Odorants of Virgin Olive Oils with Different Flavor Profiles

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The potent odorants of virgin olive oils from Italy (I), Spain (S), and Morocco (M) were screened by aroma extract dilution analyses and gas chromatography olfactometry of headspace samples. After quantification, odor activity values (OAVs) were calculated by dividing the concentrations of the odorants in the oil samples by their nasally and retronasally determined odor threshold values in sunflower oil. On the basis of the nasal thresholds, the following compounds showed high OAVs in the oils given in parentheses: acetaldehyde (I, S, M), acetic acid (I, S), propanal (I), 1-penten-3-one (I), (*E*,*Z*)-2,4-decadienal (I, M), *trans*-4,5-epoxy-(*E*)-2-decenal (I, S, M), (*Z*)-3-hexenal (I, M), (*E*)-2-hexenal (I), (*Z*)-3-hexenyl acetate (I), 4-methoxy-2-methyl-2-butanethiol (S), ethyl 2- and 3-methylbutyrate (S, M), 2- and 3-methylbutanal (S), ethyl cyclohexylcarboxylate (M), and ethyl isobutyrate (M). Higher OAVs were additionally found for hexanal (I) and (*Z*)-2-nonenal (I, M) when retronasal odor thresholds were used as the basis. The potent odorants were dissolved in a refined plant oil in the concentrations found in the three olive oil samples. The flavor profiles of the models obtained were very close to those of the real samples, indicating that the different notes in the flavor profiles of these oils could be reproduced, e.g., green, fruity, black currantlike. Models missing one or several compounds with the same odor quality gave an insight into the importance of the odorants contributing to the flavor profiles of the oil samples.

Keywords: *Aroma extract dilution analysis; flavor analysis; flavor differences; flavor imitation; flavor profile analysis; isotope dilution asssay; sensory study; virgin olive oil*

INTRODUCTION

Flavor is an important criterion for the quality of virgin olive oils. Therefore, great effort has been made to identify the compounds causing the flavor.

Flath et al. (1973), who identified 77 volatile compounds, performed a sensory study. They added the identified compounds in different ratios to an odorless safflower oil and compared the odor of these models with that of a virgin olive oil. The odor of the best mixture containing five odorants approached that of olive oil but still lacked certain components essential to provide the fruity note.

Guth and Grosch (1991, 1993a), Blekas et al. (1994), and Blekas and Guth (1995) have evaluated the potent odorants of olive oils with different flavors by dilution analyses. After quantification of the odorants by stable isotope dilution analysis (IDA), odor activity values (OAVs, ratio of concentration to odor threshold) were calculated to show the actual contribution of each odorant to the olive oil flavors. It was concluded from the results that the following compounds mainly contributed to the flavor notes given in parentheses: (*Z*)-3-hexenal (green); ethyl 2-methylbutyrate, ethyl isobutyrate, ethyl cyclohexylcarboxylate (fruity); (*Z*)-2-nonenal (fatty); and 4-methoxy-2-methyl-2-butanethiol (black currantlike).

Morales and Aparicio (1993) as well as Morales at al. (1994, 1995, 1996) analyzed the volatiles of olive oils using dynamic headspace gas chromatography. They identified 55 compounds and evaluated their odor qualities by gas chromatography−olfactometry. Statistical methods were applied to correlate the volatiles with sensory attributes, e.g., the attribute "banana" was correlated with (*E*)-2-hexenal, hexan-1-ol, and (*E*)-2-hexen-1-ol.

However, the only way to verify that the identified odorants contribute to the flavor of olive oils is through experiments analogous to those reported by Flath et al. (1973). Therefore, olive oil samples from Italy (I), Spain (S), and Morocco (M) with different flavor profiles were analyzed as outlined in Table 1. On the basis of the quantitative results and calculated OAVs, the potent odorants were established. To check whether the results of the instrumental analyses were correct, sensory studies were performed. The odorants were dissolved in a refined plant oil in the concentrations found in the three olive oil samples. These model mixtures were compared to the original oils for similarities in the flavor profiles. Additionally, changes in the overall flavor of the models were evaluated after omission of one or more odorants. On the basis of the results, reduced models containing less odorants were evaluated.

EXPERIMENTAL PROCEDURES

Olive Oils. The Italian virgin olive oil (I), originating from Umbria, was from retail trade. The Spanish virgin olive oil (S) was purchased from a german oil mill. The Moroccan virgin olive oil (M) was obtained from Morocco directly. The oil samples were stored at −30 °C until use. The fatty acid composition (cf. Table 2) was determined by gas chromatography after transesterification of the olive oil samples (Christie, 1982). An odorless plant oil (Union Deutsche Lebensmittelwerke, Hamburg, Germany) was used as the basis for the model mixtures.

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Table 1. Outline of the Analytical Procedure

	identification of potent odorants
I	isolation of volatile compounds by distillation of the oil samples
II	analysis of volatiles by high-resolution gas chromatography (HRGC) and localization of potent odorants in the gas chromatograms by aroma extract dilution analysis (AEDA)
III	localization of highly volatile potent odorants in gas chromatograms by gas chromatography−olfactometry of headspace samples (GCOH)
IV	enrichment of potent odorants by separation of the volatiles in neutral and acidic compounds (AF, acidic fraction), by column chromatography (CC) of the neutral compounds, and by multidimensional gas chromatography (MDGC)
V	localization of potent odorants in the gas chromatogram of each of the five CC fractions by HRGCO
VI	analysis of AF and the CC fractions by HRGC and mass spectrometry (MS) using the MS system MAT95S
VII	identification of the potent odorants by comparison of HRGC and MS data and odor quality with the corresponding properties of authentic substances
	quantification
VIII	spiking of the oil samples with known amounts of labeled internal standards
IX	isolation and enrichment of the odorants and the standards according to points I and IV and by silver ion chromatography
X	enrichment of trace compounds by reversible covalent chromatography and by MDGC
XI	determination of the odorants and their standards by HRGC−MS
XII	determination of highly volatile odorants, e.g., acetaldehyde, by headspace analysis and HRGC−MS

Table 2. Fatty Acid Composition of Virgin Olive Oil Samples from Italy (I), Spain (S), and Morocco (M)

	wt %		
fatty acid	I	S	M
14:0	nd[a]	nd	nd
16:0	10.7	8.6	10.9
16:1	0.5	0.5	0.8
18:0	2.4	4.4	2.5
18:1	79.0	81.4	70.4
18:2 *n-6*	6.6	4.3	14.3
18:3 *n-3*	0.4	0.5	1.0
20:0	0.5	0.4	0.2
20:1	0.3	0.2	0.2

[a] nd, not detectable.

Chemicals. The following pure samples of the compounds listed in Tables 3 and 4 were obtained commercially: **1**−**5**, **8**−**12**, **16**, **18**, **21**, **24**−**27**, **30**, **34**−**36**, **38**, **39**, **41** (Aldrich, Steinheim, Germany); **6**, **14**, **17**, **22**, **28** (Lancaster, Mühlheim, Germany); **19** (Serva, Heidelberg, Germany); **32** (Haarmann and Reimer, Holzminden, Germany); **33**, **37** (Merck, Darmstadt, Germany); **40** (Fluka, Neu-Ulm, Germany). (^{13}C)Acetic acid (**c-33**) was from Sigma−Aldrich, Deisenhofen, Germany; ($^{13}C_2$)acetaldehyde (**c-36**) was from Promochem, Wesel, Germany. Acetone and silver nitrate were from Merck; butyl butyrate, 2-methyl-1-pentanol, pentanal, and 1-phenylethanol were purchased from Aldrich; (*E*)-2-decenal was from Alfa Products, Karlsruhe, Germany. Deuterium oxide, lithium aluminum deuteride, 1-pentyn-3-ol, and pyridinium chlorochromate, used for the synthesis of **d-3**, were obtained from Aldrich. Florisil (magnesium trisilicate) was purchased from Serva. Silica gel 60 (0.002−0.2 mm, Merck) was treated with HCl (Esterbauer, 1968) and dried to a water content of 1.5% by mass. The following reference substances were synthesized according to the literature cited: **7**, **15** (Ullrich and Grosch, 1988a); **13** (Guth and Grosch, 1991); **20**, **23** (Ullrich and Grosch, 1988b); **31** (Schieberle and Grosch, 1991). (*E*,*Z*)-2,4-Decadienal (**29**), a secondary product of **30**, was isolated according to Gassenmeier and Schieberle (1994). Ethyl (*S*)-2-methylbutyrate was a gift of E. Fuhrmann (DFA, Garching, Germany).

The internal standards used for IDAs were labeled either with deuterium (**d**) or with carbon-13 (**c**).

($^2H_2$)1-Penten-3-one (d-3). 1-Pentyn-3-ol was reduced with lithium aluminum deuteride/deuterium oxide (Grant and Djerassi, 1974) to ($^2H_2$)1-penten-3-ol, which was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975) to **d-3**. 1-Pentyn-3-ol (10 mmol) dissolved in anhydrous tetrahydrofuran (20 mL) was added dropwise to a solution of lithium aluminum deuteride (13 mmol) in tetrahydrofuran (20 mL). The mixture was refluxed for 1 h and then cooled to 0 °C. Deuterium oxide (1 mL) was added slowly for hydrolysis. HCl (15%) was added until the precipitate of aluminum hydroxide was just dissolved. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (3 × 5 mL). The combined organic layers were washed with saturated aqueous NaCl and then dried over anhydrous Na_2SO_4. The solvent was distilled off. The residue was dried in a slight nitrogen stream and afterward dissolved in anhydrous dichloromethane (15 mL). The solution was added to a suspension of pyridinium chlorochromate (15 mmol) and anhydrous sodium acetate (3 mmol) in dichloromethane (15 mL). The mixture was stirred for 2 h at room temperature under nitrogen atmosphere. Diethyl ether (40 mL) was added, and the suspension was filtered through a Florisil column (20 × 2 cm). The product was eluted with diethyl ether (200 mL). The solution was concentrated to a volume of 2 mL and applicated onto a water-cooled column (30 × 2 cm) packed with silica gel. Elution of **d-3** was performed with *n*-pentane/diethyl ether (90/10, v/v, 250 mL). MS-EI: 57 (100%), 58 (30%), 86 (M^+, 22%), 40 (8%), 43 (6%), 41 (5%), 56 (5%), 87 (5%). MS-CI: 87 ($M^+ + 1$, 100%), 88 (30%), 86 (8%).

The following internal standards were synthesized according to the literature cited: **d-1** (Schieberle and Grosch, 1992); **d-4**, **d-9**, **d-10**, **d-13**, **d-17**, **d-22** (Guth and Grosch, 1993a); **d-6**, **d-19**, **d-27** (Guth and Grosch, 1993b); **d-7**, **d-12**, **d-14**, **d-15**, **d-23**, **d-24**, **d-29**, **d-30**, **d-31** (Guth and Grosch, 1990); **d-16** (Blekas and Guth, 1995); **d-32** (Sen et al., 1991); **d-34** (Guth and Grosch, 1994); **d-38** (Milo and Grosch, 1993); **d-40** (Schieberle, 1991). ($^2H_3$)Ethyl butyrate was a gift of P. Schieberle (DFA, Garching, Germany), and ($^2H_3$)ethyl (*E*)-cinnamate was a gift of H. Guth (DFA).

High-Resolution Gas Chromatography (HRGC). HRGC was performed with a Carlo Erba gas chromatograph 4200 (Carlo Erba, Hofheim, Germany) using the following fused silica capillaries: DB-5 (SE-54; 30 m × 0.32 mm, 0.25-μm film thickness) supplied from J&W Scientific, Folsom, CA; DB-1701 (30 m × 0.25 mm, 0.25-μm film thickness; J&W Scientific); FFAP−CB (25 m × 0.32 mm, 0.3-μm film thickness) supplied from Chrompack, Frankfurt, Germany; CP-Wax 52 CB (DB-Wax; 50 m × 0.32 mm, 1.2-μm film thickness; Chrompack). The samples were applied by the on-column injection technique at 35 °C (Ullrich and Grosch, 1988b). After 2 min, the temperature of the oven was raised to 50 °C (60 °C for FFAP−CB) at a rate of 40 °C/min, held isothermally for 1 min, and then raised by 6 °C/min (4 °C/min for DB-5) to 230 °C. Using the DB-Wax capillary, the initial temperature of 35 °C was held for 5 min and then raised at a rate of 4 °C/min to 230 °C. A CP−FFAP−CB capillary (25 m × 0.53 mm, 1-μm film thickness; Chrompack) was used for the determination of the fatty acid composition. The starting temperature of 60 °C was held for 2 min, then raised by 15 °C/min to 190 °C, held isothermally for 1 min, then raised at a rate of 4 °C/min to 230 °C, held isothermally for 10 min, and finally raised by 10 °C/min to 250 °C.

HRGC−Olfactometry (HRGCO). Aroma extract dilution analysis (AEDA) was performed according to Ullrich and Grosch (1987) with the gas chromatograph and the thin film capillaries DB-5 and FAPP−CB used for HRGC.

Gas Chromatography−Olfactometry of Headspace Samples (GCOH). Olive oil (40 g) was placed into a vessel (240 mL) sealed with a septum and then held for 1 h in a water bath of 40 °C. The headspace volumes detailed in Table 4 were drawn

Table 3. Potent Odorants in Different Virgin Olive Oil Samples[a]

no.	odor descriptn	CC frac.[b]	RI on SE-54	RI on FFAP	compound	FD factor I	FD factor S	FD factor M
1/2	malty	II	650	913	3-/2-methylbutanal[c]	<8	8	8
3	green, pungent	II	682	1015	1-penten-3-one[c]	8	<8	8
4	fruity	I	757	961	ethyl isobutyrate[c]	16	32	2048
5	fruity	I	777	1007	methyl 2-methylbutyrate[c]	nd[f]	<8	32
6	green	II	800	1077	hexanal[c]	8	<8	16
7	leaflike	II	800	1140	(*Z*)-3-hexenal[c]	16	<8	128
8	cheesy, fruity	I	804	1023	ethyl butyrate[c]	nd	16	128
9	green applelike	II	850	1215	(*E*)-2-hexenal[c]	32	nd	nd
10	fruity	I	851	1046	ethyl 2-methylbutyrate[c]	32	128	4096
11	fruity	I	855	1065	ethyl 3-methylbutyrate[c]	nd	16	1024
12	leaf-like	IV	858	1380	(*Z*)-3-hexenol[c]	<8	<8	16
13	black currantlike, catty	II	917	1207	4-methoxy-2-methyl-2-butanethiol[d]	nd	128	nd
14	mushroomlike	II	979	1300	1-octen-3-one[c]	32	8	64
15	geranium-like	II	985	1375	(*Z*)-1,5-octadien-3-one[d]	32	nd	8
16	citruslike	II	1004	1287	octanal[c]	32	8	8
17	banana-like	I	1009	1316	(*Z*)-3-hexenyl acetate[c]	16	<8	nd
18	aromatic, fruity	I	1057	1373	methyl cyclohexylcarboxylate[c]	nd	nd	32
19	phenolic, burnt	IV	1090	1867	guaiacol[c]	64	32	128
20	green, harsh	II	1093	1529	(*Z*)-3-nonenal[d]	8	<8	8
21	citruslike	II	1105	1390	nonanal[c]	<8	<8	16
22	aromatic, fruity	I	1131	1414	ethyl cyclohexylcarboxylate[c]	8	<8	4096
23	green, fatty	II	1149	1505	(*Z*)-2-nonenal[c]	32	16	64
24	paperlike, fatty	II	1160	1536	(*E*)-2-nonenal[c]	16	8	16
25	paprika-like	III/IV	1181	1521	2-isobutyl-3-methoxypyrazine[d]	8	8	8
26	green, fruity	I	1200	1432	ethyl octanoate[c]	nd	<8	16
27	deep-fried	II/III	1215	1708	(*E*,*E*)-2,4-nonadienal[c]	8	nd	16
28	spicy, sweet	IV	1278	2033	4-ethylguaiacol[c]	<8	<8	16
29	deep-fried	II/III	1294	1750	(*E*,*Z*)-2,4-decadienal[c]	<8	<8	16
30	deep-fried	II/III	1318	1808	(*E*,*E*)-2,4-decadienal[c]	16	8	64
31	metallic	IV/V	1378	2006	*trans*-4,5-epoxy-(*E*)-2-decenal[c]	16	16	128
32	boiled applelike	II	1388	1817	(*E*)-β-damascenone[c]	8	8	1024
33	like acetic acid	AF[e]		1453	acetic acid[c]	16	16	16
34/35	sweaty	AF[e]		1668	3-/2-methylbutyric acid[c]	16	16	<8

[a] Odorants showing an FD factor ≥ 8 at least in one of the three oil samples are listed. [b] The fraction refers to the applied column chromatography (CC) on silica gel (cf. isolation of the volatiles). [c] The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on capillaries given in the table, mass spectra obtained by MS-EI and MS-CI, and odor quality perceived at the sniffing port. [d] The MS signal was too weak to obtain a spectrum. The compound was identified on the basis of RI on capillaries SE-54, FFAP, and DB-1701 and of odor quality at the sniffing port. Compounds **13** and **15** were clearly identified in the procedure used for quantification. [e] AF, acidic fraction. [f] nd, not detectable.

Table 4. Smallest Headspace Volumes Required To Perceive the Odorant at the Sniffing Port in GCOH of Different Virgin Olive Oil Samples

no.[a]	odor descriptn	RI on SE-54	compound	volume (mL) I	volume (mL) S	volume (mL) M
36	pungent, sweet	<600	acetaldehyde[b]	0.5	1	2.5
37	alcoholic	<600	ethanol[b]	10	10	>20
38	sweet, pungent	<600	propanal[b]	1	5	20
39	malty	<600	methylpropanal[b]	>20	10	>20
1	malty	650	3-methylbutanal[b]	5	5	10
2	malty	661	2-methylbutanal[b]	>20	20	>20
3	green, pungent	682	1-penten-3-one[b]	10	>20	20
4	fruity	757	ethyl isobutyrate[c]	10	5	1
5	fruity	777	methyl 2-methylbutyrate[c]	>20	20	10
6/7	green, leaflike	800	hexanal[b]/(*Z*)-3-hexenal[b]	10	20	20
9	green applelike	850	(*E*)-2-hexenal[b]	10	nd[d]	nd
10	fruity	851	ethyl 2-methylbutyrate[c]	5	0.5	1
11	fruity	855	ethyl 3-methylbutyrate[c]	nd	10	2.5
13	black currantlike, catty	917	4-methoxy-2-methyl-2-butanethiol[c]	nd	5	nd
14	mushroomlike	979	1-octen-3-one[c]	5	5	10
15	geranium-like	985	(*Z*)-1,5-octadien-3-one[c]	10	nd	>20
16	citruslike	1004	octanal[b]	10	5	10
19	phenolic, burnt	1090	guaiacol[c]	>20	10	>20
22	aromatic, fruity	1131	ethyl cyclohexylcarboxylate[c]	>20	>20	2.5
23	green, fatty	1149	(*Z*)-2-nonenal[c]	20	10	>20
24	paperlike, fatty	1160	(*E*)-2-nonenal[c]	>20	5	>20
31	metallic	1378	*trans*-4,5-epoxy-(*E*)-2-decenal[c]	>20	20	10

[a] Numbers **1**−**7**, **9**−**11**, **13**−**16**, **19**, **22**−**24**, and **31** refer to Table 3. [b] Refers to footnote *c* in Table 3. [c] The MS signal was too weak to obtain a spectrum. The compound was identified on the basis of RI on capillary SE-54 and of odor quality at the sniffing port considering the results obtained by AEDA. [d] nd, not detectable.

by a gastight syringe, injected into the purge and trap system TCT/PTI 4001 connected to a CP-9001 gas chromatograph (Chrompack, Frankfurt, Germany), and analyzed on capillary RTX-5 (SE-54; 30 m × 0.53 mm, 1.5-μm film thickness)

Table 5. Selected Ions, Thin Film Capillaries, and Calibration Factors for Mass Chromatography of the Odorants

odorant[a,b]	selected ion (*m*/*z*)	int std[c]	selected ion (*m*/*z*)	capillary	calibr factor[d]
1	69	**d-1**	70−71	DB-Wax	0.85
2	87	**d-1**	70−71	DB-Wax	1.05
3	85	**d-3**	87	FFAP	0.80
4	117	**d-4**	120	SE-54	1.05
6	83	**d-6**	85−87	FFAP	0.86
7	81	**d-7**	83	DB-1701	1.05
8	117	**d-8**	120	SE-54	0.97
9	99	**d-9**	101	FFAP	0.50
10	131	**d-10**	134	FFAP	1.15
11	131	**d-10**	134	FFAP	1.09
12	83	**d-12**	85	FFAP	0.73[f]
13	101	**d-13**	104	FFAP + SE-54[e]	0.99[f]
14	127	**d-14**	129	SE-54	0.62
15	125	**d-15**	127	FFAP + SE-54[e]	0.96
16	111	**d-16**	113−115	SE-54	0.86
17	143	**d-17**	146	SE-54	0.95
19	125	**d-19**	128	FFAP	0.94[g]
22	157	**d-22**	160	FFAP + SE-54[e]	0.96
23	123	**d-23**	125	FFAP	0.84
24	141	**d-24**	143	SE-54	0.74
27	139	**d-27**	141	SE-54	0.61
29	153	**d-29**	157	DB-1701	0.35[h]
30	153	**d-30**	157	SE-54	0.60
31	169	**d-31**	173−174	FFAP	0.80
32	191	**d-32**	196−198	SE-54	0.73
33	61	**c-33**	62	FFAP	1.00
34/35	103	**d-34**	105	FFAP	0.56
36	45	**c-36**	47	SE-54	1.00
38	59	**d-38**	62	DB-Wax	1.03
2-phenylethanol (**40**)	105	**d-40**	106	FFAP	0.75
ethyl (*E*)-cinnamate (**41**)	177	**d-41**	180	FFAP	1.03

[a] The numbering of the compounds refers to Tables 3 and 4. [b] Compounds **17**, **27**, and **31** were determined with their internal standards by the MS system MAT 95S, compounds **36** and **38** were determined by the MS system Incos XL, and the remaining compounds were determined by the ion trap detector ITD 800. [c] Abbreviation of the labeling: c, carbon-13; d, deuterium. [d] Calibration factors were determined in mixtures of equal amounts of unlabeled odorants and corresponding labeled standards (Guth and Grosch, 1990). [e] Compounds **13**, **15**, and **22** were determined using the MDGC system connected to the ion trap detector ITD 800. [f−h] The calibration factors marked were obtained from the following sources: (*f*) Guth and Grosch (1993a); (*g*) Semmelroch et al. (1995); (*h*) Wagner (personal communication).

supplied from Amchro, Sulzbach, Germany, as described earlier (Guth and Grosch, 1993c).

HRGC−Mass Spectrometry (MS). The MS system MAT 95S (Finnigan, Bremen, Germany) was used in combination with a GC 5890 Series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany). Mass spectra in the chemical ionization mode (MS-CI) were generated at 115 eV using isobutane as the reagent gas. The INCOS XL system (Finnigan) worked as described by Milo and Grosch (1995) with methane as the reagent gas. The ion-trap detector ITD 800 was used with methanol as the reagent gas under the conditions reported earlier (Semmelroch et al., 1995). Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV independent of the MS system. The capillaries mentioned above were used in tandem with the mass spectrometers. For quantification of the odorants, the abundances of selected ions (Table 5) were evaluated.

Isolation of the Volatiles. The olive oil sample (400 g for AEDA, 1 kg for the identification experiments) was diluted with diethyl ether (1 + 1, w/v). The volatile fraction was stripped off together with the solvent in high vacuum (pressure, 6 mPa; *T*, 34 °C) using the apparatus described previously (Guth and Grosch, 1989; Jung et al., 1992). The condensate obtained was concentrated to a volume of 2 mL for AEDA by distillation on a Vigreux column (50 × 1 cm) and to a volume of 200 μL for column chromatography by microdistillation (Bemelmans, 1979). The acidic fraction (AF) was separated by treating the condensate with aqueous sodium carbonate (Guth and Grosch, 1993d).

Column Chromatography (CC). The volatile compounds of 300 g of olive oil maximum were fractionated per one water-cooled (12 °C) column (30 × 1 cm) packed with a slurry of silica gel in *n*-pentane. After application of the condensate, the column was washed with *n*-pentane (70 mL). Elution was performed successively with the following *n*-pentane/diethyl ether mixtures (v/v): 97.5/2.5 (70 mL; fraction I), 95/5 (100 mL; fraction II), 90/10 (100 mL; fraction III), 70/30 (150 mL; fraction IV), 0/100 (150 mL; fraction V). The corresponding fractions obtained from several columns were combined and concentrated to a final volume of 100 μL.

Silver Ion Chromatography. Silica gel containing silver nitrate (10%, w/w) was used for the isolation of (*Z*)-3-hexenal (**7**). The concentrated extract was applied onto the water-cooled column (30 × 1 cm) and then eluted successively with the following *n*-pentane/diethyl ether mixtures (v/v): 97.5/2.5 (200 mL), 95/5 (100 mL), 90/10 (100 mL). Finally, **7** was eluted with diethyl ether (100 mL).

Reversible Covalent Chromatography. Odorant **13** and its standard **d-13** were enriched by reversible covalent chromatography of the oil volatiles using Affi-Gel 501 (Full and Schreier, 1994; Semmelroch and Grosch, 1996). The resulting thiol fraction was separated from the displacing reagent dithiothreitol under high vacuum (pressure, 6 mPa, *T*, 22 °C) according to Sen et al. (1991).

Multidimensional Gas Chromatography (MDGC). Odorants **13** (enriched by reversible covalent chromatography) and **15** and **22** (purified by CC) as well as the enantiomeric distribution of the 2-alkyl-branched ester (cf. below) were analyzed by MDGC using the moving capillary stream switching (MCSS) system (Fisons Instruments, Mainz-Kastell, Germany) which was installed into a gas chromatograph of the HRGC MEGA 2 series (Fisons Instruments) and controlled via a personal computer. The MCSS outlet had a flame ionization detector; the main column outlet was connected to the HRGC−MS system with the ion-trap detector described above. A DB−FFAP and a DB-5 fused silica capillary (30 m × 0.32 mm, 0.25-μm film thickness) supplied from J&W Scientific were used as the precolumn and main column, respectively. The carrier

gas was helium at 2 mL/min for the precolumn and main column each. The samples (0.5 μL) were applied by the on-column injection technique at 35 °C. The initial temperature was held for 2 min, then raised by a rate of 40 °C/min to 60 °C, held isothermally for 1 min, and finally raised by 6 °C/min to 230 °C. The HRGC effluent of the precolumn eluting between 5 and 7 min was cut out, cryofocused with liquid nitrogen, and then transferred onto the main column. After the start, a temperature of 40 °C was held for 1 min, raised by 40 °C/min to 50 °C, and then raised by a rate of 6 °C/min to 230 °C.

Enantiomeric Distribution of Ethyl 2-Methylbutyrate. The enantiomers ethyl (*R*)- and (*S*)-2-methylbutyrate were separated on the fused silica capillary BGB-176 (30 m × 0.25 mm, 0.25-μm film thickness) obtained from BGB Analytik AG, Rothenfluh, Switzerland, possessing the chiral phase heptakis-(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD). The capillary BGB-176 was used as the main column in MDGC. After application of CC fraction I onto the DB−FFAP precolumn, the temperature of the oven was held at 35 °C for 2 min, raised by 40 °C/min to 60 °C, held isothermally for 2 min, and then raised by 4 °C/min to 230 °C. The cut of the HRGC eluate was set at 4.6−6.1 min. The cryofocused sample was injected onto the main column at 50 °C. The oven temperature was raised by 40 °C/min to 70 °C, held isothermally for 1 min, and then raised at a rate of 3 °C/min to 230 °C. The main column was connected to the ion-trap detector ITD 800 (Finnigan, Bremen, Germany) used in the electron impact mode. Mass chromatograms were recorded at *m*/*z* 88 and 102.

Quantification. *Concentrations of the Deuterated Compounds.* The concentrations of compounds **d-3**, **d-6**, **d-7**, **d-9**, **d-12**, **d-14**, **d-15**, **d-16**, **d-19**, **d-22**, **d-23**, **d-24**, **d-32**, **d-34**, and **d-41** were determined by HRGC with methyl octanoate as the internal standard using the apparatus and conditions reported above. Correction factors were calculated by HRGC analysis of mixtures consisting of known amounts of methyl octanoate and of the unlabeled compounds. The concentrations of the following compounds were determined by HRGC without correction factors using the internal standards given in parentheses: **d-1** (pentanal); **d-4** (ethyl butyrate); **d-8** (ethyl isobutyrate); **d-10** (ethyl 3-methylbutyrate); **d-13** (2-methyl-1-pentanol); **d-17** (butyl butyrate); **d-27** ((*E*)-2-nonenal); **d-29**, **d-30**, **d-31** ((*E*)-2-decenal); **d-38** (acetone); **d-40** (1-phenylethanol).

The olive oil sample (10−1000 g) was spiked with known amounts of the labeled compounds (Guth and Grosch, 1990) except for compounds **d-13**, **c-36**, and **d-38**. After dilution with diethyl ether (10−1000 mL), the volatiles were isolated as described above, including separation of the acids by CC and silver ion chromatography. The samples were analyzed by HRGC−MS-CI under the conditions shown in Table 5. For the determination of compound **13**, a separate sample of S was treated (cf. Reversible Covalent Chromatography).

Compounds **36** and **38** were analyzed in separate charges as follows: The internal standard was pipetted into the oil sample (10 g) deposited in a sealed vessel (140 mL). The mixture was stirred for 1 h at 40 °C. A headspace volume of 10 mL was drawn by a gastight syringe and analyzed by HRGC−MS-CI as reported by Milo and Grosch (1995). Compound **36** was determined on capillary DB-5 operating with the temperature program used for GCOH. Compound **38** was analyzed on capillary DB-Wax starting at 20 °C. After 2 min, the temperature was raised at a rate of 6 °C/min to 230 °C.

Sensory Analyses. Sensory evaluations were performed in an isolated sensory panel room as described by Guth and Grosch (1993a). The test panel consisted of nine experienced assessors, seven males and two females, aged 25−35 years. The samples (15 g each) were presented in covered glass beakers (diameter, 40 mm; capacity, 45 mL) at 21 ± 1 °C after having been stirred for 30 min. The glass cover was removed, and the sample was sniffed by the panelist (nasal evaluation). Then the sample was rinsed into the mouth (retronasal evaluation). All samples were prepared shortly before sensory analysis.

Table 6. Concentrations and Odor Activity Values of Potent Odorants in Different Virgin Olive Oil Samples

	concentration[b]			odor act. value (n)[c]			odor act. value (rn)[d]		
odorant[a]	I	S	M	I	S	M	I	S	M
1	62	102	na[e]	12	19	na	5.7	9.4	na
2	na	70	na	na	32	na	na	8.5	na
3	26	na	na	36	na	na	8.2	na	na
4	1.4	4.2	71	1.1	3.4	59	1.9	5.6	95
6	1770	137	na	5.9	<1	na	24	1.9	na
7	36	na	20	21	na	12	30	na	17
8	na	27	35	na	<1	1.3	na	7.7	10
9	6770	na	na	16	na	na	26	na	na
10[f]	2.1	14	96	8.1	55	367	3.9	26	178
11	na	5.3	32	na	8.5	52	na	10	63
12	684	na	na	<1	na	na	1.9	na	na
13	na	4.3	na	na	253	na	na	179	na
14	1.4	1.5	4.1	<1	<1	<1	4.7	5.0	14
15	0.05	na	na	<1	na	na	1.7	na	na
16	382	99	na	6.8	1.8	na	6.8	1.8	na
17	2250	na	na	11	na	na	3.0	na	na
19	28	7.5	38	1.8	<1	2.4	2.2	<1	2.9
22	0.54	0.36	33	3.4	2.2	206	9.0	6.0	550
23	28	1.3	6.9	6.3	<1	1.5	47	2.2	12
24	91	24	52	<1	<1	<1	1.4	<1	<1
27	49	na	na	<1	na	na	<1	na	na
29	255	9.7	185	26	<1	18	13	<1	9.2
30	422	127	918	2.3	<1	5.1	10	3.1	22
31	32	22	20	25	17	15	11	7.3	6.7
32	0.38	0.54	6.4	<1	<1	<1	<1	<1	1.7
33	6830	1840	na	55	15	na	18	4.9	na
34/**35**[g,h]	81	32	na	3.7	1.5	na	3.1	1.2	na
36	587	410	208	2668	1864	945	83	58	29
38	409	75	62	44	8.0	6.6	6.0	1.1	<1
40	843	na	na	4.0	na	na	6.9	na	na
41	16	264	na	<1	1.8	na	<1	6.9	na

[a] Numbers of the compounds refer to Tables 3 and 4. [b] Values in micrograms per kilogram of olive oil. The data are mean values of at least duplicates. [c,d] The odor activity values were calculated by dividing the concentrations of the odorants by their (*c*) nasally ((*d*) retronasally) determined detection thresholds in refined sunflower oil (cf. Table 7). [e] na, not analyzed. [f,g] The odor activity values were calculated on the basis of the detection thresholds found for (*f*) ethyl (*S*)-2-methylbutyrate (**10a**) and (*g*) 3-methylbutyric acid (**34**) (cf. Table 7). [h] The sum was determined as 3-methylbutyric acid.

Models. With the exception of (*E*,*Z*)-2,4-decadienal (**29**) and *trans*-4,5-epoxy-(*E*)-2-decenal (**31**), the odorants listed in Table 6 were dissolved in ethanol for the preparation of models I0, S0, and M0. Aliquots containing the odorants in the amounts presented in Table 6 were dissolved in 1 L of the plant oil, and the mixtures were stirred at room temperature for 30 min. The concentration of ethanol in each model was not higher than 0.5 mL/kg.

Flavor Profile Analyses. At first, attributes for the description of flavor characteristics of the olive oils were selected by qualitative descriptive analysis of the different virgin olive oils. Then the panelists were trained with solutions of reference stimuli in sunflower oil (μg/kg), i.e., with acetaldehyde (2) for the pungent odor, with ethyl 2-methylbutyrate (10) for the fruity, with (*Z*)-3-hexenal (20) for the green, with (*E*,*E*)-2,4-decadienal (2000) for the fatty, with (*E*)-2-hexenal (5000) for the green applelike, with 4-methoxy-2-methyl-2-butanethiol (0.2) for the black currantlike, and with black olives for the black olive-like odor. In two further sessions (nine persons each), the intensities of the odor characteristics of the olive oils were scored nasally and retronasally on a category scale of 0, 0.5, 1.0, ..., 3.0. After an outlier test, Grubbs results are expressed as means ± standard deviations. Means were compared by Duncan's multiple range test.

Similarity Tests. Models I1−I9, S1−S5, and M1−M9 were prepared as reported for I0, S0, and M0, each missing one or several compounds with the same odor quality. The overall similarity in the odor was scored comparing the reduced

Table 7. Odor Detection Thresholds of the Compounds Dissolved in Refined Sunflower Oil

	threshold determined	
compound[a]	nasally	retronasally
1	5.4[b]	10.8[b]
2	2.2	8.2
3	0.73	3.2
4	1.2	0.75[c]
6	300[d]	73[d]
7	1.7	1.2
8	28[b]	3.5[b]
9	424	257
10	0.72	0.75[c]
10a	0.26	0.54
11	0.62	0.51
12	1100	364
13	0.017	0.024
14	10[d]	0.3[d]
15	0.45[d]	0.03[d]
16	56	56
17	200	750[c]
19	16	13
22	0.16	0.06
23	4.5[d]	0.6[d]
24	900[d]	66[d]
27	2500[e]	460[e]
29	10[f]	20[g]
30	180[d]	41[d]
31	1.3[d]	3[d]
32	11	3.7
33	124	378
34	22	26
36	0.22	7.1
38	9.4	68
40	211	122
41	150	38

[a] Numbers of the compounds refer to Tables 3 and 4. [b–g] The threshold values marked were obtained from the following sources: (*b*) Preininger and Grosch (1994); (*c*) Guth and Grosch (1993a); (*d*) Guth and Grosch (1990); (*e*) Meijboom (1964), threshold values determined in paraffin oil; (*f*) Gassenmeier (1994); (*g*) Badings (1970), threshold value determined in paraffin oil.

models with I0, S0, and M0, respectively. The results of nine panelists were treated as described above. Analogously, models containing the most important flavor compounds of each oil, selected by the experiments described above, were compared with I0, S0, and M0, respectively.

Threshold Values. The odorants listed in Table 7 were dissolved in refined sunflower oil as reported by Guth and Grosch (1993a). Odor detection thresholds were determined nasally and retronasally by the triangle test using refined sunflower oil as a blank. The initial concentrations, dependent on the substances, were determined in preliminary experiments. Sets of five to six samples diluted 1:3 (w/w) were offered for the nasal and the retronasal evaluations. The samples were presented in order of decreasing concentration. Threshold values were calculated according to the Bundesgesundheitsamt (1993).

RESULTS

The fatty acid compositions of oils I and S were similar (Table 2). Only the minor component stearic acid was higher, and linoleic acid was lower in S than in I. Oil M differed from I and S by a lower content of oleic acid, which was compensated by a higher linoleic acid content.

As detailed in Table 8, the flavor profiles of the three oils were different. Apple-like and green notes were characteristic for oil I, whereas oil S smelled intensely like black currants and the flavor of oil M reminded us of eating black olives.

Table 8. Flavor Profiles of the Virgin Olive Oils and Their Aroma Models

	intensity[a]											
	oil I		model I0		oil S		model S0		oil M		model M0	
attribute	n	rn	n	rn	n	rn	n	rn	n	rn	n	rn
pungent	2.1 ± 0.4^{a}	$1.1 \pm 0.5^{a,b,c}$	$2.1 \pm 0.4^{a,b}$	$1.1 \pm 0.5^{a,b}$	$1.5 \pm 0.5^{a,b}$	1.4 ± 0.3^{a}	1.7 ± 0.4^{a}	1.4 ± 0.5^{a}	$1.3 \pm 0.5^{a,b,c}$	$1.0 \pm 0.4^{a,b,c}$	$1.3 \pm 0.5^{a,b}$	$0.8 \pm 0.3^{a,b,c}$
fruity	1.7 ± 0.4	$1.2 \pm 0.5^{d,e,f}$	2.0 ± 0^{e}	$1.1 \pm 0.4^{c,d}$	$2.2 \pm 0.5^{a,c,d}$	$2.0 \pm 0.5^{b,c}$	1.8 ± 0.4^{b}	1.5 ± 0.3^{b}	$2.3 \pm 0.3^{a,d,e}$	$1.8 \pm 0.5^{a,d,e}$	$2.0 \pm 0^{a,c}$	$1.7 \pm 0.5^{a,d,e}$
green, leaf-like	1.6 ± 0.5	$1.9 \pm 0.3^{a,d}$	$1.4 \pm 0.4^{a,c}$	1.6 ± 0.4	$1.2 \pm 0.4^{c,e}$	$1.3 \pm 0.5^{b,d}$	1.2 ± 0.4^{c}	1.2 ± 0.3^{c}	$1.0 \pm 0.4^{d,f,g}$	$1.3 \pm 0.5^{f,g}$	$1.2 \pm 0.5^{c,d}$	$1.0 \pm 0.5^{d,f,g}$
fatty	$1.4 \pm 0.4^{a,b}$	$2.2 \pm 0.6^{b,e}$	$1.3 \pm 0.4^{b,d,e}$	$2.1 \pm 0.6^{a,c}$	$1.2 \pm 0.3^{d,f}$	1.8 ± 0.6	1.3 ± 0.3^{d}	1.8 ± 0.6^{d}	$2.0 \pm 0.4^{b,f,h}$	$2.6 \pm 0.5^{b,d,f}$	2.1 ± 0.6	$2.8 \pm 0.4^{b,e,f}$
applelike	2.4 ± 0.6^{b}	$2.1 \pm 0.5^{c,f}$	$2.5 \pm 0.5^{c,d}$	$2.3 \pm 0.6^{b,d}$								
black currantlike					$2.8 \pm 0.4^{b,e,f}$	$2.7 \pm 0.5^{a,c,d}$	$2.9 \pm 0.2^{a,b,c,d}$	$2.8 \pm 0.4^{a,b,c,d}$				
black olivelike									$2.7 \pm 0.2^{c,e,g,h}$	$2.5 \pm 0.3^{c,e,g}$	$2.4 \pm 0.6^{b,d}$	$2.5 \pm 0.6^{c,g}$
similarity[b]			2.6 ± 0.4	2.5 ± 0.3			2.7 ± 0.3	2.5 ± 0.4			2.5 ± 0.5	2.8 ± 0.4

[a] The intensity of the attributes was nasally (n) and retronasally (rn) scored on the scale 0 (absent) to 3 (strong). Means ± standard deviations are given in the table. Values followed by the same common letter in a column are significantly different at $P \leq 0.05$. [b] The similarity in the overall odor was evaluated on the scale 0 (no similarity) to 3 (identical with the original).

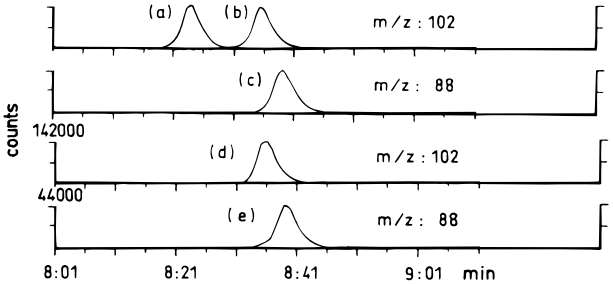

Figure 1. HRGC−MS chromatograms of ethyl methylbutyrates: (a) ethyl (*R*)-2-methylbutyrate, (b) ethyl (*S*)-2-methylbutyrate, (c) ethyl 3-methylbutyrate, (d) ester **10**, and (e) ester **11** from oil M

Flavor analysis was started by AEDA (Table 1). The results in Table 3 indicated that 35 odorants were perceived altogether by HRGCO of the volatile fractions obtained from the 3 oils. To identify the odorants, the volatile fractions were preseparated by CC and then each of the five fractions obtained was analyzed by HRGC−MS. The CC fractions in which the odorants were detected are detailed in Table 3. The enantiomeric purity of ester **10** was determined using MDGC. A comparison of the mass chromatograms shown in Figure 1 indicated that ethyl (*R*)- (peak a) and ethyl (*S*)-2-methylbutyrate (peak b) were separated on the chiral capillary used as main column in MDGC. However, ethyl (*S*)-2-methylbutyrate (peak b) partly coeluted with ethyl 3-methylbutyrate (peak c), which was also present in olive oils. To differentiate between the two esters, mass chromatograms of ions *m*/*z* 102 and 88 were recorded, indicating ethyl 2-methylbutyrate and ethyl 3-methylbutyrate, respectively. According to Figure 1, ester **10** (peak d) coeluted with ethyl (*S*)-2-methylbutyrate and ester **11** (peak e) with ethyl 3-methylbutyrate. This means that oil M contained enantiomerically pure ethyl (*S*)-2-methylbutyrate.

Most of the potent odorants found in I and S had already been identified by Guth and Grosch (1991, 1993a) in other olive oil samples originating from Italy and Spain. In oil I, these odorants were the esters **4**, **10**, **17**, and **22**, the aldehydes **7**, **9**, **16**, **23**, **24**, **30**, and **31**, 1-octen-3-one (**14**), guaiacol (**19**), *β*-damascenone (**32**), and acetic acid (**33**). Only 1-penten-3-one (**3**), (*Z*)-1,5-octadien-3-one (**15**), 2-isobutyl-3-methoxypyrazine (**25**), and 3-/2-methylbutyric acid (**34**/**35**) were detected here for the first time as potent odorants of oil I.

Oil S differed from I in the appearance of 4-methoxy-2-methyl-2-butanethiol (**13**) smelling black currantlike. This character impact flavor compound of some Spanish virgin olive oils has also been previously reported (Guth and Grosch, 1991, 1993a). In addition, the flavor dilution (FD) factor of the fruity ester **10** was 4 times higher and those of **14**, **15**, and octanal (**16**) were 4 times lower in S than in I (Table 3). The highest FD factors were found for the ethyl esters **4**, **10**, **11**, and **22** and for **32** in oil M (Table 3).

GCOH was performed to complete screening for potent odorants (Table 1). The results (Table 4) indicated that only one odorant, acetaldehyde (**36**), was perceived when the small headspace volume of 0.5 mL drawn from oil I was analyzed. In addition to **36,** propanal (**38**) was found after an increase of the sample volume to 1 mL, and five odorants including ethyl 2-methylbutyrate (**10**), 3-methylbutanal (**1**), and **14** were detected after an increase to 5 mL.

Odorants **1**, **10**, **14**, **36**, and **38** were also perceived in sample volumes between 0.5 and 5 mL drawn from the headspace of oil S. However, the ranking was different. Ester **10** was detected in the smallest volume of 0.5 mL, indicating its importance for the fruity note, which was stronger in S than in I. Furthermore, oil S differed from the other two oils in the appearance of aldehydes **16** and **24** in a smaller headspace sample (Table 4).

The detection of the four fruity smelling esters **4**, **10**, **11**, and **22** in a headspace volume of 2.5 mL underlines their contributions to the fruity note in the flavor profile of oil M. In addition, **36**, which accompanied the esters, belonged to the prominent highly volatile odorants of M.

The odorants selected by AEDA and GCOH were quantified in the three oils. According to the results shown in Table 6, oil I contained high amounts of the green-smelling C_6 aldehydes **6** and **9**, of ester **17**, and of acetic acid (**33**). Oil I differed from the other oils in higher concentrations of (*Z*)-3-hexenal (**7**), **16**, (*Z*)- and (*E*)-2-nonenal (**23**, **24**), and (*E*,*Z*)-2,4-decadienal (**29**).

The levels of the fruity esters **4**, **8**, **10**, and **11** in oil S surpassed those in I and were even higher in oil M. The latter differed clearly from the other oils in the concentration of ethyl cyclohexylcarboxylate (**22**), as this ester was 60 and 90 times higher in M than in I and S, respectively.

The odor thresholds of some compounds dissolved in sunflower oil were determined nasally and retronasally. The results are compiled in Table 7. The list of threshold values of all compounds selected for quantification was completed by data from the literature (references in Table 7).

The nasal threshold value was at least by a factor of 2 lower than the retronasal one in the case of the low molecular aldehydes **1**, **2**, **36**, and **38**, **3**, epoxydecenal (**31**), and **33**. The reverse was found for **6**, (*Z*)-3-hexenol (**12**), vinyl ketones **14** and **15**, ester **22**, **23**, **24**, and (*E*,*E*)-2,4-decadienal (**30**).

OAVs were calculated on the basis of the threshold values listed in Table 7. To explain the results in Table 6, we assume that compounds with OAVs greater than five on the basis of either nasal or retronasal odor threshold values contribute strongly to the flavor of an oil sample. 3-Methylbutanal (**1**), **3**, the green-smelling aldehydes **6**, **7**, and **9**, **16**, esters **10** and **22**, **23**, **29**, **30**, **31**, **33**, **36**, **38**, and 2-phenylethanol (**40**) belong to this group of odorants in oil I. In oil S, the OAV of the trace component **13** smelling black currantlike was high due to its very low odor threshold (Table 7). In addition, methylbutanals **1** and **2**, esters **4**, **8**, **10**, **11**, and **22**, **31**, **33**, **36**, **38**, and ethyl cinnamate (**41**) surpassed an OAV of five in oil S. The OAVs of the esters **4**, **8**, **10**, and **11** and in particular that of ester **22** were the highest in oil M, except for the OAV of **36**. Furthermore, **7**, **23**, decadienals **29** and **30**, epoxide **31**, **36**, and **38** were elucidated as important odorants of M.

In Table 8, the flavor profiles of the three virgin olive oils I, S, and M are confronted with those of the corresponding models I0, S0, and M0. In model I0 containing 25 odorants, 2 of the compounds showing higher OAVs (Table 6), (*E*,*Z*)-2,4-decadienal (**29**) and *trans*-4,5-epoxy-(*E*)-2-decenal (**31**), were omitted. Experiments not detailed here indicated that addition of the decadienal isomer to model I0 could not be perceived by the panelists, whereas addition of the epoxide led to

Table 9. Odor of Models for Oils I, S, and M as Affected by the Absence of Compounds

model[a]	omitted compd[b]	similarity[c]
I1	**36**, **38**	2.6 ± 0.2[a]
I2	**6**	2.2 ± 0.5
I3	**33**	2.2 ± 0.4
I4	**9**	2.1 ± 0.6
I5	**23**	2.0 ± 0.4
I6	**27**, **30**	2.0 ± 0.4
I7	**17**	2.0 ± 0.5
I8	**3**	1.9 ± 0.2[a]
I9	**7**, **12**	1.8 ± 0.5
S1	**36**/**38**	2.7 ± 0.3[b]
S2	**33**	2.4 ± 0.2[c]
S3	**1**, **2**	2.3 ± 0.4[d]
S4	**10**, **11**	2.3 ± 0.3[e]
S5	**13**	0.9 ± 0.2[b,c,d,e]
M1	**36**, **38**	2.5 ± 0.3[f]
M2	**24**	2.5 ± 0[g]
M3	**7**	2.2 ± 0.4
M4	**23**	2.2 ± 0.3[h]
M5	**29**	1.9 ± 0.6
M6	**14**	1.9 ± 0.5
M7	**22**	1.8 ± 0.6
M8	**8**, **4**, **10**, **11**	1.7 ± 0.6
M9	**19**	1.5 ± 0.3[f,g,h]

[a] The preparation of the models is detailed under Experimental Procedures. [b] The numbering of the compounds refers to Tables 3 and 4. [c] The similarity in the overall odor was determined nasally when comparing each reduced model with the corresponding complete model. Rating scale: 0 (no similarity) to 3 (identical with the complete model). Means ± standard deviations are given in the table. Values followed by the same common letter differ significantly ($P \leq 0.05$) from each other.

a slight off-odor in I0. These results prompted us to omit the two aldehydes also in models S0 and M0.

The flavor profile of model I0 was similar to that of the original (Table 8) because the green and applelike notes which were characteristic for oil I were clearly reproduced by the model.

Statistical evaluation of the data in Table 8 indicated that the black currantlike note in oil S as well as the note reminding us of black olives in oil M differed significantly from the other attributes which were used to describe the flavor profiles of the olive oil samples. These characteristic attributes of the flavor profiles of oils S and M were imitated by the corresponding models S0 and M0 containing 21 (S0) and 14 (M0) odorants, respectively. Of the three models, M0 was the closest to the original because it did not significantly differ ($P \leq 0.05$) when both were retronasally compared.

Models in which odorants were omitted were compared with the corresponding complete model for similarity. These omittance experiments, of which the results are listed in Table 9, were performed to evaluate the compounds causing the characteristic notes in the flavor profiles of the oils. Statistical tests indicated that the flavor of each of the reduced models presented in Table 9 differs significantly ($P \leq 0.05$) from the corresponding reference model I0, S0, or M0. However, these tests revealed also that the majority of the reduced models of one series did not differ significantly from each other. Therefore, in these cases, only tendencies can be discussed.

In models I1−I9 (Table 9), one or two components causing the same odor quality were omitted. The absence of **36** and **38** in I1 was noticed, but the similarity in the overall odor of I1 compared with I0 was only reduced by a score of 0.4 (Table 9). This result was surprising as **36** showed the highest OAV of all potent odorants occurring in oil I (Table 6). The absence of **6** and **33** in models I2 and I3 had a greater impact on the flavor. The similarity with the complete model I0 was lowered to a score of 2.2 (Table 9). A further decrease in similarity to scores of 1.9 and 1.8 was found when **3** and the two green-smelling C6 compounds **7** and **12** were omitted in models I8 and I9, respectively. In fact, **3** and **7** belonged to the odorants showing relative high OAVs in oil I (Table 6).

The odors of models S1−S5 lacking one or two odorants were compared with that of S0 (Table 9). Omission of **36** and **38** in S1, **33** in S2, the malty-smelling aldehydes **1** and **2** in S3, and the fruity esters **10** and **11** in S4 reduced the similarity to a score of 2.3 or higher. This means that the flavor was altered but the typical character of oil S was still recognizable. However, the absence of **13** in S5 caused a strong negative effect, indicating that this black currantlike smelling thiol, the concentration of which amounted to only 4.3 μg/kg in oil S (Table 6), was of paramount importance for the flavor of this oil sample. This conclusion was underlined by the statistical test indicating that model S5, in which thiol **13** was lacking, differed significantly from models S1−S4 (Table 9). The absence of **19** in M9 had the greatest effect on the flavor of M0 (Table 9). The similarity was strongly lowered because the note reminding us of eating black olives was not more clearly perceived. Among the autoxidation products of unsaturated fatty acids, which were checked in models M1−M6, **29** and **14** most strongly influenced the odor profile. These and other odorants omitted in models M2−M6 most likely contributed to the fatty character of oil M. The absence of **22** in M7 and of the fruity esters **4**, **8**, **10**, and **11** in M8 reduced the similarity with M0 to scores of 1.8 and 1.7, respectively (Table 9).

The successive combination of the compounds, whose absence had the strongest effect on the flavor of the model mixtures, revealed rather high scores for similarity between models containing 7, 6, or 9 substances and the complete model I0, S0, and M0 containing 25, 21, or 14 odorants, respectively. A similarity score of 2.2 was obtained when comparing I0 nasally with a model containing **7**, **3**, **17**, **30**, **23**, **9**, and **35**. The highest score for similarity (2.7) was obtained when comparing S0 with a mixture of **13**, **10**, **11**, **1** and **2** as well as **35**. A mixture of **19**, **4**, **10**, **11**, **22**, **14**, **30**, **23**, and **7** imitated M0 with a score of 2.3. Nevertheless, the typical black olive-like odor note had the same intensity as in M0 (2.4, cf. Table 8).

DISCUSSION

The three oil samples differed in their origin and also strongly in their flavor profiles. Nevertheless, the results presented here indicate that most of the potent odorants were identified in each oil. The great differences in the flavor profiles of the oils are mainly caused by concentration differences of these odorants. Oil M, for example, was the richest and oil I the poorest in the fruity esters **4**, **8**, **10**, **11**, and **22**. On the other hand, the green-smelling C6 aldehydes **6**, **7**, and **9** as well as **23** played a greater role in oil I than in the other oils. The levels of **36** and **38** were also higher in oil I. The potent odorant **13**, which has been identified by Rigaud et al. (1986) as the key flavor compound of black currant buds, occurred only in oil S, and on the basis of its high OAV and its black currantlike odor quality, it belonged

to the key odorants. This result and the statement that most of the potent odorants are detectable in virgin olive oils of different origin are supported by the results of previous studies (Guth and Grosch, 1991, 1993a; Blekas and Guth, 1995).

Of the potent odorants reported here and in earlier studies cited above, only **3**, the green-smelling aldehydes **6**, **7**, and **9**, **12**, **17**, and **33** have been identified by Morales et al. (1994, 1995, 1996) in 32 virgin olive oil samples of 6 European varieties. Morales et al. (1995, 1996) perceived a fruity note in the flavor profiles of the oil samples. By using statistical methods, they correlated fruitiness with 3-methylbutanal, 2-methylbutyl propanoate, 2-butanone, 2-nonanone, and ethenylbenzene. Of these compounds, only the ester smells clearly fruity, and the ketones smell soapy and fruity. The concentration of 2-butanone in the oil was 4 μg/kg. This value is so far below its odor threshold in water (23 mg/kg (Schnabel et al., 1988)) that 2-butanone cannot contribute to the flavor of olive oils. 2-Nonanone and the ester reported by Morales et al. (1995, 1996) were not confirmed as potent odorants of olive oils in our studies. On the other hand, the fruity esters **4**, **10**, and **11** identified here and in previous studies as important odorants of olive oils were not detected by Morales et al. (1995, 1996).

Furthermore, **16**, **24**, and the decadienals **29** and **30** were overlooked by Morales et al. (1994, 1995, 1996) in fresh virgin olive oils, although the concentrations of these aldehydes in oil samples are frequently so high that they appear as peaks in our gas chromatograms. In a recent study, Morales et al. (1997) detected these odorants in oxidized virgin olive oils as contributors to rancid off-odors. However, these odorants are also present in fresh oils. This conclusion is based on the results of Blekas et al. (1994), who had followed the formation of some potent odorants during maturation of olives. The olives were harvested in Greece at different stages of ripeness, the oils were immediately pressed, and the odorants were quantified by IDAs. It was found that **30** increased continuously in the oils between September and December from 37 to 440 μg/kg. The values of 422 (oil I) and 127 μg/kg (oil S) for **30** reported here lie in the concentration range of this odorant in a vigin olive oil obtained from ripe fruits. The amount of **30** was higher only in oil M (918 μg/kg).

Several potent odorants such as thiol **13** or C_8 vinyl ketones **14** and **15** as well as odorants **23**, **31**, and **32** occurred in the oil samples in such low concentrations that they did not appear as peaks in the gas chromatograms of the volatile fractions. These compounds were only detected by their odors which were perceived during HRGCO. Identification of these compounds afforded enrichment, e.g., by column chromatography and MDGC (cf. Table 1). Morales et al. (1994, 1995) have performed HRGCO, but no efforts were undertaken to enrich the odorants, which they perceived, and to identify them unequivocally by comparison with reference substances. The authors have not examined whether the reference substances can be detected by HRGCO at the concentration levels occurring in the volatile fraction of the oil samples. Only such an experiment will confirm that the odor perceived during HRGCO is indeed caused by the identified compound and not by an intense odorant which is present in such low concentrations that it is completely masked by the peak of the identified volatile compound.

Conclusions. The results indicate that the characteristic notes in the flavor profiles of the virgin olive oils I, S, and M could be imitated by mixing the potent odorants which were identified by instrumental analyses. Although the flavor profiles of the three oils were very different, most of the potent flavor compounds occurred in all oils but in different concentrations. It can be derived from this result and from those of previous studies that these odorants are generally present in virgin olive oils and that the differences in the flavor profiles are caused by concentration differences of these volatiles. Only some oils from Spain are excepted, as their special black currantlike flavor is caused by a thiol not occurring in other olive oils.

The identified potent odorants or mixtures of them are recommended as reference stimuli for the training of sensory test panels with regard to the perception of fruity, green, applelike, fatty, pungent, and black currantlike notes in the flavor profiles of olive oils.

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