Volatile Compounds from Three Cultivars of *Olea europaea* from Italy

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The chemical compositions of the volatile fractions from three *Olea europaea* L. cultivars (Leccino, Frantoio, and Cipressino) were examined by GC and GC-MS. The results showed that the cultivars can be distinguished on the basis of the volatile fraction compositions.

Keywords: *Olea europaea L.; cultivars; Frantoio; Leccino; Cipressino; volatile compounds; aldehydes; terpenes*

INTRODUCTION

In the literature some papers concerning the study of fatty oil and olive fruits' volatile fractions of *Olea europaea* L. (Oleaceae) are present, but they focused on only the composition (*1*, *2*) or only the antimicrobial activity of the components and their involvment in the likely defense mechanism of the plant against patogens (*3*).

Literature data showed that aldehyde contents in green and black olives are 50 and 75%, respectively, whereas hexanal, (*E*)-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol are the major volatile compounds of olive oils. The studied cultivars were Canino, Frantoio, and Moraiolo from Italy. Moreover, the volatiles of olive fruits of three further cultivars (Leccino, Dritta, and Caroleo) were reported: (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, ethanol, (*E*)-2-hexenal, and *n*-octane were the main compounds (*2*).

Among aldehydes (*E*)-2-hexenal and (*E*)-2-heptenal showed activity against a large number of microorganisms (*3*).

The present paper is the first step of a wider investigation on phylogeny and biodiversity in *O. europaea*. To get further information on the problem of origin and cultivar certification, we have analyzed the volatile fraction of the leaves of three cultivars (Frantoio, Leccino, and Cipressino).

MATERIALS AND METHODS

Plant Material. Fresh leaves of cultivars Frantoio, Leccino, and Cipressino of *O. europea* (clonal propagated trees) were collected in the same experimental field of the Dipartimento di Coltivazioni e Difesa delle Specie Legnose, Facoltà di Agraria, University of Pisa, on November 15, 2000. The three cultivars were never submitted to agronomical treatment, and Cipressino was used as windbreak only.

Extraction and Identification. The fresh material (200 g) was hydrodistilled in a Clevenger-like apparatus for 2 h, and volatile compounds were collected in *n*-hexane (HPLC grade). The analyses were carried out on two plants for each cultivar.

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

GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness of 0.25 μm) 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 at 1 mL/min; injection, 0.2 μL (10% hexane solution); split ratio, 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples 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 (*4*−*9*). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

Statistical Analysis. The means of the compounds shared by three or two cultivars were separated on the basis of the LSD test only when the *F* test of the ANOVA treatment was significant at the 0.01 probability level (Figures 1 and 2) (*10*).

RESULTS AND DISCUSSION

Forty-one compounds have been identified, and their relative amounts are listed in Table 1. Among these, some have been already detected in previous papers, particularly saturated and unsaturated nonterpenic aldehydes (*1*, *2*). However, our samples contained also many mono- and sesquiterpenes that represented the main constituents.

The compositions of the two samples of Frantoio (samples 1 and 2) were very similar, particularly from

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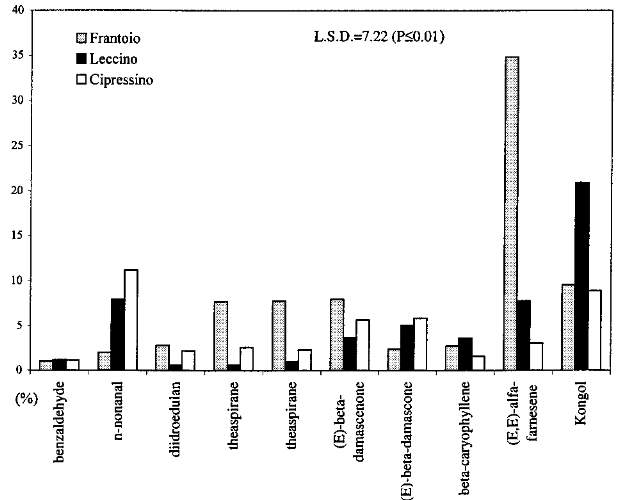

Figure 1. Effects of the interaction between cultivars (Frantoio, Leccino, and Cipressino) and commom constituents of the volatile fractions.

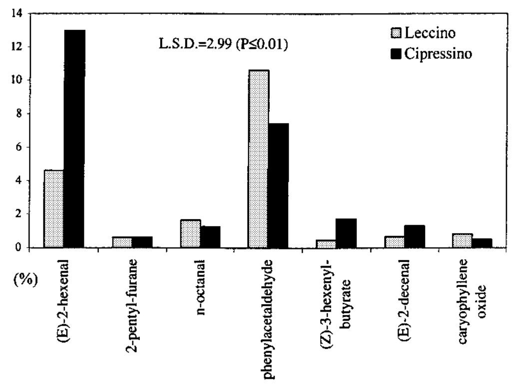

Figure 2. Effects of the interaction between cultivars (Leccino and Cipressino) and common constituents of the volatile fractions.

the qualitative point of view. The components were almost the same except for (*E*)-nerolidol and (*Z*)-3-hexenyl benzoate; the former was contained only in sample 1, whereas the latter was found only in sample 2. The main components in both plants were (*E*,*E*)-α-farnesene, kongol (Figure 3), theaspiranes (Figure 4), and (*E*)-*β*-damascenone. From a quantitative point of view the larger variability was found for (*E*,*E*)-α-farnesene, (*E*)-*β*-damascenone, and kongol.

Also for the two samples of Leccino (samples 3 and 4) the compositions of the volatile fractions were very similar from a qualitative point of view. The main components in both plants were kongol, benzenacetaldehyde, nonanal, (*E*,*E*)-α-farnesene, (*E*)-2-hexenal, (*E*)-*β*-damascone, (*E*)-*β*-damascenone, and *β*-caryophyllene. The larger quantitative variability was observed for kongol, (*E*,*E*)-α-farnesene, benzenacetaldehyde, (*E*)-2-hexenal, and (*E*)-*β*-damascenone.

Also for the two samples of Cipressino (samples 5 and 6) the compositions of the volatile fractions were very similar. The main components in both plants were (*E*)-2-hexenal, nonanal, kongol, benzenacetaldehyde, (*E*)-*β*-damascone, (*E*)-*β*-damascenone, (*E*,*E*)-α-farnesene, and (*E*)-2-hexen-1-ol.

The composition of the volatile fraction of Frantoio and Leccino was very different because of the higher number of components identified in the latter. In the two samples of Leccino we identified the aldehydes (*E*)-2-hexenal, heptanal, 2-heptenal, octanal, benzeneacetaldehyde, and (*E*)-2-decenal. These compounds were absent in the samples of Frantoio (1 and 2); nonanal and benzaldehyde were the only aldehydes present also in Frantoio. Furthermore, 2-pentylfuran, 1-hydroxycumene, (*Z*)-3-hexenyl butyrate, α-humulene, germa-

Table 1. Composition of the Volatile Fractions from Frantoio (Samples 1 and 2), Leccino (Samples 3 and 4), and Cipressino (Samples 5 and 6) Cultivars

		samples					
constituent	KI	1	2	3	4	5	6
(*E*)-2-hexenal	854			6.1	3.1	13.2	12.7
(*E*)-2-hexen-1-ol	862	1.0	tr	0.6	tr	3.0	2.5
n-heptanal	900			0.5	0.3	0.5	tr
benzaldehyde	962	2.0	tr	1.1	1.2	1.4	0.8
2-heptenal[a]	964			0.4	tr	0.6	tr
2,3-dehydro-1,8-cineole	993					0.3	tr
2-pentylfuran	994			0.7	0.5	0.5	0.7
n-octanal	1002			1.5	1.8	1.1	1.4
(*E*,*E*)-2,4-heptadienal	1017					0.5	0.2
phenylacetaldehyde	1043			12.3	8.9	6.9	7.9
(*E*)-2-octenal	1064			tr		0.5	0.3
1-undecene	1075			tr	tr	2.3	1.9
o-hydroxycumene	1089			0.6	3.6	tr	0.7
p-cymenene	1091			tr		0.4	tr
linalool	1099					0.9	1.2
n-nonanal	1102	2.2	1.8	8.2	7.6	10.2	12.1
4-terpineol	1178			tr	0.3		
(*Z*)-3-hexenyl butyrate	1187			0.4	0.5	1.6	1.8
α-terpineol	1190					1.2	2.0
hexyl butyrate	1193					0.7	0.6
(*E*)-2-hexenyl butyrate	1195					1.4	1.1
n-decanal	1205					0.6	0.9
β-cyclocitral	1223					0.7	0.5
(*E*)-3-caren-2-ol	1227					1.2	1.4
(*E*)-2-decenal	1263			1.0	0.3	2.6	2.3
diidroedulan I	1292	3.3	2.3	0.6	tr	2.1	2.3
theaspirane[b]	1298	7.9	7.5	1.1	0.1	2.5	2.7
theaspirane[b]	1315	8.3	7.2	1.3	0.7	2.1	2.5
(*E*)-*β*-damascenone	1381	10.5	5.4	5.1	2.3	5.3	6.0
(*E*)-*β*-damascone	1410	2.2	2.6	4.2	5.9	5.5	6.2
β-caryophyllene	1420	2.7	2.7	4.1	3.1	1.5	1.6
α-humulene	1456			0.8	0.5	tr	tr
germacrene D	1481			1.1	0.8	tr	tr
β-selinene	1485			0.6	tr		
(*E*,*E*)-α-farnesene	1509	31.1	38.5	11.9	3.6	2.7	3.3
liguloxide	1532					0.7	0.5
(*E*)-nerolidol	1564	3.7		0.8	1.3		
(*Z*)-3-hexenyl benzoate	1571		1.1	1.6	1.4		
caryophyllene oxide	1583			0.8	0.8	0.3	0.2
(*E*)-2-hexenyl benzoate	1584			2.6	2.1		
kongol	1654	7.3	11.8	13.7	28.0	9.1	8.5

[a] Correct isomer not identified. [b] Natural theaspirane is a mixture of the (6*R*) and (6*S*) compounds (*11*).

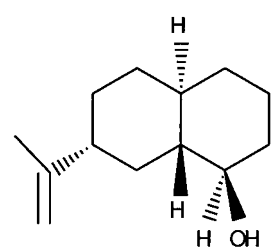

Figure 3. Structure of kongol.

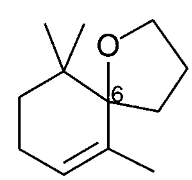

Figure 4. Theaspiranes, 6*S* and 6*R* enantiomer.

crene D, *β*-selinene, caryophyllene oxide, and (*E*)-2-hexenyl benzoate were distinctive for Leccino plants and were completely absent in Frantoio, whereas the compounds identified in Frantoio were always present in Leccino. From a quantitative point of view the larger variability in the common compounds of these two cultivars was found for (*E*,*E*)-α-farnesene, kongol, theaspiranes, nonanal, (*E*)-*β*-damascenone, and (E)-*β*-damascone.

The volatile fraction composition of Cipressino (samples 5 and 6) was more similar to that of Leccino than of Frantoio owing to the same mixture of nonterpenic aldehydes, a high percentage of (*E*)-2-hexenal and nonanal, low contents of (*E*,*E*)-α-farnesene, and low contents of theaspiranes.

Linalool, α-terpineol, decanal, (*E*)-3-caren-2-ol, hexyl butyrate, (*E*)-2-hexenyl butyrate, *β*-cyclocitral, liguloxide, 2,3-dehydro-1,8-cineole, and (*E*,*E*)-2,4-heptadienal, not identified in Frantoio and Leccino, were the compounds that qualitatively characterised the cv. Cipressino.

Besides the nonterpenic aldehydes, most of the compounds present in Leccino and absent in Frantoio [2-pentylfuran, 1-hydroxycumene, (*Z*)-3-hexenyl butyrate, α-humulene, germacrene D, and caryophyllene oxide] were found at least in trace amounts in Cipressino.

Our results showed that, as confirmed by the statistical treatment, the three cultivars can be distinguished on the basis of their volatile fraction composition. Further investigations on other cultivars could be necessary to generalize the obtained data.

Studies on the same cultivars growing in different habitats are in progress and will allow us to discuss the environmental effects.

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