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Identification of Phenolic Acids and Flavonoids Compounds from Some Varieties *Triticum Aestivum* and *Triticum Durum*

Sanak, R. N., Allam, M. A. and El-Shazely, A. S. I.

Food Technol. Res. Inst., Agric. Res. Center, Giza- Egypt

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ABSTRACT

This investigation was carried out to evaluate of some varieties from *Triticum aestivum* and *Triticum durum* seeds using identification of phenolic acids and flavonoids compounds before and after hydrolysis using HPLC. Contents of the phenolic acids and flavonoids compounds are expressed as mg of compounds per 100g of dry weight of bread and durum wheat seeds. The experimental material consisted of seven wheat bread (*Triticum aestivum* L.) varieties Misr 1, Giza 167, Sakha 93, Gemaza 7, Gemaza 10, Sads 12 and Sads 13 and three wheat durum (*Triticum durum* L.) varieties Beni Sewaf 4, 5 and 6, respectively. The identification of phenolic acids before and after hydrolysis for wheat bread and durum at different varieties the results showed that the phenolic acids were the highest in wheat bread and durum for some different varieties after hydrolysis than wheat bread and durum different varieties before hydrolysis. The highest amounts of phenolic acids in wheat bread and durum different varieties after hydrolysis may be caused the free and conjugated phenolic acids has contained and it was calculated in wheat bread and durum different varieties after hydrolysis. Identification of flavonoids compounds in bread and durum wheat before hydrolysis was determined and the resultant could be illustrated that the luteolin compound was predominate in all bread wheat varieties and also hisperidin compound was rich amounted in all bread wheat varieties except Sads 12 was not detected. Quercetin and hispertin flavonoids compounds were predominate in all bread wheat varieties. The identification of flavonoids compounds in some different varieties durum wheat after hydrolysis the resultant could be observed that luteolin, hisperidin, quercetin and hispertin were the highest amounts in all durum wheat varieties. From the obviously results it could be suggest that there are opportunities for developing the wheat varieties from high phenolic acids, flavonoids compounds and enhanced phytochemical content.

Key words: Phenolic acids, flavonoids compounds, *Triticum aestivum*, *Triticum durum*

Introduction

Wheat has traditionally been selected for functionality, for example, baking or biscuit values, while the nutritional value of the grain has been almost neglected. However, during the last 10 years much more attention has been paid to the phytonutrients of wheat as potential antioxidants acting on the health benefits (Fardet *et al*., 2008).

Antioxidant properties of wheat derive mainly from phenolics and lipid-soluble compounds, carotenoids and tocopherols. The most common phenolic compounds found in whole grains are phenolic acids and flavonoids with ferulic acid as the predominant phenolic acid (Moore *et al*., 2005). The beneficial effects derived from phenolic compounds could be a major determinant of the antioxidant potentials of foods (Heim *et al*., 2002) and could therefore be a natural source of antioxidants. Generally, the free phenolic compounds are proanthocyanidins or flavonoids, while the bound phenolic compounds are ester-linked to cell-wall polymers (Bonoli *et al*., 2004). Phenolic compounds commonly present in whole grains are phenolic acids and flavonoids. The common phenolic acids found in whole grains are ferulic acid, vanillic acid, caffeic acid, syringic acid and pcoumaric acid (Sosulski *et al*., 1982), while flavonoids are flavonols, flavan-3-ols, flavones and flavanones. Ferulic acid has been known as an antioxidant which is effective with respect to antiinflammation and as an inhibitor of tumor initiation and as being a preservative (Adom and Liu, 2002).

Phenolic compounds can be grouped into three broad categories: polyphenols, phenolic acids, and a miscellaneous group. Polyphenols can be arranged into two broad classes: tannins (condensed and hydrolysable) or flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidins). Similarly, phenolic acids can be further subdivided into two main subgroups, hydroxycinnamic and hydroxybenzoic

Corresponding Author: Sandak, R. N., Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
E-mail: raafat_naguib@yahoo.com

acids and the miscellaneous group comprises lignans, coumarins, stilbenes, and other phenolic compounds not included in the other two subgroups (Luthria, 2006 a,b). Phenolic acids are known to exhibit significant biological activity (Robbins and Bean, 2004).

Phenolic compounds, exhibit a wide range of physiological properties, such as antiallergenic, anti-artherogenic, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effect (Pupponen-Pimiä *et al*., 2001). It has been recognized that health benefits can be achieved from consuming high levels of fruits and vegetables.

Flavonoids are some of the most common phenolics, widely distributed in plant tissues, and often responsible alongside the carotenoids and chlorophylls for their blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, proanthocyanidins and catechins Ferreira and Pinho (2012). All flavonoids are derived from the aromatic amino acids, phenyalanine and tyrosine, and have three-ringed structures Routray and Orsat (2012). Variation in flavonoid structure arises from the scale and pattern of hydroxylation, prenylation, alkalinization and glycosylation reactions that alter the basic molecule Stalikas (2007).

Flavonoids are a class of secondary plant phenolics having potential beneficial effects on human health with significant antioxidant and chelating properties in the human diet. Over the years, they have been found to be an important part of the human diet and are considered to be active principles in some medicinal plants. The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O2·-), hydroxyl (OH·), peroxyl (ROO·) and alcohoxyl (RO·) radicals Wang *et al*. (2003).

Reversed phase-high performance liquid chromatography (RP-HPLC) has been accepted as the most useful tool for the qualitative and quantitative analyses of phenolic compounds (Zhou *et al*., 2004). Since phenolic compounds possess antioxidant activities, it is worthwhile to investigate their antioxidant activities along with their quantities. The DPPH assay is the commonly used method for the evaluation of free radical scavenging activity (Chew *et al*., 2008).

The objective of the present study has been carried out to evaluate and compared between some varieties from *Triticum aestivum* and *Triticum durum* seeds using identification of phenolic acids and flavonoids compounds before and after hydrolysis.

Materials and Methods

Materials:

The experimental material consisted of seven wheat bread (*Triticum aestivum* L.) varieties Misr 1, Giza 167, Sakha 93, Gemaza 7 and 10, Sads 12 and 13 and three wheat durum (*Triticum durum* L.) varieties Beni Seuf 4, 5 and 6 were purchased from Field Crops Research Institute, Agricultural Research Center, Giza-Egypt. All seeds were milled in a Laboratory Mill Junior to give a fine power wholemeal.

Authentic samples of phenolic acids and flavonoids compounds were purchased from Sigma Co. (St. Louis, MO, USA). All other solvents and chemicals were of analytical grade

Methods:

Identification of phenolic acids compounds before hydrolysis using HPLC:

Phenolic compounds were determined by HPLC according to the method of Goupy *et al.* (1999) as follow: 5 g of sample were mixed with ethanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Agilant (series 1200) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 350C. Gradient separation was carried out with ethanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. phenolic acid standard from sigma Co, were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate the phenolic compounds concentration by the data analysis of HEWLLET Packared software. Standard compounds were prepared as stock solutions at 2 mg mL–1 in 80% ethanol. The stock solutions were stored in darkness at 18 ºC and remained stable for at least 3 months. All samples were analyzed in duplicate unless otherwise stated, and concentrations of individual phenolic acids were expressed in mg/100 of dry matter.

Identification of phenolic acids compounds after hydrolysis using HPLC:

Free phenolic acids were extracted using an 80:20 chilled ethanol/water solvent mixture. Insoluble bound phenolic acids were released via alkaline hydrolysis (6 M NaOH, 16 h) of the residue from the initial ethanol/water extraction, followed by acid hydrolysis. Both phenolic acid fractions extracted by cold diethyl ether (DE) and ethyl acetate (EA, 1:1 v/v) after alkaline and acid hydrolysis were acidified to pH 2 to enable extraction into organic solvent. Individual phenolic acids in the flour extracts were analyzed by an Agilent 1100 Series high-performance liquid chromatography (HPLC), equipped with a UV detector at 280 nm and an Agilent TC-C18 (250 _ 4.60 mm, 5 mm) column (Irmak *et al*., 2008). The injection volume was 20 mL, and the flow rate was set to 1 mL min_1. A gradient elution program was utilized, as described by Zhang *et al*. (2012), and incorporated a mobile phase of water with 0.05% trifluoroacetic acid (solvent A) and 30% acetonitrile, 10% methanol, 59.95% water and 0.05% trifluoroacetic acid (solvent B). Identification and quantification of phenolic acids in samples were performed by comparison with chromatographic retention times and areas of external standards. Calibration curves of phenolic acid standards were constructed using authentic standards that had undergone the same extraction procedure to ensure that losses due to the extraction were accounted for. Standard compounds were prepared as stock solutions at 2 mg mL–1 in 80% ethanol. The stock solutions were stored in darkness at 18 ^{o}C and remained stable for at least 3 months. All samples were analyzed in duplicate unless otherwise stated, and concentrations of individual phenolic acids were expressed in mg/100 of dry matter.

Identification of flavonoids compounds before hydrolysis using HPLC:

Flavonoid compounds were determined by HPLC according to the method of Mattila *et al.,* (2000) as follows: 5 g of sample were mixed with ethanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1 - 3 ml was collected in a vial for injection into HPLC Hewllet Packared (series 1050) equipped with auto-sampling injector, solvent degasser, ultraviolet (UV) detector set at 330 nm and quarter HP pump (series 1050). The column temperature was maintained at 350C. Gradient separation was carried out with ethanol and acetonitrile as a mobile phase at a flow rate of 1 ml/min. Flavonoids acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of HEWLLT Packared software.

Identification of flavonoids compounds after hydrolysis using HPLC:

The standard procedure used for acid hydrolysis of free flavonoids compounds in different bread and durum wheat verities has been described by Hertog *et al*. (1993). Acidified methanol (25 mL) containing 1% (v/v) HCl and 0.5 mg mL^{-1} TBHQ was added to different bread and durum wheat verities (25 g). HCl (1.2 M, 5 mL) was added and the mixture was stirred at 90°C under reflux for 2 h to obtain the aglycons by hydrolysis of the flavonol glycosides. The extract was cooled to room temperature and leave for 3 min, to remove oxygen, before injection. The final extract was filtered through a 0.45-µm (Acrodisc) filter then through a 0.5 µm (A crodisc) filter. The filtrate was injected into the HPLC.

HPLC analysis of flavonoids compounds from different bread and durum wheat verities with UV detection were performed by a modification of a method published elsewhere Hertog *et al*. (1993). Compounds were separated on a 250 mm × 4.6 mm i.d., 5-µm particle, Hypersil-ODS column (Phenomenex, CA, USA) with 25:75 (v/v) acetonitrile–pH 2.4 phosphate buffer (25% acetonitrile in 0.025 M NaH2PO4) as mobile phase at a flow rate of 1.2 mL min –1. The HPLC equipment comprised Hewlett–Packard (HP) 1050 Chem Station Software, an HP model 35900 interface units, an HP 9000 Series 300 computer, and an HP DeskJet 500 Printer. A Waters 486 tunable absorbance detector was operated at 266 nm; detector sensitivity was 0.05 AUFS and the column oven temperature was 30°C.

Results and Discussion

Identification of phenolic acids in bread and durum wheat before hydrolysis using HPLC.

Distribution of the polyphenolic and related compounds in bread and durum wheat before hydrolysis and the results are shown in Tables (1 and 2). Contents of the polyphenolic compounds are expressed as

mg of compounds per 100g of dry weight of wheat seeds. From the results in Table (1) it could be noticed that the phenolic acids pyrogllol and salycilic acids were the highest amounted in Misr 1, Giza 167, Sakha 93, Gemaza 7 and 10, Sads 12 and 13 (2.139, 1.57, 11.076, 2.904, 2.435, 1.571 and 2.031 mg/100g, respectively in pyrogllol and 2.626, 2.751, 3.845, 3.249, 3.778, 2.968 and 3.022 mg/100g, respectively in salycilic acid). Whereas, syrinigc and ferulic were detected and the highest in Sakha 93 and Sads 13 (9.521 and 1.656 mg/100g). Moreover, the all phenolic acids are reported in Table (1) were detected in the obviously bread wheat varieties and it was amounted less than 1mg/100g. Phenolic compounds in wholemeal deserve much more attention because the total phenolic content strongly correlates with the total antioxidant activity (Verma *et al.,* 2008). Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun *et al*., 2009).

Table 1: Identification of phenolic acids in bread wheat before hydrolysis using HPLC (mg/100g).

Phenolic compounds	Misr 1	Giza 167	Sakha 93	Gemaza 7	Gemaza 10	Sads 12	Sads 13
Syrinigc	0.371	0.221	9.521	0.967	0.435	0.162	0.964
Pyrogllol	2.139	1.570	11.076	2.904	2.491	1.571	2.031
Gallic	0.185	0.148	0.288	0.165	0.186	0.168	0.132
Protocatechuic	0.719	0.291	0.213	0.510	0.559	0.508	0.406
Catechol	0.351	0.276	0.131	0.226	0.112	--	0.169
4- Amino-benzoic	0.0101	0.015	0.019	0.023	0.041	0.34	0.17
Catechein	0.320	0.091	0.086	0.082	0.075	0.079	0.50
Chlorogenic	0.384	0.296	0.761	0.371	0.380	0.199	0.139
P-OH-benzoic	0.158	0.178	0.229	0.115	0.187	0.151	0.161
Epicatechein	0.580	0.466	0.867	0.297	0.378	0.357	0.762
Caffeic	0.052	0.035	0.079	0.067	0.060	0.037	0.035
Vanillic	0.024	0.083	0.073	0.035	0.054	0.029	0.013
Caffeine	0.237	0.139	--	--	--	--	--
Ferulic	0.392	0.365	0.298	0.304	0.643	0.697	1.656
Benzoic	--	--	--	--	--	--	--
Salycilic	2.626	2.751	3.845	3.249	3.778	2.968	3.022
Coumarin	-	0.123	0.0151	0.221	0.146	--	0.068
Ellagic	0.903	0.576	0.312	--	0.293	--	0.345
Cinnamic	0.095	0.094	0.071	0.075	0.169	0.555	0.066

The results from identification of the phenolic acids in the durum wheat before hydrolysis are tabulated in Table (2). The resultant illustrated that the phenolic acid salycilic higher in Beni Suef 4 1.658 mg/100g. Syrinigc, pyrogllol and benzoic acids were the highest amounted in Beni Suef 5 (2.307, 10.226 and 1.600 mg/100g, respectively). Moreover, syrinigc, salycilic and ferulic acids were the highest amounted in Beni Suef 6 (1.631, 1.591 and 2.193 mg/100g, respectively) and all phenolic acids resultant were less than 1 mg/100g. Different concentrations of these compounds were found in the anatomic parts of the grain. Phenolics are highly concentrated in bran layers (Li *et al*., 2005). Therefore, the whole grain products could be considered as food products with maximum health benefits.

The health benefits of wheat are derived from the importance in bran and germ such as dietary fiber or phenolic acids. The consumption of dietary fiber has been related to reducing the risk of heart disease and the prevention of colorectal cancer, and metabolic and inflammatory bowel diseases such as diabetes and diverticulitis (Topping and Clifton, 2001), while phenolic acids play an important role in combating oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000). Phenolic acids exist in cereal grains in free soluble conjugate and insoluble bound forms. The previous studies reported that phenolic acids in wheat grains are mostly in the bound form and exist in bran associated with cell wall materials (Liyana-Pathirana and Shahidi, 2006).

Free phenolic acids made up the least abundant class, contributing only about 6% of the grain phenolic acids determined, in agreement with Fernandez-Orozco *et al*. (2010). The average percentage contributions to total free and bound phenolic acid concentrations varied with components and cultivars. Syringic acid represented the most abundant class of free phenolic acids, contributing from 29.8% to 36.8% of the total concentrations among cultivars; whereas ferulic represented the most abundant class of the total bound phenolic acid, contributing from 46.0% to 55.8% of the concentrations among cultivars. Therefore, the free phenolic extracts of the grain were mostly contributed by syringic rather than ferulic, whereas ferulic acid primarily existed in bound form (Hung *et al*., 2009). Moreover, the bound form contributed 95.8% of the grain ferulic acid concentration determined, similar to the previous results (Zhang *et al*., 2012). Ferulic acid was, as expected, the dominant component in the bound fraction, consistent with

previous reports by Verma *et al*. (2009. The second most abundant phenolic acids in whole grain were caffeic and p- coumaric, largely in agreement with Li *et al*. (2008).

Table 2: Identification of phenolic acids in durum wheat before hydrolysis using HPLC (mg/100g).

Phenolic compounds	Beni Suef 4	Beni suef 5	Beni Suef 6
Syrinigc	0.760	2.307	0.723
Pyrogllol	0.764	10.226	1.631
Gallic	0.124	0.100	0.100
Protocatechuic	0.338	0.256	0.241
Catechol	0.236	--	0.126
4- Amino-benzoic	0.012	--	0.007
Catechein	0.171	0.317	0.146
Chlorogenic	0.121	0.200	0.124
P-OH-benzoic	0.145	0.350	0.100
Epicatechein	0.681	--	0.456
Caffeic	--	0.085	0.052
Vanillic	0.023	0.050	0.039
Caffeine	0.361	0.271	0.118
Ferulic	--	0.788	2.193
Benzoic	0.107	1.600	--
Salycilic	1.658	--	1.591
Coumarin	0.153	0.106	--
Ellagic	0.443	0.467	0.334
Cinnamic	0.102	0.527	0.237

Identification of phenolic acids in bread and durum wheat after hydrolysis using HPLC:

The results from Tables (3 and 4) showed that the identification of phenolic acids in bread and durum wheat after hydrolysis using HPLC. From the resultant in Table (3), it could noticed that the bread wheat Misr 1 variety had contained rich amounts in protocatechuic, chlorogenic, salycilic and epicatechein (57.106, 33.41, 32.313 and 19.125 mg/100g). Pyrogllol, protocatechuic, chlorogenic, P-OH-benzoic, epicatechein, salycilic and ellagic acids were predominant in Giza 167 variety. Pyrogllol, catechol, epicatechein, benzoic, salycilic and ellagic were the highest phenolic acids amounted in Sakha 93 variety. Protocatechuic, benzoic, salycilic, epicatechein, ferulic and P-OH-benzoic were predominant phenolic acids in Gemaza 7 variety. Whereas, the highest phenolic acids in Gemaza 10 variety were pyrogllol, protocatechuic, patechol, phlorogenic, ferulic, salycilic and ellagic acids. The phenolic acids pyrogllol, epicatechein, ferulic and salycilic were predominate in Sads 12 variety and pyrogllol, protocatechuic, P-OH-benzoic and salycilic were the highest in Sads 13 wheat bread variety. Moreover, the all phenolic acids resultant were less than 10 mg/100g.

Table 3: Identification of phenolic acids in bread wheat after hydrolysis using HPLC (mg/100g).

Phenolic compounds	Misr 1	Giza 167	Sakha 93	Gemaza 7	Gemaza 10	Sads 12	Sads 13
Syrinigc	0.180	--	0.440	1.949	1.918	1.548	0.605
Pyrogllol	--	80.441	107.426	7.076	26.517	18.874	20.935
Gallic	5.381	6.451	3.759	2.232	--	2.327	3.649
Protocatechuic	57.106	143.986	10.765	66.998	36.810	--	11.728
Catechol	7.919	--	13.616	--	15.545	7.358	6.580
4- Amino-benzoic	1.693	--	-	--	--	--	--
Catechein	--	--	2.298	--	--	3.403	--
Chlorogenic	33.410	14.603	9.462	--	21.641	9.717	8.824
P-OH-benzoic	--	26.815	7.224	12.917	--	--	28.798
Epicatechein	19.125	20.612	22.798	17.117	3.233	22.571	--
Caffeic	2.811	4.257	2.729	2.619	2.698	2.850	2.343
Vanillic	2.200	5.015	1.505	4.048	3.586	3.659	2.376
Caffeine	1.784	2.211	2.051	1.398	2.105	2.336	1.543
Ferulic	8.627	6.957	11.357	12.497	15.744	9.481	9.037
Ferulic	2.106	8.732	33.211	36.951	32.498	32.103	--
Salycilic	32.313	11.363	46.358	42.343	55.014	45.276	20.955
Coumarin	2.982	0.978	4.213	3.427	3.902	1.373	1.148
Ellagic	9.257	14.901	13.476	6.191	14.944	6.510	8.034
Cinnamic	6.577	6.284	2.232	5.520	6.813	1.481	7.568

Table (4) showed that the identification of phenolic acids from durum wheat varieties and the results reported that the Beni Suef 4 was the highest in phenolic acids pyrogllol, protocatechuic, catechol, P-OH-benzoic, epicatechein, ferulic, benzoic and salycilic. Meanwhile, Beni Suef 5 and 6 were predominating in phenolic acids pyrogllol, epicatechein and salycilic. Moreover, the all phenolic acids resultant were less than 10 mg/100g.

Table 4: Identification of phenolic acids in durum wheat after hydrolysis using HPLC (mg/100g).

Phenolic compounds	Beni Suef 4	Beni Suef 5	Beni Suef 6
Syrinigc	1.422	0.506	0.566
Pyrogllol	11.532	43.709	13.379
Gallic	2.444	4.316	2.925
Protocatechuic	21.049	6.424	--
Catechol	11.962	8.287	5.857
4- Amino-benzoic	--	--	--
Catechein	4.173	3.014	--
Chlorogenic	5.834	2.138	--
P-OH-benzoic	20.229	7.267	6.101
Epicatechein	33.041	23.938	15.753
Caffeic	4.048	2.130	2.171
Vanillic	6.670	1.115	--
Caffeine	2.696	0.984	--
Ferulic	13.030	9.773	6.235
Benzoic	30.357	27.779	15.192
Salycilic	44.854	32.088	31.856
Coumarin	5.489	2.832	--
Ellagic	9.533	10.416	5.350
Cinnamic	0.816	2.571	4.460

From the identification phenolic acids results before and after hydrolysis for wheat bread and durum different varieties. It could be noticed that the phenolic acids were the highest in wheat bread and durum different varieties after hydrolysis than wheat bread and durum different varieties before hydrolysis. The highest amounts of phenolic acids in wheat bread and durum different varieties after hydrolysis may be caused the free and conjugated phenolic acids has contained and it was calculated in wheat bread and durum different varieties after hydrolysis.

Among phenolic acids, predominant phenolics in cereals are benzoic acid derivatives such as gallic, vanillic and syringic acid and cinnamic acid derivatives such as ferulic, p-coumaric and caffeic acid, of which, ferulic acid is the most potent in all cereals and makes from 70 to 90% of total Moore *et al*. (2006) and Žilić *et al*. (2012b). On the other hand, ferulic acid and diferulates, as the most abundant phenolics and major contributors to the in vitro antioxidant capacity of cereals grain, are not present in significant quantities in some fruits and vegetables. Antioxidant properties of phenolic acids stem from the reactivity of their phenol moiety, i.e. from the reactivity of hydroxyl substituent on the aromatic ring. The hydroxylation and methoxylation in the aromatic ring affect the radical-quenching ability and hence different antioxidant activities of phenolic acids. It is considered that radical scavenging via the hydrogen atom donation can be the predominant mode of the phenolic acids antioxidant activity Shahidi *et al*. (1995).

The aleuronic layer is a wheat fraction with the highest antioxidant activity, followed by the bran Liyana-Pathirana and Shahidi (2006). According to results of Žilić *et al*. (2012a), debranned flour from bread and durum wheat had 3.7 and 2.4-fold lower antioxidant capacity than the respective bran fractions. The significant correlations between total phenolic contents and ABTS radical scavenging activities, point to their high contribution to antioxidant capacity. However, the variation in the antioxidant capacity, as well as in the content of phenolic compounds in bran and flour, highly depend on milling processes.

Identification of flavonoids compounds in bread and durum wheat before hydrolysis using HPLC:

Isolation and identification of flavonoids compounds in bread and durum wheat before hydrolysis and the results are shown in Tables (5 and 6). Contents of the flavonoids compounds are expressed as mg of compounds per 100g of dry weight of wheat seeds. Table (5) showed that the determination of the flavonoids compounds in bread wheat before hydrolysis. From the resultant it could be noticed that the variety Misr 1 had rich contained from luteolin, rutin, quercetin and hisperidin which ranged from 1.087 to

7.048 mg/100g. Whereas, Sakha 93 variety had contained at 1.343 mg/100g hisperidin and Gemaza 7 variety was predominate in luteolin, hisperidin and hispertin (3.901, 2.239 and 1.054 mg/100g, respectively). Gemaza 10 variety was rich in luteolin and rutin (3.089 and 1.287 mg/100g) and also Sads 12 bread wheat variety was predominate at luteolin and Sads 13 bread wheat variety was contained at hisperidin flavonoids compounds. Moreover, the all flavonoids compounds in bread wheat before hydrolysis resultant were less than 1 mg/100g.

Table 5: Identification of flavonoids compounds in bread wheat before hydrolysis using HPLC (mg/100g).

Flavonoid compounds	Misr 1	Giza 167	Sakha 93	Gemaza 7	Gemaza 10	Sads 12	Sads 13
Luteolin	7.048	0.620	0.852	3.901	3.089	1.955	0.439
Rutin	1.932	0.347	0.532	0.483	1.287	0.175	0.356
Hisperidin	1.786	0.795	0.434	2.239	0.132	0.688	1.050
Rosmarinic	0.324	0.425	0.206	0.636	0.587	0.273	0.157
Quercetrin	0.215	0.447	0.358	0.362	0.544	0.225	0.102
Quercetin	1.590	0.456	0.050	0.217	0.050	0.104	0.105
Hispertin	1.087	0.418	1.343	1.054	0.206	0.172	0.320
Kampferol	0.136	0.268	0.086	0.268	0.082	0.013	0.054
Apegnin	0.243	0.102	0.048	0.104	0.102	0.005	0.029
7-Hydroxyflavone	0.047	0.011	0.020	0.045	0.034	0.002	0.006

Table (6) showed that the results from the identification of flavonoids compounds in durum wheat before hydrolysis. From the resultant it could be observed that the Beni Suef 4 is a good source of hisperidin compound (1.419 mg/100g) and Beni Suef 5 had contained 2.835 mg/100g from luteolin compound. Moreover, the compounds contained in Beni Suef 6 and all flavonoids compounds in durum wheat before hydrolysis resultant were less than 1 mg/100g. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).

Although some investigators have suggested that flavonoid glucosides may utilize the sodium-dependent glucose transporter for uptake by the gut, it has been shown that the h-glycosides genistin and daidzin, and by implication other flavonoid glucosides, are hydrolyzed in the gut wall by lactose phlorizin hydrolase, an enzyme in the apical membrane of the villi of the small intestine Day *et al*. (2000) and by intestinal microflora that convert them into aglycone forms Barnes *et al*. (2003). The flavonoid algycones that are produced by hydrolysis are then absorbed into the intestinal cells by passive mechanisms. This is followed by a reconjugation step in the intestinal cell with glucuronic acid by the phase II enzyme UDP-glucuronosyl transferase. Those aglycones that escape this initial metabolism pass into the circulation and are converted to glucuronidated, methylated, and sulfated phase II metabolites by enzymes in the liver and other organs Sfakianos *et al*. (1997).

The flavonoid phase II metabolites are taken up from the blood by the liver and are excreted in bile, thus transporting them back into the intestines. Intestinal hglucuronidases and sulfatases then release the aglucones these can be reabsorbed or enter the bacterial rich large bowel for further metabolism. For example, reduction (daidzein to equol), ring opening (daidzein to O-desmethylangolensin), and ring cleavage [daidzein to p-ethylphenol and/or 2-(4-hydroxyphenyl)- propionic acid] of the heterocyclic ring of the isoflavonoids can occur Coldham *et al*. (1999). Flavonoids are converted to several other phenolic acids. Some of these metabolites have shown higher anti-oxidative and estrogenic activities (measured in vitro) than their parent compounds, for instance equol compared with daidzein Rimbach *et al*. (2003).

Table 6: Identification of flavonoids compounds in durum wheat before hydrolysis using HPLC (mg/100g).

Flavonoid compounds	Beni Suef 4	Beni Suef 5	Beni Suef 6
Luteolin	0.765	2.835	0.938
Rutin	0.495	0.534	0.770
Hisperidin	1.419	0.100	0.389
Rosmarinic	0.040	0.201	0.155
Quercetrin	0.050	0.128	0.454
Quercetin	0.096	0.200	0.137
Hispertin	0.221	0.428	0.670
Kampferol	0.058	0.2122	0.085
Apegnin	0.044	0.042	0.028
7-Hydroxyflavone	0.010	0.007	0.037

Identification of flavonoids compounds in bread and durum wheat after hydrolysis using HPLC:

Identification of flavonoids compounds in bread and durum wheat after hydrolysis was determined using HPLC and the results are reported in Tables (7 and 8). The results from Table (7) showed that the identification of flavonoids compounds in some different varieties (Misr 1, Giza 167, Sakha 93, Gemaza 7 and 10, Sads 12 and 13) bread wheat after hydrolysis. From the resultant it could be illustrated that the luteolin compound was predominate in all bread wheat varieties and also hisperidin compound was rich amounted in all bread wheat varieties except Sads 12 was not detected. Quercetin and hispertin flavonoids compounds were predominate in all bread wheat varieties. Moreover, the all flavonoids compounds in bread wheat after hydrolysis resultant were less than 10 mg/100g.

Table 7: Identification of flavonoids compounds in bread wheat after hydrolysis using HPLC (mg/100g).

Flavonoid compounds	Misr 1	Giza 167	Sakha 93	Gemaza 7	Gemaza 10	Sads 12	Sads 13
Luteolin	11.458	11.458	13.714	16.847	19.575	14.093	15.831
Rutin	7.843	--	9.356	--	5.598	3.524	3.949
Hisperidin	33.969	64.278	14.214	57.769	16.509	-	97.929
Rosmarinic	4.462	6.256	2.831	2.855	3.443	2.329	3.505
Quercetrin	3.757	3.744	0.486	2.976	8.133	4.755	7.361
Quercetin	12.259	76.809	30.456	76.628	67.387	55.847	55.902
Hispertin	16.352	5.514	13.381	17.489	16.778	20.033	16.083
Kampferol	2.105	2.233	3.151	3.211	0.983	5.058	4.017
Apegnin	0.799	3.123	0.598	0.632	1.187	0.853	0.491
7-Hydroxyflavone	0.550	0.206	0.752	0.274	0.378	0.657	--

Table (8) showed that the identification of flavonoids compounds in some different varieties (Beni Suef 4, 5 and 6) durum wheat after hydrolysis. From the resultant it could be observed that luteolin, hisperidin, quercetin and hispertin were the highest amounts in all durum wheat varieties. Similar to phenolic acids, biochemical activities of flavonoids depend on their chemical structures and the relative orientation of various moieties in the molecules. Flavan nucleus, consisting of two benzene rings combined by an oxygen-containing pyran ring, makes basic structure of flavonoids Aherne and O'Brien (2002). The ability of flavonoids to be effective antioxidants depends on their metal-chelating potential, the presence of hydrogen-/electron-donating substituents in the molecules and the ability of the flavonoid to delocalize the unpaired electron Shahidi and Ambigaipalan (2015). In addition to a soluble conjugated form, flavonoids are present in cereals in the free form, as the respective aglycines, and the insoluble bound form. The results of Lee *et al*. (2005) showed that flavonoide aglycones had greater antioxidant activities than their glucosides when the potency was assessed using the LDL oxidation assay. Cereals grain mainly contains flavones. Further, colored whole grain of cereals contains a larger number of anthocyanins that are identified mostly as derivatives of cyanidin, pelargonidin, delfinidin and peonidin Žilić *et al*. (2012b). Delphinidin is known to be responsible for the bluish color, whereas cyaniding and pelargonidin are responsible for purple and red colors.

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).

Table 8: Identification of flavonoids compounds in durum wheat after hydrolysis using HPLC (mg/100g).

Flavonoid compounds	Beni Suef 4	Beni Suef 5	Beni Suef 6
Luteolin	17.231	18.776	17.508
Rutin	--	--	5.341
Hisperidin	55.413	69.477	21.448
Rosmarinic	1.106	2.394	2.444
Quercetrin	3.478	3.489	2.471
Quercetin	80.106	87.040	12.457
Hispertin	13.813	10.756	11.779
Kampferol	5.057	2.314	1.921
Apegnin	1.223	2.637	0.745
7-Hydroxyflavone	0.498	0.410	0.382

In this study, for most of the traits, considerable variations within bread and durum wheat seeds were found. Because grains were collected at the full maturity stage from plants grown under the equal conditions in a field-trial at the same location during the growing season 2014-2015, the influence of

environmental factors could be ignored. Moreover, the identification of phenolic acids and flavonoids compounds were significantly higher in durum than bread wheat seeds. Therefore, it could be suggest that there are opportunities for developing the wheat varieties with high phenolic acids, flavonoids compounds and enhanced phytochemical content.

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