

*Journal of Mining and Metallurgy 44 B (2008) 125 - 131*

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Journal of  
Mining and  
Metallurgy

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## INFLUENCE OF PRESSURE AND TIME ON EXTRACTION PROCESS USING SUPERCRITICAL CO<sub>2</sub>

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*(Received 01 February 2008; accepted 26 May 2008)*

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### Abstract

*The supercritical fluid extraction (SFE) by carbon dioxide (CO<sub>2</sub>) of *Salvia officinalis* L. was investigated. SFE by CO<sub>2</sub> 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*

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### 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. CO<sub>2</sub> 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 CO<sub>2</sub>, 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 CO<sub>2</sub> at liquid or supercritical conditions should also be considered. Some authors have suggested the use of liquid CO<sub>2</sub> 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 CO<sub>2</sub> extracts. Other investigators, have used CO<sub>2</sub> at supercritical conditions to avoid the simultaneous extraction of essential oil and high – molecular – weight compounds.

Therefore, supercritical CO<sub>2</sub> can show higher selectivity compared with liquid CO<sub>2</sub> since its density varies from about 0.2 to 0.9gcm<sup>-3</sup> for many SFE conditions (temperatures from 40 to 60°C, pressures from 80 to 300bar). By contrast, the density of liquid CO<sub>2</sub> can be varied only between about 0.7 and 1.0 gcm<sup>-3</sup> at the extraction conditions typically used for natural product extraction. Therefore, in processes performed at high CO<sub>2</sub> 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 CO<sub>2</sub>, the extraction of essential oils at CO<sub>2</sub> 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 $\mu$ 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 $\mu$ 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-CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> flow rate was 97.72 dm<sup>3</sup>/h. Separator conditions were 15 bar and 25°C.

## 3. Results and Discussion

The supercritical fluid extraction (SFE) by carbon dioxide (CO<sub>2</sub>) of *Salvia officinalis* L. was investigated. SFE by CO<sub>2</sub> 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 officinallis*, 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                     |

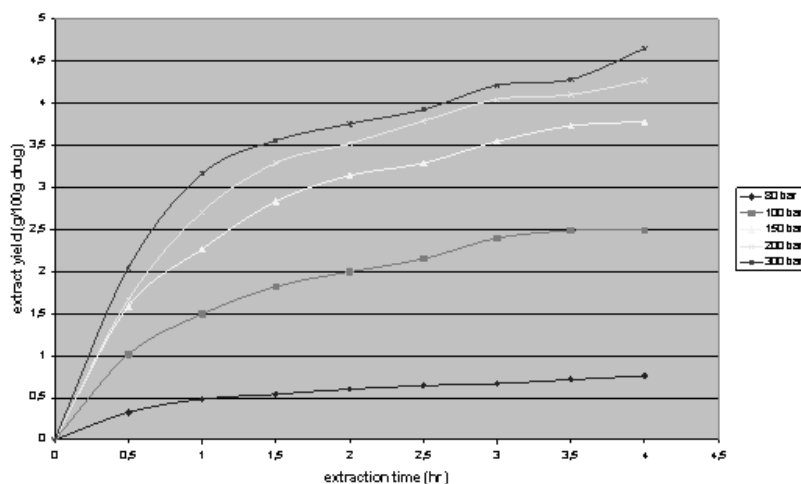


Fig. 1. Extract yield vs extraction time at different pressure

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), EO<sub>2</sub> (p=100 bar), EO<sub>3</sub> (p=150 bar), EO<sub>4</sub>

(p=200 bar), EO<sub>5</sub> (p=300 bar) are given in Tab.3.

The relative contents of the identified components of extracts E<sub>1</sub> (p=80 bar),

E<sub>2</sub> (p=100 bar), E<sub>3</sub> (p=150 bar), E<sub>4</sub> (p=200 bar), E<sub>5</sub> (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 – CO<sub>2</sub> method are shown in Fig. 2. and Fig. 3.

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

| N <sub>0</sub> | t <sub>R</sub> (min) | Compound                   | Content (%)     |                 |                 |                 |                 |
|----------------|----------------------|----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                |                      |                            | Essential oils  |                 |                 |                 |                 |
|                |                      |                            | EO <sub>1</sub> | EO <sub>2</sub> | EO <sub>3</sub> | EO <sub>4</sub> | EO <sub>5</sub> |
| 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<sub>R</sub> – retention timesTable 4. Composition of the CO<sub>2</sub> extracts of *salvia officinallis*

| N <sub>0</sub> | t <sub>R</sub> (min) | Compound              | Content (%)     |                 |                 |                 |                 |
|----------------|----------------------|-----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                |                      |                       | Extracts        |                 |                 |                 |                 |
|                |                      |                       | EO <sub>1</sub> | EO <sub>2</sub> | EO <sub>3</sub> | EO <sub>4</sub> | EO <sub>5</sub> |
| 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  $\alpha$  – thujone, camphor, isoborneol, bornylacetate, phyllocladene. The main components in extract were camphor, isoborneol,  $\gamma$  – 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 ( $\alpha$  – 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  $\gamma$  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,  $\gamma$  – elemene, selina – 3,7 (11) – diene, phyllocladene) represent

66.8508% of the extract.

#### Acknowledgment

The Authors are thankful to Mrs Vlatka Vajs for help that analyses were done.

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