Multidimensional Capillary GC–GC for the Analysis of Real Complex Samples. 3. Enantiomeric Distribution of Monoterpene Hydrocarbons and Monoterpene Alcohols of Mandarin Oils

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The enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, and α-terpineol in mandarin oils has been determined using a fully-automated, multidimensional, double-oven GC–GC system. This system allows fractions to be multitransferred during the same GC analysis and the use of the two GCs independently when the multitransfer option is not used. The results obtained allowed the characterization of mandarin essential oil and the determination of extraneous oils added to or contaminating the oil.

Keywords: *Multidimensional GC; enantioselective gas chromatography; mandarin oil; monoterpene hydrocarbons; monoterpene alcohols*

INTRODUCTION

The determination of the enantiomeric distribution of citrus essential oil components by gas chromatographic analysis using capillary columns coated with modified cyclodextrins has been studied extensively.

Enantioselective gas chromatography on the whole essential oil can represent the single step of the analytical process (Takeoka et al., 1990; König et al., 1990, 1992, 1994; Cotroneo et al., 1992; Dugo et al., 1992a,b, 1993; Werkhoff et al., 1993; Bicchi et al., 1994; Ravid et al., 1995) or the final step of a more complex analytical process that permits the separation of the compounds of interest that can be, therefore, analyzed by GC analysis with a chiral column. On-line HPLC–HRGC systems have been rarely used (Dugo et al., 1994a,b; Mondello et al., 1996), while multidimensional GC–GC systems have been more extensively used (Hener et al., 1990a,b; Mosandl et al., 1990; Kreis et al., 1991; Schubert and Mosandl, 1991; Weinrich and Nitz, 1992; Rocca et al., 1992; Werkhoff et al., 1993; Mosandl, 1995; Wang et al., 1995).

LC–GC systems permit transfer of compounds of the same polarity from the LC to the GC for further separation and analysis. The GC–GC systems, instead, allow us to transfer fractions containing one or more compounds of the same volatility belonging to different chemical classes.

The LC–GC systems allow transfers from the HPLC to the GC of more than one LC fraction, but each LC fraction subsequent to the first can be transferred and analyzed by GC only after the end of the previous GC run (Munari et al., 1990). The GC–GC systems permit several fractions to be transferred from the precolumn to the analytical column and analyzed during the same GC run. Therefore, the multidimensional GC systems prove to be more versatile, allowing the chiral analysis, during the same LC and GC run, even for a large number of compounds (Mondello et al., 1997a,b).

The early multidimensional GC systems were based on Deans' principle (Deans, 1968), while systems working with mechanical valves, proposed earlier, were not considered reliable, since the valves available at that time did not have adequate thermal stability and memory effects were likely to occur. Technological progress of valve design rendered miniaturized connectors available for the assembly of multidimensional GC systems, eliminating unswept volumes. These mechanical valves are stable at high temperatures, can be used to set up multidimensional GC systems without any drawbacks, and are easier to operate than those based on Deans' principle (Mondello et al., 1997a,b).

The enantiomeric distribution of some monoterpene hydrocarbons in mandarin essential oils was studied by Mosandl's research group (Mosandl et al., 1990; Hener et al., 1990b; Kreis et al., 1991; Mosandl, 1995), Dugo et al. (1992a,b), Rocca et al. (1992), and Casabianca et al. (1995), while the distribution of some monoterpene alcohols was the subject of research carried out by Bicchi et al. (1994), Dugo et al. (1994a,b), Mondello et al. (1996), and Casabianca and Graff (1996). Table 1 shows the results reported in the earlier literature on the enantiomeric distribution of the components of mandarin oil.

Most of the results of the research is limited to one sample only or a very small number of samples, and the data obtained are not related to the extraction technologies or to the harvest time and the cultivar of the fruits.

This paper reports the values obtained for the enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, and α-terpineol, in mandarin essential oil, obtained by a fully automated multidimensional GC, assembled in our laboratories, which allows the chiral analysis of these six components with only one run (Mondello et al., 1997a,b).

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Table 1. Enantiomeric Distributions of Some Components of Mandarin Oil Reported in the Literature

		Mosandl et al., 1990; Hener et al., 1990b; Mosandl, 1995	Kreis et al., 1991	Rocca et al., 1992	Dugo et al., 1992a,b	Bicchi et al., 1994	Dugo et al., 1994a		Dugo et al., 1994b	Casabianca et al., 1995	Casabianca and Graff, 1996
							a	*b*	*c*		
α-pinene	1*R*,5*R* (+)	57–68	45–100	52						68–96	
	1*S*,5*S* (−)	43–32	55-tr	48						32–4	
β-pinene	1*R*,5*R* (+)	84–97	63–99	96						76–78	
	1*S*,5*S* (−)	16–3	37–1	4						24–22	
limonene	4*S* (−)	tr-1	tr-1	2	1.8–2.6					tr-0.5	
	4*R* (+)	100–99	100–99	98	98.2–97.4		16–18	16–20	12–30	100–99.5	4.0–6.5
linalool	3*R* (−)					16.1–16.8	84–82	84–80	88–70		96.0–93.5
	3*S* (+)					83.9–83.2	16–18	16–20	12–30		
terpinen-4-ol	4*S* (+)					11.3–11.9	12–14	25–30			
	4*R* (−)					88.7–88.1	88–86	75–70			
α-terpineol	8*S* (−)					74.8–76.7					
	8*R* (+)					25.2–23.3					

[a] Cold-pressed oils. [b] Distilled oils. [c] Uruguayan oils (cv. Malvasio, Ellendale, Ortanique, Comun, and Malaquina).

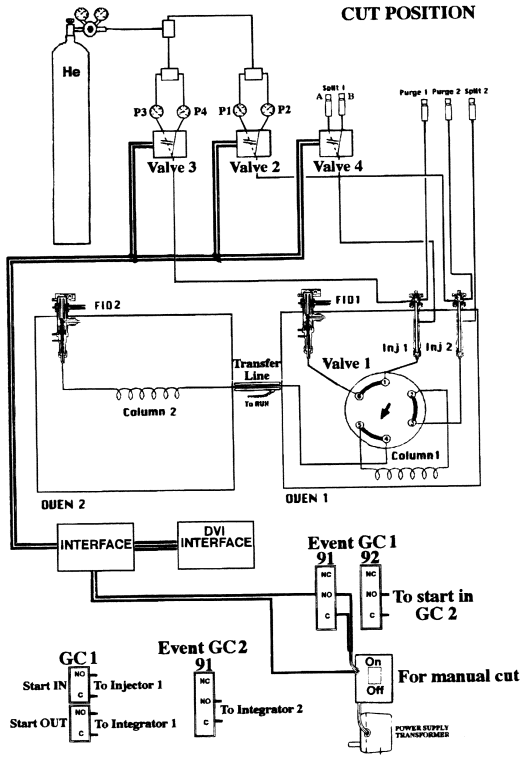

Figure 1. Pneumatic and electronic scheme of the GC–GC system in the cut position.

Table 2. Mandarin Oil Samples Analyzed

no. of samples	period of prodn	color of the oils	technol of extrctn
7	Oct 1996	green	Pelatrice
48	Oct–Nov 1996	yellow	Torchi (screw-press)
5	Dec 1996	red	Torchi (screw-press)
3	Dec 1996–Jan 1997	red	FMC
7	Dec 1996–Feb 1997	red	laboratory-extracted
4	Nov–Dec 1996	white or pale yellow	distilled oils
11			commercial oils
4			mixtures

MATERIALS AND METHODS

The analyses were carried out on genuine cold-pressed mandarin essential oils, produced during the 1996–1997 season with the most commonly used extraction techniques; on laboratory-extracted mandarin oils; on distilled oils obtained from the residue of the extraction of the cold-pressed oils; on commercial oils; on mixtures of cold-pressed mandarin oil with sweet orange, lemon, and clementine tangerine oils; and on sweet orange, lemon, and clementine oils.

The laboratory-extracted oils were obtained by applying manual pressure on the rind so as to cause the breaking of the utricules and the release of the oil itself, which was collected on a watch glass, transferred to a test tube, and centrifuged.

The samples of mandarin oils analyzed are described in Table 2.

All the samples were analyzed by multidimensional GC injecting 1 μL of a 10% (v/v) solution of essential oil in pentane with a split ratio of 1:10.

The multidimensional system used in this study was a developmental model, formerly described in detail (Mondello et al., 1997a,b), which consisted of two Shimadzu 17A gas chromatographs, a transfer line, and two Shimadzu C-R3A

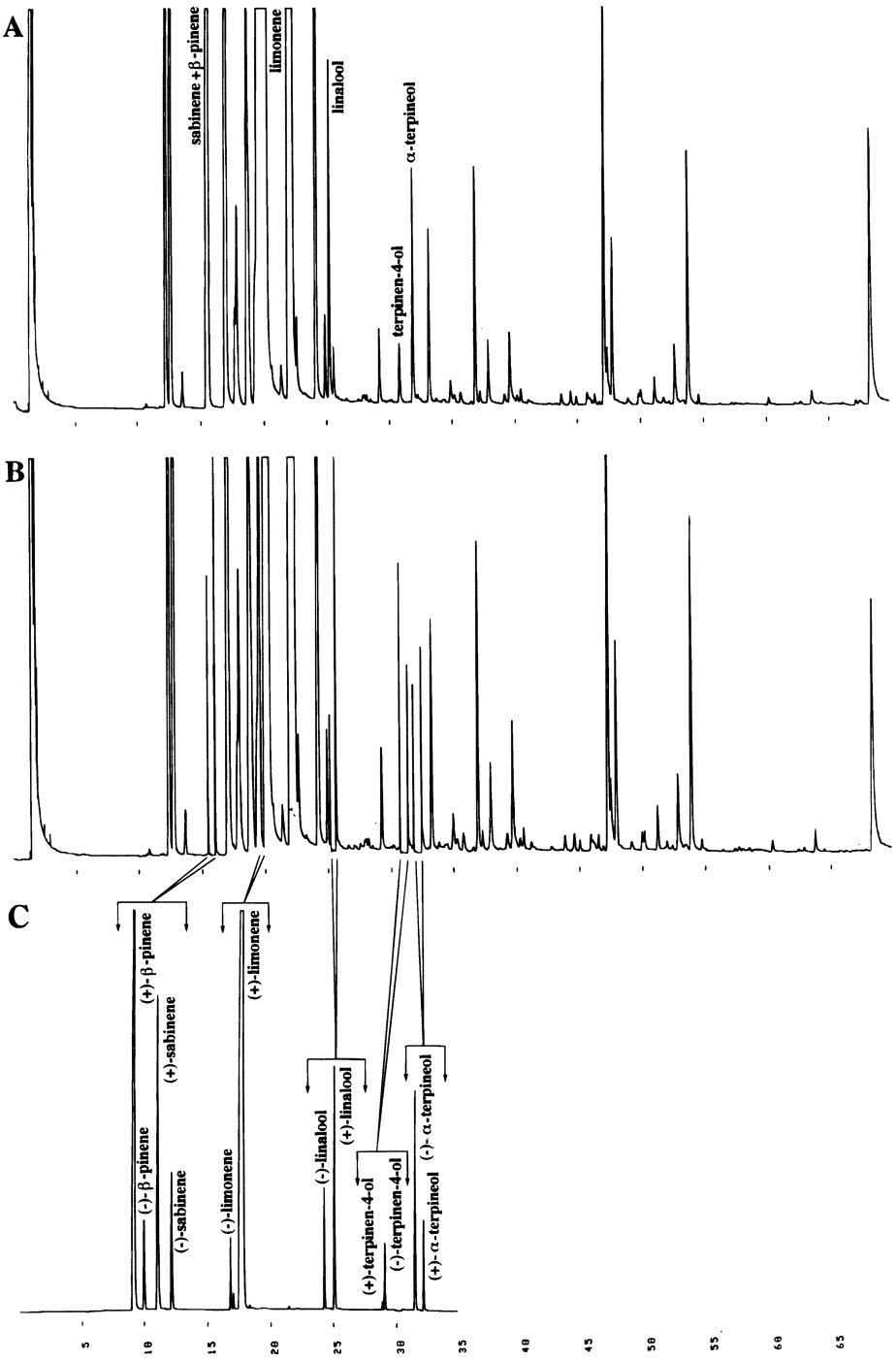

Figure 2. (A) GC chromatogram of a cold-pressed mandarin oil obtained with the SE-52 column. (B) GC chromatogram of a cold-pressed mandarin oil obtained with the SE-52 column with the five heartcuts. (C) GC–GC chiral chromatogram of the transferred components.

integrators. The instrumental setup and the experimental conditions used are described below (see Figure 1).

Gas Chromatograph 1. The conditions were as follows: two split/splitless injectors at 250 °C with two manual flow controllers (injectors 1 and 2) and a FID at 250 °C (FID 1); a SE-52 capillary column, 30 m × 0.32-mm i.d., 0.40–0.45-μm film thickness (Mega, Legnano, Italy), temperature program: 45 °C for 6 min and then to 240 °C at 2.0 °C/min, carrier He

Table 3. Percentage Compositions of the Transferred Components in the Analyzed Oils and Transfer Windows

	mandarin oil					
	industrial (Cotroneo et al., 1994)	laboratory-extracted	distilled	commercial	lemon oil (Trozzi et al., 1993)	mandarin 90%, lemon 10%
β-pinene + sabinene	1.62–3.96	1.21–1.74	1.42–2.24	1.67–2.67	10.58–20.59	3.86
limonene	65.30–74.53	69.45–78.51	67.05–74.69	61.38–92.95	59.57–71.06	68.25
linalool	0.04–0.19	0.08–0.20	0.07–0.33	0.05–0.58	0.05–0.18	0.12
terpinen-4-ol	0.01–0.21	0.03–0.05	0.03–0.39	0.01–0.07	0.01–0.08	0.07
α-terpineol	0.04–0.54	0.09–0.22	0.07–0.44	0.04–0.23	0.06–0.28	0.21

	mandarin 99%, lemon 1%	sweet orange oil (Dugo et al., 1994c)	mandarin 80%, Sweet orange 20%	clementine oil	mandarin 90%, clementine 10%	transfer windows, min
β-pinene + sabinene	1.98	0.26–1.20	1.59	1.10	1.76	15.30–16.00
limonene	71.45	93.93–95.90	75.53	93.20	73.37	20.00–20.20
linalool	0.10	0.25–0.67	0.16	0.19	0.11	25.00–25.55
terpinen-4-ol	0.04	<0.01–0.01	0.04	<0.01	0.04	30.60–31.20
α-terpineol	0.14	0.03–0.10	0.13	0.04	0.14	31.60–32.35

Table 4. Enantiomeric Distributions of Some Components in Laboratory-Extracted Mandarin Oils from cv. Avana Comune and Tardivo di Ciaculli

		Avana Comune			Tardivo di Ciaculli				
		1	2	3	1	2	3	4	range
β-pinene	1*R*,5*R* (+)	98.7	98.3	98.8	98.5	98.8	98.7	98.8	98.3–98.8
	1*S*,5*S* (−)	1.3	1.7	1.2	1.5	1.2	1.3	1.2	1.7–1.2
sabinene	1*R*,5*R* (+)	79.9	82.9	80.7	81.1	82.9	83.4	81.3	79.9–83.4
	1*S*,5*S* (−)	20.1	17.1	19.3	18.9	17.1	16.6	18.7	20.1–16.6
limonene	4*S* (−)	1.9	1.5	1.8	1.6	1.5	1.5	1.6	1.5–1.9
	4*R* (+)	98.1	98.5	98.2	98.4	98.5	98.5	98.4	98.5–98.1
linalool	3*R* (−)	13.3	15.6	13.7	13.6	18.2	19.3	12.7	12.7–19.3
	3*S* (+)	86.7	84.4	86.3	86.4	81.8	80.7	87.3	87.3–80.7
terpinen-4-ol	4*S* (+)	10.5	17.5	13.6	11.7	13.0	12.9	11.3	10.5–17.5
	4*R* (−)	89.5	82.5	86.4	88.3	87.0	87.1	88.7	89.5–82.5
α-terpineol	4*S* (−)	75.1	67.8	72.1	72.9	68.9	67.8	71.5	67.8–75.1
	4*R* (+)	24.9	32.2	27.9	27.1	31.1	32.2	28.5	32.2–24.9

90 kPa (2.7 mL/min); a Valco six-port ($^1/_{16}$-in.) two-position W-type valve (valve 1) with a right-angle drive (A3RADN6WT) (Valco Europe); a digital valve interface (DVI-220) (Valco Europe) connected to EVENT 91 on gas chromatograph 1; a solenoid valve (valve 2) to change the carrier pressure (P1, 110 kPa) (standby position, column 2) to higher pressure (P2, 195 kPa) (cut position, columns 1 and 2) connected to EVENT 91 on gas chromatograph 1 (this ensures that the correct retention times are obtained on column 1 even for those components eluted after more than one transfer); a solenoid valve (valve 3) to change the carrier pressure (P3, 90 kPa) (standby position, column 1) to lower pressure (P4, 5 kPa) (cut position, injector 1 and FID 1) connected to EVENT 91 on gas chromatograph 1 (this allows us to maintain a constant flow in the detector FID 1 and protects it from flow surges due to the absence of column 1 in the flow path when the system is in the cut position); a solenoid valve (valve 4) which allows the use of two splitter valves (A and B) with different ratios in the injector 1 (this valve allows splitter 1A to be used in the standby position (split ratio for the sample introduction) and splitter 1B in the cut position (high split ratio to rapidly establish pressure P4); a Shimadzu C-R3A integrator connected to start and out signals on gas chromatograph 1.

Transfer Line. An aluminum thermoregulated block was equipped with a heater assy and a thermocouple assy connected to the AUX2 exit on gas chromatograph 1.

Gas Chromatograph 2. The conditions were as follows: a MEGADEXDETTBSβ (diethyl-*tert*-butylsilyl-β-cyclodextrin), 25 m × 0.25-mm i.d., 0.25-μm film thickness (Mega, Legnano, Italy), temperature program: 45 °C × 6 min and then to 180 °C at 2.0 °C/min, carrier He 110 kPa (1.9 mL/min); the GC program started with the first cut; a FID at 250 °C (FID 2); a Shimadzu C-R3A integrator connected to EVENT 91 on gas chromatograph 2.

As shown in Figure 1, the system is completely automated by the use of the external events of the gas chromatograph. The time the valve should be switched to begin the cuts can be determined from a preliminary analysis. After this step, a fully-automated analysis is possible by programming the valve events.

RESULTS AND DISCUSSION

The essential oils were first analyzed with the SE-52 precolumn to determine the concentrations of the components of interest and their retention times, maintaining the multidimensional system in the standby position. Depending on the retention times and the concentration of each component, different transfer windows were chosen and automatically programmed so that well-resolved peaks would be obtained on the chiral column either for components present in the oils at high concentrations and for those present at very low concentrations.

Figure 2 reports the chromatogram of a cold-pressed mandarin oil obtained with the SE-52 column and the system in the standby position, the chromatogram of the same oil obtained with the SE-52 column and the system in the cut position (on this chromatogram, the cuts are shown), and the chromatogram obtained with the chiral column for the fractions transferred from the SE-52 precolumn.

To obtain on the chiral column well-resolved peaks either for the compounds present in large amounts and for the components present as traces, limonene in all the oils and β-pinene + sabinene in lemon oils were only partially transferred because of their high concentrations; while the other components analyzed were quantitatively transferred since they were present in lower amounts.

The percentage compositions of the transferred components in the analyzed oils and the transfer windows are shown in Table 3.

Table 5. Enantiomeric Distributions of Some Components in Cold-Pressed Mandarin Oils Grouped According to Month and Technology of Production

		October			December			all (63 samples)	
		Pelatrice (7 samples)	Torchi (32 samples)	Nov Torchi (16 samples)	Torchi (5 samples)	FMC (2 samples)	Jan FMC (1 sample)	$\bar{X}$	range
β-pinene	1*R*,5*R*(+)	98.3	98.4	98.0	97.7	97.6	97.4	98.2	97.0–98.8
	1*S*,5*S* (−)	1.7	1.6	2.0	2.3	2.4	2.6	1.8	3.0–1.2
sabinene	1*R*,5*R*(+)	79.2	79.2	78.8	78.7	78.6	78.8	79.0	76.2–80.5
	1*S*,5*S* (−)	20.8	20.8	21.2	21.3	21.4	21.2	21.0	23.8–19.5
limonene	4*S* (−)	2.2	2.2	2.2	2.2	2.1	2.0	2.2	2.0–2.3
	4*R* (+)	97.8	97.8	97.8	97.8	97.9	98.0	97.8	98.0–97.7
linalool	3*R* (−)	16.8	16.8	15.7	14.1	14.0	13.1	16.1	13.1–19.8
	3*S* (+)	83.2	83.2	84.3	85.9	86.0	86.9	83.9	86.9–80.2
terpinen-4-ol	4*S* (+)	12.7	14.1	12.8	11.0	11.5	11.4	13.2	10.0–19.2
	4*R* (−)	87.3	85.9	87.2	89.0	88.5	88.6	86.8	90.0–81.8
α-terpineol	4*S* (−)	74.7	75.7	76.3	76.1	71.3	71.1	75.6	69.6–76.8
	4*R* (+)	25.3	24.3	23.7	23.9	28.7	28.9	24.4	30.4–23.2

Laboratory-Extracted Oils. "Avana Comune" (harvesting earlier) and the "Tardivo di Ciaculli" (harvesting in the second half of the season) are the most common varieties in Sicily and Calabria; in Italy, therefore, they represent the raw material for the extraction of mandarin oils. The Avana comune cultivar is more common than the Tardivo di Ciaculli in Sicily, and the latter represent the predominant cultivar in some Calabrian areas. Since the fruits are carried to the factories by the warehouses and not by the direct producers, it is common that during the whole season the transformed fruits are mixtures of both cultivars.

Table 4 reports the enantiomeric distribution of the analyzed compounds in laboratory-extracted mandarin oils cultivar Avana Comune and Tardivo di Ciaculli.

As can be inferred from Table 4, the laboratory-extracted oils of the two varieties show similar values of enantiomeric distribution and therefore, the industrial samples' compositions are not affected by the proportions of each variety.

Industrial Cold-Pressed Oils. Table 5 reports the enantiomeric distribution of industrial cold-pressed mandarin oils.

As can be seen from Table 5, the values of the enantiomeric distribution of limonene are in accordance with the data reported in the literature and present constant values from October to January (see Table 1). The enantiomeric ratios of the other components present different ranges; these variations do not seem to be related to the extraction technologies used. The variation of the enantiomeric ratios of α-terpineol is irregular, while the enantiomeric ratios of β-pinene, sabinene, terpinen-4-ol, and linalool vary regularly during the season, as shown in Figure 3, where the enantiomeric excess of the analyzed components is plotted as a function of the month of production of the oil.

The ratios of β-pinene are in accordance with the results obtained by Rocca et al. (1992) and differ from most of the values reported in the literature, as can be seen in Table 1. The ratio between (−)- and (+)-β-pinene higher than 3:97 could be probably due to a contamination with other citrus oil, as can be deduced from the data reported in Table 6. Monoterpene alcohols show enantiomeric distributions in accordance with the values reported in the literature (Dugo et al., 1992b, Bicchi et al., 1994) but greatly differ from the values reported for linalool by Casabianca and Graff (1996).

The results reported by Casabianca and Graff for linalool seem to be closer to the data reported for clementine tangerine or sweet orange oil than to the data for a mandarin oil.

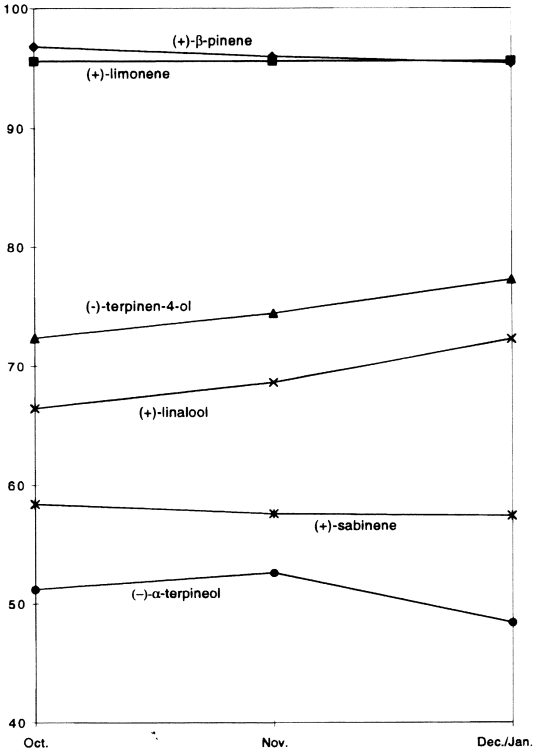

Figure 3. Variations of the enantiomeric excesses of (1*R*,5*R*)-(+)-β-pinene, (4*R*)-(+)-limonene, (4*R*)-(−)-terpinen-4-ol, (3*S*)-(+)-linalool, (1*R*,5*R*)-(+)-sabinene, and (4*S*)-(−)-α-terpineol.

The enantiomeric distribution of sabinene in mandarin oil has never been determined so far; therefore, its values cannot be compared with literature data.

Distilled Oil. Table 7 reports the enantiomeric distribution in distilled mandarin oil. Distilled oils, as reported in Table 7, show similar values of enantiomeric ratios for most of the components investigated to those determined in cold-pressed oils. An exception is terpinen-4-ol, which shows a relative percentage of the dextrorotatory isomer that is always higher than the highest values determined for cold-pressed oils, as already reported earlier (Dugo et al., 1994a). This difference is due to the extraction technologies; in fact, distilled oils are obtained from the aqueous acid residues

Table 6. Enantiomeric Distributions of Some Components of Cold-Pressed Lemon, Sweet Orange, and Clementine Oils and of Their Mixtures with Cold-Pressed Mandarin Oil

		lemon	mandarin 90%, lemon 10%	mandarin 99%, lemon 1%	sweet orange	mandarin 80%, sweet orange 20%	clementine	mandarin 90%, clementine 10%	range[a]
β-pinene	1*R*,5*R* (+)	5.1	50.2	90.3	42.2	97.7	22.9	95.8	97.0–98.8
	1*S*,5*S* (−)	94.9	49.8	9.7	57.8	2.3	77.1	4.2	3.0–1.2
sabinene	1*R*,5*R* (+)	15.1	45.6	75.0	96.8	85.8	90.1	82.5	76.2–83.4
	1*S*,5*S* (−)	84.9	54.4	25.0	3.2	14.2	9.9	17.5	23.8–16.6
limonene	4*S* (−)	1.9	2.2	2.0	0.6	1.7	0.7	1.9	1.5–2.3
	4*R* (+)	98.1	97.8	98.0	99.4	98.3	99.3	98.1	98.5–97.7
linalool	3*R* (−)	71.5	26.9	19.3	4.9	12.4	7.5	17.1	12.7–19.8
	3*S* (+)	28.5	73.1	80.7	95.1	87.6	92.5	82.9	87.3–80.2
terpinen-4-ol	4*S* (+)	19.7	15.7	14.4	64.9	15.0	46.4	15.1	10.0–19.2
	4*R* (−)	80.3	84.3	85.6	35.1	85.0	53.6	84.9	90.0–81.8
α-terpineol	4*S* (−)	77.4	74.4	73.5	8.2	69.2	13.1	71.8	67.8–76.8
	4*R* (+)	22.6	25.6	26.5	91.8	30.8	86.9	28.2	32.2–23.2

[a] Industrial and laboratory-extracted cold-pressed mandarin oils.

Table 7. Enantiomeric Distributions of Some Components in Distilled Mandarin Oils

		distilled oils				
		1	2	3	4	range
β-pinene	1*R*,5*R* (+)	97.4	97.3	96.1	97.7	96.1–97.7
	1*S*,5*S* (−)	2.6	2.7	3.9	2.3	3.9–2.3
sabinene	1*R*,5*R* (+)	78.5	78.8	77.8	79.9	77.8–79.9
	1*S*,5*S* (−)	21.5	21.2	22.2	20.1	22.2–20.1
limonene	4*S* (−)	2.2	2.1	2.0	1.7	1.7–2.2
	4*R* (+)	97.8	97.9	98.0	98.3	98.3–97.8
linalool	3*R* (−)	16.3	17.7	17.3	20.4	16.3–20.4
	3*S* (+)	83.7	82.3	82.7	79.6	83.7–79.6
terpinen-4-ol	4*S* (+)	25.3	27.3	25.6	28.7	25.3–28.7
	4*R* (−)	74.7	72.7	74.4	71.3	74.7–71.3
α-terpineol	4*S* (−)	71.1	68.4	73.4	61.2	61.2–73.4
	4*R* (+)	28.9	31.6	26.6	38.8	38.8–26.6

Table 8. Enantiomeric Distributions of Some Components of Commercial Mandarin Oils

		1	2	3	4	5	6	7	8	9	10	11	range[a]
β-pinene	1*R*,5*R* (+)	**29.3**	**40.3**	**71.1**	**94.2**	**84.0**	**94.6**	**56.8**	**49.6**	97.0	97.8	97.4	97.0–98.8
	1*S*,5*S* (−)	**70.7**	**59.7**	**28.9**	**5.8**	**16.0**	**5.4**	**43.2**	**50.4**	3.0	2.2	2.6	3.0–1.2
sabinene	1*R*,5*R* (+)	82.2	**37.6**	**58.3**	78.6	82.2	**96.6**	**86.2**	**75.1**	80.3	81.3	**85.5**	76.2–83.4
	1*S*,5*S* (−)	17.8	**62.4**	**41.7**	21.4	17.8	**3.4**	**13.8**	**24.9**	19.7	18.7	**14.5**	23.8–16.6
limonene	4*S* (−)	**4.5**	1.9	2.0	1.8	1.7	**0.7**	**4.6**	**5.0**	2.3	2.0	2.0	1.5–2.3
	4*R* (+)	**95.5**	98.1	98.0	98.2	98.3	**99.3**	**95.4**	**95.0**	97.7	98.0	98.0	98.5–97.7
linalool	3*R* (−)	**26.5**	19.2	15.3	14.0	12.4	**3.5**	18.0	19.1	17.6	13.7	**8.4**	12.7–19.8
	3*S* (+)	**73.5**	80.8	84.7	86.0	87.6	**96.5**	82.0	80.9	82.4	86.3	**91.6**	87.3–80.2
terpinen-4-ol	4*S* (+)	17.3	14.8	12.4	14.6	14.9	**38.9**	**24.5**	**25.8**	16.4	16.6	18.1	10.0–19.2
	4*R* (−)	82.7	85.2	87.6	85.4	85.1	**61.1**	**75.5**	**74.2**	83.6	83.4	81.9	90.0–81.8
α-terpineol	4*S* (−)	**43.9**	75.0	75.1	**46.1**	**53.2**	**24.2**	**36.2**	**46.2**	68.0	69.2	**57.4**	67.8–76.8
	4*R* (+)	**56.1**	25.0	24.9	**53.9**	**46.8**	**75.8**	**63.8**	**53.8**	32.0	30.8	**42.6**	32.2–23.2

[a] Industrial and laboratory-extracted cold-pressed oils.

of the cold extraction so that the temperature and pH cause the hydration of monoterpenes and the increase of the alcohols, especially terpinen-4-ol, while the linalool content is not influenced to a great extent in distilled citrus oils (Dugo et al., 1983; Verzera et al., 1992). The monoterpene hydration is not a stereospecific reaction so that it modifies the enantiomeric ratio of the alcohols leading this ratio toward the racemate (Dugo et al., 1994a).

Mixtures. The most common adulteration of mandarin oil is obtained by mixing the genuine oil with a certain percentage of reconstituted oil from sweet orange terpenes. The most common causes of contamination for mandarin oil are due to the presence of lemon or clementine oil. The contamination with lemon oil can occur during the extraction of the oil, since it is possible that the mandarin fruits are processed with the same machines previously used for lemon without being cleaned. The contamination determined by the presence of clementine oil is due to a nonadequate selection of fruits before the transformation that, therefore, are mixed in variable percentage with clementine.

In Table 6, the enantiomeric distribution of the same components analyzed for mandarin oil is reported, determined for lemon, clementine tangerine and sweet orange oils and for mixtures obtained by adding such oils to a cold-pressed mandarin oil.

The mixture of mandarin with lemon is obtained by adding 1% of the contaminant, since this may be the average quantity of this extraneous oil in mandarin oil due to processing of a small stock of mandarin with the machines previously used for the processing of lemon. The mixture with clementine tangerine oil is obtained with 10% of this oil since this appears to be the smallest amount that produces a variation to the organoleptic properties of mandarin oil; the mixture with sweet orange oil was prepared with 20% of the extraneous oil, considering that such adulteration can yield economical advantages.

As can be seen in Table 6, the contamination with lemon oil can be evidenced by the enantiomeric ratio of β-pinene and sabinene; the contamination caused by the presence of clementine oil is detected by observing the enantiomeric ratio of β-pinene, while the presence of sweet orange terpenes is evidenced by the enantiomeric ratio of sabinene.

The variation of the enantiomeric distribution of the components of mandarin oil contaminated by clementine and sweet orange oils is relatively modest. Although the enantiomeric distribution of the considered components is different in sweet orange and clementine oils, the content of these substances in such oils is much lower than that in mandarin oil.

Commercial Oils. From the 11 commercial oils analyzed (Table 8), samples 9 and 10 show values of the enantiomeric ratios compatible with a genuine mandarin oil. All the other samples show at least two of the ratios (marked character in Table 8) out of range for a genuine mandarin oil. For some of these samples, it may be possible to conjecture the nature of the extraneous substances: considering the enantiomeric ratios of β-pinene and sabinene in samples 2 and 3, they may be contaminated by different amounts of lemon oil, as can be deduced by comparison with the data reported in Table 6 relative to the mixtures obtained by the addition of known amounts of lemon oil to genuine cold-pressed mandarin oil. In fact, the volatile fraction chromatograms for these two oils show the presence of α-bergamotene, neral, geranial, and neryl and geranyl acetate at a higher level than a genuine mandarin oil, confirming, therefore, our hypothesis. Samples 1, 7, and 8 could be reconstituted oils from sweet orange terpenes added with (−)-limonene, to correct the rotation value, and with some oxygenated components extraneous to the oil. In sample 1, the addition of β-pinene mostly levorotatory can also be noticed. The volatile fraction chromatograms of these three samples reveal the presence of δ-3-carene, a typical component of sweet orange oil, confirming the nature of the adulteration.

Samples 4 and 5, because of the enantiomeric distribution of β-pinene, may be contaminated with small amounts of lemon oil, and they also contain α-terpineol, whose origin should be different from mandarin. Sample 6 is certainly a reconstituted oil, since all the enantiomeric ratios are inconsistent with a genuine mandarin oil, but it was not possible to determine the origin of this sample.

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