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SUPERCRITICAL CO_2 EXTRACTION OF VOLATILE COMPONENTS FROM LEAVES OF *Laurus nobilis L.**

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Comparative investigation of the chemical compositions of volatile components from leaves of Laurus nobilis *obtained by supercritical CO_2 extraction and hydro- and steam distillation was carried out.*

A number of methods have been utilized to obtain essential oils from plant materials. They include water distillation, steam distillation, solvent extraction, and trapping techniques. Essential oils are produced by distillation. Although this method has been widely used, it has some disadvantages from the analytical point of view, such as heat instability, etc. [1].

Supercritical Fluid Extraction (SFE) is an interesting technique for the extraction of flavor and fragrance compounds from natural materials. It can constitute an industrial alternative to steam distillation and solvent extraction processes because relatively rapid extractions can be carried out under wild conditions and at a low temperature. In addition, there is no need to evaporate organic solvents. Also supercritical CO_2 extraction allows continuous modification of solvent power and selectivity by changing the solvent density [1-3].

Laurus nobilis L., an evergreen tree or shrub, is cultivated in many temperate and warm parts of the world. It grows abundantly in the Mediterranean region and along the coastal line of Turkey [4, 5]. Dried laurel leaves are exported in amounts exceeding 6.000 tons a year [6].

Laurel leaves are mainly used as a condiment and a source of laurel leaf oil. The oil is a light yellow to yellow liquid, having an aromatic and spicy odor [7, 8]. It is an important raw material for the flavor and fragrance industries. For this reason, the extraction of this oil using SFE could have an industrial impact.

The main objective of the present study was to investigate the chemical compositions of supercritical CO_2 extracts and to compare them with products obtained by hydro- and steam distillation.

Laurel leaf oil was obtained by hydrodistillation, steam distillation, and supercritical CO_2 extraction. SFE experiments were conducted at P=80, 100 and 150 bar and T=40 and 50°C using the single-step SFE separation technique, i.e., single-stage depressurization of the supercritical solutions.

The compositions of the oil obtained by hydro- and steam distillation, and supercritical CO_2 extraction at 40°C/80 bar and 50°C/100 bar, are given in Table 1.

Supercritical CO_2 extraction experiments confirmed that at none of the operative conditions was it possible to obtain pure essential oils, since large quantities of cuticular waxes were present in the supercritical CO_2 extract. This experimental evidence has been explained by Reverchon and Senatore [9] as follows: cuticular waxes that are located on the surface of the plant material are more readily extracted than essential oil components which are located at the internal part of the leaf. Therefore cuticular waxes are extracted by simple leaching under all extraction conditions.

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TABLE 1. Composition of Laurel Leaf Oil Obtained by Hydro-, Steam Distillation, and Supercritical CO_2 Extraction*

Compounds	KIP	Distillation, (%)		SFE, (%)	
		Hydro-	Steam	40°/80 bar	50°/80 bar
α-pinene	1032	4.5	3.0	0.6	0.2
α-thujene	1035	0.4	0.3	<0.1	-
camphene	1076	0.2	-	-	-
β-pinene	1118	3.7	<0.1	0.9	0.6
sabinene	1132	5.6	3.0	2.3	1.6
butylbenzene	1138	-	5.2	-	-
myrcene	1174	0.1	0.2	-	<0.1
α-terpinene	1188	0.5	0.3	-	-
dehydro-1,8-cineole	1195	1.0	0.5	0.1	<0.1
limonene	1203	0.8	0.8	0.9	0.5
1,8-cineole	1213	49.7	54.2	43.0	40.2
γ-terpinene	1255	0.9	0.8	0.2	0.2
p-cymene	1280	2.6	3.1	1.8	1.8
terpinolene	1290	0.2	0.1	-	-
trans-sabinene hydrate	1474	0.8	0.8	10.3	1.5
δ-elemene	1479	-	-	-	0.2
α-ylangene	1472	-	-	0.1	0.2
α-campholene aldehyde	1500	-	-	0.1	0.7
linalool	1553	0.1	0.2	0.2	0.1
cis-sabinene hydrate	1556	0.7	0.6	2.9	1.4
trans-p-ment-2-en-1-ol	1571	0.2	0.2	0.2	0.2
pinocarvone	1586	0.9	1.0	0.8	0.8
bornylacetate	1591	0.2	0.2	0.2	0.3
β-elemene	1600	-	-	0.2	0.2
terpinen-4-ol	1611	3.3	2.4	2.3	3.3
2-methyl-6-methylene-3,7-octadien-2-ol	1628	0.2	0.1	0.2	0.2
cis-p-menth-2,8-dien-1-ol	1638	0.6	0.3	0.4	0.4
thuj-3-en-10-al	1641	0.4	0.4	-	0.5
myrtenal	1648	0.8	0.9	0.7	0.8
sabinaketone	1651	0.3	0.2	0.5	0.5
trans-pinocarveol	1671	1.0	1.0	1.0	1.1
δ-terpineol	1683	0.8	0.7	1.4	1.5
cryptone	1687	-	-	0.2	0.2
α-terpineol	1707	2.0	1.4	2.2	2.5
α-terpinylacetate	1709	5.3	7.6	10.8	13.8
trans-p-ment-2-en-1,8-diol	1740	0.2	0.2	-	-
β-selinene	1740	-	-	0.5	0.6
trans-2-hydroxy-1,8-cineole	1742	0.4	-	-	-
carvone	1755	0.3	-	-	-
cis-piperitol	1758	0.1	0.1	0.1	0.1
γ-cadinene	1776	-	-	-	0.2
cis-p-mentha-2-en-1,8-diol	1779	0.2	0.1	0.1	0.1
myrtenol	1779	0.7	0.6	0.8	0.8
cuminaldehyde	1804	0.1	0.2	0.1	0.1
trans-p-mentha-1(7), 8-dien-2-ol	1811	0.5	0.2	0.2	0.2
p-mentha-1,5-dien-7-ol	1815	0.3	0.3	0.4	0.4
trans-carveol	1845	0.3	0.1	0.1	0.1
p-cimen-8-ol	1864	0.1	0.1	0.1	0.1
ascaridol	1889	0.1	<0.1	0.1	0.2
cis-p-mentha-1(7), 8-dien-2-ol	1896	0.5	0.1	0.2	0.2
(Z)-3-hexenyl nonanoate	1949	-	-	0.4	0.2
caryophyllene oxide	2008	0.3	0.3	0.4	0.4
methyleugenol	2029	0.4	0.3	0.7	0.8
p-mentha-1,4-dien-7-ol	2065	0.2	0.1	0.2	0.1
cumin alcohol	2113	0.3	-	3.1	2.4
spathulenol	2114	-	-	0.1	-
hexahydrofarnesyl acetone	2131	-	-	0.7	-
cinnamil acetate	2170	-	0.1	-	-
eugenol	2192	0.3	0.3	0.8	0.7
trans-methyl isoeugenol	2200	0.1	0.1	-	-
carvacrol	2246	0.2	0.2	0.3	0.2
β-eudesmol	2255	0.3	0.2	0.3	0.3
intermedeol	2257	0.1	<0.1	-	-
selina-11-en-5-α-ol	2273	0.1	-	-	-
caryophylla-2(12), 6(13)-dien-5β-ol	2312	-	-	0.1	0.1
caryophylla-2(12), 6(13)-dien-5α-ol	2316	0.1	0.1	0.2	-
caryophylla-2(12), 6-dien-5α-ol	2392	0.1	-	-	-
caryophylla-2(12), 6-dien-5β-ol	2396	0.1	<0.1	-	-
vanilin	2600	-	-	0.2	0.1
phytol	2622	-	-	0.4	0.3
hexadecanoic acid	2931	-	-	0.3	0.3

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* Compounds are listed >0.1%.

TABLE 2. Comparison of the Composition of the Oils and Supercritical CO_2 Extracts

	Distillation		Supercritical CO_2 extracts	
	Hydro-	Steam	40°/80 bar	50°/80 bar
Yield	2.6	1.9	1.3	1.1
Monoterpene hydrocarbons (MT)	19.5	16.5	6.8	5.1
Oxygenated monoterpenes (OMT)	73.6	70.7	75.6	76.4
Sesquiterpene hydrocarbons (ST)	-	-	0.8	1.4
Oxygenated sesquiterpenes (OST)	-	-	1.1	0.8
1,8-cineol	49.7	54.2	43.0	40.2
α-terpinylacetate	5.3	7.6	10.8	13.8

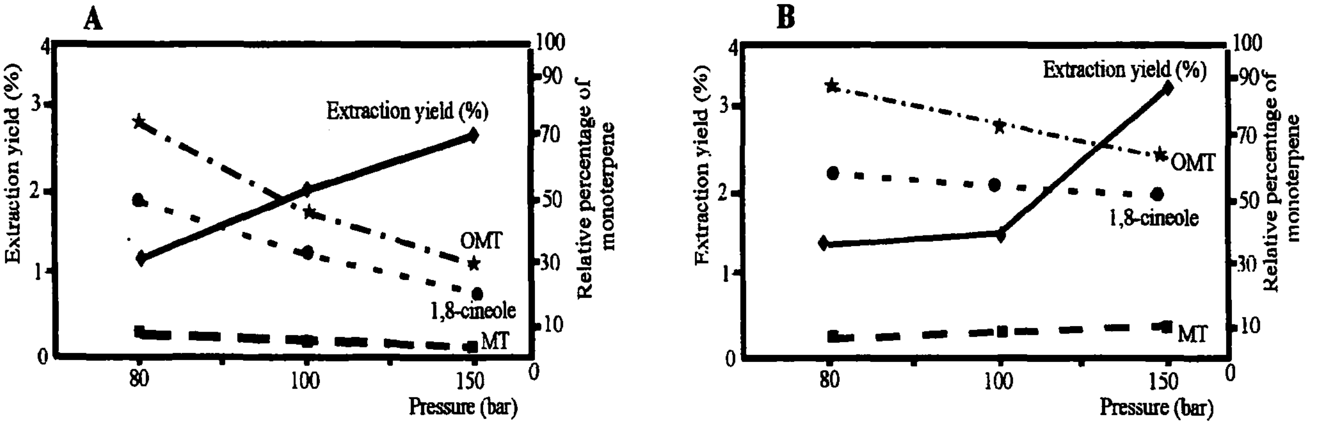

Fig. 1. Extraction yields and composition of the SFE extracts at 40°C (A) and 50°C (B).

Although we know this, we aimed to obtain, if possible, an oil more similar in composition to the oils obtained by distillation techniques in a single-stage extraction procedure in the range of supercritical CO_2 regions. Results showed that the composition of the extracts obtained at 40°C/80 bar (d=0.259 g/L) and 50°C at 80 bar (d=0.221 g/L) were the most similar extracts to the oils obtained by distillation techniques (Table 1). On the other hand, P=80 bar at 40°C and 50°C minimized the co-extraction of unwanted compounds but increased the yield of the volatile compounds.

The oil yields (on a dry weght basis) were 2.6 % for hydro- and 1.9 % for steam distillation. The extraction with supercritical CO_2 yielded 1.34 % and 1.13 % for 40°C/80 bar and 50°C/80 bar, respectively.

The oil obtained by distillation was a mixture of monoterpene hydrocarbons (16.9-19.5 %), oxygenated monoterpenes (73.6-75.9 %), and oxygenated sesquiterpenes (0.8-1.1 %). Monoterpenes are considered to be the main constituents responsible for the aroma of laurel leaf oil, such as 1,8-cineole (49.7-54.2 %), α-pinene (1.4-2.0%), α-terpinylacetate (5.3-7.6%), terpinen-4-ol (2.4-3.3%), α-pinene (3.0-4.5%), β-pinene (0.1-3.7%), sabinene (3.0-5.6%), and *p*-cymene (2.6-3.1%).

The supercritical CO_2 extracts contained mainly oxygenated monoterpene compounds (75.5-76.4%), the main component being 1,8-cineole (40.2-43.0%), α-terpinylacetate (10.8-13.8%), and α-terpineol (2.2-2.5%). A comparison of the composition of two oils and the supercritical CO_2 extracts is summarized in Table 2.

A decrease in the content of monoterpene hydrocarbons was noticeable in supercritical CO_2 extracts (6.8-5.1%) in comparison to the hydro- (19.5%) and steam (16.5%) distilled oils. Thus, the use of supercritical CO_2 extraction present an additional advantage, due to the fact that the extract so obtained is devoid of hydrocarbons which are undesirable components for odor quality.

In the investigated SFE conditions, it was observed that, while extraction yields were increased with the densities of the fluid, the monoterpene hydrocarbon and oxygenated monoterpene contents seemed to decrease due to the enhanced leaching of cuticular waxes and other undesirable compounds. Results are given in Fig.1.

EXPERIMENTAL

GC and GC/MS: GC/MS analysis was carried out using a Hewlett-Packard GCD system. Innowax column (60 m × 0.25 mm i.d. with 0.25 µm film thickness) was used with helium as the carrier gas. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, then kept constant at 220°C for 10 min and then 240°C at a rate of 1°C/min. The injector temperature was at 250°C. The split ratio was adjusted at 50:1. MS were taken at 70 eV in the EI mode. Mass range was from m/z 35 to 425. Library search was carried out using the Wiley GC/MS Library and the TBAM Library of Essential Oil Constituents.

GC analysis was carried out with the Hewlett-Packard GC-5890 system. The same column employed in GC analysis with the same conditions was used, with nitrogen as the carrier gas.

Dried leaves of *Laurus nobilis* were provided by a local supplier. Dried leaves were chopped and crushed before extraction. Liqueified CO_2 used was of 99.99 purity.

Hydrodistillation was performed for 3 h using a Clevenger type distillation apparatus. Oil yield obtained by hydrodistillation was 2.64% on a dry weight basis. Leaves were also subjected to steam distillation in a 30 L capacity stainless steel pilot plant to yield 1.9%.

Supercritical CO_2 extraction was carried out using an ISCO SFX-220 extractor in 10 ml polystyren extraction cells loaded with about 2g sample of laurel leaves. Pure CO_2 was passed into an ISCO Model 100DX syringe pump. Extraction conditions at P=80, 100 and 150 bar and T=40, 50°C were maintained for 20 min under static conditions. Dynamic extraction was taken then carried out at a CO_2 flow of approximately 1 ml/min for 20 min by allowing the fluid to first pass through the cell and then through a fused-silica back-pressure restrictor. Extraction materials was trapped by bubbling the CO_2 effluent in a tube containing 10 ml of n-hexane placed in the dry CO_2 ice bath. After extraction, n-hexane was evaporated under nitrogen at room temperature.

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