

Volatile Constituents and Key Odorants in Leaves, Buds, Flowers, and Fruits of Laurus nobilis L.

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The volatiles of fresh leaves, buds, flowers, and fruits from bay (Laurus nolilis L.) were isolated by solvent extraction and analyzed by capillary gas chromatography-mass spectrometry. Their odor quality was characterized by gas chomatography-olfactometry-mass spectrometry (HRGC-O-MS) and aroma extract dilution analysis (AEDA). In fresh bay leaves 1,8-cineole was the major component, together with α -terpinyl acetate, sabinene, α -pinene, β -pinene, β -elemene, α -terpineol, linalool, and eugenol. Besides 1,8-cineole and the pinenes, the main components in flowers were α -eudesmol, β -elemene, and β -caryophyllene, in fruits (E)- β -ocimene and biclyclogermacrene, and in buds (E)- β -ocimene and germacrene D. The aliphatic ocimenes and farnesenes were absent in leaves. By using HRGC-O-MS 21 odor compounds were identified in fresh leaves. Application of AEDA revealed (Z)-3-hexenal (fresh green), 1,8-cineole (eucalyptus), linalool (flowery), eugenol (clove), (E)-isoeugenol (flowery), and an unidentified compound (black pepper) with the highest flavor dilution factors. Differences between buds, flowers, fruits, and leaves with regard to the identified odor compounds are presented.

KEYWORDS: Laurus nobilis L.; bay; key odorants; volatile compounds; HRGC-olfactometry-MS; AEDA; FD factors

INTRODUCTION

Apart from synthetic formulations in the markets, plants are the main natural source of odor compounds. Bay (Laurus nobilis L.) is an industrial plant used in foods, drugs, and cosmetics. Dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups, and fishes (1, 2). Antimicrobial and insecticide activities are other factors for bay to be used in the food industry as a food preservative (3, 4). The essential oil is also used as a folk medicine, especially for rheumatism and dermatitis. With respect to its dosage, care has to be taken because of its allergic effect (5). Because of their high fatty acid content berries are generally utilized for the production of perfumed soaps and candle manufacture. The soaps are good for acne and have an antidandruff activity (6,7).

The chemical composition and the antimicrobial activity of both leaves and berries of bay were extensively studied by different researchers (8-17). However, to date there is not much work on the odor-contributing compounds of L. nobilis L. responsible for the characteristic odor of this herb.

The aim of this study was to determine the odor-relevant compounds of fresh bay leaves, buds, flowers, and fruits. With regard to the preservation of the original composition, solvent

extraction proved to be the best method for isolation of the volatile components from bay leaves (18). High-resolution gas chromatography-olfactometry-mass spectrometry (HRGC-O-MS) was used to identify the odor-contributing compounds in the extracts. The key odorants of leaves were analyzed by means of aroma extract dilution analysis (AEDA) (19, 20), and their flavor dilution (FD) factors were determined.

MATERIALS AND METHODS

Plant Material. Fresh bay leaves (L. nobilis L.) were collected at the beginning of March through October 2000 on every 15th day of the month from the North Black Sea region in Turkey. The specimens were taken from lower (old shoots) and upper (young shoots) parts of the trees. Bud, flower, and fruit parts were sampled in February, May, and September 2000, respectively.

Sample Preparation. In each experiment 2 g of plant material was used. They were ground in liquid nitrogen and extracted three times with pentane/dichloromethane (PDM 2:1, total volume = 350 mL). Before concentration on a Vigreux column (40 °C), 1 mL of methyl octanoate (internal standard) solution and Na₂SO₄ were added. Extracts not used immediately for GC and GC-MS analyses were stored at -20

HRGC-MS Analyses. For the identification of volatile compounds each sample was analyzed by using a Finnigan-MAT 8200 mass spectrometer directly coupled with an HP 5890 gas chromatograph equipped with an SE-54 (J&W) 30 m \times 0.25 mm i.d. (0.25 μ m film thickness) fused silica capillary column; carrier gas was 1.15 mL of He/min; temperature program was 60 °C (5 min) raised at 2 °C/min to

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Table 1. Chemical Composition of Bay (L. nobilis L.) Leaves Harvested in July

compound	RI, DB5 0.25μ	old leaves (%)	young leaves (%)	ID^a	compound	RI, DB5 0.25μ	old leaves (%)	young leaves (%)	ID^a
toluene	776	0.1	0.1	b	(E)-pinocarvyl acetate	1297	Х	Х	(27)
hexanal/(Z)-3-hexenal	799	0.2	0.3	b	δ -terpinyl acetate	1313	0.4	0.2	(21)
(E)-2-hexenal	848	0.1	0.2	b	2-acetoxy-1,8-cineole	1339	0.1	0.3	b
(<i>Ž</i>)-3-hexenol	852	0.4	0.2	b	α-terpinyl acetate	1348	6.5	4.8	b
hexanol/(E)-2-hexenol	865	Х	0.1	b	eugenol	1354	1.6	0.1	b
tricyclene	923	Х	XX	b	neryl acetate	1365	Х	X	b
α-thujene	928	0.3	0.3	b	α-ylangene	1367	XX	0.2	b
α-pinene	935	3.9	5.0	b	α-copaene	1371	0.1	0.3	b
camphene	949	0.6	1.1	b	iso-βċ-elemene	1379	0.1	0.1	b
sabinene	975	7.6	7.1	b	β -cubebene	1384	Х	0.1	b
β -pinene	977	3.0	3.8	b	β -elemene	1386	1.4	1.8	b
2,3-dehydro-1,8-cineole	989	0.1	Х	b	tetradecene?	1391	Х	Х	С
myrcene	991	0.9	1.4	b	vanillin	1392	0.1	X	b
α-phellandrene	1002	0.2	0.1	b	eugenol methyl ether	1399	1.2	0.2	b
Δ^3 -carene	1008	XX	0.2	b	β -caryophyllene	1409	0.3	0.8	b
α-terpinene	1016	XX	Х	b	α-guaiene	1429	0.1	0.1	b
<i>p</i> -cymene	1025	0.1	X	b	sesquiterpene (MW 204)	1434	0.1	0.2	С
limonene	1029	2.5	2.0	b	sesquiterpene (MW 204)	1439	0.1	0.1	С
1,8-cineole	1032	32.1	24.2	b	(E)-isoeugenol	1444	0.1	0.6	b
γ -terpinene	1059	0.2	0.1	b	α -humulene	1446	XX	XX	b
(E)-sabinene hydrate	1071	0.5	0.3	b	alloaromadendrene	1449	Χ	X	b
terpinolene	1086	Х	0.1	b	sesquiterpene (MW 204)	1466	XX	0.3	С
2-nonanone	1092	X	X	b	germacrene D	1472	0.4	0.6	b
(Z)-sabinene hydrate	1094	0.4	0.1	b	β -selinene	1478	0.1	0.1	b
linalool	1098	0.7	1.5	b	bicyclogermacrene	1486	Х	Х	b
p-1,3,8-menthatriene	1109	X	X	b	germacrene A	1495	0.4	1.2	b
(<i>E</i>)- <i>p</i> -menth-2-en-1-ol	1118	XX	X	b	γ -cadinene	1502	0.1	0.3	b
(E)-pinocarveol	1135	XX	Х	b	7-epi- α -selinene	1506	Χ	Χ	b
sabinol	1141	Х	Х	(27)	δ -cadinene	1511	0.1	0.3	b
sabina ketone	1153	Х	Х	b	cubebol	1520	Х	Х	(27)
pinocarvone	1158	Х	Х	b	homovanillyl alcohol	1526	Х	0.2	С
borneol	1162	0.3	t	b	elemicine	1545	Х	Х	b
δ -terpineol	1164	XX	XX	b	germacrene D-4-ol	1568	0.2	0.2	b
terpinen-4-ol	1175	0.7	0.3	b	spathulenol	1568	Х		b
3-thujen-10-al	1179	Х	Х	(27)	caryophyllene oxide	1571	Х		b
2,6-dimethyl-3,7-octa-	1183	Х	Х	b	humulene epoxid II	1596	Х	Х	b
diene-2,6-diol	4400				(E)-isoelemicine	1644	Х	Х	b
α-terpineol	1188	1.3	1.8	b	eudesmol acetate	1780	X	X	С
(E)-sabinene hydrate acetate	1200	X	0.6	b	sesquiterpene lactone	1788	1.1	0.5	С
2-hydroxy-1,8-cineole	1219	0.1	Х	b	(MW 230)	4040			
nerol	1228	Х	Х	b	neophytadiene	1810	0.3	0.2	С
3-hydroxy-1,8-cineole	1239	0.1	Х	b	6,10,14-pentadecanone	1834	Х	Х	С
linalyl acetate	1255	0.1	Х	b	sesquiterpene lactone	4040	, ,	0.0	
geranial	1267	Х	Х	b	(MW 230)	1849	6.6	8.2	С
octenyl acetate?	1272	X	Х	C (0.7)	phytadiene	1856	X	X	С
4-thujen-2-yl acetate	1273	0.1	Х	(27)	sesquiterpene lactone	1903	1.5	1.7	С
2,6-dimethyl-1,7-octa-	1274	0.1	Х	b	(MW 230)	4044	0.0	0.7	(4.0)
diene-3,6-diol	1001	0.7	1.1	6	spirafoliolide	1944	2.2	3.7	(18)
bornyl acetate	1281	0.6	1.1	b	dehydrocostunolide	1963	X	X 1.2	(18)
p-cymen-7-ol	1290	X	Х	b	sesquiterpene lactone	2265	Х	1.2	С
menthadien-8-ol?	1291	X	X	C	(MW 246)	2700			h
2-undecanone	1292	Х	Х	b	squalene	2790	Х	Х	b

^a Identification remarks: *b*, identification based on comparison of mass spectral and retention data with those of authentic reference compounds; *c*, tentatively identified on the basis of comparison of mass spectral data (x, <0.01%; xx, <0.05%).

260 °C, injector temperature was 250 °C, transfer line temperature was 230 °C, and 1 μ L was injected (split 1:10). Quantitative data were obtained with an HP 5890 GC equipped with the same column under the same GC conditions (FID temperature was 260 °C). Mean standard deviation in triplicate experiments were in the typical range expected for GC analysis (5–10%).

HRGC-O-MS. Sensorial characterization of volatile compounds was done by means of a Siemens SiChromat II gas chromatograph directly coupled through a Live-T effluent splitter (1:1) to a Finnigan-MAT 8222 mass spectrometer and a sniffing port. An SE-54 30 m \times 0.53 mm i.d. (1.5 μ m film thickness) fused silica capillary column was used. The thick-film column was chosen to obtain a better resolution of lowboiling substances and to get a second set of retention indices for identification purpose. The injector temperature was 250 °C, and the sniffing module was 250 °C. The temperature program was 100 °C raised at 5 °C/min to 250 °C, ionization chamber was 200 °C, carrier gas was 3 mL of He/min, and split ratio was 1:5. Sniffing analyses were done between 10:00 and 11:00 a.m. and were stopped after 30 min, because preliminary tests showed that no sensorially active compounds eluted at higher retention times. Because of the 1:1 effluent split at the end of the column, 2 μ L was injected.

Identification of odor compounds detected by HRGC-MS and HRGC-O-MS analyses was done by comparing mass spectra and retention indices (RI) on both GC-MS systems with our own MS/RI library created from commercially available (Merck, Fluka, Sigma-Aldrich Chemie GmbH) reference substances and compounds isolated from natural sources or literature data as indicated in **Table 1**.

RESULTS AND DISCUSSION

The chemical composition of fresh bay leaves is listed in **Table 1**. The percentages given in this table are from the plant material collected in July 2000, when the highest yield of volatile compounds was found. Details of the seasonal variations and their comparison will be published elsewhere.

The volatile compounds in bay leaves mainly consist of mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. Besides phenolic compounds, also sesquiterpene lactones derived from the germacranolide costunolide can be found. As seen from **Table 1**, 1,8-cineole is the major component, ranging between 24.2 and 32.1%, followed by α -pinene (3.9–5.0%), β -pinene (3.0–3.8%), sabinene (7.1–

Table 2. Volatile Compounds of Buds, Flowers, and Fruits of L. nobilis L. (Percent)

compound	RI^a	bud ^b flower ^b fruit ^b	flower ^b	fruit ^b compound	compound	RI^a	bud^b	flower ^b	fruit ^b
tricyclene	923	0.1	Х	XX	eugenol	1354	0.3		Х
α-thujene	928	0.1	0.2	0.1	α-ylangene	1367	0.5	0.9	0.2
α-pinene	935	7.0	5.1	3.3	α-copaene	1371	0.2	0.3	0.1
camphene	949	3.4	2.4	1.7	iso-β-elemene	1379	0.1	0.4	0.1
sabinene	975	2.4	1.7	1.7	β -cubebene	1384			Х
β -pinene	977	4.6	3.7	2.1	β -elemene	1386	2.6	5.4	2.0
myrcene	991	0.7	0.6	0.5	eugenol methyl ether	1399	0.3		0.1
α-phellandrene	1002	Χ	0.1	XX	(<i>E</i>)- β -caryophyllene	1409	0.9	5.1	0.3
Δ^3 -carene	1008		0.4		(E)-isoeugenol	1444	0.3	0.5	0.2
p-cymene	1025			0.1	α-humulene	1446	0.2	0.5	0.1
limonene	1029	Х	Х	Х	alloaromadendrene	1449	0.1		0.1
1,8-cineole	1032	16.8	8.8	9.5	(E)- β -farnesene	1453	0.2	0.1	0.1
(Z) - β -ocimene	1038	0.1	0.3		γ-muurolene	1469			XX
phenylacetaldehyde	1041	0.1			germacrene D	1472	6.6	2.4	1.5
(E) - β -ocimene	1048	8.1	2.7	22.1	β -selinene	1478	0.1	0.3	0.1
(E)-sabinene hydrate	1071	0.1	XX	XX	, bicyclogermacrene	1486	1.2	2.2	4.5
linalool	1098	0.8			α-farnesene	1489	0.8	1.3	0.3
pinocarvone	1158			0.1	germacrene A	1495	0.8	1.1	0.6
borneol	1162	0.7	0.4	0.3	ν-cadinene	1502			0.3
α-terpineol	1188		Х	0.4	δ –cadinene	1511	Х		0.1
linalyi acetate	1255	0.7		0.2	ni (sesquiterpene)	1537	5.5	3.4	0.9
bornyl acetate	1281	2.0	2.1	1.1	elemol	1541	0.4		
2-undecanone	1292			0.1	germacrene D-4-ol	1568	0.7		0.5
δ-terpinyl acetate	1313	0.2	0.3	0.1	α-eudesmol	1644	2.7	11.8	
α-terpinyl acetate	1348	1.6	1.8	1.2	costunolide	2058			2.9

^a RI determined on the 0.25 μ m SE-54 column. ^b x, <0.01%; xx, <0.05%.

Table 3. Odor Compounds of Fresh Leaf, Bud, Flower, and Fruit of $\it L.$ nobilis L.

TIODIIIS E.						
compound	RIª	leaf	bud	flower	fruit	odor description
hexanal/(Z)-3-hexenal	796	+	+	+	+	fresh green
2/3-methylbutyric acid	820	_	+	_	+	oily, rancid
ethyl 2-methylbutanoate	845	+	+	+	_	sweet apple
ni ^b	886	+	+	_	_	roasted nut
ni	899	+	+	_	_	salted fish
methional	910	+	+	+	_	baked potato
α -pinene	952	_	+	_	_	green
1-octen-3-one	983	+	+	+	+	mushroom
octanal	1005	+	_	_	_	fresh, lemon
ni	1045	+	_	_	_	plastic
1,8-cineole	1051	+	+	+	+	eucalyptus
(<i>E</i>)- β -ocimene	1055	_	+	+	_	mushroom
phenylacetaldehyde	1058	_	+	_	_	flowery
ni	1060	_	_	_	+	oily
linalool	1106	+	+	+	+	flowery
(Z)-sabinene hydrate	1125	+	+	_	+	tobacco
borneol	1195	_	_	+	+	earthy
(E,Z)-2,4-nonadienal	1200	+	+	+	+	oily
(E,E)-2,4-nonadienal	1232	+	_	_	_	oily
(E,Z)-2,4-decadienal	1309	_	_	_	+	olive oil
(E,E)-2,4-decadienal	1330	+	+	+	+	olive oil
α-terpinyl acetate	1365	+	_	_	_	fresh flowery
eugenol	1378	+	+	+	+	clove, flowery
ni	1392	+	+	+	+	fresh bean
vanillin	1430	+	+	+	+	vanilla
(E)-isoeugenol	1474	+	+	+	+	flowery
ni	1500	+	+	+	_	wet paper
ni	1634	+	+	_	_	pepper
ni	1731	+	+	+	+	pepper
ni	1743	+	+	+	_	pepper

 $[^]a\,\text{RI}$ determined on the 1.5 μm SE-54 column. $^b\,\text{Not}$ identified.

7.6%), α -terpinyl acetate (4.8–6.5%), α -terpineol (1.3–1.8%), linalool (trace–1.5%), eugenol (1.6–0.1%), and β -elemene (1.4–1.8%). The amount of δ -terpinyl acetate, identified as a new natural component in bay oil by Brauen et al. (21), was determined to be 0.2–0.4%.

With regard to the amount of volatiles, there is a significant difference between leaves from young and old shoots of the tree. Whereas higher amounts of α -pinene, β -pinene, linalool, α -terpineol, 2-hydroxy-1,8-cineole, and some sesquiterpenes can be found in the young shoot leaves, 1,8-cineole, sabinene, sabinene hydrates, terpinene-4-ol, α -terpinyl acetate, eugenol,

Table 4. AEDA Results of Fresh Bay Leaves

			FD factor		
no.	substance	odor description	lower part, Oct	upper part, July	
1	(Z)-3-hexenal	fresh green	1024	64	
2	ethyl 2-methylbutanoate	sweet apple	4		
3	ni (RI 886)	roasted nut	4		
4	ni (RI 899)	salted fish	8		
5	methional	baked potato	1	1	
6	1-octen-3-one	mushroom	2	4	
7	ni (RI 1045)	plastic	128	64	
8	1,8-cineole	eucalyptus	512	128	
9	linalool	flowery	512	64	
10	(Z)-sabinene hydrate	tobacco	4		
11	(É,Z)-2,4-nonadienal	oily	4	1	
12	(E,E)-2,4-decadienal	olive oil	32	8	
13	α-terpinyl acetate	fresh flowery	1	4	
14	eugenol	flowery, clove	1024	128	
15	ni (RI 1392)	oily, fresh bean	4	32	
16	vanillin	vanilla	32	0	
17	(E)-isoeugenol	flowery	256	1024	
18	ni (RI 1500)	wet paper	2	4	
19	ni (RI 1634)	pepper	16		
20	ni (RI 1731)	pepper	32		
21	ni (RI 1743)	pepper	2048	64	

and eugenol methyl ether were found to be in higher amounts in the old shoot leaves.

The volatile content of fresh leaves in the younger shoots of the tree showed to be be highest in July and August (21.4 and 23.3 mg/g, respectively), mainly due to the presence of only weak or non-odor-contributing monoterpene hydrocarbons. The corresponding amount of volatiles in the older leaves was less and amounted to 14.3 and 19.7 mg/g, respectively. The monthly content of essential oil of *L. nobilis* leaves decreased steadily after August, amounting in October to ~50% of the value determined in August. Similar observations were reported by Yoshida (22), who studied the behavior of essential oil content in bay leaves and claimed that their essential oil reached a maximum in late July and constantly decreased thereafter.

Volatiles determined in bud, flower, and fruit parts are listed in **Table 2**. (*E*)- β -Ocimene, (*Z*)- β -ocimene, (*E*)- β -farnesene, α -farnesene, and α -eudesmol are not present in leaves (see **Table 1**). Similar results were obtained by Fiorrini et al. from the essential oil of French bay (9).

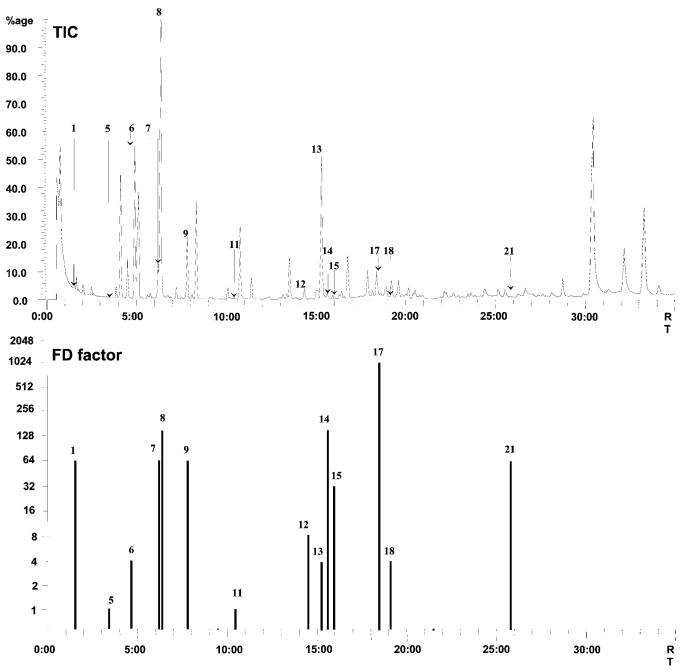


Figure 1. Total ion chromatogram (TIC) and FD factors of bay leaves from young shoots (harvested in July). Numbering corresponds to Table 4.

1,8-Cineole, (E)- β -ocimene, some monoterpene hydrocarbons (such as α -pinene, camphene, sabinene, and β -pinene), α -terpinyl acetate, bornyl acetate, and some sesquiterpenes (germacrene D and bicyclogermacrene) are the major compounds in these parts. The content of other sesquiterpene compounds such as β -elemene, β -caryophyllene, and α -eudesmol is highest in flowers. During maturation the main compound changes from 1,8-cineole (16.8%) in buds to (E)- β -ocimene (22.1%) in fruits.

Sensorial characterization of volatiles mentioned above was done by using HRGC-O-MS. Odor compounds identified in leaves, buds, flowers, and fruits are listed with their odor descriptions in **Table 3**. Some of them could not be identified because of their low concentration.

Oxidation of fatty acids in leaves, buds, and fruits could be an explanation for the occurrence of nonadienals, decadienals, hexenal, and 1-octen-3-one. Strecker aldehydes such as methional and phenylacetaldehyde can be regarded as artifacts. Three substances with a characteristic pepper odor could not be identified due to their low concentration. Although these substances were registered during sniffing analyses with a remarkable odor intensity, definite mass spectral data for identification purposes could not be obtained due to coeluting odorless oxygen-containing sesquiterpene compounds. The same peppery odorants have also been detected in flavor extracts of pepper and cloves as part of the key odorants (23–25).

AEDA results of the extracts from fresh leaves from young (harvested in July) and old shoots (harvested in October) are listed in **Table 4**. A comparison of the total ion chromatograms (TIC) obtained by GC-MS and the corresponding FD factors of the identified odor-contributing compounds are summarized in **Figures 1** and **2**, respectively.

On the basis of their high FD factors (>128), (Z)-3-hexenal (fresh green), 1,8-cineole (eucalyptus), linalool (flowery), eugenol (clove), (E)-isoeugenol (flowery), and an unidentified

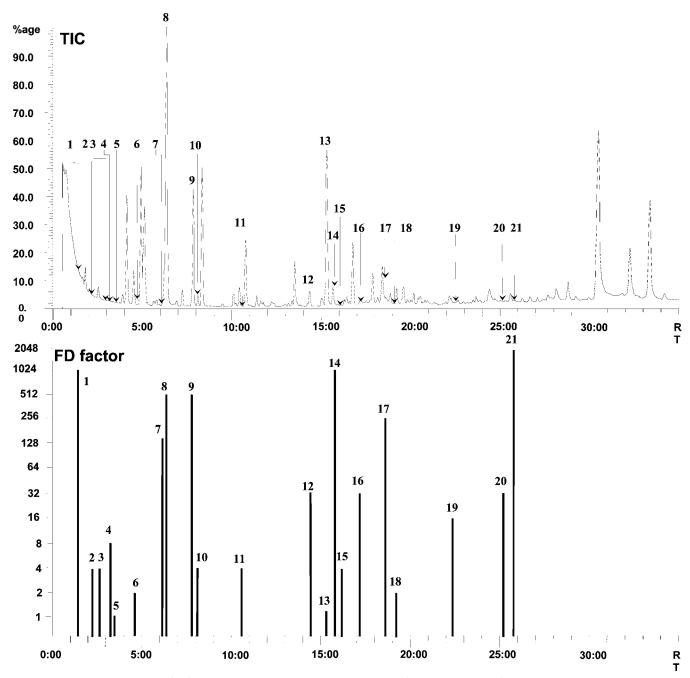


Figure 2. Total ion chromatogram (TIC) and FD factors of bay leaves from old shoots (harvested in October). Numbering corresponds to Table 4.

compound (black pepper) showed to be the most odor-active among the 21 aroma compounds characterized in fresh bay leaves. With regard to the main components only 1,8-cineole and linalool contribute essentially to the odor quality of the fresh leaves. This is in accordance with the findings of Buttery et al. (26), who stated that 1,8-cineole is the major aroma component for Mediterranean bay oil, followed by linalool. In addition, substances present in lower concentration such as eugenol and (E)-isoeugenol, and especially the nonidentified compounds at trace level possessing a pepper-like odor, have to be considered as key aroma compounds with a marked influence on the overall odor and flavoring quality of the leaves. Research work is actually in progress to identify the latter compounds, who seem to have a remarkably low odor threshold. The results obtained with the AEDA (see Figures 1 and 2) also show that the FD values of the majority of the odor-contributing substances from the old leaves harvested in October are higher than those of the fresh leaves in July, especially those for (Z)-3-hexenal, linalool, eugenol, and one of the compounds with a pepper-like odor.

As mentioned before, the high yield of essential oil in July is mainly due to nearly odorless monoterpene hydrocarbons, and their concentration decreases steadily from July/August to October. In contrast, the concentration of the identified odorcontributing compounds increases in autumn (e.g., linalool, 2-4-fold; eugenol, 4-10-fold). As a consequence of this finding it could be assumed that despite their lower essential oil content, the leaves harvested in October might have a better flavoring quality. Additional experiments will be necessary to confirm this assumption. (*E*)-Isoeugenol was shown to have a higher FD factor in the AEDA analysis of the young leaves in July, and quantitative analyses revealed that these leaves contain up to 6-8-fold amounts of this substance when compared to old leaves.

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