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Supercritical extraction of *Salvia officinalis* L.

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Abstract - In this paper supercritical fluid extraction with carbon dioxide at different pressure was investigated and the total yield of the extract was measured. With increasing pressure from 80 to 300 bar extraction yield was increasing. It was due to the fact that supercritical carbon dioxide density increases by increasing pressures. Qualitative and quantitative analyses obtained extracts and essential oils obtained from these extracts were done using GC/MS and GC/FID analyses. Also, investigation of the extraction kinetics in the function of pressure (80, 100 and 200 bar), where every point of kinetic curve with new sample of drug in extractor was obtained is performed. The highest extract yield was obtained at pressure 200bar (4.17g/100g drug). At the end of the extraction process, CO₂ – extract has been removed, and content of residual essential oil in the drug was determined. Essential oil content in drug after supercritical extraction was the highest at pressure 80 bar.

I. INTRODUCTION

With increasing public interest in herbal medicine and natural products, the conventional methods such as hydro-distillation and solvent extraction were found unsatisfactory. 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 either organoleptic and/or health reasons. Besides, organic solvents are insufficiently selective, so that, in addition to the active substances, they also dissolve some concomitant compounds. An alternative extraction technique with better efficiency and selectivity is highly require, in order to eliminate solvents, avoid the degradation or loss of sensitive and thermo-labile substances and to decrease the high energy and manpower input of conventional processes [1, 2]. To improve efficiency and selectivity of the extraction, alternative extraction techniques as supercritical extraction started to be developed.

The broad interest in supercritical CO₂ extraction (SFE) of essential oils is proved by large number of scientific literature published on this argument. These studies were undertaken in view of a possible industrial application of the process.

Supercritical extraction is not widely used yet, but as new technologies are coming there are more and more viewpoints that could justify it, as high purity, residual solvent content and environment protection [1, 2, 3]. The use of supercritical

fluids (SCF) as reaction or separation media offers the opportunity to replace conventional organic solvents and also to optimize and potentially control the effects that solvent properties can have on selectivity [2]. Carbon dioxide is most widely used in SFE because it is simple to use, inexpensive, non-flammable, nontoxic, chemically stable, shows great affinity to volatile (lipophilic) compounds, and can be easily and completely removed from any extract [2, 4, 5, 6]. By changing pressure and /or temperature above critical point of carbon dioxide (T_c=31.3°C, P_c=72.8 bar,) a pronounced change in the density and dielectric constant, i.e. solvent power of supercritical carbon dioxide can be achieved.

The special properties of supercritical fluids bring certain advantages to chemical separation processes. Several applications have been fully developed and commercialized [6, 7]. The biggest application is the decaffeination of tea and coffee. A process for removal of caffeine from coffee using supercritical carbon dioxide was patented in the United States in 1974, and a commercial plant went on stream in the FRG in 1978. Other important areas are the extraction of essential oils and aroma materials from spices. Brewery industry uses SFE for the extraction of hop. The method is used in extracting some edible oils and producing cholesterol-free egg powder.

In this paper, SFE of essential oil from sage (*Salvia officinalis* L.) was investigated.

II. PLANT MATERIAL

For this experiments *Salvia officinalis* L. from Berkovici, near Trebinje gathered 2008 was used.

III. CHEMICALS

Commercial carbon dioxide (99% purity, Tehno-gas, Novi Sad, Serbia) as the extracting agent was used. All other chemicals were of analytical reagent grade.

Chromatographic procedures:

MS, Finnigan – MAT 8230 BE geometry, resolution 1000, EI – CIU source at 200°, EI 70 eV, 0.5 mA; CI, 1 mtorr of isobutane 150 eV 0.2 mA.

GC/MS, Varian 3400 GC equipped with Split/Splitless injector (1:99) operated at 244°. Column J&W Scientific DB-5ms-ITD 30m, 0.25mm id, 0.25µm film. Carrier gas hydrogen, 1 ml/min measured at 210°. Column temperature was linearly

programmed from 40° to 285° at 4.3°/min. Transfer line at 270°, coupled to Finnigan-MAT 8230 BE mass spectrometer, ion source temperature 1700, EI, 70eV 0.1 mA. Scan range 33-333 / 1 sec.

GC, HP5890 series II 3400 GC equipped with Split/Splitless injector (1:99) operated at 244°. Column J&W Scientific DB-5ms-ITD 30m, 0.25mm id, 0.25µm film. Carrier gas hydrogen, 1 ml/min measured at 210°. Column temperature was linearly programmed from 40° to 285° at 4.3°/min.

IV. SUPERCRITICAL FLUID EXTRACTION

SFE by CO₂ was carried out with a laboratory – scale high – pressure extraction plant (NOVA – Swiss, Effretikon, Switzerland), shown in Fig. 1. 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 (Pmax = 250bar), and maximum CO₂ mass flow rate of approximately 5.7 kg/h.

The mass of Salvia sample in extractor was 60g at the investigated value of pressure and at temperature 40°C, and the CO₂ flow rate was 97.72 dm³/h. Separator conditions were pressure 15 bar and temperature 25°C.

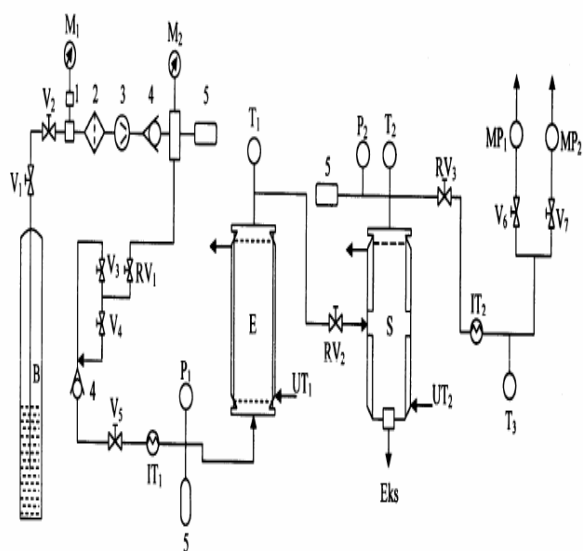


Figure 1. Laboratory scale high pressure SFE plant

1- measuring connector; 2 – filter; 3 – diaphragm compressor;
4 – control valve; 5 – safety valve; RV – regulation valve; V – valve;
HE – heat exchanger; B – carbon dioxide bottle; P – pressure gauge;
T – thermometer; UT – ultrathermostat; M – manometer; E – extractor;
S – separator; FM – flow meter;
Exs – extract

V. RESULTS AND DISCUSSION

The supercritical fluid extraction (SFE) of *Salvia officinalis* by carbon dioxide was investigated in order to obtain the best conditions. Experiments were performed at different pressures, all other extraction conditions were same (Table 1). In order to prevent thermal decomposition of some volatile oil compounds, the temperature of supercritical fluid extraction of 40°C was selected. The pressure range of 80 – 300 bar (a pronounced change in the density and dielectric constant, i.e. solubility power of carbon dioxide) for SFE of *Salvia officinalis* L. was investigated. The selections of the pressures ranges are based on the fact that a great change in the density and dielectric constant of CO₂ occurs between pressure 80 and 150 bar.

Investigations of extraction kinetics of sage in the function of pressure are obtained results which were shown in the Fig. 2.

After extraction time of 4h the amounts of obtained extracts were measured and the extraction yields were calculated. Essential oils were isolated from obtained CO₂ extracts, and their contents in extracts were calculated (Table 2).

TABLE 1. Supercritical CO₂ extraction conditions

Sample	d (mm)	extraction time (h)	CO ₂ flow rate (kg/min)	t (°C)	p (bar)
F1	0.32	4	3.23·10 ⁻³	40	80
F2	0.32	4	3.23·10 ⁻³	40	100
F3	0.32	4	3.23·10 ⁻³	40	150
F4	0.32	4	3.23·10 ⁻³	40	200
F5	0.32	4	3.23·10 ⁻³	40	300

d - mean particle diameter

t – temperature

p - pressure

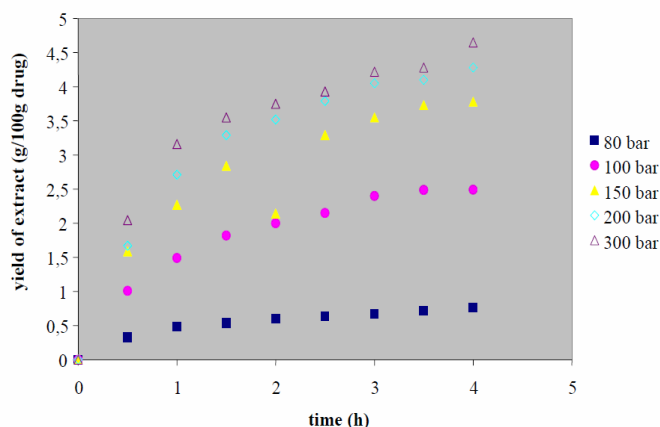


Figure 2. Plots yield of extract vs. extraction time

Table 2. Yield of CO₂ – extract and essential oils in extract

pressure (bar)	yield of CO ₂ – extract (g/100g drug)	Content of essential oils in CO ₂ - extract (ml/100g CO ₂ - extract)
80	0.76	58.79
100	2.49	47.87
150	3.78	40.00
200	4.28	29.93
300	4.65	29.90

Results showed that increasing pressure from 80 to 300 bar enhanced the extraction yield, which was due to the increased SCCO₂ density at higher pressures. Also extract obtained at pressure 80 bar has the highest content of essential oil (58.79%), while the contents in other extracts were from 29.90 to 47.87%.

CO₂ extracts and essential oils obtained from CO₂ extracts were detailed identification and quantification using GC/MS and GC/FID analyses. The results of qualitative and quantitative analyses were given in table 3 and table 4.

It can be seen from table 3 that the extracts obtained at pressure 150 and 200 bar consists β – thujone while other extracts does not consists it. Dominant components are phyllocladene, γ – elemene, isoborneole, selina – 3,7 (11) diene, camphor, 1,11 – epoxyhumulene. In extract component contents varying in the function of pressure. Content of phyllocladene in extract obtained at pressure $p = 80$ bar is 10.42%, while in other extracts is from 21.99 to 30.64%. At the pressure $p = 80$ bar content of camphor is least (1.43%), at other pressures it is from 11.37 to 15.24%. Extract obtained at pressure $p = 80$ bar, contents γ – elemene 24.98%, in other extracts content is from 7.02 to 9.73%. Also, we have seen that the extract obtained at pressure $p = 80$ bar contents a lot of 1,11 – epoxyhumulene (8.99%), in other extracts (4.56 – 5.87%). Selina 3,7 (11) diene content is approximately equal in all obtained extracts (11.25 - 13.83%).

Qualitative contents of essential oils obtained from CO₂ extracts at pressure 80, 100, 150 and 200 bar) is same, while essential oil obtained at pressure 300 bar is different. This essential oil has not β – pinen. All essential oils have similar quantitative contents in regard dominated components. Dominated component are: α – thujone, camphor, isoborneole, γ – elemene. From results shown in table 4, we can conclude that investigated essential oils content α – thujone (15.63 – 27.38%) in high percentage. Also, essential oil from extract obtained at pressure 300 bar there is phyllocladene in high percentage (23.37%). In other essential oils there are phyllocladene from 4.19 to 6.90%. In essential oil from extract obtained at pressure 80 bar γ – elemene is the highest (15.52%), and in other essential oils (7.46 – 9.86%). Camphor content is similar in all essential oils (16.03 – 23.45%).

Table 3. Qualitative and quantitative content of CO₂ – extract

Component	Component content (% m/m)				
	CO ₂ extract				
	Pressure (bar)				
	80	100	150	200	300
α – thujone	0.66	4.44	4.18	3.77	5.15
β - thujone	-	-	0.19	0.70	-
camphor	1.43	11.93	11.37	14.88	15.24
isoborneole	11.29	7.39	6.80	9.52	8.17
terpineol L - 4	2.08	0.32	0.25	0.33	0.30
bornyl – acetate	5.90	3.59	2.01	4.62	3.96
sabiny – acetate	1.05	0.53	0.41	0.64	0.42
isocaryophyllene	2.74	1.18	0.84	1.30	1.17
α – gurjunene	1.45	0.54	0.44	0.62	0.55
γ - elemene	24.98	9.31	7.02	9.73	9.00
selina – 3,7 (11) diene	11.25	12.17	13.83	12.51	12.14
1,11 – epoxyhumulene	8.99	4.56	5.87	4.96	4.92
caryophyllene oxide	2.76	2.66	2.64	2.39	2.46
phyllocladene	10.42	26.06	30.64	21.99	24.60
Total	85.00	84.70	86.50	88.00	88.10

Table 4. Qualitative and quantitative content of essential oil

Component	Component content (% m/m)				
	essential oil				
	Pressure (bar)				
	80	100	150	200	300
β – pinene	0.23	0.20	0.33	0.62	-/-
1,8 - cineole	0.47	1.77	1.06	2.88	0.96
α – thujone	19.56	26.28	23.48	27.38	15.63
β - thujone	3.30	4.11	3.57	4.17	2.44
camphor	19.56	22.95	23.45	23.06	16.03
isoborneole	9.12	9.89	12.11	7.94	8.16
terpineol L - 4	0.78	0.75	0.89	0.62	0.54
bornyl – acetate	6.81	4.33	4.82	3.74	3.51
sabiny – acetate	0.86	0.51	0.55	0.43	0.43
isocaryophyllene	2.10	1.21	1.39	1.05	1.01
α – gurjunene	0.88	0.42	0.63	0.49	0.34
γ - elemene	15.52	8.66	9.86	7.46	7.76
selina – 3,7 (11) diene	5.60	5.61	6.46	5.85	8.64
1,11 – epoxyhumulene	3.67	2.01	2.12	1.98	/2.85
caryophyllene oxide	0.97	0.87	0.93	0.97	1.73
phyllocladene	4.19	6.27	4.75	6.90	23.37
Total	93.70	96.10	96.40	95.50	93.50

In continue of investigation, the extraction of kinetics in the function of pressure (80, 100 and 200 bar) was performed. Now every point of kinetic curve was obtained with new sample of drug in extractor. Results of investigation of kinetics extraction are shown on the Fig 3.

From Fig. 3 we show that the extraction yield dependent from extraction pressure and extraction time, and increase by increasing pressure and extraction time.

Essential oils content in CO₂ extract in the function of extraction time for different pressures are given in table 5.

We see that the highest essential oil (26.03 – 75.85%) was obtained at pressure $p = 80$ bar. Similar results were obtained at pressure $p = 100$ bar (21.36 - 75.25%). With increasing pressure to 200bar, content of essential oil in obtained extracts for investigated extraction time was from 17.39 to 61.06%.

At the end of the extraction process, CO₂ – extract has been removed, and content of essential oil in residual drug was determined. Results are shown in table 6.

Content of essential oil in residual drug which firstly extracted by carbon dioxide at pressure $p = 80$ bar is from 0.2 to 0.66% and it is significant amount in regard to drug extracted on pressure $p = 100$ bar (0.04%) and pressure $p = 200$ bar (0.02%).

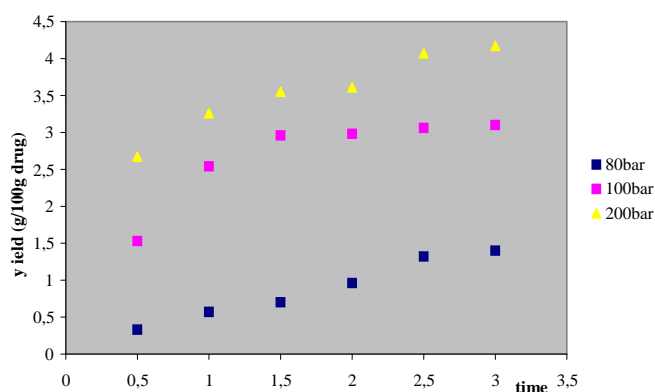


Figure3. Plot total extract yield vs extraction time (conditions: $T = 313K$; $d = 0.22mm$; $w = 3.23 \cdot 10^{-3} kg/min$)

Table 5. Essential oil content in CO₂ extract (%)

Extraction time (h)	Pressure (bar)		
	80	100	200
	Content of essential oil in CO ₂ extract (% w/w)/extract yield (g/100g drug)		
0.5	75.85/0.33	75.25/1.53	61.06/2.67
1.0	72.50/0.57	65.81/2.54	48.43/3.26
1.5	70.01/0.70	62.70/2.96	40.23/3.55
2.0	63.45/0.96	58.59/2.98	36.49/3.61
2.5	41.26/1.32	51.56/3.06	32.40/4.07
3.0	26.03/1.40	21.36/3.10	17.39/4.17

From results (table 5) we can see that extract yield of sage is increasing and essential oil content is decreasing at defined pressure during the extraction of sage. The essential oil content is decreasing with the time because the extract yield is increasing with the time.

Table 6. Essential oil content in residual drug which first extracted by carbon dioxide (extraction conditions: $T = 40^{\circ}C$; $w = 3.23 \cdot 10^{-3} kg/min$; $d = 0.22mm$)

Extraction time (h)	Content of essential oil in residual drug (% w/w)		
	Pressure (bar)		
	80	100	200
0.5	0.66	0.40	0.04
1.0	0.54	0.04	0.02
1.5	0.50	0.04	0.0
2.0	0.36	0.0	0.0
2.5	0.30	0.0	0.0
3.0	0.20	0.0	0.0

Conclusion

The solubility of investigated sage is equal to their yield. So it could be concluded that sage solubility increase by increasing of carbon dioxide pressure, i.e. density or solubility power of extragent. According to the results of this study, SFE offered more choices (pressures level) for the extraction of different components.

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