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Effects of freeze drying on retention of essential oils, changes in glandular trichomes of lemon balm leaves

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Abstract

The effect of chamber pressure of a freeze dryer on essential oil contents, drying kinetics, drying characteristics lemon balm leaves and morphology of lemon balm glandular trichomes (oil reservoirs) were investigated. It was found that overall freeze drying (FD) carried out at high (FD-HP) and low pressure (FD-LP) settings consist of sublimation rate, first falling rate and second falling rate periods. Drying rate of FD-LP dried Lemon Balm leaves are higher than FD-HP dried samples, where the drying rates ranged from 0.063 to 0.449 g H_2O/ g DM. s and 0.0365 to 0.395 g H_2O/ g DM. s, respectively. 3rd order Polynomial model was found to be the best fit model for both drying kinetics. In terms of product quality, eight (8) major constituents of lemon balm leaves essential oil were quantified. Further to this, electro-microscope was used to observe the peltate glandular hairs structure. Product quality analysis showed that FD-HP retained higher amount of essential oil, shape of glandular hairs, but no positive effect on the freeze drying duration.

Introduction

Lemon balm (*Melissa officinalis* L.) belongs to family of *Lamiaceae*. It is an upright perennial plant and it can reach a meter height. The hairy leaves are 2 to 8 cm long with heart-shape (Zargari, 1995). Lemon balm has been traditionally used for different medicinal purposes as tonic, juice or tea, for digestives, gastrointestinal disorders, catarrhs, migraines, high blood pressures, nerve pains, stiff necks, toothaches, earaches, sedative-hypnotics, strengthening the memory, stress-induced headaches (Blumenthal *et al*., 2000). Melissa oils are used as an anti-tumeral agent as a potential for cancer remedy or prevention (Sorensen, 2000). The volatile oils of lemon balm leaves can also be used as an anti-virus agent that is capable of exerting a direct antiviral effect on herpes viruses (Schnitzler *et al*., 2008). The lemon balm leaves essential oils are used in the production of perfumes and cosmetics, beverages and ice creams, confectionary and backed food products (Burt, 2004).

More than seventy compounds are found in lemon balm leaves essential oils (Abuhamdah and Chazot, 2008). Major compounds are monoterpene (>60%), aldehydes, citral (geranial + neral) (20 to 30%) and citronellal (30 to 40%) as well sesquiterpenes (>30%): β-caryophyllene and β-caryophyllene oxide, germacerne D; monoterpene alcohols: nerol, geraniol and citronellol (Newall *et al*., 1996). Lemon balm essential oil can be obtained from fresh or dried flower, leaf and branches by supercritical carbon dioxide extraction, water steam distillation or chemical extraction (Mandana *et al*., 2011; Saeb and Gholamrezaei, 2012). The parts that are mostly used are dried lemon balm leaves (Leung and Foster, 2003). The composition and quantity of volatile oil from a particular species could be markedly affected by harvesting season, geographical environment and other agronomical factors (Jordán *et al.*, 2006).

Drying is used to preserve freshly harvest lemon balm leaves before subjecting to extraction process. According to Garg and Kumar (2001), drying is commonly used for post-harvest preservation of herbs to improve the quality of plants and prevent contamination and losses. Currently, the most popular drying method is convective air drying. However, increase in the hot-air temperature and drying duration usually results in decrease of product quality of medicinal herbs viz. colour deterioration, essential

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oil losses and etc. (Argyropoulos and Müller, 2001; Diaz-Maroto *et al*., 2002; Arabhosseini *et al*., 2009). Therefore, suitable drying technique has to be used in order to avoid quality degradation. Freeze drying (FD) is known for its ability to retain food quality such as minimising loss of flavour and aroma compounds as well as bio-active ingredients, reducing shrinkage and etc. through vacuum and low-temperature drying.

Freeze drying has been used in producing product with high nutritional and marketing value (Anghel *et al*., 2005). According to Ratti (2001), it is due to the absence of liquid water and low temperature deactivated most of the microbiological reactions and gives a final product of excellent quality. Currently, research related to freeze drying kinetics and product quality of lemon balm leaves essential oil is rather scarce. The objectives of this study are to investigate the influence of chamber pressure (high and low) of a freeze dryer on essential oil contents, product temperature during freeze drying, drying kinetics, drying characteristics of lemon balm leaves and morphology of lemon balm glandular trichomes (oil reservoirs).

Materials and Methods

Preparation of lemon balm leaves extracts

Lemon balm samples were collected before flowering from an organic farm in Nyíregyháza (Hungary), in 15 June 2011. The samples were obtained by cutting the plant manually. After that, harvesting samples was stored in plastic bags and kept in a refrigerator (Husqvarna, QT 4609 RW, Hungary) at temperature, frozen duration and relative humidity of 5°C, 4 h and 88 - 92%, respectively. Prior to freeze drying process, lemon balm leaves were separated from the stems.

Freeze drying of lemon balm leaves

About 50 g of lemon balm leaves were dried using a freeze drying method. The leaves were frozen at -28°C in a laboratory freeze dryer (FT33, Armfield Ltd., England). The samples were dried at pressure of 250 - 300 Pa for 14 hours (freeze drying at high pressure, FD-HP) and 50 - 80 Pa for 12 hours (freeze drying at low pressure, FD-LP). The self-temperature was set at temperature of 18°C and -50 to -55°C as condenser temperature. Data collection was performed through weighing samples at 1 hour intervals using a digital balance (EMALOG, PAB-01, Hungary) with accuracy of 5000 ± 0.1 g built in under a samples tray. Samples were freeze dried at high pressure (FD-HP) and low pressure (FD-LP) with three replications.

Chemical extractions of lemon balm leaves

About 10 g of dried leaves were added to a volumetric flask filled with chloroform/hexane solvents (1:1, 600 ml). The steps of extraction procedure: 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 Gas Chromatograph (GC). The amount of extracted essential oil was measured and expressed in mg per 100 g dry matter.

Chemical analysis

A capillary column, TG-5SILMS (30 m × 0.25 mm, 0.25 µm film thickness) was fitted to a Gas Chromatograph (Clarus 500, PerkinElmer, USA). The injection port and detector (FID, PerkinElmer, USA) temperatures were set at 245°C and 250°C, respectively. The column temperature was programmed at 40°C for 1 minute and increased to 220°C for 1 minute at a rate of 15°C/min, total analysis duration is 14 minute. Carrier gas used was helium, adjusted to linear velocity of 1.5 ml/min. The samples were injected using splitless method. Analytical standards of the flavour principles were obtained from Sigma-Aldrich (Sigma, St. Louis, MO, USA). Quantitative data was obtained from electronic integration of peak areas without the use of correction factors. Analysis was done in triplicate.

Gas chromatography-mass spectrometry (GC-MS) is a useful tool for quantitative and qualitative analysis of a wide range of relatively volatile compounds and the technique has been widely applied in medicinal, biological and food research (Saeb and Gholamrezaei, 2012). GC/MS (Trace GC Ultra, Thermo Scientific, USA) analysis was performed at electronic impact ionisation of 70eV. It was equipped with a TG-5SILMS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) with Helium as a carrier gas. The splitless injection setting for temperature, split flow and splitless time are -250°C, -10 ml/min and -1 minute, respectively. The temperature at Mass Spectrometry (MS) transfers line is 270°C. The components of the oils were identified by both retention times and MS spectra. Mass spectra were recorded with a scan rate of 0.58 s, covering a mass

range of 50 - 650 m/z for GC/MS. Three replicates were used in this analysis.

Morphological evaluation

Structures of the fresh and dried glandular hairs were examined using an electro-microscope (Bresser Biolux Al, 20x - 1280x, Bresser, Germany). The samples were prepared little piece by cutting using razor blade. Each sample was viewed and images with a magnification of 40× were acquired. Analysis was done in triplicate. All the microstructure examinations and micrographs were performed at 20°C.

Moisture content

The moisture content before and after freeze drying of the leaves was determined by the gravimetric method (105°C for 4 h) in triplicate and the average value was recorded. The experiments were conducted using a hot-air laboratory drier (LP-306, LABOR-MIM, Hungary).

In an environment containing moisture, dry material will absorb moisture until it is in equilibrium with the surrounding atmosphere. Similarly, saturated material, when placed in an atmosphere of l ower relative humidity (RH), will lose moisture until equilibrium is attained. If the sample is placed in an environment where the RH is stable, it will attain constant moisture content (MC), known as the equilibrium moisture content (EMC) (Akyildiz and Ates, 2008). The EMC is usually determined by weighing the sample periodically until a constant weight is reached.

Mathematical modelling of drying

The moisture ratio (MR) of lemon balm leaves during the drying experiments was calculated using equation (1). The M_e values were a bit lower than final moisture content of dried samples. Such low values of M_e had a negligible effect on MR, which depended mainly on the values of M and M_o (Calín-Sánchez *et al*., 2013).

$$MR = \frac{M - M_e}{M_0 - M_e} \quad (1)$$

where MR = Moisture ratio, M = Moisture content (g H_2O/g DM), M_e = Equilibrium moisture content (g H_2O/g DM), M_0 = Initial moisture content (g H_2O/g DM).

The final moisture content of samples was determined using equation (2):

$$M = \frac{W_t - W_k}{W_k} \quad (2)$$

where W_t = Sample weight at a specific time (kg), W_k =Sample dry weight (kg).

The experimental data were fitted to the following a third-degree polynomial model (Eq. 3), Newton model (Eq. 4), Page model (Eq. 5), Henderson and Pabis model (Eq. 6) and Logarithmic model (Eq.7) (Page, 1949; Henderson and Pabis, 1961; O'Callaghan *et al*., 1971; Liu and Bakker-Arkema, 1997; Yagcioglu *et al*., 1999).

$$MR = at^3 + bt^2 + ct + 1$$

The values of parameters *a*, *b*, *c* 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).

$$MR = \exp(-kt) \quad (4)$$

$$MR = \exp(-kt^n) \quad (5)$$

$$MR = a\exp(-kt) \quad (6)$$

$$MR = a\exp(-kt) + c \quad (7)$$

where MR = Moisture ratio; *a*, *c* and *n* = constants in models, *k* = drying constant (h^{-1}), *t* = drying time (h).

To find the best fit model, two statistical indicators are used and shown in Equation (8) and (9). Model with the highest coefficient of determination (R^2) and the lowest Root Mean Square Error (RMSE) values indicate the best fit model.

$$R^2 = \frac{\sum_{i=1}^{N}(MR_{pre_i} - MR_{mean})^2}{\sum_{i=1}^{N}(MR_{\exp_i} - MR_{mean})^2} \quad (8)$$

$$RMSE = \sqrt{\frac{1}{N}\cdot\sum_{i=1}^{N}(MR_{\exp_i} - MR_{pre_i})^2} \quad (9)$$

where *i* = Sequence number of observation, $MR_{exp,I}$ = Experimental moisture ratio at observation, $MR_{pre,I}$ = Predicted moisture ratio at observation, *N* = Number of observations.

Statistical Analysis

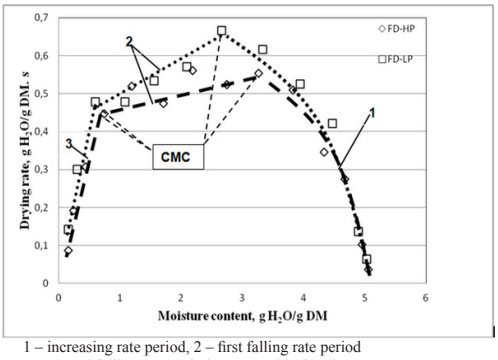
ANOVA Tukey's test was used to determine significant differences ($p < 0.05$) among the dried and fresh essential oils content of lemon balm. The statistical package chosen for analysis was PASW Statistics version 18.0 (SPSS Inc., Chicago, Illinois, USA). In addition, non-linear regression analysis was performed using Excel Solver program (Microsoft Office Excel, 2007) in the numerical calculations.

Results and Discussion

Figure 1 shows the drying characteristics of lemon balm leaves dried using high pressure and low pressure settings. Drying rates of lemon balm leaves dried at lower pressure (FD-LP) are higher compared to high pressure (FD-HP), where the drying rates ranged from 0.063 to 0.449 g H_2O/g DM. s (DM is dry matter) and 0.0365 to 0.395 g H_2O/g DM. s, respectively. FD-HP lemon balm leaves samples required 2 hours longer drying time to achieve the same final moisture content compared to FD-LP lemon balm leaves samples. Antal *et al.* (2011) reported that the FD-LP can reduce the drying time. On the other hand, the FD-HP samples need longer drying time. Liapis and Bruttini (2007) reported that at high pressure, the mean free path of gas molecules within the void spaces of the dried layer become substantially less than the dimension of void space and vice versa. This is also in agreement with Rim *et al.* (2009). The authors reported that the sublimation rate is inversely proportional to the mass transfer resistance. Therefore, drying rate at higher pressure is lower compared to low pressure during freeze drying. In addition, Pikal (2000) and Fissore *et al.* (2008) reported that sublimation duration increased with pressure as a result of low driving force for mass transfer.

Typical freeze drying process starts with sublimation followed by desorption. Drying rate of lemon balm leaves samples dehydrated using the FD-LP method increased from 0.063 to 0.67 g H_2O/g DM. s for the first 6 hours. At this stage, outer layer of ice of the samples was removed completely and formed a dry layer. Drying rate started to decrease after the critical moisture content due to removal of water vapor from the interior ice sublimation has to overcome the dry layer which is a barrier to the vapor transport (Wang *et al*., 2009). Sagara and Ichiba (1994) reported that drying rate decreased gradually with the increase of resistance of the dried layer to heat transfer.

Figure 1 shows that both drying curves exhibit an initial transient period and two distinctive falling rate periods. The initial transient period is rather long if compared to typical drying curves obtained from convective drying. During the initial transient period of FD-LP, drying rate increased from 0.03 to 0.68 g H2O/g DM. s while moisture content was dropped from 5.1 to 2.8 g H_2O/g DM. Moisture content of 2.8 g H_2O/g DM is the first critical point of falling rate period, it marks the beginning of falling rate. The drying rate decrease linearly to a second critical moisture content which is 0.8 g H_2O/g DM. In the



1 – increasing rate period, 2 – first falling rate period
3 – second falling rate period, CMC- critical moisture content

Figure 1. Drying characteristics of lemon balm (*Melissa officinalis* L.) leaves

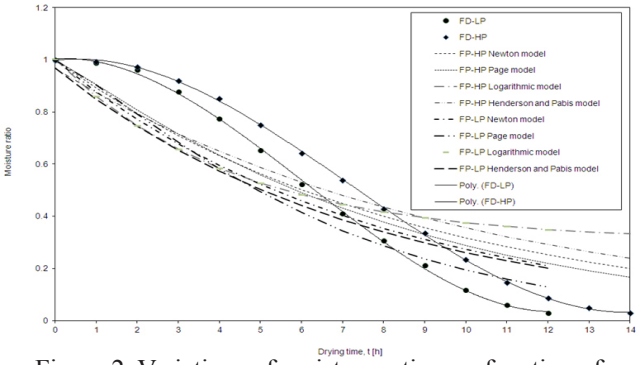


Figure 2. Variations of moisture ratio as a function of time for freeze drying of lemon balm

second falling rate period where the MC dropped from 0.8 to 0.1 g H_2O/g DM, the drying rate dropped sharply if compared to the first falling rate period. According to Wang *et al.* (2009), desorption occurred after complete removing of ice. Bound water is difficult to remove. It can be clearly observed from Figure 1 where the drying rate decreases appreciably to 0.16 g H_2O/g DM. s in the second falling rate. For samples dehydrated at the FD-HP setting, drying rate also increased at the first 6 hours from 0.036 to 0.554 g H_2O/g DM. s. The critical moisture content for the first falling rate period and second falling rate period are 3.27 and 0.79 g H_2O/g DM, respectively.

Mathematical modelling

The dimensionless moisture content also known as moisture ratio (MR) changes during freeze drying. The moisture ratio profile (moisture ratio versus time) is similar to moisture content profile (moisture content versus time). The moisture ratio profile is presented in Figure 2. The equilibrium moisture content (EMC) of dried Lemon Balm leaves is obtained when the profile becomes flat at the end of the drying process. From Figure 2, the EMC is 0.157 g H_2O/g DM for both FD-LP and FD-HP samples, after drying for 12 and 14 hours, respectively. The drying kinetics was modelled using third-degree polynomial model, Newton model, Page model, Henderson and Pabis model and Logarithmic model. Table 1 shows the

Table 1. Coefficients and evaluations of the models

Drying settings	Model coefficients and statistical parameters					
	a	b	c		R^2	RMSE
Polynomial [hp]	0.0008	-0.0177	0.0187	-	0.9998	0.00524
Polynomial [lp]	0.0012	-0.0213	0.0089	-	0.9994	0.01022
	k	n	a	c	R^2	RMSE
Newton [hp]	0.1147	-	-	-	0.9163	0.1494
Newton [lp]	0.1298	-	-	-	0.9245	0.0174
Page [hp]	0.1000	1.0947	-	-	0.9270	0.1384
Page [lp]	0.1000	1.2151	-	-	0.9432	0.1320
Logarithmic [hp]	0.2268	-	0.6981	0.3035	0.7907	0.2085
Logarithmic [lp]	0.7000	-	0.3035	2.55e10	0.8349	0.1836
Henderson & Pabis [hp]	0.9676	-	0.1000	-	0.9295	0.1590
Henderson & Pabis [lp]	0.9676	-	0.1310	-	0.9236	0.1405

[hp] means Freeze drying at high pressure
[lp] means Freeze drying at low pressure

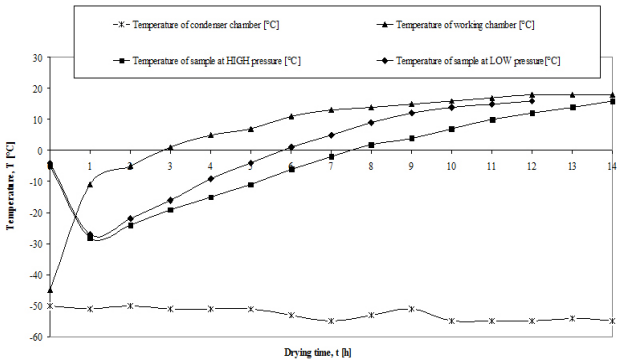


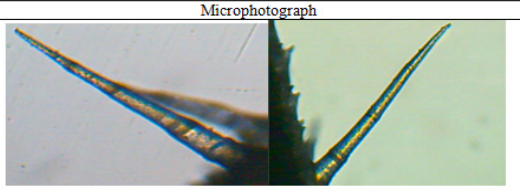
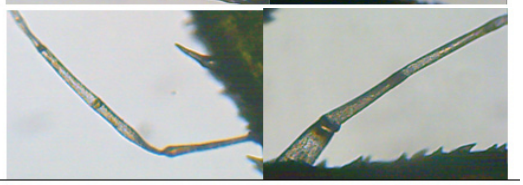
Figure 3. Change of temperature at freeze-drying of lemon balm leaves (shift the horizontal axis)

models coefficients and R^2 and RMSE coefficient obtained from statistical analysis when fitting the experimental data to the selected drying models. The best fit model is the third-degree polynomial model where the R^2 and RMSE values are 0.9994 and 0.9998, 5.24×10^{-3} and 1.022×10^{-2}, for both samples dehydrated by FD-LP and FD-HP methods.

Temperature of sample is a key parameter for the control of freeze drying process as it influences the elementary mechanisms of different freeze drying steps of freeze dried product (Rim *et al.*, 2009). After the freezing stage, the pressures in the freeze drying chamber reduced to 250 - 300 Pa for FD-HP and 50 - 80 Pa for HP - LP. Figure 3 shows the profile of temperature in the drying chamber, condenser and samples at FD-LP and FD-HP versus time. It was found that the temperature for both samples decreased in the first hour from -5 to -28°C. When sublimation occurred, significant amount of latent heat of sublimation which is about 2840 kJ/kg ice causes the samples temperature to decrease (Liapis and Bruttini, 2007). Referring to Figure 1, sublimation process occurred in the first 6 hours of drying and the FD-HP and FD-LP samples temperature profile in Figure 3 shows that after 6 hours of sublimation process, drying temperature of samples change to -6 and 1°C, respectively.

Bound water is removed during second stage of freeze drying. According to Liapis and Bruttini (2007), bound water exist is due to physical

Table 2. Microphotograph of fresh glandular hairs, glandular hairs subjected to FD-LP and FD-HP

	Microphotograph	
Fresh		
FD-LP		
FD-HP		

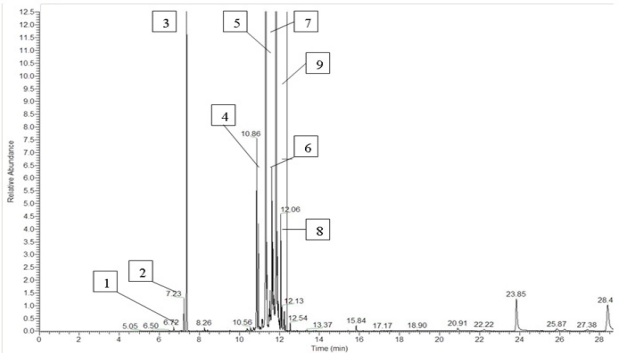


Figure 4. Chromatogram of the GC/MS analysis of volatile oils of the *Melissa* extract

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

adsorption, chemical adsorption. This water is removed by heating from the samples under vacuum. When material temperature is close to the temperature of the heating plate (working chamber), the drying process is complete.

Morphology of glandular hairs of lemon balm leaves

Two major types of hairs can be found from mature vegetative organs of lemon balm leaves viz. glandular and no-glandular (Pădurariu *et al*., 2009). Glandular hairs have a unicellular or a bicellular stalk and a head with one or two cells (Pădurariu *et al*., 2009). In this study, microphotographs were captured before and after freeze drying to investigate whether there was any structural change 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 organ of lemon balm leaves contain glandular needle-shaped trichomes (Cuervo-Andrade, 2011). Table 2 shows glandular hairs with oil reservoirs on a fresh leaf of lemon balm, glandular hairs subjected to FD-LP and FD-HP with 40×

Table 3. Concentration of the main constituents in lemon balm leaves oil

	Concentration (mg/100g db)		
Components	Fresh	FD-HP	FD-LP
Citral	123.65[a]±1.22[*]	108.34[a] ±1.48[*]	81.59[c]±1.09[*]
Citronellal	77.33±1.04[a]	66.56±1.01[b]	54.90±0.89[c]
Geraniol	29.12±0.52[a]	28.02±0.56[a]	16.88±0.48[b]
Limonene	23.79±0.41[a]	17.89±0.54[b]	12.13±0.29[c]
β-citronellol	14.87±0.33[a]	10.32±0.27[b]	9.64±0.25[b]
β-pinene	11.48±0.25[a]	10.62±0.32[ab]	6.91±0.17[b]
Linalool	10.23±0.18[a]	8.74±0.20[ab]	6.85±0.14[b]
Terpineol	2.53±0.09[a]	2.27±0.11[a]	2.09±0.13[a]
Total [%]	0.293[a]	0.252[b]	0.191[c]

[*] Standard deviation
[abc] treatment means of the ANOVA test, statistically different at $p < 0.05$, Tukey's multiple-range test.

magnifications. Essential oils are found on the surface of the leaves in the peltate glandular trichomes. When samples are freeze dried at low pressure (50 - 80 Pa), few of the glandular trichomes appeared slightly split open, which indicates loss of essential oil because most of them appeared to be damaged. The reduction in essential oil yield is associated with the observed loss of glandular contents attributed to the evaporation of volatile components. The highest losses in volatiles occurred in the FD-LP dried samples. The low pressure in freeze dryer chamber strongly affects the glandular hairs and results in significant loss of essential oils from the trichomes. The glandular trichomes remained relatively plump and the change of shape is minimum for samples dried using FD-HP (250 - 300 Pa). Thus, the trichome structure in these samples is similar to the fresh samples.

Volatile composition of the essential oil

Eight volatile compounds were identified from the GC analysis. A typical GC/MS chromatogram profile of row Melissa extract is showed in Figure 4. The principal components of volatile oil of the lemon balm leaves are citral, beta-caryophyllene, beta-cis-ocimene, and alpha-calacorene. Referring to the results of GC analysis, the main ingredient is a citral.

The concentration of the main constituents of lemon balm leaves oil extracted from fresh and dehydrated samples are presented in Table 3. The major components are Citral, Citronellal, Geraniol and Limonene, other components present in appreciable amount are: β-citronellol, β-pinene, Linalool and Terpineol. Carnat *et al.* (1998) identified 20 constituents in leaf oil of lemon balm which are citral (48%), citronellal (40%) and β-caryophyllene (2%). The citral, citronellal and geraniol as major chemical compositions of the essential oil of the lemon balm have been previously reported (Sari and Ceylan, 2002). Meftahizade *et al.* (2010) also 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. The composition of the leaf essential oil obtained in this study is in agreement with the findings reported the various researchers.

Total oil content in fresh material was approximately 0.3 mg/100gram DM. This value is similar to the value reported in the literature for *Melissa officinalis* (Argyropoulos and Müller, 2001). The total concentrations of volatile compounds in fresh, FD-HP and FD-LP lemon balm leaves samples were 0.293, 0.252, and 0.191% respectively ($p < 0.05$). Freeze dried lemon balm leaves at high pressure exhibited higher content of essential oils compared to freeze dried at low pressure. Referring to Table 3, majority compounds such as citronellal, limonene, β-citronellol, β-pinene and linalool for fresh and FD-HP samples are significantly different ($p < 0.05$). However, geraniol and terpeineol of fresh and FD-HP samples are not significantly different ($p > 0.05$). This is similar to the finding reported by de Torres *et al.* (2010) in comparing the trans-Geraniol and terpinen-4-ol compounds of fresh and freeze dried grape skin. Other compounds such as β-citronellol, β-pinene, linalool and terpineol obtained from samples dehydrated by FD-HP and FD-LP were not statistically different ($p < 0.05$). According to Baranauskienė *et al.* (2006), loss of volatile component is dependent on polarity of compounds; for instance, the vapour pressure of a non-polar compound, p-cymene is 10 times higher than a polar compound, linalool. In addition, loss of essential oils can be due to large reduction of pressure. It was found that retained volatile compounds such as citral, citronellal, geraniol, limonene of FD-HP and FD-LP are significantly different ($p < 0.05$). According to Antal *et al*. (2011) large reduction in pressure resulted in loss of some essential oils into the environment, where some of the compounds were found in the condensed water.

Conclusions

This study focused on the chemical composition characteristics of essential oils extracted from fresh and freeze dried lemon balm leaves. The essential oil of the fresh material was found higher than the dried ones. The freeze dried leaves at high pressure showed a higher quality when compared to low pressure. Low pressure caused significant essential oil losses from lemon balm leaves but its drying time was shorter. A decrease in drying chamber pressure decreased the freeze-drying time but increased the release of volatile compounds. Further to this, the

electro-microscope pictures after freeze drying at high pressure showed that there were little changes (moderate) in the glandular trichomes, thus retained the volatile compounds within the structure indicating lesser loss. On the other hand, at low pressure, hairs exhibited significant changes in their structures such as split open which resulted in volatile oils evaporated to the air. This confirms the findings obtained from this study that the amount oil losses is dependent on the pressure of freeze drier. Lyophilized samples at FD-HP retained higher amount of volatile oils compared to FD-LP. Drying using FD-HP is recommended in lemon balm due to significant retention in the volatile content. In addition, it was found that the polynomial model gave the best fitting of the experimental data.

Acknowledgement

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References

Abuhamdah, S. and Chazot, P.L. 2008. Lemon balm and lavender herbal essential oils: Old and new ways to treat emotional disorders? Current Anaesthesia & Critical Care 19: 221-226.

Akyildiz, M. H. and Ates, S. 2008. Effect of heat treatment on equilibrium moisture content (EMC) of some wood species in Turkey. Research Journal of Agriculture and Biological Sciences 4(6): 660-665.

Anghel, G., Mnerie, D. and Tucu, D. 2005. Mechanical engineering aspects for food's lyophilisation technology. Annals of the Oradea University. Fascicle of Management and Technological Engineering 562-565.

Antal, T., Figiel, A., Kerekes, B. and Sikolya, L. 2011. Effect of drying methods on the quality of the essential oil of spearmint leaves (*Mentha spicata* L.). Drying Technology 29(15): 1836-1844.

Arabhosseini, A., Padhye, S., Huisman, W., van Boxtel, A. and Müller, J. 2009. Effect of drying on the color of tarragon (*Artemisia dracunculus* L.) leaves. Food and Bioprocess Technology 1: 1-9.

Argyropoulos, D. and Müller, J. 2001. Effect of convective drying on quality of lemon balm (*Melissa officinalis* L.). Procedia Food Science 1: 1932-1939.

Baranauskienė, R., Venskutonis, P.R., Dewettinck, K. and Verhé, R. 2006. Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices. Food Research International 39: 413-425.

Blumenthal, M., Goldberg, A. and Brinckmann, J. 2000. Herbal medicine-expanded commission E monographs, p. 230-232. Boston: Integrative Medicine Communications.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods. A review. International Journal of Food Microbiology 94(3): 223-253.

Calín-Sánchez, Á., Figiel, A., Lech, K., Szumny, A. and Carbonell-Barrachina, Á. A. 2013. Effects of drying methods on the composition of thyme (*Thymus vulgaris* L.) essential oil. Drying Technology 31: 224-235.

Carnat, A.P., Carnat, A., Fraisse, D. and Lamaison, J.L. 1998. The aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. subs. *officinalis*) tea. Pharmaceutica Acta Helvetiae 72: 301-305.

Cuervo-Andrade, S.P. 2011. Quality oriented drying of lemon balm (*Melissa officinalis* L.). Witzenhausen, Germany: University of Kassel, Ph.D dissertation.

de Torres, C., Díaz-Maroto, M.C, Hermosín-Gutiérrez, I. and Pérez-Coello, M.S. 2010. Effect of freeze-drying and oven drying on volatiles and phenolic composition of grape skin. Analytica Chimica Acta 660: 177-182.

Diaz-Maroto, M.C., Perez-Coello, M.S. and Cabezudo, M.D. 2002. Effect of different drying methods on the volatile components of parsley (*Patroselinum crispum* L.). European Food Research and Technology 215: 227-230.

Fissore, D., Velardi, S.A. and Barresi, A.A. 2008. In-line control of a freeze-drying process in vials. Drying Technology 26: 685–694.

Garg, H.P. and Kumar, R. 2001. Developments in solar drying. In Proceedings of the 2nd Asian-Oceania Drying Conference. Pulau Pinang: Malaysia.

Henderson, S.M. and Pabis, S. 1961. "Grain drying theory, II: Temperature effects on drying coefficients". Journal of Agricultural Engineering Research 6: 169-174.

Jordán, M.J., Martinez, R.M., Goodner, K.L., Baldwin, E.A. and Sotomayor, J.A. 2006. Seasonal variation of *Thymus hyemalis* lange and spanish *Thymus vulgaris* L. essential oils composition. Industrial Crops Products 24: 253-263.

Leung, A.Y. and Foster, S. 2003. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. New York: John Wiley and Sons.

Liapis, A.I. and Bruttini, R. 2007. Freeze drying. In Mujumdar, A.S. (Ed.). Handbook of industrial drying, 5th Eds., p. 275-278. New York: CRC press.

Liu, Q. and Bakker-Arkema, F.W. 1997. Stochastic modelling of grain drying, part 2: model development. Journal of Agricultural Engineering Research 66: 275-280.

Mandana, B., Russly, A. R., Ali, G. and Farah, S. T. 2011. Antioxidant activity of spearmint (*Mentha spicata* L.) leaves extracts by Supercritical Carbon Dioxide (SC-CO_2) extraction. International Food Research Journal 18: 543-547.

Meftahizade, H., Moradkhani, H., Naseri, B., Lofti, M. and Naseri, A. 2010. Improved *in vitro* culture and micropropagation of different *Melissa officinalis* L.

genotypes. Journal of Medicinal Plants Research 4(3): 240-246.

Newall, C.A., Anderson, L.A. and Pillipson, J.D. 1996. Herbal medicine: a guide for healthcare professionals. London: Pharmaceutical Press.

O'Callaghan, J.R., Menzies, D.J. and Bailey, P.H. 1971. Digital simulation of agricultural dryer performance. Journal of Agricultural Engineering Research 16: 223–244.

Page, G.E. 1949. Factors influencing the maximum rates of air drying shelled corn in thin layers. M.Sc. thesis. Department of Mechanical Engineering, Purdue, USA.

Pădurariu, C., Gales, R., Preotu, A., Zamfirache, M., Toma, C. and Boz, I. 2009. Distribution and morphology of hairs in *Melissa officinalis* L.,vegetative organs. Analele ştiintifice ale Universităţii "AI.I. Cuza" Iaşi Tomul LV, fasc. 2, s.II a. Biologie vegetală 21-25.

Pikal, M.J. 2000. Heat and mass transfer in low pressure gases: Applications to freeze drying. In Amidon, G.L., Lee, P.I., Topp, E.M. (Eds.). Transport processes in pharmaceutical systems, p. 632. New York: Marcel Dekker Inc.

Ratti, C. 2001. Hot air and freeze-drying of high-value foods: a review. Journal of Food Engineering 49: 311-319.

Rim, D., Séverine, V., Julien, A. and Oliver, M. 2009. Sublimation kinetics and sublimation end-point times during freeze drying of pharmaceutical active principle with organic co-solvent formulations. Chemical Engineering Research and Design 87: 899-907.

Saeb, K. and Gholamrezaei, S. 2012. Variation of essential oil composition of *Melissa officinalis* L. leaves during the different stages of plant growth. Asian Pacific Journal of Tropical Biomedicine 1: 1-3.

Sagara, Y. and Ichiba, J. 1994. Measurement of transport properties for the dried layer of coffee solution undergoing freeze drying. Drying Technology 12: 1081-1103.

Sari, A. O. and Ceylan, A. 2002. Yield characteristics and essential oil composition of lemon balm (*Melissa officinalis* L.) grown in the Aegean Region of Turkey. Turkish Journal of Agriculture and Forestry 26: 217-224.

Schnitzler, P., Schuhmacher, A., Astani, A. and Reichling, J. 2008. *Melissa officinalis* oil affects infectivity of enveloped herpesviruses. Phytomedicine 15:734-740.

Sorensen, J.M. 2000. *Melissa officinalis.* The International Journal of Aromatherapy 10(1-2): 7-15.

Zargari, A. 1995. Medicinal plants. 5th ed. Tehran: Tehran University Press.

Wang, R., Zhang, M., Mujumdar, A.S. and Sun, J. 2009. Microwave freeze-drying characteristics and sensory quality of instant vegetable soup. Drying Technology 27: 962-968.

Yagcioglu, A., Degirmencioglu, A. and Cagatay, F. 1999. Drying characteristic of Laurel leaves under different conditions. In: Proceedings of the 7th International Congress on Agricultural Mechanization and Energy, Faculty of Agriculture, Adana, Turkey, 26–27 May, pp. 565–569.