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CHANGES IN TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY DURING DOMESTIC PROCESSING OF SOME CEREAL GRAINS

Ramadan, B.R.[1]; M. A. Sorour[2] and M. A. M. Kelany[2]
[1]Food Sci. & Tech. Dept., Fac. of Agric., Assiut University, Egypt.
[2]Food Sci. Dept., Fac. of Agric., Sohag University, Egypt

ABSTRACT

The aim of this work was to investigate the changes in total phenolic compounds 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). Total phenolic compounds in wheat, sorghum and corn raw grains were 381.4, 288.5 and 204 mg/100g, respectively. Soaking and germination processes showed significant decrease in total phenolic compounds. Losses of total phenols during soaking for 12 hr were 15.18, 14.9 and 5.96 % of its initial values in wheat, sorghum and corn raw materials, respectively. Germination process for 48 hr led to decrement of total phenols ranged from 39.3 - 43.95 % of its initial values in studied raw cereal grains. The DPPH radical scavenging activity decreased during soaking and germination processes of cereal grains.

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). Recently, El-Refai et al (2012) found that germination process had positive effect to improve the bioactive compounds content as total phenolic anf flavonoids of barely and oat grains. 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 was 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 cultivars, namely wheat *(Triticum aestivum L.*) Sids 1, corn *(Zea maiys L.)* Hybrid 310 and sorghum *(Sorghum bicolor L.)* Giza 15 collected from Sohag Governorate, Egypt.

MATERIALS AND METHODS

Materials:

Grains: Three cereal grains, including wheat *(Triticum aestivum L.*) Sids 1, corn *(Zea maiys 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-Ciocalteau 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) at room temperature for 12 hr, water was changed every 6 hr (Abdel-Gawad, 1993).

Germination: Soaked grains samples were geminated 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:

Moisture, protein, fat and ash contents was determined according to AOAC (2000).

Total carbohydrate content of grains was calculated by difference.

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

Extraction of total antioxidants:

Ten grams of dry sample were ground fine using a coffee grinder, then weighed and transferred into a test tube (25 x 150 mm). For extraction; 40 mL of methanol were 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 kepte 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-Ciocalteau reagent (Velioglu *et al.,* 1998).

DPPH radical scavenging determination:

The antioxidant activity of phenolic compounds was measured in terms of hydrogen donating or scavenging ability, using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) according to the colorimetric method described by Brand-Williams et al (1995).

Statistical analysis:

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

RESULTS AND DISCUSSION

Gross chemical composition of studied grains:

The results in Table (1) show the chemical composition and minerals of studied cereal grains cultivars. The data reveale 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).

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

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

Effect of soaking and germination on phenolic compounds content:

The results presented in Table (2) and Fig. (1) show the effect of soaking as phenolic contents of wheat, corn and sorghum. Wheat has higher phenolic content those 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 phenolic 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). 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 compounds 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 increase the activity of polyphenol oxidase and other catabolic enzymes as observed by (Kruger, 1976) for wheat. On contrary; El-Refai et al (2012) found that increasing germination period of barely and oats grains increased total phenolic compounds, antioxidants activity and flavonoids content.

Effect of soaking and germination on antioxidant activity:

The results presented in Table (3) and Fig. 2 show the effect of soaking as free radical scavenging activity in wheat, corn and sorghum grains. In raw samples, sorghum has higher antioxidant activity than that of 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 of 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 with those of Afify *et al.*

(2012) they reported that DPPH scavenging activity in raw sorghum varied from 21.72 to 27.69%.

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

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

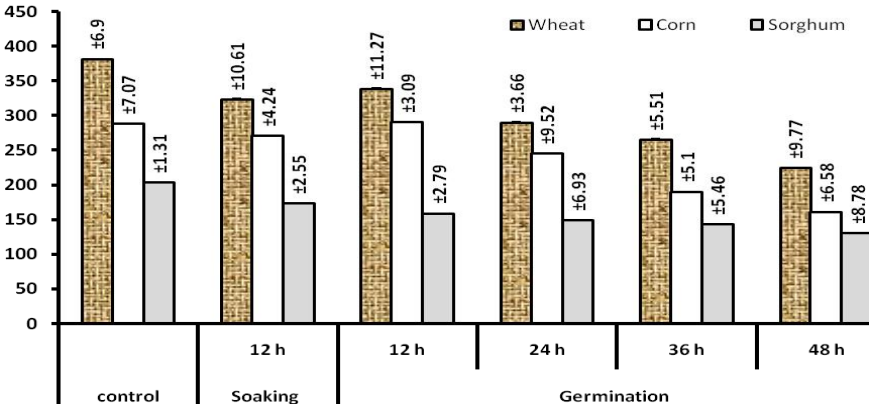

Fig. (1): Effect of soaking and germination time in phenolic content *mg (GAE)/100g* in Wheat, Corn and Sorghum.

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, Lo´pez-Amoro´s, *et al.* (2006) found that peas and beans undergo a significant increase in antioxidant activity after germination, and El-Refai et al (2012) found also that the antioxidant activity was increased by increasing the germination period of barely and oats grains.

Table (3): Effect of soaking and germination process in %DPPH scavenging activity in wheat, corn and sorghum.

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

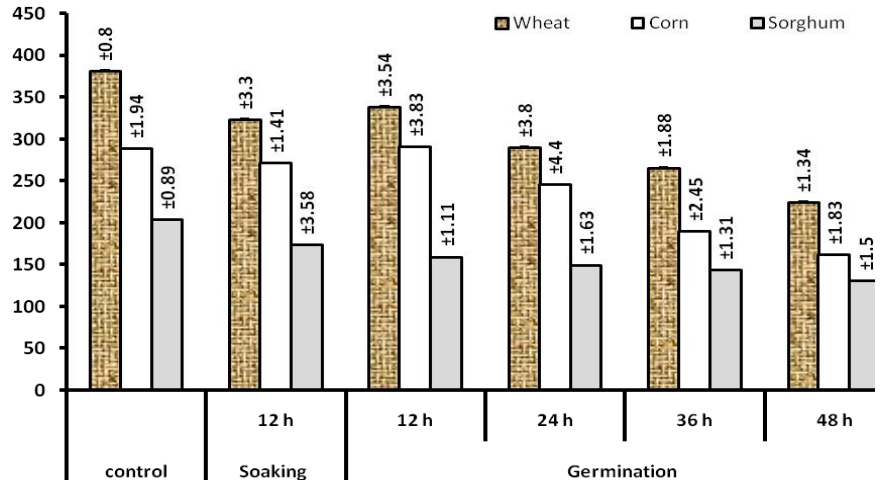

Fig. (2): Effect of germination time in % DPPH scavenging activity in wheat, corn and sorghum.

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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التغير فى محتوى الفينولات الكلية والنشاط المضاد للأكسدة اثناء معاملات التصنيع لبعض بذور الحبوب الغذائية

بلبل رمضان رمضان[1] ، محمد عبد الحميد سرور[2] و محمد على محمود كيلانى[2]

[1]قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة أسيوط

[2]قسم علوم الأغذية - كلية الزراعة جامعة سوهاج

يهدف هذا البحث إلي دراسة التغير فى المحتوى الكلى للفينولات وكذلك النشاط المضاد للأكسدة بإستخدم دليل DPPH خلال عمليتى النقع والإنبات لبذور ثلاثة من محاصيل الحبوب : قمح (سدس 1)، ذرة شامية (هجين ثلاثى 310 أبيض، ذرة رفيعة (جيزة 15). وكان المحتوى الكلى للفينولات 381.4 و 288.5 و 204 مليجرام/100 جم من بذور القمح، الذرة الشامية والذرة الرفيعة الخام على التوالى. وقد أظهرت عمليات النقع والإنبات تناقص معنوي فى المحتوى الكلى للفينولات. وقد كان الفقد فى المحتوى الكلى للفينولات بعد النقع لمدة 12 ساعة 15.18، 14.9 و 5.96 % من قيمتها قبل النقع لكل من القمح، الذرة الرفيعة والذرة الشامية على التوالى. وقد أدت عملية الإنبات لمدة 48 ساعة إلى تناقص فى محتوى الفينولات الكلية تراوحت نسبتة بين 39.3 الى 43.95 % من قيمتة قبل الإنبات للحبوب تحت الدراسة. ولقد أتضح تناقص قيم النشاط المضاد للأكسدة خلال عمليتي النقع والإنبات للحبوب بإستخدام دليل DPPH.

بتحكيم البحث

أ.د / احمد عبد العزيز الرفاعى	**كلية الزراعة – جامعة المنصورة**
أ.د / عبد الله صالح عبد الجواد	**كلية الزراعة – جامعة اسيوط**