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Characterisation of 39 varietal virgin olive oils by their volatile compositions

G. Luna [a], M.T. Morales [b,*], R. Aparicio [a]

[a] *Unidad Asociada, "Análisis y Caracterización de Alimentos Grasos", Instituto de la Grasa (CSIC), Avda. Padre García Tejero 4, 41012, Seville, Spain*
[b] *Unidad Asociada "Análisis y Caracterización de Alimentos Grasos", Department of Analytical Chemistry, Faculty of Pharmacy, University of Seville, c/Prof. García González, 2 – 41012, Seville, Spain*

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Abstract

Thirty-nine single virgin olive oils from eight producer countries, but cultivated in the same orchard under identical agronomic and pedoclimatic conditions, were characterised by 64 volatile compounds quantified by dynamic headspace-gas chromatography. The method was validated by analysing the relative standard deviation in repeatability and intermediate reproducibility of each volatile compound. Data on the total content of volatiles and C6 compounds – responsible for the green odour perception-, and the total content of hydrocarbons, aldehydes, alcohols, ketones and esters are displayed and discussed for each individual virgin olive oil. Fifty volatile compounds were characterised by their sensory attributes, determined by olfactometry. The Student *t*-test was used to determine the level of significance of each volatile compound characterising the varieties. The odour threshold and the maximum concentration of the most notable volatiles are also displayed. Finally, the Brown-Forsythe test and stepwise linear discriminant analysis were used to check the ability of the volatiles to cluster the varietal virgin olive oils according to their autochthonous geographical origin.

Keywords: Varietal olive oil; Volatile compounds; DHS-GC; Odour activity value; Olfactometry; Chemometrics

1. Introduction

Virgin olive oil, extracted from the fruit of the olive tree, *Olea europea L.*, is consumed without further refining to retain non-volatile and volatile minor compounds. The non-volatile compounds determine the purity while the volatiles are responsible for the flavour sensory attributes. These attributes qualify the virgin olive oil and have an influence on its appreciation by consumers. Virgin olive oils qualified by undesirable attributes are obviously rejected by every consumer while those ones characterised by a pleasant sweet-green odour, with a slight pungent taste, are cherished by almost all the consumers (Aparicio & Morales, 1995).

The volatile compounds responsible for virgin olive oil flavour come from the olive fruit. These volatiles can be considered to be direct metabolites produced in plant organs by intracellular biogenic pathways. Some of the volatiles found in virgin olive oil are present in the intact tissue of the fruit, and others are formed during disruption of cell structure during the virgin olive oil production due to enzymatic reactions in the presence of oxygen. The main precursors of volatile compounds are fatty acids (particularly linoleic and alpha-linolenic) and amino acids (leucine, isoleucine and valine) (Morales & Tsimidou, 2000). Some differences can be found in the fatty acid content of varietal virgin olive oils (Aparicio,

* Corresponding author. Tel.: +34 954 55 38 04; fax: +34 954 55 67 49.
E-mail address: tmorales@cica.es (M.T. Morales).

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2000; Aparicio & Luna, 2002) but they do not vary so much as to be determinants of the profile of volatiles. In fact, it has been reported that the concentrations of volatile compounds depend on the enzymatic activity (Salas, Sánchez, García-González, & Aparicio, 2005) though external parameters (e.g. climate, soil, harvesting and extraction conditions) may alter the inherent olive oil sensory profile (Aparicio, Ferreiro, & Alonso, 1994; Aparicio & Morales, 1998; Morales & Aparicio, 1999).

Various studies have been carried out on the characterisation of virgin olive oils by quantification of the volatile compounds (Aparicio, Morales, & Alonso, 1997; Aparicio & Luna, 2002; Luna, 2003; Vicchi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003) but no study has been carried out on the exclusive effect of the cultivar on the concentrations of the volatile compounds of varietal virgin olive oils.

The aim of this work is the global characterisation of reputed varietal virgin olive oils, cultivated in an orchard, by quantification of the volatile compounds present in their aroma. To avoid the influence of other factors in the characterisation, olive trees were cultivated under the same agronomic (i.e. fertilization, irrigation) and pedoclimatic conditions, olive fruits were picked at the same stage of ripeness, and their oils were extracted with the same processing system.

2. Materials and methods

2.1. Samples

Thirty-nine varietal virgin olive oils, native to several producer countries (Bartolini, Prevost, Messeri, & Carignani, 1998), were studied (Table 1). Varieties were selected regarding: (i) significance in the national production of virgin olive oil (e.g., Picual, Coratina, Koroneiki, Picholine Marocaine, Memecik); (ii) significance within some Mediterranean regions (e.g. Frantoio, Empeltre, Nevadillo, Negro); and (iii) acceptability by non-habitual consumers (e.g. Arbequina, Cornicabra, Cima di Bitonto). These olive varieties were collected from the botanic garden of Hacienda Guzman (Seville, Spain), where the olive trees are cultivated under identical agronomic and pedoclimatic conditions. Three samples were collected from each variety at the same stage of ripeness.

Olive oils were extracted from fresh and healthy fruits of good quality at a maturity index of 5–6 (Frías-Ruiz et al., 1991). The extraction process was carried on an analytical scale using an experimental oil mill (Abencor, Comercial Abengoa, Seville, Spain). Seven hundred grammes of olives of each sample were crushed. The temperature and the time of malaxation were 25 °C and 30 min, respectively (Martínez-Suárez, Muñoz-Aranda, Alba-Mendoza, & Lanzón-Rey, 1975). After the centrifugation, the oil was obtained by decantation and immediately stored at −18 °C prior to analysis by DHS-HRGC.

Table 1
Varietal virgin olive oils and their native geographical origin

Code	Varietal virgin olive oil	Geographic origin
1	Adramytini	Greece
2	Arbequina	Spain
3	Cañivano	Spain
4	Chami	Syria
5	Chetoui	Tunisia
6	Chemlal of Kabylie	Argelia
7	Chorruo	Spain
8	Cima di Bitonto	Italy
9	Coratina	Italy
10	Cornicabra	Spain
11	Empeltre	Spain
12	Frantoio	Italy
13	Hojiblanca	Spain
14	Imperial	Spain
15	Konservalia	Greece
16	Koroneiki	Greece
17	Leccino	Italy
18	Lechín	Spain
19	Manzanilla	Spain
20	Manzanillo Cordobés	Spain
21	Mastoides	Greece
22	Megaritiki	Greece
23	Memecik	Turkey
24	Moraiolo	Italy
25	Morruda	Spain
26	Negro	Spain
27	Nevadillo	Spain
28	Nevado	Spain
29	Nisjot	Greece
30	Ogghiaredda	Italy
31	Picholine Marocaine	Morocco
32	Picual	Spain
33	Picudo	Spain
34	Redondilla	Spain
35	Santa Caterina	Italy
36	Sourani	Syria
37	Tsounati	Greece
38	Verdial de Huévar	Spain
39	Zaity	Syria

2.2. Reagents

Butan-2-one, butyl acetate, ethanol, ethyl acetate, ethyl benzene, ethyl propanoate, *E*-2-hexenal, *E*-2-hexen-1-ol, *E*-3-hexen-1-ol, *Z*-3-hexen-1-ol, *Z*-2-hexen-1-ol, hexyl acetate, heptan-2-one, hexanal, hexan-1-ol, 2,4-hexadienal, *Z*-3-hexenal, 3-hexenyl acetate, isobutyl acetate, 2-methylbutyl acetate, 3-methyl-butanal, *E*-2-methyl-2-butenal, 2-methyl-3-buten-2-ol, 6-methyl-5-hepten-2-one, 3-methyl-2-butenyl acetate, methyl acetate, 2-methyl propan-1-ol, 3-methyl butan-1-ol, 4-methyl pentan-2-one, nonan-2-one, octane, octan-2-one, *E*-2-octenal, pentan-1-ol, pentan-3-one, *E*-2-pentenal, *Z*-2-pentenal, *Z*-2-penten-1-ol, 1-penten-3-ol, 1-penten-

3-one, and 1,2,4-trimethylbenzene were purchased from Fluka–Sigma–Aldrich (St. Louis, MO).

2.3. Dynamic headspace-gas chromatography

Volatile compounds were analysed by a modified dynamic headspace technique previously reported (Morales, Aparicio, & Ríos, 1994; Morales, Luna, & Aparicio, 2005). Samples of 0.5 g were heated at 40 °C and swept with N_2 (200 ml/min) for 15 min and the volatiles adsorbed onto a Tenax TA trap (Chrompack, Middleburg, The Netherlands) at room temperature. The volatiles were condensed onto a fused-silica trap, cooled at −10 °C with liquid nitrogen for 5 min just before injection, which was carried out by flash heating of the cold trap at 170 °C, where it was held for 5 min. The volatiles were transferred onto a fused silica DB-Wax column (60 m × 0.25 mm *id* × 0.25 μm thickness) (J&W Scientific, Folsom, CA). The carrier gas was hydrogen. The oven temperature was held at 40 °C for 4 min and programmed to rise at 1 °C/min to a temperature of 91 °C, and then to rise at 10 °C/min to a final temperature of 201 °C, where it was held for 10 minutes. A Hewlett–Packard 5890 Series II (Palo Alto, CA) with a flame-ionization detector was employed. Volatiles were isolated and analysed in duplicate.

The internal standard used for the quantification of volatile compounds was isobutyl acetate (Aparicio & Morales, 1994). The internal standard was used to diminish the influence of hypothetical variations of the concentration step on the results, so allowing a better comparison between the volatiles produced by every varietal virgin olive oil.

2.4. Linearity and repeatability of the internal standard

The linearity was studied by preparing eight dilutions of the internal standard (isobutyl acetate) in refined sunflower oil (Ucasol, Spain). Concentrations varied from 0.5 to 4.0 mg/kg. The regression coefficient was $r = 0.9993$. The repeatability was investigated by consecutively analysing 12 samples of Ogghiaredda variety spiked with 3.33 mg/kg of isobutyl acetate. The relative standard deviation was 6.0%.

2.5. Repeatability and intermediate reproducibility of the method

The study of the precision of the method (Boqué, Maroto, Riu, & Rius, 2002) was carried out with the Ogghiaredda variety. Three replicates were analysed in a unique analytical session to determine the repeatability while the intermediate reproducibility was determined by analysing the sample in 12 non-consecutive analytical sessions.

2.6. Identification of volatiles

The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards if available (see reagents). The identification by GC–MS was carried out using conditions identical to those used for the GC with the exception of the carrier gas that was helium. A Fisons Mass Detector MD800, coupled to a GC 8000 series, was employed. The identity of the volatiles was obtained by comparison of their mass spectral data with the information from the Nist version 1.7 library. The volatile compounds were also identified using the relative retention times of the standards with respect to the internal standard (isobutyl acetate).

2.7. Sensory properties of volatile compounds by GC-olfactometry

An HRGC-sniffing technique was applied to virgin olive oil samples in order to assess the aroma notes corresponding to olive oil volatile compounds (Morales, Alonso, Ríos, & Aparicio, 1995). The effluent of the GC column was split 1–10 to the detector and the sniffing port, respectively. The odour-active regions of the eluate were evaluated and their aroma notes assigned by five assessors. The odour descriptions were noted on a form with a pre-printed time scale; assessors did not see the chromatogram. Assessors basically agreed on the odours of volatile compounds, although different semantic terms were used. A consensus-building discussion was held with assessors to decide the final sensory descriptors.

2.8. Odour threshold of volatile compounds

A refined olive oil was the matrix used for the evaluation. The absence of volatile compounds in this matrix was checked by the DHS-GC procedure already described. The sensory evaluation was carried out according to the virgin olive oil assessment (IOOC, 1987). All the testing sessions were on Friday mornings, by four assessors. Three samples were presented to the assessors, following the triangle test method, and the results were statistically analysed. Fifteen millilitres of each sample were kept in standardised tasting glasses at 29 ± 2 °C for 15 min and then tested (IOOC, 1987).

The odour activity values (OAVs) (ratio of the concentration to the odour threshold) (Rothe & Thomas, 1963; Aparicio & Morales, 1998; Buettner & Schieberle, 2000) of volatile compounds were calculated to determine their sensory significance. Thus, the concentration of each volatile found in the oil samples was divided by its corresponding odour threshold value, previously determined as described above.

2.9. Mathematical analysis

The whole set of data was imported to Excel from the HPChemstation program (Agilent Technologies, Palo Alto, CA), and Statistica release 6.0 (Statsoft, 2001) was used to perform the data processing by means of the following statistical procedures.

Cluster analysis was applied to determine the natural conformation of groups of varietal olive oils gathered round an objective category (i.e. geographical origin). A Box–Whisker plot was used for a visual inspection of the variability of the volatile compounds from the samples conforming to the clusters. The statistical study of the differences between the clusters of virgin olive oils was carried out by analysing each one of their volatile compounds independently. The Brown and Forsythe test was used to perform the analysis on the deviations from the group medians since it gives quite accurate error rates, even when the underlying distributions for the raw scores deviate significantly from the normal distribution (Olejnik & Algina, 1987).

This univariate information was used to select the initial set of variables (volatiles) for the stepwise linear discriminant analysis (SLDA), which was applied under the strictest conditions in order to diminish over-optimistic models. Thus, tolerance was fixed at 0.01 while *F*-to-Enter value was obtained from the *F*-distribution table at $F(F) = 0.995$, taking into account the number of groups and the group with the minimum number of samples (Tabacknick & Fidell, 1983).

3. Results and discussion

Fig. 1 shows the chromatographic localization of the identified volatiles in the virgin olive oil samples var. Picual and Imperial while Table 2 shows their names and relative retention times. Table 2 also shows the relative standard deviation, in terms of repeatability (RSD_r) and intermediate reproducibility (RSD_R) of all the volatiles identified and quantified in all the varieties, with the exception of *Z*-2-hexenal whose concentration was always at trace level. An analysis of the values showed that 45 of the volatiles (71.4%) showed an RSD_r lower than 10% (20 lower than 5%), 11 volatiles (17.5%) presented values of 10–15% and only seven volatiles (11.1%) had values higher than 15%. These values were, obviously, worse in reproducibility. Thus, only 39.7% of the volatiles had RSD_R values lower than 10%, 23 volatiles (36.5%) presented values of 10–15% and unfortunately 15 volatile compounds (23.8%) had RSD_R values above 15% (8 above 20%). The results were good enough if we take into account that the volatile compounds selected for the classification of the varieties and contributing to the olive oil aroma (OAV > 1.0) presented good values of RSD in repeatability and intermediate reproducibility.

Table 2 also shows the sensory characterisation of the volatile compounds with the exception of a few that were mostly hydrocarbons. Although a consensus was held on the sensory descriptors, the assessors were suggested to limit the description of the sensory perceptions to the attributes defined by the International Olive Oil Council (IOOC, 1987) when possible.

Table 3 shows basic information on the chemical compounds of the selected varietal virgin olive oils. The table indicates interesting quantitative differences. The total volatile content of the analysed varieties ranged from 9.83 to 35 mg/kg, although 74% of the varieties were in the range 15 to 25 mg/kg. Only six varieties (Cornicabra, Hojiblanca, Konservalia, Picual, Redondilla and Verdial de Huévar) had values below 15 mg/kg, four varieties (Cañivano, Coratina, Leccino and Lechín) being between 25 mg/kg and 30 mg/kg, and the highest values corresponded to the other two varieties, Chemlal of Kabylie with 35.0 mg/kg and Nevado Azul, with 32.9 mg/kg. These different amounts of total volatiles should be exclusively related to the cul-

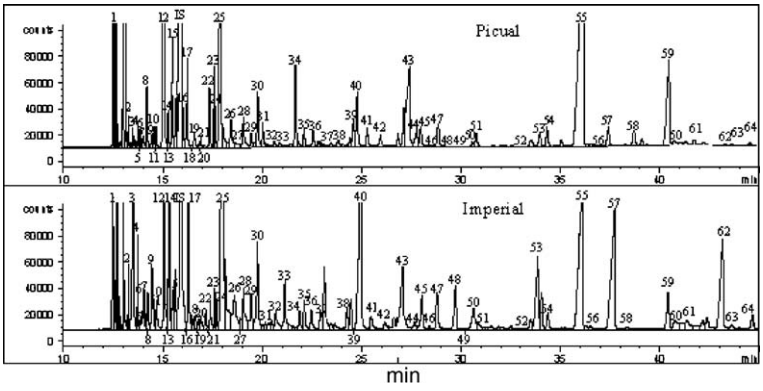

Fig. 1. Chromatographic localization of the volatile compounds identified and quantified in the Spanish varieties Picual and Imperial. Codes are described in Table 2. Note: IS, internal standard.

Table 2
Information on the relative retention time, the relative standard deviation and the sensory attributes characterising the volatile compounds quantified in the varietal virgin olive oils

Code	t_{RR}	Volatile	RSD_r	RSD_R	Sensory atributes
1	0.82	Hydrocarbon	8.0	17.0	–
2	0.84	Methyl acetate	14.8	18.7	Ethereal, sweet
3	0.85	Octane	15.3	18.5	Solvent
4	0.86	Ethyl acetate	2.4	6.8	Sweet, aromatic, ethereal
5	0.88	Hydrocarbon	15.4	19.3	–
6	0.89	Butan-2-one	2.2	9.9	Fragant, pleasant, ethereal
7	0.90	3-Methyl-butanal	4.9	14.4	Sweet, fruity
8	0.91	Hydrocarbon	16.8	21.4	–
9	0.92	Ethanol	14.6	19.7	Apple, sweet
10	0.93	Ethyl furan	21.7	24.1	Sweet, ethereal
11	0.93	Ethyl propanoate	5.0	9.8	Strawberry, apple, fruity, sweet
12	0.95	Pentan-3-one	8.9	9.7	Sweet, fruity
13	0.97	Pentan-2-one	12.7	14.8	Sweet
14	0.98	2-Methyl butanal	5.0	14.1	Sweet
15	0.99	4-Methyl-pentan-2-one	8.5	9.1	Strawberry, fruity, sweet, ethereal
16	1.01	1-Penten-3-one	3.1	5.1	Pungent, mustard
17	1.02	Hydrocarbon	10.0	28.6	Apple, sweet
18	1.03	Dimethyl nonadiene	4.8	13.1	Sweet, apple
19	1.04	2-Methyl-3-buten-2-ol	4.5	12.2	Grassy, earth, oily
20	1.05	Unknown	9.9	13.2	–
21	1.06	Unknown	10.8	7.2	–
22	1.07	Hydrocarbon	5.2	11.1	–
23	1.10	Butyl acetate	3.5	7.2	Green, fruity, pungent, sweet
24	1.11	3-Methyl pentanal	3.8	12.0	–
25	1.12	Hexanal	3.7	7.9	Green apple, grass
26	1.14	Hydrocarbon	3.0	6.0	–
27	1.16	*E*-2-Methyl-2-butenal	11.0	12.2	Green fruit, aromatic
28	1.17	2-Methyl propan-1-ol	3.7	9.8	Penetrating, solvent
29	1.20	Ethylbenzene	3.3	6.1	Strong
30	1.21	2-Methyl-butyl acetate	5.1	9.7	Fruity, green, banana
31	1.24	*Z*-2-Pentenal	5.6	13.4	Green, pleasant
32	1.26	*E*-2-Pentenal	3.8	5.2	Green apple, tomato, pungent
33	1.31	*E*-3-Hexenal	6.1	12.8	Artichoke, green, flowers
34	1.35	*Z*-3-Hexenal	10.2	18.6	Green leaves, cut grass
35	1.37	1-Penten-3-ol	9.4	12.5	Butter, soft green
36	1.40	2-Methyl-4-pentenal	11.1	25.0	–
37	1.43	Heptan-2-one	4.0	9.9	Sweet, fruity, cinnamon
38	1.48	Ester	10.7	16.8	Banana
39	1.53	*Z*-2-Hexenal	–	–	Fruity
40	1.55	*E*-2-Hexenal	3.1	4.7	Bitter almonds, green- fruity, sharp, bitter, astringent
41	1.58	3-Mehyl butanol	5.6	8.4	Unpleasant
42	1.67	Dodecene	11.3	14.7	–
43	1.70	Ethenyl benzene	8.7	15.0	–
44	1.76	Pentan-1-ol	3.5	8.3	Pungent, strong, sweet, balsamic
45	1.77	3-Methyl-2-butenylacetate	1.6	5.2	Pungent
46	1.79	1,2,4-Trimethylbenzene	9.0	15.0	–
47	1.81	Hexyl acetate	7.0	7.3	Sweet, green, fruity, apple
48	1.89	Octan-2-one	8.0	8.5	Mould, overripe
49	1.90	3-Octen-2-one	20.2	25.1	–
50	1.92	3,4-Dimethyl 3-pentenyl furan	17.2	21.2	Paint
51	1.93	3-Hexenyl acetate	8.3	14.5	Green banana, green leaves, fruity
52	2.02	*E*-2-Octenal	8.7	12.5	Green, grassy
53	2.05	*Z*-2-Penten-1-ol	7.7	14.8	Banana
54	2.12	Hydrocarbon	19.2	23.4	–
55	2.19	6-Methyl-5-hepten-2-one	3.8	7.8	Green-fruity, grass, pungent
56	2.23	Unknown	8.6	23.8	Fruity
57	2.36	Hexan-1-ol	2.6	8.1	Fruity, soft, aromatic, alcoholic, rough
58	2.38	*E*-3-Hexen-1-ol	5.5	7.4	Astringent, bitter
59	2.52	*Z*-3-Hexen-1-ol	6.1	6.5	Banana, fresh, green grass
60	2.53	Nonan-2-one	9.6	14.8	Fruity, floral
61	2.59	2,4-Hexadienal	11.3	12.2	Fresh, green, floral, citric
62	2.65	*E*-2-Hexen-1-ol	9.2	13.8	Green grass, leaves, fruity, astringent, bitter
63	2.69	Unknown	3.8	14.9	Strong, sweet, balsamic
64	2.75	*Z*-2-Hexen-1-ol	8.6	13.0	Almond, grass, astringent

Note: t_{RR}, relative retention time to the internal standard (isobutyl acetate).

Table 3
Total content (mg/kg) of volatiles and "green" volatile compounds (C_6), and total concentrations of hydrocarbons, aldehydes, alcohols, ketones and esters in the varietal virgin olive oils

Code	Varietal virgin olive oil	Total volatiles	Total C_6 compounds	Total hydrocarbons	Total aldehydes	Total alcohols	Total ketones	Total esters
1	Adramytini	15.6	11.1	3.40	10.0	1.00	0.85	0.23
2	Arbequina	19.0	12.6	2.72	10.0	4.40	1.17	0.69
3	Cañivano	28.6	12.8	8.50	6.45	9.94	2.06	1.48
4	Chami	23.7	11.8	8.77	5.01	7.69	1.09	1.08
5	Chetoui	15.0	4.88	6.06	4.88	1.89	1.59	0.55
6	Chemlal of Kabylie	35.0	17.6	9.14	11.3	11.3	1.78	1.26
7	Chorruo	16.8	7.13	4.79	4.34	4.79	1.80	1.07
8	Cima di Bitonto	19.7	9.88	4.41	9.46	4.48	0.85	0.38
9	Coratina	27.0	15.4	4.30	13.0	7.47	1.73	0.44
10	Cornicabra	14.7	2.52	3.42	1.59	1.65	5.32	2.60
11	Empeltre	17.8	11.0	1.84	0.82	11.8	2.67	0.61
12	Frantoio	19.0	10.9	4.60	7.17	5.73	1.08	0.31
13	Hojiblanca	9.83	4.25	2.22	2.05	3.15	0.80	0.64
14	Imperial	16.4	6.38	4.54	2.97	3.77	3.44	1.49
15	Konservalia	10.7	6.26	3.35	4.66	1.56	0.83	0.28
16	Koroneiki	17.7	6.78	5.56	5.46	4.58	1.48	0.51
17	Leccino	25.3	16.8	5.48	16.2	1.90	1.29	0.44
18	Lechín	27.5	17.4	4.84	17.1	2.78	2.13	0.51
19	Manzanilla	18.6	10.9	4.42	8.15	4.20	0.72	1.09
20	Manzanillo Cordobés	20.3	12.1	4.63	9.52	3.34	1.23	1.50
21	Mastoides	19.6	12.3	3.31	9.66	4.54	1.57	0.50
22	Megaritiki	21.2	16.2	3.79	14.3	1.90	0.67	0.44
23	Memecik	16.4	8.03	7.29	2.02	4.75	1.51	0.66
24	Moraiolo	24.9	12.5	8.34	8.99	3.72	1.53	2.18
25	Morruda	16.9	10.2	2.40	9.81	2.87	1.29	0.50
26	Negro	22.1	13.8	4.43	11.3	2.93	1.43	1.92
27	Nevadillo negro	18.4	8.86	4.31	4.52	4.76	3.74	0.71
28	Nevado Azul	32.9	18.1	7.82	14.1	5.72	1.83	3.37
29	Nisjot	18.0	11.9	3.76	10.8	2.22	0.73	0.41
30	Ogghiaredda	22.5	14.0	4.93	13.1	2.66	1.10	0.75
31	Picholine Marocaine	16.4	5.63	7.86	2.13	3.54	1.93	0.88
32	Picual	12.3	2.76	3.34	1.61	2.25	4.26	0.61
33	Picudo	19.3	7.32	6.48	7.42	2.62	2.34	0.38
34	Redondilla	10.1	5.78	2.65	4.87	1.33	0.74	0.28
35	Santa Caterina	24.3	12.6	8.08	10.3	3.60	1.61	0.69
36	Sourani	15.52	8.12	2.74	2.50	8.20	1.64	0.38
37	Tsounati	17.3	7.80	3.90	5.77	5.93	0.97	0.59
38	Verdial de Huévar	13.0	5.72	3.92	3.89	2.01	1.78	1.22
39	Zaity	15.1	6.70	5.56	3.01	4.66	0.97	0.85

tivar, as the extraction system, the olive ripeness and the pedoclimatic and agronomic conditions were the same for all the cultivars.

Considering the geographical origin of the olive trees cultivated in the Spanish botanical garden, Italian virgin olive oils displayed the highest values. Their concentrations ranged from 19.0 to 27.0 mg/kg, Coratina being the variety with the highest value and Frantoio with the lowest one. The Spanish samples showed the widest concentration range (9.83–32.9 mg/kg), Nevado Azul being the variety with the highest value and Hojiblanca with the lowest one. With respect to the Greek virgin olive oils, their concentrations ranged from 10.7 to 21.2 mg/kg. Finally, the non-European samples showed a total content of volatiles below 20 mg/kg, with the exception of Chemlal of Kabylie.

Because of the importance of the green perception in the olive oil flavour (Aparicio & Morales, 1995), the C_6 compounds – also called "green volatiles" (Aparicio & Morales, 1998) – were quantified in all of the varieties. These compounds are formed through the lipoxygenase biochemical pathway (Morales, Aparicio, & Calvente, 1996; Sánchez & Salas, 2000) and the differences in the concentration should be mostly related to the variety as there were no external variables that might unequally affect the enzyme activity of a particular variety (e.g., latitude, weather, fertilizers, ripeness, extraction conditions).

Table 3 shows that the range of concentrations varied from 2.52 to 18.1 mg/kg although most varieties (64%) had concentrations within the range 6–14 mg/kg, *E*-2-hexenal being the major contributor. Cluster analysis

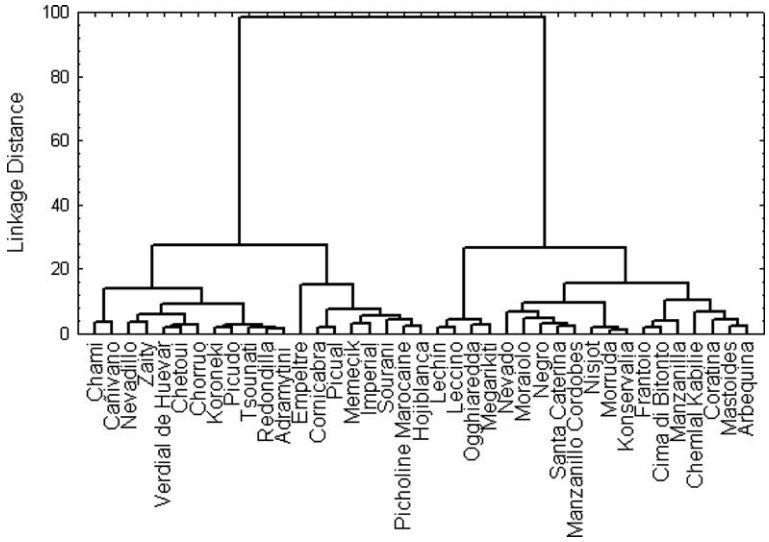

Fig. 2. Cluster analysis of the green volatile compounds of the 39 varieties analysed.

was applied to the whole set of green volatile compounds, produced through the lipoxygenase pathway, and the result showed four main groups (Fig. 2), each one of them characterised by different volatile profiles. Thus, the first group is constituted by varieties of different geographical origin (Arbequina, Coratina, Cima di Bitonto, Chemlal of Kabylie, Frantoio, Manzanilla, Manzanillo Cordobés, Mastoides, Moraiolo, Morruda, Negro, Nevado, Nisjot, Santa Caterina and Konservalia). These varieties have, in common, a high content of *E*-2-hexenal, mean contents of alcohols and esters and a low concentration of hexanal. The second group is constituted by the varieties Leccino, Lechin, Megaritiki and Ogghiaredda that also have a high concentration of *E*-2-hexenal, a low concentration of alcohols, a high concentration of esters and a concentration of hexanal similar to the alcohols. The varieties Cornicabra, Empeltre, Hojiblanca, Imperial, Picual, Memecik, Picholine Marrocaine and Sourani constitute the third group of the cluster plot, and they are characterised by high concentrations of hexan-1-ol and *Z*-3-hexen-1-ol, very low concentrations of *E*-2-hexenal and the esters, and a concentration of hexanal higher than *E*-2-hexenal. The fourth group, constituted by 12 single virgin olive oils (Adramytini, Cañivano, Chami, Chetoui, Chorruo, Koroneiki, Nevadillo, Picudo, Redondilla, Tsounati, Verdial de Huévar and Zaity), is characterised by high concentrations of *E*-2-hexenal and hexanal, a mean concentration of alcohols and a low concentration of esters.

This study, however, does not mean that all the varieties clustered within each one of the previous groups have homogeneous profiles but only that they share basic information of the volatile composition. Fig. 1 shows, as an example, the difference between the chromatograms of Spanish Picual and Imperial varieties that were, however, clustered within the third group of the cluster plot. *Z*-3-Hexenal, 6-methyl-5-hepten-2-one and *Z*-3-hexen-1-ol are at higher concentrations in the Picual variety than in the Imperial variety while, on the contrary, the Imperial variety has higher amounts of *E*-2-hexenal, octan-2-one, *Z*-2-penten-1-ol, hexan-1-ol and *E*-2-hexen-1-ol than Picual.

Table 3 shows the total concentrations of hydrocarbons (12), aldehydes (13), alcohols (12), ketones (13), and esters (6) in each variety studied. The lowest concentrations of hydrocarbons were found in three varieties, Empeltre, Hojiblanca and Morruda, all of them proceeding from Spain. The highest value corresponded to Chemlal of Kabilie and Chami, both of them autochthonous from non-European countries. These compounds have scarce sensory significance (Table 2). Aldehyde content was very variable, ranging from 0.82 to 17.1 mg/kg. The lowest values were quantified in Spanish varieties Empeltre, Cornicabra and Picual while the highest values were found in Lechin and Leccino, varieties native to Andalusia (Spain) and Italy, respectively. Aldehydes are usually characterised by intense sensory descriptions associated with green, fruity and sweet sensory notes (Table 2).

The total content of alcohols varied between 1.00 and 5.93 mg/kg for 85% of the varieties but three varieties (Chami, Coratina and Sourani) had a content around 8 mg/kg. Spanish virgin olive oils, Empeltre and Cañivano, that are cultivated in Northern (Huesca) and Southern (Córdoba) Spain, and the Algerian variety, Chemlal of Kabylie, showed the highest values of alcohols. These compounds have less sensory significance than aldehydes because of their higher odour threshold values (Table 4); their sensory descriptions being associated with fruity, soft green and aromatic sensory notes. The highest concentrations of ketones were quantified in the Spanish varieties, Cornicabra, Picual, Nevadillo,

Table 4
Characterisation of the virgin olive oil varieties by the Student *t*-test at the significance levels $\alpha = 0.05$ or $\alpha = 0.10$. Characterisation of single olive oils by the odour activity value (OAV) of the volatile compounds. Information on the odour thresholds of the volatiles, varieties with OAVs greater than 1.00 and the variety with the maximum volatile concentration

Code	Volatile	Student $t\ \alpha = 0.05$	Student $t\ \alpha = 0.10$	Threshold (μg/kg)	Varieties with OAV >1.0	Variety (max. μg/kg)
2	Methyl acetate	Cornicabra	–	2,000	None	10 (400)
4	Ethyl acetate	Manzanilla	–	940	None	39 (287)
6	Butan-2-one	Tsounati	–	40,000	None	37 (204)
7	3-Methyl-butanal	Tsounati	–	5.4	All	37 (223)
9	Ethanol	Hojiblanca	–	30,000	None	13 (917)
11	Ethyl propanoate	Cornicabra	–	100	6, 9, 10, 20, 31, 33	10 (249)
14	2-Methyl butanal	Coratina	–	2.2	All	31 (510)
15	4-Methyl-pentan-2-one	Picudo	–	300	3, 5–7, 18, 20, 24–26, 28, 33, 36	33 (899)
16	1-Penten-3-one	Picudo	–	0.7	All	33 (1689)
19	2-Methyl-3-buten-2-ol	Lechín	–	480	None	18 (147)
23	Butyl acetate	Picudo	–	100	2–3, 5–7, 9, 16–18, 20–21, 23–26, 28–29, 31–33, 35–36	33 (455)
25	Hexanal	Cañivano	–	75	All	3 (5353)
27	*E*-2-Methyl-2-butenal	Zaity	–	400	39	39 (404)
30	2-Methyl-butyl acetate	Imperial	–	2,000	None	14 (358)
31	*Z*-2-Pentenal	–	Moraiolo	1,500	None	24 (585)
32	*E*-2-Pentenal	–	Moraiolo	300	3, 7, 20, 24, 27, 29, 31–32, 34, 36–37	24 (3822)
33	(*E*)-3-Hexenal	Imperial	–	3.0	All	14 (232)
34	*Z*-3-Hexenal	Picudo	–	1.7	All	33 (581)
35	1-Penten-3-ol	–	Santa Caterina	400	14	14 (342)
37	Heptan-2-one	Negro	–	300	None	10 (75)
40	*E*-2-Hexenal	–	Lechín	424	All excepting 10 and 11	18 (15,459)
41	3-Methyl butanol	Coratina	–	100	3, 6, 8–10, 12, 16, 32, 37	3 (5357)
44	Pentan-1-ol	Sourani	–	470	None	36 (211)
47	Hexyl acetate	–	–	1,040	None	19 (368)
48	Octan-2-one	Koroneiki	–	500	None	16 (334)
51	*Z*-3-Hexenyl acetate	Chorruo	Arbequina	200	7, 16, 19, 31	19 (476)
52	*E*-2-Octenal	Nevadillo	–	4	All	2 (651)
53	*Z*-2-Penten-1-ol	–	Moraiolo	250	3–6, 12–14, 16, 19–20, 24–28, 35, 38–39	24 (1313)
55	6-Methyl-5-hepten-2-one	Picual	–	1,000	7, 10, 14, 27, 32, 38	32 (2337)
57	Hexan-1-ol	Empeltre	–	400	2–4, 6, 8–14, 19–21, 23, 27–28, 31, 35–37, 39	11 (3297)
59	*Z*-3-Hexen-1-ol	Sourani	–	1,100	11, 13, 19–20, 23, 28, 31, 35–36	36 (2764)
60	Nonan-2-one	–	Cañivano	<100	3, 10, 24, 26, 32	3 (728)
61	2,4-Hexadienal	Imperial	–	2,000	None	14 (92)
62	*E*-2-Hexen-1-ol	–	Empeltre	5,000	6, 9, 11	11 (4795)
64	*Z*-2-Hexen-1-ol	–	Nevado	1,000	None	24 (190)

Imperial and Empeltre, while the rest of the varieties presented amounts that varied between 0.67 and 2.34 mg/kg. These compounds have been mostly associated with fruity, pungent and ethereal sensory perceptions (Table 2).

Esters showed the lowest values. Almost all the varieties ranged from 0.23 to 1.50 mg/kg, all the Greek virgin olive oils having very low values. Nevado Azul, Cornicabra, Moraiolo and Negro were the varieties with the highest contents of esters, compounds associated with fruity sensory notes (Table 2).

The previous study indicated that there were quantitative differences between the volatile profiles of the varieties analysed, in spite of having been cultivated in the same orchard and under identical agronomic conditions. An analysis of histograms allowed determination of the dispersion of the values of each individual volatile compound among the varieties. The Student t-test, at the significance levels, $p = 0.10$ and $p = 0.05$, was applied to each volatile of any variety that seemed to be significantly different from the rest of the varieties. Table 4 shows the results of applying the test to the whole data set of volatile compounds. Only 21 of the 39 varieties seem to have been quantitatively characterised by one or more volatile compounds. However, a volatile compound contributes to the aroma of a virgin olive oil when its OAV > 1.0 which means that the concentration of the volatile in the oil is higher than its odour threshold.

Table 4 shows (in its first column) the code of the volatile compound (second column) that characterises a varietal virgin olive oil at $\alpha = 0.05$ or $\alpha = 0.10$, its odour threshold according to the assessors, the varietal virgin olive oils where the volatile compound presented an OAV > 1 and the varietal virgin olive oil with the highest concentration of this compound, together with the

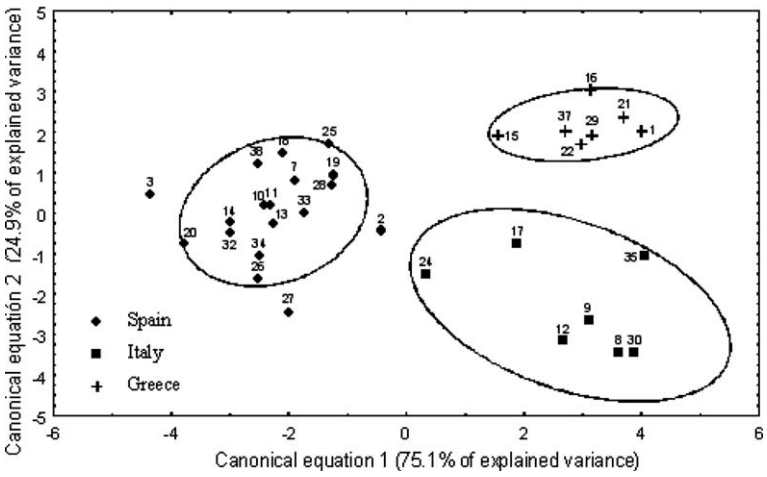

Fig. 3. Results of applying SLDA to the European samples. The ellipses of confidence were plotted at a probability of 0.95. Codes are described in Table 1.

concentration (within parenthesis). OAV showed that the contributions of the volatile compounds to the aroma of the varieties, and hence to their sensory characterisation, were very different. Thirteen of the volatiles described in Table 4 did not contribute to the aroma of the varieties since their OAVs were lower than 1.0, while seven volatiles contributed to the aroma of all of the varieties. Thirteen volatiles contributed only to a certain number of varieties, varying from 3 (*E*-2-hexen-1-ol) to 37 varieties (*E*-2-hexenal). It is notable that the volatiles characterising a variety showed a maximum concentration in that variety, excepting ethyl acetate, 1-penten-3-ol, heptan-2-one and (Z)-2-hexen-1-ol.

Because of the grouping trend observed in the varieties originally cultivated in the same country, statistical procedures were applied to the volatile compounds to see whether it was possible to differentiate the olive oil samples according to their native geographical origin. The results of the Brown and Forsythe test were not optimal as only a few compounds were useful at $p < 0.05$. These compounds constituted the set of variables submitted to SLDA. The objective was to determine their ability to discriminate the varieties native to the main producer countries (Spain, Italy and Greece). SLDA analysis showed that the compounds selected to differentiate the Spanish and Greek olive oils were hexyl acetate, ethyl benzene and an ester (38) partially identified. These compounds allowed 100% of correct classifications. The equation to distinguish Spanish oils from the Italian oils was based on 2-methyl butyl acetate, 2-methyl-4-pentenal, hexan-1-ol and the ester. These compounds allowed correct classification of 100% of the Spanish samples but only 71.4% of the Italian samples. Pentan-3-one, *Z*-3-hexenal and a hydrocarbon (26) allowed to be distinguished the Italian and Greek samples with 100% correct classifications.

In the simultaneous classification of the samples from Spain, Italy and Greece, the procedure selected the most discriminant volatile compounds obtained in the previous studies – hexyl acetate, ethyl benzene, 2-methyl-4-pentenal and an ester- and added five new compounds – ethyl furan, *E*-2-pentenal, 1,2,4-trimethylbenzene, 3-methyl-butan-1-ol and a hydrocarbon – to reach 100% correct classifications, although the level of strictness was not high enough (Fig. 3).

All the described results have shown out there is a wide variability in the chemical and sensory characteristics of the virgin olive oils because of the diversity of the varieties. Nevertheless, there is a certain tendency to the clustering of varieties native to the same country but this is not enough to qualify all the oils from a country with similar sensory and chemical characteristics.

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