Determination of Enantiomeric and Stable Isotope Ratio Fingerprints of Active Secondary Metabolites in Neroli (Citrus aurantium L.) Essential Oils for Authentication by Multidimensional Gas Chromatography and GC-C/P-IRMS

Aurélien Cuchet, Anthony Anchisi, Frédéric Schiets, Yohann Clément, Pierre Lantéri, Christelle Bonnefoy, Patrick Jame, Elise Carénini, Hervé Casabianca

Determination of Enantiomeric and Stable Isotope Ratio Fingerprints of Active Secondary Metabolites in Neroli (*Citrus aurantium* L.) Essential Oils for Authentication by Multidimensional Gas Chromatography and GC-C/P-IRMS

Authors

Aurélien Cuchet[a,b,*]; Anthony Anchisi[b]; Frédéric Schiets[b]; Yohann Clément[b]; Pierre Lantéri[b]; Christelle Bonnefoy[b], Patrick Jame[b]; Elise Carénini[a]; Hervé Casabianca[b]

Affiliations

[a]Albert Vieille SAS, 629 Route de Grasse, 06220 Vallauris, France

[b]Université de Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France

***Corresponding author**

Aurélien Cuchet, aurelien.cuchet@isa-lyon.fr; aurelien.cuchet@gmail.com

Abstract

Neroli essential oil (EO), extracted from bitter orange blossoms, is one of the most expensive natural products on the market due to its poor yield and its use in fragrance compositions, such as cologne. Multiple adulterations of neroli EO are found on the market, and several authentication strategies, such as enantioselective gas chromatography (GC) and isotope ratio mass spectrometry (IRMS), have been developed in the last few years. However, neroli EO adulteration is becoming increasingly sophisticated, and analytical improvements are needed to increase precision. Enantiomeric and compound-specific isotopic profiling of numerous metabolites using multidimensional GC and GC-C/P-IRMS was carried out. These analyses proved to be efficient for geographical tracing, especially to distinguish neroli EO of Egyptian origin. In addition, $\delta^{2}H$ values and enantioselective ratios can identify an addition of 10% of petitgrain EO. These results demonstrate that enantioselective and stable isotopic metabolite fingerprint determination is currently a necessity to control EOs.

Keywords

Isotope ratio mass spectrometry, Multidimensional gas chromatography, Enantioselective analysis, Stable isotope analysis, Neroli essential oil, Authentication

1. Introduction

The essential oil (EO) of neroli is produced via hydrodistillation or steam distillation of bitter orange (*Citrus aurantium* L.) blossoms. This EO is highly expensive due to its very low production yield (0.1%) [1]: at least one metric ton of fresh orange blossoms is required to produce only one kilogram of pure EO. The price of EO can fluctuate between 2,500 and 6,000 €/kg depending on the country and the year. The quantity of EO produced worldwide is estimated between 4.5 to 6.5 MT per year. Neroli EO is nowadays an iconic product in the fragrance industry, due to its olfactive profile (Floral, orange blossom, green, methyl-like, and honeyed) in demand in the perfume industry [2]. Previous pharmacological studies have reported neurotonic properties [3] and antimicrobial, antioxidant [4,5], anticonvulsant [6], analgesic and anti-inflammatory activities [7] for this EO. Currently, this EO *(C. aurantium ssp. amara var. pumilla)* is mainly produced in Morocco, Tunisia, and Egypt but also in Spain, and Lebanon. The EO of another variety (*C. aurantium* L *ssp amara* var. *daidai)* is produced in China.

Adulteration is a common problem in the EO industry, especially with expensive EOs such as neroli. These adulterations are generally carried out to meet standard requirements, increase artificial EO quantities, or develop economic strategies. Orange blossom harvesting requires caution, and the production quality is a function of the weather and temperature conditions throughout the year. In the case of neroli EO, the main adulterations include blending with several known adulterations have been reported in the literature: blending with inexpensive EOs such as sweet orange EO (*Citrus sinensis*) [8], bitter orange EO [8], and petitgrain EO [9]; addition of synthetic components such as linalool and linalyl acetate; and dilution with vegetable oils or solvents, such as sunflower oil [10] or ethanol [11].

Typical identification and characterization techniques, such as gas chromatography (GC) quality control or olfactive panel tests, cannot always differentiate a natural molecule from various origins (species or geographical) or a natural molecule from its synthetic equivalent. EO authentication requires the combination of several advanced analytical techniques to accurately identify adulteration with high precision. Currently, several methods are commonly used, such as the determination of synthetic residual impurities and natural markers [12], enantioselective analyses [13,14], radiocarbon activity assessment [12,15], and stable isotope analyses [12,13,16–19].

Enzymatic synthesis of asymmetric carbon molecules provides stereoselectivity. In most cases, one enantiomer is significantly in excess compared to the other enantiomer. However, organic synthesis will lead to a racemic mixture of both enantiomers. Adulteration of an EO can also be determined with a chiral study if some enantiomer proportions are reversed between the two EOs. Analysis of chiral molecules with enantioselective GC, monitored on cyclodextrin-based capillary columns, enables efficient determination of the enantiomeric ratios of compounds of interest. Chiral analysis is an effective tool to authenticate neroli EO purity. Several studies have been performed to determine the enantiomeric ratios of major metabolites in neroli EO, including linalool and linalyl acetate [14], α-pinene, β-pinene, limonene [8,19], and other components [20].

Isotope ratio mass spectrometry (IRMS) is another powerful tool used to highlight the addition of synthetic molecules to an EO, blending with another natural extract, or assessment of its geographical origin. GC – combustion/pyrolysis – IRMS (GC-C/P-IRMS) allows for the determination of compound-specific $\delta^{13}C$ and $\delta^{2}H$ isotope ratios. IRMS has been recently used to identify adulterations of food products such as honey [21,22] or wine [23]. This analytical

technique is especially useful in the EO field, which presents complex matrices with several metabolites in nonnegligible quantities. GC-C/P-IRMS has been successfully used in the past for the authentication of several essential oils, including rose [17], oregano and thyme [24], *Allium* species [16], lemongrass and citronella [13], and lemon [18]. Several studies have also determined stable isotopic ratios on several secondary metabolites in neroli EOs, such as linalool and linalyl acetate [25], and other components (β-pinene, myrcene, limonene, terpinen-4-ol, α-terpineol, nerol, neryl acetate, geranyl acetate, β-caryophyllene, (E)-nerolidol, and (E)-(E)-farnesol) [20,26].

In the present study, chiral and isotopic data of some compounds of neroli EOs have been used in combination with chemometrics to trace their geographical origin and to assess their authenticity. The first part of this study illustrates the use of these data to geographically discriminate neroli EO samples. Geographical traceability is relevant because an EO is defined by its extraction process, its species, and its location. Furthermore, prices vary with the geographical origin of harvest. The second part of this study is a focus on the purity of neroli EO samples. Although EO naturalness can be easily authenticated by ^{14}C activity assessment or chiral studies, purity is much harder to detect, especially the addition of petitgrain EO, which is also manufactured from bitter orange. Petitgrain EO is obtained from the distillation process of bitter orange leaves and twigs. Neroli and petitgrain EOs are mainly composed of monoterpenic hydrocarbons and oxygenated monoterpenoids. The principal components of neroli and petitgrain EOs are linalool, limonene, linalyl acetate, β-pinene, (E) β-ocimene, (E)-nerolidol, (E) (E) farnesol, and α-terpineol [27]. Neroli and petitgrain EOs share similar compounds, except for nitrogen-containing trace compounds (indole and methyl anthranilate), which have only been reported in neroli EO [28,29].

2. MATERIALS AND METHODS

2.1. Essential Oil Information

All studied samples (*C. aurantium* L *ssp amara var. pumilla*) were provided by Albert Vieille SAS (Vallauris, France). The traceability and provenance of the EOs were certified.

Neroli EOs came from Morocco (26 samples), Lebanon (4 samples), Tunisia (16 samples), and Egypt (22 samples) via several official producers.

In addition, Spanish samples (9 samples) were directly manufactured by Albert Vieille SAS from their aromatic raw material production facility localized in Sevilla region (Spain). Flowers of *Citrus aurantium* were mechanically harvested at the beginning of spring. Fresh flowers were manually sorted to remove leaves and twigs and quickly conveyed to production. Two different processes of distillation were undertaken. Steam distillation conditions applied were as follows: 1 ton of fresh flowers were loaded in a 4000 L still apparatus, with a water steam flow set between 100 and 150 L/h. After 3 hours of distillation, obtained yields comprised between 0.03 and 0.06%. Hydrodistillation conditions applied were as follows: 800 kg of fresh flowers were loaded in a 4000 L still apparatus, with a water flow set between 120 and 135 L/h. After 3 hours of distillation, obtained yields comprised between 0.01 and 0.04%.

Petitgrain EOs were from Madagascar (3 samples) and Paraguay (6 samples). More information can be found in **Supplementary Table S1** including the year of production, harvest location details, the vegetative stage at time of harvest, and the extraction process.

2.2. Enantioselective Analyses

2.2.1. Sample Preparation

Two hundred microliters of each EO sample were settled on silica gel 60A (35-70 µm particle size, Carlo Erba Reagents, Val-de-Reuil, France). A primary fraction was collected by elution with 200 mL of pentane. This nonpolar fraction, largely composed of monoterpene and sesquiterpene molecules, was called Fraction 1. A second fraction was obtained by elution with 200 mL of diethyl ether. This polar fraction, mainly composed of alcohols and esters, was called Fraction 2. Fractions 1 and 2 were then evaporated with a rotary evaporator (Rotavapor R200, Büchi, Villebon-sur-Yvette, France) and diluted in 1 mL of hexane. This sample preparation was applied to dispose of 1-8 cineole from Fraction 1, which can interfere with limonene and β-phellandrene detection in Enantio-GC.

2.2.2. Multidimensional Gas Chromatography (MDGC) Analyses

MDGC analyses were performed on a 7890A GC gas chromatograph system (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) operating at a detector temperature of 250°C.

One microliter of each Fraction 1 was injected into the GC in split mode (120:1). Helium was used as the carrier gas with a constant inlet pressure of 26.4 psi. The flow rate was set to 0.7 mL/min in the first column and 2 mL/min in the second column. Separation of volatile compounds was realized on an HP-1 primary capillary column (15 m x 0.200 mm x 0.5 µm film thickness, Agilent Technologies) combined with a Dean Switch system (heart cutting program) with a second Cyclosil β column (30 m x 0.250 mm x 0.25 µm film thickness, Agilent Technologies) coated with cyclodextrin stationary phase. The initial oven temperature was 55°C held for 15 min, followed by an initial increase at 1°C/min to reach a temperature of 85°C. An additional increase in temperature at 10°C/min was then applied to reach a final temperature of 200°C and held for 10 min. Enantiomeric ratios were determined for α-

146 thujene, α-pinene, camphene, sabinene, β-pinene, δ-3-carene, limonene, and β-phellandrene,
147 as reported in **Figure 1a**.

148 An aliquot 0.2 µL in volume of each Fraction 2 was injected into the GC in split mode (60:1).
149 Helium was used as the carrier gas (18.8 psi applied inlet pressure). The flow rate was set to
150 0.7 mL/min in the primary column and 1.7 mL/min in the secondary column. Separation of
151 volatile compounds was realized on an HP-1 column (15 m x 0.200 mm x 0.5 µm film thickness,
152 Agilent Technologies) combined with a DEtTBuSilBetaCDX PS086 cyclodextrin stationary phase
153 column (25 m*0.25 mm*0.25 µm, Mega, Legano, Italy) and a Dean Switch system (heart
154 cutting program). The oven temperature program was as follows: the initial temperature was
155 held at 60°C for 5 min, increased at a rate of 1°C/min to reach a temperature of 95°C, and held
156 for 9 min. An additional increase at 1°C/min was applied to reach 150°C. Then, a final increase
157 at 10°C/min was carried out to reach 200°C, which was held for 10 min. Enantiomeric ratios
158 were determined for linalool, terpinen-4-ol, α-terpineol, linalyl acetate, and (E)-nerolidol, as
159 reported in **Figure 1b**.

160 **2.3. Isotopic Analyses**

161 **2.3.1. Data Expression**

162 Isotopic results are expressed in delta (δ) units according to the following formula [30]:

163 $$\delta^{i}\mathrm{E} = \frac{({}^{i}\mathrm{R}_{Sample} - {}^{i}\mathrm{R}_{Reference})}{{}^{i}\mathrm{R}_{Reference}}$$

164 where i is the mass number of the heavy isotope of element E, R_{Sample} is the isotope ratio of
165 the sample, and $R_{Reference}$ is the isotope ratio of a relevant internationally recognized reference
166 material. The δ values were multiplied by 1000 and are expressed in units "per mil" (‰).

High-purity gases of carbon dioxide (99.998%, 4.8 Messer) and hydrogen (99.999%, 5.0 Messer) were used as the working reference gases injected with each sample. Helium (99.999% purity) was used as a carrier gas.

All studied metabolites in the EOs of interest were calibrated with a mixture of molecules (β-pinene, limonene, (E)-β-ocimene, linalool, linalyl acetate, α-terpineol, neryl acetate, geranyl acetate, nerol, geraniol, and (E)-nerolidol) purchased from Sigma Aldrich (Saint Louis, MO, USA) and TCI (Tokyo, Japan). These molecules were previously calibrated against international standards in elemental analysis – IRMS (EA-IRMS).

International reference materials IAEA CH7 (polyethylene foil: $\delta^{13}C$ (‰) -32.15, $\delta^{2}H$ (‰) -100) and USGS40 (L-glutamic acid: $\delta^{13}C$ (‰) -26.39), supplied by the International Agency of Atomic Energy (IAEA, Vienna, Austria), were used to calibrate each molecule in the mixture, which allowed us to express our $\delta^{13}C$ results relative to the Vienna Pee Dee Belemnite (VPDB) scale and our $\delta^{2}H$ results relative to the Standard Mean Ocean Water (VSMOW) scale. Each sample was analyzed versus a monitoring gas for which two pulses were admitted with each analyte. All sample values were analyzed in duplicate and verified by bracketing the analytical sequence with international reference materials every six analyses. The $\delta^{13}C$ values are expressed within a maximum standard deviation of ± 0.28‰ and those of $\delta^{2}H$ within SD = ± 5‰. $\delta^{13}C$ isotopic analyses of the standards molecules were conducted using a Flash HT elemental analyzer connected to a Delta V Plus via a Conflo V interface. The $\delta^{2}H$ measurements were carried out using a TC-EA elemental analyzer linked to a Delta V Plus via a Conflo V interface (all Thermo Fisher Scientific). The method is fully explained in a previous study [12]

2.3.2. Gas Chromatography - Mass Spectrometry - Combustion/Pyrolysis - Isotope Ratio Mass Spectrometry (GC-MS-C/P-IRMS)

The $\delta^{13}C$ and $\delta^{2}H$ values of targeted neroli and petitgrain EO metabolites were determined by GC-IRMS using a GC Trace 1310 simultaneously connected to an ISQ MS quadrupole mass spectrometer and to a Delta V Plus isotope ratio mass spectrometer via a GC Isolink II interface. (Thermo Fisher, Bremen, Germany). This equipment enabled the identification of compounds via the ISQ MS instrument and specific compound isotopic ratio measurements of $\delta^{13}C$ and $\delta^{2}H$ via IRMS. Isotopic ratios were determined for β-pinene, limonene, (E)-β-ocimene, linalool, linalyl acetate, α-terpineol, neryl acetate, geranyl acetate, nerol, geraniol and (E)-nerolidol.

One microliter of diluted EO (1 µL into 100 µL of ethanol) was injected using a Tri Plus RSH autosampler in a Trace 1310 gas chromatograph equipped with an Agilent HP-Innowax column (60 m * 0.250 mm * 0.50 µm internal diameter film thickness) used for chromatographic separation. The injection port was held at 250°C, fitted with a split liner containing glass wool and operated in split mode (120:1 for linalool and linalyl acetate assessment, 10:1 for the other compounds). The oven temperature program started at 60°C for 2 min, increased to 100°C at a rate of 2°C/min, increased to 150°C at a rate of 5°C/min, increased to 200°C at a rate of 2°C/min, and increased to 260°C at a rate of 1°C/min (which was for 5 min). Components were separated at a flow rate of 1.2 mL/min and divided in 2 ways using a Sil Flow connector.

The combustion of carbon in carbon dioxide was achieved using an alumina tube containing nickel oxide maintained at 1000°C in a furnace. Pyrolysis of the sample for ^{2}H measurement in H_2 was carried out at a high temperature (1400°C) using an alumina conversion tube (Thermo

212 Fischer Scientific). Undecane, $C_{11}H_{24}$ (Sigma Aldrich), was regularly added to regenerate the
213 alumina pyrolysis tube.

214 **2.4. Statistical analysis**

215 Multivariate statistical analyses were performed using R version 4.0.3 (R Core Team, Vienna,
216 Austria) [31] with corrplot [32] , ropls [33], Factoextra [34], and FactoMiner [35] packages. The
217 Pearson correlation test was carried out to determine pairwise correlations between isotope
218 ratio values. Univariate analysis was realized to determine significant differences between
219 species, within a confidence level p-value < 0.05.

220 For multivariate data analyses, data were first analysed with unsupervised method such as
221 principal component analysis (PCA) [36] and clustering. PCA was used in this study to describe
222 and to assess the quality and homogeneity of the 90 samples of interest (81 neroli EO and 9
223 petitgrain EO). An autoscaling was realized prior to PCA and Ward's minimum variance
224 clustering method was applied to perform the hierarchical clustering [34].

225 Supervised methods were then carried out, including orthogonal partial least square–
226 discriminant analysis (OPLS-DA), to link models and variables responsible for the separation
227 observed on the unsupervised method [37].

228 A 7-fold stratified cross validation was repeated 50 times to avoid overfitting the small number
229 of data-points. Features were ranked according to the variable importance projection (VIP)
230 from OPLS-DA. S-plot verifies the selectivity and the specificity of the selected features. The
231 model's quality was evaluated based on the residuals (R^2X, R^2Y) and the models predictive
232 ability parameter (Q^2_{cum}) [38]. The statistical significances of models were also assessed using
233 confusion matrix.

3. Results and Discussion

3.1. Enantiomeric Ratio Fingerprint

3.1.1. Neroli Essential Oils

Enantiomeric ratio determination was conducted in this study to minor chiral compounds in neroli EOs to obtain a complete enantiomeric signature of the studied EOs to improve authentication. This study was achieved using a MDGC approach. This technique was previously applied to authenticate other EOs, such as rosemary EO [39]. Thirteen chiral components were identified in the EO: α-thujene, α-pinene, camphene, β-pinene, δ-3-carene, sabinene, limonene, β-phellandrene, linalool, linalyl acetate, terpinen-4-ol, α-terpineol, and (E)-nerolidol.

The results are depicted in **Table 1**. The neroli EOs from Morocco, Tunisia and Lebanon showed the same chiral profile with the dominance of levorotary (-) form: α-thujene, α-pinene, camphene, sabinene, β-pinene, δ-3-carene, β-phellandrene, linalool, linalyl acetate, and terpinen-4-ol. Limonene, α-terpineol, and (E)-nerolidol were predominantly dextrorotary (+). The α-pinene, β-pinene, limonene, linalool, linalyl acetate, terpinene-4-ol, α-terpineol and (E)-nerolidol chiral results were in good agreement with those of previous enantioselective studies of neroli EOs [8,14,19].

Several samples from Egypt featured different chiral signatures: racemization of α-pinene and β-phellandrene was observed, and reversions of sabinene and terpinen-4-ol enantiomer amounts were also detected. These results were in good agreement with those found in the literature [20], except for a contradiction with the α-terpineol enantiomeric ratio previously indicated in the literature, as we reported the R enantiomer as the major enantiomer. These results suggest differences in varieties between neroli EO samples from Egypt and other

geographical origins. It is also hypothesized that Egyptian samples were produced from flowers of a citrus hybrid *aurantium x sinensis*, based on our harvested area data. Sweet orange *Citrus sinensis* cultivars are the most widely grown among *Citrus* species in Egypt (accounting for 69% of the total cultivated area) [40] and bitter orange *Citrus aurantium* tree is the main C*itrus* rootstock used in Egypt.

Several enantiomeric discrepancies were detected for minor compounds (α-pinene, sabinene, terpinen-4-ol, and β-phellandrene), which were all present at less than 2% of the total composition of neroli EOs. Differences between neroli EOs of Egyptian and other origins would have been undetected if only chiral analyses of major compounds were conducted. This highlights the interest in carrying out a complete enantiomeric profiling approach to authenticate EOs. These results are compelling, as neroli EOs from Egypt also present perceptible olfactive profiles. Indeed, EO is more fresh, lemony, and less methylated than Morocco origin and more green and less honeyed than Tunisian.

3.1.2. Petitgrain Essential Oils

Petitgrain EO is a common adulterant in neroli EO. This adulteration can be unintentional if producers are not meticulous enough during harvest, as they must harvest only flowers of *C. aurantium* and not the leaves. It can be tricky to detect such adulterations, as major compounds, including linalool and linalyl acetate, are similar between the two types of hydrodistilled *C. aurantium* EOs. Enantiomeric fingerprints of chiral metabolites can also highlight EO blending. Several studies have established chiral databases on petitgrain, sweet, and bitter orange EOs [8,19,41]. By comparison with chiral data for sweet orange and bitter orange EOs, adulteration of neroli EO can be detected. However, literature databases suggest that the petitgrain chiral profile is similar to that of neroli.

The petitgrain EO chiral signature is detailed in Table 1. Enantiomeric ratios were determined in 9 samples from Madagascar and Paraguay. α-pinene, β-pinene, limonene, linalool, linalyl acetate, terpinen-4-ol, and α-terpineol chirality were consistent with previous investigations [8,19,42].

No significant differences were observed between petitgrain and neroli profiling, except for limonene and nerolidol (+) forms, which were less predominant in the petitgrain profile than in the neroli profile. Enantiomeric profiles of petitgrain and neroli EOs are not sufficiently distinct to highlight an addition of petitgrain EO in neroli EO. A complementary analysis is required to detect such adulteration.

3.2. $\delta^{13}C$ and $\delta^{2}H$ Isotopic Composition

Multiple isotope (^{13}C and ^{2}H) analysis of multiple components results in a large amount of information about the samples: compound-specific isotope analysis with GC-C/P-IRMS enables the assessment of the complete isotope fingerprint of neroli EO samples via determination of the $\delta^{13}C$ and $\delta^{2}H$ ratios of major metabolites.

Carbon isotope ratios are dependent on the metabolic pathways for carbon fixation in photosynthesis. *C. aurantium* is a C3 plant (Calvin cycle [43]). Its $\delta^{13}C$ is between -20 and -33 ‰ [44]. By comparison, a C4 plant (Hatch and Slack pathway [45]), such as lemongrass [13], has a $\delta^{13}C$ value slightly more positive, between -9 and -16 ‰ [46]. The $\delta^{13}C$ stable isotopic ratios of several metabolites of the samples are detailed in **Table 2**. These values are in line with the C3 plant range stated in the literature. The results were also consistent with those of previous $\delta^{13}C$ analyses conducted with neroli EOs [20]. Furthermore, the results were not significantly different among geographical origins. It was expected that all the samples were neroli EOs and came from the same *C. aurantium* natural plant variety.

Hydrogen isotopic ratios are predominantly influenced by the geographical origin of a plant due to the hydrological cycle [47], resulting in δ^2H being dependent on the geographical area. Neroli EO δ^2H values are detailed in **Table 3**. Several differences were observed among the different geographical origins. It is important to note that the samples from Egypt were different than other samples, with mean δ^2H values higher than those of samples with other origins. According to Hör et al. (2001) [25], neroli EOs have δ^2H values (measured in 4 samples) ranging between -234‰ and -253‰ for linalool and -270‰ and -272‰ for linalyl acetate. In this study, δ^2H values of the 86 samples were higher than the previously reported values, with mean values between -200‰ (Egypt) and -214‰ (Tunisia) for linalool and between -218‰ (Egypt) and -240‰ (Tunisia) for linalyl acetate.

In conclusion, compound-specific stable isotopic data are relevant because they provide a stable isotopic fingerprint that provides geographical information, as the samples from Egypt were clearly different than those from Morocco, Lebanon, and Tunisia.

3.3. Chemometric Analyses

3.3.1. Geographical Origin Authentication

Principal component analysis (PCA) was carried out on the entire sample data set (83 samples of neroli), with 33 parameters as variables (enantiomeric ratios and $\delta^{13}C$ and δ^2H isotopic ratios) to obtain a visual representation of the collected data (**Figure 2a**). Two different clusters could be observed: one comprising neroli EOs from Morocco, Lebanon, and Tunisia, and another including neroli EOs from Egypt. Most Spanish samples were in the first cluster, but two commercial samples were found in the second cluster. The Moroccan and Tunisian samples were also slightly separated from each other.

Hierarchical clustering was then realized to distinguish the clusters with more accuracy. This clustering technique calculates the difference in distance between each sample. The cluster plot (**Figure 2b**) helps to distinguish three different clusters: one comprising samples from Spain, Morocco, Tunisia, and Lebanon (Cluster 1) and two clusters including samples from Egypt (Clusters 2 and 3). Both Egyptian clusters corresponded to different providers. Geographical location differences might explain these differences: Cluster 2 includes only samples from the specific Egyptian region of Gharbia Governorate, whereas Cluster 3 regroups other samples from Egypt.

As described in **Supplementary Table S1**, neroli essential oils were extracted from two different several processes: steam distillation and hydrodistillation. Based on the clustering, the extraction process does not affect the enantiomeric and isotopic compositions, as no differences were observed between steam distilled and hydrodistilled samples. The vegetative stage at time of harvest, the type of resources (wild or cultivated flowers), and the year of production do not impact chirality and isotopy of neroli EO metabolites too.

This study highlights the differences in quality between Egyptian and other neroli EOs. Authentication criteria need to be different among the two groups of EO samples to avoid misinterpreting analytical results from Egypt samples.

3.3.2. Purity Authentication

An additional PCA process was employed, this time including petitgrain samples. **Figure 3a** depicts the score plot of the principal components F1 and F2, which accounted for 49% of the total variance in the data. Differences between neroli and petitgrain EOs were successfully visualized.

Orthogonal partial least squares – discriminant analysis (OPLS-DA) was conducted to identify discriminant variables. This model was built from all samples using all parameters. The projection of the individual samples is represented in **Figure 3b**: neroli and petitgrain are significantly separated on the OPLS-DA score plot. The S-Plot visualization represented in **Figure 3c** highlights discriminant variables. The S-plot represents the variable confidence (correlation) against the variable contribution (covariance). Variables with the highest contributions and reliability are δ^2H stable isotope values, including nerol, geranyl acetate, geraniol, and α-terpineol. The geographical origins of petitgrain samples (Madagascar, Paraguay) probably affecting this discrimination, but this large isotopic fractionation is also explained by the differences between leaves and flowers. (E)-Nerolidol and limonene enantiomeric distributions also present high contributions. The $\delta^{13}C$ parameter did not seem to contribute to clustering based on this S-Plot representation.

Finally, petitgrain was added to one Moroccan neroli EO sample. Three levels of adulteration were applied (10%, 20%, and 50% adulteration). PCA (**Figure 4**) was then performed to observe the effects of this addition on the data. Since neroli EOs presented geographical variations, as seen earlier, only Moroccan samples were represented. As observed in the figure, adulterated samples were no longer in the neroli cluster. Even 10% adulteration can be detected with the determination of enantiomeric and stable isotope ratio fingerprints of the major metabolites of neroli EO.

This study shows the importance of determining the most complete enantiomeric and isotopic signature to investigate adulterations, especially if the matrices are similar to those of neroli and petitgrain EOs.

Conclusion

Measurements of enantiomeric and isotopic ratios of major metabolites of neroli essential oils were performed using MDGC and GC-C/P-IRMS. The data banks were treated with chemometric models, leading to geographical and authentication of varieties.

Geographical discrimination was achieved using PCA and hierarchical clustering models.

We demonstrated that an adulteration of 10% of petitgrain could be highlighted in neroli essential oil.

The work carried out in this study highlighted the interest to use simultaneously enantiomeric and isotopic compound-specific analyses to authenticate geographical and botanical origins of an essential oil sample. This type of multianalytical approach can be reproduced to other type of complex essential oils in the future to improve authentication and detect fraudulent products.

Acknowledgments

This work was conducted in partial fulfilment of the PhD requirements for Aurélien Cuchet who received a grant from the Association Nationale de la Recherche et de la Technologie (ANRT, Paris, France). The research was supported by a contract between Albert Vieille SAS and ISA (Institut des Sciences Analytiques, Villeurbanne, France).

References

[1] L. Peyron, Peyron, L., 2002. Medicinal and Aromatic Plants, Industrial Profiles. 26, p.148, in: 2002.

[2] R.G. Berger, ed., Flavours and fragrances: chemistry, bioprocessing and sustainability, Springer, Berlin ; New York, 2007.

[3] L. Duval, Les huiles essentielles à l'officine, UFR de Medecine et de Pharmacie de Rouen, 2012. https://dumas.ccsd.cnrs.fr/dumas-00713158.

[4] H. Değirmenci, H. Erkurt, Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of Citrus aurantium L. flowers, J. Infect. Public Health. 13 (2020) 58–67. https://doi.org/10.1016/j.jiph.2019.06.017.

[5] N. Dosoky, W. Setzer, Biological Activities and Safety of Citrus spp. Essential Oils, Int. J. Mol. Sci. 19 (2018) 1966. https://doi.org/10.3390/ijms19071966.

[6] T. Azanchi, H. Shafaroodi, J. Asgarpanah, Anticonvulsant activity of Citrus aurantium blossom essential oil (neroli): involvment of the GABAergic system, Nat. Prod. Commun. 9 (2014) 1615–1618.

[7] P. Khodabakhsh, H. Shafaroodi, J. Asgarpanah, Analgesic and anti-inflammatory activities of Citrus aurantium L. blossoms essential oil (neroli): involvement of the nitric oxide/cyclic-guanosine monophosphate pathway, J. Nat. Med. 69 (2015) 324–331. https://doi.org/10.1007/s11418-015-0896-6.

[8] G. Dugo, L. Mondello, eds., Citrus oils: composition, advanced analytical techniques, contaminants, and biological activity, CRC Press, Boca Raton, FL, 2011.

[9] W.A. König, D.H. Hochmuth, Enantioselective Gas Chromatography in Flavor and Fragrance Analysis: Strategies for the Identification of Known and Unknown Plant Volatiles, J. Chromatogr. Sci. 42 (2004) 423–439. https://doi.org/10.1093/chromsci/42.8.423.

[10] W. Mbogning Feudjio, H. Ghalila, M. Nsangou, Y. Majdi, Y. Mbesse Kongbonga, N. Jaïdane, Fluorescence Spectroscopy Combined with Chemometrics for the Investigation of the Adulteration of Essential Oils, Food Anal. Methods. 10 (2017) 2539–2548. https://doi.org/10.1007/s12161-017-0823-4.

[11] R. Singhal, P. Kulkarni, D. Rege, Quality indices for spice essential oils, in: Handb. Herbs Spices, CRC Press, Boca Raton, 2001: pp. 22–23.

[12] A. Cuchet, P. Jame, A. Anchisi, F. Schiets, C. Oberlin, J.-C. Lefèvre, E. Carénini, H. Casabianca, Authentication of the naturalness of wintergreen (Gaultheria genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment, Ind. Crops Prod. 142 (2019) 10. https://doi.org/10.1016/j.indcrop.2019.111873.

[13] T.-T. Nhu-Trang, H. Casabianca, M.-F. Grenier-Loustalot, Authenticity control of essential oils containing citronellal and citral by chiral and stable-isotope gas-chromatographic analysis, Anal. Bioanal. Chem. 386 (2006) 2141–2152. https://doi.org/10.1007/s00216-006-0842-2.

[14] H. Casabianca, J.B. Graff, V. Faugier, F. Fleig, C. Grenier, Enantiomeric Distribution Studies of Linalool and Linalyl Acetate. A Powerful Tool for Authenticity Control of Essential Oils, J. High Resolut. Chromatogr. 21 (1998) 107–112. https://doi.org/10.1002/(SICI)1521-4168(19980201)21:2<107::AID-JHRC107>3.0.CO;2-A.

[15] H. Gershon, A. Lykkeberg, F. Goren, S. Mason, Identifying Fraudulent Natural Products: A Perspective on the Application of Carbon-14 Analysis, J. Agric. Food Chem. 67 (2019) 13393–13399. https://doi.org/10.1021/acs.jafc.9b01821.

[16] A. Cuchet, A. Anchisi, P. Telouk, Y. Yao, F. Schiets, F. Fourel, Y. Clément, P. Lantéri, E. Carénini, P. Jame, H. Casabianca, Multi-element (13C, 2H and 34S) bulk and compound-specific stable isotope analysis for authentication of Allium species essential oils, Food Control. 126 (2021) 108086. https://doi.org/10.1016/j.foodcont.2021.108086.

[17] F. Pellati, G. Orlandini, K.A. van Leeuwen, G. Anesin, D. Bertelli, M. Paolini, S. Benvenuti, F. Camin, Gas chromatography combined with mass spectrometry, flame ionization detection and elemental analyzer/isotope ratio mass spectrometry for characterizing and detecting the authenticity of commercial essential oils of *Rosa damascena* Mill.: GC/MS, GC/FID and GC/C/IRMS analysis of *Rosa damascena* essential oil, Rapid Commun. Mass Spectrom. 27 (2013) 591–602. https://doi.org/10.1002/rcm.6489.

[18] L. Schipilliti, P. Dugo, I. Bonaccorsi, L. Mondello, Authenticity control on lemon essential oils employing Gas Chromatography–Combustion-Isotope Ratio Mass Spectrometry (GC–C-IRMS), Food Chem. 131 (2012) 1523–1530. https://doi.org/10.1016/j.foodchem.2011.09.119.

[19] A. Mosandl, Enantioselective capillary gas chromatography and stable isotope ratio mass spectrometry in the authenticity control of flavors and essential oils, Food Rev. Int. 11 (1995) 597–664. https://doi.org/10.1080/87559129509541063.

[20] I. Bonaccorsi, D. Sciarrone, L. Schipilliti, A. Trozzi, H.A. Fakhry, G. Dugo, Composition of Egyptian nerolì oil, Nat. Prod. Commun. 6 (2011) 1009–1014.

[21] H. Dong, K. Xiao, Y. Xian, Y. Wu, Authenticity determination of honeys with non-extractable proteins by means of elemental analyzer (EA) and liquid chromatography (LC) coupled to isotope ratio mass spectroscopy (IRMS), Food Chem. 240 (2018) 717–724. https://doi.org/10.1016/j.foodchem.2017.08.008.

[22] H. Dong, D. Luo, Y. Xian, H. Luo, X. Guo, C. Li, M. Zhao, Adulteration Identification of Commercial Honey with the C-4 Sugar Content of Negative Values by an Elemental Analyzer and Liquid Chromatography Coupled to Isotope Ratio Mass Spectroscopy, J. Agric. Food Chem. 64 (2016) 3258–3265. https://doi.org/10.1021/acs.jafc.6b00691.

[23] H. Wu, L. Tian, B. Chen, B. Jin, B. Tian, L. Xie, K.M. Rogers, G. Lin, Verification of imported red wine origin into China using multi isotope and elemental analyses, Food Chem. 301 (2019) 125137. https://doi.org/10.1016/j.foodchem.2019.125137.

[24] T.-T. Nhu-Trang, H. Casabianca, M.-F. Grenier-Loustalot, Deuterium/hydrogen ratio analysis of thymol, carvacrol, γ-terpinene and p-cymene in thyme, savory and oregano essential oils by gas chromatography–pyrolysis–isotope ratio mass spectrometry, J. Chromatogr. A. 1132 (2006) 219–227. https://doi.org/10.1016/j.chroma.2006.07.088.

[25] K. Hör, C. Ruff, B. Weckerle, T. König, P. Schreier, Flavor Authenticity Studies by $^{2}H/^{1}H$ Ratio Determination Using On-line Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry, J. Agric. Food Chem. 49 (2001) 21–25. https://doi.org/10.1021/jf000829x.

[26] A. Mosandl, D. Juchelka, Advances in the Authenticity Assessment of Citrus Oils, J. Essent. Oil Res. 9 (1997) 5–12. https://doi.org/10.1080/10412905.1997.9700706.

[27] O. Boussaada, R. Chemli, Chemical Composition of Essential Oils from Flowers, Leaves and Peel of Citrus aurantium L. var. amara from Tunisia, J. Essent. Oil Bear. Plants. 9 (2006) 133–139. https://doi.org/10.1080/0972060X.2006.10643484.

[28] D. De Rovira, Dictionary of Flavors, 3rd Edition, Wiley.Com. (2017). https://www.wiley.com/en-fr/Dictionary+of+Flavors%2C+3rd+Edition-p-9781118856413 (accessed January 10, 2019).

[29] A.M. Grumezescu, A.M. Holban, Soft Chemistry and Food Fermentation, in: 2017.

[30] W.A. Brand, T.B. Coplen, J. Vogl, M. Rosner, T. Prohaska, Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report), Pure Appl. Chem. 86 (2014) 425–467. https://doi.org/10.1515/pac-2013-1023.

[31] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2020. https://www.R-project.org/.

[32] T. Wei, V. Simko, R package "corrplot": Visualization of a Correlation Matrix, 2017. https://github.com/taiyun/corrplot.

[33] E.A. Thévenot, A. Roux, Y. Xu, E. Ezan, C. Junot, Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses, J. Proteome Res. 14 (2015) 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354.

[34] A. Kassambara, F. Mundt, factoextra: Extract and Visualize the Results of Multivariate Data Analyses, 2020. https://CRAN.R-project.org/package=factoextra.

[35] S. Lê, J. Josse, F. Husson, FactoMineR: An R Package for Multivariate Analysis, J. Stat. Softw. 25 (2008) 1–18. https://doi.org/10.18637/jss.v025.i01.

[36] I.T. Jolliffe, Principal Component Analysis - Second Edition, Springer Ser. Stat. (2002). https://doi.org/10.1007/b98835.

[37] M. Tenenhaus, La régression PLS: théorie et pratique, Editions TECHNIP, 1998.

[38] A. Levet, C. Bordes, Y. Clément, P. Mignon, H. Chermette, P. Marote, C. Cren-Olivé, P. Lantéri, Quantitative structure–activity relationship to predict acute fish toxicity of organic solvents, Chemosphere. 93 (2013) 1094–1103. https://doi.org/10.1016/j.chemosphere.2013.06.002.

[39] L. Mondello, A. Casilli, P.Q. Tranchida, M. Furukawa, K. Komori, K. Miseki, P. Dugo, G. Dugo, Fast enantiomeric analysis of a complex essential oil with an innovative multidimensional gas chromatographic system, J. Chromatogr. A. 1105 (2006) 11–16. https://doi.org/10.1016/j.chroma.2005.08.013.

[40] W.F. Abobatta, Citrus Varieties in Egypt: An Impression, Int. Res. J. Appl. Sci. (2019) 4.

[41] I. Bonaccorsi, D. Sciarrone, A. Cotroneo, L. Mondello, P. Dugo, G. Dugo, Enantiomeric distribution of key volatile components in Citrus essential oils, Rev. Bras. Farmacogn. 21 (2011) 841–849. https://doi.org/10.1590/S0102-695X2011005000123.

[42] D. Juchelka, A. Steil, K. Witt, A. Mosandl, Chiral Compounds of Essential Oils. XX. Chirality Evaluation and Authenticity Profiles of Neroli and Petitgrain Oils, J. Essent. Oil Res. 8 (1996) 487–497. https://doi.org/10.1080/10412905.1996.9700674.

[43] M. Calvin, J.A. Bassham, The Photosyntheis of Carbon Compounds, Benjamin, New York, 1962. https://doi.org/10.2172/910351.

[44] M.H. O'Leary, Carbon Isotopes in Photosynthesis, BioScience. 38 (1988) 328–336. https://doi.org/10.2307/1310735.

[45] M.D. Hatch, C.R. Slack, Photosynthetic CO2-Fixation Pathways, Annu. Rev. Plant Physiol. 21 (1970) 141–162. https://doi.org/10.1146/annurev.pp.21.060170.001041.

[46] K.A. van Leeuwen, P.D. Prenzler, D. Ryan, F. Camin, Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry for Traceability and Authenticity in Foods and Beverages: GC-C-IRMS for authentication in foods…, Compr. Rev. Food Sci. Food Saf. 13 (2014) 814–837. https://doi.org/10.1111/1541-4337.12096.

[47] J. Hoefs, Stable Isotope Geochemistry, 8th ed., Springer International Publishing, 2018. https://doi.org/10.1007/978-3-319-78527-1.

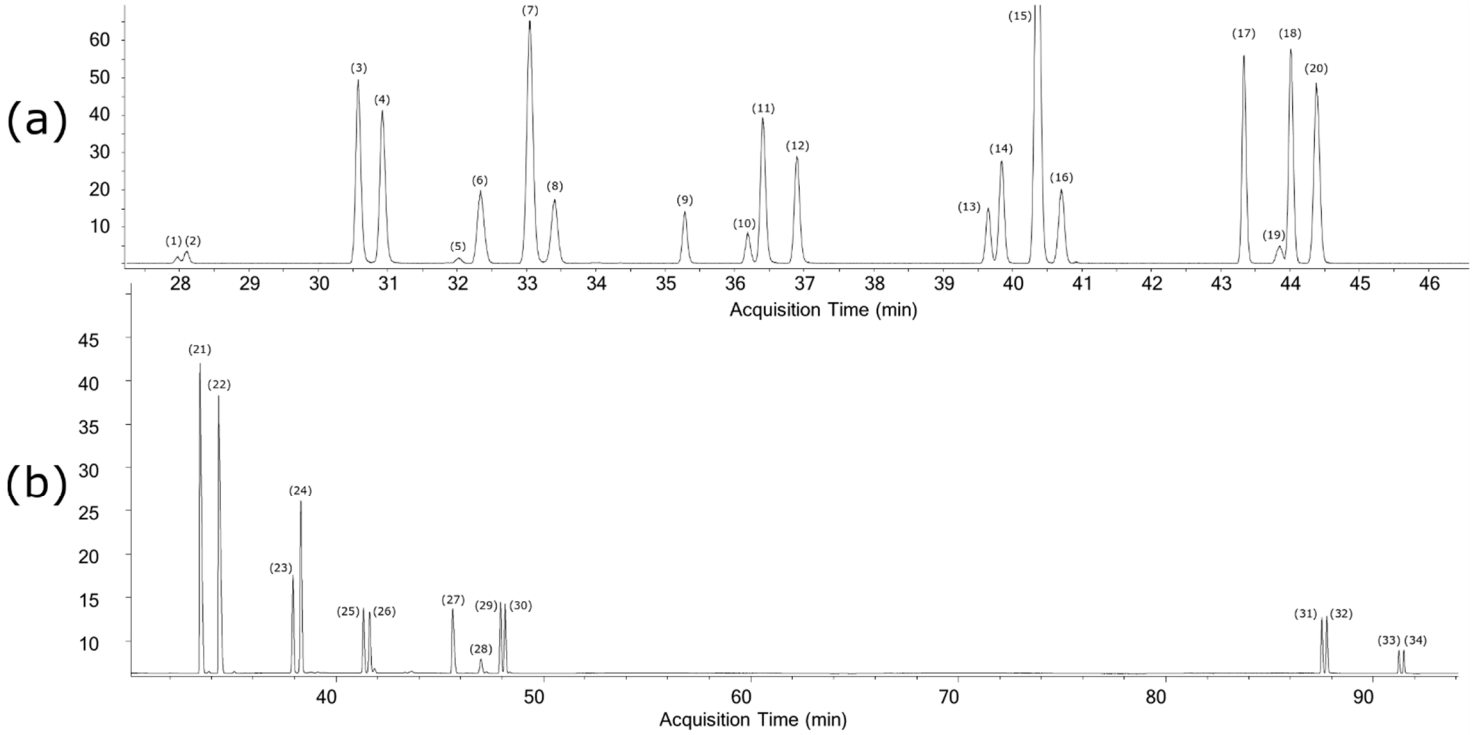


Figure 1. (a) Second dimension chromatographic profile obtained with MDGC (Columns HP-1 15m*0.200mm*0.5um. combined by Dean Switch system with a second column Cyclosil β 30m*0.250mm*0.25um). This method enables the separation of 10 chiral metabolites: α-thujene 1(+), 2(-); α-pinene 3(-), 4(+); α-fenchene 5(+), 6(-); camphene 7(-), 8(+); sabinene 9(+), 10(-); β-pinene 11(+), 12(-); α-phellandrene 13(-), 14(+); δ-3-carene 15(+), 16(-); limonene 17(-), 18(+); β-phellandrene 19(-), 20(+). **(b)** Second dimension chromatographic profile obtained with MDGC (HP-1 column 15m*0.200mm*0.5um, combined by Dean Switch system, with a second column DEtTBuSilBetaCDX PS086 25m*0.25mm*0.25µm). This method enables the separation of 7 chiral metabolites: linalool 21(+), 22(-); borneol 23(-), 24(+); terpinen-4-ol 25(+), 26(-); α-terpineol 27(-), 28(+); linalyl acetate 29(+), 30(-). (Z)-nerolidol 31(+), 32(-); (E)-nerolidol 33(-), 34(+).

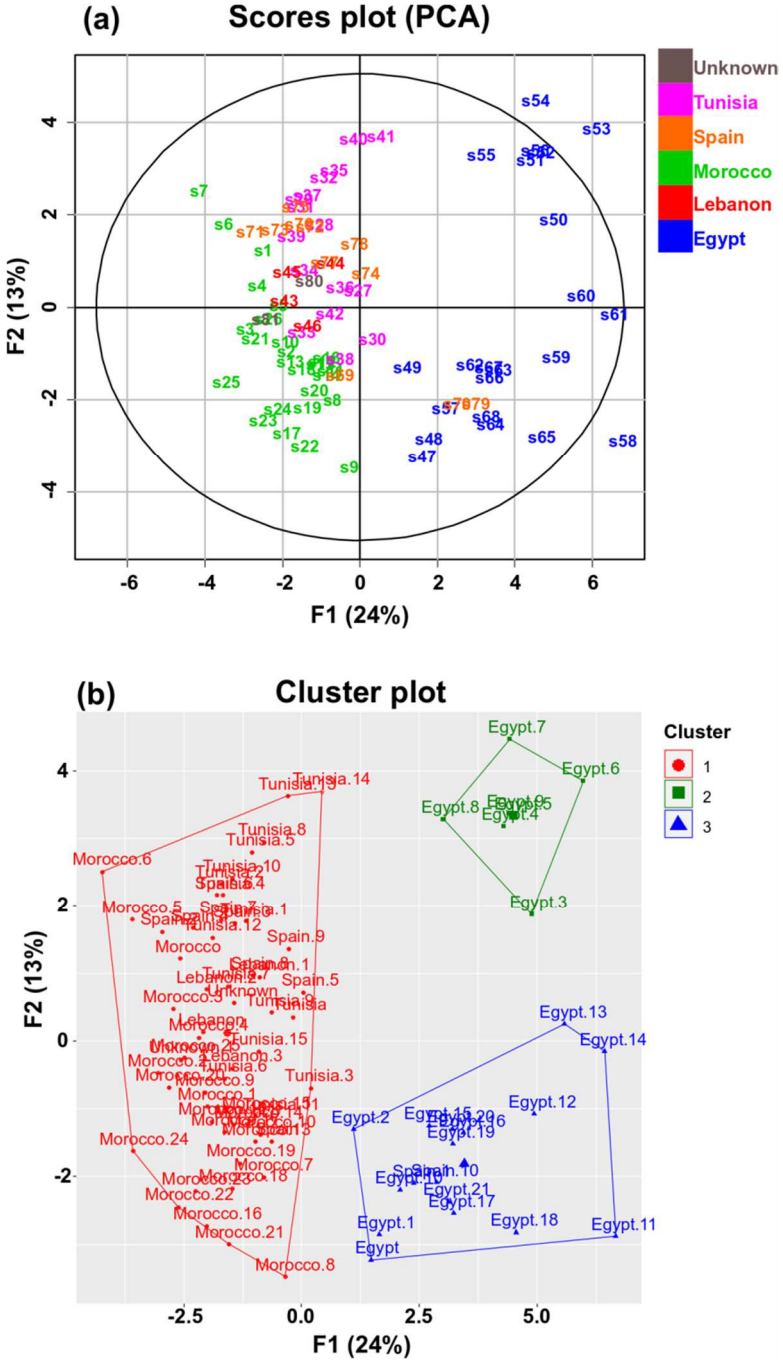
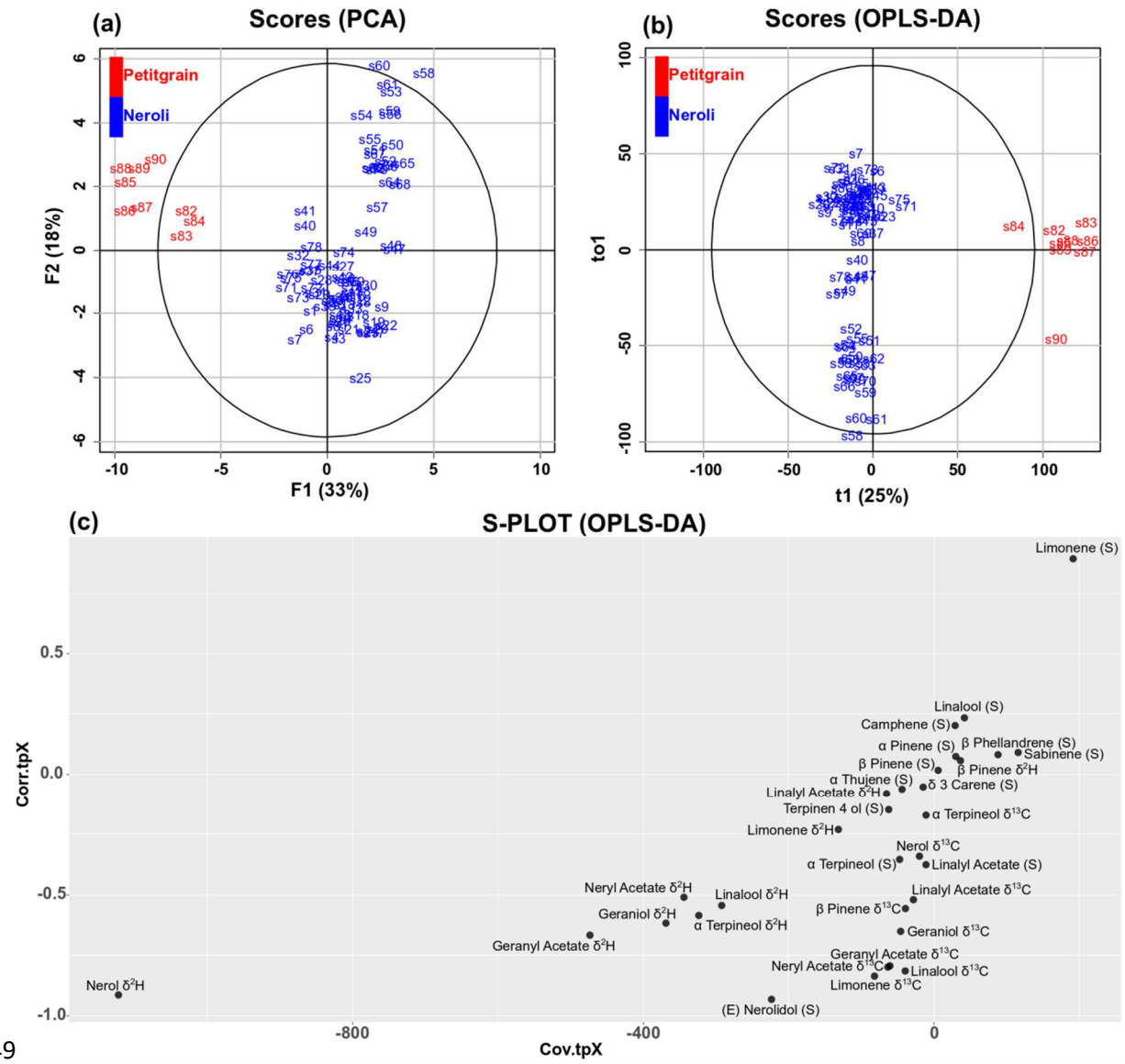


Figure 2. (a) Score plot of principal component F1 (24%) and F2 (13%) of Principal Component Analysis (PCA) model developed from enantiomeric ratio percentages and $\delta^{13}C$ and $\delta^{2}H$ compound specific isotope analysis (CSIA) values determined on neroli essential oils from different geographical origins: Morocco (in green), Egypt (in blue), Tunisia (in pink), Lebanon (in red), and Spain (in orange). Samples with unknown geographical origins are represented in grey. Clusters are represented via **(b)** hierarchical cluster plot**.** Cluster 1 (in red) includes

547 Moroccan, Lebanese, Tunisian, and most Spanish samples. Cluster 2 (in green) includes only

548 Egyptian samples. Cluster 3 (in blue) comprises some Egyptian and two Spanish samples.



549

550 **Figure 3. (a)** Score plot of principal component t1 (33%) and t2 (18%) of Principal Component
551 Analysis (PCA) model developed from enantiomeric ratio percentages and $\delta^{13}C$ and $\delta^{2}H$
552 compound specific isotope analysis (CSIA) values determined on neroli (in blue) and petitgrain
553 (in red) essential oils. **(b)** Orthogonal Partial Least Square – Discriminant Analysis (OPLS-DA)
554 score plot of enantiomeric ratio percentages and $\delta^{13}C$ and $\delta^{2}H$ CSIA values. **(c)** OPLS-DA
555 loadings S-Plot. This representation shows the correlation against the covariance.

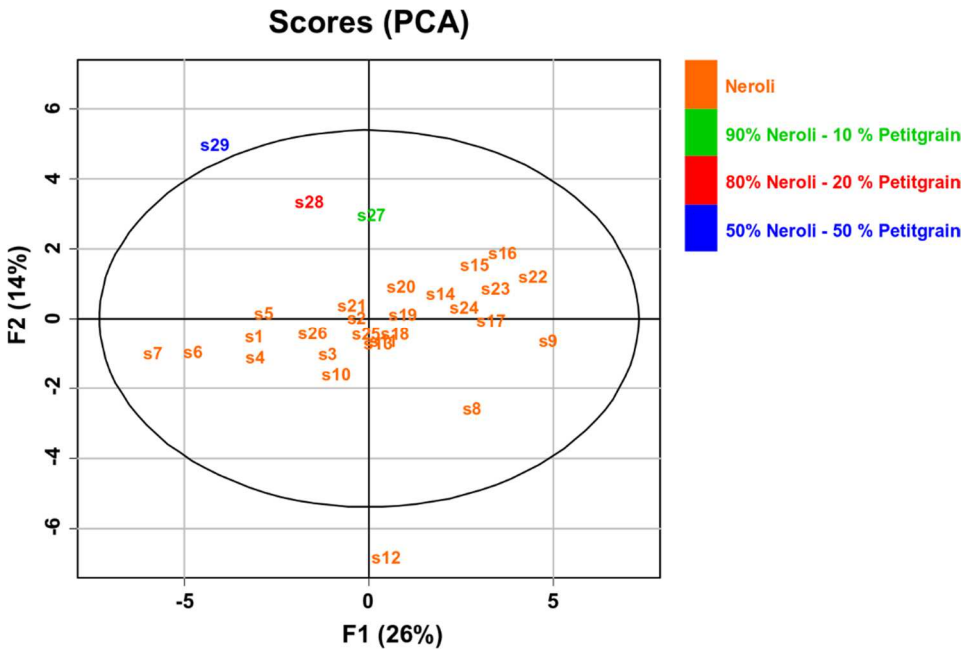


Figure 4. Principal Component Analysis representation of the addition of petitgrain in neroli essential oil. Three levels of adulteration were undertaken: 10% (green), 20% (red), and 50% (blue) additions of petitgrain in 100% pure and natural Moroccan neroli essential oils (Samples represented in orange).

Table 1: Enantiomeric distribution of chiral secondary metabolites of neroli and petitgrain essential oils from different geographical origins: Morocco, Tunisia, Lebanon, Egypt, Spain, Madagascar, and Paraguay.

Essential oil type	Geographical origin	Number of Samples	Enantiomeric distribution (%)																									
			α-Thujene S-(+)		α-Pinene S-(-)		Camphene 1S, 4R-(-)		Sabinene S-(-)		β-Pinene S-(-)		δ-3-Carene 1S-(+)		Limonene S-(-)		β-Phellandrene S-(+)		Linalool S-(+)		Terpinen-4-ol S-(+)		α-Terpineol S-(-)		Linalyl Acetate S-(+)		(E)-Nerolidol S	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Neroli	Morocco	26	0.0	0.0	87.9	2.5	98.6	1.0	93.3	6.9	99.2	1.9	0.6	2.9	1.9	1.0	25.8	10.8	26.7	1.4	35.7	1.8	30.8	1.3	1.9	0.9	98.6	0.4
Neroli	Tunisia	16	3.3	6.9	88.9	3.1	97.9	1.3	79.5	16.4	99.1	1.0	9.6	16.6	2.6	0.9	23.6	14.0	23.0	1.3	40.4	5.2	31.9	0.9	1.2	0.4	98.2	0.4
Neroli	Lebanon	4	0.0	0.0	90.8	2.6	100.0	0.0	95.4	0.8	99.8	0.1	11.3	22.7	1.7	0.3	27.3	15.2	20.7	1.2	36.6	1.4	30.0	0.4	1.4	0.4	98.8	0.2
Neroli	Egypt	22	23.4	26.7	65.9	9.6	94.3	6.1	24.9	20.8	94.6	17.3	0.3	1.1	2.5	0.6	53.1	18.1	18.6	4.0	59.8	6.2	32.5	6.2	0.7	0.1	97.5	1.5
Neroli	Spain	11	12.0	26.7	86.5	9.5	97.7	3.5	77.1	30.9	99.5	0.8	0.0	0.0	2.2	0.8	40.0	18.3	28.2	3.4	42.7	9.9	29.7	2.5	1.4	0.3	98.4	1.0
Petitgrain	Madagascar	3	0.0	0.0	92.7	3.0	100.0	0.0	92.0	0.6	98.2	0.9	0.0	0.0	9.2	2.8	18.3	16.7	24.4	4.3	30.9	7.8	27.6	1.2	0.4	0.3	81.0	5.5
Petitgrain	Paraguay	6	5.5	6.1	81.0	4.4	100.0	0.0	73.9	27.7	98.4	0.6	0.9	0.8	24.0	2.3	53.5	4.9	28.1	3.4	42.3	10.2	27.0	0.2	0.4	0.2	77.0	6.2

Table 2: Stable isotope $\delta^{13}C$ distribution of secondary metabolites of neroli and petitgrain essential oils from different geographical origins: Morocco, Tunisia, Lebanon, Egypt, Spain, Madagascar, and Paraguay.

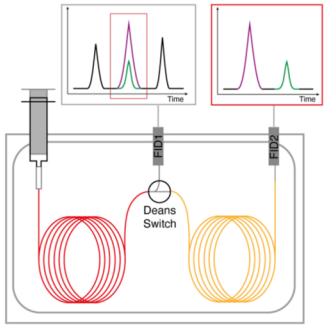
Essential oil type	Geographical origin	Number of Samples	Isotopic Distribution (‰)																	
			β-Pinene $\delta^{13}C$		Limonene $\delta^{13}C$		Linalool $\delta^{13}C$		Linalyl Acetate $\delta^{13}C$		α-Terpineol $\delta^{13}C$		Neryl Acetate $\delta^{13}C$		Gernayl Acetate $\delta^{13}C$		Nerol $\delta^{13}C$		Geraniol $\delta^{13}C$		(E) Nerolidol $\delta^{13}C$	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Neroli	Morocco	26	-26.32	0.97	-26.77	1.20	-25.47	0.95	-25.08	1.19	-26.64	1.90	-27.96	0.88	-26.77	0.98	-26.35	1.13	-26.58	0.78	-29.64	2.05
Neroli	Tunisia	16	-26.99	2.32	-27.33	1.02	-26.03	0.47	-26.15	1.23	-26.99	2.19	-29.30	2.01	-28.87	1.31	-27.39	1.36	-28.09	2.33	-30.65	2.45
Neroli	Lebanon	4	-26.99	1.08	-27.83	0.63	-26.37	0.65	-25.54	0.35	-28.10	1.69	-28.41	0.42	-27.38	0.60	-27.18	0.51	-27.19	0.75	-29.44	0.65
Neroli	Egypt	22	-26.71	1.57	-27.54	1.19	-25.96	0.88	-26.35	0.84	-27.88	1.54	-28.68	0.77	-27.67	0.89	-26.45	1.70	-27.02	1.00	-29.03	0.73
Neroli	Spain	11	-27.29	0.87	-28.31	0.94	-26.85	0.35	-27.54	1.24	-28.39	0.72	-28.99	0.72	-28.42	0.61	-27.17	0.80	-28.63	1.48	-27.98	1.17
Petitgrain	Madagascar	3	-28.01	1.31	-35.52	0.60	-29.73	0.21	-29.43	0.36	-28.31	0.08	-33.80	1.14	-33.38	0.82	-27.98	3.07	-30.77	0.47	N/A	N/A
Petitgrain	Paraguay	6	-31.51	2.92	-34.21	2.97	-29.48	0.23	-28.40	0.57	-28.21	1.68	-34.20	0.51	-32.89	0.85	-28.62	1.37	-31.99	1.16	N/A	N/A

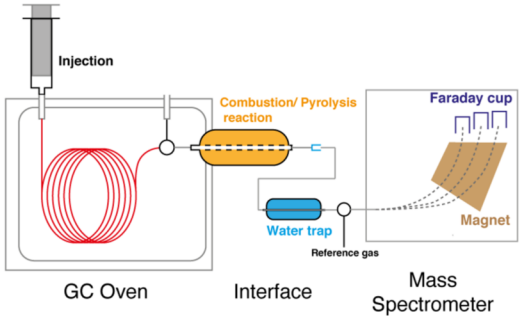
Table 3: Stable isotope δ^2H distribution of secondary metabolites of neroli and petitgrain essential oils from different geographical origins: Morocco, Tunisia, Lebanon, Egypt, Spain, Madagascar, and Paraguay.

Essential oil type	Geographical origin	Number of Samples	Isotopic Distribution (‰)																	
			β-Pinene δ^2H		Limonene δ^2H		Linalool δ^2H		Linalyl Acetate δ^2H		α-Terpineol δ^2H		Neryl Acetate δ^2H		Geranyl Acetate δ^2H		Nerol δ^2H		Geraniol δ^2H		(E) Nerolidol δ^2H	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Neroli	Morocco	26	-213	12	-221	12	-210	7	-223	24	-224	12	-210	9	-203	14	-196	11	-201	11	-102	8
Neroli	Tunisia	16	-214	12	-223	8	-210	6	-237	17	-228	15	-208	11	-204	10	-189	9	-204	15	-106	8
Neroli	Lebanon	4	-218	4	-229	5	-209	7	-227	3	-222	5	-202	8	-203	16	-198	7	-205	4	-103	4
Neroli	Egypt	22	-208	32	-216	17	-199	16	-217	15	-217	14	-187	16	-182	9	-177	15	-188	9	-84	11
Neroli	Spain	11	-211	6	-212	21	-210	16	-220	32	-223	7	-214	11	-205	12	-198	17	-194	10	-101	14
Petitgrain	Madagascar	3	-212	15	-217	3	-219	5	-212	6	-239	3	-221	3	-229	2	-271	14	-220	1	N/A	N/A
Petitgrain	Paraguay	6	-205	4	-239	10	-239	5	-240	8	-255	4	-240	5	-241	4	-291	12	-232	17	N/A	N/A

Neroli essential oil sample

Enantiomeric and Stable Isotope Ratio Fingerprints





Enantio-MDGC

GC-C/P-IRMS

Chemometric models

Purity authentication

Geographical differenciation