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SCIENCE DIRECT®
Food Chemistry 91 (2005) 639–644
Food Chemistry
www.elsevier.com/locate/foodchem

Comparison of essential oils compositions of *Ferula assa-foetida* obtained by supercritical carbon dioxide extraction and hydrodistillation methods

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Received 12 April 2004; received in revised form 22 June 2004; accepted 22 June 2004

Abstract

Essential oil of *Ferula assa-foetida*, cultivated in Iran, was obtained by hydrodistillation and supercritical (carbon dioxide) extraction methods. The oils were analysed by capillary gas chromatography using flame ionization and mass spectrometric detections. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters, such as pressure, temperature, modifier volume and extraction time, on the supercritical fluid extraction (SFE) of *Ferula assa-foetida* oil were investigated. The results showed that, under a pressure of 300 atm, temperature 35 °C, 5% methanol and dynamic extraction time of 25 min, extraction was more selective for the *E*-1-propyl sec-butyl disulfide. Twenty five compounds were identified in the hydrodistilled oil. The major components of *Ferula assa-foetida* were *E*-1-propenyl sec-butyl disulfide (40.0%) and germacrene B (7.8%). However, by using supercritical carbon dioxide under optimum conditions, only two components constituted more than 70% of the oil. The extraction yield, based on hydrodistillation, was 1.13% (w/w). Extraction yield, based on the SFE, varied in the range of 0.8–5.5% (w/w) under different conditions. The results show that, in Iranian *Ferula assa-foetida* oil, *E*-1-propyl sec-butyl disulfide is a major component.

Keywords: *Ferula assa-foetida*; Supercritical carbon dioxide; Hydrodistillation; Essential oil; *E*-1-propenyl sec-butyl disulfide

1. Introduction

Ferula assa-foetida grows in Iran, Afghanistan and Kashmir. It is herbaceous and perennial and grows up to 2 m high (Abd El-Razek, Ohta, Ahmed, & Hirata, 2001). *Ferula assa-foetida* has several medicinal properties including antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelminitic, aphrodisiac and antiseptic properties (www.egregore.com).

The essential oils of plants have usually been isolated by either hydrodistillation or solvent extraction. The disadvantages of all these techniques are: low yield, losses of volatile compounds, long extraction times, toxic solvent residues and degradation of unsaturated compounds, giving undesirable off-flavour compounds, due to heat (Doneanu & Anitescu, 1998; Illes, Daood, Perneczki, Szokonya, & Then, 2000; Poiana, Sicari, &

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0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.06.033

Mincione, 1998; Yamini, Sefidkon, & Pourmortazavi, 2002).

Supercritical fluid extraction (SFE) has received increasing attention in a variety of fields due to the following features: (a) supercritical fluids provide high solubility and improved mass-transfer rates; (b) operation can be manipulated by changing the pressure or temperature. Carbon dioxide is used as the supercritical fluid mainly because it is a safe, noncombustible, inexpensive, odourless, colourless, tasteless, nontoxic, and readily available solvent. Its low viscosity enables it to penetrate the matrix to reach the material being extracted, and its low latent heat of evaporation and high volatility mean that it can be easily removed without leaving a solvent residue. By varying the temperature and pressure of the CO_2 during extraction, the flavour or odour components can be selectively extracted (Abdullah, Young, & Games, 1994; Cassel et al., 2000; Dugo, Mondello, Bartle, Clifford, & Breen, 1995; Floch, Tena, Rios, & Valcarcel, 1998; Morales, Berry, Mcintyre, & Aparicio, 1998; Reverchon, Ambruosi, & Senatore, 1994; Roy, Goto, & Hirose, 1996).

Often, SFE methods involve the investigation of many variables, which may affect the efficiency of extraction. Selection of these variables and their levels is critical. Several statistical techniques, such as simplex optimization and factorial design, were employed for the optimization of analytical methods. Factorial design has some advantages over simplex optimization in that global optimum can be provided, large amounts of quantitative information can be extracted and both discrete and continuous factors can be estimated. One obvious disadvantage of the factorial design is the large number of experiments required when several variables are examined. However, the number of the experiments can be considerably reduced by the use of orthogonal array design (Lan, Wong, Chen, & Sin, 1994; Lan, Wong, Lee, & Sin, 1995).

The aim of the present work is the investigation of the effects of different parameters, such as pressure, temperature, modifier volume and dynamic extraction time, on the supercritical fluid carbon dioxide extraction of *Ferula assa-foetida*. The essential oil obtained by hydrodistillation was used for comparison. To the best of our knowledge, no report has yet appeared on the SFE of *Ferula assa-foetida.*

2. Material and methods

2.1. Plant material

The plant materials were collected from the mountains of Kerman (Kerman, Iran) in October, 2002. Immediately prior to SFE, the sample was ground in a blender to produce powder.

2.2. Reagents

HPLC grade dichloromethane and methanol were purchased from Aldrich. Carbon dioxide (99.99% purity), contained in a cylinder with an eductor tube, was obtained from Sabalan Co. (Tehran, Iran).

2.3. Hydrodistillation

The plant (40 g of dried material) was submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile distillate was collected over anhydrous sodium sulphate and refrigerated prior to analysis. The yield of the oil was 1.13% (w/w) based on dry plant weight.

2.4. Supercritical fluid extraction

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions. The extraction vessel was a 10-ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 100, 200 and 300 atm and temperatures of 35, 45 and 55 °C for a duration of 20 min, static, followed by 15, 25 or 35 min, dynamic. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restrict point was warmed electrically. The supercritical CO_2 flow rate through the Duraflow restrictor was approximately 0.3–0.4 ml/min (compressed). Plant powder (3.0 g) was well mixed with 2 mm diameter glass beads, and was then charged into the 10-ml extraction vessel. The essential oil was extracted from the plant using supercritical CO_2 under various conditions according to the Taguchi method (Roy, 1990). Table 1 shows the experimental conditions for each of the SFE runs. The extracted analytes were collected in dichloromethane in a 5.0 ml volumetric flask. The final volume of the extract was adjusted to 5.0 ml with dichloromethane at the end of the extraction. In order to improve the collection efficiency, the 5.0 ml volumetric flask was placed in an ice bath during the dynamic extraction stage. For all the modifier studies, methanol was spiked directly into the extraction vessel with charged sample prior to the extraction.

Four millilitres of solution were poured into a 20 ml beaker. Bubbling of the solution was done by using argon gas in order to evaporate the solution. Then the weight of essential oil was measured. Finally, the extraction yield was calculated.

2.5. GC and GC/MS analyses

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a FID and a

Table 1
SFE experimental conditions and extraction yields for *Ferula assa-foetida*

Run No.	Pressure (atm)	Temperature (°C)	Dynamic time (min)	Modifier volume (μl)	Extraction yield (w/w)
1	100	35	15	0	1.01
2	100	45	25	250	1.19
3	100	55	35	500	0.82
4	200	35	25	500	4.31
5	200	45	35	0	2.66
6	200	55	15	250	1.82
7	300	35	35	250	5.23
8	300	45	15	500	5.50
9	300	55	25	0	3.65

DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed 50 °C for 5 min, and then increased to 250 °C at a rate of 4 °C/min. Injector and detector temperatures were 250 and 265 °C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The SFE samples (1 μl) were injected into GC (without any further dilution) using the split mode with a split ratio of 1/60. Hydrodistilled extracts were diluted 30 times and 1μl of diluted solution was injected into GC with the same split ratio. The GC/MS analysis was carried out on a Varian 3400 equipped with a DB-1 column with the same characteristics as the one used in GC. The transfer line temperature was 260 °C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–300 amu. The percentages of compounds were calculated by the area normalization method without considering, response factors. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds. Data obtained were confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature (Sandra & Bicchi, 1987).

3. Results and discussion

3.1. Extraction efficiency

The hydrodistillation process has been traditionally used for the extraction of essential oils on a laboratory scale. In this study, we intend to compare the efficiency of this process with its relationship to the volatile composition of the extracts from *Ferula assa-foetida* obtained by SFE.

3.2. Optimization of the experimental conditions

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the development of a SFE method. In fact, pressure and temperature of the fluid, percentage of the modifier and the extraction times are generally considered as the most important factors. The optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows different conditions of experiments carried out with SFE for extractions of *Ferula assa-foetida* according to the Taguchi experimental design. All the selected factors were examined using a three-level orthogonal array design with an OA_9 (3^4) matrix. In this study, interactions among variables were not incorporated in the matrix and focus was placed on the main effects of the four most important factors. The results of the SFE experiments, based on the extraction yields, are given in Table 1. The mean values of the extraction yields for the corresponding factors at each level were calculated according to the assignment of the experiment (Fig. 1). For example, the extraction yields of the three trials at 300 atm were evaluated as mean values of the corresponding three runs. The mean values of the three levels of each factor (e.g., pressure) reveal how the extraction yield will change when the level of that factor is changed. Fig. 1 shows the variations in extraction yield as a function of change in different levels of the factors studied. For the complete recoveries of the main components of the plant, higher pressures are necessary. This is because raising the extraction pressure, at constant temperature, leads to higher fluid density, which increases the solubility of the analytes. To obtain quantitative recoveries of analytes, they must be efficiently partitioned from the sample matrix into the supercritical fluid. The influence of temperature on the composition of the extracts was studied. For all the analytes, the temperature of the supercritical fluid was found not to be significant as the main effect. The influence of the dynamic extraction time on the composition of the extracts was studied. Extraction was performed with supercritical carbon dioxide at the static extraction step of 20 min, followed by 15, 25 and 35 min of dynamic extractions. Results showed that increasing dynamic extraction time to 25 min enhanced the extraction of most components.

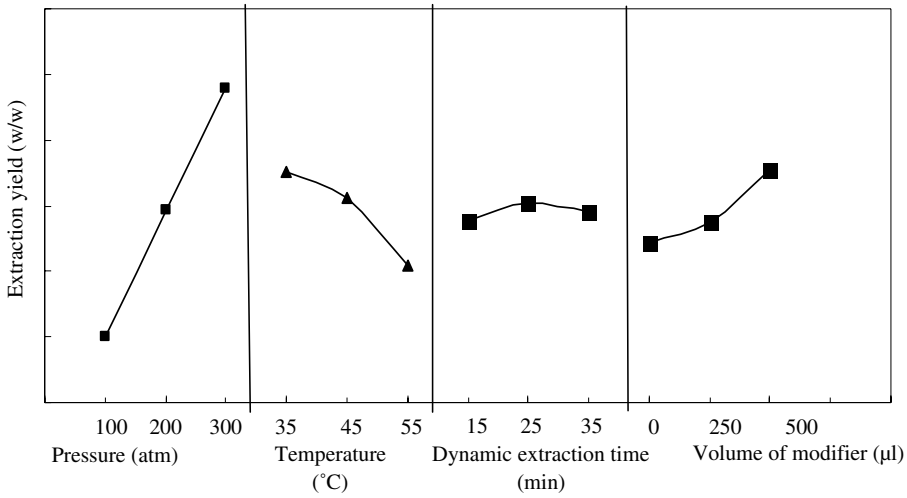

Fig. 1. Effects of temperature, pressure, dynamic extraction time and volume of modifier on the extraction yield. In all of the SFE runs static extraction time, flowrate of CO_2 (compressed), and weight of plant material were 20 min, 0.35 ± 0.05 ml/min and 3.0 g, respectively.

An essential drawback in the use of supercritical CO_2 is its low polarity, making the extraction of polar analytes difficult. Nevertheless, this limitation may be overcome by adding small amounts of polar modifiers, such as methanol or ethanol to the supercritical CO_2, in order to increase its solvation power. In the present work, the

Table 2
Ferula assa-foetida oils obtained (%) by SFE and hydrodistillation (the compounds are listed in order of elution time from a DB-1column)

No.	Compound	R.I.[a]	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	HD[b]
1	α-Pinene	938	1.4[c]	1.8	2.0	1.2	1.5	1.5	1.2	1.2	1.2	5.9
2	β -Pinene	979	1.0	1.4	1.5	0.9	1.0	1.1	1.0	0.9	1.0	5.0
3	Myrcene	990	1.1	1.2	1.4	0.9	1.0	1.1	1.0	1.0	1.0	0.8
4	α-Phellandrene	1004	0.7	0.8	0.9	0.6	0.7	0.7	0.6	0.6	0.7	0.7
5	1,4-Cineole	1016	0.5	0.6	0.7	0.8	0.6	–	–	0.6	0.6	1.4
6	*p*-Cymene	1025	–	0.5	0.5	0.4	–	–	–	–	–	0.7
7	Limonene + β-phellandrene	1030	1.7	1.8	2.2	1.4	1.7	1.8	1.5	1.4	1.6	2.0
8	(*Z*)-β-ocimene	1038	3.3	3.6	4.1	2.9	3.4	3.6	3.2	3.1	3.3	1.3
9	(*E*)-β-ocimene	1049	–	5.4	6.1	4.4	5.0	5.2	5.0	4.9	4.9	2.6
10	1-Propyl sec-butyl disulfide	1172	0.7	0.7	0.7	0.7	0.7	–	0.6	0.6	0.6	0.6
11	(*Z*)-1-propenyl sec-butyl disulfide	1177	5.1	5.2	5.2	4.9	5.4	5.4	5.3	5.2	5.5	8.7
12	(*E*)-1-propenyl sec-butyl disulfide	1181	54.2	53.8	54.0	50.3	57.6	59.4	58.2	56.1	59.0	40.0
13	Bis(1-methyl propyl) disulfide	1220	1.2	1.3	1.3	1.2	1.2	1.3	1.2	1.4	1.3	0.8
14	Bis(1-methyl propenyl) disulfide	1263	2.1	2.0	1.9	2.1	2.4	2.3	2.2	2.1	2.3	3.4
15	*trans* Anethole	1281	1.9	–	–	–	–	–	–	–	–	–
16	β-Coryophyllene	1418	–	–	–	–	–	–	–	–	–	1.4
17	Bis(1-methyl thio)propyl disulfide	1426	3.2	3.1	2.5	3.4	3.5	3.3	3.6	3.4	3.4	–
18	α-Humulene	1452	0.9	0.9	–	1.0	–	–	–	–	–	1.0
19	γ-Gurjunene	1472	1.1	1.0	0.9	1.1	1.0	1.0	0.9	0.9	–	1.9
20	Germacrene D	1479	–	–	–	2.1	–	–	–	1.1	–	–
21	Virdiflorene	1492	–	–	–	3.1	–	–	–	1.8	–	0.5
22	Cuparene	1501	–	–	–	–	–	–	–	–	–	1.0
23	γ-Cadinene	1512	–	–	–	–	–	–	–	–	–	0.8
24	*cis*-γ-Bisabolene	1514	–	–	–	1.0	–	–	–	–	–	–
25	δ-Cadinene	1522	–	–	–	–	–	–	–	–	–	1.5
26	*trans*-γ-Bisabolene	1532	1.3	1.1	–	1.0	1.4	–	1.2	0.9	1.3	–
27	Germacrene B	1554	10.4	10.0	7.7	11.0	10.9	10.7	11.7	11.4	11.3	7.8
28	Eudesmol (10-epi-γ)	1619	–	–	–	–	–	–	–	–	–	2.7
29	γ-Eudesmol	1629	–	–	–	–	–	–	–	–	–	0.6
30	Hinesol	1636	–	–	–	–	–	–	–	–	–	0.9

[a] Kovats retention indices on DB-1 column.
[b] Hydrodistillation.
[c] Percent of component based on the area normalization.

Table 3
ANOVA of the experiments (at 95% confidence)

Source of variance	Sum of squares	Degrees of freedom	Mean square	F^avalue
Pressure	21.51	2	10.76	12.23
Temperature	3.22	2	1.61	
Dynamic extraction time	0.11	2	0.06	
Methanol percentage	1.95	2	0.973	
Pooled error	5.28	6	0.88	
Total	32.07	14		

[a] $F_{critical} = 5.14$.

modifier (methanol) enhanced the solubility of solutes in supercritical CO_2 and thus efficiency of extraction increased.

The SFE extracts and hydrodistillates of *Ferula assa-foetida* showed a relatively simple GC–MS chromatographic pattern. Detailed identification and quantitation of the compounds found in *Ferula assa-foetida* seed oil, produced by SFE under different conditions, were performed by GC–MS, as shown in Table 2. Products obtained by hydrodistillation were also analysed by GC–MS. The results are also shown in Table 2, for comparison. The major compounds were: α-pinene (5.9%), β-pinene (5.0%), germacrene B (7.8%), myrcene (0.8%), *p*-cymene (0.7%), α-phellandrene (0.7%), 1,4-cineole (1.4%), (*Z*)-β-ocimene (1.3%), (*E*)-β-ocimene (2.6%), 1-propyl sec-butyl disulfide (0.6%), (*Z*)-1-propenyl sec-butyl disulfide (8.7%), (*E*)-1-propenyl sec-butyl disulfide (40.0%), bis(1-methyl propyl) disulfide (0.8%), bis(1-methyl propenyl) disulfide (3.4%), γ-gurjunene (1.9%), α-humulene (1.0%), virdiflorene (0.5%), limonene + β-phellandrene (2.0%), β-coryophyllene (1.4%), cuparene (1.0%), γ-cadinene (0.8%), δ-cadinene (1.5%), eudesmol (10-epi-γ) (2.7%), γ-eudesmol (0.6%) and hinesol (0.9%). It is noteworthy that the oil extracted by SFE under Run 4 conditions has a composition similar to that of the oil obtained by hydrodistillation, but a marked difference in the *E*-1-propyl sec-butyl disulfide content between the SFE (Runs 5, 6, 7, 8 and 9) and the hydrodistillation product can be noted from Table 2. As shown in Table 2, SFE offers a rapid method for the extraction of *Ferula assa-foetida* oil, as well as high selectivity for *E*-1-propyl sec-butyl disulfide, depending on the extraction conditions (Runs 5, 6, 7, 8 and 9). These extracts are richer in *E*-1-propyl sec-butyl disulfide. However, the recovery of *E*-1-propyl sec-butyl disulfide in SFE is better than that by hydrodistillation. Finally, SFE shows various results in comparison with the conventional hydrodistillation procedure. Furthermore, SFE gives a better selectivity for compounds of interest; changing extraction variables is less tedious, and it has a shorter extraction time. The major disadvantage of the oil obtained by SFE is the presence of co-extracted cuticular waxes.

Table 3 shows analysis of variance (ANOVA) results for calculated models. However, the ANOVA results of this experiment indicate that the pressure of the SFE plays an important role in the SFE of *Ferula assa-foetida*. In fact, it appears to be significant for all the analytes. This means that extraction recovery is enhanced as the pressure increases. The pressure increase causes an increase of the fluid density and it could thus have an important effect: increase of the solvating power of the supercritical fluid, responsible for quantitative recoveries.

4. Conclusion

The supercritical fluid extraction of *Ferula assa-foetida* was studied, and the results, were compared with essential oil composition obtained by hydrodistillation. The SFE method offers many important advantages over hydrodistillation. SFE requires shorter extraction times (25 min vs. 4 h for hydrodistillation). Energy cost is rather higher for performing hydrodistillation than that required for reaching SFE conditions.The possibility of manipulating the composition of the oil, by changing the parameters of the extraction (pressure, temperature, modifier volume and dynamic extraction time) is more attainable in SFE. Although compositions of the oils obtained by SFE and hydrodistillation are not qualitatively different, they do differ quantitatively. We obtained a higher selectivity in SFE than by the hydrodistillation method.

The flexibility in the management of the variables involved in the SFE process allows one to optimize the experimental conditions, considering the selectivity of a substance or classes of substances of interest. The selectivity of supercritical CO_2 allows maximization of the concentrations of selected compounds, with this process being more advantageous than hydrodistillation, as demonstrated for *Ferula assa-foetida*.

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