

Screening for Key Odorants in Moroccan Green Olives by Gas Chromatography–Olfactometry/Aroma Extract Dilution Analysis

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“Spanish style” Moroccan green table olives were screened for potent odorants by gas chromatography–olfactometry/aroma extraction dilution analysis of a representative Likens–Nickerson extract. (*Z*)-3-Hexenal [flavor dilution factor (FD) = 256], (*E,E*)-2,4-decadienal (FD = 128), and (*E,Z*)-2,4-decadienal (FD = 64) were revealed to confer green and coriander/paraffin oil odors to both fruit and oil extracts, whereas guaiacol (FD = 128) imparted a bad olive, phenolic note. Methional (3-methylthiopropionaldehyde, FD = 128) and several terpenes (FD ≤ 64) such as α -farnesene, *trans*-nerolidol, nerol acetate, limonene, α -, β -, and γ -terpineol, linalool, and β -myrcene were detected in the fruit extract, although they were not reported as olive oil constituents.

KEYWORDS: Green olive; flavor; aroma extract dilution analysis, AEDA; aldehydes

INTRODUCTION

Olive tree cultures (*Olea europaea*) have a great socio-economic impact in Morocco, where they account for up to 50% of the tree-covered area (1). Among table olives, which are produced from fruits harvested at various ripening stages and subjected to diverse types of processing, “Spanish-style” green olives are usually recognized as the most famous on international markets. Because of the bitterness of oleuropein (a heterosidic ester of elenolic acid and 3,4-dihydroxyphenylethanol), raw olives require processing to make them suitable for human consumption. After oleuropein hydrolyzation with sodium hydroxide solutions (lyes) and washing, the fruits are stored in a sodium chloride brine, where lactic fermentation takes place (2). Fruit ripeness, fruit size, and lye concentration greatly influence the texture and flavor of the final product (3–6).

Although table olive texture has been extensively studied (3–5), data on the major odorants present in Spanish-style green olives are scarce. Kubo et al. (7) have identified linear aldehydes (e.g., hexanal, nonanal, 2-decenal) and terpenes (β -farnesene, copaene) in hexane extracts of fresh green olives. After supercritical fluid extraction, Morales et al. (8) found various aldehydes [3-methylbutanal, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, octanal, 2-undecenal], ketones (3-methyl-2-butanone, 6-methyl-5-hepten-2-one), alcohols [1-pentanol, (*E*)-3-hexen-1-ol, 1-heptanol, 1,3-butanediol, phenylethyl alcohol], and esters [hexyl acetate, (*Z*)-3-hexenyl acetate, ethyl 3-hydroxybutanoate].

Most of these compounds have been also identified in other olive-linked products such as fresh leaves (9) and olive callus cultures (10). In the latter case, the authors measured hexanal levels 6-fold greater in their Spanish Picual cultures than in the other two cultivars studied (Koronoeiki and Coratina). Other aldehydes and alcohols were also more concentrated in Picual.

As far as odorants are concerned, virgin olive oil has been much more investigated (11–14). Reiners and Grosch (14) identified 4-methoxy-2-methyl-2-butanethiol as a key flavor of Spanish virgin olive oils (FD = 128), although this compound was undetected in olive oils of Moroccan and Italian origins. With an odor/flavor threshold of ~17–24 ppt (14), this mercaptan can impart a strong black currant-like odor to olive oils, where its concentration can reach 4.3 ppb (15). Using the aroma extract dilution analysis (AEDA) method, the same authors identified various other olive-oil odorants, sometimes very different according to the geographical origin. In Moroccan products, fruity odorants such as ethyl 2-methylbutyrate, ethyl cyclohexylcarboxylate (FD = 4096), ethyl isobutyrate (FD = 2048), ethyl 3-methylbutyrate, and β -damascenone (FD = 1024) emerged with the highest FD values. Also worth stressing is the presence in the aromagram of several aldehydes [FD = 128, (*Z*)-3-hexenal and (*E*)-4,5 epoxy-(*E*)-2-decenal; FD = 64, (*Z*)-2-nonenal and (*E,E*)-2,4-decadienal; FD = 16, hexanal, (*Z*)-2-nonenal, (*E,E*)-2,4-nonadienal, and (*E,Z*)-2,4-decadienal; FD = 8, octanal, 3-methylbutanal, and (*Z*)-3-nonenal] and also guaiacol and 1-octen-3-one (FD = 128 and 64, respectively).

In European virgin olive oils, C6 aldehydes [e.g., hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal] as well as C6 alcohols [e.g., hexanol, (*E*)-2-hexenol, (*Z*)-3-hexenol] contribute to the typical green sensory perception. Those European oils differ from the

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Australian ones, in which (*E*)-2-hexenal is very often a minor compound (16–20). Produced via the lipoxygenase (LOX) pathway from polyunsaturated fatty acids (α -linolenic and linoleic acids), hexanal and (*E*)-2-hexenal accumulate in virgin olive oils during physical extraction procedures (21). The latter is issued from (*Z*)-3-hexenal, which undergoes isomerization to a more stable compound that can then be further reduced to (*E*)-2-hexen-1-ol. Aparicio et al. (22) have proposed using flavor composition to authenticate European virgin olive oils. Factors that significantly influence oil aromatic quality include olive cultivar, climate, soil type, fruit ripeness, technological processes, and equipment (23–31).

The aim of the present work was to determine the flavor determinants of Spanish style Moroccan fruit (Picual cultivar) resulting from a long fermentation process. Comparison with Moroccan virgin olive oils (roughly defined as “juice of fresh olives”) will be attempted, keeping, however, in mind that the fruit variety, the ripening stage, the harvesting practice, the storage time, and the fermentation process strongly influence aroma composition.

MATERIALS AND METHODS

Chemicals. Dichloromethane (>99.8%) was purchased from Lab-Scan (Dublin, Ireland) and redistilled twice prior to use. The water used was ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA). *n*-Heptanol (98%), heptanal (98%), (*Z*)-2-heptenal (95%), (*Z*)-3-hexenal (50%), *n*-octanal (99%), nonanal (97%), (*Z*)-3-hexen-1-ol (98%), (*E*)-nerolidol (90%), and β -caryophyllene (<80%) were obtained from Aldrich (Bornem, Belgium); 1-hepten-3-ol (99%), 1-octen-3-ol (99%), and 1-chloroheptane were from Acros Chimica (Geel, Belgium). 9-Hexadecanoic acid (99%), *n*-octane (99%), benzaldehyde (99%), phenylacetaldehyde (95%), α - and β -terpineol (97%), linalool (98%), and 2,4-decadienal (99%) were purchased from Aldrich-Chemie (Steinheim, Germany). *n*-Hexanal (98%), phenylethyl acetate (99%), (*E*)-2-nonenal (99%), and (*E*)-2-decenal (99%) were from Fluka Chemika (Buchs, Switzerland). 3-Methylbutanal (98%), 2-methylbutanal (98%), 2-methylbutanol (98%), 3-methylbutanol (98%), and methional (98%) were purchased from Janssen Chimica (Geel, Belgium) and toluene (99%) and 1-hexanol (97%) from UCB Chemical (Leuven, Belgium). Ethylphenyl alcohol (99.5%) was purchased from Gesundheitsschädlich beim Verschlucken. α -Farnesene was obtained from Bedoukian (Research Inc.), and 4-ethylphenol was purchased from TCI Europe nv (Zwijndrecht, Belgium).

Olive Fruit Samples. Three samples of green Spanish style olive fruits (*O. europaea*, Picual cultivar) from Morocco were supplied by SICOPA (Fes, Morocco). The Spanish style procedure consists of treatment for 6–7 h with 2% NaOH lye, aqueous washing for 12–14 h, and finally lactic fermentation in an 8% sodium chloride solution (pH 4.5) for 3 months or more.

Extraction of Volatiles. Ten grams of destoned and cut olive fruits was mixed with 50 mL of water. Volatiles were further extracted according to the Likens–Nickerson procedure previously optimized by Bousseta and Collin (32). The method allows the extraction of most volatiles with recovery factors >70% and variation coefficients <8%. After the addition of 10 μ L of a 2078 mg/L chloroheptane solution as external standard (EST) in the organic phase, the dichloromethane extract was concentrated until 0.5 mL at 46 °C with a Kuderna–Danish concentrator (final EST concentration = 41.5 mg/L).

Gas Chromatography Coupled with Dual Flame Ionization and Olfactometric Detection (GC-FID-O). GC was performed using a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 225 °C and opened after 0.50 min. Compounds were separated using a 50 m \times 0.32 mm wall-coated open tubular (WCOT) apolar CP-SIL-5CB capillary column (1.2 μ m film thickness) connected to a flame ionization detector. The oven temperature, maintained at 36 °C for 2 min, was programmed to rise to 85 °C at 20 °C/min, then to 145 °C at 1 °C and to 250 °C at 3 °C/min, and finally kept at 250 °C for 30 min. A fused silica T-junction was

Table 1. First Descriptor Given by 21 Assessors for the Likens–Nickerson Extract of Green Table Olive Fruits

descriptor	no. of assessors
green olive	7
green	6
fruity	3
olive oil	3
olive with tomato	2

connected at the end of the capillary column. Fifty percent of the eluent was sent to the FID maintained at 250 °C and connected to the Spectra Physic Chromjet DP-700 integrator while the other part was directed to the GC odor port crossed by a strong flow of humidified air (20 mL/min to be sure not to accumulate odors in the funnel). Two microliters of the olive extract was injected. A 25 m \times 0.32 mm WCOT polar FFAP-CB capillary column (0.3 μ m film thickness) has been used to check the organoleptic impact of (*Z*)-3-hexenal that coelutes with hexanal on CP-SIL-5CB.

Gas Chromatography–Mass Spectrometry Analysis. Volatiles were identified using a ThermoFinnigan Trace MS mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph. The column and the analysis conditions were the same as those described above. Electron impact mass spectra were recorded at 70 eV (*m/z* 40–380) and compared to the NIST library for identification attempt.

Aroma Extract Dilution Analysis. The flavor dilution factors [FD = 2^{n-1} , where *n* is the number of dilution (factor 2) until no odor was perceived] of the odorants were determined at the sniffing port according to the AEDA method (33). All odor qualities were defined by three assessors, whereas stepwise dilution analysis (dilution factor 2) was performed only one time.

RESULTS AND DISCUSSION

Spanish style Moroccan green table olives were screened for potent odorants by GC–O/AEDA of a Likens–Nickerson extract.

The extraction procedure, previously optimized to obtain very representative extracts of unifloral honeys, again yielded very nice samples, as suggested by the list of descriptors given by 21 assessors (see **Table 1**). Three different samples from the same supplier were fully extracted. The three extracts showed indistinguishable overall sensory quality, FID chromatograms, and odors perceived at the sniffing port. Therefore, only one was kept for the complete AEDA.

Table 2 lists the 73 compounds identified in this extract on the basis of their mass spectrum (consultation of the NIST database), their retention index, and their odor (injection of pure compounds). To provide a first idea of the concentrations found in olives, the “area_{aroma}/area_{EST}” ratio was calculated (the EST concentration was close to 2 mg/L when a 100% recovery factor was applied).

The chromatogram and the aromagram of the extract are depicted in **Figures 1** and **2**, respectively. Forty potentially identified odors and 14 unknowns with FD values <64 were perceived at the sniffing port when a 20-times-concentrated extract (10 g transferred into 0.5 mL of dichloromethane) was injected.

Aldehydes [(*Z*)-3-hexenal, FD = 256; methional and (*E,E*)-2,4-decadienal, FD = 128; (*E,Z*)-2,4-decadienal and (*E*)-2-decenal, FD = 64] emerged as the most important family. Injection of our extract on an FFAP-CB column allowed us to exclude the sensorial impact of hexanal, which coelutes with (*Z*)-3-hexenal on the CP-SIL-5CB column (although quantitatively the major compound, as proved by GC-MS).

Although undetected in oil, methional (3-methylthiopropionaldehyde) proved to be, with guaiacol, the strongest odor (FD

Table 2. Volatile Compounds Identified in Green Table Olive Fruits

compound	PN ^a	RI ^b	identification ^c	FD ^d	area/area _{EST} ^e	odor (GCO)
3-methylbutanal	19	650	MS, RI, GCO	2	0.031	almond
2-methylbutanal	20	661	MS, RI, GCO	2	0.016	hazelnut
ethyl propanoate	23	703	MS		0.221	
2-methylpentane	24	707	MS		0.091	
3-methylbutanol	25	715	MS, RI, GCO	2	0.003	almond
2-methylbutanol	25'	718	MS, RI, GCO	2		varnish
2-methylpropanoic acid	26	731	MS		0.020	
pentanol	27	740	MS, RI, GCO		0.006	
toluene	28	746	MS, RI, GCO	4	0.028	gaz
(Z)-3-hexenal (hexanal) ^f	29	764	MS, RI, GCO	256	0.095	strong green
dimethyl sulfoxide	31	780	MS, RI, GCO	2	0.006	unpleasant
1-octene	32	788	MS	4	0.225	floral
2-hydroxyethyl propanoate	33	793	MS		0.030	
octane	34	800	MS, RI, GCO	2	0.003	unpleasant
furfural	35	803	MS		0.006	
3-methylbutanoic acid	36	807	MS, RI, GCO	64	0.005	cheese, sweat
(Z)-3-hexen-1-ol	39	836	MS, RI, GCO	8	0.068	vanilla, green
1-hexanol	41	850	MS, RI, GCO	8	0.022	cutted grass
1-hepten-3-ol	42	857	RI, GCO	8	0.012	earthy
methional	43	870	MS, RI, GCO	128	0.163	boiled potato
styrene	44	895	MS	4	0.006	melted plastic
1-butoxy-2-propanol	47	923	MS	2	0.123	unpleasant
(Z)-2-heptenal	48	927	MS, RI, GCO	8	0.009	solvent
benzaldehyde	49	930	MS, RI, GCO	8	0.018	almond
α -pinene	51	941	MS, RI, GCO	2	0.089	plant, lemon
1-heptanol	52	947	MS, RI, GCO	64	0.010	unpleasant, sewer
1-octen-3-one	53	953	MS, RI, GCO	64	0.050	mushroom
6-methyl-5-hepten-2-one	55	960	MS		0.031	
β -pinene	56	973	MS, RI, GCO	4	0.135	fresh green
octanal	57	975	MS, RI, GCO		0.039	green, citrus
β -myrcene	58	984	MS, RI, GCO	8	0.026	floral, pungent
hexyl acetate	59	992	MS, RI, GCO	2	0.027	pungent
decane	60	1000	GC, MS		0.032	
benzylalcohol	61	1003	MS, RI, GCO	8	0.022	grass
phenylacetaldehyde	62	1007	MS, RI, GCO	32	0.132	floral, hyacinth
<i>m</i> -cymene	63	1010	MS, RI, GCO	2	0.242	unpleasant, hazelnut
limonene	64	1020	MS, RI, GCO	32	2.265	greenery, fruity
2-methylundecanethiol	65	1026	MS		0.062	
3,7-dimethyl-1,3,7-octatriene	66	1031	MS		0.078	
γ -terpinene	70	1056	MS	2	0.267	pungent
guaiacol (2-methoxyphenol)	71	1064	MS, RI, GCO	128	0.006	bad olive, phenolic
9-hexadecanoic acid	72	1065	MS	16	0.027	tangerine, fruity
α -dimethyl- <i>p</i> -styrene	73	1070	MS	32	0.023	very spicy
nonanal	74	1076	MS, RI, GCO	8	0.732	pungent, greenery
β -linalool	75	1080	MS, RI, GCO	8	0.016	coriander
phenylethylalcohol	76	1093	MS, RI, GCO	8	0.274	floral, fruity
γ -terpineol	77	1100	MS, RI, GCO	32	0.287	floral, lemon
(Z)-2-nonenal	81	1130	MS, RI, GCO	8	0.089	cucumber
4-ethylphenol	82	1136	MS, RI, GCO	2	0.028	old
β -terpineol	83	1143	MS	8	0.042	ferment
1-nonanol	85	1156	MS		0.06	
4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	86	1161	MS		0.894	
α -terpineol	87	1170	MS, RI, GCO	8	1.397	ferment
tridecene	88	1178	MS	8	0.079	peanut
α -lormene	91	1199	MS		2.473	
bornene	92	1205	MS		0.179	
unknown 1 ^g	93	1215	MS	64	0.081	grass
(E)-2-decenal	94	1229	MS, RI, GCO	64	0.072	coriander, grass
phenylethyl acetate	95	1233	RI, GCO	64	0.422	floral
nonanoic acid	96	1241	MS	16	0.011	vanilla, coriander
1,3-bis(1,1-dimethylethyl)benzene	97	1245	MS		0.153	
2-decen-1-ol	98	1250	MS		0.058	
1-decanol	99	1261	MS		0.082	
(E,Z)-2,4-decadienal	101	1272	MS	64	0.045	oil of paraffin
(E,E)-2,4-decadienal	103	1286	MS, RI, GCO	128	0.047	coriander, oil of paraffin
2-methyl-1-hexadecanol	107	1313	MS	2	0.023	medical
2,6,10-trimethyltetradecane	111	1339	MS	8	0.101	henne
nerol acetate	113	1352	MS	64	0.034	coriander
unknown 2 ^g	115	1364	MS		0.037	
copaene	118	1383	MS		0.021	
β -caryophyllene	122	1423	MS, RI, GCO	8	0.040	frying
α -farnesene	129	1489	MS, RI, GCO	64	0.665	soft cooking of vegetable
(E)-nerolidol	135	1551	MS, RI, GCO	32	0.085	oil of car

^a Peak number (see order of elution in Figure 1). ^b Retention index on CP-SIL5-CB. ^c Compound tentatively identified by GC-MS and consultation of the NIST database (MS); GC-MS identification confirmed by comparison of the retention index of the commercial product suspected and the aroma present in the extract (RI); GC-MS identification confirmed by comparison of the odor of the commercial product suspected and the aroma present in the extract (GCO). ^d Dilution factor = 2^{n-1} , where n is the number of dilution (factor 2) until no odor was perceived. ^e Estimated concentration was 41.5 mg/L in the final 0.5 mL volume; factor concentration of 50 from olive to dichloromethane extract. ^f Sensorial data have been determined on an FFAP-CB column because hexanal and (Z)-3-hexenal coelute on a CP-SIL-5CB column. Although GC-MS analysis revealed that hexanal is quantitatively a major compound, the persistent green odor is well due to the presence of (Z)-3-hexenal. ^g 69 (100), 41 (98), 68 (20), 93 (15), 67 (15), and 69 (100), 41 (75), 43 (75), 68 (40), 93 (25) are, respectively, the five major MS fragments [m/z (relative percentage)] of unknowns 1 and 2.

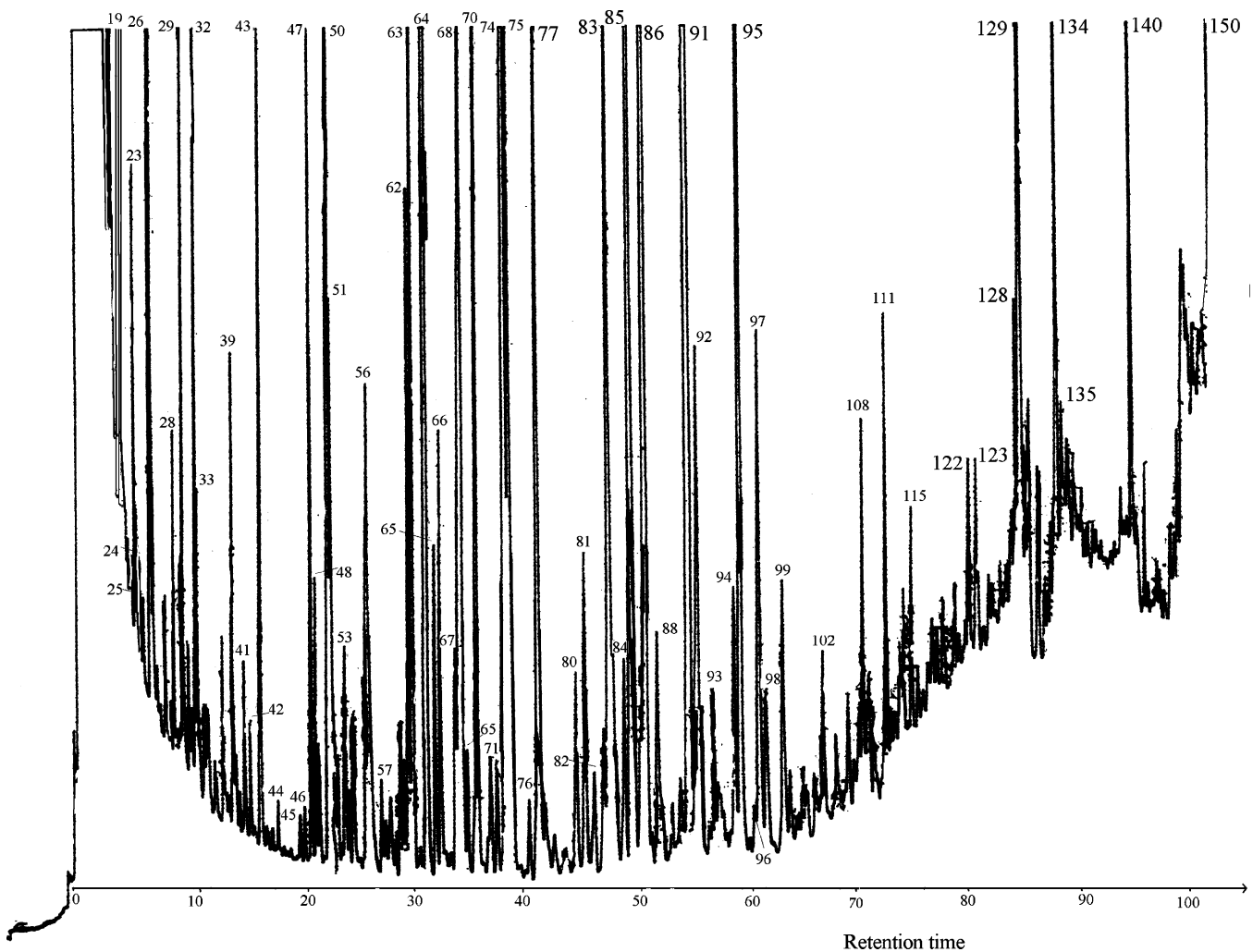


Figure 1. Typical gas chromatogram of an extract from green table olives.

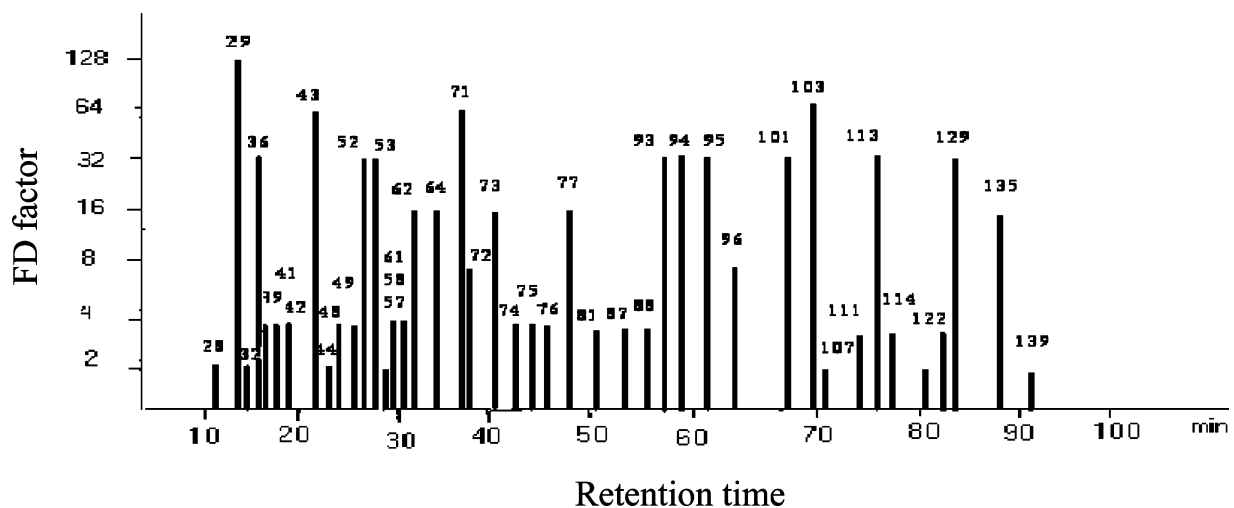


Figure 2. Aromagram of the extract from green table olives.

= 128) in the extract after (*Z*)-3-hexenal. Issued from methionine degradation, methional is known to be very sensitive to heat and light, easily generating other strong flavors such as methanethiol and polysulfides.

(*E,E*)-2,4-Decadienal and, to a lesser extent, (*E,Z*)-2,4-decadienal confer strong coriander/paraffin oil odors to both olive fruits and olive oils.

Ethyl isobutyrate, ethyl 2-methylbutyrate, and ethyl cyclohexylcarboxylate, previously described as the main contributors

to the flavor of virgin olive oil samples extracted from over-ripe fruits, were revealed to have no sensorial impact in our Moroccan Spanish-style green olive sample (see **Table 3**).

On the other hand, terpenes such as α -farnesene, nerol acetate, (*E*)-nerolidol, limonene, γ -terpineol (FD = 32), α - and β -terpineol, linalool, β -myrcene (FD = 8), and the unidentified peak 93 (probably a terpene, FD = 64) were detected only in olive fruits. Note that α -farnesene cannot be mistaken for its isomer β -farnesene, previously found in olive by Kubo et al. (7),

Table 3. Comparison between the Odorants Present in Green Olives and Those Previously Found in Olive Oils

compound	PN ^a	RI ^b	FD ^c in olive extract	FD ^d olive oil extract (olives from Italy, Spain, or Morocco)
3-methylbutanal	19	650	2	(<8, 8, 8)
2-methylbutanal	20	661	2	(<8, 8, 8)
1-penten-3-one			nd ^e	(8, <8, 8)
ethyl isobutyrate			nd	(16, 32, 2048)
methyl 2-methylbutyrate			nd	(nd, <8, 32)
acetic acid		750	nd	(16, 16, 16)
hexanal	29	764	nd	(8, <8, 16)
(Z)-3-hexenal	29	764	256	(16, <8, 128)
ethyl butyrate			nd	(nd, 16, 128)
3-methylbutanoic acid	36	807	64	(16, 16, <8)
2-methylbutanoic acid			nd	(16, 16, <8)
(E)-2-hexenal			nd	(32, nd, nd)
ethyl 2-methylbutyrate			nd	(32, 128, 4096)
ethyl 3-methylbutyrate			nd	(nd, 16, 1024)
(Z)-3-hexenol			nd	(<8, <8, 16)
methional	43	870	128	(nd, nd, nd)
4-methoxy-2-methyl-2-butanethiol			nd	(nd, 128, nd)
1-heptanol	52	947	64	(nd, nd, nd)
1-octen-3-one	53	953	64	(32, 8, 64)
(Z)-1,5-octadien-3-one			nd	(32, nd, 8)
octanal	57	975	4	(32, 8, 8)
(Z)-3-hexenyl acetate			nd	(16, <8, nd)
phenylacetaldehyde	62	1007	32	(nd, nd, nd)
limonene	64	1020	32	(nd, nd, nd)
methyl cyclohexyl-carboxylate			nd	(nd, nd, 32)
guaiaicol (2-methoxyphenol)	71	1064	128	(64, 32, 128)
(Z)-3-nonenal			nd	(8, <8, 8)
9-hexadecanoic acid	72	1065	16	(nd, nd, nd)
α -dimethyl- p -styrene	73	1070	32	(nd, nd, nd)
nonanal	74	1076	8	(<8, <8, 8)
ethylcyclohexylcarboxylate			nd	(8, <8, 4096)
γ -terpineol	77	1100	32	(nd, nd, nd)
(Z)-2-nonenal	81	1130	8	(32, 16, 64)
(E)-2-nonenal			nd	(16, 8, 16)
α -terpineol	87	1170	8	(nd, nd, nd)
2-isobutyl-3-methoxy-pyrazine			nd	(8, 8, 8)
ethyl octanoate			nd	(nd, <8, 16)
(E,E)-2,4-nonadienal			nd	(8, nd, 16)
unknown 1	93	1215	64	(nd, nd, nd)
2-decenal	94	1229	64	(nd, nd, nd)
phenylethyl acetate	95	1233	64	(nd, nd, nd)
nonanoic acid	96	1241	16	(nd, nd, nd)
4-ethylguaiaicol			nd	(<8, <8, 16)
(E,Z)-2,4-decadienal	101	1272	64	(<8, <8, 16)
(E,E)-2,4-decadienal	103	1286	128	(16, 8, 64)
nerol acetate	113	1352	64	(nd, nd, nd)
(E)-4,5-epoxy-(E)-2-decenal			nd	(16, 16, 128)
(E)- β -damascenone			nd	(8, 8, 1024)
α -farnesene	129	1489	64	(nd, nd, nd)
(E)-nerolidol	135	1551	32	(nd, nd, nd)

^a Peak numbering (see order of elution in Figure 1). ^b Retention index on CP-Sil5-CB. ^c Dilution factor = 2^{n-1} , where n is the number of dilution (factor 2) until no odor was perceived. ^d Data obtained in 1998 by Reiners and Grosch (14)—solvent extraction and high vacuum. ^e Not detected.

because both its retention index and its mass spectrum are significantly different (RI β -farnesene = 1458 vs 1498, a much higher m/z 69 peak in the mass spectrum). Quantitatively, limonene and α -lornene were prominent in the FID and MS chromatograms (see peaks 64 and 91 in Figure 1), despite a low flavor impact (FD = 32 and undetected, respectively), but according to recent data obtained on samples from another Moroccan geographical region (data not shown), the limonene peak intensity may be strongly sample-dependent.

Phenylalanine degradation leads to the occurrence in olive fruits of some additional floral/fruity odorants: phenylacetaldehyde (FD = 32), 2-phenylethyl alcohol (FD = 8), and phenylethyl acetate (FD = 64).

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