

INFLUENCE OF PRESSURE AND TIME ON EXTRACTION PROCESS USING SUPERCRITICAL CO₂

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Abstract

*The supercritical fluid extraction (SFE) by carbon dioxide (CO₂) of *Salvia officinalis* L. was investigated. SFE by CO₂ was performed at different pressure (80, 100, 150, 200 and 300 bar) and constant temperature of 40°C (all other extraction conditions, such are flow rate, particle diameter of *Salvia officinalis*, extraction time were kept constant.*

The GC – MS method was used for determination of qualitative and quantitative composition of obtained extracts and essential oils.

*Keywords: *Salvia officinalis* L., essential oil, supercritical fluid extraction, extraction pressure, GC – MS, carbon dioxide*

1. Introduction

The extraction by gases under pressure has been more and more used, representing a good alternative to the classic extraction procedures.

The advantages of the supercritical fluid

extraction (SFE) technique are well known by now and it is often regarded as an alternative to the traditional methods. CO₂ is most widely used in SFE because it is simple to use, inexpensive, nonflammable, nontoxic, chemically stable, shows great

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affinity to volatile (lipophilic) compounds and can be easily and completely removed from any extracts. With critical point at 31.3°C and 72.8 bar, carbon dioxide can be used at temperatures and pressures which are relatively safe, convenient and particularly appropriate for extraction of a range of more volatile and/or heat – labile compounds [1], [3], [4].

Supercritical fluid extraction is widely used in natural products, foods and flavors, pharmaceuticals, nutraceuticals, polymers, chemicals, and parts cleaning industries.

Natural materials contain various extractable fractions. Among these, essential oils are particularly relevant since they represent the basic material for many foods, cosmetics, and pharmaceutical preparations.

Several studies have been devoted to the extraction of essential oils and of related products by supercritical CO₂, as reviewed by Stahl et al. and by Reverchon et al. Nevertheless [1], the influence of major operating parameters of the extraction process has rarely been studied. For example, it is known that the extraction time can play a main role in determining the extract composition if the families of compounds constituting the essential oil show different diffusion times during supercritical extraction.

Relative merits of using CO₂ at liquid or supercritical conditions should also be considered. Some authors have suggested the use of liquid CO₂ for the extraction of essential oils, although it has been observed that somewhat different products can be obtained when compared with essential oils produced by traditional techniques, like steam distillation. Indeed, they noted the presence of higher molecular weight

compounds in liquid CO₂ extracts. Other investigators, have used CO₂ at supercritical conditions to avoid the simultaneous extraction of essential oil and high – molecular – weight compounds.

Therefore, supercritical CO₂ can show higher selectivity compared with liquid CO₂ since its density varies from about 0.2 to 0.9 gcm⁻³ for many SFE conditions (temperatures from 40 to 60°C, pressures from 80 to 300 bar). By contrast, the density of liquid CO₂ can be varied only between about 0.7 and 1.0 gcm⁻³ at the extraction conditions typically used for natural product extraction. Therefore, in processes performed at high CO₂ densities, the lower process selectivity associated with the higher extraction yield can result in the simultaneous extraction of several compound families and the co extraction of compounds that do not contribute to fragrance formation. The latter compounds are generally, fatty acids, fatty acid methyl esters, pigments, and other higher molecular weight compounds. Since the odoriferous compounds, such as terpenes, oxygenated terpenes, sesquiterpenes, and oxygenated sesquiterpenes are readily soluble in supercritical CO₂, the extraction of essential oils at CO₂ high densities is neither necessary, nor desirable [5], [6], [7].

Much work has recently been done on the composition of *Salvia officinalis* essential oil obtained by traditional techniques [1], [2], [3].

In this paper, the influence of extraction time, as well as of carbon dioxide pressure in SFE of *Salvia officinalis* by carbon dioxide is described.

2. Experimental

2.1. Plant Materials

For this experiments *Salvia officinalis* from Berkovici, near Trebinje gathered 2006 was used. The mean particle radius (r) were $d=0.3157$ mm. After the grinding using sieve sets (Erweva Apparatebau GmbH, Germany), the mean particle radius was calculated as

$r=100/\Sigma(m_i/Q_m)$, where m_i is the fraction (%) after sieving, and Q_m is the mean aperture diameter of two adjacent sieves.

2.2. 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.

2.3. Chromatographic procedures

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

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, 0.25 μ m 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 170°, 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, 0.25 μ m film. Carrier gas hydrogen, 1 ml/min measured at 210°. Column temperature was linearly programmed from 40° to 285° at 4.3°/min.

2.4. Supercritical Fluid Extraction

SFE-CO₂ was carried out with a laboratory – scale high – pressure extraction plant (NOVA – Swiss, Effretikon, Switzerland). 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 ($P_{max}=250$ bar), 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 40°C, and the CO₂ flow rate was 97.72 dm³/h. Separator conditions were 15 bar and 25°C.

3. Results and Discussion

The supercritical fluid extraction (SFE) by carbon dioxide (CO₂) of *Salvia officinalis* L. was investigated. SFE by CO₂ was performed at different pressure (80, 100, 150, 200 and 300 bar) and constant temperature of 40°C (all other extraction conditions, such are flow rate, particle diameter of *Salvia officinalis*, extraction time were the same)

The extract yields and essential oils yield are given for different pressures in Table 1. By increasing the pressure from 80 to 300 bar at 40°C increasing extract yields and decreasing the essential oil content in the total extract.

Table 1. The results of the extraction of *Salvia officinalis* L. by supercritical carbon dioxide

P (bar)	Extract yields (g/100g drug)	Essential oil yield ml/100g TE
80	0.76	58.79
100	2.49	47.87
150	3.78	40
200	4.28	29.93
300	4.67	29.9

Now the influence of extraction time on supercritical extraction of *Salvia officinalis* L. at different pressure was investigated.

Table 2. Parameters of Eq. 1 (coefficients a, b, c and correlation coefficient r)

Pressure (bar)	Parameters of equation (1)			Correlation coefficient r
	a	b	c	
80	-0.0556	0.3785	0.0858	0.972
100	-0.1922	1.3193	0.2124	0.986
150	-0.3043	2.037	0.3383	0.984
200	-0.3673	2.3888	0.3496	0.984
300	-0.3755	2.4291	0.5591	0.964

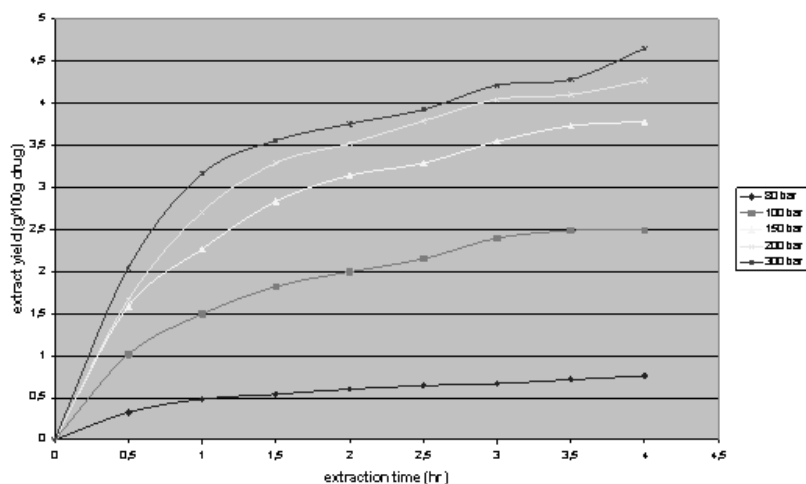


Fig. 1. Extract yield vs extraction time at different pressure

Obtained results are shown on Fig. 1.

In the first 2.5 hours of extraction by supercritical carbon dioxide, extract yield is the highest. Dependence extract yield on extraction time at pressure 80, 100, 150, 200 and 300bar can be shown by polinomial second order $y = ax^2 + bx + c$ approximately (Eq. 1). Calculated values paramerters at investigated pressures, as well as values correlation coefficient r are given in Table 2.

The relative contents of the identified components of essential oil EO1 (p=80 bar), EO₂ (p=100 bar), EO₃ (p=150 bar), EO₄

(p=200 bar), EO₅ (p=300 bar) are given in Tab.3.

The relative contents of the identified components of extracts E₁ (p=80 bar),

E₂ (p=100 bar), E₃ (p=150 bar), E₄ (p=200 bar), E₅ (p=300 bar) are given in Tab. 4.

The GC – MS method was used for determination of qualitative and quantitative composition (Table 3. and Table 4.). As an illustration GC chromatograms of *Salvia officinallis* extract and essential oil obtained by SFE – CO₂ method are shown in Fig. 2. and Fig. 3.

Table 3. Composition of essential oils of *Salvia officinallis* L.

N ₀	t _R (min)	Compound	Content (%)				
			Essential oils				
			EO ₁	EO ₂	EO ₃	EO ₄	EO ₅
1	3.965	β-Pinene laevo	0.2285	0.2963	0.3288	0.3722	-
2	4.535	β-Pinene	-	0.2054	-	0.251	-
3	5.585	Cymene o	-	-	-	0.1763	-
4	5.685	Cineole (1,8) + Limonene D	0.4689	1.7655	1.0649	2.8835	0.9651
5	7.731	α-Thujone (cis-)	19.5582	26.2801	23.4822	27.3852	15.6292
6	8.012	β-Thujone (trans)	3.3018	4.1147	3.5739	4.1749	2.4436
7	8.815	Camphor	19.5582	22.9525	23.4468	23.0601	16.0307
8	9.463	IsoBorneol	9.118	9.8896	12.1106	7.9536	8.1606
9	9.8	Terpineol L-4	0.7794	0.7523	0.8919	0.6222	0.54
10	13.178	Bornyl acetate, (-)	6.8141	4.3262	4.8249	3.7393	3.506
11	13.438	Sabinyl acetate trans	0.8621	0.5145	0.5532	0.4275	0.4344
12	17.124	Isocaryophyllene (-)	2.0994	1.2077	1.3918	1.0479	1.0138
13	17.696	α-Gurjunene (-)	0.8763	0.4318	0.6318	0.4895	0.3438
14	18.16	γ-Elemene	15.5249	8.6621	9.8638	7.4577	7.7604
15	22.102	Selina-3,7(11)-diene	5.5956	5.6147	6.4641	5.8521	8.6445
16	22.563	1,11-epoxyhumulene	3.6751	2.0129	2.1192	1.9823	2.8553
17	23.194	Caryophyllene oxide	0.9678	0.8702	0.9356	0.9743	1.7266
18	33.599	phyllocladene	4.1894	6.268	4.7461	6.8986	23.3673
Total			93.6177	96.1645	96.4296	95.7482	93.4213

Note: EO – essential oil, t_R – retention timesTable 4. Composition of the CO₂ extracts of *salvia officinallis*

N ₀	t _R (min)	Compound	Content (%)				
			Extracts				
			EO ₁	EO ₂	EO ₃	EO ₄	EO ₅
1	10.87	α-Thujone (cis-)	0.6634	4.4449	4.1828	3.7691	5.1528
2	11.22	β-Thujone (trans)	-	-	0.1952	0.7016	-
3	12.175	Camphor	1.4333	11.9289	11.3719	14.877	15.2387
4	13.001	IsoBorneol	11.2873	7.3889	6.8036	9.5228	8.1666
5	13.214	Terpineol L-4	2.0814	0.3207	0.2483	0.3324	0.2981
6	16.769	Bornyl acetate, (-)	5.9046	3.5859	2.0146	4.6214	3.9588
7	16.946	Sabinyl acetate trans	1.0535	0.5336	0.4057	0.6429	0.4215
8	20.979	Isocaryophyllene (-)	2.7452	1.1787	0.8389	1.2999	1.168
9	21.549	α.-Gurjunene (-)	1.4476	0.5444	0.4394	0.6215	0.5494
10	22.085	γ-Elemene	24.9816	9.3086	7.0177	9.7308	9.0012
11	226.155	Selina-3,7(11)-diene	11.2528	12.1679	13.8268	12.5074	12.1414
12	26.574	1,11-epoxyhumulene	8.9888	4.5565	5.8688	4.9579	4.9207
13	27.22	Caryophyllene oxide	2.7553	2.6625	2.6398	2.3898	2.4564
14	37.783	phyllocladene	10.42	26.0565	30.6452	21.9899	24.6029
Total			850,148	84.678	86.4987	87.9644	88.0765

Note: E – extract

4. Conclusions

As expected from the studies on *Salvia officinalis*, the main compounds in essential oils were α – thujone, camphor, isoborneol, bornylacetate, phyllocladene. The main components in extract were camphor, isoborneol, γ – elemene, selina – 3,7 (11) – diene, phyllocladene. The composition of main compounds in essential oils is not largely influenced by extraction pressure. Indeed, chemical analysis of sage oil at different pressure evidenced composition variations along the extraction process but it is not large. The composition of main compounds in extract at pressure $p=80$ bar is largely different from composition at other pressure.

In the first 2.5 hours of extraction, extract yield is the highest.

Eighteen compounds of essential oil EO4 were identified, representing 95.7482 % of the total essential oil, and seventeen compounds of essential oil EO2 were identified representing 96.1645% of the total essential oil. The five most abundant compounds (α – thujone, camphor, isoborneol, bornylacetate, phyllocladene) represent 69.7164% of the essential oils.

Fourteen compounds of extract E3, E4 were identified, and thirteen compounds of extract E1, E2 and E5. The content of phyllocladene and camphor in extract E1 was considerably lower than in other extracts, and content of γ elemene and isoborneol was considerably higher than in other extract.

Thirteen compounds of extract E2 representing 84.678% of the total extract. The five most abundant compounds (camphor, isoborneol, γ – elemene, selina – 3,7 (11) – diene, phyllocladene) represent

66.8508% of the extract.

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