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Comparative study of different extraction techniques for the analysis of virgin olive oil aroma

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Abstract

Headspace solid-phase microextraction (HS-SPME), simultaneous distillation/extraction (SDE) and closed-loop stripping analysis (CLSA) coupled to gas chromatography/mass spectrometry (GC/MS) were used to study virgin olive oil, with the goal of detecting large numbers of characteristic volatile and semi-volatile compounds. More than one hundred compounds were detected in the olive oil extracts, and their percent amounts obtained by each technique were calculated. Qualitative and quantitative differences of virgin olive oil volatile profiles were observed applying the three extraction techniques. SPME showed a higher affinity for alcohols and ketones, while CLSA achieved the highest percentages of esters and hydrocarbons. Finally, the highest extraction of total terpenoid compounds occurred with SDE and CLSA, where CLSA allowed extracting the highest percentages of the most of them. SDE extraction caused the thermal degradation of the oil sample, which resulted in a high percentage of aldehydic compounds.

Keywords: Olive oil; Volatile compounds; SPME; CLSA; SDE; Gas chromatography; Mass spectrometry

1. Introduction

Virgin olive oil has a characteristic flavour that distinguishes it from other edible vegetable oils. It is obtained from the olive fruit by mechanical processes only (EC 2568/91) and no further treatments are required before consumption. The absence of refining processes helps preserve the organoleptic characteristics and the nutritional properties that distinguish virgin olive oil from other edible oils. Olive oil's characteristic aroma and, in particular, its green and fruity attributes depend on many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway, which occurs during the oil extraction process (Angerosa, Mostallino, Basti, & Vito, 2000; Montedoro, Bertuccioli, & Anichini, 1978; Morales, Alonso, Ríos, & Aparicio, 1995). Variable amounts of hexanal, hexanol and hexylacetate derive from the degradation of linoleic acid, while (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate result from the enzymatic degradation of linolenic acid (Olías, Pérez, Ríos, & Sanz, 1993; Williams, Morales, Aparicio, & Harwood, 1998).

In recent years, the need for analytical procedures to evaluate the quality of virgin olive oil quality has led to several studies addressing its volatile fraction. Various analytical methods have been developed to examine these volatile compounds. In this way, a large number of components that contribute to the aroma of olive oil have been identified. Distillation methods have traditionally been applied in the analysis of plant materials. Steam distillation (SD),

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simultaneous distillation/extraction (SDE) and microwave-assisted extraction (MAE) were used for this purpose (Marriott, Shellie, & Cornwell, 2001). Among these distillation techniques, SDE appeared to afford the most favourable uptake for mono- and sesquiterpenes, as well as their oxygenated analogues (Marriott et al., 2001). Hydrodistillation has been applied for the analysis of leaf, fruit and virgin oil volatiles of an Italian olive cultivar (Flamini, Cioni, & Morelli, 2003).

With hydrodistillation (SD), the volatiles in the steam distillate are heavily diluted by water when collected in cold traps. This is overcome in simultaneous distillation/extraction (SDE) via solvent extraction of the distillate.

Dynamic headspace techniques have been used to correlate the composition of the olive oil headspace to sensory attributes (Angerosa, Di Giacinto, Vito, & Cumitini, 1996; Angerosa et al., 2000; Morales et al., 1995; Servili, Conner, Piggott, Withers, & Paterson, 1995) and off-flavors or "defects" (Angerosa, Di Giacinto, & Solinas, 1992; Morales, Rios, & Aparicio, 1997).

More recently, the solid-phase microextraction (SPME) technique has been introduced as an alternative to the dynamic headspace technique as a sample preconcentration method prior to chromatographic analysis. Among other applications, SPME allowed the characterization of virgin olive oils from different olive varieties and geographical production areas (Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003a), and the evaluation of varietal and processing effects (Dhifi et al., 2005; Tura, Prenzler, Bedgood, Antolovich, & Robards, 2004). However, as SPME uptakes are strictly dependant on the distribution coefficient of analytes between the sample matrix, the gas phase and the fibre coating (Pawliszyn, 1999), some compounds present in virgin olive oil could remain undetected by this technique. Otherwise, in the case of other techniques such as SDE, the recovery of analytes should be mainly related to their volatility.

In the present study, headspace solid-phase microextraction (HS-SPME), simultaneous distillation/extraction (SDE), and closed-loop stripping analysis (CLSA) coupled to gas chromatography/mass spectrometry (GC/MS) were applied for the study of virgin olive oil, with the aim of obtaining sufficiently rich olive oil extracts enabling the detection of a large number of characteristic volatile and semi-volatile compounds. Furthermore, the percentage areas of distinct families of olive oil compounds extracted by these techniques were compared.

2. Materials and methods

2.1. Reagents, materials and samples

1-Hexanol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, 1-octen-3-ol, 1-heptanol, 1-octanol, 1-nonanol, phenol, pentanal, hexanal, heptanal, (*E*)-2-hexenal, octanal, nonanal, decanal, benzaldehyde, (*E*)-2-nonenal, 6-methyl-5-hepten-2-one, aliphatic hydrocarbons, toluene, hexylacetate, hexenylacetate, α-pinene, limonene and *p*-cymene, were purchased from Sigma–Aldrich (St. Louis, Missouri, USA); farnesene (isomers mixture), ethylbenzene, *o*-, *m*- and *p*-xylene, 1,3,5-, 1,2,3- and 1,2,4-trimethylbenzene, 2-and 3-ethyltoluene, butylbenzene, and 1,2,3,4-tetramethylbenzene were obtained from TCI (Tokyo, Japan).

The SPME fibre used was a Divinylbenzene/Carboxen/Polydimethylsiloxane 50/30 μm, 2 cm long (DVB/CAR/PDMS), from Supelco (Bellefonte, PA, USA). Before use the fibre was conditioned as recommended by the manufacturer.

The analyzed olive oil pertained to the "extra virgin" commercial class, according to the EC regulations (EC 2568/91; EC 1513/2001; EC 796/2002). It was produced in Siurana (Spain) from Arbequina olives harvested in 2003/2004. It was analysed in duplicate.

2.2. SPME extraction

SPME analysis was carried out according to Vichi et al. (2003). Briefly, 2 g of virgin olive oil were placed into a 10 ml vial fitted with a silicone septum, and then into a water bath at 40 °C where the oil was maintained under magnetic stirring. After 2 min of sample conditioning, the SPME fibre was exposed for 30 min to the sample headspace and immediately desorbed in the gas chromatograph injector.

2.3. SDE extraction

For SDE extraction, 38,920 g of oil and 450 ml of bidistilled water were placed in the flask of a Likens–Nickerson apparatus. A second flask with a 5 ml mixture of pentane and dichloromethane (2:1) (SDS, Peypin, France) was used as the organic phase, and the mixture was then boiled for 4 h. The mixture of pentane and dichloromethane was chosen as organic solvent with the aim of obtaining different solvent polarities without exceeding the water density. In this way, the original arrangement of the extraction system could be maintained. After cooling, the extract fraction was collected and dried with anhydrous Na_2SO_4. One microlitre of the extract was then injected in the gas chromatograph in the split mode (1:60).

2.4. CLSA extraction

CLSA extraction was carried out in a commercial apparatus (Brechbüler, Zurich, Switzerland) in accordance with the standard method 6040 B (APHA, 1995). Five hundred and ninety milligrams of olive oil dissolved in 1.2 ml of acetone (Burdick & Jakson) were added to 0.95 l of double-distilled water. The samples were air-stripped for 70 min in a bath at 45 °C, and the volatile organic compounds were adsorbed on 5 mg of activated carbon filter at 55 °C. The filters were then extracted with 40 μl of carbon

disulphide (SDS, Peypin, France). Conditions were similar to those applied by Eggers, Kenefick, Richardson, Wigglesworth, and Girard (2003) in wine model solution, although the absorbent trap was a filter of 5 mg of activated charcoal. In these conditions it was not expected any saturation of the trap.

2.5. GC–MS analysis

Identification of compounds was performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a Supelcowax-10 (Supelco) 30 m × 0.25 mm ID, 0.25 mm film thickness. Column temperature was maintained at 40 °C for 3 min, increased to 75 °C at 4 °C/min, then to 250 °C at 8 °C/min, and held constant for 5 min. The injector temperature was 260 °C and the desorption time of the fibre into the injection port was fixed at 5 min. Helium was used as carrier gas at a linear velocity of 38 cm/s.

The temperature of the ion source was 175 °C and the transfer line, 280 °C. Electron impact mass spectra were recorded at 70 eV ionization energy, 2 scan/s.

GC–MS analysis in the complete scanning mode (SCAN) within the 40–300 μm mass range, was performed to allow the identification of compounds in oil samples.

2.6. Characterization of volatile compounds in virgin olive oil

Compounds were identified by comparing their mass spectra and retention times with those of standard compounds, or by comparing the mass spectrum with those of the mass spectra library, Wiley 6th. Kováts' indices were calculated and compared with retention indices available in the literature. Compounds were quantified as area percentages of total volatiles.

3. Results and discussion

Fig. 1 shows the chromatographic profiles of the virgin olive oil volatile fraction isolated by SPME, SDE and CLSA. Peak identifications are detailed in Table 1. A total of more than one hundred volatile and semi-volatile compounds were identified in virgin olive oil headspace using the three extraction techniques. The percentage concentrations of compounds observed with each technique is also reported in Table 1. Relative standard deviations (RSD) of absolute areas ranged from 1.1% to 12.2% for SPME, from 2.3% to 13.0% for SDE and from 3.4% to 9.1% for CLSA. RSD values of results expressed as percent areas were in general lower than 0.1%, ranging from 0.003% to 0.184% (data not shown).

As was expected, the volatile profile of virgin olive oil closely depended on the method of extraction used. Both qualitative and quantitative differences were observed in the chromatographic profiles obtained after extraction by SPME, SDE and CLSA (Fig. 1). The profiles obtained by SPME can depend on the type of fibre used. In this study, the three-phases coating PDMS/Car/DVB was chosen on the basis of its affinity for compounds of both low and medium molecular weight (Mani, 1999) and because in comparison with other fibres it gives among the highest uptakes of virgin olive oil volatiles (Vichi et al., 2003).

A similar number of characteristic olive oil compounds were identified by means of SPME and CLSA (77 and 80 compounds, respectively), while SDE extraction led to the identification of 89 compounds (Table 1). As can be seen in Fig. 2, relevant differences were observed in terms of percentages of the major families of volatiles. SPME proved more efficient in extracting alcohols (42.7%) and ketones (5.2%) than did both SDE (20.6% and 1.5%, respectively) and CLSA (10.1% and 1.0%, respectively). SDE extraction resulted in a higher percentage of aldehydes (37.0%) than did both SPME (17.0%) and CLSA (14.0%), and attained a higher percentage of terpenoids (21.6%) than did SPME (4.3%), though it was comparable to that of CLSA (18.7%). Finally, CLSA extracts were the richest in hydrocarbons (36%) and esters (18.5%), exceeding both SPME extracts (22.0% and 8.7%, respectively) and SDE extracts (12.2% and 7.2%, respectively).

These different sampling techniques offer a number of individual advantages, but also suffer from specific limitations. In the sample extracted by SDE, higher percentages of aldehydic compounds not deriving from the lipoxygenase pathway were observed. Compounds such as nonanal, octanal, 2-pentenal, 2-heptenal, and 2,4-heptadienal were shown to be significantly correlated with the oxidative status of virgin olive oil (Morales et al., 1997; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003b). Likewise, hexanal amounts may be derived from either lipoxygenase action on polyunsaturated fatty acids or from chemical oxidation. Also this aldehyde was found in higher percentage in SDE extract. On the contrary, (*E*)-2-hexenal, which is derived from the LOX pathway and which is inversely related to the oxidation degree of virgin olive oil (Vichi et al., 2003b), was found to be present in lower amounts in SDE and CLSA extracts compared with SPME (Table 1). These results indicate that the higher percentage of aldehydes observed in the SDE extract likely stem from to oxidative alteration of the sample rather than from a higher extraction efficiency. The thermal alteration of the oil sample extracted by SDE was also confirmed by the presence of the unidentified compound which was characterized by the mass spectral fragments m/z 81 and 124. The latter has been shown to be strictly related with the oxidative degradation of virgin olive oil (Vichi et al., 2003b).

Regarding terpenoids extraction, α-zingiberene and α-farnesene were the only compounds detected at a higher percentage by SDE, while the rest were detected at higher percentages by SPME and CLSA (Table 1). In particular, most sesquiterpenic compounds showed higher percentages in the CLSA extract (Table 1). CLSA also showed

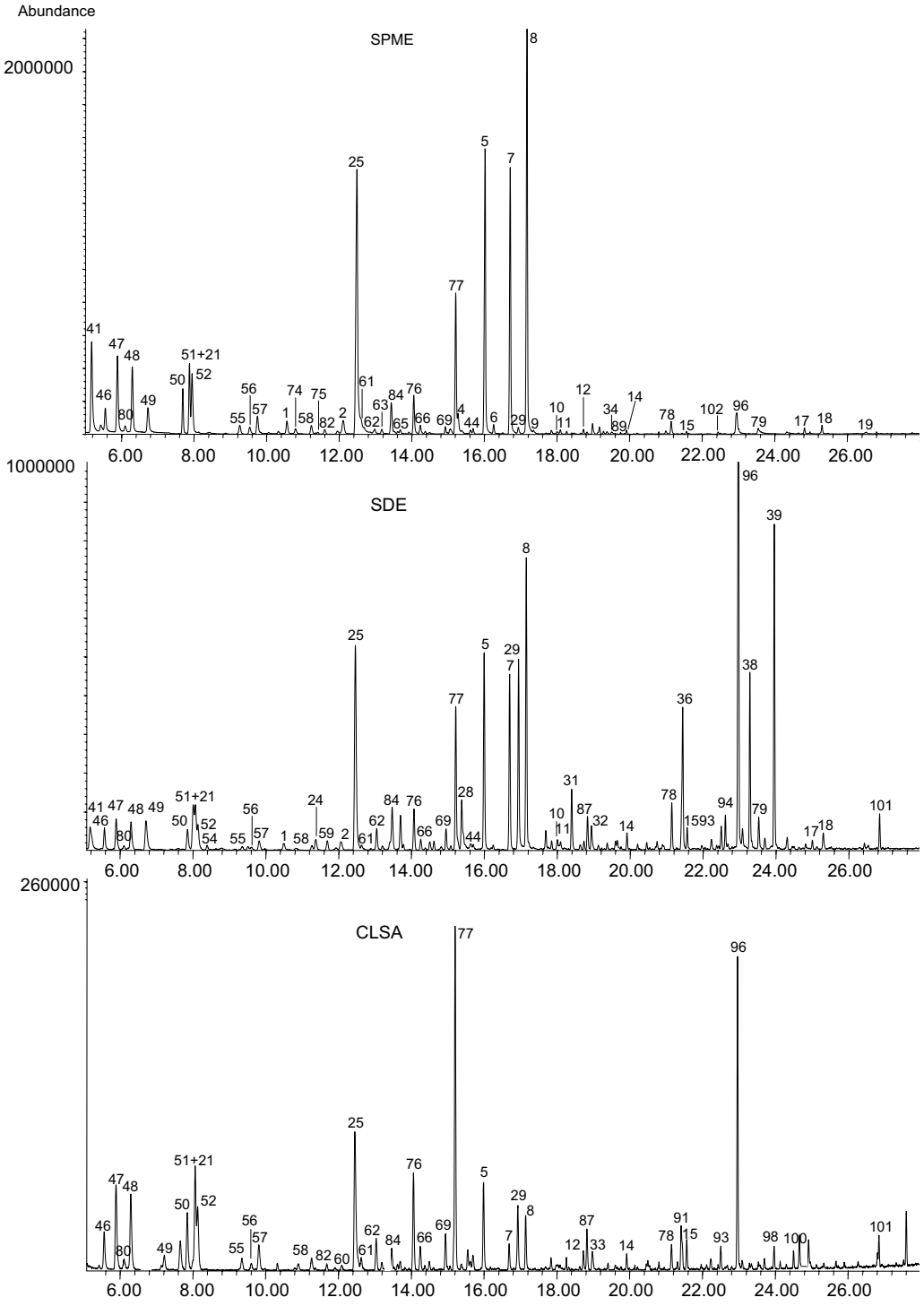

Fig. 1. Chromatographic profiles of virgin olive oil volatile fraction obtained by SPME, SDE and CLSA extraction followed by GC/MS analysis. The separation was carried out on a Supelcowax-10 capillary column. The identification numbers correspond to those reported in Table 1.

greater efficiency in the extracting esters and hydrocarbons. Esters principally consisted of hexylacetate and hexenylacetate from the lipoxygenase pathway (Olías et al., 1993; Williams et al., 1998), while hydrocarbons mainly included compounds such as pentene dimers (corresponding to the isomeric compounds 47, 48, 51–53 shown in Table 1), which are products of bioformation of virgin olive oil aroma (Angerosa, Camera, d'Alessandro, & Mellerio, 1998) and alkylated benzenes. The latter are environmental pollutants previously documented in virgin olive oil volatile fraction (Biedermann, Grob, & Morchio, 1995, 1996; Olías-Jiménez, Gutierrez-Rosales, Dobarganes-García, & Gutierrez Gonzáles-Quijano, 1980; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2005).

Table 1
Characterization and mean percent amounts of volatile compounds of virgin olive oil extracted by SPME, SDE and CLSA

	Compounds	ID[a]	KI[b]	%[c] SPME	% SDE	% CLSA
	Alcohols					
1	1-Penten-3-ol	MS[d], KI[e]	1166	0.65	0.28	0.23
2	Isoamyl alcohol	MS, KI	1215	1.03	0.44	–
3	(*E*)-2-Pentenol	MS, KI	1320	0.14	–	–
4	(*Z*)-2-Pentenol	MS, KI	1329	0.96	0.40	–
5	1-Hexanol	STD[f], MS, KI	1362	11.70	4.94	3.32
6	(*E*)-3-Hexenol	STD, MS, KI	1372	0.35	0.12	0.08
7	(*Z*)-3-hexenol	STD, MS, KI	1392	10.44	4.47	1.03
8	(*E*)-2-Hexenol	STD, MS, KI	1414	15.92	7.41	1.92
9	(*Z*)-2-Hexenol	STD, MS, KI	1423	0.17	0.06	0.06
10	1-Octen-3-ol	STD, MS, KI	1457	0.09	0.24	–
11	1-Heptanol	STD, MS, KI	1462	0.13	0.21	0.19
12	2-Ethyl-1-hexanol	MS	1495	0.16	0.22	0.64
13	(*Z*)-Hepten-2-ol	MS	1518	0.05	0.11	–
14	1-Octanol	STD, MS, KI	1563	0.13	0.36	0.48
15	1-Nonanol	STD, MS, KI	1665	0.08	0.53	0.97
16	(*Z*)-6- or 4-nonenol	MS	1690	0.02	0.11	0.20
17	Benzenemethanol	MS, KI	1889	0.21	0.12	0.88
18	Benzeneethanol	MS, KI	1926	0.36	0.45	0.12
19	Phenol	STD, MS, KI	2020	0.09	–	–
20	Ethylphenol	MS, KI	2188	–	0.12	–
	Aldehydes					
21	Hexanal	STD, MS, KI	1086	0.47	1.63	0.39
22	(*Z*)-3-Hexenal	MS, KI	1115	0.07	0.12	–
23	(*E*)-2-Pentenal	MS, KI	1138	–	0.15	–
24	Heptanal	STD, MS, KI	1190	0.06	0.45	0.14
25	(*E*)-2-Hexenal	STD, MS, KI	1228	15.63	7.68	7.99
27	Octanal	STD, MS, KI	1296	0.08	0.27	0.44
28	(*E*)-2-Heptenal	MS, KI	1334	0.16	1.92	0.30
29	Nonanal	STD, MS, KI	1402	0.34	5.03	2.47
30	(*E*,*Z*)- or (*E*,*E*)-2,4-hexadienal	MS, KI	1441	–	0.45	0.14
31	(*E*,*Z*)-2,4-Heptadienal	MS, KI	1478	0.05	1.50	–
32	(*E*,*E*)-2,4-Heptadienal	MS, KI	1506	–	0.70	–
33	Decanal	STD, MS, KI	1509	–	–	0.86
34	Benzaldehyde	STD, MS, KI	1540	0.12	0.05	–
35	(*E*)-2-Nonenal	STD, MS, KI	1548	–	0.23	0.11
36	(*E*)-2-Decenal	MS, KI	1657	0.04	4.09	0.77
37	Undecenal	MS	1766	–	0.46	0.24
38	(*E*,*Z*)-2,4-Decadienal	MS	1780	–	4.00	0.18
39	(*E*,*E*)-2,4-Decadienal	MS	1827	–	7.19	–
40	Vinylbenzaldehyde	MS	2066	0.02	–	–
	Ketones					
41	3-Pentanone+pentanal	STD, MS, KI	983	4.82	1.26	–
42	2-Octanone	MS, KI	1292	0.10	0.09	0.26
43	4-Octanone	MS	1300	–	–	0.14
44	6-Methyl-5-hepten-2-one	STD, MS, KI	1347	0.20	0.17	0.63
45	Phenylethanone	MS	1669	0.05	–	–
	Hydrocarbons					
46	Decane	STD, MS, KI	1001	1.26	0.71	1.81
47	1,5-Octadiene, 3-ethyl (E or Z)	MS, KI	1013	3.73	1.16	4.31
48	1,5-Octadiene, 3-ethyl (E or Z)	MS, KI	1027	3.18	1.06	3.89
49	Toluene	STD, MS, KI	1043	1.40	1.41	4.03
50	Ni hydrocarbon (*m*/*z* 41, 57, 76, 113)	–	1060	–	–	0.75
51	3,7-Decadiene (*EE* or *ZZ* or *EZ*)	MS, KI	1076	1.60	0.77	2.73
52	3,7-Decadiene (*EE* or *ZZ* or *EZ*)	MS, KI	1083	2.81	1.58	5.58
53	3,7-Decadiene (*EE* or *ZZ* or *EZ*)	MS, KI	1085	2.91	0.94	2.56
54	Undecane	STD, MS, KI	1100	0.05	0.17	–
55	Ethylbenzene	STD, MS, KI	1127	0.53	0.17	0.73
56	*m*-Xylene	STD, MS, KI	1135	0.43	0.16	0.32
57	*p*-Xylene	STD, MS, KI	1142	1.05	0.39	1.46

(*continued on next page*)

Table 1 (*continued*)

	Compounds	ID[a]	KI[b]	%[c] SPME	% SDE	% CLSA
58	*o*-Xylene	STD, MS, KI	1187	0.50	0.19	0.60
59	Dodecane	STD, MS, KI	1200	–	0.35	–
60	Propylbenzene	MS, KI	1214	–	–	0.33
61	3-Ethyltoluene	STD, MS, KI	1231	0.71	0.19	0.81
62	Ni hydrocarbon (*m*/*z* 55, 69, 97, 126)	–	1245	0.23	0.70	1.48
63	1,3,5-Trimethylbenzene	STD, MS, KI	1251	0.21	0.16	0.41
64	Styrene	STD, MS, KI	1266	0.10	0.03	0.29
65	2-Ethyltoluene	STD, MS, KI	1268	0.19	–	0.43
66	1,2,4-Trimethylbenzene	STD, MS, KI	1287	0.41	0.34	1.05
67	Tridecane	STD, MS, KI	1300	–	0.27	–
68	Ni hydrocarbon (*m*/*z* 55, 70, 83, 119)	–	1309	–	0.12	–
69	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	MS, KI	1314	0.28	0.58	1.45
70	Butylbenzene	STD, MS, KI	1319	0.18	0.11	0.22
71	1,2,3-Trimethylbenzene	STD, MS, KI	1344	0.15	0.21	0.37
72	Unsaturated C4-alkylbenzene	MS	1449	0.15	0.22	0.39
73	Biphenyl	MS, KI	2012	–	0.15	–
	Esters					
74	Methyl pentanoate	MS	1173	0.29	0.11	0.17
75	Methyl hexanoate	MS	1192	0.09	–	–
76	Hexylacetate	STD, MS, KI	1281	1.67	1.17	3.98
77	(*Z*)-3-Hexenylacetate	STD, MS, KI	1326	5.56	4.01	13.20
78	Methylbenzoate	MS	1638	0.52	1.13	0.89
79	Methylsalicilate	MS, KI	1798	0.52	0.78	0.22
	Terpenic and sesquiterpenic compounds					
80	α-Pinene	STD, MS, KI	1020	0.31	0.13	0.63
81	δ-3-Carene	MS, KI	1143	0.10	0.05	0.09
82	Limonene	STD, MS, KI	1198	0.26	–	0.33
83	*p*-Mentha-1,5,8-triene	MS, KI	1210	0.23	0.11	0.20
84	(*E*)-β-Ocimene	MS, KI	1260	1.49	1.38	0.85
85	*p*-Cymene	STD, MS, KI	1277	0.08	0.03	0.13
86	Cyclosativene	MS, KI	1491	0.04	0.16	0.10
87	α-Copaene	MS, KI	1500	0.08	0.79	1.36
88	α-Cedrene	MS, KI	1545	0.04	0.21	0.14
89	Linalool	STD, MS, KI	1554	0.05	0.06	0.09
90	α-Bergamotene	MS, KI	1595	–	0.17	0.16
91	(*Z*)-β-Farnesene	STD, MS, KI	1654	–	0.32	1.17
92	β-Acoradiene	MS, KI	1708	–	0.33	0.40
93	Eremophyllene	MS, KI	1727	0.05	0.58	0.75
94	α-Zingiberene	MS, KI	1735	0.05	0.78	0.12
95	α-Muurolene	MS, KI	1738	–	0.14	0.17
96	(*E*,*E*)-α-Farnesene	STD, MS, KI	1757	1.53	14.92	9.47
97	Oxygenated sesquiterpene (*m*/*z* 189, 207, 222)	MS	1808	–	0.27	0.37
98	Pulegone	MS	1828	–	–	0.73
99	Oxygenated sesquiterpene (*m*/*z* 189, 207, 220)	MS	1852	–	0.36	0.13
100	Geranylacetone	MS	1866	–	0.09	0.52
101	Farnesol	MS	2045	–	0.73	0.76
	Others					
102	Ni (*m*/*z* 81, 124)	–	1272	–	1.04	–
103	γ-Hexalactone	MS	1722	0.08	–	0.15
104	Ni (*m*/*z* 165, 180, 221, 236)	–	2106	–	–	1.55

[a] Identification method.
[b] Kováts' indices on Supelcowax-10 capillary column.
[c] Percent amount of volatile compounds in virgin olive oil, calculated on the basis of chromatographic areas.
[d] Tentatively identified by mass spectra.
[e] Tentatively identified by retention index.
[f] Identified by comparison with standard compounds.

The higher presence of alkylated benzenes in the CLSA extract could stem from the high proportion of water that was present during the stripping of olive oil. However, the uptake of the remaining hydrocarbons indicated that CLSA boasted greater efficiency in extracting this family of compounds.

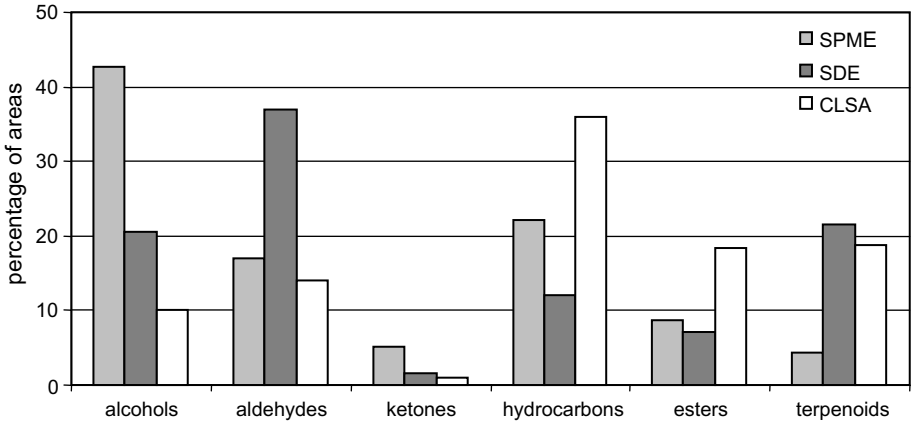

Fig. 2. Percentages of virgin olive oil's major families of volatiles extracted by SPME, SDE and CLSA.

The characteristic volatile compounds of virgin olive oil that derive from the lipoxygenase pathway were identified by each type of extraction method, although their percentage areas revealed relevant differences (Table 1). The highest percentage of LOX-derived compounds was achieved by SPME extraction. In fact, the sum of hexanal, (*Z*)-3- and (*E*)-2-hexenal, (*E*)- and (*Z*)-2- and 3-hexenol, hexylacetate and hexenylacetate corresponded to the 62.0%, 31.6% and 32.0% of total compounds for SPME, SDE and CLSA, respectively. The proportion of alcohols, aldehydes, and esters from the LOX pathway were more similar between SPME and SDE extracts than between those of the latter and the CLSA extract (Fig. 3). CLSA extraction resulted in a much higher proportion of esters and lower proportion of alcohols from LOX. In addition, SPME showed a higher proportion of alcohols, while the percentage of aldehydes proved comparable when applying SPME, SDE or CLSA. Besides obtaining the highest percentage of LOX products (in particular, of alcohols), SPME allowed for more effective extraction of ketones, principally represented by 3-pentanone.

The last consideration regards the amounts of oil from which the extraction is performed, which largely differed by the distinct methods: for SDE analysis the largest amount of sample was needed (38.92 g), while for SPME, and in particular for CLSA, small quantities of oil were sufficient to allow the detection of a high number of compounds (2 g and 590 mg, respectively).

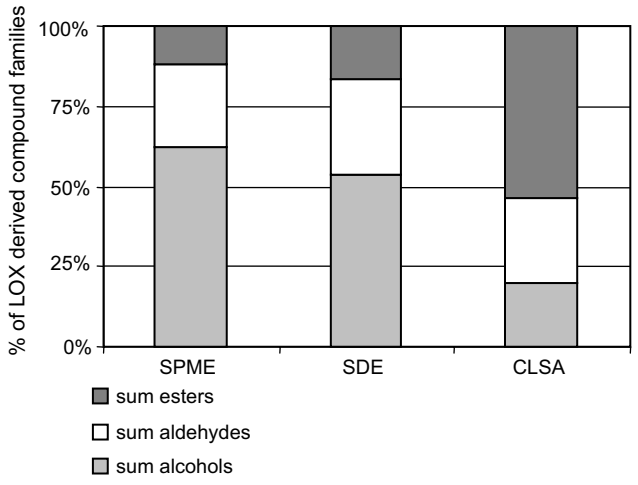

Fig. 3. Proportions of alcohols, aldehydes, and esters from the LOX pathway extracted by SPME, SDE and CLSA.

In conclusion, and as was expected, the volatile profile of virgin olive oil closely depended upon the method of extraction used. The three techniques tested (SPME, SDE and CLSA) resulted in both qualitative and quantitative differences of the virgin olive oil volatile profiles. Thermal degradation of the oil sample was observed when applying SDE. SPME demonstrated higher extraction efficiency for characteristic compounds of virgin olive oil aroma, such as LOX-derived compounds, particularly alcohols. CLSA extracted the highest percentages of esters and hydrocarbons, comprising products of bioformation of virgin olive oil aroma. Finally, the highest extraction of total terpenoid compounds was obtained with SDE and CLSA, where CLSA allowed extracting the highest percentages of the most of them.

On the basis of these results, a specific extraction technique among those tested in this study could be taken into consideration according to the class of volatile compounds to be determined in virgin olive oil.

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