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Uruguayan essential oils. Part X. Composition of the oil of *Citrus clementine* Hort.†

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ABSTRACT: The composition of the essential oil of Uruguayan *Citrus clementine* Hort., prepared in the laboratory from the fruit of Nules and Comune cultivars, has been studied. The volatile fraction was analysed by HRGC and HRGC–MS (quadrupole); 69 components were identified; the enantiomeric distribution of β-pinene, sabinene, limonene, linalol and α-terpineol was studied by multidimensional HRGC–HRGC. Polymethoxylated flavones present in the non-volatile residue were analysed by normal-phase HPLC. The results were compared with those of Italian clementine oil. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: *Citrus clementine* Hort.; clementine; Rutaceae; Nules; Comune; volatile fraction composition; enantioselective gas chromatography; polymethoxylated flavones

Introduction

In recent years, *Citrus clementine* Hort. has become the most popular mandarin variety in the Mediterranean region, with production expanding particularly in Morocco and Spain. Clementines have been developed especially in Spain, and many new mutations have been discovered recently. This has extended the clementine season in Europe from its former 2-month period, from November to December, to cover the period from mid-October to mid-February.[2] In a previous paper we analysed the composition of *Citrus clementine* oil from the cultivars Monreal, Oroval and Comune grown in Italy. The composition of the volatile fraction and non-volatile residue was reported. This composition was then compared with that of Italian sweet orange and mandarin oil.[3]

In the context of research on Uruguayan citrus essential oils, we report here the results relative to the composition of the oil of two clementine cultivars oil grown in Uruguay: Nules and Comune. The cultivar Nules was discovered in Casrellon Province, Spain, in 1953 where it constitutes around half of the current plantings.[2]

In recent years Uruguay clementine production has increased and the export of clementine fruits grew from 71,420 g in 1995 to 86,340 g in 1996. The production period for clementine fruit in Uruguay extends from April to July. The extraction of this essential oil is not an industrial process but we like to check the composition of these oils which could provide new and interesting material for the food industries.

In the literature there are no reports about Uruguayan clementine oil, while only a few papers deal with Italian[4–7] and Algerian oils.[8]

Experimental

Research was carried out on two samples of Nules clementine oil and two samples of Comune clementine oil. Fruity were picked from April to May 1996 in Estacion Experimental INIA-Salto Grande, Departemento de Salto in North Uruguay. Extraction of the essential oil was carried out in the laboratory by applying manual pressure on the rind so as to cause the breaking of the utricles and the release of the oil itself, which was collected on a watch glass, transferred to a test tube, centrifuged and analysed.

GC Analysis

The volatile fraction was analysed by HRGC, under the same conditions used for Italian clementine oils,[3] on a

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Table 1. Percentage composition as single components and as classes of substances of Uruguayan and Italian clementine oils (*Citrus clementine* Hort.)

Date of sample		Uruguayan oils				Italian oils		
		Nules		Comune		Oroval	Monreal	Comune
		23/4/96	10/5/96	23/4/96	10/5/96	$\bar{X}$	$\bar{X}$	$\bar{X}$
1.	α-Thujene	0.01	0.01	0.01	0.01	0.01	tr	0.01
2.	α-Pinene	0.56	0.55	0.58	0.58	0.50	0.49	0.35
3.	Camphene	tr	tr	tr	tr	tr	0.20	tr
4.	Sabinene	0.94	0.99	1.09	0.98	0.68	0.29	1.04
5.	β-Pinene	0.21	0.22	0.26	0.21	0.17	0.07	0.05
6.	Myrcene	2.10	1.99	2.00	2.00	1.87	1.89	1.70
7.	Octanal	0.31	0.18	0.50	0.58	0.31	0.31	0.48
8.	α-Phellandrene	0.03	0.03	0.03	0.03	0.03	0.02	0.03
9.	δ-3-Carene	0.04	0.03	0.03	0.06	0.05	0.05	0.04
10.	α-Terpinene	tr	tr	tr	tr	tr	tr	0.01
11.	Limonene	93.50	93.78	92.94	93.29	93.85	94.50	92.36
12.	(*Z*)-β-Ocimene	0.01	0.01	tr	tr	0.01	0.02	0.01
13.	(*E*)-β-Ocimene	0.07	0.10	0.04	0.05	0.05	0.02	0.10
14.	γ-Terpinene	tr	tr	tr	tr	0.01	tr	0.01
15.	*cis*-Sabinene hydrate	0.03	0.03	0.03	0.03	0.02	tr	0.05
16.	Octanol	0.03	tr	0.11	0.01	0.01	tr	0.01
17.	Terpinolene	0.01	0.01	0.01	0.02	0.02	0.01	0.02
18.	*trans*-Sabinene hydrate	tr	tr	tr	tr	tr	tr	0.01
19.	Linalol	0.55	0.46	0.67	0.63	1.06	1.08	1.04
20.	Nonanal	0.02	0.01	0.01	0.01	0.01	0.01	0.03
21.	Heptyl acetate	0.01	0.01	tr	0.01	0.01	0.01	0.01
22.	*cis*-Limonene oxide	0.01	0.01	tr	0.01	0.01	0.01	0.02
23.	*trans*-Limonene oxide	0.03	0.01	tr	0.01	0.01	0.01	0.01
24.	Citronellal	0.06	0.04	0.06	0.07	0.07	0.07	0.08
25.	Terpinen-4-ol	0.01	tr	0.01	tr	tr	tr	0.01
26.	α-Terpineol	0.07	0.05	0.06	0.06	0.05	0.06	0.11
27.	Decanal	0.40	0.36	0.40	0.40	0.25	0.24	0.46
28.	Octyl acetate	tr	tr	0.01	tr	tr	0.01	0.01
29.	*cis*-Carveol	0.01	0.01	0.01	0.01	tr	0.01	0.04
30.	Nerol	0.02	0.02	0.02	0.01	0.01	0.01	0.03
31.	Neral	0.04	0.03	0.01	0.02	0.02	0.01	0.05
32.	Carvone	tr	tr	tr	tr	tr	tr	tr
33.	Geraniol	tr	tr	tr	tr	tr	tr	tr
34.	(*E*)-Dec 2-en-1-al	0.01	0.01	tr	0.01	0.01	0.01	0.01
35.	Geranial	0.02	0.03	0.03	0.03	0.03	0.04	0.08
36.	Perillaldehyde	0.02	0.02	0.03	0.04	0.04	0.04	0.03
37.	Perillalcohol	0.01	0.01	0.01	0.01	0.01	0.01	0.03
38.	Undecanal	0.01	0.01	tr	tr	tr	tr	0.01
39.	(*E*,*E*)-2,4-Decadienal	0.01	0.01	0.01	0.01	0.01	0.01	0.02
40.	α-Terpinyl acetate	tr	tr	tr	tr	tr	tr	tr
41.	Neryl acetate	tr	tr	tr	tr	tr	tr	tr
42.	α-Copaene	0.04	0.04	0.03	0.03	0.02	0.02	0.04
43.	Geranyl acetate	tr	tr	tr	tr	tr	tr	0.01
44.	β-Cubebene	0.04	0.04	0.03	0.03	0.02	0.02	0.04
45.	β-Elemene	0.01	0.01	0.01	0.01	0.01	0.01	0.02
46.	Dodecanal	0.08	0.08	0.06	0.08	0.05	0.05	0.10
47.	Methyl *N*-methylanthranilate	tr	tr	tr	tr	tr	0.01	0.01
48.	(*E*)-Caryophyllene	0.01	0.01	0.01	0.01	0.01	0.01	0.02
49.	β-Gurjunene	0.02	0.02	0.01	0.01	0.02	0.01	0.03
50.	*trans*-α-Bergamotene	tr	tr	tr	tr	tr	tr	tr
51.	(*Z*)-β-Farnesene	0.01	0.01	0.01	0.01	0.01	0.01	0.02
52.	α-Humulene	0.01	0.01	0.01	0.01	tr	0.01	0.01
53.	(*E*)-β-Farnesene	0.01	0.02	0.01	0.02	0.02	0.01	0.08
54.	(*E*)-Dodec-2-en-1-al	0.01	0.01	0.01	0.01	0.02	0.02	0.03
55.	γ-Muurolene	tr	tr	0.01	0.02	tr	tr	0.01
56.	Germacrene D	0.03	0.04	0.02	0.03	0.02	0.02	0.03
57.	Valencene	0.01	0.01	0.01	0.01	0.01	0.01	0.03
58.	Biciclogermacrene	tr	0.01	tr	tr	tr	tr	0.01
59.	(*E*,*E*)-α-Farnesene	0.04	0.04	0.03	0.04	0.05	0.03	0.11
60.	β-Bisabolene	tr	tr	tr	tr	tr	tr	tr
61.	δ-Cadinene	0.04	0.05	0.03	0.04	0.03	0.03	0.05
62.	Tridecanal	tr	tr	tr	tr	tr	tr	0.01
63.	(*E*)-Nerolidol	0.01	tr	tr	tr	0.01	0.01	0.01
64.	Tetradecanal	tr	tr	0.01	tr	tr	tr	0.01
65.	Tetradecanol	tr	tr	tr	tr	tr	tr	0.01

(*Table continues opposite*)

Table 1. *Continued*

Date of sample		Uruguayan oils				Italian oils		
		Nules		Commune		Oroval	Monreal	Comune
		23/4/96	10/5/96	23/4/96	10/5/96	$\bar{X}$	$\bar{X}$	$\bar{X}$
66.	β-Sinensale	0.04	0.07	0.05	0.05	0.06	0.03	0.16
67.	α-Sinensale	0.21	0.28	0.23	0.24	0.30	0.26	0.56
68.	Nootkatone	tr	tr	0.03	0.01	0.01	0.01	0.01
	Hydrocarbons	97.72	98.02	97.20	97.49	97.49	97.56	96.23
	Monoterpenes	97.46	97.71	96.99	97.23	97.26	97.36	95.73
	Sesquiterpenes	0.26	0.31	0.20	0.26	0.23	0.19	0.50
	Oxygenated compounds	2.00	1.75	2.37	2.35	2.41	2.36	3.52
	Carbonyl compounds	1.21	1.14	1.44	1.54	1.19	1.12	2.14
	Alcohols	0.73	0.58	0.91	0.77	1.19	1.18	1.31
	Esters	0.02	0.02	0.02	0.02	0.01	0.02	0.04
	Linalol/Decanal	1.4	1.3	1.7	1.6	4.2	4.5	2.3

Fisons Mega Series 5160 gas chromatograph equipped with a Shimadzu data processor C-R3A and a fused silica SE-52 column (30 m × 0.32 mm i.d., film thickness, 0.40–0.45 μm, Mega, Legnano (MI), Italy); column temperature, 45°C (6 min) to 180°C at 3°C/min; injection mode, split; detector, FID; injector and detector temperature, 280°C; carrier gas, He 95 kPa; injected volume, 1 μl of neat oil.

GC–MS Analysis

Samples were analysed by GC–MS (EI) on a Fisons MD 800 (Milan, Italy) system coupled with Adams' library[9] and FFC bank;[10] GC conditions were: a DB-5 fused silica column (30 m × 0.25 mm, 0.25 μm film thickness); column temperature, 45°C (6 min) to 240°C at 3°C/min; carrier gas, He at constant pressure of 83 kPa. Aquisition parameters, full scan; scan range, 41–300 amu; temperature transfer line, 220°C; 1 μl of solution (0.33% v/v essential oil/pentane) was injected on a cold on-column system fitted with an automated actuator. The MS scan conditions were: source temperature, 200°C; interface temperature, 260°C; E energy, 70 eV; mass scan range, 39.00–350.00 amu.

Chiral Analysis

Enantiomeric ratios of some monoterpene hydrocarbons (β-pinene, sabinene, limonene) and of some monoterpene alcohols (linalol, α-terpineol) were obtained by multidimensional gas chromatography, using a developmental model[11] set up with two GC ovens, the first equipped with a column coated with SE-52 and the second with a chiral column coated with a derivatized β-cyclodextrin, a hot interface, a rotary switching valve and a system to maintain a constant

Table 2. Enantiomeric ratios for β-pinene, sabinene, limonene, linalol and α-terpineol in Uruguayan clementine oils (*Citrus clementine* Hort.)

Date of sample	Uruguayan oils			
	Nules		Comune	
	23/4/96	10/5/96	23/4/96	10/5/96
β-Pinene				
1R,5R (+)	58.1	61.1	60.1	60.1
1S,5S (−)	41.9	38.9	39.9	39.9
Sabinene				
1R,5R (+)	97.5	97.6	97.6	97.6
1S,5S (−)	2.5	2.4	2.4	2.4
Limonene				
4S (−)	0.6	0.6	0.6	0.6
4R (+)	99.4	99.4	99.4	99.4
Linalol				
3R (−)	6.7	6.7	5.0	5.3
3S (+)	93.3	93.3	95.0	94.7
α-Terpineol				
8S (−)	2.7	2.5	2.5	2.6
8R (+)	97.3	97.5	97.5	97.4

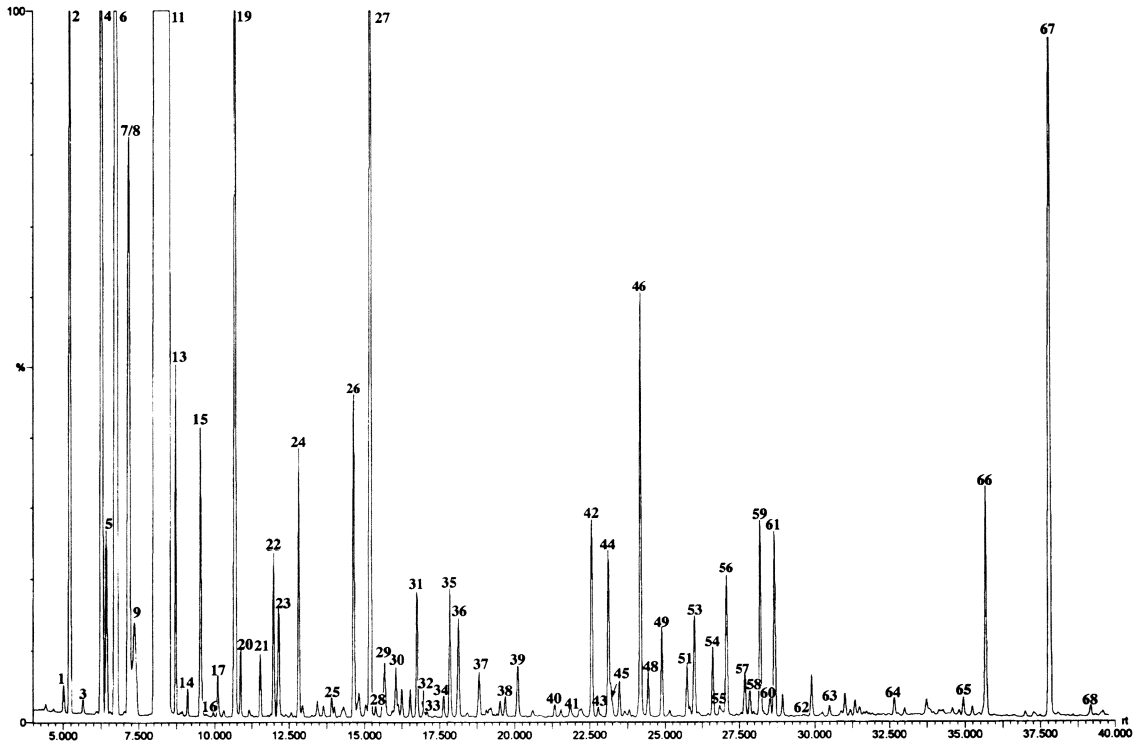

Figure 1. Total ion chromatogram of the volatile fraction of a Nules clementine oil. For peak identification see Table 1

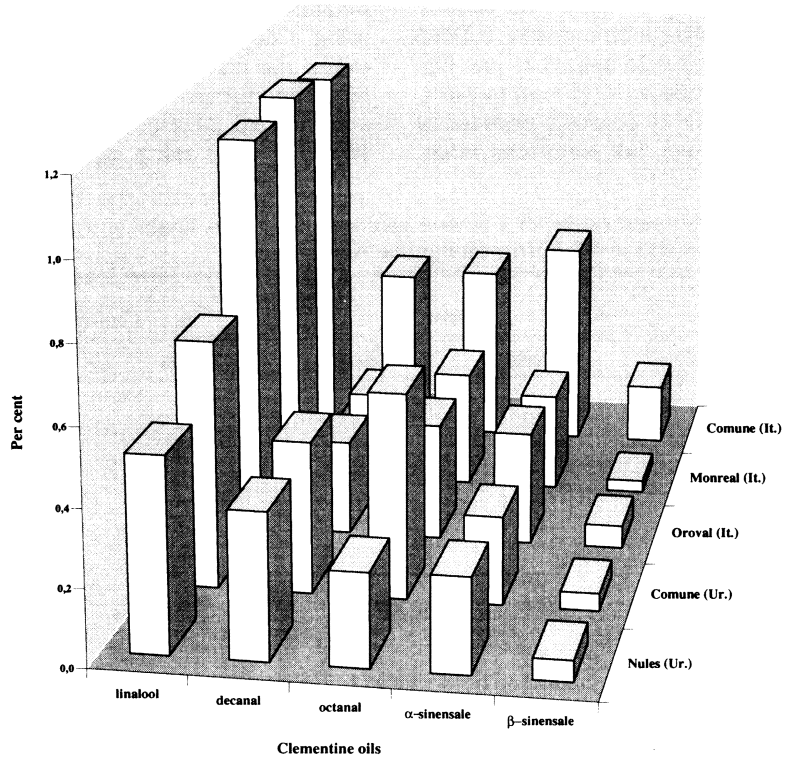

Figure 2. Linalol, decanal, octanal, α-sinensal and β-sinensal content of Uruguayan and Italian clementine oils

Table 3. Content (g/100 of oil) of Polymethoxylated Flavones in Uruguayan and Italian clementine oils (*Citrus clementine* Hort.)

	Uruguayan oils				Italian oils		
	Nules		Commune		Monreal	Oroval	Comune
	23/4/96	10/5/96	23/4/96	10/5/96	$\bar{X}$	$\bar{X}$	$\bar{X}$
Tangeretin	0.13	0.15	0.17	0.14	0.10	0.10	0.17
3,3′,4′,5,6,7,8-Heptamethoxyflavone	0.53	0.32	0.38	0.36	0.27	0.29	0.51
Nobiletin	0.14	0.12	0.11	0.10	0.09	0.09	0.09
Tetra-*O*-methylscutellarein	0.07	0.07	0.08	0.07	0.05	0.04	0.06
3,3′,4′,5,6,7-Hexamethoxyflavone	0.06	0.04	0.04	0.04	0.02	0.02	0.02
Sinensetin	0.01	0.01	0.00	0.01	tr	tr	tr

flow during the transfer. With this system a heart-cut of the relevant fractions can be made and these fractions transferred from the non-chiral column to the chiral one in the following experimental conditions: pre-column, fused silica SE-52 column (30 m × 0.32 mm i.d., film thickness 0.40–0.45 μm, Mega, Legnano (MI), Italy); column temperature 45°C (6 min) to 220°C at 2°C/min; analytical column, fused silica capillary column 25 m × 0.25 mm i.d., coated with a diethyl *tert*-butylsilyl-β-cyclodextrin, Mega, Legnano (MI), Italy; column temperature, 40–180°C at 2°C/min; interface temperature, 200°C; detector FID, 250°C (for both chromatographs).

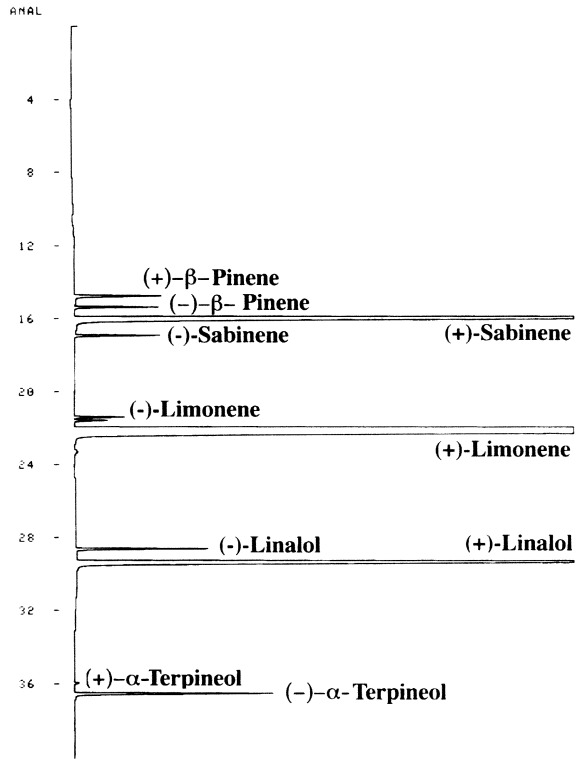

Figure 3. Chiral gas chromatogram of a Nules clementine oil

HPLC Analysis

All samples were analysed by normal-phase HPLC, using Waters Associates (WA) equipment composed of a model 519 pump; a 600 E gradient controller, a Rheodyne 9125 injector and a photo-diode array detector model 996. Peak integration and quantitative calculations were performed by Millenium 2010 (WA) system using a calibration curve obtained for each standard component against a coumarin standard.[12] The column was a Zorbax silica column (25 cm × 4.6 mm i.d.; particle size, 7 μm); mobile phase, hexane:ethyl alcohol, 95:5; flow rate, 1.6 ml/min; injection volume, 20 μl of a solution obtained by diluting about 50 mg of each oil and 0.1 ml of a coumarin solution of known concentration in 1 ml of hexane:ethyl acetate (75:25). Detection was by UV absorbance at 315 nm. The UV spectra of eluting peaks were monitored with the PDA detector in the region 200–400 nm.

Results

Volatile Fraction

Figure 1 shows the total ion chromatogram of a Nules clementine oil; the composition as classes of substances and as single components is reported in Table 1. Sixty-nine components were identified in each oil: the same number were identified in Italian oils.

The oils were characterized by a high content of limonene (more than 93%) and, among oxygenated compounds, of linalol (0.5–0.7%) and decanal (0.4%), by the presence of δ-3-carene, of a large number (16) of sesquiterpenes and of β-sinensal and α-sinensal (about 0.3% together). Among the oxygenated compounds, the main class was carbonyl compounds followed by alcohols; esters were less represented.

The composition of the oil of the two cultivars was very similar. Small differences were due to the alcohol and carbonyl compound content which were higher in

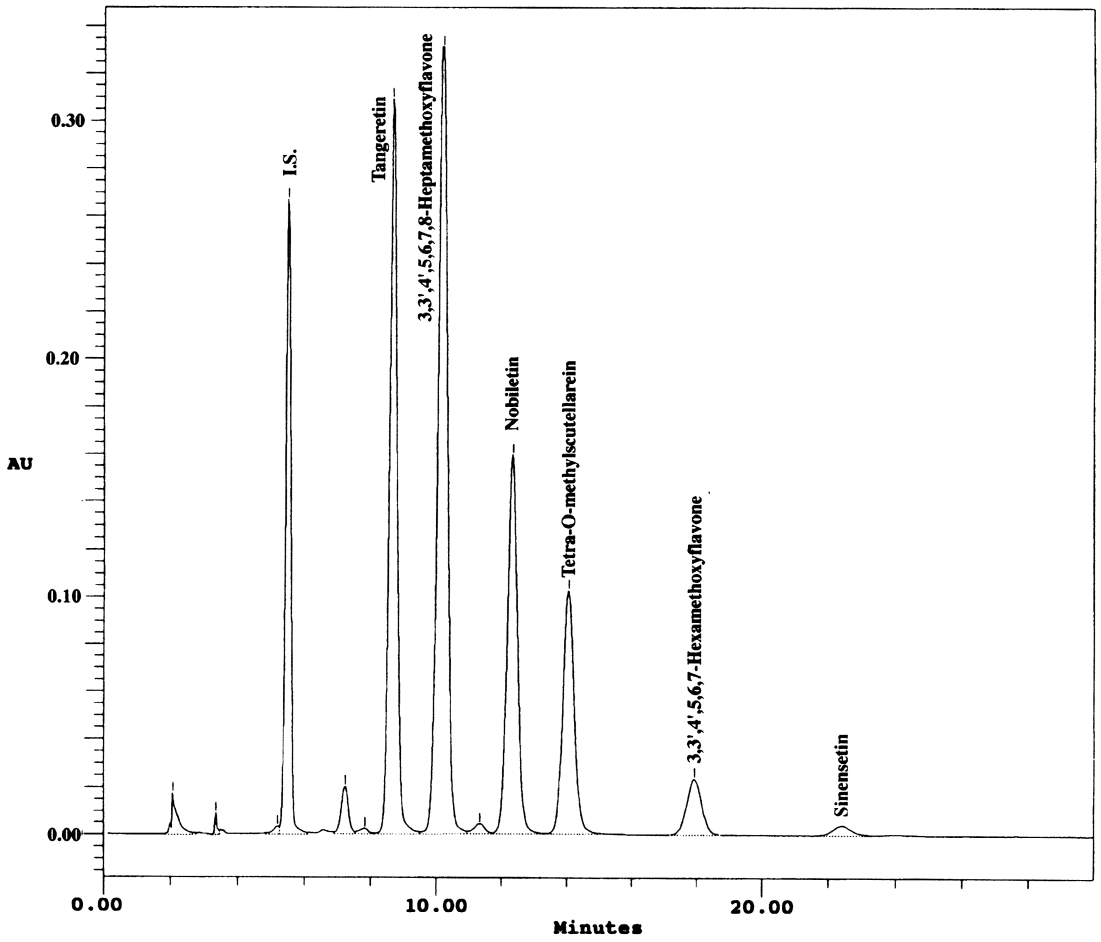

Figure 4. HPLC chromatogram of a Nules clementine oil

Comune oil than in that of Nules. Limonene showed an opposite behaviour.

Table 1 also shows the composition of Italian clementine oils. Comparing Italian and Uruguayan oils, the major differences concern the alcohol and carbonyl compound content. These differences are more evident from Figure 2, which shows the average content of linalol, decanal, octanal, α-sinensal and β-sinensal in Uruguayan and Italian oils. In the last line of Table 1, the ratio between linalol and decanal is reported. The value of this ratio could be used to distinguish Italian clementine oils from those of Uruguay.

Enantiomeric Ratios

The enantiomeric ratio of five components was determined by four subsequent transfers during the same analysis. Figure 3 shows the chiral chromatogram. Enantiomeric ratios of the components analysed (Table 2) are similar for Nules and Comune oils. The most noticeable difference was observed for linalol, which showed a (−)/(+) enantiomeric ratio of 7:93 in Nules oils and 5:95 in Comune oils. These values were intermediate to those reported for linalol in Italian clementine oils (Monreal and Oroval, 3:97; Comune, 9:91).[3]

Polymethoxylated Flavones

Figure 4 shows the HPLC chromatogram of a Nules clementine oil. Five polymethoxylated flavones have been identified in the oil analysed, viz. tangeretin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tetra-O-methylscutellarein, 3,3′,4′,5,6,7-hexamethoxyflavone and sinensetin, the same components found in Italian oils.[3]

Table 3 reports the content (g/100 g of oil) of polymethoxylated flavones in Nules and Comune clementine oil. The same table also reports the average content of polymethoxylated flavones in the clementine oils

of the three Italian cultivars previously analysed. As in the Italian oils, the main component was 3,3′,4′,5,6,7,8-heptamethoxyflavone which ranges from 0.32 to 0.53 g/100 in the oils analysed. As regard polymethoxyflavones, Italian and Uruguayan clementines appear very similar.

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