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ORIGINAL ARTICLE

Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran

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Abstract Two wheat varieties grown in Upper and Delta Egypt were compared for their total phenolic content and antioxidant activities. Three solvent systems have been used to prepare the antioxidant extracts from whole wheat and its bran fraction. The three solvent systems included 50% acetone (*v*/*v*), 70% methanol (*v*/*v*) and 70% ethanol (*v*/*v*). Antioxidant activities were tested using DPPH radical scavenging activity and total flavonoid content. The results showed that the extraction solvents and wheat varieties significantly altered the total phenolics and antioxidant activity of whole wheat and bran, and 50% acetone is a recommended solvent for extracting phenolic compounds from the tested wheat and bran. Also data indicated that the bran fraction was rich in total phenolic content and high power for radical scavenging activity than whole wheat. These results showed that wheat bran could be considered as a potential source of antioxidant agent. Therefore, durum wheat variety (Beni-suef-3) showed high level of total phenol contact and antioxidant properties in bran fraction than common wheat variety (Gemiza-9). So, whole meal wheat products maximize health benefits and strongly recommended for use in food processing.

Introduction

Common wheat (*Triticum aestivum* L.) is an important component of the human diet, and is used in the production of many food products, including bread, noodles, steamed bread, and cakes, providing energy based on the high contents of protein and carbohydrate. Wheat products contain high levels of antioxidants, which confer protection against cancer and heart diseases mostly coming from phenolics (Adom et al., 2005; Ward et al., 2008). Synthetic antioxidants, such as butylated

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hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT), which are suspected of being carcinogenic and causing liver damage (Ratnam et al., 2006). It is believed that an increased intake of food, which is rich in natural antioxidants, is associated with a lower risk of degenerative diseases, particularly cardiovascular diseases and cancer (Perez-Jimenez et al., 2008).

In wheat grain, most of the phenolic compounds are located in the bran, which constitutes the outermost parts of the grain. Traditionally, the milling of the wheat grain aimed at removing the bran or outer layers of the grain to obtain the refined white flour. Nowadays, it is well known that the outer layers contain phytochemicals with potential bioactivities, suggesting the use of wheat grain as whole instead of refined (Hemery et al., 2007). On the other hand, phenolic compounds are secondary metabolites which synthesize in plants. They possess biological prosperities such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Han et al., 2007). Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2008). The total flavonoid content of different solvent extracts from the studied wheat cultivars was measured using aluminum chloride colorimetric method (Hung and Morita 2008). The total flavonoid content was expressed as the rutin equivalent.

Antioxidant rich extracts have been obtained from wheat using various solvents including water, ethanol, methanol and an aqueous ethanol solution (Vaher et al., 2010; Zielinski and Kozowska, 2000). It is noted that a solvent system for extraction is selected according to the purpose of extraction such as preparation or analysis. Also, it was chosen according to the nature of interested components, the physicochemical properties of the matrix, the availability of reagents and equipments, cost, and safety concerns. Absolute ethanol and 50% acetone have been used to prepare antioxidant extracts from wheat and wheat-based cereal products and 70% methanol widely accepted solvents for extracting phenolic compounds (Yu et al., 2002).

The aim of the present study was determination of the phenolic content and antioxidant activity in the two varieties of whole wheat and bran fractions extracted by different solvent systems, as well as study the effect of growing locations on antioxidant activities.

Materials and methods

Materials

Egyptian wheat cultivars originating from two different eco-geographic areas were procured from Wheat Department Agriculture Research Centre. The two varieties were grown in conventional conditions. The cultivars were selected to represent the range of place of origin, i.e., Upper and Delta Egypt (Table 1).

Methods

Extraction of wheat antioxidants

The extraction of antioxidants assay was conducted according to (Moore et al., 2006). Two grams of whole wheat and bran samples were ground to 80 mesh and extracted for 15 h with 20 ml of 50% acetone (*v*/*v*), 70% ethanol (*v*/*v*) and 70% methanol (*v*/*v*) at ambient temperature, respectively. The antioxidant extracts were kept in the dark until further assays.

Total phenolics content

The total phenolic contents in the wheat extracts were estimated using Folin–Ciocalteu reagent (Yu et al., 2003). In brief, the reaction mixture contained 50 μl of whole and bran extract, 250 μl of freshly prepared Folin–Ciocalteu reagent, 0.75 ml of 20% sodium carbonate, and 3 ml of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard.

DPPH radical scavenging activity assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacities of wheat extracts were estimated by the reduction of the reaction color between DPPH solution and sample extracts as previously described by Huang et al. (2005). A final concentration of DPPH solution used was 0.15 mM for wheat phenolic extracts instead of 0.075 mM for wheat extracts. DPPH solution (3.9 ml) was mixed with sample solution (0.1 ml). The mixture was kept in the dark at ambient temperature. The absorbance of the mixtures was recorded at 515 nm for exactly 30 min. Blank was made from 3.9 ml of DPPH and 0.1 ml methanol and measured absorbance at $t = 0$. The scavenging of DPPH was calculated according to the following equation (Liyana-Pathiran and Shahidi, 2007):

$$\%\ \text{DPPH scavenging} = (\text{Abs t} = 0 - \text{Abs t} = 30)/\text{Abs t} = 0 \times 100$$

where Abs($t = 0$) = (absorbance of DPPH radical + methanol) at $t = 0$ min

Abs($t = 30$) = (absorbance of DPPH radical + phenolic extracts) at $t = 30$ min.

Total flavonoid contents

Flavonoid contents of wheat fractions were assayed using the aluminum chloride colorimetric method of Chang et al. (2002). The appropriate dilution of extracts (0.5 ml) were mixed with

Table 1 Wheat varieties investigated.

Variety	Type	Location	Thousand Kernel Weight (g)	Production yield (Ard./Fed.)[a]
Gemiza-9	Common	Delta region	36.44	18.61
Beni-suef-3	Durum	Upper Egypt	40.34	21.00

[a] *Source:* Agriculture Directorates of Governorates, Economic Affairs Sector, Ard./Fed. → Ardab/Feddan.

1.5 ml of 95% ethanol, followed by 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UNICO UV/VIS-2100A spectrophotometer (Dayton, USA). The flavonoid content was calculated using a standard calibration of rutin solution and expressed as micrograms of rutin equivalent (RE) per gram of sample.

Statistical analyses

The data obtained in this study were expressed as the mean of triplicate determinations. Statistical comparisons were made with Duncan's test which were analyzed with SPSS (SPSS for Windows, Version Rel. 10.0.5., 1999, SPSS Inc.). P values <0.05 were considered to be significant.

Results and discussion

Total phenolics content

The recovery of phenolic contents in different samples is influenced by the polarity of extracting solvents and the solubility of this compound in the solvent used for the extraction process (Alothman et al., 2009; Sulaiman et al., 2011). Therefore, it is hard to select an appropriate solvent for the extraction of phenolic contents from all samples. The content of total phenolics in whole wheat and bran extracts were determined using the Folin–Ciocalteu assay, expressed as gallic acid equivalents (GAE). Measured Table 2 illustrates that significant difference ($p < 0.05$) in total phenolic contents was observed. Also results showed that the total phenolic compounds varied greatly among different solvents, this indicated the possible influence of extracting solvent on total phenolic contents. Furthermore, among all the wheat extracts, 50% acetone was found to be the most efficient solvent for extracting phenolic compounds when compared with the other solvent systems (Junli et al., 2012) reported that the highest total phenolic content was found in the 50% acetone extract from wheat flour.

From the same table it is clear that bran of Gemiza-9 was the highest phenolic content (4.66 mg of GAE/g) when compared with other extracts, followed by Beni-suef-3 bran (3.88 mg of GAE/g), Gemiza-9 whole wheat (2.57 mg of GAE/g), and Beni-suef-3 whole wheat (1.78 mg of GAE/g). These results are in agreement with that observed by Vaher et al. (2010) who found that the bran layers have the highest content of total phenolics content, when stated that the Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties.

DPPH radical scavenging activity assay

The DPPH method is commonly used for determination of free radical scavenging activity of antioxidant. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a very stable organic free radical and presents the ability of accepting an electron or hydrogen radical. The capacity of wheat extract to scavenge the stable DPPH radical is shown in Table 3 which summarizes the results for quenching of important reactive oxygen species (ROS) such as hydroxyl radical ($HO^{\bullet}$) and superoxide radical anion ($O_2^{\bullet-}$) as well as hydrogen peroxide (H_2O_2). For both variety Gemiza-9 and Beni-suef-3 extracts, bran samples showed strong DPPH free radical scavenging activities (28.07–31.75% and 33.84–36.82%, respectively). These results were supported by those of Liyana-Pathiran and Shahidi (2007) who found that the ability to scavenge DPPH radicals in wheat fractions was in the order of bran > shorts > feed flour > whole grain > flour, for both wheat cultivars. In addition, it can be seen that the solvent systems used affected the

Table 2 Total phenolics content of (mg/g) of whole wheat and bran fraction extracted by different solvents.

Solvents/tests	Gemiza-9 (common wheat)		Beni-suef-3 (durum wheat)	
	Whole	Bran	Whole	Bran
Acetone (50%)	2.57[a]	4.66[a]	1.78[a]	3.88[a]
Methanol (70%)	1.11[b]	2.28[b]	1.05[b]	2.94[ab]
Ethanol (70%)	1.12[b]	1.99[b]	1.21[b]	2.64[b]

TPC → Total Phenol Content as GAE mg/g (Gallic Acid Equivalent).
Each value was an average of three determinations.
Means within a column showing the same small letter are not significantly different ($P \geqslant 0.05$).

Table 3 Radical DPPH scavenging activity (%) of whole wheat and bran fraction extracted by different solvents.

Solvents/tests	Gemiza-9 (common wheat)		Beni-suef-3 (durum wheat)	
	Whole	Bran	Whole	Bran
Acetone (50%)	22.62[a]	28.07[c]	17.44[b]	33.84[b]
Methanol (70%)	22.97[a]	29.11[b]	18.76[ab]	34.45[b]
Ethanol (70%)	23.90[a]	31.75[a]	20.79[a]	36.82[a]

DPPH → 1,1-diphenyl-2-picrylhydrazyl as % DPPH inhibition.
Each value was an average of three determinations.
Means within a column showing the same small letter are not significantly different ($P \geqslant 0.05$).

Table 4 Total flavonoid contents (μg/g) of whole wheat and bran fraction extracted by different solvents.

Solvents/tests	Gemiza-9 (common wheat)		Beni-suef-3 (durum wheat)	
	Whole	Bran	Whole	Bran
Acetone (50%)	208.02[b]	205.56[b]	296.77[ab]	249.54[b]
Methanol (70%)	197.40[c]	195.40[b]	278.57[b]	223.93[c]
Ethanol (70%)	239.18[a]	223.96[a]	330.57[a]	258.04[a]

TFC → Total Flavonoid Content as μg rutin equivalent (RE)/g.
Each value was an average of three determinations.
Means within a column showing the same small letter are not significantly different ($P \geqslant 0.05$).

different wheat extracts DPPH as can be observed in. The antioxidant activity of 70% ethanol >70% methanol ≈50% acetone and extracts was carried out using DPPH radical-scavenging activity assay. These results indicated that whole wheat Gemiza-9 had strangler scavenging activity on DPPH than whole wheat Beni-suef-3. However, the scavenging activity DPPH of bran Beni-suef-3 was higher than that of Gemiza-9. Finally these results suggested that the 70% ethanol is a good solvent for highest scavenging activity on DPPH radicals.

Total flavonoid contents

For all the wheat varieties, significant difference ($p < 0.05$) in total antioxidant activity was observed between the different solvents (Table 4). These results indicated that possible influence of extracting solvent on total flavonoid content for all the wheat extracts. In fact, extraction into Ethanol/H_2O (70:30, v/v) was the highest total antioxidant activity for all wheat cultivars. In Gemiza-9 bran, it was noticed that there is no significant difference between 70% methanol and 50% acetone. Nevertheless, the other extracts showed that 50% acetone containing higher level of total flavonoid content than methanol. Moreover, it can be observed that the whole wheat extracts have a higher level of total flavonoid than bran extracts, and the Upper Egypt variety (Beni-suef-3) were higher than Delta Egypt (Gemiza-9). The highest flavonoid concentration was observed in the ethanolic extract for whole wheat of Beni-suef-3 (330.57 μg RE/g). Flavonoid content was determined in order as: ethanol > acetone > methanol, for all tasted samples. The acetone extracts did not significantly differ in some fractions for the methanol extracts, and the range of data of total flavonoid content in bran layer was (195.40–223.96 and 223.93–258.04 μg RE/g) in Gemiza-9 and Beni-suef-3, respectively. These results are in harmony with those of Brewer et al. (2014) who revealed that the TFC in wheat bran layer at different particle size (177.05–206.74 μg/g).

Conclusion

The results of this study revealed that the total phenolics and antioxidant activity in bran increased significantly than whole wheat. In contrast, the higher content of flavonoids existed in whole wheat than bran. On the other hand, the data recommended that the fifty percent acetone (v/v) was the better solvent to extract phenolic compounds. However, ethanol 70% is the best effective solvent for extracting antioxidant and flavonoids from whole wheat and bran. These results indicated that wheat bran may replace synthetic antioxidant in food formulations and play a major role in human health.

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