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Yield and composition of supercritical fluid extracts of different *Lamiaceae* herbs: *Satureja hortensis* L., *Ocimum basilicum* L. and *Melissa officinalis* L.

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Summary: In our work the effectiveness of supercritical carbon dioxide extraction was studied on volatile and nonvolatile components of *Lamiaceae* herbs. The aim of our investigations was to determine the optimal extraction parameters yielding high amount of volatiles and nonvolatiles in a desirable composition. As plant materials, dried and powdered cude drugs of *Satureja hortensis, Ocimum basilicum* and *Melissa officinalis* were chosen. Pressure (8–50 MPa), temperature (35–60 °C), time (5–60 min) and the ratio of modifier (5–50% methanol) of extraction were regulated. Results obtained by supercritical fluid extraction (SFE) were compared to that of the conventional extraction procedure, hydrodistillation (HD). In the case of *Satureja hortensis, Ocimum basilicum* and *Melissa officinalis* extract yield of SFE was comparable to the essential oil amount obtained by hydrodistillation from the same drug. Essential oil rich extracts were analysed by GC-FID, while extracts rich in nonvolatile compounds were analysed by HPLC. We have found that yield and quality of SFE extracts highly depend on the conditions of extraction.

Key words: supercritical fluid extraction, *Lamiaceae, Satureja hortensis, Ocimum basilicum, Melissa officinalis,* hydrodistillation, modifier, GC-FID, HPLC

Introduction

In the field of the research of medicinal and aromatic plants, great efforts are made in order to find and to isolate the active substances as well as to define their exact way of action. Volatile and nonvolatile compounds of the species belonging to the *Lamiaceae* family play an important role in phytotherapy, aromatherapy and as food additives, too. Summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum* L.) and lemon balm (*Melissa officinalis* L.) are aromatic herbs. They are used extensively to add a distinctive aroma and flavour to food. Fresh or dried leaves of them can be used as spices. Essential oil extracted from fresh leaves and flowers can be utilized as additives in food or in cosmetics as well as in pharmaceuticals.

Supercritical fluid extraction is an extraction method using supercritical fluids as extraction solvent instead of normal liquids. It has been shown that SFE with supercritical carbon dioxide can be far superior to conventional methods of extraction (such as liquid extaction or Soxhlet) in a variety of ways: speed of extraction, completeness of extraction, eliminating the solvent concentration step, simplified procedure, selectivity, reduced environmental hazard/ nontoxicity, cost savings, suitability for thermal sensitive samples, easiness of coupling to GC for on-line SFE/GC.

A solvent is considered to be a supercritical fluid when both its temperature and pressure equal or exceed the specific supercritical point. For CO_2, the critical temperature and pressure are 31.1 °C and 7.38 MPa (=1050 psi), respectively.

Supercritical fluids possess almost similar density and solvating power to liquid organic solvents, but have extremely rapid diffusion characteristics and their viscosity is similar to those of the gases. The density of a gas can be varied and controlled by regulating pressure, temperature or both. The solvent capacity of supercritical CO_2 can be increased or decreased by varying pressure and temperature. It should be noted that identical densities may be achieved at different pressure/ temperature relationship, but supercritical fluids at higher temperature and pressure have higher solvating power. An increase in the pressure and temperature enables supercritical CO_2 to dissolve compounds of higher polarity (*McNally & Wheeler,* 1988). If the elevation of pressure and temperature increases are not sufficient to dissolve the analyte, a small amount of modifier such as methanol, isopropanol, acetonitrile, water or benzene can be added to the supercritical CO_2 to increase its solvating power as well as the polarity *(Brennecke & Eckert,* 1989). Thus combination of pressure/temperature/ modifier control contributes to extract of a large variety of analytes by supercritical CO_2 extraction (*Schantz & Chesler*, 1986).

The hydrodistillation process has been traditionally used in the extraction of essential oils on a laboratory scale. In this work, we are intended to compare the efficiency of this process with its relationship to the volatile and nonvolatile composition of the supercritical CO_2 extracts.

Considering previous data obtained by SFE extraction of *Satureja hortensis*, *Pluhár* et al. (1996) concluded that the yields were lower than int he case of hydrodistillation. The highest value was obtained at 50 °C and 50 MPa, while the best, near natural volatile composition was reached at 10 MPa and 40 °C.

The citral compounds could be extracted from lemon balm using SFE more efficiently and faster than with hydrodistillation (hydrodistillation lasted to 40 min to perform, while SFE needed only 20 min). Increased amounts of citronellal, neral, neryl acetate, and caryophyllene oxide were found in hydrodistillation, while SFE yielded increased ratio of geranial and of caryophyllene. In addition, SFE yielded a greater total amount of compounds detected as compared to hydrodistillation (*Rozzi* et al., 2002).

Linalool is the main constituent in the basil extract. Despite the higher total yield of basil at 25.5 MPa, compared to pressure 17.2 MPa, the percentage of the major components (except 1.8-cineole), were less. It means that at higher pressures, additional components were extracted and the amount of extract changed substantially (*Menaker* et al., 2004).

Materials and methods

Dried leaves of summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum* L.) were grown and surveyed in Budapest, Hungary in 2006. Dried lemon balm (*Melissa officinalis* L.) was purchased from Herbária Zrt. (Budapest).

The plant (100 g of dried material of savory; 200 g of basil and of lemon balm) was subjected to hydrodistillation (HD) for 1,5 h, using Clevenger-type apparatus, according to the Hungarian Pharmacopoeia VII. (1986). The volatile distillates were analysed by GC-MS in the case of all species.

Supercritical fluid extraction (SFE): optimal parameters in the respect of extract amount and of the composition were investigated, where the pressure (8–50 MPa), temperature (35–60 °C), time (5–60 min) and modifier (5–50% methanol) were regulated. During pressure, time or temperature experiments the other two parameters were constant. In the case of modifier experiment, methanol was used between 5–50% and 30–40–50 MPa (at constant 30 min 40 °C).

Isco SFX 2–10 type laboratory extractor with an Isco Model 260D pump module was used. The major features of the 260D pump include: high volume capacity (260 ml), wide flow range with excellent accuracy and precision (1 l to 90 ml/min), high pressure range with excellent accuracy and precision (7500 psi), high corrosion resistance and wide solvent compatibility of components, smooth DC motor operation with stepper motor-like resolution and quartz locked speed control, pressure gradient and flow rate composition gradient programmability, eliminating the need for an external computer, 4-line LCD menu type displayer, memory for multiple methods (up to 99 total steps), ability of one controller to operate up to 3 „D" series pumps simultaneously (or independently) and built-in modifier addition program (Myer et al, 1991). The SFE experiments were performed by carbon dioxide (purity 99.995% (w/w), supplied by Linde, Hungary).

In the case of the analysis of volatile compounds of savory, basil and lemon balm, GC-FID analysis was performed, using Agilent Technologies 6890N GC System: injector temperature: 250 C, split rate: 22,6:1; colonna: HP-50 50% Phenyl Methyl Siloxane, length: 30 m, diameter: 350 µm, film thickness: 0,25 µm; carrier gas: helium, linear velocity: 0,5 ml/min, constans flow. Temperature program was as follows: start temperature of 50 °C/0,5 min, then 4 °C/min till 150 °C, kept up to 10 min. Detector (FID) temperature was 250 °C. Volatile compounds were identified by comparing their retention time to those of the authentic standards. Standards were purchased at Sigma Aldrich.

GC-MS analysis was carried out by Agilent Technologies GC 6890N using a HP-5 MS colonna, where detector was Agilent Technologies MS 5975. Colonna length: 30 m, diameter: 250 µm, film thichness: 0,25 µm, carrier gas: helium, constans flow rate of 0,5 ml/min. Injector and detector temperature: 250 °C. Temperature program: 50 °C /0,5 min, then 4 °C/min – till 150 °C, then 12 °C/min to 220 °C/10 min.

Component identification was based on mass spectra (ionisation energy: 70 eV), NIST library, own volatile library as well as on retention time.

Nonvolatile compounds were analyzed at the HPLC laboratory of the Department of Pomology Corvinus University of Budapest. After methanol extraction, cleaning and filtering, 20 µl of the extract was used for determination with a WATERS HPLC (717 plus auto-sampler, 1525 binary HPLC pump, 2487 dual absorbance detector, 350 nm, SYMMETRY C18 5µm 4.6X150 column). Analyzing conditions: 2.5% acetic acid in water (350 ml), MeOH (50 ml), acetonitrile (100ml) as mobile phase; flow-rate was 1 ml/min; column pressure was 1750 ± 10 psi. The analysis was assisted by EMPOWER 2TM software (*Meixner* et al., 2007; *Végvári & Brunori*, 2007).

Statistical analysis was performed by Statistica 7.0 program.

Results

The extract yield of SFE was comparable to the essential oil amount obtained by (hydrodistilled) HD from the same drug.

Yield and composition of SFE extracts were different, when comparing our data to the literature. In the case of **summer savory** the extraction was carried out using different pressures (between 8 and 30 MPa at constant 40 °C and 30 min). In this interval an increasing tendency was observed regarding extraction yields. The highest value was detected at 27 MPa (0.78 g/100g dry material). In the temperature optimizing experiment (using different temperatures and constant 12 MPa for 30 min) the highest extract yield was detected at 50 °C (0.37 g/100g dry material)

(*Figure 1*). When studying the effect of extraction time (at constant 12 MPa and 40 °C) the highest extract yield was produced during 55 min (0.50 g/100g dry material). The effect of the extraction time was significant (p=0.00058). However, none of these results reached the amount of the destillate (1.12 g/100 g DW). In the volatile rich fraction of SFE extracts ratio of α-terpinene was lower but the ratio of carvacrol higher than in the HD extract. According to the literature, 12 Mpa and 60 min were recommended (Esquivel et al., 1999), however, based on our results, the parameters of 27 MPa and 55 min seem to be more advantageous obtaining highest extract yields. During pressure optimization for non-volatile compounds (applying pressures set between 30 and 50 MPa), the highest extract yield was measured at 43 MPa (1.00 g/100g DW). Quercetin was detected in each extract, the highest amount was observed at the pressure of 40 MPa (0.44 mg/ml). At pressures of 40 and 45 MPa eriodyctiol was also detected in the amounts of 1.336 mg/ml and 1.910 mg/ml, respectively. Luteolin was detected at pressure 35. 40 and 45 MPa, with the highest value at 45 MPa (1.06 mg/ml). When using methanol as modifier solvent the obtained yields were higher at 40 and 50 MPa. For establishing the optimal extraction parameters, more experiments are need to be done. Simlarly to the simple SFE extracts quercetin was detected in each samples in higher amounts (up to 0,33 mg/ml at 40 MPa and 40% methanol). Luteolin was measured in each sample, with the highest value at 40 MPa and 40% methanol (2.94 mg/ml). Ursolic acid was detected in the highest quantity in our samples. The best value was reached at 50 MPa and 10% methanol (3129.15 mg/ml). In the other samples was the ratio of ursolic acid prominent.

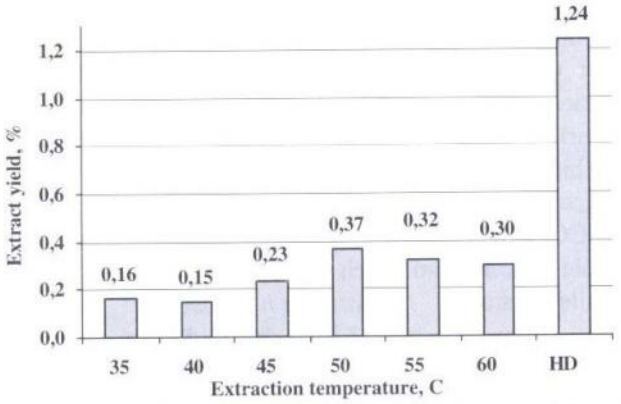

Figure 1 Optimization of extraction temperature in the case of *Satureja hortensis* (constant 10 MPa and 40 °C)

In the case of **basil,** the extraction was carried out using different pressures (between 8 and 30 MPa at constant 40°C and 30 min). In this interval, an increasing tendency was observed regarding extract yields. The highest values were detected at the pressure of 20 MPa (0.31 g/100g dry material) and of 27 MPa (0.39 g/100 g DW). In the temperature optimizing experiment (using different temperatures and constant 12 MPa for 30 min) the highest extract yield was detected at 50°C (0.30 g/100g dry material). When studying the extraction time (at constant 12 MPa and 40°C) the highest extract yield was produced during 40 min (0.33 g/100g dry material). However, none of these results reached the amount of the destillate (0.67 g/100 g DW). In the volatile rich fraction of SFE extracts ratio of linalool and estragol was lower then in the distilled essential oil. According to the literature, 12 MPa, 30 min and 40 °C were previously recommended (Consuelo Díaz-Maroto et al., 2002), however, based on our results, the parameters of 11 MPa, 50°C and 40 min seem to be more advantageous obtaining higher extract yields. During pressure optimization for nonvolatile compounds (applying pressures set between 30 and 50 MPa), the highest extract yield was measured at 40 MPa (0.71 g/100g DW) (*Figure 2*). Luteolin was detected in each extract, the highest amount was observed at the pressure of 45 MPa (105.99 mg/ml). At pressures of 35 and 45 MPa ursolic acid was also detected in the amounts of 161.23 mg/ml and 153.51 mg/ml, respectively. When using methanol as modifier solvent the obtained yields were higher at 40 and 50 MPa (0.49 and 0.43 g/100g dry material). For establishing the optimal SFE parameters, more experiments are need to be done. Simlar to the simple SFE extracts luteolin was detected in each samples in rather different amounts (0.49–100.71 mg/ml). The highest quantity was measured at 40 MPa with 40% methanol as modifier. The solvent modifier had no effect on the solubility of other non-volatile compounds accumulated by the plants.

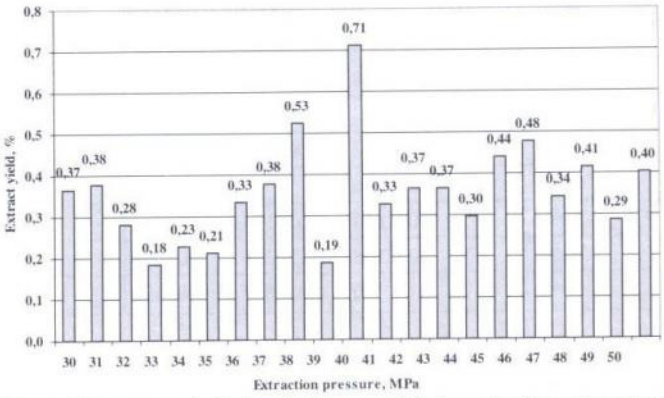

Figure 2 Pressure optimisation in the case of *Ocimum basilicum* (constant 40 °C and 30 min)

In the case of time optimalization, significant correlation was detected between the time period and the ratio of linalool and estragol in the volatile fraction (p=0.000924 and p=0.000049).

In the case of **lemon balm** the same extraction parameters were tested. During pressure optimization (at constant 40 °C and 30 min) the highest extract yield could be measured at 28 MPa (0.26 g/100g DW), and this value was much higher than that of the distilled oil (0.06 ml/100g DW). Within the volatile fraction of SFE extracts, the ratio of the main components (geranial, neral and citronellal) was lower than in the distilled essential oil. When optimizing extraction time (by constant 12 MPa and 40 °C) the best result was detected after 30 min (0.11 g/100g dry material), this value was almost twice higher than that of the HD yield. After 30

min the amount of geranial increased considerably. After the 60 min extraction the ratio of caryophyllene oxide has also increased in the volatile fraction of SFE extracts. In the temperature optimalization the highest extract yield was detected at 40 °C (0.11 g/100g dry material). In general, the ratio of the main components in the SFE extracts was lower than within the essential oil of HD. Using SFE, the citral compounds can be extracted from lemon balm more

Table 1 Nonvolatile compounds in the case of *Melissa officinalis* (mg/ml) (constant 40°C and 30 min)

Pressure (MPa)	Rosmarinic acid	Eryodictiol	Luteolin	Ursolic acid
30	0.002792	0	0	0
35	0	0	0	0
40	0.00431	0	0	0
45	0.001208	0	0	3.060052
50	0.003012	0	0	0.63885
Methanol (%) (30 MPa)				
10%	0.005898	0	0	0
20%	0	0	0	0
30%	0	0	0	0
40%	0	0.007482	0.002424	0
50%	0	0	0	0
Methanol (%) (40 MPa)				
10%	0	0	0	0
20%	0.012455	0.041528	0	0
30%	0	0.279067	0.013724	0
40%	0	0	0.00545	0
50%	0.024308	0	0	0
Metanol (%) (50 MPa)				
10%	0	0.007243	0.001236	0
20%	0.029946	0	0	0
30%	0.021614	0.024114	0.004811	0
40%	0.037738	0.259294	0.03229	0
50%	0	0.037559	0.00859	0

efficiently and faster than with hydrodistillation (hydrodistillation lasted to 40 min, while SFE needed only 20 min). Increased amounts of citronellal, neral, neryl acetate, and caryophyllene oxide were found in hydrodistillation, while SFE yielded increased proportion of geranial and caryophyllene. In addition, SFE yielded a greater total amount of compounds detected when compared to hydrodistillation (*Rozzi* et al., 2002). In the pressure optimalization for non-volatile compounds (between 30 and 50 MPa), high extract yields were measured at 31 and 45 MPa (0.31 and 0.40 g/100g DW), where the best value was obtained at 47 MPa (0.749 g/100g DW). Highest amount of rosmarinic acid was observed at 30 and 50 MPa (0.00028 and 0.00030 ml/mg) (*Table1*). Ursolic acid was measurable only at 45 and 50 MPa (0.30601 and 0.06389 ml/mg). In the case of lemon balm, the modifier solvent had no significant effect on the solvent power, the extract yields were almost the same as in the case of SFE-CO_2. The highest yields were obtained at 50 MPa pressure applying methanol (30%) as modifier (0.48 ml/100g DW), at 30 MPa applying methanol (5 and 30%) as modifier (0.332 and 0.32 ml/100g dry material), and at 40 MPa applying methanol (30, 40 and 45%) as modifier (0.33; 0.33 and 0.35 g/100g dry material). The composition of these extracts were various. Rosmarinic acid was detected in the highest amount at 30 MPa pressure with 10% methanol as modifier (0.00589 mg/ml), at 40 MPa with 50% methanol as modifier (0.02431 and 0.02995 mg/ml) and at 50 MPa with 20% methanol as modifier. Luteolin was detected in low quantity in all of these extracts. The highest amount of eriodictiol was obtained at 40 MPa with 30% methanol as modifier (0.27907 mg/ml).

In the case of pressure optimalization, significant effect of the pressure was found on the amount of geranial ($p=0.030617$) and by applying modifer on the extract yield ($p=0.000383$).

Conclusions

It has been demonstrated that SFE can produce superior quality products characterized by the absence of artefacts and by a better reproduction of the original flavour or fragrance.

Concerning *optimalization experiments*, the main effects of the four optional parameters – pressure, temperature, time and modifier – were evaluated with relevance to the yield and to the composition of extracts. It was established that the effect of pressure with or without modifier was the most significant on the above mentioned characters. In some cases, the SFE extract yields reached or were higher than the amount of essential oil obtained by hydrodistillation. Regarding different temperatures of extraction, it can be concluded that the results failed to reach the essential oil level of HD. The effect of extraction time was not proven to be really significant in the case of basil and lemon balm. In few cases, similarly to the previous parameter, the yield of SFE extracts were higher than the essential oil amount of HD.

According to our results, the following conditions of SFE-CO_2 can be determined as optimal parameters to obtain volatile or non-volatile-rich extracts:

Volatile fraction:	*Satureja hortensis:* 27 MPa, 50 °C, 55 min
Ocimum basilicum:	25–27 MPa, 45–50 °C, 40–55 min
Melissa officinalis:	13 MPa, 40 °C, 30 min
Nonvolatile fraction:	*Satureja hortensis*: 40–45 MPa
yield:	40–45 MPa (10–50% methanol)
composition:	*depends on the component*
Ocimum basilicum:	35–45 MPa
yield:	50 MPa, 5–50% modifier,
composition:	40 MPa, 40% modifier
Melissa officinalis:	45–47 MPa
yield:	30–50 MPa, 5–50% modifier,
composition:	depends on the component

The parameters determined can serve as a basis for higher scale extraction of *Lamiaceae* drugs. More research work is still necessary in all aspects related to the supercritical fluid extraction of essential oils.

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