ISOLATION OF THE OILS FROM *LAURUS NOBILIS* OF TUNISIA AND ALGERIA BY SUPERCRITICAL CARBON DIOXIDE EXTRACTION:

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Dried and ground leaves of *Laurus nobilis* from Algeria and Tunisia were used as a matrix for supercritical extraction of volatile oil with CO_2. Operative conditions were: extractor, 90 bar and 50°C for 240 min; first separator, 90 bar and –10°C; second separator, 20 bar and 15°C. GC/MS analysis of the leaves volatile oil revealed that it mainly consisted of: 1,8-cineole, linalool, α-terpenyl acetate, methyl eugenol, and sabinene.

The comparison with the hydrodistilled oil did not reveal any big difference. Collection of samples at different extraction times during supercritical extraction, allowed to monitor the change of the oil composition.

INTRODUCTION

Laurel (*Laurus nobilis L.*) is an evergreen tree up to 20 m high, native to the Mediterranean region [1]. It is the only European representative of the Lauraceae family [2]. It is also know as sweet bay, bay, bay laurel, Grecian laurel, true bay and Mediterranean bay [1]. The dried leaves are used extensively in home cookery [3] and the essential oil is used mainly in the flavouring industry. Laurel essential oil said also laurel leaf oil or sweet bay essential oil, was reported to be used in the preparation of hair lotion for its antidandruff activity and for the external treatment of psoriasis [4]. This oil is generally obtained by hydro or steam distillation. This technique, even when do not induce extensive phenomena of hydrolysis and thermal degradation, give in any case a product with a characteristic off odour [5]. Solvent extraction can give an oil, but on account of a high content of waxes and/or other high molecular mass compounds, often give rise to a concrete with a scent very similar to the material from which it was derived. A further drawback of this technique is that small amounts of organic solvents can pollute the extraction product. These limitations can be overcome using supercritical fluid CO_2 extraction (SFE). Indeed supercritical fluid CO_2 extraction can be performed at relatively low pressure, at near room temperature and CO_2 is completely eliminated from the product at the end of the extraction process [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 CO_2 extraction for the isolation of essential oils from herbaceous matrixes is a very promising technique. Unfortunately, supercritical CO_2 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 CO_2 extraction adopting a fractional separation at least in two stage. Choosing the optimal pressure an temperature, it is possible to precipitate the undesirable compounds in the first separator and the essential oil in the second one [9-10].

In recent years, Ozek et al. 1998 [11] studied the extraction of the laurel essential oil, by means of a micro SFE apparatus, and identified a large number of components. They obtained however the oil always mixed with large quantities of cuticular waxes. The present study was undertaken to verify the possibility to obtain, in a single stage, a pure laurel essential oil by means of supercritical carbon dioxide extraction.

i - Materials and Methods

Materials. Leaves of laurel were air-dried at room temperature in the shade for some weeks. They had a final moisture content of 10.0 % on dry basis. Before using, the vegetable matter was ground and the particles sizes, were in the range (300-800) μm. CO_2 (purity 99%) was supplied by Air Liquide Italia, Cagliari, Italy.

SFE apparatus. Supercritical CO_2 extractions were performed in a laboratory apparatus, equipped with a 320 cm^3 extraction vessel and two separator vessels of 300 and 200 cm^3 respectively connected in series. 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 20 bar and 15°C. Extraction were carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. About 200 g of material were charged in each run (210 of tunisiaen laurel and 250 of algerian laurel).

Hydrodistillation. Hydrodistillation was performed in a circulatory Clevenger-type apparatus, for four hours, up to the point where the oil contained in the matrix was exhausted. About 100 g of material belonging to the same batch employed in SFE were charged.

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 [12], FLAVOUR and LIBR (TP) [13] 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 [14]. Identifications were made by matching their mass spectra and I_K with those reported in the literature or those of pure compounds whenever possible.

DISCUSSION

The first step in the supercritical extraction of essential oils is to optimize the operating pressure and temperature to obtain an efficient extraction of terpenic compounds that are responsible for the aroma and to avoid the co-extraction of undesired compounds (fatty acids, their methyl esters and some colouring matter). On the basis of work previously performed by our research group [15-17] we chose as extraction conditions 90 bar and 50 °C (CO_2 density of 0.287 g/cm^3). These conditions allow the extraction of volatile oil without undesired compounds, except waxes. The presence of waxes is due to the different mass transfer mechanisms which characterize the extraction of terpenes and paraffins. Therefore, it was necessary to resolve the extract using a fractional separation technique. Indeed, at temperature around 0°C the solubility of paraffins in liquid CO_2 is near to zero, whereas terpenes are completely miscible under these conditions. Therefore paraffins solubilized during the extraction can be precipited in the first separator set at about 0°C and the essential oil compounds can be collected in the second separator, where a large pressure reduction induces the evaporation of the CO_2.

A good fractionation was obtained by cooling the first separator at -10°C with an operating pressure of 90 bar and depressuring the second separator at 20 bar and 15°C. The chosen temperature in first separator allowed the complete recovery of paraffins whereas the temperature in the second separator allowed the release of the terpenes from the gaseous CO_2, minimizing the loss of volatile compounds

Laurus nobilis volatile oil recovered in the second separator was a yellow liquid, whereas waxes recovered in the first separator were white, solid and odourless.

The present study was carried out on samples harvested from different locations in Algeria and in Tunisia. In Table 1 we report the detailed identification and the area percentages of compound found in the volatile oil recovered in the second separator shows the composition of the compounds identified in the two 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 1,8-cineole, which represented 31% of the oil in Tunisia samples, yet is 17% of Algeria oil. While the Algeria oils contain linalool in the biggest quantity, 13.4% versus 2.2%. In the tunisian oil the content of monoterpenes (

hydrocarbon and oxygenated) was higher than in the Algerian oil while the Algerian oil was rich in sesquiterpenses (hydrocarbon and oxygenated). . From these data, it can be seen that the chemical composition of the Tunisian oil is peculiar and rather different from that of the oils obtained from Algerian *Laurus nobilis.*

The asymptotic *Laurus* volatile oil yield was measured at the end of an exhaustive run at the extraction and fractionation conditions previously indicated. It was 1.6% for the *Laurus* tunisian and 1.3% for the *Laurus* algerian.

Laurus essential oil was also extracted by hydrodistillation performed on the same starting material and a comparison between the oils obtained by SFE and by hydrodistillation revealed that their chemical differences were not relevant.

The yield (Y%) of each fraction of the supercritical extraction (SFE) and of the hydrodistillation (HD), as percent w : w, with respect to the charged material, are reported in Table 3. In the same chart is enclosed also the amount of CO_2 consumed in the process, expressed as the specific mass of solvent, m_s/m_0 (m_0 is the mass of leaves charged in the extractor).

In figure 1 the yield (%) of four compound families (hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes) in which the volatile oil was divided is plotted against the extraction time. The overall volatile oil is also give for comparison. The hydrocarbon terpenes (mono- and sesqui-) yield was small,0.5%, (0.3% for the algerien oil) if compared to the overall yield in essential oil,1.6% (1.3% for the algerien oil). The oxygenated terpenes yield was 1.1% (1.0% for the algerien oil). Sesquiterpenes started to be extracted in high quantities after the first 90 min of extraction and when the monoterpene hydrocarbons extraction was nearly complete. Therefore the extraction time plays an important role in the composition of the *laurus* volatile oil obtained.

The volatile oils of Tunisia and Algeria, as well as the oil of Sardinia [18] with respect to some literature data [19-21] are less rich in 1,8-cineole but shows the highest content of methyl eugenol. Ozek et al., 1998 [11] performed some SFE experiments at different conditions using a single-step separation. At none of the tested conditions was it possible to obtain a pure essential oil since large quantities of cuticular waxes were present in the extract. The author did not report the percentage of waxes but only the composition of the essential oil and its yield (1.13 % on dry basis). They identified 71 compounds and among them 31 have been found also in our samples. They found in the extract at 80 bar and 40 °C (ρ_{CO2} = 0.221 g cm^{-3}) as main constituents: 1,8-cineole, 40.2%, α-terpenyl acetate, 13.8% and terpinyl-4-ol, 3.3%. In the first separator we found a small quantity of extract that was solubilized in $CHCl_3$ then analysed. It was composed by the essential oil constituents and by long chain alkanes: tricosane, pentacosane, octacosane, hentriacontane and tritriacontane. In the HD column, of Table 1 are shown the area percentages of the components of the hydrodistilled oil (Y = 0.90%). The chemical composition did not reveal any big difference with respect to that of the SFE oil.

These difference could be the basis of further research work aimed at determining whether this variability is caused by endogenous or exogenous factors.

The main difference between SFE and HD oils was the content of monoterpenes which are higher in the HD products while the sesquiterpenes are higher in the SFE products. This result is rationalized as follows. Essential oil compounds are only slightly soluble in water, therefore HD mainly induces migration of these compounds from the inside of the leaf up to its surface, followed by their subsequent evaporation [22]. Therefore only low molecular weight compounds are taken from the vegetable matrix. Supercritical CO_2 emulates an organic solvent characteristics with changes in extraction conditions (temperature and pressure). Also, by using this process, high molecular weight compounds can be extracted from the *Laurus* particles. The extend of extraction of these compounds must be optimize for SFE.

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Table 1. Retention time and Area Percentages of Compounds Found in Laurel essential oil extracted by Supercritical fluid (SFE) at 90 bar and 50°C and Hydrodistillation (HD):

T_R	Compound	SFE_A	HD_A	SFE_T	HD_T
5.19	tricyclene	tr	Tr	0.2	0.3
5.37	α-pinene	0.9	2.7	2.6	2.9
6.37	sabinene	2.2	4.6	6.8	7.2
6.48	β-pinene	0.8	2.1	2.2	2.5
6.83	myrcene	tr	tr	0.4	0.6
7.64	Δ^2-carene	tr	tr	Tr	0.3
8.12	1,8-cineole	17.6	16.3	31.0	32.1
9.05	γ-terpinene	tr	0.7	Tr	0.5
9.33	cis-sabinene hydrate	0.4	tr	0.4	0.3
10.56	linalool	13.4	10.9	2.2	2.1
13.14	para-mentha-1,5-dien-8-ol	0.4	tr	0.4	0.4

13.56	terpin-4-ol	1.3	2.5	0.6	1.3
14.11	α-terpineol	2.2	2.6	2.1	2.3
15.32	cis-sabinene hydrate acetate	tr	tr	0.5	tr
16.87	linalool acetate	1.2	tr	Tr	tr
17.55	n.i.	0.6	tr	Tr	tr
18.11	bornyl acetate	tr	tr	Tr	0.4
19.41	iso-3-thujyl acetate	0.4	tr	0.4	0.5
20.78	α-terpinyl acetate	10.6	16.6	15.3	15.6
21.07	eugenol	2.3	tr	1.6	tr
21.64	α-ylangene	0.6	0.8	0.4	tr
22.44	β-cubebene	tr	tr	1.1	0.6
22.51	β-longipinene	1.3	1.6	2.4	1.9
23.08	methyl eugenol	10.6	11.0	10.2	10.6
23.21	α-gurjunene	0.4	tr	0.8	0.6
23.60	(E)-caryophyllene	4.2	6.4	3.0	2.9
24.39	α-guaiene	1.2	1.0	0.7	0.6
24.58	cis-muurola-3,5-diene	0.4	tr	Tr	tr
24.79	α-himachalene	0.5	tr	Tr	tr
24.97	α-humulene	0.6	1.1	0.5	0.4
25.09	allo-aromadendrene	0.6	0.9	Tr	tr
26.07	germacrene D	1.1	1.3	1.1	0.9
26.27	cis-β-guaiene	1.2	1.3	0.4	0.4
26.70	bicyclogermacrene	1.9	0.9	3.3	3.2
27.08	α-bulnesene	1.5	tr	1.6	1.8
27.39	trans-cadinene	1.2	1.7	0.6	0.8
27.77	δ-cadinene	0.4	1.4	0.5	1.1
28.02	n.i.	0.6	tr	Tr	tr
28.29	n.i.	0.6	tr	Tr	tr
29.12	elemicin	3.2	2.0	1.9	1.0
29.75	Germacrene D-4-ol	tr	tr	1.8	0.8
29.81	spathulenol	2.8	4.0	0.5	0.8
30.01	caryophyllene oxide	2.0	4.4	Tr	tr
30.34	globulol	0.4	tr	Tr	0.3
30.77	n.i.	0.4	tr	0.4	tr
31.34	n.i.	0.7	tr	Tr	tr
31.77	n.i.	0.9	tr	Tr	tr
32.22	1-epi-cubenol	tr	tr	Tr	0.6
32.38	n.i.	0.5	tr	Tr	tr
32.51	β-eudesmol	0.5	tr	Tr	tr
32.66	α-cadinol	1.3	1.2	Tr	1.1
32.98	n.i.	0.5	tr	0.5	tr
33.18	n.i.	0.2	tr	Tr	tr
33.84	n.i.	0.3	tr	Tr	tr
33.98	5-isocedranol	0.6	tr	1.3	0.8
34.93	(Z)-trans-α-bergamotol	0.5	tr	Tr	tr
35.80	14-hydroxy-α-humulene	0.5	tr	Tr	tr
36.71	drimenol	0.3	tr	Tr	tr
38.09	α-bisabolol acetate	0.6	tr	Tr	tr
38.89	epi-β-bisabolol acetate	0.4	tr	Tr	tr
38.97	iso-longifolol acetate	0.4	tr	Tr	tr

(A, Algerian Laurel; T, Tunisian Laurel).

Table 2. Overall chromatographic area percentages of the four main classes: hydrocarbon monoterpenes, HM; oxygenated monoterpenes, OM; hydrocarbon sesquiterpenes, HS and oxygenated sesquiterpenes, OS, in which it is possible to group the constituents of the laurel oil.

Class	**SFE-T**	**HD-T**	**SFE-A**	**HD-A**
HM	12.2	14.4	3.9	8.9
OM	64.7	65.6	60.7	60.7
HS	18.1	15.5	21.5	20.7
OS	5.0	4.5	13.9	9.7

(A, Algerian Laurel; T, Tunisian Laurel).

Table 3. Percent yield of the supercritical extraction, SFE, and of the hydrodistillation, HD. The specific mass of CO_2, m_s/m_0, consumed in the process is also reported.

Quantity	**SFE-T**	**HD-T**	**SFE-A**	**HD-A**
Yield %	1.6	2.1	1.3	0.50
m_s/m_0	19.0	-	16.0	-

(A, Algerian Laurel; T, Tunisian Laurel).

Figure 1
Evolution of *Laurus nobilis* essential oil composition with the extraction time. Cumulative quantities are expressed as yield (%) of the extracted compound families at different extraction times. The overall volatile oil yield is also reported for comparison: •, hydrocarbon monoterpenes; ▲ , oxygenated monoterpenes; ◆ , hydrocarbon sesquiterpenes; ▼ , oxygenated sesquiterpenes; ■ , overall yield.