EXTRACTION OF *FERULA COMMUNIS L.* VOLATILE CONCENTRATE BY SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT
Flowerheads of *Ferula communis L.* was used for supercritical extraction with carbon dioxide to isolate the corresponding volatile concentrate. The vegetable fragrances were isolated by supercritical CO_2 extraction coupled to a fractional separation technique. The process was carried out operating at 100 bar and 50 °C in the extraction vessel, at 100 bar and below –5 °C in the first separator to selectively precipitate the cuticular waxes and at a pressure between 15 and 20 bar and temperatures in the range (15 – 21) °C in the second one to recover the volatile oil. GC-MS analysis of the extract allowed the identification of the oil composition.
The main constituents in the extract were α-gurjunene, β-gurjunene, α-selinene and α-selinene. A comparison with the oil obtained by hydrodistillation is also given.
The differences observed between the composition of the SFE volatile concentrate and of the hydrodistilled oil one were relevant. Indeed, the HD essential oil has a blue colour due to presence of chamazulene, obtained by thermal transformation from matricine [1], in SFE extract it is absent because this extraction process does not produce any thermal trasformation.

INTRODUCTION
The genus *Ferula* belongs to the Apiaceae family, it is widespread in Central Asia and in Mediterranean region [2]. The *F. communis L.* also grow wild in Sardinia, expecially in the mountainous central region and was reported to be highly toxic to animals and to humans. In the present work we show the results concerning the obtaining of volatile concentrate from the flowerheads of *F. communis L.* by means of supercritical carbon dioxide extraction, in a single extraction stage.
Supercritical fluid extraction, SFE, is a valid alternative for the production of flavours and fragrances from natural materials. Compressed CO_2 is able to solubilize hydrocarbon and oxygenated mono- and sesquiterpenes [3], the main constituents of essential oils. The separation of the extractant is easy and the oil is devoid of residues that pose a risk for human use. Conventional processes such as distillation, solvent extraction etc., often require additional steps such as separating the extractant, and are usually inferior to CO_2 with respect to their selectivity. In addition, the lower temperature in the SFE avoids thermal degradation and the low water content limits hydrolitic process. So essential oil obtained by SFE are devoid of fatty acids, resins, waxes and coloring matters normally coextracted by conventional solvent extraction, and exhibit a scent more similar to the starting material they were derived from, than those obtained by means of steam-distillation. The almost exclusive use of compressed carbon dioxide to extract oils or aroma substances destined to human nutrition and in the pharmaceutical and perfume industries is due to its chemical and physical

properties: it is safe, no toxic, non-combustible, inexpensive and its critical temperature and pressure are not high (31.06 °C and 73.82 bar) [4].

MATERIALS AND METHODS

Materials. *Ferula communis* was collected on the Gennergentu massif of Sardinia (Arzana, Nuoro). The plant was identified by Prof. Mauro Ballero, Department of Botanic Sciences, University of Cagliari, and a voucher specimen was deposited in the University's Herbarium. The aerial parts of the plant were collected during full blossom and then air-dried in the shade for several days. Before utilization the material was comminuted. CO_2 (purity 99%) was supplied by SIO (Società Italiana Ossigeno, Cagliari, Italy).

SFE apparatus. Supercritical CO_2 extractions were performed in a laboratory apparatus equipped with a 400 cm^3 extraction vessel, which operated in a single-pass mode by passing CO_2 through the fixed bed of vegetable particles. Two fractions of the extract were recovered in two separator vessels (300 and 200 cm^3) which were connected in series. The cooling of the first separator was achieved by using a thermostatic bath (Neslab, Model CC-100II, accuracy of 0.1 °C). The use of the second separator allowed the discharge of the liquid product at desired time intervals. In this section, the temperature was maintained at the desired value using two methods. First by the utilisation of a heating ribbon wrapped around the piping dividing the two separators, and secondly, by means of a water thermostatic system connected to the second separator. A high pressure diaphragm pump (Lewa, Model EL 1) with a maximum capacity of 6 kg/h, pumped liquid CO_2 at the desired flow rate. The CO_2 was then heated to the extraction temperature in a thermostatic oven (accurate to 0.02 °C). Extraction was carried out in a semi-batch mode: batch charging of vegetable matter and continuous flow solvent. The flow of CO_2 was monitored by a calibrated rotameter (Sho-rate, Model 1355) positioned after the last separator. The total CO_2 delivered during an extraction was measured by a dry test meter. Temperatures and pressures along the extraction apparatus were measured by thermocouple and Bourdon-tube test gauges, respectively. Pressure was regulated by high pressure valves under manual control, located on different points of the apparatus.

Hydrodistillation. Hydrodistillation was performed in a circulatory Clevenger-type apparatus for 5 h on 100 g of material belonging to the same samples employed in the SFE experiments.

GC/MS analysis. A Hewlett-Packard 5890 Series II gas chromatograph, GC, was used for analysis of the extracts. It was equipped with a split-splitless injector and a DB5-MS fused silica column of 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm. The GC conditions used were: programmed heating from 60 to 280 °C at 3 °C/min, followed by 30 min under isothermal conditions. The injector was maintained at 250 °C. Helium, at 1.0 mL/min, was the GC carrier gas; the sample (1 μL) was injected in split mode (1:20). The GC was fitted with a quadrupole mass spectrometer, MS, Model HP 5989 A. MS conditions were as follows: ionization energy 70 eV; electronic impact ion source temperature, 200 °C; quadrupole temperature, 100 °C; scan rate, 1.6 scan/s; and mass range 40-500 amu. Software to handle mass spectra and to record chromatogram was MS ChemStation (Hewlett-Packard) using NIST98, and LIBR(TP) [5] mass spectra libraries. Run samples were diluted in chloroform at a dilution ratio of 1:100 (w/w). Chromatographic results were expressed as area-percentages, calculated without applying any response factor, and are reported as a function of retention times, t_R. Identifications were made by matching both their mass spectra and I_K values, with those reported in the literature and those of pure compounds, whenever possible.

RESULTS AND DISCUSSION

The aerial parts volatiles of *F. communis L.* was obtained by SFE at the following conditions: P=100 bar, T=50 °C and ϕ_{CO2} = 0.6 kg/h. The total volatiles yield, after an extraction lasting 3 hours, was 0.14% and the main constituents (Table 1) were: α-gurjunene (48.9%), β-gurjunene (8.2%), α-selinene (2.5%) and β-selinene (3.3%). By hydrodistillation was been obtained a blue viscose essential oil traces. The GC-MS analysis has allowed to identify with chamazulene the compound responsible for the color of essential oil. The chamazulene derives from precursor compound by thermal transformation. The volatile concentrate obtained by SFE has also a very different appearance with respect to the HD product. Matricine was no converted to chamazulene, as demonstrated by the pale yellow color of this extract. Moreover, viscosity was lower, since paraffins were pratically absent.

The *Ferula* concentrate volatile producted by SFE has a yellow color because the this extraction process (low temperature) does not produce any thermal trasformation. *Reverchon et al.*[1] and *Ness et al.*[6] has discovered the same phenomenon studying the chamomile essential oil and he has identified the chamazulene in the HD essential oil and the matricine in the SFE extract

After the exhaustive extraction step at 100 bar and 50°C, a second step of extraction was performed on the same charge by operating at 300 bar for 3 hours, and the previously quoted values of T and ϕ. The scope of this second run was to extract the high molecular weight compounds present in the aerial parts volatiles of *Ferula. communis L.* . The high-pressure step gave in the separator a product (yield of 0.7% by weight of the charge) that is greenish semi-solid mass without the characteristic *Ferula* aroma. It consisted of some paraffins and high percentage of long-chain hydrocarbon .alcohols, fatty acid and FAME's. The composition of this extract confirms that the extraction pressure and temperature used in the first step of the process were opportunely chosen to avoid the coextraction of the long-chain compounds that give to the extract an unpleasant fragrance. Thus, by increasing the extraction pressure, it is possible to increase the yield of the process, but a less valuable *Ferula* oil is obtained.

Our results for the chemical composition of the extracts disagree with those reported by *Rustaiyan et al*. [7], *Baser et al*. [8] and *Filippini et al.* [2] . The difference in qualitative and quantitative analysis between our results and literature may depend on ambient and climatic condition and different vegetative stages.

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Table 1: Retention index, R, and percentage composition of the SFE extract, obtained at 100 bar, 50 °C and ϕ_{CO2} = 0.6 kg/h, and of the oil of flowerheads of *Ferula communis L*.

R	Compound	Extract	Oil	Waxes
914	α-pinene	0.4	1.0	-
957	sabinene	0.3	-	-
966	myrcene	1.4	3.4	-
1004	β-phellandrene	1.1	0.4	-
1010	(Z)- β-ocimene	0.2	-	-
1069	linalool	0.6	0.9	-
1073	N-nonanal	-	1.1	-
1352	α-cubebene	0.5	-	-
1366	β-elemene	0.8	1.1	-
1394	α-gurjunene	48.9	40.7	-
1402	β-gurjunene	8.2	7.1	-
1412	aromadendrene	0.6	0.7	-
1425	geranyl acetone	0.3	-	-
1428	α-humulene	0.3	-	-
1429	(E)- β-farnesene	1.8	0.6	-
1445	allo-aromadendrene	2.0	1.3	-
1453	γ-muurolene	0.6	1.1	-
1459	ar-curcumene	0.5	2.3	-
1462	β-selinene	2.5	-	-
1466	α-selinene	3.4	3.9	-
1472	α-muurolene	0.4	-	-
1479	germacrene A	0.4	-	-
1479	(E,E)- α-farnesene	0.9	1.3	-
1482	β-curcumene	-	-	-
1488	(Z)- γ-bisabolene	1.5	2.0	-
1491	δ-cadinene	1.3	-	-
1523	elemol	-	1.6	-
1535	(E)-nerolidol	0.9	-	-
1545	caryophyllene oxide	1.8	0.9	-
1572	humulene epoxide II	0.2	-	-
1600	vulgarone B	0.2	-	-
1623	selin-11-en-4 α-ol	0.4	-	-
1624	cubenol	-	1.4	-
1692	chamazulene	-	2.3	-
1719	aristolone	4.2	-	-
1737	hinesol acetate	0.2	-	-
1800	(E,E)-farnesyl acetate	0.8	-	-
1886	cyclopentadecanolide	-	3.9	-
1894	methyl hexadecanoate	0.2	-	-
1946	ethyl hexadecanoate	2.0	4.1	-
1968	N-eicosane	-	-	0.6
1975	hexadecyl acetate	0.2	-	-
2058	methyl linoleate	0.5	-	-
2062	N-heneicosane	-	-	0.6
2260	N-tricosane	-	0.7	2.7
2361	N-tetracosane	-	-	0.5
2458	N-pentacosane	-	1.5	3.0
2691	octacosane	-	-	5.2
2740	nonacosane	-	1.2	11.5

2971	triacontane	-	-	9.7
3020	hentriacontane	-	-	32.7
3201	dotriacontane	-	-	30.0
3215	11-decyl-docosane	-	-	3.5