Supercritical CO_2 extraction of mentha (*Mentha piperita* L.) at different solvent densities

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Abstract: The chemical composition of mentha essential oil and mentha extracts obtained at different pressures/temperatures by supercritical fluid extraction (SFE) were studied by GC–MS. The menthol content was also determined spectrophotometrically. The predominant compounds in the essential oil and in the CO_2 extract obtained at 100 bar were L-menthon and menthole but at higher pressures (from 150 to 400 bar), squalene was dominant. The equation of Naik *et al*. was used for modelling the mentha–supercritical CO_2 system.

Keywords: *Mentha piperita* L.; essential oil; supercritical fluid extraction; extraction pressure and temperature; GC–MS.

INTRODUCTION

The *Labiatae* family has several members with significant essential oil contents. Mentha essential oil is an important material for perfumery, as a flavour, for liquors, *i.e*., in cosmetics, in toothpastes, as well as spices in the food industry. Parts of the mint-family plant, mainly dry leaves, are used for tea worldwide. Commercial oils could be classified by their menthol or carvone content.[1–3]

The classical procedures for the separation of the active substances from plant material, *i.e*., steam distillation and extraction with organic solvents (*e.g*., dichloromethane) have serious drawbacks. The distillation procedure allows only the separation of volatile compounds (essential oils), which, to a greater or lesser extent, are transformed under the influence of the elevated temperature. On the other hand, extraction with organic solvents can hardly render an extract free of traces of the organic solvent, which are undesirable for organoleptic and/or health reasons. In addition, organic solvents are insufficiently selective, hence, in addi-

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tion to the active substances, some concomitant compounds are also dissolved. For these reasons, supercritical fluid extraction (SFE) with carbon dioxide (CO_2) has recently gained in importance as an alternative to the classical procedures. Extraction procedures involving supercritical CO_2 belong to "clean technologies", with no secondary products polluting the environment. CO_2 is the most widely used medium in SFE because it is simple to use, inexpensive, non-flammable, non-toxic, chemically stable, shows great affinity to volatile (lipophilic) compounds and can be easily and completely removed from any extract. By changing the pressure and/or temperature above the critical point of CO_2 ($t_c = 31.3$ °C; $p_c = 72.8$ bar; $d_c = 0.467$ g/ml), a pronounced change in the density and dielectric constant, *i.e.*, solvent power, of supercritical CO_2, can be achieved.[4–7] SFE of the essential oil from different mentha varieties, as well as GC analysis of the mentha essential oil and other extracted compounds have been investigated by many authors.[1–3,8–10]

In this study, the chemical composition of mentha essential oil (EO) and the mentha extracts obtained at different pressures and temperatures by supercritical fluid extraction (SFE) using CO_2 were studied. The chemical composition of the mentha EO and CO_2 extracts were compared. The SFE–CO_2 kinetics was described by the well-known model equation of Naik *et al*.[11]

EXPERIMENTAL

Plant material and chemicals

Mentha (*Mentha piperita* L.) was grown by the Institute of Field and Vegetable Crops, Novi Sad, Serbia (year 2004). The dry and milled (mean particle size 0.60 mm) leaves of mentha were used in this work.

Standard sample of (–)-menthol (Fluka AG, Switzerland) was used. Commercial carbon dioxide (Tehno-gas, Novi Sad, Serbia) was employed as the extracting agent. All other chemicals were of analytical reagent grade.

Procedures

The mentha essential oil (EO) content (3.19 %; v/w) was extracted by steam distillation, using the prescribed procedure in Ph. Jug. IV.[12]

The supercritical fluid extraction (SFE) with carbon dioxide was performed using a previously described laboratory-scale high pressure extraction plant – HPEP (Nova-Swiss, Effretikon, Switzerland).[13] The main parts and characteristics (manufacturer's specification) of the plant are: a diaphragm-type compressor (up to 1000 bar), extractor with an internal volume of 200 ml ($p_{max} = 700$ bar), separator with an internal volume 200 ml ($p_{max} = 250$ bar) and maximum CO_2 flow rate of about 5.7 kg/h. The mass of mentha (mean particle size 0.6 mm) in the extractor was 50 g and a CO_2 flow rate of 3.225 g/min was employed. The pressure and temperature were varied (see Tables I and II). The separator conditions were: pressure: 15 bar and temperature: 25 °C.

Analyses

A basic standard solution of menthol (10 mg/100 ml in 95 % ethanol) was used for obtaining the solutions with different menthol concentrations (from 0.05 to 0.70 mg/10 ml). To each standard solution (2 ml), a 0.10 % solution (3 ml) of *p*-dimethylaminobenzaldehyde

in cc. H_2SO_4 was added. After 10 min, absorbance of solution was measured at 510 nm. Obtained values were used for the construction of a calibration curve for the menthol content.

TABLE I. Operating conditions and extraction yields obtained at 40 °C

Extract No.	Pressure, bar	CO_2 density, g/ml	Extraction time, h	Extraction yield, %
1	100	0.630	2.5	3.57
2	150	0.790	0.5	0.44
3	200	0.840	0.5	0.40
4	300	0.910	0.5	0.23
5	400	0.957	0.5	0.21

TABLE II. Operating conditions, extraction yield and menthol content in the extracts obtained at different extraction temperatures (pressure: 100 bar, extraction time: 2.5 h)

Extract No.	Temperature, °C	CO_2 density g/ml	Extraction yield, %	Menthol content in extract %
1	35	0.735	2.94	32.04
2	40	0.630	3.57	28.54
3	45	0.520	2.71	26.53
4	50	0.375	2.91	33.62

The dependence of the menthol mass (m_M in mg) on the absorbance (A) could be expressed as:

$$m_M = \frac{A + 3.71 \times 10^{-3}}{6.8625} \tag{1}$$

with a linear regression coefficient was 0.987. The content of menthol in the oil and extracts was calculated using Eq. (1).

The GC–MS measurements were realise using a GCD HP G 1800 A (Hewlett-Packard, Palo Alto, CA, USA) instrument with HP-5 MS column (30.0 m×0.25 mm i.d., film thickness 0.25 μm). The carrier gas was helium at a flow rate of 0.80 ml/min. The temperatures of the injector and detector (45-425 D) were 250 and 280 °C, respectively. The column was heated from the initial 50 °C (3 min) with a linear increase of 20 °C/min to 130 °C (1 min) and 9 °C/min until the final 280 °C (10.33 min). The total analysis time was 35 min. The injected volume of sample solution in dichloromethane was 1 μL. The compounds were identified using the Wiley data base.

All investigations were performed in triplicate and the given results are mean values.

RESULTS AND DISCUSSION

The menthol content in the mentha essential oil as determined by the spectrophotometric method was 30.67 %.

The GC–MS method was used to determine the qualitative and quantitative composition of obtained mentha essential oil (EO) (Table III). The contents of the compounds the EO are expressed as % on the basis of the menthol content obtained by the spectrophotometric method, as well as by the external standard method using standard sample of (–)-menthol. The content of menthol determined by these two methods for quantification was similar and for calculation of other compounds, the mean value of the menthol content was used.

TABLE III. Composition of the essenial oil (EO) obtained by steam distillation and extracts obtained at 40 °C under different extraction pressures

| Compound | Content of compound, % | | | | | |
| | EO | Pressure, bar | | | | |
		100	150	200	300	400
D,L-Limonene	0.46	0.23	–	–	–	–
Limonene	0.98	0.54	–	–	–	–
Cineole	4.04	2.32	–	–	–	–
β-Terpineol	2.90	2.73	0.40	0.35	–	–
L-Menthone	37.15	27.08	2.18	1.98	1.74	–
Iso-menthone	10.33	8.79	0.79	0.76	–	–
Menthol	30.67	28.54	5.47	6.54	6.09	3.39
4-Terpineol	1.48	1.22	–	–	–	–
α-Terpineol	0.48	0.70	–	–	–	–
Pulegone	2.40	2.22	0.22	–	–	–
Piperitone	0.95	0.76	–	–	–	–
Menthyl acetate	5.46	4.67	0.69	0.84	0.72	–
β-Caryophyllene	0.76	4.34	0.80	1.05	0.90	–
Nerolidol	–	0.58	–	–	–	–
Germacrene-D	–	5.03	0.86	0.97	0.92	–
Bicyclogermacrene	–	1.65	1.31	0.35	–	–
β-Bisabolene	–	0.35	–	–	–	–
β-Elemene	–	0.23	–	0.15	–	–
Viridiflorol	–	0.39	–	0.15	–	–
trans-Pinene	–	0.17	0.19	0.50	0.43	1.62
Palmitic acid	–	–	1.16	0.98	0.83	0.80
9,12,15-octadecatriene-1-ol	–	–	3.77	3.81	5.57	8.96
Linoleic acid	–	–	0.47	0.31	0.41	–
Diethylmethylborane	–	–	3.08	1.90	–	–
Vitamin K_1	–	–	–	2.68	–	–
14-β-Pregnane	–	–	0.98	0.34	5.49	6.99
Hexacosane	–	0.59	7.29	7.27	16.20	18.60
Octacosane	–	0.28	1.71	1.09	1.39	1.50
Vitamine E	–	–	16.20	2.38	11.72	16.91
8-Hydroxysclerodine	–	–	5.95	2.54	0.73	1.30
Neophytadiene	–	0.49	0.60	2.08	2.27	3.60
Tricosane	–	–	29.26	28.32	3.00	–
Tetratriacontane	–	–	–	0.45	1.00	1.73
Heptacosane	–	–	0.92	0.68	1.88	1.85
Stigmast-5-en-3-ol	–	–	–	5.18	–	–
Ergost-5-en-3-ol	–	–	–	5.16	–	–
Squalene	–	1.76	4.92	11.40	25.67	23.55
Nonadecane	–	–	3.17	3.78	4.29	4.33
Triacontane	–	0.52	4.32	2.27	3.27	0.54
Total	98.06	96.18	96.71	96.26	94.53	95.67

L-Menthone was present in the highest amount (37.15 %) in the mentha EO. The most important mentha compound, menthol had a content of 30.67 %. In addition, the other predominant compounds of the mentha EO were iso-menthone (10.33 %) and menthyl acetate (5.46 %). These four compounds made up 83.61 % of the mentha EO.

The SFE of mentha by CO_2 was performed at a pressure of 100 bar and a temperature of 40 °C for 2.5 h (*i.e.*, the same time as prescribed for steam distillation by Ph. Jug. IV[12]), whereby the first extract was obtained (extract No. 1). The same mentha sample was extracted at a higher pressure (150 bar) for 0.5 h whereby the second extract was separated (extract No. 2). The extraction yields obtained under different extraction conditions are shown in Table I.

The results of the GC–MS analysis of the extracts No. 1 to No. 5 are shown in Table III.

The extraction yield of extract No. 1 (obtained at a pressure of 100 bar and 40 °C; solvent density 0.630 g/ml) was higher (3.57 %) by about 12 % than the amount of EO obtained by steam distillation (3.19 %). Extract No. 1 had a similar composition to mentha EO. However, because of the co-extraction of compounds not present in the EO obtained by steam distillation, the contents of L-menthone and menthol were lower (27.08 and 28.54 %, respectively). In this extract, the total % of the four main compounds (menthone, iso-menthone, menthol and menthyl acetate) was also lower (69.08 %) than in the EO (83.61 %).

By increasing the pressure to 150 bar (and subsequently to 200, 300 and 400 bar), an attempt was made to perform selective extractions, *i.e.*, to determine the remaining components in the mentha sample without the EO. The yield of the remaining components obtained at 150 bar was only 0.44 % (Table III shows the content the compounds in extract No. 2). The predominant compounds of extract No. 2 were tricosane (29.26 %) and Vitamin E (16.20 %), with traces of the mentha EO compounds. Primarily, the yields of menthol and menthone show the incomplete extraction of EO (*i.e.*, only about 24 mg of menthol and 10 mg of menthone were extracted in the steam distillation in comparison to 1019 mg of menthol and 967 mg of menthone obtained from 100 g of menthe present in extract No. 1).

By increasing the pressure to 200 bar, the extraction yield was a lower (0.40 %) than that obtained at 150 bar (0.44 %). The predominant compounds in extract No. 3 were tricosane (28.32 %) and squalene (11.40 %). Menthol (6.54 %) and menthone (1.98 %) were still present in this extract. Then, the extraction was continued at a pressure of 300 bar and extract No. 4 was obtained in very low yield (0.23 %). Menthol (6.09 %) and menthone (1.74 %) were detected, but the predominant compounds of extract No. 4 were squalene (25.67 %) and hexacosane (16.20 %), as well as Vitamin E (11.72 %). Finally, the extraction at 400 bar yielded in 0.21 % of dry extract (extract No. 5). Menthone was not detected in

the final extract, but menthol was (3.39 %). This percent of menthol corresponded to about 7 mg/100 g of mentha leaves. The predominant compounds of extract No. 5 were squalene (23.55 %), hexacosane (18.60 %) and Vitamin E (16.91 %). The total % of the four important mentha compounds (menthone, iso-menthone, menthol and menthyl acetate) in extracts No. 2 to No. 5 were 9.13, 10.12, 8.55 and 3.33 %, respectively.

For the investigation of the influence of temperature on the SFE of mentha, a pressure of 100 bar was chosen. The temperatures of extraction were 35, 40, 45 and 50 °C. The extraction procedure, as well as extraction yield and menthol content in the obtained extracts are shown in Table II.

The highest extraction yield was 3.57 % at a temperature of 40 °C (*i.e.*, 1019 mg/100 g of mentha leaves of menthol were extracted). The highest content of menthol in the extract obtained at 50 °C (33.62 %) corresponds to a menthol extraction yield of 980 mg/100 g of mentha leaves. The effect of operating temperature on the extraction yield, as well as on menthol yield, could be explained through the solvent density obtained at the investigated temperatures. The solubility of the mentha compounds was the highest at a CO_2 density of 0.630 g/ml, *i.e.*, at a temperature of 40 °C and a pressure of 100 bar than at the other investigated densities, *i.e.*, temperatures (Table II).

For modelling of the investigated extraction system, mentha–supercritical carbon dioxide, the simple equation of Naik *et al.*[11] was employed:

$$y = \frac{y_\infty t}{b + t} \tag{2}$$

where: y is the extraction yield after time t; y_∞ is the maximum extraction yield and b is a constant.

The linear form of Eq. (2):

$$\frac{1}{y} = \frac{b}{y_\infty}\frac{1}{t} + \frac{1}{y_\infty} \tag{3}$$

was used for calculating the parameters of Eq. (2). The obtained values for modelling the system through total extract yield and menthol yield for SFE of mentha are shown in Tables IV and V, respectively.

TABLE IV. Values of y_∞ and b for mentha total extract

t / °C	y_∞ / %	b	Correlation coefficient, r	Mean relative deviation (MRD[a]), %
35	6.29	101.96	0.977	14.75
40	5.47	62.32	0.990	7.72
45	3.62	42.31	0.999	1.95
50	5.59	91.86	0.978	10.85

[a]$MRD = \dfrac{\sum \left| \dfrac{y_c - y_e}{y_e} \right| 100}{n}$, where y_c is the calculated value, y_e the experimental value and n the number of points

TABLE V. Values of y_∞ and b (Eq. (3)) for menthol extraction

t / °C	y_∞ / %	b	Correlation coefficient, r	Mean relative deviation (MRD), %
35	1.85	101.93	0.976	14.60
40	1.56	62.32	0.990	7.73
45	1.09	42.16	0.999	1.88
50	1.84	110.40	0.979	12.56

The obtained model equations, as well as experimental points, are shown in Figs. 1 and 2 for the total yield of extract and for the menthol yield, respectively.
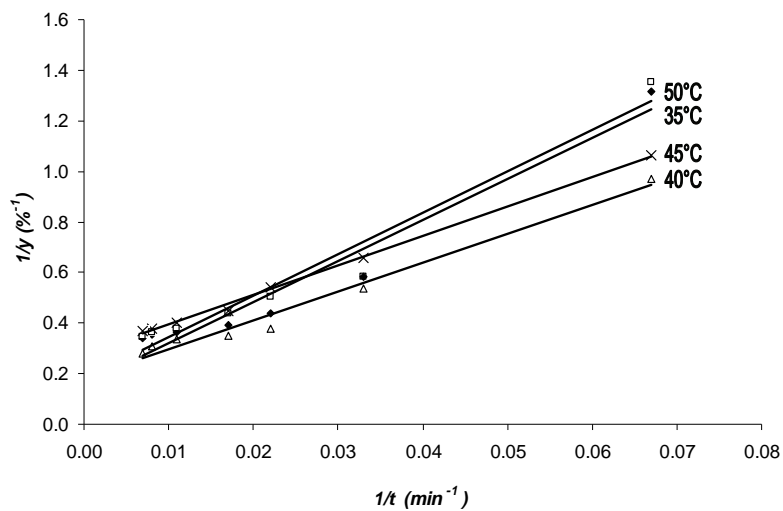
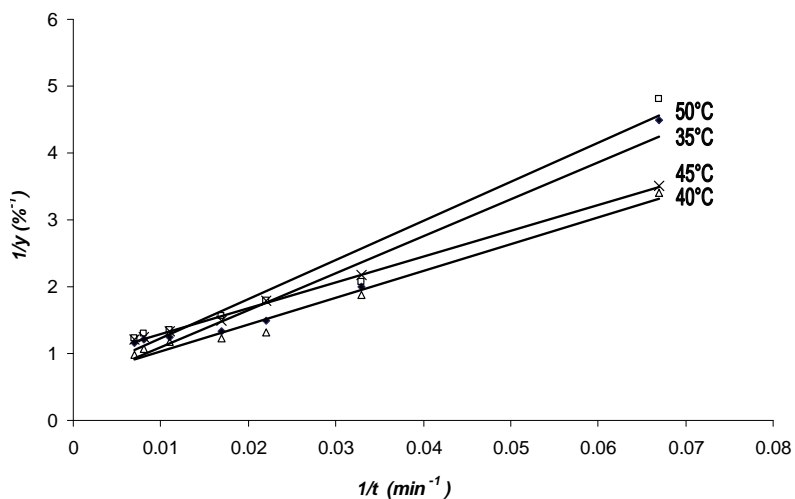Fig. 1. Dependence of $1/y$ on $1/t$ for mentha total extract.

Fig. 2. Dependence of $1/y$ on $1/t$ for menthol extraction. Legend: ♦ – 35 °C; Δ – 40 °C; × – 45 °C and □ – 50 °C.

The obtained values of the mean relative deviation (Tables IV and V) show the possibility of using the equation of Naik *et al.*[11] for the extraction process of the investigated system mentha–supercritical carbon dioxide through the total extract yield and menthol yield. The applied equation shows a good fit for the extraction process at the temperatures of 40 and 45 °C, but for the temperatures of 35 and 50 °C, some another model equation, with a better fit of the experimental results should be applied.

CONCLUSIONS

The content of mentha essential oil (EO) in mentha leaves was 3.19 %, containing menthol (30.67 %) and L-menthone (37.15 %). These values are in the good agreement with the results of other authors. The SFE conditions, such as the operating temperature and pressure, *i.e.*, solvent density, are very important for an optimum extraction process. Due to this, different combinations of pressure and temperature were investigated in this work. For the production of a mentha most similar to the mentha EO obtained by steam distillation, the extraction conditions are 100 bar, 40 °C and an extraction time of 2.5 h.

For the investigations of the influence of temperature on the SFE mentha with CO_2, the temperatures of 35, 40, 45 and 50 °C (no thermal decomposition of the mentha compounds present), at a pressure of 100 bar, were chosen. The extraction kinetics through the total extract yield, as well as menthol yield, was described by the model equation of Naik *et al.* The applied equation showed a good fit only for the extraction process at the temperatures of 40 and 45 °C.

И З В О Д

ЕКСТРАКЦИЈА МЕНТЕ (*Mentha piperita* L.) СУПЕРКРИТИЧНИМ CO_2 ПРИ РАЗЛИЧИТИМ ГУСТИНАМА РАСТВАРАЧА

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Квалитативни и квантитативни састав етарског уља и екстраката менте добијених на различитим притисцима/температурама екстракцијом суперкритичним флуидом су одређени методом GC–MS. Садржај ментола одређен је спектрофотометријски. Доминантне компоненте у етарском уљу су L-ментол и ментон, као и у CO_2 екстракту добијеном при притиску од 100 bar, док је у екстрактима добијеним при већим притисцима (од 150 до 400 bar) доминантна компонента сквален. Za моделовање испитиваног екстракционог система мента–суперкритични угљендиоксид примењена је једначина Naik-а и сарадника.

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