JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Flavonol Glucoside Profile of Southern Italian Red Onion (*Allium cepa* L.)

PAOLA BONACCORSI,* CORRADO CARISTI, CLAUDIA GARGIULLI, AND UGO LEUZZI

Dipartimento di Chimica Organica e Biologica, Università di Messina, Salita Sperone 31, 98166 S. Agata di Messina, Italy

High-performance liquid chromatography−diode array detector (HPLC−DAD) coupled with electron spray mass spectrometry (ESI−MS−MS) was used to determine the flavonol profile in southern Italian red onions (*Allium cepa* L.). This on-line technique allowed the identification of seven flavonols in southern Italian red onion, quercetin 4′-glucoside and quercetin 3,4′-diglucoside being the most abundant components. Five minor flavonols have been recognized, offering a characteristic profile of such compounds in red onions under study. Quercetin 3-glucoside, quercetin 7,4′-diglucoside, quercetin 3,7,4′-triglucoside, and isorhamnetin 4′-glucoside have been previously reported as minor flavonoid components in *Allium cepa*, while isorhamnetin 3,4′-diglucoside was previously found in *Allium ascalonicum*. Traces of isorhamnetin 3-glucoside and free quercetin were also detected.

KEYWORDS: ***Allium cepa*; flavonols; quercetin; isorhamnetin; HPLC−DAD; ESI−MS−MS**

INTRODUCTION

Since ancient times, many *Allium* species have been used as foods, spices, and medicinal plants in widespread areas of the world. The curative properties of these species have been often attributed to sulfur-containing volatile compounds such as allicin and its derivatives, which have been examined as potential anticarcinogens, antimutagens, and antimicrobial (*1*) and antioxidant molecules (*2*, *3*). However, the antioxidant activity of some members of the *Allium* species, such as onion (*Allium cepa* L.), cannot be ascribed just to these sulfur-containing compounds (*4*, *5*). Indeed, onions represent a significant source of flavonoids (*6*), the antioxidant activity of which is well-known.

Evidence of flavonoid benefits on human health has stimulated the application of several analytical techniques for their identification in onions (*7*) and for the development of methodologies for their quantification (*8*−*10*). In particular, HPLC−MS chromatography and NMR spectroscopy have been used to conduct qualitative and quantitative analyses of the flavonoid content. Earlier studies have shown that the major flavonoid found in onions is quercetin, present mainly as the 4′-*O*-glucoside and 3,4′-*O*-diglucoside (*11*), which together account for about 80% of the total content of flavonoids. Hydrolysis of glycosides has offered a practical method for the quantitative determination of flavonoids. Usually, their content in onion is expressed as μg/g of quercetin fresh weight (*12*). Dietary intake of flavonoids can be ascribed only to the consumption of fruits and vegetables, which are rich in these compounds. Therefore, numerous studies (*13*−*16*) have been conducted on the effects that storage and domestic processing, such as boiling or frying, have on the content and composition of flavonol glucosides in onions.

Few authors have focused their attention on quercetin derivatives, which differ from quercetin 4′-*O*-glucoside and 3,4′-*O*-diglucoside and are secondary constituents of the flavonoid profile in onions. However, these minor components can be significant in the characterization and differentiation of a variety and/or a cultivar. Andersen (*7*) reported the isolation and identification of quercetin 3,7,4′-*O*-β-triglucopyranoside from the pigmented scales of *A. cepa* var. Red Baron. Park and Lee (*17*) were able to isolate and characterize isorhamnetin 4′-glucoside as a secondary flavonoid component of some varieties of onion, and Brandwein (*11*) determined the presence of a small amount of quercetin 3-*O*-glucoside (isoquercitrin). To the best of our knowledge, however, there exist no reports in which a systematic identification of flavonoids in onion (*A. cepa* L.) has been conducted.

The present work focuses on the characterization of the flavonoid profile by direct analysis of extracts of a southern Italian red onion (*A. cepa* L.) variety, employing the HPLC−DAD ESI−MS−MS on-line technique. The present methodology allowed a fast, qualitative, quantitative, and reproducible determination of seven flavonol glucosides (**Figure 1**) present in the edible portion of the analyzed onions.

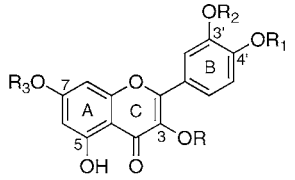
MATERIALS AND METHODS

Materials. The investigation was carried out, from November 2003 to March 2004, on 20 samples of red onion purchased from a local market and directly processed.

Reagents and Standard Solutions. HPLC-grade acetonitrile, methanol, and dimethylformamide (DMF) were supplied by Sigma Aldrich. Quercetin 4′-*O*-glucosides (spiraeoside) and isorhamnetin 3-*O*-glucoside

* To whom correspondence should be addressed. Tel.: +39.090.6765187; fax: +39.090.393895; e-mail: paolab@isengard.unime.it.

10.1021/jf048152r CCC: $30.25
Published on Web 03/05/2005



	R	R_1	R_2	R_3
1 Quercetin 3,7,4'-triglucoside	Glu	Glu	H	Glu
2 Quercetin 7,4'-diglucoside	H	Glu	H	Glu
3 Quercetin 3,4'-diglucoside	Glu	Glu	H	H
4 Isorhamnetin 3,4'-diglucoside	Glu	Glu	Me	H
5 Quercetin 3-glucoside	Glu	H	H	H
6 Quercetin 4'-glucoside	H	Glu	H	H
7 Isorhamnetin 4'-glucoside	H	Glu	Me	H

Figure 1. Flavonol glucosides found in southern Italian red onions.

(quercetin 3′-*O*-methyl-3-*O*-glucoside) were purchased from Extrasynthèse, France, and quercetin and isorhamnetin were supplied by Sigma Aldrich. Standards were dissolved in DMF (1 mg/mL) and stored at −18 °C. Calibration curves were obtained using DMF solutions of quercetin 4′-glucoside of known concentration (10−150 mg/L).

Sample Preparation. The outer dry layer of onions was manually removed, and the bulb was cut into small pieces. The pieces (10 g) were dipped in methanol (100 mL), left at 4 °C overnight, to allow the penetration of the solvent into tissues of onion, then removed from the methanolic phase and homogenized with a domestic electric blender. The methanolic phase was kept apart, whereas homogenized onions were stirred in MeOH (2 × 100 mL) for 30 min at room temperature, and the mixture was centrifuged. The three methanolic phases were combined, reduced to a volume of 10 mL, and stored at −18 °C until their use. Methanolic extracts (1 mL) were diluted with DMF (1 mL) and filtered through an Iso-Disk P-34, 3 mm diameter poly(tetrafluoroethylene) (PTFE) membrane, 0.45 μm pore size supplied by Supelco.

Hydrolysis. Acid hydrolysis was carried out on methanolic extracts of red onions, following Hertog's procedure (*8*). 6 M HCl (4 mL) in a methanol (8 mL)/water (6 mL) solution was added to 2 mL of methanolic extracts of onion, to give a 20 mL solution of 1.2 M HCl in 50% aqueous methanol. Ascorbic acid (50 mg) was used as an antioxidant. After refluxing at 90 °C for 4 h while being stirred, the solution was allowed to cool at room temperature and produced up to 25 mL with MeOH. DMF (1 mL) was added to the hydrolyzed methanolic extracts (1 mL), and the mixture was filtered through an Iso-Disk P-34, 3 mm diameter PTFE membrane, 0.45 μm pore size, prior to the injection.

LC−MS−MS Analysis of Flavonoids. LC−MS−MS analyses of samples were carried out with a ThermoQuest Model LCQ-Duo equipped with a diode array spectrophotometer and an ion trap mass spectrometer with electrospray ionization source (ESI). Separation of flavonoids was performed on a 250 mm × 4.6 mm i.d., 5 μm, Discovery C18 column, supplied by Supelco, equipped with a 20 mm × 4.0 mm guard column. The discovery C18 guard column was placed in a column oven set at 30 °C. The injection loop was 20 μL, and the flow-rate was 1.0 mL/min. The mobile phase consisted of a linear gradient of acetonitrile in H_2O as follows: 5−20% (0−15 min); 20−30% (15−20 min); 30−50% (20−30 min); 50−100% (30−35 min); 100% (35−40 min); and 100−5% (40−50 min). UV spectra were recorded between 200 and 450 nm, and simultaneous detection by diode array was performed at 350 nm.

Operating parameters of the mass spectrometer were: capillary temperature 250 °C, spray needle voltage set at 4.50 kV, and ES capillary voltage +3 and −47 V for positive and negative polarity, respectively. Nitrogen was used as a sheath gas with a flow of 50 arbitrary units. Mass analysis was carried out in full-scan mode from 80 to 900 amu, both in positive and in negative mode. The negative MS−MS spectra were obtained by collision-induced dissociation (CID), with an applied collision energy of 28 and 30% of instrumental maximum for quercetin and isorhamnetin aglycones, respectively.

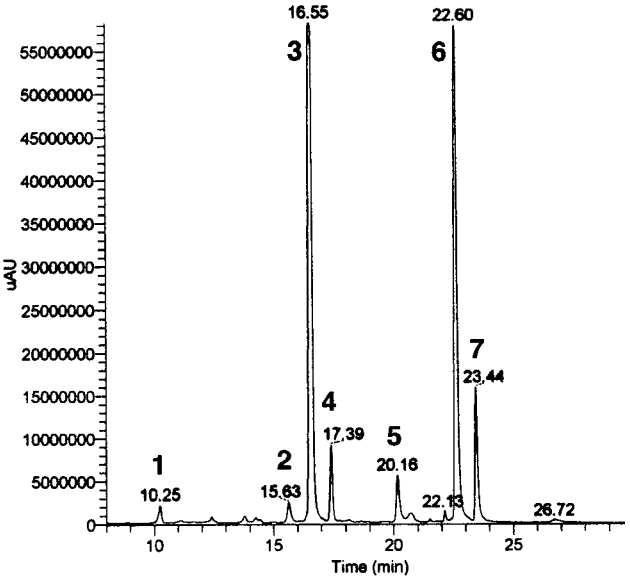


Figure 2. Typical chromatogram of southern Italian red onion at 350 nm. Components **1**–**7** were identified as follows: **1**, quercetin 3,7,4′-triglucoside; **2**, quercetin 7,4′-diglucoside; **3**, quercetin 3,4′-diglucoside; **4**, isorhamnetin 3,4′-diglucoside; **5**, quercetin 3-glucoside; **6**, quercetin 4′-glucoside; and **7**, isorhamnetin 4′-glucoside.

Each sample was tested three times and gave superimposable chromatograms.

RESULTS AND DISCUSSION

A typical DAD chromatogram, recorded at 350 nm, of methanolic extracts of southern Italian red onion (*A. cepa* L.) is shown in **Figure 2**. Seven peaks were detected, two of which, peaks 3 and 6, are the major flavonoid components of the onion extracts under study (**Figure 1**). The HPLC−DAD ESI−MS−MS apparatus allowed the simultaneous acquisition of DAD chromatograms at 350 nm, and UV spectra and ESI−MS spectra, both in positive and in negative ion mode, corresponding to each chromatographic peak. UV spectra of components **1**−**7** and quercetin standard are reported in **Figure 3**. **Figures 4**−**8** show MS and MS−MS spectra of components **1**, **2**, **4**, **5**, and **7**.

Acid hydrolysis of methanolic extracts of samples was conducted following Hertog's procedure (*8*). HPLC analysis of the hydrolyzed extracts produced two peaks that were identified as quercetin and isorhamnetin, respectively, by comparison with their commercial standards.

In the UV spectrum of component **6** (**Figure 3F**), the typical band I, associated with the absorption due to the B-ring cinnamoyl system, gave a maximum in the region of 365 nm, while band II, associated with absorption involving the A-ring benzoyl system, was detected at 253 nm. As previously stated (*18*), band I of flavonols occurs in the range of 352−385 nm, thus suggesting the flavonolic nature of component **6**. On-line ESI−MS in the positive ion mode exhibited a spectrum with two strong peaks, one centered at *m*/*z* 465 $[M + H]^+$ and the other at *m*/*z* 303 $[M + H - 162]^+$, this last peak consistent with the loss of a glycosyl moiety. Analogously, ESI−MS in negative ion mode produced two significant peaks, one at *m*/*z* 463 $[M - H]^-$ and the other at *m*/*z* 301 $[M - H - 162]^-$. Negative ESI−MS−MS analysis was carried out on the ion at *m*/*z* 301 and showed a fragmentation pattern in line with previous results proposed for a flavonol of the quercetin type (*19*). The presence of quercetin in the HPLC chromatogram of

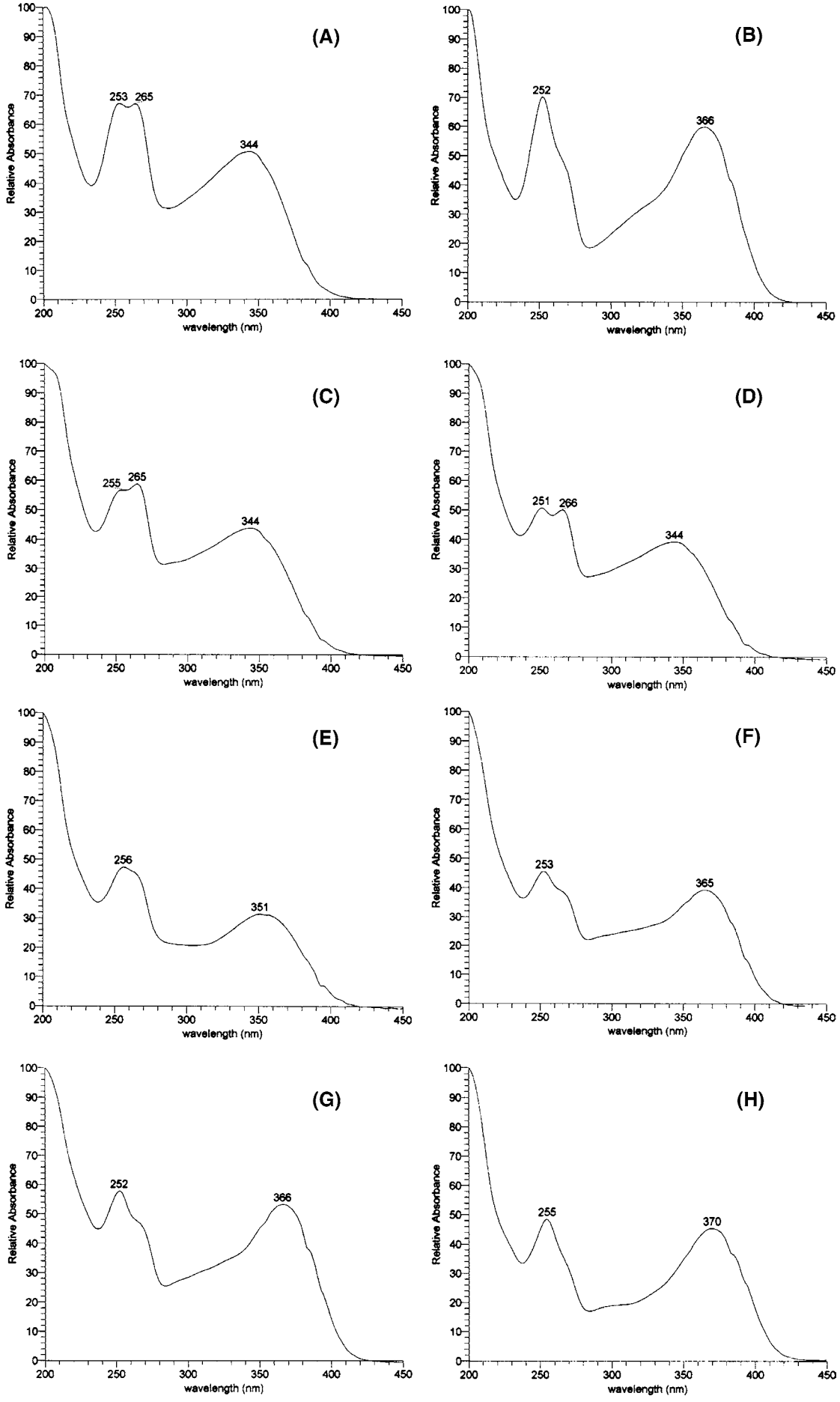


Figure 3. UV spectra of components **1**–**7** (**A**–**G**) and quercetin aglycone (**H**).

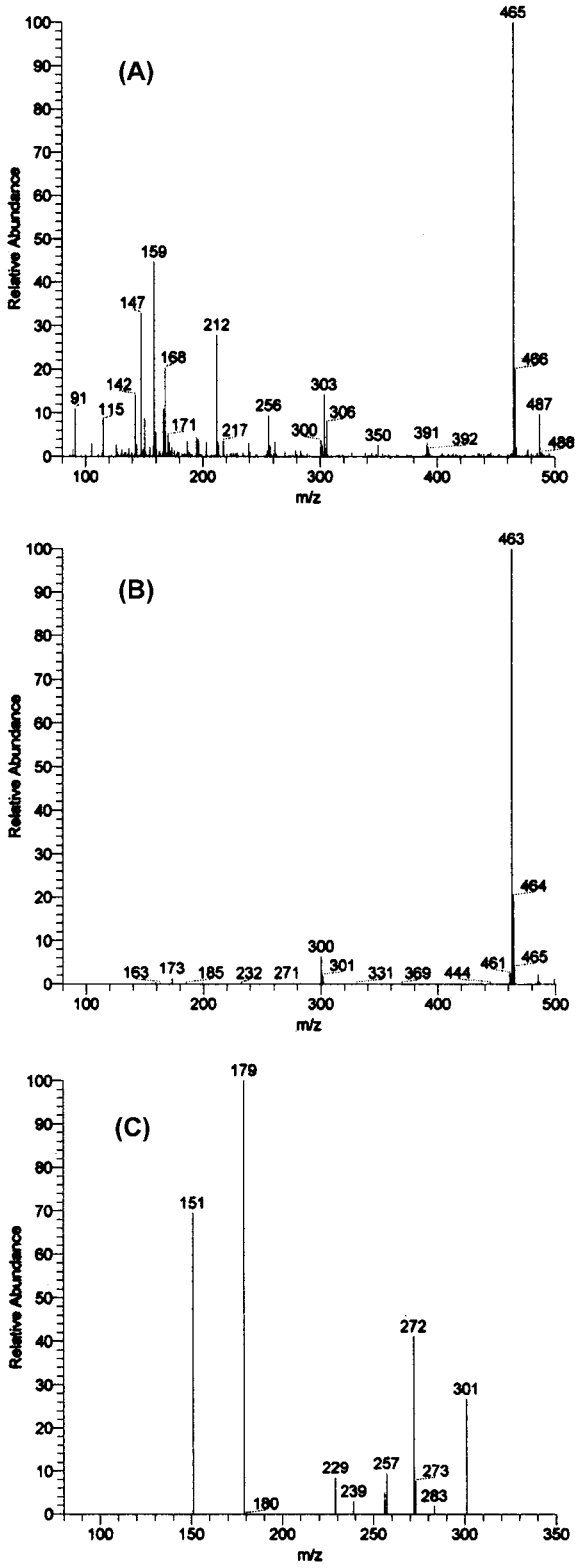


Figure 4. ESI–MS spectra in (**A**) positive and (**B**) negative ion mode and (**C**) negative ESI–MS–MS spectrum of component **5** (quercetin 3-glucoside).

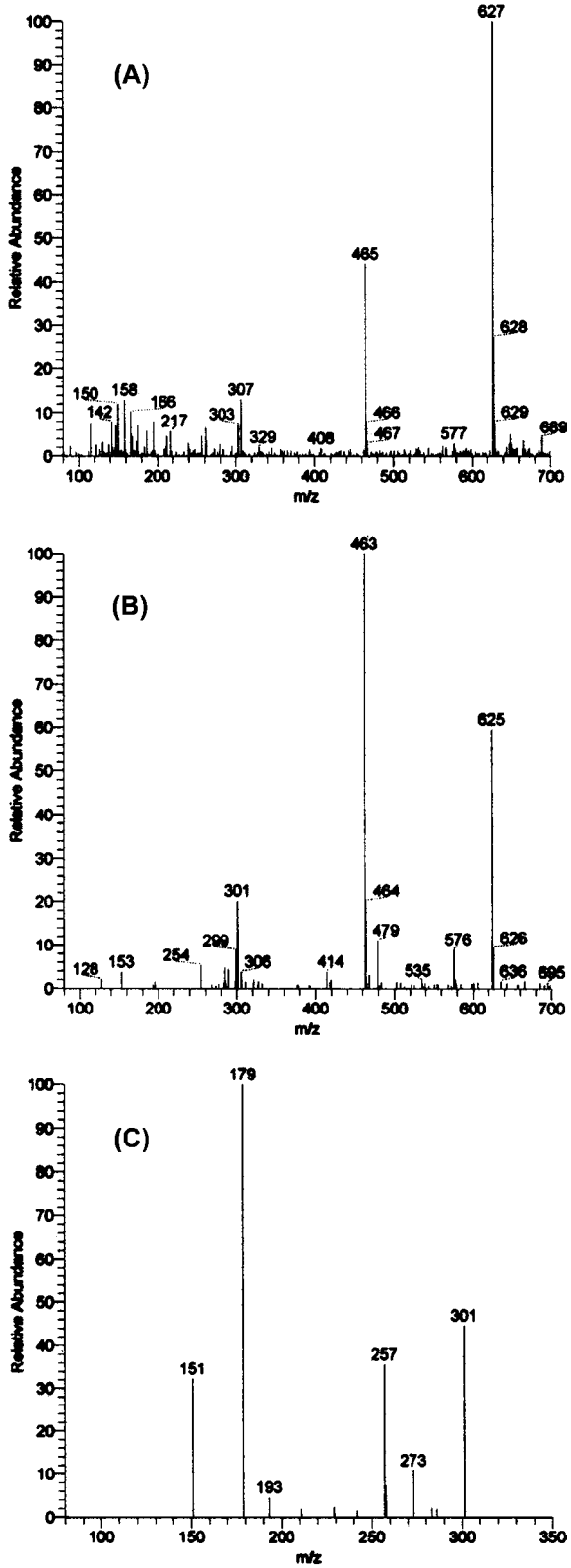


Figure 5. ESI–MS spectra in (A) positive and (B) negative ion mode and (C) negative ESI-MS-MS spectrum of component **2** (quercetin 7,4′-diglucoside).

the hydrolyzed extracts represented a further proof of its identification. HPLC−DAD ESI−MS−MS analysis of a standard solution of quercetin-4′-glucoside and its comparison with our results allowed us to unambiguously assign this structure to component **6**, in accordance with literature data that indicate quercetin-4′-glucoside (**Figure 1**) as one of the two major flavonols found in onions.

In the positive ESI−MS spectrum of component **5** (**Figure 4A**) peaks at *m*/*z* 465 $[M + H]^+$ and at *m*/*z* 303 $[M + H - 162]^+$ indicated the loss of a glycosyl moiety, showing a monoglycosylated flavonoid derivative for component **5**. Negative ESI−MS−MS on the ion at *m*/*z* 301 provided fragments that are typical of quercetin aglycone (*19*). The on-line diode

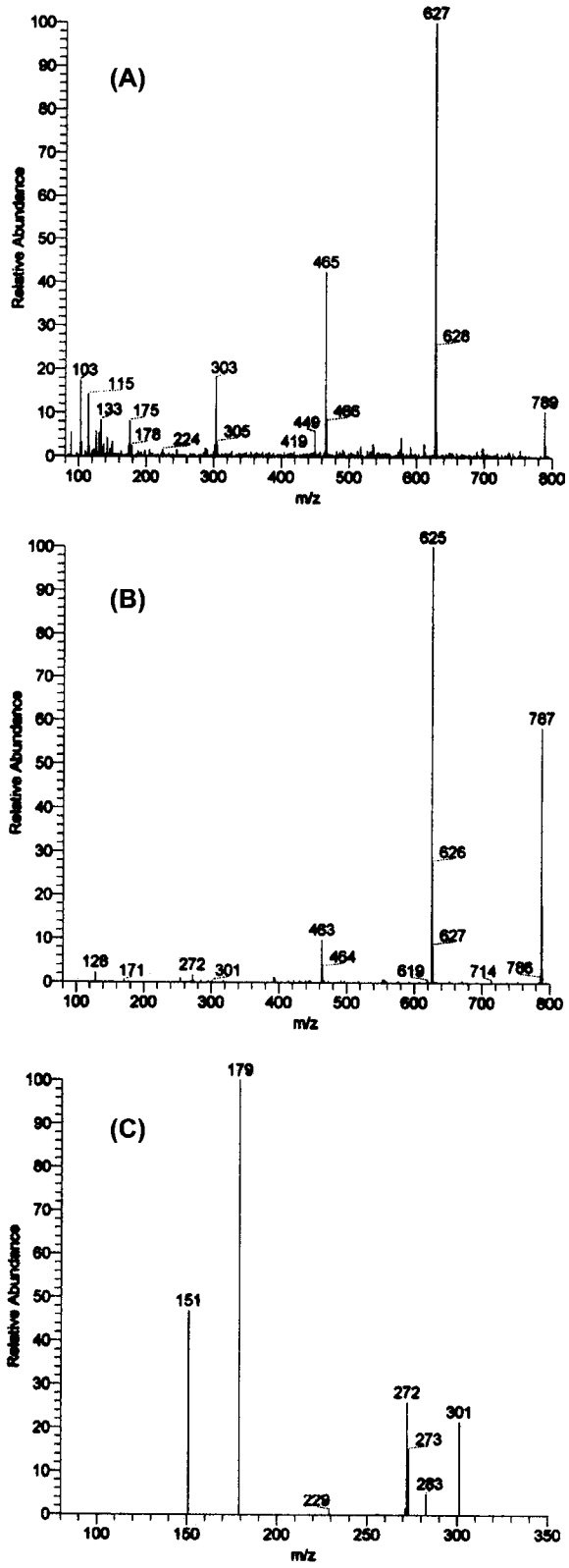


Figure 6. ESI–MS spectra in (A) positive and (B) negative ion mode and (C) negative ESI–MS–MS spectrum of component **1** (quercetin 3,7,4′-triglucoside).

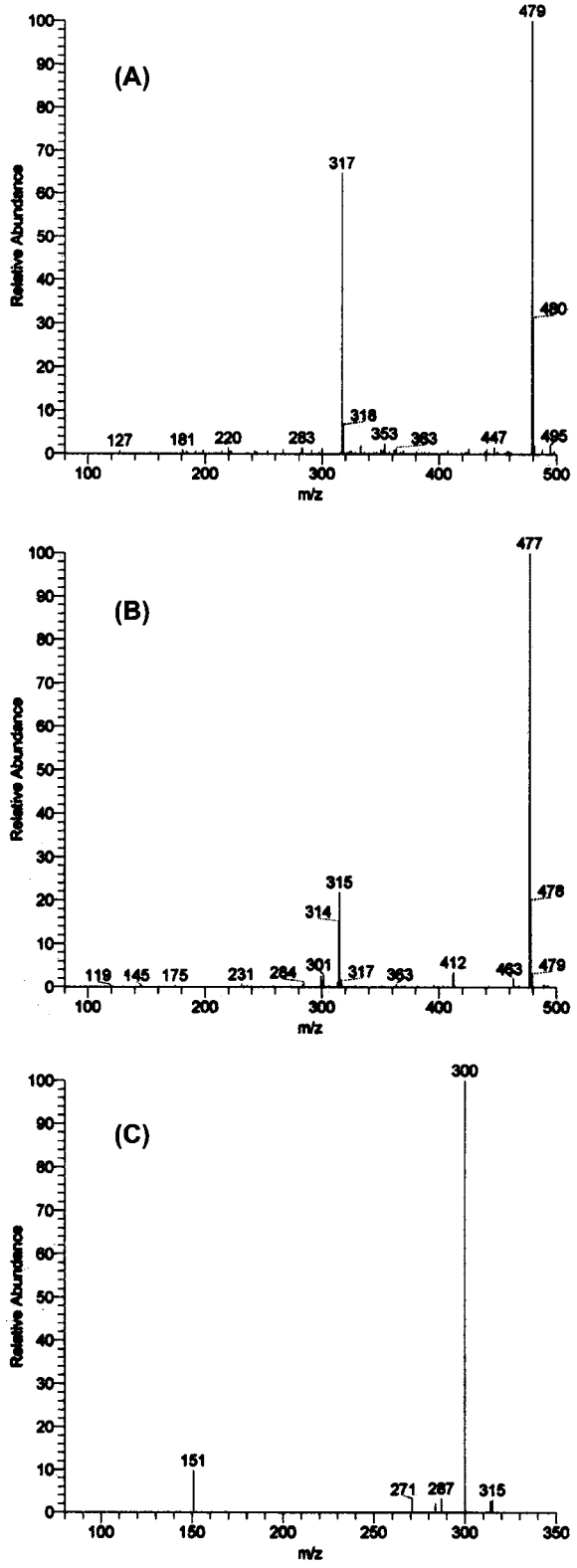


Figure 7. ESI–MS spectra in (A) positive and (B) negative ion mode and (C) negative ESI–MS–MS spectrum of component **7** (isorhamnetin 4′-glucoside).

array UV spectrum exhibited two major absorption peaks, band II at 256 nm and band I at 351 nm, with an hypsochromic shift (19 nm) of band I with respect to values for the aglycone, quercetin (**Figure 3E**,**H**). The hypsochromic shift to shorter wavelengths is normally associated with the substitution of one or more hydroxyl groups in the 3, 4′, or 5 positions of the flavone or flavonol skeleton. Substitution of other hydroxyl groups, such as the 7-hydroxyl group in the A-ring, has no effect on the UV spectrum. The shift associated with the substitution of the 5-hydroxyl group is usually of 5−15 nm in both band I and band II (*18*). In the specific case of component **5**, the hypsochromic shift was associated exclusively with band I, thus excluding substitution of the 5-hydroxyl group. On the other

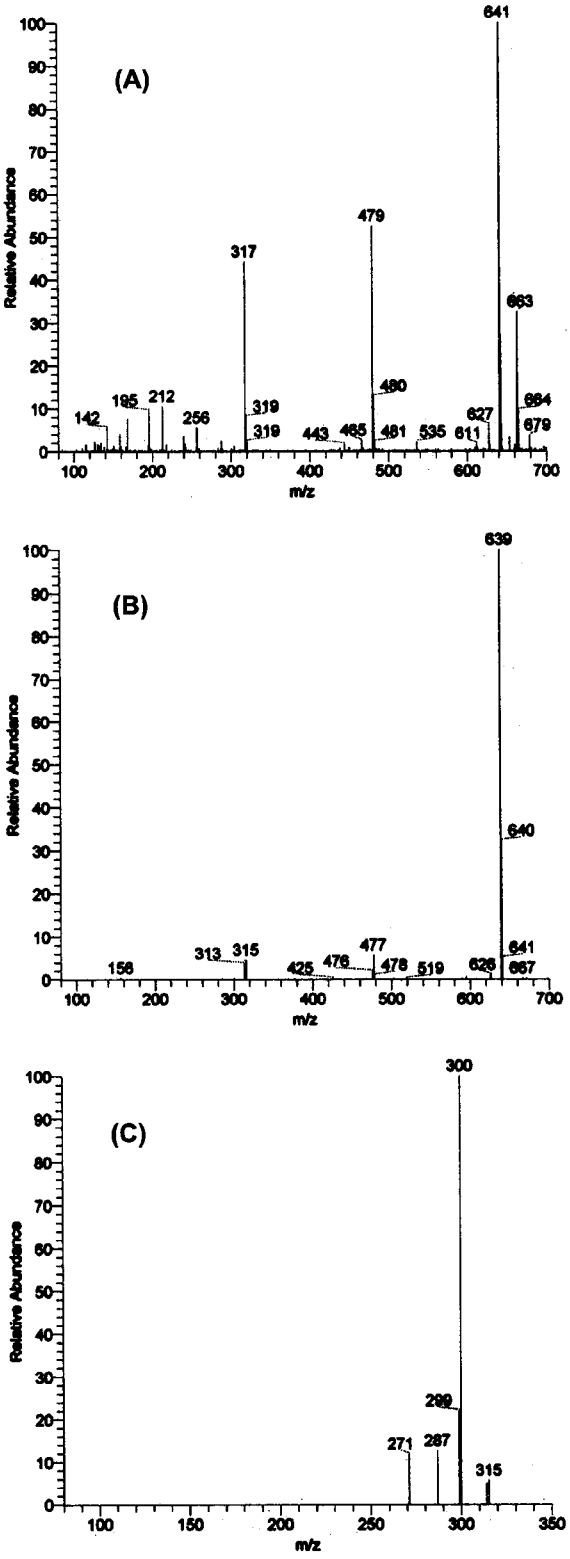


Figure 8. ESI–MS spectra in (A) positive and (B) negative ion mode and (C) negative ESI–MS–MS spectrum of component **4** (isorhamnetin 3,4′-diglucoside).

hand, the substituted 4′ position is characterized by a hypsochromic shift of 3−10 nm, a value lower than the one found for component **5.** The hypsochromic shift of 19 nm appeared in accordance with the hypothesis of a quercetin glycosylated at the 3 position of the aglycone skeleton. We have evidence for component **5** to be quercetin 3-glucoside (**Figure 1**), in accordance with previous results (*11*).

ESI−MS in positive mode of component **3** produced a strong peak centered at *m*/*z* 627 $[M + H]^+$ and two fragments at *m*/*z* 465 $[M + H - 162]^+$ and at *m*/*z* 303 $[M + H - 162 - 162]^+$. This fragmentation emphasized the diglycoside nature of compound **3**. ESI−MS−MS fragmentation was superimposable with those of components **5** and **6** and was previously reported for MS−MS degradation of quercetin (*19*). Component **3** presented an UV absorption peak centered at 255 nm (band II) with a shoulder at 265 nm and a peak at 344 nm (band I) (**Figure 3C**). A comparison of these values with those proposed for the aglycone quercetin (**Figure 3H**) showed a consistent hypsochromic shift (26 nm) of band I in component **3**. Unchanged values of band II excluded a substitution of the 5-hydroxyl group leading to the attribution of quercetin 3,4′-diglucoside to component **3** (**Figure 1**). Quercetin 3,4′-diglucoside has already been detected in red onions and regarded as one of the major flavonoids (*7*).

In positive and negative ESI−MS spectra (**Figure 5A,B**), component **2** exhibited a fragmentation typical of a diglycoside flavonoid. Negative ESI−MS−MS on the ion at *m*/*z* 301 provided fragments that are characteristic of quercetin aglycone (*19*). UV absorption values of component **2** showed a hypsochromic shift (4 nm) of band I with respect to values of the aglycone quercetin (**Figure 3B**,**H**). The shift associated with substitution of the 3-hydroxyl group is usually of 12−17 nm in band I, and the value found for component **2** (366 nm) excluded glycosyl substitution at the 3 position of the C-ring. On the other hand, the hypsochromic shift observed for component **2** was associated exclusively with band I, thus indicating that the 5-hydroxyl group was not substituted. We could then assume for component **2** a glycosyl substitution onto the 4′ position of the B-ring and the other one onto the 7 position of the A-ring. Quercetin 7,4′-diglucoside, which we identified as component **2**, has been already detected as a secondary constituent of flavonoids in red onions (*20*).

Component **1**, the first eluted (**Figure 2**), exhibited an ESI−MS spectrum in the positive ion mode with four significant peaks, one centered at *m*/*z* 789 $[M + H]^+$, the second at *m*/*z* 627 $[M + H - 162]^+$, the third at *m*/*z* 465 $[M + H - 162 - 162]^+$, and the last one at *m*/*z* 303 $[M + H - 162 - 162 - 162]^+$ (**Figure 6A**). Four analogous peaks were produced in the negative ESI−MS analysis (**Figure 6B**). The loss of three glycosyl units shown in the ESI−MS spectra and the fragmentation pattern shown in the negative ESI−MS−MS analysis on the ion at *m*/*z* 301 (**Figure 6C**) were indicative of a quercetin triglycoside. The hypsochromic shift (26 nm) of band I with respect to the quercetin value (**Figure 3A**,**H**), observed in the UV spectrum of component **1**, excluded substitution onto the 5-position of the A-ring, leading to the conclusion that the first eluted compound was quercetin 3,7,4′-triglucoside (**Figure 1**) (*7*).

Figure 7 shows the MS spectra of component **7**, which was eluted just after quercetin-4′-glucoside (**Figure 2**). The positive fragmentation pattern, ions at *m*/*z* 479 $[M + H]^+$ and *m*/*z* 317 $[M + H - 162]^+$, indicated the loss of a glycosyl unit, corroborated by mass spectrometric results in negative ion mode (*m*/*z* 477 $[M - H]^-$ and *m*/*z* 315 $[M - H - 162]^-$) (**Figure 7A,B**). Component **7** is, indeed, a flavonoid monoglycoside. The UV absorptions (**Figure 3G**) at 252 nm for band II, and at 366 nm for band I, confirmed its nature as a flavonol derivative. The hypsochromic shift of 5 nm of band I, with respect to values of quercetin (**Figure 3H)**, excluded a glycosyl substitution at the 3, 5, and 7 positions of aglycone, leading to the hypothesis of a 4′-substituted flavonol. ESI−MS−MS spectrum on the ion

Table 1. Range Values in mg/kg Fresh Weight of Flavonol Glucosides in Southern Italian Red Onions

compound	flavonol glucosides	content (mg/kg)
1	quercetin 3,7,4′-triglucoside[a]	6–9
2	quercetin 7,4′-diglucoside[a]	7–11
3	quercetin 3,4′-diglucoside[a]	254–274
4	isorhamnetin 3,4′-diglucoside[a]	21–25
5	quercetin 3-glucoside[a]	17–23
6	quercetin 4′-glucoside	208–230
7	isorhamnetin 4′-glucoside[a]	41–49

[a] Expressed as quercetin 4′-glucoside.

at *m*/*z* 315 (**Figure 7C**) exhibited an intense peak (100%) at *m*/*z* 300 $[M - H - 15]^-$ originating from the loss of a methyl moiety, followed by small but significant peaks at *m*/*z* 287, 271, and 151. Peaks at *m*/*z* 287 and 271 are associated with the neutral losses of CO and CO_2, involving the C-ring, and the relative fragments have already been observed in flavonols such as quercetin (*19*). The ion at *m*/*z* 151 can be regarded as a fragment coming from a process of retrocyclization of the aglycone as anion (*m*/*z* 315), followed by the loss of CO, or otherwise, from a retro Diels−Alder process, involving the anion flavonol of the isorhamnetin type (*19*). We observed a similar fragmentation pattern in the ESI−MS−MS spectrum of the standard isorhamnetin that we have used to identify its presence in the hydrolyzed extracts of samples under study. All these results allowed us to attribute the structure of isorhamnetin 4′-glucoside (**Figure 1**) to component **7**.

On-line positive ESI−MS of component **4**, shown in **Figure 8A**, exhibited a spectrum with three significant peaks, one centered at *m*/*z* 641 $[M + H]^+$, the second at *m*/*z* 479 $[M + H - 162]^+$, and the third at *m*/*z* 317 $[M + H - 162 - 162]^+$. The loss of two glycosyl units clearly indicated the flavonoid diglycoside nature of component **4**. Analysis of the negative MS−MS on the ion at *m*/*z* 315 and comparison with the fragmentation of the isorhamnetin standard demonstrated that component **4** is an isorhamnetin diglycoside. UV absorption at 251 nm, 266 nm (band II), and 344 nm (band I) (**Figure 3D**) led to the attribution of the two substituents in the 3 and 4′ positions of aglycone, as discussed for component **3**. Accordingly with these results, we propose component **4** to be isorhamnetin-3,4′-diglucoside (**Figure 1**). Isorhamnetin-3,4′-diglucoside has been isolated from *Zea mays* pollen (*21*), and its identification has been determined by UV and NMR spectra and characterization of its hydrolysis products. Some cultivars of *Crocus chrysanthus-biflorus* (Iridaceae) have also shown the presence of isorhamnetin 3,4′-diglucoside, which has been isolated by HPLC and recognized by UV, FAB−MS, and ^{1}H NMR (*22*). Recently, an extensive phytochemical analysis of the polar extracts from bulbs of shallot (*A. ascalonicum* Hort.) led to the isolation, in high concentration, of isorhamnetin 3,4′-diglucoside (*23*). Finally, the very small peaks with retention times of 22.13 and 26.72 min, respectively, have been identified as isorhamnetin 3-glucoside and free quercetin by comparison with their standards.

Contents of flavonols in red onions under study are reported in **Table 1** and are expressed as quercetin 4′-glucoside. The two major components, quercetin 4′-glucoside and quercetin 3,4′-diglucoside, ranged from 208 to 230 mg/kg and from 254 to 274 mg/kg fresh weight, respectively. These values together with the total amount of flavonols, which range between 554 and 621 mg/kg, are in good agreement with values reported in the literature (*7*, *10*, *12*, *15*, *16*). Among minor components, which represent about 18% of the total amount of flavonols, isorhamnetin 4′-glucoside is the most consistent (41−49 mg/kg), followed by isorhamnetin 3,4′-diglucoside, quercetin 3-glucoside, quercetin 7,4′-diglucoside, and quercetin 3,7,4′-triglucoside.

In conclusion, we have demonstrated that the on-line HPLC−DAD ESI−MS−MS technique constitutes an accurate and easy methodology for the qualitative and quantitative analysis of flavonoids in *A. cepa* varieties. It allowed the identification of seven flavonols in southern Italian red onion, quercetin 4′-glucoside and quercetin 3,4′-diglucoside being the most abundant components. We have recognized five minor flavonols among which quercetin 3-glucoside, quercetin 7,4′-diglucoside, quercetin 3,7,4′-triglucoside, and isorhamnetin 4′-glucoside have been previously reported as minor flavonol components in *A. cepa*, while to the best of our knowledge, isorhamnetin 3,4′-diglucoside was previously found in *A. ascalonicum*. These results are relevant not only from a chromatographic point of view but also for the possibility of offering a characteristic profile of flavonoids in red onions under study and more generally in vegetables that are rich in these significant compounds.

LITERATURE CITED

(1) Block, E. The organosulfur chemistry of the genus *Allium*—Implications for the organic chemistry of sulfur. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1135−1178.

(2) Xiao, H.; Parkin, K. L. Antioxidant Functions of Selected Allium thiosulfinates and *S*-alk(en)yl-L-cysteine sulfoxides. *J. Agric. Food Chem.* **2002**, *50*, 2488−2493.

(3) Higuchi, O.; Tateshita, K.; Nishimura, H. Antioxidative activity of sulfur-containing compounds in *Allium* species for human low-density lipoprotein (LDL) oxidation in vitro. *J. Agric. Food Chem.* **2003**, *51*, 7208−7214.

(4) Yin, M. C.; Cheng, W. S. Antioxidant activity of several *Allium* members. *J. Agric. Food Chem.* **1998**, *46*, 4097−4101.

(5) Nuutila, A. M.; Puupponen-Pimia, R.; Aarni, M.; Oksman-Caldentey, K. M. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.* **2003**, *81*, 485−493.

(6) Sellappan, S.; Akoh, C. C. Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *J. Agric. Food Chem.* **2002**, *50*, 5338−5342.

(7) Fossen, T.; Pedersen, A. T.; Andersen, Ø. M. Flavonoids from red onion (*Allium cepa*). *Phytochemistry* **1998**, *47*, 281−285.

(8) Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992**, *40*, 1591−1598.

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

(10) Rhodes, M. J.; Price, K. R. Analytical problems in the study of flavonoid compounds in onions. *Food Chem.* **1996**, *57*, 113−117.

(11) Brandwein, B. J. The pigments in three cultivars of the common onion (*Allium cepa*). *J. Food Sci.* **1965**, *30*, 680−685.

(12) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.

(13) Price, K. R.; Bacon J. R.; Rhodes, J. C. Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J. Agric. Food Chem.* **1997**, *45*, 938−942.

(14) Ewald, C.; Fjelkner-Modig, S.; Johansson, K.; Sjoholm, I.; Akesson, B. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chem*. **1999**, *64*, 231−235.
(15) Makris, D. P.; Rossiter, J. T. Domestic processing of onion bulb (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): Effect on flavonol content and antioxidant status. *J. Agric. Food Chem.* **2001**, *49*, 3216−3222.
(16) Gennaro, L.; Leonardi, C.; Esposito, F.; Salucci, M.; Maiani, G.; Quaglia, G.; Fogliano, V. Flavonoid and carbohydrate contents in Tropea red onions: Effects of homelike peeling and storage. *J. Agric. Food Chem.* **2002**, *50*, 1904−1910.
(17) Park, Y.-K.; Lee, C. Y. Identification of isorhamnetin 4′-glucoside in onions. *J. Agric. Food Chem.* **1996**, *44*, 34−36.
(18) Mabry, T. J.; Markham, K. R.; Thomas, M. B. The ultraviolet spectra of flavones and flavonols. In *The systematic identification of flavonoids*; Springer-Verlag: Berlin, 1970; pp 41−164.
(19) Fabre, N.; Rustan, I.; de Hoffmann, E.; Quetin-Leclercq, J. Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 707−715.
(20) Harborne, J. B.; Baxter, H. In *The handbook of Natural Flavonoids*; John Wiley and Sons: Chichester, England, 1999; Vol. 1, p 373.
(21) Ceska, O.; Styles, E. D. Flavonoids from *Zea mays* pollen. *Phytochemistry* **1984**, *23*, 1822−1823.
(22) Nørbæk, R.; Nielsen, J. K.; Kondo, T. Flavonoids from flowers of two *Crocus chrysanthus-biflorus* cultivars: "Eye-catcher" and "Spring Pearl" (Iridaceae). *Phytochemistry* **1999**, *51*, 1139−1146.
(23) Fattorusso, E.; Iorizzi, M.; Lanzotti, V.; Taglialatela-Scafati, O. Chemical composition of shallot (*Allium ascalonicum* Hortic.). *J. Agric. Food Chem.* **2002**, *50*, 5686−5690.

Received for review November 5, 2004. Revised manuscript received January 14, 2005. Accepted February 2, 2005.

JF048152R