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Characterization of Odor-Active Compounds in Extracts Obtained by Simultaneous Extraction/Distillation from Moroccan Black Olives

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"Greek-style" Moroccan black table olives were screened for potent odorants by GC/olfactometry/aroma extract dilution analysis of representative Likens−Nickerson extracts and compared with "Spanish-style" green fruits. (*Z*)-3-Hexenal, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,4-decadienal, guaiacol, and methional were found in both green and black olives, but with significant differences in concentration according to the fruit ripening degree (the first was lower and the last two were higher in black fruits). Specific compounds not previously detected in green olives (γ-deca- and dodecalactones, δ-decalactone, and 2-methyl-3-furanthiol) proved to be, with methional, the strongest odors in black olive extracts. These extracts were also distinguishable from green olive extracts by the presence of new sulfur compounds and fewer terpenes.

KEYWORDS: Black olive; green olive; flavor; AEDA; lactone; aldehydes

INTRODUCTION

In Morocco, olive tree cultures (*Olea europaea*) account for 50% of the national arboricultural area, yielding around 120,000 tons of table olives and only 48,000 tons of olive oil (*1*). Such a distribution is very unusual by comparison with other Mediterranean countries such as Algeria or Tunisia, where olive oil is the main olive-derived commercial product.

Among table fruits, "Greek-style" black olives are just salted (sometimes after light bitterness removal treatment), whereas "Spanish-style" green olives require strong hydrolysis of oleuropein (a heterosidic ester of elenolic acid and 3,4-dihydroxyphenylethanol) with sodium hydroxide solutions (lyes) and washing before fermentation in a sodium chloride brine. Table olive fermentation is due to composite microflora, consisting mainly of lactic bacteria, in particular *Lactobacillus plantarum*, and yeasts (*2, 3*). In bitter olives, lactic bacteria are inhibited due to the presence of phenolic compounds derived from oleuropein.

In 2005, Iraqi et al. (*4*) screened for odorants in "Spanish-style" Moroccan green table olives by GC/olfactometry/aroma extract dilution analysis. Previously detected in olive oils, (*Z*)-3-hexenal (dilution factor, FD = 256), (*E*,*E*)-2,4-decadienal (FD = 128), (*E*,*Z*)-2,4-decadienal (FD = 64), and guaiacol (FD = 128) emerged as key flavors. 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 also detected in fruit extracts, although they are not reported as olive oil constituents (*5–15*).

In black olives, texture and phenols have been extensively studied (*16–18*) but data on their volatile constituents are scarce. The aim of the present work was therefore to determine their flavor determinants and to compare them with those found in green olives, which undergo much shorter fruit ripening but much longer technological processing.

MATERIALS AND METHODS

Chemicals. Dichloromethane was purchased from Romil (Prosan, Belgique) and redistilled twice prior to use. Ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA) was used. (*Z*)-2-Heptenal, (*Z*)-3-hexenal, nonanal, (*Z*)-3-hexen-1-ol, (*E*)-nerolidol, diacetyl, 2-methylpropanoic acid, 3-methylbutanoic acid, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 2-hydroxybenzoate, ethyl 3-methylbutanoate, 2-methyl-3-furanthiol, 3-methylbutyl acetate, styrene, 2,3-dimethylpyrazine, benzyl alcohol, limonene, eucalyptol, 2-methoxyphenol, phenylethyl alcohol, 2,4-hexadienoic acid, ethyl 2,4-hexadienoate, 1,2-dimethoxybenzene, γ-octalactone, 3-hydroxybutan-2-one, 1-decanol, 4-ethylguaiacol, vanillin, β-damascenone, ethyl decanoate, γ-decalactone, δ-decalactone, 2-methylbutanoic acid, (*E*)-ethyl cinnamate, α-farnesene, and γ-dodecalactone were obtained from Sigma-Aldrich (Bornem, Belgium). 1-Hepten-3-ol, 1-octen-3-ol, and 1-chloroheptane came from Acros Chimica (Geel, Belgium). *n*-Octane,

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Table 1. Volatile Compounds Identified in Black Table Olive Fruits (B_1, B_2, and B_3): Comparison with Data from Reference *4* for Green Olives (G)

			B_1		B_2		B_3				G	
PN[a]	RI[b]	compound	FD[c]	[ ]$_{EST\ equ}$[d]	FD	[ ]$_{EST\ equ}$[d]	FD	[ ]$_{EST\ equ}$[d]	odor (GC-O)	identification[e−g]	FD	[ ]$_{EST\ equ}$[d]
1b	613	diacetyl			**2048**		**256**		buttery, almond	GCO, RI_{REF}		
19	650	3-methylbutanal			**2**		**2**		almond, green	GCO, RI_{REF}	**2**	0.06
20	661	2-methylbutanal			**2**				hazelnut	GCO, RI_{REF}	**2**	0.19
2b	687	3-hydroxy-butan-2-one		1.97						MS, RI_{REF}		
25	715	3-methylbutanol +	**8**	1.49	**4**	0.85	**32**	2.59	fruity, green	GCO, MS, RI_{REF}	**2**	
25′	718	2-methylbutanol		1.02		0.54		1.18		GCO, MS, RI_{REF}		
3b	727	ethyl 2-methylpropanoate	**64**		**256**	0.08	**128**	0.02	fruity, strawberry	GCO, MS RI_{REF}		
26	731	2-methylpropanoic acid				0.22				MS, RI_{REF}		0.02
28	746	toluene			**16**	0.43	**2048**	1.24	green, solvent	GCO, MS, RI_{REF}	**4**	0.02
4b	749	unknown	**2048**		**64**		**4**		strong olive, fruity			
29	764	(*Z*)-3-hexenal (hexanal)[h]	**32**	0.17	**4**	0.17	**8**	0.27	strong green	GCO (MS, RI_{REF})	**256**	0.14
5b (34)	803	$S_{unknown}$ (octane)[h]	**8**		**2**	0.54		0.43	unpleasant, pharmacy	PFPD (MS, RI_{REF})	**2**	0.04
36	807	3-methylbutanoic acid +	**8**		**64**	0.27	**4**		cheese, sweat, drugstore	GCO, MS, RI_{REF}	**64**	0.03
	818	2-methylbutanoic acid				0.12						
6b	821	2-ethenyl-2-butenal	**128**	0.58				0.48	solvent	MS, RI_{REF}		
7b	831	ethyl 3-methylbutanoate +	**8**		**2048**	0.04	**4096**	0.14	candy, sweet	GCO, MS RI_{REF}		
	833	ethyl 2-methylbutanoate							fruity	GCO, MS, RI_{REF}		
39	836	(*Z*)-3-hexen-1-ol			**4**	0.23	**2**	0.14	green	GCO, MS, RI_{REF}	**8**	0.02
41	850	1-hexanol		0.25		0.06		0.31	cutted grass	MS, RI_{REF}	**8**	0.02
8b	851	3-methylbutyl acetate				0.04		0.02		MS, RI_{REF}		
9b	858	2-methyl-3-furanthiol	**8192**		**8192**		**4096**		oxo, smoked, salted	GCO, RI_{REF}, PFPD		
43	870	methional	**16384**		**2048**	0.04	**512**	0.04	boiled potato	GCO, MS, RI_{REF}, PFPD	**128**	0.04
44	895	styrene		0.56		0.56		0.49	melted plastic	MS, RI_{REF}	**4**	0.02
10b	906	2,3-dimethylpyrazine	**512**		**128**		**64**		almond, popcorn	GCO, RI_{REF}		
11b	925	$S_{unknown}$	**2**				**32**		catty	PFPD		
48	927	(*Z*)-2-heptenal		0.06		0.04	**2**	0.14	solvent	GCO, MS, RI_{REF}	**8**	0.02
49	930	benzaldehyde		0.25		0.31	-	0.12	almond	MS, RI_{REF}	**8**	0.04
12b	945	$S_{unknown}$	**64**		**64**		**32**		unpleasant	PFPD		
61	1003	benzyl alcohol		0.43		0.66		0.19	grass	MS, RI_{REF}	**8**	0.04
62	1007	phenylacetaldehyde	**128**	0.14	**16**	0.31	**4**		floral, hyacinth	GCO, MS, RI_{REF}	**32**	0.27
64	1020	limonene				0.49		2.19	greenery, fruity	MS, RI_{REF}	**32**	4.69
13b	1022	eucalyptol	**2**		**8**	0.10			mint	GCO, MS, RI_{REF}		
66	1031	$T_{unknown}$	**8**	0.43	**8**	0.14	**2**	0.97	strong green			0.17
14b	1056	2,4-hexadienoic acid[e]				1.41				MS		
71	1064	guaiacol	**2048**	0.31	**512**		**1024**	0.31	oxo, smoked, salted	GCO, MS, RI_{REF}	**128**	0.02
15b	1066	ethyl 2,4-hexadienoate[e]		0.06		7.37		0.14		MS		
74	1076	nonanal		0.39		1.74			pungent. greenery	MS, RI_{REF}	**8**	1.51
76	1093	phenylethyl alcohol	**32**	0.70	**128**	2.09	**256**	2.34	floral. fruity	GCO, MS RI_{REF}	**8**	0.56
16b	1098	2-ethenyl-1,1-dimethyl-3-methylene cyclohexane[e]	**16**	0.43	**4**	0.48	**4**	0.29	unpleasant	MS		
17b	1109	1,2-dimethoxybenzene	**16**		**128**	0.39	**512**	0.06		GCO, MS RI_{REF}		
18b	1115	tetrahydro-1,3-oxazine-2-thione[h]	**128**	0.04	**2**		**32**	0.06	oil	MS, PFPD		
87	1170	α-terpineol			**2**	0.25		0.27	floral	GCO, MS, RI_{REF}	**32**	0.60
19b	1205	unknown	**256**		**32**		**64**		oil			
94	1229	(*E*)-2-decenal	**32**	0.39	**2**	0.35	**32**	0.91	oil	GCO, MS, RI_{REF}	**64**	0.14
20b	1250	γ-octalactone	**32**	0.02				0.02	rotted fruit	GCO, MS, RI_{REF}		
21b	1255	ethyl 2-hydroxybenzoate			**16**	0.06	**2**	0.12	tarragon	GCO, MS, RI_{REF}		
99	1261	1-decanol		0.02				0.02		MS, RI_{REF}		0.17
22b	1263	4-ethylguaiacol	**2**		**8**	0.06	**256**	0.02	smoked, salted, unpleasant	GCO, MS, RI_{REF}		
101	1272	(*E*,*Z*)-2,4-decadienal	**2048**	0.97	**16**		**512**	1.47	oil of paraffin, green	GCO, MS, RI_{REF}	**64**	0.08
103	1286	(*E*,*E*)-2,4-decadienal	**256**	2.05	**512**	0.33	**128**	2.45	coriander, oil of paraffin	GCO, MS, RI_{REF}	**128**	0.10
23b	1295	2,4-decadien-1-ol	**16**	0.06			**32**	0.04	smoked, salted	MS, RI_{REF}		
24b	1352	vanillin	**64**		**2**				vanilla	GCO, RI_{REF}		
25b	1358	β-damascenone			**64**	0.06	**4**	0.02	cooked apple	GCO, MS, RI_{REF}		
26b	1373	ethyl decanoate	**256**	0.89	**2148**	0.02	**512**	0.04	grenade syrup	GCO, MS, RI_{REF}		
27b	1428	γ-decalactone	**16**	0.10	**128**	0.27	**8**	0.06	fruity	GCO, MS, RI_{REF}		
28b	1437	*E*-ethyl cinnamate		0.08		0.02				MS, RI_{REF}		
29b	1456	δ-decalactone	**512**	0.02	**16**	0.02	**512**	0.06	nuts	GCO, MS, RI_{REF}		
129	1489	α-farnesene		3.21				7.49	soft cooking of vegetable	MS, RI_{REF}	**64**	1.37
30b	1528	β-sesquiphellandrene[e]		0.04				0.10		MS		
135	1551	(*E*)-nerolidol		0.89		0.54		2.68	oil of car	MS, RI_{REF}	**32**	0.17
31b	1655	γ-dodecalactone	**512**	0.12	**32768**	5.87	**512**	0.17	fruity, olive	GCO, MS, RI_{REF}		

[a] PN, peak numbering (see order of elution in **Figure 1**; as in ref *4* if present in green olives; otherwise, PNb). [b] RI, retention index on CP-SIL5-CB. [c] FD, dilution factor $= 2^{n-1}$, where *n* is the number of dilution (factor 2) until no odor was perceived. [d] Approximate concentration in mg/L calculated in EST equivalents, taking into account a 100% recovery factor. [e] Compound tentatively identified by GC-MS and consultation of the NIST database (MS). [f] Identification confirmed after co-injection, by comparison of the retention index of the commercial product suspected and the aroma present in the extract (RI_{REF}). [g] Identification confirmed by comparison of the odor of the commercial product suspected and the aroma present in the extract (GCO). [h] Major compound (MS spectum) in parentheses, but the odor is brought by the former.

benzaldehyde, phenylacetaldehyde, α-terpineol, and 2,4-decadienal were purchased from Aldrich-Chemie (Steinheim, West Germany). *n*-Hexanal, phenylethylacetate, (*E*)-2-nonenal, and (*E*)-2-decenal were from Fluka Chemika (Buchs, Switzerland). 3-Methylbutanal, 2-methylbutanal, 2-methylbutanol, 3-methylbutanol, and methional were supplied by Janssen Chimica (Geel, Belgium). Toluene and 1-hexanol came from UCB Chemical (Leuven, Belgium). Ethylphenyl alcohol was supplied by Merck. 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 black Greek-style olive fruits (*Olea europaea*, Picual cultivar) from Morocco were bought at public

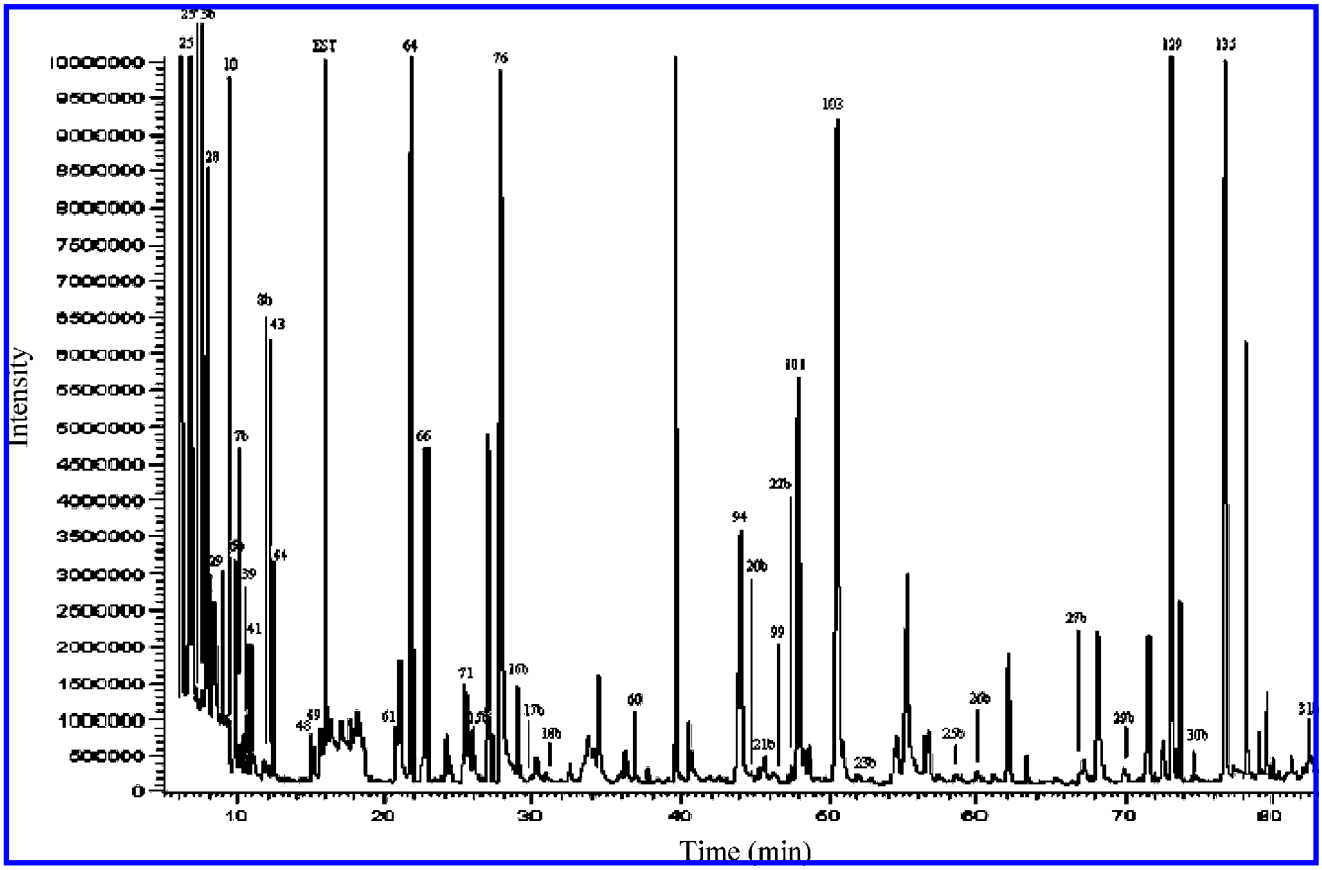

Figure 1. GC-MS chromatogram of an extract from black table olives (sample B_3).

markets (B_1 and B_3, Fes, Morocco) or in a Belgian supermarket (B_2, Delhaize, Belgium, glass conditioning). The Greek-style procedure consisted of treatment with very diluted NaOH lye, salt addition, and final storage in a 15% sodium chloride solution until sold. Potassium sorbate was used as a preservative in B_2.

Extraction of Volatiles. Ten grams of destoned and cut olive fruits were mixed with 30 mL of water. Volatiles were further extracted according to the Likens−Nickerson procedure previously optimized by Bouseta and Collin (*19*). The method allows most of the volatiles to be recovered with factors of >70% and variation coefficients of <8%. After adding 10 μL 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). Concentrations have been approximated in EST equivalents, taking into account a 100% recovery factor (2.05 mg/L when the area ratio = 1).

Gas Chromatography Coupled with Olfactometric Detection (GC-O). GC was performed using a ThermoFinigan 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 × 0.32 mm Wall-Coated Open Tubular (WCOT) apolar CP-SIL-5CB capillary column (1.2 μm film thickness). The oven temperature, initially at 40 °C, was programmed to rise to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and to 250 °C at 3 °C/min and was finally kept at 250 °C for 30 min. The GC odor port was crossed by a strong flow of humidified air (20 mL/min to ensure that odors did not accumulate in the funnel). Two microliters of the olive extract was injected. A 25 m × 0.32 mm WCOT polar FFAP-CB capillary column (0.3 μm film thickness) was 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 dilutions (factor 2) until no odor was perceived) of the odorants were determined at the sniffing port according to the AEDA method (*20*). All odor qualities were defined by three assessors, whereas stepwise dilution analysis (dilution factor 2) was performed only one time.

RESULTS AND DISCUSSION

Moroccan black table olives were screened for potent odorants by GC-O/AEDA. For each sample, both Likens−Nickerson extractions yielded very representative extracts, with indistinguishable overall sensory quality, FID chromatograms, and odors perceived at the sniffing port. A mixture of duplicates was therefore kept for the complete AEDA.

Table 1 lists the main compounds identified in samples B_1, B_2, and B_3, on the basis of the observed mass spectra (consultation of the NIST database), retention indices, and/or odors (injection of pure compounds). Twenty potentially identified odors and 2 unknowns with an FD > 64 in at least one sample were perceived at the sniffing port when a 20-times-concentrated extract (10 g transferred into 0.5 mL of dichloromethane) was injected. The chromatogram obtained for B_3 is depicted in **Figure 1**. To provide a first idea of the concentrations found in olives, values in EST equivalents were calculated.

Several lactones (FD up to 32768) and 2-methyl-3-furanthiol (FD = 4096–8192) proved to be, with methional (3-methylthiopropionaldehyde, FD = 512–16384), the main contributors to the aroma of the black olive extracts. None of these lactones were detected in green Spanish-like olives or olive oils. Among them, γ-lactones were surprisingly prominent, especially γ-dodecalactone (FD = 512–32768, olive descriptor) and γ-decalactone

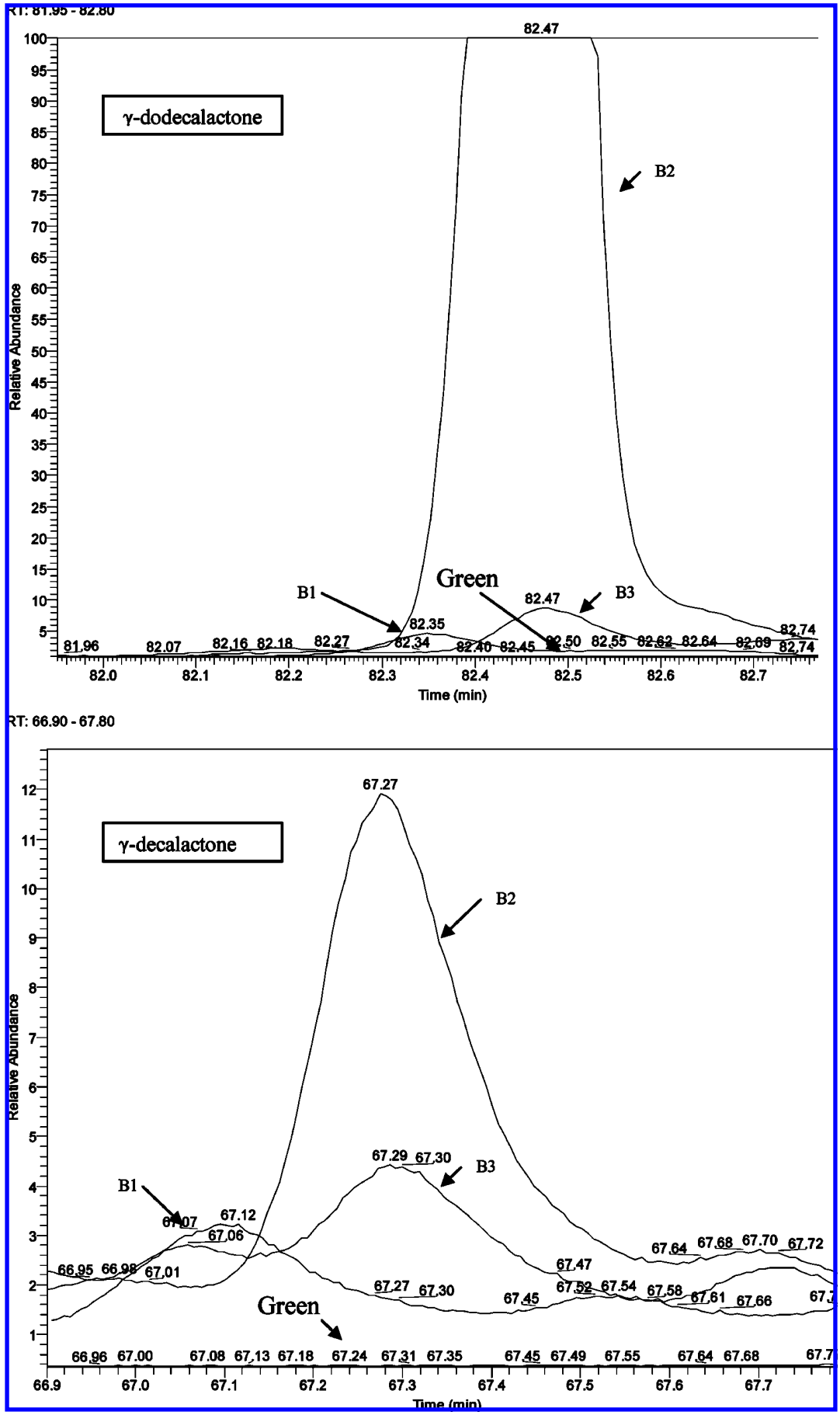

Figure 2. Zoom of the GC-MS chromatograms of black (B_1, B_2, and B_3) and green olive samples in the elution area of some lactones.

(FD = 8–128, fruity). δ-Decalactone (FD = 16–512, nuts) was the sole δ-lactone found in the black olive extracts. Many microorganisms are able to biosynthesize γ-lactones. In particular, the *Sporidiobolus* genus is known to accumulate γ-decalactone, using ricinoleic acid as precursor (*21, 22*). In malt whiskey, Wanikawa et al. (*23*) showed that γ-dodecalactone and γ-decalactone were issued from the interaction between lactic acid bacteria and yeasts. A similar biosynthesis could take place in black olives, which contain high levels of oleic acid, a fatty acid hydroxylation inducer (*23, 24*). 10-Hydroxystearic acid and 10-hydroxypalmitic acid could be produced by lactic bacteria from oleic acid and palmitoleic acid, respectively. As glucose suppresses the hydroxylation activity (*23, 24*), hydroxyl fatty acids are most probably generated by *Lactobacillus* bacteria when glucose has been consumed. The hydroxyl acids could be further catabolized by yeasts. The reduction of the aliphatic chains by three β-oxidation cycles leads after lactonization to γ-dodecalactone and γ-decalactone, respectively. Both γ-lactones gave rise to much higher MS peaks (**Figure 2**) in the chromatogram of B_2, where potassium sorbate was used as a preservative [confirmed by additional or higher peaks for ethyl-2,4-hexadienoate (15b) and 2,4-hexadienoic acid (sorbic acid, 14b)]. By comparison of the EST area, up to 5.9 mg/L of γ-dodecalactone was calculated for this sample (flavor threshold in butter = 1 mg/L according ref *25*). Although decreasing the yeast population, Turantas et al. (*3*) showed that potassium sorbate used in black olive fermentation had a slight stimulatory effect on the growth of lactic acid bacteria, suspected here to be involved in the γ-lactone biosynthesis.

A significant difference with respect to green olives was the even higher impact of methional in all three black samples, the FD for this compound reaching 16384 in B_1 (Likens−Nickerson extractions in all cases). Methional has never been reported as an olive oil constituent, although it is issued from black olives. Unidentified sulfur compounds (PFPD signals 5b, 11b, and 12b, for example) perceived in B_1, B_2, and B_3 extracts but absent from green olives might have been generated from methional, known to be very sensitive to heat and light.

Guaiacol (2-methoxyphenol) is another odorant, already present in green samples, but more concentrated after fruit ripening (FD = 512–2048 vs 128). Probably also derived from ferulic acid, 4-ethylguaiacol, with an analogous smoked odor, was not present at all in green olives but showed FD values from 2 to 256 in B_1−B_3. Reiners and Grosch (*13*) report its presence in various olive oil extracts. Also worth stressing is the presence of 1,2-dimethoxybenzene in the aromagram of black olives only (FD = 16–128 in B_1−B_3).

Recently, Iraqi et al. (*4*) mentioned the absence in green olives of ethyl 2-methylpropanoate, ethyl 2- or 3-methylbutyrate, and ethyl cyclohexylcarboxylate, previously described as the main contributors to the flavor of virgin olive oils (*9*). Except for the latter, these fruity branched esters were detected in all black olive samples investigated here (FD up to 256 for ethyl 2-methylpropanoate and 4096 for ethyl 2- or 3-methylbutyrate). To be also emphasized is the occurrence in black olives of ethyl decanoate (FD = 256–2148, pleasant pomegranate odor).

β-Damascenone has also emerged as having in olive oils (*13*) among the highest FD values, especially in Moroccan oils. Although this carotenoid-derived compound was not detected in green Spanish-like olives, its FD was as high as 64 in B_2.

The strong green-like odorant (*Z*)-3-hexenal proved to be less intense in black olive samples (FD = 4–32 vs 256) than in green olive samples. Injection of the extracts onto an FFAP-CB column enabled us to exclude the sensorial impact of hexanal, which coelutes with (*Z*)-3-hexenal from the CP-SIL-5CB column (although hexanal is quantitatively the major compound, as proved by GC-MS). On the other hand, (*E*,*Z*)- and (*E*,*E*)-2,4-decadienal (oil of paraffin/green/coriander) were characterized by high FD values, whatever the ripening state (FD = 16–2048 and 64–128 in black and green olive extracts, respectively).

Many terpenes previously found in green olives (nerol acetate, β- and γ-terpineol, linalool, β-myrcene) but not reported as olive oil constituents were absent from black olives.

The impact of potassium sorbate on lactones and the strong olive odorant at RI = 749 (FD = 4–2048; no MS peak) are being further investigated.

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