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Yield and composition of supercritical fluid extracts of different *Lamiaceae* herbs

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Summary: In our work the effectiveness of supercritical carbon dioxide extraction was studied on volatile components of *Lamiaceae* herbs. The aim of our investigations was to determine the optimal extraction parameters yielding high amount of volatiles in a desirable composition. As plant materials, dried and powdered cude drugs of *Thymus vulgaris, Thymus pannonicus, Satureja hortensis, Ocimum basilicum, Rosmarinus officinalis* and *Salvia officinalis* were chosen. Pressure (8–30 MPa), temperature (35–60 °C) and time (5–60 min) of extraction were regulated. Results obtained by supercritical fluid extraction (SFE) were compared to that of the conventional extraction procedur, hydrodistillation (HD). In the case of *Thymus vulgaris, Thymus pannonicus* and *Salvia officinalis,* extract yield of SFE was comparable to the essential oil amount obtained by hydrodistillation from the same drug. Essential oil rich thyme extracts were analysed by GC-FID. We have found that yield and quality of SFE extracts highly depend on the conditions of extraction.

Key words:supercritical fluid extraction, *Lamiaceae, Thymus pannonicus, Thymus vulgaris, Satureja hortensis, Ocimum basilicum, Rosmarinus officinalis, Salvia officinalis*

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 non-volatile compounds of the species belonging to the *Lamiaceae* family play an important role in phytotherapy, aromatherapy and as food additives, as well. Garden thyme (*Thymus vulgaris* L.), Hungarian thyme (*Thymus pannonicus* All.), summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus officinalis L.*) and sage (*Salvia 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 (*Javanmardi* et al., 2002). Essential oil extracted from fresh leaves and flowers can be utilized as additives in food or in cosmetics as well as in pharmaceuticals (*Senatore*, 1996).

Supercritical fluid extraction (SFE) is an extraction method using supercritical fluids as extraction solvent instead of normal liquids. It has been shown that SFE with supercritical carbon dioxide (SFE-CO_2) 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 31,1C and 7,38 MPa (=1050 psi), respectively.

Supercritical fluids have densities and solvating powers similar to liquid 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 composition of the supercritical CO_2 extracts.

Oszagyán et al. (1996) appointed that in the case of garden thyme the SFE method the decreased the content of thymol and increased the ratio of carvacrol compared to HD.

Aleksovski et al. (2001) investigated wild thyme. They found that SFE extracts contain water and cuticule wax and their amount increasing by the extraction time and pressure was increased in the extracts.

Regarding Satureja hortensis, is considered SFE as alternative investigation method, although the SFE extraction yields are obtained lower than HD yield. The highest yield of SFE was obtained at 50 °C and 50 MPa. Extract composition reached at the pressure value of 10 MPa and temperature of 40 °C was near to nature composition (*Pluhár* et al., 1996).

Materials and methods

Dried leaves of garden thyme (*Thymus vulgaris* L.), Hungarian thyme (*Thymus pannonicus* All.), summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) were grown and surveyed in Budapest, Hungary in 2006.

The plant (100 g of dried material of rosemary, sage, savory and garden thyme; 200 g of Hungarian thyme and basil) 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 both thyme species.

Supercritical fluid extraction (SFE): optimal parameters in the respect of extract amount and of the composition were investigated, where the pressure (8–30 MPa), temperature (35–60 °C) and time (5–60 min) were regulated. During pressure, time or temperature experiments the other two parameters were constant.

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 garden and Hungarian thyme, GC analysis was performed. GC-FID analysis was 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.

Results and Discussion

We have opportunity in case of *garden thyme* and *Hungarian thyme* for investigating composition of the volatile oils, the other experiments are in progress.

In the *pressure optimization* experiment (performed at constant 40 °C and 30 minutes) of **garden thyme** we found that the yield of SFE extracts reached the amount obtained by HD (1,848 ml/100 g) in some cases (at 13, 17, 22 and 28 MPa). The main components of HD extracts were thymol, γ-terpinene and p-cymene, however, the SFE extracts were mainly characterised only by thymol and p-cymene, while other components were found just in very small concentrations (*Figure 1*).

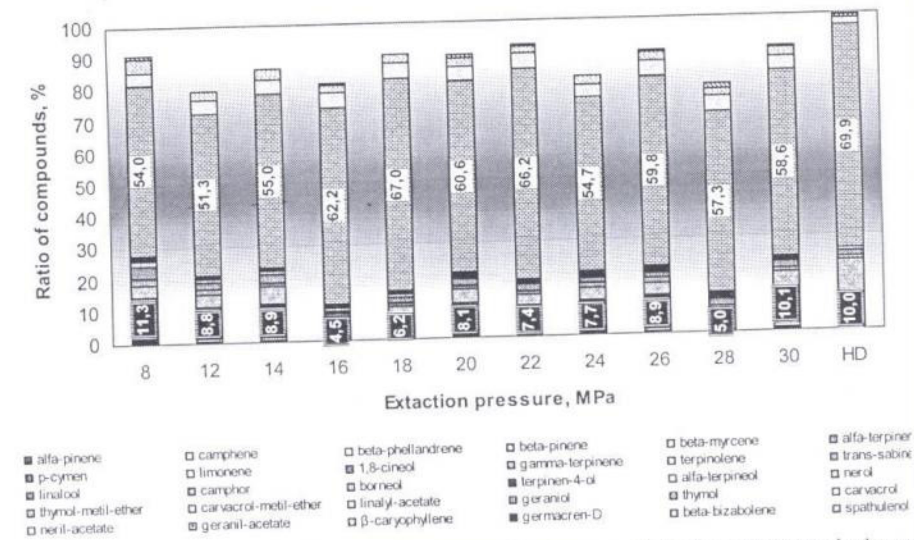

Figure 1 Changes in the volatile oil composition during the pressure optimization experiment in the case of *Thymus vulgaris* (constant 40 °C and 30 minutes)

In the case of *temperature optimization* (performed at constant 10 MPa and 30 minutes) SFE extracts did not reach the amount of HD extract, the highest results was observed at 45 C (1,133 ml/100 g) (*Figure 2*). In these SFE extracts lower ratio of thymol and p-cymene and a little higher carvacrol percent were determined, comparing them to distilled essential oil.

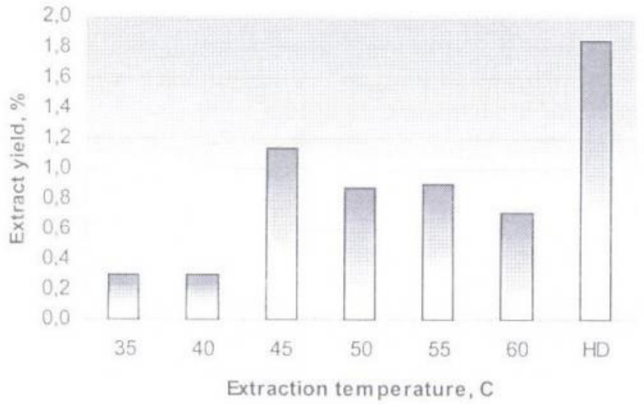

Figure 2 Optimization of extraction temperature in the case of *Thymus vulgaris* (constant 10 MPa and 30 minutes)

In *time experiment* (performed at constant 10 MPa and 40 °C), extraction time of 30 minutes have been found to be the best, as it resulted in the highest yield (1,16 ml/100 g). When comparing the main components of SFE extracts to hydrodistilled oils, it can be concluded that the proportion of thymol was nearly identical in both extracts, while carvacrol ratio was higher and p-cymene ratio was lower in SFE than in the HD extract.

In the case of *pressure experiment* (performed at constant 40 °C and 30 minutes) of **Hungarian thyme**, SFE extraction did not reach the yield of the hydrodistillation (0,926 ml/100 g), though, results obtained at 24 and 26 MPa were near to it (performed at constant 40 °C and 30 minutes). The volatile-rich SFE extracts of Hungarian thyme contained thymol and α-bisabolene as main components, while HD produced an essential oil containing thymol, γ-terpinene, thymol-methylether, carvacrol-methylether and p-cymene (*Figure 3*).

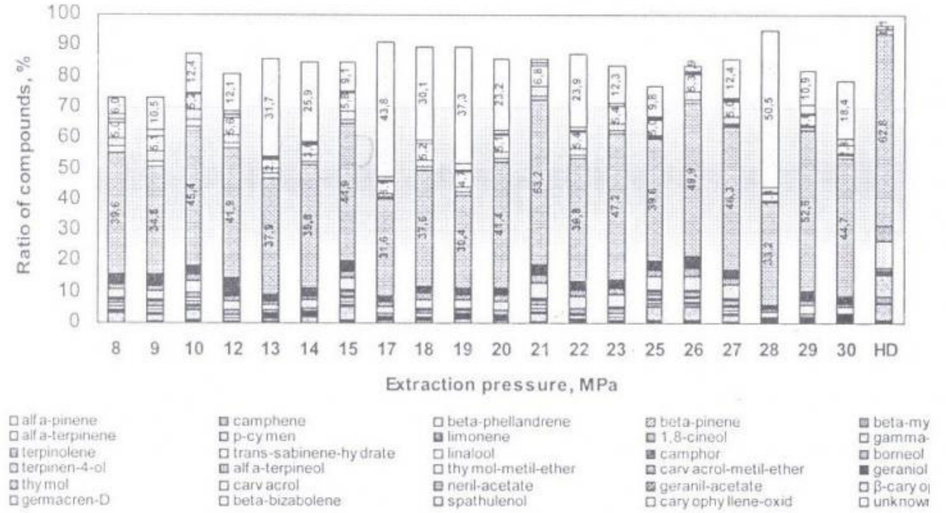

Figure 3 Changes in the volatile oil composition during the pressure optimization experiment in the case of *Thymus pannonicus* (constant 40 °C and 30 minutes)

In the *temperature experiment* (performed at constant 10 MPa and 30 minutes), temperature of 55 °C yielded the highest extract amount (0,42 ml/100 g). In the case of 45 °C, ratio of thymol was higher, while the proportion of other components (γ-terpinene, p-cymene, carvacrol-methylether, tymol-methylether) were lower than in the HD extract.

During optimization of *extraction time* (performed at constant 10 MPa and 40 °C) 20 minutes resulted in the highest yields (0,34 ml/100 g) (*Figure 4*). In these SFE extracts the ratio of thymol was much lower, while the amount of β-bisabolene and β-caryophyllene were slightly higher than in the HD extract.

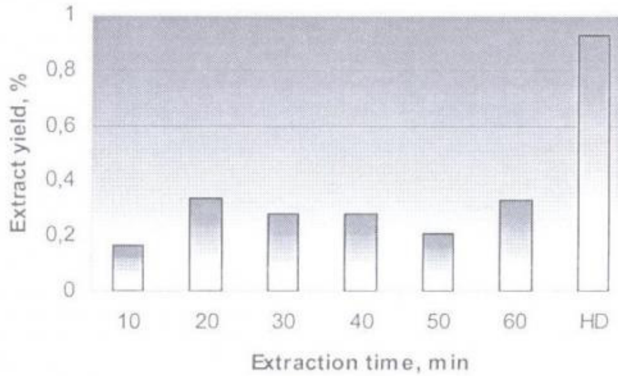

Figure 4 Optimization of extraction time in the case of *Thymus pannonicus* (constant 10 MPa and 40 °C)

At **summer savory**, the yield of hydrodistillation was 1,24 ml/100 g. This value cannot be exceeded by SFE extraction, where the highest extract yield (0,59 ml/100g) was obtained during *pressure experiment* (performed at constant 40 °C and 30 minutes), at 11 MPa. During optimizing *extraction time* (performed at constant 12 MPa and 40 °C), the highest values could be reached at 60 minutes (0,50 ml/100 g), while in the case of *temperature experiment* (performed at constant 120 MPa and 30 minutes), at 55 °C (0,50 ml/100 g) (*Figure 5*).

When extracting **basil** herb (performed at constant 40 °C and 30 minutes), the best result was detected at 24 MPa, however, this was much lower than the yield of HD (0,67 ml/100 g). During the *temperature optimization* (performed at constant 12 MPa and 30 minutes) the highest value was obtained at 60 °C (0,21 ml/100g). Regarding *extraction time* (performed at constant 12 MPa and 40 °C), 35 minutes was chosen as optimal value (0,87 ml/100g) (*Figure 6*).

Concerning **rosemary** leaves, 1,648 ml/100g essential oil was extracted by HD. The best SFE results were obtained by a *pressure* (per-

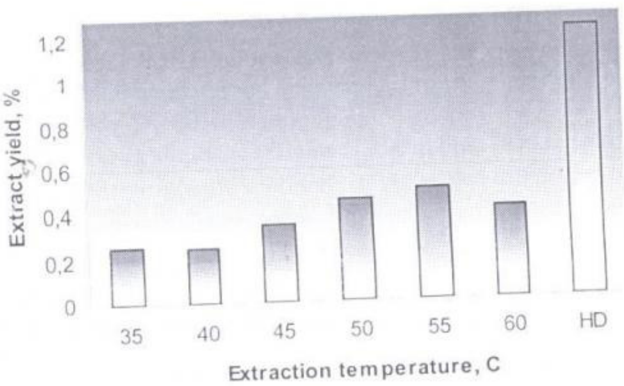

Figure 5 Optimization of extraction temperature in the case of *Satureja hortensis* (constant 12 MPa and 30 minutes)

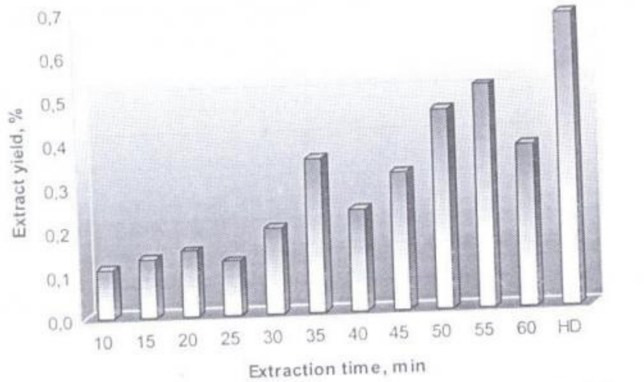

Figure 6 Optimization of extraction time in the case of *Ocimum basilicum* (constant 12 MPa and 40 °C)

formed at constant 40 °C and 30 minutes) of 29 MPa (0,946 ml/100 g), at the *temperature* (performed at constant 11 MPa and 30 minutes) of 60 °C (1,44 ml/100 g) and during 55 *minutes* (1,66 ml/100 g) (performed at constant 11 MPa and 40 °C).

According to the results of the *pressure optimization* (performed at constant 45 °C and 30 minutes) of **sage**, the highest yield could be reached at 28 MPa (1,99 ml/100 g) and this result was higher than that of the HD (1,767 ml/100 g). During the *time optimization* experiment (performed at constant 11 MPa and 45 °C) high values were obtained at 30 and 60 minutes, though none of these values reached the yield of HD extract. Considering the *temperature optimization* (performed at constant 11 MPa and 30 minutes), the optimal value (1,71 ml/100 g) was measured at 45 °C.

Conclusions

Applying SFE, in the case of thyme herbs resulted in extracts containing wider spectra of monoterpenes and sesquiterpenes than the paralelly analysed HD essential oils.

Concerning *optimalization experiments*, the main effects of the three optional parameters – pressure, temperature and time of extraction – were evaluated with relevance to the yield of extracts. It was established that the effect of pressure was the most significant on the above mentioned character. In some cases the results reached or were higher than the essential oil amounts 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 garden thyme, Hungarian thyme, summer savory and basil. We observed influence of this parameter only on rosemary. In a 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-rich extracts:

- *Thymus vulgaris*: 17 MPa, 45 °C, 30 minutes
- *Thymus pannonicus*: 24 MPa, 55 °C, 20 minutes
- *Satureja hortensis*: 11 MPa, 55 °C, 60 minutes
- *Ocimum baasilicum*: 24 MPa, 60 °C, 55 minutes
- *Rosmarinus officinalis*: 29 MPa, 60 °C, 55 minutes
- *Salvia officinalis*: 28 MPa, 45 °C, 30 minutes.

The parameters determined can serve as a basis for higher scale extraction of *Lamiaceae* drugs.

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