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SOLID-PHASE MICROEXTRACTION IN THE ANALYSIS OF THE VOLATILE FRACTION OF EXTRA-VIRGIN OLIVE OIL FROM FOURTEEN CULTIVARS GROWN IN THE LAKE GARDA REGION (ITALY)

SULLA FRAZIONE VOLATILE DI QUATTORDICI OLI EXTRA-VERGINI
DI OLIVA PRODOTTI DA CULTIVARS DEL LAGO DI GARDA

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ABSTRACT

Forty-one volatile compounds were identified in virgin olive oils from fourteen cultivars grown near Lake Garda, Italy, using headspace solid-phase microextraction coupled with gas chromatography/mass spectrometry. Analyses of oils from olives with comparable ripeness indices showed that C6 volatile compounds, enzymatically produced through the lipoxygenase pathway, accounted for 77.4-94.3% of the total volatile fraction. The C5 volatile compounds, which are not derived from the lipoxygenase pathway, ac-

RIASSUNTO

In questo lavoro gli Autori riportano i risultati ottenuti dalla valutazione dello spazio di testa della frazione volatile di olio extra vergine di oliva ottenuto da 14 cultivars differenti impiantate su terreno situato a sud-ovest del Lago di Garda. Le analisi effettuate hanno mostrato che i composti volatili C6 prodotti attraverso la via della lipossigenasi, per oli prodotti da olive con valore comparabile dell'indice di maturazione, rappresentano 77,4-94,3% della frazione volatile: viene inoltre riportata la distribuzione di questi composti volatili.

-Key words: headspace solid-phase microextraction, Lake Garda oils, virgin olive oil, volatile compounds-

counted for 1.6-8.4% of the total volatile fraction.

I composti volatili C5, che non derivano dalla via lipossigenasi, rappresentano 1,6-8,4% della frazione volatile.

INTRODUCTION

In the Lombard area, most olive trees are grown along the shores of Lakes Garda, Iseo, and Como. Over the past 15 years, the level of oil production has increased from 300 tons/year to almost 700 tons/year (PEDO' *et al.*, 2003). Extra-virgin olive oil from the Lake Garda Riviera has recently become an important niche product.

Various authors (ANGEROSA *et al.*, 1999; ANGEROSA, 2002; BENINCASA *et al.*, 2003; OLIAS *et al.*, 1993) have reported that the lipoxygenase (LOX) pathway is responsible for production of the most abundant volatile compounds found during the oil extraction process and the enzymatic activity of this pathway is known to be related to agronomic and technological factors. The influence of environmental conditions on the production of volatile compounds has also been demonstrated (APARICIO and MORALES, 1998; VICHI *et al.*, 2003; WILLIAMS *et al.*, 1998).

Many analytical procedures have been used to identify and quantify the volatile compounds that characterize olive oil aroma. Among the extraction techniques, headspace solid-phase microextraction (HS-SPME) is the simplest technique for differentiation studies based on profile comparison. Numerous studies have been reported on characterization of the volatile fraction of olive oil (ANGEROSA *et al.*, 1999, 2004; ANGEROSA, 2002; BACCOURI *et al.*, 2007; CAVALLI *et al.*, 2004; DHIFI *et al.*, 2005; HADDADA *et al.*, 2007; JIMENEZ *et al.*, 2004; LUNA *et al.*, 2006; MILDNER-SZKUDLARS *et al.*, 2003; MONTEODORO and SERVILI, 1992; TURA *et al.*, 2002, 2004; VICHI *et al.*, 2003).

The aim of this study was to obtain initial information concerning the volatile compound fraction of olive oils derived from local cultivars growing in the Lake Garda region of Italy.

MATERIALS AND METHODS

Samples

Olives were harvested from fourteen cultivars (Regina, Miniol 2, Trepp, Less, Baia, Pendolino, Maurino, Grignano, Gargnà, Casaliva, Rossanello, Mitria, Favarol 1, Leccino) that were growing in an 8,000 m² experimental plot at an altitude of 140-215 m asl near the village of Raffa di Puegnago southwest of Lake Garda. The olives were all cultivated under the same conditions and were harvested within the month of November, 2003. Each individual cultivar was stored separately for no longer than one week in open plastic containers with a 10 kg capacity at 7±2°C to simulate real storage conditions.

The ripeness indices were determined according to the indications of the Estación de Olivicultura y Elaiotecnica, Jaén (HERMOSO *et al.*, 1991). Oil was extracted separately from each of the fourteen cultivar using a standard discontinuous procedure. In total, 18 kg of olives per cultivar were ground with an Inox hammer crusher and malaxed at 28°C for 30 min. The oil was extracted using a hydraulic press (maximum 200 bars) and separated by centrifugation at 2,000 rpm.

Three 10 mL aliquots of each oil were taken as samples, centrifuged at 5,000 rpm for 10 min, then stored for two

months at 10°C in the dark and analyzed within 10 days. Three series of fourteen analyses were performed. The data reported in Table 1 are the means of three series of analyses. The oils were classified on the basis of acidity, peroxide index, UV light absorption (K_{232} and K_{270}), and fatty acid and sterol content, following the analytical methods described in Regulations EEC/2568/91 and EEC/1429/92 of the European Union Commission. All the samples were categorized as extra virgin olive oil according to the European Commission EU Regulation 796/2002 (EU 2002), which was in force at the time of the experiment.

Analysis of volatile compounds

Each olive oil sample (0.2 kg derived from approximately 2.5 kg) was spiked with an internal standard (4-methyl-2-pentanol; Sigma Aldrich, Milan, Italy) to a concentration of 1.0 µg/g. Three 5-g aliquots of each sample were weighed and placed in a 40-mL headspace vial fitted with a Teflon-lined septum. The sample was placed in a water bath at 40°C with a magnetic stirrer. After 15 min, an SPME fiber covered with 2 cm of 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (Supelco, Milan, Italy) was inserted into the headspace of the vial for 60 min. The volatile compounds were desorbed for 10 min at 220°C in a gas chromatograph with a split/splitless injection port. The injector operated in split mode (1:5). Gas chromatography/mass spectrometry (GC/MS) analyses were performed in a Shimadzu 2010 gas chromatograph coupled to a Shimadzu QP-2010 MSD quadrupole mass spectrometer (Shimadzu, Milan, Italy). The gas chromatograph was equipped with an EquityTM-5 column (30 m x 0.25 mm internal diameter x 0.25 µm film thickness, Supelco, Milan, Italy).

The operating conditions for the GC/MS were as follows: helium flow, 1.0

mL/min; oven temperature 50°C for 1 min increased to 240°C at a rate of 3°C/min. The temperature of the ion source was 200°C, the electron energy was 70 eV, and the interface temperature was 260°C. Mass spectra were acquired over the mass range 40-300 amu.

Before sampling, the fiber was reconditioned for 10 min in the GC injection port at 250°C, and blank runs were carried out periodically during the study. The efficiency of the fiber was periodically verified by monitoring the signal of the internal standard.

Volatile compounds in the headspace of the virgin olive oils under study were identified by matching their mass spectra with the reference mass spectra of a private library and the NIST 147 library.

Quantitative analysis was carried out using the internal standard method. The concentration of the main LOX pathway volatiles was calculated using relative experimental response factors. The remaining compounds were quantified by considering the relative response factor to be 1. For compounds that were not completely resolved, a quantitative evaluation was performed using single or multiple ions for each peak and adopting specific factors derived from mass spectra. This information is useful for calculating the TIC (Total Ion Chromatogram) signal from the SIM (Single Ion Monitoring) signal.

RESULTS AND DISCUSSION

Ripeness indices and data from the HS-SPME analysis characterizing the volatile compounds present in the fourteen virgin olive oils are shown in Table 1. The ripeness indices of eleven of the cultivars ranged from 2 to 3. The other three ripening indices were 1.54 for the cultivar Rossanello, 4.19 for the cultivar Baia and 5.08 for the cultivar Leccino. These exceptions were due to the difficulty of har-

Table 1 - Volatile compounds (mg/kg \pm standard deviation) in fourteen virgin olive oils from different cultivars grown on a single plot in the Lake Garda region.
n.d.: compound not detected, tr: trace (<0.01 mg/kg); ^aRI, Kovats retention index.

	RI ^a	Regina	Miniol 2	Trepp	Less	Baia
ethanol	554	0.38 \pm 0.06	0.11 \pm 0.03	0.15 \pm 0.03	0.05 \pm 0.02	1.02 \pm 0.10
acetic acid	620	0.51 \pm 0.07	0.31 \pm 0.05	0.28 \pm 0.06	0.25 \pm 0.06	0.49 \pm 0.07
3-methyl butanal + 2-methyl butanal	638	0.11 \pm 0.03	0.05 \pm 0.02	0.07 \pm 0.02	0.04 \pm 0.01	0.07 \pm 0.02
1-penten-3-ol	663	0.80 \pm 0.10	0.43 \pm 0.07	0.20 \pm 0.05	0.15 \pm 0.03	0.40 \pm 0.07
1-penten-3-one	673	1.19 \pm 0.11	0.49 \pm 0.07	0.27 \pm 0.06	0.19 \pm 0.03	0.29 \pm 0.06
pentan-3-one	677	0.54 \pm 0.07	0.11 \pm 0.02	0.06 \pm 0.02	0.03 \pm 0.02	0.96 \pm 0.12
pentanal	700	0.05 \pm 0.02	0.09 \pm 0.02	0.05 \pm 0.02	0.08 \pm 0.03	0.15 \pm 0.03
3-methyl-butan-1-ol + 2-methyl-butan-1-ol	722	0.06 \pm 0.01	0.07 \pm 0.01	tr.	tr.	0.08 \pm 0.01
(E)-2-pentenal	724	0.20 \pm 0.06	0.07 \pm 0.01	0.03 \pm 0.01	tr.	0.10 \pm 0.04
pentan-1-ol	741	0.13 \pm 0.02	0.02 \pm 0.01	tr.	n.d.	0.16 \pm 0.03
toluene	743	0.14 \pm 0.02	0.11 \pm 0.03	0.10 \pm 0.03	0.23 \pm 0.06	0.38 \pm 0.07
(Z)-2-penten-1-ol	747	1.17 \pm 0.10	1.23 \pm 0.10	0.59 \pm 0.07	0.38 \pm 0.06	1.64 \pm 0.10
(Z)-3-hexenal	774	15.84 \pm 0.92	16.53 \pm 1.20	0.24 \pm 0.06	0.34 \pm 0.07	4.36 \pm 0.32
hexanal	777	1.52 \pm 0.12	0.79 \pm 0.10	1.38 \pm 0.10	0.82 \pm 0.10	1.18 \pm 0.10
(E)-2-hexenal	827	8.84 \pm 0.70	4.18 \pm 0.12	45.00 \pm 1.30	43.25 \pm 1.30	5.83 \pm 0.50
(Z)-3-hexen-1-ol	834	11.31 \pm 1.05	11.88 \pm 0.05	0.86 \pm 0.05	2.41 \pm 0.14	15.02 \pm 1.10
(E)-2-hexen-1-ol	849	1.22 \pm 0.12	0.10 \pm 0.03	0.82 \pm 0.05	0.82 \pm 0.05	1.58 \pm 0.12
hexanol	854	1.23 \pm 0.12	0.20 \pm 0.01	0.21 \pm 0.03	0.20 \pm 0.03	2.71 \pm 0.20
p-xilene	868	0.16 \pm 0.02	0.10 \pm 0.03	0.10 \pm 0.03	0.25 \pm 0.03	0.26 \pm 0.03
styrene	871	0.03 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.03 \pm 0.01	0.05 \pm 0.01
heptan-2-one	874	0.47 \pm 0.06	0.40 \pm 0.06	0.04 \pm 0.01	0.02 \pm 0.01	0.26 \pm 0.03
heptanal	883	0.07 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.01	0.13 \pm 0.02	0.14 \pm 0.02
(E,E)-2,4-hexadienal	887	0.17 \pm 0.03	0.10 \pm 0.02	0.12 \pm 0.02	tr.	0.12 \pm 0.02
3-ethyl-1,5-octadiene (E or Z)	907	0.26 \pm 0.02	0.18 \pm 0.02	0.06 \pm 0.01	0.02 \pm 0.01	0.21 \pm 0.02
3-ethyl-1,5-octadiene (E or Z)	912	0.84 \pm 0.10	0.48 \pm 0.06	0.35 \pm 0.05	0.20 \pm 0.03	0.92 \pm 0.10
(E)-2-heptenal	931	1.76 \pm 0.10	1.10 \pm 0.10	0.67 \pm 0.04	0.42 \pm 0.06	1.83 \pm 0.10
5-ethyl-2(5H) furanone	940	n.d.	n.d.	0.04 \pm 0.01	tr.	0.07 \pm 0.01
heptan-1-ol	961	0.72 \pm 0.05	0.68 \pm 0.05	n.d.	n.d.	0.57 \pm 0.04
hexanoic acid	965	0.07 \pm 0.01	0.06 \pm 0.01	0.16 \pm 0.02	0.09 \pm 0.02	n.d.
6-methyl-5-hepten-2-one	970	n.d.	tr.	Tr.	n.d.	n.d.
octan-2-one	974	n.d.	n.d.	n.d.	n.d.	n.d.
octanal	981	0.05 \pm 0.01	0.06 \pm 0.01	0.15 \pm 0.02	0.03 \pm 0.01	n.d.
(Z)-3-hexenyl acetate	994	0.22 \pm 0.02	0.51 \pm 0.06	0.05 \pm 0.01	0.07 \pm 0.01	n.d.
hexyl acetate	999	n.d.	0.05 \pm 0.01	0.04 \pm 0.01	0.02 \pm 0.01	n.d.
p-cymene	1010	0.02 \pm 0.01	n.d.	tr.	n.d.	n.d.
limonene	1016	0.23 \pm 0.05	0.24 \pm 0.05	0.28 \pm 0.03	0.16 \pm 0.02	1.00 \pm 0.10
(E)- β -ocimene	1040	0.04 \pm 0.01	0.03 \pm 0.01	tr.	0.02 \pm 0.01	0.13 \pm 0.02
nonanal	1087	0.09 \pm 0.02	0.17 \pm 0.02	0.18 \pm 0.02	0.06 \pm 0.01	0.11 \pm 0.02
phenylethyl alcohol	1102	0.02 \pm 0.01	0.02 \pm 0.01	0.03 \pm 0.01	n.d.	0.05 \pm 0.01
α -copaene	1390	n.d.	n.d.	n.d.	n.d.	0.04 \pm 0.01
α -farnesene	1513	0.03 \pm 0.01	n.d.	n.d.	n.d.	n.d.
% C6/total volatile compounds		79.6	83.4	92.2	94.3	72.7
% C5/total volatile compounds		8.4	6.3	2.4	1.8	9.2
Degree of ripeness		2.56	2.38	2.72	2.40	4.19

Table 1 - Volatile compounds (mg/kg \pm standard deviation) in fourteen virgin olive oils from different cultivars grown on a single plot in the Lake Garda region.
n.d.: compound not detected, tr: trace (<0.01 mg/kg); ^aRI, Kovats retention index.

Pendolino	Maurino	Grignano	Gargnà	Casaliva	Rossanello	Mitria	Favarol	Leccino
0.11±0.03	0.29±0.06	0.07±0.01	0.35±0.06	0.18±0.04	3.41±0.10	0.17±0.03	0.27±0.05	1.82±0.10
1.00±0.10	0.06±0.01	0.26±0.06	0.27±0.06	0.25±0.06	0.18±0.03	0.37±0.06	0.27±0.04	0.52±0.07
n.d.	n.d.	n.d.	0.18±0.03	0.10±0.03	0.04±0.01	0.24±0.05	0.10±0.03	0.07±0.02
0.24±0.05	0.43±0.07	0.33±0.06	0.34±0.06	0.31±0.06	0.55±0.10	0.51±0.08	0.24±0.06	0.15±0.03
0.35±0.06	1.11±0.10	0.49±0.07	0.24±0.06	0.31±0.06	0.44±0.07	0.55±0.05	0.17±0.03	0.13±0.03
0.12±0.03	0.15±0.03	0.09±0.03	0.15±0.02	0.06±0.02	0.26±0.03	0.20±0.06	0.02±0.01	0.03±0.01
0.19±0.02	0.15±0.06	0.11±0.02	0.22±0.06	0.18±0.06	0.20±0.05	0.17±0.04	0.27±0.05	0.04±0.01
tr.	tr.	0.03±0.01	0.05±0.01	0.03±0.01	0.14±0.03	0.08±0.01	0.02±0.01	0.09±0.01
0.06±0.01	0.19±0.03	0.07±0.01	0.05±0.01	0.03±0.01	0.06±0.01	0.06±0.01	0.03±0.01	0.06±0.01
n.d.	n.d.	n.d.	0.04±0.01	tr.	0.07±0.01	0.05±0.01	n.d.	n.d.
0.28±0.07	0.14±0.03	0.31±0.03	0.23±0.06	0.22±0.05	1.15±0.1	0.15±0.04	0.13±0.06	0.16±0.03
0.41±0.05	0.69±0.04	0.31±0.06	0.79±0.10	0.70±0.10	1.22±0.10	1.40±0.10	0.57±0.05	0.42±0.07
0.34±0.06	16.18±0.98	12.39±1.00	0.32±0.07	0.32±0.06	14.92±1.02	0.49±0.07	0.17±0.03	0.13±0.04
1.56±0.10	2.00±0.12	1.44±0.10	2.10±0.13	1.74±0.10	2.09±0.10	1.65±0.11	1.08±0.09	0.93±0.10
64.59±1.70	7.94±0.18	7.54±0.18	45.58±1.25	56.30±1.30	4.78±0.25	56.85±1.30	40.83±1.30	34.14±1.20
5.71±0.52	3.37±0.20	4.09±0.42	1.89±0.09	2.62±0.22	13.25±1.07	3.56±0.22	0.90±0.10	0.86±0.10
3.41±0.12	0.14±0.03	0.09±0.02	4.69±0.15	1.66±0.10	0.17±0.04	7.18±0.12	0.66±0.05	1.55±0.12
2.46±0.18	0.37±0.04	0.50±0.05	2.11±0.10	0.46±0.05	0.42±0.05	3.42±0.13	0.19±0.03	0.49±0.05
0.34±0.07	0.14±0.03	0.11±0.02	0.22±0.05	0.21±0.05	0.91±0.10	0.16±0.03	0.16±0.03	0.14±0.03
0.03±0.01	0.02±0.01	tr.	0.03±0.01	0.05±0.01	0.05±0.01	0.02±0.01	0.04±0.01	0.04±0.01
0.06±0.01	0.05±0.01	0.44±0.05	0.13±0.03	0.11±0.03	0.18±0.04	0.11±0.02	0.04±0.01	0.07±0.01
0.18±0.02	0.06±0.010	0.05±0.01	0.10±0.03	0.10±0.03	0.22±0.02	0.08±0.02	0.08±0.02	0.07±0.01
0.07±0.01	0.17±0.03	0.08±0.01	0.06±0.01	0.04±0.01	0.09±0.01	0.05±0.01	tr.	n.d.
0.06±0.01	0.06±0.01	0.19±0.03	tr.	tr.	0.21±0.02	0.13±0.03	tr.	0.15±0.03
0.39±0.05	0.92±0.10	0.32±0.04	0.26±0.03	0.32±0.05	0.27±0.03	0.46±0.04	0.20±0.03	0.14±0.03
0.97±0.10	2.51±0.18	0.97±0.10	0.38±0.06	0.49±0.05	0.46±0.05	0.71±0.08	0.34±0.05	0.26±0.03
0.04±0.01	0.08±0.01	0.03±0.01	n.d.	0.04±0.01	tr.	0.07±0.01	n.d.	0.02±0.01
0.14±0.02	0.55±0.05	0.56±0.05	n.d.	n.d.	0.14±0.02	0.04±0.01	n.d.	n.d.
0.04±0.01	0.09±0.02	n.d.	tr.	tr.	tr.	0.06±0.01	0.05±0.01	0.02±0.01
n.d.	0.04±0.01	0.02±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.02±0.01	0.03±0.01	tr.	0.02±0.01	n.d.	0.03±0.01	0.02±0.01	tr.	tr.
0.05±0.01	0.04±0.01	0.02±0.01	0.05±0.01	0.03±0.01	0.06±0.01	0.04±0.01	0.04±0.01	0.02±0.01
0.06±0.01	0.03±0.01	0.42±0.06	0.07±0.01	0.15±0.02	0.26±0.03	n.d.	n.d.	n.d.
n.d.	n.d.	0.14±0.02	0.17±0.02	0.06±0.01	n.d.	n.d.	n.d.	n.d.
n.d.	0.02±0.01	0.02±0.01	tr.	n.d.	0.02±0.01	tr.	0.02±0.01	0.02±0.01
0.14±0.02	0.31±0.05	0.40±0.06	0.35±0.05	0.28±0.04	0.70±0.05	0.14±0.02	0.43±0.06	0.26±0.04
tr.	0.23±0.02	0.20±0.02	tr.	0.07±0.01	n.d.	tr.	tr.	0.28±0.02
0.11±0.02	0.21±0.02	0.17±0.02	0.16±0.02	0.05±0.01	0.05±0.01	0.06±0.01	0.10±0.02	0.06±0.01
0.02±0.01	0.04±0.01	0.05±0.01	tr.	0.02±0.01	0.04±0.01	0.09±0.02	0.08±0.02	0.03±0.01
n.d.	n.d.	0.03±0.01	n.d.	tr.	n.d.	n.d.	tr.	n.d.
n.d.	0.02±0.01	0.02±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
93.5	77.4	82.2	92.3	93.7	76.2	92.2	92.2	88.2
1.6	7.0	4.4	3.3	2.6	6.4	4.1	3.0	2.3
2.91	2.98	2.67	1.97	2.95	1.54	1.91	2.77	5.08

vesting all of the cultivars with comparable ripeness values in one month. Data corresponding to the eleven oils derived from the olive cultivars with ripeness indices of 2-3 are presented below.

Forty-one volatile compounds were identified. The C6 and C5 compounds were quantified and the percentages calculated in relation to the total quantity of volatile compounds. The quantity of C6 volatile compounds ranged from 77.4 to 94.3%, and the quantity of C5 volatile compounds, not produced by the LOX pathway, ranged from 1.6 to 8.4%. The pentene dimers were not detected and are not included in the data reported as "C5 volatile compounds".

The data show a prevalence of compounds with linoleic acid (LA) and linolenic acid (LnA) precursors: hexanal, hexanol, and hexyl acetate from LA and (Z)-3-hexenal, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-3-hexen-1-ol, and (Z)-3-hexenyl acetate from LnA. Higher levels of C5 volatile compounds were accompanied by lower levels of C6 compounds. The cultivars differed not only in terms of the level of the various C6 compounds, but also in terms of the level of various C5 compounds which contribute to olive oil aroma and flavor due to their well-defined sensory profiles and threshold levels (pentan-3-one, 1-penten-3-one, 1-penten-3-ol, pentan-1-ol, (Z)-2-penten-1-ol, 3- and 2-methyl butanal) (KALUA *et al.*, 2007) as well as others (phenylethyl alcohol and (E)- β -ocimene).

Levels of C6 aldehydes, alcohols and esters from the LOX pathway are shown in Table 2. Detailed data are reported for all volatile C6 compounds derived from the metabolism of 13-hydroperoxides of LA and LnA. Examination of the compounds derived from the LOX cascade shows that all samples contained higher levels of volatile compounds from LnA than those from LA, which is consistent with the literature (ANGEROSA *et al.*, 1999; ANGEROSA, 2002; BENINCASA *et al.*, 2003; OLIAS *et al.*, 1993).

Table 2 - C6 compounds (mg/kg) produced via the LOX pathway isolated from fourteen virgin olive oils from different cultivars grown on a single plot in the Lake Garda region.

	Regina	Miniol 2	Trepp	Less	Baia	Pendolino	Maurino	Grignano	Gargnà	Casaliva	Rossanello	Mitria	FavaroI	Leccino
hexanal	152	0.79	1.38	0.82	1.18	1.56	2.00	1.44	2.10	1.74	2.09	1.65	1.08	0.93
hexanol	123	0.20	0.21	0.20	2.71	2.46	0.37	0.50	2.11	0.46	0.42	3.42	0.19	0.49
hexyl acetate	n.d.	0.05	0.04	0.02	n.d.	n.d.	n.d.	0.14	0.17	0.06	n.d.	n.d.	n.d.	n.d.
Σ C6 compounds from LA	2.75	1.03	1.64	1.05	3.89	4.02	2.37	2.08	4.38	2.26	2.51	5.07	1.27	1.42
(E)-2-hexenal	8.84	4.18	45.00	43.25	5.83	64.59	7.94	7.54	45.58	56.30	4.78	56.85	40.83	34.14
(Z)-3-hexenal	15.84	16.53	0.24	0.34	4.36	0.34	16.18	12.39	0.32	0.32	14.92	0.49	0.17	0.13
(E)-2-hexen-1-ol	1.22	0.10	0.82	0.82	1.58	3.41	0.14	0.09	4.69	1.66	0.17	7.18	0.66	1.55
(Z)-3-hexen-1-ol	11.31	11.88	0.86	2.41	15.02	5.71	3.37	4.09	1.89	2.62	13.25	3.56	0.90	0.86
(Z)-3-hexenyl acetate	0.22	0.51	0.05	0.07	n.d.	0.06	0.03	0.42	0.07	0.15	0.26	n.d.	n.d.	n.d.
Σ C6 compounds from LnA	37.42	33.19	46.97	46.88	26.78	74.10	27.66	24.53	52.55	61.06	33.38	68.08	42.56	36.69
% C6 aldehydes/ Σ C6	65.2	62.8	95.9	92.7	37.1	85.1	87.0	80.3	84.3	92.2	60.7	80.6	96.0	92.4
% C6 alcohols/ Σ C6	34.2	35.6	3.9	7.1	62.9	14.8	12.9	17.6	15.3	7.5	38.6	19.4	4.0	7.6
% C6 esters/ Σ C6	0.5	1.6	0.2	0.2	0.0	0.1	0.1	2.1	0.4	0.3	0.7	0.0	0.0	0.0

These results allow the cultivars examined to be differentiated by several different criteria. The major components in the oil samples were aldehydes: the percent of C6 aldehydes/ Σ C6 ranged from 62.8% (Miniol cultivar) to 96.0% (Favarol 1 cultivar).

The olive oils produced from the Regina, Miniol 2, Maurino and Grignano cultivars showed non-negligible amounts of (Z)-3-hexenal (12.39-16.53 mg/kg) compared with the other olive oils. In these samples, (E)-2-hexenal was present in the range of 4.18-8.84 mg/kg. All other cultivar samples (Trepp, Less, Pendolino, Gargnà, Casaliva, Mitria and Favarol 1) contained higher levels of (E)-2-hexenal, ranging from 40.83 mg/kg to 64.59 mg/kg.

The percentage of alcohols, and to a lesser extent the percentage of esters, differed among the samples: the percent of C6 alcohols/ Σ C6 was in the range of 4.0-35.6%. The olive oils from the Regina, Miniol 2, Maurino, and Grignano cultivars, containing a high level of (Z)-3-hexenal, had a (Z)-3-hexen-1-ol content ranging from 3.37 to 11.88 mg/kg.

The total quantity of aldehydes exceeded that of alcohols. The data in Table 2 suggest that (Z)-3-hexenal is not negligible for various cultivars. This finding contrasts with a previous study in which only a low level of this compound was detected (Guth and Grosch, 1993).

Other minor volatile compounds were observed in the virgin olive oils. The aldehydes heptanal, octanal, nonanal and (E)-2-heptenal were present in small quantities due to auto-oxidation reactions. Of the saturated aldehydes, hexanal was present in all samples at levels that did not exceed those found in high quality virgin olive oils.

Under the conditions adopted, the results show apparent differences among the varieties, both at the level of single volatile compounds and in the distribution of C6 volatile compounds derived from the metabolism of 13-hydroperox-

ides of LA and LnA. Considering the cultivars that had comparable ripeness indices, four (Regina, Miniol 2, Maurino and Grignano) showed that the predominant volatile compounds were (E)-2-hexenal, (Z)-3-hexenal, and (Z)-3-hexen-1-ol, with lower levels of hexanal, hexanol, (E)-2-hexen-1-ol, and (Z)-3-hexenyl acetate. In oils from seven cultivars (Trepp, Less, Pendolino, Gargnà, Casaliva, Mitria and Favarol 1), the predominant volatile compounds were (E)-2-hexenal and (Z)-3-hexen-1-ol, with lower levels of hexanal, hexanol, (Z)-3-hexenal, (E)-2-hexen-1-ol, and (Z)-3-hexenyl acetate. These two groups of cultivars differed mainly with respect to the total content of C6 aldehydes and C6 alcohols. The content of C5 compounds was higher for the first group of cultivars. Our data show that all the cultivars can be differentiated and the volatile compound composition is sufficiently distinct.

REFERENCES

- Angerosa F., Basti C. and Vito R. 1999. Virgin olive oil volatile compounds from lipoxygenase pathway and characterization of some Italian cultivars. *J. Agric. Food Chem.* 47: 836.
- Angerosa F. 2002. Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. *Eur. J. Lipid Sci. Technol.* 104: 639.
- Angerosa F., Servili M., Selvaggini R., Taticchi A., Esposto S. and Montedoro G.F. 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *J. Chromatogr. A* 1054:17.
- Aparicio R. and Morales M.T. 1998. Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J. Agric. Food Chem.* 46:1116.
- Baccouri B., Ben Temime S., Campeol E., Cioni P.L., Daoud D. and Zarrouk M. 2007. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. *Food Chem.* 102:850.
- Benincasa C., De Nino A., Lombardo N., Perri E., Sindona G. and Tagarelli A. 2003. Assay of aroma active components of virgin olive oils from southern Italian regions by SPME-GC/Ion Trap Mass Spectrometry. *J. Agric. Food Chem.* 51:733.

- Cavalli J.F., Fernandez X., Lizzani-Cuvelier L. and Loiseau A.M. 2004. Characterization of volatile compounds of French and Spanish virgin olive oil by HS-SPME: identification of quality-freshness markers. *Food Chem.* 88:151.
- Dhifi W., Angerosa F., Serraiocco A. and Oumar I. 2005. Virgin olive oil aroma: Characterization of some Tunisina cultivars. *Food Chem.* 93:697.
- EU 2002. Commission Regulation N. 796/2002 of 6 May 2002 amending Regulation N. 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis and the additional notes in the Annex to Council Regulation N. 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff. *Official Journal L* 128, 15/0/2002 p. 8.
- Guth H. and Grosch W. 1993. Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.* 70:513.
- Haddada F.M., Manai H., Daoud D., Fernandez X., Lizzani-Cuvelier L. and Zarrouk M. 2007. Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. *Food Chem.* 103:467.
- Hermoso M., Uceda M., Garcia A., Morales B., Frias M.L. and Fernandez A. 1991. Elaboracion de aceite de calidad. *Consejeria de Agricultura y Pesca. Serie Apuntes* 5/92, Sevilla.
- Jiménez A., Beltrán G. and Aguilera M.P. 2004. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils. *J. Chromatogr. A* 1028:321.
- Kalua C.M., Allen M.S., Bedgood, D.R. Jr., Bishop A.G., Prenzler P.D. and Robards K. 2007. Olive oil volatile compounds, flavour development and quality: A critical review. *Food Chem.* 100:273.
- Luna G., Morales M.T. and Aparicio R. 2006. Characterization of 39 varietal virgin olive oil by their volatile compositions. *Food Chem.* 98:243.
- Mildner-Szkudlarz S., Jeleń H.H. and Zawirska-Wojtasiak R. 2003. Application of headspace-solid phase microextraction and multivariate analysis for plant oil differentiation. *Food Chem.* 83:515.
- Montedoro G.F. and Servili M. 1992. I parametri di qualità dell'olio di oliva ed i fattori agronomici e tecnologici che li condizionano. *Riv. Ital. Sostanze Grasse* 69:563.
- Olias J.M., Pérez A.G., Rios J.J. and Sanz L.C. 1993. Aroma of virgin olive oil: biogenesis of the "green" odor notes. *J. Agric. Food Chem.* 41:2368.
- Pedò S., Mariani L. and Minelli R. 2003. La coltura dell'olivo in Lombardia. In "Il Germoplasma dell'Olivo in Lombardia. Descrizione Varietale e Caratteristiche degli Oli" - Quaderni della Ricerca n. 25. Bassi, D. (Ed.); p. 1. Regione Lombardia Publishing, Milan, Italy. Available at http://www.agricoltura.regione.lombardia.it/admin/rla_Documenti/1-1292/monografia_olivo_lombardia.pdf.
- Tura D., Failla O., Bassi D. and Serraiocco A. 2002. Sensory and chemical analysis of monovarietal olive oils from Lake Garda (northern Italy). *Acta Hort.* 586:595.
- Tura D., Prenzler P.D., Bedgood D.R., Antolovich M. and Robards K. 2004. Varietal and processing effects on the volatile profile of Australian olive oils. *Food Chem.* 84:341.
- Vichi S., Pizzale L., Conte L.S., Buxaderas S. and Lopez-Tamames E. 2003. Solid-phase microextraction in the analysis of virgin olive oil volatile fraction: Characterisation of virgin olive oil from two distinct geographical areas of northern-Italy. *J. Agric. Food Chem.* 51:6572.
- Williams M., Morales M.T., Aparicio R. and Harwood J.L. 1998. Analysis of volatiles from callus cultures of olive *Olea europaea*. *Phytochemistry* 47:1253.

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