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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),