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Volatile Compounds Characterizing Tunisian Chemlali and Chétoui Virgin Olive Oils

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A total of 33 virgin olive oil samples of the two main Tunisian cultivars, Chemlali and Chétoui, were characterized by their volatile compounds. The olive oil samples were obtained from olives harvested at four stages of ripeness in costal and inland farms of different geographical places. Major volatiles, mostly C6 and C5 compounds produced from linolenic and linoleic acids through the lipoxygenase cascade, were quantified by solid-phase microextraction–gas chromatography. Mathematical procedures allowed for the determination of the volatiles that not only are able to discriminate the olive oils by their olive cultivar (hexanal, *E*-2-hexenal, and total ketones) and ripeness (pentanal and 1-penten-3-one) but also contribute to their distinctive aroma. Finally, an electronic nose based on metal oxide sensors was checked for a rapid and at-line implementation of Tunisian olive oil varietal traceability. The classification of the samples by the sensors was explained by their sensitivity to volatiles *E*-2-hexanal, hexanal, 1-penten-3-one, ethanol, and *Z*-3-hexenol. Multivariate procedures of discriminant analysis and principal component analysis were used in the study.

KEYWORDS: Olive oil; volatiles; SPME–GC; MOSs; variety; ripeness

INTRODUCTION

The globalization of food markets and the relative ease with which olive oil is transported through and between countries mean that the producers are increasingly interested in new methods of oil authenticity and traceability. A complete farm-to-fork traceability of virgin olive oil involves the chemical characterization of the oils obtained from the main cultivars in each producer zone. Several series of chemical compounds have been used for traceability and variety characterization (*1*), with volatile compounds being one of them (*2*). Thus, the volatile fraction is not only responsible for the virgin olive oil odor attributes (*3, 4*) and the classification of the oils into official categories (*5, 6*), but its profile also characterizes monovarietal virgin olive oils (*7, 8*), as the authors found, analyzing 39 single virgin olive oils from the main producer countries (*9*). The influence of the cultivar on the olive oil volatile profile depends upon the activity of enzymes involved in several pathways, mostly the lipoxygenase cascade (*10*). Recent biochemical studies on other vegetables have allowed for the elucidation of the role of the enzymes in the total contents of C5 and C6 compounds and their influence on olive oil sensory quality (*11, 12*). Furthermore, the volatile compound present in the oil and its concentration not only depend upon the initial level of the enzymes involved in volatile synthesis but also the processes of olive deterioration (*5*) and olive oil natural oxidation (*13*). Thus, the olive oil traceability requires the study of changes of the most remarkable volatile compounds in relation to the cultivar and ripeness.

Several analytical techniques have been used for the quantification of volatile compounds (*14, 15*), with all of them being based on the preconcentration of volatiles prior to the analysis. One of them is gas chromatography with a previous headspace process of preconcentration of volatiles in solid-phase microextraction fibers (SPME–GC). The application of this procedure is easy, solvent-free, and rapid but not to be applied online. A second alternative, which has been widely adopted in many fields of virgin olive oils, is based on the use of metal oxide sensors (MOSs) (*16*). This low-cost approach can be applied online because it does not need sample pretreatment.

In Tunisia, the second virgin olive oil exporter and producer after the European Union, the main cultivar is Chemlali, which is cultivated in central and southern areas of Tunisia, while the second is Chétoui, which is cultivated in northern areas (*17*). These varieties mean more than 85% of Tunisian olive oil production. This paper is focused on the characterization of these varietal olive oils by means of their volatile compounds analyzed by SPME–GC and MOSs. The aim of the work is to contribute to a future traceability of Tunisian virgin olive oils by means of the information supplied by the volatile compounds that are responsible for aroma, a sensory quality very appreciated in Tunisian olive oils and the main reason of its successful international market.

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MATERIALS AND METHODS

Samples. Two main Tunisian varietal virgin olive oils, Chemlali and Chétoui, have been studied. To consider different environmental conditions, 17 samples of olives var. Chemlali were harvested in an inland farm (Sidi Bouzid) and three in costal farms (Enfidha and two around Sfax), while 8 samples of olives var. Chétoui were harvested in two different provinces, Sfax and Benikhalled. The validation set was constituted by 8 samples of commercial olive oils (3 samples of Chétoui and 5 samples of Chemlali), from the same geographical origins.

Olives were harvested, from three olive trees of each selected farm, at four different stages of the maturity index (*18*), according to the color skin (1, green; 2, green–violet; 3, violet; 4, black).

The extraction process was carried out at laboratory scale in an experimental mill. The procedure was as follows. Batches of 7 kg of olives were crushed, and the resulting paste was malaxed for 30 min at 25 °C with water. After centrifugation, the oil was obtained by decantation in graduated cylinders and immediately stored in the dark at 0 °C until its analysis by SPME–GC. No sample was stored longer than 3 months.

Reagents. Hexanal, hexan-1-ol, hexyl acetate, *E*-2-hexenal, *E*-2-hexen-1-ol, *Z*-3-hexen-1-ol, *Z*-3-hexenyl acetate, *E*-3-hexen-1-ol, 1-penten-3-ol, pentan-1-ol, *Z*-2-penten-1-ol, pentan-3-one, pentanal, 1-penten-3-one, ethyl acetate, methyl acetate, 4-methyl-pentan-2-one, nonan-2-one, ethanol, 2-methyl-butan-1-ol, heptane, and 4-methyl-2-pentanol (internal standard) were purchased from Fluka-Sigma-Aldrich (St. Louis, MO). All standards had a gas chromatography (GC) purity of 98% or higher.

Concentration of Volatile Compounds. Olive oil samples (1 g) spiked with 2.6 mg/kg of internal standard were placed in a 20 mL glass vial, tightly capped with polytetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with a solid-phase microextraction (SPME) needle and the fiber was exposed to the headspace for 40 min. When the process was completed, the fiber was inserted into the injector port of the GC. The temperature and time were automatically controlled in a Combipal (CTC Analytics AG, Zwingen, Switzerland) by the software Workstation version 5.5.2 (Varian, Walnut Creek, CA).

The SPME fiber was purchased from Supelco (Bellefonte PA), and it was endowed with the Stable Flex stationary phase (50/30 μm film thickness) of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber was previously conditioned following the instructions of the supplier.

GC System. The volatiles absorbed by the fiber were thermally desorbed in the hot injection port of a GC for 5 min at 260 °C with the purge valve off (splitless mode) and deposited onto a TR-WAX capillary column (60 m × 0.25 mm i.d., 0.25 μm coating; Teknokroma, Barcelona, Spain) of a Varian 3900 gas chromatograph with a flame ionization detector (FID). The carrier gas was hydrogen, at a flow rate of 1.5 mL/min. The oven temperature was held at 40 °C for 10 min and then programmed to rise 3 °C/min to a final temperature of 200 °C, where it was held for 10 min to eliminate the memory effect of the capillary column. The signal was recorded and processed with the WorkStation (version 5.5.2) software. Each sample was analyzed in duplicate.

The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards (see the Reagents section). The identification by GC–mass spectrometry (MS) was carried out using conditions identical to those used for the GC with the exception of the carrier gas that was helium (head pressure of 15 psi). A MD800 Fisons mass detector (Fisons, Manchester, U.K.) coupled to a GC8000 (Carlo Erba, Milano, Italy) gas chromatograph was employed. The identity of the volatiles was obtained by a comparison of their mass spectral data with the information from the National Institute of Standards and Technology (NIST) library version 1.7. The volatiles were also identified using the relative retention times of the standards with respect to the internal standard (4-methyl-2-pentanol).

Linearity, Response Factors, and Repeatability of Volatiles. The linearity of the internal standard was studied by preparing eight dilutions in refined sunflower oil (Ucasol, Spain). Concentrations varied from 0.5 to 4.0 mg/kg. The regression coefficient was $r = 0.993$. The repeatability was investigated by consecutively analyzing 12 samples of var. Picual (Spain) spiked with 2.6 mg/kg of 4-methyl-2-pentanol. The relative standard deviation was 4.5%.

The linearity of the response of this fiber as a function of the concentration was studied in the range of 0.1–2.5 mg/kg. The regression coefficient was higher than 0.95 for C_6 and C_5 compounds, with the exception of *Z*-2-pentenol. The concentration values of volatile compounds were corrected by the response factors obtained in each calibration.

Repeatability, expressed as % relative standard deviation (RSD), of each individual volatile was determined by analyzing 26 samples (**Table 1**). Values of % RSD were lower than 10% for all of the volatiles, except two compounds (pentanal and 2-methyl-butan-1-ol), with the minimum, average, and maximum values being 0.26, 4, and 13.8%, respectively.

Sensory Perception of Volatile Compounds. To assess the aroma notes corresponding to olive oil volatile compounds, a GC-sniffing technique was applied to virgin olive oil samples. The effluent of the GC column was split 1–10 to the detector and the sniffing port, respectively. Five assessors, full-trained for virgin olive oil (*19*), carried out the evaluation. The descriptions of the odor-active regions were noted on a form with a preprinted time scale. The assessors did not see the chromatogram during the analysis. Although different semantic terms where used to describe the odor of volatiles released from the sniffing port, a consensus-building discussion was held with assessors to decide the final sensory descriptors.

A fully refined and deodorized olive oil was the matrix for the assessment of the odor threshold values; the absence of volatile compounds in the matrix was checked by the SPME–GC procedure described above. The sensory evaluation was carried out in accordance with the official method for the olive oil sensory assessment (*19*). A total of 15 mL of each sample was kept in standardized glasses at 29 ± 2 °C for 15 min and then evaluated by five assessors. Three samples were presented to the assessors following the triangle test (*5*, *20*), whose results were statistically analyzed. Results (**Table 1**) agree with previous studies carried out by the research group.

Because the sensory perception qualifying each volatile compound can vary depending upon its concentration in virgin olive oil, its sensory characterization has been carried out by sniffing each volatile compound at the same and double concentration of its odor threshold (**Table 1**). Volatiles were dissolved in fully refined olive oil and presented to three assessors following the official olive oil sensory assessment (*19*). Samples were randomly presented to assessors. **Table 1** shows the sensory attributes that the assessors think to be more significant qualifying the volatiles.

Analysis by MOSs. Olive oil samples (5 g) were heated at 34 °C inside a controlled thermostat-sampling chamber for a headspace generation time of 600 s. Then, the volatiles were pumped into a Fox 4000 (AlphaMOS, Toulouse, France) equipped with 18 metal oxide sensors (6 Cr_2O_3 sensors and 12 SnO_2 doped with Pd and Pt) by means of a carrier gas (air) at a flow rate of 100 mL/min for 90 s, the so-called injection time. After the injection time, a valve was switched and only carrier gas was blown into the sensor chambers to return to the baseline of the sensor signals as soon as possible. The computer starts to collect data immediately after the headspace generation time for 600 s: 90 s of injection time and 510 s of desorption time. The time between subsequent analyses was 900 s. The flow rate was kept at 500 mL/min during the first 10 min of this nonmeasurement time. These conditions ensured that the baseline had indeed been recovered before performing the next analysis. Samples were analyzed in duplicate. Standards for calibration of the sensor array were measured at programmed times to control the aging of sensors that did not affect the measurements.

To reduce the large data set of sensor responses to a reasonable size, a data compression method was applied to the data matrix prior to applying further statistical procedures. The response of the sensors was processed to produce five data per sensor by means of the windowing time slicing (WTS) algorithm (*21*). The WTS data are obtained by applying five functions in different ranges along the

Table 1. Volatile Compounds Quantified by SPME–HSGC: Information of Their Kovat's Index (KI), Percentage of Their Relative Standard Deviation in Repeatability (RSD_r), Their Odor Threshold (OT), and Their Concentration Mean Values (Mean ± Standard Deviation) of Chétoui and Chemlali Varieties Together with the *p* Value for the Brown–Forsythe Test[a]

volatile	KI	% RSD_r	OT[b]	Chétoui[b]	Chemlali[b]	*p*	sensory attributes
heptane	700	4.18	0.67	0.13 ± 0.02	0.20 ± 0.04	0.20	alkane
methyl acetate	828	1.92	0.20	0.49 ± 0.05	0.40 ± 0.05	0.27	ethereal, sweet
ethyl acetate	892	3.11	0.94	0.17 ± 0.03	0.36 ± 0.04	*0.01*	pleasant, sweet
ethanol	932	1.14	30.00	19.99 ± 5.05	24.24 ± 4.57	0.58	alcoholic
pentan-3-one	977	3.49	70.00	0.81 ± 0.05	0.51 ± 0.02	*0.00*	sweet, fruity
pentanal	979	10.04	0.24	0.36 ± 0.03	0.37 ± 0.04	0.84	woody, bitter, almond
4-methyl-pentan-2-one		0.26	0.30	0.09 ± 0.01	0.10 ± 0.01	0.41	strawberry, fruity, ethereal
1-penten-3-one	1016	0.37	0.001	0.48 ± 0.05	0.39 ± 0.04	0.17	pungent, mustard
hexanal	1074	0.33	0.08	1.13 ± 0.18	2.21 ± 0.09	*0.00*	green, apple
1-penten-3-ol	1164	3.14	0.40	0.49 ± 0.05	0.46 ± 0.04	0.70	butter, soft green
2-methyl-butan-1-ol	1210	13.75	0.48	0.01 ± 0.00	0.01 ± 0.00	0.45	winey, whiskey, wood
E-2-hexenal	1216	5.27	0.42	2.87 ± 0.67	10.59 ± 1.21	*0.00*	green, almonds
pentan-1-ol	1250	9.68	0.47	0.12 ± 0.01	0.11 ± 0.01	0.58	pungent, strong, balsamic
hexyl acetate	1274	8.48	1.04	0.26 ± 0.04	0.03 ± 0.00	*0.00*	sweet, green, fruity, apple
Z-3-hexenyl acetate	1316	3.82	0.20	0.30 ± 0.03	0.33 ± 0.02	0.39	fruity, green leaves
Z-2-penten-1-ol	1320	3.24	0.25	0.37 ± 0.03	0.32 ± 0.02	0.21	alcoholic, banana
hexan-1-ol	1357	1.57	0.40	0.55 ± 0.07	0.96 ± 0.13	*0.04*	fruity, banana, undesirable[c]
E-3-hexen-1-ol	1366	2.30	1.00	0.28 ± 0.04	0.15 ± 0.02	*0.02*	green
Z-3-hexen-1-ol	1385	0.60	1.10	0.10 ± 0.01	0.09 ± 0.00	*0.04*	green, banana
nonan-2-one		5.68	0.10	2.37 ± 0.37	0.53 ± 0.07	*0.00*	fruity, floral
E-2-hexen-1-ol	1408	6.95	5.00	0.44 ± 0.07	1.51 ± 0.33	*0.03*	green, grassy, undesirable[c]
total C_5		2.33		2.62 ± 0.19	2.16 ± 0.13	*0.01*	
total C_6		8.12		5.93 ± 0.89	15.87 ± 1.33	*0.00*	
total C_6 from LA[d]		0.20		1.94 ± 0.21	3.21 ± 0.17	*0.00*	
total C_6 from LnA[e]		0.12		3.71 ± 0.70	12.51 ± 1.24	*0.00*	
total alcohols[f]		10.07		2.35 ± 0.17	3.59 ± 0.46	0.07	
total aldehydes		6.60		4.36 ± 0.84	13.17 ± 1.29	*0.00*	
total esters		3.45		1.22 ± 0.07	1.12 ± 0.06	0.19	
total ketons		6.69		3.74 ± 0.36	1.53 ± 0.10	*0.00*	

[a] Mean values of total concentrations by a series of compounds are also shown. All of the compounds were identified by GC–MS and standards. [b] Units in mg/kg. [c] At high concentrations (*14*). [d] LA = linoleic acid. [e] LnA = linolenic acid. [f] Ethanol quantity has not been computed.

response curve of the sensor (*22*). Therefore, these five data represent an average value of the sensor response during the adsorption and desorption processes of the volatiles on the sensor hot surface.

The repeatability study, either between days (for 6 months) or during the day, has been investigated by consecutively collecting the sensor results of the same sample of virgin olive oil (*23*). The maximum % RSD of the repeatability study carried out during the day was 12.0%, with the mean being 6.1%, while the mean results of the between days repeatability study was 11.7%, with a maximum (22.2%) being far higher.

Chlorophyll Analysis. The method adopted for the quantification of chlorophylls was that described by Wolff (*24*). This method is based on the absorbance measurement at 610, 630, and 670 nm using carbon tetrachloride as a control. The content of chlorophylls is expressed as mg/kg.

Statistical Analysis. The whole set of data was imported to Excel from the HP-Chemstation program (Agilent Technologies, Palo Alto, CA), and Statistica release 6.0 (StatSoft, Tulsa, OK) was used to perform the data processing by means of the following statistical procedures. A univariate and two multivariate procedures were used for the pretreatment of the data prior to apply a multivariate unsupervised procedure to the variables selected in the data pretreatment step.

The Brown–Forsythe univariate test was used to perform the analysis on the deviations from the group medians because it gives quite accurate error rates, even when the underlying distributions for the raw scores deviate significantly from the normal distribution (*25*). This first screening of the variables (volatile compounds or MOSs) was followed by the multivariate statistical procedure of the stepwise linear discriminant analysis (SLDA) to diminish the number of variables. The objective was to select only those variables that provide multivariate information able to classify the samples into varieties and maturity stages. SLDA was applied under the strictest conditions to avoid the possibility of hyperoptimistic results. The criterion for the selection of variables was the *F*-to-enter value obtained from the *F* distribution table ($F > 0.95$), taking into account the number of groups and the number of samples from the smallest group. Tolerance was fixed at 10^{-3}.

Despite SLDA being performed under the strictest statistical conditions, the unsupervised statistical procedure of the principal component analysis (PCA) was used to check if the selected variables (volatiles or MOSs) are indeed able to distinguish the virgin olive oils by their cultivar or ripeness.

Canonical correlation was also used to determine the explanation of the variance of volatile compounds by the sensor responses.

RESULTS AND DISCUSSION

Aroma is the most important criterion for distinguishing varietal virgin olive oils, as proven in previous studies (*26*). Because the volatile compounds are mainly responsible for odor perception, the individual quantification of the volatile fraction is key information for the quality and traceability control of virgin olive oils. **Table 1** shows information about the volatile compounds quantified by SPME–HSGC in oils from Chétoui and Chemlali cultivars. The total amount of volatile compounds in the oils from Chemlali cultivars is almost double with respect to var. Chétoui, which agrees with a previous study (*27*).

The study of the differences in the concentration of individual compounds between both varieties was based on aldehydes, alcohols, ketones, and esters, with most of them being C_6 compounds that are abundant in virgin olive oils (*3*, *5*). This series has been supplemented with the quantification of C_5 compounds that are not only responsible for appreciated sensory attributes but they might modify the olive oil sensory profile if the activity of some liquid oxygen (LOX) enzymes is depleted (*11*, *12*). The amounts of C_6 and C_5 compounds are determinant in distinguishing the two major Tunisian cultivars after applying the Brown–Forsythe test ($p < 0.05$). The total concentration of C_6 compounds is higher in Chemlali cultivar, which agrees with previous studies (*7*), while this study has

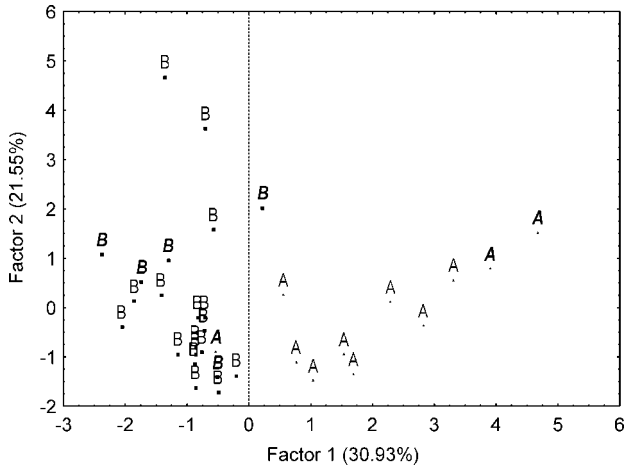

Figure 1. PCA based on four variables (hexyl acetate, hexanal, *E*-2-hexenal, and the sum of ketones) characterizing the whole set of 33 samples. The letters indicate the varieties Chétoui (A) and Chemlali (B), and bold italic letters mean the commercial oils.

found a slightly higher concentration of C_5 compounds in Chétoui cultivar after analyzing six individual C_5 volatiles. The ratio between C_6 and C_5 compounds is, however, 3 times higher in Chemlali than Chétoui. The latter result indicates that an additional branch of the LOX, which leads to the production of C_5 compounds (*11*, *12*), is promoted in the var. Chétoui. Furthermore, the ratio between the total amount of the volatiles produced through the oxidation of linolenic acid (LnA) (*E*-2-hexenal, *Z*-3-hexenyl acetate, *Z*-3-hexenol, and *E*-2-hexenol) and the linoleic acid (LA) (hexanal, hexyl acetate, and hexan-1-ol) is approximately half in var. Chétoui (1.9 versus 3.9) (**Table 1**).

In terms of series, the total concentrations of aldehydes and ketones allow for the characterization of the varieties as well. The total concentrations of the first two series are higher in the Chemlali variety, while ketones were quantified in a higher amount in Chétoui. No significant differences were observed in the total content of esters. The analysis of individual compounds shows that all C_6 compounds, except *Z*-3-hexenyl acetate, and only pentan-3-one within C_5 compounds discriminate between cultivars.

The profile of the predominant volatiles of these varieties is different among them and with respect to ones cultivated in other countries (*9*). However, not all of the volatile compounds with *p* values lower than 0.05 have the same ability to distinguish the varieties, but there may be a subset of variables that, combined among them, provides valuable multivariate information. Thus, a SLDA was applied (*F*-to-enter = 8.0) to filter irrelevant volatile compounds, distinguishing the varieties prior to applying the unsupervised PCA. Three volatile compounds (hexyl acetate, hexanal, and *E*-2-hexenal) and the total concentration of ketones were the variables of the canonical equations that are able to distinguish these varieties.

Figure 1 shows the PCA plot with the four variables (hexyl acetate, hexanal, *E*-2-hexenal, and the sum of ketones) selected by SLDA. All of the samples collected in the farms were classified correctly by the first two principal components. The equations classified the commercial oils in their correspondent group with the exception of the samples produced in Mjez Elbeb (var. Chétoui) and Enfidha (var. Chemlali). Five assessors evaluated the first sample by two successive triangle tests to determine in which varietal group this sample, var. Chétoui, would be classified. The sample was not neatly classified as var. Chétoui (57 versus 43%). No explanation was found after the sensory assessment of the sample from Enfidha (Chemlali). This sample was obtained in the region of Sousse, which is placed in northern Tunisia near the coast, while the rest of Chemlali samples were mostly collected in Sidi Bouzid and Sfax regions, which lie in southern areas.

The mathematical selection of the volatiles for distinguishing varieties does not mean the selected compounds contribute to distinguish them by their sensory perception unless the selected volatiles also contribute to aroma. The odor activity value (OAV) of a volatile compound (ratio between its concentration and its odor threshold) is the parameter commonly used to determine its contribution to aroma (OAV $\geq$ 1.0). Four volatiles (hexanal, *E*-2-hexenal, hexan-1-ol, and nonan-2-one) show *p* values lower than 0.05, distinguishing both varietal virgin olive oils, and their OAV are higher than 1.0. The data for Chétoui and Chemlali, respectively, were 14.1 and 27.6 for hexanal, 6.83 and 21.21 for *E*-2-hexenal, 23.7 and 5.3 for nonan-2-one, and 1.4 and 2.4 for hexan-1-ol, calculated with respect to the average concentrations displayed in **Table 1**. However, there are other volatiles (methyl acetate, pentanal, 1-penten-3-one, *Z*-3-hexenyl acetate, and *Z*-2-penten-1-ol) that also contribute to aroma, although their concentrations in both varietal oils are not too different. Thus, if the sensory qualification of the volatile compounds (**Table 1**) are considered, it can be concluded that the Chétoui variety is mostly qualified by floral, pungent, and throat-catching sensory perceptions and the Chemlali variety is mostly qualified by green and fruity sensory attributes. These results agree with the information that qualifies virgin olive oils from the Chétoui variety with fruity, green leaf, bitter, and pungent attributes (*28*), while the Chemlali variety is generally characterized by a high fruity odor (*29*)

Because maturity is one of the factors that most affect the virgin olive oil aroma (*30*), the same process was used to analyze the evolution of the volatile compounds with ripeness. The maturity evolution of the Chemlali cultivar was study in four different geographical origins (Bouslim, SidiBouzid, Enfidha, and Taous), while the Chétoui cultivar was studied in two places (Benikhalled and Sfax). The total content of chlorophylls was quantified in each one of the samples to verify if harvesting was carried out at different and increasing stages of ripeness. Although the content of chlorophylls varies among cultivars, in each variety, their total amount gradually decreases along ripening (*31*). **Table 2** shows the total content of chlorophylls and a series of volatile compounds of var. Chemlali cultivated in the different geographical locations. First of all, the means of the volatile compounds of the olives harvested at the four stages of ripeness regardless of the cultivar were calculated. The results showed a net decrease in the total content of C_6 and C_5 compounds in the last ripening stage. However, the total content of alcohols increases with the ripeness, mostly because of hexan-1-ol, which partially agrees with a previous study of several European varieties (*30*), and *E*-2-hexen-1-ol, which disagrees with those results, although the latter compound abruptly decreases in the last maturity stage. Because these compounds are qualified as undesirable at high concentrations (*3*), the harvest of over-ripe olives might produce low-quality virgin olive oils. The total content of esters also increases although because of two compounds (methyl and ethyl acetates) that are not involved in the lipoxygenase cascade. These compounds contribute to the sweet perception at high concentrations (*5*, *9*) that is habitual in virgin olive oils from over-ripe fruits (*32*). Ethanol, finally, doubles its concentration at the over-ripe stage with respect to the under-ripe stage. It is well-established this compound not only retards the olive senescence and is a

Table 2. Mean Concentrations (mg/kg) of the Chlorophylls and the Series of Compounds of the Lipoxygenase Pathway at Four Stages of Ripeness According to the Olive Color of the Chemlali Olive Oils Cultivated in Four Different Geographical Places

	stages of ripeness[a]			
series of compounds	1	2	3	4
chlorophylls	11.38	6.33	2.00	0.36
alcohols[b]	2.51	2.82	2.90	6.29
aldehydes	14.21	16.97	14.05	7.35
esters	1.01	0.88	1.15	1.37
ketons	1.48	1.98	1.64	1.12
C_5 compounds	2.53	3.01	1.88	1.39
C_6 compounds	15.93	18.93	19.61	9.87
C_6 from linoleic acid	2.81	2.98	4.13	2.96
C_6 from linolenic acid	13.11	15.95	14.48	6.91
ethyl acetate[c]	0.22	0.14	0.35	0.64
pentanal[c]	0.64	0.47	0.24	0.18
1-penten-3-one[c]	0.42	0.65	0.31	0.22
Z-3-hexenol[c]	0.09	0.09	0.08	0.08
4-methyl-2-pentanone	0.11	0.12	0.10	0.08
methyl acetate	0.37	0.24	0.47	0.50
ethanol	20.03	14.50	14.66	43.05
hexan-1-ol	0.56	0.63	1.64	1.02
E-2-hexen-1ol	0.65	0.75	3.52	1.18

[a] Green (1), green–violet (2), violet (3), and black (4) olives. [b] The ethanol quantity has not been computed. [c] Volatile compounds selected by SLDA for the classification based on ripeness.

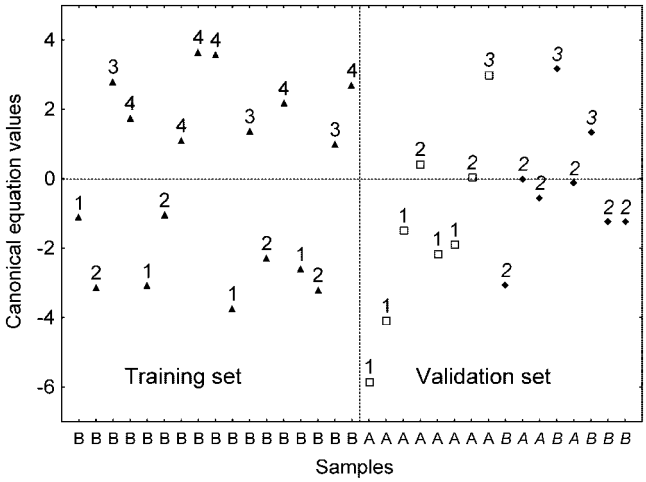

Figure 2. SLDA based on four variables (ethyl acetate, pentanal, 1-penten-3-one, and *Z*-3-hexen-1-ol) characterizing the samples. The letters indicate the varieties Chétoui (A) and Chemlali (B); the numbers (1–4) mean the stages of ripeness; and the markers are Chemlali (▲) and Chétoui (□) oils from local farms and commercial oils (♦).

precursor of aroma compounds but also increases during the maturation of fruits (*33*).

Figure 2 shows the classification of Chemlali samples according to their ripening stages by mean of a canonical equation (F-to-enter = 6.0) based on four volatile compounds (ethyl acetate, pentanal, 1-penten-3-one, and *Z*-3-hexen-1-ol). This SLDA canonical equation, built exclusively with Chemlali samples, was applied to Chétoui and commercial samples for its validation. Thus, the SLDA equation was validated with external samples belonging to another Tunisian variety collected in other geographical locations and eight of them (commercial oils) obtained at industrial scale. The plot shows there are two clear stages of ripeness (1 and 2) versus (3 and 4), whichever the sample analyzed. This result partially agrees with the ripeness (under-ripe, normal-ripe, and over-ripe) study of European varieties (*30, 34*). The reduction in the ripeness stages

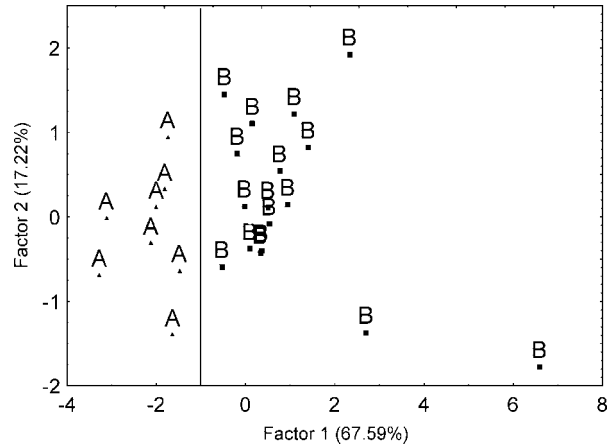

Figure 3. PCA of the responses of five sensors (1, 9, 14, 17, and 18). The letters indicate the varieties Chétoui (A) and Chemlali (B).

to two may also be explained by the little differences on climate between seasons in semidesert geographical zones.

The odor thresholds of the chemical compounds selected for classifying the ripeness stages show that two of them (ethyl acetate and *Z*-3-hexen-1-ol) do not contribute to the olive oil aroma. The other two compounds, pentanal and 1-penten-3-one, contribute to bitter and pungent sensory perceptions, respectively. The concentration of these compounds diminishes with the ripeness (**Table 2**) up to the point the pentanal amount is lower than its odor threshold for the last two stages of maturity, while 1-penten-3-one shows a bell-shaped distribution, with the lowest concentration being in the olive oils from over-ripe olives. These values agree with the empirical fact that the bitter and pungent sensory perceptions are smaller in olive oils from ripe and over-ripe olives.

This analytical procedure, although easy and rapid, cannot be applied online and requires expert analysts to identify the chemical compounds in the chromatograms, partially because of the large number of chemical compounds (*14*). An alternative is based on the use of metal oxide sensors that have been applied with success in several aspects of olive oil research (*22*). This analytical procedure is based on the resistance changes that occur as a result of the interaction of the whole set of volatiles and a semiconducting material. Thus, the characterization of aroma by a sensor system is much more rapid compared to SPME–HSGC.

The hypothesis is that the sensor response depends upon the amount and composition of volatile compounds in the sample (*21*); hence, the quantitative differences in the two varietal olive oils observed by the SPME–GC analyses may also be detected by the sensor array. Thus, the first step was to evaluate if the chromatographic information and the responses of the sensors agree from a mathematical viewpoint. The canonical correlation was the statistical procedure used for this objective. The overall canonical R is highly substantial (0.94) and statistically significant ($p < 0.001$). Furthermore, 60.3% of the variance of volatile data accounts for sensor responses. These results suggest a fairly strong overall relationship between volatile concentrations and the sensor responses.

The statistical procedure used with the data of volatile compounds has been used with the sensor responses as well. **Table 3** shows the sensors whose responses are able to discriminate between varietal olive oils ($p < 0.05$) according to the Brown–Forsythe test. **Figure 3** shows the PCA, with the responses of the five sensors (*1, 9, 14, 17, 18*). This unsupervised procedure fairly distinguished samples of varietal virgin olive oils.

Table 3. Values of the Responses of MOSs Distinguishing Samples According to the Variety and Ripeness Stages ($p < 0.05$)[a]

	variety			ripeness stage		
sensor	Chetoui	Chemlali	*p*	1–2	3–4	*p*
S1	172.79 ± 1.81	163.35 ± 1.33	*0.00*	167.19 ± 1.77	165.14 ± 2.28	0.48
S3	5429.17 ± 108.13	5542.00 ± 61.96	0.34	5600.55 ± 62.34	5363.90 ± 82.74	*0.03*
S4	3903.73 ± 69.30	3955.59 ± 43.88	0.52	4010.94 ± 42.11	3831.08 ± 51.71	*0.01*
S5	3146.48 ± 64.35	3095.26 ± 47.34	0.54	3205.95 ± 39.32	2970.19 ± 46.86	*0.00*
S9	589.56 ± 8.41	527.34 ± 13.35	*0.01*	563.04 ± 8.51	523.56 ± 23.31	0.08
S14	1482.72 ± 6.57	1390.84 ± 15.49	*0.00*	1449.33 ± 10.21	1376.61 ± 25.82	*0.01*
S17	1577.69 ± 8.99	1498.70 ± 15.46	*0.00*	1550.73 ± 10.20	1483.85 ± 24.68	*0.01*
S18	1644.14 ± 9.31	1560.66 ± 16.28	*0.00*	1615.18 ± 11.02	1545.67 ± 25.89	*0.01*

[a] WTS values (*22*).

The univariate statistical analysis based on the Brown–Forsythe test also allowed for the determination of the sensors that were able to discriminate between two stages of ripeness (**Table 3**). Sensors 3, 4, 5, 14, 17, and 18 were selected for this classification. The SLDA applied to responses of these sensors was able to classify the samples into two ripeness stages (1 and 2 versus 3 and 4), and no misclassification was observed in all of the oils collected from the farms, including the Chétoui samples used as a validation set. However, the procedure failed when it was also validated with the commercial samples, because of the great influence of the extraction system (laboratory scale versus industrial mill) on the overall olive virgin oil aroma (*2*). This result points out that the MOSs are able to classify the virgin olive oil variety and ripeness if the classification model is designed and validated with the same extraction system.

The responses of the sensors show significant correlations with the volatile compounds that were able to discriminate the cultivars. To check the partial sensitivity of the sensors to volatile compounds, several dilutions of standards of the compounds in refined oil (0, 0.2, 0.4, 1.0, and 1.5 mg/kg) were prepared and their headspace was analyzed by the sensors. All of the selected sensors were very sensitive to *E*-2-hexenal and hexanal (**Figure 4**), which were previously selected in the chromatography study for variety classification. However, the sensor responses for hexyl acetate were very low or null in all of the sensors. With concern over the compounds involved in the ripeness classification, the highest responses in sensors 1–4 (Cr_2O_3 sensors) corresponded to 1-penten-3-one. This fact explained the selection of sensors 3, 4, and 5 for this classification task. On the contrary, the highest responses of sensors 14–18 (SnO_2 sensors doped with Pd and Pt) were observed for ethanol. The concentration of this compound increases in the last stage of ripeness, as previously stated (**Table 2**). Nevertheless, it is noticeable that the responses of these three sensors (*14*, *17*, *18*) to ethanol easily reach saturation once the concentration of 4 mg/kg is exceeded; hence, its contribution to the classification results may be based on the responses of the sensors 3–5 rather than sensors 14–18 (**Figure 4**). High responses were also observed for *Z*-3-hexen-1-ol, while only sensors 14–18 showed moderate responses to ethyl acetate, also involved in the ripeness classification (**Table 2**).

The chemical characterization of Chétoui and Chemlali varieties proves their differences on sensory characteristics. The study on ripeness mostly agreed with other studies carried out with other European varieties by the authors (*34*), although some disagreements were observed, probably because of both climate and variety differences. The agreement found between the results by SPME–GC and MOSs shows that the latter can be considered for a traceability system for Tunisian oils based on variety and ripeness. Although the sensors are exposed to all of the volatile compounds present in virgin olive headspace, a study revealed that the classification results are mainly explained by their sensitivity to *E*-2-hexenal and hexanal, in the case of variety classification, and 1-penten-3-one, ethanol, and *Z*-3-hexen-1-ol, when they are classified by the ripeness stage. Nevertheless, special care should be taken with the extraction system because it greatly affects the virgin olive oil aroma and, hence, the sensor responses. This effect was not observed in the SPME–GC results, although this method is more lengthy and expensive.

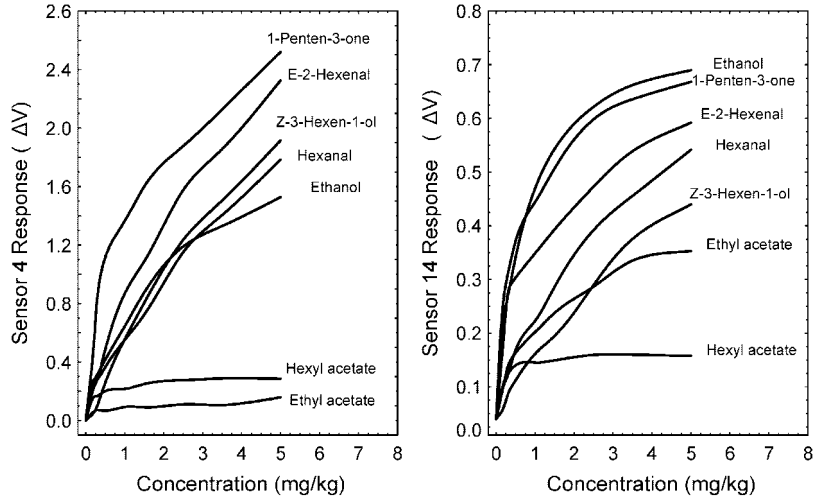

Figure 4. Responses of sensors 4 (Cr_2O_3 sensor) and 14 (SnO_2 sensor doped with Pd and Pt) to standards of volatile compounds diluted in refined oils to concentrations ranging from 0 to 5 mg/kg.

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