Carbon Isotope Ratio Analysis of Authentic and Commercial Essential Oils of Lavandin (Lavandin hybrida)

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Introduction

Although a variety of analytical methods for essential oil and flavor compound analysis exists, authenticity assessment of essential oils remains a challenging task. Synthetic analogues of many essential oil compounds are commercially available and in addition expensive oils are often blended with cheaper oils. With respect to these facts, specific methods for the determination of the origin of essential oils are becoming more and more interesting. One of the most promising tools for authenticity assessment is the determination of the stable isotopic composition performed by isotope ratio mass spectrometry (IRMS). Investigation of the stable isotopic composition of essential oil compounds is performed by a specialized IRMS, comprising an online combustion linked to a gas chromatograph (GC-C-IRMS). Lavandin (*Lavandin hybrida*) is a hybrid of two lavender sorts *Lavandula angustifolia* (lavender) and *Lavandula latifolia* (spike). Main compounds of the essential oil of lavandin are linalyl acetate, linalool, eucalyptol (1, 8-cineole) and camphor.

Methods

Several commercial and authentic essential oils of lavandin (Lavandin hybrida) were investigated for the carbon isotopic composition of their main (linalool and linalyl acetate) and minor compounds (eucalyptol and camphor) by GC-C-IRMS. Authentic samples of lavandin were distilled in 2007. Genuine dried plant material from two different companies (authentic sample 1 and 2) was distilled with a ten liters distillation plant of the type UMWEX 100–1000 with a maximum of 1 kilogram per batch. Distillation time was app. 80 minutes. In addition, commercially available samples of the individual main compounds of the oil with proclaimed synthetic and natural origins were investigated for their carbon isotopic composition by GC-C-IRMS and EA-C-IRMS (element analyzer coupled via combustion module to an isotopic ratio mass spectrometer) to compare the investigated carbon isotopic values. All oils were analyzed by GC-MS and GC-FID (with an HP 5 ms capillary column) to enable the identification of the compounds in the GC-C-IRMS chromatograms and to investigate the quantity of the single essential oil compounds. Data were interpreted regarding application for authenticity assessment of the analyzed essential oil samples.

The results are expressed in standard delta notation (δ) as per mil (‰) deviation from the V-PDB standard (Vienna-Peedee Belemnite) as:

$$\delta^{Y}X(‰) = \left(\frac{R_{sample}}{R_{std}} - 1\right) \times 1000$$

Formula 1: *Formula to express $\delta^{13}C_{PDB}$ notation*

^{Y}X is ^{13}C, and R_{sample} and R_{std} are $^{13}C/^{12}C$ ratios of sample and standard, respectively. Positive $\delta^{13}C$ values express an enrichment of the heavy isotope in the sample material with respect to the standard; negative $\delta^{13}C$ values mean a lighter composition of the sample compared to the standard. [1]

Results

Synthetic linalool standards showed $\delta^{13}C$ values of -31.13 and -28.24 ‰, whereas the natural labeled standard L-linalool showed a value of -24.85 ‰. For the authentic oils, $\delta^{13}C$ values of -25.63 and -25.53 ‰ for linalool were determined (n = 2). The commercial lavandin oil samples indicated $\delta^{13}C$ values between -27.77 and -24.76 ‰ for linalool (n = 17). The synthetic linalyl acetate standard showed $\delta^{13}C$ values of -35.62 and -35.71 ‰, the natural standard -29.41 ‰. Authentic samples of lavandin oils presented $\delta^{13}C$ values of -25.56 and -25.29 ‰ for linalyl acetate (n = 2), commercial oil samples values between -27.49 and -24.44 ‰ (n = 17). Synthetic standards of eucalyptol amounted to $\delta^{13}C$ values of -29.56 to -28.72 ‰ (no natural standard available), authentic lavandin oils to -27.13 and -26.38 ‰ (n = 2) and the commercial lavandin oils from -29.10 to -27.42 ‰ (n = 15). $\delta^{13}C$ values of -27.89 ‰ for the natural standard of camphor and -28.69 and -28.15 ‰ for the synthetic standards were measured. One authentic lavandin oil showed a value of -25.72 ‰ (n = 1), commercial oil samples -28.04 to -26.39 ‰ (n = 16).

Image 1: *Authentic plant material from lavandin used for distillation and essential oil sample (Authentic oil sample 2)*

Conclusion

$\delta^{13}C$ data for linalool and linalyl acetate of the natural standards and the authentic and commercial oils cover the same carbon isotopic ratio ranges. The synthetic standards are in both cases more negative. In addition it is known, that the (R)-Linalool content in natural lavandin samples is higher than 94 % and this applied for all commercial and authentic samples investigated by enantiomeric gas chromatography. In Figure 1 $\delta^{13}C$ values of linalool and linalyl acetate are compared, because in the biosynthetic pathway in the plant linalool is the precursor of linalyl acetate. Because of that the isotopic ratios of natural linalool and linalyl acetate are close. For the commercial samples the highest difference was identified with 1 ‰, which is negligible. All values determined for linalool were more negative than the values for linalyl acetate.[2]

Regarding all these facts, it can be concluded, that all commercial samples of the essential oil of lavandin investigated, are of natural origin.

For the two minor compounds, eucalyptol and camphor, no clear differentiation could be made due to the lack of standards and the minor concentration (max. 15 %) of the compounds.

The determination of the carbon isotopic composition by GC-C-IRMS is obviously a very powerful tool in authenticity assessment of essential oils, especially concerning the differentiation between C_3 and C_4 plants. Lavandin is a C_3 plant – all values are below -24.00 ‰.

Image 2: *GC-C-IRMS-System (Jozef Stefan Institute, Department of Environmental Sciences, Ljubljana)*

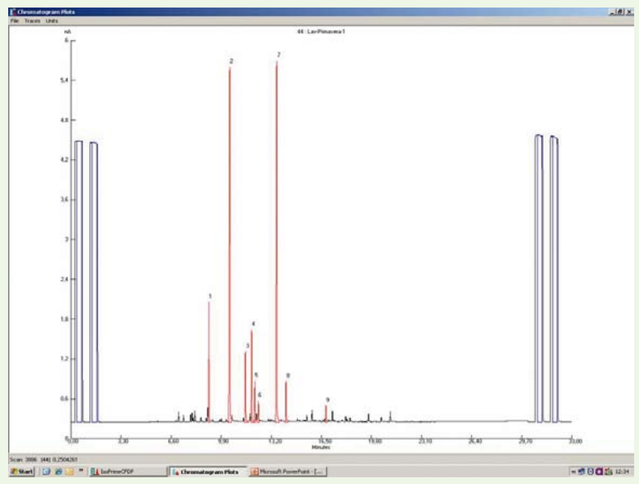

Image 3: *GC-C-IRMS chromatogram of one essential oil sample of lavandin with the main compounds*
1 … eucalyptol
2 … linalool
3 … camphor
4 … borneol
5 … 4-terpineol
6 … α-terpineol
7 … linalyl acetate
8 … β-myrcene
9 … β-caryophyllene

Table 1: *Carbon isotopic composition of main compounds of authentic and commercial essential oils of lavandin*
*(**n.i.** … Not identified, because of absence or low concentration (peak height lower than 1 nA) of the compound, each sample was measured in triplicates)*

No.	Origin	Eucalyptol	Linalool	Camphor	Borneol	Linalyl acetate
		δ¹³C ratio (GC-C-IRMS) ± SD in ‰ (n = 3)				
1	Authentic oils 2007	-26.38 ± 0.10	-25.53 ± 0.03	-25.72 ± 0.06	n.i.	-25.29 ± 0.05
2		-27.13 ± 0.08	-25.63 ± 0.06	n.i.	n.i.	-25.56 ± 0.10
3	Commercial oils	-28.80 ± 0.03	-27.66 ± 0.11	-27.80 ± 0.37	-26.88 ± 0.12	-26.92 ± 0.16
4		-28.49 ± 0.08	-27.68 ± 0.05	-27.94 ± 0.11	n.i.	-27.33 ± 0.04
5		-27.42 ± 0.10	-27.11 ± 0.06	-26.98 ± 0.04	n.i.	-26.61 ± 0.04
6		-27.84 ± 0.15	-26.78 ± 0.17	-27.06 ± 0.23	n.i.	-26.73 ± 0.13
7		-28.23 ± 0.08	-26.25 ± 0.12	-26.81 ± 0.14	n.i.	-25.87 ± 0.07
8		-28.40 ± 0.10	-26.26 ± 0.06	-26.89 ± 0.15	n.i.	-25.82 ± 0.10
9		-27.72 ± 0.09	-25.93 ± 0.09	-26.64 ± 0.09	n.i.	-25.30 ± 0.13
10		-28.51 ± 0.12	-27.76 ± 0.08	-27.79 ± 0.06	n.i.	-27.38 ± 0.09
11		n.i.	-24.76 ± 0.14	n.i.	-23.47 ± 0.21	-24.44 ± 0.13
12		-27.86 ± 0.05	-26.21 ± 0.07	-26.84 ± 0.17	n.i.	-25.24 ± 0.10
13		-27.58 ± 0.07	-25.83 ± 0.17	-26.60 ± 0.30	n.i.	-25.28 ± 0.04
14		-28.77 ± 0.16	-27.77 ± 0.04	-28.04 ± 0.25	n.i.	-27.49 ± 0.09
15		n.i.	-26.20 ± 0.14	-26.39 ± 0.29	n.i.	-25.95 ± 0.27
16		-28.62 ± 0.26	-27.43 ± 0.17	-27.45 ± 0.38	n.i.	-26.42 ± 0.10
17		-28.44 ± 0.24	-27.71 ± 0.32	-27.66 ± 0.37	n.i.	-26.93 ± 0.35
18		-28.40 ± 0.18	-27.36 ± 0.04	-27.60 ± 0.10	n.i.	-26.99 ± 0.10
19		-29.10 ± 0.21	-26.75 ± 0.06	-27.14 ± 0.10	n.i.	-26.36 ± 0.11

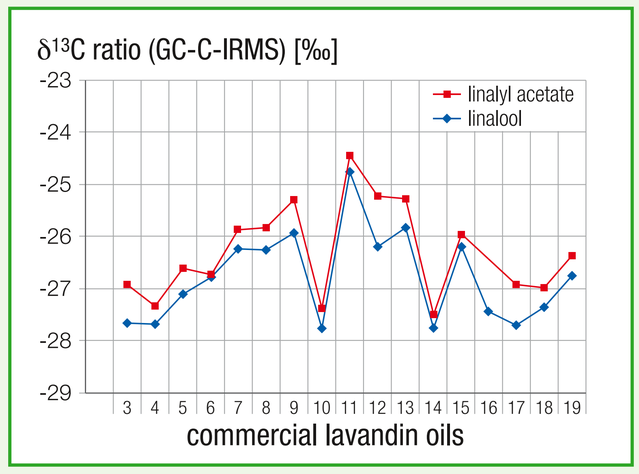

Figure 1: *Comparison of $\delta^{13}C$ values of linalool and linalyl acetate of the commercial lavandin oil samples*

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