Identification of Trace Volatile and Phenolic Compounds in Olive Oils with Trees Growing in Different Area Conditions: Using SPME/GC–MS

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Abstract

This research work was performed on olive orchards (*Olea europaea L.*, cv. Chemlali) of different origin areas of olive trees exposed to atmospheric contamination and those growing in a relatively clean area, during two successive years (2015/2016 and 2016/2017). Volatile profile of olive oil samples was carried out by headspace solid-phase micro-extraction coupled with gas chromatography/mass spectrometry (HS-SPME-GC/MS). A total of 93 volatile compounds, belonging to several classes of compounds, such as hydrocarbons, aldehydes, ketones, alcohols, terpenoids, and esters, were identified. Indeed, the analysis of phenols was performed by high-performance liquid chromatography with absorbance and fluorometric detection. Discriminant analysis confirmed that the pattern of volatile compounds includes enough information to predict sample variety amongst discrete monovarietal olive oil. The results indicate that climatic factors strongly effect volatile formation and phenolic composition and are supposed to be suitable markers of the geographic origin.

Keywords Olive oil · Geographical area · Volatile compounds · Phenolic compounds · Chemometrics

Introduction

Volatile compounds responsible for green attributes of virgin olive oils have been proved to be produced through the enzymatic oxidation of linolenic and linoleic acids (Cherfaoui et al. 2018). In addition to their health benefits, extra virgin olive oil (EVOO) is highly appreciated by consumers due to their intense color and aromatic characteristics (Ben Brahim et al. 2018). The chemistry and flavor of a specific EVOO, which define its quality and uniqueness, besides being strongly dependent on the nature of the cultivar used for its production, are also affected by several other factors like climatic and edaphic conditions, agricultural practices, extraction methods, processing techniques, and storage

conditions (Peres et al. 2017). The genetic effect related to the cultivar is one of the most important aspects of volatile composition of olive oil (Ben Brahim et al. 2018).

However, climatic and agronomic conditions of olive growing may affect the volatile composition of olive oils obtained by the same cultivar. Within this framework, the relationships between the water availability over fruit ripening and the volatile composition has been studied (Peres et al. 2017). Results revealed that, in the climatic conditions of central Italy, the rainfall impact is pre-eminent with respect to temperature and that some compounds, such as hexanal and isobutyl acetate were negatively correlated to rainfall.

In conclusion, we can consider that several agronomic and climatic parameters can influence the volatile composition of olive oils. For this reason, volatile compounds could be thought out as markers, with sensory impact, that can be used to differentiate them. On the other hand, the oxidative degradation indices assess these primary and secondary products. They are in use as surrogate measures for oil quality and oxidative stability (Frankel 2010; Cherfaoui et al. 2018), especially creating unpleasant flavor and reducing the shelf-life of edible oils. Some of volatile aldehyde compounds, particularly E,E-2,4-decadienal, E,E-2,4-heptadienal, are correlated with common lipid degradation

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parameters, thus regarded as deterioration "markers" (Molina-Garcia et al. 2017).

Others, as acrolein, are considered to have high relevance, by dint of their capacity to induce toxicological effects (Domínguez et al. 2014). Moreover, unsaturated aldehydes, such as alkenals and alkandienals, show more severe toxicity than alkanals (Domínguez et al. 2014). Thus, understanding the formation mechanisms of these volatiles will help in perceiving the involved reactions occurring due to pollution, showing a useful tool for the evaluation of the existing aroma compounds, the quantitative determination of contaminants, and oil degradation study (Sghaier et al. 2016). The volatile profile is in part responsible for oil flavor, and the sensory attributes have an impact on the appreciation of the oil by consumers (Sghaier et al. 2016). Phenolic and volatile compounds are also responsible for the virgin olive oil (VOO) flavor and, hence, the resulting sensory assessment (Drira et al. 2020). Besides the volatile compounds considered in plant organs of the intact olive fruit as secondary products, other volatile compounds are formed very quickly during disruption of the cell structure due to enzymatic reactions in the presence of oxygen. They are the main compounds responsible for the "green" and fruity desirable aroma attributes of VOO (Zhu et al. 2016). The main precursors for volatile formation are fatty acids (linoleic and a-linolenic), mainly by means of the lipoxygenase cascade (LOX), and amino acids (Aparicio and Harwood 2013). On the contrary, the low-quality VOO have complex profiles composed of a large number of volatiles responsible for off flavors like rancid, mustiness, fusty, and muddy sediment. The volatile compounds responsible for the VOO aroma contain components with diverse chemical natures and molecular masses, and they are present at very low concentrations. But the influence of each volatile on the sensory quality depends on its concentration and odor threshold.

Successive studies, obtained using HS-SPME–GS/MS, confirmed the relationships between the volatile composition of virgin olive oil and their origin area. In this study, the volatile and phenolic composition of oils from olive plants exposed to atmospheric chemical pollution in an area near a fertilizer factory has been investigated. The results were compared with those of olive trees growing in a clean area, not directly exposed to chemical pollution.

Materials and Methods

Chemicals and Reagents

All chemicals were of analytical grade hydroxytyrosol, tyrosol, oleuropein, gallic acid, catechin, vanillic acid, p-coumaric acid, 3.4-dihydroxy phenylacetic acid, chlorogenic acid, and epicatechin and were obtained from Sigma (St.

Louis MO, USA). The polyphenolic compound 2,5-dihydroxybenzaldehyde was used as internal standard at concentration 34.4 mg/l. HPLC-grade methanol, acetic acid, diethyl ether, and ethanol were obtained from Merck (Darmstadt, Germany). Hydrochloric acid was obtained from Merck. Ultrapure water from Milli-Q system (Millipore, Bedford, MA, USA) with resistivity of 18 MΩ cm was used in all cases. All solution was filtered through 0.45-mm membranes (Millipore) and degassed prior to use.

Samples

The analyses were run on six virgin olive oils from two different geographical areas. Olive oils were obtained from unwashed and washed olive fruits (Olea europaea L.cv. Chemlali) manually picked in olive groves located in a land plot at 0.7 km from the Industrial Society of Phosphoric Acid and Fertilizers (Société Industrielle d'Acide Phosphorique et d'Engrais, SIAPE) close to Sfax city, and they are referred as "polluted oils" (PO). On the other hand, the control plot (with control oils, CO) was located in Menzel Chaker region, 45 km north of the "SIAPE," in an inland rural area, without any industries. Sampling was performed during two successive crops (2015/2016 and 2016/2017).

For each sample, about 2 kg of handpicked fresh olives were used for olive oil production. Each sample from polluted oils was divided into two portions. One of them was washed and rinsed with deionized water and is referred as "washed polluted olive oils" (WPOO), and the other one is referred as "unwashed polluted olive oils" (UWPOO). Cold extractions were realized in laboratory by oleodoseur system (composed of crusher, vertical malaxator, and centrifuge). Then, the produced oils were filled in dark bottles and conserved for analysis at 4 °C.

Preparation of the Samples: Headspace-SPME Extraction (HS-SPME)

The HS-SPME extractions were performed using a carboxen/divinylbenzene/polydimethylsiloxane fiber (CAR/DVB/PDMS, 1 cm, 50-/30-μm film thickness (d_f)) supplied from Supelco, Bellefonte, PA, USA. Prior to use, the fiber was conditioned following the manufacturer's recommendations. Fiber blanks were run periodically to ensure the absence of contaminants and/or carryover. The samples, with 4.0-mL volume each, were introduced in a 22.0-mL vial, sealed with a Teflon Lined rubber septum/magnetic screw cap. The vial was equilibrated for 10 min at 50 °C and then extracted for 50 min at the same temperature. The thermal desorption of the analytes was carried out by exposing the fiber to the GC injection port at 260 °C for 3 min in a splitless mode.

GC/MS Conditions

The analyses were performed on a GC/MS system consisting of a Bruker GC 456 with a Bruker mass-selective detector Scion TQ. An automatic sampler injector was used: CTC Analysis auto-sampler CombiPAL. Data were acquired with MSWS 8.2 Bruker and analyzed with Bruker MS Data Review 8.0. The chromatographic separation was achieved on a ZB-WAX PLUS capillary column (60 m × 0.32 mm i.d., 1.0 μm d_f) supplied by Phenomenex, Torrance, CA, USA. The oven temperature program began at 40 °C hold for 5 min, raised at 4 °C min⁻¹ up to 240 °C, and hold for 5 min. Helium was used as carrier gas constant pressure of 35.0 psi at the electronic flow control (EFC 21) and 23.0 psi at the EFC 24. The MS transfer line and source temperatures were set at 240 °C and 220 °C, respectively. The spectra were matched by NIST MS Search Program, version 2.0 g. To determine characteristic mass fragments, electron ionization (EI) at 70 eV mass spectra of the analytes was recorded at full scan, from 40 to 450 Da. Linear retention indices (LRIs) were calculated using a commercial hydrocarbon mixture (C8–C20) and compared with the LRIs described in the literature.

High-Performance Liquid Chromatography with Absorbance and Fluorimetric Detection

Extraction of the Phenolic Fraction

Sample extraction was made according to Gargouri et al. (Gargouri et al. 2013). Four grams of the oil sample was added to 2 mL of *n*-hexane and 4 mL of a methanol/water (60:40, v/v) solution in a 20-mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min at 1490 g. The hydroalcoholic phase was collected, and the organic phase was re-extracted twice with 4 mL of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 4 mL of *n*-hexane to remove the residual oil, and then concentrated and dried by evaporative centrifuge in a vacuum at 35 °C.

Chromatographic Analysis of Phenols

The analyses were carried out in a Waters liquid chromatography (Agilent Infinity) equipped with two pumps (Model 510), an automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20-ml loop), a programmable fluorescence detector (Model 470), and a tunable absorbance detector (Model 486). Baseline Workstation 810 software (Waters) and a personal computer were employed for data storage and evaluation. The analytical column was a Waters (Mildford, MA, USA) Nova-Pak C_{18} 3, 9 × 150 mm particle diameter. A Nova-Pak C_{18} precolumn was employed

to protect the analytical column and kept at ambient temperature (30 °C) with an injection volume of 10 μL. The mobile phase comprised of 2% acetic acid in water (solvent A)/methanol (solvent B) at a flow rate of 0.5 mL/min, with the steps as follows: 0 min, 100% A; 15 min, 85% A; 25 min, 50% A; and 34 min, 30% A. A wavelength of 280 nm was used for absorbance detector. The fluorescence detector was operated at an excitation wavelength set at 280 nm and emissions at 320 and 350 nm (Selvaggini et al. 2006).To check the peak purity of each compound, a Beckman diode array detector (Model 168) controlled by the Beckman System Gold software was used. Peaks were considered pure when there was a match factor ≥ 99.5. Injected samples were interspersed with standards to ensure accurate quantitation. Chromatographic peaks were identified by comparing retention times of samples with those of standard compounds. Quantitation was carried out by internal standardization. The hydroxytyrosol, tyrosol, and oleuropein standards were prepared in 10% methanol + 2% CH_3COOH, the remaining standards in 50% MeOH. The determination of the signal of interest was done by comparing retention times between the samples and the injected standards at 280 nm (Rodrıguez-Delgado et al. 2001).

Statistical Analysis

For all the experiments, six samples were analyzed, and all the assays were carried out in triplicate. The significant differences between the values of all parameters were identified at $p < 0.05$ following the ANOVA: Student–Newman–Keuls test by means of SPSS Statistics 17.0 for Windows (SPSS Inc. 2008). The aim of applying non-standardized principal component analysis (PCA) was to demonstrate the effect of all investigated parameters (n = 93) on the olive oil samples using the varimax rotation together with Kaiser normalization. The PCA type was Pearson (n), the biplot type was correlation biplot, and the coefficient was automatic. A uniform hierarchical cluster analysis (HCA) methodology was applied on data. Each cluster was determined by the following parameters: similarity, Euclidean distance matrix, and the Ward's method, generating a dendogram for olive oil samples. All the chemometric analyses were carried out using XLSTAT software for Windows (v.2013.2.03, Addinsoft, NY, USA).

Results and Discussion

Identification of Volatile Compounds in Oil Samples

Concentrations of volatile compounds, whether major or minor, are crucial to virgin olive oil quality. Consequently, the identification of the compounds causing the flavor or

off-flavor is therefore the key for quality control. Virgin olive oil has a delicate and unique flavor (Cherfaoui et al. 2018). The volatile compounds identified in oil and linear retention index values on the multidimensional chromatographic system for these compounds are presented in Table 1. The identification was made by comparing the obtained spectra with MS NIST version 2.0 (Johnson 2014). A total of 93 volatile compounds were isolated and identified, of the following chemical families: 8 alkanes, 11 alkenes, 8 alkylbenzenes, 9 alkanals, 7 alkenals, 2 alkandienals, 22 alcohols, 4 esters, 8 ketones, 12 terpenes, and 2 other compounds. Most of these components represented groups of characteristic volatiles produced through the lipoxygenase pathway. In the six oleaster oils, the isolated and identified compounds are mainly alcohols such as 1-hexanol at 32.13 min (LRI 1391) ($[M+H]^+$, m/z 102) (Fig. 2d), 2-hexen-1-ol (isomer 1) at 34.16 min (LRI 1443) ($[M+H]^+$, m/z 100) (Fig. 2f), and 3-hexen-1-ol (isomer 2) (Fig. 2e) at 33.50 min (LRI 1426) ($[M+H]^+$, m/z 100). Among the aldehyde compounds identified, we can mention hexanal (Fig. 2b) at 21.96 min (LRI 1105) ($[M+H]^+$, m/z 100) and the other linear aldehydes such as pentanal, heptanal, octanal, and nonanal. In addition, the monounsaturated aldehydes were also identified in our oil samples, as 3-hexenal at 24.76 min (LRI 1173) and (($[M+H]^+$, m/z 98) and 2-hexenal (isomer 1) at 27.15 min (LRI 1231) ($[M+H]^+$, m/z 98) and 2-hexenal (isomer 2) at 27.97 min (LRI 1250) ($[M+H]^+$, m/z 98) (Fig. 2c). In addition, the volatile compounds of the virgin olive oil do not contribute to its whole aroma with the same importance. Chemical factors such as volatility and the hydrophobic character, size, shape, conformational structure, type, and position of functional groups seem to be more related to the odor intensity of a volatile compound than its concentration (Cherfaoui et al. 2018). Such compounds do not originate from the LOX pathway, and they are mostly the result of other, mostly undesirable processes (Purcaro et al. 2014). Ethyl acetate, at 13.10 min (LRI 903) ($[M+H]^+$, m/z 88), which are responsible for winey-vinegar defect and, together with 3-methylbutanol, clearly indicated that olives underwent fermentation (ben Hammouda et al., 2017). Non-LOX C_4 and C_5 branched compounds are known to derive from the conversion of certain amino acids, while linear acids, esters, and ketones originate from fatty acid metabolism (Cherfaoui et al. 2018). All of the mentioned processes are commonly linked to more or less degraded raw olive fruit material, due to physical damage, inadequate sanitary conditions, or unsuitable storage of fruit before processing, and they are often found in VOOs with sensory defects (Ben Mansour et al. 2015; Polari et al. 2018). The possibility that particular non-LOX volatile compounds were formed and/or increased in concentration as a result of various oxidative processes, as shown previously by other authors (ben Hammouda et al. 2017), should not be neglected.

Determination and Comparison of the Composition of the Volatile Compounds in Oil Samples

Figure 1 shows the typical GC/MS chromatogram data for the volatile's compounds extracted from olive oils by HS-SPME. The mean values (%) of the GC analyses of triplicate extractions and standard deviations are reported in Table 2. A total of 93 volatile compounds were isolated and tentatively identified: 27 hydrocarbons families, 8 alkanes, 11 alkenes, and 8 alkylbenzenes; 18 total aldehydes with 9 alkanals, 7 alkenals, and 2 alkandienals; 22 alcohols; 4 esters; 9 ketones; 12 terpenes, and 2 other compounds. Comparing the six oleasters collected at the same stage of ripeness, we can observe that there were some differences in the constituents of the volatile fraction. Only in control olive oils (CO), 2-pentanol; 1-butanol, 3-methyl; 1-nonanol; 1-decanol; 8-heptadecene; and 2,6-dimethyl-1,3,5,7-octatetraene were present, whereas 1-pentanol; 2-buten-1-ol; 1,5-hexadien-3-ol; 1-octanol; 1-penten-3-one; o-xylene; β-phellandrene; β-Myrcene; and decane were present only in polluted olive oils (PO) (Table 2). The most important mechanisms responsible for these volatile compounds are the thermal degradation of lipid (mainly oxidation), Maillard reaction, interaction between Maillard reaction products with lipid-oxidized products, and vitamin degradation (Van Ba et al. 2012). Among the volatile compounds, aldehydes might play an important role in the flavor because of the low odor threshold values, and their sensory characteristics are mainly associated with a fatty aroma (Song et al. 2011; ben Hammouda et al. 2017).

Alkanes, Alkenes, and Alkylbenzenes

The hydrocarbons of olive oils have been studied by different authors as possible markers to distinguish virgin olive oil from different olive varieties or different geographical origins (Molina-Garcia et al. 2017). The hydrocarbons octane; hexane; undecane; 1,7-nonadiene, 4,8-dimetyl; 1-octene, 6-methyl-; 1.3-pentadiene; 1.4-pentadiene; and 3-ethyl-1.5-octadiene have been detected in the aroma fractions of the tested oils. Mono-ring aromatic hydrocarbons (MAHs), such as ethylbenzene, styrene, and 1,2,4-trimetylbenzene, were observed in tested EVOOs at low levels. The presence of very small traces of benzene derivatives could be explained by environmental contaminants present in the fruit. On the other hand, styrene could be formed by decarboxylation of trans-cinnamic acid which is produced by L-phenylalanine deamination. Thereafter, ethylbenzene could be formed by reduction of styrene (Sabatini et al. 2009).

Table 1 RT, LRI, and the mass fragment of the volatile compounds

N° peaks	Name of compounds	RT (min)	Molecular formula	LRI	LRI*	Av*	m/z (M⁺)	m/s-m/s			
Alkanes
1	Butane, 2-methyl-	4.078	C5H12				72	43	41	42	57
2	Hexane	4.109	C6H14		600	600	86	57	41	43	56
4	Cyclopropane, ethyl-	4.366	C5H10				70	55	42	41	70
11	Octane	9.061	C8H18	800	800	800	114	43	41	57	56
16	Cyclopropane, pentyl-	10.837	C8H16	846	846		112	43	55	41	70
30	Decane	17.302	C10H22	1011	993–1000	998	142	43	57	41	42
38	Undecane	21.630	C11H24	1122	1098–1100	1099	156	57	43	41	71
56	Dodecane	25.868	C12H26	1230	1200–1201		170	57	43	71	41
Alkenes
5	1,3-Pentadiene	5.989	C5H8				68	67	68	53	41
8	1,4-Pentadiene	6.307	C5H8				68	67	68	53	41
26	3-Ethyl-1,5-octadiene	16.109	C10H18	981	907–1033	982	138	41	68	69	67
35	Cyclobutene, 2-propenylidene-	20.273	C7H8	1087			92	91	92	65	63
37	1,7-Nonadiene, 4,8-dimethyl-	21.546	C11H20	1120			152	41	69	67	68
50	1-Pentene, 4,4-dimethyl-	24.641	C7H14	1199			98	57	41	55	58
69	1-Octene, 6-methyl-	27.581	C9H18	1274			126	55	57	41	69
70	1-Tridecene	27.586	C13H26	1274			182	55	57	41	56
75	1,3,6-Octatriene, 3,7-dimethyl-, (isomer)	28.748	C10H16	1304	1257	1257	136	93	91	79	77
107	2,6-Dimethyl-1,3,5,7-octatetraene (isomer)	35.758	C10H14	1484			134	119	91	41	77
129	8-Heptadecene	44.054	C17H34	1696	1606–1642	1624	238	55	69	83	57
Alkylbenzenes
36	Toluene	20.277	C7H8	1067	952–1040	1016	92	91	92	65	63
64	Benzene, 1,3-dimethyl-	26.710	C8H10	1214	1134	1134	106	91	106	105	77
65	Ethylbenzene	26.730	C8H10	1214	1056–1139	1106	106	91	106	77	51
72	Benzene, 1-ethyl-3-methyl-	28.254	C9H12	1249	1216	1216	120	105	120	91	79
73	Benzene, 1-ethyl-2-methyl-	28.270	C9H12	1250	1234	1234	120	105	120	79	77
82	Styrene	29.587	C8H8	1280	1199	1199	104	104	78	103	51
87	Benzene, 1,2,3-trimethyl-	30.631	C9H12	1304			120	105	77	79	91
96	Benzene, 1,2,4-trimethyl-	32.874	C9H12	1355	1223–1290	1256	120	105	120	51	77
Alkanals
9	Acetaldehyde (ethanal)	6.624	C2H4O		690–744	713	44	44	43	42	41
21	Butanal, 2-methyl-	14.386	C5H10O	932	850–925	901	86	41	57	58	43
23	Butanal, 3-methyl-	14.569	C5H10O	937	911–929	915	86	44	41	43	42
31	Pentanal	17.379	C5H10O	1001	896–988	951	86	44	41	43	42
39	Hexanal	21.962	C6H12O	1105	1000–1099	1073	100	44	41	57	72
59	Heptanal	26.341	C7H14O	1204	1174–1200	1188	114	41	57	43	42

Table 1 (continued)

N° peaks	Name of compounds	RT (min)	Molecular formula	LRI	LRI*	Av*	m/z (M⁺)	m/s-m/s			
86	Octanal	30.449	C8H16O	1298	1231–1298	1283	128	43	57	41	56
99	Nonanal	34.326	C9H18O	1386	1344–1423	1392	142	57	41	56	44
122	Benzaldehyde	39.691	C7H6O	1508	1496–1541	1523	106	105	77	106	51
Alkenals											
43	2-Pentenal, (isomer)-	24.214	C5H8O	1160	1119–1130	1111	189	55	83	41	53
52	3-Hexenal	24.764	C6H10O	1173	1113–1215	1153	98	41	69	55	42
67	2-Hexenal (isomer 1)-	27.159	C6H10O	1231	1160-1228	1205	98	55	83	41	69
71	2-Hexenal (isomer 2)	27.974	C6H10O	1250	1230	1230	98	41	55	69	83
109	2-Octenal, (isomer)-	35.929	C8H14O	1442	1367–1441	1420	126	55	41	70	83
120	2-Nonenal, (isomer)-	39.495	C9H16O	1528	1504–1573	1551	140	43	41	70	83
121	2-Nonenal, (isomer)-	39.511	C9H16O	1528	1548	1548	140	41	55	70	43
Alkandienals											
101	2,4-Hexadienal, (isomer)-	34.805	C6H8O	1437	1349–1411	1386	96	81	53	67	41
113	2,4-Heptadienal, (isomer)-	37.127	C7H10O	1503	1476–1508	1485	110	81	53	41	68
Alcohols											
24	Ethanol	14.959	C2H6O	951	900–955	927	46	45	43	46	42
41	2-Pentanol	22.881	C7H16O	1154	1109–1153	1127	88	45	55	44	
46	1-Pentyn-3-ol	24.224	C5H8O	1188			84	55	83	41	53
61	1-Butanol, 3-methyl-	26.547	C5H12O	1248	1201-1229	1201	88	41	55	57	70
62	1-Pentanol	26.561	C5H12O	1248	1248–1268	1243	88	55	41	70	43
88	2-Penten-1-ol, (isomer 1)-	30.734	C5H10O	1355	1250–1329	1305	86	57	41	44	55
89	2-Heptanol	30.782	C11H18	1356	1316–1316	1317	116	69	41	81	135
90	2-Penten-1-ol, (isomer 2)-	31.016	C5H10O	1362	1281–1325	1305	86	57	68	41	
91	2-Buten-1-ol	31.120	C4H8O	1365	1215	1215	72	57	41	43	72
93	1-Hexanol	32.139	C6H14O	1391	1290–1378	1343	102	56	55	41	43
95	3-Hexen-1-ol, (isomer 1)-	32.642	C6H12O	1404	1320–1384	1362	100	67	41	55	69
97	3-Hexen-1-ol, (isomer 2)-	33.505	C6H12O	1426	1322–1394	1377	100	67	41	55	82
98	2-Hexen-1-ol, (isomer 1)-	34.167	C6H12O	1443	1348–1420	1401	100	57	41	67	82
103	1-Heptanol	35.015	C7H16O	1465	1400–1462	1449	116	41	55	70	42
105	1,5-Hexadien-3-ol	35.542	C6H10O	1478			98	57	43	41	55
106	1-Octen-3-ol	35.544	C8H16O	1478	1392–1456	1442	128	57	43	72	58
110	5-Methyl-1-hexanol	36.369	C7H16O	1499	1466	1466	116	193	73	341	57
119	1-Octanol	39.230	C8H18O	1572	1424–1541	1406	130	43	55	42	41
127	1-Nonanol	42.434	C9H20O	1654	1640–1668	1653	144	56	55	41	43
134	1-Decanol	45.689	C10H22O	1738	1745–1787	1764	158	55	41	56	43
137	Benzyl alcohol	49.766	C7H8O	1842	1846–1912	1877	108	79	108	77	107

Table 1 (continued)

N° peaks	Name of compounds	RT (min)	Molecular formula	LRI	LRI*	Av*	m/z (M⁺)	m/s-m/s			
138	Phenylethyl alcohol	50.743	C8H10O	1867	1890–1919	1913	122	91	92	65	122
Esters											
14	Acetic acid, methyl ester	10.723	C3H6O2	843	843		74	43	74	42	59
20	Ethyl acetate	13.109	C4H8O2	903	878–903	891	88	43	45	61	42
81	Acetic acid, hexyl ester	29.482	C8H16O2	1276	1268–1276	1271	144	43	56	61	55
92	4-Hexen-1-ol, acetate, (isomer)-	31.259	C8H14O2	1316			142	67	43	82	41
Ketones											
13	Acetone	10.235	C3H6O	830	808–830	819	58	43	58	42	41
29	3-Pentanone	17.219	C5H10O	1009	970–992	977	86	57	86	42	58
33	1-Penten-3-one	19.260	C5H8O	1061	932–1019	988	84	55	84	83	57
49	5-Hepten-3-one, 5-methyl-	24.634	C8H14O	1199			126	57	41	69	55
58	2-Heptanone	26.181	C7H14O	1238	1123–1200	1179	114	43	58	71	59
78	3-Octanone	29.065	C8H16O	1312	1242–1245	1243	128	43	57	72	99
85	2-Octanone	30.277	C8H16O	1343			128	43	58	41	42
94	5-Hepten-2-one, 6-methyl-	32.267	C8H14O	1394	1285–1488	1356	126	43	41	55	108
Terpenes											
45	β-Phellandrene	23.648	C10H16	1144	1185–1218	1202	136	93	91	41	77
54	p-Xylene	24.788	C8H10	1170	1128–1185	1165	106	91	106	105	51
55	β-Myrcene	25.079	C10H16	1177	1146–1174	1156	136	41	93	69	91
57	β-Ocimene	26.028	C10H16	1198	1186–1266	1230	136	93	105	91	79
63	o-Xylene	26.702	C8H10	1214	1125–1153	1118	106	91	106	105	77
66	Limonene	27.022	C10H16	1221	1139–1219	1192	136	67	68	93	79
74	trans-β-Ocimene	28.745	C10H16	1261	1246	1246	136	93	91	79	77
79	3-Carene	29.367	C10H16	1275	1130	1130	136	93	91	79	77
83	o-Cymene	29.981	C10H14	1289	1254	1254	134	119	91	134	117
84	p-Cymene	29.986	C10H14	1289	1244–1288	1271	134	119	91	134	117
131	α-Farnesene	45.424	C15H24	1642	1752	1755	204	93	41	79	69
132	α-acorenol	45.499	C15H26O	1644	1774		390	43	91	119	105
Others											
124	1-(3-Oxobutyl)-3,3-dimethyldiaziridine	39.970	C7H14N2O	1591	1583	1583	142	57	86	85	44
126	Dimethyl Sulfoxide	40.947	C2H6OS	1621	1621	1573	78	63	78	45	61

LRI*, linear retention index in literature; AV*, average in literature. Rt, retention time; Each value represents the mean of three determinations [n = 3]
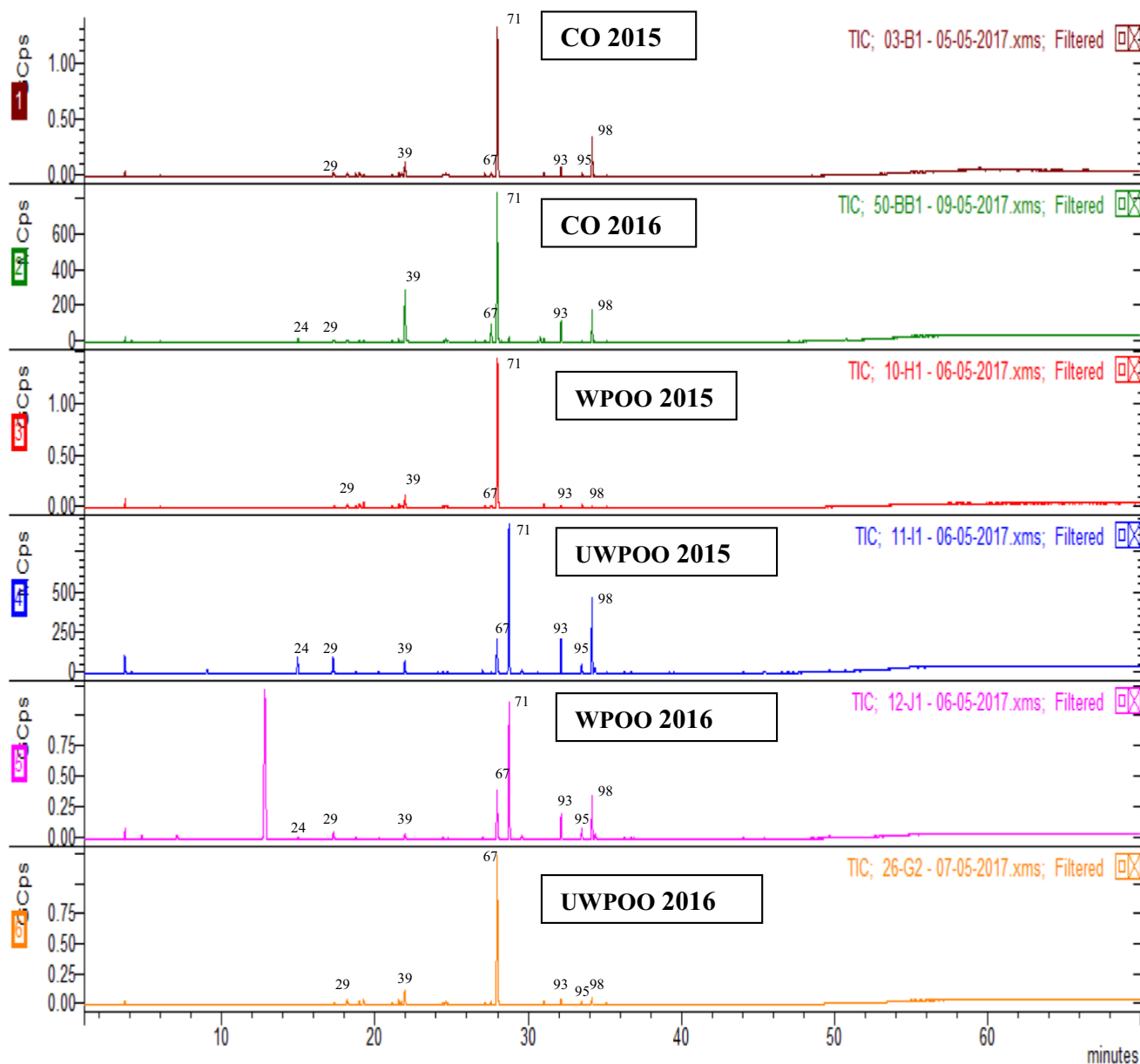
Fig. 1 Comparison of chromatograms of all samples. GC/MS chromatograms of all samples. Gcps denote giga counts per second. CO, control olive oils; WPOO, washed polluted olive oils; UWPOO, unwashed polluted olive oils

Aldehydes

As observed in Table 2, taking into consideration the effect of the growing area conditions, it is important to note that control oil samples (CO) had the highest level of aldehydes comparing to samples grown under the vicinity of phosphoric acid factory (PO). These compounds increased distinctly for the WPOO from 30.696% (2015) to 31.269% (2016), whereas they clearly increased for the UNPOO from 11.423% (2015) to 15.691% (2016). Furthermore, alkanals, alkenals, and alkandienals are the family of volatiles presenting a greater and more marked rise when oils are

subjected to pollution (Table 2). They were of most concern from health point of view (Molina-Garcia et al. 2017). Among alkanals, with 5 to 10 carbon atoms present in the volatile fraction of oil samples were butanal, pentanal, hexanal, heptanal, octanal, and nonanal, which have been generated from lipid oxidation. This result is consistent with those found by Martins et al. (2020), which have shown that lipids generally have the greatest influence on the production of aroma components. In fact, hexanal (Fig. 2b) and 2-hexenal (isomer 2) (Fig. 2c) are the major volatile compounds detected in the oil blends by the SPME method. Thus, the hexanal and 2-hexenal (isomer

Table 2 Volatile compounds determined by SPME-GC/MS

N° pics	Name of compounds	CO 2015	CO 2016	WPOO 2016	UWPOO 2016	WPOO 2015	UWPOO 2015
Alkanes							
1	Butane, 2-methyl-	n.d	0.104	n.d	n.d	0.248	n.d
2	Hexane	0.096	0.135	0.08	0.047	n.d	0.064
4	Cyclopropane, ethyl-	n.d	n.d	0.023	n.d	n.d	n.d
11	Octane	0.157	0.109	0.222	0.058	1.17	0.106
16	Cyclopropane, pentyl-	0.05	n.d	n.d	n.d	n.d	n.d
30	Decane	n.d	n.d	0.259	n.d	n.d	n.d
38	Undecane	0.655	n.d	0.517	n.d	0.326	0.168
56	Dodecane	n.d	n.d	n.d	n.d	0.126	n.d
	Total	0.958	0.348	1.101	0.105	1.87	0.338
Alkenes							
5	1,3-Pentadiene	0.317	0.162	0.259	0.3	n.d	0.096
8	1,4-Pentadiene	0.084	0.076	0.115	0.087	n.d	n.d
26	3-Ethyl-1,5-octadiene	0.185	0.064	0.177	0.147	n.d	n.d
35	Cyclobutene, 2-propenylidene-	n.d	n.d	0.121	0.134	n.d	0.295
37	1,7-Nonadiene, 4,8-dimethyl-	0.845	n.d	0.502	0.869	0.259	n.d
50	1-Pentene, 4,4-dimethyl-	n.d	n.d	0.765	n.d	n.d	n.d
69	1-Octene, 6-methyl-	n.d	2.046	0.685	0.398	0.3	n.d
70	1-Tridecene	0.5	n.d	n.d	n.d	n.d	n.d
75	1,3,6-Octatriene, 3,7-dimethyl-, (isomer)	n.d	n.d	n.d	n.d	33.011	n.d
107	2,6-Dimethyl-1,3,5,7-octatetraene (isomer)	n.d	n.d	n.d	n.d	0.309	0.166
129	8-Heptadecene	n.d	n.d	n.d	n.d	0.197	0.204
	Total	1.931	2.348	2.624	1.935	34.076	0.761
Alkylbenzenes							
36	Toluene	0.143	0.118	n.d	n.d	0.629	n.d
64	Benzene, 1,3-dimethyl-	0.089	0.078	n.d	n.d	0.198	n.d
65	Ethylbenzene	0.011	n.d	n.d	n.d	n.d	0.076
72	Benzene, 1-ethyl-3-methyl-	0.099	0.173	0.08	0.023	0.239	0.068
73	Benzene, 1-ethyl-2-methyl-	n.d	n.d	0.002	0.029	n.d	n.d
82	Styrene	0.241	0.155	0.04		1.088	0.688
87	Benzene, 1,2,3-trimethyl-	0.199	0.218	0.127	0.126	0.387	0.126
96	Benzene, 1,2,4-trimethyl-	0.025	0.101	0.021	0.149	0.08	0.043
	Total	0.807	0.843	0.27	0.327	2.621	1.001
Alkanals							
9	Acetaldehyde (ethanal)	n.d	0.018	0.024	n.d	n.d	n.d
21	Butanal, 2-methyl-		0.009	0.031	n.d	n.d	
23	Butanal, 3-methyl-	n.d	0.062	0.039	n.d	n.d	n.d
31	Pentanal	n.d	0.275	0.232	0.405		
39	Hexanal	2.931	7.196	2.55	2.28	3.107	0.973
59	Heptanal	n.d	n.d	n.d	0.015	n.d	n.d
86	Octanal	0.062	0.022	0.044	0.033	0.256	
99	Nonanal	0.225	0.207	0.243	0.174	2.104	1.112
122	Benzaldehyde	0.029	n.d	n.d	n.d	0.068	0.049
	Total	3.247	7.789	3.163	2.907	5.535	2.134
Alkenals							
43	2-Pentenal, (isomer)-	0.031	n.d	0.041	0.045	n.d	n.d
52	3-Hexenal	0.874	n.d	0.4	0.33	0.456	0.116
67	2-Hexenal (isomer 1)-	0.538	0.163	0.291	0.447	n.d	0.133
71	2-Hexenal (isomer 2)	26.357	17.793	25.369	26.838	9.425	8.948
109	2-Octenal, (isomer)-	0.006	0.012	n.d	n.d	n.d	n.d

Table 2 (continued)

N° pics	Name of compounds	CO 2015	CO 2016	WPOO 2016	UWPOO 2016	WPOO 2015	UWPOO 2015
120	2-Nonenal, (isomer)-		n.d	n.d	n.d	0.142	0.031
121	2-Nonenal, (isomer)-	0.078					
	Total	27.884	17.968	25.741	27.66	10.023	9.228
Alkandienals							
101	2,4-Hexadienal, (isomer)-	0.13	0.189	0.082	0.111	n.d	0.061
113	2,4-Heptadienal, (isomer)-	0.008	0.047	0.034	0.018	0.133	n.d
	Total	0.138	0.236	0.116	0.129	0.133	0.061
Alcohols							
24	Ethanol	n.d	0.511	0.111	n.d	n.d	0.52
41	2-Pentanol	n.d	n.d	n.d	n.d	n.d	0.026
46	1-Pentyn-3-ol	0.192	n.d	n.d	n.d	n.d	0.225
61	1-Butanol, 3-methyl-	n.d	n.d	n.d	n.d	0.314	n.d
62	1-Pentanol	0.012	0.049	n.d	n.d	n.d	n.d
88	2-Penten-1-ol, (isomer 1)-	0.104	n.d	0.068	0.13	n.d	n.d
89	2-heptanol	0.043	n.d	n.d	n.d	0.186	0.076
90	2-Penten-1-ol, (isomer 2)-	0.509	0.46	0.645	0.572	0.209	0.087
91	2-Buten-1-ol	n.d	n.d	n.d	0.051	n.d	n.d
93	1-Hexanol	1.56	2.42	0.937	0.347	7.177	3.652
95	3-Hexen-1-ol, (isomer 1)-	0.067	0.022	0.013	n.d	0.084	n.d
97	3-Hexen-1-ol, (isomer 2)-	0.529	0.317	0.45	0.505	2.176	1.697
98	2-Hexen-1-ol, (isomer 1)-	5.692	3.255	1.149	0.412	14.844	6.284
103	1-Heptanol	0.332	n.d	n.d	n.d	n.d	n.d
105	1,5-Hexadien-3-ol	n.d	n.d	n.d	0.3	n.d	n.d
106	1-Octen-3-ol	0.026	0.053	0.027	n.d	0.123	0.038
110	5-methyl-1-hexanol	0.056	n.d	0.028	0.022	n.d	0.038
119	1-Octanol	n.d	n.d	n.d	n.d	n.d	n.d
127	1-Nonanol	n.d	n.d	n.d	n.d	0.161	n.d
134	1-Decanol	n.d	n.d	n.d	n.d	0.095	n.d
137	Benzyl alcohol	n.d	0.079	n.d	n.d	0.372	n.d
138	Phenylethyl alcohol	n.d	0.231	0.068	0.057	0.363	n.d
	Total	9.263	7.397	3.496	2.396	26.104	12.643
Esters							
14	Acetic acid, methyl ester	n.d	0.064	n.d	n.d	n.d	0.067
20	Ethyl acetate	n.d	0.018	n.d	n.d	n.d	n.d
81	Acetic acid, hexyl ester	n.d	n.d	0.02	n.d	0.138	0.088
92	4-Hexen-1-ol, acetate, (isomer)-	0.056	n.d	0.029	n.d	0.181	0.111
	Total	0.056	0.082	0.049	0	0.319	0.266
Ketones							
13	Acetone	0.048	0.046	0.025	0.013	0.178	0.097
29	3-Pentanone	1.03	0.308	0.165	0.143	n.d	1.546
33	1-Penten-3-one	0.449	0.257	1.064	1.116	n.d	n.d
49	5-Hepten-3-one, 5-methyl-	n.d	0.478	n.d	0.644	0.249	n.d
58	2-Heptanone	n.d	0.095	n.d	n.d	0.13	0.049
78	3-Octanone	0.072	n.d	n.d	n.d	0.25	0.094
85	2-Octanone	n.d	n.d	n.d	n.d	0.215	0.104
94	5-Hepten-2-one, 6-methyl-	0.009	0.011	n.d	n.d	0.094	n.d
	Total	1.608	1.195	1.254	1.916	1.116	1.89
Terpenes							
45	β-Phellandrene	n.d	n.d	0.011	0.177	n.d	n.d
54	p-Xylene	n.d	0.265	0.199	0.003	n.d	n.d

Table 2 (continued)

N° pics	Name of compounds	CO 2015	CO 2016	WPOO 2016	UWPOO 2016	WPOO 2015	UWPOO 2015
55	β-Myrcene	0.032	n.d	n.d	n.d	n.d	n.d
57	β-Ocimene	n.d	0.599	n.d	n.d	n.d	0.069
63	o-Xylene	0.552	n.d	0.196	0.072	n.d	0.246
66	Limonene	n.d	n.d	n.d	0.028	0.692	0.49
74	trans-β-Ocimene	0.13	n.d	0.035	n.d	n.d	22.028
79	3-Carene	n.d	n.d	n.d	n.d	0.208	0.128
83	o-Cymene	0.008	0.009	n.d	n.d	n.d	n.d
84	p-Cymene	n.d	n.d	n.d	n.d	n.d	0.051
131	α-Farnesene	n.d	n.d	n.d	n.d	0.156	0.115
132	α-acorenol	n.d	n.d	n.d	n.d	0.225	0.104
	Total	0.722	0.873	0.441	0.28	1.281	23.231
Others							
124	1-(3-Oxobutyl) -3,3-dimethyldiaziridine	0.045	n.d	n.d	0.028	n.d	n.d
126	Dimethyl Sulfoxide	n.d	n.d	n.d	0.042	n.d	n.d
	Total	0.045	0	0	0.07	0	0

n.d., not detected; unit, percentage %; *CO*, control olive oils; *WPOO*, washed polluted olive oils; *UWPOO*, unwashed polluted olive oils

2) levels for polluted olive oils in the year of 2016 reached 2.55% and 25.369%, respectively, for WPOO and 2.28% and 26.838%, respectively, for UWPOO. These compounds are reported as the products of lipid oxidation of linoleic acid and linolenic acid (Zhu et al. 2016). The predominance of hexanal over the other compound in the volatile profile can be attributed to the multiplicity of its synthesis pathways. These results are in accordance with those described in the literature, which have reported that hexanal is the major aldehyde in oil samples and can be generated from oleic acid, linoleic and arachidonic acids, and through the degradation of other unsaturated aldehydes, such as 2,4-decadienal (Nieto et al. 2011; ben Hammouda et al. 2017). Among the other alkanals and alkenals which were detected in the oil samples, but at lower amount, 3-hexenal, 2-hexenal (isomer1) and nonanal can be mentioned; their levels for polluted olive oils in the year of 2016 reached 0.4%, 0.291%, and 0.243%, respectively, for WPOO and 0.33%, 0.447%, and 0.174%, respectively, for UWPOO. On the other hand, alkandienals were significantly present. In fact, 2,4-hexadienal (isomer) is formed in a greater amount for WPOO and UWPOO from 0.082 to 0.111% in 2016. These results are in agreement with those mentioned in the literature (Veneziani et al. 2018), which have proven that 2,4-hexadienal is one of the important unsaturated aldehydes, especially for the fat aroma and play some part in species characteristic flavor. In general, unsaturated aldehydes, such as alkenals and alkandienals, show much more severe toxicity than alkanals (Molina-Garcia et al. 2017).

Alcohols

Alcohols with six carbon atoms are associated with sweetness and contribute favorably to the aroma of extra-virgin olive oil (Reboredo-Rodríguez et al. 2012). These were among the other volatile compound affected by pollution. In fact, the highest compounds formed for oil samples WPOO and UWPOO were 2-hexen-1-ol (isomer1) (Fig. 2f) 1.149% and 0.412%, respectively (Table 2). This compound was found to contribute to a mild, fruit, and balsamic odor (Zhu et al. 2016). Indeed, as observed in Table 2, an important formation of 1-octen-3-ol, heptan-1-ol, and octan-1-ol was obtained. Both compounds were two cleavage products from methyl oleate hydroperoxides (Molina-Garcia et al. 2017).

Esters and Ketones

Nine ketones were detected in the oil samples, and they underwent a decrease in UWPOO. The content of 3-pentanone (Fig. 2a) is by far the major C_5 ketone compound in all studied oils. Two unsaturated ketones, 1-penten-3-one and 6-methyl-5-hepten-2-one, were detected by GC/MS. To 1-penten-3-one, a mushroom aroma is described (Martins et al. 2020), whereas 6-methyl-5-hepten-2-one affected sweet, fruity, and green odors (Reboredo-Rodríguez et al. 2012). Esters were formed in small quantities in polluted oils, being totally absent in control olive oils (Table 2). These results are in agreement with a recent study (Bubola et al. 2014), which has demonstrated that an increase in C_5 ketones have been observed in olive oil stored in the dark, and these compounds were proposed as markers of virgin

(mainlib) 3-Pentanone

(mainlib) Hexanal

(mainlib) 2-Hexenal

(mainlib) 1-Hexanol

(mainlib) 3-Hexen-1-ol

(mainlib) 2-Hexen-1-ol, (E)-
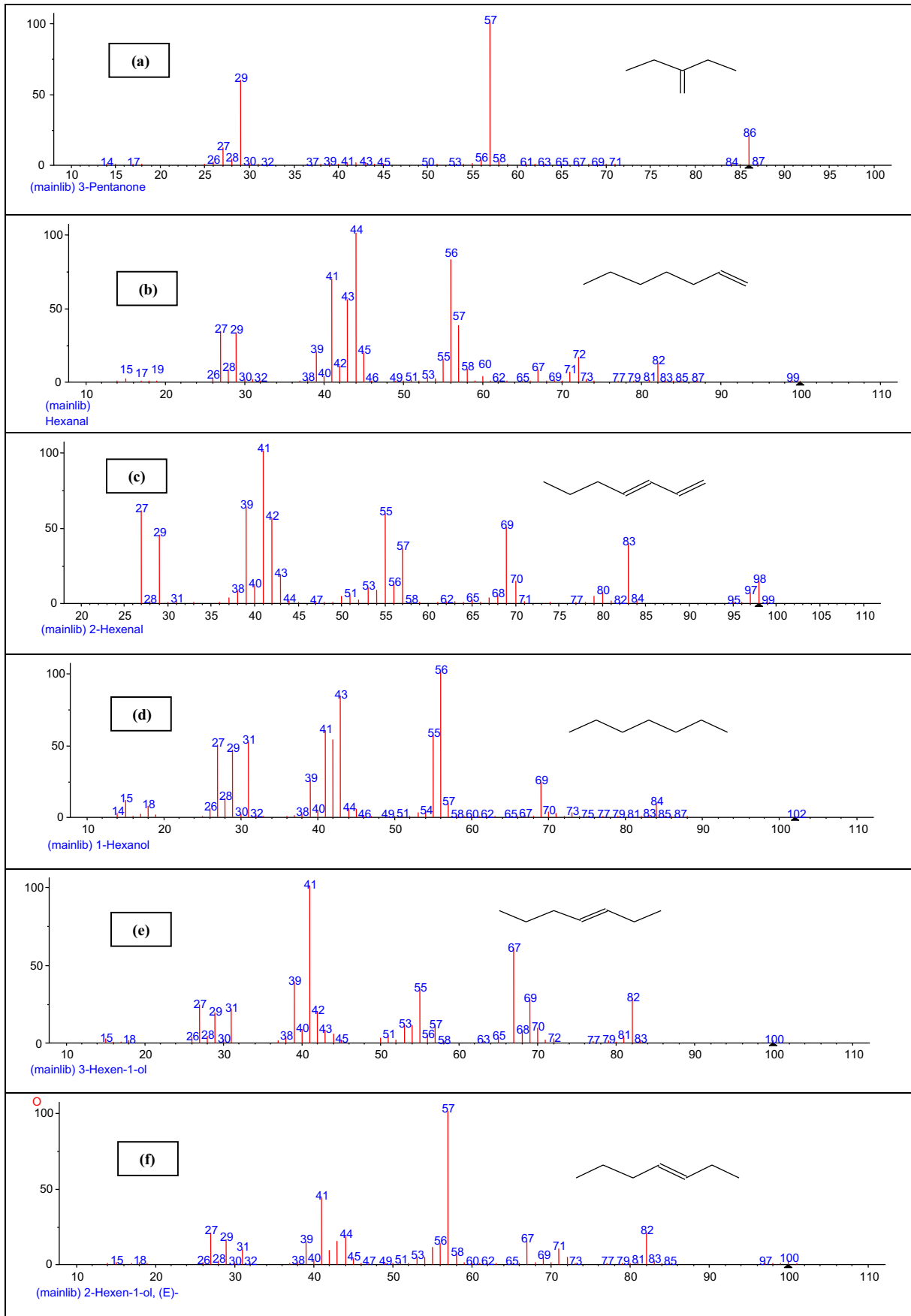
◄**Fig. 2** Major volatile compounds: **a** 3-pentanone (29); **b** hexanal (39); **c** 2-hexenal (71), **d** 1-hexanol (93); **e** 3-hexen-1-ol (isomer 2) (97); **f** 2-hexen-1-ol (isomer 1) (98). CO, control olive oils; WPOO, washed polluted olive oils; UWPOO, unwashed polluted olive oils

olive oil quality freshness. Esters, compounds associated with fruity sensory notes (Zhu et al. 2016) as acetic acid, methyl ester, and acetic acid, hexyl ester, were present in aroma of control olive oils, but there are minor components compared with aldehydes or alcohols (Table 2). The low level of esters in samples indicates a lower content of alcohol acetyl transferase (AAT) in these olive oils (Peres et al. 2017).

Terpenes

Several terpene hydrocarbons (mono- and sesquiterpenes) were often detected. Most of them are minor compounds of the volatile fraction not derived from fatty acid transformations; however, even if they occur in EVOO below their olfactory threshold, they may provide useful quality markers (Ben Brahim et al. 2018). Interestingly, the anti-microbial activity of EVOO sesquiterpene hydrocarbons against a range of different microorganisms was evidenced (Cecchi and Alfei 2013). Among sesquiterpenes, molecular formulas $C_{15}H_{24}$, 3-carene, p-xylene, and o-xylene were present in the largest amounts with respect to the other sesquiterpenes (a-farnesene, α-acorenol, trans-β-ocimene, and β-ocimene) in almost all of the oils analyzed. According to previous reports, terpenoid hydrocarbons show large differences that depend on both the olive variety and geographical origin (Pouliarekou et al. 2011; Ben Mansour et al. 2015). Actually farnesene, which vary among samples in the present study, was often reported to be the most representative terpenic compound in EVOOs from different geographical region (Ben Brahim et al. 2018).

Phenolic Compounds Content

Phenolic compounds are a group of polar components, which contain one or more aromatic hydroxylated rings, including functional derivatives and are part of non-glycerin components in virgin olive oil (Seker 2010). The amount of phenolic compounds in EVOO is an important factor when evaluating its quality, given that the natural phenols improve its resistance to oxidation and, to certain extent, are responsible for its sharp bitter taste (Bubola et al. 2014). The quantity of phenols is influenced by the cultivar, the climatic conditions during growth, and the degree of ripening (Gargouri et al. 2013; Ammar et al. 2014). In all of the investigated EVOO samples, 10 phenols were identified and quantified (Table 3) when using a wavelength of 280 nm for detection. Moreover, the molecular ions of each compound

Table 3 Quantification of major phenolic compounds (mg/L)

	Gallic Ac	Hydroxytyrosol	3,4-Dihydroxy phenilacetic Ac	Catechin	Tyrosol	Chlorogenic Ac	Vanillic Ac	Epicatechin	p-Coumaric Ac	Oleuropein
Rt (min)	2.7	3.6–3.7	4.0	5.1	5.4–5.7	6.7–7.0	7.7	10.4	14.8–15.4	33.8
CO 2015	n.d	n.d	9.6±1.75[b]	n.d	40.4±5.74[a]	n.d	2.7±0.02[c]	n.d	11.9±0.55[b]	n.d
CO 2016	n.d	51±5.04[b]	n.d	12.5±1.75[c]	109.3±15.59[a]	n.d	15±0.63[c]	11±0.88[c]	123.4±2.46[a]	7.3±2.43[c]
WPOO2016	n.d	458±45.90[b]	13.2±2.15[g]	n.d	775.6±110.82[a]	82.2±4.71[d]	64.1±3.07[e]	44.5±5.21[f]	133±2.63[c]	n.d
UWPOO2016	277.3±9.28[b]	1331.5±133.61[a]	n.d	n.d	1151.2±164.50[a]	133.8±7.91[c]	89.2±4.31[d]	32.1±3.61[e]	126.7±2.52[c]	n.d
WPOO2015	n.d	38.3±3.76[c]	10±1.80[e]	n.d	215.9±30.83[a]	50.9±2.77[c]	99.1±4.80[b]	14.4±1.32[d]	243.1±4.51[a]	n.d
UWPOO2015	n.d	n.d	12±2.02[d]	n.d	157±22.41[a]	30.5±1.51[c]	88.7±4.29[d]	10.4±0.80[d]	131.4±2.60[d]	n.d

Rt, retention time; each value represents the mean of three determinations [n=3] ±standard deviation. [a-g] Different letters in the same line concerning all samples of olive oil fruits indicating significantly different values [$p < 0.05$]. n.d., not detected. CO, control olive oils; WPOO, washed polluted olive oils; UWPOO, unwashed polluted olive oils

were used to confirm the identification of the analytes. The use of a fluorescence detector in series with absorbance detector allowed increasing selectivity and sensitivity for the determination of catechin, vanillic acid, chlorogenic acid, epicatechin, and 3.4-dihydroxy phenylacetic acid in oil samples. Differences in bioactive and antioxidant compounds between oils from the polluted and control olive oils samples (PO and CO, respectively) were recorded.

Moreover, differences were observed comparing the analytical data of oils of the same field in the two consecutive years. Generally, the most abundant was tyrosol, followed by hydroxytyrosol. A higher concentration was observed in UWPOO and WPOO for 2016, while the WPOO for 2015 was distinguished by higher amount of p-coumaric Ac. The effects of the environment and pedo-climatic conditions on these compounds were clearly observed based on the diverse behaviors of each growing area (Rekik et al. 2019).

Method Validation

To assess the validity of the method, validation tests were run. All test parameters were carefully chosen to cover the range of samples and concentrations involved (Table 4). The linearity of standard curves was expressed in terms of the determination coefficient from plots of the integrated peak area versus concentration of the same standard (mg/L). These equations were obtained over a wide concentration range in accordance with the levels of these compounds found in the olive samples. Details are given in Table 4. The system was linear in all cases ($R^2 > 0.99$). LOD and LOQ were studied to check the sensitivity of the methods under the working conditions proposed. These limits, referring to the concentrations in olive oil needed if they were to be detected and quantified, were of the order of milligrams. The method has excellent sensitivity (Table 4).

Chemometric Analysis

In the preliminary analysis of the olive oil data, PCA was performed to investigate any potentially, existing clustering of samples on the basis of geographical origin and the year of harvest. PCA was applied to the dataset of volatile compounds of VOO from the different samples studied (Fig. 3). The first principal compound (PC) accounted for 44.47% of variance, while the second PC contributed 17.35%, resulting 65% of total variance. As Fig. 3a and b shows, using this approach, the olive oils were divided in three groups. The formed clusters indicated a large variation in the profiles of olive oil volatiles between the two harvest years, possibly caused by different weather conditions during the growing season. The majority of the investigated aldehydes and ketones that derive from the LOX pathway, including the major ones, such as 2-hexenal (isomer 1 and 2) (v67 and v71) and 3-hexenal (v52), as well as 1-penten-3-one (v33), 3-pentanone (v29) (Fig. 2a), 5-hepten-3-one, 5-methyl- (v49), and unsaturated hydrocarbons, were characteristic for monovarietal EVOO, washed and unwashed olive oils samples for the year 2016, and they could have contributed to generating positive green and fruity notes (Ben Brahim et al. 2018).

The application of cluster analysis to the volatiles divided all the samples into three groups: UWPOO 2016, WPOO 2016, CO 2015, and CO 2016 comprised the first group, the second group consisted of UWPOO 2015, while WPOO 2015 belonged to the thirrd group. The dendrogram of Fig. 3c shows that washing olive samples has no relevant influence on the production of volatiles since samples of the same variety and the same year of harvest 2016 have a great similarity. UWPOO 2015 and WPOO 2015 presented an extreme dissimilarity in their composition not only between each other, but also in comparison with the other samples. In fact, these samples can be distinguished from the others by the high levels of alkenes, alkylbenzenes, esters, and terpenes, whose contents

Table 4 Linearity of phenolic compound standards and sensitivity of the HPLC method

Compounds	Concentration range (mg/L)	Linear regression	R^2 [a]	LOD [b] (mg/L)	LOQ [c] (mg/L)
Gallic acid	0.51–10.33	y = 28.561x – 2.5246	0.9999	1.131	3.427
Hydroxytyrosol	5–200	y = 10.366x – 1.0078	0.9999	0.318	0.964
3.4-DHPA	5.63–112.7	y = 9.2014x – 9.4	0.9999	0.311	0.943
Catechin	0.50–10.15	y = 7.5718x – 0.7306	0.9957	0.208	0.630
Tyrosol	5–100	y = 6.9976x + 0/1499	1	0.098	0.297
Chlorogenic acid	1.02–51	y = 14.9x + 2.1975	0.9997	0.248	0.752
Vanillic acid	2.52–50.42	y = 20.168x + 2.2155	0.9998	0.213	0.647
Epicatechin	0.98–49	y = 7.6066x – 4.4754	0.9857	1.479	4.484
Coumaric Acid	0.51–10.21	y = 58.434x – 20.441	0.9978	0.149	0.452
Oleuropein	0.92–18.57	y = 2.41x + 1.4544	0.9966	0.441	1.336

[a] Correlation coefficients of the regression equation. [b] *LOD*, limit of detection. [c] *LOQ*, limit of quantification

Fig. 3 Chemometric analysis. Principal component analysis (PCA) obtained from the volatile compounds (listed in Tables 1 and 2) on PC1 and PC2 of all olive oil samples analyzed, considering 93 variables presented in the loading plot (**a**) and 6 observations shown in the score plot (**b**). Hierarchical cluster analysis [HCA]: dendrogram showing the clustering of the all analyses performed on oils from washed and unwashed polluted olives under two seasons 2015–2016 and 2016–2017 in the score plot (**c**)
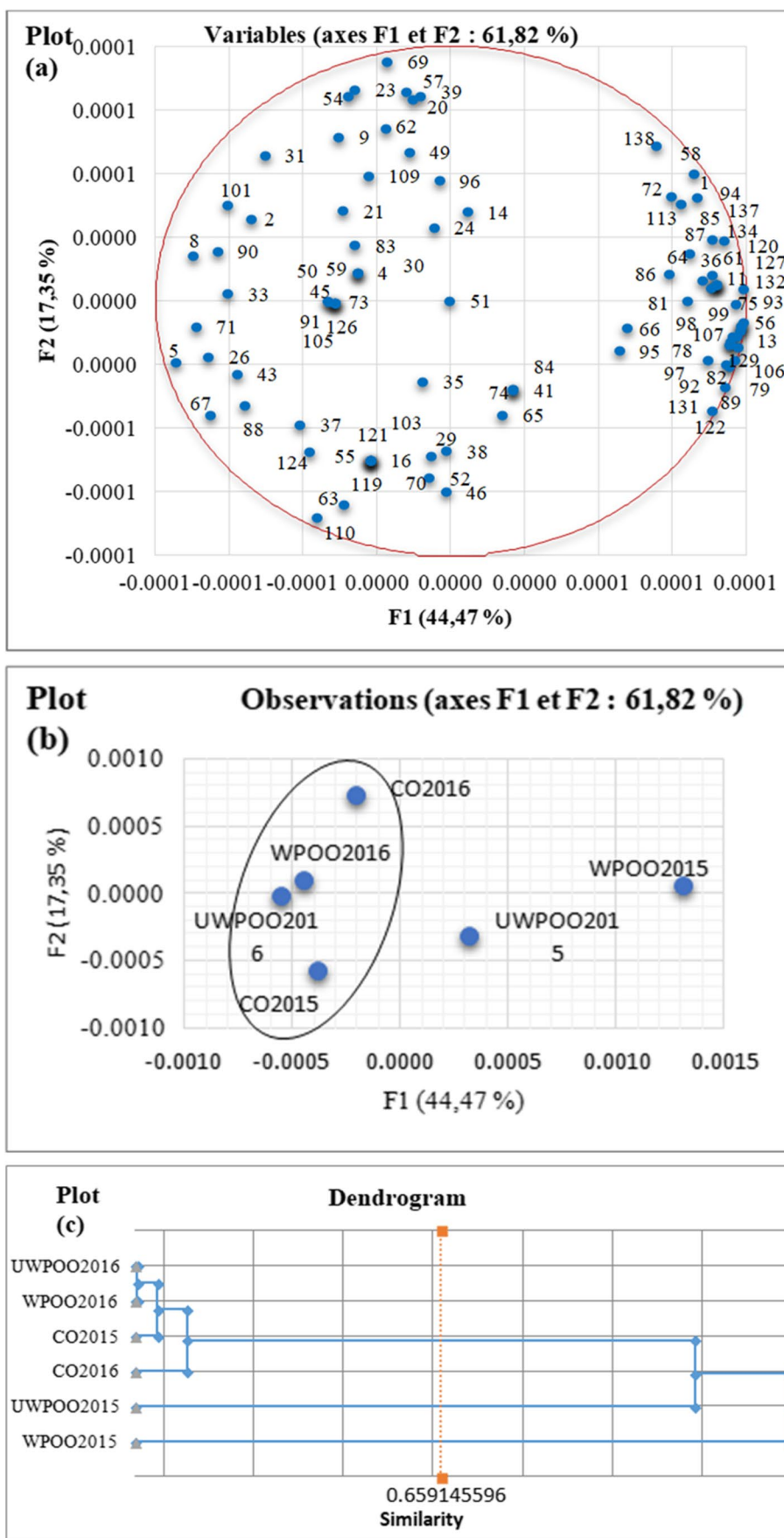
are by far the highest in WPOO 2015 samples. Nonetheless, in the same group, there is a high similarity between polluted oils in the year 2016 and CO 2015. It can be explained by the levels of alkenals in both. The geographic origin of oils plays a fundamental role in defining the volatile compound profile of virgin olive oils. Early studies conducted by Ben Mansour et al. (Ben Mansour et al. 2015) show the ability of volatiles, sampled with the static headspace, in discriminating different oils coming from different Tunisian regions.

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 geographical origins. The results indicate that besides the genetic factor, environmental conditions influence the volatile profiles. 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. The semi-quantitative determination of the volatile compounds can provide very useful information on EVOO quality and its control. A method for analysis of 10 phenolic compounds in oil samples by HPLC using fluorescence and absorbance detectors in series is presented. Even that the number of samples analyzed were limited, the influence of the pollution on volatile profiles of Chemlali virgin olive oils should be considered preliminary.

Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable.

Conflict of Interest Ons Rekik declares that he has no conflict of interest. Amir ben Mansour declares that he has no conflict of interest. M.D.R. Gomes Da Silva declares that he has no conflict of interest. Mohamed BOUAZIZ declares that he has no conflict of interest.

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