JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Separation of Flavanone-7-*O*-glycoside Diastereomers and Analysis in Citrus Juices by Multidimensional Liquid Chromatography Coupled with Mass Spectrometry

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The major flavanone-7-*O*-glycoside constituents in citrus fruit juices (naringin, hesperidin, neohesperidin, narirutin, and eriocitrin) were separated as diastereomers by multidimensional liquid chromatography. The method consisted of coupling two HPLC columns: a reversed-phase (RP_{18}) column was used for the separation of flavanone glycosides, which were, then, individually switched into a carboxymethylated β-cyclodextrin (β-CD)-based column and resolved as the corresponding stereoisomers. The method was used for the full analysis of flavanone glycosides in fresh hand-squeezed and commercial fruit juices by combining the quantitative estimation with the diastereomeric analysis. Quantitative data were in general consistent with previously reported data in this field. CC-LC isomer analysis was carried out by coupling the β-CD column with a mass spectrometer operated with negative ion electrospray ionization (ESI-MS). The results showed that hesperidin was present in orange juices almost exclusively as the 2*S* isomer, whereas narirutin had mainly the 2*R* configuration. In grapefruit juices (2*S*)-naringin prevailed with the respect to the 2*R* isomer, whereas the opposite was true for narirutin. Lemon juices contained eriocitrin stereoisomers in equal amount (50% each), but hesperidin was almost exclusively found as the 2*S* isomer. Significant differences of the diastereomeric ratios were observed between freshly squeezed juices and juices from commercial sources.

KEYWORDS: Flavanone-7-*O*-glycosides; diastereomer separation; multidimensional liquid chromatography; ESI-MS detection; citrus fruit juices

INTRODUCTION

Flavanone-7-*O*-glycosides, an important class of naturally occurring compounds, are specifically distributed in *Citrus* (*1*). Due to their polyphenolic structure, these compounds have health-related properties, which are based on their antioxidant activity including anticancer, antiviral, and antiinflammatory activities (*2*, *3*). Naringin, narirutin, hesperidin, neohesperidin, and eriocitrin are the most abundant flavonoids in the edible part of many species of citrus fruits (*4*) and are known to greatly influence the quality of both the fresh fruit and processed products, thus playing an important role in nutritional and pharmacological fields. As is well documented, naringin is the most representative flavanone glycoside in grapefruit (*5*), and hesperidin and narirutin have been determined in common sweet orange (*6*), whereas neoeriocitrin and eriocitrin are typical to sour orange (*7*) and lemon juices (*1*). Owing to this biodiversity, flavanone glycosides have been used as chemotaxonomic markers in quality control to identify adulterated processed juices (*8*, *9*).

The stereochemistry of flavanone glycosides shows that these compounds can exist as pairs of diastereomers, due to the chirality of both the aglycon (C2) and the sugar moieties. The stereoisomeric composition of naringin during the maturation process of grapefruit has been studied by circular dichroism and NMR spectroscopy by Gaffield et al. (*10*, *11*). These authors observed a prevalent presence of (2*S*)-naringin in immature grapefruit and an increase of the 2*R* isomer during ripening. These findings led to a new interest in separation methods of flavanone glycosides as isomeric forms either to obtain insights into the biosynthesis of polyphenolic compounds or as a powerful tool for the characterization of quality and safety of processed products.

Krause and Galensa (*12*, *13*) studied the diastereomeric composition of some flavanone glycosides in citrus fruit juices by HPLC on native β-cyclodextrin (β-CD) (Cyclobond I) and cellulose triacetate derivative stationary phases, but only three flavanones, that is, naringin, narirutin, and neohesperidin, were fully resolved into the corresponding stereoisomers. Gel-Moreto et al. (*14*) have reported the separation of diastereomeric flavanone glycosides in *Citrus* by capillary electrophoresis (CE). More recently, Aturki et al. (*15*) described fast baseline

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10.1021/jf0400967 CCC: $27.50
Published on Web 07/30/2004

separation of stereoisomers for five flavanone glycosides in citrus fruit juices by CE using sulfobutyl ether-β-CD in the background electrolyte (BGE). This method also allowed pairs of diastereomers to be separated from each other. Despite CE's being shown to be very effective for the separation of flavanone glycoside stereoisomers, serious problems due to interference of the additive in the BGE with analytes could arise when this technique is coupled with a mass spectrometer detector. At present, more conventional HPLC-MS is the preferred mode for the analysis of flavonoids in foods.

Multicolumn or coupled column approaches by liquid chromatography (CC-LC) have efficient application in bioanalysis, because it combines two or more different chromatographic systems with different chromatographic selectivities in the same analysis (*16*). In addition, the column switching mode is attractive because it is often possible to employ a less time-consuming sample workup than would be otherwise required because only the analytes of interest are transferred into the second column. In multidimensional chromatography the eluents required for the different chromatographic stages have to be broadly compatible, but the fact that each system is regulated by an individual delivery pump permits selection of optimal mobile phase conditions for both separations.

The objective of the present study was to develop a CC-LC method easily coupled to electrospray ionization mass spectrometry (ESI-MS) for the simultaneous quantitative analysis and the determination of the diastereomeric composition of some major flavanone glycosides constituents (hesperidin, narirutin, eriocitrin, naringin, and neohesperidin) (**Figure 1**) of citrus fruit juices. Freshly hand-squeezed and processed fruit juices from different sources were analyzed.

MATERIALS AND METHODS

Chemicals and Reagents. Naringin (naringenin-7-*O*-neohesperidoside) and hesperidin (hesperetin-7-*O*-rutinoside) were purchased from Aldrich (Steinheim, Germany). Neohesperidin (hesperetin-7-*O*-neohesperidoside) was from Sigma (St. Louis, MO). Narirutin (naringenin-7-*O*-rutinoside), eriocitrin (eriodictyol-7-*O*-rutinoside), and rhoifolin (apigenin-7-*O*-neohesperidoside) were supplied from Extrasynthese (Lyon, France). HPLC-grade methanol and formic acid were obtained from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade.

Chromatographic Procedure. Experiments were carried out using a coupled column system, as described in **Figure 2**. The apparatus consisted of two model 410 (Perkin-Elmer, Norwalk, CT) HPLC delivery pumps, one of which was equipped with a model 7125 (10 or 100 μL sample loops) injection valve (Rheodyne), and two prepacked columns connected by means of a model 7000 (Rheodyne) six-port valve. Each column outlet was connected to a model 2550 (Varian, Walnut Creek, CA) UV detector set at a 280 nm wavelength. A 30 μL portion of the effluent (1 mL/min) from the β-CD column was diverted to the ES source of a mass spectrometer, whereas the rest of the mobile phase was delivered to the UV detector. A Fisons VG Platform benchtop mass spectrometer (Fisons Instruments/VG BioTech, Milan, Italy) equipped with a pneumatically assisted electrospray LC-MS interface and a single quadrupole was used for analyzing target diastereomers in the column effluent. This was introduced into the ES interface through a 30 cm length of 75 μm diameter poly(ether ether ketone) (PEEK) capillary tube. The mass spectrometer was operated in the negative-ion mode by applying to the capillary a voltage of 4 kV, whereas the skimmer cone voltage was set at 30 V. Mass spectra collected in full-scan mode were obtained by scanning over the range $120 \leq m/z \leq 650$. The source temperature was maintained at 120 °C. Ions were generated using highly pure nitrogen as drying and nebulizing gas. The optimum flow rates of the drying and nebulizing gas were found to be 12 mL/min and 300 L/h, respectively. Data were processed by the manufacturer's software.

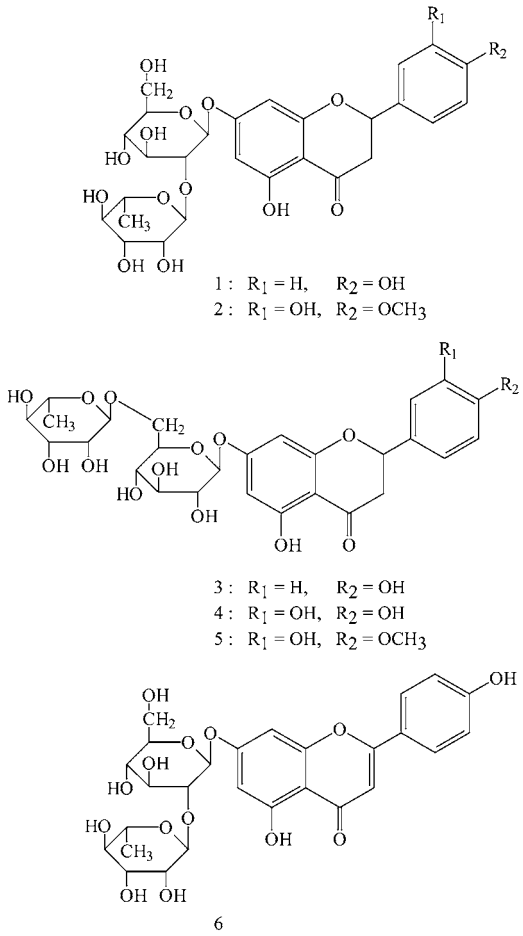

Figure 1. Structures of the studied flavanone glycosides: **1**, naringin (naringenin-7-*O*-neohesperidoside); **2**, neohesperidin (hesperitin-7-*O*-neohesperidoside); **3**, narirutin (naringenin-7-*O*-rutinoside); **4**, eriocitrin (eriodictyol-7-*O*-rutinoside); **5**, hesperidin (hesperetin-7-*O*-rutinoside); **6**, rhoifolin (apigenin-7-*O*-neohesperidoside).

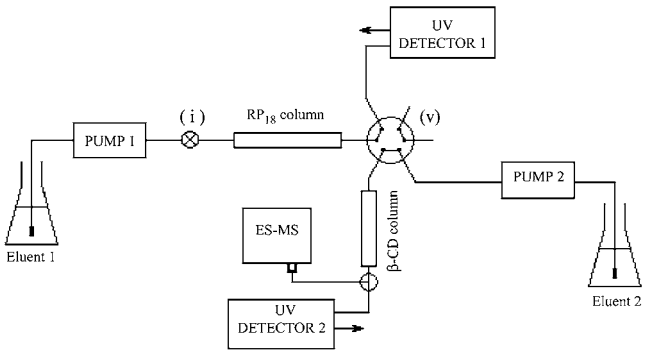

Figure 2. Scheme of the used two-dimensional HPLC system: (i) injection valve; (v) six-port valve.

Samples were injected into a 250 × 4.6 mm i.d., 5 μm RP-18 column (Restek, Chemtek Analytica, Milan, Italy) (system 1), and the separated flavanone glycosides were individually switched into a derivatized-β-CD-bonded column (system 2) and resolved as the corresponding diastereomer pairs.

In system 1, the mobile phase consisted of mixtures of 5 mM aqueous formic acid (pH 3)/methanol (95:5 v/v) (eluent A) and 100% methanol (eluent B). Gradient elution was performed in two steps: (1) 95% A, 5% B for 2 min and (2) from 5 to 30% B in 30 min; both steps were performed at a flow rate of 1 mL/min.

In system 2, the mobile phase consisted of a mixture of 5 mM aqueous formic acid (pH 3)/methanol (8:2 v/v). Water was filtered

Table 1. Determination of Flavanone-7-*O*-glycosides in Citrus Fruit Juices

		concn (mg/100 mL)				
sample	fruit juice	eriocitrin	naringin	narirutin	hesperidin	neohesperidin
1	orange juice	nd[a]	4.3 ± 0.14	5.5 ± 0.21	36.4 ± 1.44	1.5 ± 0.05
2	orange juice	nd	4.8 ± 0.22	6.7 ± 0.25	25.6 ± 1.02	0.4 ± 0.01
3	orange juice	nd	nd	5.8 ± 0.22	42.0 ± 1.68	0.6 ± 0.02
4	hand-squeezed orange juice	nd	2.3 ± 0.07	1.5 ± 0.06	38.0 ± 1.52	0.5 ± 0.02
5	(red) grapefruit juice	nd	34.1 ± 1.12	17.0 ± 0.63	2.0 ± 0.08	2.0 ± 0.07
6	(white) grapefruit juice	nd	26.2 ± 1.0	15.0 ± 0.56	0.3 ± 0.01	0.8 ± 0.03
7	hand-squeezed (red) grapefruit juice	nd	23.0 ± 0.8	12.3 ± 0.46	3.3 ± 0.13	0.9 ± 0.03
8	lemon juice	26.5 ± 0.8	nd	nd	20.0 ± 0.8	nd
9	lemon juice	25.5 ± 0.8	nd	nd	19.2 ± 0.77	nd

[a] Not detected.

through Millipore (Bedford, MA) type GS (0.22 μm) filter disks. Eluents were degassed by ultrasonic treatment prior to use.

Separations of flavanone glycoside diastereomers were studied by isocratic elution on three different β-cyclodextrin (β-CD) silica bonded stationary phases: (a) carboxymethyl-β-CD-bonded column 150 × 4.6 mm i.d., 5 μm Orpak CDBS-453 (Shodex, Tokyo, Japan); (b) acetylated β-CD, 250 × 4.6 mm i.d., 5 μm Cyclobond I 2000 (Alltech-Italia, Milan, Italy); and (c) permethylated β-CD, 200 × mm i.d., 5 μm Nucleodex β-PM (Chemtek Analytica, Bologne, Italy).

Sample Preparation. Commercial citrus fruit juices (lemon, orange, and grapefruit) from different producers as well as fresh fruits such as oranges (Italy) and white and red grapefruits (Jaffa, Israel) were purchased in supermarkets.

Flavanone glycosides of freshly hand-squeezed and commercial citrus fruit juices were extracted following, with minor modifications, the procedure described in the literature (*17*). Juices (5 mL) were diluted in 10 mL of dimethylformamide and 10 mL of 50 mM ammonium oxalate solution, and then 1 mL of a 1.5 mg/mL methanol solution of rhoifolin was added as an internal standard. The mixture was heated at 90 °C for 10 min. After cooling, the solutions were rinsed into volumetric flasks and made up to 50 mL. All solutions were centrifuged during 20 min at room temperature and 3500 rpm. The clarified sample solutions were filtered through 0.45 mm HA type filter disks (Millipore Corp., Bedford, MA), and then 50−100 μL of sample was injected into the chromatographic system. Extracts were stored in the dark at −18 °C.

Assay Validation. Interday and intraday repeatabilities of the retention factor k as well as interday repeatability of peak area were determined for each flavanone glycoside by assaying seven control samples and three control samples after 7 days, respectively, at the sample concentration of 0.1 mg/mL. Assay quantitative precision was assessed by calculating the corresponding percent relative standard deviation (%RSD) values. The linearity of the assay was calculated over the concentration range of 3.12×10^{-3}−0.4 mg/mL by assaying calibration standards in duplicate at seven separate concentrations. Calibration curves were obtained by plotting the peak area values of each compound/internal standard ratio versus concentration.

RESULTS AND DISCUSSION

The quantitative as well as the stereoisomeric analyses of five flavanone-7-*O*-glycosides in citrus fruit juices were carried out by a two-dimensional chromatographic system. The qualitative/quantitative analysis of flavanones was performed on a reversed-phase column (system 1) according to the optimal chromatographic conditions previously described in the literature (*18*, *19*). Successively, by a series of injections, the separated flavanone glycosides were switched individually from the reversed-phase column into the β-CD column (system 2) to obtain discrimination as the corresponding diastereomers.

Determination of Flavanone-7-*O*-glycosides in Citrus Fruit Juices. The identification of five flavanone glycosides (naringin, hesperidin, neohesperidin, narirutin, and eriocitrin) was made by comparing their retention times and MS spectra (data not reported) with those of standards. Quantitative data (expressed as milligrams per 100 mL) for a number of citrus juices are summarized in **Table 1**. The qualitative analysis of predominant flavanone glycosides was in general consistent with previous investigations (*19*, *20*).

In freshly hand-squeezed juices, orange juice was characterized by a high content of hesperidin and, to a minor extent, by the presence of naringin and narirutin. Neohesperidin was also found in a small amount. Because it is known that neither naringin nor neohesperidin is a normal constituent of sweet oranges (*Citrus sinensis*) (*6*), the analyzed orange sample, the variety of which was not declared on the product, was probably an orange/tangerine or an orange/sour orange cross. Specifically, the hesperidin, naringin, and narirutin contents were found to average 38.0, 2.3, and 1.5 mg/100 mL, respectively. Amounts of naringin, narirutin, hesperidin, and neohesperidin in red grapefruit were 23.0, 12.3, 33.0, and 0.9 mg/100 mL, respectively. Eriocitrin and hesperidin contents in lemon juices were 25.5 and 19.2 mg/100 mL, respectively.

The flavanone glycoside levels in squeezed orange juices found here are, with the exception of naringin and neohesperidin, in the same general range of values reported in most previous publications on the subject. Hesperidin in sweet orange juice has been quantified at 7.64−21.9 (*21*), 29.3−91.5, 23.5−40.7 (*5*), and 73.0−76.9 mg/100 g (*22*). Narirutin levels in sweet orange juices have been reported at 2.63−5.42 (*21*), 3.69, 3.97, and 3.0−8.4 mg/100 g in different varieties of this fruit (*5*). Naringin in red grapefruit juice has been quantified at 21.0 (*23*), 30.6 (*24*), 35.5−46.7 (*25*), 13.8−22.7 (*7*), 33.1, 20.5, and 11.3−48.1 mg/100 g (*5*). Narirutin in white grapefruit juice has been reported at 12.4 (*24*), 10.6 (*5*), and 10.3−2.2 mg/100 g (*7*). Eriocitrin in lemon juices has been determined at 47−94 mg/L (*5*). Obviously, the large variability in concentration depends on the variety and on the maturation degree of the fruits (*26*). In the present study only one variety of sample fruit was examined.

In commercial orange juices the narirutin and naringin contents were found to be higher than in fresh fruits, ranging between 5.5 and 6.7 mg/100 mL and between 4.3 and 4.8 mg/100 mL, respectively. Again, it has to be noted that naringin is not normally considered to be a constituent of sweet orange, and its presence in commercial orange juices can indicate adulteration of the product. Unexpected high values of hesperidin (42.0 mg/mL) and narirutin (5.8 mg/100 mL) were observed in sample 3, a drink with orange flavor (orangeade). Grapefruit juice samples 5 (red) and 6 (white) showed similar contents of the predominant flavanone glycosides, that is, narirutin and naringin [34.1 and 26.2 (red) and 17.0 and 15.0 mg/100 mL (white) respectively] but a lower content of hesperidin in sample 6 (0.3 mg/100 mL). The lemon juice

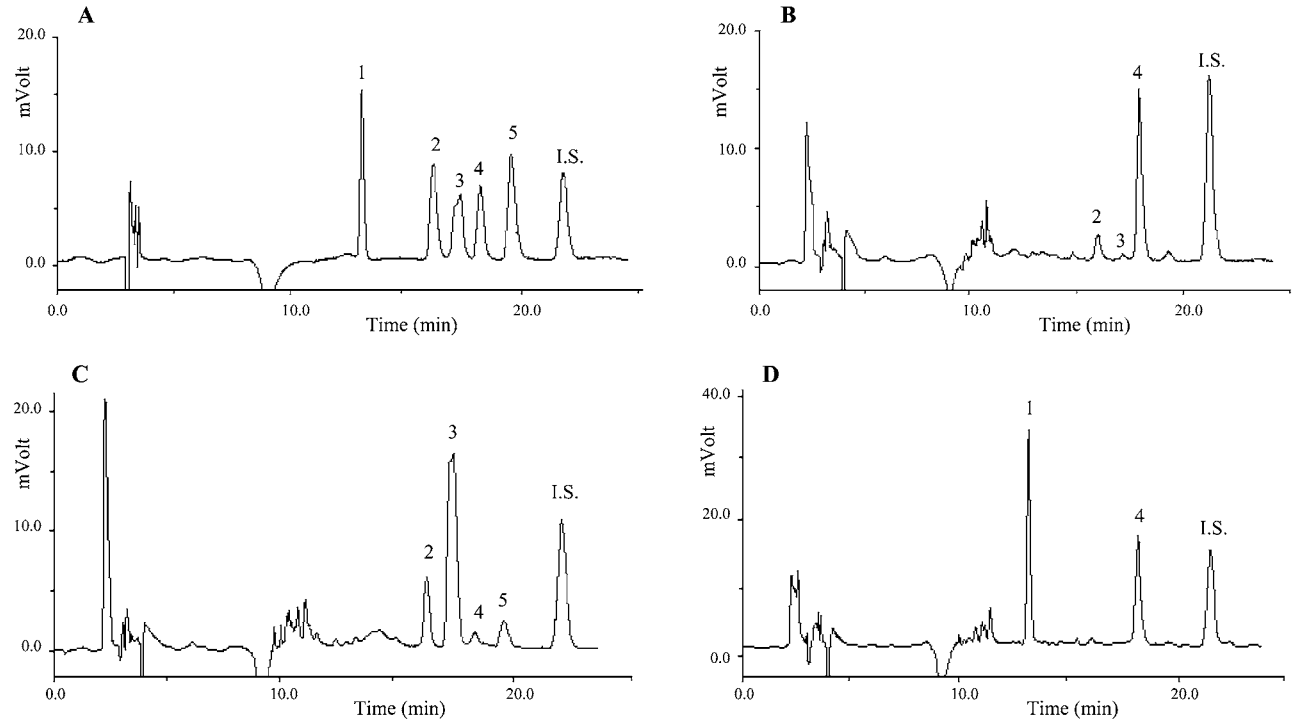

Figure 3. Separation of flavanone glycosides by reversed phase chromatography: (**A**) standard mixture (0.1 mg/mL each component); (**B**) orange (sample 2); (**C**) white grapefruit (sample 6); (**D**) lemon (sample 8) juice extracts. Peaks: (1) eriocitrin; (2) narirutin; (3) naringin; (4) hesperidin; (5) neohesperidin; (I.S.) rhoifolin.

(sample 8) showed eriocitrin and hesperidin contents at 26.5 and 20.0 mg/100 mL, respectively.

The narirutin-to-hesperidin and naringin-to-neohesperidin ratios have been proposed for quality control of orange and grapefruit commercial juices. The ratios obtained for the studied samples were compared to the values published by Rouseff (*27*). In orange juices the narirutin-to-hesperidin ratios were in all cases (0.138−0.262) in accordance with the lower limit reported by Rouseff; that is, this ratio has to be <0.339 for authentic orange juices (*28*). Squeezed juice showed the lowest value (0.04). The naringin-to-hesperidin ratios found for commercial grapefruit juices were 17.1 and 32.7 for samples 5 and 6, respectively, and 25.6 in fresh squeezed juice (sample 7), and all values were within the wide range of authenticity, that is, 14−83, indicated by Rouseff (*28*).

Representative chromatograms of orange, grapefruit, and lemon juice extracts are shown in **Figure 3**. Rhoifolin, the internal standard added to the sample juices, is known to occur in grapefruit and sour orange peel (*29*), but none was observed in citrus fruit juices unless added to the sample.

Intraday and interday repeatabilities of the capacity factor *k* as well as the coefficients (r^2) obtained by linear regression of the curves for quantitative analysis of each flavanone glycoside indicated good performance of the method, showing *k* interday and day-to-day variations below 0.2 and 0.4%, respectively. Interday peak area variation was below 4.0%. Main recovery of flavanones from juices, assessed by spiking samples with 20 μL of a 1.5 mg/mL standard solution of rhoifolin, was 98.2 ± 2.7%.

Determination of the Diastereomeric Composition of Flavanone-7-*O*-glycosides in Citrus Fruit Juices by CC-LC-MS. Flavanone glycosides have similar structures, containing a sugar moiety, which presents very low affinity toward ODS stationary phases. Therefore, these compounds cannot be resolved as the corresponding diastereomers by conventional reversed phase chromatography on a RP_{18} silica bonded column. The stereoisomer separation was investigated on three different derivatized β-CD-bonded packings. The chromatographic data are shown in **Table 2**. β-CD derivatized with carboxymethyl groups (CM-β-CD) exhibited higher selectivity, providing satisfactory discrimination of all the studied compounds. The different selectivities observed between β-CDs can be explained on the basis of a previous study regarding the analysis of flavanone glycoside diastereomers in citrus fruit juices by CE using sulfobutyl ether−β-CD in the background electrolyte (*15*). It was reasonably assumed that in the presence of cyclodextrins, flavanone glycosides fit the CD cavity with the aglycon unit, so finding proper conditions for hydrophobic interactions, whereas the sugar moiety or a part of it interacts by H-bonding with the groups bonded at the edge of the CD cavity. This latter interaction seems to be stronger with carboxymethyl than with permethyl or acetyl groups, and, in particular, for neohesperidoside disaccharides (neohesperidin and naringin), which exhibit a higher concentration of hydroxyl groups in the proximity of C7, than for rutinoside disaccharides (hesperidin and nariturin). Interactions of the substituent groups at C3′ and C4′ with the inner walls of CD should also stabilize the host−guest complexes by increasing discrimination, as in the case of neohesperidin.

Table 2. Separation of Flavanone Glycoside Diastereoisomers on β-CD-Bonded Stationary Phases: Permethylated β-CD (PM-β-CD), Acetylated β-CD (AC-β-CD), and Carboxymethylated β-CD (CM-β-CD)

	PM-β-CD		AC-β-CD		CM-β-CD	
compound	$k_{(2S)}$[a,b]	α[c]	$k_{(2S)}$[d]	α	$k_{(2S)}$[d]	α
naringin	9.96	1.0	2.60	1.0	11	1.20
narirutin	10.5	1.04	na[e]	na	14.0	1.09
hesperidin	11.2	1.09	7.97	1.0	15.1	1.16
neohesperidin	10.6	1.0	5.16	1.0	8.78	1.54
eriocitrin	na	na	na	na	12.7	1.19

[a] *k*, capacity factor of the more retained diastereoisomer. [b] Thirty percent methanol. [c] α, selectivity factor. [d] Twenty percent methanol. [e] Not analyzed.

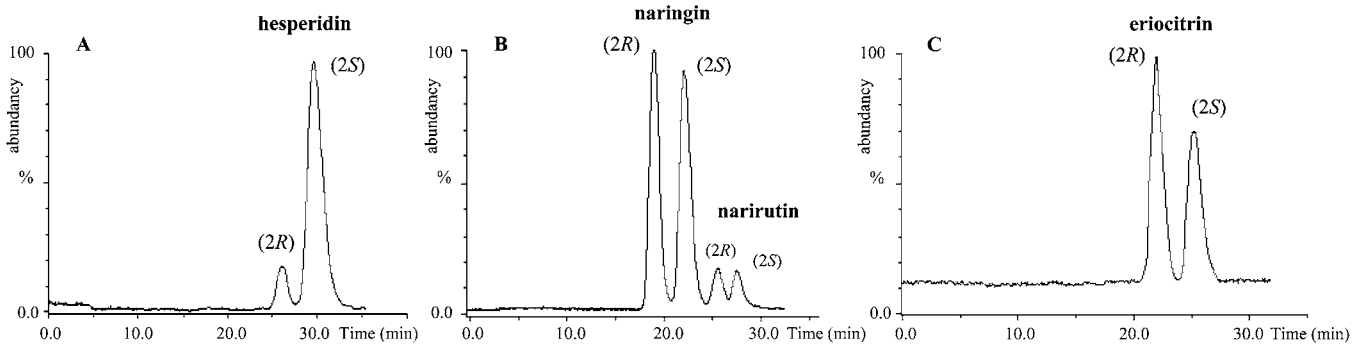

Figure 4. Separation by CC-MS of flavanone glycoside diastereomers in citrus juice extracts on columns packed with ODS/CM-β-CD-bonded stationary phases: (**A**) orange juice (sample 1); (**B**) (red) grapefruit juice (sample 5); (**C**) lemon juice (sample 8).

Table 3. Diastereoisomeric Ratios [(2*S*)/(2*R*)] of Flavanone Glycosides in Different Citrus Juices Determined by LC-LC-MS[a]

sample	type of juice	eriocitrin	naringin	narirutin	hesperidin
1	orange juice	nd[b]	na[c]	1.01	10.4
3	orange juice	nd	nd	0.96	6.42
4	hand-squeezed orange juice	nd	na	0.73	15.4
5	(red) grapefruit juice	nd	1.08	0.97	na
6	(white) grapefruit juice	nd	1.03	0.85	na
7	hand-squeezed (red) grapefruit juice	nd	1.32	1.31	na
8	lemon juice	0.72	nd	nd	19.5
9	hand-squeezed lemon juice	0.77	nd	nd	25.6

[a] Relative standard deviations for the calculated ratios were between 1 and 0.5%. [b] Not detected. [c] Not analyzed.

On CM-β-CD, with the exception of naringin and narirutin, all of the pairs of diastereomers eluted with similar retention times; thus, such a column does not fit for the simultaneous analysis of flavanone glycosides in complex mixtures. On the contrary, full isomer discrimination of mixtures of hesperidin, narirutin, naringin, and eriocitrin was accomplished by CC-LC mode. The diastereomeric 2*S*/2*R* ratios for eight citrus juices are reported in **Table 3**. Only the major flavanone glycoside constituents in the extracts were analyzed. The separated isomers were detected using an electrospray mass spectrometer in selected ion monitoring (SIM) mode. In accordance with data reported in the literature (*22*), the MS spectra were obtained by flow injection analysis in full scan mode. Hesperidin, narirutin, and eriocitrin (containing a rutinose moiety) showed intense molecular ions $[M - \text{sugar} - H]^-$ at *m*/*z* 609.5, 579.3, and 595.3, respectively, and intense aglycon molecular ions $[M - H]^-$ at *m*/*z* 301.3, 271.4, and *m*/*z* 287.3, respectively, as the result of the elimination of the sugar moiety. Naringin and neohesperidin (isomers with neohesperidose) showed only the glycosylated molecular ion.

Orange juices contained predominately flavanone rutinosides such as hesperidin and narirutin (**Table 1**). In an orange/sour orange cross freshly squeezed juice the isomeric ratio 2*S*/2*R* for hesperidin was 15.4, indicating that this flavanone was present almost exclusively as the 2*S* isomer (92%). In both commercial orange juices (samples 1 and 3) the ratio values were found to be lower, that is, 10.4 and 6.42, respectively. **Figure 4A** shows the separation of hesperidin diastereomers from an orange juice extract (sample 1). Contrary to hesperidin, narirutin in freshly squeezed juice showed a negligible difference in the stereoisomer composition, whereas in commercial products the two diastereomers were found in equal amount.

Grapefruit juices contained the flavanone rutinoside narirutin and the flavanone neohesperidoside naringin. On CM-β-CD the two pairs of stereoisomers corresponding to naringin and narirutin eluted with different elution times; thus, the four species were separated in a single chromatographic run without using the coupled-column system. Freshly squeezed grapefruit juice contained 56% (2*S*)-naringin, whereas a lower percentage of this isomer was found in commercial juices. A similar isomer composition of naringin in squeezed grapefruit juice has been reported by Krause and Galensa (*13*), who investigated the separation of flavanone glycoside diastereomers in citrus juices. However, these authors showed only partial discrimination of narirutin. Here, we estimated 55% (2*S*)-narirutin in fresh juice and an almost equal amount of isomers in commercial products (samples 5 and 6). **Figure 4B** illustrates the separation of both narirutin and naringin diastereomers in a grapefruit juice extract (sample 5).

The two lemon juices showed similar diastereomeric compositions for both eriocitrin and hesperidin. Eriocitrin was present as the 2*R* isomer at 58% (sample 8) and 56% (sample 9), whereas hesperidin was present almost exclusively as the isomer with 2*S* configuration (96%). **Figure 4C** shows the separation of the eriocitrin stereoisomers in a lemon juice extract (sample 8).

It has to be noted that the diastereomeric ratios can be affected by racemization reactions during the sample preparation. It has been assumed that the extent of interconversion of flavanone glycoside isomers could be related to the substituents in the aglycon unit; thus, hesperidin and neohesperidin, which both possess a 4′-methoxy group, should be more stable than narirutin and naringin (*13*).

Finally, it was considered of interest for method validation to determine the recovery of flavanone glycosides after the compounds were switched from one column to the other one. The recovery, calculated for naringin by triplicate injection of a grapefruit juice extract (sample 5), was 86.0 ± 2.8%.

ACKNOWLEDGMENT

We are indebted to Antonio Berti for technical assistance.

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Received for review February 27, 2004. Revised manuscript received May 21, 2004. Accepted May 26, 2004.

JF0400967