FLAVOUR AND FRAGRANCE JOURNAL, VOL. 12, 163–172 (1997)

On the Genuineness of Citrus Essential Oils. Part LII. Chemical Characterization of Essential Oil of three Cultivars of *Citrus clementine* Hort.

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The composition of *Citrus clementine* Hort. essential oil, laboratory-prepared from fruits of different cultivars, has been studied. The following cultivars were examined: Comune, Oroval and Monreal. The volatile fraction was analysed by HRGC and HRGC–MS (quadrupole); 69 components were identified. The composition as single components and as classes of substances for each sample and the average composition for each cultivar are reported. The enantiomeric distribution of linalol was studied by HRGC with β-cyclodextrin columns. Polymethoxylated flavones, present in the non-volatile residue, were analysed by normal phase HPLC. Six components were identified: tangeretin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tetra-*O*-methylscutellarein, sinensetin and 3,3′,4′,5,6,7-hexamethoxyflavone. Clementine oil composition was then compared with that of Italian sweet orange and mandarin oil. © 1997 by John Wiley & Sons, Ltd.

Flavour Fragr. J., **12**, 163–172 (1997) (No. of Figures: 4 No. of Tables: 6 No. of Refs: 17)

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

INTRODUCTION

Citrus clementine Hort. is considered a mandarin (clementine fruit is similar to mandarin) and the following cultivars are grown: Comune, Monreal, Oroval and Nules. Clementine production has been increasing in recent years and Italian industries have started to process these fruits with the same production line used to obtain mandarin oil.

Initial research on the volatile fraction composition of Italian clementine oil, using gas chromatography, was carried out on an experimental industrial clementine oil; 17 components were identified and the main component was limonene (83.03%).[2] Dugo *et al.* reported the composition of three laboratory prepared clementine oils when the main components were limonene ($\bar{X} = 95.01\%$) and myrcene ($\bar{X} = 1.63\%$).[3] The composition of a clementine oil volatile fraction, produced in Algeria, was analysed by GC–MS and 24 components were identified.[4] The composition of a Sicilian clementine oil was also reported in the context of a research on the essential oil of a new hybrid obtained by cross-breeding *C. clementine* and *C. limon*.[5] Finally, Mondello *et al.* analysed monoterpenes and sesquiterpenes present in the volatile fraction of an industrial clementine oil by coupled HPLC–HRGC–MS(ITD).[6] No information has been reported about the non-volatile residue; other researches refer to the juice composition.[7–10]

Overall, information reported in literature is scant and refers to a limited number of samples, of which the cultivar has never been indicated.

In this paper the composition of the volatile and non-volatile fraction of clementine oil from different cultivars is studied in order to obtain more detailed knowledge of a product which could be a new and interesting material for the cosmetic and food industries.

EXPERIMENTAL

Research was carried out on three samples of Comune clementine oil, three samples of Oroval

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Contract grant sponsor: Ministero dell'Università e della Ricerca Scientifica, Italy.

CCC 0882–5734/97/030163–10$17.50

Received 29 June 1996
Accepted 12 August 1996

clementine oil and three samples of Monreal clementine oil.

Fruits were picked from December 1995 to January 1996 near Reggio, Calabria. 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

Volatile fraction was analysed by HRGC, under the same conditions used for mandarin and sweet orange oils,[11–13] on a gas chromatograph Fisons Mega Series 5160 equipped with a Shimadzu data processor C-R3A; fused silica column SE-52 (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 temperatures, 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[14] and FFC banks;[15] GC conditions were: fused silica column SE-52 (60 m × 0.32 mm i.d., film thickness, 0.40–0.45 μm, Mega, Legnano (MI), Italy); column temperature 45°C (6 min) to 111°C at 3°C/min, then to 160°C at 2°C/min and to 300°C at 3°/min and held for 15 min; carrier gas, He, was delivered at constant pressure of 70 kPa (40.5 cm/sec). 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

The enantiomeric distribution of linalol was carried out by GC on a gas chromatograph Fisons Mega series 5160, equipped with a Shimadzu data processor C-R3A; fused capillary column (25 m × 0.25 mm i.d., coated with diethyl *tert*-butylsilyl-β-cyclodextrin, Mega, Legnano (MI), Italy);[16] column temperature, 40–140°C at 2.5°C/min; injection mode, split; detector, FID; injector and detector temperatures, 280°C; carrier gas, hydrogen, 0.70 kg/cm^2; injected volume, 1 μl of a solution obtained by diluting 3 μl of clementine oil in 1 ml of pentane.

HPLC Analysis

All samples were analysed by normal-phase HPLC, using Waters Associates (W.A.) 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 (W.A.) system, using a calibration curve obtained for each standard component against a coumarin standard.[17] 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

Sixty-nine components were identified in each oil. The quantitative data were divided according to the fruit cultivar (Oroval, Monreal, Comune); for each cultivar the average ($\bar{X}$) and the standard deviation (s) of all components were calculated. These data are reported in Tables 1–3. Moreover for each sample, the total amounts of hydrocarbons, oxygenated compounds, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols and esters were also calculated.

Figure 1 shows, for example, the chromatogram of a Comune clementine oil. With respect to previous researches[2–6] on clementine oil, the following additional components have been identified: heptyl acetate, *cis*-limonene oxide, *trans*-limonene oxide, octyl acetate, carvone, geraniol, (*E*)-dec-2-en-1-al, *trans*-α-bergamotene, (*Z*)-β-farnesene, (*E*)-dodec-2-en-1-al, bicyclogermacrene, β-bisabolene, tridecanal, (*E*)-nerolidol, tetradecanal and tetradecanol.

These oils were characterized by the presence of a large number of sesquiterpenes (17), of δ-3-carene,

Table 1. Percentage composition as classes of substances and as single components of Oroval clementine oils

	1 Dec '95	2 Dec '95	3 Jan '96	$\bar{X}$	s
1 α-Thujene	0.01	0.01	t	0.01	0.001
2 α-Pinene	0.50	0.49	0.51	0.50	0.010
3 Camphene	t	t	t	t	–
4 Sabinene	0.86	0.52	0.67	0.68	0.170
5 β-Pinene	0.22	0.13	0.17	0.17	0.045
6 Myrcene	1.90	1.77	1.95	1.87	0.093
7 Octanal	0.39	0.36	0.17	0.31	0.119
8 α-Phellandrene	0.04	0.04	0.02	0.03	0.012
9 δ-3-Carene	0.05	0.04	0.06	0.05	0.010
10 α-Terpinene	t	t	t	t	–
11 Limonene	92.98	94.11	94.46	93.85	0.774
12 (*Z*)-β-Ocimene	0.02	0.01	0.01	0.01	0.006
13 (*E*)-β-Ocimene	0.07	0.04	0.04	0.05	0.017
14 γ-Terpinene	0.01	0.01	t	0.01	0.004
15 *cis*-Sabinene hydrate	0.03	0.02	0.01	0.02	0.010
16 Octanol	0.01	0.01	t	0.01	0.002
17 Terpinolene	0.02	0.01	0.02	0.02	0.006
18 *trans*-Sabinene hydrate	t	t	t	t	–
19 Linalol	1.21	1.21	0.77	1.06	0.254
20 Nonanal	0.01	0.01	0.01	0.01	0.000
21 Heptyl acetate	0.01	0.01	t	0.01	0.004
22 *cis*-Limonene oxide	0.02	0.01	0.01	0.01	0.006
23 *trans*-Limonene oxide	0.01	0.01	0.01	0.01	0.000
24 Citronellal	0.09	0.06	0.07	0.07	0.015
25 Terpinen-4-ol	0.01	t	t	t	–
26 α-Terpineol	0.07	0.06	0.03	0.05	0.021
27 Decanal	0.31	0.21	0.22	0.25	0.055
28 Octyl acetate	t	t	t	t	–
29 *cis*-Carveol	t	t	t	t	–
30 Nerol+Citronellol	0.02	0.01	0.01	0.01	0.006
31 Neral	0.02	0.02	0.01	0.02	0.006
32 Carvone	t	t	t	t	–
33 Geraniol	t	t	t	t	–
34 (*E*)-Dec-2-en-1-al	0.01	t	0.01	0.01	0.003
35 Geranial	0.03	0.04	0.02	0.03	0.010
36 Perilla aldehyde	0.04	0.05	0.02	0.04	0.015
37 Perilla alcohol	0.02	0.02	0.01	0.01	0.006
38 Undecanal	0.01	t	t	t	–
39 (*E*, *E*)-Deca-2,4-dienal	0.01	t	0.01	0.01	0.004
40 α-Terpinyl acetate	t	t	t	t	–
41 Neryl acetate	t	t	t	t	–
42 α-Copaene	0.03	0.02	0.02	0.02	0.006
43 Geranyl acetate	t	t	t	t	–
44 β-Cubebene	0.03	0.02	0.02	0.02	0.006
45 β-Elemene	0.01	0.01	0.01	0.01	0.000
46 Dodecanal	0.06	0.05	0.05	0.05	0.006
47 Methyl N-methyl anthranilate	t	t	t	t	–
48 (*E*)-Caryophyllene	0.01	0.01	0.01	0.01	0.000
49 β-Gurjunene	0.02	0.02	0.02	0.02	0.000
50 *trans*-α-Bergamotene	t	t	t	t	–
51 (*Z*)-β-Farnesene	0.01	0.01	0.01	0.01	0.000
52 α-Humulene	t	t	0.01	t	–
53 (*E*)-β-Farnesene	0.03	0.02	0.02	0.02	0.006
54 (*E*)-Dodec-2-en-1-al	0.02	0.01	0.02	0.02	0.006
55 γ-Muurolene	t	t	t	t	–
56 Germacrene-D	0.03	0.02	0.02	0.02	0.006
57 Valencene	0.02	0.01	0.01	0.01	0.006
58 Bicyclogermacrene	t	t	t	t	–
59 (*E*, *E*)-α-Farnesene	0.06	0.04	0.04	0.05	0.012

Table 1 continues on next page

Table 1. Continued

	1 Dec '95	2 Dec '95	3 Jan '96	$\bar{X}$	s
60 β-Bisabolene	t	t	t	t	–
61 δ-Cadinene	0.03	0.02	0.03	0.03	0.006
62 Tridecanal	t	t	t	t	–
63 (*E*)-Nerolidol	0.01	0.01	0.01	0.01	0.000
64 Tetradecanal	0.01	t	t	t	–
65 Tetradecanol	0.01	t	t	t	–
66 β-Sinensal	0.09	0.04	0.05	0.06	0.026
67 α-Sinensal	0.39	0.25	0.27	0.30	0.076
68 Nootkatone	0.01	0.01	0.01	0.01	0.000
Hydrocarbons	96.96	97.38	98.13	97.49	0.593
Oxygenated compounds	2.93	2.50	1.80	2.41	0.570
Monoterpenes	96.68	97.18	97.91	97.26	0.619
Sesquiterpenes	0.28	0.20	0.22	0.23	0.042
Carbonyl compounds	1.50	1.12	0.94	1.19	0.286
Alcohols	1.39	1.33	0.84	1.19	0.302
Esters	0.01	0.01	t	0.01	0.004

$\bar{X}$ = average content; s = standard deviation.

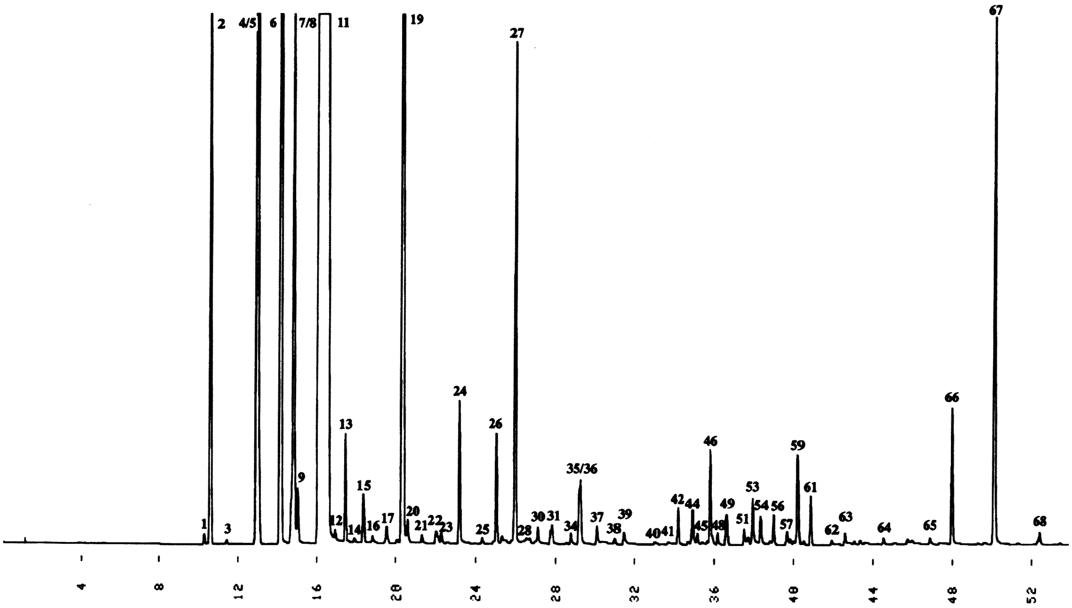

Fig. 1. Gas chromatogram of the volatile fraction of a Comune clementine oil. For peak identification see Table 1.

of β-sinensal, of α-sinensal and of nootkatone. The content of β-sinensal (0.02–0.19) and of α-sinensal (0.24–0.68) and the ratio between these sesquiterpene aldehydes distinguish clementine oil from the other citrus oils.

Monoterpenes were present in quantities which vary (95.01 to 97.91%), with limonene the main component (over 91%).

Carbonyl compound content varied from 0.94 to 2.38%; octanal, decanal and α-sinensal were the most abundant.

Alcohol content varied from 0.84 to 1.81% with linalol the main component. Ester content was very low: from trace to 0.05%.

Looking at the composition as single components and as classes of substances, quantitative

Table 2. Percentage composition as classes of substances and as single components of Monreal clementine oils

	1 Dec. '95	2 Dec. '95	3 Jan. '96	$\bar{X}$	s
1 α-Thujene	t	t	t	t	–
2 α-Pinene	0.48	0.50	0.48	0.49	0.012
3 Camphene	t	t	t	t	–
4 Sabinene	0.30	0.28	0.29	0.29	0.010
5 β-Pinene	0.08	0.07	0.07	0.07	0.006
6 Myrcene	1.79	1.85	2.03	1.89	0.125
7 Octanal	0.32	0.29	0.33	0.31	0.021
8 α-Phellandrene	0.02	0.02	0.02	0.02	0.000
9 δ-3-Carene	0.04	0.07	0.04	0.05	0.017
10 α-Terpinene	t	t	t	t	–
11 Limonene	94.72	94.78	94.00	94.50	0.434
12 (*Z*)-β-Ocimene	0.02	0.01	0.02	0.02	0.006
13 (*E*)-β-Ocimene	0.03	0.02	0.02	0.02	0.006
14 γ-Terpinene	t	t	0.01	t	–
15 *cis*-Sabinene hydrate	t	t	t	t	–
16 Octanol	t	t	t	t	–
17 Terpinolene	0.01	0.02	0.01	0.01	0.006
18 *trans*-Sabinene hydrate	t	t	t	t	–
19 Linalol	0.95	0.93	1.35	1.08	0.237
20 Nonanal	0.01	0.01	0.02	0.01	0.006
21 Heptyl acetate	0.01	0.01	0.01	0.01	0.000
22 *cis*-Limonene oxide	0.01	0.01	0.01	0.01	0.000
23 *trans*-Limonene oxide	0.01	0.01	0.01	0.01	0.000
24 Citronellal	0.07	0.07	0.08	0.07	0.006
25 Terpinen-4-ol	t	t	t	t	–
26 α-Terpineol	0.06	0.05	0.06	0.06	0.004
27 Decanal	0.21	0.20	0.32	0.24	0.067
28 Octyl acetate	t	0.01	0.01	0.01	0.004
29 *cis*-Carveol	0.01	t	0.01	0.01	0.004
30 Nerol+Citronellol	0.02	0.01	0.01	0.01	0.006
31 Neral	0.02	0.01	0.01	0.01	0.006
32 Carvone	t	t	t	t	–
33 Geraniol	t	t	t	t	–
34 (*E*)-Dec-2-en-1-al	0.01	0.01	0.01	0.01	0.000
35 Geranial	0.04	0.03	0.04	0.04	0.006
36 Perilla aldehyde	0.04	0.03	0.04	0.04	0.006
37 Perilla alcohol	0.01	0.01	0.01	0.01	0.000
38 Undecanal	t	t	0.01	t	–
39 (*E*, *E*)-Deca-2,4-dienal	0.01	0.01	0.01	0.01	0.000
40 α-Terpinyl acetate	t	t	t	t	–
41 Neryl acetate	t	t	t	t	–
42 α-Copaene	0.02	0.02	0.03	0.02	0.006
43 Geranyl acetate	t	t	t	t	–
44 β-Cubebene	0.02	0.02	0.02	0.02	0.000
45 β-Elemene	0.01	0.01	0.01	0.01	0.000
46 Dodecanal	0.04	0.04	0.07	0.05	0.017
47 Methyl *N*-methyl anthranilate	0.01	t	0.01	0.01	0.004
48 (*E*)-Caryophyllene	0.01	0.01	t	0.01	0.004
49 β-Gurjunene	0.01	0.01	0.01	0.01	0.000
50 *trans*-α-Bergamotene	t	t	t	t	–
51 (*Z*)-β-Farnesene	0.01	0.01	0.01	0.01	0.000
52 α-Humulene	0.01	0.01	0.01	0.01	0.000
53 (*E*)-β-Farnesene	0.01	0.01	0.01	0.01	0.000
54 (*E*)-Dodec-2-en-1-al	0.02	0.02	0.03	0.02	0.006
55 γ-Muurolene	t	t	t	t	–
56 Germacrene-D	0.02	0.02	0.02	0.02	0.000
57 Valencene	0.01	0.01	0.01	0.01	0.000
58 Bicyclogermacrene	t	t	t	t	–
59 (*E*, *E*)-α-Farnesene	0.04	0.03	0.03	0.03	0.006

Table 2 continues on next page

Table 2. Continued

	1 Dec. '95	2 Dec. '95	3 Jan. '96	X̄	s
60 *β*-Bisabolene	t	t	t	t	–
61 *δ*-Cadinene	0.03	0.03	0.03	0.03	0.000
62 Tridecanal	t	t	t	t	–
63 (*E*)-Nerolidol	0.01	0.01	0.01	0.01	0.000
64 Tetradecanal	t	t	t	t	–
65 Tetradecanol	t	t	t	t	–
66 *β*-Sinensal	0.03	0.03	0.02	0.03	0.006
67 α-Sinensal	0.29	0.26	0.24	0.26	0.025
68 Nootkatone	0.01	0.01	0.01	0.01	0.000
Hydrocarbons	97.69	97.81	97.17	97.56	0.340
Oxygenated compounds	2.25	2.08	2.74	2.36	0.342
Monoterpenes	97.49	97.62	96.98	97.36	0.338
Sesquiterpenes	0.20	0.19	0.19	0.19	0.006
Carbonyl compounds	1.12	1.02	1.23	1.12	0.105
Alcohols	1.07	1.02	1.46	1.18	0.241
Esters	0.02	0.02	0.03	0.02	0.006

X̄ = average content; s = standard deviation.

differences appear, which are related to the three cultivars. Comune oils had a lower average content of hydrocarbons and monoterpenes than Oroval and Monreal oils. Among monoterpenes, each cultivar showed a very different value for sabinene; the same behavior was observed for sweet orange oils of different cultivars.[13] As regards sesquiterpenes, Comune oils showed the highest value, mainly due to the (*E*)-*β*-farnesene and (*E*, *E*)-α-farnesene content.

Oxygenated compound average content was about the same for Monreal and Oroval oils while Comune oils showed higher values. The main difference in oxygenated compound content was due to carbonyl compound content; aliphatic and terpene aldehydes showed higher values in Comune oils than in Monreal and Oroval ones. The values of octanal, decanal, dodecanal, α-sinensal and *β*-sinensal in Comune oils are shown in Figure 2. Comune oils also showed the highest alcohol and ester content. Each alcohol showed a higher content in Comune oils, except linalol, whose content was about the same in the different cultivars.

Comparing the volatile fraction of clementine oil with that of mandarin[11] or sweet orange,[12–13] it is similar to that of sweet orange oil in terms of limonene, decanal and ester content and of the large number of sesquiterpenes while the α-sinensal content is the only characteristic common to mandarin oil.

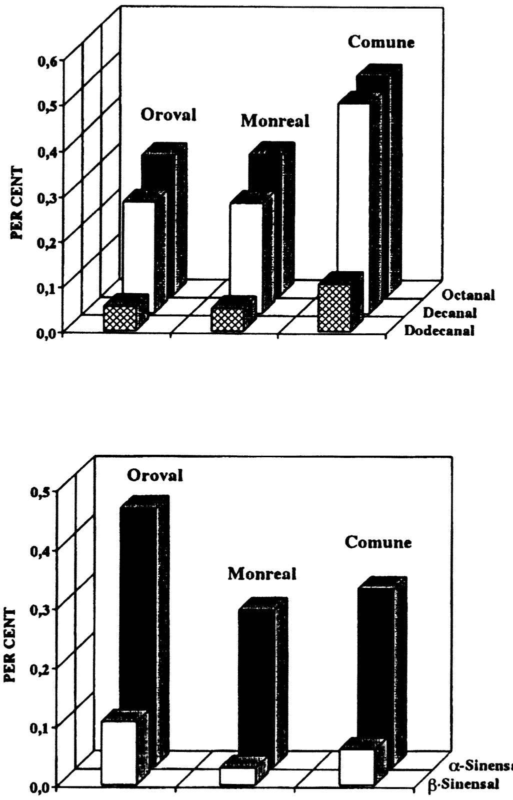

Fig. 2. Octanal, decanal, dodecanal, *β*-sinensal and α-sinensal average content for Monreal, Oroval and Comune clementine oils.

Table 3. Percentage composition as classes of substances and as single components of Comune clementine oils

	1 Dec. '95	2 Dec. '95	3 Jan.'96	$\bar{X}$	s
1 α-Thujene	0.01	0.01	0.01	0.01	0.000
2 α-Pinene	0.30	0.37	0.39	0.35	0.047
3 Camphene	t	t	t	t	–
4 Sabinene	1.26	0.64	1.22	1.04	0.347
5 β-Pinene	0.07	0.03	0.06	0.05	0.021
6 Myrcene	1.61	1.68	1.80	1.70	0.096
7 Octanal	0.53	0.40	0.52	0.48	0.072
8 α-Phellandrene	0.03	0.02	0.03	0.03	0.006
9 δ-3-Carene	0.03	0.04	0.04	0.04	0.006
10 α-Terpinene	t	0.01	0.01	0.01	0.004
11 Limonene	91.50	93.57	92.00	92.36	1.080
12 (*Z*)-β-Ocimene	0.01	t	0.02	0.01	0.009
13 (*E*)-β-Ocimene	0.16	0.05	0.10	0.10	0.055
14 γ-Terpinene	0.01	0.01	0.02	0.01	0.006
15 *cis*-Sabinene hydrate	0.07	0.03	0.06	0.05	0.021
16 Octanol	0.02	0.01	0.02	0.01	0.006
17 Terpinolene	0.02	0.02	0.02	0.02	0.000
18 *trans*-Sabinene hydrate	0.01	t	0.01	0.01	0.003
19 Linalol	1.52	0.64	0.97	1.04	0.445
20 Nonanal	0.03	0.02	0.03	0.03	0.006
21 Heptyl acetate	0.02	0.01	0.01	0.01	0.006
22 *cis*-Limonene oxide	0.03	0.02	0.02	0.02	0.006
23 *trans*-Limonene oxide	0.01	0.01	0.01	0.01	0.000
24 Citronellal	0.10	0.06	0.09	0.08	0.021
25 Terpinen-4-ol	0.01	0.02	0.01	0.01	0.006
26 α-Terpineol	0.13	0.10	0.11	0.11	0.015
27 Decanal	0.46	0.46	0.47	0.46	0.006
28 Octyl acetate	0.01	t	0.01	0.01	0.001
29 *cis*-Carveol	0.03	0.06	0.02	0.04	0.021
30 Nerol+Citronellol	0.03	0.04	0.02	0.03	0.010
31 Neral	0.06	0.04	0.04	0.05	0.012
32 Carvone	t	t	t	t	–
33 Geraniol	t	t	t	t	–
34 (*E*)-Dec-2-en-1-al	0.01	0.01	0.02	0.01	0.006
35 Geranial	0.10	0.08	0.07	0.08	0.015
36 Perilla aldehyde	0.04	0.03	0.03	0.03	0.006
37 Perilla alcohol	0.04	0.02	0.03	0.03	0.010
38 Undecanal	0.01	t	0.01	0.01	0.004
39 (*E*, *E*)-Deca-2,4-dienal	0.01	0.02	0.02	0.02	0.006
40 α-Terpinyl acetate	0.01	t	t	t	–
41 Neryl acetate	t	t	t	t	–
42 α-Copaene	0.03	0.04	0.04	0.04	0.006
43 Geranyl acetate	0.01	0.01	0.01	0.01	0.000
44 β-Cubebene	0.03	0.04	0.05	0.04	0.010
45 β-Elemene	0.01	0.02	0.02	0.02	0.006
46 Dodecanal	0.11	0.09	0.11	0.10	0.011
47 Methyl *N*-methyl anthranilate	0.01	0.01	0.01	0.01	0.000
48 (*E*)-Caryophyllene	0.02	0.01	0.02	0.02	0.006
49 β-Gurjunene	0.04	0.03	0.03	0.03	0.006
50 *trans*-α-Bergamotene	t	t	t	t	–
51 (*Z*)-β-Farnesene	0.02	0.02	0.03	0.02	0.006
52 α-Humulene	0.01	0.01	t	0.01	0.001
53 (*E*)-β-Farnesene	0.11	0.04	0.10	0.08	0.038
54 (*E*)-Dodec-2-en-1-al	0.03	0.03	0.04	0.03	0.006
55 γ-Muurolene	0.01	0.01	t	0.01	0.004
56 Germacrene-D	0.03	0.03	0.04	0.03	0.006
57 Valencene	0.02	0.04	0.04	0.03	0.012
58 Bicyclogermacrene	0.01	0.02	0.01	0.01	0.006
59 (*E*, *E*)-α-Farnesene	0.15	0.06	0.11	0.11	0.045

Table 3 continues on next page

Table 3. Continued

	1 Dec. '95	2 Dec. '95	3 Jan.'96	$\bar{X}$	s
60 β-Bisabolene	t	0.01	t	t	–
61 δ-Cadinene	0.05	0.05	0.06	0.05	0.006
62 Tridecanal	0.01	0.01	0.01	0.01	0.000
63 (*E*)-Nerolidol	0.01	0.01	0.01	0.01	0.000
64 Tetradecanal	0.01	0.01	0.01	0.01	0.000
65 Tetradecanol	0.01	0.01	0.01	0.01	0.000
66 β-Sinensal	0.19	0.11	0.19	0.16	0.046
67 α-Sinensal	0.68	0.41	0.59	0.56	0.137
68 Nootkatone	0.01	0.01	0.02	0.01	0.006
Hydrocarbons	95.54	96.88	96.27	96.23	0.671
Oxygenated compounds	4.28	2.75	3.52	3.52	0.765
Monoterpenes	95.01	96.45	95.72	95.73	0.720
Sesquiterpenes	0.53	0.43	0.55	0.50	0.064
Carbonyl compounds	2.38	1.78	2.25	2.14	0.316
Alcohols	1.81	0.91	1.21	1.31	0.458
Esters	0.05	0.03	0.03	0.04	0.012

$\bar{X}$ = average content; s = standard deviation.

Table 4. Enantiomeric ratio of linalol in clementine, sweet orange and mandarin oils

	(−)-Linalol	(+)-Linalol
Clementine		
Oroval	2.4–2.6	97.4–97.6
Monreal	2.6–2.8	97.2–97.4
Comune	8.1–9.0	91.0–91.9
Sweet orange	4–11	89–96
Mandarin	16–18	82–84

The Enantiomeric Ratio of Linalol

The value of (+)/(−)-linalol in the oils analysed compared with those of Italian sweet orange and mandarin oils[16] can be seen in Table 4. Oroval and Monreal oils showed a similar ratio of (+)/(−)-linalol while Comune oils showed a ratio which was included in the range reported for Italian sweet orange oil.

The chromatogram of a Comune clementine oil obtained by GC using chiral columns is reported in Figure 3.

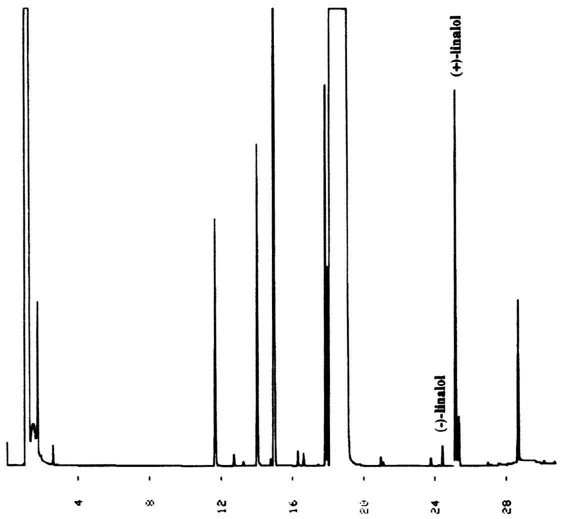

Fig. 3. Chiral gas chromatogram of a Comune clementine oils

Polymethoxylated Flavones

Five polymethoxylated flavones have been identified in the oils analysed, namely tangeretin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tetra-*O*-methylscutellarein, sinensetin and 3,3′,4′,5,6,7-hexamethoxyflavone.

Table 5 reports the content (g/100 g of oil) of polymethoxylated flavones in Monreal, Oroval and Comune clementine oils.

Figure 4 shows the HPLC chromatogram of polymethoxylated flavones in an Oroval clementine oil.

The main component is 3,3′,4′,5,6,7,8-heptamethoxyflavone which ranges from 0.20 to 0.57 g/100 g in the oils analysed. Comune oils showed a higher content of tangeretin and 3,3′,4′,5,6,7,8-heptamethoxyflavone than Oroval and Monreal oils. Oroval and Monreal oils showed a similar composition.

Table 6 reports the average composition of polymethoxylated flavones for each clementine

Table 5. Content (g/100 g of oil) of polymethoxylated flavones in clementine oils

Cultivar	Monreal			Oroval			Comune		
	1 Dec. '95	2 Dec. '95	3 Jan. '96	1 Dec. '95	2 Dec. '95	3 Jan. '96	1 Dec. '95	2 Dec. '95	3 Jan. '96
Tangeretin	0.10	0.10	0.09	0.12	0.09	0.10	0.15	0.15	0.21
3,3′,4′,5,6,7,8-Heptamethoxyflavone	0.30	0.32	0.20	0.35	0.24	0.28	0.57	0.44	0.53
Nobiletin	0.09	0.09	0.08	0.10	0.08	0.08	0.11	0.06	0.10
Tetra-*O*-methylscutellarein	0.05	0.04	0.05	0.05	0.04	0.04	0.08	0.03	0.06
3,3′,4′,5,6,7-Hexamethoxyflavone	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sinensetin	tr	tr	tr	tr	tr	tr	tr	tr	tr

Table 6. Average content (g/100 g of oil) of polymethoxylated flavones in clementine, mandarin and sweet orange oils

	Clementine oils						Sweet orange oils		Mandarin oils	
	Monreal		Oroval		Comune		$\bar{X}$	s	$\bar{X}$	s
	$\bar{X}$	s	$\bar{X}$	s	$\bar{X}$	s				
Tangeretin	0.10	0.006	0.10	0.015	0.17	0.028	0.05	0.008	0.21	0.036
3,3′,4′,5,6,7,8-Heptamethoxyflavone	0.27	0.064	0.29	0.056	0.51	0.054	0.08	0.014	0.04	0.012
Nobiletin	0.09	0.006	0.09	0.011	0.09	0.022	0.05	0.011	0.07	0.029
Tetra-*O*-methylscutellarein	0.05	0.006	0.04	0.007	0.06	0.021	0.03	0.005	–	–
3,3′,4′,5,6,7-Hexamethoxyflavone	0.02	–	0.02	–	0.02	–	0.01	0.004	0.01	0.002
Sinensetin	tr	–	tr	–	tr	–	0.01	0.003	0.01	0.001

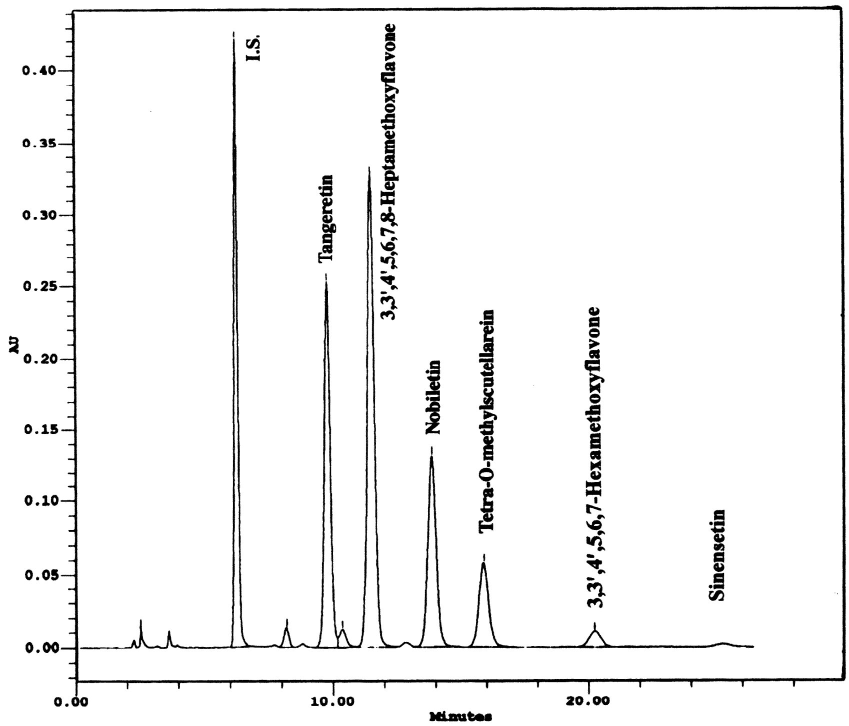

Fig. 4. HPLC chromatogram of an Oroval clementine oil.

cultivar, together with that of sweet orange and mandarin, reported in literature.[17] Although clementine oils showed the same polymethoxylated flavones present in sweet orange and mandarin oils, the content of these components was different from that obtained from sweet orange and mandarin ones.

Acknowledgments — This research was supported by Ministero dell'Università e della Ricerca Scientifica of Italy (60% and 40% research funds). Coordinator of the research group: Prof. Giovanni Dugo.

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