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ABSTRACT

The influence of carbon dioxide flow rate and grinding degree of thyme on supercritical fluid extraction of thyme was investigated. The extraction rate of the pharmacologically important compound of thyme-thymol can be successfully expressed by analogy to first-order reaction kinetics. To model extraction of the thyme–supercritical carbon dioxide system, we used the Reverchon-Sesti Osseo equation as well as our modified equation.

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INTRODUCTION

The strongly antiseptic and antifungal activities of thyme (*Thymus vulgaris* L.), i.e. through thyme essential oil, is mainly due to the presence of phenolic compounds, especially thymol and carvacrol. The yield of essential oils from thyme is 0.3–6.3% (1–5). The Yugoslav pharmacopoeia suggests that the minimal yield is 1.5% (6). The amount of thymol in thyme essential oil is much higher than is the amount of carvacrol (up to 60% to 6%, respectively) (7,8). Thymol shows 30 times higher antiseptic effect and 4 times lower toxic effect than does phenol (9). The thyme phenolic compounds can form oxygen free radicals (10).

The classical procedures for separating active substances from plant material, such as steam distillation and extraction with organic solvents, 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. In addition, traces of organic solvent found in extracted samples are undesirable for either organoleptic and/or health reasons. Besides, organic solvents are insufficiently selective, so they dissolve some concomitant compounds along with the active substances. For these reasons supercritical fluid extraction (SFE) with supercritical carbon dioxide (CO_2) has been recently investigated as an important alternative to classical procedures. Extraction procedures involving supercritical CO_2 are considered clean technologies because no secondary products from the process pollute the environment. CO_2 is most widely used in SFE because it is simple to use, inexpensive, nonflammable, nontoxic, chemically stable, shows great affinity to volatile (lipophilic) compounds, and can be easily and completely removed from any extract. By changing 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., the solvent power of supercritical CO_2, can be achieved.

SFE of essential oil from wild thyme (*Thymus serpyllum*) has been investigated (11). The high-performance liquid chromatography (HPLC) method for determination of thymol and carvacrol (7,12–16), i.e., gas chromatography (GC) analysis of thyme essential-oil compounds (8,16,17), has been used by many researchers.

In this paper, the influence of carbon dioxide flow rate, as well as of thyme particle size, in SFE of thyme by carbon dioxide is described.

EXPERIMENTAL

Plant Material

Thyme was produced in village Sanad near Ĉoka, Vojvodina, Yugoslavia, in 1996. Besides a sample of thyme *in toto* (grinding degree d_o), after milling, thyme samples subjected to 2 grinding degrees were investigated (Table 1).

Table 1. Grinding Degree of Thyme and Mean Particle Radius (r)

Grinding Degree	Mean Particle Radius, r (mm)
d_o	1.66
d_1	0.73
d_2	0.35

After thyme milling and sieving using sieve set (Erweka Appatarebau GmbH, Germany), a mean particle radius was calculated as $r = \dfrac{100}{\Sigma\left(\dfrac{m_i}{o_m}\right)}$ where m_i is the fraction (%) after sieving, and o_m is the mean aperture diameter of 2 adjacent sieves.

Chemicals

Standard samples of thymol (Kemika, Zagreb, Croatia) and commercial carbon dioxide (Tehno-gas, Novi Sad, Yugoslavia) as the extracting agent were used. All other chemicals were of analytical reagent grade.

Chromatographic Procedures

HPLC

The HPLC instrument was a Waters 600E Multisolvent Delivery System with Waters Multiwavelength Detector (Millipore Corp, Waters Chromatography Division, Milford, Mass, USA) and an HP 3396 Series Integrator (Hewlett-Packard GmbH, Waldbronn, Germany). A column NovaPak C_{18} (Waters) (of 4 μm in a 3.9 mm I.D. $\times$ 15 cm column) and a precolumn Waters Guard-Pak^{TM}/Resolve^{TM} (10 μm) were used. The mobile phase was acetonitrile-water (50:50; vol/vol) (isocratic elution) used with a flow rate of 0.8 mL/min. After filtration (0.45-μm Millipore filter, Milipore, Bedford, Mass, USA), 10 μL of each sample was used. Detection was carried out at 276 nm. The recorder chart speed was 0.4 cm/min. The quantitative determination was carried out through the use of the external standard method (16).

Gas Chromatography–Mass Spectrometry

The GC instrument was a GCD HP G 1800 A (Hewlett-Packard, Palo Alto, Calif, USA). A column HP-5 MS (30.0 m $\times$ 0.25 mm) was used. The helium flow rate was 0.8 mL/min. The injector temperature was 250°C; the detector was set at 280°C; it was set initially at 50°C and was increased linearly at 20°C per minute to 130°C (1 minute) and then was increased 9°C per minute until the final temperature of 280°C (8.33 minutes) was reached. Total analysis time was 30 minutes. The injected volume of sample solution in *n*-pentane (40–50 mg/mL) was 5 μL. The detector was set to 45–425 D. The compounds were identified using the Wiley database and the quantitative determination was carried out by the quasi-internal standard method (the content of thymol determined in sample by HPLC) (16).

Supercritical Fluid Extraction

SFE-CO_2 was carried out with a laboratory-scale high-pressure extraction plant (NOVA-Swiss, Effretikon, Switzerland) described previously (18). The main parts and characteristics (manufacturer specification) of the plant were as follows: the diaphragm-type compressor (up to 1000 bar), extractor with an internal volume of 200 mL ($p_{max} = 700$ bar), separator with internal volume of 200 mL ($p_{max} = 250$ bar), and maximum CO_2 mass flow rate of approximately 5.7 kg/h. The mass of thyme sample in the extractor was 50 g at 100 bar at 40°C; the CO_2 flow rate was the investigated value; and the total extraction time was 2.5 hours. Separator conditions were 15 bar and 25°C.

RESULTS AND DISCUSSION

The content of essential oil, determined by the procedure of Yugoslav Pharmacopoeia (6), was 1.75% (vol/wt). The predominant compound of the essential oil is thymol (50.06% wt/wt), while carvacrol is present in traces (1.15% wt/wt). The amount of *n*-tetradecane in the thyme essential oil (16.11% wt/wt) is higher than that of other paraffins (16).

To prevent thermal decomposition of essential oil compounds, the SFE-CO_2 was used at 40°C. The pressure of 100 bar was selected for investigation of the CO_2 flow rate during SFE as was thyme particle size.

Investigated flow rates of carbon dioxide (Q_V and Q_M) and corresponding values of velocities W_A and W_{SA}, as well as of the specific mass flow rate of carbon dioxide, are given in Tables 2 and 3 (see Appendix).

The values of mean specific velocity of carbon dioxide are in intervals of extraction equilibrium (19,20). The specific mass flows could be used as indicators of carbon dioxide flow for obtaining equilibrium of extraction for different flow rates, pressures, temperatures, and extractor cross-section surfaces (21).

The results of qualitative and quantitative extract analyses obtained by SFE-CO_2 (100 bar, 40°C, 2.5 hours) of thyme grinding degree, d_o, at investigated flow

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Table 2. Carbon Dioxide Flow Rates (Q_V and Q_M) and Values of Velocity (W_A and W_{SA})

Flow Rate Mark	Volume Flow Rate Q_V (dm^3/h)	Mass Flow Rate Q_M (g/h·g thyme)	W_A (mm/s)	W_{SA} (10^2) (mm/s)
Q_1	48.08	1.90	10.63	3.34
Q_2	97.72	3.87	21.60	6.79
Q_3	139.90	5.54	30.92	9.72

Flow rate ratio Q_1:Q_2:Q_3 is 1:2.03:2.91.
$p = 100$ bar ($d_{CO_2,\,E} = 630\ g/dm^3$; $F_d = 318.18$).

rates, are shown in Table 4. A quantitative extraction of thyme essential oil was not achieved. The most pharmacologically important compound thymol (T) was the most prevalent compound in the CO_2 extract as measured through the flow rate Q_2.

The extraction kinetics of T are expressed by analogy to first-order reaction kinetics defined as

$$-\ln\left(1 - \frac{c_E}{c_o}\right) = k\tau \tag{1}$$

where c_E is the mass of extracted T after extraction time t (appropriate concentration); c_o is the total mass of T (i.e., 800 mg/100 g thyme); t is the extraction time (hours); and k is the extraction rate constant (per hour).

The following equations for investigated flow rates of carbon dioxide were obtained:

Flow Rate Q_1

$$-\ln\left(1 - \frac{c_E}{c_o}\right) = (-0.175 \times 10^{-2}) + 0.0858\tau \tag{2}$$
$$r = 0.9989$$

Table 3. Mass Flow Rate (Q_M) and Specific Mass Flow Rate (Q_{MS}) of Carbon Dioxide

Flow Rate Mark	Q_M (g/min)	Q_{MS} (10^3) (g/s·cm^2)
Q_1	1.59	2.10
Q_2	3.22	4.28
Q_3	4.62	6.12

Table 4. Results of GC-MS Analysis of Compounds Obtained by Extraction of Thyme critical Carbon Dioxide (*TE*)

	Flow Rate Q_1			
	Extracted		Remained	
Compound	Thyme (mg/100 g)	TE (%)	Thyme (mg/100 g)	EO (%)
Camphor	12.66	0.86	19.83	1.98
L-Menthol	45.88	3.11	76.13	7.61
n-Dodecane	42.94	2.91	27.11	2.71
Thymol	174.88	11.84	545.27	54.53
n-Tetradecane	624.48	42.42	24.07	2.41
β-Caryophyllene	26.84	1.82	4.30	0.43
2-Methyldecane	18.28	1.24	3.61	0.36
n-Pentadecane	85.65	5.80	21.66	2.17
n-Hexadecane	73.33	4.96	27.64	2.76
n-Heptadecane	55.58	3.76	24.35	2.43
n-Octadecane	30.40	2.06	14.17	1.42
Phytol	—	—	21.12	2.11
Essential oil content (%; vol/wt):			0.925	

Remaining compounds of essential oil were obtained by steam distillation.

Flow Rate Q_2

$$-\ln\left(1-\frac{c_E}{c_o}\right) = (-0.1733 \times 10^{-2}) + 0.2209\tau \qquad (3)$$

$r = 0.9880$

Flow Rate Q_3

$$-\ln\left(1-\frac{c_E}{c_o}\right) = (-6.946 \times 10^{-2}) + 0.1177\tau \qquad (4)$$

$r = 0.9934$

where r is the correlation coefficient.

The corresponding graphical presentation of Eqs. (2–4), and experimental values are given in Fig. 1.

The obtained results show that the extraction rate of thymol can be successfully expressed by analogy to first-order reaction kinetics. The highest value of the extraction rate constant (k) 0.2209 h^{-1} for the flow rate of supercritical carbon

(Grinding Degree d_o) and Remaining Compounds in Essential Oils (EO) Through Super-

Flow Rate Q_2				Flow Rate Q_3			
Extracted		Remained		Extracted		Remained	
Thyme (mg/100 g)	TE (%)	Thyme (mg/100 g)	EO (%)	Thyme (mg/100 g)	TE (%)	Thyme (mg/100 g)	EO (%)
5.02	0.38	6.33	0.63	14.18	1.01	5.32	0.53
73.85	5.66	17.00	1.70	69.26	4.92	13.58	1.36
25.09	1.92	17.94	1.80	25.12	1.78	17.64	1.76
389.08	29.84	533.84	53.38	198.11	14.07	464.37	46.44
430.43	33.01	239.63	23.96	445.58	31.65	221.27	22.13
17.75	1.36	12.20	1.22	20.05	1.42	12.42	1.24
11.63	0.89	5.62	0.56	11.69	0.83	5.07	0.51
60.57	4.64	33.51	3.35	65.42	4.65	32.35	3.32
52.66	4.11	29.80	2.98	58.42	4.15	29.78	2.98
38.52	2.95	15.37	1.54	30.12	2.14	22.05	2.20
20.07	1.54	10.95	1.09	22.49	1.60	11.69	1.17
8.40	0.64	—	—	10.17	0.72	—	—
		1.050				0.950	

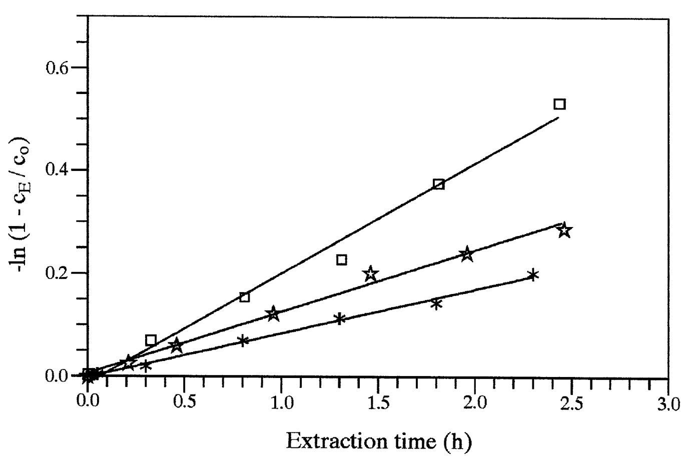

Figure 1. $-\ln(1 - c_E/c_o)$ vs. extraction time. ★ flow rate Q_1, □ Q_2, ☆ Q_3.

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dioxide of 97.72 dm^3/h (Q_2) was obtained and is considered the best flow rate for thyme extraction by supercritical carbon dioxide.

The SFE-CO_2 of thyme at grinding degrees d_1 and d_2 and selected CO_2 flow rates were investigated and the obtained results are given in Table 5.

A quantitative extraction of thyme essential oil by supercritical carbon dioxide (at 100 bar, 40°C, and 2.5 hours) was obtained at grinding degree d_2 (mean particle radius 0.35 mm). The composition of this extract was very similar to that of the essential oil obtained by steam distillation (16).

To model the extraction of the thyme–supercritical carbon dioxide system, we used the final form of the Reverchon-Sesti Osseo equation (22):

$$Y = 100\left[1 - \exp\left(-\frac{t}{t_i}\right)\right] \tag{5}$$

where Y is the normalized extraction yield (%); t is the extraction time (seconds); and t_i is the internal diffusion time (seconds).

Table 5. Results of GC-MS Analysis of Compounds Obtained by Extraction of Thyme Grinding Degree d_1 and d_2

	Grinding Degree d_1				Grinding Degree d_2	
	Extracted		Remained		Extracted	
Compound	Thyme (mg/100 g)	TE (%)	Thyme (mg/100 g)	EO (%)	Thyme (mg/100 g)	TE (%)
Camphor	10.28	0.55	3.10	0.56	10.18	0.49
L-Menthol	66.90	3.55	6.57	1.19	59.49	2.84
n-Dodecane	31.49	1.67	9.38	1.70	38.18	1.83
Thymol	559.32	29.71	217.96	39.63	779.80	37.29
n-Tetradecane	543.26	28.86	156.89	28.52	555.39	26.56
β-Caryophyllene	25.66	1.36	11.63	2.11	29.02	1.38
2-Methyldecane	14.06	0.75	3.30	0.60	13.82	0.66
n-Pentadecane	75.26	4.00	22.62	4.11	78.54	3.76
n-Hexadecane	68.03	3.61	19.99	3.63	70.13	3.35
n-Heptadecane	51.16	2.72	14.24	2.59	49.66	2.37
n-Octadecane	28.12	1.49	7.60	1.38	26.53	1.27
Phytol	28.31	1.50	21.12	2.11	28.98	1.39
Essential oil content (%, vol/wt):			0.550			

TE is the total extract obtained by supercritical extraction, and EO is the essential oil obtained by steam distillation.

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Table 6. Coefficient of Internal Mass Transfer (D) and Internal Diffusion Time (t_i) of Total Extract and Thymol for Investigated Grinding Degrees of Thyme

Grinding Degree	Total Extract		Thymol	
	D (10^{12}) (m^2/s)	t_i (seconds)	D (10^{12}) (m^2/s)	t_i (seconds)
d_o	9.77	1.8826×10^4	8.94	2.0564×10^4
d_1	2.52	1.4229×10^4	4.37	8.214×10^3
d_2	0.84	9.753×10^3	2.49	3.281×10^3

Villermaux (23) showed the equivalence between t_i and the internal mass-transfer coefficient D for different particle geometries by the following equation:

$$t_i = \mu \frac{l^2}{D} \tag{6}$$

where μ is the particle geometry factor (in the case of spherical particles $\mu = 3/5$), and l is the particle characteristic dimension of V_p/A_p, where V_p is particle volume (m^3), and A_p is a particle surface area (m^2). In the case of spherical particles $l =$ r/3, and r is the mean particle radius (m). The values of D (m^2/s) for the total extract (TE) and T were calculated using the following equation (24):

$$D = \frac{r^2\left(\log a_1 - \log \dfrac{q_i}{q_o}\right)}{0.434\, b_1 t} \tag{7}$$

where r is the mean particle radius (m); $a_1 = 6/\pi^2$ and $b_1 = \pi^2$ in the case of spherical particles; q_o is total content of matter extracted from thyme (e.g., total content of thymol); q_i is content of matter extracted from thyme (e.g., thymol) remaining in the thyme after t ($q_i = q_o - q_i'$); q_i' is the content of matter extracted from thyme (e.g., thymol) after t; t is the extraction time (seconds).

The calculated values of D and t_i of total extract and thymol for investigated grinding degrees of thyme are shown in Table 6.

The graphical presentation of Eq. (5) for SFE-CO_2 is shown in Figs. 2 and 3. The TE and T is denoted by the solid line in each figure.

To avoid the evaluation of an internal diffusion time, Eq. (5) was modified (25) based on the assumption that for a certain extraction system, t_i could be approximated as a constant. This assumption allows one to assert that

$$-\frac{t}{t_i} = at + b \tag{8}$$

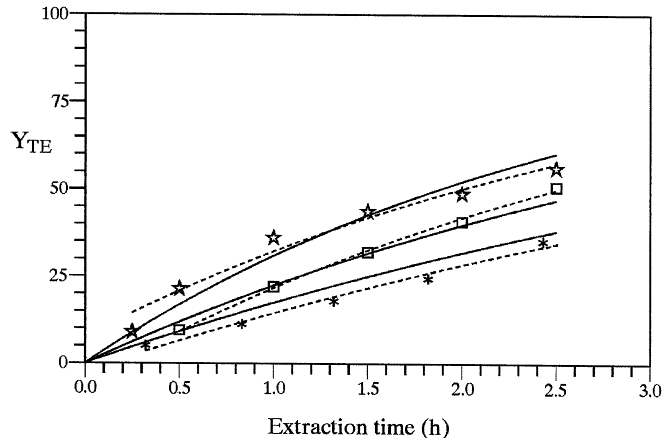

Figure 2. Graphical presentation of Eq. (5) by solid line and Eq. (9) by dashed line for total extract (*TE*). ★ grinding degree d_o, □ d_1, ☆ d_2.

Note that $-t/t_i = Z$; a is a constant; t is the extraction time (hours); and b is a correction term, and Eq. (5) can be written as

$$Y = 100\,[\,1 - \exp(at + b)] \tag{9}$$

Z is defined as

$$Z = \ln\left(1 - \frac{Y}{100}\right) \tag{10}$$

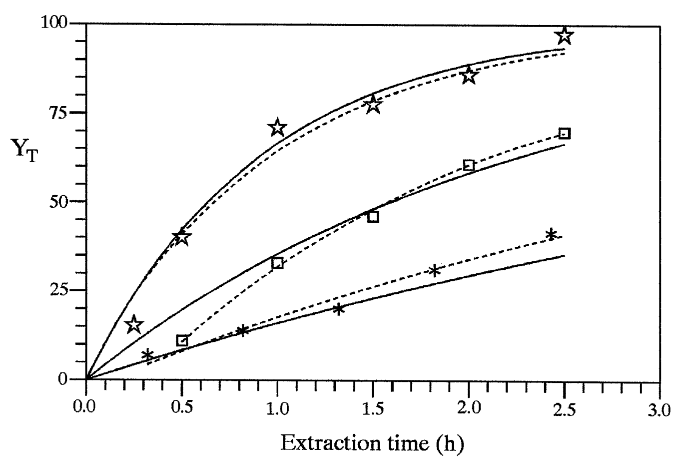

Figure 3. Graphical presentation of Eq. (5) by solid line and Eq. (9) by dashed line for thymol (*T*). ★ grinding degree d_o, □ d_1, ☆ d_2.

Table 7. Modified Eq. (9) for Calculating Normalized Yield of Total Extract (Y_{TE}) and Normalized Yield of Thymol (Y_T)

Grinding Degree	Correlation Coefficient (r) of $Z = at + b$	Equation
d_o	0.9910	$Y_{TE} = 100\,[1 - \exp(-0.177t + 0.0218)]$
	0.9885	$Y_T = 100\,[1 - \exp(-0.221t + 0.0263)]$
d_1	0.9983	$Y_{TE} = 100\,[1 - \exp(-0.297t + 0.0539)]$
	0.9989	$Y_T = 100\,[1 - \exp(-0.541t + 0.1570)]$
d_2	0.9865	$Y_{TE} = 100\,[1 - \exp(-0.308t - 0.0793)]$
	0.9853	$Y_T = 100\,[1 - \exp(-1.012\mathrm{t} - 0.0210)]$

Table 8. Standard Error of Regression ($S_{Y,X}$) of Model Equations for Supercritical Extraction of Thyme by Carbon Dioxide

	$S_{Y,X}$					
	d_o		d_1		d_2	
Equation	Thymol	Total Extract	Thymol	Total Extract	Thymol	Total Extract
(5)	3.480	3.578	2.068	4.527	3.645	4.727
(9)	1.165	1.984	0.716	0.997	2.945	4.893

$S_{Y,X} = \sqrt{\dfrac{\Sigma(Y_E - \mathrm{Y_i})^2}{n}}$, where Y_E is experimental value; Y_i is the calculated value; and n is the number of experimental points.

where Y is the normalized extraction yield

$$Y = \frac{Y_{ext}}{Y_{max}} \times 100 \qquad (11)$$

where Y_{ext} is the extraction yield (g/100 g of thyme for *TE*, i.e. mg/100 g of thyme for *T*), Y_{max} is the maximal extraction yield (g/100 g, i.e. mg/100 g).

The equations in Table 7 were obtained on the basis of modified Eq. (9) for calculating normalized yield of total extract (Y_{TE}) and normalized yield of thymol (Y_T) in SFE-CO_2 of thyme at noted grinding degrees. The graphical presentations of the obtained equations are shown in Figs. 2 and 3 by the dashed line.

The calculated values of the standard error of regression ($S_{Y,X}$), given in Table 8, show that the modified Eq. (9) is generally a better fit for Y_{TE} and Y_T than was the original Eq. (5).

APPENDIX

On the basis of extractor diameter ($R = 4$ cm) and the carbon dioxide flow rate used (Q_V, dm^3/h), the velocity flow W_A (mm/s), and the mean specific velocity W_{SA} (mm/s), the extragent could be calculated as:

$$W_A = \frac{Q_V}{A} \tag{A.1}$$

and

$$W_{SA} = \frac{W_A}{Fd} \tag{A.2}$$

where

$$Fd = \frac{d_{CO_2,E}}{d_{CO_2}} \tag{A.3}$$

A is the extractor cross-section surface (12.566 cm^2); $d_{CO_2,E}$ is the CO_2 density at extraction pressure and 40°C; and d_{CO_2} is the CO_2 density at atmospheric pressure and 20°C (1.98 g/dm^3).

The specific mass flow rate of carbon dioxide (Q_{MS}) could be used as an indicator of equilibrium conditions of extraction:

$$Q_{MS} = \frac{Q_M}{A} \tag{A.4}$$

where Q_M is the mass flow rate of carbon dioxide calculated based on the total mass of thyme in the extractor (g/min).

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