EFFECT OF FREEZE DRYING ON THE CHEMICAL COMPOSITION OF LEMON BALM LEAVES
(Melissa officinalis, L.)

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REZUMAT. Cercetarea se concentrează pe analiza componenței chimice a uleiului esențial de lămâie. Uleiul esențial din frunze a fost obţinut prin extracţie. Componentele chimice ale uleiului esenţial de lămâie au fost analizate cu un GC capilar şi GC/MS, 8 substanţe fiind identificate. Calitatea produsului final depinde, în mare măsură, de operaţia de uscare prin îngheţare (FD). Conţinutul de ulei esenţial a fost influenţat mai mult de presiunea camerei (înaltă şi joasă). Camera de înaltă presiune are tendinţa de a prelungi timpul de uscare a compuşilor de lămâie aromatici volatili. Determinări comparative ale uleiului esenţial pentru un material proaspăt şi unul uscat, la presiune mare, au arătat un conţinut uşor mai mare de ulei în materialul proaspăt. Efectul liofilizării pe structura glandulară a firelor a fost observat prin electro-microscopie.

Cuvinte cheie: Melissa officinalis L, liofilizare, ulei esenţial, compoziţie chimică, microscop.

ABSTRACT. This research focuses on the analysis of the chemical component of essential oil of lemon balm. The essential oil of leaves was obtained by extraction. The chemical components of the essential oil of lemon balm were analyzed by capillary GC and GC/MS and 8 substances were identified. The quality of the final product depends heavily on the operation of freeze drying (FD). It was found that essential oil content was more influenced by chamber pressure (high and low). Higher chamber pressure tended to lengthen the drying time but major volatile compounds of lemon balm. Comparative determinations of the essential oil in fresh and dried material at high pressure showed slightly higher content of the oil in the fresh one. The effect of freeze drying method on peltate glandular hairs structure was observed by electro-microscopy.

Keywords: Melissa officinalis L., freeze drying, essential oil, chemical composition, micrograph

1. INTRODUCTION

Lemon balm (*Melissa officinalis*, L.) is known as an officinal herb of a long tradition and a large variety of uses. Lemon balm, member of Lamiaceae is a perennial bushy plant growing up to 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped. This species originates from southern Europe, Asia and southern parts of North America [11].

More than forty compounds were recognized in melissa oil, only some of them, however, occur in the significant quantities. These are monoterpene aldehydes: citral (geranial + neral) and citronellal as well sesquiterpenes: β-caryophyllene and β-caryophyllene oxide; monoterpene alcohols: nerol, geraniol and citronellol [8].

Lemon balm is used for several purposes such as an additive in food, a herb tea, an ingredient in cosmetics, an ornamental and a medicine. The essential oil is currently used in medicine and pharmacology (anti-tumor, anti-bacterial, antimicrobial, antihistaminic, antispasmodic, antioxidant, antiulcerogenic, moderate Alzheimer's disease, against anti HIV-1) [10,1]. In addition, lemon balm has traditionally been used due to its memory enhancing properties, but using of which is currently more widely used as sedative or calm, spasmolytic and antibacterial agent and sleep aid has been more popular recently [6]. Because the essential oil rate of lemon balm is quite low, the production cost and price of the oil are very high [9].

Herbal products are traded as fresh or dry products. Drying of medicinal herbs should take place as soon as possible after harvesting, otherwise insects and fungi, which thrive in most conditions, render them unusable. Conventional drying methods are not suitable, bring undesirable changes in medicinal plants. Moreover, they may not be reliable and environmentally safe [5]. Therefore, the adequate drying technique is the most important operation in post harvest processing to avoid quality

losses. The freeze drying (FD) process is known for its ability to sustain food quality during low-temperature drying due to the minimum loss of flavour and aroma, negligible shrinkage, loss of valuable components, etc.

In the present study we have evaluated the qualitative and quantitative composition of volatile oil of the lemon balm. The effect of the pressure (high and low) of the freeze-drying procedure was also investigated. No report has been found detailing the effect of freeze drying technique on essential oil content of Melissa leaves.

2. MATERIAL AND METHODS

2.1. Raw material

The lemon balm was cultivated locally in Nyíregyháza and the plants were harvested just before flowering in June 2011. Fresh leaves were separated from the stem and only leaves were used for the drying and extraction of the oil. Essential oil was separately extracted from fresh leaves, freeze dried leaves at high pressure and freeze dried leaves at low pressure with three replications. The oil was identified by the staff at the Agrarian and Molecular Research Institute College of Nyíregyháza. The total amount of essential oil recovery was calculated in mg/100g based on the dry matter. The moisture content of fresh lemon balm was 5,09 kg water/kg dry matter (83,58% moisture content wet basis)

2.2. Moisture content determination

To determine the moisture content of *Melissa officinalis* before and after freeze drying, the oven method (LP-306, LABOR-MIM, Hungary) was used. In this method the sample is placed inside and oven at 105°C for 24 h and the loss of mass is registered in order to determine the moisture content of the lemon balm. The test was carried out for triplicate.

2.3. Preparation and analysis of essential oil

Determinations of the volatile oil contents were done by chemical extraction. About 50 g of fresh and ~9,5 g of dried plant leaves were subjected separately to extraction. The extraction procedure consisted of adding chloroform/hexane solvents (1:1, 600 ml) to the lemon balm samples, then mixing, blending and the ultrasonic homogenization of the sample (1 h, 40°C), followed by filtration and the release of solvent by rotating vacuum evaporation. The solvent was diluted with chloroform/hexane (1:1, 5 ml) to allow for the release of chlorophyll with Al_2O_3. The remaining steps involved spraying the mixture with nitrogen gas, diluting the solvent with hexane (1 ml). A total of 1 µl of the extract was injected into the GC.

The oil was analyzed by Gas Chromatography (GC) and Gas Chromatography-mass Spectrometry (GC/MS). GC analysis was carried out on a Thermo Scientific Trace GC Ultra TG-5SILMS capillary column (30m×0.25mm, 0.25µm film thickness). The chromatographic conditions were as follows: The oven temperature increased from 40 (1 min) to 220°C (1 min) at a rate of 15°C/min, analysis time 14 min. The injector and detector temperature was 250°C. Helium used as the carrier gas was adjusted to linear velocity of 1,5 ml/min. The samples were injected using CT splitless method. Analytical standards of the flavor principles were obtained from Sigma-Aldrich. Quantitative data was obtained from electronic integration of peak areas without the use of correction factors.

GC/MS analysis was performed on a Thermo Scientific Trace GC Ultra-MS ITQ 1100 operating at 70eV ionization energy. Equipped with a TG-5SILMS capillary column (30m×0.25mm, 0.25µm film thickness) with He as the carrier gas and a parameters of constant splitless injection: Temperature – 250°C, split flow – 10 ml/min, splitless time – 1 min. The temperature of MS transfer line: 270°C. Components of the oils were identified by both retention times and MS spectra. The result was an average of three determinations.

2.4. Lyophilisation of *Melissa officinalis* L.

The lemon balm leaves were lyophilized by freeze drying (FT33, Armfield, England). The product was dried for a period of 14 h at 200-300 Pa (FD-HP) and for 12 h at 50-80 Pa (FD-LP) with the heating plate kept at 18°C. The condenser temperature was kept at –50 to –55°C. A special digital weighing apparatus (EMALOG, Hungary, accuracy of 5000 ±0.1 g) measures the mass loss of the product during the freeze drying process. During each drying experiment, the weight of the samples on the tray was measured. The tests were repeated three times. The final moisture content of dried lemon balm leaves: 0,157 kg water/kg dry matter (10,92% moisture content wet basis).

2.5. Microstructure imaging

Fresh and freeze dried leaves of lemon balm were examined by BRESSER BIOLUX AL type electro-microscope. The leaves were broken into smaller pieces. We took photos of (4× and 10×) magnification with the program named MicrOcular. Through the camera attached to the microscope we transmitted the photos to the computer.

2.6. Statistical analysis

Tukey's test was used to determine significant differences ($p<0,05$) between the three types of lemon balm (fresh, FD at high pressure, FD at low pressure). The statistics package chosen was PASW Statistics version 18.0 (SPSS Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Chemical characterisation of lemon balm oil

The analysis of the oil components performed by Gas Chromatography (GC) and GC/MS showed that each of the samples contained components typical for the oil from *Melissa officinalis* such as: citral (neral and geranial), citronellal, geraniol, limonene, β-citronellol, β-pinene, linalool , terpineol.

The chromatogram of identified volatile oils of fresh lemon balm is shown in Fig. 1.

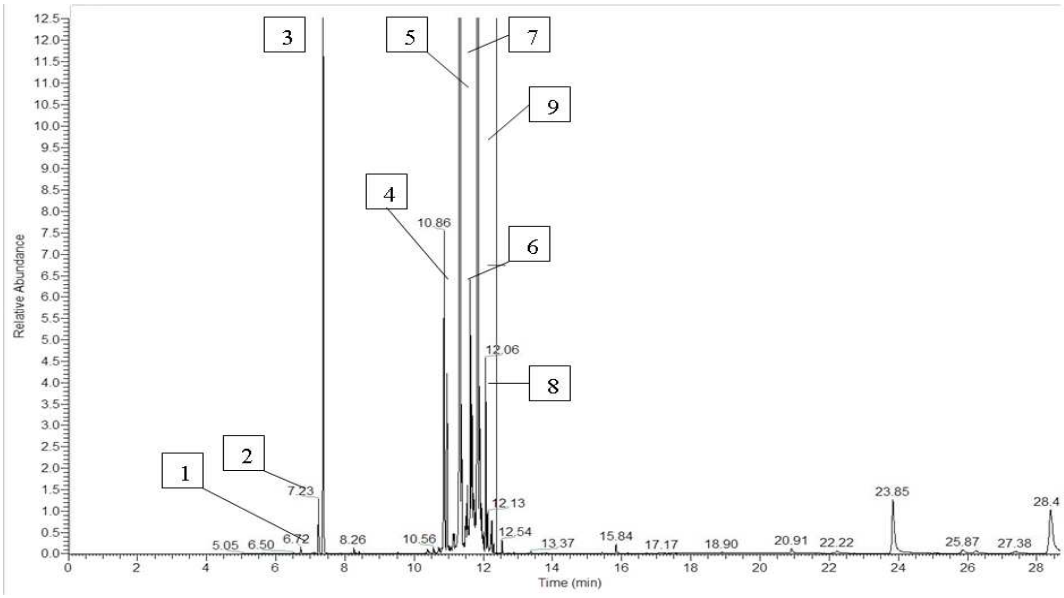

Fig. 1. *Gas-chromatogram of 'Melissa officinalis' essential oil*
The peaks correspond to identified compounds: (1) β-pinene, (2) limonene, (3) β-cis-ocimene, (4) α-cubebene, (5) β-caryophyllene, (6) α- caryophyllene, (7) citral, (8) δ-cadinene, (9) α-calacorene

The principal components of essential oil of the leaves are presented on Table 1.

Table 1. *Essential oil composition in fresh and freeze dried 'Melissa officinalis'*

Compound [mg/100g db]	**Concentration**		
	Fresh	**FD-HP[1]**	**FD-LP[2]**
Citral	123,65[a]	108,34[b]	81,59[c]
Citronellal	77,33[a]	66,56[b]	54,90[c]
Geraniol	29,12[a]	28,02[a]	16,88[b]
Limonene	23,79[a]	17,89[b]	12,13[c]
β-citronellol	14,87[a]	10,32[b]	9,64[b]
β-pinene	11,48[a]	10,62[ab]	6,91[b]
Linalool	10,23[a]	8,74[ab]	6,85[b]
Terpineol	2,53[a]	2,27[a]	2,09[a]
Total [%]	0,293[a]	0,252[b]	0,191[c]

[1] Freeze dried at high pressure
[2] Freeze dried at low pressure
[abcd] treatment means of the ANOVA test, statistically different at $p<0.05$, Tukey's multiple-range test.

The total concentrations of volatile compounds in fresh, FD-HP and FD-LP lemon balm samples were 0.293, 0.252, and 0.191 respectively.

The citral, citronellal and geraniol as major chemical compositions of the essential oil of the lemon balm have been previously reported [9]. Meftahizade et al.[7], reported that the main constituents of the essential oil are citral, citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, comprising 96% of the oil ingredients. Also Carnat et al. [4], reported the chemical composition of essential oil of lemon balm, and found that major components are citral representing 48,2% of the essential oil, followed by citronellal with 39,7% and caryophyllene with 2,37%. The composition of the leaf essential oil agreed with the data of the previous authors.

Comparing the hot air dried (0,14%) and freeze dried (0,211%) essential oil content of lemon balm leaves, significant differences between hot air drying and freeze drying have been found [2].

The result is in agreement with [3]: Drying caused a reduction of essential oil compound in the examined lemon balm leaves. Our study revealed that FD-HP samples retained most of the compounds significantly better than FD-LP samples. This means that the concentrations of essential oils in the FD-processed herb decreased with a reduction of pressure in the drying chamber of the freeze dryer.

3.2. Morphology of glandular hairs (leaves)

Microscope pictures were taken of Melissa leaves both before and after freeze drying. This was to investigate whether or not there are any structural changes in the oil reservoirs during freeze drying.

The essential oil is located in oil reservoirs, such as peltate glands and glandular hairs shed. The vegetative organs of *Melissa officinalis* (leaves) are containing glandular needle-shaped trichomes. This statement was in agreement with literature [5]. Figure 2. shows the glandular hair on fresh leaf of lemon balm (10× magnification). The essential oil is found on the surface of the leaves in peltate glandular trichomes.

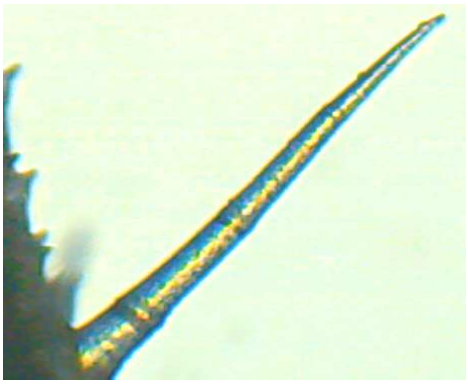

Fig. 2. *Microphotograph of raw glandular trichome*

When freeze dried at low pressure (50-80 Pa) occur a few of the glandular trichomes appeared slightly split open, suggesting oil loss, while most of them appeared to be damaged (Fig. 3.). The reduction in essential oil yield is associated with the observed loss of glandular contents, is attributed to the evaporation of volatile components. This is in agreement with above chapter, the highest losses in volatiles occurred in the freeze dried (at low pressure) samples.

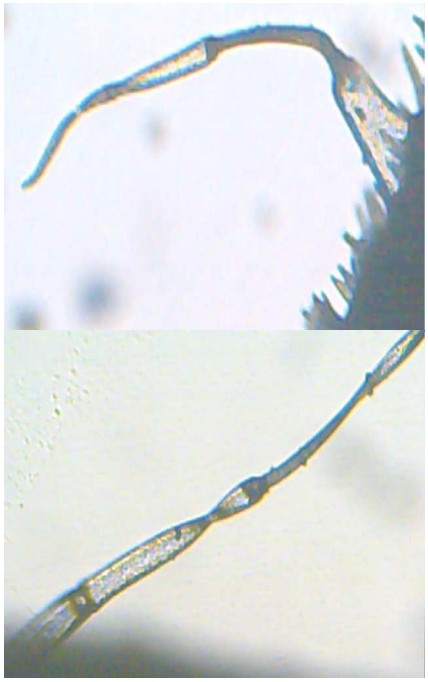

Fig. 3. *Microphotograph of glandular hairs subjected to freeze drying at low pressure*

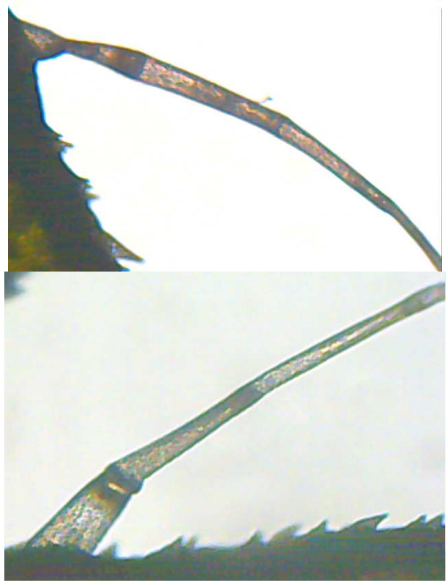

Fig. 4. *Microphotograph of glandular hairs subjected to freeze drying at high pressure*

The glandular trichomes remained relatively plump and the change of shape is minimum for the freeze drying at high pressure (200-300 Pa). Thus, the trichome structure in these samples is similar to that in the fresh samples. The Figure 4. demonstrates the effect of high pressure on glandular hairs by microscopic view (10× magnification).

4. CONCLUSIONS

The volatiles composition of freeze dried lemon balm under different conditions of pressure has been studied. Essential components for the *Melissa officinalis* were detected in fresh samples as well as in dried ones. The essential oil of the fresh material was higher than of the dried one. The quality of lemon balm extract obtained from FD-HP (freeze dried at high pressure) samples was found to be superior, as compared to that of FD-LP (freeze dried at low pressure) samples. A decrease in drying chamber pressure significantly decreased the freeze-drying time of the lemon balm leaves but considerably increased the release of volatile compounds.

The electro-microscope pictures before and after freeze drying at high pressure showed that there were little changes (moderate) in the glandular trichomes. After freeze drying at low pressure, hairs exhibited significant changes in their structures such as split open and volatile oils evaporate to the air. This confirm the statement of our, that the amount released is dependent on the pressure of freeze drier.

The reported results in the present work prove that the freeze drier at high vacuum pressure is recommended for cosmetic and medicine industrial use.

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