Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Modeling of the Thyme-Supercritical Carbon-Dioxide Extraction System. II. The Influence of Extraction Time and Carbon Dioxide Pressure

Zoran Zeković[a]; Žika Lepojević[a]; Aleksandar Tolić[a]
[a] Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Novi Sad, Yugoslavia

Online publication date: 20 February 2003

To cite this Article Zeković, Zoran , Lepojević, Žika and Tolić, Aleksandar(2003) 'Modeling of the Thyme-Supercritical Carbon-Dioxide Extraction System. II. The Influence of Extraction Time and Carbon Dioxide Pressure', Separation Science and Technology, 38: 3, 541 — 552
To link to this Article: DOI: 10.1081/SS-120016650
URL: http://dx.doi.org/10.1081/SS-120016650

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 38, No. 3, pp. 541–552, 2003

Modeling of the Thyme–Supercritical Carbon-Dioxide Extraction System. II. The Influence of Extraction Time and Carbon Dioxide Pressure

Zoran Zeković,* Žika Lepojević, and Aleksandar Tolić

Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Novi Sad, Yugoslavia

ABSTRACT

The influence of extraction time and carbon dioxide pressure (i.e., carbon dioxide density) on supercritical fluid extraction of thyme was investigated. For determination of obtained extracts, HPLC and GC-MS methods were used. To model extraction of the thyme–supercritical carbon dioxide system, we used the Reverchon-Sesti Osseo equation as well as our modified equation.

*Correspondence: Zoran Zeković, Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Yugoslavia; E-mail: zzekovic@tehnol.ns.ac.yu and E-mail: zekovic@EUnet.yu.

DOI: 10.1081/SS-120016650 0149-6395 (Print); 1520-5754 (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

INTRODUCTION

The strongly antiseptic and antifungal activities of thyme (*Thymus vulgaris* L.), i.e., thyme essential oil, is mainly due to the presence of phenolic compounds, namely 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]

The 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 the first part of the same work on supercritical fluid extraction (SFE) of thyme (*Thymus vulgaris* L.), the influence of flow rate of carbon dioxide and grinding degree of thyme were reported.[11] In this paper, the influence of extraction time, as well as of carbon dioxide pressure in SFE of thyme by carbon dioxide is described.

EXPERIMENTAL

Plant Material

Thyme was produced in the village of Sanad, near Čoka, Vojvodina, Yugoslavia, 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 (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, MA 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-Pak™/Resolve™ (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^{-1}. After filtration [0.45 μm Millipore filter (Milipore, Bedford, MA)], 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]

GC-MS

The GC instrument was a GCD HP G 1800 A (Hewlett-Packard, Palo Alto, CA). A column HP-5 MS (30.0 m × 0.25 mm; film thickness 0.25 μm) was used. The helium flow rate was 0.8 mL/min. The injector temperature was 250°C and the detector was set at 280°C. The detector was set initially at 50°C and was increased linearly at 20°C/min to 130°C (1 min) and then was increased 9°C/min 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 quasiinternal standard method (the content of thymol determined in sample by HPLC).[9]

Supercritical Fluid Extraction

SFE-CO_2 was carried out with a laboratory-scale high-pressure extraction plant (NOVA-Swiss, Effretikon, Switzerland) described previously.[13] The main parts and characteristics (manufacturer specification) of the plant were as follows: a 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 extractor was 50 g at the investigated value of pressure and at 40°C, and the CO_2 flow rate was 97.72 dm^3/h. Separator conditions were 15 bar and 25°C.

RESULTS AND DISCUSSION

After investigations on the influence of carbon dioxide flow rate and grinding degree of thyme, the flow rate of supercritical carbon dioxide of 97.72 dm^3/h and thyme mean particle radius of 0.35 mm were obtained and considered the best parameters for thyme supercritical fluid extraction (SFE) by carbon-dioxide.[11] For modeling of investigated thyme–supercritical carbon dioxide extraction system in this paper, we again used the final form of the Reverchon-Sesti Osseo equation.[14]:

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

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]:

$$Y = 100[1 - \exp(at + b)] \tag{2}$$

where Y is the normalized extraction yield ($Y = \frac{Y_{ext}}{Y_{max}} \times 100$; 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.

The composition of extract obtained after 2.5 hours of extraction by supercritical carbon dioxide at 100 bar and 40°C was a very similar to that of the essential oil obtained by steam distillation. In this way, a quantitative extraction of thyme essential oil was obtained, too.[11]

For investigation of the extraction time influence on thyme SFE by carbon dioxide, extraction time was prolonged. After 10 hr of extraction, the extraction yield was 3.7% (wt/wt). The results of qualitative and quantitative extract analyses are shown in Table 1.

By increasing of the extraction time from 2.5 hr up to 10 hr, the content of the pharmacologically important compound of thyme-thymol decreases from 37.3% to 21.8%. The consequence is a lesser quality of obtained extract because of the coextraction of other, not-so-important thyme compounds by prolonged extraction time. In the point of a view of extract qualitative and quantitative characteristics, it could be concluded that the extraction time of 2.5 hr is the best for obtaining the thyme extract.

For modeling of the extraction results by Eqs. (1) and (2), the values of internal mass-transfer coefficient (D), i.e., internal diffusion time (t_i), and a constant a and correction term b, obtained previously,[11] were used.

Table 1. Results of GC-MS analysis of compounds obtained by extraction of thyme through supercritical carbon dioxide (100 bar; 40°C; 10 hr of extraction).

	Content in relation to:	
Compound	T (mg/100 g)	TE (%)
Camphor	10.2	0.3
L-Menthol	36.2	1.0
n- Dodecane	34.9	0.9
Thymol	810.3	21.8
n-Tetradecane	687.7	18.5
β-Caryophyllene	32.6	0.9
2-Methyldecane	22.9	0.6
n-Pentadecane	102.8	2.8
n-Hexadecane	93.4	2.5
n-Heptadecane	25.5	0.7
n-Octadecane	38.1	1.0
Phytol	16.6	0.4

The following equations for total extract (TE) and thymol (T) normalized extraction yields were obtained:

$$Y_{TE} = 100\left[1 - \exp\left(-\frac{t}{9.753 \times 10^3}\right)\right] \tag{3}$$

$$Y_T = 100\left[1 - \exp\left(-\frac{t}{3.281 \times 10^3}\right)\right] \tag{4}$$

and

$$Y_{TE} = 100\left[1 - \exp(-0.308t - 0.0793)\right] \tag{5}$$

$$Y_T = 100\left[1 - \exp(-1.012t - 0.0210)\right] \tag{6}$$

The corresponding graphical presentation of Eqs. (3–6), and experimental values are given in Fig. 1.

The calculated values of the standard error of regression ($S_{Y,X}$), given in Table 2, show that the modified Eq. (2) is generally a better fit for Y_{TE} and Y_T than was the original Eq. (1).

Investigated pressures and corresponding densities of carbon dioxide are given in Table 3.

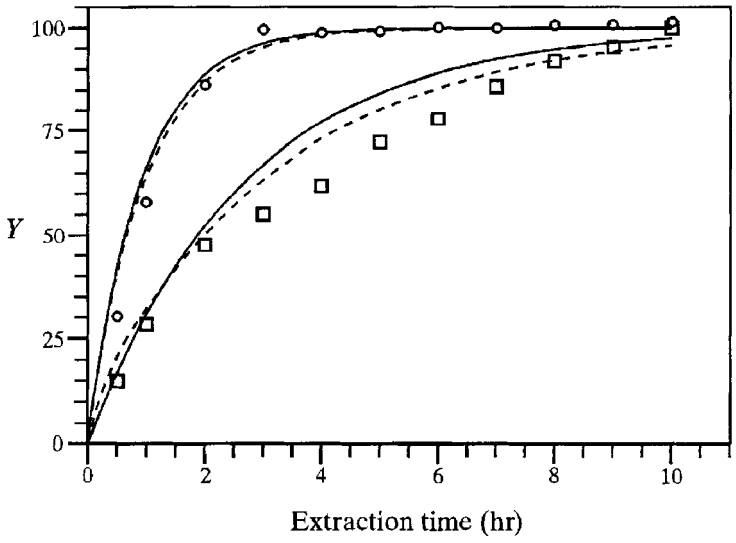

Figure 1. Graphical presentation of Eqs. (3) and (4) by solid line and Eqs. (5) and (6) by dashed line for total extract (□) and thymol (○).

The results of extraction yield and content of extracted compounds, obtained after 2.5 hr of extraction at each pressure, are shown in Table 4.

The extraction by low carbon-dioxide solubility power (80 bar; 40°C; 0.192 g/cm^3) did not yield in quantitative extraction of essential oil (after 2.5 hr, only 21% of thymol total content was extracted). The obtained results show that by increasing of carbon dioxide pressure (i.e., density or solubility power), the extraction yield of thyme compounds, especially paraffins, increases.

Table 2. Standard error of regression ($S_{Y,X}$) of investigated model equations for total extract *(TE)* and thymol (*T*).

	$S_{Y,X}$[a]	
Equation	*TE*	*T*
(1)	8.189	4.877
(2)	6.061	4.210

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

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Table 3. Investigated pressures (p) and densities (d) of carbon dioxide at 40°C.

P (bar)	d (g/cm^3)
80	0.192
100	0.630
150	0.790
200	0.845
400	0.983

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

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

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 ($q_i = q_o - q_i{}'$); $q_i f'$ is the content of matter extracted from thyme (e.g., thymol) after t; and t is the extraction time (seconds),

$$ti = \mu \frac{I^2}{D} \tag{8}$$

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).

Eq. (1) was modified 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

$$-\frac{t}{ti} = at + b \tag{9}$$

Note that for $-t/t_i = Z$, a is a constant; t is the extraction time (hours), and b

Table 4. Extraction yield and content of extracted compounds at investigated pressures.

Pressure (bar)	80		100[a]		150		200		400	
Extraction yield (%; wt/wt)	0.7		2.1		3.2		3.8		3.9	
	Content in relation to:									
Compound	*Th*[b]	*TE*[c]	*Th*	*TE*	*Th*	*TE*	*Th*	*TE*	*Th*	*TE*
Camphor	3.7	0.5	10.2	0.5	22.0	0.7	24.0	0.6	25.5	0.6
L-Menthol	53.6	7.6	59.5	2.8	73.0	2.3	72.8	1.9	75.7	1.9
n-Dodecane	13.1	1.8	38.2	1.8	65.4	2.0	79.7	2.1	84.7	2.1
Thymol	167.6	23.7	779.8	37.3	833.5	26.0	854.2	22.3	788.7	20.0
n-Tetradecane	209.9	29.7	555.4	26.6	1,095.9	34.2	1,437.3	37.5	1,533.9	38.9
β-Caryophyllene	26.7	3.8	29.0	1.4	33.2	1.0	36.4	0.9	38.6	1.0
2-Methyldecane	6.7	0.9	13.8	0.7	33.4	1.0	43.7	1.1	41.8	1.1
n-Pentadecane	31.2	4.4	78.5	3.8	156.5	4.9	202.9	5.3	207.1	5.2
n-Hexadecane	26.9	3.8	70.1	3.3	140.8	4.4	179.5	4.7	183.0	4.6
n-Heptadecane	13.7	1.9	49.7	2.4	99.9	3.1	127.6	3.3	129.7	3.3
n-Octadecane	9.8	1.4	26.5	1.3	53.0	1.6	67.3	1.8	68.8	1.7
Phytol	–	–	29.0	1.4	11.5	0.4	–	–	14.9	0.4

[a] Published results.[11]
[b] Thyme (mg/100 g).
[c] Total extract (%; wt/wt).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Table 5. Parameters of Eq. (1) (internal mass transfer coefficient D and internal diffusion time t_i) and Eq. (2) (coefficients a and b, correlation coefficient γ) and standard error of regression ($S_{Y,X}$) for applied equations.

	Total extract					Thymol				
p (bar)	80	100[a]	150	200	400	80	100[a]	150	200	400
Parameters of Eq. (1)										
D (10^{12}) (m^2/s)	1.93	0.836	2.45	2.86	3.45	1.14	2.49	3.03	3.89	3.97
t_i (10^{-3}) (s)	4.219	9.753	3.329	2.855	2.361	7.127	3.281	2.691	2.095	2.055
Parameters of Eq. (2)										
a	−0.921	−0.308	−1.034	−1.157	−1.798	−0.507	−1.012	−1.615	−1.226	−1.165
b	−0.009	−0.079	−0.052	−0.045	0.204	0.042	−0.021	0.264	−0.205	−0.349
|r|	0.993	0.986	0.994	0.999	0.999	0.999	0.985	0.999	0.984	0.987
Standard error of regression ($S_{Y,X}$)										
Eq. (1)	4.307	3.645	2.955	1.274	2.671	2.998	4.727	6.413	4.223	4.160
Eq. (2)	3.241	2.945	4.152	1.095	2.488	0.676	4.893	1.156	5.016	3.329

[a]Published results.[11]

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

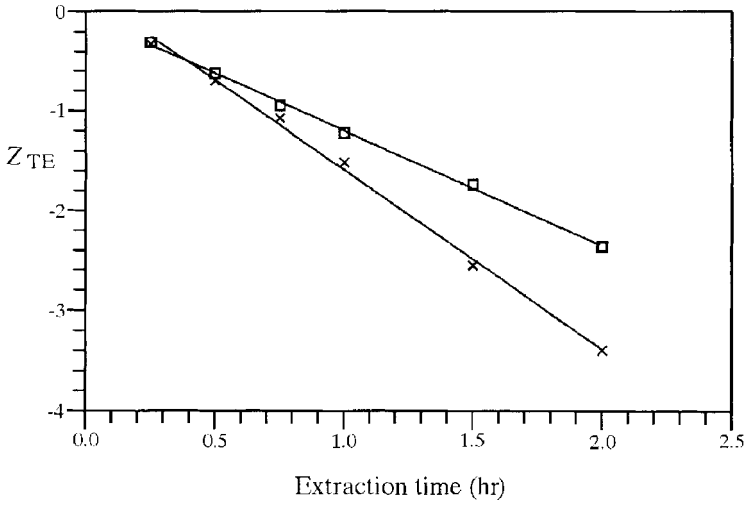

Figure 2. Z_{TE} vs. extraction time. □ 200 bar, × 400 bar.

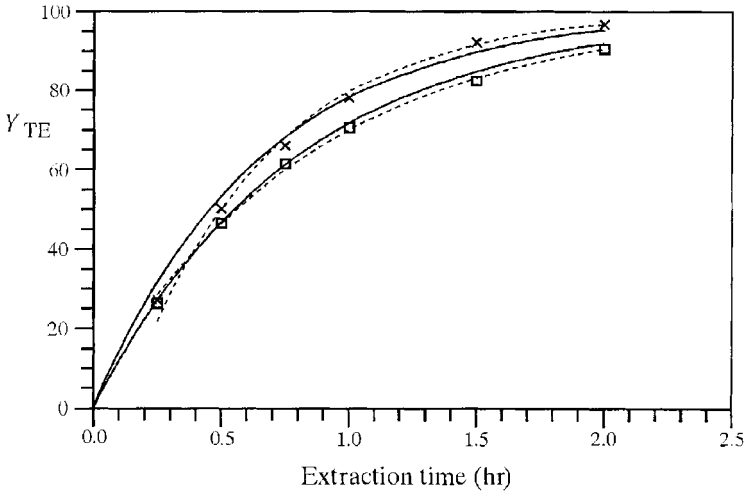

Figure 3. Graphical presentation of Eq. (1) by solid line and Eq. (2) by dashed line for total extract (*TE*). □ 200 bar, × 400 bar.

is a correction term. Z is defined as

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

where Y is the normalized extraction yield.[1]

In Table 5 are given calculated values of Eqs. (1) and (2) parameters for modeling of the thyme–supercritical carbon dioxide extraction system at investigated pressures, as well as values of the standard error of regression ($S_{Y,X}$) for obtained equations.

As an illustration, the dependence of Z_{TE} and Y_{TE} on the extraction time at pressures of 200 bar and 400 bar are shown in Figs. 2 and 3, respectively.

The calculated values of the standard error of regression ($S_{Y,X}$), given in Table 5, show that the modified equation (2) is generally a better fit for Y_{TE} and Y_T than was the original Eq. (1).

ACKNOWLEDGMENT

The authors are thankful to Mr. Lazar Oluški for thyme sample.

REFERENCES

1. Chevallier, A. *The Encyclopedia of Medicinal Plants*; Dorling Kindersley Limited: London, 1996; 142.
2. Kišgeci, J.; Adamović, D.; Soldatovic, M. Sistematski prikaz privredno značajnih lekovitih, aromatičnih i začinskih biljaka i njihov izbor za gajenje u Vojvodini. Bilten za hmelj, sirak i lekovito bilje **1985**, *17* (49–50), 44–71, (in Serbian).
3. Pekić, B. *Hemija i tehnologija farmaceutskih proizvoda (alkaloidi i etarska ulja)*; (in Serbian) Tehnološki fakultet Univerziteta u Novom Sadu: Novi Sad, 1983; 223.
4. *The Merck Index*, 10th Ed.; Merck & Co, Inc: Rahway, N.J, USA, 1983; 978.
5. Wagner, H.; Bladt, S.; Zgainski, E.M. *Plant Drug Analysis*; Scott, A., Ed.; Trans. Springer-Verlag: Berlin, 1984; 13.
6. *Pharmacopoeia Jugoslavica, Editio quarta* (Ph. Jug. IV), Savezni zavod za zdravstvenu zaštitu, Beograd, 1984 (in Serbian).

7. Pekić, B.; Lepojević, Ž.; Zeković, Z.; Pekić, L. Ispitivanje sadŕaja etarskog ulja i fenolnih jedinjenja u nekim biljnim vrstama roda Thymus i Satureja. Proc. Fac. Technol. (Novi Sad) **1991**, *22*, 87 (in Serbian).
8. Fehr, D.; Stenzhorn, G. Untersuchungen zur Lagerstabilität von Pfefferminzblättern, Rosmarinblättern und Thymian. Pharmazeutische Zeitung **1979**, *124* (46), 2342 (in German).
9. Zeković, Z.; Lepojević, Ž.; Vujić, Dj. Supercritical Extraction of Thyme (Thymus vulgaris L.). Chromatographia **2000**, *51* (3/4), 175.
10. Zeković, Z.; Lepojević, Ž.; Milošević, S.; Tolić, A. Thyme (*Thymus vulgaris* L.) compounds in SFE. Proceedings 6th Conference on Supercritical Fluids and Their Applications, Maiori, Italy, September, 9/10, 2001; 209.
11. Zeković, Z.; Lepojević, Ž.; Tolić, A. Modelling of the Thyme-Supercritical Carbon Dioxide Extraction System; I. The Influence of Carbon Dioxide Flow Rate and Grinding Degree of Thyme. Sep. Sci.Technol. **2001**, *36* (15), 3459.
12. Aleksovski, S.; Sovová, H.; Poposka, F.; Curapova, B. Supercritical fluid extraction of essential oils from peppermint and wild thyme and its comparison with hydrodistillation. XIII CHISA '98 (13th International Congress of Chemical and Process Engineering), Praha, Aug 23–28, 1998; Summaries 4, 118.
13. Pekić, B.; Zeković, Z.; Petrović, L.; Tolić, A. Behavior of (-)-(-bisabolol and (-)-(-bisabololoxides A and B in camomile flower extraction with supercritical carbon dioxide. Sep. Sci. Technol. **1995**, *30* (18), 3567.
14. Reverchon, E.; Sesti Osseo, L. Modelling the supercritical extraction of basil oil. Proc. 3rd Int. Symp. Supercritical Fluids, **1994**, *2*, 189.

Received December 2001
Revised July 2001