

Isolation of *Crithmum Maritimum* Volatile Oil By Supercritical Carbon Dioxide Extraction

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Dried and ground leaves of *Crithmum maritimum* were used as a matrix for supercritical extraction of volatile oil with CO₂. Operative conditions were: extractor, 90 bar and 50 °C for 240 min; first separator, 90 bar and -10°C; second separator, 15 bar and 15 °C. GC/MS analysis of the leaves volatile oil revealed that it mainly consisted of: para-cymene, β-phellandrene, γ-terpinene, thymol methyl ether and dillapiole.

INTRODUCTION

Supercritical fluid extraction is an interesting technique for the extraction of flavoring compounds from vegetable material [1-6]. SFE is a separation technique where the yield and selectivity can be controlled to some extent by changing the pressure and temperature of the fluid. Carbon dioxide has been the most used supercritical solvent for application in the food and related industries (mainly because is non-flammable, cheap and non-toxic) [7-8]. With this solvent is possible to obtain solvent-free extract and avoid the degradation of thermally labile components. Therefore, the natural odour and flavour of the initial material are maintained. So, the application of supercritical CO₂ extraction for the isolation of essential oils from herbaceous matrixes is a very promising technique. Unfortunately, supercritical CO₂ shows a high affinity not only for the essential oil, but also for many other classes of compound that exist in the vegetable matrix. If the supercritical extraction is carried out in a single stage separation, the extracts obtained show a solid consistency due to the simultaneous extraction of the oil (hydrocarbon terpenes, oxygenated terpenes and sesquiterpenes), high molecular mass compounds and the cuticular waxes. However, it is possible to obtain the essential oil by supercritical CO₂ extraction adopting a fractional separation at least in two stage. Choosing the optimal pressure and temperature, it is possible to precipitate the undesirable compounds in the first separator and the essential oil in the second one [9-10].

Crithmum maritimum L., is a perennial plant of the family Umbrelliferae, that grow spontaneously in European, Atlantic coasts, Azores, Madeira, Canarias islands, Mediterranean and Black Sea coasts, NW Africa and W Asia. It is a shrub which grows on maritime rocks, piers, and breakwaters under the influence of salt-rich wind, more rarely on sand or gravel. The whole plant is aromatic and has a powerful scent. *C. maritimum* L. is widely used in modern perfumery and medicine. Furthermore, the succulent leaves and young branches are used in folk medicine for their antiscorbutic, carminative, diuretic, digestive, purgative and vermifuge properties. It is also commonly known in the coastal areas as an excellent season salad used to accompany fish dishes [11-18].

I - MATERIALS AND METHODS

Materials. Leaves of *Crithmum maritimum* were collected from four different sites: Buggerru (Sulcis-Iglesiente SW-Sardinia) sea-area; Calamosca (Sulcis-Iglesiente, SW-Sardinia) sea-area; Figueira (Portugal); Faro S.Pedro Moel (Portugal). After harvesting leaves were air-dried in the shade. Before utilization, the vegetable matter was ground with a Malavasi mill (Bologna, Italy) taking care to avoid overheating. CO₂ (purity 99%) was supplied by SIO (Società Italiana Ossigeno, Cagliari, Italy).

SFE apparatus. Supercritical CO₂ extractions were performed in a laboratory apparatus, equipped with a 320 cm³ extraction vessel and two separator vessels of 300 and 200 cm³ respectively connected in series [1-6]. Experiments were carried out at different conditions in the extraction section. In the first separator the temperature was set at -10 °C and the pressure at the same value as the extraction section. The second separator was set at 15 bar and 10 °C. Extraction were carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. About 180 g of material were charged in each run.

GC/MS analysis. A Hewlett-Packard (Palo Alto, USA) 5890 series II gas chromatograph, GC, was employed. It was equipped with a split-splitless injector and a DB5-MS fused silica column; 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm. The used GC conditions 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 was the carrier gas at 1.0 mL/min; the sample (1 µL) was injected in the 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, mass range (40-500) amu. The software adopted to handle mass spectra and the chromatogram was ChemStation. NIST98 [19], FLAVOUR and LIBR (TP) [20] mass spectra libraries were used as references. Samples were run diluted in chloroform with a dilution ratio of 1:100. The Tables show the chromatographic results, expressed as area percentages calculated without any response factor, as a function of Kováts' Indices, I_K [21]. Identifications were made by matching their mass spectra and I_K with those reported in the literature or those of pure compounds whenever possible.

CONCLUSION

The study was carried out on samples harvested from different locations in Sardinia and in Portugal. Operative conditions were chosen on the basis of previous results on SFE of similar matrixes: extractor, 90 bar and 50 °C for 240 min; first separator, 90 bar and -10°C. The essential, of a pale yellow colour was recovered in the separator that worked at 15 bar and 10 °C. Table 1 shows the composition of the compounds identified in the four samples. The analyzed oils have not shown differences in the chemical composition but they content the major constituents in variable proportions. It is interesting to note that dillapiole, which represented 73% and 68% of the oil in Sardinia samples, yet was completely absent in the oil of Fars S. Pedro Moel and is 38% of Figueira oil. While the Portugal oils contain thymol methyl ether in the biggest quantity, 24 and 16% versus 3 and 0% respectively. From these data, it can be seen that the chemical composition of the Sardinian oil is peculiar and rather different from that of the oils obtained from Portuguese *Crithmum maritimum*. These difference could be the basis of further research work aimed at determining whether this variability is caused by endogenous or exogenous factors.

Table 1. Kovats' indices, I_K and chromatographic area percentages of compounds found in *Crithmum maritimum* essential oil extracted by SFE at 90 bar 50°C: Buggerru (SFE-1 column); Calamosca (SFE-2 column); Figueira (SFE-3 column); Fars S. Pedro Moel (SFE-4 column).

I_K	SFE-1	SFE-2	SFE-3	SFE-4	Compound
906	-	-	0.35	1.24	tricyclene
913	-	0.90	3.23	5.87	α -pinene
950	-	1.28	5.79	-	sabinene
955	-	-	Tr	2.87	β -pinene
965	-	-	0.65	1.05	myrcene
981	-	-	-	1.78	α -phellandrene
992	-	-	0.46	3.51	α -terpinene
998	3.44	3.19	5.09	15.94	para-cymene
1005	5.44	5.40	0.55	6.88	β -phellandrene
1009	0.83	1.68	1.82	1.48	(Z)- β -ocimene
1032	12.47	16.29	23.11	33.16	γ -terpinene
1042	-	-	1.61	-	cis-sabinene hydrate
1055	2.51	0.70	-	1.33	terpinolene
1145	-	-	0.91	-	trans- β -terpineol
1198	2.69	-	16.29	23.49	thymol,methyl ether
1259	-	-	-	0.42	bornyl acetate
1401	-	0.44	-	0.98	α -cis-bergamotene
1520	-	1.05	0.62	-	germacrene b
1591	72.62	68.55	38.23	-	dill apiole

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