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Solid-Phase Microextraction in the Analysis of Virgin Olive Oil Volatile Fraction: Characterization of Virgin Olive Oils from Two Distinct Geographical Areas of Northern Italy

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SPME was employed to characterize the volatile profile of virgin olive oils produced in two geographical areas of northern Italy: the region of the Gulf of Trieste and the area near Lake Garda. There are as yet no data on the headspace composition of virgin olive oils from these regions, characterized by particular conditions of growth for *Olea europaea*. Using the SPME technique coupled to GC-MS and GC-FID, the volatile components of 42 industrially produced virgin olive oil samples were identified and the principal compounds quantitatively analyzed. Significant differences in the proportion of volatile constituents from oils of different varieties and geographical origins were detected. The results suggest that besides the genetic factor, environmental conditions influence the volatile formation.

KEYWORDS: Virgin olive oil; volatiles; solid-phase microextraction; SPME, characterization; aroma

INTRODUCTION

Virgin olive oil has a characteristic flavor that distinguishes it from other edible vegetable oils. After its extraction from the fruit of *Olea europea* L., extra virgin olive oil can be consumed without refining and it preserves its typical aroma. This characteristic aroma and, in particular, the oil's green and fruity attributes depend on many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway taking place during the oil extraction process (*1*−*3*). Variable amounts of hexanal, hexanol, and hexyl acetate derive from the degradation of linoleic acid, whereas (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate result from the enzymatic degradation of linolenic acid (*4*, *5*). The volatile profiles of oils are believed to be influenced by factors such as the cultivar of the olives, climate, soil quality, degree of ripeness of the fruit, and oil extraction process (*1*, *5*−*8*).

In the present study, headspace solid-phase microextraction (HS-SPME) was employed to define the volatile profile of virgin olive oils produced in two geographical areas of northern Italy: the region of to the Gulf of Trieste and the area near Lake Garda. Although both geographical areas are situated at quite high latitudes, they are characterized by a temperate climate due to the proximity of the sea and the lake, respectively; this enables the growth and fructification of *O. europaea* plants.

Due to the particular growing conditions created by this environment, the oils produced in these regions are thought to possess a characteristic aroma profile. However, there are as yet no data on the headspace composition of oils from these regions. Using the SPME technique, the components of the volatile fraction of 42 industrially produced virgin olive oil samples were identified or tentatively identified and the principal compounds quantitatively determined.

MATERIALS AND METHODS

Samples. The virgin olive oils studied were from two different geographical areas of northeastern Italy and were produced from olives of various cultivars harvested in 2001. The harvesting period in both of the areas was from the middle of October to the middle of November, and the olives were stored during a maximum of 1 week. The oils were principally extracted by three-phase continuous systems equipped with metallic crushers. The samples of group 1 corresponded to oils obtained from olives grown in the area of the Gulf of Trieste, whereas those of group 2 were from the Lake Garda area. The samples from the Gulf of Trieste were from olives of Bianchera ($n = 8$), Frantoio ($n = 4$), and Leccino ($n = 4$) varieties and from a blend of various cultivars ($n = 7$) produced at industrial scale (mean = 1300 kg of olives for each oil stock), whereas the samples from the Lake Garda area were produced from Casaliva ($n = 5$), Frantoio ($n = 4$), Leccino ($n = 4$), and Grignano ($n = 6$) olives. All of the analyzed samples pertained to the extra virgin olive oil commercial class, according to European Commission (EU) regulations (*9*).

SPME Sampling. The most suitable SPME sampling conditions were investigated in a previous study (*10*). Briefly, the oil was spiked

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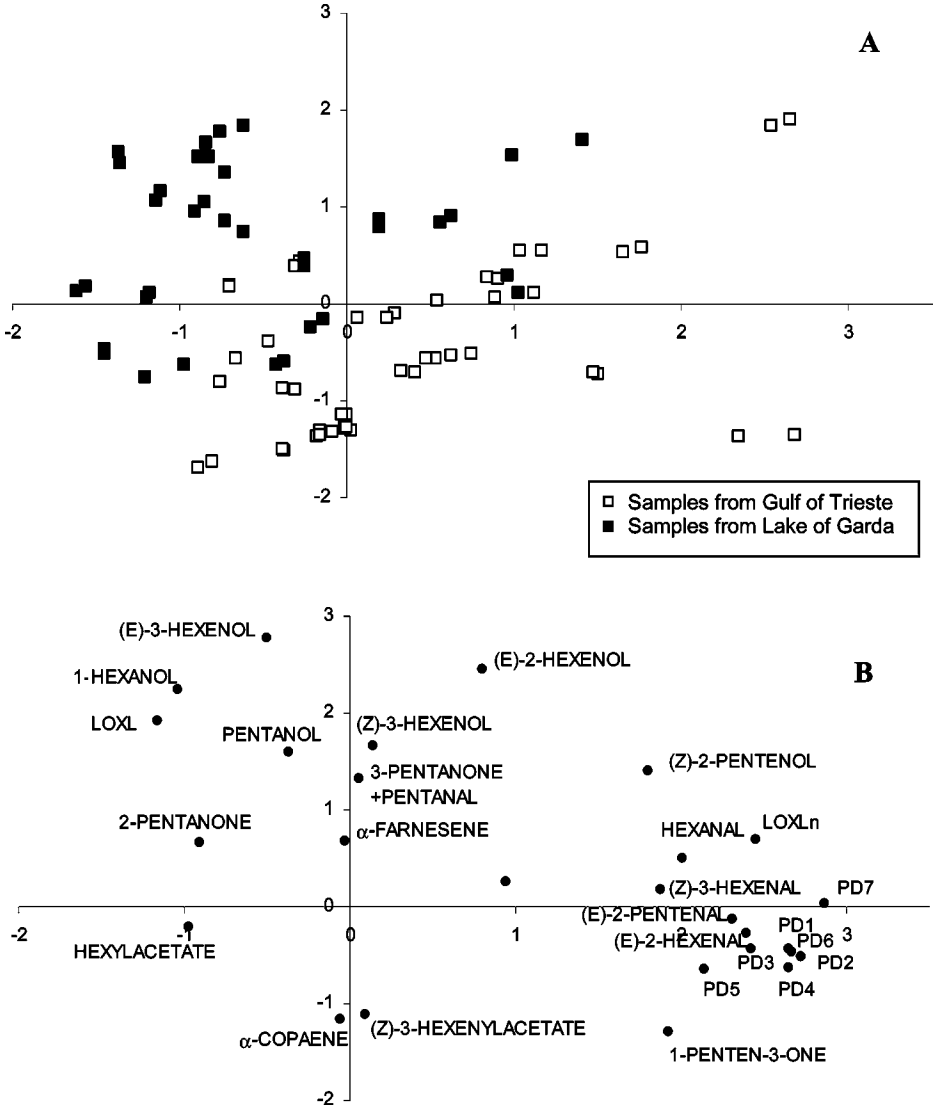

Figure 1. Principal component analysis of volatile compounds: (A) scatterplot of samples from Gulf of Trieste and Lake Garda; (B) plot of component weights. PD1, 1,5-hexadiene, 3,4-diethyl (*R*,*S* + *S*,*R*); PD2, *meso*-1,5-hexadiene, 3,4-diethyl; PD3, 1,5-octadiene, 3-ethyl (*E* or *Z*); PD4, 1,5-octadiene, 3-ethyl (*E* or *Z*); PD5, 3,7-decadiene (*EE*, *ZZ*, or *EZ*); PD6, 3,7-decadiene (*EE*, *ZZ*, or *EZ*); PD7, 3,7-decadiene (*EE*, *ZZ*, or *EZ*).

with 4-methyl-2-pentanol (Sigma-Aldrich, St. Louis, MO) to a concentration of 1.5 μg/g, and 1.5 g of the sample was weighed into a 10 mL vial fitted with a silicone septum. The sample was placed in a water bath at 40 °C under magnetic stirring, and a divinylbenzene/Carboxen/polydimethylsiloxane fiber (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) was maintained for 30 min in the sample headspace. The volatile compounds of the fiber were desorbed for 1 min at 260 °C in the gas chromatograph injection port.

GC-FID and GC-MS Analysis. GC analyses were performed in two Hewlett-Packard 5890 series II gas chromatographs, one equipped with an FID detector and one coupled to a Hewlett-Packard 5971A quadrupole mass selective spectrometer. Both were provided with a split−splitless injection port. Helium was the gas carrier, at linear velocities of 23 and 17 cm^3/s for GC-FID and GC-MS, respectively.

Separation of compounds was performed by two columns of different polarities: Supelcowax-10 and SPB-1 (both 30 m × 0.25 mm i.d., 0.25 μm film thickness), purchased from Supelco Ltd. Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C/min. The FID temperature was set at 280 °C, and the temperatures of the ion source and the transfer line were 175 and 280 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 15−250 amu mass range, 2 scan/s.

Quantitative analysis was carried out in duplicate by HS-SPME/GC-FID, and relative amounts of volatile compounds were calculated by using the internal standard method. For the main volatiles from the lipoxygenase pathway, the concentration was calculated by using the experimental relative response factors reported in ref *10*. The remaining compounds were quantified by considering the relative response factor to be 1 and were expressed as micrograms per gram equivalents of 4-methyl-2-pentanol.

Qualitative analysis of the volatile fraction was performed by means of HS-SPME/GC-MS analysis. Volatile compounds present in the headspace of the virgin olive oils under study were tentatively identified by matching their mass spectra with the reference mass spectra of the Wiley 6.0 library and checked by comparing the retention indices calculated in two capillary columns of distinct polarity with those reported in the literature (**Table 1**). In several cases, the identification was based on the comparison with standard compounds.

Seven pentene dimers were identified in accordance with the characterization proposed by Angerosa et al. (*16*), as reported in a previous study (*10*). A number of compounds were tentatively identified on the basis of their mass spectra alone.

The volatiles included a large number of hydrocarbons, aldehydes, alcohols, ketones, esters, and other heterogeneous compounds. The compounds reported in **Table 1** were detected in at least 80% of the samples except *m*-xylene, isoamyl acetate, and trichloroethylene, which were detected in 66, 64, and 54% of the samples, respectively. The sensory characteristics of quite a large number of these are well-known, and in terms of their relative odor thresholds and concentrations some

Table 1. Identification of the Volatile Compounds in Virgin Olive Oils

compound	RI[h]		RI refs		ID[h]	content[a]	
	SW[h]	SPB-1[h]	SW	SPB-1		min	max
hydrocarbons							
octane	803	800			*b*, *c*	0.057	0.687
1-octene	837	785	850 (*11*)		*c*	tr[h]	0.155
(*E*)-2-octene	864	811	880 (*12*)	811 (*14*)	*c*	0.002	0.024
nonane	901	900			*b*, *c*	0.004	0.216
1,5-hexadiene, 3,4-diethyl (*R*,*S* + *S*,*R*)	952	nd[h]			*d*	0.011	0.110
meso-1,5-hexadiene, 3,4-diethyl	959	nd			*d*	0.012	0.258
decane	999	1000			*b*, *c*	tr	1.037
1,5-octadiene, 3-ethyl (*E* or *Z*)	1006	nd			*d*	0.058	1.127
1,5-octadiene, 3-ethyl (*E* or *Z*)	1017	nd			*d*	0.059	1.511
toluene	1026	742	1042 (*13*)	756 (*15*)	*c*	0.057	0.513
3,7-decadiene (*EE*, *ZZ*, or *EZ*)	1068	nd			*d*	tr	0.456
3,7-decadiene (*EE*, *ZZ*, or *EZ*)	1076	nd			*d*	0.021	0.978
3,7-decadiene (*EE*, *ZZ*, or *EZ*)	1078	nd			*d*	0.050	1.046
undecane	1098	1100			*b*, *c*	0.001	0.167
ethylbenzene	1114	829	1116 (*11*)		*b*, *c*	tr	
m-xylene	1126	848	1140 (*12*)	860 (*14*)	*b*, *c*	tr	0.294
p-xylene	1129	848			*b*, *c*	0.033	0.593
o-xylene	1171	873	1183 (*13*)	884 (*14*)	*b*, *c*	0.023	0.417
ni[h] (hydrocarbon)	1242	1196			*c*	0.003	1.057
styrene	nd	870	1307 (*12*)	1012 (*14*)	*b*, *c*	0.011	0.537
tridecane	1300	1300			*b*, *c*	tr	0.028
(*E*)-4,8-dimethyl-1,3,7-nonatriene[e]	1303	1106			*c*	0.037	2.574
aldehydes							
3-methylbutanal	911	637	910 (*13*)	641 (*15*)	*b*, *c*	tr	0.161
2-methylbutanal	913	645	1001 (*13*)	639 (*15*)	*c*	tr	0.177
pentanal[f]	970	672	971 (*11*)		*b*, *c*	0.153	1.053
hexanal[g]	1073	771	1084 (*12*)	772 (*15*)	*b*, *c*	0.169	6.367
(*E*)-2-pentenal	1121	724	1131 (*13*)	766 (*15*)	*c*	tr	0.242
(*Z*)-3-hexenal	1133	nd	1126 (*11*)	795 (*15*)	*c*	tr	0.574
heptanal	1180	881	1186 (*12*)	885 (*15*)	*b*, *c*	tr	0.151
(*E*)-2-hexenal[g]	1211	824	1207 (*12*)	826 (*15*)	*b*, *c*	1.135	148.700
octanal	1281	984	1278 (*12*)	982 (*15*)	*b*, *c*	0.015	0.533
(*E*)-2-heptenal	1313	934	1314 (*11*)	954 (*15*)	*c*	tr	0.216
nonanal	1389	1084	1385 (*13*)	1079 (*15*)	*b*, *c*	0.058	0.639
(*E*,*Z*)-2,4-hexadienal[e]	1391	884			*c*	tr	0.706
(*E*,*E*)-2,4-hexadienal	1395	884	1395 (*11*)		*c*	tr	0.937
(*E*,*Z*)-2,4-heptadienal	1458	973	1454 (*11*)	1000 (*15*)	*c*	tr	0.375
(*E*,*E*)-2,4-heptadienal	1485	973	1483 (*11*)		*c*	tr	
benzaldehyde	1512	nd	1507 (*11*)		*c*	0.021	0.217
cinnamaldehyde[e]	nd	1179			*c*	0.004	0.024
alcohols							
ethanol	927	551	929 (*13*)	500 (*14*)	*b*, *c*	0.007	6.575
isobutanol[e]	1086	nd			*c*	tr	
1-penten-3-ol	1157	666	1157 (*13*)	673 (*14*)	*c*	0.025	0.323
IS (4-methyl-2-pentanol)	1167	737			*b*, *c*		
3-methylbutanol	1204	718	1205 (*13*)	736 (*15*)	*b*, *c*	0.004	5.149
2-methylbutanol	1204	721	1208 (*13*)	843 (*15*)	*b*, *c*	tr	2.809
1-pentanol	1248	747	1255 (*13*)	747 (*13*)	*c*	tr	0.349
(*E*)-2-pentenol	1307	nd	1327 (*14*)		*c*	tr	
(*Z*)-2-pentenol	1314	749	1336 (*14*)		*c*	0.060	0.815
1-hexanol[g]	1350	858	1360 (*13*)	858 (*13*)	*b*, *c*	0.685	50.200
(*E*)-3-hexenol	1359	836			*b*, *c*	0.010	0.310
(*Z*)-3-hexenol[g]	1378	838	1391 (*13*)	844 (*13*)	*b*, *c*	0.252	8.587
(*E*)-2-hexenol[g]	1400	853	1377 (*13*)	854 (*12*)	*b*, *c*	1.118	89.100
(*Z*)-2-hexenol	1417	855			*b*, *c*	0.002	0.195
heptanol	1451	962			*b*, *c*	tr	0.245
1-octanol	1554	1061	1553 (*13*)	1061 (*12*)	*b*, *c*	0.010	0.148
benzyl alcohol	1861	1006	1865 (*13*)	1033 (*12*)	*c*	0.001	0.305
phenylethyl alcohol	1894	1082	1859 (*12*)	1104 (*12*)	*c*	0.010	1.086
phenol[e]	1995	nd			*c*	tr	0.186
methoxyphenol[e]	nd	1058			*c*	tr	
ethylphenol[e]	nd	1145				tr	
ketones					*c*		
2-propanone[e]	816	nd			*c*	tr	0.522
2-pentanone	969	655	971 (*11*)		*c*	tr	3.200
3-pentanone[f]	970	675	984 (*13*)	619 (*13*)	*c*	0.153	1.053
1-penten-3-one	1013	662	1014 (*11*)	680 (*13*)	*c*	tr	0.634
2-heptanone	1177	872	1170 (*13*)	872 (*13*)	*c*	tr	0.093
2-octanone	1279	973	1285 (*13*)	982 (*13*)	*c*	tr	
6-methyl-5-hepten-2-one	1332	968	1336 (*13*)	965 (*13*)	*b*, *c*	0.016	0.193

Table 1 (Continued)

compound	RI[h] SW[h]	RI[h] SPB-1[h]	RI refs SW	RI refs SPB-1	ID[h]	content[a] min	content[a] max
carboxylic acids							
acetic acid	1442	617	1450 (*13*)	710 (*13*)	*b*, *c*	0.039	37.534
propanoic acid[e]	1531	nd			*c*	tr	0.561
butanoic acid[e]	1620	nd			*c*	tr	0.193
isovaleric acid[e]	1663	nd			*c*	tr	1.076
hexanoic acid	1835	nd	1850 (*13*)	890 (*13*)	*b*, *c*	0.012	0.984
(*E*)-2-hexenoic acid[e]	1969	nd			*c*	0.014	1.525
esters							
methyl acetate	826	566	813 (*12*)	513 (*12*)	*c*	tr	2.444
ethyl acetate	890	612	872 (*13*)	595 (*12*)	*c*	tr	13.390
isoamyl acetate	1118	864	1110 (*12*)	860 (*12*)	*c*	tr	0.536
hexyl acetate [g]	1269	999	1307 (*12*)	1012 (*12*)	*b*, *c*	tr	27.523
(*Z*)-3-hexenyl acetate[g]	1312	991	1300 (*12*)	988 (*12*)	*b*, *c*	0.815	55.369
methyl salicylate[e]	1760	1164			*c*	0.002	0.078
ethyl hexanoate[e]	nd	987			*c*	tr	
others							
1-methoxyhexane[e]	940	818			*c*	tr	1.600
trichloroethylene	984	680			*b*, *c*	tr	1.877
1-methyl-3-(hydroxyethyl)propadiene[e]	1194	819			*c*	tr	1.083
limonene	1186	1016	1178 (*13*)	1022 (*13*)	*b*, *c*	tr	0.156
β-ocimene	1247	1040	1242 (*13*)	1043 (*13*)	*c*	0.066	2.586
α-pinene	nd	927	1032 (*13*)	920 (*13*)	*c*	0.017	0.575
menthatriene[e]	1439	1116			*c*	tr	0.067
α-copaene	1482	1390	1488 (*15*)	1398 (*12*)	*c*	0.006	0.325
α-muurolene	1718	nd	1722 (*15*)		*c*	tr	0.058
α-farnesene	1745	1491	1751 (*15*)	1515 (*15*)	*c*	0.012	0.479

[a] Data expressed as μg/g equivalents of 4-methyl-2-pentanol where no differently specified. [b] Identified by comparison with standard compounds. [c] Tentatively identified by Wiley 6.0 mass spectra library search and comparison of retention index. [d] Tentatively identified according to the method of Angerosa et al. (*17*). [e] Tentatively identified only by means of mass spectral database. [f] Sum of pentanal and 3-pentanone. [g] Expressed as μg/g by using the experimental response factor. [h] RI, Kovats retention index; SW, polar capillary column (Supelcowax-10); SPB-1, apolar capillary column (SPB-1); ID, identification method; ni, not identified; nd, not detected; tr, traces

of these compounds have been shown to be linked to the aromatic properties of virgin olive oil (*2*, *17*).

Statistical Analysis. Statistical treatment of data was carried out using the Statistica 5.0 (StatSoft '97 edition) and the Statgraphics 4.1 (Statistical Graphic Corp. 1994−1999) packages.

Differences between the two groups of samples of different origins were identified by applying the Student *t* test for independent samples, whereas differences among the various cultivars were studied by analysis of variance (one-way ANOVA). Significant results were considered at $p \leq 0.05$.

Discriminant analysis was performed to classify the samples into groups according to their origins. The cultivar was considered as a variable in order to cover a wide range of characteristics related to the geographical origin. Thus, eight groups of oils were considered on the basis of the variety and by the geographical origin. The capability of the analytical method to provide data for predicting which group a new case is likely to fall into was evaluated, and a number of useful predictor variables were investigated. The analysis of volatile compounds could be a useful instrument of classification and autentication of virgin olive oils in terms of the definition of a brand of origin.

RESULTS AND DISCUSSION

Volatile C6 Compounds from the Lipoxygenase Pathway. The major components of the volatile fraction of virgin olive oils, the main cause of the green odor note, are the C6 compounds, which derive from the cascade of enzymatic reactions starting with the formation, by lipoxygenase action, of 13-hydroperoxides from linoleic and linolenic acid (*4*, *5*, *18*).

Table 2 shows the results of the quantitative analysis of compounds from the lipoxygenase pathway. These results were obtained by using the experimental relative response factor and are expressed in micrograms per gram.

The different accumulations of metabolites from the lipoxygenase cascade have been reported by other authors (*1*, *6*, *7*) to be highly dependent on the levels of enzymes involved, the extraction conditions, the storage time of olives, and the degree of ripening (*1*, *6*, *8*, *18*, *19*). In addition, climatic and environmental growth conditions may also influence the production of volatiles (*1*, *6*, *20*). As the harvesting period and extraction conditions were similar for the samples studied, principal component analysis of bioformation volatiles was performed to evaluate the influence of the geographical origin: Gulf of Trieste and Lake Garda.

Among the C6 compounds, hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenal distinguished the samples from the Gulf of Trieste, whereas the corresponding alcohols, 1-hexanol, (*E*)-2-hexenol, (*Z*)-3-hexenol, and (*E*)-3-hexenol, characterized the samples from Lake Garda (**Figure 1**). The overall amounts of aldehydes were higher than the sum of alcohols in group 1 samples, as reported for virgin olive oil analyzed by other authors (*7*), whereas in group 2 the sum of C6 alcohols was clearly higher than that of C6 aldehydes (**Table 2**). These results may be explained by differential activity of the enzyme alcohol dehydrogenase (ADH), which reduces the C6 aldehydic compounds in the corresponding alcohols; in the case of the samples studied here it appears to be more active in samples from Lake Garda. Thus, levels of hexanal, 1-hexenol, (*E*)-2-hexenal, (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*E*)-3-hexenol, and (*E*)-2-hexenol showed a strong dependence on geographical origin (**Figure 1**), suggesting the influence of environmental growth conditions on the activity of ADH.

(*Z*)-3-Hexenyl acetate seems to characterize the oils from the Gulf of Trieste, whereas the amounts of hexyl acetate were not significantly different between zones (**Figure 1**). It can therefore be hypothesised that levels of alcohol acetyl transferase (AAT) are less dependent on pedoclimatic conditions.

Table 2. Mean Concentrations of Compounds from the Lipoxygenase Pathway (Expressed in Micrograms per Gram); Differences between Distinct Cultivars[a]

group 1 (Gulf of Trieste)	Frantoio ($n = 4$)	Leccino ($n = 4$)	Bianchera ($n = 8$)	blend ($n = 7$)	mean
hexanal	1.6	0.7	1.3	1.9	1.4□
1-hexanol	6.4	1.5	3.8	6.2	4.5□
hexyl acetate	1.0a	1.1a	5.9b	0.9a	2.2
sum LOXL	9.0	3.3	11.0	9.0	8.1□
(*E*)-2-hexenal	84.6a	20.7b	53.6ab	69.0a	57.0□
(*E*)-2-hexenol	15.6	3.1	9.8	17.6	11.5□
(*Z*)-3-hexenol	3.5	4.0	1.5	2.3	2.8
(*Z*)-3-hexenyl acetate	4.6	9.8	14.8	7.0	9.0□
sum LOXLn	100.3a	23.6b	63.7a	86.9a	80.3□

group 2 (Lake Garda)	Frantoio ($n = 4$)	Leccino ($n = 4$)	Grignano ($n = 5$)	Casaliva ($n = 6$)	mean
hexanal	0.7	0.9	0.7	0.9	0.8●
1-hexanol	16.7	31.3	17.9	14.0	19.9●
hexyl acetate	1.9a	3.6a	10.2b	2.3a	4.5
sum LOXL	19.3	35.8	28.7	17.2	25.2●
(*E*)-2-hexenal	21.9	25.6	30.8	25.0	25.8●
(*E*)-2-hexenol	22.1a	53.8b	18.6a	20.2a	28.7●
(*Z*)-3-hexenol	3.8	3.2	3.6	1.8	3.1
(*Z*)-3-hexenyl acetate	5.9ab	3.5a	7.5b	3.3a	5.0●
sum LOXLn	53.7	86.1	60.5	50.3	62.6●

[a] Values with different letters indicate significant differences among cultivars. Values with different symbols indicate significant difference between areas of origin. LOXLn, LOXL, volatile compounds from lipoxygenase pathway having as precursors linolenic and linoleic acid, respectively.

When the sum of compounds derived from the different branches of the lipoxygenase cascade (**Figure 1**) was considered, in accordance with the findings of other authors (*7*), volatiles from linonenic acid were found in all of the samples in higher amounts than were compounds from linoleic acid (**Table 2**). However, samples from the Gulf of Trieste showed higher concentrations of compounds derived from linolenic acid than did those from Lake Garda, (*E*)-2-hexenal being the main compound found. In group 2 the formation of compounds derived from linoleic acid was favored with respect to group 1, in particular due to the formation of 1-hexanol.

The increased amounts of 1-hexanol and the low levels of C6 aldehydes and (*Z*)-3-hexenyl acetate in group 2 could be explained by a slight over-ripeness of olives, as suggested by Aparicio et al. (*6*). However, the degree of ripeness does not account for the higher amounts of (*E*)-2-hexenol in the same samples, because over-ripeness should lead to a drastic decrease in levels of this compound. Therefore, climate and environmental conditions probably not only have an indirect effect, acting on the degree of ripeness but also directly influence the production of volatiles, as some authors have suggested (*5*, *6*). Indeed, it was demonstrated that parameters such as growth temperature and pH of the medium [to be referred to the soil quality (*5*)] influence both quantitative and qualitative production of volatiles from olive tissue, reflecting an alteration in the amounts or activity of enzymes of the lipoxygenase pathway (*6*).

The effect of using different cultivars on the differences of volatile composition observed between oils of distinct geographical areas was also evaluated and considered as a variable. As shown in **Table 2**, quantitative differences were found among some cultivars, but the use of different varieties in the geographic areas under study does not seem to be the cause of the oils' differentiation according to their origins. These results confirm that the main effect on volatile compound differences is related with the geographic area of origin.

Volatile C5 Compounds. Considerable amounts of various classes of C5 volatile compounds were found in the oils examined in the present study (**Table 1**). Pentene dimers, pentenols, and C5 carbonyl compounds were proposed by Angerosa et al. (*16*) to be products of the bioformation of olive oil aroma. They are thought to derive from the hydroxylation or dimerization of pentene radicals originated by β-scission of alkoxy radicals formed from 13-hydroperoxides by an enzyme-mediated mechanism (*16*).

The quantitative analysis was carried out by applying a relative response factor equal to 1 (**Table 1**) and revealed abundant amounts of compounds identified as pentene dimers. Comparison of the groups of samples of different origin showed the levels of these dimers to be significantly higher in oils from the Gulf of Trieste, as pointed out by principal component analysis (**Figure 1**). (*E*)-2-Pentenal and 1-penten-3-one were also characteristic of group 1 samples, whereas pentanol, 2-pentanone, and the sum of 3-pentanone and pentanal proved to be significantly more abundant in samples of group 2 (**Figure 1**). No differences were observed for these compounds with respect to the cultivar in either group, suggesting that the factors related to the geographical growth area are the main influence on the formation of these compounds.

Minor Volatile Compounds. The hydrocarbons of olive oil have been studied by different authors as possible markers to distinguish virgin olive oil from different olive varieties or geographical origin (*15*, *21*, *22*). In oils from different varieties great differences were found mainly in the content of α-copaene, α-farnesene, and hydrocarbons with >20 carbon atoms (*21*). Moreover, pedoclimatic conditions seem to influence the contents of α-copaene (*22*). In oils from the Gulf of Trieste (**Figure 1**) the amounts of α-copaene were significantly higher than in oils from Lake Garda (mean values of 0.074 and 0.027 μg/g equiv of 4-methyl-2-pentanol, respectively), whereas no differences in α-farnesene content were observed between oils from different geographical areas. Also, the levels of the alkanes from C_9 to C_{13} found in the samples did not allow their differentiation.

Other minor volatile compounds were observed in the virgin olive oils studied. Among them, the hydrocarbons octane and octene and the aldehydes heptanal, octanal, nonanal, (*E*)-2-heptenal, and 2,4-heptadienal isomers are due to the autoxidation reactions (*23*) that inevitably start after the virgin olive oil has been extracted. However, in the extra virgin olive oils analyzed the amounts of compounds formed from oxidation reactions were quite low (**Table 1**).

Some products deriving from sugar fermentation and amino acid transformation were also found. They were ethanol, ethyl acetate, and acetic acid (*24*) and branched aldehydes, alcohols, and acids, respectively. The latter are thought to be produced by molds during olive fruit storage (*24*).

Quite low amounts of volatile aromatic hydrocarbons such as toluene, ethylbenzene, xylene isomers, and styrene were found in the headspace of olive oils. The origin of these compounds in virgin olive oil is largely unknown; some of them are components of vehicular and industrial emissions, classified as priority environmental pollutants by the U.S. Environmental Protection Agency (EPA). Some studies on the presence of these aromatic hydrocarbons in virgin olive oil have been carried out

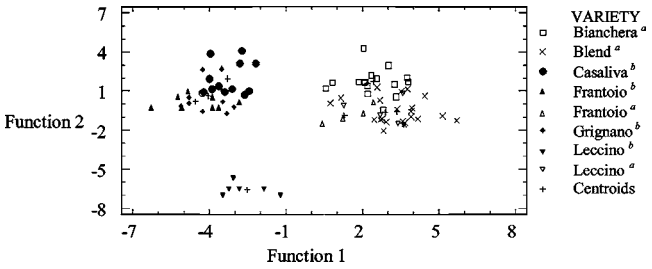

Figure 2. Discriminant functions used to classify the virgin olive oils by variety and geographical origin according to their volatile composition. [a] Samples from Gulf of Trieste. [b] Samples from Lake Garda.

by other authors, revealing that they could possibly arise from both exogenous contamination and endogenous pathways (*25*, *26*).

Classification of Virgin Olive Oils According to Their Origin. Figure 2 displays the plot of the discriminant analysis. The oils from a distinct geographical origin were clearly distinguished. The percentages of correct classification of the samples in base on their geographical origin were 100%. Even if the classification of some cultivar was not 100% correct (data not shown), the prediction was into the correct group of geographical origin.

In conclusion, the application of SPME to the analysis of virgin olive oil headspace allowed the detection of significant differences in the proportion of volatile constituents from oils of different varieties and geographical origins. The results indicate that besides the genetic factor, environmental conditions influence the volatile production. However, the study of a larger number of samples from various years of production would lend support to the results obtained by this first screening.

LITERATURE CITED

(1) Montedoro, G.; Bertuccioli, M.; Anichini, F. Aroma analysis of virgin olive oil by headspace (volatiles) and extraction (polifenols) techniques. In *Flavor of Food and Beverages: Chemistry and Technology*; Charalambous, G., Inglett, G. E., Eds.; Academic Press: New York, 1978; pp 247−281.

(2) Morales, M. Y.; Alonso, M. V.; Ríos, J. J.; Aparicio, R. Virgin Olive Oil Aroma: Relationship between Volatile Compounds and Sensory Attributes by Chemometrics. *J. Agric. Food Chem.* **1995**, *43*, 2925−2931.

(3) Angerosa, F.; Mostallino, R.; Basti, C.; Vito, R. Virgin olive oil odour notes: their relationship with volatile compounds from lipoxygenase pathway and secoiridoid compounds. *Food Chem.* **2000**, *68*, 283−287.

(4) Olías, J. M.; Pérez, A. G.; Ríos, J. J.; Sanz, L. C. Aroma of Virgin Olive Oil: Biogenesis of the "Green" Odor Notes. *J. Agric. Food Chem.* **1993**, *41*, 2368−2373.

(5) Williams, M.; Morales, M. T.; Aparicio, R.; Harwood, J. L. Analysis of volatiles from callus cultures of olive *Olea europaea*. *Phytochemistry* **1998**, *47*, 1253−1259.

(6) Aparicio, R.; Morales, M. T. Characterization of Olive Ripeness by Green Aroma Compounds of Virgin Olive Oil. *J. Agric. Food Chem.* **1998**, *46*, 1116−1122.

(7) Angerosa, F.; Basti, C.; Vito, R. Virgin Olive Oil Volatile Compounds from Lipoxygenase Pathway and Characterization of Some Italian Cultivars. *J. Agric. Food Chem.* **1999**, *47*, 836−839.

(8) Morales, M. T.; Aparicio, R. Effect of Extraction Conditions on Sensory Quality of Virgin Olive Oil. *J. Am. Oil Chem. Soc.* **1999**, *76*, 295−300.

(9) European Commission. Regulation 796/2002. *Off. J. Eur. Communities* **2002**.

(10) Vichi, S.; Castellote, A. I.; Pizzale, L.; Conte, L. S.; Buxaderas, S.; Lopez-Tamames, E. Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. *J. Chromatogr. A* **2003**, *983*, 19−33.

(11) Horiuchi, M.; Umano, K.; Shibamoto, T. Analysis of Volatile Compounds Formed from Fish Oil Heated with Cysteine and Trimethylamine Oxide. *J. Agric. Food Chem.* **1998**, *46*, 5232−5237.

(12) Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980.

(13) http://nysaes.cornell.edu/fst/faculty/acree/flavornet.

(14) Shimoda, M.; Peralta, R. R.; Osajima, Y. Headspace Gas Analysis of Fish Sauce. *J. Agric. Food Chem.* **1996**, *44*, 3601−3605.

(15) Bortolomeazzi, R.; Berno, P.; Pizzale, L.; Conte, L. S. Sesquiterpene, Alkene, and Alkane Hydrocarbons in Virgin Olive Oils of Different Varieties and Geographical Origins. *J. Agric. Food Chem.* **2001**, *49*, 3278−3283.

(16) Angerosa, F.; Camera, L.; d'Alessandro, N.; Mellerio, G. Characterization of Seven New Hydrocarbon Compounds Present in the Aroma of Virgin Olive Oils. *J. Agric. Food Chem.* **1998**, *46*, 648−653.

(17) Reiners, J.; Grosch, W. Odorants of Virgin Olive Oil with Different Flavor Profiles. *J. Agric. Food Chem.* **1998**, *46*, 2754−2763.

(18) Angerosa, F.; d'Alessandro, N.; Basti, C.; Vito, R. Biogeneration of Volatile Compounds in Virgin Olive Oil: Their Evolution in Relation to Malaxation Time. *J. Agric. Food Chem.* **1998**, *46*, 2940−2944.

(19) Salas, J. J.; Sánchez, J. The Decrease of Virgin Olive Oil Flavor Produced by High Malaxation Temperature is due to Inactivation of Hydroperoxyde Lyase. *J. Agric. Food Chem.* **1999**, *47*, 809−812.

(20) Servili, M., et al. Volatile Compounds of Virgin Olive Oil Evaluated by Solid-Phase Microextraction: an Application in the Discrimination of Virgin Olive Oils According to the Cultivar and Area. In *Flavour and Fragrance Chemistry*; Lanzotti, V., Taglialatela-Scafati, Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; Vol. 46, pp 211−220.

(21) Guinda, A.; Lanzón, A.; Albi, T. Differences in Hydrocarbons of Virgin Olive Oils Obtained from Several Olive Varieties. *J. Agric. Food Chem.* **1996**, *44*, 1723−1726.

(22) Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 614−627.

(23) Morales, M. T.; Ríos, J. J.; Aparicio, R. Changes in the Volatile Composition of Virgin Olive Oil during Oxidation: Flavors and Off-Flavors. *J. Agric. Food Chem.* **1997**, *45*, 2666−2673.

(24) Angerosa, F. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 639−660.

(25) Biedermann, M.; Grob, K.; Morchio, G. On the origin of benzene, toluene, ethylbenzene and xylene in extra virgin olive oil. *Z. Lebensm. Unters. Forsch.* **1995**, *200*, 266−272.

(26) Morchio, G.; Spadone, J.; Braco, U. Volatile aromatic hydrocarbons (VAHs) in edible vegetable oils with particular reference to virgin olive oil. *Riv. Ital. Sostanze Grasse* **1994**, *71*, 491−502.

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