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Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography–mass spectrometry

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Abstract

Essential oils from fruit peel and leaves of colombian lemon (*Citrus volkameriana*), mandarin (*C. reticulata*) and orange (*C. sinensis*) were obtained by steam distillation and/or cold pressing. The extracts were analysed by high-resolution gas chromatography using either a flame ionization detector or a mass selective detector (electron impact ionization, 70 eV). The oil constituents were identified according to their mass spectra and Kováts retention indices determined on both polar and non-polar stationary phase capillary columns. The concentration of volatile secondary metabolites was maximum when the citrus fruits were at an intermediate maturation stage characterized by a greenish yellow coloration (45–75% green). While citrus peel oils contained from 94.01 to 98.66% of monoterpenes ($C_{10}H_{16}$), limonene as a major component and from 0.82 to 5.84% of oxygenated compounds, the extracts from citrus leaves contained only 65.26, 31.23 and 79.43% of monoterpenes ($C_{10}H_{16}$) in lemon, mandarin and orange, respectively. Oxygenated compounds in these oils represented 33.08, 68.47 and 16.38%, respectively.

1. Introduction

Citrus oils constitute the largest sector of the world production of essential oils. The study of the dependence of citrus oil composition on variables that affect the raw plant material, such as freshness, climate, location and harvest time, is a necessary step in the development of their production on a large scale [1–6]. We recently established that the concentration of volatile compounds in lemon (*Citrus volkameriana*) peel was maximum when fruits were at their intermediate maturation stage [7]. We now report a comparative high-resolution (HR) GC and GC–MS study of the incidence of maturation on the composition of oils extracted from the leaves and fruit peels of Colombian lemon (*C. volkameriana*), mandarin (*C. reticulata*) and orange (*C. sinensis*).

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2. Experimental

2.1. Plant material

The various citrus fruits were gathered from the same plantation, situated 30 km north of Bucaramanga (Santander, Colombia). Oil extractions used lemon, mandarin and orange peels chopped manually into ca. 4-cm^2 pieces. Fruits belonging to different harvesting periods (December 1993, February 1994 and April 1994) had

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different colorations, which were used as a ripeness indicator. Thus, the plant materials were classified as completely green (I), 45–75% green (intermediate maturity stage) (II) and yellow-orange, fully ripe fruits (III). The leaves used for oil extraction from lemon, mandarin and orange trees were collected at the same time as the respective fruits. Fresh plant material was employed in all extractions.

2.2. Essential oil extraction

Essential oils from citrus fruit peels were isolated by both steam distillation (A) and cold pressing (B). Secondary metabolites from the citrus leaves were obtained only by steam distillation. Cold pressing was used to isolate the essential oils from 2.0–2.5 kg of lemon, mandarin or orange fruit peel in different stages of ripeness (IB, IIB and IIIB). Steam distillation was carried out by passing steam (1 kg/h; 96–100°C) at 1.1 atm (1 atm = 101 325 Pa) for 3 h through a 5-*l* round-bottomed flask containing 1.0–1.5 kg of chopped lemon, mandarin or orange fruit peel in different stages of maturity (IA, IIA and IIIA), or 500–700 g of the respective leaves cut into small pieces. The condensed volatile oils were decanted from brine and dried over anhydrous Na_2SO_4. Three extractions were performed for each type of plant material.

The yields for lemon, mandarin and orange essential oils obtained by steam distillation and cold pressing were 0.19, 0.21, 0.17% and 0.60, 0.71 and 0.79%, respectively. The yields of volatile oils obtained from citrus leaves were 0.18, 0.54 and 0.34% for lemon, mandarin and orange, respectively. The reported data are averages of three extractions for each type of plant material.

2.3. Instrumental analysis

HRGC analysis of the samples was performed on a Hewlett-Packard (HP) (Palo Alto, CA, USA) 5890A Series II gas chromatograph equipped with a split–splitless injector (250°C, splitting ratio 1:30) and a flame ionization detector operated at 250°C. Chromatographic data were processed with an HP ChemStation 3365-II. The columns used were a DB-1 (J&W Scientific, Folsom, CA, USA) cross-linked fused-silica capillary column (60 m × 0.25 mm I.D.) coated with polydimethylsiloxane (0.25-μm phase thickness) and a DBWAX (J&W Scientific) fused-silica capillary column (60 m × 0.25 mm I.D.) coated with Carbowax 20M (0.25-μm phase thickness). The oven temperature was programmed from 50°C (10-min hold) to 150°C (20-min hold) at 2°C min^{-1} for the DBWAX column and from 70°C (5-min hold) to 270°C at 2.5°C min^{-1} for the DB-1 column. Helium (AGA, 99.995%) was used as the carrier gas (inlet pressure 152 kPa) with linear velocity 19 cm s^{-1} for both columns. Air and hydrogen flow-rates were maintained at 300 and 30 ml min^{-1}, respectively. Nitrogen was used as a make-up gas at 30 ml min^{-1}. The injection volume was 0.5 μl of a 20% (v/v) solution of citrus oil in dichloromethane (chromatography-grade reagent, Merck), using *n*-tetradecane (reference substance for gas chromatography, Merck) as an internal standard. Peak areas from different chromatograms were compared after they had been normalized with this standard.

An HP 5890A Series II gas chromatograph interfaced to an HP 5972 mass-selective detector with an HP MS ChemStation data system was used for identification of the GC components. The column used was a DB-1 (J&W Scientific) cross-linked fused-silica capillary column (30 m × 0.25 mm I.D.) coated with polydimethylsiloxane (0.25-μm phase thickness). The oven temperature was programmed from 50°C (5-min hold) at 3.5°C min^{-1} to 250°C. The helium inlet pressure was 78 kPa, with a linear velocity of 20 cm min^{-1} (splitting ratio 1:10). The injector temperature was kept at 250°C and the volume injected was 0.5 μl. The temperatures of the ionization chamber and of the transfer line were 180 and 280°C, respectively. The electron energy was 70 eV. Mass spectra and reconstructed total ion chromatograms were obtained by automatic scanning in the mass range m/z 30–350 at 2.2 scans s^{-1}. Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms obtained for the characteristic fragment ions (e.g., m/z 136, 121 for monoterpenes; m/z 154, 139 for monoterpenols; m/z 204, 189 for sesquiterpenes). A C_7–C_{19} hydrocarbon mixture (Bio-Rad, Sadtler Division) was used to determine Kováts retention indexes.

3. Results and discussion

3.1. Citrus peel essential oils

A typical profile of citrus essential oils from lemon, mandarin and orange isolated by steam distillation is shown in Fig. 1. Table 1 gives the compositions found for citrus oils extracted by steam distillation (A) and cold-pressing (B) from fruits at different stages of ripeness (I, II and III). The various compounds were identified by comparison of their Kováts retention indices [8], determined utilizing a non-logarithmic scale on both polar (DBWAX) and non-polar (DB-1) stationary phase columns, and by comparison of the mass spectra of each GC component with those of standard and reported data [9–11].

Thirty components were detected in the orange peel extracts and the volatile mixtures isolated from mandarin and lemon peels were composed of 35 and 41 compounds respectively. Using both chromatographic (retention indices) and spectroscopic (mass spectra) criteria, 90, 97 and 90% of the detected compounds were fully identified in lemon, mandarin and orange peel oils, respectively.

Whereas the essential oils extracted by either steam distillation or cold pressing from lemon, mandarin and orange fruit peel differed considerably both qualitatively and quantitatively, the oils extracted from the fruits at different stages of maturity changed only quantitatively (Table 1). Limonene was the main component in all samples, with a mass concentration that varied in the ranges 77.27–79.36, 83.45–84.29 and 91.03–92.57% for lemon, mandarin and orange oils obtained by steam distillation, respectively. These values for mandarin and

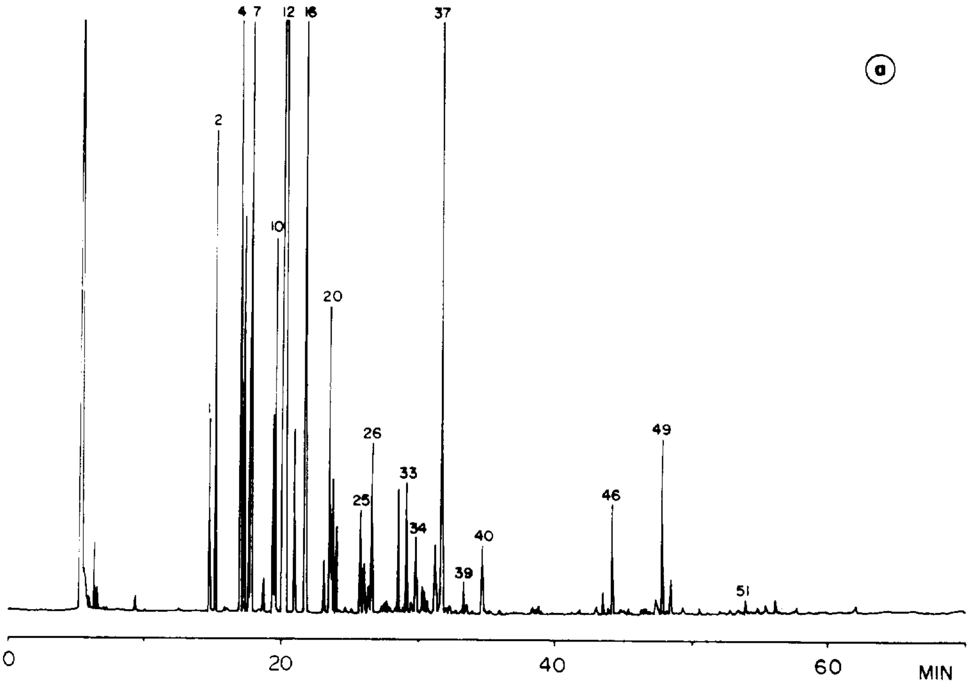

Fig. 1 (continued on p. 504).

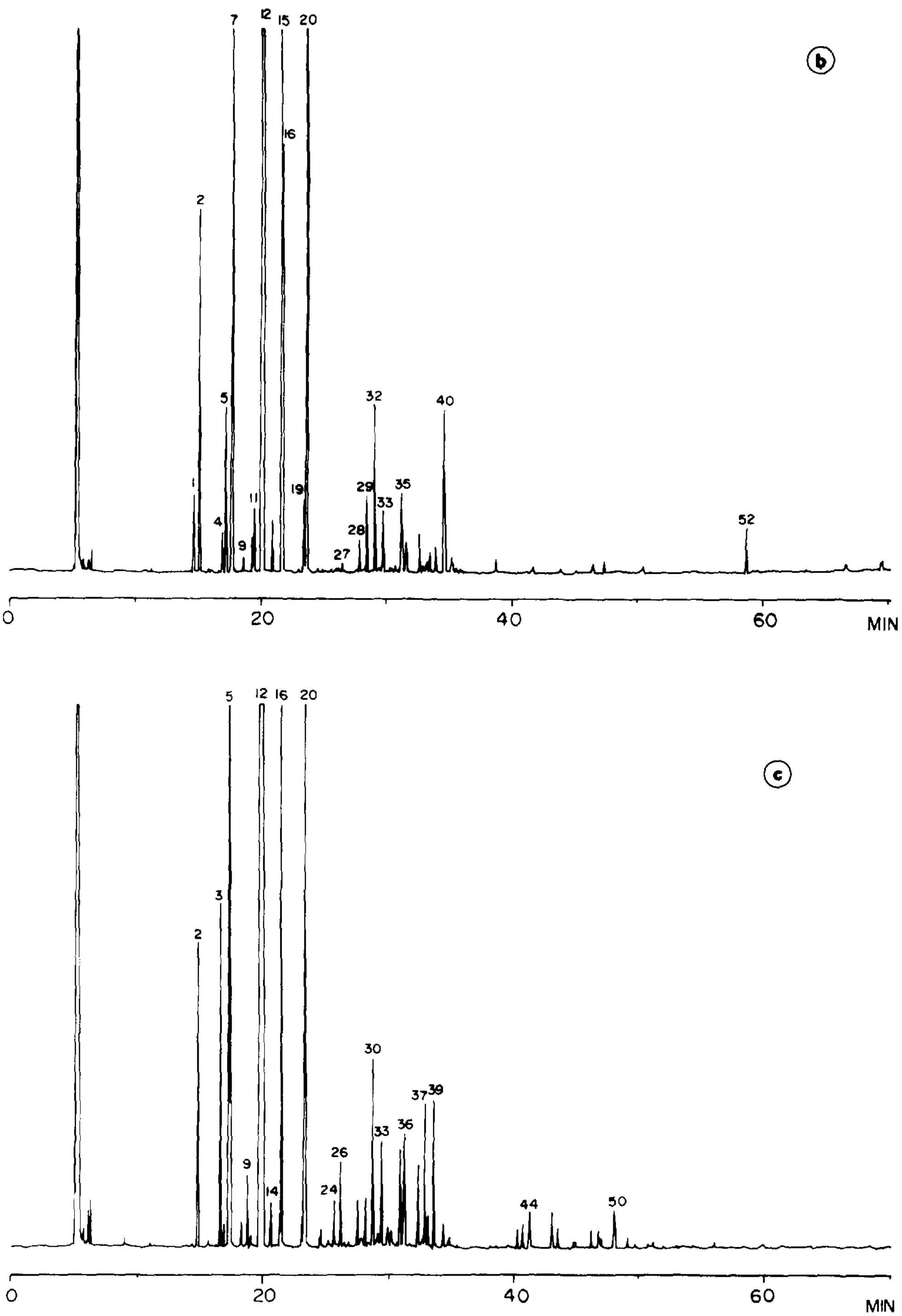

Fig. 1. Typical gas chromatograms of (a) lemon (*C. Volkameriana*), (b) mandarin (*C. reticulata*) and (c) orange (*C. sinensis*) peel oils obtained by steam distillation on a DB-1 cross-linked fused-silica column (60 m × 0.20 mm I.D.) coated with polydimethylsiloxane (0.25-μm phase thickness). Column temperature programmed from 70°C (5-min hold) to 270°C at 2.5°C min^{-1}. Splitting ratio, 1 : 30. Flame ionization detector. Carrier gas, helium; inlet pressure, 152 kPa. See Table 1 for peak identification.

Table 1
Chemical compositions of citrus peel essential oils from fruits at different stages of maturity

Peak No. (DB-1)	Compound	I_K[a]		GC peak area (%)[b]																	
		DB-1	DBWAX	Lemon						Mandarin						Orange					
				I	S.D.	II	S.D.	III	S.D.	I	S.D.	II	S.D.	III	S.D.	I	S.D.	II	S.D.	III	S.D.
1	α-Thujene	924	1025	0.32[c]	$8\cdot10^{-2}$	0.31	$3\cdot10^{-2}$	0.34	$3\cdot10^{-2}$	0.12	$2\cdot10^{-2}$	0.11	$2\cdot10^{-2}$	0.19	$1\cdot10^{-1}$	–	–	–	–	–	–
				0.47[d]	$3\cdot10^{-2}$	0.33	$2\cdot10^{-1}$	0.36	$2\cdot10^{-1}$	0.19	$3\cdot10^{-3}$	0.17	$1\cdot10^{-2}$	0.16	$1\cdot10^{-2}$	–	–	–	–	–	–
2	α-Pinene	932	1021	1.00	$2\cdot10^{-1}$	0.80	$8\cdot10^{-2}$	0.90	$6\cdot10^{-2}$	0.54	$1\cdot10^{-1}$	0.53	$1\cdot10^{-1}$	0.39	$2\cdot10^{-1}$	0.28	$2\cdot10^{-2}$	0.32	$1\cdot10^{-2}$	0.32	$2\cdot10^{-2}$
				1.53	$3\cdot10^{-2}$	1.50	$3\cdot10^{-3}$	1.42	$3\cdot10^{-2}$	0.93	$9\cdot10^{-3}$	0.86	$4\cdot10^{-2}$	0.87	$3\cdot10^{-2}$	0.53	$1\cdot10^{-2}$	0.29	$2\cdot10^{-1}$	0.52	$1\cdot10^{-2}$
3	Camphene	962	1083	–												0.27	$2\cdot10^{-1}$	0.36	$1\cdot10^{-4}$	0.35	$1\cdot10^{-2}$
				–	–	–	–	–								0.52	$2\cdot10^{-2}$	0.46	$6\cdot10^{-2}$	0.42	$1\cdot10^{-2}$
4	Sabinene	967	1120	3.55	$3\cdot10^{-1}$	1.39	$3\cdot10^{-1}$	1.15	$6\cdot10^{-1}$	0.11	$5\cdot10^{-3}$	0.17	$9\cdot10^{-2}$	0.15	$6\cdot10^{-2}$	0.02	$1\cdot10^{-2}$	0.02	$6\cdot10^{-4}$	0.02	$6\cdot10^{-4}$
				4.87	$9\cdot10^{-1}$	3.40	$1\cdot10^{-1}$	2.25	$4\cdot10^{-1}$	0.15	$1\cdot10^{-3}$	0.14	2.103	0.14	$2\cdot10^{-3}$	0.03	$1\cdot10^{-3}$	0.02	$1\cdot10^{-3}$	0.03	$6\cdot10^{-4}$
5	β-Pinene	973	1107	0.80	$4\cdot10^{-2}$	0.65	$7\cdot10^{-2}$	0.73	$3\cdot10^{-2}$	0.30	$3\cdot10^{-2}$	0.46	$2\cdot10^{-1}$	0.29	$4\cdot10^{-3}$	0.63	$4\cdot10^{-1}$	1.05	$2\cdot10^{-1}$	0.89	$9\cdot10^{-2}$
				0.99	$3\cdot10^{-2}$	0.94	$5\cdot10^{-4}$	0.90	$4\cdot10^{-2}$	0.36	$4\cdot10^{-3}$	0.33	$1\cdot10^{-2}$	0.32	$2\cdot10^{-2}$	0.63	$1\cdot10^{-1}$	0.76	$6\cdot10^{-2}$	0.72	$2\cdot10^{-2}$
6	*n*-Octanal	977	1270	0.20	$1\cdot10^{-1}$	0.22	$2\cdot10^{-2}$	0.25	$1\cdot10^{-1}$	0.83	$6\cdot10^{-1}$	0.39	$3\cdot10^{-1}$	1.05	$3\cdot10^{-1}$	1.50	$1\cdot10^{-2}$	1.60	$4\cdot10^{-2}$	1.64	$3\cdot10^{-2}$
				0.12	$6\cdot10^{-4}$	0.17	$6\cdot10^{-3}$	0.14	$3\cdot10^{-2}$	0.49	$3\cdot10^{-1}$	0.28	$1\cdot10^{-2}$	0.26	$3\cdot10^{-2}$	1.55	$5\cdot10^{-1}$	1.87	$1\cdot10^{-2}$	1.89	$1\cdot10^{-2}$
7	β-Myrcene	982	1162	1.74	$1\cdot10^{-1}$	1.68	$5\cdot10^{-2}$	1.65	$4\cdot10^{-2}$	1.49	$2\cdot10^{-1}$	1.49	$7\cdot10^{-2}$	1.45	$9\cdot10^{-2}$	0.01	$9\cdot10^{-3}$	–	–	–	–
				2.00	$1\cdot10^{-2}$	1.96	$2\cdot10^{-2}$	1.55	$5\cdot10^{-1}$	1.86	$7\cdot10^{-3}$	1.84	$4\cdot10^{-3}$	1.86	$3\cdot10^{-3}$	–	–	–	–	–	
8	α-Phellandrene	994	1177	0.08	$3\cdot10^{-2}$	0.07	$5\cdot10^{-3}$	0.07	$4\cdot10^{-3}$	0.04	$7\cdot10^{-4}$	0.04	$2\cdot10^{-3}$	0.04	$9\cdot10^{-4}$	0.02	$1\cdot10^{-2}$	0.04	$2\cdot10^{-3}$	0.04	$1\cdot10^{-4}$
				0.06	$7\cdot10^{-4}$	0.06	$2\cdot10^{-3}$	0.06	$3\cdot10^{-4}$	0.04	$3\cdot10^{-4}$	0.04	$2\cdot10^{-3}$	0.04	$1\cdot10^{-3}$	0.04	$2\cdot10^{-3}$	0.05	$1\cdot10^{-2}$	0.04	$3\cdot10^{-4}$
9	3-Carene	1000	1150	–	–	–	–	–	–	–	–	–	–	–	–	0.04	$3\cdot10^{-2}$	0.08	$1\cdot10^{-2}$	0.08	$7\cdot10^{-3}$
				–	–	–	–	–	–	–	–	–	–	–	–	0.05	$1\cdot10^{-2}$	0.07	$2\cdot10^{-2}$	0.07	$7\cdot10^{-3}$
10	1,4-Cineole	1009	1177	0.33	$7\cdot10^{-2}$	0.36	$5\cdot10^{-2}$	0.34	$2\cdot10^{-2}$	0.11	$2\cdot10^{-2}$	0.09	$1\cdot10^{-2}$	0.11	$7\cdot10^{-3}$	0.02	$6\cdot10^{-4}$	–	–	–	–
				0.19	$8\cdot10^{-3}$	0.13	$9\cdot10^{-2}$	0.20	$5\cdot10^{-3}$	0.09	$1\cdot10^{-2}$	0.08	$8\cdot10^{-3}$	0.08	$2\cdot10^{-4}$					–	–
11	*p*-Cymene	1013	1284	1.28	$3\cdot10^{-1}$	0.65	$1\cdot10^{-1}$	0.92	$3\cdot10^{-1}$	0.20	$4\cdot10^{-2}$	0.24	$8\cdot10^{-2}$	0.36	$2\cdot10^{-1}$	–	–	–	–	–	–
				0.59	$2\cdot10^{-2}$	0.50	$7\cdot10^{-2}$	0.55	$1\cdot10^{-1}$	0.08	$1\cdot10^{-2}$	0.18	$1\cdot10^{-1}$	0.24	$9\cdot10^{-2}$	–	–	–	–	–	–
12	*d*-Limonene	1026	1201	77.27	$8\cdot10^{-1}$	79.36	$7\cdot10^{-1}$	78.78	$8\cdot10^{-1}$	84.29	$2\cdot10^{+0}$	83.71	$2\cdot10^{+0}$	83.45	$2\cdot10^{+0}$	91.63	$9\cdot10^{-1}$	91.03	$6\cdot10^{-1}$	92.57	$4\cdot10^{-1}$
				77.10	$9\cdot10^{-1}$	78.90	$1\cdot10^{-1}$	78.21	$6\cdot10^{-1}$	89.25	$2\cdot10^{-1}$	89.54	$3\cdot10^{-1}$	90.05	$3\cdot10^{-1}$	94.55	$1\cdot10^{-1}$	93.77	$6\cdot10^{-1}$	94.42	$2\cdot10^{-1}$
13	*cis*-β-Ocimene	1028	1235	tr[e]		tr		tr		tr		tr		tr						–	–
				tr		tr		tr		tr		tr		tr		–	–	–	–	–	–
14	*trans*-β-Ocimene	1035	1252	0.32	$6\cdot10^{-2}$	0.24	$5\cdot10^{-2}$	0.21	$4\cdot10^{-2}$	0.14	$2\cdot10^{-2}$	0.14	$3\cdot10^{-2}$	0.13	$1\cdot10^{-2}$	0.02	$2\cdot10^{-2}$	0.03	$9\cdot10^{-3}$	0.03	$3\cdot10^{-3}$
				0.32	$1\cdot10^{-2}$	0.21	$4\cdot10^{-2}$	0.18	$1\cdot10^{-2}$	0.13	$1\cdot10^{-2}$	0.13	$2\cdot10^{-2}$	0.10	$2\cdot10^{-2}$	0.02	$5\cdot10^{-4}$	0.02	$7\cdot10^{-4}$	0.02	$8\cdot10^{-4}$
15	Not identified	1045	–	–	–	–	–	–	–	–	–	–	–	–	–	0.01	$1\cdot10^{-2}$	0.02	$9\cdot10^{-3}$	0.01	$6\cdot10^{-3}$
			–	–	–	–	–	–	–	–	–	–	–	–	–	0.02	$3\cdot10^{-3}$	0.01	$9\cdot10^{-4}$	0.02	$1\cdot10^{-3}$
16	γ-Terpinene	1051	1248	7.81	$9\cdot10^{-2}$	8.85	$2\cdot10^{-1}$	8.75	$3\cdot10^{-1}$	4.27	$2\cdot10^{-1}$	3.85	$4\cdot10^{-1}$	3.63	$2\cdot10^{-1}$	0.41	$3\cdot10^{-1}$	1.09	$4\cdot10^{-1}$	0.64	$4\cdot10^{-2}$
				7.32	$1\cdot10^{-1}$	7.87	$3\cdot10^{-1}$	7.92	$2\cdot10^{-1}$	3.87	$4\cdot10^{-2}$	3.64	$2\cdot10^{-1}$	3.35	$1\cdot10^{-2}$	0.02	$2\cdot10^{-3}$	0.04	$7\cdot10^{-3}$	0.03	$3\cdot10^{-4}$
17	*trans*-Sabinene hydrate	1054	1463	0.06	$5\cdot10^{-2}$	0.01	$8\cdot10^{-3}$	0.04	$2\cdot10^{-2}$	0.11	$6\cdot10^{-4}$	0.33	$3\cdot10^{-1}$	0.19	$1\cdot10^{-1}$	–	–	–	–	–	–
				0.16	$2\cdot10^{-2}$	0.08	$5\cdot10^{-2}$	0.08	$4\cdot10^{-2}$	–	–	–	–	–	–	–	–	–	–	–	–
18	*n*-Octanol	1074	1507	0.12	$4\cdot10^{-2}$	0.08	$2\cdot10^{-2}$	0.10	$2\cdot10^{-2}$	–	–	–	–	–	–	0.02	$1\cdot10^{-2}$	0.03	$4\cdot10^{-3}$	0.03	$5\cdot10^{-4}$
				0.06	$1\cdot10^{-3}$	0.05	$6\cdot10^{-3}$	0.06	$5\cdot10^{-3}$	–	–	–	–	–	–	0.02	$3\cdot10^{-3}$	0.04	$8\cdot10^{-4}$	0.02	$1\cdot10^{-3}$

Table 1 (continued)

Peak No. (DB-1)	Compound	I_K [a] DB-1	I_K [a] DBWAX	GC peak area (%) [b] Lemon I	S.D.	II	S.D.	III	S.D.	Mandarin I	S.D.	II	S.D.	III	S.D.	Orange I	S.D.	II	S.D.	III	S.D.
19	Not identified	1078	– –	– –	– –	– –	– –	– –	–	0.22 0.20	$3\cdot10^{-2}$ $2\cdot10^{-3}$	0.18 0.19	$2\cdot10^{-2}$ $7\cdot10^{-3}$	0.21 0.17	$9\cdot10^{-3}$ $3\cdot10^{-3}$	– 0.06	– $1\cdot10^{-4}$	– 0.04	– $2\cdot10^{-2}$	 0.07	– $1\cdot10^{-3}$
20	Terpinolene	1080	1291	0.53 0.42	$1\cdot10^{-2}$ $7\cdot10^{-3}$	0.55 0.43	$4\cdot10^{-2}$ $4\cdot10^{-3}$	0.54 0.42	$2\cdot10^{-2}$ $6\cdot10^{-3}$	4.55 1.46	$8\cdot10^{-1}$ $1\cdot10^{-1}$	5.95 1.57	$1\cdot10^{+0}$ $5\cdot10^{-2}$	5.96 1.41	$1\cdot10^{+0}$ $2\cdot10^{-1}$	2.61 0.58	$6\cdot10^{-1}$ $4\cdot10^{-1}$	2.54 0.78	$4\cdot10^{-1}$ $5\cdot10^{-2}$	1.83 0.75	$2\cdot10^{-1}$ $9\cdot10^{-3}$
21	Nonanal	1082	1382	0.08 0.09	$4\cdot10^{-2}$ $8\cdot10^{-3}$	0.12 0.13	$2\cdot10^{-2}$ $1\cdot10^{-3}$	0.12 0.11	$5\cdot10^{-2}$ $3\cdot10^{-2}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
22	Linalool	1083	1553	0.43 0.28	$3\cdot10^{-1}$ $3\cdot10^{-2}$	0.16 0.23	$1\cdot10^{-1}$ $2\cdot10^{-2}$	0.24 0.18	$1\cdot10^{-1}$ $6\cdot10^{-2}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
23	*cis*-Sabinene hydrate	1088		0.05 0.07	$3\cdot10^{-2}$ $9\cdot10^{-2}$	0.16 0.10	$3\cdot10^{-2}$ $5\cdot10^{-2}$	0.18 0.21	$7\cdot10^{-2}$ $6\cdot10^{-2}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
24	*cis*-Epoxylimonene	1118	–	0.07 0.12	$6\cdot10^{-2}$ $2\cdot10^{-1}$	0.25 0.17	$5\cdot10^{-2}$ $8\cdot10^{-2}$	0.31 0.38	$1\cdot10^{-1}$ $9\cdot10^{-2}$	 –	– –	0.02 –	$3\cdot10^{-3}$ –	0.02 –	$7\cdot10^{-3}$ –	0.04 0.02	$4\cdot10^{-2}$ $6\cdot10^{-3}$	0.04 0.37	$1\cdot10^{-2}$ $5\cdot10^{-3}$	0.02 –	$3\cdot10^{-3}$ –
25	1,2-Dihydrolinalool	1121	1449	0.04 0.03	$1\cdot10^{-2}$ $4\cdot10^{-2}$	0.08 0.05	$1\cdot10^{-2}$ $2\cdot10^{-2}$	0.10 0.10	$3\cdot10^{-2}$ $1\cdot10^{-2}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
26	Not identified	1127	–	0.06 0.05	$9\cdot10^{-3}$ $2\cdot10^{-3}$	0.05 0.04	$1\cdot10^{-2}$ $3\cdot10^{-3}$	0.05 0.04	$4\cdot10^{-3}$ $2\cdot10^{-3}$	– –	– –	– –	– –	– –	– –	0.02 0.09	$1\cdot10^{-2}$ $1\cdot10^{-1}$	0.06 0.02	$3\cdot10^{-2}$ $6\cdot10^{-4}$	0.03 0.02	$8\cdot10^{-4}$ $1\cdot10^{-3}$
27	Citronellal	1131	1472	0.40 0.36	$2\cdot10^{-1}$ $5\cdot10^{-3}$	0.30 0.41	$1\cdot10^{-2}$ $8\cdot10^{-3}$	0.33 0.40	$1\cdot10^{-1}$ $3\cdot10^{-2}$	0.08 0.06	$4\cdot10^{-2}$ $2\cdot10^{-2}$	0.07 0.04	$2\cdot10^{-2}$ $7\cdot10^{-3}$	0.13 0.06	$2\cdot10^{-2}$ $4\cdot10^{-3}$	– –	– –	– –	– –	– –	– –
28	Isopulegol	1146	1565	0.40 0.03	$2\cdot10^{-1}$ $5\cdot10^{-3}$	0.27 0.03	$2\cdot10^{-2}$ $1\cdot10^{-3}$	0.29 0.03	$2\cdot10^{-2}$ $5\cdot10^{-3}$	– –	 –	0.05 –	$2\cdot10^{-2}$ –	0.02 –	$8\cdot10^{-3}$ –	0.03 –	$2\cdot10^{-2}$ –	0.05 –	$9\cdot10^{-4}$ –	0.05 –	$4\cdot10^{-4}$ –
29	Nonanol	1161	– –	– –	– –	– –	– –	– –	– –	0.09 –	$9\cdot10^{-3}$ –	0.13 –	$4\cdot10^{-2}$ –	0.10 –	$3\cdot10^{-2}$ –	 –	– –	– –	 –	– –	– –
30	Terpinen-4-ol	1168	1610	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	0.11 0.23	$9\cdot10^{-2}$ $2\cdot10^{-1}$	0.18 0.07	$4\cdot10^{-2}$ $2\cdot10^{-2}$	0.13 0.06	$2\cdot10^{-2}$ $7\cdot10^{-4}$
31	Not identified	1173	–	0.46 0.29	$2\cdot10^{-1}$ $3\cdot10^{-2}$	0.31 0.23	$5\cdot10^{-2}$ $2\cdot10^{-2}$	0.35 0.18	$9\cdot10^{-2}$ $7\cdot10^{-2}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
32	α-Terpineol	1179	1740	0.05 0.04	$3\cdot10^{-2}$ $3\cdot10^{-3}$	0.02 0.03	$3\cdot10^{-3}$ $5\cdot10^{-3}$	0.03 0.03	$8\cdot10^{-3}$ $3\cdot10^{-3}$	0.26 0.10	$4\cdot10^{-2}$ $2\cdot10^{-3}$	0.35 0.09	$5\cdot10^{-2}$ $4\cdot10^{-3}$	0.35 0.09	$1\cdot10^{-1}$ $1\cdot10^{-3}$	– –	– –	– –	– –	– –	– –
33	Decanal	1183	1482	0.34 0.30	$2\cdot10^{-1}$ $1\cdot10^{-3}$	0.19 0.28	$9\cdot10^{-3}$ $2\cdot10^{-2}$	0.21 0.26	$8\cdot10^{-2}$ $3\cdot10^{-2}$	0.14 0.14	$8\cdot10^{-2}$ $3\cdot10^{-3}$	0.13 0.17	$6\cdot10^{-3}$ $2\cdot10^{-2}$	0.10 0.17	$8\cdot10^{-2}$ $8\cdot10^{-3}$	0.11 0.26	$8\cdot10^{-2}$ $4\cdot10^{-2}$	0.25 0.39	$1\cdot10^{-1}$ $7\cdot10^{-3}$	0.35 0.38	$3\cdot10^{-2}$ $2\cdot10^{-2}$
34	Methylthymol	1196	–	0.05 0.01	$2\cdot10^{-2}$ $8\cdot10^{-3}$	0.05 0.01	$9\cdot10^{-3}$ $4\cdot10^{-3}$	0.05 0.00	$1\cdot10^{-2}$ $6\cdot10^{-3}$	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
35	Citronellol	1203	1751	0.04 0.02	$0\cdot10^{+0}$ $2\cdot10^{-2}$	0.08 0.03	$3\cdot10^{-2}$ $2\cdot10^{-2}$	0.08 0.06	$2\cdot10^{-2}$ $3\cdot10^{-3}$	0.14 –	$5\cdot10^{-2}$ –	0.23 –	$9\cdot10^{-2}$ –	0.19 –	$8\cdot10^{-2}$ –	– –	– –	– –	– –	– –	– –
36	Nerol	1208	1832	0.24 0.05	$1\cdot10^{-1}$ $4\cdot10^{-3}$	0.23 0.06	$9\cdot10^{-2}$ $1\cdot10^{-3}$	0.27 0.07	$2\cdot10^{-1}$ $5\cdot10^{-3}$	0.08 –	$4\cdot10^{-4}$ –	0.07 –	$8\cdot10^{-3}$ –	0.10 –	$6\cdot10^{-3}$ –	0.07 –	$5\cdot10^{-2}$ –	0.13 –	$3\cdot10^{-2}$ –	0.10 –	$1\cdot10^{-2}$
37	Neral	1215	1705	2.11 1.19	$6\cdot10^{-1}$ $2\cdot10^{-2}$	1.83 0.98	$4\cdot10^{-1}$ $1\cdot10^{-1}$	1.85 0.99	$3\cdot10^{-1}$ $2\cdot10^{-2}$	0.28 0.10	$1\cdot10^{-1}$ $2\cdot10^{-3}$	0.13 0.08	$4\cdot10^{-2}$ $2\cdot10^{-2}$	0.18 0.08	$1\cdot10^{-2}$ $4\cdot10^{-3}$	0.12 0.08	$9\cdot10^{-2}$ $9\cdot10^{-3}$	0.15 0.08	$4\cdot10^{-4}$ $2\cdot10^{-4}$	0.12 0.08	$2\cdot10^{-2}$ $2\cdot10^{-3}$

Table 1 (continued)

Peak No. (DB-1)	Compound	I_K [a]		GC peak area (%) [b]																	
		DB-1	DBWAX	Lemon						Mandarin						Orange					
				I	S.D.	II	S.D.	III	S.D.	I	S.D.	II	S.D.	III	S.D.	I	S.D.	II	S.D.	III	S.D.
38	Geraniol	1237	1789	–	–	–	–	–	–	0.17	$1\cdot10^{-1}$	0.12	$6\cdot10^{-3}$	0.13	$3\cdot10^{-2}$	0.14	$1\cdot10^{-1}$	0.17	$6\cdot10^{-3}$	0.14	$2\cdot10^{-2}$
										–	–	–	–	–	–	0.11	$1\cdot10^{-2}$	0.11	$1\cdot10^{-3}$	0.10	$2\cdot10^{-3}$
39	Geranial	1248	1735	0.11	$7\cdot10^{-2}$	0.08	$2\cdot10^{-2}$	0.07	$4\cdot10^{-2}$	0.12	$5\cdot10^{-2}$	0.05	$3\cdot10^{-2}$	0.12	$5\cdot10^{-2}$	0.15	$1\cdot10^{-1}$	0.21	$4\cdot10^{-2}$	0.23	$3\cdot10^{-3}$
				0.06	$6\cdot10^{-3}$	0.04	$1\cdot10^{-2}$	0.03	$6\cdot10^{-3}$	0.05	$5\cdot10^{-3}$	0.04	$4\cdot10^{-3}$			0.02	$1\cdot10^{-3}$	0.02	$8\cdot10^{-4}$	0.02	$3\cdot10^{-4}$
40	Perillaldehyde	1265	–	0.18	$1\cdot10^{-1}$	0.11	$2\cdot10^{-2}$	0.13	$2\cdot10^{-2}$	0.43	$7\cdot10^{-2}$	0.36	$6\cdot10^{-2}$	0.36	$1\cdot10^{-1}$	0.01	$1\cdot10^{-2}$	0.03	$2\cdot10^{-3}$	0.02	$5\cdot10^{-3}$
				0.08	$2\cdot10^{-2}$	0.06	$2\cdot10^{-2}$	0.05	$2\cdot10^{-2}$	0.11	$2\cdot10^{-2}$	0.09	$2\cdot10^{-2}$	0.08	$5\cdot10^{-3}$	–	–	–	–	–	–
41	Decanol	1270	1729	0.04	$3\cdot10^{-2}$	0.03	$6\cdot10^{-3}$	0.04	$1\cdot10^{-2}$	0.07	$1\cdot10^{-2}$	0.09	$2\cdot10^{-3}$	0.10	$2\cdot10^{-2}$	–	–	–	–	–	–
				0.03	$8\cdot10^{-3}$	0.03	$1\cdot10^{-3}$	0.03	$4\cdot10^{-3}$		–	–	–	–	–					–	–
42	Thymal	1286	2100	0.02	$1\cdot10^{-2}$	0.02	$2\cdot10^{-2}$	0.03	$2\cdot10^{-2}$									–	–		
				0.03	$2\cdot10^{-4}$	0.05	$2\cdot10^{-3}$	0.04	$9\cdot10^{-3}$	–	–	–	–	–	–	–	–	–	–	–	–
43	Citronellyl acetate	1335	1645	–	–	–	–	–	–	0.10	$5\cdot10^{-2}$	0.07	$9\cdot10^{-4}$	0.05	$6\cdot10^{-3}$	–	–	–	–	–	–
					–	–	–	–		0.09	$5\cdot10^{-3}$	0.07	$7\cdot10^{-3}$	–	–	–	–	–	–	–	–
44	δ-Elemene	1384	1475	–	–					0.10	$6\cdot10^{-3}$	0.05	$8\cdot10^{-3}$	0.02	$9\cdot10^{-4}$	0.07	$5\cdot10^{-2}$	0.09	$3\cdot10^{-2}$	0.09	$5\cdot10^{-3}$
				–	–	–										0.03	$1\cdot10^{-3}$	0.07	$1\cdot10^{-2}$	0.08	$4\cdot10^{-3}$
45	β-Caryophyllene	1424	1654	0.07	$3\cdot10^{-2}$	0.04	$1\cdot10^{-2}$	0.04	$0\cdot10^{-0}$	0.11	$7\cdot10^{-4}$	0.04	$5\cdot10^{-4}$	0.04	$3\cdot10^{-3}$	0.01	$1\cdot10^{-2}$	0.02	$3\cdot10^{-3}$	0.02	$7\cdot10^{-4}$
46	α-Bergamotene	1435	1589	0.06	$5\cdot10^{-3}$	0.03	$6\cdot10^{-3}$	0.03	$2\cdot10^{-3}$	–					–	–	–	–	–	–	–
				0.22	$4\cdot10^{-2}$	0.20	$3\cdot10^{-2}$	0.19	$3\cdot10^{-2}$	–	–	–	–	–	–	–	–	–	–	–	–
				0.21	$2\cdot10^{-3}$	0.18	$2\cdot10^{-2}$	0.20	$9\cdot10^{-3}$					–	–	–	–	–	–	–	–
47	Germacrene D	1477	1714	–	–	–	–			0.08	$4\cdot10^{-2}$	0.06	$7\cdot10^{-3}$	0.03	$2\cdot10^{-3}$	0.02	$1\cdot10^{-2}$	0.02	$2\cdot10^{-3}$	0.15	$9\cdot10^{-4}$
				–	–	–	–	–		0.08	$2\cdot10^{-3}$	0.07	$6\cdot10^{-3}$	0.05	$2\cdot10^{-3}$	0.02	$1\cdot10^{-3}$	0.02	$7\cdot10^{-4}$	0.02	$6\cdot10^{-4}$
48	β-Bisabolene	1495	1770	0.09	$4\cdot10^{-2}$	0.04	$1\cdot10^{-2}$	0.04	$3\cdot10^{-3}$	0.09	$4\cdot10^{-2}$	0.06	$1\cdot10^{-2}$	0.05	$5\cdot10^{-3}$	–	–	–	–	–	–
				0.07	$2\cdot10^{-2}$	0.02	$8\cdot10^{-3}$	0.03	$1\cdot10^{-3}$	0.06	$5\cdot10^{-3}$	0.06	$7\cdot10^{-3}$	0.05	$8\cdot10^{-3}$	–	–	–	–	–	–
49	α-Muurolene	1502	1730	0.33	$8\cdot10^{-2}$	0.12	$2\cdot10^{-1}$	0.29	$6\cdot10^{-2}$	–	–	–	–	–	–	–	–	–	–	–	–
				0.28	$3\cdot10^{-3}$	0.24	$3\cdot10^{-2}$	0.26	$1\cdot10^{-2}$	–	–	–	–	–	–	–	–	–	–	–	–
50	δ-Cadinene	1511	1766	–	–	–	–	–	–	–				–	–	0.04	$3\cdot10^{-2}$	0.04	$4\cdot10^{-3}$	0.03	$4\cdot10^{-3}$
				–	–	–	–	–	–	–	–	–	–	–	–	0.03	$2\cdot10^{-3}$	0.03	$1\cdot10^{-3}$	0.03	$1\cdot10^{-3}$
51	Sesquiterpenol	1650	–	0.02	$8\cdot10^{-3}$	0.02	$1\cdot10^{-2}$	0.03	$4\cdot10^{-3}$	–	–	–	–	–	–	–	–	–	–	–	–
				0.03	$2\cdot10^{-3}$	0.01	$7\cdot10^{-3}$	0.02	$1\cdot10^{-2}$	–	–	–	–	–	–	–	–	–	–	–	–
52	α-Sinensal	1725	–	–	–	–	–	–	–	0.23	$1\cdot10^{-1}$	0.15	$3\cdot10^{-2}$	0.13	$2\cdot10^{-2}$	–	–	–	–	–	–
			–	–	–	–	–	–	–	0.20	$7\cdot10^{-3}$	0.20	$2\cdot10^{-2}$	0.18	$3\cdot10^{-2}$		–	–		–	–
53	Sesquiterpenol	1780	–	0.02	$5\cdot10^{-3}$	0.02	$1\cdot10^{-2}$	0.03	$7\cdot10^{-3}$	–	–	–	–	–	–	–	–	–	–	–	–
			–	0.03	$6\cdot10^{-3}$	0.03	$2\cdot10^{-3}$	0.03	$6\cdot10^{-3}$	–	–	–	–	–	–	–	–	–	–	–	–

[a] Experimentally determined Kováts retention indices.
[b] Percentages were calculated from the peak areas on the DB-1 column (flame ionization detector).
[c] Steam distillation.
[d] Cold pressing.
[e] Traces.

orange oils are slightly higher in the extracts isolated by cold pressing (Table 1).

Nonanal (0.08–0.13%), linalool (0.16–0.43%), *cis*-sabinene hydrate (0.05–0.21%), 1,2-dihydrolinalool (0.03–0.10%), thymol (0.02–0.05%), methylthymol (0.01–0.05%), α-bergamotene (0.18–0.22%) and α-muurolene (0.12–0.33%) were found only in lemon oils. Among characteristic secondary metabolites, nonanol (0.10–0.13%), citronellyl acetate (0.05–0.10%) and α-sinensal (0.13–0.23% were present only in mandarin oil and camphene (0.27–0.52%), 3-carene (0.04–0.08%) and δ-cadinene (0.03–0.04%) only in orange oil.

In order to appreciate the influence of fruit maturity on the chemical composition of the essential oils obtained, the results from Table 1 were grouped into compound families, as shown in Fig. 2. For quantitative evaluation, all GC peak areas were compared relative to the internal standard. Monoterpenes ($C_{10}H_{16}$) represented the main compound family in all extracts. Their abundance varied in the oils isolated by steam distillation (cold pressing) in the ranges 94.01–94.40% (94.19–96.36%) in lemon, 95.92–97.01% (98.43–98.66%) in mandarin and 95.94–96.72% (96.25–97.03%) in orange.

Mandarin fruits possessed the highest content of monoterpenes ($C_{10}H_{16}$) when at their intermediate (II) and full (III) maturation stages. At any stage of fruit ripeness, lemon and mandarin essential oils had 1.5–2 times more oxygenated compounds than the respective distilled or cold-pressed orange oils. The sesquiterpene ($C_{15}H_{24}$) contents were found to be 2–3 times higher in lemon than in mandarin and orange essential oils, respectively.

The generation of the total volatile secondary metabolites grew initially during the fruit maturation and achieved the highest concentration at the intermediately matured stage (II, 45–75% green), but then decreased as the fruits ripened completely (III) and their peel colaration turned yellow-orange (Fig. 2).

3.2. Citrus leaf essential oils

Fig. 3 shows typical gas chromatograms of the oils extracted by steam distillation from lemon, mandarin and orange leaves. Kováts retention indices calculated for chromatographic peaks on both polar (DBWAX) and non-polar (DB-1) columns were used together with the mass spectra in the identification of the essential oil components. Table 2 contains the results of the triplicate analysis of lemon, mandarin and orange leaf oils. The chemical composition of these extracts differed more sharply than for those isolated from the respective citrus peels (Table 1 and 2).

Whereas only 37 compounds (97% positively identified) were detected in mandarin leaf oil, 50 and 52 constituents (96% positively identified in both oils) were found in the extracts distilled

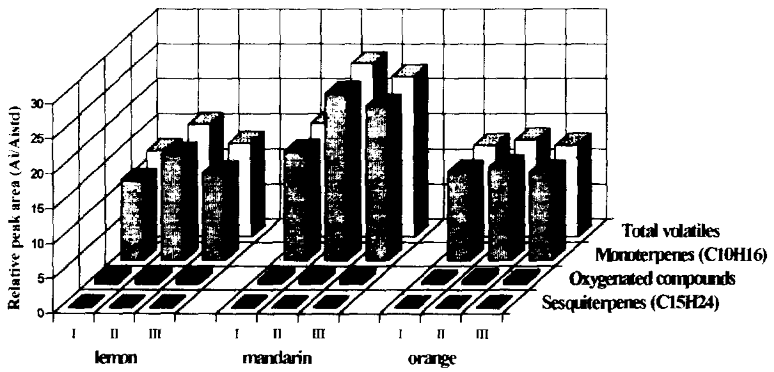

Fig. 2. Compositional variation of citrus peel oils obtained by steam distillation from fruits at different stages of ripeness (I = 100% green; II = 45–75% green; III = fully matured, yellow-orange fruits).

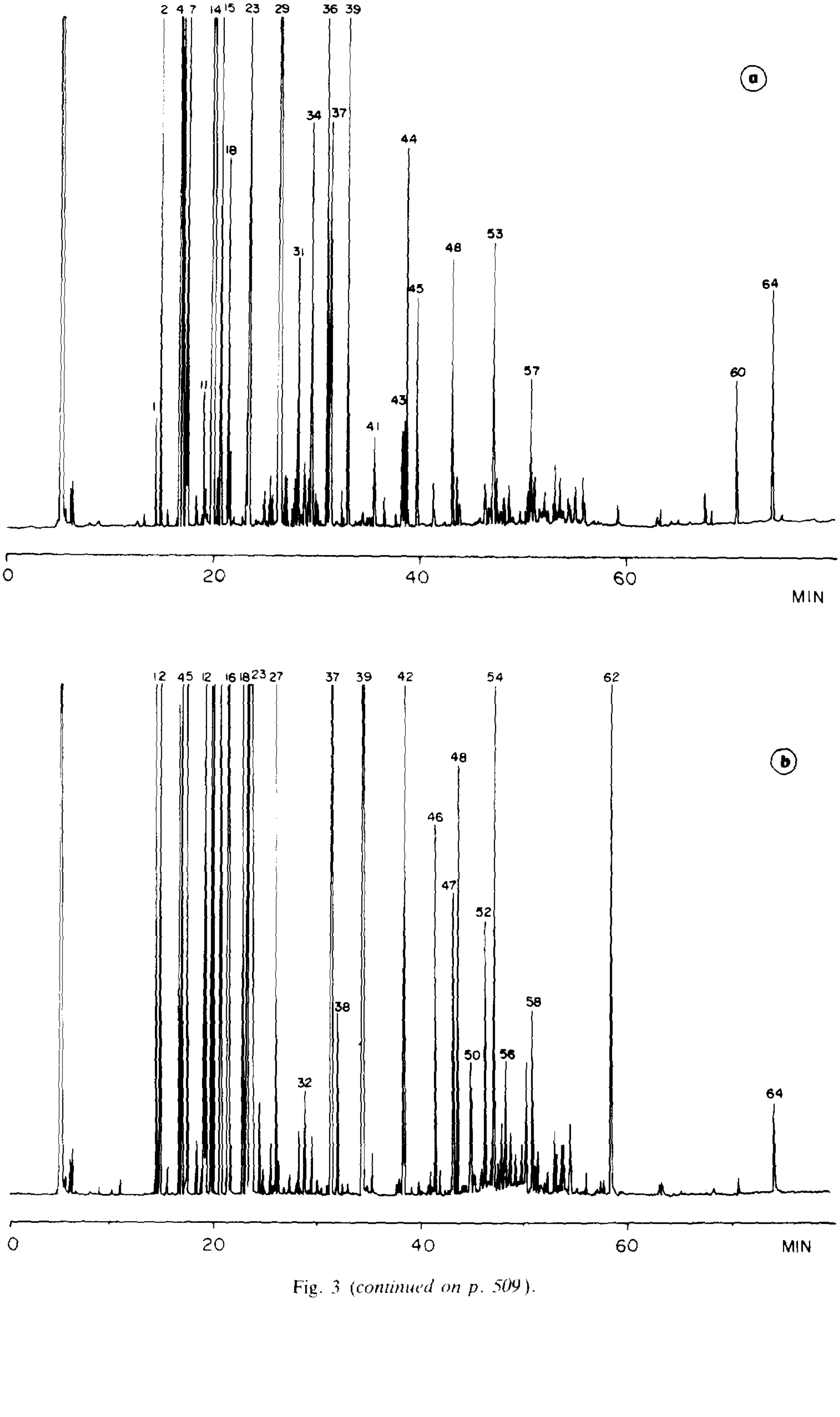

Fig. 3 (continued on p. 509).

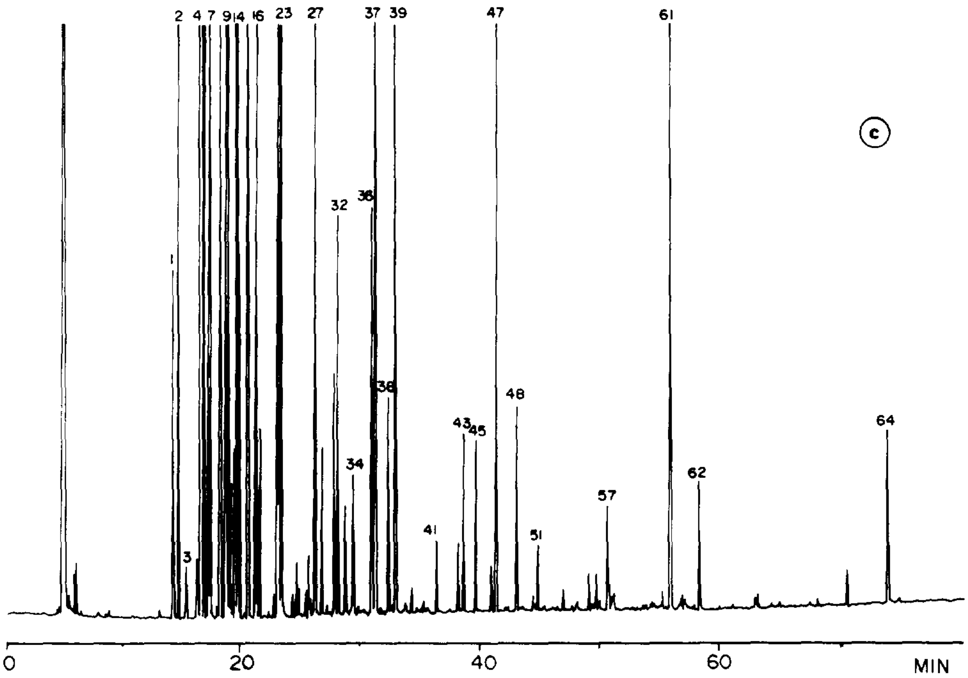

Fig. 3. Typical gas chromatograms of (a) lemon (*C. Volkameriana*), (b) mandarin (*C. reticulata*) and (c) orange (*C. sinensis*) leaf oils obtained by steam distillation on a DB-1 cross-linked fused-silica column (60 m × 0.20 mm I.D.) coated with polydimethylsiloxane (0.25-μm phase thickness). See Fig. 1 for GC conditions. See Table 2 for peak identification.

from lemon and orange leaves, respectively. The second most abundant compound in lemon leaf oil was citronellal (22.21%) (limonene was the most abundant, 41.74%), followed by sabinene (16.16%), *trans*-β-ocimene (3.88%) and linalool (3.12%). In the mandarin leaf oil linalool (52.66%) was found to be the major compound, together with limonene (8.32%), *trans*-β-ocimene (7.87%), neral (6.05%) and geranial (7.45%), which were presented in large amounts. Orange leaf oil possessed sabinene (47.68%) as the main compound, followed by *trans*-β-ocimene (8.31%), 3-carene (7.38%), limonene (5.95%) and linalool (4.38%). Whereas citrus peel oils were constituted mainly by $C_{10}H_{16}$ monoterpenes (94.01–98.66%), the essential oils distilled from lemon, mandarin and orange leaves contained 65.26, 31.23 and 79.43% of monoterpenes, respectively.

Fig. 4 shows comparative chemical compositions of citrus leaf oils, based on the compound group classification. Mandarin leaf oil contained 2 and 3.5 times more oxygenated compounds than oils distilled from lemon and orange leaves, respectively. Based on the high content of linalool, neral and geranial (total 66.16%), mandarin leaf essential oil could be developed as an important raw material for the flavour and fragrance industries.

Fig. 5 shows the comparative chemical compositions of oxygenated compounds found in the citrus leaf oils analysed. Aldehydes were 2 and 3.5 times more abundant in lemon leaf oil (27.59%) than in mandarin and orange leaf oils, respectively. Alcohols were the predominant (70%) oxygenated compounds in mandarin leaf oil and prevailed almost six times over the total alcohol concentration in lemon and orange leaf oils.

4. Conclusions

The various constituents of the essential oils from Colombian lemon (*C. volkameriana*), mandarin (*C. reticulata*) and orange (*C. sinensis*) were isolated by cold pressing or/and by steam distillation from the fruit peel and leaves, and

Table 2
Chemical compositions of citrus leaf essential oils

Peak No. (DB-1)	Compound	I_K[a]		GC peak area (%)[b]					
		DB-1	DBWAX	Lemon	S.D.	Mandarin	S.D.	Orange	S.D.
1	α-Thujene	924	1025	0.11	$6\cdot10^{-2}$	1.26	$1\cdot10^{-2}$	0.30	$1\cdot10^{-2}$
2	α-Pinene	932	1021	0.53	$1\cdot10^{-1}$	2.66	$7\cdot10^{-2}$	0.55	$6\cdot10^{-1}$
3	Camphene	962	1083	0.04	$1\cdot10^{-2}$	0.37	$7\cdot10^{-2}$	0.02	$7\cdot10^{-4}$
4	Sabinene	967	1120	16.16	$3\cdot10^{+0}$	2.81	$1\cdot10^{-1}$	47.68	$2\cdot10^{+0}$
5	β-Pinene	973	1107	0.69	$1\cdot10^{-1}$	0.86	$9\cdot10^{-2}$	2.00	$7\cdot10^{-2}$
6	*n*-Octanal	977	1270	0.13	$6\cdot10^{-3}$	–	–	0.04	$5\cdot10^{-3}$
7	β-Myrcene	982	1162	1.56	$3\cdot10^{-1}$	0.05	$6\cdot10^{-3}$	3.73	$2\cdot10^{-1}$
8	α-Phellandrene	994	1177	0.06	$6\cdot10^{-3}$	–	–	0.69	$3\cdot10^{-2}$
9	3-Carene	1000	1150	–	–	0.04	$4\cdot10^{-3}$	7.38	$3\cdot10^{-1}$
10	1,4-Cineole	1009	1177	–	–	0.27	$8\cdot10^{-3}$	0.48	$3\cdot10^{-2}$
11	α-Terpinene	1012	1183	0.11	$4\cdot10^{-2}$	1.95	$3\cdot10^{-1}$	0.12	$1\cdot10^{-2}$
12	*p*-Cymene	1013	1284	–	–	2.35	$3\cdot10^{-1}$	0.02	$1\cdot10^{-3}$
13	1,8-Cineole	1018	1228	–	–	0.70	$2\cdot10^{-1}$	0.16	$6\cdot10^{-3}$
14	*d*-Limonene	1026	1201	41.74	$2\cdot10^{+0}$	8.32	$2\cdot10^{+0}$	5.95	$2\cdot10^{-1}$
15	β-Phellandrene	1027	1213	–	–	–	–	0.33	$3\cdot10^{-2}$
16	*trans*-β-Ocimene	1035	1252	3.88	$4\cdot10^{-1}$	7.87	$9\cdot10^{-1}$	8.31	$4\cdot10^{-1}$
17	Not identified	1045	–	–	–	–	–	0.13	$6\cdot10^{-3}$
18	γ-Terpinene	1051	1248	0.27	$8\cdot10^{-2}$	1.20	$2\cdot10^{-1}$	0.65	$4\cdot10^{-2}$
19	*trans*-Sabinene hydrate	1054	1463	0.07	$3\cdot10^{-2}$	–	–	0.16	$7\cdot10^{-3}$
20	*n*-Octanol	1074	1507	–	–	–	–	0.27	$1\cdot10^{-2}$
21	Terpinolene	1080	1291	0.11	$1\cdot10^{-2}$	1.49	$2\cdot10^{-1}$	1.57	$7\cdot10^{-2}$
22	Nonanal	1082	1382	0.16	$3\cdot10^{-2}$	–	–	–	–
23	Linalool	1083	1553	3.12	$7\cdot10^{-1}$	52.66	$3\cdot10^{+0}$	4.38	$2\cdot10^{-1}$
24	*cis*-Sabinene hydrate	1088	–	–	–	0.08	$9\cdot10^{-3}$	0.02	$2\cdot10^{-3}$
25	Myrcenol	1104	1581	–	–	–	–	0.05	$2\cdot10^{-3}$
26	Methyl *n*-octanoate	1106	1372	–	–	–	–	0.12	$1\cdot10^{-1}$
27	*cis*-Epoxylimonene	1118	–	0.07	$3\cdot10^{-2}$	0.59	$1\cdot10^{-1}$	0.05	$3\cdot10^{-3}$
28	1,2-Dihydrolinalool	1121	1449	0.05	$1\cdot10^{-2}$	0.03	$8\cdot10^{-3}$	0.02	$9\cdot10^{-4}$
29	Citronellal	1131	1472	22.21	$3\cdot10^{+0}$	–	–	2.83	$1\cdot10^{-1}$
30	Isopulegol	1146	1565	0.04	$1\cdot10^{-2}$	–	–	0.13	$2\cdot10^{-2}$
31	Isoborneol	1157	1654	0.18	$1\cdot10^{-1}$	–	–	0.20	$2\cdot10^{-2}$
32	Nonanol	1161	–	0.17	$1\cdot10^{-2}$	0.19	$6\cdot10^{-2}$	0.37	$1\cdot10^{-2}$
33	α-Terpineol	1179	1740	0.13	$2\cdot10^{-2}$	0.06	$5\cdot10^{-3}$	0.10	$4\cdot10^{-3}$
34	Decanal	1183	1482	0.96	$1\cdot10^{-1}$	–	–	0.15	$6\cdot10^{-3}$
35	Methylthymal	1196	–	0.02	$2\cdot10^{-2}$	–	–	–	–
36	Citronellol	1203	1751	1.43	$5\cdot10^{-1}$	–	–	0.53	$2\cdot10^{-2}$
37	Neral	1215	1705	0.91	$6\cdot10^{-1}$	6.05	$1\cdot10^{-0}$	1.87	$7\cdot10^{-2}$
38	Geraniol	1237	1789	0.04	$2\cdot10^{-3}$	0.16	$1\cdot10^{-2}$	0.21	$8\cdot10^{-3}$
39	Geranial	1248	1735	1.26	$5\cdot10^{-1}$	7.45	$1\cdot10^{-1}$	2.61	$1\cdot10^{-1}$
40	Linalyl acetate	1264	1519	0.02	$8\cdot10^{-3}$	–	–	0.03	$2\cdot10^{-3}$
41	Thymol	1286	2100	0.07	$4\cdot10^{-2}$	–	–	0.07	$3\cdot10^{-3}$
42	Citronellyl acetate	1335	1645	0.11	$2\cdot10^{-2}$	0.43	$3\cdot10^{-1}$	0.06	$2\cdot10^{-3}$
43	Neryl acetate	1340	1729	0.13	$3\cdot10^{-2}$	–	–	0.17	$7\cdot10^{-3}$
44	Not identified	1341	–	0.60	$1\cdot10^{-1}$	–	–	–	–
45	Geranyl acetate	1357	1746	0.37	$8\cdot10^{-2}$	–	–	0.16	$6\cdot10^{-3}$
46	Longifolene	1380	1642	0.07	$3\cdot10^{-3}$	0.23	$1\cdot10^{-1}$	0.04	$2\cdot10^{-3}$
47	δ-Elemene	1384	1475	0.05	$2\cdot10^{-3}$	0.22	$1\cdot10^{-1}$	0.63	$2\cdot10^{-2}$
48	β-Caryophyllene	1424	1654	0.36	$1\cdot10^{-2}$	0.24	$2\cdot10^{-1}$	0.21	$8\cdot10^{-3}$

(*Continued on p. 512.*)

Table 2 (*continued*)

Peak No. (DB-1)	Compound	I_K [a]		GC peak area (%) [b]					
		DB-1	DBWAX	Lemon	S.D.	Mandarin	S.D.	Orange	S.D.
49	Isoeugenol	1431	–	0.06	$1 \cdot 10^{-2}$	–	–	–	–
50	α-Bergamotene	1435	1589	0.06	$2 \cdot 10^{-3}$	0.11	$2 \cdot 10^{-2}$	–	–
51	α-Humulene	1452	1676	0.04	$1 \cdot 10^{-3}$	0.08	$3 \cdot 10^{-2}$	0.06	$3 \cdot 10^{-3}$
52	Germacrene D	1477	1714	0.04	$2 \cdot 10^{-2}$	0.17	$1 \cdot 10^{-1}$	–	–
53	β-Bisabolene	1495	1770	0.31	$9 \cdot 10^{-2}$	–	–	0.03	$2 \cdot 10^{-3}$
54	β-Selinene	1500	1756	0.08	$1 \cdot 10^{-2}$	1.01	$4 \cdot 10^{-1}$	–	–
55	α-Muurolene	1502	1730	–	–	0.08	$5 \cdot 10^{-2}$	–	–
56	δ-Cadinene	1511	1766	0.03	$2 \cdot 10^{-3}$	0.08	$1 \cdot 10^{-2}$	–	–
57	Germacrene D	1564	1550	0.15	$6 \cdot 10^{-2}$	0.10	$4 \cdot 10^{-2}$	0.12	$5 \cdot 10^{-3}$
58	α-Bisabolol	1570	2022	–	–	0.29	$5 \cdot 10^{-2}$	–	–
59	δ-Cadinol	1615	2150	0.03	$8 \cdot 10^{-3}$	0.06	$8 \cdot 10^{-3}$	–	–
60	Sesquiterpenol	1650	–	0.05	$2 \cdot 10^{-2}$	0.09	$1 \cdot 10^{-2}$	0.03	$6 \cdot 10^{-3}$
61	Not identified	1672	–	–	–	–	–	0.75	$4 \cdot 10^{-2}$
62	α-Sinensal	1725	–	–	–	0.50	$2 \cdot 10^{-1}$	0.13	$8 \cdot 10^{-3}$
63	$C_{20}H_{40}O$	1918	–	0.03	$3 \cdot 10^{-3}$	–	–	–	–
64	Phytol	2066	–	0.66	$2 \cdot 10^{-1}$	0.13	$1 \cdot 10^{-2}$	0.22	$9 \cdot 10^{-3}$

[a] Experimentally determined Kováts retention indices.
[b] Percentages were calculated from the peak areas on the DB-1 column.

were identified and quantified by means of HRGC with flame ionization or mass spectrometric detection. DB-1 and DB-WAX fused-silica capillary columns (both 60 m × 0.25 mm I.D.) were successfully applied to resolve completely all volatile compounds present in the extracts. Thus, under the conditions employed in this work, prefractionation of the citrus essential oils was not required for their complete GC analysis. The highest concentration of the volatile secondary metabolites was observed in the extracts isolated either by steam distillation or cold pressing from the citrus peel (*lemon, mandarin or orange*) at the intermediate stage of fruit maturity (45–75% green). No pronounced qualitative or quantitative differences in composition were detected between the expressed and distilled citrus peel oils studied. In the Colombian citrus peel oils, the total mass concentration of citral (neral + geranial) and linalool, which are considered to be the most potent aroma compounds in citrus oils [12], did not exceed 3.0%

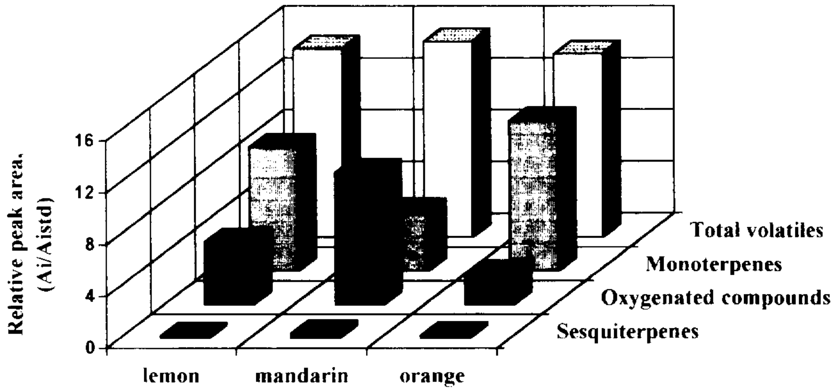

Fig. 4. Comparative chemical compositions of citrus leaf oils obtained by steam distillation.

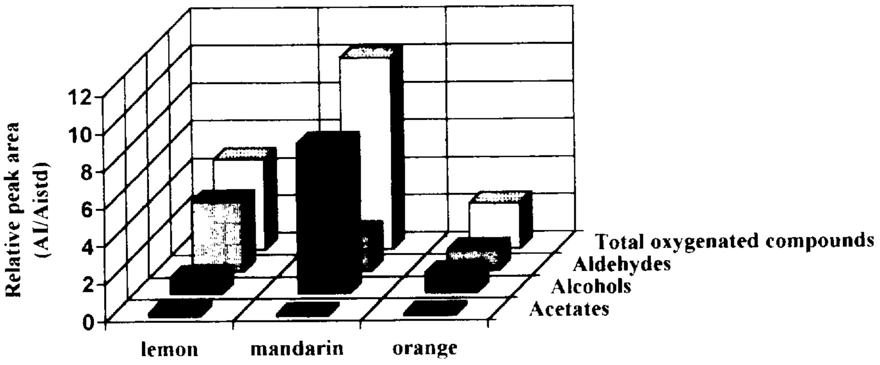

Fig. 5. Comparative chemical composition of the oxygenated compounds in the oils distilled from citrus leaves.

(lemon), 0.4% (mandarin) and 0.35% (orange). The content of limonene, suggested to be more important than citral to convey the fresh lemon aroma [13], reached 78.90, 90.05 and 94.55% in lemon, mandarin and orange peel oils, respectively. The high mass concentrations of citronellal (22.21%), linalool (52.66%) and citral (13.50%) in the steam-distilled lemon and mandarin leaf oils, respectively, make these oils very attractive raw materials for the flavour industry.

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