



Journal of Applied Sciences

ISSN 1812-5654

science
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Supercritical Extraction of *Salvia officinalis* L.

¹V. Mičić, ²Ž. Lepojević, ¹M. Jotanoviæ, ¹G. Tadić and ¹B. Pejović

¹Department of Chemical Engineering, Faculty of Technology Zvornik,
University of East Sarajevo, Karakaj bb 75400 Zvornik, Bosnia and Herzegovina

²Department of Pharmaceutical Engineering, Faculty of Technology,
University of Novi Sad, 21000 Novi Sad, Serbia

Abstract: The wild sage (*Salvia officinalis* L.) growing in municipality Trebinje, Bosnia and Herzegovina was extracted with supercritical carbon dioxide at flow rate $3.23 \cdot 10^{-3}$ kg min⁻¹, temperature 313 K under varying pressures in order to determine yields and compositions of extracts. Supercritical fluid extraction was carried out with a laboratory-scale high pressure extraction plant-HPEP (Nova-Swiss, Switzerland). With increasing pressure from 80 to 300 bar extraction yield increased. It was due to the fact that supercritical carbon dioxide density increases with increasing pressures. In the supercritical CO₂ extracts the major components were phyllocladene (10.42-30.64%), γ -elemene (7.02-24.98%), isoborneole (6.80-11.29%), camphor (1.43-15.24%). Essential oils were isolated by hydro distillation from CO₂ extracts. The major components were α -thujone (15.63-27.38%), camphor (16.03-23.45%), γ -elemene (7.46-15.52%). In continue of investigations, the kinetics of extraction where each point of the kinetic curves obtained with the new sample of drug in extractor at pressures 80, 100 and 200 bar were studied. With increasing pressure extract yield increased (1.40-4.17 g/100 g drug). Qualitative and quantitative analyses extracts and essential oils were done using GC/MS and GC/FID analyses. The composition of sage extracts and essential oils were different. Supercritical fluid extraction by CO₂ allowed optimization of the experimental conditions for selection of the substances of interest.

Key words: Supercritical extraction, essential oil, *Salvia officinalis* L., carbon dioxide, pressure

INTRODUCTION

The past few years have seen a growing interest in natural foods, with increased demand for non-synthetic, natural antioxidants. The use of synthetic antioxidants in the food industry is severely restricted by low as to both application and level of use. Valued traditionally as a spice, sage is now being studied because of its antioxidant properties (Bozin *et al.*, 2007; Ali, 2011).

Salvia officinalis L. is valuable medicinal plant which is used widely in traditional medicine. This plant is very rich in biologically active compounds and many studied have indicated their increasing practical importance (Velickovic *et al.*, 2007). The presence of flavonoids in the sage has already been confirmed by Lu and Foo (2002) and Valant-Vetschera *et al.* (2003). They have therapeutic effects because of their inhibitory effects on certain enzymes and antioxidant activity. They have been shown to possess antibacterial (Harborne, 1986), antifungal (Weidenbornen and Jha, 1994), antiviral and anti-inflammatory activities (Abad *et al.*, 1997). Their ant

allergic, antioxidant and anti- mutagen activities (Yanishlieva *et al.*, 1999; Hossain and Ismail, 2011) also been proven.

The demand of the food industry for natural antioxidant prepared with safe solvents has directed attention to more efficient extraction methods, such as Supercritical Fluid Extraction (SFE). Now, the conventional methods such as hydro-distillation and solvent extraction are 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 (Koukos *et al.*, 2000). 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 (Damjanovic *et al.*, 2005). 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 (Sovova, 2005; Stampar *et al.*, 2006; Bendahou *et al.*, 2007). 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 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 (Zekovic *et al.*, 2001). 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.

Carbon dioxide is most widely used in Supercritical Fluid Extraction (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 (Reverchon and Senatore, 1992; Cheng *et al.*, 2000; Adeib *et al.*, 2010). By changing pressure and/or temperature above critical point of carbon dioxide ($T_c = 31.3^\circ\text{C}$, $P_c = 72.8 \text{ bar}$), a pronounced change in the density and dielectric constant, i.e., solvent power of supercritical carbon dioxide can be achieved (Vargaftik, 1975; Ried *et al.*, 1987). The balance between solvent power and selectivity of a supercritical solvent is perhaps the most important aspect to be optimized. Higher densities induce higher solvent power, however, solvent selectivity towards compounds characterized by similar polarities and different molecular weights decrease with this increase in solvent power. Therefore, supercritical CO₂ can show high selectivity compared with liquid CO₂ since its density varies from about 0.2 to 0.9 g cm⁻³ for many SFE conditions (temperatures from 40 to 60°C, pressures from 80 to 300 bar) (Vukalovich and Altunin, 1968). 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. 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 (Reverchon *et al.*, 1995).

The special properties of supercritical fluids bring certain advantages to chemical separation processes. Several applications have been fully developed and commercialized (Takeuchi *et al.*, 2008; Wenqiang *et al.*, 2007). 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 (McHugh and Krukonis, 1994). Other important areas are the extraction of essential oils and aroma materials from spices. Brewery industry uses SFE for the extraction of hop (Zoran *et al.*, 2007).

The influence of CO₂ density and extraction time on the essential oil composition was studied.

MATERIALS AND METHODS

Plant material: Sage leaves (*Salvia officinalis* L.) air dried in the shade were supplied in Berkovići, near municipality Trebinje. Leaves of growing wild sage were collected manually from the same collection site in the Berkovići region in 2008.

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: GC analyses were performed using GC 5890 Series II (Hewlett-Packard, Palo Alto, Calif, USA) gas chromatograph equipped with a FID and a DB-5 capillary column (30 m×0.25 mm, film thickness 0.25 μm). The hydrogen was used as a carrier gas with flow rate 1 mL min⁻¹. Injector and detector temperature were 244 and 285°C, respectively. Oven temperature was programmed at 40°C for 2 min and then increased linearly to 285°C at a rate of 4.3°C min⁻¹. A split flow of 1 mL min⁻¹ was used. The constituents of essential oil and extracts were identified by comparing their retention times with those of available standards and their mass was calculated from a predetermined peak area response factor.

The GC-MS analyses were carried on a capillary GC (Varian, model 3400) interfaced with an ion-trap detector (Finnegan Mat, model BE 8230) with the same column and same characteristics as the one used in GC. The samples, previously dissolved in chloroform : methanol (3:1), were injected (1 μL) in split mode with split ratio of 1:99 and the flow rate of carrier gas (hydrogen) was 1 mL min⁻¹. MS conditions were: ionization voltage, 70 eV scanning interval 1.0 sec, detector voltage 1.3 kV and m/z range of

33-333. The components were identified by computer searching and comparing their mass spectral data with available standards and those in the WILLEY 229 mass spectra libraries.

Supercritical fluid extraction: SFE by 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} = 700 bar), separator with internal volume of 200 mL (P_{max} = 250 bar) and maximum CO₂ mass flow rate of approximately 5.7 kg h⁻¹.

For each extraction test 60 g of sage with a mean diameter of 0.32 mm were charged into the extractor. The mean diameter of particles was determined by mechanical sieving. Flow rate of CO₂ was 3.23×10⁻³ kg min⁻¹, temperature 313 K, the total extraction time was 4 h (samples were taken every half hour). Separator conditions were pressure 15 bar and temperature 25°C.

RESULTS AND DISCUSSION

In order to prevent thermal decomposition of sage compounds, especially thermal degradation of some volatile oil compounds, the temperature of 40°C was selected for supercritical fluid extraction with carbon dioxide (Reverchon *et al.*, 1995; Zoran *et al.*, 2007). The selections of the pressures ranges were based on the fact that a great change in the density and dielectric constant of CO₂ occurs between pressure 80 and 300 bar (Roy *et al.*, 2006). The pressure varied from 80 to 300 bar variations in the solvent density and the transport properties of the system were observed.

At lower solvent densities (80 bar) very low total yield of extract was observed. The increase of pressure was accompanied by a significant increase of total yield of extract. At the end of extraction process (extraction time 4 h) essential oils isolated by hydro-distillation from CO₂ extracts and their contents were calculated (Table 1).

The content of sage essential oil was determined by an official procedure (Pharmacopoeia SFRJ, 1984).

The essential oil content was the highest in extract obtained at pressure 80 bar (58.79%), the contents in other essential oils varied from 29.90 to 47.87%.

CO₂ extracts and essential oils were detailed identification and quantification using GC/MS and GC/FID analyses. The results of qualitative and quantitative analyses are shown in Table 2 and 3.

Table 1: Yield of CO₂-extract and essential oil in extract

Pressure (bar)	Yield of CO ₂ -extract (g/100 g drug)	Content of essential oils in CO ₂ -extract (mL/100 g 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

Table 2: Qualitative and quantitative content of CO₂-extract

Component	Component content (% m m ⁻¹)				
	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
Sabinyl-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 3: Qualitative and quantitative content of essential oil

Component	Component content (% m m ⁻¹)				
	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
Sabinyl-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

The predominant component in extract obtained at 80 bar was γ-elemene (24.98%). The isoborneole (11.29%), selina-3,7 (11) diene (11.25%), 1,11-epoxyhumulene(8.99%) were detected in significant amount. The main component in extracts obtained at 100, 150, 200 and 300 bar was phyllocladene (21.99-30.64%). These extracts show high content of camphor (11.37-15.24%), selina-3,7 (11) diene (12.14-13.83%), γ-elemene (7.02-9.73%), isoborneole (6.80-9.52%).

Table 4: Essential oil content in CO₂ extract and residual drug

Time (h)	Pressure (bar)		
	80	100	200
0.5	75.85/0.66	75.25/0.40	61.06/0.04
1.0	72.50/0.54	65.81/0.04	48.43/0.02
1.5	70.01/0.50	62.70/0.04	40.23/-
2.0	63.45/0.36	58.59/-	36.49/-
2.5	41.26/0.30	51.56/-	32.40/-
3.0	26.03/0.20	21.36/-	17.39/-

The main components in essential oils were α -thujone (15.63-27.38%), camphor (16.03-23.45%), isoborneole (7.94-12.11%), γ -elemene (7.46-15.52%), phyllocladene (4.19-23.37%).

In continue of investigations, the kinetics of extraction where each point of the kinetic curves obtained with the new sample of drug in extractor at pressures 80, 100 and 200 bar were studied.

After supercritical extraction for defined time, essential oil isolated by hydro-distillation from CO₂ extract and residual drug (samples of sage residue after extraction). Results are shown in Table 4.

It can be seen that content of essential oil obtained from extract and residual drug (drug after extraction) decreased with pressure and time.

CONCLUSION

In this study, wild growing sage from Berkovici region were used to obtain extracts. Therewith, in order to achieve higher selectivity of the SC-CO₂ extraction and thus higher antioxidant activity of the SC-CO₂ extracts, no modifier were used in this study. This could also be the reason for the lower yields of antioxidant extracts reported in this study. Flavonoids, fatty acids, terpenes and diterpenoids were identified in the SC-CO₂ extracts of sage. The main difference between extracts and essential oils was the content of sesquiterpenes and diterpenes which are higher in the extracts.

Samples of sage were extracted by varying CO₂ pressure from 80 to 300 bar. Other extraction parameters were constant: temperature 313 K, flow rate 3.23×10^{-3} kg min⁻¹, mean particle diameter 0.32 mm. The composition of sage extracts and essential oils obtained by supercritical extraction is largely influenced by solvent density. Supercritical extraction by CO₂ allowed optimization of the experimental conditions for selection of substances of interest, namely sesquiterpenes and diterpenes.

According to the results of this investigation, SFE offered more choices (pressures level) for the extraction of different components. On the basis of

these results, it could be concluded that the extracts of the investigated sage are different.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Technology the Republic of Srpska.

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