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Identification of metabolomic markers of lavender and lavandin essential oils using mid-infrared spectroscopy

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ABSTRACT

Lavender *(Lavandula angustifolia)* and lavandin (sterile hybrid of *L. angustifolia* P. Mill. × *Lavandula latifolia* (L.f.) Medikus) are widely cultivated in the Mediterranean area for produce essential oils. In this study, 80 lavandin and 55 lavender essential oil samples from various varieties were analyzed. Firstly, a chemometric treatment of mid-infrared spectra was used to evaluate the capacity of Partial Least Squares Discriminant Analysis (PLS-DA) regression to discriminate French lavandin and lavender essential oil (EO) samples and their varieties (Abrial, Fine, Grosso, Maillette, Matherone, Sumian and Super), and secondly, to quantify the main compounds such as linalyl acetate, linalool, eucalyptol and camphor by PLS regression using reference data from gas chromatography. The examination of PLS and PLS-DA regression coefficients allowed the identification of metabolomic markers. The lavender/lavandin EOs and their varieties were very well classified (100% for lavender/lavandin EOs and between 98 and 100% for varieties). The calibration models obtained by PLS regression for the determination of the main compound contents revealed good correlation ($\geq$0.86) between the predicted and reference values. This method can be used to control the authenticity and traceability of lavender/lavandin and their varieties. Finally, mid-infrared and Raman spectroscopy results were compared.

1. Introduction

Thirty nine lavandula species plus a number of hybrids and intraspecific taxa, mostly of Mediterranean origin, compose the lavandula family (Lamiaceae). Among them, *Lavandula angustifolia* (lavender) and its hybrid (lavandin) differ essentially in their chemical composition. Lavender/lavandin essential oils are obtained from lavender/lavandin by hydro-distillation. The quality of the oil depends on three main factors: the quality of the plant, the time of harvest, and the distillation process.

Lavender essential oils are used in perfumes and aromatheraphy. The essential oil produced from *L. angustifolia* is the most suitable for use in perfumes, due to it high linalool content [1,2]. Lavandin essential oils are used in soap, detergents and cosmetics, because of their high camphor content [2–8].

Lavandin is a hybrid between *L. angustifolia* P. Mill. and *Lavandula latifolia* (L.f.) Medikus. Among its varieties (Abrial, Grosso, Sumian and Super), Grosso is the most famous for its essential oil yield. Among the main lavender varieties (Fine, Maillette and Matherone), the Fine lavender is the most famous for the same reason, while the Matherone variety is currently less cultivated.

As has been said, Lavender species differ in their chemical composition, and logically so do their hybrids and the essential oils they produce when distilled. Lavender and lavandin oils contain more than one hundred compounds, including linalyl acetate, linalool, camphor, borneol, eucalyptol and β-caryophyllene, each contributing to the chemical and sensory properties of the oil. The major distinction between the two essential oils lies in their relative contents of linalyl acetate, linalool, eucalyptol and camphor.

The chemical composition can be determined using gas chromatography and gas chromatography-sniffing [9,10]. These methods are usually applied for the purpose of quality control and selection of high-quality plants, but they are very time-consuming, and attempts have been made to find alternative analytical methods.

In this context, vibrational spectroscopy methods (near-infrared, mid-infrared (MIR) and Raman spectroscopies) combined with chemometric treatments have been successfully introduced for a non-destructive determination of metabolites present in essential oils [11–22]. Momentarily, it is only by applying statistical methods that EOs near-infrared spectra can be interpreted,

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whereas the bands that are characteristic of the individual compounds can easily be seen in the MIR and Raman spectra, therefore making it possible to discriminate between essential oils. MIR spectroscopy is a tool for research and data analysis which is well-known and widely used. During the past ten years, MIR spectroscopy has increasingly been used in the food industry. Vegetable oils including olive oil constitute an important group of food products for which MIR spectroscopy has successfully been applied to characterize French olives, to authenticate vegetable oils and to distinguish the geographic origin of extra virgin olive oils [23–26]. Whatever its acquisition mode, attenuated total reflection (ATR) or transmission cell accessories for Fourier-transform infrared (FT-IR) spectroscopy can be used to quantify, authenticate, identify and classify fats, fatty oils or essential oils.

Currently in various studies, analytical data have been treated using chemometric methods such as Principal Component Analysis (PCA) [27,28], Soft Independent Modelling of Class Analogies (SIMCA) [13] and Partial Least Squares (PLS) Regression [29,30].

The first part of this paper considers the potential of MIR spectroscopy for discriminating between the two EOs and the seven varieties. Then in the second part, the combination of MIR spectroscopy and chemometric methods to quantify terpenoids in EOs is presented, along with the identification of the metabolomic markers of the varieties. In the last part, a comparison with previous results from Raman spectroscopy used to determine the original varieties of lavender and lavandin OEs [31] is given.

2. Materials and methods

2.1. Essential oil samples

In total, 135 samples were analyzed including 80 lavandin oil samples and 55 lavender oil samples from several varieties. The lavender and lavandin were *L. angustifolia* Miller and its hybrid, i.e. *L. angustifolia* Miller × *L. latifolia* Linnaeus fils Medikus, French type, which had been harvested in 2012, 2013 and 2014 in various French collection areas (unknown department (00), Alpes-de-Haute-Provence (04), Ardèche (07), Drôme (26) and Vaucluse (84)). Samples were divided into varieties: Fine (FI, $n = 19$), Maillette (MA, $n = 24$) and Matherone (MT, $n = 12$) varieties for lavender samples and Abrial (AB, $n = 15$), Grosso (GR, $n = 30$), Sumian (SU, $n = 16$) and Super (SP, $n = 19$) varieties for lavandin samples.

2.2. Pure standard substances

Pure standard substances – 3-octanone, lavandulyl acetate, linalyl acetate, linalool, borneol, camphor, β-caryophyllene and eucalyptol – were purchased from Sigma Aldrich (Steinheim, Germany), Adrian (Aix-les-Milles, France), Lavender France (Montguers, France), Fluka (Buchs, Switzerland), Alpha Aesar (Karlsruhe, Germany), Alpha Aesar (Karlsruhe, Germany), TCI Europe (Zwijndrecht, Belgium) and Merck (Schuchardt, Germany) respectively.

2.3. Gas chromatography (GC)

2.3.1. GC–MS analysis

GC–MS analyses were performed on a 7890A GC system coupled with a 5975C VL mass spectrometer detector (Agilent Technologies) equipped with a HP-5MS capillary column (J&W Scientific, 30 m × 0.25 mm, 0.25 μm film thickness). Data acquisition and processing were performed using the MSD Chemstation E.01.01.335 (Agilent) software. 1 μL of diluted essential oil (80 μL in 1.5 mL of ethanol) was injected. The experimental conditions developed in the laboratory were: solvent delay, 2 min; programmed column temperature: 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then 200 °C to 260 °C (20 °C/min), final temperature held for 5 min; injector (split ratio 60) and detector temperature: 250 °C; carrier gas: helium (flow rate 1.2 mL/min); ionisation voltage 70 eV; electron multiplier, 1 kV.

2.3.2. GC-FID analysis

GC analyses were performed on a 7890A GC (Agilent Technologies) system with a flame ionisation detector (FID) equipped with a HP5 capillary column (J&W Scientific, 30 m × 0.25 mm, 0.25 μm film thickness). Data acquisition and processing were performed using the Chemstation B.04.03-SP1 (87) (Agilent) software. The experimental conditions were the same as given for the GC–MS analyses. Hydrogen was the carrier gas at a flow rate of 1.2 mL/min. Linear retention indices were calculated with reference to *n*-alkanes (C8–C28).

2.4. Spectroscopy

2.4.1. MIR spectroscopy

The spectra of each lavender or lavandin oil sample were recorded within the 1800–650 cm^{-1} spectral range with 4 cm^{-1} resolution and 64 scans, on a Nicolet Avatar spectrometer equipped with a MCT/A detector, an Ever-Glo source, and a KBr/germanium beam splitter. The MIR spectrometer was situated in an air-conditioned room (21 °C). Samples were deposited without preparation on an Attenuated Total Reflection (ATR) accessory provided with a diamond crystal. Air was taken as reference for the background spectrum collected before each sample under the same conditions. After each spectrum, the ATR plate was cleaned with ethanol solution, allowing the ATR crystal to dry. Cleanliness was verified by collecting a background spectrum and comparing it with the previous background spectrum.

2.4.2. Raman spectroscopy

Spectra were collected with an Almega (Thermo-fisher Scientific Nicolet) Raman spectrometer equipped with a Nd: YVO_4 diode-pumped solid-state (DPSS) laser (532 nm). The minimum and maximum powers at the output of the laser head were 15 and 150 mW, respectively. All spectra were taken using the 180° backscattering geometry. The detector was a charge coupled device (CCD). Samples were placed in a quartz cell (2 mm) and the spectra were recorded with 2 cm^{-1} resolution and two accumulations of 15 s each at full laser power in the range 4000–90 cm^{-1} using the Omnic 7.2 software (Thermo-fisher Scientific Nicolet). The Raman spectrometer was situated in an air-conditioned room (21 °C).

During data preprocessing, the Extended Multiplicative Signal Correction (EMSC) was used to correct unwanted variation effects, such as diffusion effect. In this study samples are not filtered. Some of them contain airborne particles and are very slightly colored. EMSC contributes to making subsequent calibration model simpler and statiscally more robust. The number of factors used for modelling is reduced and RMSEP is slightly improved [32]. All EMSC-pretreated spectra, constitute the matrix used to perform PCA and most PLS regressions.

2.5. Chemometric analysis

PCA [33] is an unsupervised modelling method, also known as projection method, and it is often the first step in exploratory data analysis aiming to find patterns in the data. The procedure establishes a linear spectral model which allows original and correlated variables (absorbance) to be converted into uncorrelated variables called principal components or loading. These latent variables contain the main information and are calculated from