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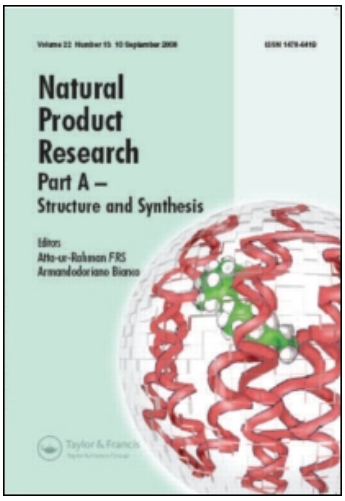

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Profiles of volatile compounds from nine new hybrids obtained by controlled crossings on olive *Chemlali* cultivar and Mediterranean varieties

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Virgin olive oil is a unique oil that can be consumed directly without any refining process. This particularity is due to the exceptional quality and flavour formed by the presence of more than 100 volatile compounds. Nine new hybrids obtained by controlled crossing of the *Chemlali* and seven ancient Mediterranean varieties cultivated in the same orchard under identical agronomic and pedoclimatic conditions were characterised by their main volatile compounds quantified by dynamic headspace-gas chromatography-MS. More than 40 volatile compounds from the main chemical groups, aldehydes, alcohols, ketones and esters, were identified by GC-MS and confirmed by their Linear Retention Index (LRI). Compounds produced from the lipoxygenase pathway were studied to determine the genetic potential and the influence on each crossing. Finally, Ward's method test and Pearson PCA analysis were used to check the ability of the volatiles to cluster the varietal virgin olive oils according to their genetics origin.

Keywords: olive oil; crossing; hybrid; volatile compounds; lipoxygenase

1. Introduction

Extra virgin olive oil (EVOO), obtained from the fruit of *Olea europea* L., is a unique oil that can be consumed directly without any refining process because it retains the entire volatile and non-volatile compounds that determine the quality and the authenticity of this oil. The volatile fraction of EVOO consists of a complex mixture of more than one hundred compounds (Morales, Aparicio, & Rios 1994; Vichi et al., 2003). These compounds are characterised by a low molecular weight (<300 Da) and can be considered to be metabolites directly produced in plant organs by intracellular biogenic pathways, such as the lipoxygenase pathway. Some of the volatiles found in EVOO are present in the intact tissue of the fruit, and others are formed during the disruption of cell structure during the EVOO production due to enzymatic reactions in the presence of oxygen (Angerosa, 2002). The major volatile compounds reported in olive oil aroma are

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attributed to the same C5 and C6 compounds from major classes: aldehydes, alcohols, esters, hydrocarbons, ketones, furans and, probably, others as yet unidentified. This fraction includes: hexanal, *trans*-2-hexenal, hexan-1-ol and 3-methylbutan-1-ol (Angerosa, 2002; Aparicio, Morales, Alonso, & Agri, 1997). Cultivar, geographic region, fruit maturity, processing methods, and parameters play an important role in the process of the creation of the volatile compounds (Angerosa, Mostallino, Basti, Vito, & Serraiocco, 2000; Di Giovacchino, Costantini, Serraiocco, Ranalli & Angerosa, 1996; Ranalli, Costantini, De Mattia, & Ferrante, 2000; Ranalli, De Mattia, Patumi, & Proietti, 1999; Ranalli, Tombesi, Ferrante, & Dematti, 1998; Surricchio & Basti, 2001). In fact, fruit from different cultivars grown under the same environmental conditions produce oils with different volatile compounds, as do fruits of the same cultivars grown in different geographic regions.

The aim of this work is the characterisation of some virgin olive oils from new hybrids compared to these ancient cultivars, cultivated in an orchard, by quantification of the volatile compounds present in their aroma and to find out the genetic factor effect in the volatile fraction. To avoid the influence of other factors on the characterisation, olive trees were cultivated under the same agronomic and pedoclimatic conditions, olive fruits were picked at the same stage of ripeness, and their oils were extracted immediately with the same processing system.

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2. Material and methods

2.1. *Plant material*

Seventeen samples of EVOO composed of five introduced Mediterranean cultivars (*Arbequina*, *Picholine*, *Souri*, *Coratina*, *Koroneiki*), three autochthonous Tunisian cultivars (*Chemlali*, *Chemchali*, *Kotti*), and nine hybrids were obtained beteewn four cultivars used as pollen acceptors and five cultivars as pollinators for a total of nine genetic combination progenies. Six year old hybrids were obtained often from seed germination and plant breeding (Trigui & Fiorino, 1995). Table 1 shows details of these new hybrids obtained through controlled intervarietal crossings. All olive varieties were collected from the botanic orchard of Taoues (Sfax, Tunisia), where the olive trees are cultivated under identical agronomic and pedoclimatic conditions.

Table 1. Selected progenies hybrids details obtained by cross-breeding from Tunisian and Mediterranean introduced cultivars.

	Chemlali cv. ♀	*Coratina* cv. ♀	*Arbequina* cv. ♀	*Chemchali* cv. ♀	Total progenies selected
Chemchali cv. ♂	CCH13; CCH73; CCH235	–	CA32	–	4
Kotti cv. ♂	CK725	–	–		1
Chemlali cv. ♂	–	CC325	–	CCH225	2
Arbequina cv. ♂	CA21	–	–	–	1
Coratina cv. ♂	CC45	–	–	–	1
Total progenies selected	6	1	1	1	9

Notes: ♀Cultivars used as pollen acceptors; ♂cultivars used as pollinators.

2.2. *Olive oil processing*

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 the same degree of maturity index. The extraction process was carried out on laboratory scale using an experimental oil mill. 920 g of olives of each sample were crushed. The temperature and the time of malaxation were 35°C and 40 min, respectively. After the centrifugation, the oil was obtained by decantation.

2.3. *Volatile compound extraction process*

Volatile compounds were analysed by a modified dynamic headspace technique previously reported by Dhifi et al. (2004) and Angerosa et al. (1999). Samples of 25 g each of oil were heated at 37° and swept by nitrogen (0.2 L min^{-1}) for 2 h into 120 mL Drechel gas washing bottles with a porous distributor. Volatile compounds were trapped in 50 mg of activated charcoal (0.5–0.85 mm, 20–35 mesh ASTM) from E. Merck (Schuchardt, Germany) and eluted with 1 mL of diethyl ether.

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2.4. *GC and GC-MS analysis*

The analytes were separated using a HP5890 series II gas chromatograph equipped with a HP-INNOWAX capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The separation was performed in splitless mode as follows: helium was used as gas carrier at a linear velocity of 1 mL min^{-1}; injector and detector temperatures were 40 and 200°C, respectively. Initial oven temperature was 40°C for 10 min, and subsequently programmed from 40 to 280°C at a rate of 5°C min^{-1} where it was held for 5 min. The compounds were analysed by an HP5972A-MSD. Mass spectra were obtained in the electron impact mode (70 eV). The operating conditions were as follows: interface temperature 280°C (transfer-line), ion source temperature 230°C and the quadrupole 150°C. The mass range varied from 30 to 350 amu, the solvent delay was 6 min, the threshold 150 and scan speed 4.45 scan s^{-1}. The identification of compounds was based on computer matching against commercial libraries (Wiley 6th ed. and ADAMS) and from a library built from pure substances and compounds of known oils and literature data (Kanavouras, Kiritsakis, & Hernandez, 2005; Morales, Luna & Aparicio, 2005; Ranalli, Contento, Schiavone, & Simone, 2001). Their characterisations were confirmed by calculation of retention times with those of pure and authentic standards and by means of their linear relative index (LRI) relative to the *n*-hydrocarbons. Compounds were quantified as area percentages of total volatiles.

2.5. *Statistical analysis*

The results are reported as the mean of triplicate determinations. Hierarchical cluster analysis was performed on volatile compounds data using Ward's method. Data were also processed by principal component analysis (PCA) under the following conditions: Kaiser's normalisation, varimax rotation and tolerance limits for matrix inversion (0.0001). Statistical analysis was performed using the XLSTAT 7.5.2 (Addinsoft, Paris, France).

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Table 2. Mean volatile compounds identified (%) by dynamic headspace of EVOO from new nine hybrids obtained by cross-breeding between Tunisian and Mediterranean introduced cultivars.

			Parental varieties								New hybrids								
			Mediterranean					Tunisian											
Volatile compounds	LRI[a]	Aroma attributes[b]	*Arbequina*	*Coratina*	*Koroneiki*	*Picholine*	*Souri*	*Kotti*	*Chemcheli*	*Chemlali*	CCH13	CK725	CCH225	CC325	CA21	CA32	CC45	CCH73	CCH235
Hexanal	1080	Green, sweet	0.074	0.434	–	–	–	0.165	0.261	0.332	0.068	1.176	0.017	–	0.286	0.622	0.124	0.211	0.227
Methyl Pentanoate	1133	Fruit, song	0.045	–	2.654	0.065	0.075	0.087	0.048	0.412	0.015	0.394	0.132	0.032	0.201	0.161	0.019	0.103	–
Cis-3-hexenal	1146	Green, apple-like	0.034	0.076	0.085	0.018	–	0.071	–	0.161	0.018	0.074	0.043	0.032	0.162	0.034	0	0.086	–
2-Pentenoic acid methyl	1159	Pungent, sour	3.992	1.724	4.785	3.636	1.304	3.922	0.567	14.823	–	6.048	2.192	1.706	1.784	–	0.252	1.737	4.253
Heptanal	1172	Oily, fatty, woody	0.136	0.066	0.073	0.05	0.155	0.066	0.016	0.016	0.015	0.075	0.039	0.04	0.122	–	–	0.081	–
1, 8-Cineole	1183		–	0.166	0.731	–	–	0.248	0.834	1.138	–	0.269	0.034	–	0.327	0.12	–	0.576	0.212
2-Methyl butanol	1211	Winey, spicy	0.095	0.079	–	–	–	0.081	–	2.079	–	0.337	1.313	–	–	–	0.163	–	0
Tans-2-hexenal	1216	Green, apple-like	12.466	2.265	33.806	6.759	15.67	15.106	8.475	35.657	20.726	11.683	46.403	8.533	18.524	32.479	15.683	35.522	13.447
2-Hexanol	1219	Fruit, banana, soft	0.082	0.083	0.062	0.081	–	0.226	0.019	0.026	–	0.39	0.045	0.231	0.159	–	0.069	0.023	–
1-Pentanol	1233	Fruity	1.651	–	–	16.868	3.021	14.613	2.548	4.922	2.843	3.479	1.558	5.152	21.535	–	14.274	4.391	5.258
Cis-β-ocimene	1236	Warm. mouldy	–	3.776	4.054	0.248	–	0.025	–	0	–	–	–	0.014	–	6.401	–	–	–
3-Octanone	1237	Herb, butter	0.35	0.278	–	0.189	0.609	1.599	0.053	0.278	–	0.317	1.819	0.669	0.034	–	0.366	0.033	–
Hexyl acetate	1274	Green, fruity	0.254	0.097	3.44	–	0.529	2.679	6.057	0.664	0.038	2.009	0.056	0.035	0.18	1.703	–	1.564	–
Octanal	1264	Fatty, sharp	–	0.056	0.71	0.044	0.174	0.072	0.023	0.019	–	–	0.153	0.014	0.201	0.281	0.019	–	–
3-Heptanol	1270		0.091	0.024	0.057	0.027	0.051	0.08	0.035	0.046	–	–	0.096	0.1	0.451	0.1	0.038	–	–
Heptanol	1289		0.188	0.1	0.036	0.112	0.059	0.216	0.092	0.049	–	0.184	1.035	0.251	0.203	0.218	0.105	0.056	0.199
Cis-3-hexyl acetate	1295	Green	–	–	–	–	–	0.111	1.397	0.4	0.136	0.072	0.107	0.372	–	–	0.064	1.158	0.395
2-Heptenal	1298	Oxidised, tallowy, pungent	0.631	0.178	2.209	0.292	0.804	4.542	3.691	2.679	–	3.844	1.309	–	1.3	1.169	0.16	–	–
Trans-2-pentenol	1304	Perfumery, woody	1.263	1.397	6.483	2.467	0.522	1.283	0.315	2.508	0.442	4.959	1.901	1.649	2.417	1.249	0.727	1.231	1.031
Cis-2-pentenol	1317		0.047	–	0.268	–	0.057	0.097	0.016	–	–	0.168	0.141	0.153	0.34	0.335	–	0.018	–
6-Methyl-5-hepten-2-one	1328	Pungent, green	–	0.04	–	0.062	–	0.013	–	–	–	–	–	0.021	0.012	–	0.068	–	–

(*continued*)

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Table 2. Continued.

			Parental varieties								New hybrids								
			Mediterranean					Tunisian											
Volatile compounds	LRI[a]	Aroma attributes[b]	*Arbequina*	*Coratina*	*Koroneiki*	*Picholine*	*Souri*	*Kotti*	*Chemcheli*	*Chemlali*	CCH13	CK725	CCH225	CC325	CA21	CA32	CC45	CCH73	CCH235
1-Hexanol	1339	Fruit, banana, soft	18.447	36.442	4.542	18.999	4.886	5.695	13.393	7.18	1.715	7.897	10.032	20.188	17.89	18.682	9.102	11.885	8.117
2-Methyl-2-cyclo-penten1-one	1342		–	–	0.91	–	–	0.016	–	0.988	–	–	0.553	–	0.123	–	0.089	–	–
Tans-3-hexen-1-ol	1350	Green	0.085	0.243	–	0.138	–	0.024	0.014	–	–	0.116	0.047	0.16	0.12	0.212	0.366	0.036	–
Cis-3-hexen-1-ol	1367	Green	1.65	0.805	2.294	2.441	1.005	1.784	1.552	3.151	0.114	1.575	3.579	1.43	0.895	2.584	0.969	0.78	–
Nonanal	1374	Fatty, waxy, pungent	1.02	0.771	–	0.097	0.184	0.251	0.488	0.297	0.188	2.004	0.578	0.172	2.49	0.613	0.889	0.529	–
2, 4-Hexadienal	1382	[c]Fresh, green, floral	0.131	0.188	0.213	0.124	0.052	0.125	0.017	0.156	–	0.325	0.093	0.418	0.216	0.11	0.064	0.536	0.709
Tans-2-hexenol	1394	Green grass, leaves	17.604	31.926	1.445	32.575	4.253	11.922	2.859	5.548	–	7.723	9.106	31.311	2.982	2.826	25.593	2.383	8.624
2-Octanol	1409	Earthy, fatty	0.084	0.081	–	–	0.062	0.031	0.067	0.271	–	0.047	0.035	0.031	0.121	–	0.039	–	–
Trans-2-octenal	1424	[c]Herbaceous, spicy	1.845	2.692	0.849	0.319	2.47	3.224	1.201	3.218	4.46	2.669	0.147	0.611	1.465	2.73	3.638	2.386	7.614
δ-Cadinene	1450		0.06	0.136	0.397	0.076	0.176	0.057	0.019	–	0.04	0.409	–	0.03	0.091	–	–	0.011	–
α-Copaene	1464		0.019	0.29	–	0.268	–	–	–	0.009	–	–	0.016	0.067	0.011	0.081	–	–	–
p-Cymene	1467		–	0.241	4.034	–	–	0.006	–	–	–	0.11	–	–	0.008	–	1.62	–	–
2-Ethyl-1-hexanol	1471		0.426	0.703	0.547	0.237	0.37	1.015	0.764	–	1.421	1.446	0.303	0.491	0.957	0.339	0.041	0.744	1.04
Propanoic acid	1514	Pungent, sour	0.092	0.189	0.923	0.091	0.249	0.077	0.287	2.15	0.131	0.304	0.259	0.111	1.504	1.652	0.231	0.287	0.269
Methyl decanoate	1567	[d]Fresh	0.113	0.091	–	0.017	–	0.059	0.019	–	–	–	0.019	0.022	0.042	–	0.018	–	0.194
Hexyl hexanoate	1579		–	0.028	–	–	–	–	–	–	–	0.41	–	–	–	–	–	–	–
Cis-2-decenal	1587		0.07	0.055	–	–	–	–	0.048	–	0.016	0.274	0.064	–	0.064	0.039	–	0.027	0.196
Tans-2-decenal	1609	Painty, fishy, fatty	0.117	0.033	–	–	0.681	–	–	1.626	0.011	0.059	0.038	0.023	0.156	–	0.062	0.347	1.808
2-Octenal-2-butyl	1633		0.055	0.101	0.043	0.018	–	0.078	0.118	0.027	–	0.701	0.6	0.046	0.283	0.13	0.068	0.038	0.215
2-Dodocen1-al	1712		0.02	0.025	–	–	–	–	0.014	–	–	0.554	0.045	–	0.035	–	0.032	0.02	–
Trans-cis-2.4-decadienal	1730	Strong, fatty	0.024	0.065	0.051	–	0.121	–	0.032	0.14	–	–	0.066	–	–	–	0.073	–	–
Hexanoic acid	1862	Rancid, pungent	0.029	0.163	–	–	0.05	0.008	0.028	0.075	0.12	0.25	0.017	0.028	0.031	–	0.032	–	–
Methyl heptanoic acid	1933		0.011	–	–	–	–	–	–	0.061	–	0.264	–	–	0.068	–	0.069	–	–
Nonanoic acid	2144		–	–	–	0.191	–	0.007	–	–	–	0.486	–	0.012	–	–	0.027	–	–
Hexadecanoic acid	2428		0.101	0.24	–	0.067	–	0.073	0.02	–	4.447	0.296	–	0.031	0.01	–	–	–	–

Notes: [a]Linear retention indices as determined on HP-INNOWax column using the homologous series of *n*-alkanes. [b]Volatile compounds attributes reported by Morales et al., 2005. [c]Volatile compounds attributes reported by Luna, Morales, and Aparicio, 2006. [d]Volatile compounds attributes reported by Kanavouras et al., 2005.

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3. Results and discussion

The composition of volatile components in virgin olive oils is one of the most important aspects in defining EVOO sensory quality. Table 2 shows some basic information on the aroma compounds, identifying their name, their LRI and also the sensory attribute from the headspace extract of the selected EVOO. Those compounds are especially divided in four principal chemical groups: ketones, aldehyde, alcohols and esters, and their most important attribute note groups: green, fruity, winey and fatty. The main chemical groups identified are alcohols (4–71% of total volatile fraction), aldehyde (2–46%) and esters (0–7.45%). However, the ketone fraction was very weak and was identified only in a few oils, like CA21, CC325, *Kotti*, CC45, *Coratina* and *Picholine cvs*. The low level of esters observed in all studied oils indicates a lower content or activity of alcohol acetyl transferase in these culivars as reported by Cavalli, Fernandez, Lizzani-Cuvelier and Loiseau (2004). According to the chemical grouping of the volatile fractions, we can easily differentiate four group types of EVOO (Figure 1). The first is characterised by aldehyde fraction up to 30% composed of the following hybrids: CCH225, CCH73, CA32, and the *Chemlali cv*. The second is formed by CC325, *Picholine* and *Coratina cvs*. that have a particular composition formed by ester $<20\%$ and the alcohols up to 60%. The third group is formed by CA21, CC45 and *Arbequina cv*.; their volatile fraction is composed of alcholols up to 40%. Finally the last group is composed of the hybrid CK725 and the *Chemcheli cv*. that have an ester fraction less than 10% (Figure 1).

Comparing the EVOO of the nine hybrids and the Tunisian and Mediterranean varieties collected at the same stage of ripeness, we observe that there were some differences in the constituents of the volatile fraction (Table 2). The *trans*-2-hexenal is the most represented compound of the volatile fraction and it ranges from 2.265% for the *Coratina* variety to 46.4% for the CCH225 sample. The second most abundant compound, *trans*-2-hexenol, ranges from 2.32% to 32% for the *Coratina* variety. The major constituents of the volatile fraction of *Chemlali* oils were *trans*-2-hexanal (35.66%) and 2-pentoic acid methyl (14.82%). The volatile fraction of CCH225 oil was characterised by the dominance of three compounds: *trans*-2-hexanal (46.4%), *trans*-2-hexan-1-ol (9.11%)

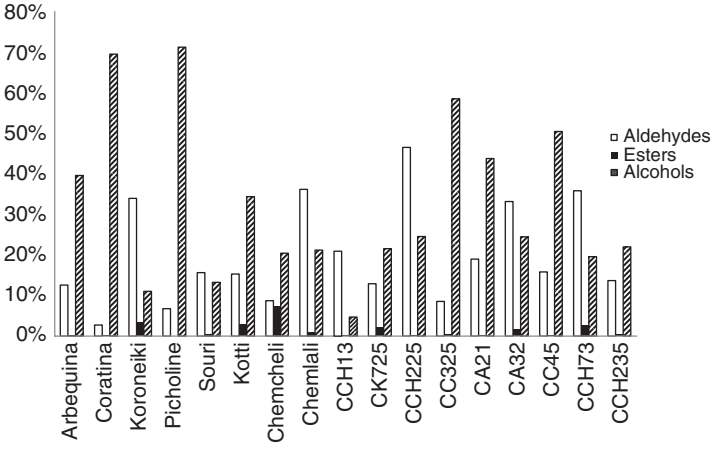

Figure 1. Principal chemical group identified from the volatile fraction by dynamic headspace in EVOO from the nine hybrids and the ancient Tunisian and Mediterranean varieties.

and *cis*-3-hexen-1-ol (3.58) (Table 2). The main constituents that characterise the volatile fraction of CCH235 oil are *trans*-2-hexanal (13.45%) and *trans*-2-hexan-1-ol (8.62%). The CA32 is distinguishable from the others by its higher content of *trans*-2-hexanal (32.48%) and the presence in a relatively high concentration of *cis*-β-ocimene (6.4%). The CCH13 volatile fraction is characterised by the following constituents *trans*-2-hexanal (20.72%) and 2-octenal (4.45%). CK725 is obvious from the others by its higher content of *cis*-2-pentenol (4.95%) and the presence in a relatively high concentration of 2-pentenoic acid methyl (6.04%) and the volatile fraction of the CC325 is composed essentially by the following compounds: *trans*-2-hexenol (31.48%) and 1-hexanol (20%). Finally, *trans*-2-hexenal (35.52%) and 1-hexanol (11.88%) were the two compounds that qualitatively characterised the volatile fraction of CCH73 (Table 2).

The study of the C6-compounds present in all cases is the main fraction of the compounds from the lipoxygenase pathway (Table 3). *Trans*-2-hexenal, the most abundant compound of the whole C6-fraction, proves the high specificity of the enzymatic system of the olive fruit for the production of 6-carbon unsaturated aldehydes from the 13-hydroperoxides of linolenic acid (Angerosa & Basti, 2003). Table 3 also shows the close dependence on the genetic potential of each variety. In effect, the accumulation of each metabolite from the lipoxygenase pathway in the oils obtained from olives of a single cultivar was different as reported by Angerosa et al. (1999). The amounts of each single C5-compound did not seem to be significantly affected by cultivar factor. In fact, we can observe relatively the same fraction in all the oils studied. However, the C5/C5+C6 ratio indicates that there are three distinguished groups: the first characterised by high fraction formed by the CK725 and *Koroneiki cv*., the second formed by the CCH235, CA21, *Picholine*, *Chemlali* and *Kotti cvs*. which have a content ranged between 3.24 and 6.34, and finally the last group formed by the rest that have the ratio less than 3.

To determine the genetic potential on the volatile fraction and due to the high specificity of the lipoxygenase pathway, we have investigated the activity of those enzymes by the study of the volatile compounds formed exclusively by this pathway, and the results were subjected to the cluster analysis using Ward's method. Figure 2 shows four groups of subjects. The first is composed of the CC45, CC325, *Coratina* and *Picholine cvs*. which have a similar hydroperoxide lyase activity but a reduced one in alcohol dehydrogenase activity due to the presence of the *trans*-2-hexanol in relative high proportion (25–32%) and the *trans*-2-hexen-1-ol in weak one (0.13–0.36%). The second group is composed only of the CCH73 and Chemcheli variety, where the aromatic fraction is distinguished from the other variety and hybrids by the presence of high proportion of the *cis*-3-hexyl-acetate and the same level of 1-hexanol. This group is characterised by the pre-eminence of the activity of the alcohol acetyl transferase in the hexanal/hexanol pathway compared to the *trans*-hex-2-enal/*trans*-hex-2-enol pathway. The third group is composed of *Arbequina*, *Kotti cvs*. and its respective hybrids obtained with the *Chemlali cv*. This group is characterised by a lower content of alcohol acetyl transferase by reason of the presence of *trans*-2-hexenal (13–35%) and *trans*-2-hexenol (8.62%). The last group is composed of the *Chemlali*, *Koroneiki cvs*. and the hybrids CK725, CCH225 and CA32. This group is characterised by the presence of a high proportion of the *trans*-2-hexenal (18.52–46%), 1-hexanol (10–18%) and of a weak level of *cis*-3-hexen-1-ol (0.89–3.57%). This special composition implies that the *trans*-hex-2-enal/*trans*-hex-2-enol and the hexanal/hexanol pathway have the same importance in the volatile compound formation.

To determine the genetic influence degree of the ancient cultivars on the hybrids, the PCA treatment has been performed on the volatile compound data obtained from the

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Table 3. Concentrations of C6 and C5 compounds, as %, from lipoxygenase pathway in EVOO from the nine new hybrids, Tunisian cultivars and Mediterranean varieties introduced.

	Parental varieties								New hybrids								
	Mediterranean				Tunisian												
Volatile compounds	*Arbequina*	*Coratina*	*Koroneiki*	*Picholine*	*Souri*	*Kotti*	*Chemcheli*	*Chemlali*	CCH13	CK725	CCH225	CC325	CA21	CA32	CC45	CCH73	CCH235
Hexanal	0.07	0.43	0.00	0.00	0.00	0.16	0.26	0.33	0.07	1.18	0.02	0.00	0.29	0.62	0.12	0.21	0.23
1-hexanol	18.45	36.44	4.54	19.00	4.89	5.70	13.39	7.18	1.71	7.90	10.03	20.19	17.89	18.68	9.10	11.88	8.12
Hexyl acetate	0.25	0.10	3.44	0.00	0.53	2.68	6.06	0.66	0.04	2.01	0.06	0.04	0.18	1.70	0.00	1.56	0.00
Compounds from linoleic	18.77	36.97	7.98	19.00	5.41	8.54	19.71	8.18	1.82	11.08	10.11	20.22	18.36	21.01	9.23	13.66	8.34
Cis-3-hexyl acetate	0.00	0.00	0.00	0.00	0.00	0.11	1.40	0.40	0.14	0.07	0.11	0.37	0.00	0.00	0.06	1.16	0.40
Trans-2-hexenal	12.47	2.27	33.81	6.76	15.67	15.11	8.47	35.66	20.73	11.68	46.40	8.53	18.52	32.48	15.68	35.52	13.45
Cis-3-hexen-1-ol	1.65	0.81	2.29	2.44	1.01	1.78	1.55	3.15	0.11	1.57	3.58	1.43	0.89	2.58	0.97	0.78	0.00
Trans-2-hexenol	17.60	31.93	1.45	32.57	4.25	11.92	2.86	5.55	0.00	7.72	9.11	31.31	2.98	2.83	25.59	2.38	8.62
Compounds from linolenic	31.72	35.00	37.54	41.77	20.93	28.92	14.28	44.76	20.98	21.05	59.20	41.64	22.40	37.89	42.31	39.84	22.47
Trans-2-pentenol	1.26	1.40	6.48	2.47	0.52	1.28	0.31	2.51	0.44	4.96	1.90	1.65	2.42	1.25	0.73	1.23	1.03
Cis-2-pentenol	0.05	0.00	0.27	0.00	0.06	0.10	0.02	0.00	0.00	0.17	0.14	0.15	0.34	0.33	0.00	0.02	0.00
C5 compounds from linolenic	1.31	1.40	6.75	2.47	0.58	1.38	0.33	2.51	0.44	5.13	2.04	1.80	2.76	1.58	0.73	1.25	1.03
Σ C6 compounds	50.49	71.97	45.53	60.77	26.34	37.46	33.99	52.93	22.80	32.14	69.30	61.87	40.76	58.90	51.54	53.50	30.81
C5+C6 compounds	51.80	73.37	52.28	63.24	26.92	38.84	34.32	55.44	23.24	37.26	71.34	63.67	43.51	60.48	52.26	54.75	31.84
C5/C5+C6	2.53	1.90	12.91	3.90	2.15	3.55	0.96	4.52	1.90	13.76	2.86	2.83	6.34	2.62	1.39	2.28	3.24

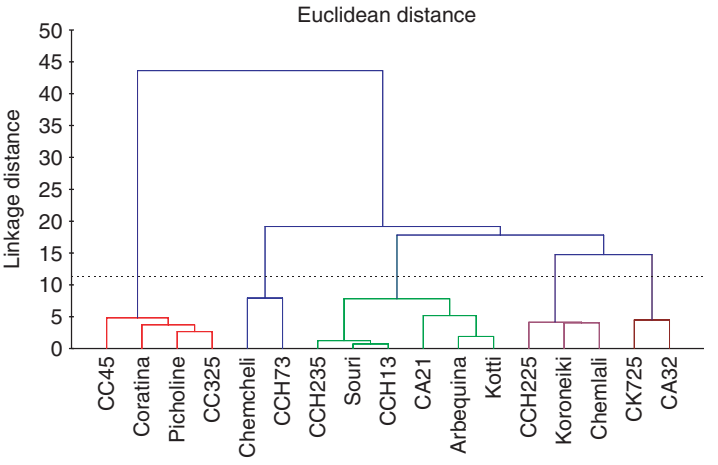

Figure 2. Dendrogram, using Ward method with rescaled distance cluster combine, showing clustering of volatile compounds produced by the lipoxygenase pathway dataset from EVOO extracted from the new hybrids, Tunisian cultivars and Mediterranean introduced varieties.

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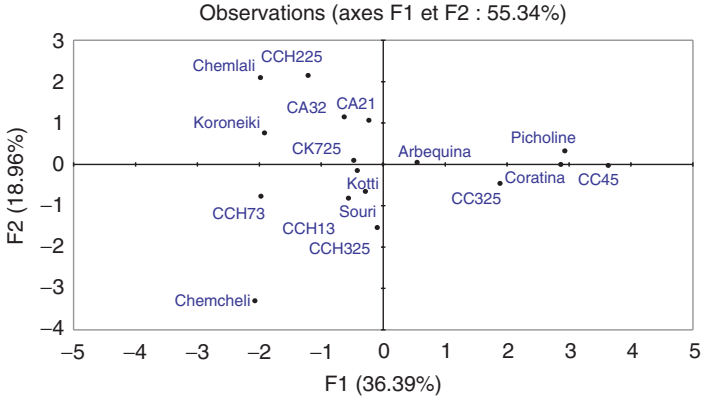

Figure 3. Score plot by dimensions 1 and 2 from PCA, based on the volatile data set obtained from lipoxygenase pathway, of the EVOO extracted from new hybrids, Tunisian cultivars and Mediterranean introduced varieties.

lipoxygenase pathway. All analysed oils were compared with each other in relation to all used odour attributes: green and fruity (major odour attributes resulted from the lipoxygenase pathway). The PCA analysis shows the similarity of the aromatic attribute of the volatile fraction between the hybrids and these ancient cultivars (Figure 3). In fact, we can see the evident similarity of this volatile fraction of the hybrids (CA32; CA21; CCH225) and their ancient cultivars (*Arbequina* and *Chemlali*). For the hybrids CCH73, CCH13 and CCH235 obtained through the crossing of the *Chemlali* x *Chemchali cvs*. their volatile fraction is characterised by attributes most nearly to the *Chemlali* attribute to the *Chemchali*, but similar to the *Kotti* and *Souri* flavour. For the hybrids obtained from the *Chemlali* x *Coratina* crossing (CC325 and CC45), their volatile fraction seems to be similar to the *Coratina* attributes and almost odourless, characterised only by a weak sweet.

These results can be justified by the influences of the genetic potential of the variety on the volatile compound formation. In fact, those compounds depend on the levels and activity of the enzymes involved in the various pathways (Angerosa et al., 1999) which are genetically determined (Campeol, Flamini, Chericoni, Catalano, & Cremonini, 2001). That is why the genetic potential of the *Coratina* variety seems to be dominant over the *Chemlali* one and affect those of the new hybrids obtained by the crossing of the *Chemlali* cv.

4. Conclusion

Analysis of the nine new hybrids and Mediterranean EVOO by dynamic headspace enabled us to identify 48 compounds, representing 38.7–91.42% of the chemical composition. The differences between hybrids and these ancient cultivars were mainly quantitative, because most compounds were present in all the olive oils analysed. As the pedoclimatic conditions, harvesting period and extraction conditions were similar for all samples studied, the results indicate that only genetic factors have an influence on the volatile fraction of the virgin olive oils. All the described results have shown that there is a wide variability in the chemical and sensory characteristics of the virgin olive oils because of the diversity of the genetic factor. Nevertheless, there is a certain tendency towards the clustering of varieties native from the same crossing but this is not enough to qualify all the oils from a crossing with similar sensory and chemical characteristics.

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