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Flavone-di-*C*-glycosides in *citrus* juices from Southern Italy

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Abstract

6,8-Di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin were detected by HPLC–DAD–ESI–MS–MS in a variety of Southern Italian *Citrus* juices (orange, lemon, bergamot, citron, mandarin, clementine). In some juices, the amounts of *C*-glycosides detected were significant. 6,8-Di-*C*-glucopyranosylapigenin is characteristic of orange juice, while 6,8-di-*C*-glucopyranosyldiosmetin is the most important *C*-glycoside in lemon and citron juice. In bergamot juice the concentrations of 6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin are similar. Clementine juice is distinctive as the amounts of both *C*-glycosides present are negligible.

Keywords: *Citrus* juices; Flavonoids; *C*-glycosides; HPLC–DAD–ESI–MS–MS

1. Introduction

Flavonoids constitute one of the most important groups of naturally occurring phenols that are beneficial, to plants as protective agents (Arena, Fallico, & Maccarone, 2001; Harborne & Williams, 2000; Robards, Prenzler, Tucker, Swatsitang, & Glaver, 1999) and to human health (Benavente-García, Castillo, Marin, Ortuño, & Del Río, 1997; Kurowska et al., 2000).

Their profile in fruits is characteristic of the different species and depends on the cultivar, the degree of ripeness and the pedoclimatic environment, and presents a typical pattern in various parts of the same fruit. Flavonoids found in different parts of *Citrus* fruits usually occur as glycosides, flavanone or flavone-*O*-glycosides. Polymethoxylated flavones are also present (Bocco, Cuvelier, Richard, & Berset, 1998; Leuzzi, Caristi, Panzera, & Licandro, 2000; Ooghe, Ooghe, Detavernier, & Huyghebaert, 1994; Robards & Antolovich, 1997).

Naringin and narirutin, together with small amounts of hesperidin and neohesperidin, characterize grapefruit species (Rouseff, Martin, & Youtsey, 1987); naringin, neohesperidin and, to some extent, neoeriocitrin are distinctive of bitter orange (Marini & Balestrieri, 1995); hesperidin, eriocitrin, and diosmin are present in lemon juice (Caristi et al., 2003; Miyake, Yamamoto, Morimitsu, & Osawa, 1997); hesperidin, narirutin and small amounts of didymin are present in sweet orange (Leuzzi et al., 2000; Mouly, Gaydou, Faure, & Estienne, 1997) together with traces of luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, quercetin 3-*O*-rutinoside, narirutin-4′-glucoside and neoponcirin (Robards, Li, Antolovich, & Boyd, 1997). Bergamot is a natural hybrid fruit from bitter orange and lemon which is produced exclusively in the region of Reggio Calabria (Italy). Studies have focused on its essential oil as this constitutes a raw material for the perfume and food industries. However, the presence of neoriesperidin, naringin, and neoeriocitrin in its juice has also been reported (Gionfriddo, Postorino, & Bovalo, 1996; Kawaii, Tomono, Katase, Ogawa, & Yano, 1999).

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An analytical differentiation of *Citrus* juices, based on the differing concentrations of certain minor components, including flavonoids, plays an important role in determining chemotaxonomic markers to ascertain the authenticity of commercial products (Hammond, 1996). Investigations in this field are performed more and more by LC–MS analysis using a diode array detector (Hakkinen & Auriola, 1998; He, 2000; Hedin & Phillips, 1992; Hughes, Croley, Metcalfe, & March, 2001; Pupin, Dennis, & Toledo, 1998; Stobiecki, 2000; Swatsitang, Tucker, Robards, & Jardine, 2000; Waridel et al., 2001).

In a recent paper we identified a di-*C*-glycoside of diosmetin as a novel component in the lemon juice of various Sicilian cultivars (*Femminello comune*, *Monachello*, and *Interdonato)* by means of HPLC–DAD–ESI–MS–MS where the structure was assigned by ^{1}H NMR spectra analysis (Caristi et al., 2003). Previously, a flavone-*C*-glycoside was reported to be present in the juices (Gil-Izquierdo, Gil, Ferreres, & Thomás-Barberán, 2001; Ogawa et al., 2001) but a more extensive investigation of the presence of *C*-glycosides in juices from different *Citrus* species is not available in the literature.

The aim of the present paper is to evaluate the flavone-di-*C*-glycoside content in the juice from cultivars of various *Citrus* species (orange, lemon, bergamot, citron, mandarin and clementine) produced in Southern Italy by direct analysis of unprocessed juice, employing HPLC–DAD–ESI–MS–MS techniques in order to highlight the importance of the flavone-di-*C*-glycosides as characteristic minor components of the juices of different *Citrus* species.

2. Materials and methods

2.1. Materials

The hand-squeezed juices of cultivars were obtained from commercially ripe fruits grown in different areas of Sicily – Italy (orange (*Citrus sinensis*) *Tarocco* and *Moro;* lemon (*Citrus lemon*) *F. comune*, *Monachello*, *Interdonato*; citron (*Citrus medico*) *Diamante*; mandarin (*Citrus deliciosa*) *Avana*; clementine (*Citrus clementine*) *Comune*) and, in Reggio Calabria, (bergamot (*Citrus bergamia*) *Fantastico*, *Femminello* and *Castagnaro*). Industrially processed bergamot juice was handled by the Consorzio del Bergamotto in Reggio Calabria The study was carried out using 10 juice samples of each of the cultivars obtained during the 2002–2003 *Citrus* fruit season. Each juice was obtained from 20 squeezed fruits.

2.2. Reagents and standard solutions

HPLC-grade acetonitrile and acetic acid were supplied by Sigma–Aldrich, dimethylformamide (DMF) by Carlo Erba 6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin were separated from orange and citron juice, respectively, as described in the preparative HPLC paragraph below and were used as standards. The calibration lines were obtained using methanol solutions of known concentration (10–100 mg/l).

2.3. Preparation of samples

The peeled fruits were carefully squeezed by hand to avoid the juices being contaminated by components in the peel. DMF (10.0 ml) was added to the juice samples (10.0 ml) and the resulting solutions were filtered through ISO-DISC P-34, 3 mm diameter PTFE membrane, 0.45 μm pore size. The peels from bergamot cultivars (20.0 g) were extracted in methanol (100 ml). The mixtures were stirred for 2 h at room temperature and centrifuged. DMF (10.0 ml) was added to the samples of surnatant liquid (10.0 ml) and the resulting solutions were filtered through ISO-DISC P-34, 3 mm diameter PTFE membrane, 0.45 μm pore size.

2.4. Liquid chromatography–mass spectrometry

HPLC–MS analyses of flavonoids were carried out on a ThermoQuest Model ECQ-DUO ion trap mass spectrometer with electrospray ionization source (ESI) equipped with a diode array to perform a MS–MS analysis. HPLC with gradient elution was used: detection by diode array was performed at 325 nm. The UV spectra were recorded between 200 and 450 nm. Analytical conditions were identical to those described in previously published work (Caristi et al., 2003). Each sample was tested three times and gave superimposable chromatograms.

2.5. Preparative HPLC

Preparative high-performance liquid chromatography, with isocratic elution, was earned out using an HPLC instrument (Shimadzu Model LC 8 A) equipped with a UV detector. The column used was Discovery C18 Supelco 250 × 21.2 mm, particle size 5 μm. The injection loop was 1.0 ml and the flow-rate was 10.0 ml/min at room temperature (20 °C). The mobile phase consisted of water (80%) and acetonitrile (20%). 6,8-Di-*C*-glucopyranosylapigenin was eluted at RT 7.9 min from *Moro* orange juice and collected (about 4 ml) in RT 7.8–8.0 min. The combined fractions were rotavapor-dried at room temperature. The residue was treated overnight with P_2O_5 in a vacuum dryer and then weighed (7.5 mg). 6,8-di-*C*-glucopyranosyldiosmetin was eluted at RT 8.65 min. from citron juice and collected (about 5 ml) in RT 8.3–8.8 min. The combined fractions were rotavapor-dried at room temperature. The residue was treated overnight with P_2O_5 in a vacuum dryer and then weighed (6.0 mg).

2.6. *Acid hydrolysis*

Ten milliliters of 6 M HC1 in a methanol (25 ml)/water (10 ml) mixture were added to 5 ml of each juice to give a 50 ml solution of 1.2 M HC1 in 50% aqueous methanol. The solution was refluxed at 90 °C for 2 h. After cooling, the solution was dried under vacuum at room temperature. The residue was diluted with 5 ml of water/DMF, 1:1, and analyzed by HPLC.

2.7. ^{1}*H NMR*

^{1}H spectra were recorded in dimethylsulfoxide (DMSO), containing tetramethylsilane as internal standard, using a Varian Gemini 300 spectrometer at 300 MHz.

3. Results and discussion

3.1. *General*

The crude hand-squeezed *Citrus* juices investigated were analyzed by HPLC–DAD–ESI–MS–MS technique. Fig. 1 shows the DAD chromatograms of *Citrus* juices at 325 nm resulting from a 17 min HPLC run. Peaks numbered 1 and 2 are present in all chromatograms.

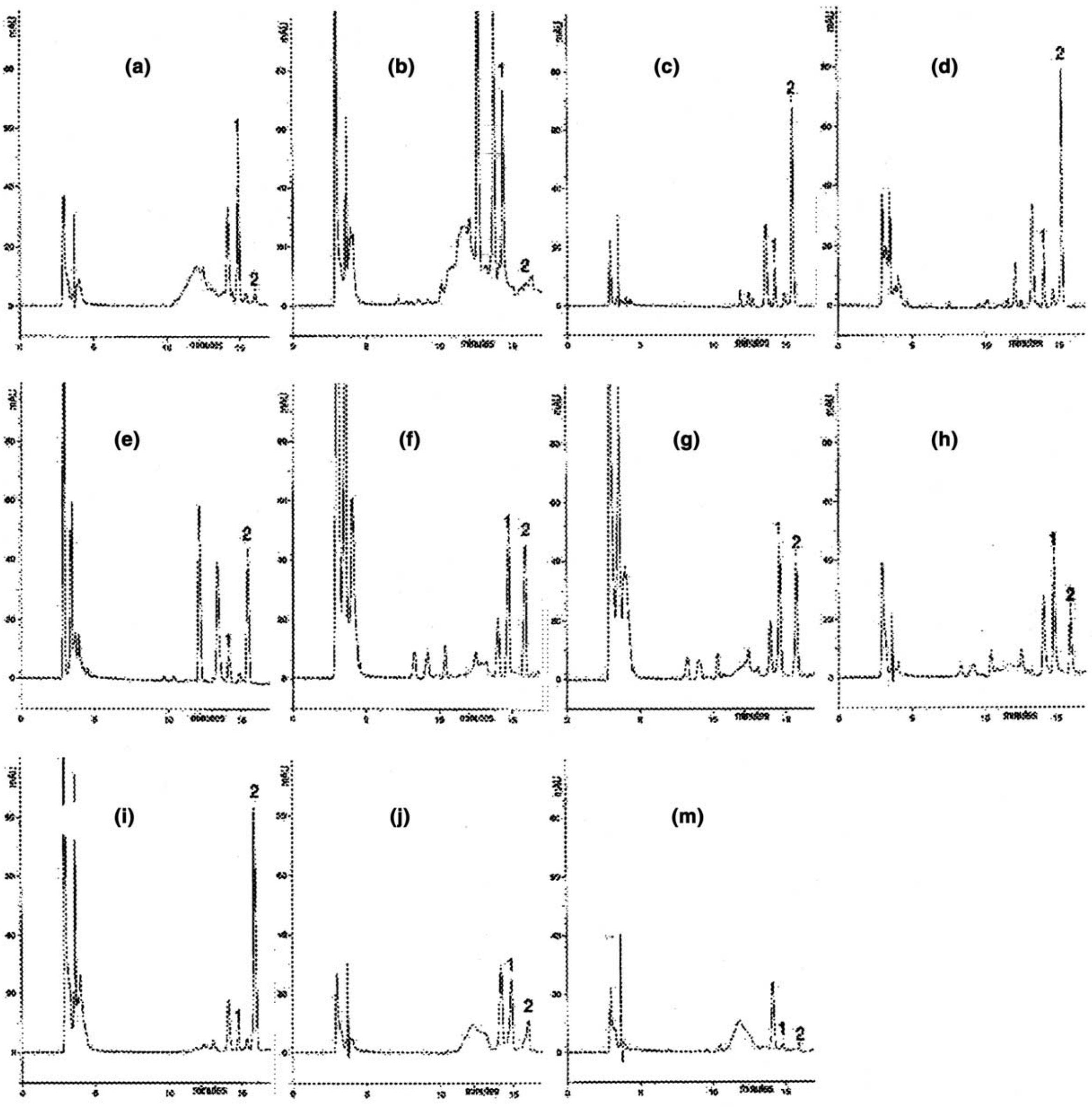

Fig. 1. Typical chromatograms of *Citrus* juices at 325 nm: (a) orange, *Tarocco*; (b) orange, *Moro*; (c) lemon, *Femminello commune*; (d) lemon, *Monachello*; (e) lemon, *Interdonato*; (f) bergamot, *Fantastico*; (g) bergamot, *Femminello*; (h) bergamot, *Castagnaro*; (i) citron; (j) mandarin; (m) clementine. Peak 1: 6,8-di-*C*-glucopyranosylapigenin; Peak 2: 6,8-di-*C*-glucopyranosyldiosmetin.

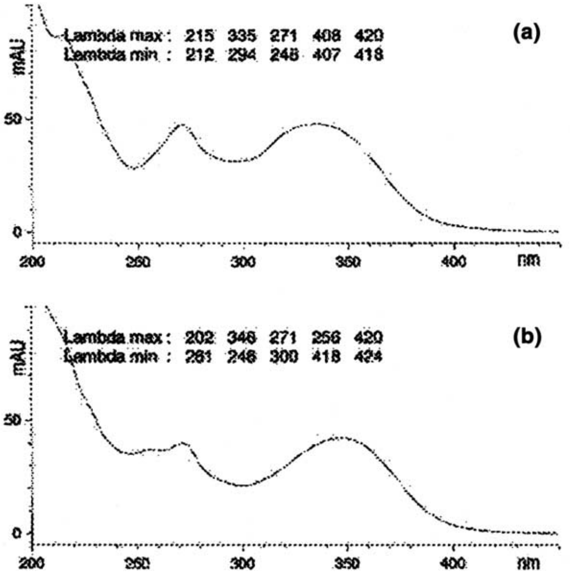

Fig. 2. UV spectra recorded in correspondence with peak 1 (a) and peak 2 (b).

The UV spectra, recorded in correspondence with peaks 1 and 2, showed absorptions centred at 271 and 335 nm (peak 1) and 271 and 346 nm (peak 2) which can be attributed to bands II and I, respectively, of a flavone structure. Typical UV spectra are shown in Fig. 2 for the components related to peak 1 and 2.

The MS spectra recorded in correspondence with peak 1 show a molecular $[M-H]^-$ peak centred at *m*/*z* 593 in all chromatograms, and the ESI–MS–MS spectrum shows fragments generated by typical losses of 18, 60, 90 and 120 mass units from the $[M-H]^-$ ion of a *C*-glycoside (Fig. 3(a)). In fact, the fragmentation related to a sugar moiety rather than the loss of a whole sugar unit as in the *O*-glycosides. The compound corresponding to peak 1 was obtained from juice of the *Moro* orange by means of preparative HPLC.

The ^{1}H NMR spectrum presented characteristic resonance of aromatic protons of ring B [8.02 δ (2H, d, $J_{2',6'} = 8.8$ Hz, H-2′ and H-6′); 6.89 δ (2H, d $J_{3',5'} = 8.8$ Hz, H-3′ and H-5′)], and the H-3 proton appeared as a singlet centred at 6.78 δ. The two anomeric protons of both glucose units appeared as a based singlet centred at 4.93 δ. Both UV and ^{1}H NMR data are superimposable with those previously reported for 6,8-di-*C*-glucopyranosylapigenin (Fig. 4) isolated from *Navel* oranges (Gil-Izquierdo et al., 2001).

DAD–UV, MS and MS/MS spectra (Fig. 3(b)), recorded on one chromatographic run of each crude juice in correspondence with peak 2, are highly consistent with the spectral data of 6,8-di-*C*-glucopyranosyldiosmetin (Fig. 4). Furthermore, the compound corresponding to peak 2 was obtained from *citron* juice by means of preparative HPLC. The ^{1}H NMR spectrum was superimposable on the those previously reported

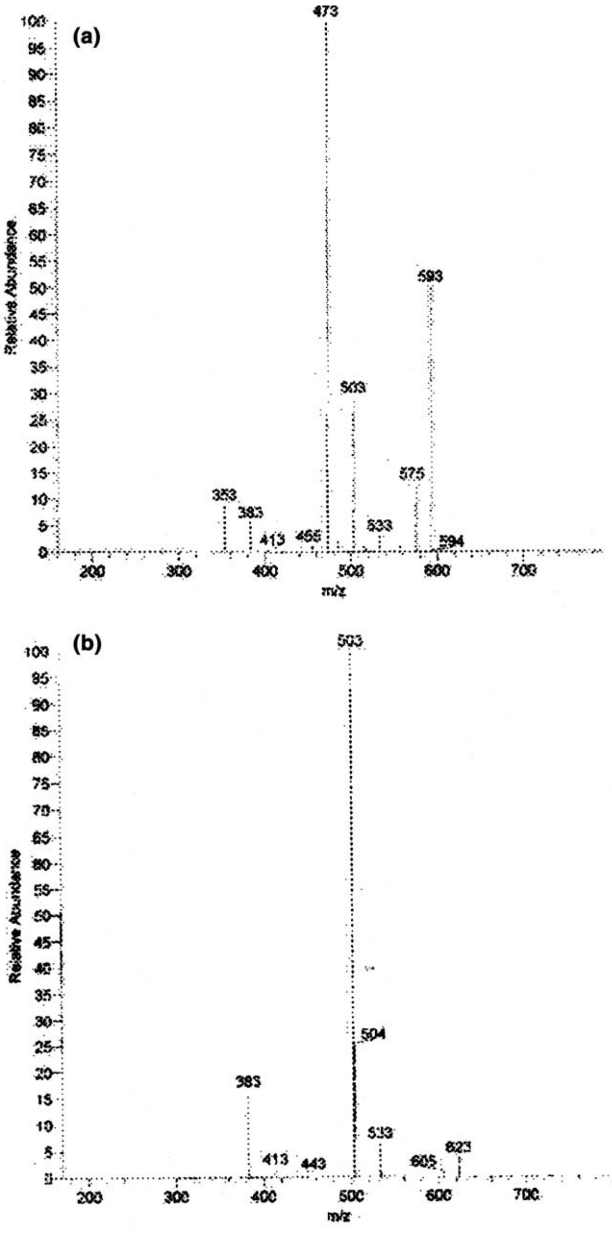

Fig. 3. ESI–MS–MS spectrum recorded in correspondence with peak 1 (a) and peak 2 (b).

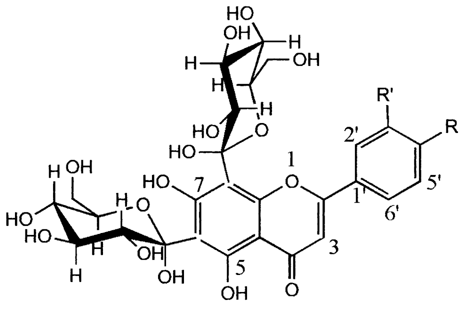

Fig. 4. *C*-Glycosidestructures. 6,8-di-*C*-glucopyranosylapigenin R = OH R′ = H 6,6-di-*C*-glucopyranosyldiosmetin R = OMe R′ = OH.

for 6,8-di-*C*-glucopyranosyldiosmetin (Caristi et al., 2003).

In order to confirm the nature of *O*-sugar or *C*-sugar bonds in the two glycosides from which the above mentioned peaks 1 and 2 originate, each juice was refluxed with an HCl methanol solution for 2 h. The resultant hydrolyzed juices were then analyzed by HPLC–DAD–ESI–MS–MS under the same experimental conditions as the corresponding crude starting materials. Because both peaks 1 and 2 remain unaltered, only C-sugar bonds may be claimed to be occurring in the two glycosides (6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin) detected in all the juices investigated.

As an example, Fig. 5 compares the chromatograms of the crude juice of *F. comune* lemon before and after hydrolysis. All peaks previously attributed to the *O*-glycosy-flavonoids (Caristi et al., 2003) disappeared while peaks 1 and 2 appeared to be hydrolysis-resistant.

The two *C*-glycosides, obtained as described above, were raised as standards in order to quantify, via the calibration curves described in the experimental section, the respective amounts of 6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin present in the different *Citrus* Juices.

Table 1 shows the amounts detected. In particular, orange juices are rich in 6,8-*C*-glucopyranosylapigenin while 6,8-di-*C*-glucopyranosyldiosmetin is only present

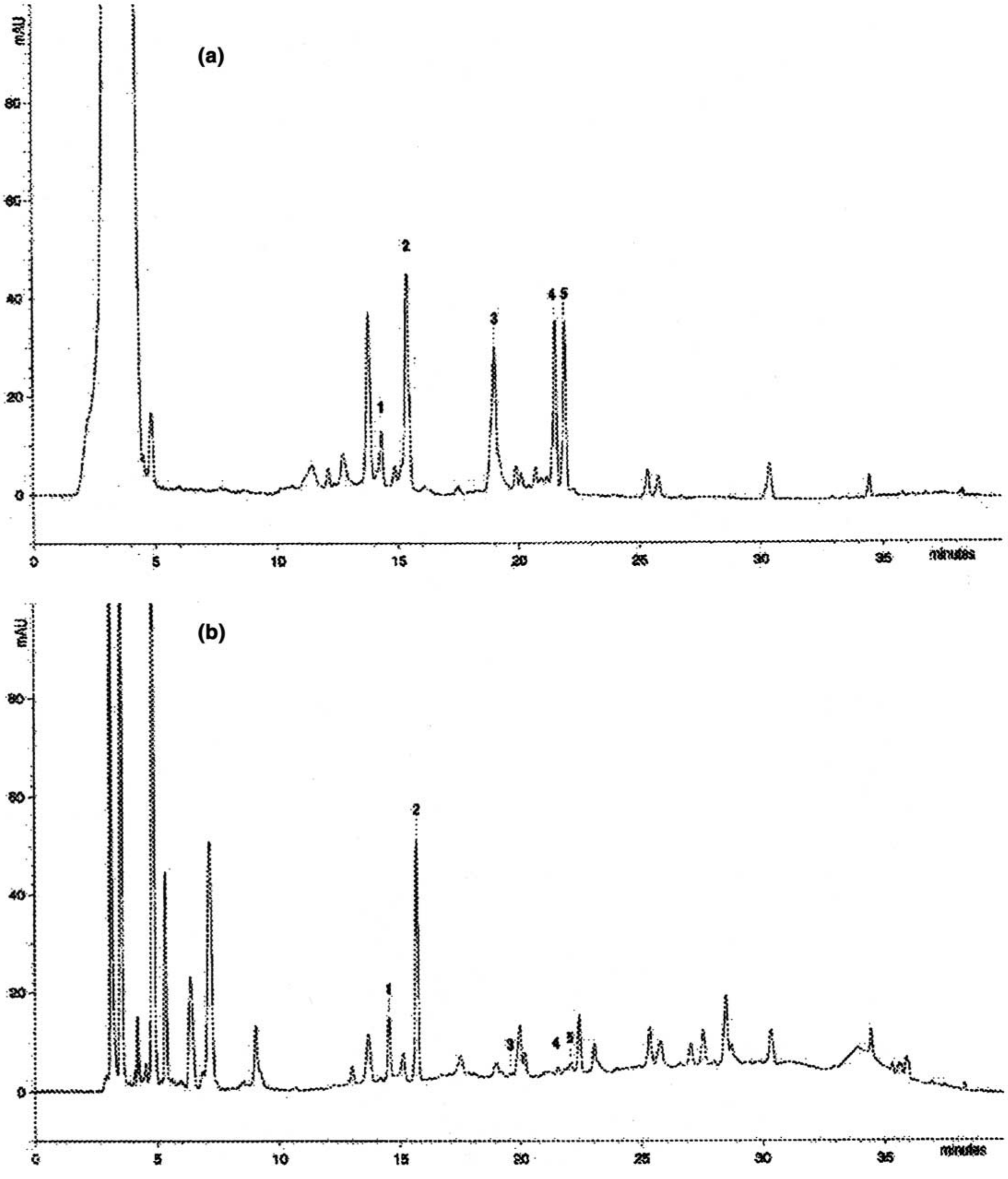

Fig. 5. Chromatograms, at 325 nm, of crude lemon juice (a) and after acid hydrolysis (b). 1 – 6,8-di-*C*-glucopyranosylapigenin; 2 – 6,8-di-*C*-glucopyranosyldiosmetin; 3 – eriocitrin; 4 – diosmin; 5 – hesperidin.

Table 1
Di-*C*-glycoside contents (mg/l) as range values of *Citrus* juices

	6,8-Di-*C*-glucopyranosylapigenin	6,8-Di-*C*-glucopyranosyldiosmetin
Tarocco[a]	39–44	4–5
Moro[a]	47–53	2–3
Femminello comune[b]	9–11	48–52
Monachello[b]	13–16	56–60
Interdonato[b]	9–12	36–45
Fantastico[c]	48–55	44–48
Femminello[c]	42–47	37–44
Castagnaro[c]	38–42	29–35
Diamante[d]	6–8	61–68
Avana[e]	23–27	6–8
Comune[f]	4–6	1–3

[a] *Citrus sinensis.*
[b] *C. lemon.*
[c] *C. bergamia.*
[d] *C. medica.*
[e] *C. deliciosa.*
[f] *C. clementine.*

in negligible amounts. In contrast, lemon juice shows an abundance of 6,8-di-*C*-glucopyranosyldiosmetin compared to 6,8-di-*C*-glueopyranosylapigenin.

Citron juice is characterized by a large amount of 6,8-di-*C*-glucopyranosyldiosmetin. Small amounts of the two *C*-glycosides were found in mandarin juice when compared with the other juices investigated and the concentration of 6,8-di-*C*-glucopyranosylapigenin was over three times more than that of 6,8-di-*C*-glucopyranosyldiosmetin. In clementine juice, the amounts of both *C*-glycosides were negligible.

All the Bergamot cultivars studied showed comparable levels of the two *C*-glycosides. This is probably due to the hybrid nature of this species, which originated as a cross between orange and lemon. We also investigated industrially processed bergamot juice. This juice presents an apparent anomaly because of the high content of 6,8-di-*C*-glucopyranosylapigenin. In fact, we found a 6,8-di-*C*-glucopyranosylapigenin content in the range of 70–76 mg/l in the different samples of industrially-produced juice while the concentrations of 6,8-di-*C*-glucopyranosyldiosmetin were in the range 44–49 mg/l. This can be attributed to the juice being polluted by peel components during the industrial juice preparation process. Indeed, we found a concentration of 6,8-di-*C*-glucopyranosylapigenin in bergamot peel of the cultivars *Fantastico*, *Femminello* and *Castagnaro* that was 2–2.5 times higher than that of 6,8-di-*C*-glucopyranosyldiosmetin.

4. Conclusion

The present paper demonstrates, for the first time, that 6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin are present in significant amounts in the juices of *Citrus* species grown in Southern Italy. In some juices, the amounts are comparable with the *O*-glycoside content. In fact, the concentration of 6,8-di-*C*-glucopyranosyldiosmetin (36–60 mg/l) in lemon juice was comparable with the diosmin content (32–67 mg/l) (Caristi et al., 2003), Pigmented orange juice is characterized by a large presence of 6,8-di-*C*-glucopyranosylapigenin (39–53 mg/l); the concentration of 6,8-di-*C*-glucopyranosylapigenin in orange juice is comparable with the narirutin (50-80 mg/l) and didymin (26–41 mg/l) concentrations (Leuzzi et al., 2000). From the above results it appears that 6,8-di-*C*-glucopyranosylapigenin may be considered a characteristic component of orange juices while 6,8-di-*C*-glucopyranosyldiosmetin characterizes lemon juices.

The concentrations of the two *C*-glycosides in citron juice indicate the proximity of the citron fruit to the lemon.

The role of *C*-glycosides is remarkable in demonstrating that bergamot cultivars are hybrids of lemon and orange, since the two *C*-glycosides in bergamot juices have similar concentrations of, 6,8-di-*C*-glucopyranosylapigenin and 6,8-di-*C*-glucopyranosyldiosmetin to those found in oranges and lemons, respectively.

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References

Arena, R., Fallico, B., & Maccarone, E. (2001). Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage. *Food Chemistry, 74*, 423–427.

Benavente-García, O., Castillo, I., Marin, F. R., Ortuño, A., & Del Río, J. A. (1997). Uses and properties of *Citrus* flavonoids: a review. *Journal of Agricultural and Food Chemistry, 45*, 4505–4515.

Bocco, A., Cuvelier, M.-E., Richard, H., & Berset, C. (1998). Antioxidant activity and phenolic composition of *Citrus* peel and seed extracts. *Journal of Agricultural and Food Chemistry, 46*, 2123–2129.

Caristi, C., Bellocco, E., Panzera, V., Toscano, G., Vadalá, R., & Leuzzi, U. (2003). Flavonoids detection by HPLC–DAD–MS–MS in lemon juices from Sicilian cultivars. *Journal of Agricultural and Food Chemistry, 51*, 3528–3534.

Gil-Izquierdo, A., Gil, M. I., Ferreres, F., & Thomás-Barberán, F. A. (2001). In vitro availability of flavonoids and other phenolics in orange juice. *Journal of Agricultural and Food Chemistry, 49*, 1035–1041.

Gionfriddo, F., Postorino, E., & Bovalo, F. (1996). I flavanoni glucosidici nel succo di bergamoto. *Essenze e Derivati Agrumari, 4*, 404–416.

Hakkinen, S., & Auriola, S. (1998). High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones glyeosides in berries. *Journal of Chromatography A, 829*, 91–100.

Hammond, D. A. (1996). Authenticity of fruit juices, jams and preserves. In P. R. Ashurst & M. J. Dennis (Eds.), *Food authentication* (pp. 32–36). London, UK: Chapman & Hall.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992: a review. *Phytochemistry, 55*, 481–504.

He, X. G. (2000). On-line identification of phytochemical constituents in botanical extracts by combined high-performance liquid chromatography-diode array detection-mass spectrometric techniques. *Journal of Chromatography A, 880*, 203–232.

Hedin, P. A., & Phillips, V. A. (1992). Electron impact mass spectral analysis of flavonoids. *Journal of Agricultural and Food Chemistry, 40*, 607–611.

Hughes, R. J., Croley, T. R., Metcalfe, C. D., & March, R. E. (2001). A tandem mass spectrometric study of selected characteristic flavonoids. *International Journal of Mass Spectrometry, 210/211*, 371–385.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., & Yano, M. (1999). Quantitation of flavonoid constituents in *Citrus* fruits. *Journal of Agricultural and Food Chemistry, 47*, 3565–3571.

Kurowska, E. M., Spence, J. D., Jordan, J., Wetmore, S., Freeman, D. J., Piché, L. A., et al. (2000). HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia. *American Journal of Clinical Nutrition, 72*, 1095–1100.

Leuzzi, U., Caristi, C., Panzera, V., & Licandro, G. (2000). Flavonoids in pigmented orange juice and second-pressure extracts. *Journal of Agricultural and Food Chemistry, 48*, 5501–5506.

Marini, D., & Balestrieri, F. (1995). Multivariate analysis of flavanone glycosides in citrus juices. *Italian Journal of Food Science, 3*, 255–264.

Miyake, Y., Yamamoto, K., Morimitsu, Y., & Osawa, T. (1997). Isolation of *C*-glucosylflavone from lemon peel and antioxidative activity of flavonoid compounds in lemon fruit. *Journal of Agricultural and Food Chemistry, 45*, 4619–4623.

Mouly, P. P., Gaydou, E. M., Faure, R., & Estienne, J. M. (1997). Blood orange juice authentication using cinnamic acid derivates. Variety differentiations associated with flavanone glycoside content. *Journal of Agricultural and Food Chemistry, 45*, 373–377.

Ogawa, K., Kawasaki, A., Omura, M., Yoshida, T., Yoshinori, I., & Yano, M. (2001). $3',5'$-Di-*C*-β-glucopyranosylphloretin, a flavonoid characteristic of the genus *Fortunella*. *Phytochemistry, 57*, 737–742.

Ooghe, W. C., Ooghe, S. J., Detavernier, C. M., & Huyghebaert, A. (1994). Characterization of orange juice (*Citrus sinensis*) by flavanone glycosides. *Journal of Agricultural and Food Chemistry, 42*, 2183–2190.

Pupin, A. M., Dennis, M. G., & Toledo, M. C. F. (1998). Flavanone glycosides in Brazilian orange juice. *Food Chemistry, 61*, 275–280.

Robards, K., & Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids: a review. *Analyst, 122*, 11R–34R.

Robards, K., Li, X., Antolovich, M., & Boyd, S. (1997). Characterization of citrus by chromatographic analysis of flavonoids. *Journal of the Science of Food and Agriculture, 75*, 87–101.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glaver, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Rouseff, Ri. L., Martin, S. F., & Youtsey, C. O. (1987). Quantitative survey of narirutin, narmgin, hesperidin and neohesperidin in *Citrus*. *Journal of Agricultural and Food Chemistry, 35*, 1027–1030.

Stobiecki, M. (2000). Application of mass spectrometry for identification and structural studies of flavonoid glycosides. *Phytochemistry, 54*, 237–256.

Swatsitang, P., Tucker, G., Robards, K., & Jardine, D. (2000). Isolation and identification of phenolic compounds in *Citrus sinensis*. *Analytica Chimica Acta, 417*, 231–240.

Waridel, P., Wolfender, J. L., Ndjoko, K., Hobby, K. R., Major, H. I., & Hostettmanan, K. (2001). Evaluation of quadrupole time-of-flight tandem mass spectrometry and ion-trab multiple-stage mass spectrometry for differentiation of *C*-glycosidic flavonoid isomers. *Journal of chromatography A, 926*, 29–41.