

## EFFECT OF LYOPHILIZATION ON VOLATILES OF LEMON BALM LEAVES (*Melissa officinalis*, L.)

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### ABSTRACT

*Melissa officinalis* is a part of the herbs with the high amount of volatile compounds. The losses of essential oils in lemon balm under freeze-drying depend mainly on drying parameters and conditions.

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 mainly on the operation of freeze dryer (FD). It was found that essential oil content was more influenced by chamber pressure (high and low). The higher chamber pressure causes a longer drying period but results better quality 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 drying data were fitted to thin-layer mathematical model (third-degree polynomial). The performance of this model was investigated by comparing the determination of coefficient ( $R^2$ ) and root mean square error (RMSE) between the observed and predicted moisture ratios.

### 1. INTRODUCTION

Lemon balm (*Melissa officinalis*, L.) a member of Lamiaceae family is a perennial herb native to southern climates of Europe and North America (Sharafzadeh et al., 2011). The leaves of *Melissa officinalis* contain relatively low essential oil, but the production cost and price of the oil are very high (Sari and Ceylan, 2002). The volatile oil content depending on provenances, nutrient, light intensity, variety, plant part age, harvesting stages, harvesting hours, oil isolation method and the drying method (Moradkhani et al., 2010, Cuervo-Andrade, 2011). Conventional drying brings undesirable changes in medicinal herbs. It is caused by several modifications in the material (chemical, physical, biological) as a consequence of the removal of moisture and increase of temperature during the drying procedure. According to Enjalbert et al. (1983), the oil from dried lemon balm was darker in color, exhibited relatively lower concentrations of neral and geranial compared with the oil from fresh herb. Air-drying (temperature of 25-32°C for 10 days) caused a great reduction of antioxidant capacity of *Melissa*, reported by Capecka et al. (2005).

The development of new drying methods for medicinal herbs that allow reaching the required quality parameters. For example, the freeze drying can be used to avoid damage caused by heat, producing a product with superior physical and chemical qualities, it is considered a costly and time consuming process (Yousif et al., 2000).

The chemical constituents of volatile oil have been extensively studied. More than forty compounds were recognized in *Melissa* oil (Patora et al., 2003). Meftahizade et al. (2010) reported, that the main component of the essential oil in lemon balm leaves are citral (geranial and neral), citronellal, geraniol, beta-pinene, alpha-pinene, beta-caryophyllene, comprising 96% of the oil ingredients. The major compounds were citronellal and citral, accompanied by beta-caryophyllene, germacrene D, ocimene and citronellol, reported by Nykanen and Nykanen (1986).

Lemon balm has been traditionally used for different medical purposes, food preparation, cosmetics.

There are many previous studies conducted on drying of various products. However, studies on drying characteristics of the lemon balm leaves are scarce in the literature. Moreover none of them reports about freeze drying kinetics and the effects of this drying technique in terms of chemical components of essential oil.

The aim of this study was to evaluate the qualitative and quantitative composition of volatile oil of the lemon balm. The effect of the pressure (high: FD-HP and low: FD-LP) of the freeze-drying procedure was also investigated.

## 2. MATERIALS 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. Determination of moisture content

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. Gas chromatography of the essential oil

Determinations of the volatile oil contents were done by chemical extraction (Moradkhani et al., 2010). 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<sub>2</sub>O<sub>3</sub>. 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 flavour 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. It was equipped with a TG-5SILMS capillary column

(30m×0.25mm, 0.25µm film thickness) with *Helium* 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. The components of the oils were identified by both retention times and MS spectra. The result was an average of three determinations.

#### 2.4. Drying procedure

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: 0,15 kg water/kg dry matter (10.92% moisture content wet basis).

#### 2.5. Mathematical modelling of drying curve

The third-degree polynomial equation (1) is the most convenient mathematical model of freeze drying kinetics and has been used in thin layer drying studies (Antal, 2011).

$$MR = at^3 + bt^2 + ct + d \quad (1)$$

The values of parameters  $a$ ,  $b$ ,  $c$ ,  $d$  of the third-degree polynomial depend on the characteristics of the material, including variety, freezing rate, ripeness, and tendency to lose water (Antal et al., 2011).

The moisture ratio (MR) of lemon balm during drying experiments was calculated using the following equations (2):

$$MR = \frac{M - M_e}{M_0 - M_e} \quad (2)$$

where,  $MR$  is dimensionless moisture ratio,  $M$  is moisture content dry basis (kg water/kg dry matter),  $M_e$  is equilibrium moisture content dry basis (kg/kg db),  $M_0$  is initial moisture content dry basis (kg/kg db).

The sample moisture content  $M$  was calculated on a dry basis (db) according to equation (3):

$$M = \frac{W_t - W_k}{W_k} \quad (3)$$

where,  $M$  is moisture content dry basis (kg water/kg dry matter),  $W_t$  is sample weight at a specific time (kg),  $W_k$  is sample dry weight (kg).

The correlation coefficient ( $R^2$ ) was primary criterion for selecting the best equation to describe the drying curve equation. In addition to  $R^2$ , the root mean square error analysis (RMSE) was used to determine the goodness of the fit (4, 5).

$$R^2 = \frac{\text{Residual sum of squares}}{\text{Corrected total sum of squares}} \quad (4)$$

$$RMSE = \sqrt{\frac{1}{N} \cdot \sum_{i=1}^N (MR_{\text{exp}, i} - MR_{\text{pre}, i})^2} \quad (5)$$

where,  $MR_{exp,i}$  is the experimental moisture ratio at observation,  $MR_{pre,i}$  is the predicted moisture ratio at this observation,  $N$  is number of observations. The lower RMSE values and the higher  $R^2$  values indicate the high fit of the model.

## 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). The software package Microsoft Office Excel 2007 was used in the numerical calculations.

## 3. RESULTS AND DISCUSSION

### 3.1. Drying kinetics

The dimensionless moisture content (MR) change during freeze drying is presented in Figure 1. In this figure the experimental data and the mathematical modelling of the drying kinetics experimental data for lemon balm samples are also shown.

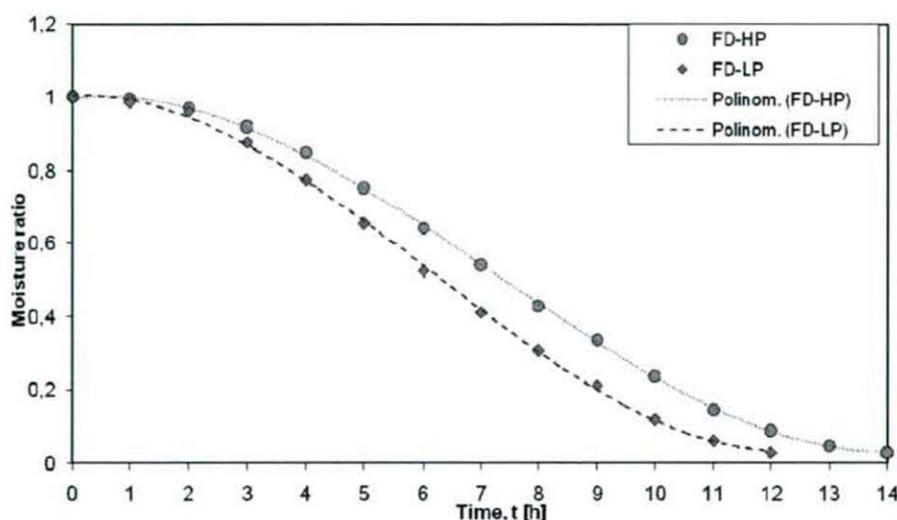


Figure 1. Moisture ratios versus time at various chamber pressures

This figure shows higher drying rate when the sample is dried at low chamber pressure (FD-LP). The final moisture content for *Melissa officinalis* was found to be about 0,15 kg water/kg dry matter (db) after 12 and 14 hours of drying time for FD-LP and FD-HP samples, respectively. The final dimensionless moisture content of the products for FD-LP and FD-HP samples were 0.029. Thus, FD-HP samples required 2 hours longer drying time to achieve the same final moisture content compared to FD-LP *Melissa* samples.

The Table 1 shows the drying model coefficients and the comparison criteria used to assess quality of fit. The statistical values,  $R^2$  and RMSE, are also shown in Table 1. The  $R^2$  and RMSE values changed between 0.9994 and 0.9998,  $5,24 \times 10^{-3}$  and  $1,022 \times 10^{-2}$ , respectively. So, the model could be used to describe the drying of lemon balm leaves.

The drying kinetics results showed that there is a good agreement between the experimental data and thin-layer modelling equations. As shown in Table 1, the highest value

of coefficient of determination ( $R^2 > 0.999$ ) and the lowest value of the root mean square error less than  $1.022 \times 10^{-2}$  predicted by third-degree polynomial's model for *Melissa* samples.

**Table 1. Effect of drying conditions on model parameters**

| Drying method      | Model name | Model parameters |         |        |        |                |         |
|--------------------|------------|------------------|---------|--------|--------|----------------|---------|
|                    |            | a                | b       | c      | d      | R <sup>2</sup> | RMSE    |
| FD-HP <sup>1</sup> | Polynomial | 0,0008           | -0,0177 | 0,0187 | 0,998  | 0,9998         | 0,00524 |
| FD-LP <sup>2</sup> |            | 0,0012           | -0,0213 | 0,0089 | 1,0046 | 0,9994         | 0,01022 |

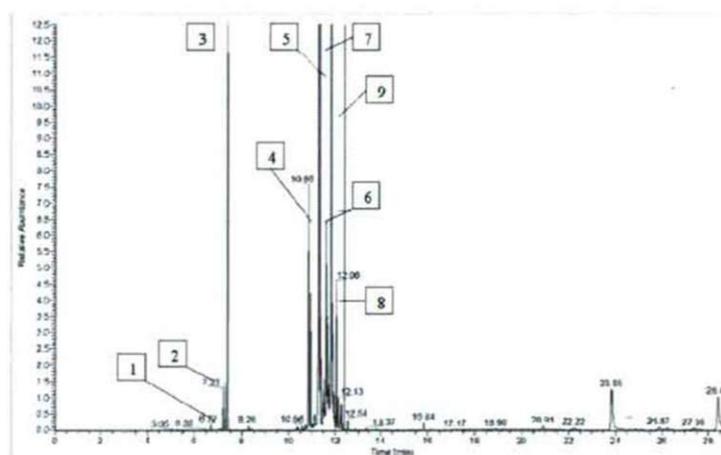
<sup>1</sup> Freeze dried at high pressure

<sup>2</sup> Freeze dried at low pressure

This model can be used satisfactorily to predict the experimental values of the moisture ratio values. Our previous results indicate that in the case of the freeze drying the polynomial model gives the most accurate fit (Antal et al., 2011).

### 3.2. Identification of essential oil components

The chemical composition of lemon balm oil was characterized by Gas Chromatography (GC) and GC/MS analysis. In Figure 2 the chromatogram of fresh lemon balm leaves essential oil are presented from mass spectral data. The principal components of volatile oil of the leaves: citral, beta-caryophyllene, beta-cis-ocimene, alpha-calacorene.



**Figure 2. Gas-Chromatogram of volatiles extract from fresh 'Melissa officinalis'**

The peaks correspond to identified compounds: (1)  $\beta$ -pinene, (2) limonene, (3)  $\beta$ -cis-ocimene, (4)  $\alpha$ -cubebene, (5)  $\beta$ -caryophyllene, (6)  $\alpha$ -caryophyllene, (7) citral, (8)  $\delta$ -cadinene, (9)  $\alpha$ -calacorene

Table 2 presents the results of GC in the fresh and lyophilized lemon balm oil. The major volatiles found were citral, citronellal, geraniol and limonene. The main composition of the fresh leaf essential oil agreed with the data of the previous authors (Carnat et al., 1998, Schnitzler et al., 2008).

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 components of *Melissa* oil were identified by authentic terpene standards (we have only eight standards).

Table 2. Volatile compounds of lemon balm leaves

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

<sup>1</sup> Freeze dried at high pressure

<sup>2</sup> Freeze dried at low pressure

<sup>abcd</sup> Different letters in the same row indicate statistical differences at the 0,05 level according to the Tukey's test.

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.

#### 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. From the drying kinetics result, the FD-LP lemon balm leaves had shorter drying time than the FD-HP 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 third-degree polynomial equation has shown a very well fit to experimental data. The model gave  $R^2$  of 0.9998 and RMSE  $5.24 \times 10^{-3}$  for FD-HP samples and  $R^2$  of 0.9994 and RMSE  $1.022 \times 10^{-2}$  for FD-LP samples in the thin layer freeze drying process. According to the results, the polynomial equation could adequately describe freeze drying characteristics of lemon balm leaves.

We recommend the drying of lemon balm leaves by freeze drying and the pressure in the drying chamber should not be too low.

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