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Red Chicories as Potent Scavengers of Highly Reactive Radicals: A Study on Their Phenolic Composition and Peroxyl Radical Trapping Capacity and Efficiency

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Eight varieties of *Cichorium* genus vegetables (five heavily red colored, one red spotted, and two fully green) were investigated for their phenolic content (by HPLC and UV−vis spectrophotometry) and for their antioxidant activity. In particular, the capacity (that is, the amount of trapped peroxyl radicals) and the efficiency (that is, the amount of antioxidant necessary to halve the steady-state concentration of peroxyl radicals) were measured. All of the studied chicories are characterized by the presence of a large amount of hydroxybenzoic and hydroxycinnamic acids, whereas the red color is due to cyanidin glycosides. The presence of these phenolics in red chicories confers to them an exceptionally high peroxyl radical scavenging activity in terms of both capacity and efficiency, particularly in their early stage of growth, and makes this popular and low-cost foods comparable or superior to many foods having well-known antioxidant properties such as red wine, blueberry, and tomato.

KEYWORDS: Antioxidants; polyphenols; chicory; lipid peroxidation; anthocyanins

INTRODUCTION

Italian horticultural production is characterized for the relevant number of vegetables. These vegetables represent a patrimony of genetic variability and food diversification combining good taste with excellent health and nutritional properties. Typical products, extensively cultivated in northeastern Italy, are red or variegated chicories, called "radicchio", being valuable high-quality crops. They may have different destinations—fresh or cooked. In particular, they are mostly consumed as raw salad in winter time when most vegetables are not available. In fact, red chicories are vegetables particularly resistant to low temperatures. The nice and typical taste of some of these varieties such as Treviso red chicory and Verona red chicory is enjoying great success, with a consequent increase of the market demand, the local production being ∼250 000 tons per year. In recent years the growing and marketing of these chicories have been extended to all of central Europe and recently also in the United States (*1*).

Chicory, or *Cichorium*, a typical Mediterranean plant indigenous to Europe, Western Asia, and North America, varies in color from white to red (*2*, *3*). In particular, the genus *Cichorium* is made up of two cultivated species, *C. intybus* L. (chicory) and *C. endivia* L. (lettuce). All of the red types of chicories now being cultivated derive from red-leafed individuals belonging to the botanical variety *foliosum*, whereas the types with spotted or variegated leaves derived from spontaneous or controlled crosses between these individuals and plants of the species *C. endivia* L. var. *latifolium* (*4*).

The red color is mostly caused by the presence of water-soluble anthocyanin pigments, and some papers on such a type of red chicories have shown the presence of noteworthy antioxidant activity, which was assessed by using the DPPH$^{\bullet}$ assay or the micellar system linoleic acid−β-carotene (*5*−*7*).

The presence of water-soluble flavonoids in chicories is of special interest because recent epidemiological studies have described many beneficial health effects of flavonoids for protection against cancer, cardiovascular diseases, and aging (*8*−*11*). In this paper we report on the phenolic composition and peroxyl trapping efficiency and capacity of some varieties of green, red spotted, and red chicories and compare these characteristics with those of some fruits and vegetables well-known for their antioxidant properties.

MATERIALS AND METHODS

Reagents. The reagents were purchased from Fluka (Buchs, Switzerland) and were of the highest available quality (for HPLC or

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standard for GC). 2,2′-Azobis[2-(2-imidazolin-2-yl)propane] (ABIP) was a kind gift of Wako Chemicals (Neuss, Germany). All of the aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA) and, when necessary, were passed through a column of Chelex-100 (Bio-Rad, Richmond, CA) to minimize the concentration of heavy metal ions. Phenolic acid standards were purchased from Sigma-Aldrich (Milano, Italy), and cichoric acid was obtained from PhytoLab GmbH & Co. KG (Hamburg, Germany). Anthocyanins were obtained from Extrasynthese (Genay Cedex, France). Vegetables and fruits were from the experimental fields of Padova University or were purchased from the Padova main vegetable market between November 2003 and February 2005. Wines were obtained from a local supermarket.

Growth. The influence of growing conditions on the phenolics content and on the antioxidant properties of red chicories was studied in the experimental fields of Padova University.

Plantlets of *Chioggia red* chicory were transplanted in an open field in August. A nonfertilized control was compared to 10 treatments providing different nitrogen, phosphorus, and potassium combinations (from low to very high levels: N, 0−200 kg ha^{-1}; P, P_2O_5, 0−320 kg ha^{-1}; K, K_2O, 0−200 kg ha^{-1}) to induce different metabolic and yield responses. In January 12 plants from each treatment were analyzed to determine the total polyphenol content and antioxidant characteristics.

Vegetables and Fruits. About 200 g of vegetables or fruits was randomly sampled from 1 kg of raw material. The edible part (∼90% in the case of vegetables) was washed, weighed, cut in small pieces, and put in a nylon bag (porosity = 5 μm). The pressurized liquid extraction of polyphenols was carried out for 1.5 h in an automatic equipment (NM LAB/M Depurex 88, Limena, Padova, Italy) with 490 mL of ethanol/water solution (85:15 v/v) containing 0.1 M HCl (solution A), previously deoxygenated by flushing with nitrogen. The volume of each sample was measured to calculate the extract concentration (grams per liter). The extracts were subdivided in dark glass bottles without headspace and stored at −80 °C until the analytical measurements.

HPLC Analyses. *Hydroxycinnamic and Hydroxybenzoic Acids.* The extracts were analyzed by a HPLC (Bio-Rad 2700, Milano, Italy) equipped with a UV−vis detector. All samples were filtered on a 0.45 μm cartridge and directly injected. Hydroxycinnamic and hydroxybenzoic acids were separated using an LC-18 Suppelco-sil column at 25 °C with a flow rate of 1.5 mL/min under isocratic conditions (*12*). The absorbances at 275 and 330 nm were used as reference wavelengths. The mobile phase consisted of an aqueous solution containing 1.5% acetic acid and 18% *n*-butanol. Phenolic acid standards were chromatographed singly and in mixture. The compounds were identified according to their retention time. Each reported value is the average of three repeated analyses.

Anthocyanins. For the characterization of the anthocyanins a Thermo-Finnigan series liquid chromatograph (San Jose, CA) equipped with a photodiode array detector UV 6000LP and a Hewlett-Packard computer system was used. Separation was performed on an LC-18 Suppelco-sil (5 μm), 250 × 5 mm i.d., fitted with a Pelliguard LC-18 (Supelco, Bellefonte, PA). The binary solvent system consisted of an aqueous solution of 1% phosphoric acid, 10% acetic acid, and 6% acetonitrile (eluent A) and 100% acetonitrile (eluent B). The following conditions were applied: isocratic 100% A for 20 min, then linear increment from 0 to 10% B in 10 min and isocratic 100% B for 10 min (*13*). The flow rate was 1.3 mL/min, and sample aliquots of 20 μL were injected. The column oven temperature was set to 20 °C. The diode array spectra were obtained in the wavelength range of 250−600 nm in steps of 2 nm. Detection was at 520 nm.

Standards were used to identify peaks by retention times and cochromatography. Diode array spectral characteristics were matched to standards and to library spectra.

Demalonation. Demalonation of anthocyanins in chicory extracts was carried out as reported by Du Pont et al. (*14*). The chicory samples were diluted in a solution of methanol/acetic acid (95:5 v/v), stored at 25 °C, and analyzed at various times (0, 5, 10, and 15 days) according to the anthocyanin procedure analysis by HPLC previously described.

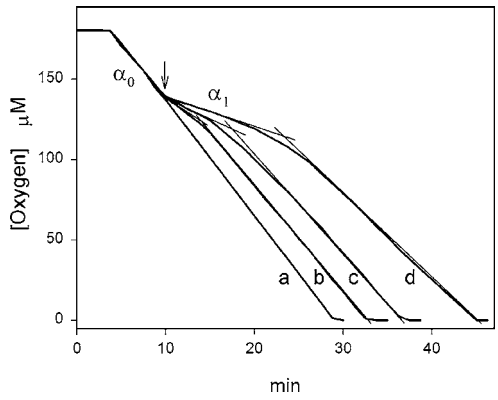

Figure 1. Representative traces of oxygen uptake of inhibited and not inhibited free radical peroxidation of 1 mM linoleic acid micelles by a chicory extract (Chioggia Red). The peroxidation of LH was carried out at 37 °C in 50 mM phosphate, pH 7.4, containing 4 mM ABIP. Curve a represents the time course in the absence of inhibitor. Curves b, c, and d represent the initial rates after addition to the model system of 0.13, 0.26, and 0.52 g/L of RC chicory extract, respectively.

Spectrophometric Analyses. Anthocyanin standard solutions were prepared in methanol and then diluted in solution A for spectrophotometric analyses by a Varian Cary 50 Scan UV−visible spectrophotometer.

Ten microliters of the aqueous ethanol solution of chicory extracts was diluted in 990 μL of solution A, and the UV−vis spectra were recorded between 200 and 800 nm. The total anthocyanin concentration in the chicory extracts was calculated from the absorbance at 535 nm using cyanidin 3-glucoside for the calibration curve.

Measurement of Peroxyl Radical Trapping Efficiency. The peroxyl radical trapping efficiency and the peroxyl radical trapping capacity of the vegetable and the fruit extracts were measured according to a slight modification of an improved method that permits the simultaneous determination of these two parameters (*15*). This method reproduces in vitro the peroxidation of linoleic acid (LH), one of the major components of low-density lipoprotein (LDL), in conditions close to those occurring in human plasma. In particular, the peroxidation of LH, induced by $ROO^{\bullet}$ radicals generated by thermal decomposition of 4 mM ABIP at 37 °C, was followed by an oxygraphic apparatus. After thermal equilibration, an aqueous solution of ABIP was added at the final concentration of 4.0 mM and incubated to establish the rate of oxygen consumption during peroxidation. Ethanol standard solutions of phenolics, or of chicory or fruit extracts, were then added, and the rate of the inhibited reaction was recorded until oxygen disappearance.

By this method it is possible to obtain the simultaneous measurement of two parameters characterizing the antioxidant activity of phenolic compounds or foods, that is, the peroxyl radical trapping capacity (PRTC), which represents the amount of radicals trapped by a given amount of antioxidant, and the peroxyl radical trapping cfficiency (PRTE), by which these radicals are trapped. The latter property is expressed as the reciprocal of the concentration of the antioxidant $(C_{1/2})^{-1}$ that halves the peroxidation rate of LH.

Typical kinetic runs show that the O_2 consumption rate (α_0) accompanying the peroxidation of LH in aqueous solution, where peroxyl radicals are generated at constant rate, is inhibited by the addition of antioxidants such as phenolics or by the addition of vegetable or fruit extracts to a much lower rate (α_1) for a given period of time or lag time (LT) (see **Figure 1**).

From the LT values measured when LH peroxidation is fully inhibited by the antioxidant, PRTC values were calculated according to equation

$$\mathrm{PRTC} = \mathrm{LT}\cdot R_0/C \qquad (1)$$

where R_0 is the rate of generation of peroxyl radicals (1.60 μM min^{-1} in our case) and therefore PRTC is the amount of peroxyl radicals trapped by a given concentration C of the added antioxidant.

The efficiency was calculated by plotting the ratio α_1/α_0 versus the initial concentration C of the antioxidant in the oxygraphic cell. This plot is fitted by an exponential function according to the equation

$$\frac{\alpha_1}{\alpha_0} = A + B \times e^{-(\ln 2)\times C/C_{1/2}} \quad (2)$$

where $C_{1/2}$ is the concentration of antioxidant that halves the rate of oxygen consumption due to the peroxidation of LH and A and B are constants obtained from the fitting procedure. Under our experimental conditions the constant A was found to be 0.26 ± 0.02 and represents the value of the α_1/α_0 ratio when LH peroxidation is fully inhibited by antioxidant. The constant B is the value of $(\alpha_1/\alpha_0 - A)$ extrapolated at $C = 0$, and usually it was found that $B = 1 - A$. In the presence of two or more antioxidants, as in the case of chicory extracts, and given the hypothesis that the scavenging of $ROO^{\bullet}$ radicals by the various antioxidants is additive, eq 2 can be rewritten as

$$\frac{\alpha_1}{\alpha_0} = A + B \times e^{-\ln 2\times \sum Ci/Ci_{1/2}} \quad (3)$$

where C_i and $C_{i\ 1/2}$ refer to the ith antioxidant and the ratio $C_i/C_{i1/2}$ represents the contribution of the ith antioxidant to PRTE (*16*).

RESULTS AND DISCUSSION

Composition and Quantification of Major Phytochemicals of Eight Varieties of Chicory. Eight varieties of chicory, five heavily red colored (Verona red, Chioggia red, Treviso early red, Treviso late red, and red Grumolo), one red spotted (Castelfranco red spotted), and two fully green (Chioggia white and green Grumolo) were characterized for their phenolic content by spectrophotometry and by HPLC.

The UV−vis spectra in the range of 200−800 nm of the eight chicory varieties show that all of the red chicories and the spotted one are characterized by a maximum in the visible region at 535 nm corresponding to the maximum of cyanidin 3-glucoside (see later). Conversely, the extracts of green varieties do not show significant absorption in the visible range.

The phenolic acid composition of the eight cultivars, expressed as micromoles per gram of fresh weight (FW), was calculated from the HPLC chromatograms carried out as reported under Materials and Methods. A typical example of the chromatogram of a red chicory is reported in **Figure 2A,B** in which the presence of a large amount of hydroxycinnamic and benzoic acids can be seen.

HPLC analyses, carried out according to the method of Pazmiño-Duràn et al. (*13*), to identify anthocyanins confirmed that the main component of this class of phytochemicals is cyanidin in various glycosylated forms (see **Figure 2C**, where the chromatogram of the anthocyanins present in juvenile Verona red chicory is reported as example). In fact, in all of the red varieties we have examined, the glycosylated cyanidins represent 83−89% of total anthocyanins, on a molar basis. Malvidin (as aglycon and 3-glucoside), ranging from 4 to 8.3% of total anthocyanins, was found as the second component of this class of phytochemicals in the red chicories. Small amounts (≤1%) of pelargonidin and peonidin were also identified.

With regard to cyanidin, the mean peak was attributed to cyanidin 3-malonylglucoside. The presence of the malonyl ester of cyanidin 3-glucoside in the HPLC chromatograms of red chicories, identified previously by NMR and mass spectroscopy analyses (*17*), was confirmed by demalonation experiments. In fact, these experiments, carried out according to the method of Du Pont et al. (*14*), showed a progressive decrease of the putative malonate peak and the corresponding increase of cyanidin 3-glucoside with time of storage of the extracts in methanol/acetic acid.

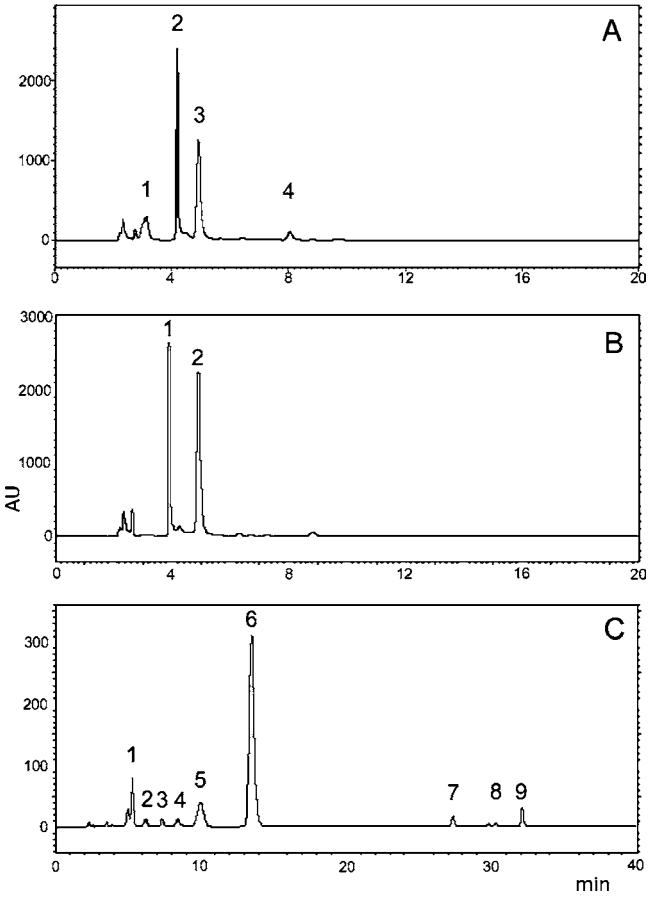

Figure 2. HPLC profiles of juvenile Verona Red chicory: (**A**) peak identifications at 275 nm (1, gallic acid; 2, protocatechuic acid; 3, chlorogenic acid; 4, caffeic acid); (**B**) peak identifications at 330 nm (1, cichoric acid; 2, chlorogenic acid); (**C**) peak identifications at 520 nm (1, cyanidin 3-*O*-glucoside; 2, cyanidin 3-*O*-rutinoside; 3, pelargonidin 3-*O*-glucoside; 4, peonidin 3-*O*-glucoside; 5, malvidin 3-*O*-glucoside; 6, cyanidin 3-malonylglucoside; 7, pelargonidin; 8, peonidin; 9, malvidin).

On the basis of the HPLC data of the studied chicories the concentration of anthocyanins was expressed as cyanidin 3-glucoside and was calculated from the absorbance at 535 nm using cyanidin 3-glucoside as standard.

The phenolic composition of the eight cultivars, expressed as micromoles per gram of FW, obtained by HPLC (phenolic acids) and by spectrophotometry (anthocyanins), is reported in **Table 1**. Furthermore, in the last two rows of this table is also reported the composition of two red chicories in their early stages of growth, that is, juvenile Verona red and juvenile Treviso late red. From this table it appears that chlorogenic acid is the main component of the eight cultivars we have studied, ranging from 37 to >60% of the total phenolics (TP), whereas gallic acid or protocatechuic acid is the second main component in most of the studied varieties. Trace amounts (<2% of TP) of syringic, vanillic, and *p*-coumaric acid were found in some chicory extracts, but their presence was neglected. In the case of heavily red colored chicories, anthocyanins are in the range of 15−17% of TP, on a molar basis, if the Treviso late red chicory is excluded. In fact, in this cultivar, which is bleached by an artificial process, the anthocyanins represent only 9% of TP. Furthermore, anthocyanins become 1.5% in the red spotted chicory (Castelfranco red spotted) and are undetectable in the green chicories (Chioggia white and green Grumolo).

Table 1. Phenolic Composition (C_i) of Some Chicory Cultivars[a]

chicory	antho-cyanins[b]	gallic acid[c]	protocatechuic acid[c]	caffeic acid[c]	chicoric acid[c]	chlorogenic acid[c]	total polyphenols[d]
Castelfranco red spotted	0.06	0.83	0.79	0.29	0.53	1.45	3.95
Verona red	1.86	0.92	1.67		0.84	7.08	12.37
Chioggia red	1.25	0.55	0.99	0.36	0.68	3.49	7.32
Chioggia white		0.75	0.87		0.46	1.22	3.30
Treviso early red	0.77	0.47	1.12		0.61	2.37	5.34
Treviso late red	0.36	0.55	0.73		0.30	1.88	3.82
Green Grumolo		2.29	1.96	0.29	1.29	4.48	10.31
Red Grumolo	3.12	1.82	1.24		1.26	12.23	19.67
juvenile Verona red	1.93	0.83	3.14	0.17	3.06	15.59	24.72
juvenile Treviso late red	1.51	4.57	2.20		1.90	3.47	13.65

[a] Standard deviation of the phenolic composition data was <10%. [b] Expressed as cyanidin-3 glucoside (μmol/g of FW), from spectrophotometric analysis. [c] Micromoles per gram of FW, from HPLC analysis. [d] Micromoles per gram of FW, calculated as the sum of the various phenolics.

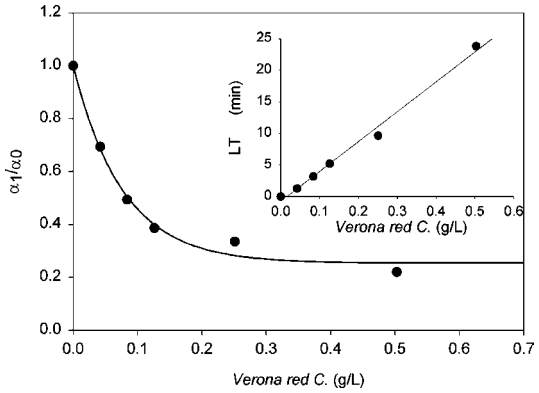

Figure 3. Inhibition of LH peroxidation by Verona Red chicory extract: solid line, fitting of experimental data to eq 2 ($r = 0.996$); (inset) dependence of the lag time from the Verona Red extract concentration; solid line, fitting of experimental data to eq 1 ($r = 0.995$).

It is interesting to observe that in juvenile chicories the concentration of TP is 2−3 times higher than that found in more aged plants (see the last rows of **Table 1**). Finally, with regard to the absolute amount of phenolics, juvenile Verona red contains up to 25 μmol of TP/g of FW (~10 g of TP/kg of FW), whereas, with regard to the anthocyanins, red Grumolo contains up to 3 μmol/g of FW (~1.5 g of anthocyanins/kg of FW).

Peroxyl Radical Scavenging Activity of Red Chicories and of Their Most Representative Antioxidants. The peroxyl free radical scavenging activity of the eight chicory varieties and of the most representative antioxidants was characterized in terms of both capacity and efficiency (see Materials and Methods).

An example of this characterization relative to Verona red is reported in **Figure 3**. From the plot of LT values versus Verona red concentrations (see **Figure 3** inset), the PRTC was calculated according to eq 1 and expressed as micromoles of peroxyl radical trapped by 1 g of FW of chicory.

From the values of α_0 and α_1, the α_1/α_0 ratio was calculated and plotted against the concentration of the chicory extract in the measurement system. **Figure 3** shows the good fit of the ratio of α_1/α_0 to eq 2 (solid line) by which $C_{1/2}$ values were calculated. The $C_{1/2}$ values of the various chicories, that is, the amount of fresh chicory (grasm of FW of chicory per liter of peroxidation system) which halves the rate of oxygen consumption due to peroxidation of LH, are reported in **Table 2**, column 2, together with the standard error. The correspondent PRTE experimental values ($PRTE_{exptl}$), that is, $(C_{1/2})^{-1}$, are also reported in **Table 2**. The $C_{1/2}$ values of the studied chicories range from a minimum value of 0.021 g/L ($PRTE_{exptl} = 47.6$ L/g), found for juvenile Verona red, that is, the cultivar with the highest efficiency in trapping ROO$^\bullet$ radicals, to a maximum $C_{1/2}$ value of 0.300 g/L ($PRTE_{exptl} = 3.33$ L/g) found for Castelfranco red spotted (the cultivar with the lowest efficiency in trapping ROO$^\bullet$ radicals). These values show that the $PRTE_{exptl}$ values of the studied cultivars span >1 order of magnitude. It is interesting to note that the $PRTE_{exptl}$ values of both juvenile varieties are ~2−4 times higher than those of the more aged chicory plants.

The $PRTE_i$ values of the major phenolic antioxidants present in chicories are also reported in **Table 2**, second row. In this case $C_{i1/2}$ is the concentration of the antioxidant *i*, expressed in micromoles per liter, that halves the rate of oxygen consumption due to peroxidation of LH. The $C_{i1/2}$ values range from a minimum value of 0.14 μmol/L ($PRTE_i = 7.14$ L/μmol) in the case of cyanidin 3-glucoside to a maximum value of 3.90 μmol/L ($PRTE_i = 0.256$ L/μmol) in the case of protocatechuic acid. In other words, according to the data of **Table 2**, it appears that cyanidin 3-glucoside and chicoric acid are by far more efficient in trapping peroxyl radicals than the other hydroxycinnamic and benzoic acids characterizing the studied chicories.

In **Table 3**, column 2, the PRTC experimental values ($PRTC_{exptl}$) of the various chicories calculated from LT measurements according to eq 1 and expressed as micromoles of peroxyl radicals trapped by 1 g of FW of chicory are reported together with the $PRTC_i$ values of the major phenolic antioxidants present in the studied chicories (see **Table 3**, second row), $PRTC_i$ being in this case the number of ROO$^\bullet$ radicals trapped by each molecule of the *i*th antioxidant. According to these data, juvenile Verona red and red Grumolo chicories are characterized by the highest $PRTC_{exptl}$ values, their trapping capacities being >100 μmol of ROO$^\bullet$ radicals/g of FW, whereas Castelfranco red spotted and Chioggia white chicories are characterized by the lowest $PRTC_{exptl}$ values (~11 μmol of ROO$^\bullet$ radicals/g of FW). With regard to the phenolic antioxidants, the $PRTC_i$ values range from a minimum value of 3.6 to a maximum value of 9.9 of ROO$^\bullet$ radicals trapped by a molecule of antioxidant, in the case of gallic and chicoric acid, respectively.

Comparison between the $PRTC_i$ and $PRTE_i$ data of the phenolics reported in **Tables 2** and **3** shows the independence of their values, as expected. In fact, the ratio $PRTC_i/PRTE_i$ ranges from a minimum value of 0.98 in the case of cyanidin 3-glucoside to 13.6 in the case of protocatechuic acid. However, according to the data of **Tables 2** and **3**, the $PRTC_{exptl}/PRTE_{exptl}$ ratios of the studied chicories show a lower variability, ranging from a minimum value of 2.02 (Treviso early red) to a maximum

Table 2. PRTE Values of Some Chicory Cultivars and Contribution of the Main Phenolics

chicory	$C_{1/2}$[a]	$PRTE_{exptl}$[b]	antho-cyanins[c]	gallic acid[c]	protocatechuic acid[c]	caffeic acid[c]	chicoric acid[c]	chlorogenic acid[c]	$PTRE_{calcd}$[d]	$PTRE_{calcd}/PRTE_{exptl}$
$PRTE_i$[e]			7.14[f]	1.25	0.25	0.57	4.37	0.77		
					$C_i \cdot PRTE_i$					
Castelfranco red spotted	0.300 ± 0.030	3.33	0.41	1.04	0.20	0.17	2.30	1.12	5.24	1.57
Verona red	0.053 ± 0.008	18.87	13.27	1.15	0.43		3.66	5.49	23.99	1.27
Chioggia red	0.078 ± 0.010	12.82	8.91	0.68	0.25	0.21	2.96	2.70	15.72	1.23
Chioggia white	0.184 ± 0.050	5.43		0.94	0.22		1.99	0.95	4.10	0.75
Treviso Early red	0.085 ± 0.012	11.76	5.49	0.59	0.29		2.66	1.84	10.87	0.92
Treviso late red	0.186 ± 0.037	5.38	2.57	0.69	0.19		1.31	1.46	6.22	1.16
Green Grumolo	0.119 ± 0.011	8.40		2.87	0.50	0.17	5.62	3.47	12.63	1.50
Red Grumolo	0.023 ± 0.003	43.48	22.29	2.28	0.32		5.47	9.48	39.83	0.92
juvenile Verona red	0.021 ± 0.002	47.62	13.81	1.04	0.81	0.10	13.32	12.09	41.16	0.86
juvenile Treviso late red	0.043 ± 0.010	23.26	10.77	5.71	0.57		8.26	2.69	28.00	1.20

[a] Expressed as g of FW of chicory/L. [b] $PRTE_{exptl} = (C_{1/2})^{-1}$ as (g of FW of chicory/L) $^{-1}$. [c] $C_i \times PRTE_i$, where C_i is the μmol of *i*/g of FW (see **Table 1**). [d] $PRTE_{calcd} = \Sigma_i C_i \times PRTE_i$. [e] $PRTE_i = (C_{i1/2})^{-1}$ as (μmol of *i*/L)$^{-1}$. [f] PRTE of cyanidin-3 glucoside.

Table 3. PRTC Values of Some Chicory Cultivars and Contribution of the Main Phenolics

	$PRTC_{exptl}$[a]	antho-cyanins[b]	gallic acid[b]	protocatechuic acid[b]	caffeic acid[b]	chicoric acid[b]	chlorogenic acid	$PRTC_{calcd}$[a]	$PRTC_{calcd}/PRTC_{exptl}$
$PRTC_i$[d]		7.0[e]	3.6	3.5	4.8	9.9	6.4		
					$C_i \cdot PRTC_i$				
Castelfranco red spotted	11.35	0.41	2.99	2.77	1.39	5.24	9.26	22.05	1.94
Verona red	72.29	13.01	3.30	5.84	0.00	8.33	45.31	75.79	1.05
Chioggia red	41.75	8.73	1.97	3.48	1.73	6.75	22.31	44.97	1.08
Chioggia white	11.64	0.00	2.71	3.05	0.00	4.53	7.83	18.12	1.56
Treviso early red	23.85	5.38	1.69	3.93	0.00	6.06	15.19	32.25	1.35
Treviso late red	16.01	2.52	1.99	2.55	0.00	2.99	12.04	22.08	1.38
Green Grumolo	31.85	0.00	8.25	6.86	1.39	12.80	28.67	57.98	1.82
Red Grumolo	107.64	21.84	6.56	4.34	0.00	12.45	78.30	123.49	1.15
juvenile Verona red	160.00	13.54	2.99	11.00	0.84	30.33	99.80	158.50	0.99
juvenile Treviso late red	85.82	10.56	16.45	7.71	0.00	18.81	22.23	75.77	0.88

[a] Expressed as μmol of ROO•/g of FW. [b] $C_i \times PRTC_i$ where C_i is the μmol of *i*/g of FW (see **Table 1**). [d] Expressed as number of ROO• radicals trapped by a molecule of polyphenol. [e] PRTC of cyanidin-3 glucoside.

value of 3.83 (Verona red), indicating a similar phenolic composition (see **Table 1**).

Experimental versus Calculated Antioxidant Activity of Red Chicories. On the basis of the concentration of the various phenolics, C_i (μmol of *i*/g of FW of chicory), in the chicories (see **Table 1**) and of the $PRTE_i$ values of phenolics, reported in the second row of **Table 2**, the expected contribution of each phenolic, $C_i \times PRTE_i$, was calculated for the various chicories and reported in **Table 2**. The sum of these contributions is the calculated PRTE ($PRTE_{calcd}$), that is, $\Sigma C_i \times PRTE_i = PRTE_{calcd}$, when 1 g of FW of chicory is added to the measurement system. From the $C_i \times PRTE_i$ values, reported in **Table 2**, it appears that in the case of the red chicories the major contribution to $PRTE_{calcd}$ is given by anthocyanins, although these phenolics are not the main components of the studied chicories (see **Table 1**). In the case of red spotted and green varieties, chicoric acid appears to be the major contributor to $PRTE_{calcd}$, although, in the case of these chicories, chlorogenic acid is the main component.

The ratios $PRTE_{calcd}/PRTE_{exptl}$ are reported in the last column of **Table 2**. Values of $PRTE_{calcd}/PRTE_{exptl}$ below 1, as in the case of Chioggia white chicory, may indicate a synergistic effect (*18*) among the various antioxidants present in the chicory. Values of $PRTE_{calcd}/PRTE_{exptl}$ higher than 1, as in the case of Verona red chicory, may be explained by the disappearance of the phenolic radicals, generated in the scavenging of peroxyl radicals, by mutual reaction instead of their reaction with the peroxyl radicals itself.

In **Table 3** are reported the $PRTC_{exptl}$ values of each chicory and the contribution to the PRTC of the various phenolics ($C_i \times PRTC_i$) to the calculated total PRTC values, $PRTC_{calcd}$. From **Table 3** it appears that in the studied chicories chlorogenic acid is the main contributor to $PRTC_{calcd}$, its contribution being >50%, on average. $PRTC_{calcd}$ and the ratio $PRTC_{calcd}/PRTC_{exptl}$ are reported in the last columns of **Table 3**. The values of this ratio range from 0.88 in the case of juvenile Treviso late red chicory to 1.94 in the case of Verona red chicory, its average value being 1.12 for the red chicories and 1.77 for the green or white chicories. Values of the ratio $PRTC_{calcd}/PRTC_{exptl}$ much higher than 1 are a strong indication that the phenolic radicals, generated in the trapping of the highly reactive peroxyl radicals, react with other phenolics, decreasing in this way the expected capacity calculated on the basis of a simple addictive effect of PRTC values.

Dependence of the Antioxidant Activity and of the Polyphenol Content of Chicories on Growing Conditions. In the case of Chioggia red chicory the antioxidant activity was studied with reference to the type of soil (sandy, loamy, peaty) and fertilizer (a nonfertilizer control was compared to 10 treatments providing different nitrogen, phosporus, and potassium combinations, ranging from low to very high levels). No significant changes of polyphenols composition, efficiency, and capacity were found (standard deviation <10%). These results

Table 4. Antioxidant Activity of Some Fruits and Vegetables or Their Derivatives

	$C_{1/2}$[a] (g/L)	PRTE[b] $(g/L)^{-1}$	PRTC[c] (μmol/g)
American blueberry	0.029	34.48	51.3
Vaccinium myrtillus	0.012	83.33	104.1
black currant	0.031	32.25	62.6
cherry (Ferrovia)	0.055	18.18	27.5
red wine (Cabernet)	0.133	7.52	24.0
red wine (Teroldego)	0.101	9.90	31.4
apple (Golden Delicious)	1.230	0.81	26.2
tomato (Red Cerry)	0.819	1.22	5.6
tomato Ramato	3.107	0.32	1.4

[a] Expressed as g of food/L. [b] Expressed as $(g \text{ of food/L})^{-1}$. [c] Expressed as μmol of ROO•/g of food.

Table 5. Relative Absorbance and pH Values of Verona Red Chicory Extracts

extractent solution	pH of extract	A_{335nm}[a]	A_{533nm}[a]
ethanol/water 85:15 (v/v), 0.1 M HCl	2.05	1	1
0.12 M HCl	1.62	0.72	1
ethanol/water 85:15 (v/v)		0.90	0.30
water	6.02	0.46	nd

[a] All of the spectra were carried out in solution A.

confirm that different growing conditions have minimal effect on phenolic concentrations and on the antioxidant activity (*19*).

Comparison of Antioxidant Activity of Chicories with Those of Some Fruits and Vegetables. In **Table 4** PRTC and PRTE values of some fruits and vegetables or of their derivatives, well-known for their antioxidant activity, are reported for comparison purposes. From the comparison of the data of **Table 4** with those of **Tables 2** and **3**, it appears that the antiradical activity of some red chicories is similar to or higher than that of some foods which are usually present in the human diet and are acknowledged for a high antioxidant activity, such blueberry or red wine.

The antioxidant activities of some of the red chicories we have studied are orders of magnitude higher than those reported previously by Papetti et al. (*5*−*7*). This discrepancy is easily explained because in Papetti's papers the antioxidant activity tests were carried out on juices obtained by simple homogenization of vegetables, whereas in our case we measured compositions, capacities, and efficiencies of the extracts obtained using 0.12 M HCl, which is the concentration of HCl in the gastric juice after stimulation (*20*). In fact, in **Table 5** the relative amounts of phenolic acids and anthocyanins, calculated on the basis of their absorbance at 335 and 533 nm, respectively, obtained under various extraction conditions, are reported. From **Table 5** it appears that low pH values are important to achieve a high extraction yield of phenolics from this type of food. In particular, 100% of anthocyanins and 70% of phenolic acids are extracted by 0.12 M HCl, with respect to the maximum extraction yield obtained by aqueous ethanol containing 0.1 M HCl.

Due to the presence of substantial amounts of anthocyanins and phenolic acids in the red chicories, a single serving of some of these vegetables (90 g) may contribute up to 120 mg of anthocyanins and up to 600 mg of total polyphenols to the human diet. These amounts represent a substantial contribution to the daily intake of 1 g/day of polyphenols suggested in a pioneering paper by Kuhnau (*21*) and confirmed by Scalbert and Williamson (*22*).

The high concentration of phenolics present in a serving of red chicories might exert a direct topical effect within the gastrointestinal tract, scavenging highly reactive species such as reactive oxygen species (*23*). With regard to the bioavailability, it appears that chlorogenic and chicoric acids, which are present at high levels in the red chicories, can be easily hydrolyzed by esterases present in the gastrointestinal tract (*24*). In particular, chlorogenic acid was found in human plasma as caffeic acid after hydrolysis by the action of esterases at concentrations close to the micromolar range (*25*). Moreover, recent research studies have shown that beyond these compounds, anthocyanins too are adsorbed by humans by an anion translocator such as bilitranslocase (*26*, *27*). Further data suggest that absorption and excretion of anthocyanins in humans are influenced by the nature of the sugar conjugate and by the nature of the aglycon and demonstrated that cyanidin conjugated with glucose or rutinose is absorbed (*28*). The high concentration of anthocyans and in particular of cyanidin in the red chicories together with their bioavailability is very interesting because recent studies have shown that these flavonoids are beneficial in reducing age-associated oxidative stress, as well as improving neuronal and cognitive brain functions (*29*−*31*).

These findings indicate that some of the phytochemicals present in large amount in red chicories may contribute in a significant way to the daily intake of antioxidants and make these relatively low-cost foods very interesting from a nutritional point of view.

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