JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7329–7334 7329
DOI:10.1021/jf1010534

Polyphenol Content of Modern and Old Varieties of *Triticum aestivum* L. and *T. durum* Desf. Grains in Two Years of Production

Daniela Heimler,*,† Pamela Vignolini,‡ Laura Isolani,† Paola Arfaioli,† Lisetta Ghiselli,§ and Annalisa Romani‡

†Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, Università degli Studi di Firenze, P.le delle Cascine 18, 50144 Firenze, Italy, ‡Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, via U. Schiff 6, 50019 Sesto Fiorentino, Italy, and §Dipartimento di Scienze Agronomiche e Gestione del Territorio Agroforestale, Università degli Studi di Firenze, P.le delle Cascine 18, 50144 Firenze, Italy

Antiradical activity and total polyphenol and flavonoid contents of modern and old varieties of durum (9 varieties) and soft (17 varieties) wheat grains, sampled over two years (summer 2007 and 2008) in the same site, were determined in order to point out differences among varieties and between old and modern varieties. Nine compounds were identified by means of HPLC/MS, and their amount was determined. No correlation was found between antiradical activity and polyphenol or flavonoid contents. The temperature conditions in the 30 days before harvesting were the principal factor which differentiated the quantitative profile of polyphenols and the antiradical activity of the seeds ($p < 0.001$): high temperatures cause a drop in polyphenol content.

KEYWORDS: Antiradical activity; flavonoids; polyphenols; wheat grains

INTRODUCTION

Wheat is a type of grass grown all over the world for its highly nutritious and useful grain. It is one of the top three most produced crops in the world, along with corn and rice. Wheat has been cultivated for over 10,000 years and probably originates in the Fertile Crescent and in Ethiopia, along with other staple crops. *Triticum aestivum* L. (soft wheat) and *Triticum durum* Desf. (durum wheat) are cultivated in Italy, which ranks 17th in the world for the cultivation of soft wheat and second, after Canada, in the production of durum wheat, which is mostly used for the production of different kinds of pasta. In Italy, durum wheat is cultivated in about 1.7 million hectares and soft wheat in 750,000 ha. In both cases, modern varieties are used whose heights vary from about 60 to 90 cm, while old varieties may reach and even exceed 1.5 m in height. In effect, the new varieties were selected on the basis of the improvement of their functional characteristics in addition to their productivity. In the case of durum wheat, the main strategy for plant breeding programs aimed toward a better grain yield, shorter height, and early maturity (*1*). For instance, in a study on 24 durum wheat varieties (*2*), an increase of the yield was observed passing from old (<1945) to intermediate (1950−1985) and to modern varieties (1988−2000).

Regular consumption of whole grain and whole-grain derivatives might reduce the risk of developing chronic diseases such as cardiovascular diseases, type-2 diabetes, and some cancers. These beneficial effects are associated with the phytochemicals of whole grain (*3*), which include phenolics, carotenoids, vitamin E, lignans, β-glucan, phytosterols, and dietary fiber (*4*).

Among these compounds, polyphenols play an important role in contrasting oxidative stress, which is defined as the imbalance between oxidants and antioxidants and has been indentified as the cause of aging and various diseases in humans (*5*). Many papers deal with the polyphenol content and antioxidant activity of wheat grains in relation to the polishing method (*6*−*8*); in any case, antioxidant activity and polyphenol content increase from the inner parts of the grain outward. This depends on the localization of phenolics in grains: husk, pericarp, testa, and alueurone layers contain the highest concentration of polyphenols. Dinelli et al. (*9*) determined the qualitative profile of phenolic compounds (free and bound fractions) in 10 wheat varieties, comprising old and modern durum wheat genotypes from Italy, some of the same varieties analyzed in this work, harvested in a different location (Bologna), Adom, Sorrell, and Liu (*10*) studied phytochemical profiles and antioxidant activity of 11 wheat varieties, while Yu et al. (*11*) studied the antioxidant properties of wheat flours from different locations. Antioxidant activity (*12*) was determined in grains from different samples (oats, corn, wheat, and rice), and free radical scavenging properties (*13*) were studied in three hard winter wheat varieties.

In recent years, cultivation of old wheat varieties has taken place owing to consumer preferences and to exploit old varieties in peculiar zones where they have been cultivated traditionally.

The purpose of this work was to study some characteristics of old and modern soft and durum wheat varieties harvested over two years (2007 and 2008) in the same site from the standpoint of polyphenols and antiradical activity.

*Corresponding author. Tel: (+39) 553288201. Fax: (+39) 55333273. E-mail: daniela.heimler@unifi.it.

Published on Web 05/28/2010

Table 1. Modern and Old Varieties of Durum and Soft Wheat Sampled along Two Years (2007 and 2008)

durum wheat		soft wheat	
modern	old	modern	old
1 Anco Marzio	7 Kamut	10 Bolero	14 Abbondanza
2 Claudio	8 Senatore Cappelli	11 Eureka	15 Andriolo
3 Iride	9 Urria 12	12 Miieti	16 Autoonmia B
4 Levante		13 Palesio	17 Bianco Nostrale
5 Orobel			18 Carosello
6 Solex			19 Gentil Bianco
			20 Gentil Rosso
			21 Gentil rosso Mutico
			22 Inallettabile
			23 Marzuolo d'Acqui
			24 Marzuolo val Pusteria
			25 Sieve
			26 Verna

MATERIALS AND METHODS

Agricultural Conditions and Plant Material. The varietal comparisons were conducted during the 2006−2007 and 2007−2008 grain seasons on a farm near Florence, Tuscany. The wheat varieties used in this study are reported in **Table 1**. Old varieties are no longer registered in the national variety register, while modern varieties are commercial ones. All varieties were cultivated under an organic agricultural system. The field trials included plots of 1.5 m^2 with five rows within each plot. Wheat grains were sampled on 18 June, 2007 and 4 July, 2008.

Standards. Apigenin 7-*O*-glucoside, gallic acid, Folin−Ciocalteu reagent, and DPPH• (1,1-diphenyl-2- picrylhydrazil radical) were purchased from Sigma-Aldrich (St. Louis, USA).

Solvents. All solvents used were of HPLC grade purity (BDH Laboratory Supplies, Poole, United Kingdom).

Extraction of Polyphenol. Wheat grains of each variety were ground in a stone mill. The whole flour obtained was extracted with ethanol/water/formic acid (70:29.5:0.5); 6 g of flour was suspended in 25 mL of the extracting solution for 3 h. The mixture was then centrifuged at 6000*g* for 30 min in order to obtain clear solutions. The supernatants were used for subsequent analysis.

Antiradical Activity. Free radical scavenging activity was evaluated with the DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams and Cuvelier (*14*) with slight modifications. Two milliliters of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH• (0.0025 g/100 mL), and the mixture was kept at room temperature. After 20 min, the absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin-Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH• solution was checked. Antiradical activity is expressed as IC_{50}, the antiradical dose required to cause a 50% inhibition. IC_{50} was calculated plotting the ratio $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorption of the DPPH• solution, and A_{sample} is the absorption of the DPPH• solution after the addition of the sample, against the concentration of the sample. Straight lines are obtained in each case with R^2 changing from 0.8179 to 0.9981. IC_{50} is expressed as mg sample/mg DPPH•.

Total Phenolic Content. The total phenolic content was determined using the Folin−Ciocalteu method, described by Singleton et al. (*15*) and slightly modified according to Dewanto et al. (*16*). To 125 μL of the suitably diluted sample extract, 0.5 mL of deionized water and 125 μL of the Folin−Ciocalteu reagent were added. The mixture was kept for 6 min, and then 1.25 mL of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/100 g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 μg/mL (R^2 = 0.9969).

HPLC/DAD Analysis. Analyses of flavonols and hydroxycinnamic derivatives were carried out using a HP 1100 L liquid chromatograph equipped with a diode array detector (DAD) and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Analysis was carried out over a 50-min period at a flow rate of 0.65 mL min^{-1} using a 250 × 4.6 mm i.d., 5 μm Lichrosorb RP18 column (Phenomenex, USA) operating at 27 °C. A five-step linear solvent gradient system was used, starting from 100% H_2O (adjusted to pH 3.2 by HCOOH) up to 100% CH_3CN over a 45-min period. UV/vis spectra were recorded in the 190−600 nm range, and the chromatograms were acquired at 260, 280, 330, and 350 nm.

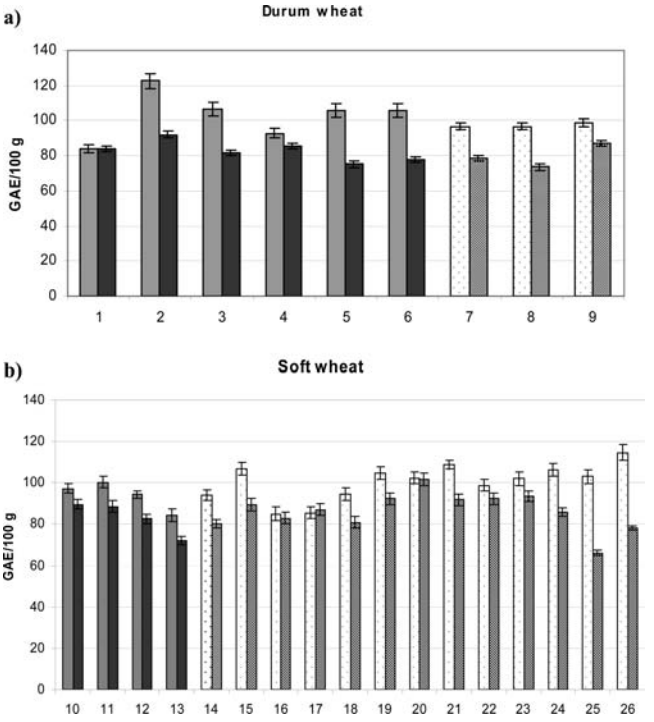

Figure 1. Polyphenol content (GAE, mg gallic acid/100 g) in 2007 (first bar) and 2008 (second bar) samples of (**a**) durum and (**b**) soft wheat. Variety numbers are as described in **Table 1**. Solid bars indicate modern varieties.

HPLC/MS Analysis. Analyses were performed using a HP 1100 L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/Electrospray interface (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer operating conditions were gas temperature, 350 °C; nitrogen flow rate, 11.0 L/min; nebulizer pressure, 40 psi; quadrupole temperature, 100 °C; and capillary voltage, 4000 V. The mass spectrometer was operated in positive mode at 120 eV.

Identification and Quantification of Individual Polyphenols. Identification of individual polyphenols was carried out using their retention times and both spectroscopic and mass spectrometric data. Quantification of individual polyphenolic compounds was directly performed by HPLC/DAD using a five-point regression curve ($r^2 \geq 0.998$) in the range 0−30 μg on the basis of standards. In particular, flavonols were determined at 330 nm using apigenin 7-*O*-glucoside as the reference compound. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight. Three samples were collected from each site so as to express the analytical results as an average with its standard deviation.

Statistical Analysis. Statistical analysis was performed with SYSTAT 9 software. Data were subjected to one-way analysis of variance. Differences at $p < 0.05$ were considered to be statistically significant. All data are the mean values of three determinations, and standard deviations never exceeded 5%.

Meteorological Data. The meteorological data were supplied by the Laboratory for Meteorology and Environmental Modeling (IBIMET-LAMMA) of the Italian Research Council (CNR).

RESULTS AND DISCUSSION

Figure 1 shows the polyphenol content of durum (6 modern and 3 old varieties) and soft (4 modern and 13 old varieties) wheat

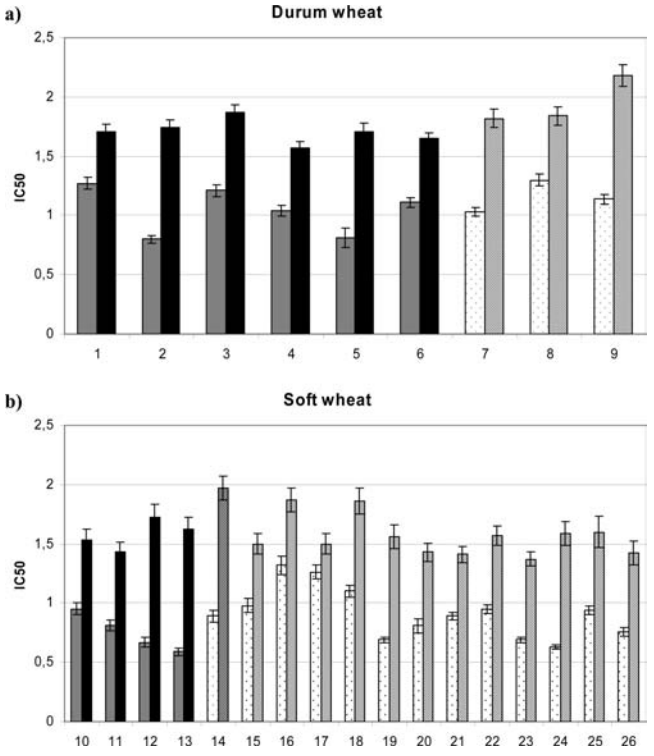

Figure 2. Antiradical activity (IC_{50} values) in 2007 (first bar) and 2008 (second bar) samples of (**a**) durum and (**b**) soft wheat. Variety numbers are as described in **Table 1**. Solid bars indicate modern varieties.

sampled in 2007 and 2008. The extraction of polyphenols was carried out without a prior hydrolysis process; therefore, the amount detected is lower than that previously reported when extraction involved a hydrolysis process (*10*, *23*) but similar to that when the hydrolysis process was lacking (*6*, *17*). This extraction was chosen for two reasons: first, for an easier and more rapid analysis; and second, to reproduce the original quali-quantitative profile. The absorption of polyphenols (free or bound) in human nutrition is a complex subject concerning specific cases and compounds (*18*−*20*). In wheat, it has been shown that most phenolics are present in insoluble wall-bound forms (*10*, *12*) and may reach the colon without any modification (*12*). In general, free or glycosilated flavonoids are absorbed in the stomach or small bowel (*21*, *22*); our study focuses mainly on these compounds. Among durum wheat modern varieties, there are some differences, and for instance, Claudio (var. number 2) exhibited the highest content in both years. In most cases, polyphenol content of 2008 seeds is lower than that of 2007 seeds. No significant differences were found either between modern and old varieties of durum and soft wheat or between durum and soft wheat. When all varieties are considered together, there are significant differences between the two years regarding modern and old varieties of durum ($p < 0.05$) and soft ($p < 0.001$) wheat. According to the data of polyphenol content, the antiradical activity of wheat in 2008 was lower (higher IC_{50} values) than that in 2007 for durum and soft modern and old varieties (**Figure 2**). Among 2007 samples, Palesio (var. number 13) and Marzuolo Val Pusteria (var. number 24) exhibited the highest antiradical activity. In the 2008 samples of durum wheat, only the low antiradical activity of Urria 12 (var. number 9) can be pointed. In general, with regard to 2008 data, very similar contents are achieved.

Total phenolics, due to the analysis method used, account also for the antioxidant activity of wheat; therefore, it is interesting to check whether a correlation can be found between polyphenol content and antiradical activity. In no cases has a relationship between antiradical activity and polyphenol content been found. Adom and Lui (*12*) found a correlation between polyphenol content and antioxidant activity in the case of bound and total extracts of grains, but the correlation was lacking in the case of free extracts, while Yu et al. (*11*) did not find any correlation between polyphenol content and antiradical activity. With DPPH as radical scavenger, different correlations between polyphenol content and antiradical activity were found depending on the salad species (*23*). It should be kept in mind that the Folin−Ciocalteu test measures the sample's reducing capacity and not its antioxidant capacity; some compounds such as simple phenols may react with the Folin−Ciocalteu reagent even if they are not effective radical scavenging antioxidants (*24*). It is not clear whether DPPH reactions occur via a hydrogen atom or an electron transfer. In the case of cinnamic acids, the reaction with the DPPH radical occurs via an electron transfer (*25*), while for Mediterranean herbs and aromatic plants, the mechanism seems to be via a hydrogen atom transfer (*26*) with a good correlation among DPPH scavenging properties and the Folin−Ciocalteu test. A previous study on hard winter wheat bran showed no correlation between DPPH and total phenolic content, while total phenolic content seems a good indicator of ABTS and ORAC scavenging capacities (*27*).

Liquid chromatography coupled with UV−vis and MS detection allows the identification of free phenolic compounds of wheat extracts. In the complex chromatographic profile of wheat extract (**Figure 3**), there were only six flavonoids that were noteworthy. Our elution method enabled us to separate four isomers: peaks 1 and 2 had the same UV/vis spectra with absorption maxima at 272 and 340 nm, respectively, even peaks 3 and 4 had the same absorption maxima at 272 and 334 nm, respectively. These compounds were similar to those isolated by Asenstorfer et al. (*28*) in Australian wheat cultivars, in particular, Asenstorfer identified two major components: the first was a mixture of apigenin-6-*C*-arabinoside-8-*C*-glucoside and apigenin-6-*C*-glucoside-8-*C*-arabinoside, and the second was a mixture of apigenin-6-*C*-arabinoside-8-*C*-galactoside and apigenin-6-*C*-galactoside-8-*C*-arabinoside. Even the MS analysis of peaks 1, 2, 3, and 4 enabled the identification of these compounds. The observed parent ions of these peaks were identical with m/z 565 $[M + H]^+$, and even the ionization pattern is quite similar to that previously reported (*28*), with a gradual loss of H_20 molecules (**Figure 4**). Peaks 5 and 6 are two isomers with the same spectra with absorption maxima at 272 and 330 nm, respectively, and with m/z 771 $[M + H]^+$ identical to the sinapic acid adduct of apigenin-*C*-diglycoside described by Asenstorfer et al (*28*). Using this elution method and LC/MS, it has been also possible to identify the presence of the other two minor components (in traces) with m/z 741 $[M + H]^+$ (peaks 7 and 8), probably the ferulic acid esters of apigenin diglycosides previously reported by Asenstorfer et al. (*28*). To confirm the presence of these compounds, in **Figure 3** are reported the extract ion currents for the masses m/z 565, 771, and 741. Besides flavonoids, ferulic acid was also identified (peak 9, retention time = 28 min).

A structured peak, probably due to the presence of phenolics in wall-bound or associated forms, is also evident in the chromatographic profile with elution times from 32 to 42 min. This band is always present in all of the varieties considered, and in particular, for durum wheat, it ranges from 39% (Orobel) to 45% (Urria 12) of a total area calculated at 280 nm UV−vis (the maximum of absorbance of UV−vis spectra of these compounds), while for soft wheat it ranges from 37% (Svevo) to 55% (Autonomia B).

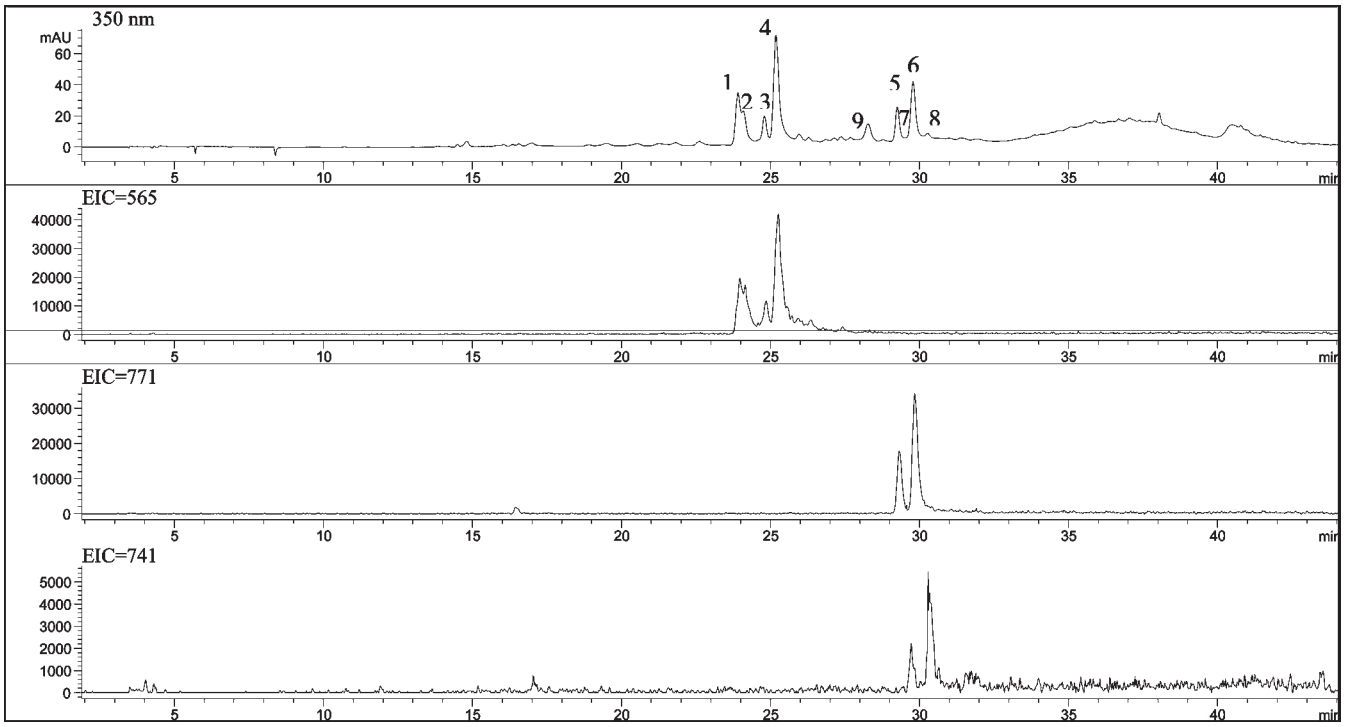

Figure 3. Chromatographic profile acquired by HPLC/DAD (350 nm) of the hydroalcoholic extract of a soft wheat (Palesio). Identified flavonols: apigenin-6-*C*-arabinoside-8-*C*-glucoside and apigenin-6-*C*- glucoside-8-*C*-arabinoside (peaks 1 and 2), apigenin-6-*C*-arabinoside-8-*C*-galactoside and apigenin-6-*C*-galactoside-8-*C*-arabinoside (peaks 3 and 4), sinapic acid esters of apigenin-*C*-diglycosides (peaks 5 and 6), acid esters of apigenin-*C*-diglycosides (peaks 7 and 8), and ferulic acid (peak 9). Extract ion currents are at *m*/*z* 565, 771, and 741.

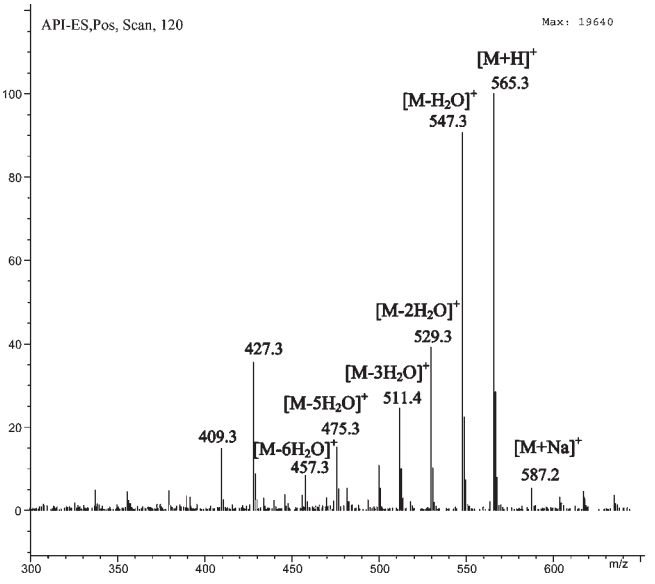

Figure 4. MS spectrum of the peak with *m*/*z* 565.

The total amounts of flavonoids calculated from HPLC data of the identified compounds for all of the wheat samples are reported in **Figure 5**; they were quantified according to the calibration curve of apigenin 7-*O*-glucoside (R^2 = 0.9987). The data agree with the trends of polyphenols and antiradical activity, i.e., the flavonoid content for both durum and soft wheat is much higher ($p < 0.001$) in the 2007 than in the 2008 samples. Claudio (var. number 2), Marzuolo d'Acqui (var. number 23), and Bolero (var. number 10) exhibit the highest flavonoid content in 2007; in 2008, Marzuolo d'Acqui maintains its highest flavonoid content. **Figure 6** shows the quantitative trend of apigenin-*C*-diglycoside

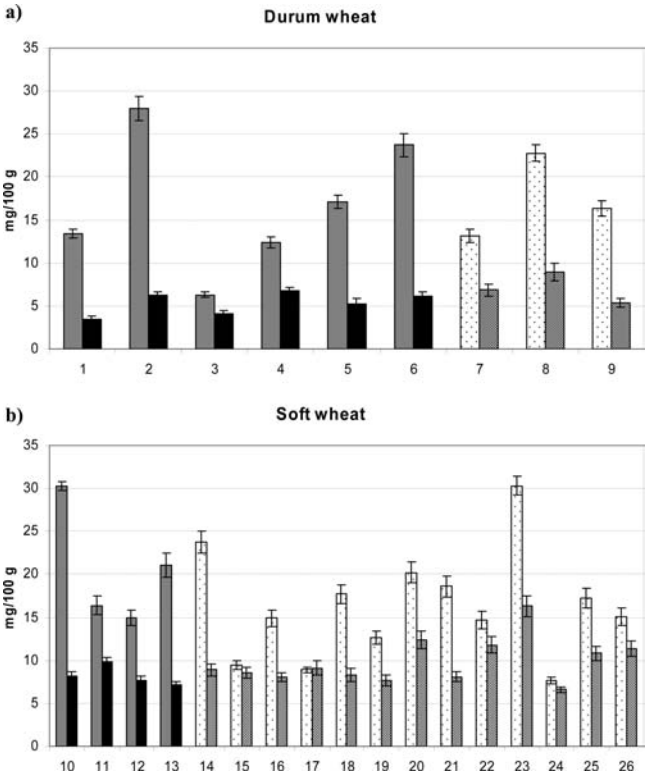

Figure 5. Flavonoid content (mg/100 g) in 2007 (first bar) and 2008 (second bar) samples of (**a**) durum and (**b**) soft wheat. Variety numbers are as described in **Table 1**. Solid bars indicate modern varieties.

(peaks 1 and 2), apigenin-*C*-diglycoside (peaks 3 and 4), and the sinapoyl derivatives of apigenin-*C*-diglycoside (peaks 5 and 6 of **Figure 3**) contents of the wheat cultivars grown in 2007 and 2008.

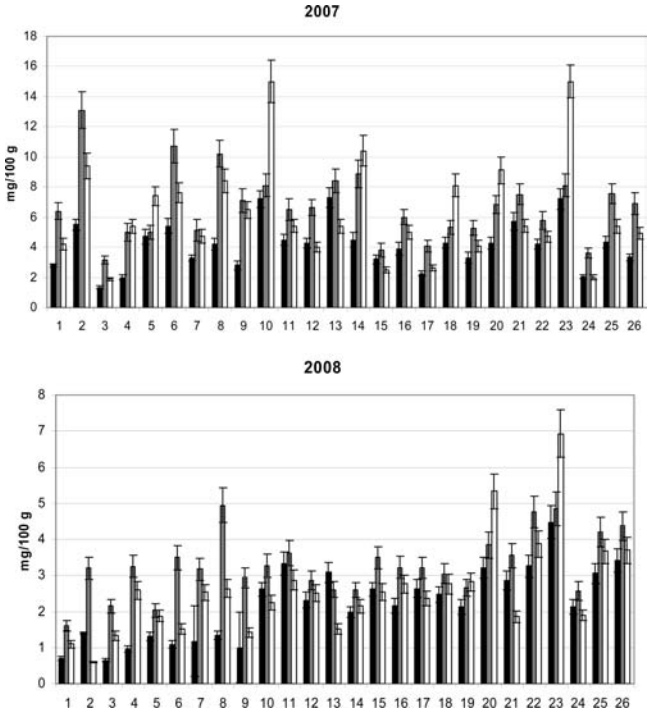

Figure 6. Content of apigenin-*C*-diglycosides (peaks 1 and 2 of **Figure 3**, first bar), apigenin-*C*-diglycosides (peaks 3 and 4, second bar), and sinapoyl derivatives of apigenin-*C*-diglycosides (peaks 5 and 6, third bar) in the wheat cultivars grown in 2007 and 2008. Numbers are as described in **Table 1**.

In particular, we can notice that samples 10 and 23 (sowed in 2007), different from all other samples, exhibit the highest content of sinapoyl derivatives of apigenin-*C*-diglycosides with respect to those of the other compounds; in the 2008 samples, Marzuolo d'acqui (sample number 23) maintains its highest content of sinapoyl derivatives together with Gentil Rosso (sample number 20).

The harvest year has a great influence on the characteristics of wheat. Polyphenol and flavonoid contents and antiradical activity are significantly different in the two years. These differences may be ascribed to the temperature and amount of rain in the 30 days before harvest. In this period, both minimum and maximum temperatures were lower in 2007 than in 2008; the mean temperature in this period changed from 16.9 to 19.8 °C for t_{min} and from 26.3 to 32.4 °C for t_{max}. It has been shown that heat stress reduced phenolic contents in spearmint (*29*) and in hawthorn (*30*), while spring-grown spinach contained more phenolics and exhibited higher antioxidant capacity than fall-grown varieties (*31*). In a study on changes in amino acid composition of durum wheat grains depending on temperature and water regimes, an increase of protein content was observed in the warmest and driest environments, while most amino acids exhibited their lowest values under very dry conditions with the exceptions of glutamine, proline, and phenylalanine whose values are statistically significantly higher under dry conditions (*32*). In our case, higher temperatures in the 30 days before harvest may have caused a lesser amount of flavonoids and reduced antiradical capacity. The amount of rainfall, considered in the 30 days before harvest, was higher in 2007 (80.2 mm) than in 2008 (29.6 mm). Water stress generally causes an increase of antioxidant capacity and phenolic content (*30*, *33*) in the present study, in which water stress has not been induced, but only the differences between the two years have been monitored, the effect seems the opposite; however, it is not possible to discriminate between heat and water deficit.

In conclusion, the cultivation of wheat in the same site for two years showed that atmospheric conditions are the main factor which causes differences in free polyphenols and antiradical activity. Differences between the two species, between old and modern varieties, and within varieties are of lesser importance with regard to free polyphenol content and antiradical activity. However, location played a key role in the total content of polyphenols (*9*, *34*), and location in this study may include atmospheric conditions.

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Received for review March 19, 2010. Revised manuscript received April 20, 2010. Accepted April 28, 2010. This research was supported by funds from Ente Cassa di Risparmio di Firenze, which contributed to the acquisition of a part of the instrumentation used for this work, from the Fondazione Monte dei Paschi di Siena, and from the MIUR (PRIN 2006).