

Volatiles from Leaves, Fruits, and Virgin Oil from *Olea europaea* Cv. Olivastra Seggiane from Italy[†]

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The volatiles produced by leaves and fruits of *Olea europaea* cv. Olivastra Seggiane have been analyzed in two different phenological stages. Furthermore, the volatiles of the virgin olive oil obtained from ripe fruits has been characterized. The volatiles were sampled by means of two different techniques: hydrodistillation and SPME. Differences were observed between the two different collection times, the different organs, and sampling techniques. The major constituents were often aldehydes, particularly (*E*)-2-hexenal (9.8–48.0%); however, also many terpenoids have been identified, mainly (*E,E*)- α -farnesene (0.2–27.0%), linalool (0–3.6%), β -caryophyllene (0–8.1%), and valencene (0–2.5%). This is the first investigation on this cultivar.

KEYWORDS: *Olea europaea*; Olivastra Seggiane; volatiles; aldehydes; terpenes; leaves; fruits; virgin olive oil; hydrodistillation; SPME

INTRODUCTION

The IGP label (Geographic Protected Indication) is referred, according to European Community Regulations 2081/92, to a particular geographic area and designates agricultural products or foodstuffs produced in that area. Geographical indications are valuable to producers from particular regions because they are source identifiers; that is, they identify goods as originating in a particular territory or in a region or locality in that territory. Geographical indications are also indicators of quality; they let consumers know that the goods come from an area where a given quality, reputation, or other characteristic of the goods is essentially attributable to their geographic origin. In addition, they are business interests because they promote the goods of a particular area. These indicators are recognized also by the World Trade Organization (WTO). The utilization of the IGP label is subordinate to severe controls of the observance of production rules.

The samples used in this study have been collected within the area that received the IGP labels CE no. 644 (20-03-98) and DM (21-07-98), and the virgin olive oil has been named "Olio extravergine di oliva Toscano 'Seggiano'". This is the sole Italian virgin oil that gained the IGP label. The production rules provide for the production of this oil to use only olives obtained from the cultivar Olivastra Seggiane.

This cultivar (syn. Seggianina, Olivastra di Montalcino, Olivo dell'Amiata) has its origins in the Monte Amiata area (southern Tuscany, Italy) (1, 2). Its diffusion is limited to the provinces of Grosseto and Siena (1, 2). It is very resistant to cold and fairly tolerant to olive leaf spot (*Spilocaea oleagina*) and olive

knot (*Pseudomonas syringae* ssp. *savastanoi*) and has a good oil yield (2).

The aim of this paper is to give a contribution to the characterization of this cultivar of *Olea europaea*, never previously studied, by means of the analyses of the volatiles obtained from leaves, fruits, and virgin oil.

MATERIALS AND METHODS

The plant material was collected from five plants ~25 years old, at 550 m above sea level, in Monte Giovi (Amiata Mount, southern Tuscany, Italy). The samples were gathered as follows: September 22, 2000, leaves and unripe fruits; November 30, 2000, leaves and ripe fruits; December 2, 2000, olive stone milling and oil production.

Hydrodistillation was performed the next day by means of a Clevenger-type apparatus for 2 h.

GC analyses were accomplished with an HP-5890 series II instrument equipped with HP-Wax and HP-5 capillary columns (30 m \times 0.25 mm, 0.25 μ m film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min to 220 °C; injector and detector temperatures, 250 °C; carrier gas, nitrogen (2 mL/min); detector, dual FID; split ratio, 1:30; injection of 0.5 μ L. The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (LRI) relative to the series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak area normalization, all relative response factors being taken as one.

GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m \times 0.25 mm; coating thickness = 0.25 μ m) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas, helium

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[†] In memory of Prof. Serena Catalano, 1945–2002.

Table 1. Composition^a of the Volatiles Obtained from Leaves, Olives, and Virgin Oil of *O. europaea* Cv. Olivastra Seggianese

constituent	LRI ^b	olive paste						
		leaves		unripe,			virgin oil	
		Sept, hydrodist	Dec, hydrodist	Sept, hydrodist	ripe, Dec, hydrodist	Dec, SPME	Dec, hydrodist	Dec, SPME
dimethylamine	426					2.2		
chloroform	469					4.2		
1-heptene	695					2.6		
toluene	750					3.4		
hexanal	802					9.1		
(<i>E,Z</i>)-2,4-hexadienal	841	5.1	8.0	5.0	8.3			
(<i>E</i>)-2-hexenal	856	36.7	48.0	14.8	9.8	30.0	11.1	18.7
(<i>E</i>)-2-hexen-1-ol	862	0.6		0.2		23.1	1.3	
2,6-dimethyl-1-heptene	866	2.4	1.4				4.2	37.0
<i>p</i> -xylene	872			1.0				
<i>m</i> -xylene	898			0.6				
heptanal	901		0.4	0.4	0.5		0.7	
(<i>E,E</i>)-2,4-hexadienal	911	tr ^c	0.5					
benzaldehyde	963	1.5	2.5	1.4	9.5	0.8	3.2	
(<i>Z</i>)-2-heptenal	964	tr		0.8			0.9	
<i>m</i> -ethyltoluene	968			0.7				
3-ethenylpyridine	975		5.9		4.7			
6-methyl-5-hepten-2-one	988	tr						
(<i>E,Z</i>)-2,4-heptadienal	990						tr	
myrcene	992			tr				
2-pentylfuran ^d	994		0.5					
2,3-dehydro-1,8-cineole	994	tr						
mesitylene	996			1.5		1.5	3.3	2.5
octanal	1003			0.4				
3-hexenyl acetate	1005	0.7	0.7		3.3		9.2	9.0
3-methyl-4-penten-1-ol acetate	1006					0.9		
hexyl acetate	1011				0.8	0.3	3.1	3.3
<i>p</i> -cymene	1027			0.1				
pseudocumene	1028						tr	
limonene	1033			0.2		3.5		
1,8-cineole	1035			tr				
(<i>Z</i>)-ocimene	1041			tr				
(<i>E</i>)-ocimene	1052	0.7		3.6		1.0	5.2	3.3
phenylacetaldehyde	1045		0.7		0.4			
(<i>E</i>)-2-octenal	1064		0.2	0.5	1.3			
<i>cis</i> -sabinene hydrate	1070					0.3		
1-undecene	1075		1.1	—	0.6			
terpinolene	1089			tr				
<i>p</i> -cymenene	1091	tr						
<i>n</i> -undecane	1100					0.1	0.7	
linalool	1101	2.2	0.1	3.6	0.5			
nonanal	1104	4.1	9.8	11.8	8.5	0.2	12.8	1.3
(<i>E,E</i>)-2,4-octadienal	1115						5.1	
(<i>E</i>)-2-nonenal	1164				0.3			
4-terpineol	1179	0.7		0.8	0.3			
naphthalene	1181			tr	0.3			
<i>p</i> -cymen-8-ol	1185	tr						
(<i>Z</i>)-3-hexenyl butyrate	1187	1.2	0.5					
α -terpineol	1191	0.3		0.2	0.3			
methyl salicylate	1192	0.9			0.4			
hexyl butyrate	1193	0.8						
methyl chavicol	1197			0.3	0.5			
decanal	1205		0.3				1.3	0.9
carvone	1244	tr						
(<i>Z</i>)-2-decenal	1252				0.3			
(<i>E</i>)-2-decenal	1263	0.7	0.8	17.5	10.0		tr	
thymol	1291		0.1	tr	0.4			
(<i>E,Z</i>)-2,4-decadienal ^d	1293				2.2			
(<i>E,E</i>)-2,4-decadienal	1316				4.0			
dihydrocarveol acetate	1344			0.3				
2-undecenal	1368			0.2	0.3			
(<i>E</i>)- β -damascenone ^d	1383	7.5	4.9					
(<i>E</i>)- β -damascone	1410	3.4	2.4					
β -caryophyllene	1420	8.1	1.0	1.0		0.3		
γ -elemene	1433					0.1		
α -humulene	1455	1.3		0.1				
germacrene D	1481	1.2		0.1				
(<i>E</i>)- β -ionone	1486		0.3					
valencene	1493				1.2	0.1	2.5	
(<i>E,E</i>)- α -farnesene	1506	10.7	0.2	20.3	25.0	1.0	27.0	7.4
<i>trans</i> -nerolidol	1564	0.3			0.5			

Table 1 (Continued)

constituent	LRI ^b	olive paste						
		leaves		unripe,			virgin oil	
		Sept, hydrodist	Dec, hydrodist	Sept, hydrodist	ripe, Dec, hydrodist	Dec, SPME	Dec, hydrodist	Dec, SPME
(Z)-3-hexenyl benzoate	1571	0.6	0.1					
caryophyllene oxide	1583	0.6						
viridiflorol ^d	1591			0.4				
monoterpenes		3.9	0.2	8.81	1.5	4.8	5.2	3.3
sesquiterpenes		22.2	1.2	21.9	26.7	1.5	29.5	7.4
fatty acid derivatives		52.9	71.8	51.6	50.2	66.3	50.4	70.2
benzenoids		2.4	3.2	5.5	11.1	5.7	6.5	2.5
nitrogen derivatives			5.9		4.7	2.2		
other derivatives		10.9	8.1			4.2		

^a Percentages obtained by FID peak area normalization (HP-5 column). ^b Linear retention indices (DB-5 column). ^c tr < 0.1%. ^d Tentative identification (no reference compound available).

at 1 mL/min; injection of 0.2 μ L (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their LRI relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built from pure substances and components of known oils and MS literature data (3–8). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas. All of the reference compounds were obtained from Aldrich Italia (either normal or flavor and fragrances catalogs) except α -humulene (Sigma Italia) and 4-terpineol and ocimene (mixture of isomers) (ChromaDex); some compounds, such as (*E*)-3-hexen-1-ol acetate, 3-methyl-4-penten-1-ol acetate, (*Z*)-3-hexenyl butyrate, and dihydrocarveol acetate, were prepared by simple synthesis, whereas (*E,Z*)-2,4-hexadienal, 2,3-dehydro-1,8-cineole, *cis*-sabinene hydrate, *p*-cymene, *p*-cymen-8-ol, γ -elemene, germacrene D, and (*E,E*)- α -farnesene were confirmed by NMR analyses of other essential oils (9).

For SPME analyses, Supelco SPME devices coated with poly(dimethylsiloxane) (PDMS, 100 μ m) were used to sample the headspace of 3 mL of olive paste or virgin olive oil inserted into a 5 mL glass vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC-MS system, operating in the same conditions as above for both quantification and identification of the constituents except that the splitless injection mode was used and the injector temperature was 250 $^{\circ}$ C.

RESULTS AND DISCUSSION

The compositions of the different samples are reported in Table 1. Seventy-four compounds were identified, accounting from 84.7–96.8% of the whole samples.

The main constituents of the volatile fraction of unripe fruits collected in September were (*E,E*)- α -farnesene (20.3%), (*E*)-2-decenal (17.5%), (*E*)-2-hexenal (14.8%), nonanal (11.8%), and (*E,Z*)-2,4-hexadienal (5.0%). Also, the ripe olives collected in December showed (*E,E*)- α -farnesene (25.0%) as principal constituent, followed by (*E*)-2-decenal (10.0%), (*E*)-2-hexenal (9.8%), benzaldehyde (9.5%), nonanal (8.5%), and (*E,Z*)-2,4-hexadienal (8.3%). It is apparent that the main compounds characterizing both qualitatively and quantitatively the two samples are the same and in the same order with the exception of benzaldehyde, which increases during olive ripening. The presence of many mono- and sesquiterpenes, even if in low amounts, in the unripe fruits must be pointed out; these compounds disappeared in ripe olives. The only monoterpene

still present in appreciable amounts in ripe fruits was linalool (0.5%), the main monoterpene in unripe olives (3.6%). The percentages of (*E*)-2-hexenal, nonanal, and (*E*)-2-decenal decreased in ripe fruits, whereas those of (*E,Z*)-2,4-hexadienal, benzaldehyde, and (*E*)-2-octenal increased. Furthermore, in ripe olives appeared also new compounds, such as the aldehydes phenylacetaldehyde, (*E*)-2-nonenal, (*Z*)-2-decenal, (*E,Z*)-2,4-decadienal, the hydrocarbon 1-undecene, the esters methyl salicylate, hexyl acetate, 3-hexenyl acetate, and the sesquiterpene valencene.

All of the aldehydes detected in the leaves harvested in September increased significantly in December; in particular (*E*)-2-hexenal increased from 36.7 to 48.0%, (*E,Z*)-2,4-hexadienal from 5.1 to 8.0%, nonanal from 4.1 to 9.8% and, in smaller amounts, benzaldehyde from 1.5 to 2.5% and (*E*)-2-decenal from 0.7 to 0.8%. Furthermore, other new aldehydes appeared in the leaves during December, that is, heptanal, phenylacetaldehyde, (*E*)-2-octenal, and decanal. Similarly to fruits, in the leaves mono- and sesquiterpenes tended to radically decrease in December; in particular (*E,E*)- α -farnesene increased from 10.7 to 0.2%, β -caryophyllene from 8.1 to 1.0%, and linalool from 2.2 to 0.1%. α -Terpineol, 4-terpineol, (*E*)-ocimene, *p*-cymene, *p*-cymen-8-ol, carvone, α -humulene, germacrene D, *trans*-nerolidol, and caryophyllene oxide disappeared completely from leaves at the time of optimal olive ripeness.

In a previous paper Campeol et al. (10) examined the volatiles obtained from leaves of the cultivars Frantoio, Leccino, and Cipressino collected in Pisa. The three cultivars showed different profiles in their volatiles that permitted the authors to distinguish among them. The cultivar Olivastra Seggianese, collected in the same vegetative stage, was characterized by a high percentage of (*E*)-2-hexenal (~50%) and the absence of theaspiranes and kongol, typical of the other three cultivars. On the contrary, (*E,Z*)-2,4-hexadienal and (*E,E*)-2,4-hexadienal were distinctive of the cultivar Olivastra Seggianese.

The volatiles of the olive paste obtained by stone milling of the ripe fruits was analyzed both by SPME and by hydrodistillation. Using the former method, the main compounds found were the aldehyde (*E*)-2-hexenal and the alcohol (*E*)-2-hexen-1-ol (30.0 and 23.1%, respectively), followed by hexanal (9.1%) and limonene (3.5%). SPME evidenced many compounds not detected by hydrodistillation, probably because their high volatility, such as dimethylamine, chloroform, 1-heptene, toluene, hexanal, (*E*)-2-hexen-1-ol, and mesitylene. On the contrary, SPME was less sensitive for the detection of higher

boiling compounds, such as many aldehydes and mono- and sesquiterpenes, with the exception of β -caryophyllene, γ -elemene, valencene, and (*E,E*)- α -farnesene, all detected in percentages lower than 1%. It must be pointed out that (*E,E*)- α -farnesene, the main constituent in hydrodistillation analysis (25.0%), was present only at 1.0% levels in SPME analysis. This finding suggests a complementary character for these techniques.

The virgin olive oil obtained by pressing the olive paste was hydrodistilled, and the identified volatiles are reported in **Table 1**. The major constituent detected was (*E,E*)- α -farnesene (27.0%, a percentage similar to that in ripe fruits), followed by nonanal (12.8%), (*E*)-2-hexenal (11.1%), 3-hexenyl acetate (9.2%), and (*E*)-ocimene (5.2%). With respect to the ripe fruits, a minor number of volatiles have been found in the virgin oil (27 vs 17 compounds). In the oil disappeared the monoterpene alcohols linalool, 4-terpineol, and α -terpineol, the phenolic derivatives thymol, methylchavicol, and methyl salicylate, the aldehydes (*E,Z*)-2,4-hexadienal, phenylacetaldehyde, (*E*)-2-octenal, (*E*)-2-nonenal, (*Z*)-2-decenal, (*E,Z*)-2,4-decadienal, (*E,E*)-2,4-decadienal, and 2-undecanal, and the sesquiterpene alcohol *trans*-nerolidol. Therefore, in the virgin oil, the number of aldehydes decreased from 12 to 7 and their total percentage from 55.1 to 35.1%. On the contrary, hexyl acetate (3.1%), (*E*)-ocimene (5.2%), mesitylene (3.3%), and some carbonylic compounds such as (*Z*)-2-heptenal, decanal, and 6-methyl-5-hepten-2-one were exclusive of the virgin oil.

The SPME analysis of the olive oil permitted us to identify, among the main volatiles, 2,6-dimethyl-1-heptene (37.0%), (*E*)-2-hexenal (18.7%), 3-hexenyl acetate (9.0%), and (*E,E*)- α -farnesene (7.4%). With respect to the hydrodistillation, this technique sampled a greater amount of 2,6-dimethyl-1-heptene (37.0 vs 4.2%) and (*E*)-2-hexenal (18.7 vs 11.1%) and lesser percentages of mesitylene, (*E*)-ocimene, nonanal, decanal, and (*E,E*)- α -farnesene. However, the SPME technique should better represent the real profile of volatiles emitted spontaneously at room temperature by the virgin olive oil.

Many papers on volatiles from olives and olive oil can be found in the literature. Some of them describe the relationships between volatile compounds and virgin olive oil odor notes (11–16). The influence of operative conditions during the storage and processing has been also evaluated (17–20), as well as their biogenesis (21–23). These chemicals have been also used to characterize the aroma of the oil obtained from new cultivars (24) or the presence of adulterants or contaminants (25). Volatiles have been also obtained from callus cultures (26, 27).

A direct comparison with literature data is not possible because of the great variability of the volatiles composition with reference to the different ripeness stages of olives, extraction techniques, and analytical methods; however, also in the cultivar *Olivastra Seggianese* the main constituents identified were aldehydes, alcohols, ketones, and esters. Furthermore, also mono- and sesquiterpene hydrocarbons have been detected among the main volatiles of this cultivar. In the literature only very few papers (28–31) report the presence of these compounds, which could play a very important role in the fragrance of this valuable food.

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Received for review August 5, 2002. Revised manuscript received November 13, 2002. Accepted November 17, 2002.

JF020854Y