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Polyphenol Content and Antioxidative Activity in Some Species of Freshly Consumed Salads

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Ten genotypes belonging to *Lactuca sativa*, *Cicorium intybus*, *Plantago coronopus*, *Eruca sativa*, and *Diplotaxis tenuifolia* and used in fresh mixed salads were investigated for their polyphenol contents. Flavonoids and hydroxycinnamic acids were characterized by high-performance liquid chromatography (HPLC)/diode array detection/mass spectrometry. Quercetin, kaempferol, luteolin, apigenin, and crysoeriol derivatives were identified; hydroxycinnamic acids were all caffeoyl derivatives. The total polyphenol content was obtained through the Folin−Ciocalteu test and from the HPLC data. The amounts ranged between 0.9 and 4.7 mg/g fresh weight. The antiradical activity was determined by the reaction with the stable DPPH• radical. The Fe^{2+} chelating activity was determined with a spectrophotometric test. From the complex of data, a quite complete picture of the characteristics of the vegetables emerges. A cultivated *C. intybus* cultivar exhibited the highest polyphenol content, while a wild *C. intybus* genotype exhibited the highest antiradical activity. In every case, the characteristics of the different salads as functional foods have been pointed out.

KEYWORDS: Flavonoids; hydroxycinnamic acid derivatives; total phenolics; antiradical activity; iron ion chelating ability; *Lactuca sativa*; *Cicorium intybus*; *Plantago coronopus*; *Eruca sativa*; *Diplotaxis tenuifolia*

INTRODUCTION

In these last years, great importance has been devoted to the consumption of fresh vegetables and fruits since they are a source of naturally occurring antioxidants; the search for vegetables that are endemic or are traditionally used as food offers wide spaces to add value to local products. In fact, plant secondary metabolites, which account for most antioxidant properties, have evolved according to their interactions with the surrounding environment.

Antioxidants prevent lipid oxidation and can act in different ways, including decreasing oxygen concentrations, scavenging initiating radicals, and binding metal ions to prevent initiating radical formation (*1*). Today, it is believed that regular consumption of dietary antioxidants may reduce the risk of several diseases (*2*). Dietary antioxidants can increase cellular defense and help to prevent oxidation damage to cellular components (*3*). Among natural antioxidants, plant polyphenols play a very important role (*4*). Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities; the protective effects can be ascribed to their capacity to transfer electron-free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (*5*).

Fresh salad is currently consumed throughout the year; in Tuscany, in winter, mixed greens are generally used with mixed salad including different varieties of lettuce, chicory, and rocket.

Mixed salads are chosen for two purposes: They can provide visual variety, because of nongreen colors or interesting shapes, and they can provide some taste variety. The nutritional value is generally neglected, but it can be a stimulus for consuming unusual greens.

We considered five varieties of lettuce (Salad bowl, Red salad bowl, Oak leaf, Canasta, and Catalogna), two of rocket (Rocket and Wild rocket), two of chicory (Spadona and Wild chicory), and Buck's-horn plantain, and we attempted to characterize their polyphenol contents, together with rapid tests to assess their antioxidant activities. The polyphenol content is affected by a large number of external factors, such as agronomic processes, light, and climatic and postharvest conditions (*6*, *7*). Therefore, we chose plant material from one farm in which climatic and agronomic conditions were the same for all landraces so that a comparison among them could be performed both on total phenolic content and on different polyphenols separated and

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Table 1. Kinds of Salads and Their Water Contents

sample	botanical name	water content (%)
1	*L. sativa* L. ssp. *capitata* L., cv. Canasta	89.42
2	*L. sativa* L. ssp. *secalina* L., cv. Catalogna	92.98
3	*L. sativa* L. ssp. *secalina* L., cv. Oak leaf	94.96
4	*L. sativa* L. ssp. *secalina* L., cv. Salad bowl	94.21
5	*L. sativa* L. ssp. *secalina* L., cv. Red salad bowl	92.77
6	*C. intybus* L., cv. Spadona	93.54
7	*C. intybus* L., cv. Wild chicory	90.78
8	*P. coronopus* L., cv. Minutina (Buck-horn's plantain)	92.67
9	*Eruca sativa* Miller (Rocket)	91.28
10	*D. tenuifolia* L. (Wild rocket)	92.37

characterized by high-performance liquid chromatography/diode array detection/mass spectrometry (HPLC/DAD/MS). Also, the antiradical activity [toward the 1,1-diphenyl-2-picrylhydrazil radical (DPPH•)] and Fe^{2+} chelating ability were compared.

Much work has been carried out on different lettuce varieties (*6*, *8*−*10*), while only a few papers deal with chicory (*11*, *12*) and rocket (*13*). No research at all has been performed on Buck's-horn plantain (*Plantago coronopus*), known in Italy as "minutina" or in Europe as "Herba Stella", an herb used in mixed salad for its mild, nutty flavor and crunchy texture.

MATERIALS AND METHODS

Plant Material. The salads (see **Table 1**) were cultivated on a small farm near Florence (Lastra a Signa) and were sowed in September 2005.

Standards. Authentic standards of rutin, chlorogenic acid, and gallic acid and Folin−Ciocalteau reagent, DPPH•, and ferrozine [3,2-(pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-tyrazine] were purchased from Sigma-Aldrich (St. Louis, MO).

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

Extraction and Analysis of Phenolic Compounds. *Extraction.* The edible part of each vegetable was frozen in liquid nitrogen and stored at −80 °C before proceeding with the analysis. Frozen tissues were then ground in a mortar with a pestle under liquid nitrogen. A quantity of 2 g of tissue was extracted in 30 mL of 70% ethanol (pH 3.2 by formic acid) overnight. This solution was used for the determination of antioxidant activity, total phenolic and flavonoid contents, and Fe^{2+} chelating ability. For HPLC analysis, the solution was evaporated to dryness under reduced pressure at room temperature by a Rotavapor 144 R, Büchi, (Switzerland) and finally rinsed with a $CH_3CN/CH_3OH/H_2O$ (pH 2 by HCOOH) 60:20:20 mixture to a final volume of 2 mL; the concentrated solution was used after an extraction step with *n*-hexane. The extraction yield (95%) was controlled by the addition of 40 μL of gallic acid (5.88 mM) as an internal standard; gallic acid is not naturally present in our samples and exhibits a retention time that falls in an empty zone of the chromatogram. Each experiment was run at least three times; all data are mean values.

Total Phenolics Determination. 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 was 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 1100L liquid chromatograph equipped with a DAD and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA). Analysis was carried out during a 30 min period at flow rate of 0.8 mL min^{-1} using a Varian Polaris C18-E (250 mm × 4.6 mm i.d., 5 μm) column operating at 27 °C. A four-step linear gradient system starting from 95% water (adjusted to pH 3.2 by formic acid) up to 100% acetonitrile during a 30 min period was used. UV/vis spectra were recorded in the 190−600 nm range, and the chromatograms were acquired at 260, 280, 330, and 350 nm.

HPLC/MS Analysis. Analyses were performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies). The mass spectrometer operating conditions were as follows: 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 and negative modes at 80−180 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 of 0−30 μg on the basis of standards. In particular, flavonols were determined at 350 nm using rutin as a reference compound. Hydroxycinnamic derivatives were determined at 330 nm using chlorogenic acid 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.

Antiradical Activity of Phenolic Extracts. Free radical scavenging activity was evaluated with the DPPH• assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams (*14*) and slightly modified. 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) vs ethanol as a blank. Each day, the absorption of the DPPH• solution was checked. The antiradical activity was expressed as IC_{50}, the antiradical dose required to cause a 50% inhibition. IC_{50} was calculated by 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 addition of the sample, against the concentration of the sample. IC_{50} was expressed as mg sample/mg DPPH•.

Fe^{2+} Chelating Ability of Phenolic Extracts. The chelating activity of samples on Fe^{2+} was measured according to Duh et al. (*17*) and the original work of Stookey (*18*) with modifications. One hundred microliters of plant extracts or 400 μL of standards (66.7 and 1 mg/mL, respectively) was diluted up to 1.5 mL with 0.25 M acetate buffer (pH 4.75), and then, 25 μL of 2 mM $FeCl_2$ and 1 mL of the same solvent in which plant extracts or standards were dissolved were added. The solution was incubated at room temperature for 20 min. After incubation, 100 μL of 5 mM ferrozine was added, the mixture was shaken, and the absorbance was measured after 5 min at 562 nm against the same mixture, without the sample, as a blank. The ability of chelating ferrous ions was calculated according to equation: chelating activity % $= [(A_0 - A_s)/A_0] \times 100$, where A_0 is the absorbance of the blank and A_s is the absorbance of the sample.

RESULTS

In **Figure 1**, three HPLC profiles of salad extracts are reported; the identified compounds are reported in **Table 2**. Three main trends can be pointed out: One concerns the five *L. sativa* cultivars and partially Spadona and Wild chicory (two *C. intybus* varieties); the second concerns Buck's-horn plantain; and the third includes Rocket and Wild rocket. In the case of *L. sativa* cultivars, quercetin and luteolin glucosides and glucuronides were found according to previous research (*6*, *7*, *19*), and apigenin glucoside and kaempferol malonylglucoside, in addition to the above-mentioned flavonoids, were found in the two *C. intybus* cultivars; in the case of the Spadona extracts,

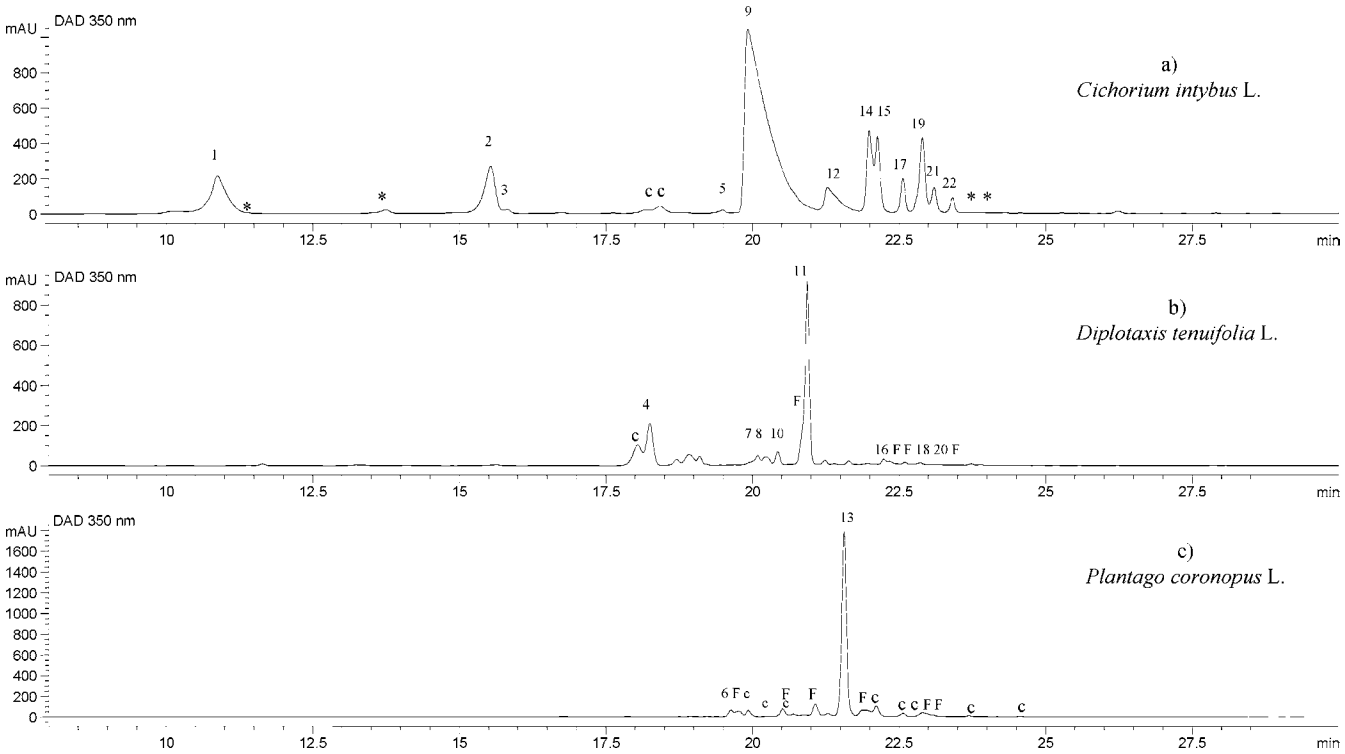

Figure 1. Chromatographic profile acquired by HPLC/DAD (350 nm) of the hydroalcholic extracts of *Cichorium intybus* cv. Spadona (**a**), *Diplotaxis tenuifolia* cv. Wild rocket (**b**), and *P. coronopus* cv. Buck's horn plantain (**c**). Identified compounds: 1, caffeoyl tartaric acid; 2, chlorogenic acid; 3, quercetin diglucoside; 4, quercetin 3,3′,4′-tri-O-β-D-glucopyranoside; 5, crysoeriol glucoside; 6, luteolin derivative; 7, quercetin diglucoside; 8, cicoric acid; 9, kaempferol diglucoside; 10, quercetin methyl ether diglucoside; 11, quercetin 3′-(6-sinapoyl-O-β-D-glucopyranosyl)-3,4′-di-O-β-D-glucopiranoside; 12, dicaffeoyl tartaric acid; 13, verbascoside; 14, luteolin glucuronide; 15, quercetin glucuronide; 16, quercetin 3-(2-sinapoyl-O-β-D-glucopyranosil)-3′-(6-sinapoyl-O-β-D-glucopyranosyl)-4′-O-β-D-glucopyranoside; 17, quercetin malonyl glucoside; 18, quercetin methyl sinapoyl glucoside; 19, dicaffeoyl chinic acid; 20, kaempferol derivative; 21, apigenin glucuronide; 22, kaempferol malonyl glucoside; F, flavonoids; C, caffeoyl derivatives; and *, unknown compounds.

a chrysoeriol glucoside was identified. Only a luteolin derivative could be identified in the Buck's-horn plantain extracts while quercetin and kaempferol derivatives were identified in Rocket and Wild rocket in agreement with previous findings (*13*, *20*). In all cases, caffeoyl derivatives were identified. The quantitative data are reported in **Table 3**. It can be pointed out that apart from Rocket and Wild rocket, caffeoyl derivatives are the main compounds; in fact, flavonoid contents range from 7.5 (Wild chicory) to 22.5% (Salad bowl).

As regards total phenolic content (**Table 4**), the values ranged from 100.08 (Rocket) to 573.57 (Wild chicory). Cano and Arnao (*10*) found lower (about two magnitude orders) polyphenol contents in lettuce varieties; this discrepancy may be linked to the different extraction conditions (aqueous extraction followed by an ethyl acetate one), since in other papers values that are comparable to our results are reported (*21*, *22*). If we consider the quantitative data of **Table 3**, there is a correlation between these data and those obtained by HPLC measurements (R^2 = 0.8936).

Table 4 presents the IC_{50} values, which may help in assessing the antiradical activity of extracts. The IC_{50} value indicates the amount of vegetable (mg) that is needed to cause a 50% inhibition of 1 mg of DPPH•. There is a broad range of variation within these data. The most antioxidant vegetable is Wild chicory, while Rocket is the least antioxidant salad among those tested. It is difficult to compare these kinds of data deriving from different measurements. However, if we compare these data with those obtained with the same procedure for turnip tops (*23*), comparable values are obtained. In the case of greens sampled in Turkey (*21*), including also chicory (*C. intybus*), lower values are generally obtained for the EC_{50} (efficient concentration) parameter; in this case, however, the total phenolic content is not known; therefore, a discussion is limited.

There is a correlation between antiradical activity and polyphenol content (R^2 = 0.9246 and 0.9193 for GAE and HPLC data, respectively) only in the case of the five *L. sativa* varieties. If all greens are taken into account, no correlation at all can be found, indicating that mechanisms and compounds operating in the radical activity depend on the different species. The absence of correlation between antioxidant activity and phenolic content has been ascribed to interferences by other substances like ascorbic acid (*21*, *25*). However, in this case, Rocket, Wild rocket, and Buck's-horn plantain exhibit a quite different phenolic pattern (see **Table 3**); therefore, the mechanism operating in the reaction with the DPPH• radical should also be investigated. **Figure 2** reports five kinetic curves. The time selected for the spectrophotometric measurement after the addition of the antioxidant is correct with the exception of Wild chicory. In fact, its antioxidant activity is high and no plateau is obtained. It should, however, be observed that in order to compare the kinetic behavior, the same concentration of the antioxidant was selected. Two possible equations can fit the experimental data:

$$\ln [\text{DPPH}^\bullet] = at + b \quad (1)$$

$$\ln [\text{DPPH}^\bullet] = a \ln t + \ln b \quad (2)$$

All of the tested greens follow eq 2, with the exception of Wild chicory for which a high correlation coefficient (0.9755) was obtained applying the exponential model of eq 1. We may

Table 2. Qualitative Composition of the Salad Extracts[a]

compound	retention time	sample 1	2	3	4	5	6	7	8	9	10
caffeoyl tartaric acid	10.87	×	×	×	×	×	×	×			
flavonoid	11.64										×
flavonoid	13.54									×	
caffeoyl chinic acid	15.5	×	×	×	×	×	×	×			
quercetin diglucosyde	15.69						×				
caffeoyl derivative	18.13		×	×	×	×	×	×			
caffeoyl derivative	18.2	×		×							
caffeoyl derivative	18.26										×
quercetin–3,3′-tri-*O*-β-D-glucopyranoside	18.49										×
caffeoyl derivative	18.7	×		×			×				
caffeoyl derivative	18.8	×	×								
flavonoid	19.02									×	
caffeoyl derivative	19.3	×	×		×						
flavonoid	19.31									×	
crysoeriol glucoside	19.4						×				
flavonoid	19.48									×	
luteolin derivative	19.7								×		
caffeoyl derivative	19.5							×			
flavonoid	19.8								×		
caffeoyl derivative	20.04								×		
quercetin diglucoside	20.05									×	×
kaempferol diglucoside	20.17									×	×
caffeoyl derivative	20.39								×		
cicoric acid	20.4	×	×	×		×	×	×			
quercetin methyletherdiglucoside	20.5									×	×
flavonoid	20.7								×		
flavonoid	20.8										×
quercetin-3′-(6-sinapoyl-*O*-β-D-glucopyranosyl)-3,4′-di-*O*-β-D-glucopyranoside	20.97									×	×
caffeoyl derivative	20.99								×		
kaempferol derivative	21.01									×	
flavonoid	21.1								×		
dicaffeoyl tartaric acid	21.22	×	×	×	×	×	×	×			
verbascoside	21.6								×		
flavonoid	21.67										×
caffeoyl derivative	21.7			×							
luteolin glucuronide	21.96	×	×	×	×	×	×	×			
flavonoid	22								×		
flavonoid	22.05										×
flavonoid	22.08									×	
quercetin glucuronide	22.1	×	×	×	×	×	×	×			
caffeoyl derivative	22.15								×		
quercetin-3-(2-sinapoyl-*O*-β-D-glucopyranosyl)-3′-(6-sinapoyl-*O*-β-D-gluco-pyranosyl)-4′-*O*-β-D-glucopyranoside	22.23										×
flavonoid	22.43										×
quercetin malonylglucoside	22.56	×	×	×	×	×	×	×			×
caffeoyl derivative	22.6								×		
flavonoid	22.61										×
quercetin-methylethersina-poylglucoside	22.77									×	
dicaffeoyl chinic acid	22.77		×	×		×	×	×			
kaempferol derivative	22.80										×
flavonoid	22.87										×
flavonoid	22.91									×	
caffeoyl derivative	22.9								×		
apigenin glucuronide	23.09						×	×			
flavonoid	23.11								×		
flavonoid	23.3								×		
kaempferol malonylglucoside	23.4			×			×	×			
caffeoyl derivative	23.7								×		
unknown	24.5						×	×			
unknown	24.6						×	×			
caffeoyl derivative	24.6								×		
flavonoids	22.89–31.09	×									

[a] Numbers of samples as in **Table 1**.

therefore conclude that in this case the IC_{50} values only partly account for the behavior of the samples in their antiradical action.

Table 3. Content of Flavonoid and Caffeoyl Derivatives (mg/g, Fresh Weight) from HPLC Measurements[a]

sample	flavonoids	caffeoyl derivatives	total phenolics
1, Canasta	0.2553	1.5422	1.7975
2, Catalogna	0.1512	0.7778	0.929
3, Oak leaf	0.4457	1.9892	2.4349
4, Salad bowl	0.4114	1.4149	1.8263
5, Red salad bowl	0.3641	1.7099	2.074
6, Spadona	0.2884	2.5874	2.8758
7, Wild chicory	0.3378	4.3638	4.7016
8, Buck-horn's plantain	0.3221	1.0241	1.3462
9, Rocket	0.6528	traces	0.6528
10, Wild rocket	0.2915	0.0716	0.3631

[a] Data are the means of three determinations (standard deviation, <3%). Traces = mg/g under detection limit.

Table 4. Total Phenolics (Folin–Ciocalteu Method) Expressed as mg Gallic Acid/100 g, Fresh Weight; IC_{50} as mg Sample (Fresh Weigh)/mg DPPH•[a]

sample	total phenolics	IC_{50}	chelating ability (%)[b]
1, Canasta	213.15 (19.67)	456.23	60
2, Catalogna	103.38 (7.45)	1007.38	74
3, Oak leaf	297.64 (28.11)	280.32	34
4, Salad bowl	238.57 (16.56)	414.78	36
5, Red salad bowl	246.80 (22.38)	270.48	32
6, Spadona	283.06 (21.37)	314.27	22
7, Wild chicory	573.57 (46.83)	145.89	34
8, Buck-horn's plantain	209.78 (16.45)	456.97	9
9, Rocket	208.11 (18.32)	3829.12	41
10, Wild rocket	100.08 (10.63)	1462.67	43

[a] Standard deviations are in parentheses. [b] Chelating ability with solutions containing 66.7 mg sample (fresh weight)/mL.

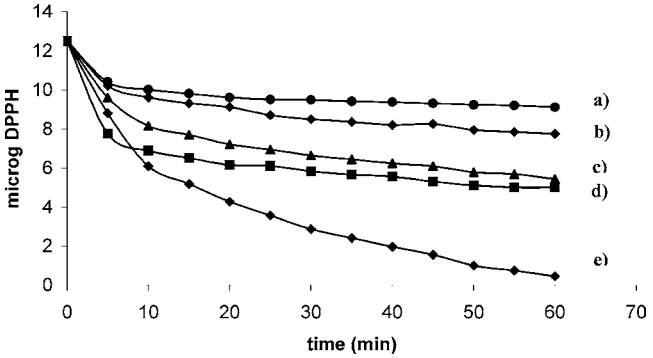

Figure 2. Kinetic curves of (a) rocket, (b) wild rocket, (c) salad bowl, (d) spadona, and (e) wild chicory. The concentration of the antioxidant = 6.7 mg/mL.

As regards the Fe^{2+} chelating ability, this test is not frequently used (*26*−*28*) even if free Fe^{2+} ions can lead to the formation of radical species. When Fe^{2+} is bound, the Fenton reaction, which involves oxygenated water with the formation of hydroxyl radicals, and the Haber−Weiss cycle, with the formation of superoxide anions, are both inhibited. These free radicals may be implicated in human cardiovascular disease (*29*); by removing and neutralizing iron ions from iron-loaded hepatocytes, flavonoids inhibit oxidative damage (*30*). Despite several papers concerning the chemical mechanism, which operates with some pure compounds (*31*, *32*), systematic research on the behavior of most common plant phenolics is lacking. Also, the procedure that is generally followed shows some problems, since, as is

Table 5. Chelating Ability (See Materials and Methods) of Standard Solutions[a]

standard	chelating ability (%)	
	1 mg/mL	0.89 μM
quercitrin	55	57
ascorbic acid	46	12
chlorogenic acid	24	15
quercetin	22	17
kaempferol	16	0
caffeic acid	16	4
isoquercitrin	13	13
kaempferol-7-neohesperidoside	11	16
gallic acid	5	0
rutin	3	18
catechin	1	5
ferulic acid	0	0

[a] Standard deviation, <5%.

reported in the original paper of Stookey (*18*), the formation of the complex is affected by the pH value of the solution. Therefore, we obtained reproducible results only using a buffer solution in the reaction mixture. **Table 5** reports the chelating activities of some standards both on a weight and on a molar basis; in both cases, the chelating activity order chlorogenic acid > caffeic acid > gallic acid is the same as that of their respective binding constants (*31*). The results show that the presence and kind of the glycosidic residue affect the behavior toward iron ions.

In **Table 4**, the chelating abilities of the samples are reported as much higher than those of standard solutions. In fact, the sample solutions (66.7 mg/mL) contain from 2×10^{-2} to 3.2×10^{-1} mg/mL of total polyphenols (see **Table 3**). In the case of red bean extracts, a high correlation level was found between the Fe^{2+} chelating ability and the anthocyanin content (*26*). Among the green, only red salad bowl may contain anthocyanins; however, its chelating ability is not particularly high. Therefore, the chelating ability must also be ascribed to other compounds, which are extracted together with polyphenols or to the quantitative composition of the polyphenol mixture. Glucosinolates (*20*) may be responsible for the quite good chelating ability of Rocket and Wild rocket; in fact, their almost low phenolic content (see **Tables 3** and **4**) cannot account for their behavior.

The results obtained show that mixed salad can be regarded as a functional food as it is used fresh and in quantities of about 100 g each serving with the functional molecules introduced in biologically significant amounts.

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LITERATURE CITED

(1) Dorman, M. J. D.; Peltoketo, A.; Hiltunen, R.; Tikkanen, M. J. Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Laminaceae herbs. *Food Chem.* **2003**, *83*, 255−262.

(2) Block, G.; Patterson, B.; Subar, A. Fruit, vegetables and cancer prevention. A review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1−29.

(3) Halliwell, B. Protection against tissue damage in vivo by desferrioxammine: What is its mechanism of action? *Free Radical Biol. Med.* **1989**, *7*, 645−651.

(4) Hertog, M. G. L.; Freskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and the risk of coronary heart disease: The Zutphen Elverly Study. *Lancet* **1993**, *342*, 1007−1011.

(5) Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* **2002**, *13*, 572−584.

(6) DuPont, M. S.; Mondin, Z.; Williamson, G.; Price, K. R. Effect of variety, processing and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agric. Food Chem.* **2000**, *48*, 3957−3964.

(7) Romani, A.; Pinelli, P.; Galardi, C.; Sani, G.; Cimato, A.; Heimler, D. Polyphenols in greenhouse and open-air grown lettuce. *Food Chem.* **2002**, *79*, 337−342.

(8) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic fllavonoids of 28 vegetables and 9 fruit commonly consumed in the Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.

(9) Llorach, R.; Tomas-Barberan, A.; Fererres, F. Lettuce and chicory by products as a source of antioxidant phenolic extracts. *J. Agric. Food Chem.* **2004**, *52*, 5109−5116.

(10) Cano, A.; Arnao, M. B. Hydrophillic and lipophilic antioxidant activity in different leaves of three lettuce varieties. *Int. J. Food Prop.* **2005**, *8*, 521−528.

(11) Innocenti, M.; Gallori, S.; Giaccherini, C.; Ieri, F.; Vincieri, F. F.; Mulinacci, N. Evaluation of the phenolic content in the aerial parts of different varieties of Cichorium intybus L. *J. Agric. Food Chem.* **2005**, *53*, 6497−6502.

(12) Salvatore, S.; Pellegrini, N.; Brenna, O. V.; Del Rio, D.; Frasca, G.; Brighenti, F.; Tumino, R. Antioxidant characterization of some Sicilian Edible wild greens. *J. Agric. Food Chem.* **2005**, *53*, 9465−9471.

(13) Weckerle, B.; Michel, K.; Balazs, B.; Schreier, P.; Toth, G. Quercetin 3,3′,4′-tri-O-β-D-glucopyranosides from leaves of *Eruca sativa* (Mill.). *Phytochemistry* **2001**, *57*, 547−551.

(14) Brand-Williams, W.; Cuvelier, M. E. Use of a free radical method to evaluate the antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.

(15) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of the Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.

(16) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010−3014.

(17) Duh, P.-D.; Yen, G.-C.; Yen, W.-J.; Chang, L. W. Antioxidant effects of water extracts from barley (Hordeum vulgare L.) prepared under different roasting temperatures. *J. Agric. Food Chem.* **2001**, *49*, 1455−1463.

(18) Stookey, L. L. Ferrozine, a new spectrophotometri reagent for iron. *Anal. Chem.* **1970**, *42*, 779−781.

(19) Hohl, U.; Neubert, B.; Pforte, H.; Schonhof, I.; Bohm, H. Flavonoid concentrations in the inner leaves of head lettuce genotypes. *Eur. Food Res. Technol.* **2001**, *213*, 205−211.

(20) Bennett, R. N.; Rosa, E. A. S.; Mellon, F. A.; Kroon, P. A. Ontogenic profile of glucosinolates, flavonoids and other secondary metobolites in *Eruca sativa* (salad rocket), *Diplotaxis erucoides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket) and *Bunias orientalis* (Turkish rocket). *J. Agric. Food Chem.* **2006**, *54*, 4005−4015.

(21) Stratil, P.; Klejdus, B.; Kuban, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables−Evaluation of spectrophotometric methods. *J. Agric. Food Chem.* **2006**, *54*, 607−616.

(22) Ninfali, P.; Mea, G.; Giorgini, S.; Rocchi, M.; Bacchiocca, M. Antioxidant capacity of vegetables, spices and dressing relevant to nutrition. *Br. J. Nutr.* **2005**, *93*, 257−266.

(23) Romani, A.; Vignolini, P.; Isolani, L.; Ieri, F.; Heimler, D. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa* L., subsp. *sylvestris* L.). *J. Agric. Food Chem.* **2006**, *54*, 1342−1346.

(24) El, S. N.; Karakaya, S. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. *Int. J. Food Sci. Nutr.* **2004**, *55*, 67−74.

(25) Heimler, D.; Vignolini, P.; Dini, M. G.; Vincieri, F. F.; Romani, A. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food Chem.* **2006**, *99*, 464−469.

(26) Chou, S. T.; Chao, W. W.; Chung, Y. C. Antioxidant activity and safety of 50% ethanolic red bean extract (*Phaseolus radiatus* L. var. Aurea). *Food Chem. Toxicol.* **2003**, *68*, 21−25.

(27) Gulcin, I.; Oktay, M.; Kirecci, E.; Kufrevioglu, O. I. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem.* **2003**, *83*, 371−382.

(28) Gulcin, I.; Sat, I. G.; Beydemir, S.; Elmastas, M.; Kufrevioglu, O. I. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb.) buds and lavender (*Lavandula stoechas* L.). *Food Chem.* **2004**, *87*, 393−400.

(29) Halliwell, B.; Gutteridge, J. M. C. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol.* **1990**, *186*, 1−85.

(30) Morel, I.; Lescoat, G.; Cogrel, P.; Sergent, O.; Pasdeloup, N.; Brissot, P.; Cillard, P. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocytes cultures. *Biochem. Pharmacol.* **1993**, *45*, 13−19.

(31) Andjelkovic, M.; Van Camp, J.; De Meulenaar, B.; Depaemelaere, G.; Socaciu, C.; Verloo, M.; Verhe, R. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.* **2006**, *98*, 23−31.

(32) Leopoldini, M.; Russo, N.; Chiodo, S.; Toscano, M. Iron chelation by the powerful antioxidant flavonoid quercetin. *J. Agric. Food Chem.* **2006**, *54*, 6343−6351.

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