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Evaluation of the Phenolic Content in the Aerial Parts of Different Varieties of *Cichorium intybus* L.

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Fresh aerial parts of different chicory varieties: green chicory (c.v. "Catalogna"), two red chicory varieties ("radicchio rosso di Chioggia" and "radicchio rosso di Treviso"), and Witloof or Belgian endive were analyzed by HPLC/DAD/MS. The chromatographic fingerprint was diagnostic for each variety. A monocaffeoyl tartaric acid, chlorogenic acid, and chicoric acid were detected in all the varieties, while cyanidin 3-*O*-glucoside, delphinidin 3-*O*-(6″ malonyl) glucoside, and cyanidin 3-*O*-(6″ malonyl) glucoside were the main phenolic compounds in the red varieties. The flavonoidic compounds, quercetin 3-*O*-glucuronide and luteolin 7-*O*-glucuronide, were absent in the Witloof sample. The phenolic compounds from total leaves were the same as those obtained from only the colored parts; nevertheless, the total amount was remarkably lower with a decrease of up to 80% for Belgian endive. Chemical stability at high temperature was observed for the phenolic fraction from the green variety after decoction at 100 °C for 30 min.

KEYWORDS: ***Cichorium intybus* L.; red varieties; Witloof; Catalogna; cichoric acid; anthocyanins; HPLC/DAD; HPLC/MS**

INTRODUCTION

Cichorium intybus L. is a member of the sunflower family, Asteraceae (Compositae), which also includes globe and Jerusalem artichokes, lettuce, and many ornamental plants. It is indigenous to Europe, western Asia, Egypt, North America, and Italy (*1*). In popular medicine, *Cichorium intybus* L. has been used to treat skin disorders, such as gout, because of its antihepatotoxic activity (*2*, *3*). Animal studies have revealed that preparations from chicory roots can lower serum and liver lipid concentration in rats (*4*). Recently, *Cichorium intybus* L. aqueous extracts from roots and aerial parts were reported for antibacterial activity (*5*). Currently, chicory is used as a vegetable, fresh or cooked, while the ground and roasted roots are widely used for blending with coffee powder.

Cichorium intybus L. consists of many varieties, having different commercial uses. Because of the large number of different chicories, there is some misunderstanding about the botanical classification of these varieties; an example is the case of Belgian or French endive. In fact, while the common name Belgian endive helps to distinguish it from the crops of *C. endivia* and helps make it a market distinction, it is not as descriptive as the common name Witloof chicory which translated means "white-leaf" chicory. The two common names are often used indiscriminately and hereafter the names Witloof chicory and Belgian endive will be used as equivalent.

Within *Cichorium intybus* L., some main groups can be indicated (*6*): the loose leaf chicories, constituted by young leaf shoots and young flower shoots either used as a salad ingredient or as greens; the heading chicories, which produce cone-shaped heads, often used as fresh salads; and some special types of heading chicory such as red chicory and Witloof also known as Belgian or French endive and the root chicories, mainly used for industrial productions.

In a previous paper, *Cichorium intybus* L. var. Silvestre or wild chicory (W) was investigated to assess the content of phenolic compounds, mainly cinnamic acids and flavonoids (*7*). The antioxidant and radical scavenger activity of phenolic compounds is well documented, in particular, caffeic acid derivatives such as echinacoside act as antiinflammatory (*8*), skin photo damage protectors (*9*) and recently chicoric acid showed anti-HIV-1 activity (*10*). Until now, an evaluation of the qualitative and quantitative content of flavonoids, anthocyanins, and caffeoyl derivatives in different commercial chicory samples, commonly used as foods, has not been available in the literature. This investigation by HPLC/DAD/MS of commercial samples of fresh aerial parts of *Cichorium intybus* L. var. *foliosum* Bishoff c.v. Catalogna (CA), two red varieties of *Cichorium intybus* L. var. *silvestre* Chioggia (CH) and Treviso (TR), and finally *Cichorium intybus* L. var. *sativus* Bishoff (*6*) or Belgian endive (B) was done to compare their phenolic compositions. Quantitative results and the yields of different extraction procedures were also determined.

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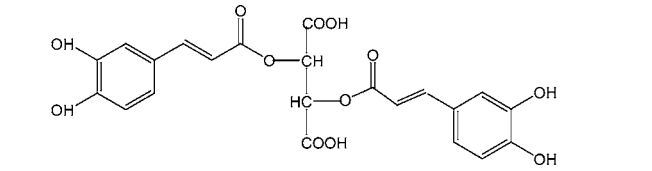
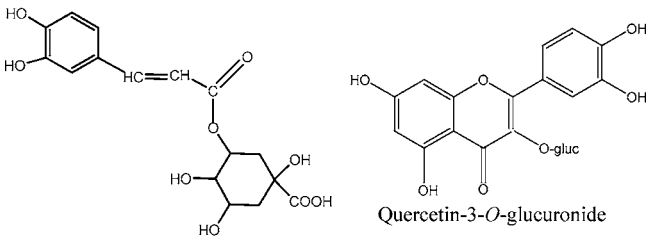
Table 1. List of the Analyzed Samples with the Ratio between Uncolored Leaf and Pigmented Part[a]

analyzed samples		uncolored leaf/ pigmented part (w/w)
CA1	Catalogna (February)	1.23
CA2		1.71
CA3		1.87
B1	Belgian endive (November)	6.4
B2		5
B3		6.6
CH1[b]	Radicchio rosso di Chioggia (November)	
CH2[b]		
CH3[b]		
TR1	Radicchio rosso di Treviso (November)	1.9
TR2		2.4
TR3		1.94

[a] Values are a mean of three determinations with an RSD below 5%. [b] The distinction between the two parts of the leaves of CH samples was not possible because of their morphology.

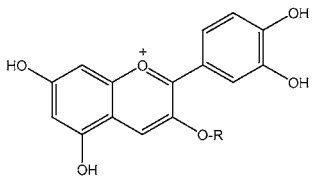


2S,3S-O-Di-caffeoyl tartaric acid (Chicoric acid).



Chlorogenic acid

Quercetin-3-*O*-glucuronide



R=(6″malonyl)-glucose

Cyanidin-3-*O*-(6″malonyl)-glucoside

Figure 1. Chemical structures of the main constituents present in the chicory varieties.

MATERIALS AND METHODS

The chicory was purchased in three different markets near Florence in February and November 2000 for a total of 12 samples (**Table 1**). All solvents used were of HPLC grade; CH_3CN and MeOH were from E. Merck, (Darmstadt, Germany) while H_2O was from Baker (J. T. Baker, Italy). The pure standards of caffeic acid, keracyanin (3-*O*-rutinoside cyanidin), and rutin were purchased from Extrasynthese (Gene-France), and the chicoric acid was previously isolated from wild chicory (*7*).

Sample Preparation. *Hydro Alcoholic Extraction.* This procedure was applied to all the samples listed in **Table 1** and was performed both on the colored parts and the whole leaves. The only exception was the "Radicchio rosso di Chioggia"; because of its specific morphology, it was impossible to distinguish and therefore to efficiently separate the colored section of the leaves that appeared almost completely pigmented. For each sample, 500 g of fresh leaves was crushed after contact with liquid nitrogen, freeze-dried, and 10 g of the obtained powder was extracted with (60 mL × 3) of EtOH/H_2O (adjusted to pH 2 by HCOOH) 7:3, v/v, up to a final volume of 200 mL. From all these samples, 10 mL was taken and extracted with hexane to completely eliminate the lipophilic components, was concentrated under vacuum, and then was dissolved in 1−2 mL of EtOH/H_2O (adjusted to pH 2 by HCOOH) 6:4, v/v, for green chicory and Belgian endive samples and with H_2O/HCOOH (95:5, v/v) for the red varieties. HPLC/DAD and HPLC/MS analyses were carried out on these samples.

Decoction. From each sample of green chicory leaves (several kilograms), 500 g was selected and submitted to a rough separation of the pigmented parts, and their ratio weight/weight was determined, ranging from 1.23 up to 2.05. To the green parts (160−220 g) and the whole leaves (≈500 g) were added 2 and 4 L, respectively, of hot water, and then the chicory was boiled for 30 min. The decoctions were filtered and the water extracts were lyophilized and then were redissolved for the HPLC/DAD and HPLC/MS analyses.

Quantitative Determination. Quantitative evaluation of each compound was performed using four-point regression curves (r^2 = 0.9998) through the use of authentic standards or isolated compounds. The chicoric acid amount was calculated at 330 nm; the caffeoyl derivatives were calculated at 330 nm using a standard of caffeic acid; the flavonoid molecules were evaluated at 350 nm using rutin; the anthocyanin amounts were calculated at 520 nm using keracyanin as a reference compound.

Apparatus. *HPLC-DAD Analysis.* The analyses were carried out using a 1090L liquid chromatograph equipped with a DAD detector (Hewlett-Packard, Palo Alto, CA). Solvents were A: H_2O/0.1% HCOOH, B: CH_3CN, and C: MeOH. For the phenolic acids and flavonoids, a 250 × 4.6 mm i.d., 5 μm LiChrosorb RP18 column (Merck, Darmstatt, Germany) equipped with a precolumn of the same phase was used. The following multistep linear gradient was applied: from 100% A to 85% A in 20 min and a plateau for 5 min; 10 min to 75% A, then a plateau of 8 min, to 100% CH_3CN in 10 min; and a final plateau of 3 min. Total time of analysis, 57 min; equilibration time, 15 min; flow rate, 1 mL min^{-1}; oven temperature, 26 °C. The anthocyanins of the red varieties were analyzed by a 150 × 3.00 mm i.d., 5 μm Luna C18 column (Phenomenex, Germany) applying the following linear solvent gradient: from 98% A to 48% A, 27% C and 25% B in 20 min, and a plateau for 4 min; 2 min to 60% MeOH and 40% CH_3CN; and then a final plateau of 2 min. Total time of analysis, 28 min; equilibration time, 15 min; flow rate, 1 mL min^{-1}; oven temperature, 26 °C. The injection volumes were in the range 10−25 μL for all the samples. UV/vis spectra were recorded in the range 220−530 nm and the chromatograms were acquired at 254, 280, 330, 350, and 520 nm.

HPLC-MS Analysis. The HPLC/MS analyses were performed using an 1100L liquid chromatograph equipped with a DAD detector and the interface was a 1100 MSD API-electrospray (Hewlett-Packard).

Negative and positive ionization modes were used with a gas temperature of 350 °C, nitrogen flow rate of 10.0 L/min, nebulizer pressure 30 psi, quadrupole temperature 30 °C, and capillary voltage 3500 V; the applied fragmentor was in the range 80−150 V.

RESULTS AND DISCUSSION

Until now, only a few reports have focused on the phytochemical investigation of chicory (*11*−*14*), and no data are available on the phenolic content in different cultivars. The aim of this research was to evaluate the amount of cichoric acid, flavonoids, phenolic acids, and anthocyanins in four widely distributed cultivars: Catalogna, two red varieties, and Witloof. From a preliminary screening, remarkable lower amounts of phenolic constituents were revealed for the nonpigmented leaves; consequently, the work focused mainly on the pigmented parts. With the aim of avoiding the enzymatic degradation, previously described for cichoric acid in Echinacea extracts (*15*), several parameters were controlled during the sample manipulation. The ground raw material was immediately frozen before the freeze-drying; low pH value and high percent of ethanol were used

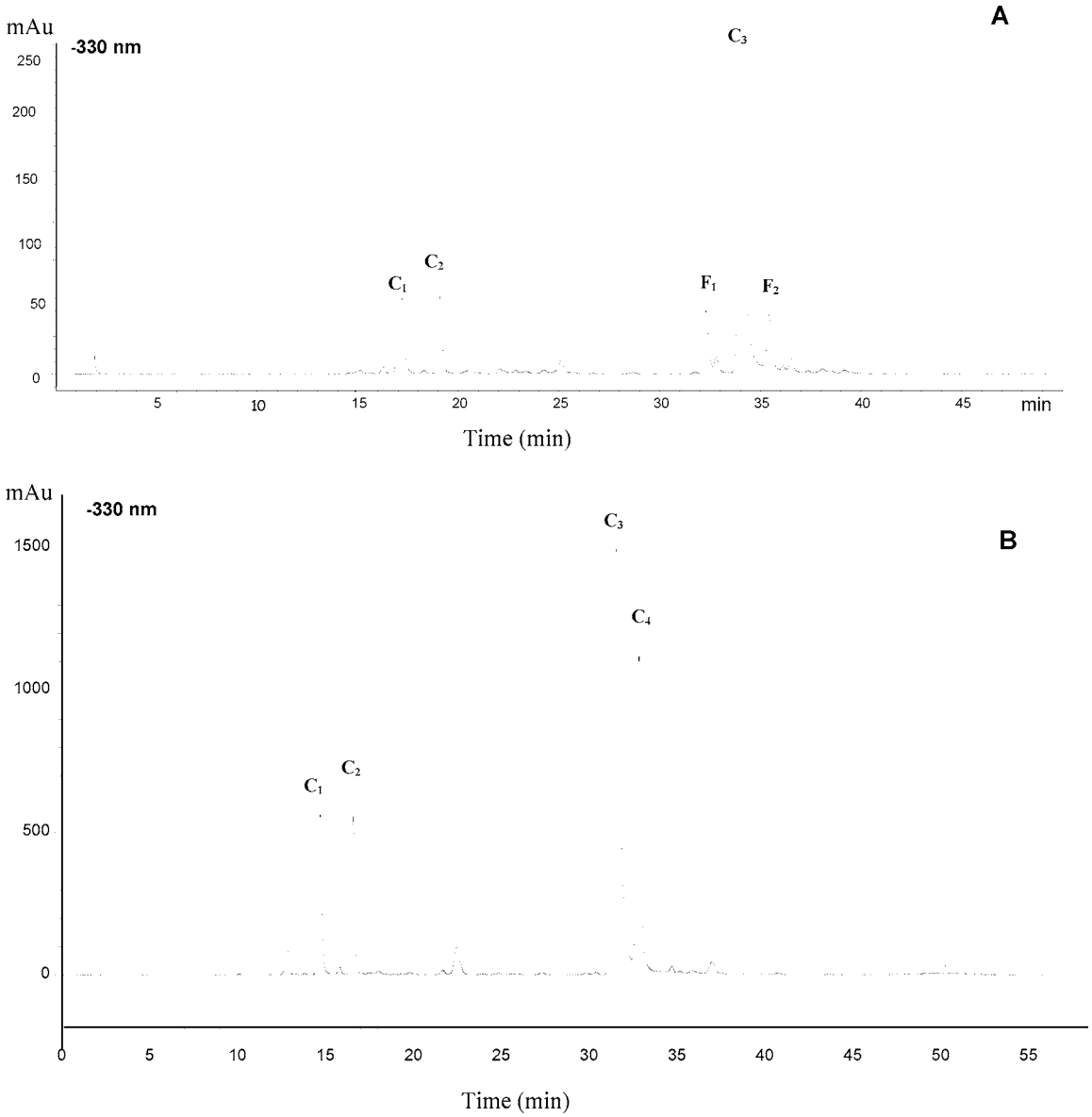


Figure 2. Representative chromatographic profile of (**A**) Catalogna sample CA1 and (**B**) Belgian endive (B1) recorded at 330 nm.

for the extractive solution. Moreover, the lag time between extraction and HPLC analysis was a few hours, and repetition of analysis of the same extract after several months of storage at −20 °C was carried out. Fresh and stored extracts showed overlapped HPLC/DAD profiles.

In **Figure 1** are shown the chemical structures of the main representative compounds. The presence of these compounds in our samples was confirmed both by UV/vis and MS spectra through the use of reference pure standards and by a comparison with previous results obtained for the wild chicory (*7*) and red varieties (*12*, *13*, *16*, *17*).

Catalogna cv. A hydro alcoholic extraction (ethanol 70%) was applied to the green part of Catalogna samples, and the analyses were performed by HPLC/DAD. A representative profile at 330 nm is shown in **Figure 2A** and the quantitative results are summarized in **Table 2** (CA1−CA3).

Chicoric acid was confirmed as one of the most abundant compounds with respect to the total phenolic content, followed by the other caffeoyl esters and by flavonoids.

Belgian Endive or Witloof. To our knowledge, no investigation on the phenolic composition of Belgian endive has been reported in the literature. The samples of Witloof were submitted to the same hydro alcoholic extraction procedure as applied to Catalogna cv. The HPLC/DAD profiles of these samples at 330 nm show similar patterns (**Figure 2B**) revealing as characteristic compounds mono- and dicaffeoyl esters of tartaric acid (C_1 and C_3) and of quinic acid (C_2 and C_4), while no traces of flavonoids were detected in this variety. A summary of the quantitative findings is presented in **Table 2** with, again, chicoric acid as the most abundant compound, ranging around 75% with respect to the total phenolic content.

Red Chicory. Two varieties were considered: "radicchio rosso di Chioggia" and "radicchio rosso di Treviso", both cultivated in northern Italy and characterized as production of geographical indication (PGI). **Figure 3** shows the HPLC/DAD profiles of a representative red chicory sample recorded at 520 nm for the anthocyanins and at 330 nm for the caffeoyl esters and flavonoids, and again the same pattern was observed for the two varieties. In all the samples, the following pigments were identified: cyanidin 3-*O*-glucoside (A_1), delfinidin 3-*O*-(6″ malonyl)-glucoside (A_2), and cyanidin 3-*O*-(6″malonyl)-glucoside (A_3) according to MS and UV/vis spectra and previous investigations (*12*, *14*, *17*). The mass spectra obtained with an API/ESI ionization both in positive for the anthocyanins and in

Table 2. Quantitative Data of the Hydro Alcoholic Extracts Expressed in μg/g Fresh Leaves of the Phenolic Content in the Catalogna (CA), Belgian Endive (B), Radicchio di Chioggia (CH), and Radicchio di Treviso (TR) Samples[d]

	C_1[a]	C_2[a]	C_3	C_4[a]	F_1[b]	F_2[b]	A_1[c]	A_2[c]	A_3[c]	total
CA1	51.2 ± 0.5	39.4 ± 0.5	246.4 ± 2.9		81.1 ± 0.9	34.7 ± 0.9				452.8
CA2	136.2 ± 1.5	56.7 ± 0.8	178.6 ± 2.0		188.5 ± 1.9	69.2 ± 1.4				629.2
CA3	222.4 ± 1.8	223.8 ± 1.8	905.6 ± 7.3		327.0 ± 2.9	128.5 ± 1.7				1807.3
B1	56 ± 1.1	50.7 ± 0.6	700 ± 3.5	145 ± 1.9						951.7
B2	151.2 ± 1.7	73.8 ± 0.7	759.5 ± 5.3	108.5 ± 1.1						1093
B3	49.1 ± 1.3	16.4 ± 0.2	346.5 ± 3.2	20.3 ± 0.3						432.3
CH 1		198 ± 4.0	606 ± 9		235.8 ± 4.7	438 ± 6.6	111.6 ± 4.5	75 ± 5.3	1452 ± 36	3116
CH 2		304.6 ± 3.1	468.3 ± 4.2		183 ± 3.7	396.5 ± 6.0	138 ± 8.3	58.8 ± 4.4	1100 ± 22	2181
CH 3		276 ± 4.1	330 ± 3.3		318 ± 6.3	354 ± 5.3	237 ± 14.2	92.4 ± 6.9	1626 ± 32	3233
TR 1		542.2 ± 5.4	1401 ± 12		1065 ± 11	885.8 ± 17	535.6 ± 35	407 ± 28	3621 ± 87	8458
TR2		151.5 ± 3.0	1535 ± 21		962 ± 7.7	1138 ± 12	139.1 ± 8.7	38.1 ± 4.0	1555 ± 37	5519
TR3		566.5 ± 11	1344 ± 12		1055 ± 12	1648 ± 13	463.5 ± 14	231.7 ± 16	4790 ± 110	10099

[a] These compounds are expressed as caffeic acid. [b] These compounds are expressed as rutin. [c] These compounds are expressed as keracyanin; C_1, monocaffeoyl tartaric acid; C_2, chlorogenic acid; C_3, chicoric acid; C_4, caffeoyl derivative; F_1, quercetin 3-*O*-glucuronide; F_2, luteolin 7-*O*-glucuronide; A_1, cyanidin 3-*O*-glucoside; A_2, delphinidin 3-*O*-(6″ malonyl)-glucoside; A_3, cyanidin 3-*O*-(6″ malonyl)-glucoside. [d] Values are a mean ± RSD of three determinations.

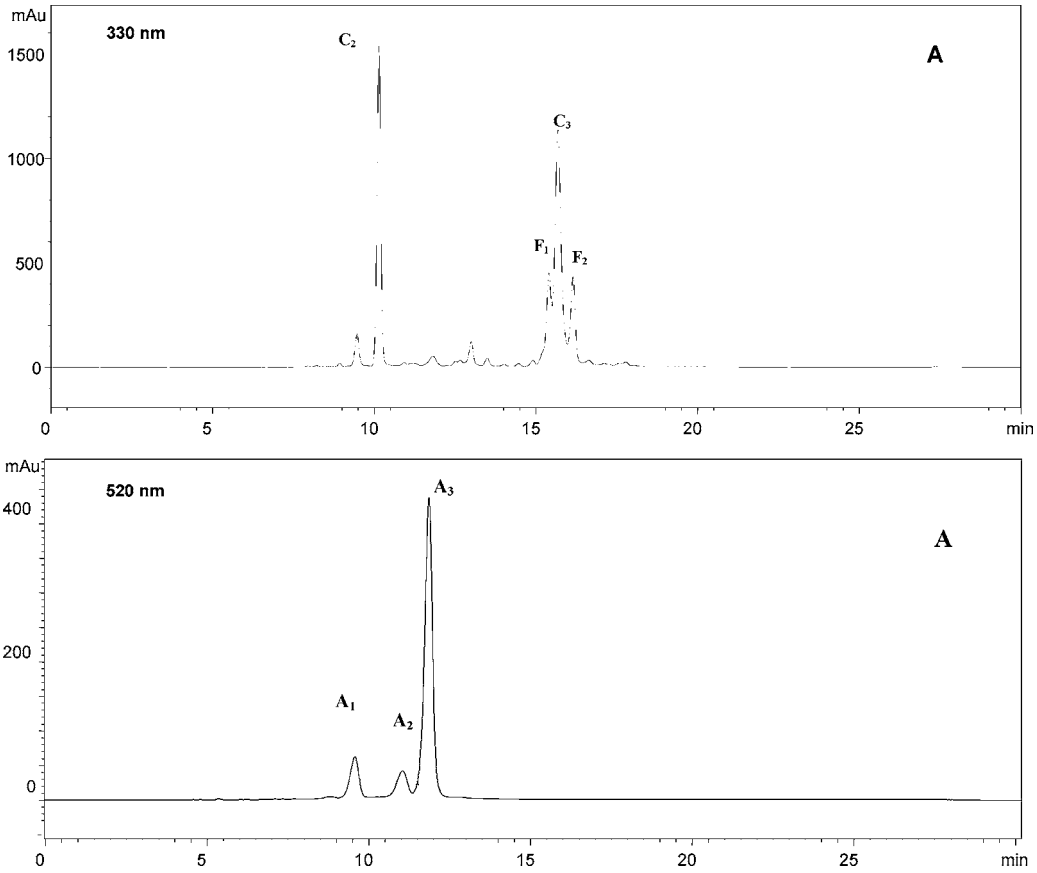


Figure 3. Chromatographic profiles of Radicchio rosso di Treviso sample, TR1, at (**A**) 330 nm and at (**B**) 520 nm.

negative mode (for the other derivatives) were particularly diagnostic for their structural characterization. The pseudo molecular ions and typical fragments were always detected with different intensity depending on the applied fragmentation energy. In these past few years, this technique has resulted to be one of the most powerful tools for the investigation of these compounds in complex mixtures.

Table 2 summarizes the quantitative results obtained applying the hydro alcoholic extraction. As expected for the red varieties, the anthocyanins are widely represented within the phenolic compounds (50−55%), and for both radicchio di Chioggia and radicchio di Treviso, cyanidin-3-*O*-malonyl glucoside was the main pigment (80−90% of the total anthocyanins), in agreement with previous studies on other red varieties (*12*). Moreover, the radicchio di Treviso was ≈60% higher in anthocyanins with respect to the other varieties.

Observing the other classes of constituents, chicoric acid was again the most abundant ranging between 10 and 15% of the total phenols, while the monocaffeoyl tartaric acid was absent. The flavonoid compounds were the same as those identified for the green chicory, with an estimated amount of quercetin and luteolin glycosides up to 21% and 38% for radicchio di Chioggia and radicchio di Treviso, respectively.

Quantitative Correlations among the Different Varieties. All the samples derived from extraction on the total leaves show HPLC profiles similar to those obtained by analysis of only the colored parts without relevant variations of the ratio between the considered molecules. Nevertheless, the phenol contents in

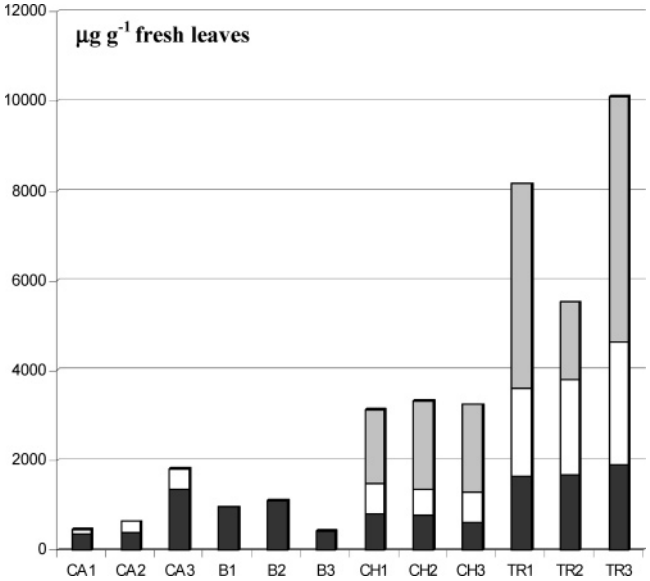


Figure 4. Comparison of the phenolic content in all the analyzed chicory samples.

the whole leaves were remarkably lower with a decrease of 65% for radicchio di Treviso, 70% for green chicory, and up to 80% for Belgian endive with respect to only the colored part. The histogram in **Figure 4** underlines the variability of the phenolic content within each variety. Radicchio di Chioggia and radicchio di Treviso are the richest varieties, and the latter attains phenolic concentrations up to 10 times more than those obtained for the green chicory and the Belgian endive, which show comparable low amounts.

Decoction of Catalogna Samples. For this variety, which is mainly consumed as a cooked vegetable, also a decoction to reproduce the normal cooking procedure was considered. The hot extraction was optimized in terms of water volume and time of cooking and was applied to both the whole leaves and only the green part of Catalogna. This procedure gave information about the thermal stability of phenolic compounds and their concentration in the cooking waters. The lyophilized decoctions obtained from the treatment of the green leaves were powdery and easy to handle, while those from the extraction of the whole leaves were more hygroscopic, presumably because of their higher amount of simple sugars. The yields obtained from decoction ranged between 4.6 and 6.7% of the dried weight of the extract.

From the HPLC/DAD investigation, the chromatographic profiles of all the samples (CA1d-CA3d) were almost identical. Some differences were evident with respect to those from wild chicory, particularly the relative ratio C_3/C_1 was higher for the Catalogna cultivar in comparison with the wild sample.

As already observed for the hydro alcoholic extracts, the total phenolic content of the whole-plant decoction was remarkably lower, with a decrease up to 70%, when compared with the concentration estimated in the colored part. Therefore, the quantitative evaluation was only applied to the extracts from the pigmented part of the leaves (**Table 3**).

Chicoric acid was confirmed as the main compound as already observed for the hydro alcoholic extract, ranging between 50 and 58% with respect to the total phenolic content, followed by the caffeoyl esters (12−17%), and the flavonoid compounds, the group with the greatest quantitative variability from 4.4 to 12% with respect to total phenols.

It is worth noting how the 30-min decoction guaranteed the chemical stability of all these phenolic constituents; in fact, the measured amounts of total phenols were of the same magnitude of order for the two extractive typologies.

Table 3. Quantitative Data of the Catalogna Samples Obtained from Decoction (d)[c]

	µg/g fresh green leaves					
	C_1[a]	C_2[a]	C_3	F_1[b]	F_2[b]	total
CA1d	180.9 ± 1.8	34.4 ± 0.5	445.8 ± 4.4	46.8 ± 0.9	19.1 ± 3.6	727
CA2d	381.4 ± 3.4	77.0 ± 1.1	701.8 ± 5.6	109.7 ± 1.1	29.0 ± 0.6	1298.9
CA3d	407.2 ± 1.9	11.3 ± 0.2	929.1 ± 9.3	68.3 ± 1.22	28.4 ± 0.6	1544.3

[a] These compounds are expressed as caffeic acid. [b] These compounds are expressed as rutin. [c] Values are a mean ± RSD of three determinations.

As expected, our findings suggest a complete loss of these polar phenols in the pot chicory used as food. At the same time, the chemical stability observed for these molecules at high temperature can suggest a potential recovery of bioactive phenolic fractions from waste decoction waters of green chicory varieties.

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