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Multidimensional enantio gas chromtography/mass spectrometry and gas chromatography–combustion-isotopic ratio mass spectrometry for the authenticity assessment of lime essential oils (*C. aurantifolia* Swingle and *C. latifolia* Tanaka)

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ABSTRACT

This article focuses on the genuineness assessment of Lime oils (*Citrus aurantifolia* Swingle and *C. latifolia* Tanaka), by Multi Dimensional Gas Chromatography (MDGC) to determine the enantiomeric distribution of α-thujene, camphene, β-pinene, sabinene, α-phellandrene, β-phellandrene, limonene, linalool, terpinen-4-ol, α-terpineol and by gas chromatography–combustion isotope ratio mass spectrometry (GC–C-IRMS) to determine the isotopic ratios of α-pinene, β-pinene, limonene, α-terpineol, neral, geranial, *β*-caryophyllene, trans-α-bergamotene, germacrene B. To the author's knowledge this is the first attempt to assess the authenticity and differentiate Persian Lime from Key lime oils by GC–C-IRMS. The results of the two analytical approaches were compared. The simultaneous use of the two techniques provides more reliable capability to detect adulteration in *Citrus* essential oils. In fact, in some circumstance only one of the two techniques allows to discriminate adulterated or contaminated oils. In cases where only small anomalies are detected by the two techniques due to subtle adulterations, their synergic use allows to express judgments. The advantage of both techniques is the low number of components the analyst must evaluate, reducing the complexity of the data necessary to deal with. Moreover, the conventional analytical approach based on the evaluation of the whole volatile fraction can fail to reveal the quality of the oils, if the adulteration is extremely subtle.

1. Introduction

The genus *Citrus* counts an extremely large number of species, mainly cultivated in subtropical regions. Limes are members of the *Citrus* family, native to Southeast Asia or India and well grown in the tropical regions, mainly Mexico, Brazil, Perù, India and Egypt. There are two main species of lime: Tahitian or Persian (*Citrus latifolia* Tanaka) and Mexican, West Indian or Key (*Citrus aurantifolia* Swingle) limes. Most of the crop of these *Citrus* species is used fresh. The rest is processed to produce lime juice to be marketed as bottled lime juice or used in carbonated beverages. The principal by-product is the essential oil, used in perfumery, cosmetics and flavouring.The techniques used to extract the essential oil can vary in function of the characteristic of the fruits and in the case of Key lime, in function of the properties to confer to the essential oil. Key lime oils type A and type B are, in fact, obtained from the same fruits extracted by different technologies. The essential oil of Key lime type A is extracted screw pressing the whole fruits, obtaining the emulsion of the juice with the essential oil. This can be centrifuged to separate the cold extracted oil (type A), or can be steam distilled to obtain the distilled lime oil. During both processes an important amount of acid catalyzed reactions of the compounds naturally present in the oil take place. Key lime fruits can also be processed by common rasping machines to obtain the essential oil avoiding contact with the juice. In this case the essential oil obtained is called Key lime type B oil. The same procedures used for Key lime are also used to extract Persian lime oil [1]. Distilled and Type A versions of Persian lime oil are however not of commercial importance [2].The composition of the essential oil is thus strictly dependent on the extraction process used. Moreover, due to the different geographic origin, and seasonal variations the composition of these oils, as it happens for other *Citrus* oils, can be subject to large ranges of variability [3].The market values of *Citrus* oils greatly vary from one to another, so it is possible to find commercial oils of the most

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valuables ones, adulterated by addition of synthetic products or more frequently by dilution with cheaper ones or with their fractions. These last frauds cannot always be detected by conventional analytical tools.The composition of volatiles and non-volatiles and to a less extent the chiral distribution of different samples of *Citrus* oils (Limes, Mandarin, Bergamot, Lemon, etc.) were extensively investigated in previous studies as reported by Dugo and Mondello, Bonaccorsi et al. and Dugo et al. [3–7]. Moreover the analytical technique applied on *Citrus* oils have been described in two recent reviews [8,9]. From the results provided in literature it is possible to highlight differences useful to characterize each *Citrus* oil. However, there is little record in literature on the isotope ratios determined in *Citrus* oils [9–19] and no record at all is found in literature on lime oils. Recently it was demonstrated that in some circumstances the discrimination against adulterated compounds can be achieved uniquely by this technique [9,18].

Gas chromatography–combustion-isotopic ratio mass spectrometry (GC–C-IRMS) is a unique tool for the determination of the ratio of the two most abundant isotopes of carbon (^{12}C and ^{13}C) [20]. These values are strictly dependent on the plant biology pathways and also strictly related to the environmental occurrence of these two isotopes. It has been proved that this technique can be used to assess the genuineness of essential oils and define their geographic origin [21].

Multidimensional chromatography could be considered the most suitable approach to analyze complex volatile samples due to the user-friendly instrumentation nowadays available and the lower costs per analysis, respect to comprehensive techniques employing cryogenic focusing gas and interfaces. MDGC finds particular application in essential oil quantitative analysis [22,23] and when applied for the chiral separation of volatile enantiomers in essential oils has been demonstrated to be the most reliable analytical tool [9,24]. In fact, chirally selective stationary phases noticeably increase the number of components to be separated, thus a higher risk of peak overlap occurs. A more reliable approach consists of a pre-separation on a conventional GC column, and the transfer to the chiral column of only the components of interest, so that the enantiomeric pairs can be separated avoiding interferences. This is particularly true when determining the enantiomeric distribution of minor components. In fact in these cases the coelution of one of these enantiomers with different components could drastically compromise the result.

This article will provide new data useful for the characterization of lime oils. The results on the enantiomeric distribution of the selected compounds will improve the information hitherto available in literature on lime oils [4,6,25]; the isotope ratios, never determined before on these *Citrus* species, will be useful to confirm the authenticity of the oils analyzed, and represent the first attempt to differentiate *C. aurantifolia* Swing., versus *C. latifolia* Tan. by means of this analytical approach.

2. Experimental

2.1. Samples

The samples analyzed in this research are 39 lime oils (genuine and commercial Key types A and B, Persian and distilled lime oils).

The genuine samples reported in Table 1 were used to determine the ranges of authenticity by MDGC and by GC–C-IRMS, grouped in function of the type of essential oils. Key lime oils type A and B were all produced during the same productive season in the same industrial plant, from fruits of Key lime cultivated in Mexico. The commercial samples are also listed and described in Table 1. Samples 25–31 are generically indicated lime oil, as reported on the original labels.

Table 1
Description of the 39 samples analyzed.

Sample no.	Sample description	Geographic origin
Authentic oils		
1–5	Cold-Pressed Type A	Mexico
6–12	Cold-Pressed Type B	Mexico
13–15	Cold-Pressed Persian lime oil	Mexico
16–18	Cold-Pressed Persian lime oil	Brazil
Commercial samples		
19	Key lime oil Type A	Mexico
20	Distilled lime oil	Mexico
21	Distilled lime oil	Ivory Coast
22	Distilled lime oil	Mexico
23	Distilled lime oil	Mexico
24	Distilled lime oil	Perù
25	Lime oil[a]	Brazil
26	Lime oil[a]	Brazil
27	Lime oil[a]	Unknown
28	Lime oil[a]	Unknown
29	Lime oil[a]	Unknown
30	Lime oil[a]	Unknown
31	Lime oil[b]	Unknown
32	Persian lime oil	Unknown
33	Persian lime oil	Unknown
34	Persian lime oil	Unknown
35	Persian lime oil	Unknown
36	Persian lime oil	Unknown
37	Persian lime oil	Brazil
38	Persian lime oil	Mexico
39	Persian lime oil[c]	Mexico

[a] Based on composition compatible with Persian lime.
[b] Based on composition this sample seems a terpene-free oil with addition of camphene.
[c] Persian lime oil concentreted 5-fold.

2.2. Multidimensional enantio-GC/MS

The MDGC system consisted of two GC2010 (defined as GC1 and GC2) gas chromatographs, equipped with a Deans switch transfer device, an MS-QP2010 quadrupole mass spectrometer, and an AOC-20i autosampler (Shimadzu). GC1 was equipped with a split/splitless injector and a flame ionization detector (FID1). The MDGC switching element, located inside the oven, was connected to an advanced pressure control (APC) system which supplied carrier gas (He) at constant pressure. GC1 column was an SLB-5MS 30 m × 0.25 mm I.D. × 0.25 μm d_f [silphenylene polymer, virtually equivalent in polarity to poly(5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy). The operational conditions were as follows: constant inlet pressure 220 kPa (300 °C), split mode 1:20 (gas carrier He); injected volume, 1.5 μl; initial linear velocity, 30 cm/s. Temperature program: 50–280 °C at 3 °C/min. The FID (300 °C) was connected, via a stainless steel retention gap, to the transfer system; sampling rate: 80 ms. APC constant pressure: 130 kPa. GC2 was equipped with a split/splitless injector and a flame ionization detector (both not used in the present research). Transfer line temperature between GC1 and GC2: 180 °C. The chiral column in GC2 was a Megadex DETTBS-β (diethyl-*tert*-butil-silyl β-cyclodextrin) 25 m × 0.25 mm I.D. × 0.25 μm d_f (Mega, Legnano, Italy). Temperature program: 40 °C, at 1 °C/min to 100 °C (20 min), to 160 °C at 3 °C/min MS detector: mass range 40–400 amu., scan speed: 2000 amu/s. Ion source temperature: 200 °C, interface temperature: 230 °C. The system and the Deans switch configuration have been previously described in detail elsewhere [22].

2.3. GC–C-IRMS

IRMS analyses, enabling $\delta^{13}C$ measurements, were performed through a combustion furnace (GC–C-IRMS), where the C atoms contained in the sample are converted into a simple gas (CO_2),

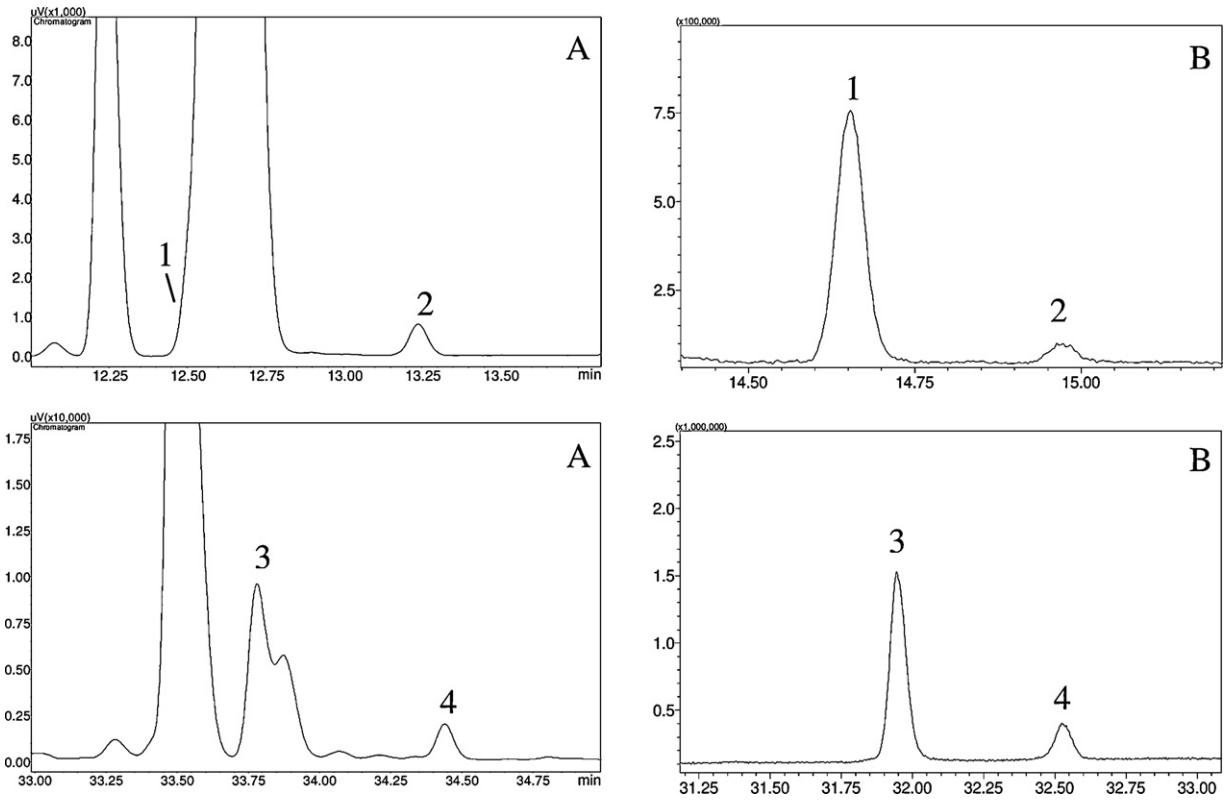

Fig. 1. Es-GC (A) and Es-MDGC (B) comparison of the enantiomeric separation of (−)-camphene (1), (+)-camphene (2), (−)-α-terpineol (3) and (+)-α-terpineol (4).

and afterwards the attained carbon isotope ratio of the unknown sample is compared to that of a calibrated CO_2 reference.

2.3.1. Instrumentation

The system consists of a Trace GC Ultra equipped with a TriPlus autosampler, retrofitted to the combustion interface GC/CIII and hyphenated to the isotope ratio mass spectrometer Delta V Advantage (all purchased from Thermo Fisher Scientific, Milan, Italy). Data are collected in triplicate by the Isodat 2.5 software (Thermo Fisher Scientific).

2.3.2. GC

Column: SLB-5 ms (silphenylene polymer) 30 m × 0.25 mm I.D., 0.25 μm d_f (Supelco, Milan, Italy); temperature program: 50–230 °C at 3 °C/min; split/splitless injector (250 °C). Inlet pressure: 167 kPa; column flow: 2.0 ml/min (constant flow mode); carrier gas: He.

2.3.3. GC/C III

Ox. reactor (Cu/Ni/Pt): 980 °C; red. reactor: 640 °C; He: 1 bar; O_2: 0.8 bar; CO_2: 0.5 bar.

2.3.4. IRMS

EI; electron voltage: 123.99 eV; electron current: 1.5 mA; 3 Faraday cup collectors at *m*/*z* 44, 45, and 46; peak center predelay and post-delay: 15 s, cup 3; reference: 50–70 s, 90–110 s, 130–150 s, 170–190 s; split: open; evaluation type: CO_2_SSH, ref. time: 155.90 s, $\delta^{13}C/^{12}C$ – 60.174‰; integration time 0.2 s.

In order to obtain adequate peak intensities, all samples were appropriately diluted in hexane. To determine the carbon isotope ratio of α-terpineol, neral, geranial, (E)-caryophyllene, trans-α-bergamotene and germacrene B, the samples were diluted 1:2 (*v*/*v*) and 2 μl of this solution were introduced into the Trace GC injector with a split ratio 1:50; the backflush was operated for the first 14.5 min (870 s) then switched off until the end of the analysis time. For the more concentrated compounds, α-pinene, β-pinene, limonene, the samples were diluted 1:2 (*v*/*v*), and 1 μl of this solution was injected with a split ratio 1:200; the backflush was kept off for the entire analysis. Data were collected in triplicate by the Isodat 2.5 software (Thermo Fisher Scientific).

2.3.5. CO_2 reference gas cylinder calibration

The attained carbon isotope ratio of the unknown sample was compared to that of a calibrated CO_2 reference. The CO_2 reference gas was calibrated by injecting 1 μl (70 ppm) of a carbon stable isotope ratio certified reference alkanes mixture, comprising C_{16} to C_{30} (Indiana University, Bloomington, USA). Alkanes were also calibrated against the VPDB standard (Vienna Pee Dee Belemnite), a secondary standard with carbon isotope ratio very similar to a fossil calcium carbonate, Belemnitilla Americana [26] characterized by a defined ^{13}C content; tricosane (C_{23}) was arbitrarily chosen as reference alkane.

Isotope ratios were expressed as δ values (‰), versus a standard.

$$\delta^{13}C_{VPDB} = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{CO_2} \times 1000}{(^{13}C/^{12}C)_{sample}}$$

3. Results and discussion

The use of the MDGC allowed the determination of the enantiomeric distribution of volatiles present at low percentages with respect to direct Es-GC in *Citrus* oils, moreover, since all the co-elutions which can occur in direct Es-GC can be avoided, the results have to be considered more accurate. In fact, in conventional Es-GC analysis, the enantiomers of (−)-camphene, (−)-α-terpineol appear partially or completely co-eluted with other compounds, (+)-α-pinene and linalyl propionate respectively, compromising the determination of their enantiomeric distribution or leading to

Table 2
Enantiomeric excess determined by MDGC in genuine Persian and Key lime oils and in commercial oils.

	Persian lime oils[a]	Commercial lime oils						Commercial Persian Lime oils							
	Range	25	26	27	28	29	30	32	33	34	35	36	37	38	39
(−)-α-Thujene	98–99	99	98	99	99	99	99	98	98	99	99	99	99	98	nd
(−)-Camphene	74–92	84	86	86	86	84	86	76	78	80	82	88	84	84	nd
(−)-β-Pinene	79–82	82	82	88	80	84	87	80	82	80	82	86	80	82	nd
(−)-Sabinene	53–68	64	66	68	66	64	66	60	62	62	68	62	62	64	nd
(−)-α-Phellandrene	4–16	10	12	16	14	2	7	8	12	4	6	6	12	8	
(−)-β-Phellandrene	6–32	8	4	19	22	18	6	2	10	2	14	6	4	9	nd
(+)-Limonene	94–99	94	96	94	96	96	96	90	95	94	96	96	96	94	nd
(−)-Linalool	8–39	30	12	34	28	30	30	−4[b]	28	20	28	20	28	26	28
(−)-Terpinen-4-ol	50–70	54	62	48	54	62	44	45	62	48	58	50	68	56	64
(−)-α-Terpineol	44–62	56	50	58	56	56	50	48	56	52	60	52	48	50	52

	Key lime oils [a]	Commercial Key lime type A	Commercial distilled lime oils					
	Range	19	20	21	22	23	24	31
(−)-α-Thujene	98–100	98	98	nd	nd	nd	nd	nd
(−)-Camphene	84–89	90	59	60	54	56	54	56
(−)-β-Pinene	91–94	93	90	nd	nd	nd	nd	nd
(−)-Sabinene	68–72	70	60	nd	nd	nd	nd	nd
(−)-α-Phellandrene	<1–17	6	18	23	25	26	25	22
(−)-β-Phellandrene	30–49	22	1	27	20	24	23	20
(+)-Limonene	94–96	95	87	86	84	86	81	86
(−)-Linalool	30–47	40	−2[b]	−4[b]	−7[b]	−11[b]	−4[b]	−18[b]
(−)-Terpinen-4-ol	41–58	40	25	15	14	18	11	16
(−)-α-Terpineol	56–71	70	23	13	8	12	6	8

[a] Ranges of authenticity were determined from the value determined in the present study and considering the results by Mondello et al. [25] and Bonaccorsi et al. [4,6].
[b] Inversion of the enantiomeric distribution.

a bogus enantiomeric ratio. In Fig. 1 it is demonstrated how, by means of multidimensional GC, the pre-separation allows to avoid this inconvenient. It is well assessed that, in some circumstances when co-elutions occur, it is possible to determine the correct enantiomeric excess by GC/MS extracting the characteristic ion of the enantiomer investigated. In the case of α-terpineol is present a characteristic ion (59 *m*/*z*) useful for this approach. However during the entire GC analysis coelution of components with similar structure can occur, thus this approach could not be applied for all of them.

Enantioselective separation carried out by multidimensional GC–MS allowed to separate ten pairs of enantiomers (α-thujene, camphene, β-pinene, sabinene, α-phellandrene, β-phellandrene, limonene, linalool, terpinen-4-ol, α-terpineol). The reproducibility of the method was excellent, with CV values determined from triplicates of the same sample below 2%. The results are reported in Table 2.

The GC–C-IRMS was performed determining first the range of variability of the isotopic ratios ($\delta^{13}C$) for the following components: α-pinene, β-pinene, limonene, α-terpineol, neral, geranial β-caryophyllene, trans-α-bergamotene, germacrene B. These components were identified based on previous studies carried out by our research group using GC/MS-LRI [17–19,24]. These compounds were selected among the most representative, with appropriate concentration levels and good chromatographic resolution to provide high reproducibility of the results. In Table 3 are reported the authenticity ranges, mean values and standard deviations obtained from twelve cold-pressed Key lime oils, five type A and seven type

Table 3
Ranges of $\delta^{13}C$ determined genuine Key lime oil (types A and B) and Persian lime oils.

	α-Pinene	β-Pinene	Limonene	α-Terpineol	Neral	Geranial	β-Caryophyllene	trans-α-Bergamotene	Germacrene B
Authenticity range (samples 1–12)									
Mexican lime type A–B									
Min lime oil type A–B	−31.17	−30.66	−29.76	−30.81	−28.97	−28.88	−31.34	−34.71	−30.83
Max lime oil type A–B	−29.30	−28.18	−28.35	−29.18	−27.23	−27.38	−29.96	−32.12	−29.13
Mean value	−30.29	−29.55	−29.03	−29.97	−28.29	−28.01	−30.92	−33.14	−30.13
σ	0.56	0.98	0.54	0.61	0.59	0.45	0.46	0.83	0.57
Authenticity range calculated with β-caryophyllene internal standard									
Min mexican lime oil type A–B i-std	−1.20	−0.54	0.51	0.33	1.21	1.65	0.00	−4.59	−0.58
Max mexican lime oil type A–B i-std	1.33	3.15	2.75	1.72	3.40	3.75	0.00	−0.88	2.10
Mean value	0.53	1.24	1.76	0.86	2.50	2.79	0.00	−2.34	0.66
σ	0.72	1.30	0.77	0.47	0.81	0.68	0.00	1.16	0.85
Authenticity range (samples 13–18)									
Persian lime									
Min Persian lime	−29.32	−27.58	−25.98	−30.22	−27.07	−26.36	−30.49	−31.16	−28.39
Max Persian lime	−28.35	−25.05	−24.42	−28.66	−25.03	−24.19	−27.34	−28.39	−26.58
Mean value	−29.12	−26.42	−25.25	−29.44	−25.94	−25.15	−28.89	−29.63	−27.39
σ	0.55	0.95	0.59	0.62	0.82	0.82	1.13	0.99	0.65
Authenticity range calculated with β-caryophyllene internal standard									
Min Persian lime i-std	−1.45	1.14	1.36	−1.79	1.90	2.70	0.00	−1.05	0.17
Max Persian lime i-std	0.71	3.74	5.06	0.60	3.75	4.59	0.00	−0.47	2.10
Mean value	−0.22	2.48	3.65	−0.54	2.96	3.75	0.00	−0.73	1.51
σ	0.87	1.14	1.44	0.92	0.71	0.72	0.00	0.22	0.80

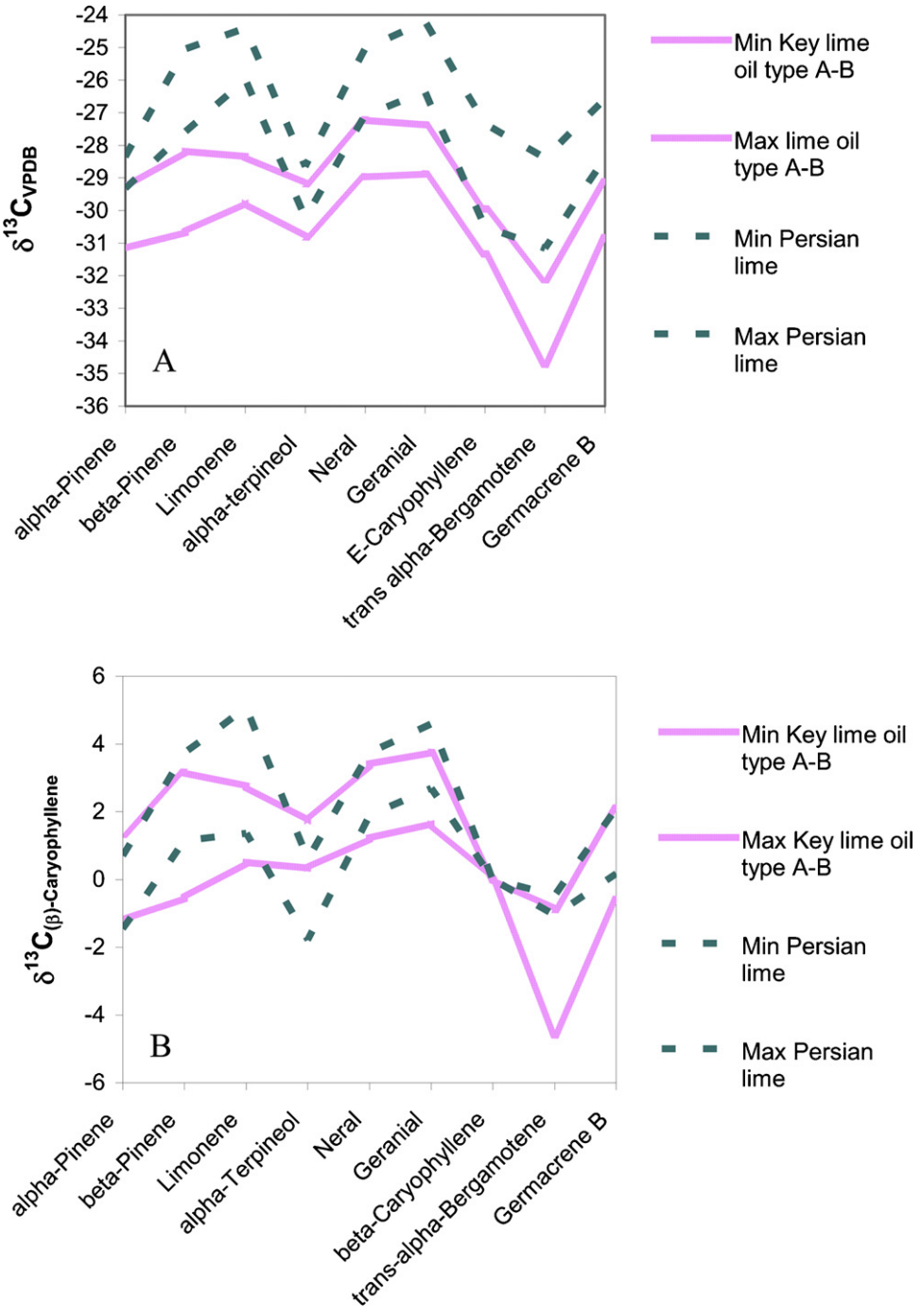

Fig. 2. Comparison of range of $\delta^{13}C$ values without (A) and with internal standard (B) of authentic Persian and Key lime oils.

B, industrially produced in Mexico and surely genuine. As predictable, the values determined for genuine Key lime types A and B were in good agreement, therefore it was assumed that they could be treated to build a single range of variability. This range was also considered for the assessment of authenticity of distilled lime oils, which are obtained from the same fruits. In the same table are reported the values relative to Persian lime oils, determined from six samples produced in Mexico and Brazil industrially cold-extracted and surely genuine. The C isotope ratio can vary in function of the climate, latitude and is generally linked to the geographic environment in which the plant develops and fix the CO_2. In addition the enzymathic pathways for the monoterpene precursor biogeneration, geranyl pyrophosphate, contribute to shift of this ratio. The use of Internal Standard (I.S.), subtracting the above mentioned effects, is often necessary to assess the authenticity of samples produced in different geographic areas [27]. In fact, this approach permits to evaluate only the variation of the isotopic ratio due to the secondary biogenetic pathways linked to the chemistry involved for the synthesis of terpenes. To choose the most appropriate I.S. the following requirements must be satisfied: it must be in sufficient amount but possess low sensorial importance; must be biogenetically related to the other compounds investigated; must be inert during sample storage. In function of all the above mentioned consideration β-cariophyllene represented a good choice for I.S., and was selected as the most suitable for lime essential oils. Due to the higher reliability of the results obtained by the use of I.S.

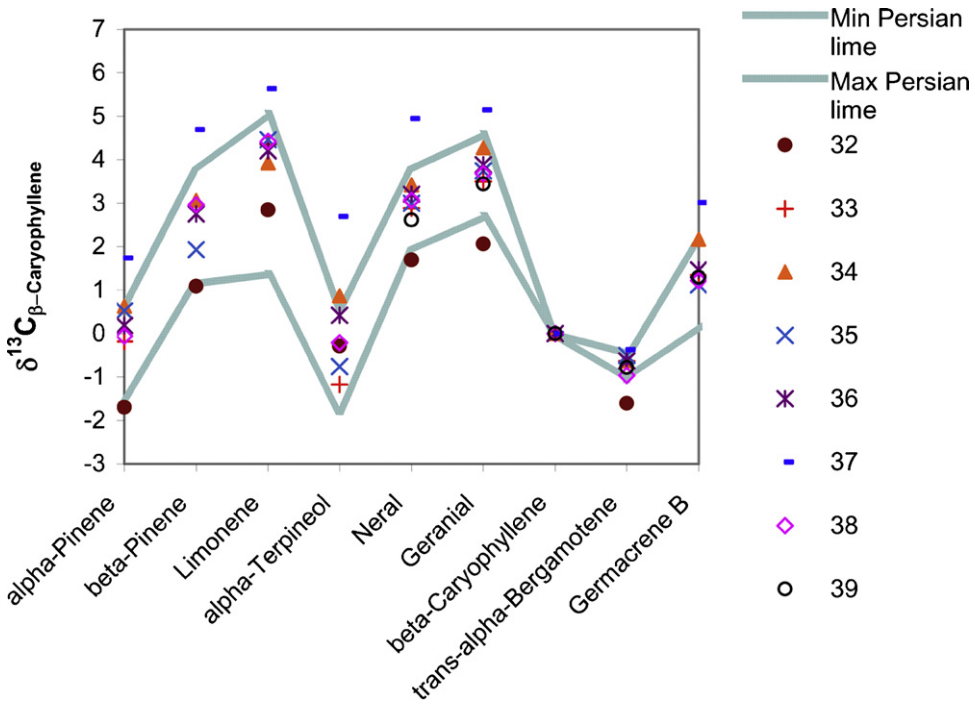

Fig. 3. Range of $\delta^{13}C$ values of authentic Persian lime oils and values determined in commercial Persian lime oils with internal standard. For sample description see Table 1.

the comparison between commercial oils and authenticity ranges is reported using these values.

Between Key and Persian lime oils (Table 2) only few differences are noticed in their enantiomeric distribution. The main difference is relative to β-pinene with significantly higher e.e. of the levorotatory enantiomer in Key lime oils. Significant differences are also noticed for the e.e. of (−)-sabinene, (−)-β-pinene and (−)-linalol also in this case higher in the oils obtained from Key lime. These differences are in good agreement with what reported in previous studies [4,6,25].

In Table 3 and in Fig. 2A and B are compared the ranges of variability determined in Key and Persian lime oils of $\delta^{13}C_{VPDB}$ and of $\delta^{13}C_{\beta\text{-caryophyllene}}$ respectively. From the table and from the figures it is possible to notice that while the ranges of variability of $\delta^{13}C_{VPDB}$ determined for the two oils are almost completely separated those relative to $\delta^{13}C_{\beta\text{-caryophyllene}}$ are for most compounds overlapped, with the exception of α-terpineol, and trans-α-bergamotene. These two compounds present their maxima in Key lime coincident with the minima determined in Persian lime oils.

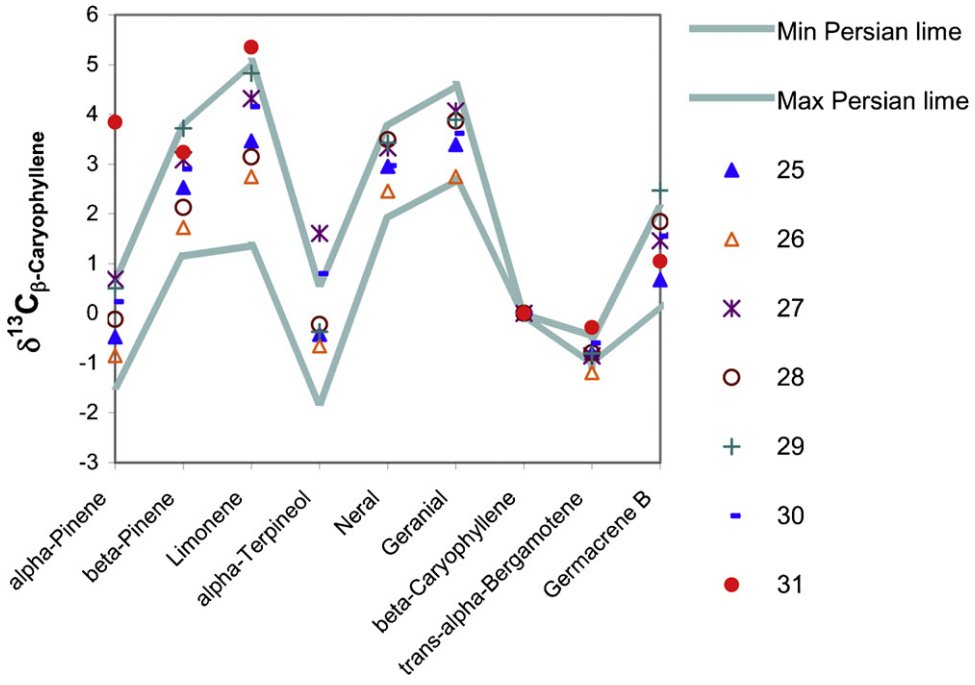

Fig. 4. Range of $\delta^{13}C$ values of authentic Persian lime and valued determined in commercial lime oils with internal standard. For sample description see Table 1.

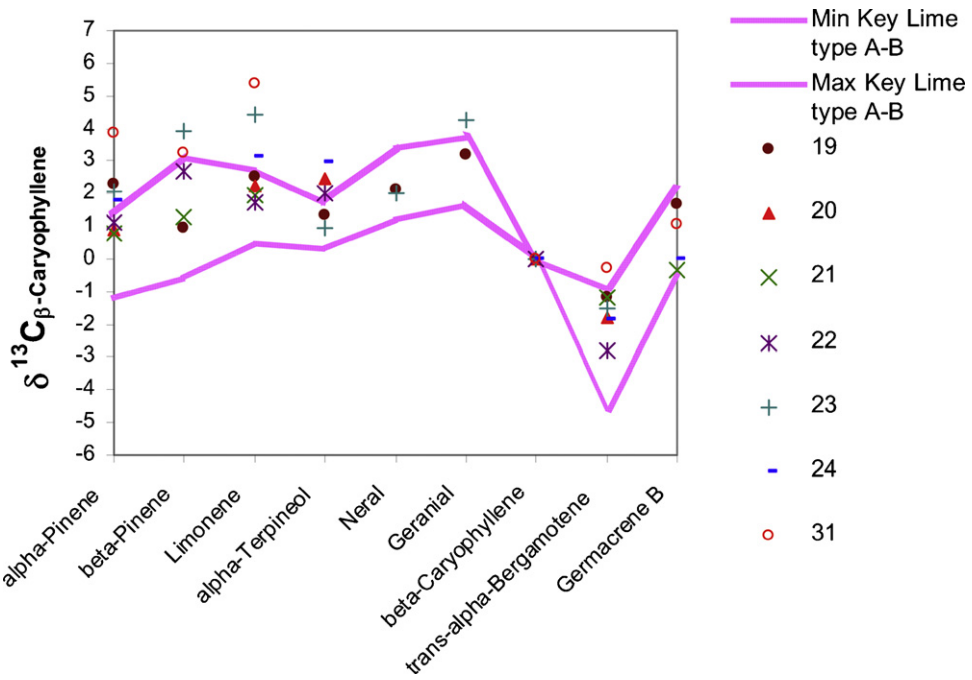

Fig. 5. Range of $\delta^{13}C$ values of authentic Key lime oils and values determined in commercial Key lime and distilled oils with internal standard. For sample description see Table 1.

In Table 2 the values of the e.e. relative to commercial samples labelled as Persian lime oils (samples 32–38) and to those labelled as lime oils (25–30) whose composition was compatible with Persian lime, based on the amount of β-pinene, γ-terpinene, limonene previously determined by GC-FID, and that relative to sample 39, labelled as concentrated lime oil (5-fold) are compared to the values determined for genuine Persian lime oils. In the same table are reported the e.e. of the sample labelled as commercial Key lime and of distilled oils compared with the range of authentic Key lime types A and B. The last column of the table reports the values determined for sample 31, which presented a composition of the volatile fraction, determined by GC-FID, compatible with a terpeneless oil added with camphene, not compatible with any of the types of oils reported in the table. The range of authenticity of Persian and Key lime are determined taking in consideration the values previously reported by Mondello et al. [25] and by Bonaccorsi et al. [4,6].

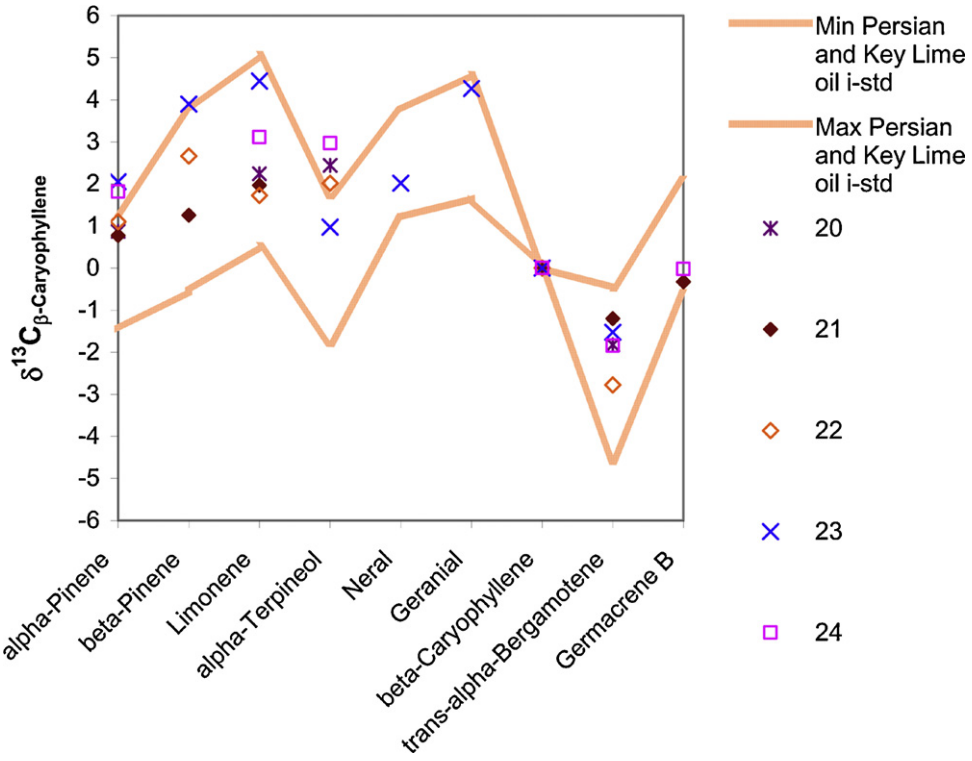

Fig. 6. Range of $\delta^{13}C$ values of authentic Key and Persian lime oils and values determined in distilled lime oils, with internal standard. For sample description see Table 1.

In Figs. 3–6 are reported the $\delta^{13}C_{\beta\text{-caryophyllene}}$ of the same oils.

The enantiomeric excess values of samples 25, 28, 33, 35, 38 and 39 fall within the range of authenticity as Persian lime oils. For samples 27, 29, 30 and 36 the enantioimeric excesses are within the ranges of authenticity with the exception of (−)-β-pinene always higher, in these samples, than that of authentic Persian lime oils and for terpinen-4-ol in samples 27 and 30, and of (−)-α-phellandrene for sample 29 lower than the minima of the authenticity range. These samples are compatible with presence of Key lime. Samples 26, 34 and 37 show values of e.e. of (−)-β-phellandrene slightly below the minimum of genuine samples and for sample 34 the same occurs for (−)-terpinen-4-ol; these results do not allow to express judgement based uniquely on the enantiomeric distribution values on these three samples.

Sample 32 show anomalous values of (−)-β-phellandrene, terpinene-4-ol and mainly limonene and linalool. These values give sufficient information to define the oil surely not authentic.

The results of $\delta^{13}C_{\beta\text{-caryophyllene}}$ (Figs. 3 and 4) of samples 25, 28, 33, 35, 38 and 39 confirm the genuineness of these oils. Sample 36 presents values of $\delta^{13}C_{\beta\text{-caryophyllene}}$ compatible with Persian lime but, as observed from the chiral analysis, these values fall, also in this case, within the range of Key lime. The values of $\delta^{13}C_{\beta\text{-caryophyllene}}$ of samples 27, 30 are compatible with the authenticity range with the exception of α-terpineol, which presents isotopic ratios higher than authentic Persian lime. However, these values, as well as all the enantiomeric ratios determined for all the components analyzed, are compatible with the presence in these samples of Key lime, as previously stated evaluating the enantiomeric excesses. Samples 26, 29 and 34 present slight discrepancy in their $\delta^{13}C_{\beta\text{-caryophyllene}}$ profiles. The first sample for trans-α-bergamotene, the second for germacrene B and the third for α-terpineol; the anomalies determined for these three samples, either for the enantiomeric distribution either for their isotopic ratios, cannot be explained by the possible presence of Key lime, but should be prudently considered sign of adulteration. Based on the results of their isotopic ratio (Fig. 3) samples 32 and 37 should be considered adulterated.

Sample 19 (Table 2) presents slight differences in its enantiomeric distribution of camphene, and of terpinen-4-ol and more evident for β-phellandrene. For what concerns the isotopic ratios the value of α-pinene is outside the range of authentic Key lime. For this value and for the results of the chiral analysis doubts rise on its authenticity.

The results on the enantiomeric distribution of distilled lime oils (Table 2) could be in agreement with predictable values obtained for oils which remained for several hours in contact with the acid juice before distillation. Thus the consequent tendency to racemize is due to the reversible hydration reactions catalyzed by the acidic medium, which are not enantioselective [28,29]. This process continues during distillation leading to partial or total racemization of the components involved in these reactions. The entity of this phenomenon is dependent on the conditions applied during the extraction procedures (length of contact water/oil, pH, distillation parameters). Literature reports values on the enantiomeric distribution on six samples analyzed by Mondello et al. [25] and to a small number of components. These appear insufficient to represent an authenticity range mainly if considering the possible differences of the entity of the transformation in function of the extraction procedure. To evaluate the authenticity of these oils must be considered, in this case, only the results obtained by GC–C-IRMS (Fig. 5). Thus sample 21 can be considered surely genuine, while samples 23 and 24 are surely adulterated. Samples 20 and 22 present the values of α-terpineol outside the range of authenticity. Values of isotopic ratios determined in distilled oils, for which it is not possible to exclude presence of either Persian and Key limes, were compared to the range of authenticity obtained from Key and Persian lime simultaneously (Fig. 6). As it can be seen from the figure results agree with those obtained from the comparison with the range of authenticity relative to only Key lime oils. With regards to sample 31 the adulteration is confirmed by both analytical approach, since the enantiomeric distribution (Table 2) and the isotopic ratios are not compatible with Persian lime oils (Fig. 4) nor with Key lime (Fig. 5).

The judgements, expressed on the commercial samples analyzed, perfectly fit for both techniques for samples 25, 28, 33, 35, 38, 39 (surely genuine samples), for samples 27, 30, 36 (mixtures of Key and Persian lime oils) and for samples 31 and 32 (surely adulterated). The evaluation of samples 19, 26, 34, 37 is similar with both techniques, therefore the two techniques support the difficulties in the evaluation of their authenticity; to evaluate some of the distilled oils (21, 23, 24) the GC–C-IRMS appears to be the most reliable analytical approach. For distilled oils 20 and 22, which present only small differences of their isotopic value of α-terpineol, it is not possible to express an univocal judgement of adulteration.

4. Conclusions

An authenticity range of $\delta^{13}C$ was determined for *C. aurantifolia* Swing. and *C. latifolia* Tan. essential oil for the first time. In addition the enantiomeric ratios here determined contribute to the few values hitherto available in literature. These information provide useful tools to characterize the different species of lime oils. The two techniques show excellent reproducibility. In both cases the analyst can assess the quality of the sample dealing with a very limited number of values to evaluate. Although up-to-date the instrumentation required is not considered conventional, this study should be indicative of the power of these two analytical approaches, to be applied for quality assessments of *Citrus* oils in general, but particularly useful for lime oils, due to the large variability of the composition of the volatile components highlighted in literature for the different types of oil. In most of the cases the results were in excellent agreement. However, chiral analysis can fail in subtle circumstances and often the evaluation of the samples is difficult due to the chemical changes caused by the extraction technology and the seasonal variation which has not been established yet.

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