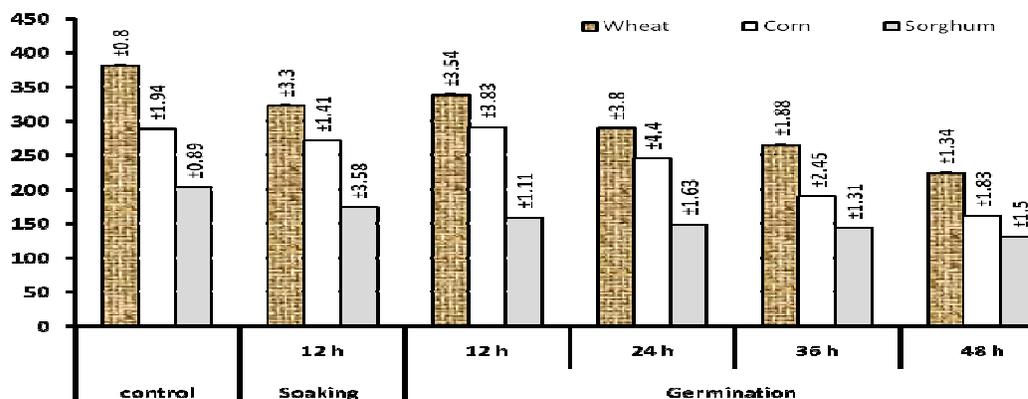


Fig. (2) Effect of germination time in % DPPH scavenging activity in wheat, corn and sorghum

Effect of soaking and germination on antioxidant activity:

The results presented in Table (3) and Fig. 2 shows the effect of soaking in free radical scavenging activity in wheat, corn and sorghum grains. In raw samples, sorghum has higher antioxidant activity than wheat and corn grains. DPPH radicals scavenging activity were 37.28, 34.44, and 33.05% for raw sorghum, wheat, and corn grains, respectively. DPPH scavenging activity in wheat, corn and sorghum was decreased during soaking period. It was decreased with about 6.85, 13.49 and 16.84% of the original value after soaking for 12 hr for wheat, corn and sorghum respectively. These results are in the same line that of Afify *et al.* (2012) they reported that DPPH scavenging activity in raw sorghum varied from 21.72 to 27.69%.

The DPPH radical scavenging activity in the studied grains was gradually decreased during germination period (Table 3 and Fig 2). these values were decreased by 21.60, 20.12 and 30.79% of its initial values in wheat, corn and

sorghum, respectively. Results revealed that the highest losses were recorded in sorghum grains compared with other grains. These results are in the line with these reported by Bolívar *et al.* (2010). They found that antioxidant activity for wheat grains decreased after germinated for 7 days. Donkor, *et al.* (2012) reported that the radical scavenging activities of the phenolic extracts were between 13% and 73% for non-germinated and 14% and 53% for germinated of seven selected commercially important grains including wheat and sorghum. In contrary, López-Amorós, *et al.* (2006) found that peas and beans undergo a significant increase in antioxidant activity after germination, whereas lentils show a decrease.

4. CONCLUSIONS

Soaking and germination processes showed significant decrease in total phenolic compounds and antioxidant activity. Losses of total phenols during soaking for 12 hr ranged from 5.96-15.18 % of its initial values of raw

materials; in wheat, sorghum and corn. Germination process for 48 hr led to decrement of total phenols ranged from 39.3 - 43.95 % in investigated cereal grains of its initial values in raw grains. DPPH radical scavenging activity decreased during soaking and germination processes.

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