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Quality Characterization of the New Virgin Olive Oil Var. Sikitita by Phenols and Volatile Compounds

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New cultivars with greater adaptability to modern irrigated (super-) high-density orchards and producing good sensory quality oils are highly demanded by an olive oil industry in continuous change. This work analyzes olive oil sensory quality, in terms of phenols and volatiles that are responsible for virgin olive oil flavor, for three cultivars: Picual, which is used for >15% of world olive oil production; Arbequina, which is cultivated worldwide; and the new progeny Sikitita, which is derived from the other two. The availability of data at three different levels of ripeness allowed quantifying the genetic and olive maturity effects on the oil composition by means of the analysis of variance (ANOVA) and principal component analysis (PCA). Phenols and volatiles varied greatly both with genotype and, to a lesser extent, with olive maturity. With regard to the phenol profile, the crossbred cultivar Sikitita showed a higher degree of similarity with the Arbequina variety. The volatile composition of var. Sikitita, however, varies significantly from that of Arbequina, in the first stages of the olive ripeness, and becomes more similar to that of Picual as the level of ripeness increases.

KEYWORDS: Olive oil; Sikitita variety; phenols; volatile compounds; sensory quality

INTRODUCTION

The traditional nonirrigated olive tree orchards have been gradually substituted, when possible, for high-density and super-high-density plantations since the 1990s. In this new agronomic practice the olive trees are planted very close; they become productive more quickly, and specific machinery allows picking large quantities of olives rapidly and with lower labor costs. This new cultivation strategy (**Table 1**) has meant a revolution in olive oil production and has affected the selection of cultivars that are better adapted to the new orchards.

The first attempt of cultivar selection has been based on oil content and slow and vigorous growth, as well as high productivity so that the trees can be planted in hedge-lines and harvested with new automated combined shaker machines. Six are the most commonly planted cultivars all over the world, Arbequina, Manzanilla, and Picual (Spain), Barnea (Israel), and Frantoio and Leccino (Italy), Arbequina being the most universal by far. These cultivars were selected from their best progenitors with desirable characteristics for plantations in hedge-lines with drip irrigation and mechanical harvesting.

Not all of the botanical varieties of olive trees are fully adaptable to the new cultivation practices. Thus, researchers in agronomy from the main olive-producing countries are developing crossbreeding programs with the most outstanding cultivars and the selection within their progenies (*1*). These programs use a rapid genetic selection technique and variability identification (*2*). As a consequence of the high level of heterozygosis of the olive cultivars, any cross combination generates features that vary in a range determined by the variability of these features in the progenitors. Thus, the selection criteria concern not only the botanical aspects but also the olive oil chemical composition.

The composition of fatty acids, the percentage of oleic acid in particular, has been the main characteristic evaluated in these studies (*3*), mainly because of its implication in nutrition and health (*4*). However, the importance of minor compounds with implications in nutrition and sensory quality (*5*) has recently turned the focus to the determination of chemical compounds as a procedure to evaluate virgin olive oil quality (*6*−*8*). Volatile compounds are mainly responsible for olive oil aroma, and their concentrations and odor threshold allow explanation of the sensory characteristics of the oils (*5*). These compounds are mainly formed through the lipoxygenase pathway (LOX), and their profile strongly depends on the cultivar (*9*). On the other hand, the bitter and pungent notes in olive oil are related to the presence of phenolic compounds (*5*). In addition to their sensory implications, the importance of these compounds lies in their antioxidant and health-promoting properties (*10*, *11*). Like volatile compounds, the concentration of phenols is strongly affected by the olive tree cultivar (*12*), which explains the variation of bitterness and pungency levels between monovarietal oils.

The characterization of phenols and volatiles is of particular interest in the chemical characterization of new botanical varieties obtained in the crossbreeding programs. One of these varieties is Sikitita, derived from hybridization between Picual (female parental) and Arbequina (male parental) cultivars. This new cultivar is characterized by high yield efficiency, small canopy adaptable to plantation in-hedge, and high oil content (*13*). This new variety has been studied in its morphological aspects and resistance and susceptibility to pests, among other agronomical

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Table 1. Main Characteristics of Traditional and (Super-)intensive Olive Tree Orchards

characteristic	traditional	(super-)intensive
geographic relief	hills and mountains	rolling and flat plains
diversity of cultivars	1275 (approx)	20 (approx)
density: trees/ha	40−300	400−2000
irrigation: m^3/ha	rain-fed	drip (1000−8000) + rain-fed
fertilization: N_2/tree	1−8 kg	150−350 kg
pesticide: treatments per year	2	10
harvesting: shaker machines	semiautomatic	combine (Colossus)
yield: kg/ha	200−3000	8000−20000

aspects. Nevertheless, little is known about the chemical characterization of its phenolic and volatile fractions in relation to its precursors and the influence of maturity over these compounds.

The aim of this work was to compare the evolution of the concentration of individual volatile and phenolic compounds, which are responsible for virgin olive oil flavor, during the ripening process of the olives of three cultivars, Arbequina, Picual, and Sikitita, the latter being the result of crossbreeding between the other two cultivars (*3*). The study of these compounds will serve as a basis to establish similarities and dissimilarities between these cultivars according to their sensory properties and will provide valuable information about the heritability behavior of olive tree crossbreeding with regard to quality.

MATERIALS AND METHODS

Plant Material. Olive tree varieties Arbequina, Picual, and Sikitita were cultivated in the same olive tree orchard in Córdoba (Spain), and olives were harvested on November 1, 15, and 30, which approximately corresponded to second, third, and fourth ripeness levels according to Frías et al. (*14*). The olive tree orchard was irrigated with 1000 m^3/ha (water on demand under regulated deficit irrigation). Rainfall was of approximately 600 mm, and the olives were processed at laboratory scale by means of the Abencor system (MC2 Ingeniería y Sistemas S.L., Sevilla, Spain) to obtain virgin olive oil (VOO). Thus, genotypic variance was the main contributor to differences among the VOO chemical compositions of the three cultivars. The harvested olives of each cultivar and each maturity level were split into three groups, so the differences in olive ripeness of each group were the main source of variability.

The study has been carried out with only one crop (2009) as previous results have showed that variance due to yearly differences was negligibly small in irrigated orchards in comparison with the genotype, which is the main contributor to total variance in the VOO chemical composition (*3*, *9*).

Determination of Phenols. A standard solution (0.5 mL) of *p*-hydroxyphenylacetic (0.12 mg/mL) and *o*-coumaric (0.01 mg/mL) acids in methanol was added to a sample of filtered VOO (2.5 g). A rotary evaporator at 40 °C under vacuum was used to evaporate the solvent, and the oily residue was dissolved in 6 mL of hexane.

The diol-bonded phase cartridge was conditioned according to the method described by Mateos et al. (*15*). After the sample was loaded, the column was washed with 6 mL of hexane and 3 mL of hexane/ethyl acetate (90:10 v/v). The final residue was extracted with 10 mL of methanol and evaporated at 40 °C under vacuum, and the extract was diluted with 500 μL of methanol/water (1:1, v/v). A filtered aliquot (20 μL) of the final colorless solution was injected onto the HPLC system (an Agilent Technologies 1100 liquid chromatographic system equipped with a diode array UV detector). The column was a Lichrospher 100RP-18 column (4.0 mm inner diameter × 250 mm; 5 μm, particle size) maintained at 30 °C. The gradient elution, at a flow rate of 1.0 mL/min, was achieved using the following mobile phases: a mixture of water/orthophosphoric acid (99.5:0.5, v/v) (solvent A) and methanol/acetonitrile (50:50, v/v) (solvent B). The change of the solvent gradient was programmed as follows: from 95% (A) and 5% (B) to 70% (A) and 30% (B) in 25 min, to 62% (A) and 38% (B) in 10 min, to 62% (A) and 38% (B) in 5 min, to 55% (A) and 45% (B) in 5 min, to 47.5% (A) and 52.5% (B) in 5 min, and to 100% (B) in 5 min, followed by 5 min of maintenance. The chromatographic signals were obtained at 235, 280, and 335 nm.

Quantification of phenols, except flavones and ferulic acid, was carried out at 280 nm using *p*-hydroxyphenylacetic acid as the internal standard. Quantification of flavones and ferulic acid was performed at 335 nm using *o*-coumaric acid as the internal standard. The response factors and recoveries were based on the procedure carried out by Mateos et al. (*15*).

Concentration of Volatile Compounds. Olive oil samples (1 g) spiked with 2.6 mg/kg of internal standard (4-methyl-2-pentanol) were placed in a 20 mL glass vial, tightly capped with a 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 StableFlex stationary phase (2 cm 50/30 μm film thickness) of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fiber was previously conditioned following the supplier's instructions.

Chromatographic Analysis of Volatiles. 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 in the 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 at 3 °C/min to a final temperature of 200 °C, at which it was held for 10 min to eliminate the memory effect of the capillary column. The signal was recorded and processed with 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 (*16*, *17*). The assessment of the aroma notes corresponding to VOO volatile compounds was already carried out in previous works (*17*, *18*).

Statistical Analysis. The data were analyzed using the analysis of variance (ANOVA) and principal component analysis (PCA). For the univariate statistical analysis, data of individual phenols and volatiles were subjected to ANOVA to test for significant differences between the effects of cultivar, ripeness, and cultivar × ripeness on their concentrations. The analysis of variance of factorial designs was used with repeated measures. The separation of the means was obtained at $p \leq 0.05$ by Duncan's test.

For a multivariate analysis of the whole information, the selected statistical procedure was PCA. PCA is an unsupervised tool oriented toward modeling the variance/covariance structure of the data matrix into a model that represents significant variations and considers the noise as an error. Thus, PCA was used for interpreting the behavior of the profiles of VOO phenols and volatiles with respect to cultivar and olive ripeness. Statistica 7.0 (StatSoft, Tulsa, OK) was used for carrying out all of the statistical analyses.

RESULTS AND DISCUSSION

Many works have shown that the olive oil chemical composition depends primarily on genetic factors, with most cultivars displaying particular chemical profiles that have been used to differentiate monovarietal olive oils (*19*) and their geographical origin (*19*, *20*). It has been described that the profile of olive oil fatty acids from a cultivar obtained by crossbreeding is mainly the consequence of the heritability of its progenitors (*3*, *21*). However, the evolution of the concentrations of olive oil minor compounds in the olive ripeness process of cultivars resulting from the crossbreeding and its comparison with their parental cultivars have been scarcely studied.

The numerous series of VOO minor compounds (sterols, alcohols, hydrocarbons, volatiles, phenols, tocopherols, chlorophylls, carotenoids, etc.) can be divided into compounds implied in authenticity, traceability, and flavor of virgin olive oil (*19*). As this work is focused on the characterization of the flavor of virgin olive oils from var. Sikitita resulting from the crossbreeding

Table 2. Concentrations of Individual Phenols (Milligrams per Kilogram, Mean ± SD) of Virgin Olive Oils from Cultivars Arbequina and Picual and Their Progeny Sikitita at Three Levels of Ripeness of Their Olives[a]

phenol	Arbequina	Sikitita	Picual	phenol	Arbequina	Sikitita	Picual
hydroxytyrosol	0.64 ± 0.18a	1.49 ± 0.34a	2.16 ± 0.16a	*p*-HPEA-EDA	71.86 ± 1.04a	99.79 ± 8.55a	35.36 ± 3.62a
	0.83 ± 0.40	0.07 ± 0.02a	0.73 ± 0.08		42.30 ± 2.00	41.95 ± 5.44	13.17 ± 1.53a
	0.94 ± 0.00	0.06 ± 0.02a	1.98 ± 0.75a		44.55 ± 2.80a	34.52 ± 5.47a	8.96 ± 1.03a
tyrosol	1.72 ± 0.13a	4.55 ± 1.04	5.02 ± 0.34	pinoresinol	2.91 ± 0.08	2.43 ± 0.72	13.74 ± 0.50a
	0.95 ± 0.09a	3.97 ± 0.55	2.51 ± 0.27		2.14 ± 0.10a	1.10 ± 0.15a	3.63 ± 0.45a
	1.05 ± 0.07a	4.89 ± 0.27a	2.77 ± 0.27a		1.77 ± 0.09	1.20 ± 0.07	4.42 ± 0.57a
vanillic acid	0.54 ± 0.18	0.78 ± 0.19	0.02 ± 0.00a	1-acetoxypinoresinol	34.96 ± 1.45a	17.11 ± 1.72a	117.30 ± 9.19a
	0.54 ± 0.04a	0.73 ± 0.08a	0.02 ± 0.00a		25.90 ± 1.31a	14.42 ± 1.52a	34.93 ± 5.39a
	0.22 ± 0.04a	0.96 ± 0.09a	0.02 ± 0.00a		20.60 ± 3.06a	13.51 ± 0.88a	40.91 ± 7.11a
ferulic acid	2.33 ± 0.77	4.31 ± 0.52a	1.82 ± 0.14	3,4-DHPEA-EA	22.47 ± 2.70	60.99 ± 6.26	390.43 ± 47.55a
	1.16 ± 0.19a	2.79 ± 0.96	3.30 ± 0.17		7.98 ± 0.44	10.77 ± 3.32	148.72 ± 13.26a
	0.82 ± 0.12a	2.16 ± 0.24	8.30 ± 5.68		8.50 ± 1.93a	16.33 ± 4.04a	174.86 ± 33.39a
hydroxytyrosol acetate	30.87 ± 1.00a	7.86 ± 1.19a	0.53 ± 0.04a	*p*-HPEA-EA	26.71 ± 7.37	30.73 ± 5.73	100.15 ± 3.54a
	27.57 ± 2.84a	17.60 ± 1.23a	2.23 ± 0.13a		9.70 ± 2.60a	22.92 ± 2.45a	19.95 ± 2.04a
	20.43 ± 1.10a	7.64 ± 0.61a	1.63 ± 0.24a		11.58 ± 0.93	13.75 ± 3.94	24.65 ± 4.44a
3,4-DHPEA-EDA	380.69 ± 17.89a	320.00 ± 26.79a	85.29 ± 7.25a	elenoic acid	56.65 ± 0.25	57.77 ± 10.43	94.96 ± 7.20a
	268.46 ± 28.66a	130.79 ± 22.93a	40.99 ± 1.82a		25.21 ± 1.79a	14.16 ± 0.78a	48.47 ± 5.42a
	254.15 ± 19.26a	76.15 ± 8.65a	29.99 ± 4.36a		40.02 ± 1.68	40.30 ± 4.87	80.78 ± 10.10a
dialdehydic form of oleuropein aglycon	tr	45.35 ± 9.77	307.47 ± 35.45a	luteolin	9.14 ± 0.16	8.69 ± 0.53	4.04 ± 0.13a
	tr	tr	116.43 ± 10.23a		11.47 ± 1.89	15.77 ± 5.78	6.21 ± 0.40a
	tr	tr	180.55 ± 45.57a		15.64 ± 0.47a	4.55 ± 0.88	6.59 ± 1.53
apigenin	2.48 ± 0.27a	2.06 ± 0.07a	12.29 ± 0.49	sum of phenols	643.95 ± 26.28	663.92 ± 22.31	1159.52 ± 78.35
	2.91 ± 0.37	3.86 ± 1.51	9.79 ± 1.12a		427.12 ± 28.70	280.90 ± 31.22	442.27 ± 37.44
	3.36 ± 0.57a	1.10 ± 0.21	10.90 ± 2.37		423.63 ± 23.02	217.11 ± 29.98a	569.48 ± 99.72

[a] For entries followed by an "a", there is a significant difference between this variety and the other two ($p \leq 0.05$).

between Picual and Arbequina, phenols and volatiles were the chemical compounds analyzed in this study.

One of the prospects of crossbreeding is increasing the concentration of phenols as recent studies have shown that some of the health benefits of consuming this edible oil can be explained by the concentration of individual phenols (*5*). It is well-established, however, that these compounds are responsible for the sensory tasting perceptions bitterness, pungency, throat-catching, and astringency, and there are consumers who do not favor an excessive degree of bitterness. Thus, the balance between both kinds of qualities, nutritional and sensory, is nowadays one of the most remarkable objectives of olive crossbreeding programs.

The concentration of phenols varies, according to the bibliography, from a few milligrams per kilogram to approximately 1200 mg/kg, and it greatly depends on the cultivar (*22*) and environmental variables such as the irrigation regimen and the olive ripeness at harvest. A previous study on crossbreeding cultivars showed that the variance can be explained by environmental factors rather than genetic heritability (*21*, *23*). This result recently was confirmed with olive oils from var.. Arbequina cultivated in Spain and Chile (*24*). The three cultivars of this study, however, were harvested at the same level of ripeness and cultivated in the same orchard under identical irrigation regimens, and therefore no other factors affected phenol content besides the cultivar.

As VOO from the Picual variety is characterized by a high concentration of phenols (*25*) and the oil from the Arbequina variety contains a medium-low concentration of total phenols (*5*), it would be expected that their crossbred progeny (Sikitita) would contain phenols between these concentrations. The result, however, is not so predictable because the level of heterozygosis and long juvenility of olive tree cultivars hinder the expression of recessive genes, and this fact makes it too difficult to predict the heritability of desired characters.

Table 2 shows the concentrations (mean ± standard deviation) of 15 phenolic compounds at each of three ripeness level (2, 3 and 4) (*14*) of the oils from var. Arbequina, Sikitita, and Picual. The most remarkable, at first sight, is the high of concentration of a dialdehydic form of oleuropein aglycon (identified at a retention time between 29.97 and 30.92) in the samples of var. Picual. This phenolic compound has not been detected in the samples of Arbequina and Sikitita cultivars, with the exception of the oil from the first ripeness level of Sikitita. The high concentration of oleuropein derivatives in var. Picual with respect to Arbequina has already been reported by other authors (*25*). According to the concentration of this compound, Sikitita is closer to Arbequina than to Picual. On the other hand, the standard deviation (SD) was generally lower in the compounds quantified at the third ripeness level due to the fact that the homogeneity in maturity of the olives increases with their ripeness, although there are some exceptions, mostly related to those phenols that do not neatly diminish when olive ripeness increases.

Taking into account the variability of the selected olives for each analysis, the concentrations of almost all of the phenols diminished with ripeness, which agrees with other authors (*26*). As a consequence, the intensity of bitterness and astringency, together with the other tasting perceptions, waned as the phenol concentrations shrank (*27*). The ripeness process greatly affected the concentration of secoiridoids (e.g., 3,4-DHPEA-EDA, *p*-HPEA-EDA, 3,4-DHPEA-EA, and *p*-HPEA-EA). The concentration

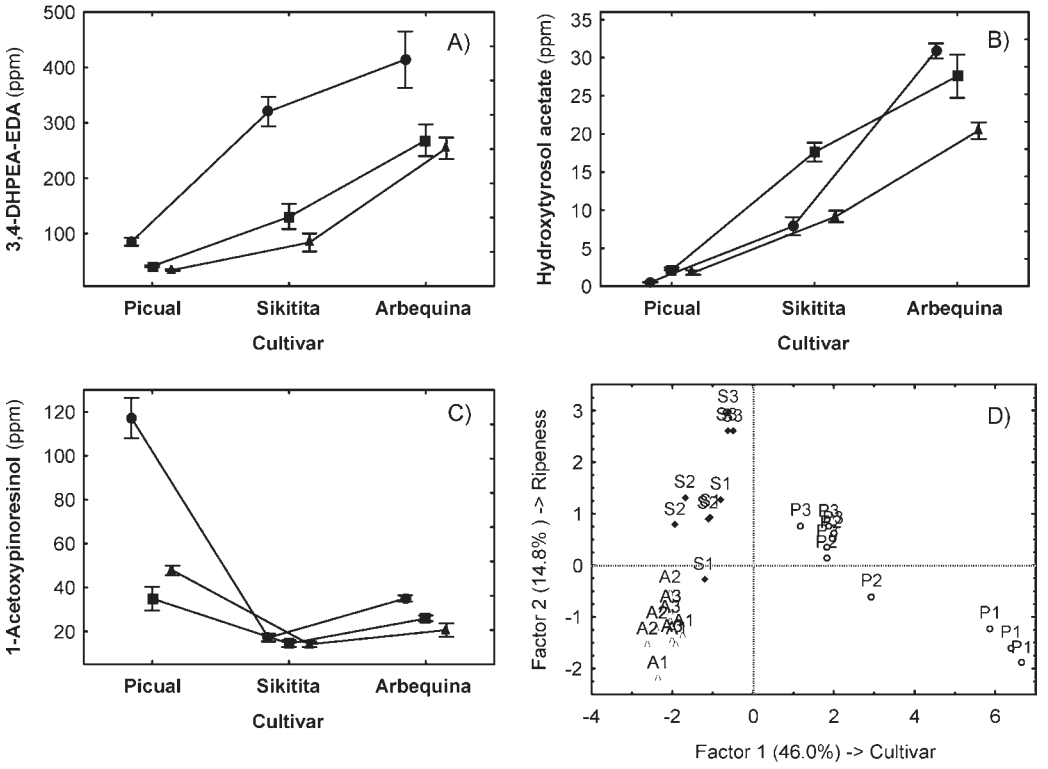

Figure 1. Mean concentrations of three phenols quantified in var. Picual and Arbequina and their progeny Sikitita at three levels of ripeness (**A**−**C**) together with PCA of the whole set of phenols quantified in the samples (**D**). Vertical bars denote 0.95 confidence intervals; ●, second level of ripeness; ■, third level of ripeness; ▲, fourth level of ripeness.

of 3,4-DHPEA-EA (or oleuropein aglycon) was higher in Picual cultivar than in Arbequina and Sikitita, the two latter having similar concentrations. The concentrations, however, decreased dramatically from the first to the second ripeness level in the three cultivars. This decrease of 3,4-DHPEA-EA during ripeness might be due to the increased activity of the hydrolytic enzymes with maturation (*22*).

The similarity in the phenolic profile of Arbequina and Sikitita cultivars is also observed in the dialdehydic form of the decarboxymethyl oleuropein aglycon (3,4-DHPEA-EDA), the concentration of which is high in both cultivars. Furthermore, the aglycon and the dialdehydic form of the decarboxymethyl ligustroside aglycon (*p*-HPEA-EA and *p*-HPEA-EDA) showed the same behavior as the oleuropein. The decrease in the concentrations of secoiridoids as the ripeness progresses has been attributed to the esterase activity that causes the degradation of the oleuropein (*22*).

With regard to simple phenols such as hydroxytyrosol and tyrosol, these compounds are more abundant in oils from Sikitita and Picual cultivars. Within this group, the *o*-diphenols, such as hydroxytyrosol, are among those phenols with significant antioxidant (*11*) and antiatherosclerotic properties (*28*). The trend of simple phenols through the three ripeness levels (e.g., hydroxytyrosol in var. Picual) is not clearly downward like that of the complex phenols, although other authors have described an increase in hydroxytyrosol concentration during ripening (*25*).

In any study characterizing olive oils from single cultivars by phenols, the qualitative differences among them are less evident than quantitative variations (*29*). For that reason, the study was assisted by ANOVA to highlight those factors that significantly influence the concentrations of individual phenols. The result of the analysis of the planned design, 3 (cultivars) × 3 (levels of ripeness) × 3 (repetitions), by ANOVA with Wilks' algorithm showed that there is no significant difference in terms of ripeness ($p = 0.126$), but the concentrations vary in terms of cultivar ($p = 0.020$) and, obviously, cultivar × ripeness ($p = 0.025$).

By analysis of the individual phenols (**Table 2**), three of them, hydroxytyrosol acetate, 3,4-DHPEA-EDA, and 1-acetoxypinoresinol, showed the highest ability in distinguishing cultivars at each of their ripeness levels (**Figure 1A**−**C**), but none of them was able to distinguish cultivars regardless their ripeness levels.

The analysis of the whole data set by PCA confirmed that the phenolic composition of VOOs from var. Sikitita is, with respect to its progenitors, more similar to VOOs from var. Arbequina than from var. Picual (**Figure 1D**). Factor 1 explains the cultivar variability by the negative correlation of the concentrations of dialdehydic form of oleuropein aglycon (−0.83), pinoresinol (−0.88), 1-acetoxypinoresinol (−0.84), and 3,4-DHPEA-EA (−0.92), whereas factor 2 explains the ripeness levels of the harvested olives by means of phenols *p*-HPEA-EDA (−0.79) and 3,4-DHPEA-EDA (−0.73).

If phenols are responsible for VOO tasting perception, volatiles are responsible for VOO aroma. The oils from the cultivars Picual and Arbequina differ in their aromas. Thus, at the same ripeness level, Picual oils are commonly characterized with a green-fresh aroma, whereas Arbequina oils are characterized with green-fruity sensory notes. These peculiarities are due to their different volatile profiles (*9*). **Table 3** shows the concentrations (mean ± SD) of the most remarkable volatiles quantified in the cultivars at the three levels of ripeness, together with their sensory characterization. Many of these compounds are formed through the lipoxygenase pathway, and the activity of the enzymes involved is greatly affected by the variety (*30*). Thirty-four volatiles allow for distinguishing the three cultivars also after applying ANOVA with Wilks' algorithm. Only 29 compounds characterized the ripeness levels, but none of them was able to distinguish the cultivars regardless their levels of ripeness, except *E*-2-hexenal (**Figure 2A**−**C**).

Table 3. Concentrations of Individual Volatiles (Milligrams per Kilogram, Mean ± SD) of Virgin Olive Oils from Cultivars Arbequina and Picual and Their Progeny Sikitita at Three Levels Ripeness of Their Olives[a]

volatile (GC-sniffing)	Arbequina	Sikitita	Picual	volatile (GC-sniffing)	Arbequina	Sikitita	Picual
octane	0.215 ± 0.091	0.134 ± 0.016	0.140 ± 0.021	limonene	0.448 ± 0.032a	0.294 ± 0.019a	0.083 ± 0.004a
(alkane)	0.278 ± 0.131	0.403 ± 0.072a	0.110 ± 0.004a	(lemon)	0.462 ± 0.012	0.366 ± 0.022	0.489 ± 0.404
	0.239 ± 0.051	0.262 ± 0.037	0.171 ± 0.061		0.313 ± 0.013	0.277 ± 0.054	0.294 ± 0.021
E-2-octene	0.035 ± 0.011	0.026 ± 0.002	0.033 ± 0.011	*E*-2-hexenal	10.371 ± 2.036a	3.119 ± 0.716a	0.146 ± 0.029a
(−)	0.009 ± 0.007a	0.032 ± 0.001	0.039 ± 0.007	(bitter almonds)	14.360 ± 1.261a	3.688 ± 0.387a	1.179 ± 0.033a
	0.049 ± 0.008a	0.033 ± 0.006	0.023 ± 0.008		7.803 ± 0.586a	3.470 ± 0.236a	1.531 ± 0.046a
methyl acetate	0.016 ± 0.008	0.022 ± 0.009	0.028 ± 0.005	3-octanone	0.224 ± 0.021a	0.308 ± 0.018a	0.198 ± 0.012a
(ethereal, sweet)	0.034 ± 0.005a	0.013 ± 0.002	0.011 ± 0.004	(mold, green)	0.207 ± 0.022	0.192 ± 0.011	0.174 ± 0.004a
	0.023 ± 0.004	0.023 ± 0.002	0.017 ± 0.005a		0.182 ± 0.016	0.204 ± 0.017	0.170 ± 0.009
ethyl acetate	0.008 ± 0.004	0.007 ± 0.005	0.003 ± 0.002	pentanol	0.017 ± 0.003	0.021 ± 0.002	0.005 ± 0.000a
(sticky)	0.002 ± 0.000a	0.009 ± 0.006	0.006 ± 0.002	(strong, sticky, balsamic)	0.032 ± 0.002	0.007 ± 0.001a	0.021 ± 0.012
	0.006 ± 0.001	0.003 ± 0.002	0.070 ± 0.064		0.013 ± 0.005	0.010 ± 0.002	0.047 ± 0.005a
2-butanone	0.587 ± 0.052a	1.508 ± 0.185a	0.394 ± 0.016a	3-hydroxy-2-butanone	0.035 ± 0.001a	0.043 ± 0.003a	0.015 ± 0.002a
(fragrant, pleasant)	0.607 ± 0.034a	1.122 ± 0.048a	0.260 ± 0.021a	(butter off-flavor)	0.040 ± 0.004	0.031 ± 0.000a	0.034 ± 0.001
	0.362 ± 0.040a	0.780 ± 0.060a	0.186 ± 0.091a		0.031 ± 0.003	0.033 ± 0.004	0.031 ± 0.005
2-methylbutanal	0.077 ± 0.027a	0.021 ± 0.002	0.015 ± 0.006	*E*-2-heptenal	0.061 ± 0.001a	0.070 ± 0.004a	0.020 ± 0.002a
(malty, almond)	0.013 ± 0.002	0.015 ± 0.003	0.005 ± 0.001a	(fatty, green, apple, spicy)	0.068 ± 0.006a	0.052 ± 0.003a	0.034 ± 0.003a
	0.044 ± 0.003a	0.010 ± 0.002a	0.005 ± 0.001a		0.048 ± 0.003	0.040 ± 0.011	0.030 ± 0.002
3-methylbutanal	0.041 ± 0.001a	0.016 ± 0.002	0.011 ± 0.004	*Z*-2-pentenol	0.160 ± 0.013	0.171 ± 0.019	0.046 ± 0.008a
(sweet, fruity)	0.024 ± 0.001a	0.038 ± 0.012a	0.009 ± 0.002a	(alcohol, banana)	0.185 ± 0.018a	0.140 ± 0.010a	0.104 ± 0.009a
	0.016 ± 0.001	0.061 ± 0.021a	0.014 ± 0.004		0.132 ± 0.006	0.127 ± 0.019	0.103 ± 0.008
ethanol	0.063 ± 0.018	0.070 ± 0.025	0.024 ± 0.005a	hexanol	1.925 ± 0.142a	1.105 ± 0.171a	0.251 ± 0.02a
(alcohol)	0.095 ± 0.003a	0.012 ± 0.003a	0.160 ± 0.054a	(fruity, banana, soft)	2.139 ± 0.056a	1.073 ± 0.033a	0.808 ± 0.017a
	0.187 ± 0.032	0.229 ± 0.024	0.365 ± 0.065a		1.619 ± 0.106a	0.938 ± 0.069	0.767 ± 0.031a
ethyl propanoate	0.124 ± 0.016a	0.182 ± 0.001a	0.083 ± 0.005a	*E*-3-hexen-1-ol	0.026 ± 0.002a	0.013 ± 0.001a	0.002 ± 0.000a
(fruity, strong)	0.118 ± 0.013	0.143 ± 0.007a	0.128 ± 0.010	(green)	0.029 ± 0.001a	0.086 ± 0.010	0.091 ± 0.005
	0.093 ± 0.010	0.096 ± 0.011	0.111 ± 0.002		0.015 ± 0.001a	0.104 ± 0.012	0.099 ± 0.003
3-pentanone/pentanal	2.205 ± 0.072	2.905 ± 0.116a	2.065 ± 0.221	*Z*-3-hexen-1-ol	0.089 ± 0.011a	0.046 ± 0.004a	0.017 ± 0.002a
(green, sweet/woody, oily)	2.271 ± 0.195	1.766 ± 0.086a	2.250 ± 0.051	(grass, banana)	0.113 ± 0.007	0.115 ± 0.004	0.134 ± 0.012a
	1.809 ± 0.131	0.880 ± 0.055a	1.791 ± 0.079		0.050 ± 0.003a	0.068 ± 0.006a	0.094 ± 0.004a
2,3-butanodione	0.129 ± 0.017a	0.164 ± 0.005a	0.083 ± 0.006a	nonanal	0.016 ± 0.002	0.019 ± 0.018	0.008 ± 0.004a
(buttery)	0.158 ± 0.010	0.131 ± 0.015	0.070 ± 0.003a	(rancid)	0.006 ± 0.004a	0.029 ± 0.006	0.025 ± 0.006
	0.151 ± 0.013a	0.114 ± 0.010a	0.089 ± 0.003a		0.032 ± 0.004	0.020 ± 0.003a	0.036 ± 0.003
4-methyl-2-pentanone	0.232 ± 0.026	0.279 ± 0.014	0.088 ± 0.012	1-octen-3-ol	0.033 ± 0.001	0.041 ± 0.007	0.008 ± 0.002a
(sweet)	0.383 ± 0.016a	0.253 ± 0.020a	0.108 ± 0.008a	(mushroom, earthy)	0.032 ± 0.008	0.031 ± 0.003	0.029 ± 0.005
	0.253 ± 0.016	0.214 ± 0.018	0.137 ± 0.017a		0.019 ± 0.002	0.018 ± 0.003	0.034 ± 0.002a
1-penten-3-one	0.449 ± 0.041	0.413 ± 0.048	0.176 ± 0.030a	2.4-hexadienal	0.090 ± 0.005a	0.061 ± 0.009a	0.008 ± 0.003a
(pungent, mustard)	0.544 ± 0.052a	0.308 ± 0.027	0.340 ± 0.006	(fresh, green, floral)	0.099 ± 0.010a	0.058 ± 0.001	0.061 ± 0.007
	0.413 ± 0.026a	0.324 ± 0.023	0.312 ± 0.002		0.066 ± 0.009	0.047 ± 0.004a	0.066 ± 0.004
1-propanol	0.035 ± 0.004a	0.046 ± 0.003a	0.010 ± 0.004	*E*-2-hexen-1-ol	0.116 ± 0.015a	0.053 ± 0.008a	0.004 ± 0.000a
(alcohol, pungent)	0.065 ± 0.010	0.068 ± 0.006	0.027 ± 0.002a	(green, grassy, fruit)	0.155 ± 0.011a	0.062 ± 0.004a	0.042 ± 0.009a
	0.051 ± 0.003a	0.032 ± 0.011	0.033 ± 0.001		0.087 ± 0.006a	0.046 ± 0.002a	0.034 ± 0.002a
butyl acetate	0.100 ± 0.011	0.107 ± 0.009	0.032 ± 0.007a	*Z*-2-hexen-1-ol	0.048 ± 0.006a	0.034 ± 0.003a	0.002 ± 0.000a
(over-ripe fruit,	0.132 ± 0.004a	0.114 ± 0.007a	0.048 ± 0.003a	(leaf, green, wine, fruit)	0.064 ± 0.017a	0.038 ± 0.001	0.040 ± 0.006
sweet, banana)	0.092 ± 0.003	0.080 ± 0.008	0.060 ± 0.007a		0.041 ± 0.002a	0.028 ± 0.003a	0.058 ± 0.002a
hexanal	5.396 ± 0.395a	3.092 ± 0.385a	0.589 ± 0.079a	octenal	0.011 ± 0.001a	0.006 ± 0.00a1	0.000 ± 0.000a
(green, strong)	8.338 ± 0.788a	4.214 ± 0.074a	2.335 ± 0.072a	(green, nut, fat)	0.175 ± 0.154	0.013 ± 0.005	0.016 ± 0.006
	5.064 ± 0.132a	3.047 ± 0.314a	2.178 ± 0.101a		0.010 ± 0.003	0.008 ± 0.002	0.006 ± 0.001
2-methyl-3-buten-2-ol	2.816 ± 0.484a	1.130 ± 0.216a	0.141 ± 0.02a	acetic acid	0.097 ± 0.006a	0.062 ± 0.005	0.066 ± 0.009
(woody, oily)	1.526 ± 1.230	0.630 ± 0.266	0.161 ± 0.111a	(sour, vinegary)	0.082 ± 0.074	0.143 ± 0.014	0.137 ± 0.003
	1.520 ± 0.527	1.164 ± 0.118	0.780 ± 0.099a		0.134 ± 0.074	0.132 ± 0.045	0.099 ± 0.042

Table 3. Continued

volatile (GC-sniffing)	Arbequina	Sikitita	Picual	volatile (GC-sniffing)	Arbequina	Sikitita	Picual
2-methyl-1-propanol	0.003 ± 0.000	0.002 ± 0.000 6	0.002 ± 0.000	octanal	0.115 ± 0.014	0.109 ± 0.004	0.033 ± 0.004a
(winey)	0.100 ± 0.097	0.166 ± 0.078	0.026 ± 0.021a	(fat, soap, lemon, green)	0.072 ± 0.006a	0.147 ± 0.010	0.173 ± 0.015
	0.244 ± 0.011a	0.060 ± 0.00	0.076 ± 0.009		0.028 ± 0.011a	0.073 ± 0.019a	0.122 ± 0.010a
Z-3-hexenal	0.082 ± 0.005a	0.052 ± 0.005a	0.037 ± 0.003a	isobutyric acid	0.096 ± 0.010	0.105 ± 0.005	0.027 ± 0.004a
(leaf, green)	0.098 ± 0.005a	0.074 ± 0.012a	0.074 ± 0.002a	(cheesy, fruity)	0.024 ± 0.011a	0.122 ± 0.010	0.146 ± 0.011
	0.069 ± 0.008	0.056 ± 0.008	0.054 ± 0.003		0.012 ± 0.002a	0.056 ± 0.018a	0.118 ± 0.012a
1-butanol	0.879 ± 0.122	0.888 ± 0.028	0.209 ± 0.053a	total volatiles	29.328 ± 2.805a	18.883 ± 2.007a	5.889 ± 0.558a
(medicine, fruit)	0.013 ± 0.012	0.892 ± 0.066a	1.001 ± 0.057		35.010 ± 0.209a	18.751 ± 0.938a	12.982 ± 0.418a
	0.101 ± 0.013a	0.572 ± 0.172a	1.396 ± 0.130a		22.812 ± 1.405a	15.248 ± 1.385	14.333 ± 0.544
1-penten-3-ol	0.433 ± 0.028	0.416 ± 0.005	0.092 ± 0.012a				
(green, light)	0.280 ± 0.004	0.300 ± 0.025	0.231 ± 0.024a				
	0.190 ± 0.008	0.238 ± 0.064	0.379 ± 0.025a				

[a] For entries followed by "a", there is a significant difference between the Sikitita variety and the other two ($p \leq 0.05$).

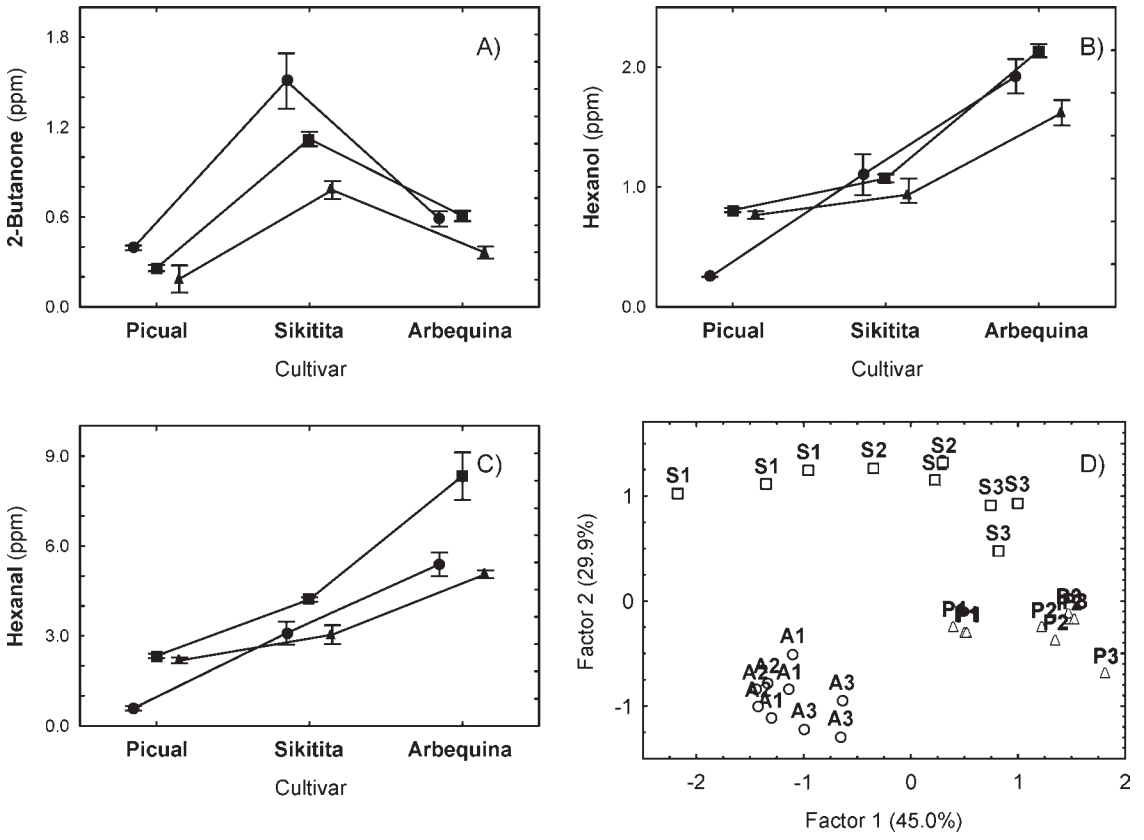

Figure 2. Mean concentrations of three volatiles quantified in var. Picual and Arbequina and their progeny Sikitita at three levels of ripeness (**A**−**C**) together with PCA of a selected set of volatiles (**Table 3**) quantified in the samples. Vertical bars denote 0.95 confidence intervals; ●, second level of ripeness; ■, third level of ripeness; ▲, fourth level of ripeness.

In general terms, the volatile composition of oils from the Sikitita cultivar was more similar to that of Picual oils than to that of Arbequina oils, overall at the first level of ripeness. The oils from Picual cultivar are characterized with low concentrations of *E*-2-hexenal and also of total volatiles, which agrees with previous studies characterizing this variety (*9*). On the contrary, the concentration of *E*-2-hexenal was up to 10 times higher in oils from var. Arbequina, which agrees with Gómez-Rico et al. (*25*), whereas the Sikitita cultivar was closer to Picual with regard to the concentration of this compound. This compound contributes to an aroma with green and bitter almond sensory notes and a bitter taste (*30*). The evolution of *E*-2-hexenal through the three ripeness levels is different from that for most of the volatile compounds. Thus, the *E*-2-hexenal concentration increases in oils from Picual and Sikitita cultivars, whereas it decreases in Arbequina. The increment of concentration through the ripening process agrees with the results of previous experiments (*30*). Low concentrations of hexanal, another aldehyde, characterize the oils from Picual and Sikitita cultivars in comparison with Arbequina. This compound is also characterized with a green aroma. Hexanal is among the volatile compounds that vary during maturation and characterizes the ripeness levels (*30*).

The concentration in the three varieties followed the same trend. Thus, the concentration of hexanal increases at the second ripeness level and decreases in the third. With regard to flavor, the similarity between Sikitita and Picual is supported by other compounds such as *E*-2-hexenal, hexanol, and *E*-2-hexen-1-ol. For all of the volatile compounds, except 2-butanone, the concentrations corresponding to Sikitita oils are between the values for Picual and Arbequina oils. Nevertheless, the concentration of 2-butanone for Sikitita oils was higher than for the oils from its two parental cultivars. This compound is characterized by a fragrant and pleasant aroma.

The similarities between the three varieties with regard to the volatile composition were studied by PCA. A PCA plot (**Figure 2D**) was obtained with those volatile compounds that show significant differences between the Sikitita variety and the other two ($p \leq 0.05$) (**Table 3**). Factor 1 (45% explained variance) is related to the ripeness of the samples, which is more noticeable in Sikitita and Picual samples. On the other hand, the variance explained by factor 2 (29.9%) allows for classifying samples between Sikitita cultivar and the other two.

These results generally agree with previous works in which VOOs from diverse cultivars and ripeness levels were independently characterized by volatiles (*9*, *17*). Contrary to phenols, not all of the volatile concentrations diminish with ripeness, but they show different trends and evolutions depending on the cultivar (*19*, *30*).

In conclusion, according to individual phenols the oils from Sikitita cultivar are closer to oils from Arbequina cultivar, whereas oils from var. Sikitita are more similar to oils from Picual cultivar with regard to volatile compounds, the similarity being more remarkable at the first level of ripeness. These results provide the chemical basis for a further study on the sensory assessment of var. Sikitita in comparison with its two progenitors, Arbequina and Picual.

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LITERATURE CITED

(1) Rallo, L. Selection and breeding of olive in Spain. *Olivae* **1995**, *59*, 46–53.

(2) Hatzopoulos, P.; Banilas, G.; Giannoulia, K.; Gazis, F.; Nikoloudakis, N.; Milioni, D.; Haralampidis, K. Breeding, molecular markers and molecular biology of the olive tree. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 574–586.

(3) León, L.; De la Rosa, R.; Gracia, A.; Barranco, D.; Rallo, L. Fatty acid composition of advanced olive selections obtained by cross-breeding. *J. Sci. Food Agric.* **2008**, *88*, 1921–1926.

(4) López-Miranda, J.; Badimón, L.; Bonanome, A.; Lairon, D.; Kris Etherton, P. M.; Mata, P.; et al. Monounsaturated fat and cardiovascular risk. *Nutr. Rev.* **2006**, *64*, S2–S12.

(5) García-González, D. L.; Aparicio-Ruiz, R.; Aparicio, R. Olive oil. In *Gourmet and Health-Promoting Oils*; Moreaou, R. A., Kamal-Eldin, A., Eds.; AOCS Press: Champaign, IL, 2009; pp 33−72.

(6) Ranalli, A.; Contento, S.; Marchegiani, D.; Pardi, Di.; Pardi, D.; Girardi, F. Effects of genetic store on the composition and typicality of extra-virgin olive oil: traceability of new products. *Adv. Hortic. Sci.* **2008**, *22*, 110–115.

(7) Baccouri, B.; Ben Temime, S.; Taamalli, W.; Daoud, D.; M'Sallem, M.; Zarrouk, M. Analytical characteristics of virgin olive oils from two new varieties obtained by controlled crossing on Meski variety. *J. Food Lipids* **2007**, *14*, 19–34.

(8) Manai, H.; Haddada, F. M.; Trigui, A.; Daoud, D.; Zarrouk, M. Compositional quality of virgin olive oil from two new Tunisian cultivars obtained through controlled crossings. *J. Sci. Food Agric.* **2007**, *87*, 600–606.

(9) Luna, G.; Morales, M. T.; Aparicio, R. Characterisation of 39 varietal virgin olive oils by their volatile compositions. *Food Chem.* **2006**, *98*, 243–252.

(10) Servili, M.; Montedoro, G. Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 602–613.

(11) Visioli, F.; Bogani, P.; Grande, S.; Galli, C. Mediterranean food and health: building human evidence. *J. Physiol. Pharmacol.* **2005**, *56*, 37–49.

(12) Skevin, D.; Rade, D.; Strucelj, D.; Mokrovcak, Z.; Nederal, S.; Bencic, D. The influence of variety and harvest time on the bitterness and phenolic compounds of olive oil. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 536–541.

(13) Rallo, L.; Barranco, D.; De La Rosa, R.; León, L. 'Chiquitita' olive. *HortScience* **2008**, *43*, 529–531.

(14) Frías, L.; García-Ortiz, A.; Hermoso, M.; Jiménez, A.; Llavero Del Pozo, M. P.; Morales, J. et al. *Analistas de Laboratorio de Almazara*; Junta de Andalucía: Sevilla, Spain, 1991.

(15) Mateos, R.; Espartero, J. L.; Trujillo, M.; Ríos, J. J.; León Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185–2192.

(16) García-González, D. L.; Tena, N.; Aparicio, R. Characterization of olive paste volatiles to predict the sensory quality of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 663–672.

(17) Tena, N.; Lazzez, A.; Aparicio-Ruiz, R.; García-González, D. L. Volatile compounds characterizing Tunisian Chemlali and Chétoui virgin olive oils. *J. Agric. Food Chem.* **2007**, *55*, 7852–7858.

(18) Morales, M. T.; Luna, G.; Aparicio, R. Comparative study of virgin olive oil sensory defects. *Food Chem.* **2005**, *91*, 293–301.

(19) Aparicio, R. Characterization: mathematical procedures for chemical analysis. In *Handbook of Olive Oil: Analysis and Properties*; Harwood, J., Aparicio, R., Eds.; Aspen Publications: Gaithersburg, MD, 2000; pp 285−353.

(20) García-González, D. L.; Luna, G.; Morales, M. T.; Aparicio, R. Stepwise geographical traceability of virgin olive oils by chemical profiles using artificial neural network models. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 1003–1013.

(21) Ripa, V.; De Rose, F.; Caravita, M. A.; Parise, M. R.; Perri, E.; Rosati, A.; Pandolfi, S.; Paoletti, A.; Pannelli, G.; Padula, G.; Giordani, E.; Bellini, E.; Buccoliero, A.; Mennone, C. Qualitative evaluation of olive oils from new olive selections and effects of genotype and environment on oil quality. *Adv. Hortic. Sci.* **2008**, *22*, 95–103.

(22) Baccouri, O.; Guerfel, M.; Baccouri, B.; Cerretani, L.; Bendini, A.; Lercker, G.; Zarrouk, M.; Ben Miled, D. D. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chem.* **2008**, *109*, 743–754.

(23) Serrilli, A. M.; Padula, G.; Ripa, V.; Petrosino, L.; Bianco, A. Searching new olive (*Olea europaea* L.) cultivars: analysis of phenolic fraction in olive selections derived from a breeding program. *Adv. Hortic. Sci.* **2008**, *22*, 104–109.

(24) García-González, D. L.; Tena, N.; Morales, M.; Aparicio, R. *Olive Oil Quality and Irrigated Olive Tree Orchards*; International Symposium on Olive Irrigation and Oil Quality, Abstract Book, Israeli Agricultural Research Organization (ARO), Nazareth, Israel, 2009 (http://www.olive-irrigation-symposium.org/)

(25) Gómez-Rico, A.; Fregapane, G.; Salvador, M. D. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Res. Int.* **2008**, *41*, 433–440.

(26) Caponio, F.; Gomes, T.; Pasqualone, A. Phenolic compounds in virgin olive oils: Influence of the degree of olive ripeness on organoleptic characteristics and shelf-life. *Eur. Food Res. Technol.* **2001**, *212*, 329–333.

(27) Morales, M. T.; Tsimidou, M. The role of volatile compounds and polyphenols in olive oil sensory quality. In *Handbook on Olive Oil: Analysis and Properties*; Harwood, J., Aparicio-Ruiz, R., Eds.; Aspen: Gaithersburg, MD, 2000; pp 393−458.

(28) Miles, E. A.; Zoubouli, P.; Calder, P. C. Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. *Nutrition* **2005**, *21*, 389–394.

(29) Andjelkovic, M.; Van Camp, J.; Pedra, M.; Renders, K.; Socaciu, C.; Verhe, R. Correlations of the phenolic compounds and the phenolic content in some Spanish and French olive oils. *J. Agric. Food Chem.* **2008**, *56*, 5181–5187.

(30) Aparicio, R.; Morales, M. T. Characterization of olive ripeness by green aroma compounds of virgin olive oil. *J. Agric. Food Chem.* **1998**, *46*, 1116–1122.

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