Enantiomeric Distribution of Volatile Components of Citrus Oils by MDGC

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The enantiomeric distribution of volatile components of citrus oils provides a useful parameter for the evaluation of genuineness and quality of the oils.[1-14] Chiral gas chromatography, using modified cyclodextrins, as a stationary phases, is the most common technique for the determination of the enantiomeric distribution of the volatile components.

The volatile fraction of citrus oils is usually a very complex matrix. Therefore the direct gas chromatographic determination of its components, though sometimes possible, is usually difficult. In fact, it requires particular attention for the choice of the most appropriate experimental condition and stationary phases, in order to avoid co-elution of enantiomers with other components.[2,3,15] Coupled systems of liquid chromatography and gas chromatography (LC-GC), and multidimensional gas chromatographic systems (MDGC), permits the pre-fractionation of the samples and the further chiral analysis of the components of interest. These techniques avoid possible peak overlaps. The LC-GC methods have rarely been used, while the MDGC systems are more commonly employed.[9,10,13,14,16-23]

The early multidimensional GC systems were based on Dean's principle, while systems working with mechanical valves, proposed earlier, were not considered reliable, because the valves available at the time did not have adequate thermal stability.[24] In addition, Memory effects were likely to occur. Technological progress of valve design rendered miniaturized connectors available for the assembly of multidimentional GC systems, eliminating upswept volumes. These mechanical valves are stable at high temperatures and can be used to set up multidimensional GC systems without any drawbacks. They are easier to operate than those based on Dean's principle.

The results relative to the enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol and α-terpineol in citrus oils determined by a multidimensional GC system developed and assembled in our laboratories are reported in this personal review.[25-30] This system, based on the use of mechanical valves, allows for the multitransfer of different fractions during the same GC analysis (cut position) and the use of the two GC independently when the multitransfer option is not used (stand-by position). Pneumatic and electronic circuits permit to maintain constant retention times in the pre-column even for the components eluted after numerous transfers. The pre-column, mounted in the first oven, was a capillary SE-52, 30 x 0.32 mm, 0.40-0.45 µm film thickness column; the main column, mounted in the second oven, was a capillary column of 2,3-di-O-ethyl-6-O-(tertbutyldimethylsilyl)-β-cyclodextrin 30% in PS 086, 25 m x 0.25 mm, 0.25 µm film thickness.

The oils were first analyzed with the SE-52 pre-column to determine the concentration of the components of interest and their retention times, maintaining the multidimensional system system in the stand-by position. Depending on the tetention times and the concentration ofeach component, different transfer windows were chosen and automatically programmed so that well-resolved peaks were obtained on the chiral columnb for all the transferred components, indepenmdently on their concentration levels. The samples of the oils analyzed are presented in Table 1. The percentage composition of the transferred components in the analyzed oils and the transfer windows are shown in Table 2.

Bergamot Oil

In Italy, the production sseason for bergamot oils is from December to March. The oil is obtained by Pelatrice machines. These wrap the peel of the fruit under streams of spouting water and then separate the oil by centrifugation from the solid residue and water. Generally, the water is recycled and used during the entire processing day. Small amounts of less valuable bergamot oils can be recovered by processing the residues of the cold-pressed extraction:

- Pulizia dischi oils: recovered by decantation or 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 (approximately 0.5% of the total oil).
- Torchiati oils: recovered by pressing the solid residues of the secondary separator after centrifucation with little hydraulic presses (approximately 3-4% of the total oil).
- Ricicli oils: recovered by centrifugation of the recycled water at the end of an entire processing day (approximately 1.0% of the total oil).
- Distilled oils: recovered by distillation of the semifluid wastes (feccie) ejected by the first separator (approximately 3.0% of the total oil).

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Table 1. Samples of essential oils analyzed by MDGC	
3	cold-pressed key lime oils type A
1	cold-pressed key lime oil type B
11	cold-pressed Persian lime oils
6	distilled lime oils
7	laboratory extracted mandarin oils
63	cold-pressed mandarin oils
4	distilled mandarin oils
11	commercial mandarin oils
148	cold-pressed lemon oils
2	distilled lemon oils
6	commercial lemon oils
101	cold-pressed bergamot oils
7	torchiati bergamot oils
7	ricicli bergamot oils
3	pulizia dischi bergmot oils
2	distilled bergamot oils
12	cold-pressed sweet orange oils
6	cold-pressed bitter orange oils

The enantiomeric distribution of linalool and linalyl acetate in bergamot oils has been extensively studied, while the data in literature regarding the enantimeric ratios of other volatile compounds are scant.[1,4,8,11,20,21,31-36] Few data on the enantimeric ratios of α-pinene, β-pinene and limonene are reported.[8,16,17,19]

Cold-pressed oils: Figure 1 shows the chromatogram of a cold-pressed bergamot oil obtained with an SE-52 column and the system in the stand-by position; the chromnatogram 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 of the fractions transferred from the SE-52 column. In Table 3, the values of the enantiomeric ratios for the cold-pressed bergamot oils gathered every two weeks can be found. Figure 2 shows the enantiomeric excess of the components analyzed during the production season.

As can be seen in Table 3 and Figure 2, the enantiomeric distribution of linalool and linalyl acetate seems to be stable during the production season for all oils analyzed. Particularly, S (+)-linalool precentage is included between 0.3% and 0.6% of the total content of linalool, while 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 literature for genuine bergamot

Table 2. Relative percentage and transfer windows of some components in the essential oil analyzed by MDGC

	sabinene + β-pinene	limonene	linalool	terpinen-4-ol	α-terpineol	linalyl acetate
lime oils						
key type A	21.8–25.5	49.3–49.9	0.2	0.4–0.7	0.2–0.3	–
key type B	24.3	49.9	0.2	0.1	0.2	–
persian	12.0–13.6	54.8–59.8	0.1–0.2	0.1	0.2–0.3	–
distilled	0.8–1.9	43.5–49.7	0.1–0.3	0.7–0.9	7.6–8.0	–
mandarin oils						
cold-pressed	1.6–4.0	65.3–74.5	t–0.2	t–0.1	t–0.5	–
lab. extracted	1.2–1.7	69.5–78.5	0.1–0.2	t	0.1–0.2	–
distilled	1.4–2.2	67.1–74.7	0.1–0.3	t–0.4	0.1–0.4	–
commercial	1.7–2.7	61.4–92.9	0.1–0.6	t–0.1	t–0.2	–
lemon oils						
cold-pressed	15.39–22.25	59.3–71.1	13.2–18.5	t–0.1	t–0.3	–
bergamot oils						
cold-pressed	4.8–12.7	25.4–45.4	3.6–22.7	t	t–0.1	21.8–41.4
pulizia dischi	7.5–9.8	29.7–33.2	5.7–10.3	t	t–0.1	35.5–40.1
torchiati	6.2–9.3	30.3–34.7	7.3–11.2	t	0.1–0.5	34.3–37.9
ricicli	6.6–8.9	27.2–34.4	7.5–12.2	t	0.1–0.3	33.2–41.8
distilled	4.2–7.9	23.1–35.1	25.4–36.9	0.4–0.5	1.3–4.0	8.9–22.8
bergapten-free	6.9–8.3	32.8–35.9	6.8–7.7	t	0.1	30.2–32.8
transfer windows (min)	15.30–16.00	20.00–20.20	25.00–25.55	30.60–31.20	31.60–32.25	36.80–37.10

oils.[1,4,8,21,32-36] The enantiomeric ratio of sabinene seems to be stable enough during the production season, while the enantiomeric ratios of limonene and β-pinene change slowly during the same period. The enantiomeric ratio of terpinen-4-ol changes irregularly during the production season. In contarast, the enantiomeric ratio of α-terpineol changes greatly during the same period. The ratios S(-)/R(+)-α-terpineol were 63.0/37.0 at the beginning of the season, and 21.7/78.3 at the end. In Table 4, the values of the enantiomeric ratios for pulizia dischi, torchiati and ricicli oils are reported. The results obtained for cold-pressed oils produced in the same period can be found in the same Table for comparative purposes.

Pulizia dischi oil: As can be seen in Table 4, the enantiomeric distribution values for all pulizia dischi oils are in the range observed for cold-pressed oils produced in the same period. Therefore, the system used for the recovery of these ouils does not appear to influence the enantiomeric distribution of the compnents analyzed

Torchiati oils: In Table 4, the enantiomeric distribution of β-pinene, sabinene, limonene, linalool and linalyl acetate for samples produced in the first and second half of February are in the range observed for the cold-pressed oils produced in the same period. The S(+)-terpinen-4-ol isomer shows values for torchiati oils produced in the first half of February, higheer than the highest value shown by cold-pressed oils. At the same time, torchiati oils produced in the second half of February show values close to the highest value shown by cold-pressed oils produced in the same period. This result implies that contact with the solid acid residues, before the oil is recovered by pressing, causes a partial racemization of terpinen-4-ol. The S(–)-α-terpineol isomer for torchiati oils produced both in the first and second half of February, reveals values much lower than the minimum values of the other cold-pressed oils.

Ricicli oils: Table 4 shows that ricicli oils behave as just as torchiati oils. In fact, terpinen-4-ol shows a slight trend to recemize. At the same time, the S(–)-isomer of

Table 3. Bi-weekly average values of the enantiomeric distribution of some components in cold-pressed bergamot oils

	December		January		February		March	All
	1–15	16–31	1–15	16–31	1–15	16–28	2–4	
	(23)[a]	(33)	(5)	(8)	(7)	(22)	(3)	(101)
	Range	Range	Range	Range	Range	Range	Range	Range
β-pinene								
R (+)	6.8–8.9	7.1–8.9	7.7–8.6	7.4–9.4	7.5–8.9	7.1–9.0	8.5–9.5	6.8–9.5
R (–)	93.2–91.1	92.9–91.1	92.3–91.4	92.6–90.6	92.5–91.1	92.9–91.0	91.5–90.5	93.2–90.5
sabinene								
R (+)	14.1–16.0	14.4–16.0	14.7–15.4	14.3–16.4	14.5–15.9	14.1–18.8	15.5–16.5	14.1–18.8
R (–)	85.9–84.0	85.6–84.0	85.3–84.6	85.7–83.6	85.5–84.1	85.9–81.2	84.5–83.5	85.9–81.2
limonene								
S (–)	2.0–2.6	2.0–2.7	2.3–2.5	2.0–2.6	2.0–2.4	2.1–2.6	1.9–2.2	1.9–2.7
R (+)	98.0–97.4	98.0–97.3	97.7–97.5	98.0–97.4	98.0–97.6	97.9–97.4	98.1–97.8	98.1–97.3
linalool								
R (–)	99.5–99.7	99.4–99.7	99.6–99.7	99.5–99.6	99.4–99.7	99.4–99.6	99.6	99.4–99.7
S (+)	0.5–0.3	0.6–0.3	0.4–0.3	0.5–0.4	0.6–0.3	0.6–0.4	0.4	0.6–0.3
terpinen-4-ol								
S (+)	12.3–26.3	11.3–25.4	10.1–13.9	10.0–16.7	12.5–16.6	9.7–20.0	15.4–16.8	9.7–26.3
R (–)	87.7–73.7	88.7–74.6	89.9–86.1	90.0–83.3	87.5–83.4	90.3–80.0	84.6–83.2	90.3–73.7
α-terpineol								
S (–)	56.1–69.4	43.4–65.5	33.6–57.7	33.7–49.0	30.6–41.9	20.7–44.1	17.5–25.9	17.5–69.4
R (+)	43.9–30.6	56.6–34.5	66.4–42.3	66.3–51.0	69.4–58.1	79.3–55.9	82.5–74.1	82.5–30.6
linalyl acetate								
R (–)	99.7–99.8	99.7–99.9	99.8–99.9	99.7–99.9	99.8–99.9	99.8–99.9	99.8	99.7–99.9
S (+)	0.3–0.2	0.3–0.1	0.2–0.1	0.3–0.1	0.2–0.1	0.2–0.1	0.2	0.3–0.1

[a]number of samples analyzed

Figure 1. GC chromatogram of a cold-pressed bergamot oil obtained with the SE-52 column (A); GC chromatogram of bergamot oil obtained with the SE-52 column with the six heart-cuts (B); GC chiral chromatogram of the transferred components (C)

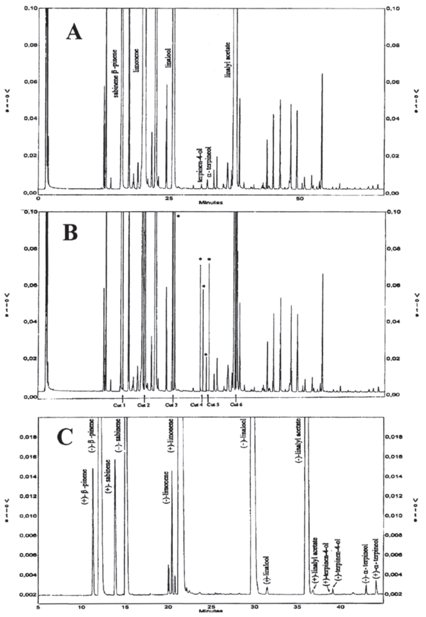
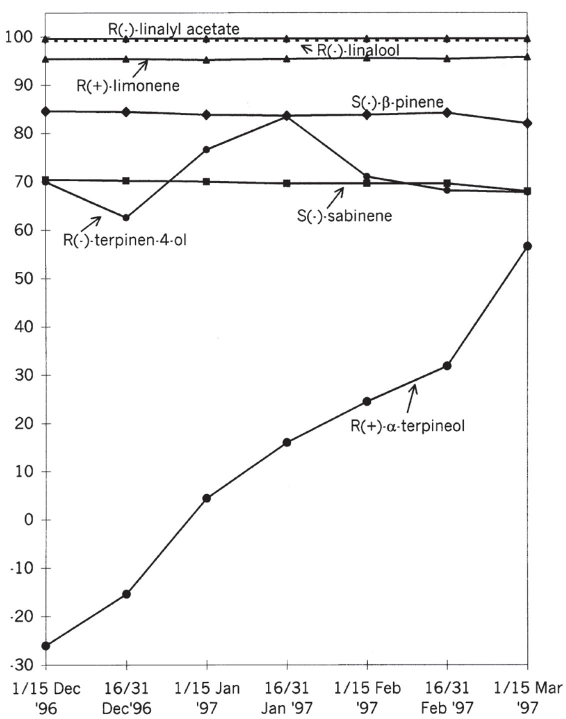


Figure 2. Variation of the bi-weekly average values of the enantiomeric excess of R(+)-limonene, S(–)-a-pinene, S(–)-sabinene, R(–)-terpinen-4-ol, S(–)-a-terpineol, R(–)-linalool, and R(–)-linalyl acetate in cold-pressed bergamot oil during the productive season



α-terpineol shows values lower than the lowest values of cold-pressed oils produced over the same period. The behavior of α-terpineol in torchiati and ricicli oils cannor be explained with the contact of the oil with the acid, solid and liquid residues before its recovery. This contact could have caused a trend to the racemization but not the increase of the enantiomeric excess of R(+)- isomer. This phenomenon can be explained with the hypothesis that (+)-α-terpineol can be produced by microorganisms. In fact, recycling water and solid residues of the cold-extraction are a good culture medium. Another hypothesis could be that of the acid or enzymatic hydrolysis of glycosidically bound R(+)-α-terpineol. The percentage of α-terpineol in torchiati and in ricicli oils analyzed varies between 0.10-0.47 and between 0.10-0.30, respectively. In cold-pressed oils produced in the same period, the range was 0.04-0.12.

Distilled oils: The values of the enantiomeric distribution of two oils recovered by distillation of the semifluid wastes ejected by the first separator can also be found in Table 4. The same Table compares the results obtained for cold-pressed bergamot oils. In Table 5, the enatiomeric distribution of β-pinene, sabinene and limonene is not influenced by the distillation process. Linallool, linalyl acetate and terpinen-4-ol show a trend to racemize. The phenomenon is more evident for the sample obtained by distillation at atmospheric pressure.

Lemon Oil

In Italy, lemon production and industrial processing occurs throughout the year. It is conventionally established that the productive season of lemon starts in October. The oils is extracted by all of the cold-press technologies commonly available, including Pelatrice, Sfumatrice, Torchi and FMC. Distilled oils are less valuable than those that are cold-extracted. These small amounts are obtained by distillation of the industrial residues from cold-extraction.

Table 4. Bi-weekly average values of the enantiomeric distribution of some components in Torchiati, Ricicli, and Pulizia dischi bergamot oils

	February 1–15			February 16–28			
	Torchiati (2)[a]	Ricicli (2)	Cold-pressed (7)	Torchiati (5)	Ricicli (5)	Pulizia Dischi (3)	Cold-pressed (22)
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
R (−)	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
R (−)	85.2–85.0	85.1–85.0	85.5–84.1	85.3–85.0	85.5–85.0	85.3–85.0	84.9–81.2
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

[a]number of samples analyzed

Cold-pressed oils: Determination of the enantiomeric distribution of the volatile components of lemon oil has been the objects of numerous studies carried out by direct GC analysis of the whole oil by LC on-line coupled with gas chromatography anmultidimensional gas chromatography.[3,10,14,15-23,37-39]

The cold-pressed oils analyzed in our laboratory have been gathered according to production month and, for each month, according to the extraction technology. For every group, the average3 value of the enantiomeric distribution has been determined.

Figure 3 shows the multidimensional chiral chromatogram of a cold-pressed lemon oils. The monthly average values of the enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol and α-terpineol are reported in Table 6, while Figure 4 shows the values of the enantiomeric excess, in accordance to the month of production of the oils.

As can be observed from Table 6 and Figure 4, limonene shows enantiomeric distribution values that are practically constant all year. The ratios between (–)- and (+)-β-pinene and (–)- and (+)-sabinene are in a limited range. Thea average values from October to march are nearly constant, while a slow increase can be noted from April onwards. Values of the enantiomeric distribution of terpinen-4-ol and α-terpineol cover a wide range. However, during the productive season, the enantiomeric excess of (–)-terpinen-4-ol and (–)-α-terpineol show several similar values and trends. Values of the enantiomeric distribution of linalool vary widely. At the beginning of the productive season the enantiomeric excess of (–)-linalool is about 33; this value decreases during the season from October to May, and the again rises, reaching values close to those in the beginning of the season in September.

In Table 7 the results obtained for oils extracted with different technologies in November, December and january are compared. As can be seen from the Table, the extraction technology does not seem to influence the enantiomeric distribution of β-pinene, sabinene, limonene, linalool and α-terpineol. Only terpinen-4-ol tends to racemize slightly in oils extracted with Sfumatrice and Torchi. These latter techniques allow for contact of the oil with the liquids coming from the peel and juice residues.

Distilled oils and commercial oils: The enantiomeric distribution values for two lemon oils obtained by distillation of the residues of the cold extraction and of six commercial oils can be seen in Table 8. For the two distilled oils analyzed, the enantiomeric distribution of all the components analyzed, except for terpinen-4-ol, are in the ranges found for cold-pressed oils. Terpinen-4-ol tends to recemize slightly because of the extraction technology used. For the commercial oil analyzed, only β-pinene had enantiomeric distribution values similar to those of the genuine lemon oils. The other components analyzed had had enantiomeric distribution values very different from those of genuine oils. The differences are so large that it was impossible to determine the products used in the reconstitution of these oils

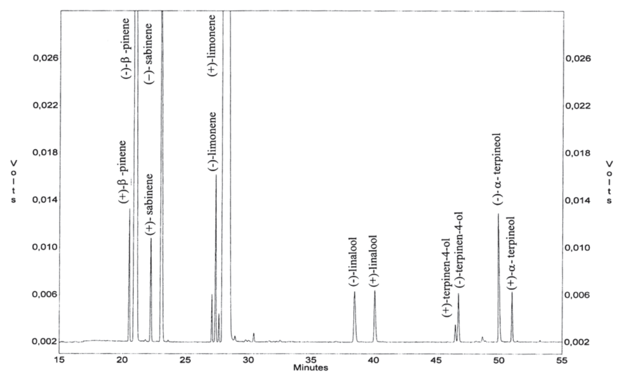
Table 5. Enantiomeric distribution of some components in distilled bergamot oils

	Feccie oils	Cold-pressed oils	
	Distillation at atmospheric pressure	Distillation at reduced pressure	Range
β-pinene			
R (+)	8.9	8.2	6.8–9.5
R (–)	91.1	91.8	93.2–90.5
sabinene			
R (+)	15.9	15.2	14.1–18.8
R (–)	84.1	84.8	85.9–81.2
limonene			
S (–)	2.0	2.3	1.9–2.7
R (+)	98.0	97.7	98.1–97.3
linalool			
R (–)	81.6	98.7	99.4–99.7
S (+)	18.4	1.3	0.6–0.3
terpinen-4-ol			
S (+)	31.8	27.1	9.7–26.3
R (–)	68.2	72.9	90.3–73.7
α-terpineol			
S (–)	26.6	11.2	17.5–69.4
R (+)	73.4	88.8	82.5–30.6
linalyl acetate			
R (–)	98.9	99.1	99.7–99.9
S (+)	1.1	0.9	0.3–0.1

Mandarin Oils

In Italy the industrial processing of mandarin oil goes from October to March. The technologies of extraction are Pelatrice, Torchi and FMC. As with lemon oils, small amounts of less valuable oils are obtained by distillation in fhe industrial residue of the cold extraction. In the literature, some data is reported on the enantiomeric distribution of the momoterpene hydrocarbons and several monoterpene alcohols of mandarin oil[2,10,15-21,37,39] Most of the results are

Figure 3. GC chiral chromatogram of the heart-cuts of sabinene + β-pinene, limonene, linalool, terpinen-4-ol, and α-terpineol of lemon oil



limited to one sample or a small number of samples. The data obtained are not related to the extraction technologies or to the harvest time and cultivar of the fruits.

Cold-pressed oils: The cold-pressed oils analyzed in our laboratory have been gathered according to the production month and, for each month, with the extraction technology. For each group, the average value of the enantiomeric distribution has been determined. Figure 5 shows the MD chromatogram of a cold-pressed mandarin oil. In Table 9, the enantiomeric distribution of industrial cold-pressed mandarin oils is reported. In Figure 6, the enatiomeric excess of the analyzed components is plotted as the function of the month of oil production.

As can be seen from Table 9 and Figure 6, the values of the enantiomeric distribution of limonene are constant from October to January. The enantiomeric ratios of the other components revealed different ranges. Upon examination of these data, it is believed that these variations do not seem to be ralated to the extraction technologies used. The variation of the enantiomeric ratios of α-terpineol is irregular, while the enantiomeric ratios of β-piene, sabinene, terpinen-4-ol, and lonalool regularly vary during the season.

Distilled oils: Table 10 shows the enantiomeric distribution in distilled mandarin oils. Distilled oils, as reported in Table 10, 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 relative percentage of the dextrorotatory isomer always higher than the highest values determined for cold-pressed oils. The difference is due to the extraction technologies; distilled oils are obtained from the acid

Table 6. Monthly average values of the enantiomeric distribution of some components in cold-pressed lemon oils

	Oct (15)[a]	Nov (28)	Dec (30)	Jan (15)	Feb (10)	Mar (10)	Apr (12)	May (11)	Jun (3)	Jul (2)	Aug (4)	Sep (6)	All (148) Range
β-pinene													
R (+)	6.0	6.4	6.4	6.3	6.2	5.9	5.5	5.1	4.5	4.6	4.6	5.1	4.2–7.0
R (–)	94.0	93.6	93.6	93.7	93.8	94.1	94.5	94.9	95.5	95.5	95.4	94.9	95.8–93.0
sabinene													
R (+)	14.6	14.7	14.7	14.6	14.4	14.3	13.9	13.4	12.7	12.8	13.3	13.1	12.5–15.3
R (–)	85.4	85.3	85.3	85.4	85.6	85.7	86.1	86.6	87.3	87.2	86.7	86.9	87.5–84.7
limonene													
S (–)	1.8	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.8	1.9	1.8	1.8	1.5–2.0
R (+)	98.2	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.2	98.1	98.2	98.2	98.5–98.0
linalool													
R (–)	66.6	61.0	58.9	55.6	54.3	53.2	53.0	52.3	53.3	60.3	60.1	63.7	56.8–71.5
S (+)	33.4	39.0	41.1	44.4	45.7	46.8	47.0	47.7	46.7	39.7	39.9	36.3	43.2–28.5
terpinen-4-ol													
S (+)	20.7	22.5	23.3	21.2	21.3	20.1	21.8	22.7	21.6	20.4	21.9	18.3	13.7–26.9
R (–)	79.3	77.5	76.7	78.8	78.7	79.9	78.2	77.3	78.4	79.7	78.1	81.7	86.3–73.1
a-terpineol													
S (–)	78.1	77.1	76.4	76.9	77.5	78.3	78.9	78.3	80.4	78.4	79.2	77.7	64.2–82.0
R (+)	21.9	22.9	23.6	23.1	22.5	21.7	21.1	21.7	19.6	21.6	20.8	22.3	35.8–18.0

[a]number of samples analyzed

aqueous residues of the cold-extraction so that the temperature and pH can cause the hydration of monoterpenes with a corresponding increase of the alcohols, especially terpinen-4-ol. The monoterpene hydration is not a stereospecific reaction so that it modifies the enantiomeric ratio of the alcohols leading this ratio towards the racemate.

Commercial oils: From the eleven commnercial oils analyzed (Table 11), samples 9 and 10 show values of the enatiomeric ratios compatible with a genuine mandarin oil. All the other samples show at least two of the ratios out of the normal range for genuine mandarin oils. For some of these samples, it may be possible to conjecture that considering the enantiomeric ratios of b-pinene and sabinene in samples 2 and 3, the nature of the extraneous substances may be contaminated by different amounts of lemon oil. This can be deduced by comparison with the data reported in Table 6 relative to lemon oil. 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 mandarin oil. In sample 1 it can also be noticed that the addition of β-pinene is mostly levorotatory. Samples 4 and 5, because of the enantiomeric distribution of β-pinene, may be contaminated with small amounts of lemon oil. They also contain a-terpineol, whose origin should be different from mandarin. Sample 6 is a reconstituted oil, because all the enatiomeric ratios are inconsistent with a genuine mandarin oil. It was not possible, however, to determine the origin of this sample.

Lime Oils

Lime oils are obtained from *Citrus aurantifolia* Swingle (Key lime) and *Citrus latifolia* Tanaka (Persian lime). The industrial products consist of four kinds of oils:[40]

- Cold-pressed Key lime oil, type A, obtained by centrifugation of the oil/juice emulsion produced by passing the whole fruit through a screw press that crushes the fruits,
- Cold-pressed Key lime, type B, obtained by rasping the peel to release the oil.
- Cold-pressed Persian lime oil, obtained with the same method as cold-pressed Key lime, type B.
- Distilled lime oil, obtained by reacting the flavor compounds of a mixture of oil/juice/crushed fruits in acid medium.

Table 7. Average values of the enantiomeric distribution of some components in pelatrice, sfumatrice, torchi and FMC cold-pressed lemon oils produced in the period November 1996–January 1997

	November (28)				December (30)				January (15)			
	P (3)[a]	S (8)	T (7)	FMC (10)	P (2)	S (12)	T (13)	FMC (3)	P (4)	S (4)	T (4)	FMC (3)
β-pinene												
R (+)	6.3	6.3	6.3	6.5	6.2	6.3	6.4	6.4	6.4	6.3	6.4	6.2
R (–)	93.7	93.7	93.7	93.5	93.8	93.7	93.6	93.6	93.6	93.7	93.6	93.8
sabinene												
R (+)	14.6	14.6	14.6	14.9	14.6	14.7	14.8	14.6	14.6	14.6	14.7	14.5
R (–)	85.4	85.4	85.4	85.1	85.4	85.3	85.2	85.4	85.4	85.4	85.3	85.5
limonene												
S (–)	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.8	1.7	1.7
R (+)	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.3	98.2	98.3	98.3
linalool												
R (–)	60.2	61.2	61.7	60.6	59.6	58.4	59.3	58.9	54.8	56.0	56.4	55.0
S (+)	39.8	38.8	38.3	39.4	40.4	41.6	40.7	41.1	45.2	44.0	43.6	45.0
terpinen-4-ol												
S (+)	16.3	24.5	24.8	21.1	18.3	24.2	23.7	20.6	16.7	23.8	23.7	20.7
R (–)	83.7	75.5	75.2	78.9	81.7	75.8	76.3	79.4	83.3	76.2	76.3	79.3
α-terpineol												
S (–)	74.1	78.2	77.0	77.2	78.3	78.2	74.2	78.3	77.6	78.4	73.5	78.6
R (+)	25.9	21.8	23.0	22.8	21.7	21.8	25.8	21.7	22.4	21.6	26.5	21.4

[a]number of samples analyzed

Table 8. Enantiomeric distribution of some components in distilled lemon oils and commercial lemon oils

	Distilled oils		Commercial oils						Cold-pressed
	1	2	1	2	3	4	5	6	Range
β-pinene									
R (+)	6.4	6.6	7.8	3.5	4.1	6.7	6.1	5.4	4.2–7.0
R (–)	93.6	93.4	92.2	96.5	95.9	92.3	93.9	94.6	95.8–93.0
sabinene									
R (+)	14.6	12.7	58.4	94.0	56.5	50.2	16.9	16.9	12.5–15.3
R (–)	85.4	87.3	41.6	6.0	43.5	49.8	83.1	83.1	87.5–84.7
limonene									
S (–)	1.7	1.7	13.7	9.6	11.6	14.5	6.9	5.0	1.5–2.0
R (+)	98.3	98.3	86.9	90.4	88.5	85.5	93.1	95.0	98.5–98.0
linalool									
R (–)	60.0	53.1	14.8	5.7	10.3	14.6	23.3	20.4	50.8–71.5
S (+)	40.0	46.9	85.2	94.3	89.7	85.4	76.7	79.6	49.2–28.5
terpinen-4-ol									
S (+)	28.5	28.4	57.2	75.4	39.9	46.7	33.9	28.9	13.7–26.9
R (–)	71.5	71.6	42.8	24.6	60.1	53.3	66.1	71.1	86.3–73.1
α-terpineol									
S (–)	76.4	77.0	39.9	8.8	48.1	34.2	67.1	65.8	64.2–82.0
R (+)	23.6	23.0	60.1	91.2	51.9	65.8	32.9	34.2	35.8–18.0

The enantiomeric distribution of some components of lime oils has been reported only in three papers.[12,16,17]

Figures 7-10 show the chiral chromatogram of β-pinene, sabinene, limonene, linalool, terpien-4-ol and α-terpineol for a cold-pressed Key lime oil type A, a cold-pressed Key lime oil type B, a cold-pressed Persion lime oil and a distilled lime oil, respectively. The enantiomeric distribution in lime oils of the components analyzed can be seen in Table 12. From analyses it can be seen that cold-pressed Key lime oils type A type B possess nearly identical enantiomeric distribution values for the analyzed components.

Cold-pressed Persian lime oils show the same value for the enantiomeric distribution of limonene, as do Key lime oils, but different values for the enantiomeric distribution of other components. (+)-β-Pinene, (+)-sabinene, (+)-linalool and (+)-α-terpineol in Persian lime oils represent a higher percentage of the total amount of the components than in Key lime oils. The converse is true for terpinen-4-ol. The differences between Persian and Key lime oils can be attributed to the natural

Figure 4. Variation of monthly average values of the enantiomeric excess of R(+)-limonene, S(–)-b-pinene, S(–)-sabinene, R(–)-terpinen-4-ol, S(–)-a-terpineol, and R(–)-linalool in cold-pressed lemon oil

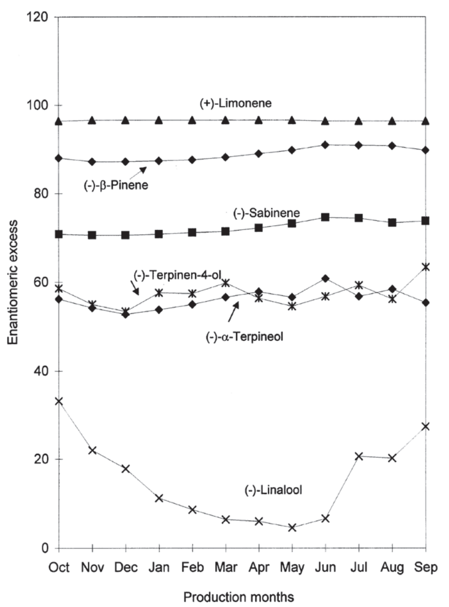
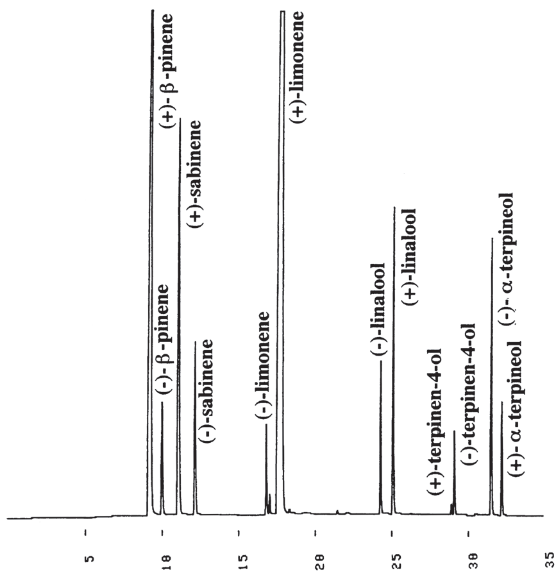


Figure 5. GC chiral chromatogram of the heart-cuts of sabinene + b-pinene, limonene, linalool, terpinen-4-ol, and a-terpineol of mandarin oil



characteristics of the two oils and not to the isolation technology. The Key lime oil type B analyzed shows similar values to Key lime oils type A, and not to Persian lime oils. However, it was obtained with the same extraction technology used for Persian lime oils.

Distilled lime oils, obtained from Key lime fruits, show values of the enantiomeric distribution of the analyzed components different from those shown by the cold-pressed oils, excepting β-pinene, which shows the same value as for cold-pressed oils. The other enantiomeric distributions tend to be racemic, and linalool shows a racemic composition. This behavior agrees with the chemistry of the distilled lime oils, described in detail by Clark and Chamblee.[40]

Table 9. Average values of the enantiomeric distribution of some components in cold-pressed mandarin oils grouped according to month and technology of production

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

[a]number of samples analyzed

Comparison of the Different Italian Cold-Pressed Essential Oils

In Table 13, the enantiomeric distribution values of Italian cold-extracted bergamot, lemon and mandarin oils that were analyzed are reported. In the same table, the preliminary data obtained for italian sweet and bitter orange oils can be found. In sweet orange oil, only the enantiomeric ratios of sabinene and limonene are reported. The enantiomeric distribution of the other components varies within a very wide range. Therefore, a large number of samples analyzed are necessary top obtain enough data for a robust interpretation. This is probably due to the numerous cultivars grown and used for the industrial processing of blond sweet orange fruit (biondo commune, valencia navel, washington navel, ovale) and of pigmented oranges (moro, tarocco, sanguinello). All these cultivars will contribute in a different manner, during the productive season, to the raw material that arrives at the processing site. Because the volatile composition of sweet orange oil is influenced by the different cultivars of the fruit processed, it is possible to foresee a smililar behavior for the enantiomeric distribution of its components.

As seen in Table 13, the enantiomeric distribution values allow for the characterization of different oils and to differentiate between them even if some components present very similar values to other oils. The enantiomeric ratios of limonene are very similar for all the oils analyzed, with the exception of sweet orange oil, where the R(+) enantiomer is more abundant. For β-pinene and sabinene, a similar behavior in bergamot, lemon and bitter orange is observed. In mandarin and sweet orange the ratio of the enantiomers of these components is inverted. The enantiomeric ratio of (–) and (+) linalool is the same in bergamot and bitter orange oils where the S(+) enantiomer is present at very lowlevels. In lemon and mandarin oil, the S(+) enantiomer is present at higher levels. In lemon oil this amount ranges between 28% and 43% of the total amount of linalool. In manadarin oils, this level reaches values up to 87%. The enantiomeric ratios of terpin-4-ol and α-terpineol swing within large ranges for all oils. For terpinen-4-ol, no substantial differences among the analyzed citrus oils were found, while in terpineol, it was possible to determine differences. The R(+) isomer is present at higher levels only in bergamot oils produced at the end of the productive season. The values of enantiomeric distribution of linalyl acetate are the same for all of the bergamot and bitter orange analyzed.

Summary

Bergamot can be differentiated from lemon oil by the different enantiomeric ratio of linalool; from mandarin oil by the different enantiomeric ratios of β-pinene, sabinene and linalool; and from sweet orange oil by the enantiomeric ratios of sabinene and limonene. Lemon

Figure 6. Variation of monthly average values of the enantiomeric excess of R(+)-limonene, S(–)-b-pinene, S(–)-sabinene, R(–)-terpinen-4-ol, S(–)-a-terpineol, and R(–)-linalool in cold-pressed mandarin oil

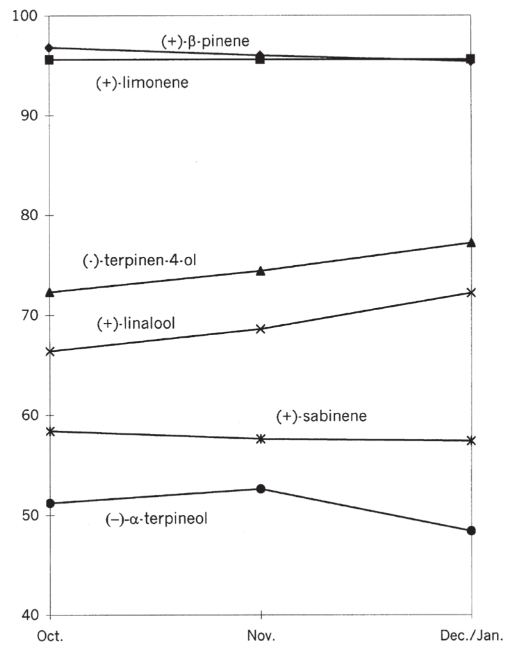


Table 10. Enantiomeric distribution of some components in distilled mandarin oils

	Range
β-pinene	
R (+)	96.1–97.7
R (–)	3.9–2.3
sabinene	
R (+)	77.8–79.9
R (–)	22.2–20.1
limonene	
S (–)	1.7–2.2
R (+)	98.3–97.8
linalool	
R (–)	16.3–20.4
S (+)	83.7–79.6
terpinen-4-ol	
S (+)	25.3–28.7
R (–)	74.7–71.3
α-terpineol	
S (–)	61.2–73.4
R (+)	38.8–26.6

Table 11. Enantiomeric distribution of some components in commercial mandarin oils

	1	2	3	4	5	6	7	8	9	10	11	Cold-pressed Range
β-pinene												
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
R (–)	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												
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
R (–)	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												
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
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												
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
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												
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
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												
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
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

oil can be differentiated from mandarin oil by the different enantiomeric ratios of β-pinene, sabinene, limonene and linalool; from sweet orange oil by the enantiomeric ratios of sabinene and limonene; and from bitter orange by that of linalool. Mandarin oil is differentiated from sweet orange oil by the enantiomeric ratios of sabinene and limonene, and from bitter orange oil by those of β-pinene, sabinene and lonalool. Sweet orange oil can be differentiated from bitter orange oil by the enantiomeric ratios of sabinene and limonene. As mentioned before, bitter orange oil is different from mandarin, lemon and sweet orange because of the enantiomeric ratios of some of the components analyzed, but has values very similar to those of bergamot oil, with the exception that bergamot oils produced at the end of the productive season where the enantiomeric distribution of α-terpineol is different. ■

Table 12. Enantiomeric distribution of some components in cold-pressed and distilled lime oils

	Cold-pressed			
	Key type A range	Key type B	Persian range	Distilled Range
β-pinene				
R (+)	3.4–3.5	3.5	9.1–10.3	3.2–4.0
R (–)	96.6–96.5	96.5	90.9–89.7	96.8–96.0
sabinene				
R (+)	15.1–15.2	15.3	18.2–23.4	–
R (–)	84.9–84.8	84.7	81.8–76.6	–
limonene				
S (–)	2.6–2.9	1.8	0.4–2.7	5.5–8.7
R (+)	97.4–97.1	98.2	99.6–97.3	94.5–91.3
linalool				
R (–)	70.2–71.5	70.0	54.4–69.3	49.8–50.0
S (+)	29.8–28.5	30.0	45.6–30.7	50.2–50.0
terpinen-4-ol				
S (+)	29.2–29.5	29.5	18.6–24.9	42.3–45.0
R (–)	70.8–70.5	70.5	81.4–75.1	57.7–55.0
α-terpineol				
S (–)	84.0–85.5	82.8	74.5–80.8	53.3–56.8
R (+)	16.0–14.5	17.2	25.5–19.2	46.7–43.2

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Figure 7. GC chiral chromatogram of the heart-cuts of sabinene + b-pinene, limonene, linalool, terpinen-4-ol, and a-terpineol of key lime oil type A

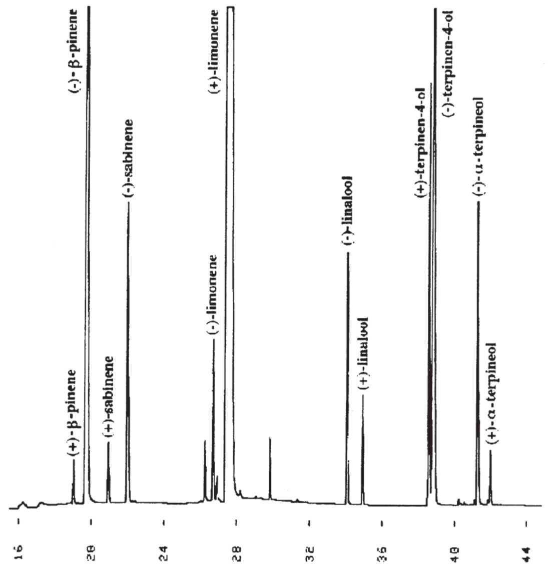

Figure 8. GC chiral chromatogram of the heart-cuts of sabinene + b-pinene, limonene, linalool, terpinen-4-ol, and a-terpineol of key lime oil type B

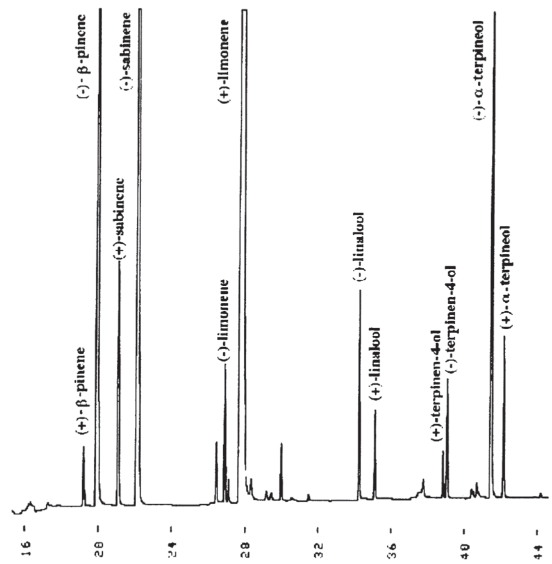

Figure 9. GC chiral chromatogram of the heart-cuts of sabinene + b-pinene, limonene, linalool, terpinen-4-ol, and a-terpineol of Persian lime oil

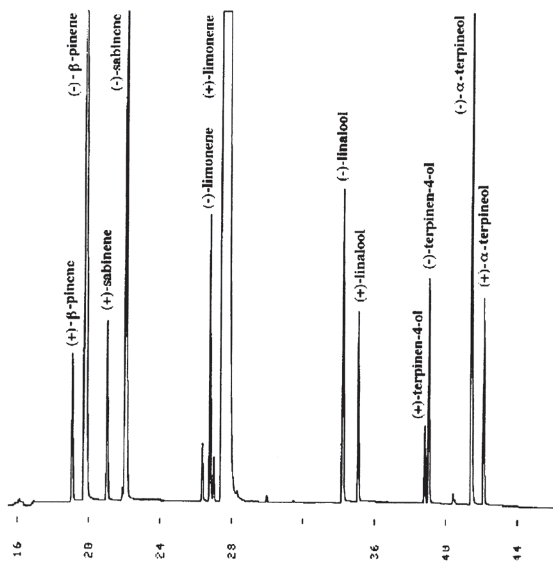

Figure 10. GC chiral chromatogram of the heart-cuts of sabinene + b-pinene, limonene, linalool, terpinen-4-ol, and a-terpineol of distilled lime oil

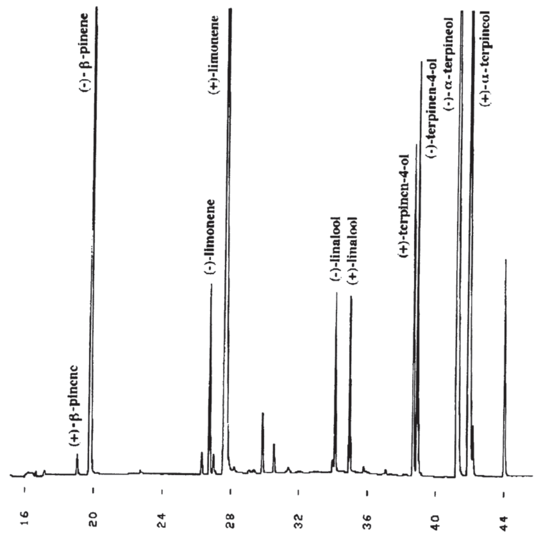

Table 13. Enantiomeric ratios of some components of cold-pressed Italian citrus oils					
	Bergamot	Lemon	Mandarin	Sweet orange	Bitter orange
β-pinene					
R (+)	6.8–9.5	4.2–7.0	97.8–98.8		6.8–8.9
R (–)	93.2–90.5	95.8–93.0	1.2–3.0		92.2–91.1
sabinene					
R (+)	14.1–18.8	12.5–15.3	76.2–80.5	94.6–97.9	14.1–16.0
R (–)	85.9–81.2	87.5–84.7	23.8–19.5	5.4–2.1	85.9–84.0
limonene					
S (–)	1.9–2.7	1.5–2.0	2.0–2.3	0.6	20.–2.7
R (+)	98.1–97.3	98.5–98.0	98.0–97.7	99.4	98.0–97.3
linalool					
R (–)	99.4–99.7	56.8–71.5	13.1–19.8		99.5–99.7
S (+)	0.6–0.3	43.2–28.5	86.9–80.2		0.5–0.3
terpinen-4-ol					
S (+)	9.7–26.3	13.7–26.9	10.0–19.2		13.4–25.4
R (–)	90.3–73.7	86.3–73.1	90.0–81.8		86.6–74.6
α-terpineol					
S (–)	17.5–69.4	64.2–82.0	69.6–76.8		49.3–68.1
R (+)	82.5–30.6	35.8–18.0	30.4–23.2		50.7–31.9
linalyl acetate					
R (–)	99.7–99.9				99.7–99.8
S (+)	0.3–0.1				0.3–0.2

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