Multidimensional Capillary GC–GC for the Analysis of Complex Samples. 5. Enantiomeric Distribution of Monoterpene Hydrocarbons, Monoterpene Alcohols, and Linalyl Acetate of Bergamot (*Citrus bergamia* Risso et Poiteau) Oils

Luigi Mondello,[†] Antonella Verzera,[†] Piero Previti,[‡] Francesco Crispo,[§] and Giovanni Dugo*,[†]

Dipartimento Farmaco-chimico and Dipartimento Farmaco-biologico, Facoltà di Farmacia, Università di Messina, viale Annunziata 98168 Messina, Italy, and Consorzio del Bergamotto, 89100 Reggio Calabria, Italy

The enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, α-terpineol, and linalyl acetate in cold-pressed, "torchiati", "ricicli", "pulizia dischi", "distilled", and "bergapten-free" bergamot 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 the cold-pressed bergamot oils, to determine the variations of the enantiomeric distribution of some components during the whole production season, and to correlate the enantiomeric ratios with the technology of oil isolation.

Keywords: *Multidimensional GC–GC; enantioselective gas chromatography; bergamot oils; Citrus bergamia Risso et Poiteau; monoterpene hydrocarbons; monoterpene alcohols; linalyl acetate*

INTRODUCTION

Bergamot *(Citrus bergamia* Risso et Poiteau) cultivation started in Italy at the beginning of the 18th century. Italian bergamot production is limited to approximately 2000 hectares in a narrow strip of the Calabrian coast, near Reggio Calabria, along the Ionian and the Thyrrenian seas. The plant is also cultivated in the Ivory Coast, Brazil, Argentina, and, more recently, Uruguay (Dellacassa et al., 1997). Bergamot is mainly cultivated for the essential oil representing an important raw material for cosmetic and food industries.

In Italy, the "Consorzio del Bergamotto di Reggio Calabria" and the private industries use "Pelatrice" machines to obtain the essential oil. The oil is obtained first by scraping the peel fruit under streams of spouting water, and then, by centrifugation it is separated from the water and the solid residues. The centrifugation of the oil is accomplished in two different steps: a first separator, where the aqueous emulsion increases in oil content up to about 70–90%; and then, in a secondary separator, the pure essential oil is recovered. Water must be recycled to avoid the loss of polar compounds, which are partly soluble in water. Generally, the same recycled water is used during the entire processing day. Small amounts of less valuable bergamot oil can also be recovered by processing the residues of the cold-pressed oil extraction. These oils are named depending on the process used:

"Pulizia dischi" oils are oils recovered by decantaction of the liquid residues and by centrifugation of the solid residues of the drum of the secondary separator at the end of an entire processing day. The yield of the oil, so obtained, is about 0.5% of the total oil.

"Torchiati" oils are oils recovered by pressing the solid residues of the secondary separator after centrifugation with little hydraulic presses. The yield is 3–4% of the total oil. This name for citrus essential oil, other than bergamot oils, indicates a different extraction process. In fact, "Torchiati oils" are usually those obtained by cold-pressing the whole fruit; therefore, an acid product is obtained at a pH depending on the citrus fruit (pH of citrus juices is always less than 3.7). In this process the oil is separated centrifuging the acid product.

"Ricicli" oils are oils recovered by centrifugation of the recycle water at the end of an entire processing day. The yield of the oil, so obtained, is about 1.0% of the total oil.

The oils recovered using these techniques can be separately marked or, more frequently, they are added to cold-pressed oils.

"Distilled" oils are oils recovered by distillation of the semifluid wastes ("feccie") ejected by the first separator which is supplied with an automated discharge. Previously, the distillation was carried out at atmospheric pressure while, presently, it is carried out at reduced pressure using the "Peratoner" method (Di Giacomo, 1974). The yield of the oil, so obtained, is about 3% of the total oil.

In our laboratory, bergamot volatile fraction composition has been widely studied (Dugo et al., 1987, 1991; Lamonica et al., 1990; Verzera et al., 1996; Mondello et al., 1994, 1998d). During the production season large quantitative differences of each component are observed. Therefore quantitative data are not always useful to determine the genuineness and the quality of a bergamot oil, as is possible for other citrus oils, such as lemon or mandarin (Dugo, 1986; Dugo et al., 1990). The quantitative determination of flavor components, to-

* To whom correspondence should be addressed (phone, +39-90-356022; fax, +39-90-6766532; e-mail, gdugo@pharma.unime.it).

† Dipartimento Farmaco-chimico.

‡ Dipartimento Farmaco-biologico.

§ Consorzio del Bergamotto.

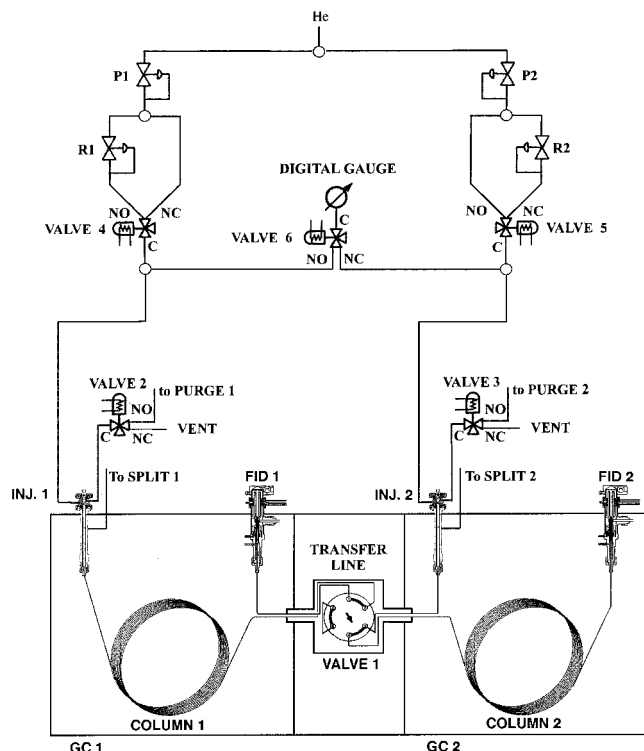**4276** *J. Agric. Food Chem.*, Vol. 46, No. 10, 1998

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

gether with their enantiomeric distribution, can provide useful information about the genuineness of the oils and their quality. For example, in genuine cold-pressed bergamot oils, the percentage of *S*(+) enantiomer, both for linalool and linalyl acetate, is lower than 1% of the total content of the two isomers (Cotroneo et al., 1992; Verzera et al., 1996; Juchelka and Mosandl, 1996; Casabianca and Graff, 1996; König et al., 1997; Mosandl and Juchelka, 1997; Dellacassa et al., 1997); in commercial oils (Mosandl and Schubert, 1990; Schubert and Mosandl, 1991; Bernreuther and Schreier, 1991; Weinrich and Nitz, 1992; Casabianca and Graff, 1994, 1996; Casabianca et al., 1995; Juchelka and Mosandl, 1996; König et al., 1997) and in oils which were obtained by "drastic" processes, such as distillation (Juchelka and Mosandl, 1996; König et al., 1997), this percentage is more than 1%.

Therefore, an *S* enantiomer content of linalool and linalyl acetate of more than 1% allows the presence of extraneous products in bergamot oil to be detected, but a value less than to 1% is not always due to a genuine bergamot oil; in fact it is possible to prepare reconstituted bergamot oils in which the enantiomeric ratio of linalool shows the same values of the genuine oils (Juchelka and Mosandl, 1996; König et al., 1997).

In literature, the enantiomeric ratios of some monoterpenes, such as α-pinene, β-pinene, and limonene are

reported for bergamot oils (Mosandl et al., 1990; Hener et al., 1990; Mosandl, 1995; Juchelka and Mosandl, 1996). These results, because of the low number of samples which are related and their similarity to other citrus essential oils are not able to be used to characterize a genuine bergamot oil.

In this paper, the results relative to the enantiomeric ratios of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, α-terpineol, and linalyl acetate for cold-pressed, recovered, and bergapten-free bergamot oils are reported. The data can be used to determine the genuineness and the quality of the bergamot oils.

Analyses were carried out using a fully automated, multidimensional double oven GC system, made in our laboratory (Mondello et al., 1998e) which is an improvement of a system previously described (Mondello et al., 1997; 1998a–c). The system, using an SE-52 precolumn and a chiral main column, allows the determination of the enantiomeric ratios of each component during a single analysis.

Previously, the advantages of the multidimensional system were widely discussed (Mondello et al., 1997; 1998b,c,d,e)

MATERIALS AND METHODS

The research was carried out on 101 genuine cold-pressed bergamot oil samples obtained by "Pelatrice" machines during the entire 1996/97 production season; 7 "torchiati" bergamot oil samples; 7 "ricicli" bergamot oil samples; 3 "pulizia dischi" bergamot oil samples; 2 "distilled" bergamot oil samples; 7 "bergapten-free" bergamot oils obtained by distillation; and 7 "bergapten-free" bergamot oil samples obtained by NaOH treatment.

The multidimensional system used in this study was an improved model (Mondello et al., 1998e), made in our laboratory, of a developmental instrument formerly described in detail (Mondello et al., 1997, 1998a–c). The instrument setup and the experimental conditions used are described below and in Figure 1.

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

Gas Chromatograph 1. Injector (Inj. 1): split/splitless injector at 250 °C; split ratio 1:10. Column (column 1): 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, 90 kPa (delivered at constant pressure) (standby position, flow path: injector 1 to column 1 through the hot transfer line to FID 1), 170 kPa (delivered at constant pressure) (Cut position, flow path: injector 1 to column 1 through the hot transfer line to column 2 and FID 2). Detector (FID 1): flame ionization detector at 280 °C connected to CLASS-VP Chromatography Data System (channel 1) (Shimadzu, Milan, Italy). Valve 2: three-way solenoid valve (SIRAI, Milan, Italy) connected to CLASS-VP Chromatography Data System (event 1) (Shimadzu, Milan, Italy). Valve 2 is normally in the opened position (NO) when the system is both in standby position and in cut position (purge flow). When the system is switched from

Table 1. Minimum to Maximum Percentage Values of the Analyzed Component in Bergamot Oils and Their Transfer Windows

| | | | | | | "bergaptene-free" | | transfer |
	"cold-pressed"	"pulizia dischi"	"torchiati"	"ricicli"	"distilled"	NaOH	distilled	windows (min)
β-pinene + sabinene	4.8–12.7	7.5–9.8	6.2–9.3	6.6–8.9	4.2–7.9	7.1–7.9	6.9–8.3	15.30–16.00
limonene	25.4–45.4	29.7–33.2	30.3–34.7	27.2–34.4	23.1–35.1	32.8–35.9	33.5–35.7	20.00–20.20
linalool	3.6–22.7	5.7–10.3	7.3–11.2	7.9–12.2	25.4–36.9	6.8–7.7	6.9–7.6	25.00–25.55
terpinen-4-ol	tr	tr	tr	tr	0.4–0.5	tr	tr	30.60–31.20
α-terpineol	tr-0.1	tr-0.1	0.1–0.5	0.1–0.3	1.3–4.0	0.1	0.1	31.60–32.35
linalyl acetate	21.8–41.4	35.5–40.1	34.3–37.9	33.2–41.8	8.9–22.8	31.3–32.6	30.2–32.8	36.80–37.10

Multidimensional GC for Chiral Separation of Bergamot Oils

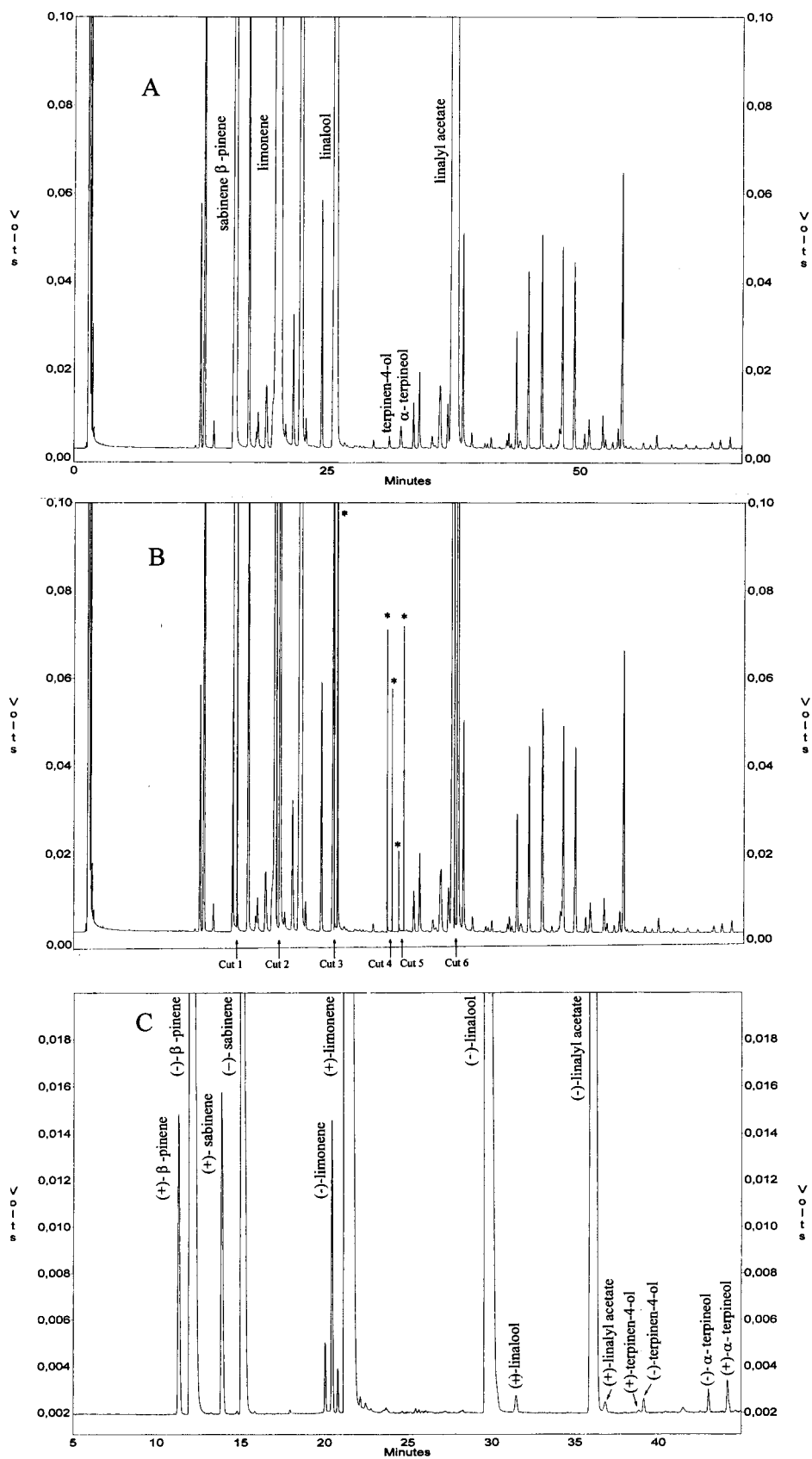*J. Agric. Food Chem.,* Vol. 46, No. 10, 1998 **4277**

Figure 2. (A) GC chromatogram of a cold-pressed bergamot oil obtained with (A) the SE-52 column, and (B) the SE-52 column with the six heart-cuts, and (C) GC–GC chiral chromatogram of the transferred components. Stars in part B show the electric spike coming from the valve switching. In the case of cut 3 (linalool) the electric spike at the beginning of the cut is not visible because the peak was partially transferred and at the end of the cut is very high because under this spike there is the nonanal peak. In the case of cut 1 (sabinene + β-pinene), cut 2 (limonene), and cut 6 (linalyl acetate), the electric spikes are not visible because the transfer of these peaks was only partial and these spikes are placed on the signals of those compounds that are not completely transferred.

Table 2. Enantiomeric Ratios of Some Components in Cold-Pressed Bergamot Oils

	December 1–15 (23 samples)		December 16–31 (33 samples)		January 1–15 (5 samples)		January 16–31 (8 samples)		February 1–15 (7 samples)		February 16–28 (22 samples)		March 2–4 (3 samples)		all: (101 samples)	
	X̄	range	X̄	range	X̄	range	X̄	range	X̄	range	X̄	range	X̄	range	X̄	range
β-pinene																
R(+)	7.7	6.8–8.9	7.8	7.1–8.9	8.1	7.7–8.6	8.2	7.4–9.4	8.1	7.5–8.9	7.9	7.1–9.0	9.0	8.5–9.5	7.9	6.8–9.5
S(−)	92.3	93.2–91.1	92.2	92.9–91.1	91.9	92.3–91.4	91.8	92.6–90.6	91.9	92.5–91.1	92.1	92.9–91.0	91.0	91.5–90.5	92.1	93.2–90.5
sabinene																
R(+)	14.8	14.1–16.0	14.9	14.4–16.0	15.0	14.7–15.4	15.2	14.3–16.4	15.2	14.5–15.9	15.2	14.1–18.8	16.0	15.5–16.5	15.0	14.1–18.8
S(−)	85.2	85.9–84.0	85.1	85.6–84.0	85.0	85.3–84.6	84.8	85.7–83.6	84.8	85.5–84.1	84.8	85.9–81.2	84.0	84.5–83.5	85.0	85.9–81.2
limonene																
S(−)	2.3	2.0–2.6	2.3	2.0–2.7	2.4	2.3–2.5	2.3	2.0–2.6	2.2	2.0–2.4	2.3	2.1–2.6	2.1	1.9–2.2	2.3	1.9–2.7
R(+)	97.7	98.0–97.4	97.7	98.0–97.3	97.6	97.7–97.5	97.7	98.0–97.4	97.8	98.0–97.6	97.7	97.9–97.4	97.9	98.1–97.8	97.7	98.1–97.3
linalool																
R(−)	99.6	99.5–99.7	99.6	99.4–99.7	99.6	99.6–99.7	99.6	99.5–99.6	99.6	99.4–99.7	99.6	99.4–99.6	99.6	99.6	99.6	99.4–99.7
S(+)	0.4	0.5–0.3	0.4	0.6–0.3	0.4	0.4–0.3	0.4	0.5–0.4	0.4	0.6–0.3	0.4	0.6–0.4	0.4	0.4	0.4	0.6–0.3
terpinen-4-ol																
S(+)	15.5	12.3–26.3	18.7	11.3–25.4	11.7	10.1–13.9	13.3	10.0–16.7	14.5	12.5–16.6	15.9	9.7–20.0	16.1	15.4–16.8	16.2	9.7–26.3
R(−)	84.5	87.7–73.7	81.3	88.7–74.6	88.3	89.9–86.1	86.7	90.0–83.3	85.5	87.5–83.4	84.1	90.3–80.0	83.9	84.6–83.2	83.8	90.3–73.7
α-terpineol																
S(−)	63.0	56.1–69.4	57.7	43.4–65.5	47.8	33.6–57.7	42.0	33.7–49.0	37.8	30.6–41.9	34.1	20.7–44.1	21.7	17.5–25.9	49.7	17.5–69.4
R(+)	37.0	43.9–30.6	42.3	56.6–34.5	52.2	66.4–42.3	58.0	66.3–51.0	62.2	69.4–58.1	65.9	79.3–55.9	78.3	82.5–74.1	50.3	82.5–30.6
linalyl acetate																
R(−)	99.8	99.7–99.8	99.8	99.7–99.9	99.8	99.8–99.9	99.8	99.7–99.9	99.9	99.8–99.9	99.8	99.8–99.9	99.8	99.8	99.8	99.7–99.9
S(+)	0.2	0.3–0.2	0.2	0.3–0.1	0.2	0.2–0.1	0.2	0.3–0.1	0.1	0.2–0.1	0.2	0.2–0.1	0.2	0.2	0.2	0.3–0.1

cut position to standby position, valve 2 is diverted to vent position for a fraction of second. This allows the gas accumulated in the injector 1 and column 1 to escape after disconnection of column 2 from the flow path. A microswitch on the injector allows the automatic start of the analysis by inserting syringe.

Gas Chromatograph 2. Injector (Inj. 2): split/splitless injector at 250 °C; split ratio 1:15. Column (column 2): 2,3-di-*O*-ethyl-6-*O*-(*tert*-butyldimethylsilyl)-*β*-cyclodextrin 30% in PS 086 capillary column, 25 m × 0.25 mm i.d., 0.25 µm film thickness (Mega, Legnano, Italy). Temperature program: 45 °C for 6 min and then to 90 °C (10 min) at 2.0 °C/min and then to 230 °C at 2 °C/min. Carrier: He, 110 kPa (delivered at constant pressure) (standby position, flow path: injector 2 through the hot transfer line to column 2 and FID 2), 5 kPa (delivered at constant pressure) (Cut position, flow path: injector 2 through the hot transfer line to FID 1). Detector (FID 2): flame ionization detector at 230 °C connected to CLASS-VP Chromatography Data System (channel 2). Valve 3: three-way solenoid valve connected to CLASS-VP Chromatography Data System (event 1). Valve 3 is normally in the opened position (NO) when the system is in standby position (purge flow). When the system is in cut position valve 3 is diverted to vent position for a fraction of second to allow the gas accumulated in the injector 2 to escape, so that the flame of FID 1 is not blown out when column 2 is disconnected from the flow path.

The analysis starts by programming the event 2 on CLASS-VP Chromatography Data System.

Multidimensional Interface. Transfer line: aluminum thermoregulated block (200 °C) equipped with a heater assay, a PT sensor, and a thermocouple assay (Shimadzu, Milan, Italy) connected to the AUX1 exit on gas chromatograph 1. Valve 1: six-port (1/16 in.) two-position W-type valve (A3C6WT) (Valco Europe, Schenkon, Switzerland) equipped with a heated valve enclosure (HVEA-220) (Valco Europe, Schenkon, Switzerland) connected to CLASS-VP Chromatography Data System (event 1). Ports 5 and 6 in valve 1 are connected together by a piece of deactivated fused silica capillary (10 cm × 0.25 mm i.d.). P1, P2, R1, R2: pressure regulators (Shimadzu, Milan, Italy) connected to CLASS-VP Chromatography Data System (event 1). Valves 4, 5, and 6: three-way solenoid valves connected to CLASS-VP Chromatography Data System (event 1). Digital gauge: a digital gauge 0–400 kPa (Generalcontrol, Milan, Italy) reading the inlet pressures of the two injectors both in standby and cut positions. In the standby position valve 4 is normally opened (NO) and the pressure regulator P1 is followed by the pressure regulator R1. This restrictor emulates column 2, so that in column 1, standby position, the component retention times are the same of those obtained with the system working in the cut position when the restrictor 1 is replaced by the column 2 and valve 4 is in the position of normally closed (NC). In the other side valve 5 is normally opened (NO) and the pressure in the inlet of column 2 is dictated from the pressure regulator P2. When the system is in cut position valve 5 is switched in the normally closed (NC) position and the pressure regulator P2 is followed by the pressure regulator R2 that acts as a restrictor with a back pressure equal of that of column 1. As result with this system, the retention times of all the compounds eluted from column 1 even those eluted immediately after the compounds that are transferred in the second GC column are consistent with the retention times of the same compounds when the mixture is injected in the system in standby position. The time at which the valves should be switched to begin the cuts can be determined from a preliminary analysis on the first column. After this a fully automated analysis is possible by adding the retention windows in the external events table of the CLASS-VP Chromatography Data System.

As previously described (Mondello et al., 1998c), under the specific experimental conditions, valve 1 does not cause any loss of the analyzed components.
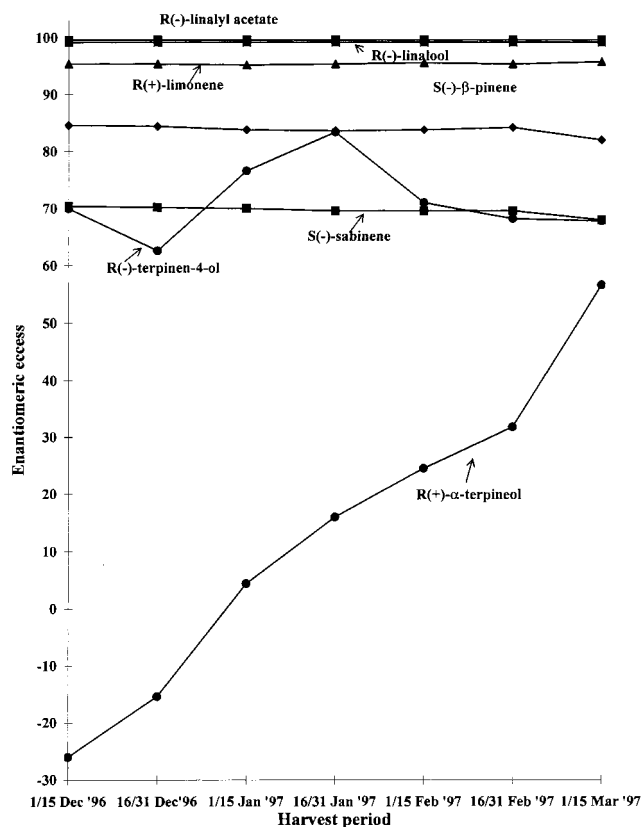
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Figure 3. Variation of the fortnightly average values of the enantiomeric excess of (4*R*)-(+)-limonene, (1*S*,5*S*)-(−)-β-pinene, (1*S*,5*S*)-(−)-sabinene, (4*R*)-(−)-terpinen-4-ol, (4*S*)-(−)-α-terpineol, (3*R*)-(−)-linalol, (3*R*)-(−)-linalyl acetate in cold-pressed bergamot oils during the productive season.

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 single component, different transfer windows were then chosen and automatically pro-

grammed so that well-resolved peaks would be obtained in the chiral column both for components present in the oils at high concentrations and for those present at very low concentrations.

Sabinene + β-pinene, limonene, linalool, and linalyl acetate were only partially transferred because of their high concentration. The other components analyzed were quantitatively transferred since they were present in lower amounts.

The percentage content of the transferred components in the analyzed oils and the transfer windows are shown in Table 1.

Figure 2 reports the chromatogram of a cold-pressed bergamot 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.

The elution order of the enantiomers was determined with standard injections.

Cold-Pressed Oils. Table 2 reports the values of the enantiomeric ratios for the cold-pressed bergamot oils divided in fortnightly periods, and Figure 3 shows the enantiomeric excess of the components analyzed during the production season.

As can be seen from Table 2 and Figure 3, the enantiomeric distribution of linalool and linalyl acetate seems to be stable during the production season for all oils analyzed. Particularly, the *S*(+)-linalool percentage is included between 0.3 and 0.6% of the total content of linalool while the *S*(+)-linalyl acetate content is included between 0.1 and 0.3% of the total content of linalyl acetate. These values agree with those reported in the literature for genuine bergamot oils (Cotroneo et al., 1992; Juchelka and Mosandl, 1996; Casabianca and Graff, 1996; Verzera et al., 1996; König et al.,1997; Mosandl and Juchelka, 1997; Dellacassa et al., 1997).

The enantiomeric ratios of limonene and β-pinene slowly changes during the production season, and these values are in agreement with Juchelka and Mosandl (1996) and Mosandl and Juchelka (1997).

Table 3. Enantiomeric Ratios of Some Components in "Torchiati", "Ricicli", and "Pulizia Dischi" Bergamot Oils

	February 1−15			February 16−28			
	"torchiati" (2 samples)	"ricicli" (2 samples)	"cold-pressed" (7 samples)	"torchiati" (5 samples)	"ricicli" (5 samples)	"pulizia dischi" (3 samples)	"cold-pressed" (22 samples)
β-pinene							
R(+)	7.7−8.5	7.8−7.9	7.5−8.9	7.6−8.0	7.4−7.8	7.8−8.0	7.1−9.0
S(−)	92.3−91.5	92.2−92.1	92.5−91.1	92.4−92.0	92.6−92.2	92.2−92.0	92.9−91.0
sabinene							
R(+)	14.8−15.0	14.9−15.0	14.5−15.9	14.7−15.0	14.5−15.0	14.7−15.0	14.1−18.8
S(−)	85.2−85.0	85.1−85.0	85.5−84.1	85.3−85.0	85.5−85.0	85.3−85.0	85.9−81.2
limonene							
S(−)	2.1−2.3	2.2−2.3	2.0−2.4	2.2−2.3	2.2−2.3	2.2−2.3	2.1−2.6
R(+)	97.9−97.7	97.8−97.7	98.0−97.6	97.8−97.7	97.8−97.7	97.8−97.7	97.9−97.4
linalool							
R(−)	99.6	99.6	99.4−99.7	99.6	99.5−99.6	99.5−99.6	99.4−99.6
S(+)	0.4	0.4	0.6−0.3	0.4	0.5−0.4	0.5−0.4	0.6−0.4
terpinen-4-ol							
S(+)	18.7−18.8	19.3−20.0	12.5−16.6	18.1−19.1	18.7−21.4	12.3−13.1	9.7−20.0
R(−)	81.3−81.2	80.7−80.0	87.5−83.4	81.9−80.9	81.3−78.6	87.7−86.9	90.3−80.0
α-terpineol							
S(−)	5.1−5.2	11.3−18.0	30.6−41.9	4.9−10.5	6.4−18.7	27.5−41.8	20.7−44.1
R(+)	94.9−94.8	88.7−82.0	69.4−58.1	95.1−89.5	93.6−81.3	72.5−58.2	79.3−55.9
linalyl acetate							
R(−)	99.8	99.8	99.8−99.9	99.7−99.9	99.7−99.8	99.7−99.8	99.8−99.9
S(+)	0.2	0.2	0.2−0.1	0.3−0.1	0.3−0.2	0.3−0.2	0.2−0.1

Table 4. Enantiomeric Ratios of Some Components in "Distilled" Bergamot Oils

	"feccie" oil		cold-pressed oils (all samples)	
	distillation at atmospheric pressure, 04/29/97	distillation at reduced pressure, 04/29/97	$\bar{X}$	range
β-pinene				
R(+)	8.9	8.2	7.9	6.8−9.5
S(−)	91.1	91.8	92.1	93.2−90.5
sabinene				
R(+)	15.9	15.2	15.0	14.1−18.8
S(−)	84.1	84.8	85.0	85.9−81.2
limonene				
S(−)	2.0	2.3	2.3	1.9−2.7
R(+)	98.0	97.7	97.7	98.1−97.3
linalool				
R(−)	81.6	98.7	99.6	99.4−99.7
S(+)	18.4	1.3	0.4	0.6−0.3
terpinen-4-ol				
S(+)	31.8	27.1	16.2	9.7−26.3
R(−)	68.2	72.9	83.8	90.3−73.7
α-terpineol				
S(−)	26.6	11.2	49.7	17.5−69.4
R(+)	73.4	88.8	50.3	82.5−30.6
linalyl acetate				
R(−)	98.9	99.1	99.8	99.7−99.9
S(+)	1.1	0.9	0.2	0.3−0.1

Table 5. Enantiomeric Ratios of Some Components in "Bergapten-Free" Bergamot Oils, Both Distilled and Treated with NaOH

			cold-pressed oils (all samples)	
	treated with NaOH	distilled	$\bar{X}$	range
β-pinene				
R(+)	8.2−8.7	8.1−9.2	7.9	6.8−9.5
S(−)	91.8−91.3	91.9−90.8	92.1	93.2−90.5
sabinene				
R(+)	15.3−15.7	15.1−16.0	15.0	14.1−18.8
S(−)	84.7−84.3	84.9−84.0	85.0	85.9−81.2
limonene				
S(−)	2.1	2.1−2.2	2.3	1.9−2.7
R(+)	97.9	97.9−97.8	97.7	98.1−97.3
linalool				
R(−)	99.6	99.5−99.6	99.6	99.4−99.7
S(+)	0.4	0.5−0.4	0.4	0.6−0.3
terpinen-4-ol				
S(+)	18.4−20.1	19.8−21.5	16.2	9.7−26.3
R(−)	81.6−79.9	80.2−78.5	83.8	90.3−73.7
α-terpineol				
S(−)	31.4−52.1	41.4−50.5	49.7	17.5−69.4
R(+)	68.6−47.9	58.6−49.5	50.3	82.5−30.6
linalyl acetate				
R(−)	99.7−99.8	99.7−99.9	99.8	99.7−99.9
S(+)	0.3−0.2	0.3−0.1	0.2	0.3−0.1

The values of the enantiomeric ratio of limonene (1.9/98.1 to 2.7/97.3) are similar to those of lemon (1.5/98.5 to 2.0/98.0) (Mondello et al., 1998e) and mandarin oils (2.0/98.0 to 2.3/97.7) (Mondello et al., 1998a), while the values of β-pinene (6.8/93.2 to 9.5/90.5) are similar to those of lemon oils (4.2/95.8 to 7.0/93.0) (Mondello et al., 1998e) but very different from those of mandarin oils (97,0/3.0 to 98.8/1.2) (Mondello et al., 1998a).

The enantiomeric ratio of sabinene seems to be stable enough during the production season; the ratio R(+)/S(−)-sabinene is included from 14.1/85.9 to 18.8/81.2. These values are similar to those of lemon oils (13.3/86.7 to 15.3/84.7) (Mondello et al., 1998e) and different from those of mandarin oils (76.2/23.8 to 80.5/19.5) (Mondello et al., 1998a), from those of sweet orange oils (94.6/5.4 to 97.9/2.1) and from those of bitter orange oils (44.4/55.6 to 54.5/45.5) (Mondello et al., 1997).

The enantiomeric ratio of S(+)/R(−)-terpinen-4-ol changes irregularly during the production season. The values of S(+)/R(−)-terpinen-4-ol enantiomeric ratio in bergamot oils (9.7/90.3 to 26.3/73.7) are similar to those

of mandarin oils (10.0/90.0 to 19.2/81.8) (Mondello et al., 1998a) and to those of lemon oils (15.9/84.1 to 26.7/73.3) (Mondello et al., 1998e).

The enantiomeric ratio of S(−)/R(+)-α-terpineol changes greatly during the production season and its fortnightly average values are included from 63.0/37.0 at the beginning of the season to 21.7/78.3 at the end of the season. Lemon and mandarin oils always have a high content of S(−)-α-terpineol isomer (Mondello et al., 1998a,e).

With regard to the values of sabinene, terpinen-4-ol, and α-terpineol enantiomeric ratios no data are reported in the literature, except for our preliminary results (Mondello et al., 1997).

Table 3 reports the values of the enantiomeric ratios for "pulizia dischi", "torchiati", and "ricicli" oils. The same table compares the results obtained for cold-pressed oils produced in the same period.

"Pulizia Dischi" Oil. As can be seen from Table 3, values of the enantiomeric distribution for all the "pulizia dischi" oils are in the ranges observed for cold-

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pressed oils produced in the same period, so the system used for the recovery of these oils does not seem to influence the enantiomeric distribution of the components analyzed.

"Torchiati" Oils. As can be seen from Table 3, the enantiomeric distribution of β-pinene, sabinene, limonene, linalool, and linalyl acetate for sample produced in the first half and in the second half of February, are in the ranges observed for the cold-pressed oils produced in the same period. The isomer $S(+)$- of terpinen-4-ol shows values for "torchiati oils" produced in the first half of February, higher than the highest value shown by cold-pressed oils, while for "torchiati oils" produced in the second half of February, it shows values near to the highest value shown by cold-pressed oils produced in the same period. This result seems to show that the contact with the solid residues, that are acidic, before the recovery of the oil by pressing, causes a partial racemization of terpinen-4-ol.

The $S(-)$ isomer of α-terpineol for "torchiati oils" produced both in the first and in the second half of February, shows values much lower than the minimum values presented by a cold-pressed oils.

"Ricicli" Oils". As can be observed in Table 3, "ricicli" oils behave as "torchiati" oils. In fact, terpinen-4-ol shows a slight trend to the racemization, while the $S(-)$ isomer of α-terpineol shows values lower than the lowest values of cold-pressed oils produced in the same period.

The behavior of α-terpineol in "torchiati" oils and "ricicli" oils cannot be explained with the contact of the oil before its recovery with the acid, solid, and liquid residues. This could have caused a trend to the racemization but not the increase of the enantiomeric excess of $R(+)$ isomer. In fact, as observed in our laboratory, the warm treatment in an aqueous medium acidic for citric acid (pH 3) of a mixture of $S(-)$- and $R(+)$-α-terpineol (30:70) led to the racemization after 4 h.

This phenomenon can be explained with the hypothesis that $R(+)$-α-terpineol can be produced by microorganisms. And in fact, recycling water and solid residues of the cold extraction are good culture mediums (Murdock et al., 1969). Another hypothesis could be that of the acid or enzymatic hydrolysis of glycosidically bonded $R(+)$-α-terpineol. In fact, the analyzed percentage of α-terpineol in "torchiati" and in "ricicli" oils varies between 0.1 and 0.47 and between 0.1 and 0.30 respectively, while in cold-pressed oils produced in the same period ranged between 0.04 and 0.12.

"Distilled" Oils. Table 4 reports values of the enantiomeric distribution of two oils recovered by distillation of the semifluid wastes ejected by the first separator. The same table compares the results obtained for cold-pressed bergamot oils.

As can be seen from Table 4, the enantiomeric distribution of β-pinene, sabinene, and limonene is not influenced by the distillation process. Linalool, linalyl acetate, and terpinen-4-ol show a trend to the racemization. For the linalool this trend was previously observed by other authors (König et al. 1997; Hener et al., 1990). The phenomenon is more evident for the sample obtained by distillation at atmospheric pressure. In this sample, $S(-)$-α-terpineol shows a value lower than the lowest values observed for the cold-pressed bergamot oils, as previously observed for "torchiati" and "ricicli" oils. In the sample obtained by distillation at reduced pressure this latter phenomenon is not evident.

"Bergapten-Free" Oils. Table 5 reports values of the enantiomeric distribution of "bergapten-free" bergamot oils, obtained by distillation or by treatment with NaOH. The same table reports the results obtained for cold-pressed bergamot oils. As can be seen from Table 5, the enantiomeric ratios of all the samples of "bergapten-free" oil are in the ranges observed for cold-pressed oils, so the systems used for the elimination of bergapten do not seem to influence the enantiomeric distribution of the components under examination.

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