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Effect of the Rootstock and Interstock Grafted in Lemon Tree (*Citrus limon* (L.) Burm.) on the Flavonoid Content of Lemon Juice

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The grafting of the rootstock with the lemon tree is an agronomical technique used to improve production and/or quality of the fruit. The interstock has been used with different fruit trees to modulate the tree size, fruit production and quality, and the aging of the tree. The lemon trees grafted with interstocks increase their longevity, lemon production and quality; interstocks are also used to decrease the thickness of the trunk at the grafting point. This enlarging of the trunk provokes a decrease of the sap flow. In our study, "Verna" lemon trees were grafted with interstock between the rootstock and the lemon tree to follow the flavonoid content of the lemon juice. The lemon juice was obtained from the lemons collected of the grafted lemon trees. Two types of rootstocks were used: *Citrus aurantium* L. and *Citrus macrophylla* L. Seven interstocks from five cultivars of orange tree, one cultivar of lime tree, and one cultivar of tangerine tree were used. "Verna" lemon trees were also grafted directly to the rootstock. The rootstock was more important agronomic factor than the interstock on the total flavonoid content of lemon juice. The interstock grafting had only a small influence on the flavonoid content of the lemon juice, and it modulated the individual flavonoid content. *Citrus aurantium* L. rootstock and "Berna" and "Washington Navel" interstocks were the most appropriate to graft in the lemon tree. This interstock grafting technique does not increase the flavonoid content of the lemon juice. Regarding the individual flavonoids, the 6,8-di-*C*-glucosyl diosmetin was the most affected flavonoid by the type of rootstock used. The interstock used is able to alter the individual quantitative flavonoid order of eriocitrin, diosmin, and hesperidin. In addition, the HPLC-ESI/MS^n analyses provided the identification of two new flavonoids in the lemon juice: Quercetin 3-*O*-rutinoside-7-*O*-glucoside and chrysoeriol 6,8-di-*C*-glucoside (stellarin-2). The occurrence of apigenin 6,8-di-*C*-glucoside (vicenin-2), eriodictyol 7-*O*-rutinoside, 6,8-di-*C*-glucosyl diosmetin, hesperetin 7-*O*-rutinoside, homoeriodictyol 7-*O*-rutinoside and diosmetin 7-*O*-rutinoside was also confirmed in lemon juice by this technique.

KEYWORDS: Lemon juice; lemon tree; interstock; rootstock; flavonoid; flavanone; flavone; mass spectrometry

INTRODUCTION

Citrus fruit is a product that offers many advantages in the lifestyles of people who are health conscious, demand convenience, and place a premium on food safety. It is one of the important horticultural crops, with worldwide agricultural production over 100 million metric tons per year (*1*). In 1996−1998, lemon production reached 9.25 million metric tons, with 7.3 millon metric tons in the fresh market and 1.9 million metric tons processed. In 2010, lemon production is projected to be 10.6 million metric tons (*2*). Lemon juice exhibits a high level of consumption per capita, but it is lower than orange juice, because it is ingested as diluted juices (soft drinks and alcoholic drinks) (*3*, *4*). This juice can be highlighted by its high content of flavonoids, especially flavanone and flavone glycosides (*5*, *6*). High concentrations of flavanone glycosides are rare in other fruits and vegetables. Only similar citrus fruits and juices (orange, lime, mandarin, and grapefruit) contribute concentrations of these compounds similar to those found in lemons and their juice (*4*). Other phenolic compounds such as hydroxycinnamic acids are present in this juice at a lower concentration than flavanones and flavones (*5*, *7*). The main flavanones detected in lemon juice are eriocitrin and hesperidin (*6*). Extensive in vivo and in vitro experiments of these flavanones showed beneficial health activities as protective agents against

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cancer (*8*) and cardiovascular (*9*), inflammatory (*10*), and allergic (*11*) disorders. It has been previously demonstrated that hesperidin and diosmin show therapeutic effects on microcirculation in chronic venous insufficiency (*12*). Eriocitrin has shown a suppressive effect against oxidative stresses caused by exercise and diabetes (*13*, *14*). Furthermore, combined treatments of diosmin and hesperidin showed beneficial properties against oxidative stress and decreased the internal hemorrhoids of pregnancy (*15*, *16*).

On the other hand, there is no disputing the importance of citrus rootstocks to desert citrus production. The ideal citrus rootstock must be compatible with the scion, be adaptable to the appropriate soil and climatic factors, and should also improve one or more of the following characteristics: pest and disease resistance, cold tolerance, harvest date, internal and external fruit quality, yield, and post-harvest quality. Ultimately, the value of a rootstock lies in its ability to improve production and/or quality of the fruit (*17*). The interstock is a segment of a tree trunk that it is grafted between the rootstock and the tree. It has been used with different fruit trees to modulate the tree size, fruit production and quality, and the aging of the tree. However, the trees grafted with interstocks are more expensive, and grafting delays the production phase (*18*). The lemon trees grafted with interstocks increase their age and lemon production and quality (lower size and peel and albedo thickness) (*19*). In the "Verna" lemon trees, the interstocks are used to decrease the thickness of the trunk at the grafting point. This enlarging of the trunk provokes a decrease of the sap flow (*19*, *20*). However, the interstock in lemon tree produces a lower chlorine absorption and impedes the accumulation of these ions (*21*, *22*). With regard to the lemon flavonoids, there is a lack of knowledge about the influence of the interstock on the nature and content of these compounds in the lemons and lemon juice. In addition, previous studies have demonstrated the capacity of rootstock to synthesize and accumulate flavonoids (*23*, *24*). For these reasons, the goal of our study was focused on the effect of the rootstocks and interstocks grafted in "Verna" lemon trees on the flavonoid content of the lemon juice. Furthermore, a qualitative analysis was carried out to investigate the nature of the flavonoids present in lemon juice by HPLC coupled to a diode array detector (DAD) and an ion trap mass spectrometry detector equipped with ionization electrospray interface.

MATERIALS AND METHODS

Material. All the assayed lemon trees (*Citrus limon* (L.) Burm.) corresponded to the "Verna" cultivar and were ordered in independent plots with identical agronomical and environmental conditions. Sour orange (*Citrus aurantium* L.) and *Citrus macrophylla* L. trees were selected as rootstocks because they present the highest affinity with the lemon tree. Seven interstocks were used, five from different cultivars of sweet orange trees (*Citrus sinensis* L.) ("White", "Washington Navel", "Berna", "Cipó", and "Sanguinelli"), one from the "Sweet Lime" tree (*Citrus aurantifolia* (Christm.) Swingle) and one from the "Cleopatra" tangerine tree (*Citrus reticulata* L.). One "Verna" lemon tree was directly grafted to the sour orange tree rootstock without interstock. Three lemon trees were grafted with "Washington Navel", "White", and "Berna" interstocks using "Sour orange" tree as rootstock. Four interstocks ("Sweet Lime", "Cleopatra", "Cipó", and "Sanguinelli") were used with *Citrus macrophylla* L. rootstock. According to the DOCE (*25*) and MAPA (*26*), the maturity stage of the lemon is fixed by the lemon juice volume. This parameter must be higher than 30% in relation to the total weight of the fruit. In our case, all the lemon juices volumes ranged 30.6−37.0%. Therefore, the maturity stage of the lemons collected in the different plots was similar.

Sample Preparation for Qualitative and Quantitative Analyses. Lemon juice obtained by hand squeezing from the lemon cultivar "Verna". A squeezer (model Citromatic, Braun Española S. A., Spain) was used to obtain the domestic squeezed juice. To ensure the reliability and reproducibility of the squeezing of lemon juice, the lemons were squeezed carefully, to obtain the juice from only the edible part of the fruit without reaching the albedo. Each juice replicate was made with 10 lemons. Three replicates were used for the quantitative analysis ($n = 3$). Juice samples were centrifuged at 12000*g* for 5 min (Sigma centrifuge 14−13, B. Braun Biotech International, Germany). The supernatant was filtered by a 0.45-μm pore size polyethersulfone filter (Millipore, Millex-HV 13 mm, France). A volume of 95 μL of the filtered sample was injected in the HPLC for flavonoid analyses.

HPLC Coupled to Diode Array Detector and Ion Trap Spectrometer for Qualitative Analyses. The HPLC correponded to an Agilent 1100 series (Agilent Technologies, Waldbronn, Germany) and was equipped with a binary pump (model G1312A), an autosampler (model G1313A) and a degaser (model G1322A). An Agilent diode array detector (DAD, model G1315B) was used for UV−Vis spectra analyses. An Agilent ion trap mass spectrometer (model G2445A) was coupled to the outlet of the DAD in order to know the molecular weight and the structure of the compounds.

For HPLC analyses, the separation of the phenolic compounds was achieved in a LiChroCART RP-18 column (250 × 4 mm, 5-μm particle size, Merck, Darmstadt, Germany) protected with a column guard (4 × 4 mm. Merck, Darmstadt, Germany). The mobile phase was water/acetic acid (A) (99:1, v/v) and methanol (B). The linear gradient started with a 15% B in A and continued with 25% B in A at 10 min. 25% B in A at 20 min, 40% B in A at 25 min, 50% B in A at 30 min to finally reach 80% B in A at 32 min. The flow rate was set at 1 mL min^{-1}. The diode array detection ranged between 250 and 400 nm. For qualitative analyses, the chromatograms were recorded at 290 and 345 nm to monitor the flavanones and flavones peaks, respectively.

The ion trap mass spectrometer was equipped with an electrospray ionization interface. The nebulizer gas was nitrogen. The pressure and the flow rate of the dryer gas were set at 65 psi and 11 L min^{-1}, respectively. The capillary temperature and voltage were 350 °C and 4 kV, respectively. The total ion chromatogram ranged at *m*/*z* 200−1500. The MS^n experiments were achieved using hellium as collision gas. The collision energy was adjusted at 1 V with ramping cycles from 30% up to 200% of this voltage value. The MS and MS^n events were recorded in the negative mode.

HPLC Equipment and Conditions for Quantitative Analyses. A Merck-Hitachi HPLC was used for the quantitative analyses (Merck, Darmstadt, Germany). It was equipped with a ternary pump (model L-6200), an autosampler (model AS-2000), a UV−vis detector (model L-7420) and an integrator (model D-2500). The mobile phase and the linear gradient were the same as those used for qualitative analyses. Sample aliquots of 90 μL were injected in the HPLC. The chromatograms were recorded at 273 nm for flavone and flavanone quantification. The flavonoids were quantified using authentic markers. The flavanones eriocitrin and hesperidin were quantified as hesperidin (Zoster, S. A., Murcia, Spain), and the flavones 6,8-di-*C*-glucosyl diosmetin and diosmin as diosmin (Genay, France).

RESULTS AND DISCUSSION

Qualitative Analysis of the Lemon Juice Flavonoids. The lemon juices analyzed obtained by hand squeezing of lemons collected from trees grafted with two rootstocks and different interstocks presented the same phenolic qualitative profile. The identification of the compounds (**Figure 1**) is detailed as follows:

Compound 1. This compound was detected at 15 min of retention time and was present in trace amounts. Its UV spectra provided three maxima at 256, 268, and 350 nm, coincident with a flavonol glycosilated in the positions 3 (**Table 1**) (*27*).

MS analyses showed a deprotonated molecule in the negative mode at *m*/*z* 771.7 (**Figure 2**). Its MS^2 fragmentation revealed a base peak at *m*/*z* 609.4 ($[M - H - glucose]^-$), and a low peak at *m*/*z* 301.3 (13% relative abundance), which corresponded to the deprotonated aglycon (quercetin). This type of fragmentation is characteristic of 3,7 di-*O*-glycosyl flavonols. Previous

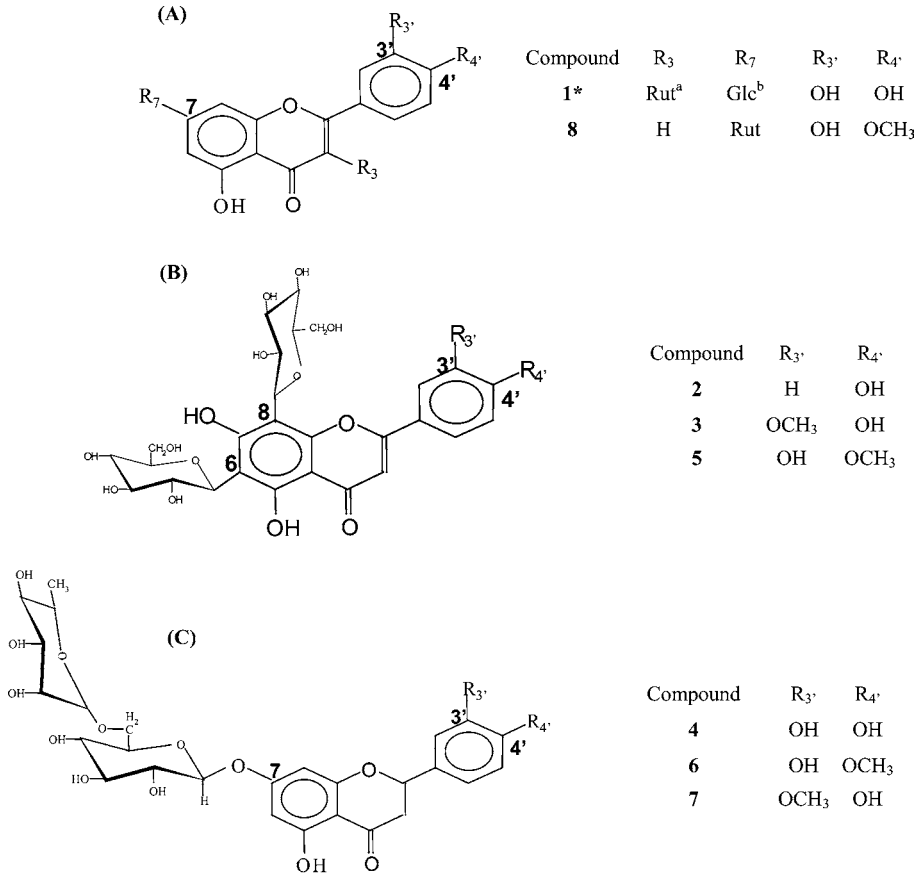

Figure 1. Structures of the compounds identified. (A) Flavone *O*-glycosides (asterisk in **1** means tentative identification; [a]Rut, rutinose; [b]Glc, glucose). (B) Flavone *C*-glycosides. (C) Flavanone *O*-glycosides.

Table 1. UV and ESI Mass Spectral Data (Negative Mode) of Lemon Juice Flavonoids

cpd	retention time (min)	UV absorbtion (nm)	$[M - H]^-$ (*m*/*z*)	MS^n (*m*/*z*)	structure attribution
1	15.2	256, 268sh,[a] 350	771.7	609.4	quercetin 3-*O*-rutinoside-7-*O*-glucoside (tentative)
				301.3	
2	17.8	270, 334	393.7	503.2	apigenin 6,8-di-*C*-glucoside
				473.3	
				383.5	
				353.7	
3	21.7	258sh, 271, 346	624.0	533.2	chrysoeriol 6,8-di-*C*-glucoside
				503.2	
				413.2	
				383.3	
4	23.0	284, 334sh	595.1	287.7	eriodictyol 7-*O*-rutinoside
5	24.5	257sh, 272, 346	623.9	533.2	diosmetin 6,8-di-*C*-glucoside
				503.3	
				413.4	
				383.5	
6	28.4	284, 334sh	610.0	301.0	hesperetin 7-*O*-rutinoside
7	29.6	284, 332sh	609.6	301.1	homoeriodictyol 7-*O*-rutinoside
8	32.5	254, 266, 348	607.4	299.1	diosmetin 7-*O*-rutinoside

[a] sh, shoulder.

studies on isolated similar flavonoids of cauliflower indicated that the first loss by HPLC-ESI/MS^n was due to a glycosilation in the position 7, and then in the position 3 (*28*). The ion at *m*/*z* 609.4 indicated the occurrence of quercetin rhamnoglucoside. In addition, the absence of intermediate ions in the MS^3 scan between the ions at *m*/*z* 609.4 and 301.3 showed a (1 → 6) interglycosidic linkage (rutinosyl) (*29*). Therefore, the compound was tentatively considered to be **quercetin 3-*O*-rutinoside-7-*O*-glucoside** (**Table 1**). Previous studies have not reported the occurrence of this compound in lemon juice. This structure has been described in other plant species.

Compound 2. HPLC-DAD analyses detected this peak at 17.8 min. The UV maxima spectra at 270 and 334 nm showed that this compound was a flavone with one hydroxyl in the ring B (*26*) and a possible *C*-glycosylation in the positions 6 and/or 8 (**Table 1**) (*27*). The compound was present in trace amounts.

Full mass scan (MS) indicated the occurrence of the deprotonated molecule at *m*/*z* 593.7. MS^2analyses (**Figure 3**) of this ion provided a typical fragmentation of di-*C*-glycosylflavones (*30*). Particularly, the ions at *m*/*z* 503.2 ($[M - H - 90]^-$) and 473.3 ($[M - H - 120]^-$, base peak) characterized the occurrence of 6,8-di-*C*-glucosides (**Table 1**). The ions at *m*/*z* 353.7

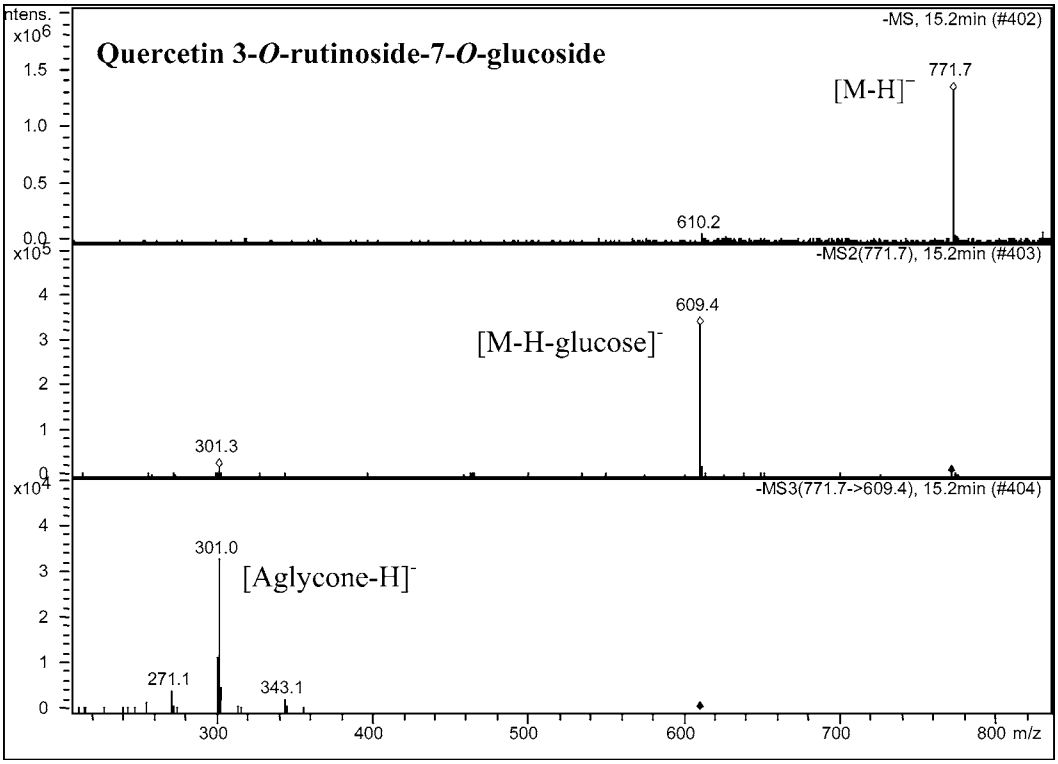

Figure 2. ESI/MS^n analyses of quercetin 3-*O*-rutinoside-7-*O*-glucoside.

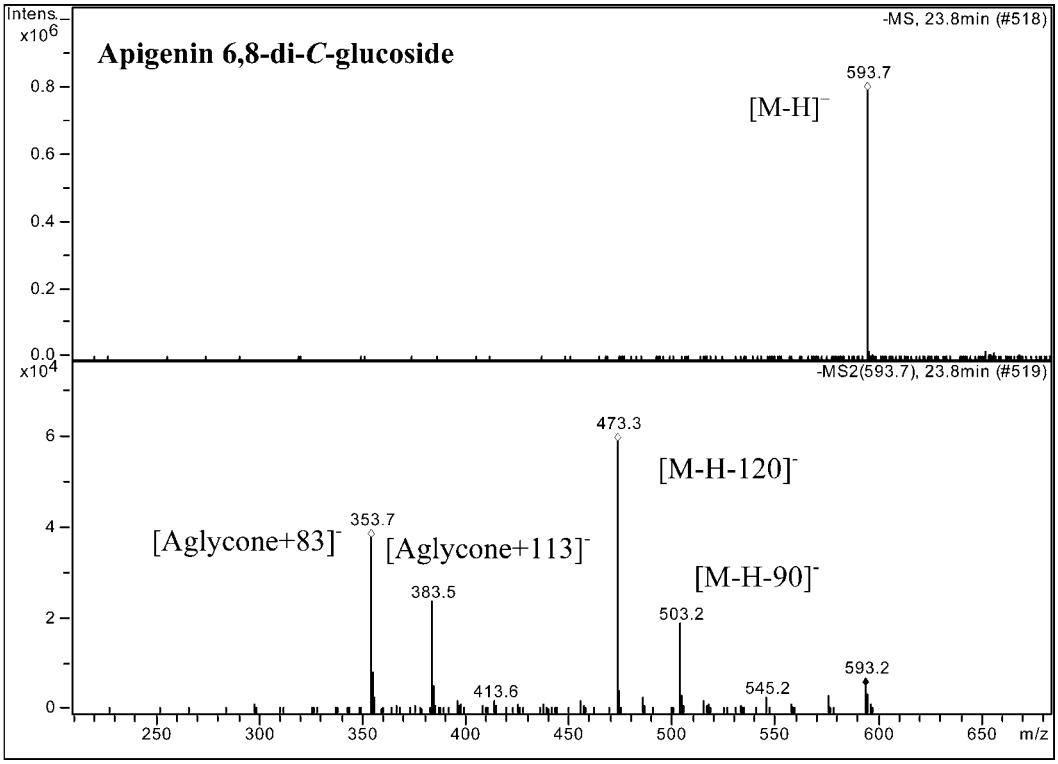

Figure 3. ESI/MS^n analyses of apigenin 6,8-di-*C*-glucoside.

([aglycone + 83]$^-$) and 383.5 ([aglycone + 113]$^-$) indicated the aglycone as a trihydroxyflavone. Therefore, this compound could be 5,7,4′-trihydroxyflavone 6,8-di-*C*-glucoside. The comparison of the sample compound with vicenin-2 isolated from *Spergularia rubra* (*31*) confirmed the presence of **apigenin 6,8-di-*C*-glucoside** (**vicenin-2**) (**Table 1**). This compound has been previously described in *Citrus limon* (L.) Burm. (*6*, *32*, *33*).

Compound 3. This compound (21.7 min) showed two UV maxima spectra at 271 and 346 nm and a shoulder at 258 nm. These UV data were in accordance to a flavone di-substituted in the ring B and *C*-glycosylated in the 6 and/or 8 positions (**Table 1**) (*27*). It was found in trace amounts and coelutes with compound **4**.

The deprotonated molecule detected by mass spectrometry in the negative mode was 624.0. MS^2 scan of the [M − H]$^-$ ion yielded four fragment ions at *m*/*z* 533.2, 503.2, 413.2 and 383.3 (**Table 1**). As in the compound **2**, this molecule showed the characteristic fragment ions of *C*-glycosyl flavones at *m*/*z*

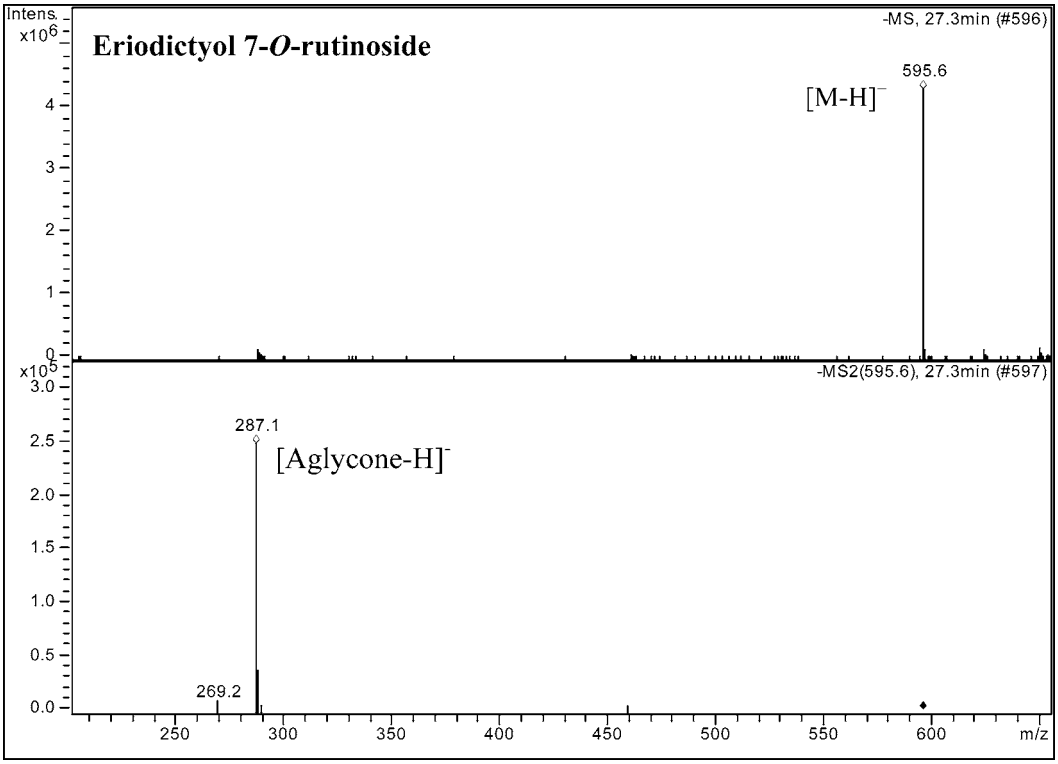

Figure 4. ESI/MS^n analyses of eriodictyol 7-*O*-rutinoside.

533.2 ([M − H − 90]$^-$) and 503.2 ([M − H − 120]$^-$, base peak). Particularly, and according to these data, this compound could be a symmetric di-*C*-hexosyl flavone. The ions at *m*/*z* 383.3 ([aglycone + 83]$^-$) and 413.2 ([aglycone + 113]$^-$) showed the mass of the aglycone, the chrysoeriol. Therefore, this compound was considered to be a 6,8-di-*C*-glucosyl-trihydroxy-methoxy flavone. The comparison with **Stellarin-2** (**chrysoeriol 6,8-di-*C*-glucoside**) isolated from *Spergularia rubra* (*31*) confirmed the identity of this compound (**Table 1**). This compound has not been previously described in the juice of *Citrus limon* (L.) Burm.

Compound 4. This intense peak showed a retention time at 23.0 min. The UV spectra (maxima at 284 nm and shoulder at 334 nm) was characteristic of a flavanone (2,3-dihydroxy-flavone) (**Table 1**) (*27*).

In the full mass scan analyses, a signal at *m*/*z* 595.6 was found as the [M − H]$^-$ ion in this peak. The MS^2 event provided a fragment ion at *m*/*z* 287.1 (M − H − rhamnose − glucose) corresponding to the aglycone mass in the negative mode (**Table 1**) (**Figure 4**). As we described above (see compound 1), the absence of intermediate ions in the MS^2 scan indicated a (1 → 6) interglycosidic bond (*29*). Taking into account all the information provided by diode array detection and ESI/MS^n and the comparison with an authentic marker of **eriocitrin** (**eriodictyol 7-*O*-rutinoside**), we concluded that it was this compound (**Table 1**). It has been previously identified in *Citrus limon* (L.) Burm. and other species of *Citrus* (*6*, *32*, *34*, *35*).

Compound 5. At 24.5 min, an intense peak was deteted. The UV spectra showed two maxima at 272 and 346 nm and a shoulder at 257 nm indicating a flavone with two substitutions in the ring B, and probably a *C*-glycosilation in the position 6 and/or 8 (**Table 1**) (*27*). The spectra was very similar to that of **3**.

Its MS analysis was also similar to that of **3**, showing a deprotonated molecule in the negative mode at *m*/*z* 623.9. The experiments yielded ions at *m*/*z* 533.2, 503.3, 413.4, and 383.5 (**Table 1**). The characteristical fragments [M − H − 90]$^-$ (*m*/*z* 533.2) and [M − H − 120]$^-$ (*m*/*z* 503.3) of di-*C*-glycosyl flavones were detected. The ions at *m*/*z* 413.4 ([aglycone + 113]$^-$) and 383.5 ([aglycone + 183]$^-$) defined the mass of the aglycone. The glycosidic fragmentation of this compound was identical to that found in **3**; therefore, it was a position isomer with regard to the substituents in the ring B. Therefore, all the information indicated the occurrence of diosmetin 6,8-di-*C*-glucoside. On the other hand, the elution order by HPLC of this pair of isomers were in accordance to the structures proposed, because the hydroxyl in the position 4′ of the compound awards higher acidity and shorter retention time than the hydroxyl in the position 3′ of **5**. As conclusion, this compound was identified as **diosmetin 6,8-di-*C*-glucoside** (**Table 1**). It has been previously described in the fruit juice of *Citrus limon* (L.) Burm. (*6*, *32*).

Compounds 6 and 7. Two peaks at 28.4 and 29.6 min were observed in the UV chromatogram. The first one was found in trace amounts and the second one was very abundant. Both compounds showed a very similar UV spectra (maxima at 284 nm and shoulder at 334 nm for **6** and maxima at 284 nm and shoulder at 332 nm for **7**) characteristic of flavanones to **4** (**Table 1**) (*27*).

In the ESI-MS analyses, both peaks showed similar deprotonated molecules (at *m*/*z* 610.0 for **6** and 609.6 for **7**) and fragment ions by MS^2 experiments (*m*/*z* 300.5 (**6**) and (**7**)) (**Table 1**). The (1 → 6) interglycosidic linkage was also detected as in **1** and **4** (*29*). Therefore, both compounds were derived from a tri-hydroxy-methoxy-flavanone. One of the compounds could be hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone 7-*O*-[rhamnosyl (1 → 6) glucoside]), typical compound in the genus *Citrus*, and the other one, an isomer of it, neohesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone 7-*O*-[rhamnosyl (1 → 2) glucoside]), or homoeriodictyol 7-*O*-rutinoside (5,7,4′-trihydroxy-3′-methoxy flavanone 7-*O*-[rhamnosyl (1 → 6) glucoside).

In the co-chromatographic study of lemon juice plus hesperidin standard (Zoster S. A., Murcia, Spain) and lemon juice

Table 2. Total Flavonoid Content of Lemon Juices (mg L^{-1}) Obtained from Lemons Grown with Seven Interstocks Grafted in Lemon Tree Using Two Types of Rootstocks and One Lemon Tree Grafted without Interstock

rootstock	interstock	total flavonoid content[a]
Citrus aurantium L.	without interstock	1457 ± 183
Citrus aurantium L.	"White" (O)[b]	1497 ± 135
Citrus aurantium L.	"Washington Navel" (O)	1158 ± 80
Citrus aurantium L.	"Berna" (O)	1613 ± 103
Citrus macrophylla L.	"Sweet Lime" (L)[c]	827 ± 95
Citrus macrophylla L.	"Cleopatra" (T)[d]	757 ± 43
Citrus macrophylla L.	"Cipó" (O)	811 ± 133
Citrus macrophylla L.	"Sanguinelli" (O)	847 ± 35

[a] Data are the mean of three values, and standard deviation (SD) was calculated (mean ± SD). [b] (O), orange tree interstock. [c] (L), Lime tree interstock. [d] (T), Tangerine tree interstock.

plus neohesperidin (Zoster, S. A.), the results indicated that the compound **6** was **hesperidin** (hesperetin 7-*O*-rutinoside) (**Table 1**). The neohesperidin eluted at 29.0 min, 0.6 min later than hesperidin. Therefore, the flavanone **7** could probably be the isomer of the eriodictyol 7-*O*-rutinoside, concretely, **homoeriodictyol 7-*O*-rutinoside** (**Table 1**). The chromatographic behavior by HPLC of both isomers compounds was in accordance to their structures and the co-chromatographic study with the authentic markers confirmed the identity of these compounds. Hesperidin and homoeriodictyol 7-*O*-rutinoside have been previously described in *Citrus limon* (L.) Burm. (*6*, *32*, *34*).

Compound 8. This intense peak was detected at 32.5 min. The UV spectra with maxima at 254, 266, and 348 nm was characteristic of a flavone di-substituted in the ring B (**Table 1**) (*27*).

Its MS analysis provided a $[M - H]^-$ at *m*/*z* 607.4. The MS^2 scan showed an ion at *m*/*z* 299.1 as unique fragment ($[M - H\text{-rutinose}]^-$) (**Table 1**). Therefore, it could be a trihydroxymethoxy flavone *O*-glycosilated with rutinose. The chromatographic comparison by retention time, UV, and MS^n analyses with **diosmetin-7-*O*-rutinoside** (**diosmin**) confirmed the occurrence of this compound (**Table 1**). This compound has been previously reported in *Citrus limon* (L.) Burm. (*6*, *32*, *36*).

Effect of the Interstocks Grafted in Lemon Tree on the Flavonoid Content of Lemon Juice. The total flavonoid content of the lemon juice was different depending on the rootstock grafted in the lemon tree. Lemon juices from lemon trees grafted with sour orange (*Citrus aurantium* L.) rootstock showed 1.8-fold higher flavonoid content than that obtained from lemon trees grafted with *Citrus macrophylla* L. rootstock (**Table 2**). In the lemon trees with sour orange rootstock, no differences in the total lemon juice flavonoid content were detected in relation to the lemon trees cultivated without interstock. The "Washington Navel" interstock lemon trees gave as result lemon juices with lower flavonoid content than that grafted with "White" and "Berna" interstocks (**Table 2**). Among the lemon trees grafted with *Citrus macrophylla* L. rootstock, the flavonoid content of lemon juices ranged 757−847 mg L^{-1}. The tangerine "Cleopatra" interstock was the only one that decreased the phenolic content of the lemon juice (**Table 2**).

Regarding the individual flavonoids, the flavones diosmin and 6,8-di-*C*-glucosyl diosmetin (DCGD) and the flavanones eriocitrin and hesperidin were studied. DCGD was the most abundant flavonoid in all the assayed lemon juices (**Figure 5**). Among the lemon trees grafted with Sour orange rootstock, only the lemon trees grafted with "Berna" and "Washington Navel" interstocks provided lemon juices with similar DCGD content than that obtained from the lemon tree without interstock. The "Washington Navel" interstock contributed to the obtaining of

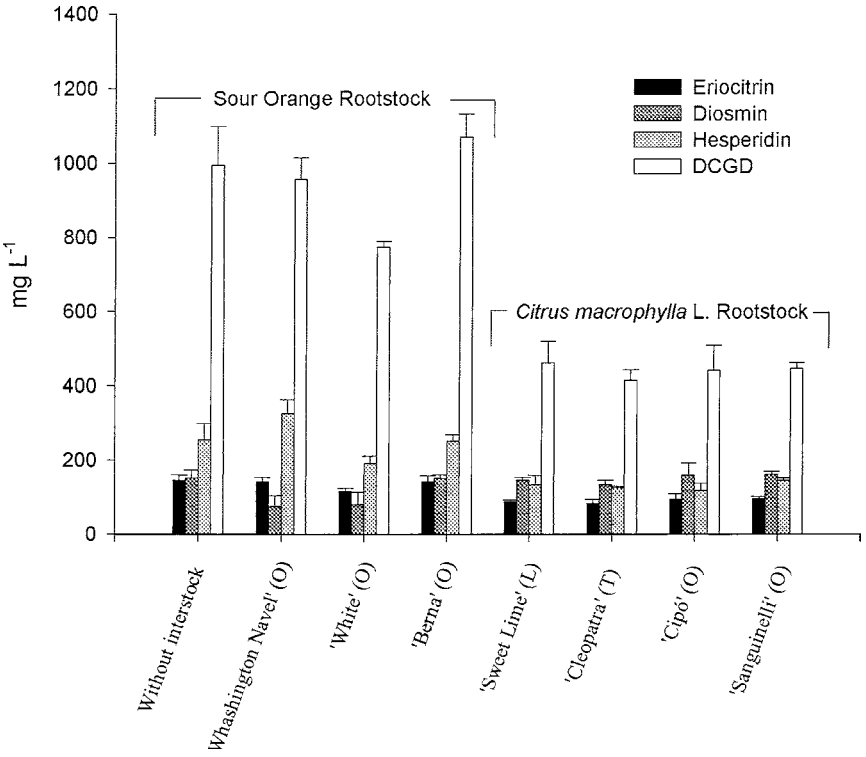

Figure 5. Eriocitrin, diosmin, hesperidin and DCGD content of lemon juices obtained from lemons grown in lemon trees grafted with seven interstocks ("White", "Washington Navel", "Berna", "Sweet Lime", "Cleopatra", "Cipó", and "Sanguinelli") and two rootstocks (*Citrus aurantium*, L. and *Citrus macrophylla*, L.). One lemon tree was grafted without interstock (*Citrus aurantium*, L. rootstock). (O), Orange tree interstock; (L), Lime tree interstock; (T), tangerine tree interstock. DCGD, 6,8-di-*C*-glucosyl diosmetin. Data are the mean of three values and standard deviation (SD) was calculated (mean ± SD).

a lemon juice richer in hesperidin than that from the lemon tree without interstock (**Figure 5**). In the case of diosmin and eriocitrin, the lemon trees with Sour orange rootstock + interstocks presented similar or lower contents in the lemon juice than the lemon tree grafted directly to the rootstock (**Figure 5**). Regarding the lemon trees with *Citrus macrophylla* L. rootstock, a lower DCGD, eriocitrin, and hesperidin content was found in all lemon juices than that were obtained from lemon trees with Sour orange rootstock. However, diosmin content was more abundant in lemon juices with *Citrus macrophylla* L. rootstock (**Figure 5**). In all the lemon juices from lemon trees with this type of rootstock and the different interstocks, no great differences were detected in the DCGD, eriocitrin, and hesperidin content. In the case of diosmin, this flavonoid was higher in lemon juices from lemon trees with "Cipó" and "Sanguinelli" interstock with respect to that with "Sweet Lime" and "Cleopatra" interstocks (**Figure 5**).

With regard to the quantitative profile of lemon juice from lemon trees with Sour orange rootstock, only the lemons collected from the Berna interstock lemon tree maintained the individual flavonoid content order of that from the lemon tree without interstock (DCGD > hesperidin > diosmin > eriocitrin) (**Figure 5**). In the lemon trees with "Washington Navel" and "White" interstocks, the eriocitrin content of the juice was higher than the diosmin content. In the case of the lemon trees grafted with "Sweet Lime", "Cleopatra", "Cipó", and "Sanguinelli" interstocks and *Citrus macrophylla* L. rootstock, the diosmin was the second most abundant flavonoid overcoming the hesperidin content (**Figure 5**).

The beneficial properties of lemon juice flavonoids advice a regular intake of this product to maintain a healthy quality of life (*13*−*16*). In human studies, 8−10% of hesperidin and other citrus flavanones are absorbed in the gastrointestinal tract in relation to the initial content of the administrated juice (*37*). A higher flavonoid content in lemon juice would improve the intake of these compounds and could increase their absorption in humans. Recent studies have demonstrated the absorption of flavanones after administration of two doses of orange juice in humans (*37*). Presently, different techniques and conditions (agronomic, postharvest, and processing) are being assayed to increase these beneficial compounds. In our study, the rootstock grafting in lemon tree largely influenced the flavonoid content of lemon juice. In fact, the flavonoid content was higher in the lemon juices from lemon trees with Sour orange rootstock than those from *Citrus macrophylla* L. rootstock. Regarding the individual flavonoids, the most affected flavonoid was the DCGD, because it presented 40−50% of variability among the different lemon juices, depending on the rootstock used. The interstock had only a small influence on the total lemon juice flavonoid content. The selection of the rootstock could have a larger effect on the flavonoid biosynthesis in the lemon fruit. On the other hand, the interstock affects the individual flavonoid content of the lemon juice. The grafting of the Sweet Lime, Cleopatra, Cipó, Washington Navel and White interstocks provoked different quantitative order of hesperidin, diosmin, and eriocitrin in the lemon juice.

In conclusion, the rootstock was a more important agronomic factor than the interstock on the total flavonoid content of the lemon juice, due to the high influence on the DCGD content. The Sour orange rootstock was found as the most appropriate. The agronomic technique of interstock grafting between the rootstock and the lemon tree only slightly influenced the total flavonoid content of lemon juice, and it modulated the individual flavonoid content. Among the assayed interstocks, Washington Navel and Berna were found the most appropriated to preserve the highest flavonoid content of lemon juice. This technique does not increase the flavonoid content of the lemon juice.

ABBREVIATIONS USED

DAD, diode array detector; DCGD, 6,8-di-*C*-glucosyl diosmetin

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