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MODELING OF THE THYME - LIQUID CARBON DIOXIDE EXTRACTION SYSTEM

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The extraction of thyme (Thymus vulgaris L.*) by liquid carbon dioxide was investigated. The obtained extracts were analyzed by HPLC and GC-MS methods, and their composition was compared with that of the essential oil obtained by steam distillation and by supercritical carbon dioxide at 100 bar and 40°C. To model extraction of the thyme - liquid carbon dioxide system, we used the Reverchon - Sesti Osseo equation, as well as our modified equation.*

KEY WORDS: thyme, extraction, liquid carbon dioxide, modeling

INTRODUCTION

The strongly antiseptic and antifungal activities of thyme (*Thymus vulgaris* L.), i.e. of thyme essential oil, is mainly due to the presence of phenolic compounds, in the first place of thymol and carvacrol. The thyme essential oil yield is 0.3-6.3% (1-6). The content of thymol in thyme essential oil is much higher compared to carvacrol content (up to 60%, and 6%, respectively) (7,8).

Supercritical fluid extraction (SFE) with supercritical carbon dioxide (CO_2) has recently gained in importance as an alternative to the classical procedures (steam distillation and extraction with organic solvents).

SFE of essential oil from thyme (*T. vulgaris*) (9-11), as well as from wild thyme (*T. serpyllum*) were investigated (12).

In a previous work on supercritical fluid extraction (SFE) of thyme (*Thymus vulgaris* L.), the influence of flow rate of carbon dioxide and grinding degree of thyme (11), as well as of extraction time and carbon dioxide pressure (13), were reported. In this paper,

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the investigations on using liquid carbon dioxide for thyme extraction and modeling of the thyme – liquid carbon dioxide extraction system are described.

EXPERIMENTAL

Plant Material

Thyme was grown in village Sanad near Čoka, Vojvodina, Serbia and Montenegro, in 1996. All extractions were performed using the thyme of the grinding degree d_2 (mean particle radius 0.35 mm) (11).

Chemicals

Standard sample of thymol (Kemika, Zagreb, Croatia) and commercial carbon dioxide (Tehnogas, Novi Sad, Serbia and Montenegro) were used. All other chemicals were of analytical reagent grade.

Supercritical Fluid Extraction

SFE-CO_2 was carried out on a laboratory-scale high pressure extraction plant (HPEP, NOVA-Swiss, Effretikon, Switzerland) described previously (14). 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 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 extractor was 50 g at the investigated value of pressure (65 bar) and temperature (23°C); the CO_2 flow rate was 97.72 $dm^3 h^{-1}$. Separator conditions were 15 bar and 25°C .

Chromatographic Procedures

The HPLC instrument was a Waters 600E Multysolvent Delivery System with Waters Multiwavelenght 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. × 15 cm column) and a precolumn Waters Guard-PakTM/Resolve^{TM} (10 μm) were used. The mobile phase was acetonitrile-water (50:50; v/v) (isocratic elution) used with a flow rate of 0.8 mL min^{-1}. After filtration (0.45 μm Millipore filter-Millipore, Bedford, Mass, USA), 10 μL of each sample were used. Detection was carried out at 276 nm. The quantitative determination was carried out through the use of the external standard method (9).

The GC instrument was a GCD HP G 1800 A (Hewlett-Packard, Palo Alto, Calif, USA). An HP-5 MS column (30.0 m × 0.25 mm; film thickness 0.25 μm) was used. The helium flow rate was 0.8 mL min^{-1}. 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 min). Total analysis time was 30 min. 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) (9).

RESULTS AND DISCUSSION

After investigating the influence of the carbon dioxide flow rate and grinding degree of thyme (11), as well as the extraction time (13), the extractant flow rate of 97.72 dm^3/h, thyme mean particle radius of 0.35 mm and extraction time of 2.5 hours were obtained and considered the best parameters for thyme extraction by liquid carbon dioxide – LCO_2 (65 bar; 23°C). To model the investigated thyme - LCO_2 extraction system, we used the final form of the Reverchon - Sesti Osseo equation (15):

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

where Y is the normalized extraction yield (%); t is the extraction time (seconds) and t_i is the internal diffusion time (seconds), as well as our modified equation (11):

$$Y = 100\,[1 - \exp\,(\boldsymbol{a}\,t + \boldsymbol{b})] \quad [2]$$

where Y is the normalized extraction yield **(** $Y = \frac{Y_{ext}}{Y_{max}} \times 100$; where Y_{ext} is the extraction yield (g/100 g of thyme for total extract - *TE*, i.e. mg/100 g of thyme for thymol - *T*); Y_{max} is the maximal extraction yield (g/100 g, i.e. mg/100 g**)**; ***a*** is a constant; t is the extraction time (hours); and ***b*** is a correction term.

Total extract (*TE*) and thymol (*T*) yields obtained by extraction using LCO_2, as well as the content of *T* in *TE,* are shown in Table 1. The obtained extract was a liquid at room temperature, and no solid like extracts were obtained by supercritical carbon dioxide.

Table 1. Total extract (*TE*; g/100 g) and thymol (*T*; mg/100 g) yields obtained by liquid carbon dioxide (65 bar; 23°C) extraction

Time (h)	TE	T	Content of *T* in *TE* (%)
0.25	0.500	304.7	61.0
0.50	1.073	369.0	34.4
0.75	1.455	453.7	31.2
1.00	1.587	566.4	35.7
1.50	1.836	686.8	37.4
2.00	2.034	732.9	36.0
2.50	2.199	786.4	35.7

GC-chromatogram of thyme *TE* obtained by LCO_2 is shown in Fig. 1, and the results of GC-MS analyse, i.e. compound contents in relation to thyme and *TE* are given in Table 2.

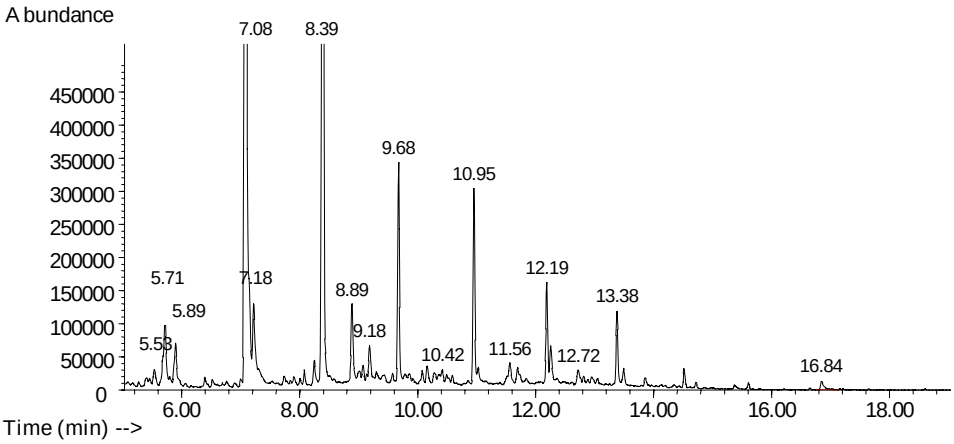

Fig. 1. GC-chromatogram of thyme *TE* obtained by liquid carbon dioxide

Table 2. Results of GC-MS analysis of thyme *TE* obtained by liquid carbon dioxide

t_R (min)	Compound	Content	
		Thyme(mg/100 g)	TE(%)
5.53	Camphor	12.71	0.58
5.71	*L*-Menthol	53.78	2.44
5.89	*n*-Dodecane	37.04	1.68
7.08	Thymol	786.40	35.75
8.39	*n*-Tetradecane	703.99	32.00
8.89	*β*-Caryophyllene	44.13	2.01
9.18	2-Methyldecane	12.73	0.58
9.68	*n*-Pentadecane	104.35	4.74
10.95	*n*-Hexadecane	95.89	4.36
12.19	*n*-Heptadecane	70.34	3.30
13.38	*n*-Octadecane	39.61	1.80
16.84	Phytol	9.51	0.43

The composition of the extract obtained after 2.5 hours of extraction by LCO_2 at 65 bar and 23°C was very similar to that of the essential oil obtained by steam distillation, i.e to the extract obtained by supercritical carbon dioxide at 100 bar and 40°C (11,13). In this way the extraction of thyme essential oil using LCO_2, gave again quantitative results (11,13).

For modeling the investigated thyme - LCO_2 extraction system by Eq. [1], the values of D (m^2/s) and t_i were calculated using the following equations:

$$D = \frac{r^2 \left(\log a_1 - \log \frac{q_i}{q_o} \right)}{0.434\, b_1\, t} \qquad [3]$$

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 the total content of matter extracted from thyme (e.g., total content of thymol); q_i is the 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),

$$t_i = \mu \frac{l^2}{D} \qquad [4]$$

where μ is the particle geometry factor (in the case of spherical particles $\mu = 3/5$); l is the particle characteristic dimension of V_p/A_p, where V_p is the particle volume (m^3); and A_p is the particle surface area (m^2). In the case of spherical particles $l = r/3$, and r is the mean particle radius (m).

Eq. [1] was modified based on the assumption that for a certain extraction system, t_i could be approximated by a constant. This assumption allows one to assert that

$$-\frac{t}{t_i} = at + b \qquad [5]$$

Note that $-t/t_i = Z$; $\boldsymbol{a}$ is a constant; t is the extraction time (hours); and $\boldsymbol{b}$ is a correction term.

Z is defined as

$$Z = \ln\left(1 - \frac{Y}{100}\right) \qquad [6]$$

where Y is the normalized extraction yield (1).

In Table 3 are given the calculated values of Eqs. [1] and [2] parameters for modeling the thyme - LCO_2 extraction system, as well as the values of standard error of regression ($S_{Y,X}$) for the obtained equations.

The corresponding graphical presentation of Eqs. [1] and [2], and experimental values for total extract (TE) normalized extraction yield (Y_{TE}), i.e. thymol (T) normalized extraction yield (Y_T), are shown in Figs. 2 and 3, respectively.

Table 3. Internal mass transfer coefficient (*D*) and parameters of used model equations for total extract (*TE*) and thymol (*T*), correlation coefficient (r) and standard error of regression ($S_{Y,X}$) for applied equations

Equation (parameter)	TE	T	|r|		Standard error of regression ($S_{Y,X}$)*	
			TE	*T*	*TE*	*T*
Eq. [1]						
$D\ 10^{12}$ (m^2/s)	2.9925	3.1383			2.866	5.100
t_i (s)	2726	2599				
Eq. [2]						
A	-1.2662	-1.3272	0.9949	0.9929	2.955	4.804
B	-0.01334	-0.00778				

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

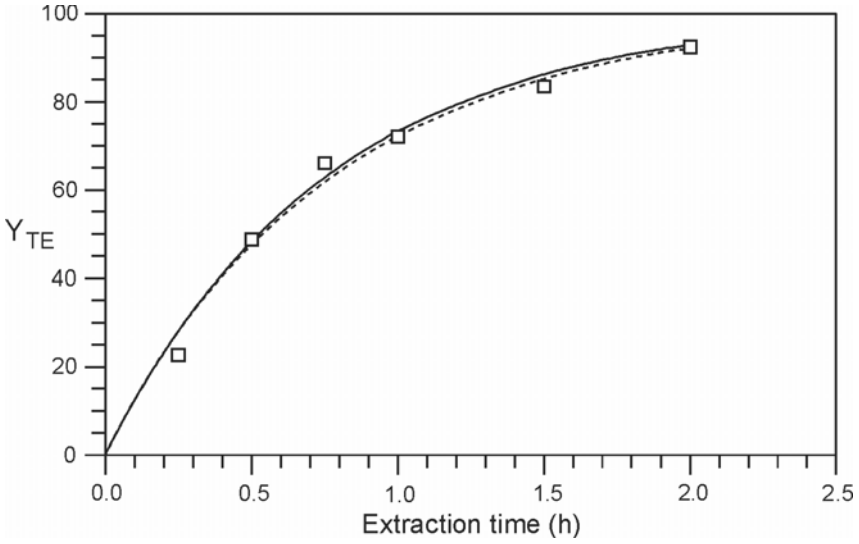

Fig. 2. Graphical presentation of Eq. [1] (solid line) and Eq. [2] (dashed line) and the experimental values (~) of total extract (*TE*) normalized extraction yield (Y_{TE})

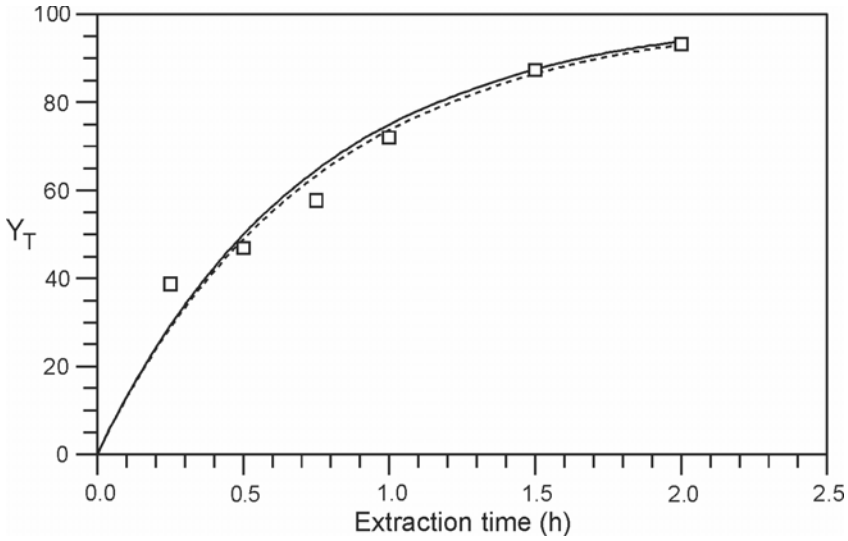

Fig. 3. Graphical presentation of Eq. [1] (solid line) and Eqs. [2] (dashed line) and the experimental values (~) of thymol (T) normalized extraction yield (Y_T)

The similarity of the calculated values of the standard error of regression ($S_{Y,X}$), given in Table 3, show that both the original Eq. [1] and the modified Eq. [2] can be used for modeling the thyme – liquid carbon dioxide extraction system.

CONCLUSION

Liquid carbon dioxide is a convenient solvent for obtaining the thyme extraction product for the pharmaceutical industry. The composition of the extract obtained by liquid carbon dioxide is very similar to that of the essential oil obtained by steam distillation, i.e to the extract obtained by supercritical carbon dioxide at 100 bar and 40°C. Thus a quantitative extraction of thyme essential oil was achieved. The similarity of the calculated values of standard error of regression show that the original equation, as well as the modified equation, can be used for modeling the thyme – liquid carbon dioxide extraction system.

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МОДЕЛОВАЊЕ ЕКСТРАКЦИОНОГ СИСТЕМА ТИМИЈАН – ТЕЧНИ УГЉЕНДИОКСИД

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Испитана је екстракција тимијана (*Thymus vulgaris* L.) применом угљендиоксида у течном стању. За квалитативну и квантитативну анализу добијених екстраката примењене су методе HPLC и GC-MS. Извршено је упоређивање квалитативног и квантитативног састава екстракта добијеног применом течног угљендиоксида са етарским уљем тимијана добијеним дестилацијом помоћу водене паре, као и са екстрактом добијеним променом угљендиоксида у суперкритичном стању (100 bar, 40°C). Моделовање испитиваног екстракционог система тимијан – течни угљендиоксид извршено је применом једначине Reverchon - Sesti Osseo-a, као и применом наше модификоване једначине.

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