

HPLC-DAD/MS Characterization of Flavonoids and Hydroxycinnamic Derivatives in Turnip Tops (*Brassica rapa* L. Subsp. *sylvestris* L.)

ANNALISA ROMANI,[†] PAMELA VIGNOLINI,[†] LAURA ISOLANI,[§]
FRANCESCA IERI,[†] AND DANIELA HEIMLER^{*,§}

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze, via U. Schiff 6, 50019 Sesto Fiorentino, Italy, and Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze, P.le delle Cascine 18, 50144 Firenze, Italy

Flavonoids and hydroxycinnamic derivatives of turnip tops (*Brassica rapa* L. subsp. *sylvestris* L.) were characterized for the first time in four samples from different origins. Turnip tops exhibit a high polyphenols content (ranging from 107 to 191 mg/100 g, fresh weight) and a good antiradical activity, determined with the DPPH[•] test. After a liquid–liquid extraction and fractionation procedures, most flavonoids (isorhamnetin, kaempferol, and quercetin glycosides) and hydroxycinnamic derivatives were identified by means of HPLC-DAD/MS techniques. Isorhamnetin glycosides were the main flavonoid derivatives, differing from that found in the vegetables belonging to the *Brassica oleracea* group.

KEYWORDS: Flavonoids; hydroxycinnamic derivatives; total phenolics; antiradical activity

INTRODUCTION

Brassica vegetables are used as food all over the world, and there is evidence that a diet rich in vegetables (and fruits) is associated with a decreased risk of some chronic diseases (1). It is generally assumed that antioxidants (such as ascorbic acid, α -tocopherol, and β -carotene) are responsible for the beneficial effects of this food (2). The antioxidant activity of phenolics, which act as reducing agents and hydrogen donors, has been studied also in relation to polyphenol content (3–5). Furthermore, the composition of the polyphenol mixture is of great importance in view of the different biological actions of its components on human metabolism (6). The polyphenol composition of members of the Brassicaceae family has been investigated, in particular, for broccoli (7), cabbage (8), white cabbage (9), and Italian kale (10). Most studies deal with the total phenolic composition as determined by HPLC or by the Folin–Ciocalteu method (11, 12). Some recent publications describe the nearly complete composition of the polyphenol mixture of *Brassica* vegetables or byproducts (13–15). All of the above-mentioned vegetables belong to the *Brassica oleracea* group.

The group *Brassica rapa* includes many significant crops such as Chinese cabbage; in Italy this group is mainly represented by turnip tops [*B. rapa* L. subsp. *sylvestris* (L.) Janch var. *esculenta* Hort.], which are used as a cooked vegetable and are known as “cime di rapa”. It is cultivated as a winter vegetable,

and it is regarded as a typical product in many Italian regions. The only report on minor components of *B. rapa* vegetable concerns the determination of glucosinolates, a group of secondary metabolites with β -thioglucose, which is characteristic of the genus *Brassica*, in Japanese “nabana” turnip rape (16). Mineral and vitamin contents have been determined (17).

The purpose of this study was to identify and characterize polyphenols from turnip tops and to assess their antiradical activity with respect to the known characteristics of members of the *B. oleracea* group.

MATERIALS AND METHODS

Plant Material. The vegetables were purchased in February 2005 from farmers selling in open markets (samples A–C) and from a supermarket (sample D) in Florence (Italy).

Standards. Authentic standards of isorhamnetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, quercetin 3-*O*-glucoside, and chlorogenic and gallic acids were purchased from Sigma-Aldrich (St. Louis, MO).

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

Extraction and Purification of Polyphenols. The edible part (leaves and flowers) of each sample was frozen in liquid nitrogen and stored at -80 °C before proceeding with the analysis. Frozen tissues were ground in a mortar with a pestle under liquid nitrogen. A quantity of 1.5 g of tissue was extracted in 20 mL of 70% ethanol (pH 3.2 with formic acid) overnight. The extracts were filtered and defatted with 3 \times 15 mL of petroleum ether. The defatted extracts were evaporated to dryness under vacuum at room temperature and finally redissolved in EtOH/H₂O (70:30), adjusted to pH 3.2 with formic acid, to a final volume of 4 mL.

Liquid–Liquid Extraction (LLE). A quantity of 14 g of frozen leaves was extracted in 200 mL of 70% ethanol (pH 3.2 with formic acid)

* Corresponding author [telephone (+39)553288201; fax (+39)-55333273; e-mail daniela.heimler@unifi.it].

[†] Dipartimento di Scienze Farmaceutiche.

[§] Dipartimento di Scienza del Suolo e Nutrizione della Pianta.

overnight. The extract was filtered and defatted with 3×100 mL of petroleum ether. The defatted extract was then evaporated under vacuum at room temperature to eliminate the organic solvent, and finally the aqueous solution was extracted with ethyl acetate (5×50 mL). The ethyl acetate extract and the aqueous solution were evaporated to dryness under vacuum at room temperature and finally redissolved in EtOH/H₂O (70:30), adjusted to pH 3.2 with formic acid, to a final volume of 25 mL.

HPLC-DAD Analysis. Analyses of flavonols and hydroxycinnamic derivatives were carried out using a HP 1100L liquid chromatograph equipped with a diode array detector (DAD) and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA). Flavonols and hydroxycinnamic derivatives were separated by using a 250×4 mm, i.d. $5 \mu\text{m}$, RP-18, LiChroCART column (Merck, Darmstadt, Germany), operating at 27 °C, with a three-step linear solvent gradient system as follows (13): from 80% H₂O (adjusted to pH 3.2 by H₃PO₄)–20% methanol to 50% at 35 min and 20% H₂O at 37 min, with a final step to wash the column, over a 42-min period, at a flow rate of 1.0 mL/min and a 150×3 mm, i.d. $5 \mu\text{m}$, RP-18, Luna RP-18 column (Phenomenex), operating at 27 °C, with a five-step linear solvent gradient system starting from 100% H₂O to 75% H₂O/25% acetonitrile, over a 70-min period, at flow rate of 0.8 mL/min. 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 Analyses. Analyses were performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies, Palo Alto, CA). 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 mode at 80–180 eV.

Flow Injection Analysis Electrospray Ionization Mass Spectrometry (FIA/ESI/MS). FIA/ESI/MS was performed using a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies). The analyses were performed by alternating both positive and negative ionization modes or the capillary voltage.

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 (such as kaempferol, quercetin, and isorhamnetin derivatives) were determined at 350 nm using isorhamnetin 3-*O*-glucoside as reference compound. Hydroxycinnamic derivatives were determined at 330 nm using chlorogenic acid as reference compound. In all cases, actual concentrations of the derivatives were calculated after corrections for differences in molecular weight had been applied. Three samples were collected from each site so as to express the analytical results as an average with its standard deviation. For the quantitative analysis high values of polyphenols recovery (>95%) were obtained. The extraction yield was controlled by adding gallic acid as internal standard. The choice of this molecule was based on its absence in our samples and on its retention time, which falls in an empty zone of the chromatogram (RT = 3.68 min).

Total Phenolic Content. The total phenolic content was determined using the Folin–Ciocalteu method, described by Singleton et al. (18) and slightly modified according to the procedure of Dewanto et al. (19). To 125 μL of the suitably diluted sample extract were added 0.5 mL of deionized water and 125 μL of the Folin–Ciocalteu reagent. The mixture was kept for 6 min, and then 1.25 mL of a 7% aqueous Na₂CO₃ 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, milligrams of gallic acid per 100 g of sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 $\mu\text{g/mL}$ ($R^2 = 0.9969$).

Antiradical Activity. Free radical scavenging activity was evaluated with the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH[•]) assay. The antiradical capacity of the sample extracts was estimated according to

the procedure reported by Brand-Williams (20) 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 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. The antiradical activity is expressed as IC₅₀, the antiradical dose required to cause a 50% inhibition. IC₅₀ was calculated by plotting the ratio $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{sample}}) \times 100$, 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. IC₅₀ is expressed as milligrams of sample per milligram of DPPH[•].

RESULTS AND DISCUSSION

The four turnip top samples extracted with ethanol/water (see Materials and Methods) gave rise to the HPLC profiles reported in **Figure 1**. Because the identification of phenolic components was the main goal of this work, a solvent and a procedure (see Materials and Methods), which are suited for the recovery of a wide range of phenolics (21), were chosen. Owing to the complicated nature of the chromatograms and its numerous peaks, a LLE was considered to be more appropriate to make a first rough separation prior to the HPLC-MS analysis. The chromatograms of the ethyl acetate and aqueous solutions are reported in **Figure 2A,B**, whereas the HPLC method was well-suited for the ethyl acetate extract; for the aqueous solutions a different method was chosen (**Figure 2C**).

From HPLC-DAD data, all flavonoids are glycosylated derivatives of three flavonols, that is, kaempferol (266, 294sh, and 349 nm for 3-glycosides and 266, 318sh, and 349 nm for 3,7-diglycosides), quercetin (255, 266sh, and 355 nm for 3-glycosides and 255, 266sh, 294sh, and 354 nm for 3,7-diglycosides), and isorhamnetin (255, 268sh, 294sh, and 354 nm for 3-glycosides and 255, 268sh, and 354 nm for 3,7-diglycosides). However, several compounds coeluted in the same peak, and therefore their UV spectra were not very useful for identification. To determine the best conditions for the recording of MS spectra, a FIA/ESI/MS analysis was performed. Under the best conditions selected, all of the flavonols and most hydroxycinnamic derivatives gave rise to fragments.

Table 1 reports all of the identified compounds with the most frequent ions after the fragmentation. The structures were assigned on the basis of the MS data, literature reports (13), retention times, and UV-vis spectra. In **Figure 3** are reported the chemical structures of the major components.

Flavonols **2**, **4**, and **12**, which were not identified, are probably quercetin derivatives on the basis of the m/z 949, 787, 625, and 463 ions. Flavonol **10**, which exhibits the same fragmentation and the same UV-vis spectrum as flavonol **11**, may be regarded as a kaempferol derivative. Under our experimental conditions, some hydroxycinnamic derivatives did not give rise to any fragmentation; among them compounds **17**, **20**, and **26** are caffeoyl derivatives on the basis of their UV-vis spectra. Compounds **31** and **32**, which gave rise to high molecular weight fragments and exhibit high retention times, can be regarded, on the basis of their UV-vis spectra, as caffeoyl derivatives.

In **Table 2** the quantitative data of the four samples analyzed are reported. No important qualitative differences were observed among the four samples; the only difference concerns the quantitative results of the phenol classes. Flavonols were always the most represented compounds; hydroxycinnamic derivatives were also found in the 5.77–52.54 mg/100 g range in all analyzed samples. It should be noted that the total polyphenol

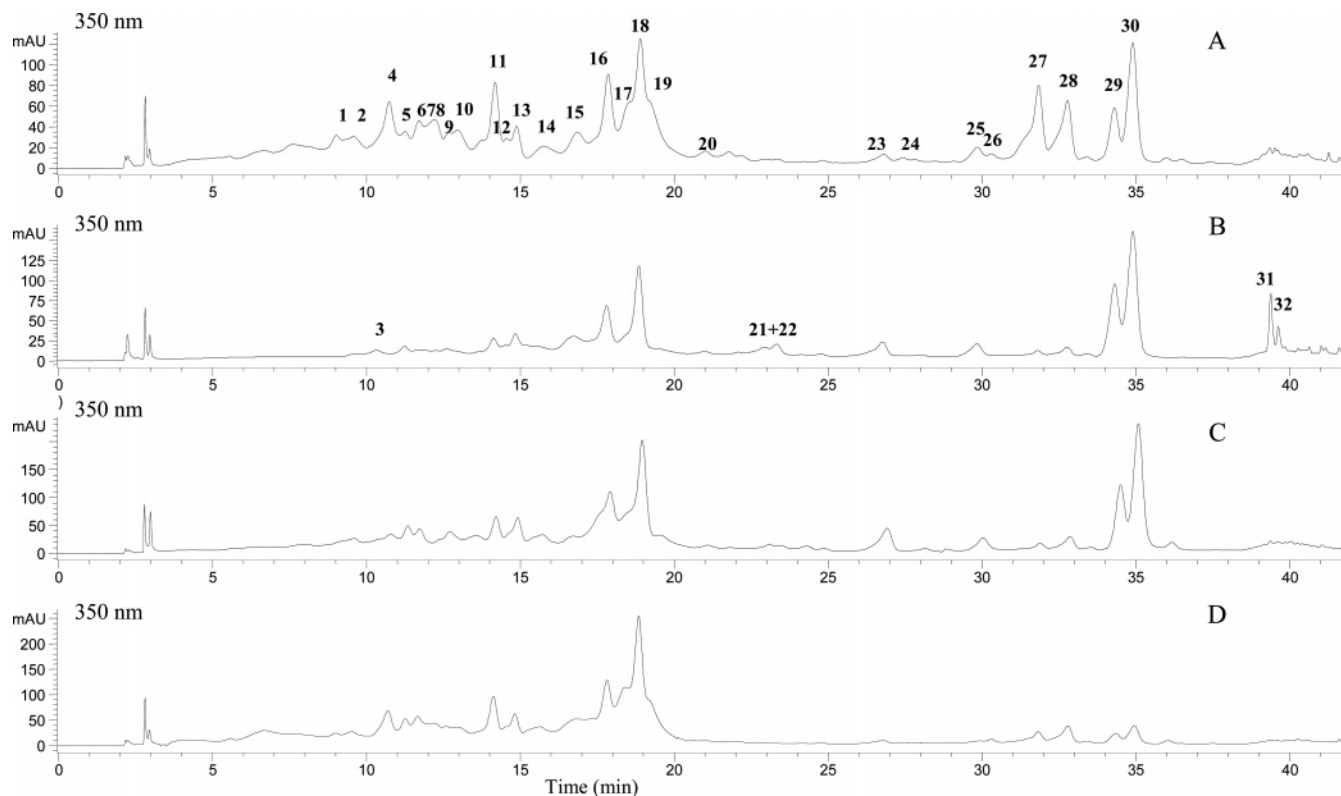


Figure 1. Chromatographic profiles acquired by HPLC-DAD (350 nm) of the hydroalcoholic (ethanol/water 70:30, pH 3.2) extracts of four samples of turnip top (A–D).

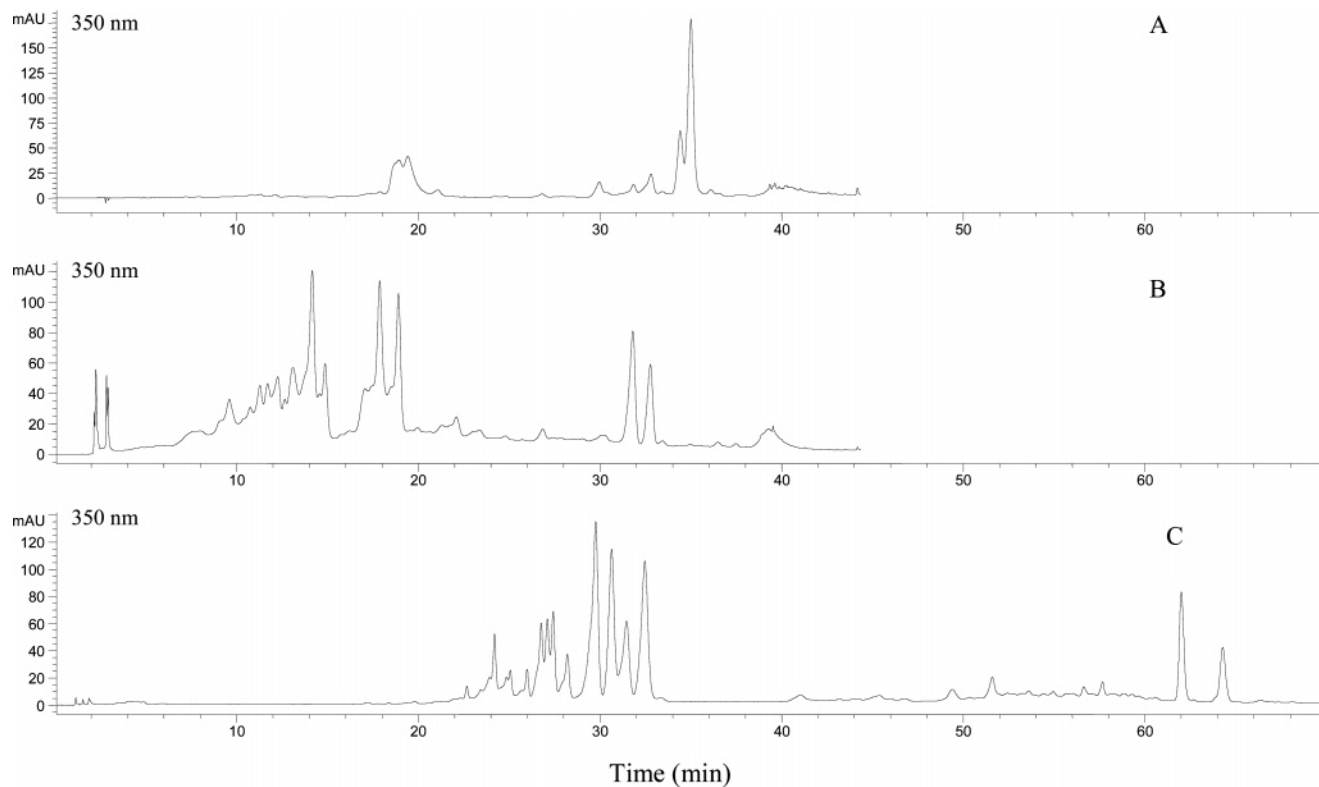


Figure 2. Chromatographic profiles acquired by HPLC-DAD (350 nm) with the LiChroCART RP-18 column of the ethyl acetate (A) and the aqueous (B) solutions and chromatographic profile acquired by HPLC-DAD (350 nm) with the Luna RP-18 column of the aqueous extract (C) of a turnip top sample.

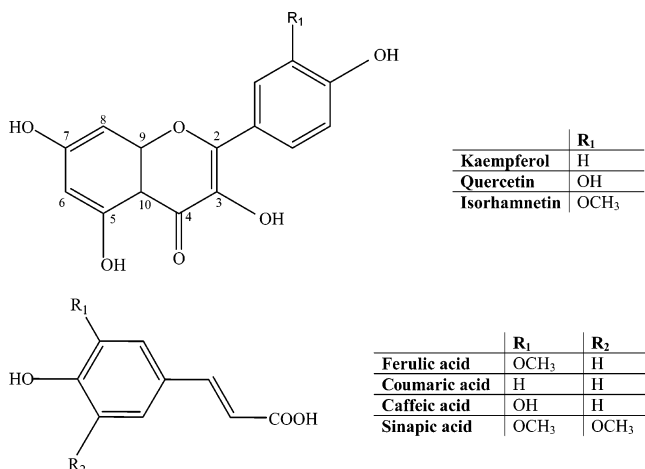
amount is quite high as compared with *B. oleracea* results (11). In contrast to other *B. oleracea* vegetables, the most abundant polyphenols were isorhamnetin derivatives (data not shown). In the case of broccoli (7, 13), cauliflower (15), kale (22), and Italian kale (10) the main flavonoids were kaempferol and

quercetin glycosides; only kaempferol glycosides were found in tronchuda cabbage (14). Isorhamnetin-3,7-*O*-di- β -D-glucoside has been found in the corolla of *B. rapa*, playing the role of nectar guide (23), suggesting that isorhamnetin glycosides may be the primary flavonoids in the *B. rapa* group. Isorhamnetin-

Table 1. Peak Numbers (Figure 1), Retention Times (t_R), Assigned Structures, Molecular Weights, and m/z of Turnip Top Extracts

peak	t_R (min)	structure ^a	MW	peaks (m/z)
1	8.9	K-3-Ome-caffeoyl-sophotr-7-gluc	1126	963, 771, 609
2	9.5	unknown flavonol		949, 787, 625, 463
3	10.3	K-3-sophotr-7-glu	934	933, 771, 609, 447, 285
4	10.7	unknown flavonol		963, 801, 625, 463
5	11.2	K-3-caffeoyl-sophotr-7-soph	1258	933, 771, 609, 447, 285
6	11.7	Q-3-p-coumaroyl-soph-7-gluc	934	933, 771, 625, 463
7	11.9	Q-3-diferoyl-soph-7-gluc	978	977, 625, 463, 301
8	12.3	Q-3-sinapoyl-sophotr-7-glu	1156	993, 787, 625, 463, 301
9	12.7	Q-3-feruloyl-sophotr-7-glu	1126	963, 949, 787, 625, 463
10	13.0	unknown flavonol		977, 815
11	14.2	K-3-sinapoyl-sophotr-7-glu	1140	977, 815
12	14.5	unknown flavonol		949, 787, 625, 463, 301
13	14.9	K-3-feruloyl-sophotr-7-soph	1272	947, 785, 609, 447, 285
14	15.8	K-3-cumaroyl-sophotr-7-soph l derivative	1242	917, 755, 609, 447, 285 639, 477, 315
15	16.8	K-3,7-diglu l derivative	610	609, 447, 285 639, 477, 315
16	17.8	K-3-gluc-7-soph l derivative	772	609, 447, 285 639, 477, 315
17	18.7	caffeoyl derivative		
18	18.9	l-3-gluc-7-gluc	640	639, 447, 315
19	19.0	caffeoyl derivative		
20	21.1	caffeoyl derivative		
21	23.4	K-3-soph	610	609, 447, 285
22	23.4	Q-3-soph	626	625, 463, 301
23	26.8	K-3-feruloyl-soph	786	785, 609, 285
24	27.4	hydroxycinnamic derivative		
25	29.8	Q-3-gluc	464	463, 301
26	30.3	caffeoyl derivative		
27	31.9	1,2-disinapoyl-gentiobiose	754	753, 529, 223, 205
28	32.7	1,2-disinapoyl-feruloyl-gentiobiose	724	723, 499, 223, 175
29	34.3	K-3-gluc	448	447, 285
30	34.9	l-3-gluc	478	477, 315
31	39.4	caffeoyl derivative		909, 879, 849, 713
32	39.6	caffeoyl derivative		923, 893, 863, 727

^a K, kaempferol; Q, quercetin; l, isorhamnetin; gluc, glucose; sophotr, sophotriose; soph, sophorose.

**Figure 3.** Chemical structures of main flavonoids and hydroxycinnamic acids.

3,7-*O*-di- β -D-glucoside was the major compound in sample C, whereas isorhamnetin-3-*O*- β -D-glucoside was the major compound in the other three samples (A, B, and D). Among kaempferol and quercetin derivatives, kaempferol-3-*O*- β -D-glucoside and quercetin-3-*O*-sinapoylsophotriose-7-*O*-glucoside are the most abundant, respectively.

Table 2. Total Flavonoids and Hydroxycinnamic Derivatives (Milligrams per 100 g, Fresh Weight) As Determined by HPLC^a

sample	total flavonoids	hydroxycinnamic derivatives	total phenolics
A	138.85 (8.19)	52.54 (2.05)	191.39
B	105.79 (7.93)	13.79 (0.63)	119.58
C	101.56 (4.97)	5.77 (0.35)	107.33
D	119.20 (6.55)	51.7 (2.69)	170.90

^a Standard deviation within parentheses. Data reported are mean values of three determinations.

Table 3. Antiradical Activity Expressed as IC₅₀ and Total Phenolic Content (Folin–Ciocalteu Method) of the Four Turnip Top Samples

sample	IC ₅₀ sample/DPPH* (mg, fresh wt/mg)	total phenolics, gallic acid/sample (mg/100 g, fresh wt)
A	549.87	250.69 (34.82)
B	600.57	236.42 (33.05)
C	528.97	221.46 (38.76)
D	516.60	243.53 (27.10)

^a Standard deviation within parentheses. Data reported are the means of three determinations.

It should be pointed out that the polyphenol content of turnip top is quite high; in the case of flavonoids the content is from about 3 to 10 times higher than that of all other Brassicaceae (5, 7, 9, 22, 24). Within one variety, as in the case of turnip top, there is a great variation of the flavonoid content; such occurrence is probably related to these peculiar products of secondary metabolism, the amount of which is affected by light, environment, and plant phytopathological conditions (25–27), making the comparison of data very difficult.

Table 3 reports the IC₅₀ values, that is, the concentration which inhibits by 50% the activity of 1 mg of DPPH*. The values are quite similar and are not correlated to the total phenolic content as obtained with the Folin–Ciocalteu method. If we consider the IC₅₀ values of ascorbic acid (0.195), quercetin (0.153), kaempferol (0.514), and quercitrin (0.294), the antiradical activity of turnip tops seems extremely low. However, on the basis of their flavonoid content, the values in **Table 3** can be modified. Assuming a mean value of 550 for the IC₅₀ parameter, if we considered only the flavonoid content, an IC₅₀ value of 0.638 was obtained, that is i.e., the same magnitude as those found for pure standards.

The results obtained in this study show that turnip tops are an appreciable source of polyphenols, especially flavonoids. Even if polyphenols undergo numerous reactions during processing and cooking (7, 24), their presence in fresh food is related to their antiradical activity and may help in promoting the cultivation of vegetables with a known geographical origin. Furthermore, in this case the presence of isorhamnetin, a flavonoid not present in the *B. oleracea* family, and of its derivatives has been pointed out, indicating that the qualitative data are important in the definition of the flavonoid mixture. In fact, isorhamnetin diglucoside, isolated from mustard leaf (*B. juncea*), showed a strong activity in reducing serum levels of glucose in diabetes mellitus through an antioxidant activity (28).

LITERATURE CITED

- (1) Liu, R. H. Protective role of phytochemicals in whole foods: implications for chronic disease prevention. *Appl. Biotechnol. Food Sci. Policy* **2003**, *1*, 39–46.

- (2) Kaur, C.; Kapoor, H. C. Antioxidants in fruits and vegetables—the millennium's health. *Int. J. Food Sci. Technol.* **2001**, *36*, 703–725.
- (3) Kaur, C.; Kapoor, H. C. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.* **2002**, *37*, 153–161.
- (4) Kahkonen, M. P.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.-P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954–3962.
- (5) Proteggente, A. R.; Pannala, A. S.; Pagana, G.; Van Buren, L.; Wagner, E.; Wiseman, S.; Van De Put, F.; Dacombe, C. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Res.* **2002**, *36*, 217–233.
- (6) Williams, R. J.; Soencer, J. P. E.; Rice-Evans, C. Flavonoids: antioxidants or signalling molecules? *Free Radical Biol. Med.* **2004**, *36*, 838–849.
- (7) Price, K. R.; Casuscelli, F.; Colquhoun, I. J.; Rhodes, M. J. C. Composition and content of flavonol glycosides in broccoli florets (*Brassica oleracea*) and their fate during cooking. *J. Sci. Food Agric.* **1998**, *77*, 468–472.
- (8) Nielsen, J. K.; Norbek, R.; Olsen, C. E. Kaempferol tetraglycosides from cabbage leaves. *Phytochemistry* **1998**, *49*, 2171–2176.
- (9) Chu, Y. H.; Chang, C. L.; Hsu, H. F. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* **2000**, *80*, 561–566.
- (10) Romani, A.; Pinelli, P.; Galardi, C.; Corti, G.; Agnelli, A.; Vincieri, F. F.; Heimler, D. Analysis of flavonoids in leaves of black cabbage (*Brassica oleracea* var. *acephala* DC. subvar. *viridis* forma *serotina*) grown on different soils and heights. *Ital. J. Food Sci.* **2003**, *15*, 197–205.
- (11) Vallejo, F.; Tomas-Barberan, F. A.; Garcia-Viguera, C. J. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *J. Sci. Food Agric.* **2002**, *82*, 1293–1297.
- (12) Zhang, D.; Hamauzu, Y. Structural characterization and detection of kale flavonoids by electrospray ionization mass spectrometry. *Food Chem.* **2004**, *88*, 503–509.
- (13) Vallejo, F.; Tomas-Barberan, F. A.; Ferreres, F. Characterization of flavonols in broccoli (*Brassica oleracea* L. var. *italica*) by liquid chromatography-UV diode-array detection-electrospray ionisation mass spectrometry. *J. Chromatogr. A* **2004**, *1054*, 181–193.
- (14) Ferreres, S.; Valentao, P.; Llorach, R.; Pinheiro, C.; Cardoso, L.; Pereira, J. A.; Sousa, C.; Seabra, R. M.; Andrade, P. B. Phenolic compounds in external leaves of Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC). *J. Agric. Food Chem.* **2005**, *53*, 2901–2907.
- (15) Llorach, R.; Gil-Izquiero, A.; Ferreres, F.; Tomas-Barberan, F. A. HPLC-DAD-MS/MS ESI characterization of unusual highly glycosylated acylated flavonoids from cauliflower (*Brassica oleracea* L. var. *botrytis*) agroindustrial byproducts. *J. Agric. Food Chem.* **2003**, *51*, 3895–3899.
- (16) Kim, S.-J.; Kawaguchi, S.; Watanabe, Y. Glucosinolates in vegetative tissues and seeds of twelve cultivars of vegetable turnip rape (*Brassica rapa* L.). *Soil Sci. Plant Nutr.* **2003**, *49*, 337–346.
- (17) Leung, W. T. W.; Butrun, R. R.; Chang, F. H. Proximate composition, mineral and vitamin contents of East Asia food. In *Food Composition Table for Use in East Asia*; Department of Health, Education & Welfare, U.S. GPO: Washington, DC, 1972.
- (18) 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.
- (19) 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.
- (20) Brand-Williams, W.; Cuvelier, M. E. Use of a free radical method to evaluate the antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25–30.
- (21) Robards, K. Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *J. Chromatogr. A* **2003**, *1000*, 657–691.
- (22) Zhang, J.; Setterfield, M. B.; Brodbelt, J. S.; Britz, S. J.; Clevidence, B.; Novotny, J. A. Structural characterization and detection of kale flavonoids by electrospray ionisation mass spectrometry. *Anal. Chem.* **2003**, *75*, 6401–6407.
- (23) Sasaki, K.; Takahashi, T. A flavonoid from *Brassica rapa* flower as the UV-absorbing nectar guide. *Phytochemistry* **2002**, *61*, 339–343.
- (24) Vallejo, F.; Tomas-Barberan, F. A.; Garcia-Viguera, C. Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking. *J. Sci. Food Agric.* **2003**, *83*, 1511–1516.
- (25) Cooper-Driver, G. A.; Bhattacharya, M. Role of phenols in plant evolution. *Phytochemistry* **1998**, *49*, 1165–1174.
- (26) Romani, A.; Pinelli, P.; Mulinacci, N.; Vincieri, F. F.; Gravano, E.; Tattini, M. HPLC analysis of flavonoids and secoroidoids in leaves of *Ligustrum vulgare* L. (Oleaceae). *J. Agric. Food Chem.* **2000**, *48*, 4091–4096.
- (27) Dixon, R.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085–1097.
- (28) Yokozawa, T.; Kim, H. Y.; Cho, E. J.; Choi, J. S.; Chung, H. Y. Antioxidant effects of isorhamnetin 3,7-di-*O*- β -D-glucopyranoside isolated from mustard leaf (*Brassica juncea*) in rats with streptozotocin-induced diabetes. *J. Agric. Food Chem.* **2002**, *50*, 5490–5495.

Received for review October 24, 2005. Revised manuscript received December 27, 2005. Accepted December 28, 2005.

JF052629X