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Characterization of Volatiles in Virgin Olive Oil Produced in the Tunisian Area of Tataouine

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The Tataouine province in southern Tunisia is well known for its severe pedoclimatic conditions. Using solid phase microextraction (SPME) and gas chromatography (GC), coupled to flame ionization and mass spectrometer detectors, we characterized virgin olive oils from Chemlali Tataouine, Fakhari Douirat, Zarrazi Douirat, and Dhokar Douirat varieties, which grow in the harsh arid region of Tataouine. Significant differences in the proportions of volatile constituents from oils of different varieties were detected. The results showed that lipoxygenase products were generally the major metabolites of the volatile fraction, and (*E*)-Hex-2-enal was the principal compound characterizing the olive oil headspace for most samples, though the absolute levels varied greatly, never exceeding 76.45 and 32.16%, respectively. The C_5 compounds were unusually abundant, comprising 42.97% of the total lipoxygenase products and a remarkably high level of penten dimers. Each autochthonous variety could thus be differentiated according to the percentage of each metabolite.

KEYWORDS: Virgin olive oil; extreme environment; headspace-solid-phase microextraction; volatile compounds; lipoxygenase pathway

INTRODUCTION

Tunisia is a Mediterranean country characterized by a wide range of edapho-climatic conditions, from a lower semiarid to arid stage. The governorate (province) of Tataouine is located in the southernmost part of Tunisia, bordered by Algeria and Libya. This special area is characterized by extreme pedoclimatic conditions. In fact, the arid climate that characterizes Tataouine distinguishes itself by the rarity and the irregularity of precipitation (between 88 and 157 mm/year), the aggressiveness of showers, violent winds, and a dramatic thermal difference between the cold winter (min 8 °C), and the long, hot, and dry summer (max 45 °C). The Sahara covers more than 70% of Tataouine, which is surrounded by mountains and characterized by calcareous soil and strong erosion. All these parameters distinguish this area from other Tunisian areas with a more temperate climate due to their proximity to the Mediterranean sea. Despite these severe conditions, olive trees in the region of Tataouine are resistant and adaptive, helping to maintain the primary source of oil for local people and the principle agricultural commodity. These trees represent the most striking feature of their agricultural landscape.

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The native olive tree varieties in this area are characterized by frost resistance, variable fruit sizes, and high productivity. Traditionally, in the Tataouine area, olives are picked by hand because of the immense dimensions of the trees. The olive trees of Tataouine are typical of that region and are among the biggest olive trees in the world.

Olive cultivars growing in restricted areas and well adapted to severe edapho-climatic conditions should be characterized and protected as possible parental trees in future breeding programs.

Detailed information about olive oil quality can be obtained by analyzing the complex mixture of its volatile compounds. Some compounds commonly associated with this volatile fraction are the six carbon aldehydes (hexanal, (*Z*)-hex-3-enal, and (*E*)-hex-2-enal), alcohols (hexanol, (*Z*)-Hex-3-enol, and (*Z*)-Hex-2-enol), and their acetyl esters (hexyl acetate and (*Z*)-Hex-3-enyl acetate); (*E*)-Hex-2-enal is the most prominent component. It is well established that the C_6 compounds, which are also constituents of the volatile fraction of many fruits, vegetables, and their products, are formed from polyunsaturated fatty acids through a cascade of biochemical reactions collectively known as the lipoxygenase (LOX) pathway (*1*). In fact, they are derived from the heterolytic breakdown of the 13-hydroperoxide of linoleic (LA) and linolenic (LnA) acid, respectively, and catalyzed by enzymes involved in the reaction

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of hydroperoxide lyase in accordance with published data (*2, 3*). The pathway having LnA as a precursor leads to some metabolites through two possible branches described by Angerosa et al. (*3*), branch (A) (including (*E*)-hex-2-enal and (*E*)-Hex-2-enol) and branch (B) (including (*Z*)-Hex-3-enol and (*Z*)-Hex-3-enyl acetate). This biochemical pathway, which in plants is stimulated by tissue damage (such as crushing), involves a series of enzymes that oxidize (lipoxygenase) and cleave (hydroperoxide lyase) polyunsaturated fatty acids to yield aldehydes, which are subsequently reduced to alcohols (alcohol dehydrogenase) and which can, in turn, be esterified (alcohol acyltransferase) to produce esters (*2*).

Various studies have been carried out on virgin olive oil (VOO) volatile compounds, and most of them are focused on the influence of geographic region (*4*), fruit maturity (*2*), extraction conditions (*5*), and product aging (*6*) on their profiles, but, to our knowledge, no references can be found regarding VOO volatile fraction produced by olive varieties grown in extremely harsh pedoclimatic conditions. Therefore, the aim of the present work is to separate, identify, and quantify VOO volatile compounds of the main autochthonous varieties grown under the same pedoclimatic conditions of the arid region of Tataouine.

MATERIALS AND METHODS

Standards. All standards are with purity ranging between 95 and 99%. Acetic acid, butan-1-ol, 3-methyl butanol, hexanal, (*Z*)-hex-3-enol, hexanol, hexanoic acid, octanal, hexyl acetate, *n*-decane, heptanoic acid, nonanal, undecane, *n*-dodecane, nonan-1-ol, tridecane, *n*-pentadecane, and *n*-hexadecane were purchased from Fluka (Buchs, Switzerland), whereas *n*-octane, (*E*)-hex-2-enal, (*E*)-hex-3-enyl acetate, and (*E*)-allocimene were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Oil Samples. Four samples of VOOs were analyzed from the following varieties: Chemlali Tataouine, Fakhari Douirat, Zarrazi Douirat, and Dhokar Douirat. All the varieties were grown in the locality of Douirat in the region of Tataouine (southern Tunisia) in an arid zone under the same pedoclimatic conditions.

It should be mentioned that Dhokar Douirat is used as a pollinator for the Chemlali Tataouine variety. Therefore, very few trees of that variety are present in the Tataouine region.

Olive samples of 5 kg each were handpicked at the same stage of maturity (fruit skin is a light violet color) all around the trees at human height. Only fresh, healthy, and undamaged drupes were selected. Ripeness index (RI) was determined according to a method developed by the Agronomic Station of Jaén, Spain, which defines the RI as a function of skin and pulp color (*7*). After harvest, the olive fruit samples were immediately transported to the laboratory mill, where the oils were extracted using an Abencor analyzer. Olives (1.5−2 kg) were crushed with a hammer mill and slowly mixed for 30 min, centrifuged without the addition of warm water, and then transferred into dark glass bottles. All samples were stored in the freezer in darkness in amber glass bottles until analysis.

Measurements of Quality Characteristics. Measurements of acidity (CV% =2.2, $n = 5$), specific extinction coefficients K_{232} (CV% = 1.4, $n = 5$), K_{270} (CV% = 2.3, $n = 5$), and peroxide index (PI) (CV% = 3.6, $n = 5$) were carried out according to the European Official Method of Analysis (*8*).

Oxidative Stability. Oxidative stability was evaluated by the Rancimat apparatus (model 743, Metrohm Co., Basel, Switzerland). Stability was expressed as the oxidation induction time (h) using 3.5 g of oil. The temperature was set at 100 °C, and the air flow rate was 10 L h^{-1}. The oxidative stability is calculated with the following formula: [($h_{\text{induction time}} \times 1000$ g/kg)/(g oil × 24 h/day)]. The repeatability of the determination of Oxidative stability was checked for five replicates and was 3.8%

Total Biophenol Content. The total biophenol content of the oils was determined colorimetrically using the Folin−Ciocalteu reagent. Two-and-a-half grams of olive oil was dissolved in 5 mL of hexane and extracted with 5 mL of a MeOH/H_2O (60:40, v/v) mixture. The mixture was then shaken vigorously with a mechanical shaker (Vortex) and centrifuged at 3500 rpm for 10 min.

An aliquot of the polar fraction (0.4 mL) isolated from VOO samples was transferred into a 10 mL volumetric flask, and subsequently, water (4.8 mL) and Folin−Ciocalteu reagent (0.5 mL) were added. At 3 min after the addition of the reagent, 1 mL of a saturated sodium carbonate solution (35%, w/w) was added to the reaction mixture. The solution was brought to 10 mL with water, and after 1 h, the absorbance at 725 nm was measured against a blank solution. The standard curve was prepared using solutions of caffeic acid in methanol/water from 0 to 1000 mg L^{-1}. Total phenol values were expressed as mg of caffeic acid per kg of oil. Repeatability of standard solutions within the same day was satisfactory ($C = 100$ mg kg^{-1}, CV% = 3.3, $n = 5$).

Triacylglycerol Composition. The analysis of triacylglycerols (TAGs) was performed according to the official chromatographic method of the European Economic Community Regulations (*8*).

Solid Phase Extraction. The optimized procedure was as follows. VOO (0.2 g) was weighed and dissolved in 0.5 mL *n*-hexane. The silica cartridge (Step-Pak cartridge, Waters Corporation, USA) was conditioned with 10 mL of *n*-hexane before the application of oil solution. The TAG fraction was obtained with subsequent elution using mixtures of 15 mL of *n*-hexan/diethylic ether (90:10, v/v), and then, the solvent of the collected fractions were evaporated to dryness.

HPLC Analysis of Triacylglycerol. Extracted TAG (0.05 g) was dissolved in 1 mL of acetone for HPLC analysis, and the injected volume was 10 μL. A Hewlett-Packard HPLC (HP 1050, Agilent Technology) quaternary pump instrument equipped with a refractometer detector was employed using a Lichrosorb RP18 column (250 × 4.6 mm, 5 μL particle size; Teknocroma, Barcelona, Spain). Settings were column oven, 45 °C; elution solvent, acetone/acetonitrile (60:40, v/v) at a flow rate of 1.2 mL/min. The percent of the equivalent carbon number (ECN) TAG groups was calculated on the basis of the total area of all TAG groups from ECN 38 to ECN 50. Repeatability of the TAG on the same day was satisfactory (CV% = 8%, $n = 5$ for ECN 46 TAG representing 22% and CV% = 7.2%, $n = 5$ for ECN 48 TAG representing 64.8%).

Volatile Compound Analyses. The fiber used for the extraction of the volatile components (purchased from Supelco Company, Bellefonte, PA, USA) was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm. Before use, the fiber was conditioned as recommended by the manufacturer (1 h at 270 °C). The olive oil (5 g) was placed in a 20 mL vial closed by a PTFE/silicone septum (Interchim). Before extraction, stabilization of the headspace in the vial was accomplished by equilibrating the sample for 60 min at 25 °C. The extraction was carried out at 25 °C (room temperature) with magnetic stirring (900 rpm/min) (*9*).

To determine the optimal adsorption time of sample headspace volatiles to the fiber, the DVB/CAR/PDMS fiber was exposed for 10, 30, 60, 90, and 120 min. A sampling time of 90 min was chosen to perform the analysis (*9*).

After exposure, injections were performed using an SPME autosampler (CTC Analytics, Switzerland). The fiber was thermally desorbed into a gas chromatograph and left in the injection port (equipped with a 0.75 mm i.d. inlet liner) for 4 min. The injector was set at 250 °C and operated in the splitless mode for 2 min unless otherwise stated. Before sampling, the fiber was reconditioned for 5 min in a washing port at 250 °C, and blank runs were included periodically during the study.

GC analyses were carried out using two Agilent 6890N gas chromatographs, one equipped with a FID and one coupled to an Agilent 5973N system mass-selective detector (quadrupole). Both were equipped with fused silica capillary columns HP-1 (PDMS, 50 m × 0.2 mm i.d., film thickness 0.33 μm for GC-FID and 0.5 μm for GCMS).

The carrier gas was helium for GC-FID and GC-MS; the oven temperature was programmed from 60 °C (5 min) to 250 °C at 2 °C/min and then held isothermally for 20 min. The FID temperature was set at 250 °C, and the temperatures of the ion source and the transfer line were 170 and 280 °C, respectively. The ionization energy was 70

Table 1. Means and Standard Deviations for the Different ECN TAG Groups, The Legal Quality Indices, The Biophenol Contents, and the Oxidative Stability Evaluated in the Studied Olive Oil Samples[a]

	ECN[b] 38	ECN 40	ECN 42	ECN 44	ECN 46	ECN 48	ECN 50
Chemlali Tataouine	0.41 ± 0.03a	ND a	0.43 ± 0.00 a	4.04 ± 0.09 a	20.10 ± 0.13 a	67.63 ± 0.13 c	7.40 ± 0.07 c
Fakhari Douirat	0.91 ± 0. 01 d	ND a	0.36 ± 0.06 a	4.10 ± 0.00 a	21.97 ± 0.01 ab	64.78 ± 0.03 b	7.90 ± 0.03 c
Zarrazi Douirat	0.65 ± 0.01 c	ND a	0.45 ± 0.05 a	4.67 ± 0.06 a	23.59 ± 0.00 b	64.05 ± 0.10 b	6.59 ± 0.08 b
Dhokar Douirat	0.09 ± 0.02 a	0.16 ± 0.06 b	3.17 ± 0.08 b	16.29 ± 0.00 b	37.28 ± 0.05 c	38.76 ± 0.08 a	4.25 ± 0.03 a

	free acid content (as oleic acid, g kg^{-1})	peroxide index (meq O_2 kg^{-1})	UV absorbance			$\Delta K \times 10^3$	total biophenols (as caffeic acid, mg kg^{-1})	induction time (h)	induction time (year)
			K_{270}	K_{232}	K_{232}/K_{270}				
Chemlali Tataouine	0.25 ± 0.03 a	4.00 ± 0.54 a	0.16 ± 0.00 a	2.30 ± 0.03 b	14.37 a	−6.0 ± 0.0 a	675 ± 5 b	91.6 ± 1.3 c	4 c
Fakhari Douirat	0.30 ± 0.05 b	5.00 ± 0.38 a	0.14 ± 0.00 a	1.95 ± 0.02 a	13.92 a	−2.0 ± 0.0 b	680 ± 67 b	72.6 ± 1.4 b	3 b
Zarrazi Douirat	0.45 ± 0.03 bc	15.46 ± 0.55 b	0.14 ± 0.02 a	1.88 ± 0.06 a	13.43 a	7.0 ± 0.1 c	820 ± 9 c	100 ± 3.8 d	4 d
Dhokar Douirat	0.64 ± 0.04 c	16.03 ± 0.69 b	0.12 ± 0.07 b	2.27 ± 0.07 b	18.92 b	−3.0 ± 0.1 b	303 ± 5 a	30.3 ± 1.4 a	1 a

[a] Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.05$). [b] ECN, equivalent carbon number; ECN=CN-2DB, where CN is the number of carbon atoms in all acyls, and DB is the number of double bonds.

eV; electron ionization mass spectra were acquired over the mass range of 35−350 amu.

GC-MS analyses on a polar column were performed on a Hewlett-Packard 5890/5970A system with HP20 M capillary column (50 m × 0.20 × 0.1 μm). Injector and transfer line temperature was 220 °C, and conditions were the same as desbribed above for the apolar column HP-1 (PDMS, 50 m × 0.2 mm i.d., film thickness 0.5 μm).

Retention indices were determined with C_5−C_{26} alkane standards as a reference (retention times determined for SPME experiment: 20 s at 50 °C). Relative amounts of individual compounds are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these values and the standard deviation were determined for each component identified.

The identification of the constituents was based on a comparison of the retention times with those of pure standards, on computer matching against commercial libraries (NIST 1998, Wiley 6N, MassFinder 2.1 Library 2001), and on a laboratory-made spectral library built from pure substances and MS literature data (*10, 11*). This identification was then confirmed by comparison of retention indices with published index data (*12, 13*). Several structures were also confirmed by standard compound injection. All chemicals were purchased from Fluka or Sigma-Aldrich (Saint Quentin Fallavier, France).

Statistical Analysis. The results are shown as the mean values and standard deviation of independent measurements. Significant differences among varieties were determined by an analysis of variance, which applied a Duncan's test. All collected data were used for multivariate analysis (principal component analysis) to obtain more information on the variables that mainly characterize each variety olive oil samples. The statistical analysis was performed using the SPSS 16.0 for Windows (SPSS Inc., 2007).

RESULTS AND DISCUSSION

Legal Quality Indices. Table 1 shows a summary of the average data and standard deviations from the chemical analysis of olive oil samples. All the olive oils complied with the category of VOO according to regulation (*14*). These results are not surprising because the raw material was carefully selected, picked, and processed. The obtained data show that varieties had significant influence on these analytical parameters.

Triacylglycerol, Oxidative Stability, and Biophenol Contents. The TAG content is a prime characteristic of olive oils and is well suited to distinguish VOO from other vegetable oils (*15*). HPLC analysis of TAG permitted the identification and quantification of four TAG groups, ECN 44, 46, 48, and 50 (**Table 1**), which are present at the highest levels in the studied VOOs. Together, these groups accounted for more than 90% of total TAGs in almost all the analyzed VOO samples. Small amounts (≤2%) of some other groups, such as ECN 38, 40, and 42 (**Table 1**), were also observed in all samples.

The TAG group compositions varied widely among VOO samples. The ECN 48 TAG group was the major fraction of TAG in Chemlali Tataouine, Fakhari Douirat, and Zarrazi Douirat with a mean value of (65%). Its level was about two times higher than that in Dhokar Douirat (38.76%). TAG of ECN 42, 46, and 44 were observed to be highest in Dhokar Douirat (3.17, 37.28, and 16.29%, respectively). Only negligible amounts of ECN TAG groups such as ECN 38 were present in all analyzed samples (<1%). Except in Dhokar Douirat VOO, ECN 40 was not detected in any VOO samples. Chemlali Tataouine, Fakhari Douirat, and Zarrazi Douirat showed small differences in the proportion of ECN 44 and ECN 50 TAG group proportions, with mean values of 4.27 and 7.30%, respectively. These results show that TAG composition is a useful parameter for discriminating between olive oil varieties ($p < 0.05$).

These results, which are in close agreement with the literature (*16*), show that TAG synthesis is not affected by the water regime of the Tataouine region. The quantified TAG groups in Chemlali Tataouine, Fakhari Douirat, and Zarrazi Douirat VOOs are similar to those found in the most Tunisian VOOs from varieties grown in different pedoclimatic conditions (*4, 17*) and to the many common oils from Spain, Italy, Greece, France, and Portugal (*18, 19*). The influence of rainfall on the synthesis of TAG by Italian cv. Frantoio grown in different geographical regions of Italy was studied by Angerosa et al. (*20*). By applying statistical procedures, the authors found that the amounts of some TAGs varied depending on the autumn temperatures, the humidity of the summer months, and the rainfall of the whole year.

According to the results of The TAG group analyzed, we suggest a high oxidative stability. The influence of the glyceridic matrix on the oxidative stability of olive oils was verified by many studies (*21*). The oxidative stability (**Table 1**) showed a great variability from one variety to another (30.3−100 h). Except Dhokar Douirat, all remaining varieties showed remarkably high stability values compared to the European ones (>70 h) (*22*). Zarrazi Douirat and Chemlali Tataouine VOOs were the most stable Tunisian varieties with an oxidative stability of 100 and 91.6 h, respectively; they are similar to the Spanish VOOs "Picual" and "Cornicabra" (*22*).

This high stability may be due not only to the particular climate of Tataouine as demonstrated by Tovar et al. (*23*), who

Table 2. Volatile Carbonyl Compounds Identified in the Analyzed Virgin Olive Oil Headspaces

code	volatile	RI HP-1	RI HP20M	ID	ref.	code	volatile	RI HP-1	RI HP20M	ID	ref.
1	ethanol	577	871	b,c	(*28, 38, 39*)	39	(*E*)-hept-2-enal	926	n.d.	b,c	(*38, 39, 40*)
2	propan-2-one	580	n.d.	b,c	(*28, 38, 39*)	40	3-ethylocta,1−5-diene [d,e]	932	1047	b	(*28, 38, 39*)
3	acetic acid	592	1400	a,b,c	(*28, 38, 39*)	41	3-ethylocta,1−5-diene [d,e]	939	1054	b	(*28, 38, 39*)
4	2-methylpentane [d,e]	593	n.d.	c	(*38*)	42	hexanoic acid	957	1798	a,b,c	(*38, 39*)
5	3-methylpentane [d,e]	596	n.d.	c	(*38*)	43	octan-3-one	962	n.d.	b,c	
6	*n*-hexane	600	595	b,c	(*38*)	44	(*E,E*)-hepta-2,4-dienal[e]	963	n.d.	c	(*38*)
7	butan-1-ol	623	n.d.	a,c		45	octane-2-one	965	n.d.	b,c	(*38, 40*)
8	3-methyl butanal[d]	628	n.d.	c	(*39, 40*)	46	octanal	973	n.d.	a,b,c	(*38, 39, 40*)
9	2-methyl butanal[d]	638	863	c	(*38, 39, 40*)	47	(*E*)-hex-3-enyl acetate	984	1285	a,b,c	(*28, 38, 40*)
10	pentanal	645	n.d.	b,c	(*38, 39, 40*)	48	deca-3,7-diene [d,e]	986	n.d.	c	(*28, 38, 39*)
11	pentan-2-one	658	n.d.	b,c	(*28, 39*)	49	deca-3,7-diene [d,e]	989	n.d.	c	(*28, 38, 39*)
12	pent-1-en-3-one	655	n.d.	b,c	(*39*)	50	hexyl acetate	992	1241	a,b,c	(*28, 38, 39*)
13	pentan-3-one[d]	669	n.d.	c	(*28, 38, 39, 40*)	51	*n*-decane	1000	n.d.	a,c	(*39*)
14	butan-2-one	675	n.d.	b,c	(*41*)	52	limonene	1012	1165	b,c	(*28, 38, 39*)
15	heptane	700	n.d.	b,c	(*28, 38*)	53	unknown	1019	n.d.		
16	3-methyl butanol	714	720	a,b,c	(*28, 38, 39, 40*)	54	(*E*)-oct-2-enal	1024	1247	b,c	(*38*)
17	(*E*)-pent-2-enal	723	n.d.	b,c	(*28, 38, 39, 40*)	55	β-ocimene	1029	n.d.	b,c	(*28, 38, 39*)
18	(*Z*)-pent-2-enol	745	1279	b,c	(*28, 38, 39*)	56	(*E*)-β-ocimen	1036	1223	b,c	
19	toluene	750	976	b,c	(*28, 38, 39*)	57	γ-terpinene[d]	1040	1214	c	(*28*)
20	(*Z*)-hex-3-enal	769	1105	b,c	(*28, 38, 39*)	58	heptanoic acid	1049	n.d.	a,b,c	(*38*)
21	hexanal	772	1035	a,b,c	(*28, 38, 39*)	59	nonanal	1080	1358	a,b,c	(*38, 39, 40*)
22	oct-1-ene	786	n.d.	b,c	(*39*)	60	undecane	1100	n.d.	a,c	(*39*)
23	*n*-octane	800	769	a,b,c	(*28, 38, 39, 42*)	61	4,8-dimethyl nonatriene	1101	n.d.	b,c	(*28, 38, 39*)
24	oct-2-ene	810	n.d.	b,c	(*38, 39*)	62	allocimene[d]	1110	n.d.	c	
25	(*Z*)-hex-2-enal	817	n.d.	b,c	(*40*)	63	(*E*)-allocimene	1128	n.d.	a,c	
26	(*E*)-hex-2-enal	824	1180	a,b,c	(*28, 38, 39*)	64	benzoic acid	1167	n.d.	b,c	
27	(*Z*)-hex-3-enol	834	1343	a,b,c	(*28, 38, 39*)	65	*n*-dodecane	1200	n.d.	a,c	(*38, 40*)
28	hexanol	848	1316	a,b,c	(*28, 38, 39*)	66	nonan-1-ol	1217	n.d.	a,b,c	(*38*)
29	*p*-xylene	853	1104	b,c	(*28, 38, 39*)	67	(*E*)-dec-2-enal	1234	1600	b,c	
30	3-methyl butyl acetate	855	n.d.	b,c		68	nonanoic acid	1255	n.d.	b,c	(*39*)
31	heptane-2-one	864	n.d.	b,c	(*38, 39*)	69	tridecane	1300	n.d.	a,c	(*38, 39, 42*)
32	heptanal	871	n.d.	b,c	(*38, 39, 40*)	70	α-copaene	1375	1462	b,c	
33	(*E,E*)-hexadiene-2,4-al	876	1353	b,c	(*38*)	71	tetradecane	1385	n.d.	c	(*39, 42*)
34	3,4-diethylhexa-1,5-diene [d,e]	893	n.d.	c	(*28, 38, 39*)	72	α-muurolene[d]	1496	1691	c	(*28, 38, 39*)
35	3,4-diethylhexa-1,5-diene [d,e]	897	n.d.	c	(*28, 38, 39*)	73	α- farnesene	1499	1726	b,c	
36	nonane	900	n.d.	a,c	(*39*)	74	*n*-pentadecane	1500	n.d.	a,c	
37	methyl hexanoate	905	n.d.	b,c		75	γ-bisabolene[d]	1582	1898	c	
38	unknown	909	n.d.			76	*n*-hexadecane	1600	n.d.	a,c	

ID, identification method; n.d., not determined; RI, retention indices; HP-1, apolar capillary column; HP20M, polar capillary column. [a]Identified by comparison with standard compounds. [b]Identified by comparison of mass spectra and order of elution according to Cavalli et al.(*9*) and Angerosa et al.(*5*). [c]Identified by commercial libraries search (NIST 1998, Wiley 6N, MassFinder 2.1 Library 2001). [d]Tentatively identified. [e]Correct isomer not characterized.

showed that the oxidative stability is affected significantly by the water regime, but also to the nature of the fatty acids present in the olives (*21*).

Additionally, the level of polyphenols, which are among the minor constituents, correlates with stability (*21*).

The total biophenols described in **Table 1** showed a wide variability (303−820 mg caffeic acid kg^{-1}) depending on the variety. Except Dhokar Douirat VOO, all remaining variety VOOs showed a remarkably high biophenol content (reaching 820 mg caffeic acid kg^{-1}). This high amount is possibly related not only to genetic factors but also to both the low rainfall accumulation in Tataouine (*23*) and to the soil type (*24*).

A correlation between biophenolic compounds and both VOO stability and shelf life was also verified in our study in all cases. Zarrazi Douirat VOO, which showed the highest biophenol content, showed the highest stability and the longest shelf life (four years) (**Table 1**), whereas Dhokar Douirat VOO, which showed the lowest biophenol content, showed the lowest stability and as a consequence the shortest shelf life (one year). This finding is in agreement with the work of Gonzalez-Quijano et al. (*25*).

Headspace Volatile Composition. The analysis of volatile fractions from the four Tunisian olive oils showed a complex mixture of more than 75 compounds (**Table 2**). Furthermore, the headspace of all oil samples was composed primarily of aldehydes (21.50−47.35% of the total peak area percentage), alcohols (5.26−11.7%), esters (0.20−7.14%), ketones (1.08−9.03%), and carboxylic acids (0.65−6.18%) (**Table 3**).

All analyzed VOO headspaces contain volatile compounds derived from the biochemical pathway of LOX (**Table 3**). LOX products showed a wide variability from one type to another. Although Chemlali Tataouine, Zarrazi Douirat, and Dhokar Douirat produced low levels of LOX pathway compounds (<50% of the total peak area percentage), oils from Fakhari Douirat varieties showed a high level of LOX products (>70% of the total peak area percentage). These levels were similar to those found in almost all Tunisian (*26, 27*) and European (*2, 28*) VOOs. The difference in LOX product concentrations is due to the influence of genetic factors as described by Angerosa et al. (*2*).

The LOX pathway allows the catalytic oxidation of 1−4 pentadienic structures to give C_6 derivatives (*1, 29*), which are the major volatile components identified and quantified in all the analyzed samples (**Table 3**). They varied from 25.27 to 49.31% of the total peak area for Zarrazi Douirat and Fakhari Douirat VOOs, respectively. This variability is mostly related to genetic factors, as no external variables could have unequally affected the enzyme activity of a particular variety (e.g., latitude, weather, ripeness, and extraction conditions).

In all oil samples, the aldehydic class represents the major

Table 3. Total Quantified Compounds and Chemical Families Present in the Analyzed Headspaces of the Analyzed VOOs (Results Expressed in % of Total Aroma)[a]

	total compounds	total LOX products (%)	total C6 compounds (%)	total monoterpene hydrocarbons (%)	total sesquiterpene hydrocarbons (%)	total alcohols (%)	total aldehydes (%)	total esters (%)	total acids (%)	total ketones (%)
Chemlali Tataouine	54c	30.98a	25.27b	3.29d	0.93b	5.26a	25.08b	7.14d	2.00b	7.25c
Fakhari Douirat	53c	76.45d	49.31d	0.84a	1.28c	5.87c	47.35d	3.49c	2.69b	9.03d
Zarrazi Douirat	46a	40.01b	22.68a	1.39b	0.27a	5.49b	21.50a	2.41b	6.18c	3.58b
Dhokar Douirat	51b	44.89c	42.32c	2.21c	2.20d	11.74d	45.08c	0.20a	0.65a	1.08a

[a] Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.05$).

Table 4. C_6 and C_5 Metabolites from LOX Pathway and Their Percent Distribution in Relation to Their Total Amount, Respectively[a]

LOX pathway components	Fakhari Douirat	Dhokar Douirat	Zarrazi Douirat	Chemlali Tataouine
C_6Compounds				
Branch LA				
%hexanal/Σ C_6	12.47 ± 0.20 a	19.12 ± 0.49 c	16.31 ± 0.53 b	29.72 ± 1.86 d
%hexanol/Σ C_6	0.41 ± 0.05 a	4.02 ± 0.34 b	0.18 ± 0.00 a	0.24 ± 0.02 a
%hexyl acetate/Σ C_6	4.73 ± 0.19 b	0.38 ± 0.02 a	6.75 ± 0.27 c	14.76 ± 0.39 d
% branch LA/ΣC_6	17.60 ± 0.44 a	23,51 ± 0.86 b	23,24 ± 0.80 b	44.72 ± 3.07 c
Branch LnA				
% (*Z*)-hex-3-enal/Σ C_6	32.63 ± 0.58 c	n.d.[b]	33.86 ± 1.32 c	2.49 ± 0.45 b
Branch A				
% (*Z*)-hex-2-enal/Σ C_6	5.31 ± 0.34 c	0.47 ± 0.06 a	3.79 ± 0.16 b	n.d.
% (*E*)-hex-2-enal/Σ C_6	38.88 ± 1.00 b	75.99 ± 2.43 c	29.50 ± 0.89 a	39.93 ± 3.51 b
Branch B				
% (*Z*)-hex-3-enol/Σ C_6	3.22 ± 0.28 b	n.d.	5.73 ± 0.15 c	n.d.
% (*E*)-hexene-3yle acetate/Σ C_6	2.35 ± 0.32 b	0.02 ± 0.00 a	3.88 ± 0.12 c	12.86 ± 0.38 d
branch LnA/Σ C_6	82.40 ± 2.51 c	76.49 ± 2.49 b	76.76 ± 2.64 b	55.28 ± 4.33 a
C_5 Compounds				
% pentanal/Σ C_5	n.d.	4.28 ± 1.03 b	n.d.	n.d.
% pentan-2-one/Σ C_5	28.41 ± 1.30 d	22.18 ± 2.17 c	n.d.	12.61 ± 0.53 b
% pent-1-en-3-one/Σ C_5	4.09 ± 0.29 c	n.d.	0.18 ± 0.06 b	n.d.
% pentan-3-one/Σ C_5	n.d.	n.d.	9.25 ± 0.59 b	15.24 ± 0.35 c
% (*E*)-pent-2-enal/Σ C_5	3.30 ± 0.34 b	n.d.	5.27 ± 0.54 c	6.30 ± 0.61 d
% (*Z*)-pent-2-enol/μ C_5	10.21 ± 0.68 b	2.72 ± 0.39 b	14.75 ± 0.63 c	10.16 ± 0.98 a
% penten dimers/Σ C_5	53.91 ± 2.26 b	70.82 ± 2.94 a	70.57 ± 3.76 c	55.69 ± 1.59 c

[a] Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.05$). [b] n.d., not determined.

C_6 fraction (**Table 4**); it varied between 72.14 and 95.58% of the whole C_6 fraction. The percentages of alcohols and esters differed for each variety, suggesting a strict dependence on the enzymatic store (*2*). As shown for some Italian (*2*), Spanish (*28*), French (*28*), New Zealand (*30*), Croatian (*31*), Australian (*32*), and Tunisian volatile VOOs (*26, 27*), the most representative C_6 compounds were the aldehydes (*E*)-hex-2-enal (29.50−75.99% of the whole C_6 fraction) followed by (*Z*)-hex-3-enal (2.49−33.86% of the whole C_6 fraction) and hexanal (12.47−29.72% of the whole C_6 fraction) (**Table 4**). The high level of (*E*)-hex-2-enal in the LOX cascade illustrates the preeminence of the (*E*)-hex-2-enal/(*E*)-hex-2-enol pathway, compared to the hexanal/hexanol pathway, in all the varieties that we tested. These results show that the LOX activity is higher for LnA than for LA. In fact, the compounds generated from the branch of the LnA part of the LOX pathway accounted for 55.28%, 76.49%, 76.76%, and 82.40% of the totality of C_6 compounds, respectively, in Chemlali Tataouine, Dhokar Douirat, Zarrazi Douirat, and Fakhari Douirat varieties (**Table 4**). Additionally, in the LnA branch, the branch A was predominant than branch B in all the varieties considered and was the principal pathway active as demonstrate in some literatures (*2*).

The level of (*E*)-hex-2-enal in the analyzed samples showed a variability from one variety to another suggesting an influence of genetic factors on the biosynthesis of this compound; this result agrees with the results of Angerosa et al. (*2*), who worked on Italian varieties grown under the same pedoclimatic conditions. It interesting to underline that the level of (*E*)-hex-2-enal in the analyzed samples is lower than that found in almost all Tunisian (*26, 27*) and European (*2, 28*) varieties, which have a level of that compound representing more than 50% of the total volatiles. We suggest that, in addition to the genetic factors, the climatic condition has also an effect on the biosynthesis of this compound, as previously demonstrated by Gómez-Rico et al. (*32*), who showed that an increase in irrigation produces an increase in (*E*)-hex-2-enal.

The high levels of hexanol in the headspace of Dhokar Douirat oil (4.02% of the whole C_6 fraction) and (*Z*)-hex-3-enol in the headspaces of both Fakhari Douirat and Zarrazi Douirat VOOs (3.22 and 5.73% of the whole C_6 fraction, respectively) can be attributed to the increased presence or activity of alcohol dehydrogenase (ADH). ADH has previously been reported as the enzyme responsible for the biosynthesis of six-carbon alcohols (hexanol, (*E*)-hex-2-enol, and (*Z*)-hex-3-enol) (*2, 3*).

It is worth noting the absence of the C_6 alcohol (*E*)-hex-2-enol in all the analyzed samples as shown in some Tunisian varieties grown in different regions of Tunisia (*26, 27, 33*). This may be due to the fact that the accumulation of (*E*)-hex-2-enal over (*E*)-hex-2-enol in all studied varieties pointed out that the isomerization of (*Z*)-hex-3-enal forms to (*E*)-hex-2-enal ones was the dominant process of the branch A (*2*), and the main step of LnA branch, especially in Dhokar Douirat VOO, which

also showed the absence of the C_6 alcohol (*Z*)-hex-3-enol. These results are in good agreement with the conclusion of Angerosa et al. (*2*) on the VOO volatiles of the Italian variety "Leccino".

In all the samples analyzed, there were also products of alcohol acetyl transferase (AAT) activity, which produces hexyl acetate (ringing between 0.38 and 14.76% of the whole C_6 fraction) from hexanol and (*E*)-hex-3-enyl acetate (ringing between 0.02 and 12.86% of the whole C_6 fraction) from the corresponding alcohol (*Z*)-hex-3-enol.

Data obtained from the olive oil of Chemlali Tataouine and Dhokar Douirat were highly interesting because it shows that ADH enzyme in Chemlali Tataouine oil has a low activity in this variety, resulting in a high concentration of (*E*)-hex-2-enal (39.93% of the whole C_6 fraction), whereas, the AAT enzyme in Dhokar Douirat showed a low activity in this variety, as previously stated in the literature (*2*), with a quit level of both hexyl acetate (0.38% of the whole C_6 fraction) and (*E*)-hex-3-enyl acetate (0.02% of the whole C_6 fraction).

C_5 compounds derived from the breakdown of the alkoxy radical are also present (*5*). The detection of C_5 compounds (**Table 4**), which include different classes of aldehydes, alcohols, and ketones, indicate the presence of an additional branch of the LOX pathway.

It is interesting to emphasize the high level of C_5 compounds, which reach 42.97% of the total LOX product in the volatile profile of almost all varieties. This amount was almost 2-fold higher than that found in some Tunisian (*26, 27*) and European (*2, 28*) varieties. This unusually high level of C_5 compounds may be related not only to genetic factors but also to the extreme conditions of the region of Tataouine as proved by the work of Kalua et al. (*34*) who showed that the differences in C_5 volatile contents of oils may be affected by the geographic region.

Among these compounds, we observed that pentanal was only detected in Dhokar Douirat oil (4.28% of the whole C_5 fraction). (*E*)-Pent-2-enal, absent in Dhokar Douirat, was present in all the remaining varieties to varying degrees (3.30−6.30% of the whole C_5 fraction). The alcohol (*Z*)-pent-2-enol is detected in appreciable amounts in all analyzed samples (2.72−14.75% from the whole C_5 fraction). The pentene dimers were also identified in the volatile fraction of the four VOOs at an unusually high level (reaching 70.82% from the whole C_5 fraction), representing a 3-fold increase over some Tunisian (*26, 27*) and European varieties (*2, 28*). Through the comparison of mass spectra and elution order according to Angerosa et al. (*5*), these C_5 compounds can be derived from the enzymatic transformation of fatty acids (*5*).

Recently, several authors have also extensively studied the hydrocarbon fraction of virgin oils and its utility in characterization. Thus, the simultaneous determination of LOX products and representative hydrocarbons might be particularly useful for determining origin (*5, 33*).

Some volatile hydrocarbons (**Table 2**), such as toluene, xylene isomers, octane, and the aldehydes heptanal, octanal, nonanal, and (*E*)-hept-2-enal, were found in low amounts in the headspaces that we examined. The origin of some of these compounds in VOOs is largely unknown or due to the autooxidation reactions, which are inevitable after the VOO has been extracted. Some studies on the volatile hydrocarbons in virgin olive oil have been carried out, suggesting that these compounds might arise from both exogenous contamination and endogenous pathways (*35*). Moreover, it has been reported that virgin olive oil could contain higher levels of volatile contaminants due to the absence of a refining process that would otherwise lead to a decrease in total volatile compounds (*36*). For this reason, the assessment of volatile contaminants in olive oil deserves special attention for some researchers.

In addition, several terpenic hydrocarbons (mono- and sesquiterpenes), which may be used to distinguish samples from different cultivar and geographical areas (*37*), were detected in all the analyzed samples (**Table 3**). Significant differences ($p < 0.05$) were detected in the proportion of terpenic compounds in VOOs obtained from the different studied olive varieties suggesting an influence of genetic factors on the biosynthesis of those compounds. Except for Fakhari Douirat VOO, all remaining varieties of VOOs showed a total amount of monoterpene hydrocarbons (ranging between 1.39 and 3.29% of the total peak area percentage) higher than that of sesquiterpene hydrocarbons (ranging between 0.27 and 2.20% of the total peak area percentage). This terpenic hydrocarbons composition of virgin olive oil may be taken into consideration to be studied as genetic or geographic markers of virgin olive oil origin (*37*).

It is notable how the qualitative and quantitative composition of accumulating products is tightly dependent on the level and activity of enzymes. Enzyme contents are genetically determined, but the production of metabolites changes in relation to the degree of ripening, time of storage, and conditions of operation during the oil extraction process (*5*). Factors such as climate and soil type are also important (*3*). Fruit from different varieties grown under the same environmental conditions produce oils with different volatile compounds, as do fruits from the same cultivar grown in different geographic regions (*2*).

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