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Supercritical Carbon Dioxide Extraction and Characterization of *Laurus nobilis* Essential Oil

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Supercritical carbon dioxide extraction allowed essential oil of *Laurus nobilis* to be obtained. Extraction conditions were as follows: pressure, 90 bar; temperature, 50 °C; and carbon dioxide flow, $\Phi = 1.0$ kg/h. Waxes were entrapped in the first separator set at 90 bar and −10 °C. The oil was recovered in the second separator working at 15 bar and 10 °C. The main components were 1,8-cineole (22.8%), linalool (12.5%), α-terpinyl acetate (11.4%), and methyleugenol (8.1%). Comparison with the hydrodistilled oil did not reveal any significant difference. Collection of samples at different extraction times during supercritical extraction allowed the change of the oil composition to be monitored. Lighter compounds such as hydrocarbon and oxygenated monoterpenes were extracted in shorter times than the heavier hydrocarbon and oxygenated sesquiterpenes.

KEYWORDS: Supercritical carbon dioxide extraction; essential oil; *Laurus nobilis*

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 known 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 flavoring industry. Laurel essential oil, also called 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 hydrodistillation or steam distillation. This technique, even when it does not induce extensive phenomena of hydrolysis and thermal degradation, gives in any case a product with a characteristic off-odor (*5*). Solvent extraction can give an oil, but due to a high content of waxes and/or other high molecular mass compounds, often gives rise to a concentrate 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. Supercritical fluid extraction (SFE) is a good technique for the production of flavors and fragrances from natural materials and can constitute a valid alternative to both of the above-mentioned processes. In fact, compressed carbon dioxide (CO_2) is able to solubilize hydrocarbon and oxygenated mono- and sesquiterpenes (*6*), the main essential oil constituents. The separation of the extractant is easy, and possible residues do not cause a risk for human health. Indeed, CO_2, besides being safe, noncombustible, and inexpensive, is nontoxic. In recent years, Ozek et al. (*7*) 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.

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MATERIALS AND METHODS

Materials. Leaves of laurel (*L. nobilis* L., Lauraceae) were collected from the locality of Porto Columbu, Sarroch, in southern Sardinia, Italy. The plant was identified by Bruno De Martis, Dipartimento di Scienze Botaniche, Università di Cagliari, and a voucher specimen was deposited in the Herbarium of that University. The leaves were air-dried at room temperature in the shade for some weeks. They had a final moisture content of 9.8% on dry basis. Before using, the vegetable matter was ground to particle sizes in the range of 300−800 μm. CO_2 (purity = 99%) was supplied by Società Italiana Ossigeno (SIO), Cagliari, Italy.

SFE Apparatus. Supercritical CO_2 extractions were performed in a laboratory apparatus (**Figure 1**) equipped with a 400 cm^3 extraction vessel, E, operating in the single-pass mode of CO_2 through the fixed bed of ground material. The two fractions of the extract were recovered in two separator vessels connected in series, S_1 and S_2, of 300 and 200 cm^3, respectively. The cooling of the first separator was achieved by using a thermostated bath (Neslab, model CC-100II, accuracy of 0.1 °C). The second separator allowed the discharge of the liquid product at desired time intervals. In this section, the temperature was maintained at the wanted value, by using a heating ribbon wrapped around the pipe between the two separators, and by means of a water thermostated system connected to the second separator. A high-pressure diaphragm pump, P (Lewa, model EL 1), with a maximum capacity of 6 kg/h, pumped liquid CO_2 at the desired flow rate. CO_2 was then heated to extraction temperature in a thermostated oven (accuracy of 0.02 °C).

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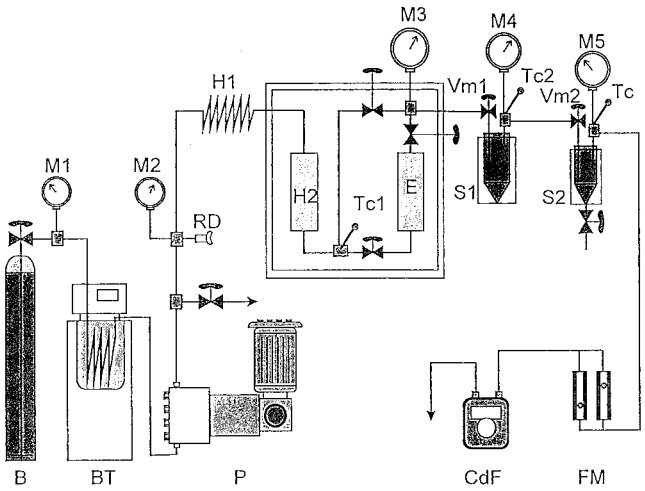

Figure 1. Schematic representation of the laboratory apparatus used in the SFE experiments: B, CO_2 bottle; BT, thermostated bath; P, diaphram pump; RD, rupture disk; H1, preheater; H2, pulsation dampening; E, extractor; S_1, S_2, separators; FM, rotameter; CdF, dry test meter; M1–M5, pressure measurement devices; Tc1, Tc2, thermocouples; Vm1, Vm2, pressure-regulating valves.

The extraction was carried out in a semibatch mode: batch charging of vegetable matter and continuous flow solvent. Carbon dioxide flow was monitored by a calibrated rotameter, FM (Sho-rate, model 1355), located after the last separator. Total CO_2 delivered during an extraction test was measured by a dry test meter, CdF. 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.

Hydrodistillation. Hydrodistillation was performed in a circulatory Clevenger-type apparatus, for 4 h, up to the point at which the oil contained in the matrix was exhausted. About 100 g of material belonging to the same batch employed in SFE was charged.

GC-MS Analysis. A Hewlett-Packard 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. GC conditions used were as follows: 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. Software adapted to handle mass spectra and chromatograms was ChemStation. NIST98, FLAVOUR, and LIBR(TP) (*8*) mass spectral libraries were used as references. Samples were run diluted in chloroform with a dilution ratio of 1:100. In the tables, chromatographic results, expressed as area percentages calculated without any response factor, are reported as a function of retention times, t_R, and Kováts indices, I_K (*9*). Identifications were made by matching their mass spectra and I_K values with those reported in the literature or those of pure compounds, whenever possible.

RESULTS AND DISCUSSION

The employed apparatus **(Figure 1)**, owing to a two-stage separation, allowed us to obtain an oil deprived of cuticular waxes. Indeed, being located on the leaves' surface, they are easily washed off with mild conditions of extraction (*10*). In all experiments reported in this paper solvent flow was 1.0 kg/h and the extractor pressure and temperature were set to 90 bar and 50 °C, respectively. Operative conditions were chosen on the basis of previous results (*11*, *12*) on SFE of similar matrices.

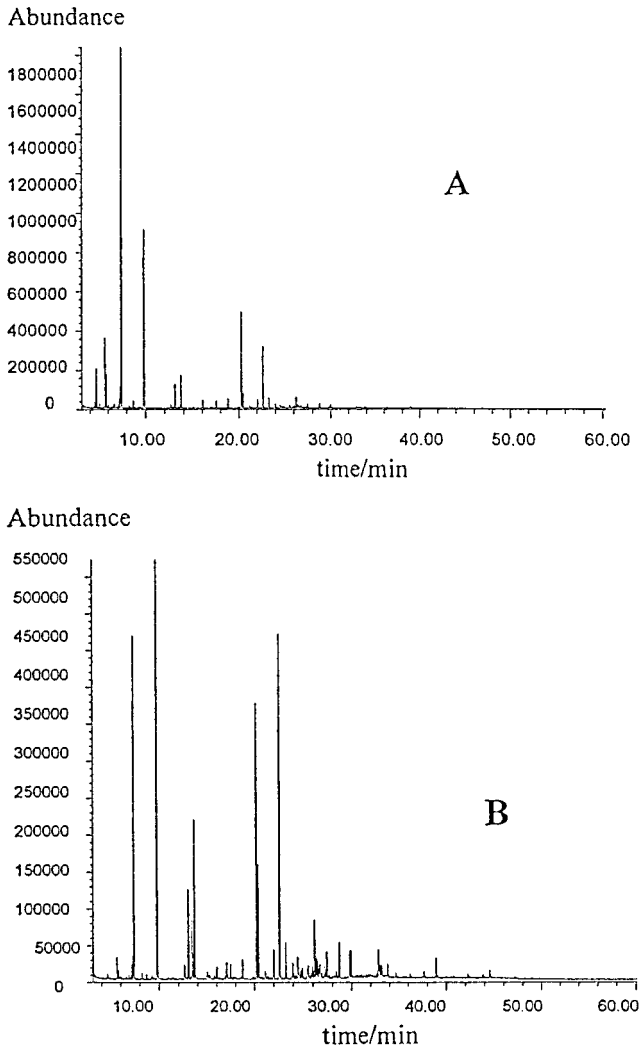

Figure 2. GC traces of two oil fractions obtained by SFE at different extraction times: (A) sample obtained after the first hour, SFE-1; (B) sample obtained between the second and third hours, SFE-3. Experimental conditions: extractor, 90 bar and 50 °C; first separator, 90 bar and −10 °C; second separator, 15 bar and 10 °C.

Waxes were entrapped in the first separator set at 90 bar and −10 °C. The oil, of a pale yellow color, was recovered in the second separator that worked at 15 bar and 10 °C. At these conditions the release of terpenes from the gaseous CO_2 is assured and the loss of volatiles is minimized. The water, which may be coextracted with the essential oil, when present, was removed from the samples by means of a syringe. We performed a preliminary run drawing the oil, in separate vials, after each hour of extraction for 4 h. In **Table 1** are reported the relative amounts of the oil constituents of each sample, SFE-1−SFE-4. The most remarkable differences in opposite direction are shown by 1,8-cineole, 30.98 versus 2.05% (in the first and fourth samples, respectively), and by methyleugenol, 6.85 versus 16.42%. In parts A and B of **Figure 2** are shown the GC traces of SFE-1 and SFE-3 respectively; the different proportions of the main components in the two oil fractions are clearly seen. Moreover, in the GC traces no traces of cuticular waxes are present. It is possible to group the oil components in four classes, hydrocarbon monoterpenes (HM), oxygenated monoterpenes (OM), hydrocarbon sesquiterpenes (HS), and oxygenated sesquiterpenes (OS), on the basis of their chemical structure or retention time for nonterpenoids or nonidentified compounds. The area percentages relative to each class are reported in **Table**

Table 1. Retention Times, t_R, Kovats Indices, I_K, and Chromatographic Area Percentages of Compounds Found in Laurel Essential Oil Extracted by SFE at 90 bar and 50 °C (SFE-T) and Hydrodistillation (HD)[a]

t_R	I_K	compound	SFE-1	SFE-2	SFE-3	SFE-4	SFE-T	HD
4.45	924	α-thujene	0.34	tr[b]	tr	tr	0.43	0.53
4.63	931	α-pinene	2.28	tr	tr	tr	2.81	3.19
5.03	947	camphene	0.32				0.39	0.51
5.61	968	sabinene	4.53	0.27	0.56	tr	4.30	4.23
5.75	973	β-pinene	2.19	tr	tr	tr	2.57	2.72
5.90	978	6-methyl-5-hepten-2-one	tr	tr			tr	tr
6.01	982	myrcene	0.24	tr	tr	tr	0.27	0.22
6.06	983	dehydro-1,8-cineole				tr	tr	tr
6.40	994	ethyl hexanoate	0.37	tr			tr	tr
6.48	996	*p*-mentha-1(7),8-diene		tr				tr
6.57	999	α-phellandrene	tr	tr	tr		tr	tr
6.61	1000	Δ^3-carene	tr	tr			0.61	0.67
6.89	1009	α-terpinene	tr	tr	tr		tr	0.30
6.96	1011	*o*-cymene	tr	tr	tr		tr	0.23
7.12	1016	*p*-cymene	0.78	1.07	0.43	0.31	0.84	0.89
7.27	1020	limonene	1.72	1.13	0.76	tr	1.18	1.23
7.47	1026	1,8-cineole	30.98	12.09	10.05	2.05	22.84	23.51
7.80	1035	*Z*-β-ocimene	tr	tr	tr		tr	tr
8.23	1046	γ-terpinene	0.27	0.26	tr	tr	0.25	0.58
8.69	1058	*cis*-linalool oxide	0.71	0.34	tr	0.35	0.74	0.55
9.21	1070	terpinolene	tr	tr	tr	tr	tr	0.21
9.29	1072	*trans*-linalool oxide	tr	tr	tr	tr	tr	tr
9.45	1075	2-nonanone	tr	tr	tr	tr	tr	tr
9.88	1085	linalool	17.65	19.47	14.59	11.86	12.46	10.57
10.72	1103	*cis*-*p*-menth-2-en-1-ol		tr			tr	tr
10.98	1111	*endo*-2-norborneol acetate				tr	tr	tr
11.39	1123	*trans*-pinocarveol	tr	tr	tr	tr	tr	tr
12.61	1157	*trans*-dihydro-α-terpineol	0.53	0.80	0.67	0.47	0.67	0.69
12.69	1159	borneol		tr	tr	tr	tr	tr
13.05	1168	terpin-4-ol	2.41	3.75	3.48	2.64	2.57	3.26
13.33	1175	*p*-cymen-8-ol	tr	tr	tr	tr	tr	tr
13.48	1179	*Z*-3-hexenyl butyrate	tr	0.32	2.41	1.04	0.29	
13.71	1185	α-terpineol	3.29	5.56	6.44	6.82	3.35	3.92
14.94	1216	nerol		tr		0.59	tr	tr
16.00	1244	linalyl acetate	0.80	0.87	0.50	0.44	1.02	0.38
16.84	1265	*Z*-cinnamyl alcohol	tr	0.24	tr	tr	0.31	tr
16.98	1268	*E*-cinnamaldehyde		tr	1.32	0.42	tr	
17.14	1272	NI				0.85	tr	
17.47	1280	bornyl acetate	0.84	0.98	0.61	0.48	0.84	0.96
17.92	1290	2-undecanone	tr	tr	tr	tr	tr	0.32
18.36	1300	*trans*-ascaridole	tr	tr		tr	tr	
18.56	1305	*E*-cinnamyl alcohol				tr	tr	
18.73	1309	*neo*-iso-isopulegol acetate	1.03	1.23	0.84	0.86	1.10	1.06
20.27	1346	α-terpinyl acetate	11.18	14.23	12.23	10.92	11.36	10.79
20.47	1350	eugenol	1.66	3.25	4.69	5.97	2.60	1.83
20.77	1357	neryl acetate	tr	tr	tr	tr	tr	tr
21.09	1364	cyclosativene	0.57	0.70	tr	tr	0.79	tr
21.12	1365	hydrocinnamyl acetate	tr	tr	tr	tr	tr	0.44
21.37	1370	α-copaene	tr	tr	tr	tr	0.31	tr
21.64	1376	geranyl acetate		tr		tr	tr	tr
21.89	1381	β-cubebene	tr	tr	tr	tr	tr	tr
21.99	1383	β-elemene	1.06	1.73	1.35	1.40	1.18	0.66
22.70	1398	methyl eugenol	6.85	11.86	16.22	16.42	8.09	9.42
23.23	1411	*E*-caryophyllene	1.26	1.92	1.71	1.45	1.36	0.92
23.86	1427	aromadendrene		tr		tr	tr	
23.93	1428	α-guaiene	0.57	0.92	0.81	0.76	0.82	tr
24.46	1441	cinnamyl acetate	0.62	1.10	1.20	1.69	0.97	1.28
24.58	1444	geranyl acetone	tr	0.34	tr	tr	0.31	tr
24.74	1448	α-humulene	tr	0.44	tr	tr	0.30	tr
24.90	1452	*allo*-aromadendrene	tr	0.76	0.81	0.80	0.62	0.64
25.41	1463	NI	0.31	0.56	0.54	0.85	0.36	0.42
25.49	1465	NI	0.39	0.59	0.64	0.84	0.40	0.61
25.70	1470	γ-muurolene	tr	tr	tr	tr	tr	tr
25.86	1474	germacrene D	tr	0.44	tr	0.53	tr	tr
25.94	1476	β-selinene	tr	0.57	0.66	0.67	0.47	0.33
26.15	1480	*cis*-β-guaiene	1.35	2.39	2.81	2.66	1.65	1.43
26.31	1484	valencene	0.47	0.80	0.95	0.97	0.75	0.59
26.44	1487	viridiflorene	0.50	0.89	1.07	1.14	0.63	0.43
26.62	1491	methyl isoeugenol	tr	0.40	tr	0.85	0.47	0.45
26.70	1493	α-bulnesene	0.43	0.76	0.90	0.96	0.46	tr
27.18	1503	*trans*-γ-cadinene	tr	0.44	0.53	0.61	0.42	0.37
27.39	1508	7-epi-α-selinene	0.54	1.00	1.33	1.29	1.01	0.79
28.11	1526	*Z*-nerolidol		tr	tr	tr	tr	tr
28.35	1531	α-calacorene		tr	tr	0.59	tr	tr

Table 1 (Continued)

t_R	I_K	compound	SFE-1	SFE-2	SFE-3	SFE-4	SFE-T	HD
28.72	1540	elemicin	0.51	1.13	1.69	1.96	0.87	0.85
29.75	1563	spathulenol	tr	0.77	1.33	1.95	0.64	1.24
29.92	1567	caryophyllene oxide	0.50	0.99	1.40	1.85	0.92	1.83
30.78	1585	β-oplopenone		tr		tr	tr	0.33
31.61	1604	NI				3.67	tr	
32.82	1640	β-eudesmol	tr	0.86	1.77	2.84	1.01	2.16
32.97	1644	selin-11-en-4-α-ol		tr	tr	0.71	tr	tr
33.10	1648	bulnesol	tr	0.65	0.77	1.92	0.62	0.93
33.78	1667	NI	tr	0.51	0.70	1.27	0.49	0.54
37.60	1772	β-eudesmol acetate		tr	tr	tr	tr	
38.91	1807	NI	tr	0.39	0.95	1.26	0.30	
42.28	1903	NI				0.66	tr	
44.58	1970	NI		tr	tr	0.94	tr	

[a] Columns SFE-1–SFE-4 refer to the oil fractions obtained after each hour of extraction. [b] tr = trace (<0.2%).

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[a]

class	SFE-1	SFE-2	SFE-3	SFE-4	SFE-T	HD
HM	12.67	2.74	1.75	0.31	13.65	15.51
OM	79.43	77.62	76.94	66.68	70.86	70.28
HS	7.45	14.91	14.11	15.52	11.53	7.19
OS	0.5	4.17	6.92	17.07	3.68	7.03

[a] Columns SFE-1–SFE-4 refer to the oil fractions collected after each hour of the supercritical extraction. SFE-T is the overall essential oil obtained by SFE, and HD is the hydrodistilled oil.

Table 3. Percent Yield and Cumulative Percent Yield of the Different Stages of the Supercritical Extraction (SFE-1–SFE–4), of the Overall Run (SFE-T), and of the Hydrodistillation (HD)[a]

quantity	SFE-1	SFE-2	SFE-3	SFE-4	SFE-T	HD
yield, %	0.27	0.28	0.22	0.05	0.82	0.90
cumulative yield, %	0.27	0.56	0.77	0.82	0.82	0.90
m_s/m_0	5.36	10.72	16.08	21.44	21.44	

[a] The specific mass of CO_2, m_s/m_0, consumed in the process is also reported (m_0 = 186.52 g).

2. In general, lighter compounds (HM) are extracted almost completely during the first extraction hour, 12.67, against 0.31% in the fourth hour. OM decreased but to a minor extent from 79.43 to 66.68%. HS and OS are present at, respectively, 15.52 and 17.07% in the fraction obtained from the 180th to the 240th min and at 7.45 and 0.50 in the first hour sample. This confirms (*10*) that a long time run is necessary to obtain an oil with a stable composition. In **Table 1** are also reported, in the SFE-T column, the analytical results concerning the sample obtained, at the above-mentioned conditions, putting together all fractions (SFE-1−SFE-4) in a single vial. The yields of each fraction of the supercritical extraction, of the overall SFE run and of the hydrodistillation, as percent w/w, with respect to the charged material, are reported in **Table 3**. In the same table is also given 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). The overall yield of the supercritical extraction was 0.82%. 1,8-Cineole (22.84%) was the major component. The other important constituents were linalool (12.46%), α-terpinyl acetate (11.36%), and methyleugenol (8.09%). We identified also a trace of dehydro-1,8-cineole, which was found occurring naturally for the first time in *L. nobilis* by Hogg et al. (*13*). With respect to some literature data (*14*, *15*) our oil is less rich in 1,8-cineole but shows the highest content of methyleugenol. Ozek et al. (*7*) 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 because 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_{CO_2} = 0.221 g cm^{-3}) as main constituents 1,8-cineole (40.2%), α-terpinyl acetate (13.8%), and terpinyl-4-ol (3.3%). In the first separator we found a small quantity of extract that was solubilized in $CHCl_3$ and then analyzed. It was composed of the essential oil constituents and 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 significant difference with respect to that of the SFE oil.

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