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A New Analytical Method for the Determination of Phenolic Compounds and Their Antioxidant Activities in Different Wheat Grass Varieties

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Abstract
In this work, the phenolic content and antioxidant capacity of wheat grass, Turkish Amber Durum wheat was studied. For this purpose Turkish Amber Durum wheat was germinated at three different times (15, 30 and 40 days). All the wheat samples sprouted in the same organic conditions and then dried with air and without sunlight and placed into an incubator at 50°C overnight. The dried and milled amber wheat grass was extracted with ethyl acetate and compared for their antioxidant capacities against the 2,2-diphenyl-1-picrylhydrazyl (DPPH.) radical, and at the same time the total phenolic content (TPC) was determined. Phenolic compounds in the extracts were examined by high performance liquid chromatography (HPLC). In this study, by using a new method, nine standards were separately detected within 50 min by using the HPLC system, as previously reported by other authors.
The major phenolics, identified in the wheat grass that consisted of ferulic acid, gallic acid, caffeic acid, p-coumaric acid, ellagic acid, benzoic acid, p-hydroxybenzoic acid, syringic acid, quercetin and bha (butylated hydroxyanisole). The highest antioxidant and antiradical activities were detected in Turkish Amber wheat that sprouted in 15 days. This study demonstrates that the importance of wheat grass is due to its high antioxidant activity. Consumption of wheat grass has positive effects on human health such as reduced risk of coronary heart diseases, certain type of cancers and stroke. Due to the high antioxidant effect of wheat grass the aim is to use it as a food preservative. When wheat grass will be used as a natural food preservative, carcinogenic effects of synthetic food preservatives for body would be preserved.
Keywords: Antioxidant activity, HPLC, phenolic compounds, scavenging of DPPH radical, total phenolic content, Wheat grass.

Farklı Buğday Çimi Çeşitlerinin Antioksidan Aktiviteleri ve Fenolik Bileşiklerin Tayini İçin Yeni Bir Analitik Metod

Özet
Bu çalışmada, Türk Amber Durum buğdayının fenolik içeriği ve antioksidan kapasitesi incelendi. Bu amaçla, Türk Amber Durum buğdayı (15, 30 ve 40 gün süre ile) üç farklı zamanda çimlendirilmiştir. Filizlenen buğdaylar, aynı organik koşullarda güneş ışığı olmadan açık havada kurutuldu ve 50°C gece boyunca inkübatör de bırakıldı. Kurutulmuş ve öğütülmüş buğday çimi etil asetat ile ekstrakte edildi ve 2,2-difenil-1-picrylhydrazyl (DPPH.), radikaline karşı antioksidan kapasiteleri kıyaslandı ve toplam fenolik içeriği (TFİ) de tayin edildi. Ekstraktlardaki fenolik bileşikler, yüksek performanslı sıvı kromatografisi (YBSK) ile incelenmiştir.
Bu çalışmada, yeni bir metod kullanılarak 10 farklı standart, yaklaşık 50 dakikalık YBSK sistemiyle, önceden diğer yazarlar tarafından rapor edildiği gibi, ayrı ayrı analiz edilmiştir. Buğday çiminde belirlenen başlıca fenolik bileşikler ferulik asit, gallik asit, kafeik asit, p-kumarik asit, ellagik asit, benzoik asit, p-hidroksibenzoik asit, şiringik asit, kuersetin ve bha'dan oluşuyordu. En yüksek antioksidan ve antiradikal aktivite 15 gün içinde çimlenmiş esmer buğday çiminde tespit edilmiştir. Bu çalışma, buğday çiminin yüksek antioksidan aktivitesine bağlı olarak önemini ortaya koymıştur. Buğday çimi tüketiminin insan sağlığı üzerinde koroner kalp hastalıkları, belirli kanser türleri ve felç riskini azaltmak gibi olumlu etkileri vardır.
Buğday çiminin yüksek antioksidan etkisi nedeniyle, gıda katkı maddesi olarak kullanımı hedeflenmektedir. Buğday çimi doğal bir gıda katkı maddesi olarak kullanıdığında, sentetik gıda koruyucu maddelerin vücut için kanserojen etkileri önlenmiş olacaktır.
Anahtar Kelimeler: Antioksidan aktivite, Buğday çimi, çimlenme, fenolik bileşikler, HPLC, toplam fenolik içeriği, DPPH radikal süpürücü.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereals in the world because of the universal use of wheat for a wide range of products such as bread,

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noodles, cakes, biscuits, cookies, etc. Wheat kernel is composed of endosperm (81-84 %), bran (14-16 %), and germ (2-3%) (Pomeranz 1988). 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 (Craig et al. 1998, 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 (Adom and Liu 2002, Liyana-Pathirana and Shahidi 2006).

They are widely distributed in medicinal plants, spices, vegetables, fruits, grains and other seeds (Stratil et al. 2007). Phenolic compounds, exhibit a wide range of physiological properties, such as anti-allergenic, 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.

Plant-derived antioxidants may function as reducing agents, scavengers of free radicals and metal ion chelators. The antioxidant activity of plasma has been shown to increase after consumption of foods high in antioxidants.

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 p-coumaric 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 anti-inflammation and as an inhibitor of tumor initiation and as being a preservative (Adom and Liu 2002).

There are a number of literature reports on phenolic compounds and their antioxidant activities in cereals, fruits and vegetables. The analysis and determination of phenolic compounds in plants is usually performed in three steps. Firstly, extraction of phenolic compounds from samples, either in free or bound form, next, clean up of the extracts to eliminate interferences or, in some cases, to preconcentrate the phenolic compounds and finally, analysis of the phenolic compounds in the extracts. All steps are important to provide accurate and precise results.

Isolation of phenolic compounds from sample matrices is generally based on extraction. A wide variety of extraction techniques have been used to extract free phenolic compounds from plant materials, such as ultrasonic assisted extraction (Hung and Morita 2008), the shake-flask technique, supercritical fluid extraction, soxhlet extraction, microwave assisted extraction and pressurized liquid extraction (Bonoli et al. 2004). On the other hand, bound phenolic compounds can be extracted from plant materials using alkaline hydrolysis and acid hydrolysis. However, most researchers determine the bound phenolic compounds in cereal flours by alkaline hydrolysis (Bonoli et al. 2004, Tian et al. 2004, Zhou et al. 2004). Whereas, in only a few reports, has the bound phenolic compounds of cereals been determined by using acid hydrolysis (Bonoli et al. 2004).

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 (Bonoli et al. 2004, 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 (Herrera and Luque de Castro 2005, Chew et al. 2008).

A review of the current scientific literature however indicates that there are no published papers investigating the composition of simple phenolic acid containing compounds and their associated antioxidant activity within spouted wheat as a function of the germination period. Therefore aim

of this study is to investigate the phenolic composition and corresponding antioxidant activities of the phenolic extracts of the sprouted wheat flours at the three different germination times (15, 30 and 40 days).

The aim of this study is the development of an analytical method for the determination of phenolic compounds and their antioxidant activities in wheat grass. The study comprises extraction of phenolic compounds using alkaline hydrolysis for bound phenolic compounds, and analysis of phenolic compounds by RP-HPLC.

There are many studies concerned with wheat and wheat grass. But in our study we used a new method for analyzing the phenolic compounds in wheat grass. The cultivation effects haven't been studied until now. The extraction steps include important differences therefore our method is new and has differences from the other studies published before.

The high-performance liquid chromatography (HPLC) is an effective method for the determination of the active components in plant extracts. Previous studies validated an analytical HPLC method for the determination of phenolic compounds in various samples, which supplied some recommendations for the compounds chromatographic separation of phenolics. The aim of this study was to optimize a new method for the determination of phenolic compounds in sprouted wheat by using the different germination samples (15, 30 and 40 days) with HPLC Technologies.

MATERIALS AND METHODS

Chemicals and Reagents

The highest purity of phenolic compound standards was used. The Gallic acid, p-hydroxybenzoic acid, vanillic acid and caffeic acid were obtained from Acros (USA); whereas, the syringic acid, and p-coumaric acid, were purchased from Fluka (Switzerland) and the Ferulic acid was obtained from Sigma (Germany). The deionized water was obtained from Hamilton (UK) and was used throughout the experiments. The methanol of HPLC grade was obtained from Lab-Scan, the acetic acid of analytical reagent (AR) grade was obtained from Carlo Erba (Italy), and the 2,2′-diphenyl-1-picryl-hydrazyl (DPPH.) was from Sigma (USA). The quartz spectrophotometer dishes (Labart), the 4 ml and 22 ml vials (Agilent, USA), the reversed phase HPLC column (Zorbaks C_{18}), and the different sized glass materials (Isotherm) were used in the analysis.

Instrumentation

The HPLC system was an Agilent 1200 and the separations were performed on a C_{18} column. The analytical column was an Agilent Zorbax C_{18} (25 cm x 4.6 mm, with a 5μm particle diameter). The HPLC system has a degasser AF, a Rheodyne injector with a sample loop of 20 μL and a photodiode array detector and temperature control system.

Wheat Samples

The Turkish amber wheat (*Triticum turgidum*), which was harvested in 2011, was obtained from the Soil Products Office. The wheat samples were stored at 4°C in a vacuum package. These seeds were placed in water for 24 hours one day prior to germination. Yang et al. (2001) reported that the wheat grains steeped for 24 h germinated up to 40 days steadily and increased their antioxidant vitamins with the increased germination time.

Sprouting Conditions

The wheat was seeded in flower soil and was left to germination in pots with sunlight. The wheat varieties were grown in same organic conditions and after 15, 30 and 40 days of germination, the wheat grass was harvested. The sprouting wheat was dried with air in the dark; to dispose of humidity before being dried in an incubator. The wheat grass was allowed to air for a minimum of 7 days at room temperature and then placed in an incubator at 50°C overnight. The dried sprouted wheat grass was ground by using a Model 3100 Laboratory blender to obtain wheat grass flour.

Phenolic Extraction

The cell wall phenolic acids were extracted as described by Sen et al. (1991) with some modifications. Dried wheat grass was directly milled by using a blender and then was pounded using a glass mortar. Approximately 200 mg (dw; dry weight) of ground sample was added to 15 mL of 4 N NaOH in a 50 mL Pyrex centrifuge tube, purged with nitrogen, and shaken for 2 h at 30°C in the dark with a wrist-action shaker and heater (GFL model 1083, Germany). After the phenolic acid liberation by alkaline hydrolysis, the samples were acidified with ice-cold 6 N HCl to reduce the pH to between 1 and 2. Samples were centrifuged at 6000g (Hettich model Eba 20, Germany), and the supernatant was decanted into a 250 mL separatory funnel. The

supernatant was extracted with ethyl acetate (3 x 50 mL) with shaking for 10 s, and the mixture was allowed to settle for 5 min between extractions. The ethyl acetate fractions were collected and pooled, and all supernatants were combined. The remaining pellet was diluted with 15 mL of distilled H_2O, vortex disrupted, and recentrifuged at 3000g. The second supernatant was re-extracted with ethyl acetate (3 x 50 mL) as before and all ethyl acetate fractions were pooled. The phenolic acids-rich ethyl acetate fraction was dried by the addition of anhydrous sodium sulfate (Sigma-Aldrich, Germany) and concentrated using a rotary vacuum evaporator (Heidolph model laborota 4001) The pooled ethyl acetate extracts were evaporated to dryness and then the bound phenolic compounds were reconstituted in 10 mL of methanol and stored at -200 C until use for analyses.

HPLC Analysis

The phenolic extract was filtered through a 0.2 μm syringe filter into a vial and 3 μL was analyzed by a high performance liquid chromatography system (Agilent 1200 HPLC system). The analytical column was an Agilent Zorbax C18 (25 cm x 4.6 mm, 5µm). The mobile phase consisted of water containing 2% acetic acid, water (solvent A) and methanol (solvent B). The programmed run was only 40 min using a constant flow rate of 1 μL/min with the following gradient: 95% A, 5% B in 0 min, 95% A to 5% B, in 5 min, 30% A, 70% B in 30 min, and 95% A to 5% B, in 40 min. The peaks of all components were detected at 280 nm. The individual phenolic compounds were quantified based upon their peak's detected area against previously determined calibration curves and the HPLC chromatograms of the standard phenolic acids are shown in Fig.1.

Total Phenolic Measurement

The total phenolic content in the control and sprouted wheat extracts was determined using Folin–Ciocalteu's method as previously described by Hung et al. (2009). The phenolic extracts were diluted to an appropriate concentration prior to use. The Folin–Ciocalteu's reagent (0.5 mL) was added to a 15 mL-centrifuge tube containing a 0.5 mL of extract. The solution was oxidized and then neutralized with a saturated sodium carbonate solution (1 mL). The volume was adjusted to 10 mL with water, thoroughly mixed and allowed to stand for 45 min at ambient temperature. The solution was centrifuged for 5 min at 4000g and the absorbance of the clear supernatant was measured at 725 nm using an Perkin Elmer Lamda 35 model UV/Vis spectrometer, USA. A standard calibration was prepared using gallic acid (0, 20, 40, 60, 80 and 100 µg/mL) and the content of total phenolics in each extract was calculated and expressed as milligrams of gallic acid equivalent (GAE) per gram of the sample.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The antioxidant capacity of the phenolic extracts in the three different germination times of the sprouted wheat flours was determined by employing the DPPH radical scavenging assay according to the method previously described by Huang et al. (2005). The DPPH solution (0.075 mM) was prepared daily prior to use. The DPPH solution in ethanol was mixed with the wheat extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 60 min at 10 min intervals. The absorbance of the remaining DPPH radicals was measured at 515 nm using a Perkin Elmer Lamda 35 model UV/Vis spectrometer. The phenolic extracts (0.2 mL) were mixed with the DPPH solution (2.5 mL), kept in the dark at ambient temperature. The absorbance of the mixtures was recorded at 515 nm for exactly 50 min. A control made from 3.9 mL of DPPH and 0.1 mL methanol was measured the absorbance at time =0. The scavenging of DPPH was calculated according to the following equation (Liyana-Pathirana and Shahidi, 2006):

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

$Abs_{(t=0)}$= absorbance of DPPH radical + methanol at t = 0 min;

$Abs_{(t=30)}$= absorbance of DPPH radical + phenolic extracts at t = *30* min.

The scavenging capacity was expressed as μg DPPH radical scavenged/g of defatted material.

Statistical Analysis

All analyses were performed in triplicate and the data reported as mean ± standard deviation, unless otherwise stated. All analyses of variance was performed using the General Linear Model of Minitab Release 14 Xtra for Windows (Minitab Inc., State College, PA). The significant differences ($P < 0.05$) among the means were determined using Tukey's multiple range test at a fixed level of a =

Table 1. Phenolic acid composition of alkaline hydrolysis of ethyl acetate extracts on sprouted Turkish Amber Durum according to HPLC results based on the dry weight of wheat grass (μg/g of sample, db).

SAMPLE(μg/g)	15 DAYS	30 DAYS	40 DAYS	R^2
GALLIC	3.45±1.7	2.10±6.7	1.3±1.3	0.997
SYRINGIC	150.8±1.3	75.8±13.2	54.7±4.2	0.994
P-HYDROXYBENZOIC	35.4±3.2	16.4±1.3	4.2±1.7	0.997
CAFFEİC	75.1±1.8	32.3±2,7	11.2±1.3	0.999
BENZOIC	65.3±2.7	54.8±1.9	2.2±0.5	0.999
QUERCETIN	115±3.7	180.2±1.4	5.95±1.7	0.998
P-COUMARIC	83.7±12,1	7.4±2.4	5.3±1.1	0.998
FERULIC	1305.8±1.7	809.4±23.5	250.4±1.5	0.999
ELLAGIC	18.3±1.3	24.2±1.1	42.2±1.6	0.998
BHA	150.4±1.8	260±1.7	12±1.2	0.999

0.05.

RESULT AND DISCUSSION

Phenolic Compounds Profiles

The chromatographic profile of phenolic compounds extracted from the sprouted wheat grass flours by the HPLC system is shown in Table 1. Nine standards were separately detected by using the HPLC. In cereal grass, the majority of phenolic acids are bound to the cell wall in ester form and present in insoluble bound form. Only a small portion exists as free phenolic acids. Therefore, the insoluble phenolic acids were recovered using alkaline hydrolysis. The identification of the monomeric phenolic acids was accomplished by comparison of the UV spectra and retention times with external standards. Nine simple phenolic acids were observed in the wheat grass. They were respectively eluted at 5.167, 14.088, 16.554, 17.968, 20.143, 21.313, 23.145, 24.314, 27.497 and 34.412 min, representing gallic acid, p-hydroxy benzoic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, ellagic acid benzoic acid, quercetin and bha. To quantify individual phenolic acids, the monitor wavelength was set at 280 nm for all phenolic acids. Table 1 summarizes the content of each phenolic acid in wheat grass which germinated at different time periods. Ferulic acid, as reported before, was found as the most dominant phenolic acid in all of the wheat grass samples (74-87 mg/100 g), constituting up to 69% of the total phenolic acids. In wheat grass flours at 15, 40 and 30 days germination the ferulic acid represented 65.25%, 56.85%, and 64.59% of the total amount of detected free phenolic compounds, respectively. However, the main bound phenolic compounds released by alkaline hydrolysis was ferulic acid, which accounted for 65.25% of the amber wheat grass flour (germinated in 15 days) and syringic acid which accounted for 26.46% of the wheat grass flours. Table 1 shows the concentration of individual phenolic compounds in amber wheat grass flours extracted with alkaline hydrolysis.

In our study, the phenolic profile of amber wheat grass flours after alkaline hydrolysis included nine compounds see Fig. 2. By using ethyl acetate extraction, nine phenolic compounds were found in amber wheat grass flours with a decreasing order of ferulic acid > syringic acid > quercetin> p-coumaric acid > caffeic acid > benzoic acid > p-hydroxybenzoic acid > ellagic acid > gallic acids (Fig. 3).

These results are not consistent with the data reported by Liyana-Pathirana and Shahidi (2006), where syringic acid was not found. These results are in agreement with those reported by Hatcher and Kruger (1997) and Mattila et al. (2005). These results are in agreement with the previous observations (Onyeneho and Hettiarachchy 1992, and Graf 1992) that ferulic acid was the predominant total phenolic acid on a per weight basis. The grain of Swiss red wheat contained 33.71 μg of extractable ferulic acid/g of seeds, which is >5 ppm (μg/g) of free and soluble bound ferulic acid (Sosulski et al 1982) but is much lower than the reported typical level of 500 μg of ferulic acid/g of ground whole wheat (Graf 1992, Sosulski et al 1982). The extractable phenolic acid is a portion of, and may account for <%10 of, the total phenolic acid presented in wheat grass. Ferulic acid has been evaluated for its potential application as an analytical parameter in the rapid determination of wheat grass carry over during germination. The antioxidant properties of ferulic acid were evaluated and reviewed by Graf in 1992. In this study, the ferulic acid content was well correlated with antioxidant activities, total phenolic content, and concentrations of other identified individual phenolic acids. Therefore, ferulic acid may serve as a marker for the quality control of wheat grass antioxidants or may be used to monitor wheat antioxidant processing.

Total Phenolic Measurement

The total phenolic content (TPC) of the amber wheat grass, germinated at different time periods (15, 30 and 40 days), was determined by the Folin-Ciocalteu's method, and expressed as gallic acid equivalent per gram of wheat grass flour. In this study, the results are in good accordance with some

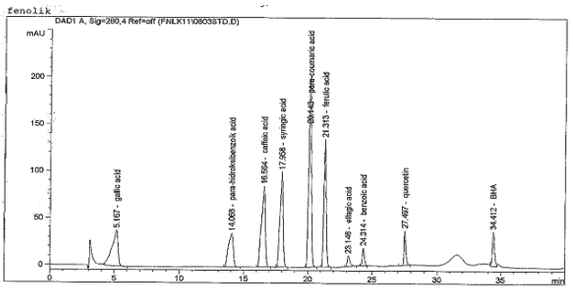


Fig. 1. HPLC chromatogram of standard phenolic acids.

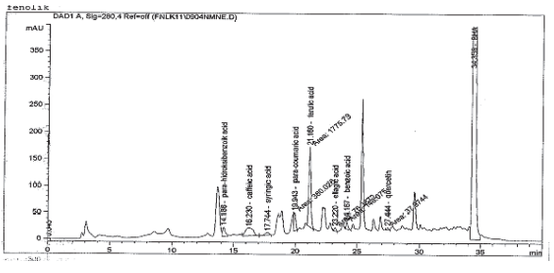


Fig. 2. HPLC chromatogram of wheat grass (germinated 30 days).

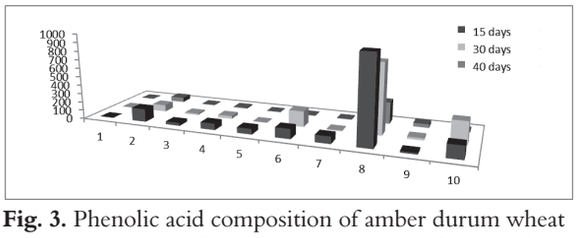


Fig. 3. Phenolic acid composition of amber durum wheat grass (μg/g of sample, db, and dry basis).1) gallic acid, 2) syringic acid, 3) p-hydroxybenzoic, 4) caffeic acid, 5) benzoic acid, 6) quercetin, 7) p-coumaric acid, 8) ferulic acid, 9) ellagic acid, and 10) BHA

previously reported data. Zielinski et al. (2000) found similar contents of total phenolics in dehulled buckwheat (269.8 mg/100 g), using this type of solvent for extraction. In this work, the level of total phenolic content varied significantly ($p \leq 0.05$) between cultivars (Table 2).

The TPC was the highest in amber wheat grass flour germinated for 15 days at 292.8 mg/100 g and the lowest in wheat grass flour germinated for 40 days at 196.5 mg/100 g, while the value for wheat grass flour germinated for 30 days was 235,0 mg/100 g (Fig. 4). Many studies confirmed that the content of phenolic compounds depends on the type of analyzed sources

In our study, the contribution of ferulic acid to the total detected phenolic compounds (56.85-65.25%) is also in agreement with the findings by Zhou et al. (2004), where the ferulic acid accounted for 57-77% of the total identified phenolic acids in Swiss red wheat. However, these findings were lower than those reported by Sosulski et al. (1982) and Hatcher and Kruger (1997). Thus, the phenolic acid profile and the concentration of individual phenolic acid are dependent on the wheat varieties and growing location (Hatcher and Kruger 1997) of the studied individual wheat varieties and not a composite of varieties as in our current study. The phenolic acid profiles in amber wheat grass flour after germination are given in Fig. 3.

DPPH Radical Scavenging

The DPPH radical scavenging method was used to determine the antioxidant activity of the phenolic extracts in this study. This method is based on the reducing ability of antioxidants toward DPPH (Huang et al. 2005, Prior et al. 2005) and widely used to evaluate the antioxidant activity of phenolic compounds extracted from fruit, vegetables, cereal grain, wine, etc. (Jimenez-Escrig et al. 2000) because of its stability and ease of use. Table 3 highlights the antioxidant activity in amber wheat grass flour extracts.

During germination, amber wheat grass exhibited an increase in their antioxidant activities. With extraction by ethyl acetate the bound and free phenolic compounds in the sprouted wheat had significantly higher DPPH radical scavenging (Fig. 5).

The data also show that the antioxidant activities of the extracts by alkaline hydrolysis were not significantly different for the sprouted amber wheat grass flours. These results are due to the increase in amount of syringic acid, caffeic acid and vanillic acid during germination, while the phenolic acids bound to the cell wall did not change. In addition Yang et al. (2001) reported that the antioxidant compounds such as vitamin C and tocopherols also increased with the length of germination, which might also increase the antioxidant activity of the sprouted wheat flours. Thus, the sprouted wheat's are shown to have more desirable nutritional values than the un-germinated wheat's and could be used to blend with commercial wheat flour to increase both the nutritional value and texture of bread (Ranhotra et al. 1977, Morad and Rubenthaler 1983).

CONCLUSION

This study demonstrates a reliable method to extract and analysis the phenolic compounds in wheat. The HPLC was used and a binary mixture of

Table 2. Total phenolic content from alkaline hydrolysis of ethyl acetate extracts of Turkish amber wheat sprouted on 15 days, 30 days, and 40 days (µg GAE/g of sample, db, and dry basis).a,b

Sample	Alkaline hydrolysis And ethyl acetate extract
15 Days Amber wheat grass	$2928 \pm 16_{a,b}$
30 days Amber wheat grass	$2350 \pm 21_{a,b}$
40 days Amber wheat grass	$1965 \pm 15_{a,b}$

a Means by the same letter in the same column is not significant difference ($p < 0.05$), n = 4. b Gallic acid Equivalents.

Table 3. DPPH radical scavenging capacity (% scavenging) of extracts obtained from sprouted Turkish amber durum.

Sample	DPPH radical scavenging capacity (% scavenging)
15 Days Amber wheat grass	52.62a
30 days Amber wheat grass	42.03a
40 days Amber wheat grass	31.14a

Full-size table
a Means by the same letter in the same column is not significant difference ($p < 0.05$), n = 4.

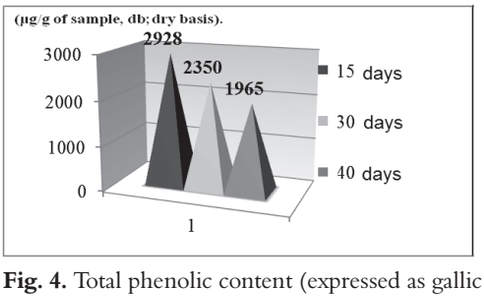


Fig. 4. Total phenolic content (expressed as gallic acid equivalent) in analyzed cultivars. Means denoted by the same letter for each cultivar are not statistically different at $p \leq 0.05$. Results are the means of four measurements (n = 4)

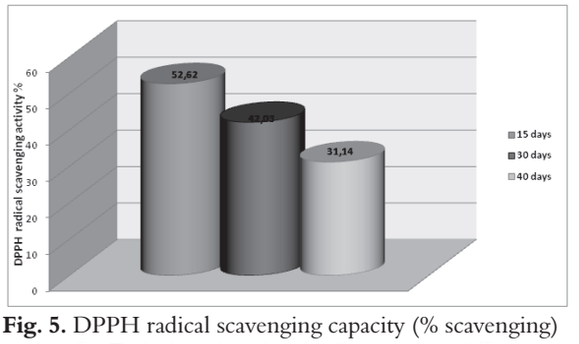


Fig. 5. DPPH radical scavenging capacity (% scavenging) for Turkish amber durum(Sprouted in different time periods).

the mobile phase was used as a chromatographic condition in which 10 phenolic compounds were separated within 40 min. In this study, a database for the presence of phenolic compounds and their amounts, as well as the antioxidant activity of wheat varieties (germinated in three different times) were reported. For the ethyl acetate extracts of all the studied samples, ten phenolic compounds were detected. Ferulic acid, p-coumaric acid and syringic acid were detected in large amounts when compared to the other phenolic acids. Total Phenolic Content and the DPPH radical scavenging activity were also determined using spectrophotometry. The TPC found in the wheat samples was in good agreement with the results obtained from HPLC. As a result, no studies have ever been found on wheat grass grown in Turkey; therefore, the current study is critically important for the Food Industry in Turkey. In following studies, the use of its as natural food preservatives in meat and meat products will be studied.

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