DOI:10.1021/jf100497c

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Evolution of Sesquiterpene Hydrocarbons in Virgin Olive Oil during Fruit Ripening

Stefania Vichi,*,† Aida Lazzez,‡ Naziha Grati Kamoun,‡ Elvira López-Tamames,† and Susana Buxaderas†

†Departament de Nutrició i Bromatologia, Xarxa de Referència en Tecnología dels Aliments (XaRTA), Facultat de Farmàcia, Universitat de Barcelona, Avda Joan XXIII, s/n, 08028, Barcelona, Spain, and ‡Unité Technologie et Qualité Institut de l'Olivier Route de l'aéroport, Km 1.5 B. P. 1087 3000 Sfax, Tunisia

Despite the potential of sesquiterpene hydrocarbons in olive oil authentication, their metabolism in *Olea europaea* is poorly understood, and little is known about their biochemical regulation in olives as a function of ripening. To ascertain some metabolic relationships between sesquiterpene hydrocarbons and olive ripening, the content of sesquiterpene hydrocarbons was assessed in virgin olive oils from two olive varieties grown in the same geographical area and produced at different harvesting periods. During the ripening, the accumulation of sesquiterpenes in the olive itself, and thus in the oil, differed according to their molecular structure: bicyclic sesquiterpenes, showed decreasing concentrations the later the harvest, while acyclic farnesene-like compounds progressively increased through the olive ripening process. This is first evidence that the accumulation of sesquiterpene hydrocarbons in olive, and hence in olive oil, is modulated during ripening. Therefore, the degree of ripening of olives should be taken into consideration when considering the sesquiterpenic profile of virgin olive oil for their authentication.

KEYWORDS: Virgin olive oil; sesquiterpene hydrocarbons; ripening; SPME

INTRODUCTION

Sesquiterpenes are widespread secondary plant metabolites that exhibit a wide range of biological properties from herbivore defense to signaling infochemicals in plant−insect interactions (*1*−*4*). Their biosynthesis in various plant organs including leaves, roots, and fruit (*5*) is developmentally regulated and takes place in response to both biotic and abiotic factors (*6*). In *Olea europaea*, the amounts and type of sesquiterpenes vary markedly between the leaves and olive fruits (*7*), and the metabolic relationship between the various parts and sesquiterpene hydrocarbon content is poorly understood. The changes that occur in olives are directly reflected in the composition of virgin olive oil, and interest in this area is related to the importance of sesquiterpenes in virgin olive oil authentication. Recent results indicate that sesquiterpene hydrocarbons could be suitable markers of virgin olive oil varieties and geographical areas of production (*8*−*12*). In comparison to other volatile substances found in olive oil, the presence of terpenic hydrocarbons should be relatively less influenced by technological factors and determined mainly by the variety and growing conditions of the olive trees (*11*). Nevertheless, as occurs for other metabolites, the accumulation of sesquiterpenes in the olive itself, and thus in the oil, can also be influenced by the ripening process. Evidence of developmental regulation of sesquiterpenes in some plants has been reported (*13*, *14*), in particular regarding specifically expressed terpene synthases at particular stages of fruit development or ripening (*6*). Despite the potential of terpenoid volatiles in olive oil authentication, little is known about the biochemical and molecular regulation of terpenoids in olives as a function of ripening. A recent study of gene expression patterns in *O. europaea* fruits reported that transcripts involved in terpenoid biosynthesis and in responses to biotic and abiotic stress are mainly expressed at early stages of fruit development (*15*). To the best of our knowledge no data are available on the relationship between olive maturation and the content of sesquiterpene hydrocarbons in the oil.

With the aim to evaluate the effect of olive ripening on the use of sesquiterpene hydrocarbons for virgin olive oil authentication and to ascertain some metabolic relationships between sesquiterpene hydrocarbons and olive development, the content of sesquiterpene hydrocarbons was assessed in virgin olive oils from two olive varieties grown in the same geographical area and produced at different harvesting periods.

MATERIALS AND METHODS

Reagents and Materials. The SPME fiber used was divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm, 2 cm long (DVB/CAR/PDMS), from Supelco (Bellefonte, PA).

Chemicals. Farnesene (isomer mixture) was from TCI (Tokyo, Japan). Indene, cyclosativene, α-copaene, and β-caryophyllene were from Sigma-Aldrich (St. Louis, MO).

Olive Oil Sampling. Thirty oil samples from Olive Tree Institute Experimental field Taous (Sfax-Tunisia) were obtained in 2008/2009 from two main Tunisian varieties: Chemlali (n = 18) and Chetoui (n = 12),

*Corresponding author. Phone: +34 93 4024508. Fax: +34 93 4035931. E-mail: stefaniavichi@ub.edu.

 Published on Web 05/10/2010

Table 1. Maturity Index (MI) of Chemlali and Chetoui Olives at the Distinct Collection Dates

harvest date	Chemlali	MI	Chetoui	MI
22/10/2008	1	1.5	1	0.8
	2	1.5	2	1.0
	3	1.8	3	1.0
11/11/2008	4	1.9	4	1.3
	5	2.0	5	1.4
	6	2.0	6	1.5
27/11/2008	7	2.5	7	2.0
	8	2.2	8	1.9
	9	2.7	9	1.8
10/12/2008	10	4.5	10	3.1
	11	5.3	11	2.9
	12	4.9	12	2.9
06/01/2009	13	5.5		
	14	5.4		
	15	5.4		
03/02/2009	16	6.0		
	17	6.2		
	18	6.5		

during six and four different harvesting periods, respectively. At each sampling point, three olive oils were extracted from olives harvested from three different plants of comparable age and vigor and located in distinct points of the same parcel. The maturation index was determined according to the "Estación de Olivicultura de Jaén" (*16*) and is reported in **Table 1**. Five kilograms of olives from each tree was processed by a pilot extraction plant Abencor MC2 (Comercial Abengoa S.A., Sevilla, Spain) equipped with a hammer crusher, a paste beater, and a pulp centrifuge. Malaxation was carried out at 28 °C for 30 min. The virgin olive oils thus obtained were then decanted, bottled, and stored in the dark at 4 °C until analysis, which were carried out in duplicate. All of the samples were classified as extra virgin olive oils according to the EU regulations (*17*, *18*).

Solid-Phase Microextraction (SPME). SPME was performed as described by Vichi et al. (*12*). Briefly, 2 g of oil spiked with indene (internal standard) was weighed into a 10 mL vial fitted with a silicone septum and placed into a silicone oil bath at 70 °C where the oil was magnetically stirred (700 rpm). After 10 min of sample conditioning, a DVB/CAR/PDMS fiber was exposed for 60 min to the sample headspace and immediately desorbed in the gas chromatograph injector. Each extraction was performed in duplicate.

Gas Chromatography/Mass Spectrometry (GC/MS). Identification and quantification of compounds were performed by gas chromatography coupled to quadrupolar mass selective spectrometry using an Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA). Analytes were separated on Supelcowax-10 (Supelco) and on DB-1 (J&W Scientific, Agilent Technologies, Palo Alto, CA) capillary columns, both 60 m × 0.25 mm i.d. and 0.25 μm film thickness. The column temperature was held at 60 °C for 3 min, increased to 160 °C at 3 °C/min and then to 270 °C at 15 °C/min, and held for 5 min. The injector temperature was 260 °C, and the time of desorption of the fiber into the injection port was fixed at 5 min. Helium was the carrier gas at a linear velocity of 38 cm/s. The temperature of the ion source was 175 °C and of the transfer line 280 °C. Electron impact mass spectra were recorded at 70 eV ionization energy, two scans/s.

GC-MS analysis in the complete scanning mode (SCAN) in the 40−300 unit mass range was performed to identify compounds in the oil samples. Quantitative assessment of sesquiterpene hydrocarbons was carried out in the selected ion monitoring (SIM) by analyzing the following ions: m/z 93, 115, 119, 69, 161, 157, 159, 204, and 202.

Characterization of Sesquiterpene Hydrocarbons in Virgin Olive Oil. Compounds were identified by comparison of their mass spectra and retention times with those of standard compounds or by comparison of the mass spectrum with those in the mass spectrum library, Wiley sixth. Nonisothermal Kovats retention indices, using the definition of Van den Dool and Kratz, were calculated and compared with those available in the literature.

Response factors of standard sesquiterpene hydrocarbons were calculated using a calibration curve by analyzing deodorized sunflower oil with different concentrations of reference compounds. Standard solutions were prepared in the range 0.1−50 mg/kg and analyzed in duplicate under the same conditions described for the samples. The internal standard (indene) concentration in the samples was maintained at 10 μg/kg.

Statistics. Data were analyzed using the package Statgraphics Plus 5.1. Simple regression was applied to relate the concentration of sesquiterpene hydrocarbons in the oils to the time of olive harvesting.

RESULTS AND DISCUSSION

Table 2 reports the sesquiterpene hydrocarbons detected in virgin olive oils from Chemlali and Chetoui olive varieties. Sesquiterpene concentrations were calculated using the α-copaene response factor when the reference compound was not available and ranged between undetectable amounts to approximately 133 mg/kg.

The inter-tree variability in sesquiterpene amounts at each sampling point depended on the compound analyzed and ranged from 1% to more than 50% in a few cases (**Figures 1** and **2**); however, the relative standard deviation (RSD) was in general lower than 10% (**Table 2**). This inter-tree variability could not be related to the small differences in the maturation index of the samples reported in **Table 1**.

The amounts of all of the detected sesquiterpenes were higher in oils from Chetoui olives than in those from Chemlali olives produced in the same area (**Table 2**). In particular, Chetoui oils were characterized by much higher levels of α-bergamotene. Other sesquiterpenes, such as cyclosativene, α-copaene, and α-muurolene, showed quite similar concentrations in the two oil varieties obtained on each harvesting date (**Figure 1**). Furthermore, these compounds presented rather high inter-tree variability: above 30% for Chemlali oils and above 15% for Chetoui oils.

Two groups of sesquiterpenes could be distinguished in the oils according to their molecular structure and their behavior during ripening: bicyclic sesquiterpenes, mostly characterized by a cadalane backbone, in most cases showed decreasing concentrations the later the harvest (**Figure 1**), while acyclic sesquiterpenes, with a farnesane skeleton, progressively increased through the olive ripening process (**Figure 2**). The only cyclic sesquiterpene found to increase during ripening (**Figure 2**) was the tentatively identified ar-curcumene. The correlation between the amount of these sesquiterpenes in the oil and the harvesting period was significant in most of the cases, except for compounds **2**, **3**, and **14** in Chetoui oils, for which the harvesting period was shorter than for Chemlali oils (**Figure 1**). On the other hand, the amounts of β-caryophyllene and β-sesquiphellandrene were not significantly correlated to the olive ripening process in either variety.

Acyclic sesquiterpenes such as α-farnesene have already been associated with late stages in the ripening process in plants other than *O. europaea*. In particular, the relationship between ripening-related ethylene synthesis and α-farnesene accumulation is well documented in apples and pears (*13*, *19*−*21*). Conversely, no previous data relating other farnesene-like sesquiterpenes to fruit maturity are currently available. Although no evidence of developmental regulation of sesquiterpene synthases has been found in olives, genes encoding farnesyl pyrophosphate synthase were reported to be highly expressed at later stages of olive development (*15*). This could be in accordance with the increase of acyclic sesquiterpenes documented in this study. On the other hand, the regulation of some plant isoprenoids by abiotic factors such as temperature has been demonstrated (*22*, *23*). This may correlate with the evolution of cyclic sesquiterpenes mentioned above, considering that environmental stress factors, such as temperature or UV emission, decrease over the course of the harvesting period. Although the data available do not allow us to

Table 2. Characterization of Sesquiterpene Hydrocarbons in Virgin Olive Oils, with Relative Standard Deviation (%) Calculated on Five Replicates, and Response Factor Used for Quantification of Each Compound[a]

							concn (mg/kg)	
		ion[b]	ID[c]	KI WAX[d]	KI-DB1[e]	RSD[f]	Chemlali oils min−max	Chetoui oils min−max
1	α-cubebene	161	*g, h*	1455	1351	5.5	0.1 ± 0.01−0.3 ± 0.04[i]	0.3 ± 0.0−1.0 ± 0.2
2	cyclosativene	161	*g, h, j*	1482	1372	5.4	1.0 ± 0.4−2.4 ± 1.2[k]	2.9 ± 0.4−3.3 ± 0.5
3	α-copaene	161	*g, h, j*	1490	1379	4.1	11.0 ± 4.4−21.1 ± 10.2[i]	23.5 ± 4.4−27.6 ± 4.2
4	ni[l] sesquiterpene	119		1530	1389	2.4	0.1 ± 0.02−0.3 ± 0.07[i]	9.7 ± 4.5−16.1 ± 2.2
5	α-bergamotene	119	*g, h*	1583	1442	3.5	0.2 ± 0.1−0.9 ± 0.4[i]	12.2 ± 6.9−25.0 ± 3.9
6	calarene	161	*g, h*	1595		3.5	0.4 ± 0.1−1.0 ± 0.3[i]	1.0 ± 0.1−1.9 ± 0.4
7	β-caryophyllene	93	*g, h, j*	1602	1427	6.4	3.5 ± 0.4−9.0 ± 0.2[m]	5.8 ± 1.0−8.1 ± 0.5
8	(*Z*)-β-farnesene	69	*g, h, j*	1646		4.7	3.3 ± 0.2−7.5 ± 0.4[i]	2.2 ± 0.9−6.3 ± 4.9
9	(*E*)-β-farnesene	69	*g, h, j*	1662	1456	4.2	0.4 ± 0.03−1.7 ± 0.03[i]	0.7 ± 0.3−1.3 ± 0.2
10	ni sesquiterpene	119		1683		7.0	0.0 ± 0.03−0.2 ± 0.02[i]	0.5 ± 0.1−1.2 ± 0.3
11	(*Z,Z*)-α-farnesene	93	*g, h, j*	1688		4.3	0.1 ± 0.04−2.8 ± 0.14[i]	0.6 ± 0.2−2.7 ± 0.9
12	γ-muurolene	161	*g, h*	1692	1479	3.5	0.3 ± 0.00−0.7 ± 0.1[i]	1.0 ± 0.1−2.2 ± 0.3
13	(*Z,E*)-α-farnesene	93	*g, h, j*	1724		5.1	0.6 ± 0.00−1.3 ± 0.1[i]	1.4 ± 0.3−2.7 ± 1.3
14	α-muurolene	161	*g, h*	1732	1498	2.4	0.1 ± 0.5−7.2 ± 0.8[i]	1.6 ± 0.7−6.9 ± 0.5
15	(*E,E*)-α-farnesene	93	*g, h, j*	1752	1499	3.3	1.4 ± 0.4−133.1 ± 3.8[i]	18.4 ± 6.8−119.5 ± 37.6
16	δ-cadinene	161	*g, h*	1767	1522	14.9	1.0 ± 0.2−2.3 ± 0.2[i]	2.3 ± 0.1−4.6 ± 0.9
17	β-sesquiphellandrene	161	*g, h*	1777		5.4	0.0 ± 0.01−0.1 ± 0.02[i]	1.0 ± 0.1−2.0 ± 0.2
18	ar-curcumene	119	*g, h*	1780	1478	9.6	2.1 ± 0.4−6.2 ± 0.8[i]	6.7 ± 1.0−15.3 ± 1.8
19	allofarnesene isomer	93	*g*	1820	1548	15.0	0.0 ± 0.01−0.7 ± 0.3[i]	0.1 ± 0.1−0.9 ± 0.1
20	calamenene	159	*g, h*	1846	1518	6.5	1.2 ± 0.6−2.4 ± 0.8[i]	2.4 ± 0.2−4.5 ± 1.1
21	allofarnesene isomer	93	*g*	1854	1565	4.6	0.0 ± 0.0−1.2 ± 0.2[i]	0.2 ± 0.03−1.3 ± 0.2
22	(*Z*)2,(*E*)4,(*E*)6-allofarnesene	93	*g, h*	1894	1588	5.5	0.1 ± 0.02−4.7 ± 0.4[i]	0.7 ± 0.02−4.3 ± 0.4
23	(*E*)2,(*Z*)4,(*E*)6-allofarnesene	93	*g, h*	1929	1612	8.6	0.1 ± 0.06−5.1 ± 0.6[i]	0.7 ± 0.2−4.7 ± 0.7
24	calacorene	157	*g, h*	1934		12.8	0.3 ± 0.0−0.5 ± 0.04[i]	0.6 ± 0.2−1.0 ± 0.1

[a] Concentrations of sesquiterpenes in oils, determined after separation on a Supelcowax capillary column, are expressed as minimum and maximum values during the olive ripening ± the standard deviation due to the inter-tree variability. [b] Ion: ion used for quantification. [c] ID: identification method. [d] KI WAX: Kováts' indices on Supelcowax capillary column. [e] KI-DB1: Kováts' indices on DB-1 capillary column. [f] RSD: relative standard deviation (%). [g] Tentatively identified by mass spectra. [h] Tentatively identified by retention indices. [i] Quantified using α-copaene response factor. [j] Identified by comparison with standard compounds. [k] Quantified using cyclosativene response factor. [l] ni: not identified. [m] Quantified using β-caryophyllene response factor.*i*

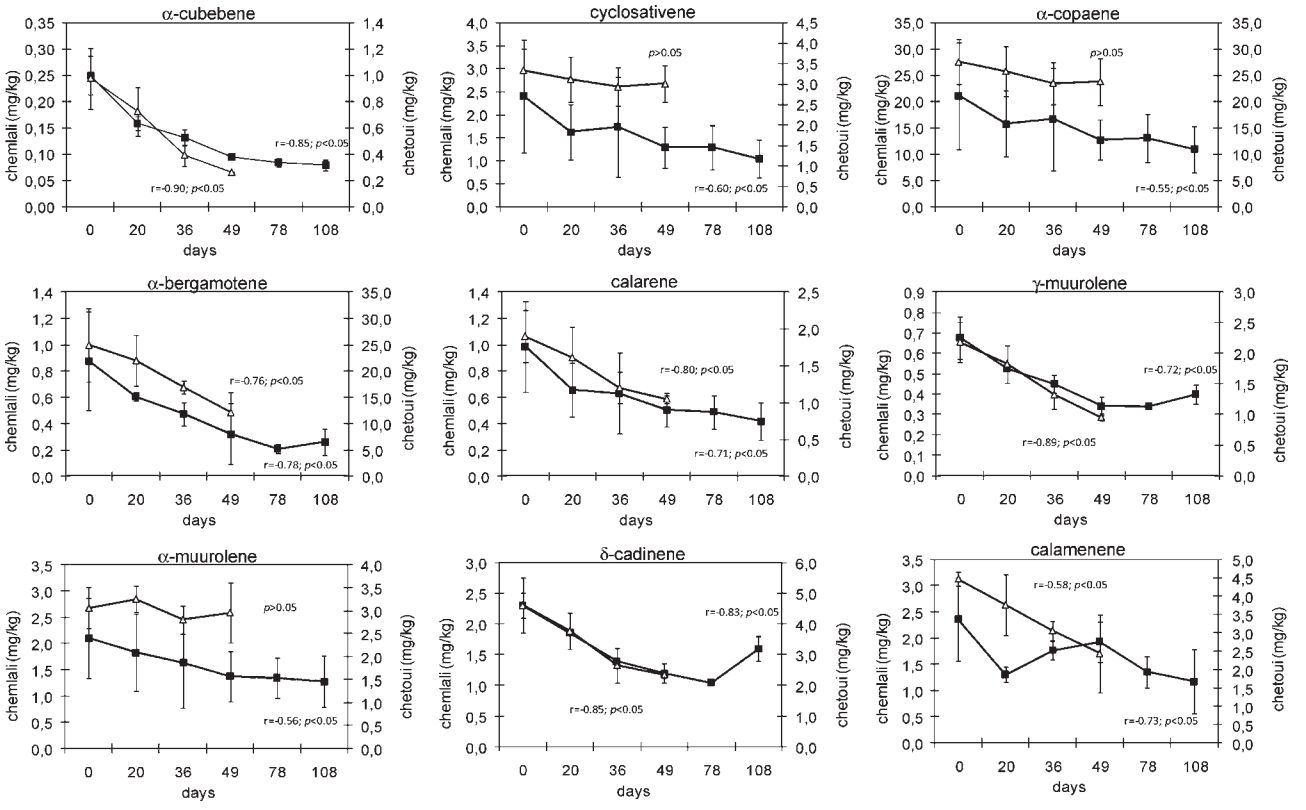

Figure 1. Decreasing evolution of cyclic sesquiterpenes identified or tentatively identified in virgin olive oils from Chemlali (■) and Chetoui (Δ) olives harvested in different periods. The harvest dates are represented as the number of days after the first collection date (22/10/2008). The linear correlation between the amount of sesquiterpenes and time (coefficient of regression, *r*) and its significance (*p* values) are reported for each compound.

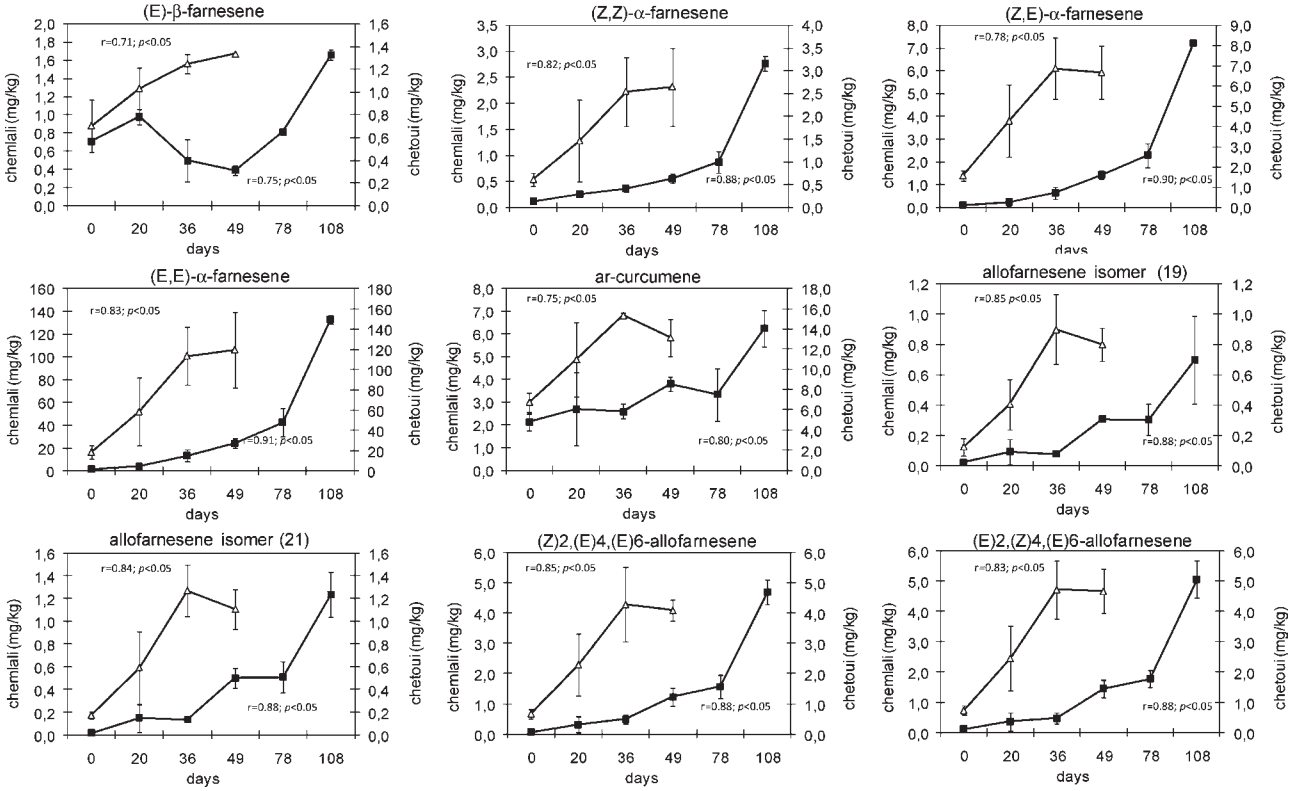

Figure 2. Increasing evolution of farnesane-like sesquiterpenes and ar-curcumene identified or tentatively identified in virgin olive oils from Chemlali (■) and Chetoui (Δ) olives harvested in different periods. The harvest dates are represented as the number of days after the first collection date (22/10/2008). The linear correlation between the amount of sesquiterpenes and time (coefficient of regression, *r*) and its significance (*p* values) are reported for each compound.

ascertain the existence of such a relationship, they represent a starting point for further research.

These results represent the first evidence that the accumulation of sesquiterpene hydrocarbons in olive, and hence in olive oil, is modulated during ripening, as occurs with other olive secondary metabolites. The role of distinct classes of sesquiterpenes in olive metabolism is largely unknown, but in view of these results it can be assumed that the accumulation of cyclic and acyclic sesquiterpene hydrocarbons in olives undergoes different regulation.

From the point of view of olive oil authentication, since the accumulation of distinct classes of sesquiterpenes significantly varies during the maturation process, the degree of ripening of olives should be taken into account when considering the sesquiterpenic profile of virgin olive oil. On the basis of these first results concerning sesquiterpene evolution during olive maturation, the proportions of the distinct sesquiterpenes in virgin olive oil could be studied in a large number of samples as potential markers of olive ripeness.

ACKNOWLEDGMENT

Special thanks to the technical staff of the research unit "Technology and Quality" at the Olive Tree Institute Arous M.N, Hbaieb M, who helped us to carry out this research project.

LITERATURE CITED

(1) Chen, F.; Tholl, D.; D'Auria, J. C.; Farooq, A.; Pichersky, E.; Gershenzon, J. Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *Plant Cell* **2003**, *15*, 481–494.

(2) Keeling, C. I.; Bohlmann, J. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytol.* **2006**, *170*, 657–675.

(3) Kessler, A.; Baldwin, I. T. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **2001**, *291*, 2141–2144.

(4) Wink, M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* **2003**, *64*, 3–19.

(5) Göpfert, J. C.; MacNevin, G.; Ro, D. K.; Spring, O. Identification, functional characterization and developmental regulation of sesquiterpene synthases from sunflower capitate glandular trichomes. *BMC Plant Biol.* **2009**, *9*, 86–103.

(6) Tholl, D. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr. Opin. Plant Biol.* **2006**, *9*, 297–304.

(7) Flamini, G.; Cioni, P. L.; Morelli, I. Volatiles from leaves, fruits, and virgin oil from *Olea europaea* Cv. Olivastra Seggianese from Italy. *J. Agric. Food Chem.* **2003**, *51*, 1382–1386.

(8) Guinda, A.; Lanzón, A.; Albi, T. Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. *J. Agric. Food Chem.* **1996**, *44*, 1723–1726.

(9) Bortolomeazzi, R.; Berno, P.; Pizzale, L.; Conte, L. S. Sesquiterpene, alkene, and alkane hydrocarbons in virgin olive oils of different varieties and geographical origins. *J. Agric. Food Chem.* **2001**, *49*, 3278–3283.

(10) Zunin, P.; Boggia, R.; Lanteri, S.; Leardi, R.; De Andreis, R.; Evangelisti, F. Direct thermal extraction and gas chromatographic−mass spectrometric determination of volatile compounds of extra-virgin olive oils. *J. Chromatogr. A* **2004**, *1023*, 271–276.

(11) Zunin, P.; Boggia, R.; Salvadeo, P.; Evangelisti, F. Geographical traceability of West Liguria extravirgin olive oils by the analysis of volatile terpenoid hydrocarbons. *J. Chromatogr. A* **2005**, *1089*, 243–249.

(12) Vichi, S.; Guadayol, J. M.; Caixach, J.; López-Tamames, E.; Buxaderas, S. Monoterpene and sesquiterpene hydrocarbons of virgin olive oil by headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry. *J. Chromatogr. A* **2006**, *1125*, 117–123.

(13) Ju, Z.; Curry, E. A. Evidence that α-farnesene biosynthesis during fruit ripening is mediated by ethylene regulated gene expression in apples. *Postharv. Biol. Technol.* **2000**, *19*, 9–16.

(14) Zeppa, S.; Gioacchini, A. M.; Guidi, C.; Guescini, M.; Pierleoni, R.; Zambonelli, A.; Stocchi, V. Determination of specific volatile organic compounds synthesised during *Tuber borchii* fruit body development by solid-phase microextraction and gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 199–205.

(15) Alagna, F.; D'Agostino, N.; Torchia, L.; Servili, M.; Rao, R.; Pietrella, M.; Giuliano, G.; Chiusano, M. L.; Baldoni, L.; Perrotta, G. Comparative 454 pyrosequencing of transcripts from two olive genotypes during fruit development. *BMC Genomics* **2009**, *10*, 399–413.

(16) Uceda, M.; Hermoso, M. La calidad del aceite de oliva. In *El cultivo del olivo*; Barranco, D., Fernández-Escobar, R., Rallo, L., Eds.; Mundi Prensa: Madrid, Spain, 2001; pp 589−614.

(17) European Commission, Off. J. European. Communities, May 6, Commission Regulation (EC) No. 796/2002 of 6 May 2002 amending Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis and the additional notes in the Annex to Council Regulation (EEC) No. 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff.

(18) European Commission, Off. J. European. Communities, July 4, Commission Regulation (EC) No. 640/2008 of 4 July 2008 amending Regulation (EEC) No. 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis.

(19) Whitaker, B. D. DPA treatment alters α-farnesene metabolism in peel of "Empire" apples stored in air or 1.5% O_2 atmosphere. *Postharv. Biol. Technol.* **2000**, *18*, 91–97.

(20) Lurie, S.; Lers, A.; Shacham, Z.; Sonego, L.; Burd, S.; Whitaker, B. Expression of α-farnesene synthase AFS1 and 3-hydroxy-3-methylglutaryl-coenzyme A reductase HMG2 and HMG3 in relation to α-farnesene and conjugated trienols in "Granny Smith" apples heat or 1-MCP treated to prevent superficial scald. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 232–236.

(21) Gapper, N. E.; Bai, J.; Whitaker, B. D. Inhibition of ethylene-induced α-farnesene synthase gene PcAFS1 expression in "d'Anjou" pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. *Postharv. Biol. Technol.* **2006**, *41*, 225–233.

(22) Loreto, F.; Förster, A.; Dürr, M.; Csiky, O.; Seufert, G. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant Cell Environ.* **1988**, *21*, 101–107.

(23) Sharkey, T. D.; Chen, X. Y.; Yeh, S. Isoprene increases thermotolerance of fosmidomycin-fed leaves. *Plant Physiol.* **2001**, *125*, 2001–2006.

Received for review February 5, 2010. Revised manuscript received April 25, 2010. Accepted May 1, 2010. This study was supported by the Agencia Española de Cooperación Internacional para el Desarrollo (AECID)—Ministerio de Asuntos Exteriores y Cooperación (MAEC), project A/017542/08, by the Ministerio de Educación y Ciencia (MEC) through the Juan de la Cierva program, and by the Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie du Gouvernement Tunisien.