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Where has all the aroma gone? Identification of aroma compounds in fresh and dried leaves of *Melissa officinalis*

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Abstract

Melissa officinalis is a popular aromatic plant, renowned for its lemon-like smell and pharmaceutical effects. Drying of *Melissa officinalis* is important for long-term storage and commercial purposes but can impact the sensory quality of the final product. Here, we aimed to characterize drying-related changes in aroma quality. In a first step, aroma profiles of fresh and dried leaves of *Melissa officinalis* were established by a trained sensory panel. In a second step, one- and two-dimensional gas chromatography-mass spectrometry/olfactometry (GC-MS/O) was performed to identify odor-active substances from fresh and dried leaves. A hay-like, straw-like and algae-like/fishy off-flavor was evident in dried leaves. GC-MS/O analyses led to the identification of known odor-active substances from *Melissa officinalis* like neral, geranial, geraniol in both fresh and dried leaves and additionally demonstrated the generation of further odor-active compounds due to drying, amongst others (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal. Based on these results, further experiments can be designed to further investigate drying-related aroma changes, for instance with regard to quantitative determination of the aroma compounds in the leaves of *Melissa officinalis*.

Keywords: Lemon balm; Hay-like; Straw-like; Fishy; Off-flavor; Gas chromatography-olfactometry.

1. Introduction

Melissa officinalis – commonly known as lemon balm - is a perennial herb belonging to the family *Lamiaceae* and is widely cultivated for culinary, cosmetic and medicinal purposes. The lemon-scented leaves are used in salads and dishes, cold and hot drinks, both for their aroma and various health-promoting effects attributed to terpenes and terpenoids but also further constituents like rosmarinic acid, caffeic acid and other polyphenols (Petrisor et al., 2022). As with other natural products, the composition of lemon balm essential oil (EO) varies with subspecies and conditions of cultivation (e.g., geographical location, soil, climate), harvesting (e.g., developmental stage, environmental factors) and post-harvest processing (Patora et al., 2003; Ayanoglu et al., 2005; Basta et al., 2005). Correspondingly, the percentage distribution of EO constituents varies. Nonetheless, the mono- and sesquiterpenes and -terpenoids neral, geranial, citronellal, caryophyllene oxide, and β-caryophyllene – which are well-known for their citrus-like smells – can be considered major constituents of lemon balm EO (Petrisor et al., 2022).

Drying of herbs and medicinal plants is a common post-harvest preservation, impeding microorganism growth and reducing transport costs by reducing water content of the plant material. Thereby, the type of drying has a major influence on the quality of the final product, notably on its appearance (e.g., color, form and size) and composition (Díaz-Maroto et al., 2002; Rababah et al., 2015; Chua et al., 2019; Thamkaew et al., 2021). Volatile constituents can be reduced through drying; in addition, new compounds can be formed as a result of oxidation processes, amongst others. Several years ago, Argyropoulos and colleagues investigated the influence of different drying conditions on the color, EO content and composition, and rosmarinic acid equivalents of lemon balm to derive optimum drying conditions, considering the quality of the product but also practical aspects such as the drying time (Argyropoulos and Müller, 2014a; b; c). Considering convective drying, temperature had a clear influence on EO loss, with a loss of 16 % at 30 °C and a loss of 73% at 75 °C (Argyropoulos and Müller, 2014a). To evaluate the impact of drying conditions on EO composition, the authors applied gas chromatography coupled to a flame ionization detector. Using drying temperatures between 30 °C and 60 °C in a convection-type laboratory dryer, a decrease in the relative amounts of citronellal, neral, and geranial, and an increase in those of citronellol and caryophyllen oxide became evident at 60 °C (Argyropoulos and Müller, 2014a). Comparing convective drying, vacuum drying, and freeze drying, the same authors found, amongst others, decreased relative amounts of neral and geranial in the vacuum drying condition(Argyropoulos and Müller, 2014b). The authors furthermore reported an earthy, hay-like flavour in vacuum dried leaves possibly due to the reduction of geranial. This off-flavor is also readily perceivable from commercially available dried Melissa products. However, to the best of our knowledge, the odor-active compounds leading to this odor impression in dried lemon balm have not yet been elucidated. Therefore, we aimed to identify odor-active compounds in fresh and dried leaves of *Melissa officinalis* to achieve first insights into the molecular basis of drying-induced off-flavor in lemon balm. To this aim, gas chromatography-olfactometry/mass spectrometry was applied.

2. Materials and methods

2.1. Chemicals

Dichloromethane (DCM) was obtained from Th. Geyer (Renningen, Germany). DCM was freshly distilled prior to use. The reference compounds (purity) were: (*E*)-4,5-epoxy-(*E*)-2-decenal (97%), (*Z*)-1,5-octadien-3-one (99%), 2-acetyl-1-pyrroline (95%) from AromaLab, Freising, Germany; (*Z*)-3-hexenal (50%) from Merck KGaA, Darmstadt, Germany; (*Z*)-4-heptenal (98%), 1-octen-3-one (50%), 2-phenylethanol (99%), 2-ethyl-3,5-dimethylpyrazine (95%), 3-(methylthio-)propanal, citronellal (95%), geranial (96%), geraniol (96%), neral (96%), octanal, phenyl acetaldehyde (90%), *trans*-β-ocimene (90%), β-ionone (96%), 1-octen-3-ol (98%), butanoic acid (99.5%), *cis*-linalool oxide (97%), linalool (97%), β-pinene (99%), nonanoic acid (97%), and alkanes from *n*-hexane to *n*-tetratriacontane from Sigma-Aldrich, Steinheim, Germany; vanillin (99%) from ABCR, Karlsruhe, Germany.

2.2. Plant material

Melissa officinalis L. (Erfurter Aufrechte cultivar) was provided by Salus Haus Dr. med. Otto Greither Nachf. GmbH & Co. KG, Bruckmühl, Germany. The plant was harvested in the middle of August and shipped to Fraunhofer IVV within two hours in a cooled transport. The leaves were removed from the stem and the stems were discarded. The moisture content of the leaves was measured using a moisture balance (Sartorius moisture balance MA40.Sartorius AG, Göttingen). The fresh leaves had a moisture content of 79.25%. The leaves were then placed in an in-house temperature cabinet (constant climate chamber, Binder GmbH, Germany) for 20 hours at 40 °C, and at a relative humidity of 20%, as suggested by Argyropoulos et al. (2011). The leaves were periodically checked for their moisture content and dried until they reached a moisture content of less than 11%. The final moisture content of the leaves when collected from the cabinet was 10.76%. The fresh and the dried leaves were vacuum packed (Vacuum chamber machine, Multivac, Germany) and stored at –80 °C until further analysis.

2.3. Sensory evaluation

The sensory evaluation was conducted in two sessions by trained panelists (age range: 20–45 years) from the Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany. The panelists were trained in assessing odorants with regard to their odor qualities according to an in-house flavour language by weekly training sessions. To establish the aroma profiles of fresh and dried lemon balm leaves, the following attributes (references) were determined by the panel in the first session: grassy, (*cis*-3-hexenal), lemon-like (citral), soapy (octanal), fatty/cardboard-like ((*E*)-2-nonenal), algae-like/fishy ((*Z*)-4-heptenal), hay-like (hay), straw-like (straw). In the second session, eleven panelists rated the intensity of these attributes on a scale from 0 (no perception) to 10 (very intense). In addition, total intensity was rated on the same scale and hedonic perception was evaluated as well (0: unpleasant, 5: neutral, 10: very pleasant). The ratings between fresh and dried leaves were compared via a Wilcoxon test.

2.4. Isolation of odor-active substances

Lemon balm leaves (3 g) were extracted for 30 min with 100 ml DCM. After filtering, solvent assisted flavor evaporation (SAFE) was performed at 50 °C. The distillate was thawed and concentrated to a final volume of 100 µl via Vigreux and micro-distillation at 50 °C.

2.5. Detection of odor-active substances via gas chromatography-flame ionization detection/olfactometry (GC-FID/O)

A Trace GC Ultra instrument (Thermo-Fisher Scientific, Dreieich, Germany) equipped with an uncoated pre-column followed by a DB-FFAP capillary column (30 m x 0.32 mm, 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) was used in cold-on-column injection mode (40 °C) with an injection volume of 2 µl. After 2 min, oven temperature was increased to 240 °C at a rate of 10 °C/min and held for 5 min. Carrier gas was helium (2.2 ml/min). At the end of the capillary column, the eluent was split 1:1 volume ratio to the FID (250 °C) and the odor detection port (ODP; 230 °C). The undiluted concentrated distillates were analysed by two trained panelists. In addition, one panelist did an aroma extract dilution analysis by stepwise (1:3) diluting the distillates and performing GC-O. Dilutions were continued until FD 2187. This way, a flavor dilution factor (FD factor) was determined for each odor-active substance, namely the highest dilution in which the respective odorant was still perceived. Retention indices were determined via a series of homologous *n*-alkanes. A capillary DB-5 column was used for complementary analyses.

2.6. Identification of odor-active substances via GC-MS/O and 2D-GC-MS/O

To identify the odor-actives substances, a Trace GC Ultra coupled to a DSQII (both Thermo-Fisher Scientific, Dreieich, Germany) equipped with a DB-FFAP capillary column (see above) and a Gerstel MPS 2 autosampler was used (Gerstel GmbH, Mülheim an der Ruhr, Germany). The eluent was split towards the mass spectrometer and an ODP. Mass spectrum recording and data analysis was performed with XCalibur Data System (version 1.4, Thermo-Fisher Scientific, Dreieich, Germany). Electron ionization (EI) mass spectra were generated in full scan mode (m/z range 35–249) at an ionization energy of 70 eV. Injection and temperature program was as with GC-FID/O with the exception of the heating rate, which was 8 °C/min.

For trace compounds and co-eluting compounds that could not be identified using GC-MS/O, a two-dimensional GC-MS/O was used. The first GC was a 7890A system (Agilent Technologies, Santa Clara, CA, USA) equipped with an MPS2 autosampler (see above) and a DB-FFAP column (30 m x 0.32 mm, film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). It was coupled via a CTS1 cryo-trap system (Gerstel, Mülheim an der Ruhr, Germany) to a second GC of the same type equipped with a DB-5 column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA) and coupled to an Agilent mass spectrometer 220 Ion Trap. Mass spectra were acquired in EI mode with an ionization energy of 70 eV and the scanned mass range was 40–400 m/z. The temperature programs were, for the first oven: 40 °C for 2 min, temperature raise at 10 °C/min until 240 °C, hold time 5 min; for the second oven: 40 °C, temperature raise at 8 °C/min until 250 °C, hold time 1 min. Helium (8.9 ml/min) was used as carrier gas and the ODPs were set at 290 °C. Injection volume was 2 µl using the on-column technique and a MPS autosampler (see above).

Identification of odor-active substances was achieved by comparison of odor quality, retention indices on two columns of different polarity, and mass spectra with those of reference compounds. If no reference compounds were available, comparison was done with NIST Mass Spectral Library (version 2.0, National Institute of Standards and Technology, USA) and literature data.

3. Results and discussion

3.1. Sensory evaluation

The panel rated the overall smell intensity of fresh leaves with an average of 7.9 whereas dried leaves were slightly reduced in intensity with an average of 6.3. Fresh leaves were evaluated to smell more pleasant than dried leaves (8.3 vs 5.3).The aroma profiles of the fresh and dried leaves are shown in Figure 1. The most intense attributes in the fresh lemon balm leaves were lemon-like (average rating of 8.7) and soapy (5.3). In contrast, the dried leaves' most intense odor attribute was hay-like (6.7). Wilcoxon tests showed that the ratings differed between fresh and dried leaves for the attributes lemon-like, which was rated more intense in fresh leaves ($Z = 2.9$, $p > 0.05$), and for the attributes hay-like and straw-like, which were rated more intense in dried leaves ($Z = 2.8$, $p < 0.05$). In addition, the attribute algae-like/fishy tended to be rated as more intense in dried compared to fresh leaves ($Z = 2.7$, $p < 0.1$). The results support that a hay-like off-flavor developed with the here applied drying procedure.

3.2. Aroma extract dilution analysis and identification of odor-active substances

The distillates obtained from fresh and dried lemon balm leaves were analysed via GC-O, GC-MS/O, and 2D-GC-MS/O. The results are summarized in Table 1.

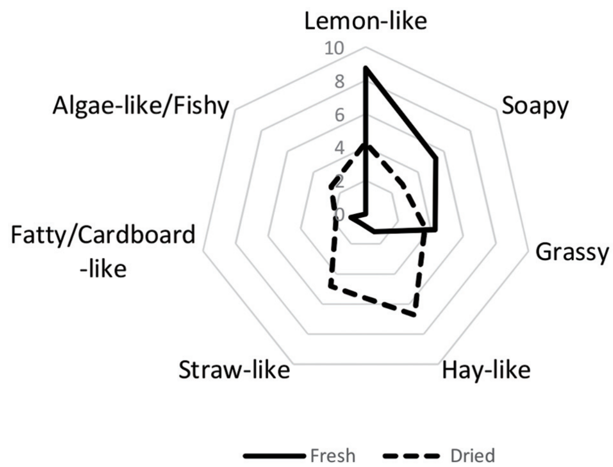

Figure 1. Aroma profiles of fresh and dried lemon balm leaves, determined by a trained panel (n = 11).

Overall, 32 odor impressions were perceived for the fresh leaves, and 35 odor impressions for the dried leaves. The compounds with the highest FD factors were, for both samples, the citrus-like smelling compounds citronellal, neral, geranial, geranyl acetate, geraniol, and an unknown compound with RIs 2440 (FFAP) and 1550 (DB-5). Since the AEDA was performed until FD 2187 only, no conclusions can be drawn on potential influence of drying on the FD factors of these substances. The grassy smelling compound (*Z*)-3-hexenal was further detected with the highest FD factor in the distillate obtained from fresh samples. Presence of this aldehyde might be due to freezing and thawing of the plant material, leading to cell wall rupture and corresponding enzymatic generation of oxidation products.

Differences in the FD factors between fresh and dried samples became evident for several substances. Evaluating those substances with differences of two or more dilution steps, it appeared that β-pinene, (*E*)-2-hexenal, and *cis*-linalool oxide were detected in both sample types yet with higher FD factors in the dried leaves' distillate, whereas the opposite pattern was evident for linalool, phenylacetaldehyde, and nonanoic acid. In addition, some substances were detected either only in distillates obtained from fresh leaves ((*Z*)-3-hexenal, vanillin) or only in distillates obtained from dried leaves ((*Z*)-4-heptenal, unknown compound #10, (*E*,*Z*)-2,6-nonadienal, butanoic acid, and β-ionone). Considering the aroma profiles and comparing them with the results of AEDA, it can be hypothesized that the algae-like/fishy off-flavor in the dried leaves resulted from reduced amounts of (*Z*)-3-hexenal, and/or increased amounts of (*Z*)-4-heptenal and (*E*,*Z*)-2,6-nonadienal. Especially the latter compounds have already previously been related to fishy off-flavours (Venkateshwarlu et al., 2004; Spitzer et al., 2010). It is also noteworthy that (*Z*)-1,5-octadien-3-one and methional were described to yield a fishy off-flavor in a concentration ratio of about 1:100 in dried spinach (Masanetz et al., 1998). Both compounds were identified in the distillates investigated here, and drying led to increased FD factors of methional, which may have led to a shift in the aroma quality of the mixture. Regarding the hay-like and straw-like odor attributes, previous research identified 3-methyl-2,4-nonanedione as a main contributor to those off-flavours (Masanetz and Grosch, 1998; Masanetz et al., 1998; Liang et al., 2022). This compound, however, was not detected

Table 1. Aroma compounds, their retention indices (RI), flavor dilution (FD) factors, and odor qualities as detected in distillates of fresh and dried leaves of *Melissa officinalis*

No.	Substance	Odor quality	RI		FD factor		Criteria for (tentative) identification
			FFAP	DB-5	fresh	dried	
1	Unknown	Glue-like, green	1018		9	27	
2	Unknown	Green	1043		9	9	
3	β-Pinene	Eucalyptus-like	1115	993	9	81	O, RI, MS, RC
4	*(Z)*-3-Hexenal	Grassy, green	1141	795	2187		O, RI, MS, RC
5	(*E*)-2-Hexenal	Fruity, apple-like, banana-like	1215	852	3	27	RI_{LIT}, MS_{NIST}
6	*trans*-β-Ocimene	Celery-like	1231	1039	81	81	O, RI, MS, RC
7	(*Z*)-4-Heptenal	Fishy, fatty	1234			9	O, RI, RC
8	1-Octen-3-one	Mushroom-like	1292	987	81	81	RI_{LIT}, MS_{NIST}
9	*cis*-β-Ocimene	Celery-like	1247	1050	81	81	RI_{LIT}, MS_{NIST}
10	Unknown	Mint-like	1252			27	
11	Octanal	Citrus-like, soapy	1282	1006	81	81	O, RI, MS, RC
12	2-Acetyl-1-pyrroline	Popcorn-like, roasty	1326	922	81	81	O, RI, RC
13	(*Z*)-1,5-Octadien-3-one	Geranium leaf-like, green, metallic	1362	982	81	81	RI_{LIT}
14	(*Z*)-3-Hexenol	Grassy, green	1382		3	9	RI_{LIT}, MS_{NIST}
15	3-Isopropyl-2-methoxypyrazine	Pea-like	1416	1097	81	81	RI_{LIT}
16	*cis*-Linalool oxide	Eucalyptus-like	1426	1099	81	729	O, RI, MS, RC
17	1-Octen-3-ol	Mushroom-like	1434	988	81	81	O, RI, MS, RC
18	3-Methylthio propanal	Cooked potato-like	1439	905	27	81	O, RI, RC
19	Citronellal	Citrus-like	1464	1143	2187	2187	O, RI, MS, RC
20	Unknown	Metallic	1525		3	3	
21	Linalool	Flowery	1539	1100	729	81	O, RI, MS, RC
22	(*E*,*Z*)-2,6-Nonadienal	Cucumber-like	1569	1145		243	O, RI, MS, RC
23	Phenylacetaldehyde	Beeswax-like, rapeseed-like, flowery	1638	1044	81	9	O, RI, MS, RC
24	Butanoic acid	Cheesy, sweaty	1624			9	O, RI, RC
25	Neral	Citrus-like	1666	1241	2187	2187	O, RI, MS, RC
26	Geranial	Citrus-like	1715	1274	2187	2187	O, RI, MS, RC
27	Geranyl acetate	Lemon-like, soapy	1752	1384	2187	2187	RI_{LIT}, MS_{NIST}
28	Geraniol	Flowery	1835	1250	2187	2187	O, RI, MS, RC
29	2-Phenylethanol	Rose-like, flowery	1891	1115	9	9	O, RI, MS, RC
30	β-Ionone	Violet-like, flowery	1930	1484		9	O, RI, MS, RC
31	*trans*-(4,5)-Epoxy-(*E*)-2-decenal	Metallic	2006	1382	9	9	O, RI, RC
32	Unknown	Leather-like	2064	1421	27	27	
33	(*E*,*Z*,*Z*)-2,4,7-Tridecatrienal	Blood-like	2097	1575	27	81	O, RI, RC
34	Nonanoic acid	Soapy, fatty, musty	2163	1259	27	3	RI_{LIT}, MS_{NIST}
35	Unknown	Leather-like	2167	1094	27	27	
36	Unknown	Soapy, citrus-like	2440	1550	2187	2187	
37	Vanillin	Vanilla-like	2563	1408	243		O, RI, MS, RC

O: odor quality, MS: mass spectrum, RC: comparison of respective data with reference compounds, RI_{LIT}: comparison of RI with literature data, MS_{NIST}: comparison of MS with NIST Library here. One possible explanation is that AEDA was performed on a DB-FFAP, and high amounts of geranial were eluting at RI 1715, which might have prevented detection of this compound. Another possible explanation is that the hay-like, straw-like odor impression results from a mixture of different compounds, analogously to the fishy off-flavor. Further experiments are needed to confirm or reject these possible explanations.

4. Conclusion

Sensory evaluation of dried lemon balm leaves confirmed presence of a hay-like, straw-like and fishy off-odor. Analysis of distillates obtained from fresh and dried lemon balm leaves via gas chromatography-olfactometry/mass spectrometry allowed identifying the most important odorants in the samples. Future qualitative and quantitative experiments together with sensory evaluation of model mixtures are needed to further resolve the aroma composition of fresh and dried lemon balm leaves. Ways towards off-flavour-free lemon balm products should be conceived to provide consumers with the full joy of experiencing aroma of fresh *Melissa officinalis*.

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