



Volatile profile and quality characteristics of the Greek “Chondrolia Chalkidikis” virgin olive oils: effect of ripening stage

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Abstract

Among the various parameters affecting olive oil quality, ripening stage is one of the most important. Optimal harvest time ensuring target quality for the final product varies in relation to the effect of many intrinsic and extrinsic factors. Therefore, its determination necessitates thorough examination of each case. The present study explores the impact of six harvest times on volatile profile and quality attributes of olive oils from “Chondrolia Chalkidikis” Greek cultivar. All samples examined were classified “Virgin Olive Oils” (VOOs) according to findings of acidity, peroxide, and K values. The low values for the principal official quality indices, the high oleic acid percentages (76–78%), the high oxidative stabilities (up to 36 h induction period), and phenols content (606–290 mg/kg) were considered nutritionally promising. Total phenols, carotenoids and chlorophylls contents, as well as oxidative stability (induction period values) decreased with ripening. Harvest time had a strong impact on HS-SPME–GC–MS volatile fingerprint. Optimal volatile profiles were related to intermediate examined ripening stages. Fatty acid composition did not show remarkable trends. Chondrolia Chalkidikis VOOs perform as interesting candidates of high quality. Findings of the study may support existing databases with scientific records for Chondrolia Chalkidikis VOOs, boost their competitiveness in the global market, and encourage worldwide exploitation of VOOs from similar cultivars (table olives oriented).

Keywords Olive oil quality · Chondrolia Chalkidikis cultivar · Volatiles · Ripening stage

Abbreviations

ANOVA	Analysis of variance
Ch	Chondrolia Chalkidikis
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DVB/CWR/PDMS	Divinylbenzene/carbon wide range/ polydimethylsiloxane

EVOO	Extra virgin olive oil
FAMEs	Fatty acid methyl esters
FFAs	Free fatty acids
GC–MS	Gas chromatography–mass spectrometry
HPLC	High-performance liquid chromatography
HS-SPME	Head-space-solid-phase micro-extraction
IS	Internal standard
MI	Maturity index
MUFA	Monounsaturated fatty acids
NIST	National Institute of Standards and Technology
OTV	Odor threshold value
PCI	Photometric color index
PTFE	Polytetrafluoroethylene
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
QC	Quality control sample
RSD	Relative standard deviation

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SFA	Saturated fatty acids
TCar	Total content of carotenoid
TChl	Total content of chlorophyll
TP	Total phenolic content
UFA	Unsaturated fatty acids
VOC	Volatile organic compound
VOO	Virgin olive oil

Introduction

Virgin olive oil (VOO) is considered “liquid gold” and “great healer” from times of Homer and Hippocrates. Additionally, it is acknowledged as a fundamental ingredient of the Mediterranean diet. Lately, it experiences global recognition and demand in favor to its bioactive constituents (e.g., oleic acid, linoleic acid, phenols, pigments, α -tocopherol, and squalene), appraised for numerous health benefits, including anti-inflammatory and antimicrobial properties [1]. Its unique composition favors overall health and may even act beneficially against new threats, such as COVID 19 and related pandemic diseases [2]. Besides, VOO is also distinguished for its exclusive sensorial attributes that are highly valued and significantly contribute to the overall product quality [3]. Consequently, there is an intense constantly increasing interest for VOOs of favorable chemical and aroma characteristics, of superior quality and differentiated character [4, 5].

Still, it is widely known that VOO chemical composition, quality, and sensorial characteristics significantly vary due to numerous and frequently interrelated pre- and post-harvest factors. The most important ones being cultivar, agro-environmental conditions, harvest time, and practices from harvest to oil processing and storage [5–7]. Therefore, knowledge on the nutritional and sensorial potential of a VOO needs comprehensive study of each individual case. When production focuses on premium quality, contribution of drupes maturation is a crucial parameter to be optimized [5, 8].

Enzymatic and chemical changes occurring during olives ripening result in oil accumulation up to a certain point. Once, the decisive factor for harvest was the point related to maximum oil yield. Later, harvest decision was based on measurements on growing olives (color, size, firmness, pulp to stone ratio, dry matter, fruit force removal, and scar formation between stem and olive) and other practical concerns (e.g., weather, availability of laborers, and mills). Lately, interest has moved to products’ quality, and therefore, critical changes in biomolecules profiles have drawn attention. The latter necessitates the need to couple empirical approaches with analytical data directly linked with quality to better approach optimal harvest time [5, 9].

There are several studies on the impact of olives ripening on VOO quality. Few examine also how the volatile profile and sensorial attributes are affected. Findings indicate extended variation due to differences associated with cultivar, site, year, pre and post-harvest practices, and outcomes for one case cannot be conveyed to another [5, 8, 10–17]. The recommended maturity indices, frequently used to define optimal harvest time, greatly vary (1–4.5) [5]. Total and major (e.g., (E)-2-hexenal) volatile organic compounds (VOCs), may increase up to a maximum, beyond which they may decrease [6, 15, 18–20]. Positive sensorial perceptions and bitter-pungent notes may decline at later maturation stages [5, 21]. Fatty acid profile usually exhibits minor variations. Chlorophyll and carotenoid pigments, phenolic compounds, and oxidative stability frequently decline as harvest time proceeds [5, 14, 22]. Nonetheless, since the effect of harvest time on VOO quality is a complex matter, deviations from the above-mentioned patterns are frequently reported [11, 13, 23].

Chondrolia Chalkidikis (Ch) olive cultivar is of medium hardness, with large-sized drupes (4–14 g), elongated with a prominent tip at the end. Although it is dual purposed, it is mainly used for table olives, accounting for more than 50% (about 90–100,000 tons) of total national production (<https://www.pemete.gr/>). Cultivation for table olives requires high labor and high amount of water. Therefore and due to the climatic change and the drying out of drillings for irrigation, many producers turn their interest to VOO. Ch is quite productive, partially self-compatible, requiring lower temperatures for flowering, resistant to average cold, sensitive to *Cycloconium oleaginum*, cancer, *Verticillium* diseases, and insects attack [24]. Its olives are hand-picked from trees while still green, so harvest begins earlier (middle September–middle October) compared to other cultivars in neighboring areas. It is related to 16–20% oil yields [24], and high-quality products [4, 25, 26]. Lately, Ch commercial extra virgin olive oils (EVOOs) have been gaining quality recognitions (Agoureleo Chalkidikis protected denomination of origin) and awards in international olive oil competitions (e.g., Mario Solinas 2021) revealing their quality potential and the need for thorough investigation of their compositional and aroma attributes [26, 27].

The aim of this work is to assess the impact of harvest time on the volatile profile and quality characteristics of Ch VOOs. To our knowledge, information related to the latter is limited [25], and no information is available for the former. Findings are expected to support existing databases with scientific records for Ch VOOs, encourage production and exploitation of high-quality traded bottled products, and boost their competitiveness in the global olive oil market. Nonetheless, outcomes of the study may also encourage exploitation of high-quality VOOs from similar cultivars (table olives oriented) worldwide.

Materials and methods

Chemicals

n-Hexane, glacial acetic acid, and chloroform were by VWR Chemicals BDH (Fontenay-sous-Bois, France). Isooctane and cyclohexane were from VWR Chemicals BDH (Leuven, Belgium). Diethyl ether was from Riedel de Haen (Honeywell, France). Starch and phenolphthalein were from PanReac AppliChem (Barcelona, Spain). Ethanol (99.8%) was from PanReac AppliChem (Darmstadt, Germany) and methanol from PanReac AppliChem (Barcelona, Spain). Gallic acid monohydrate (> 99%, HPLC) was from Sigma-Aldrich (China). Sodium carbonate anhydrous and ethyl acetate were purchased from CHEMLAB (Zedelgem, Belgium). Folin–Ciocalteu reagent was from Merck (Darmstadt, Germany) and DPPH from TCI (Tokyo, Japan). All other reagents used were of the appropriate purity from various suppliers.

Olive fruits harvest

Ch olive fruits were collected at 6 different times along the same harvest period (H1: 18/9, H2: 24/9, H3: 4/10, H4: 17/10, H5: 31/10, H6: 7/11/2019). Fruits were hand-picked from the whole perimeter of the same 3 trees (3 sampling heights, ~ 1.5 kg per tree) of an orchard placed in Néa Ténedhos, Greece (40° 19' 49.1" N 23° 14' 47.5" E). Selected trees were of similar crop load (~ 45 to 50 kg) and located in different places to minimize orientation variabilities. The site has a typical Mediterranean climate, 130 m altitude, with a long-term average annual temperature of 18 °C (ranged from – 4 to 37 °C) and an annual rainfall of 613 mm (1/9/2018–30/10/2019; [28]). Trees (35 years old) are planted at 5 × 6 m and are shaped as a free cup with 3–4 main branches. Soil is sandy type (50% sand, 30% clay, 20% silt; 1.5% organic matter). Cultivation practices follow conventional agriculture and Globalgap protocols. Briefly, sprinkle irrigation (approximately 450 L/tree/week) was applied from June to September 2019. Fertilization took place in early February 2019, according to needs of grove, by ground application of 0.6 kg N/tree, 0.3 kg P/tree, 0.35 kg K/tree, 0.1 kg Bo/tree, and 0.2 kg Ca/tree. A second application of 0.3 kg N/tree was applied by irrigation in mid-end of June 2019 and a foliar application (Pitstop Plus, Farma-Chem SA; 16% Ca, 0.2% Bo; 3 L Pistop Plus in 1000 L water/12 acres) was also employed 50–60 days after blooming.

Olive oil extraction

Collected olives were immediately placed in plastic air-ventilated crates and transferred to the lab within 2 h after harvest. Olive oil was directly extracted from each sample (containing 3 × 1.5 kg healthy drupes from the 3 trees per sampling time), with the aid of an Abencor System (MC2 Ingeniería y Sistemas, S.L., Sevilla, Spain). Olives were deleafed, washed to remove dust, dried with a paper towel, and milled with the Abencor stainless still hammer mill MM-100 (operated at 3000 rpm and a 5.5 mm sieve). Thereafter, malaxation was carried out in 700 g pulp (obtained from the pulp resulting from simultaneous milling of drupes collected from the 3 trees) for 30 min at 25 ± 1 °C with the aid of the Abencor thermo mixer TB-100. Pulp was mixed inside stainless stir jars at a constant speed of 50 rpm. Finally, must was delivered (in a 1 L volumetric tube) after centrifugation (Abencor centrifugal machine CF-100) of the kneaded pulp at 3500 rpm for 1 min. Mixing jar was rinsed with 100 mL tap water (room temperature) that was poured in centrifugal machine which was again operated for 1 min. The new resulting must was collected in the same tube previously used. Tube contents were allowed to decant for at least 30 min. Olive oil samples (Ch VOOs) collected from the upper part of tube were placed in dark glass bottles without headspace and stored at 4 °C until analysis.

Maturity index

Maturity index (MI) was based on the classification of 100 fruits randomly taken from 1 kg sample. For MI determination, the following formula (1) was employed [29]:

$$MI = \frac{(0 + n_0) + (1 + n_1) + (2 + n_2) + \dots + (7 + n_7)}{100}, \quad (1)$$

where n_0 – n_7 is total number of olives belonging to each of the following categories:

- 0 = deep or dark green color skin.
- 1 = yellow or yellowish-green color skin.
- 2 = yellowish color skin with reddish spots.
- 3 = reddish or light violet color skin.
- 4 = black skin and completely green flesh.
- 5 = black skin and violet color halfway through flesh.
- 6 = black skin and violet color almost right through the stone flesh.
- 7 = skin of which is black and the flesh is completely dark.

Quality indices

Free fatty acid content (FFAs), peroxide value (PV), and K values were determined according to methods described in [30].

Total phenolic content (TP)

TP was determined according to Mastralexi et al. [31]. Oil (2.5 g) dissolved in 5 mL *n*-hexane was extracted with 5 mL methanol/H₂O (60:40, *v/v*). The mixture was vortexed for 2 min and centrifuged at 3500 rpm for 10 min. Suitable polar extract aliquots were transferred in a 10 mL volumetric flask and, subsequently, water (5 mL) and Folin–Ciocalteu reagent (0.5 mL) were added. After 3 min, 1 mL of saturated (35%, *w/v*) sodium carbonate solution was added. The mixture was diluted with water to 10 mL and after 1 h absorbance at 725 nm was measured against a blank with a spectrophotometer. Standard solutions of gallic acid (50–400 mg/L) were used to prepare the calibration curve.

DPPH radical-scavenging activity

The determination of % scavenging activity of DPPH radical was according to a modified method described in the literature [32]. Briefly, 1 mL of the oil solution in ethyl acetate (10% *w/v*) was added to 4 mL of a freshly prepared DPPH solution (10⁻⁴ M in ethyl acetate). Reaction mixture was vigorously vortexed for 10 s and kept in dark for 30 min. Thereafter, absorbance (A_{30}) at 515 nm was measured against ethyl acetate. The absorbance of a mixture of DPPH solution diluted with ethyl acetate in the same ratio (4:1) was measured to obtain A_0 value. % Inhibition values were determined by formula (2)

$$\% \text{Inhibition} = 100 \times \frac{A_0 - A_{30}}{A_0} \quad (2)$$

Total chlorophyll and total carotenoid content

Total contents of chlorophyll (TChl) and carotenoid (TCar) pigments were spectrophotometrically determined according to Mínguez Mosquera et al. [33]. Briefly, 1.5 g of oil dissolved in cyclohexane in a 5 mL volumetric flask was spectrophotometrically recorded at 670 and 470 nm. Results were expressed as mg pigment/kg oil; (pheophytin a and lutein for TChl and TCar respectively) using the Eqs. (3) and (4)

$$\text{TChl} = \frac{(A_{670} \times 1000000)}{(613 \times 100 \times d)}, \quad (3)$$

$$\text{TCar} = \frac{(A_{470} \times 1000000)}{(2000 \times 100 \times d)}, \quad (4)$$

where A is the absorbance at the respective wavelengths and $d=1$ cm is the optical path length.

Color indices

The photometric color index (PCI) of Ch VOOs was determined according to [34] by recording absorbance values at 460, 550, 620, and 670 nm against air. PCI was calculated employing formula (5)

$$\text{PCI} = 1.29 \times (A_{460}) + 69.7 \times (A_{550}) + 41.2 \times (A_{620}) - 56.4 \times (A_{670}), \quad (5)$$

where A is the absorbance at the respective wavelengths.

The color coordinates a^* (reddish/greenish), and b^* , (yellowish/bluish), as well as the psychometric index of lightness, L^* , were recorded with the CR-400 chroma meter (Konica Minolta optics, Inc, Japan). 10 g of oil were placed in a petri dish (92 × 16 mm, Sarstedt, Nümbrecht, Germany) which was covered with its lid. The petri dish was placed over a filter paper and L , a^* and b^* values were recorded from three different places after instrument calibration.

Oxidative stability

Oxidative stability was determined as follows: 3.0 g oil was exposed to accelerated oxidation at 110 °C and a constant air flow of 20 L/h using the 892 Professional Rancimat (Metrohm, Herisau, Switzerland). Results were expressed as induction time (h) values.

GC–MS analysis of fatty acid composition

Fatty acids methyl esters (FAMES) were prepared according to a modified version of the International Olive Council method [35]. 100 µL of sample were diluted in 1 mL of hexane and analysis was performed on a Shimadzu GCMS-QP2020 instrument fitted with a BPX70 capillary column (50 m × 0.22 mm i.d. × 0.25 µm film thickness). Ultra-high-purity helium (99.9999%) was used as carrier gas at 1.33 mL/min constant flow. Injection port temperature was 240 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 35–350 atomic mass unit. The injected sample volume was 1 µL with a 80:1 split ratio. Oven temperature program started at 120 °C (hold for 0 min) and increased at a rate of 10 °C/min to 180 °C (hold for 0 min), and further increased with a rate of 3 °C/min at 240 °C and hold for 3 min. Compounds were identified by comparing the spectra obtained with mass spectra library (FAMES) and further confirmed with Supelco 37 component FAMES mixture. Samples were run

in randomized order in duplicate and runs ($n=5$) of a quality control sample (QC; prepared by mixing equal volumes of each Ch VOO) were also employed to evaluate instrument stability and analyte reproducibility. All compounds in QC presented 4–6% RSD values indicating satisfactory stability and reproducibility of the system during the analytical batch. Results were expressed as mean % area values.

HS-SPME–GC–MS analysis of VOCs

Analysis of VOCs was performed using solid-phase micro-extraction (SPME) technique. The PAL SPME (DVB/CWR/PDMS) fiber (2 cm length, 50/30 thickness) was initially conditioned according to the manufacturer's recommendations. Oil samples (2 mL) were placed in 20 mL vials and spiked with eucalyptol internal standard (IS; 4 μ L of a 1000 ppm methanolic solution); vials were closed with PTFE/silicone septum. Samples were equilibrated at 55 ± 0.1 °C for 15 min upon agitation (speed 250 rpm) in a PAL SHIMADZU autosampler unit (AOC 6000, CTC Analytics, Switzerland). After equilibration, fiber was exposed to headspace for 50 min at 55 °C for sample extraction. VOCs were analyzed using a Shimadzu GCMS-QP2020 instrument, equipped with an MEGA-5 MS capillary column (30 m \times 0.25 mm, 0.25 μ m) (MEGA, Legnano, Italy). Sample desorption in injection port (equipped with a 0.75 mm i.d. inlet liner) was 4 min and injection temperature was 260 °C. Injection was operated in split mode at a ratio of 2.0. Carrier gas was helium, running at 1.2 mL/min constant flow; oven temperature was held at 40 °C for 2.5 min, and then ramped to 230 °C at a rate of 10 °C/min, where it was held for 5 min. Fiber was pre- and post- conditioned for 10 min at 260 °C. Ion source and interface temperatures were 200 and 260 °C, respectively. Solvent cut time was 1.5 min. Mass spectrometer was operated in electron impact mode collecting signal within the range of 35–450 m/z. Identification of compounds was based on computer matching against

commercial libraries (NIST17 and FFNSC 3 Shimadzu) and by determining linear retention indices relative to a series of n-alkanes. The samples were run in randomized order in duplicate. Blank runs were performed to reveal possible carryover. A QC (prepared by mixing equal volumes of each Ch VOO) was injected ($n=5$) to evaluate instrument stability and analyte reproducibility. All compounds in QC presented RSD < 30% indicating satisfactory stability and reproducibility of the system during the analytical batch. The semi-quantitative results (mean value of two measurements) were expressed as concentration of target compound (IS equivalents, mg/L) and % areas. IS equivalents were determined by dividing peak areas of target compounds by IS peak area and multiplying this ratio by IS initial concentration.

Statistical analysis

Apart from HS-SPME–GC–MS and GC–MS analysis, all other analyses were carried out at least in triplicate and findings were expressed as mean value \pm standard deviation. Mean values were statistically compared based on one-way analysis of variance (ANOVA), followed by multiple Duncan test (0.05 significance level) using PASW statistics 18.0 software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Influence of olive ripening stage on Ch VOOs quality indices

It is widely known that free fatty acid content, and PV and K values are principal parameters checked for classification of olive oils according to standard regulations. For the EVOO category, thresholds of most standards (IOC, Codex, USDA, EU, Australian) are $\leq 0.8\%$ as oleic acid, ≤ 20.0 meq O_2 /kg oil, ≤ 2.5 , ≤ 0.22 , and ≤ 0.01 for FFAs, PV, K232,

Table 1 Maturity Index (MI), Free Fatty Acid content (FFAs, expressed as % oleic acid), Peroxide (PV, expressed as meq O_2 /kg oil), K232, K268, Δ K, Total Chlorophyll content (TChl, expressed as mg pheophetin a/kg oil), Total Carotenoid content (TCar, expressed

as mg lutein/kg oil), and Induction Period (IP, expressed as h) values of Ch VOOs extracted from olive fruits differing in ripening stage (H1–H6)

Ch VOOs	MI*	FFAs** % oleic acid	PV** meq O_2 /kg oil	K232**	K268**	Δ K**	TChl** mg pheophetin a/kg oil	TCar** mg lutein/kg oil	IP** h
H1	0.2	0.3 ± 0.0^b	7 ± 1^a	$1.75 \pm 0.19^{b,c}$	0.13 ± 0.01^c	0.00 ± 0.00^a	10.5 ± 0.8^f	5.4 ± 0.6^f	32.3 ± 1.1^b
H2	0.4	0.4 ± 0.0^c	6 ± 2^a	1.93 ± 0.00^c	0.12 ± 0.01^c	0.00 ± 0.00^a	8.6 ± 0.4^d	4.6 ± 0.2^d	31.8 ± 0.7^b
H3	0.6	0.4 ± 0.0^c	8 ± 2^a	$1.62 \pm 0.17^{a,b,c}$	0.10 ± 0.01^b	0.00 ± 0.00^a	4.5 ± 0.1^c	2.5 ± 0.1^c	35.8 ± 0.6^c
H4	1.7	0.2 ± 0.0^a	13 ± 0^b	1.39 ± 0.09^a	0.09 ± 0.01^b	0.00 ± 0.00^a	2.4 ± 0.1^b	1.4 ± 0.1^b	30.6 ± 1.8^b
H5	2.5	0.3 ± 0.0^b	13 ± 2^b	1.35 ± 0.05^a	0.09 ± 0.01^b	0.00 ± 0.00^a	$2.0 \pm 0.2^{a,b}$	$1.0 \pm 0.1^{a,b}$	23.7 ± 1.4^a
H6	3.4	0.4 ± 0.0^c	7 ± 1^a	$1.45 \pm 0.08^{a,b}$	0.07 ± 0.01^a	0.00 ± 0.00^a	1.6 ± 0.1^a	0.7 ± 0.1^a	21.7 ± 1.2^a

*Mean value of 2 measurements

**Mean value of 3 measurements \pm standard deviation; means with different letters are significantly different ($p < 0.05$) within the same column

K270, and ΔK , respectively. The Californian standard sets even lower thresholds [36]. These values (Table 1) for all examined cases were below the strictest thresholds classifying the products in the superior category and revealing a high-quality potential. Variations shown, although statistically significant in certain cases, were considered minor and in line with similar literature findings [16, 25, 37, 38]. No significant trend was observed and changes detected (i.e., the lower PV value of the last harvest) could be related to enzymatic activities in each ripening stage. K270 values showed a small gradual decrease as ripening progressed, similar to observations of Franco et al. [37]. Still, reported findings on ripening stage impact to these quality indices are controversial and are affected among other factors by cultivar [13, 16, 39]. Oils from drupes of advanced ripening may hold higher acidity levels caused by lipases' enzymatic activity, and other parameters. Probably, higher values could be recorded if later maturation stages ($MI > 3.4$) were also examined in the present study. Indeed, in the study of Mastralexi and Tsimidou [25], the acidity of VOOs from Ch cultivar slightly increases with ripening in most harvest periods examined; higher acidity was recorded for MI values 3.8–4.

Influence of olive ripening stage on Ch VOOs' phenolic antioxidant potential

Olive phenolic compounds are accredited with numerous health benefits and their levels if over 250 mg/kg enable use of relevant health claims too [40]. High phenol content entails oils with greater oxidative stability, higher pungency, and bitterness. VOOs' phenolic content greatly varies (from < 50 to > 1000 mg caffeic acid equivalents/kg) according to crop year, agro-environmental factors, cultivar, harvest time, practices from harvest to extraction, packing,

storage, and analytical conditions employed. This variability, among others, is related to phenolic glycoside amounts and enzymatic activities in fruit [5, 7, 31, 41].

As seen in Fig. 1, TP of Ch VOOs decreased with ripening from 606 ± 28 to 290 ± 11 mg/kg, in line to published findings for Frantoio and Manzanilla [10], Souri and Barnea [13], Moroccan Picholine [17], Coratina [16], and Chondrolia Chalkidikis (only for one of the four harvest periods examined) [25] cultivars, among others. Still, results are controversial [11, 16, 39] even for the same cultivar at different harvest periods [25].

The recorded TP values were considered promising for the use of Health Claim for "Polyphenols". According to Mastralexi and Tsimidou [25] and Rebordo-Rodriguez and coworkers [42] findings, such TP values should fulfill the requirement of the relevant health claim. According to the Italian scale [43] related to taste perception (50–200, 200–500, and 500–1000 mg gallic acid equivalents/kg for low, medium, and high TP, respectively), H1 and H2 Ch VOOs studied were of "high", and H3–H6 of "medium" TP. Trend in TP did not correlate with % Inhibition (Fig. 1) of DPPH radical (48 ± 7 to $87 \pm 6\%$). The latter could imply that content and synergism of individual phenols and/or other constituents (such as tocopherols and pigments) contribute to the radical-scavenging potential of the Ch VOOs [41].

Influence of olive ripening stage on Ch VOOs fatty acid composition

Fatty acid composition is one of the olive oil purity criteria established by regulatory systems. Findings on Ch VOOs fatty acid composition are shown in Table 2 and Fig. 2a, b. Percentages were within limits specified by official regulations [36], and were similar to those reported for VOOs of

Fig. 1 Evolution of Total Phenols (TP) and % Inhibition of DPPH radical in Ch VOOs extracted from olive fruits differing in ripening stages (H1–H6)

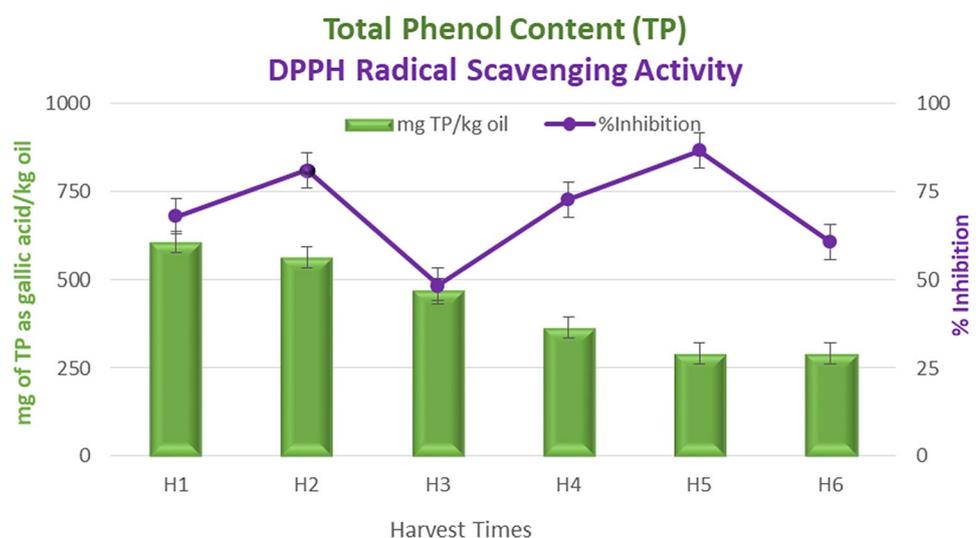
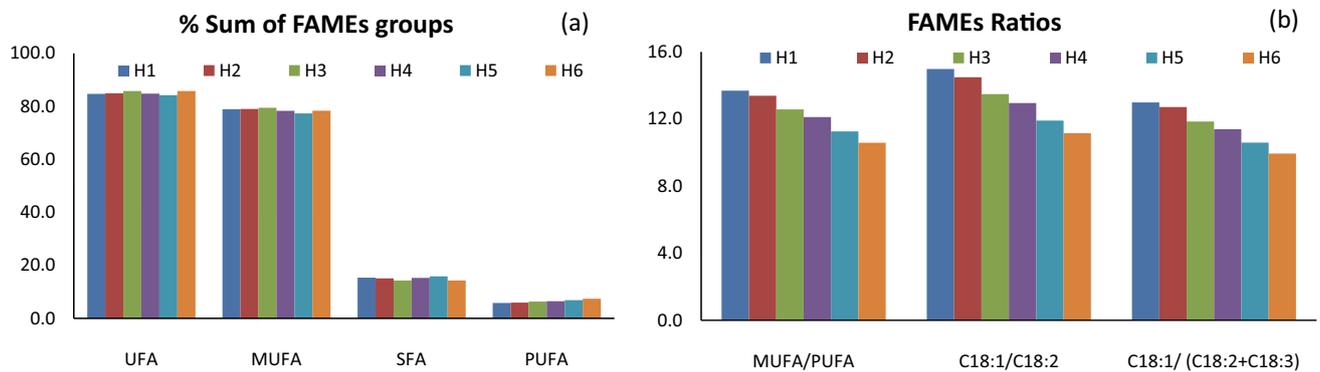


Table 2 Fatty acid composition (mean value of $n=2$) of Ch VOOs extracted from olive fruits differing in ripening stage (H1–H6)

Fatty acids	H1	H2	H3	H4	H5	H6
Oleic acid C18:1	78.0	78.0	78.3	77.1	76.2	77.1
Palmitic acid C16:0	13.4	13.0	12.2	13.2	13.8	12.2
Linoleic acid C18:2	5.2	5.4	5.8	6.0	6.4	6.9
Stearic C18:0	1.6	1.8	1.7	1.7	1.7	1.7
Palmitoleic acid C16:1	0.7	0.7	0.9	0.9	0.9	1.0
Linolenic acid C18:3	0.6	0.5	0.5	0.5	0.5	0.5
Arachidic acid C20:0	0.3	0.3	0.3	0.3	0.3	0.3
Eicosenoic acid C20:1 ω 9	0.2	0.3	0.3	0.3	0.2	0.3

**Fig. 2** Evolution of percentages (%) of unsaturated (UFA), monounsaturated (MUFA), saturated (SFA), and polyunsaturated (PUFA) fatty acids sums (a), as well as respective ratios (b) in Ch VOOs extracted from olive fruits deferring in ripening stages (H1–H6)

the same [25] and other cultivars [44–47]. Values for oleic acid were higher than 70% (76–78%) implying a high content, similar to other high-quality Greek VOOs [4, 48].

During fruit maturation, fatty acid composition depends upon cultivar, site, harvest year, and other parameters, and reported findings are controversial [10, 11, 13, 14, 22, 49, 50]. Changes in fatty acid composition were not considered significant and in most cases did not follow a clear trend during ripening. A slight decrease in oleic acid percentage and increase in percentages of linoleic and palmitoleic acids was observed. Moreover, PUFAs increased, contrary to decrease observed for MUFA/PUFA, C18:1/C18:2, oleic/(linoleic + linolenic) ratios (Fig. 2). These findings are partially in accordance with literature [5, 10, 11, 13, 14, 22, 25, 39, 49, 50]. The decrease in oleic and increase in linoleic acids, that coincides in many studies and is in line with the current findings, is related to continuing biosynthesis of triglycerides and transformation of oleic in linoleic with the action of oleate desaturase enzyme [14]. Such

transformation seems to be enhanced at low temperatures [13, 51] and therefore could be related to lower temperatures expected at later harvests.

Influence of olive ripening stage on Ch VOOs' oxidative stability

Olive oil stability depends upon synergism of various parameters such as chemical indices, fatty acid composition, type, and levels of bioactives (such as phenols, tocopherols, squalene, and pigments). Additionally, it is reported that higher MUFA, MUFA/PUFA, MUFA/SFA, C18:1/C18:2 and oleic/(linoleic + linolenic), and lower PUFA/SFA, relate to oils with higher oxidative stability [52–56]. Indeed, Ch VOOs TP content presented a moderate-positive correlation ($R^2 = 57\%$) with IP values. Moreover, best determined R^2 values ranged from 67 to 69% for correlation of IP with oleic/(linolenic + linoleic), C18:1/C18:2, PUFA, MUFA, MUFA/PUFA, and sum of C18:1. Nonetheless, these

moderate correlations were attributed to the small size of samples and small differences in recorded values. IP of Ch VOOs ranged from 21.7 ± 1.2 to 35.8 ± 0.6 (Table 1) and were in line with the published findings employing same experimental conditions [53, 57, 58]. The last two (H5 and H6) samples presented a similar performance and the lowest stability. Present findings are in line with the literature [11, 50] that also report higher oxidative stability for early in comparison to late harvested VOOs. Moreover, Ch VOOs studied were classified among products of moderate-to-high stability. According to Ceci and Carelli [53], IP values from 58 VOOs of variable characteristics ranged from 5.6 to 27.9. Nonetheless, the same values seem low if compared to Picual variety for which 77 and 103 IP values are reported [58].

Influence of olive ripening stage on Ch VOOs' pigments and color attributes

Interest in chlorophylls and carotenoids has lately revived due to their health-promoting benefits [59]. Their concentration varies according to various biotic and abiotic parameters, freshness, storage conditions, authenticity, and quality issues [5]. During ripening, they are expected to decrease, following a decrease in color coordinates too [5, 33, 60]. Nonetheless, some cultivars follow a different pattern [33]. TChl and TCar of the Ch VOOs examined (Table 1) were in line with declining trends reported in literature and absolute values were of same size as those published for various VOOs examined under same conditions [25, 33, 60, 61].

The key role of olive oil color goes beyond consumers' acceptability and relates to quality control issues, since it is correlated with its pigments [33, 59, 60]. The latter was also verified in the present study. PCI values showed an increasing trend (from -59.93 to 21.40) along with ripening, with the exception of H1, and were similar to those reported for Greek Koroneiki VOOs [62]. Additionally, with the exception of H1, rest samples presented an increasing trend for a^* (-7.57 ± 0.06 to -1.69 ± 0.02) and decreasing trend for b^* (22.69 ± 0.19 to 6.53 ± 0.03) values as ripening progressed. L^* values did not significantly vary (average 52.70 ± 0.87). Findings related to color coordinates were also similar to Minguez-Mosquera and collaborators [33].

Influence of olive ripening stage on Ch VOO VOCs

EVOO is highly appraised for its sensorial attributes, the assessment of which (panel test) is mandatory for commercialized products (e.g., IOC, EU regulation). Presence of "off flavors" or absence of "fruity" derives the "virgin"/"extra virgin" character from products with chemical parameters in line to official thresholds [21]. Still, the panel test entails limitations, and therefore, the SPME-GC-MS technique is recently applied for the determination of the volatile

fingerprint of olive oils and a better understanding of their sensorial perception [21, 63].

VOO flavors are primarily determined by enzyme content and activity related among others to cultivar, environment, ripening, and pre- and post-harvest factors [3, 5, 6, 20, 21, 64]. During ripening, variable volatile patterns evolve depended on differences in the above-mentioned parameters [23, 39, 65], as well as in the analytical conditions applied.

Of the 60 identified VOCs in the current study, mainly C6, but also C5 compounds derived from lipoxygenase (LOX) pathways [6] and considered powerful pleasant odorants [3], were present (Table 3, Fig. 3). C6 aldehydes considered in terms of quantity and quality prime influential aroma constituents [3] predominate in current study (Fig. 3). These findings indicate high product quality [64] and are in accordance with the volatile fingerprint reported of Kosma and coworkers [63] for this cultivar. Still, deviations in VOC profiles of current samples and Kosma et al., ones, such as presence and absence of esters respectively, exist. Such differences could be attributed to the advanced ripening (MI 5–6), or other differences (environmental, agronomical, and technological) of that samples compared to present ones. It has to be highlighted that the studied cultivar is considered a late maturing one and usually the fruits are harvested at $MI < 4$ according to current producers' suggestions [25] or Agoureleo Chalkidikis protected denomination of origin prerequisites.

Most VOCs and VOC classes did not follow a clear trend throughout harvest, similarly to several literature findings [12, 15, 18, 39, 65–68]. Reports on the existing trends usually arise from studies with 2–3 MI employed and overripe stage included [18, 65, 66, 68]. In the current study, where 6 harvest times with MI 0.2–3.4 are examined, a pattern is observed for several cases (e.g., sum of alcohols, ketones, furans, hexanal, 1-pentanol, (E)-2-hexen-1-ol, and 1-hexanol) from 1st to 5th harvest (Table 2). H5 possessed the higher amount of total VOCs, total LOX VOCs, and total C6 LOX VOCs (Table 2, Fig. 3), that are related with high VOO quality [12]. H5 possessed also the lower concentration in sum of carboxylic acids, which are related to sensory defects [3, 69]. Moreover, in H5, presence or higher concentration of individual VOCs (e.g., 3-methylbutanol) related to positive perceptions is also observed. During maturation, total VOCs are reported to increase up to a point, after which their amount decreases [15, 20].

Aldehydes usually predominate VOC profiles, a fact also confirmed in the current study (Table 2, 35–42 mg/L), and significantly (due to low threshold values, OTV) positively contribute to aroma [3, 70]. (E)-2-hexenal contribution, related to green and fruity attributes [6, 71], is widely discussed as this is considered the most predominant and remarkable VOO VOC [3, 6, 21]. This was also verified for the studied Ch VOOs and was also in line with Kosma

Table 3 Mean concentration ($n=2$) of identified VOCs in Ch VOOs extracted from olive fruits differing in ripening stage (H1–H6), expressed as internal standard (eucalyptol) equivalents, in mg/L

Group of VOCs	Rt	RI _{lit}	RI _{ex}	S.I	H1	H2	H3	H4	H5	H6
<i>Alcohols</i>										
1-Penten-3-ol	2.48	685	675	98	1.065	1.808	1.440	1.039	1.000	0.668
3-Methylbutan-1-ol	3.3	736	735	87	0.000	0.000	0.000	0.000	0.173	0.000
1-Pentanol	3.84	766	768	93	0.000	0.316	0.606	0.483	0.652	0.032
(Z)-2-Penten-1-ol	3.89	767	771	96	1.040	1.694	1.243	1.097	1.100	0.806
(Z)-3-Hexen-1-ol	5.45	856	853	95	0.000	0.199	0.076	0.063	0.095	0.297
(E)-2-Hexen-1-ol	5.8	862	870	85	0.000	2.225	4.132	3.980	6.593	0.408
1-Hexanol	5.88	862	873	97	0.000	2.175	8.351	9.375	20.634	1.314
1-Decanol	12.98	1280	1280	92	0.028	0.026	0.025	0.022	0.019	0.019
SUM					2.134	8.442	15.873	16.059	30.265	3.543
<i>Aldehydes</i>										
3-Methyl-butanal	2.21	650	647	94	0.438	0.000	0.000	0.000	0.000	0.000
(E)-2-Pentenal	3.46	754	746	92	0.241	0.087	0.071	0.063	0.034	0.118
(E)-2-Pentenal	3.66	754	757	93	0.402	0.407	0.125	0.141	0.115	0.325
(Z)-3-Hexenal	4.45	800	804	94	4.122	3.584	1.463	0.501	1.963	2.801
Hexanal	4.5	801	806	98	9.525	8.494	7.787	4.833	4.417	5.459
(E)-2-Hexenal	5.61	852	855	96	15.202	21.661	30.467	30.603	31.828	29.267
Heptanal	6.55	899	908	92	0.043	0.045	0.030	0.036	0.024	0.124
(E,E)-2,4-Hexadienal	6.74	916	917	96	1.328	0.816	0.353	0.261	0.476	0.898
(E,E)-2,4-Hexadienal	6.78	916	918	97	3.196	2.557	1.205	0.717	1.597	2.671
Nonanal	10.38	1111	1104	97	0.137	0.189	0.116	0.111	0.115	0.193
Decanal	12.07	1208	1213	85	0.027	0.022	0.024	0.020	0.015	0.015
SUM					34.661	37.862	41.641	37.286	40.583	41.872
<i>Ketones</i>										
1-Penten-3-one	2.52	678	679	92	3.637	3.079	0.877	1.186	0.909	3.820
3-Pentanone	2.65	694	693	95	0.000	2.642	2.945	2.020	3.193	0.000
3-Hexen-2-one	5.41	845	852	80	0.100	0.079	0.083	0.060	0.033	0.053
2,2-Dimethyl-3-heptanone	7.67	967	964	97	3.912	2.166	2.144	1.542	1.007	1.708
6-Methyl-5-hepten-2-one	8.19	985	985	92	0.146	0.114	0.183	0.129	0.096	0.140
1,4-Cyclohex-2-enedione	9.01	1032	1034	80	0.658	0.433	0.477	0.310	0.205	0.316
5-Methyl-4-hexen-3-one	9.17		1043	90	1.117	0.839	0.849	0.639	0.335	0.570
ketone derivative*	9.67		1071	89	18.700	11.234	12.509	9.075	4.186	7.881
methyl-2-cyclohex-1-one	9.84	-	1080	81	0.322	0.216	0.224	0.164	0.099	0.072
SUM					28.593	20.801	20.290	15.126	10.064	14.559
<i>Furans</i>										
2-Ethyl-furan	2.69	691	696	91	4.200	2.110	1.872	1.618	0.944	1.466
2-Vinylfuran	3.11	723	725	94	0.784	0.501	0.464	0.362	0.219	0.359
5-Ethyl-2(5H)-furanone	7.83	968	973	91	0.134	0.114	0.099	0.080	0.063	0.084
SUM					5.118	2.725	2.435	2.060	1.225	1.909
<i>Acids</i>										
Acetic acid	1.68	595	594	97	0.580	0.277	0.133	0.093	0.111	0.265
(E,E)-2,4-Hexadienoic acid	9.53	1056	1063	89	0.175	0.138	0.128	0.093	0.063	0.090
2-Ethyl-hexanoic acid	10.44	1123	1116	95	0.241	0.066	0.417	0.248	0.111	0.037
Octanoic acid	11.36	1175	1170	96	0.168	0.089	0.106	0.050	0.025	0.013
Nonanoic acid	12.91	1272	1268	95	0.296	0.234	0.270	0.096	0.058	0.047
SUM					1.459	0.803	1.054	0.581	0.367	0.451
<i>Esters</i>										
Ethyl acetate	1.86	614	612	92	0.101	0.076	0.078	0.066	0.100	0.067
(Z)-3-Hexenyl acetate	8.55	1005	1005	89	0.154	0.103	0.154	0.109	0.145	0.170

Table 3 (continued)

Group of VOCs	Rt	RI _{lit}	RI _{ex}	S.I	H1	H2	H3	H4	H5	H6
SUM					0.256	0.179	0.232	0.174	0.245	0.237
<i>Hydrocarbons</i>										
Hexane	1.73	601	600	97	1.191	1.474	1.221	1.092	0.934	0.619
Toluene	3.81	771	767	90	0.476	0.084	0.104	0.089	0.078	0.087
1-Octene	4.23	792	791	80	0.000	0.000	0.000	0.000	0.000	0.028
Octane	4.39	800	801	90	0.297	0.202	0.261	0.216	0.284	0.212
3-Ethyl-1,5-octadiene	6.3	894	894	92	0.145	0.377	0.281	0.236	0.364	0.221
3-Ethyl-1,5-octadiene	6.38	894	898	90	0.135	0.311	0.208	0.178	0.276	0.173
3-Ethyl-1,5-octadiene	7.18	939	949	98	0.743	2.087	1.228	0.977	1.550	1.057
3-Ethyl-1,5-octadiene	7.32	939	946	92	0.875	2.400	1.351	1.147	2.026	1.645
3-Ethyl-1,5-octadiene	8.26	939	949	94	0.438	1.166	0.721	0.566	0.752	0.609
4,8-Dimethyl-1,7-nonadiene	8.34	998	998	97	1.289	3.560	2.067	1.789	3.014	2.597
(E,E)-2,6-Dimethyl-1,3,5,7-octatetraene	10.81	1131	1133	85	0.060	0.061	0.058	0.048	0.023	0.033
Dodecane	11.89	1200	1201	93	0.091	0.054	0.035	0.041	0.024	0.040
(Z)-2-Dodecene	11.96	1210	1206	92	0.143	0.075	0.072	0.079	0.130	0.214
Tridecane	13.44	1300	1301	96	0.104	0.062	0.054	0.055	0.036	0.057
Tetradecane	14.91	1400	1401	91	0.073	0.064	0.061	0.062	0.041	0.052
Pentadecane	16.28	1500	1501	83	0.041	0.030	0.033	0.036	0.021	0.026
SUM					6.098	12.006	7.756	6.611	9.554	7.669
<i>Terpenes</i>										
α-Ocimene	9.29	1050	1055	84	0.016	0.027	0.026	0.013	0.069	0.106
(E)-4,8-Dimethylnona-1,3,7-triene	10.474	1117	1117	92	0.073	0.078	0.735	0.028	0.202	0.085
Copaene	14.74	1392	1389	93	0.214	0.168	0.000	0.084	0.060	0.080
α-Farnesene	16.4	1497	1509	85	0.046	0.033	0.000	0.041	0.218	0.713
p-Mentha-1,5,8-triene	22.46			90	0.000	0.000	0.030	0.048	0.000	0.000
SUM					0.348	0.306	0.791	0.214	0.549	0.984
<i>Phenolic compounds</i>										
2-Hydroxy-benzeneethanol	15.41	1431	1437	94	0.078	0.589	0.756	0.793	0.549	0.121
Eucalyptol (I.S.)	9.09	1040	1037	97	I.S	I.S	I.S	I.S	I.S	I.S
<i>Total VOCs</i>					78.747	83.714	90.828	78.905	93.403	71.345
<i>Total C6 LOX VOCs</i>					29.004	38.442	52.430	49.464	65.675	39.715
<i>Total LOX VOCs</i>					37.724	54.815	63.528	58.597	77.647	49.189

Rt retention time, S.I. Similarity Index, I.S. Internal Standard, R_{lit} Retention Index literature, R_{ex} Retention Index experimental, * ketone derivative: 2-methyl-6-methyleneoct-7-en-4-one or 2,2-dimethyl-3-heptanone

and coworkers [63] findings for Chondrolia cultivar as well as the rest 9 examined Greek cultivars. Variable are the reported trends for its evolution pattern, similarly to other VOCs [18, 19, 23]. In the current study, (E)-2-hexenal concentration increased from H1 to H3 (15–30 mg/L), and then, it remained constant from H3 to H6 (29–32 mg/L). Its evolution pattern could be related to enzymatic activities, as well as substrates and enzymatic activity inhibitors availability [23]. (Z)-3-hexenal, another key VOC due to its low OTV [3], decreased from H1 (4.1 mg/L) to H4 (0.5 mg/L). This compound correlates with leaf, green odors [72]. Hexanal, also expected to noteworthy contribute with green, apple and grassy attributes [3, 21], decreased from H1 to H5 (9.5–4.4 mg/L), and then increased again at H6 (5.5 mg/L).

A constant decrease of hexanal has been reported for Tunisian VOOs (of higher MI than those examined in the current study) [69]. Similarly to other VOCs, reports for its evolution vary [12, 39]. Nonanal, another important aroma compound [70], which high levels are often related to off-flavors [71], was stable at low levels throughout H1-H5 harvest and only increased in H6.

After aldehydes, ketones, were found to predominate (Table 2, 10–29 mg/L). Most ketones as well as ketones sum decreased from H1 to H5. 1-penten-3-one, that may significantly contribute to overall sensory attributes due to its low OTV [3, 18, 21, 71], initially decreased from H1-H2 (3.1–3.6 mg/L) to H3-H5 (0.9–1.2 mg/L), reaching its maximum concentration at H6 (3.8 mg/L). It is positively

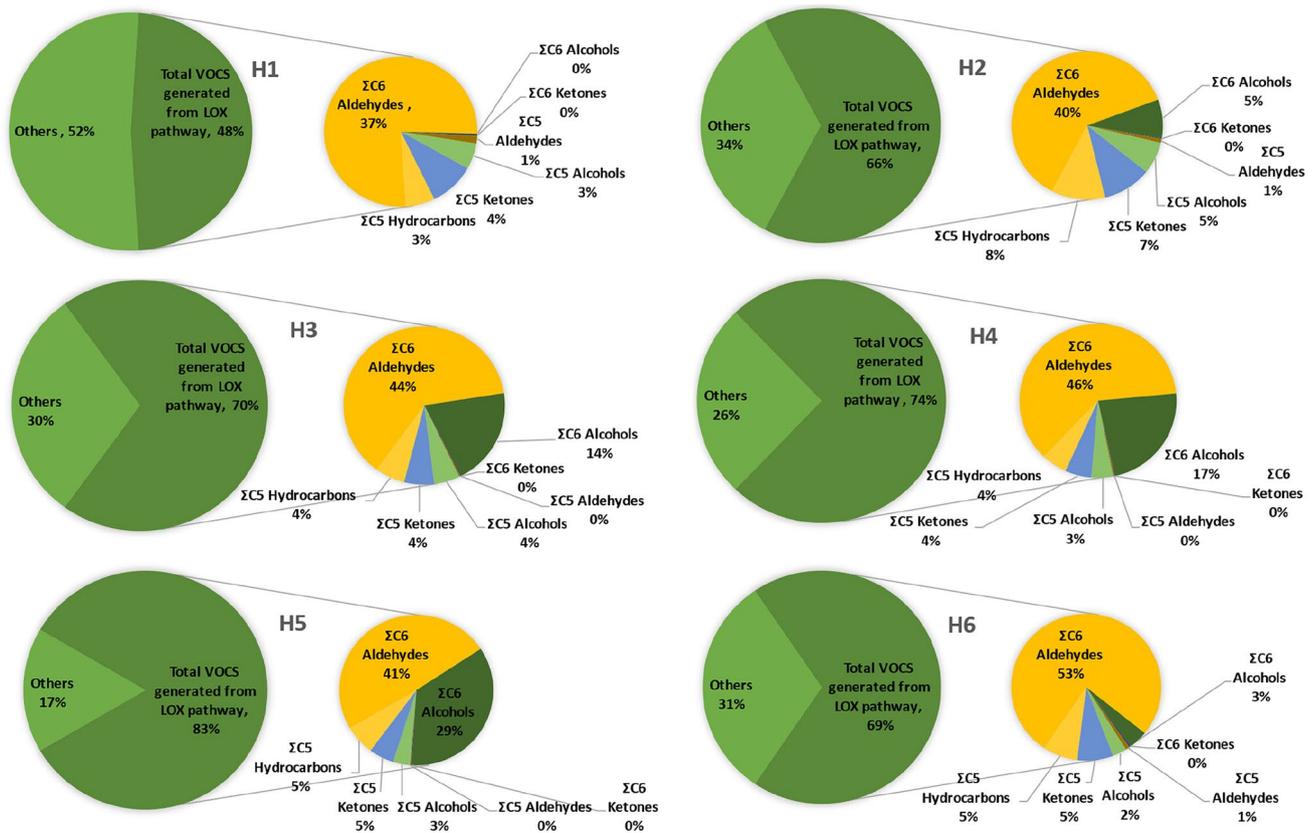


Fig. 3 Contribution (%) of different chemical groups of VOCs derived from LOX pathway from Ch VOOs extracted from olive fruits differing in ripening stages (H1-H6)

correlated to bitterness and pungency, fruity, green, and mustard-like attributes [6, 64, 71]. Still, high levels may give metallic off-flavors [6]. Another ketone (identified as 2-methyl-6-methyl-eneoct-7-en-4-one or 2,2-dimethyl-3-heptanone) with a similar decreasing evolution pattern was found at relatively high amounts (4.2–18.7 mg/L) in the examined VOOs. Still, the absence of records for its presence in olive oils, as well as OTV and sensorial characteristics did not allow further discussion.

Alcohols opposed to ketones followed an increasing trend, from 1st to 5th harvest. Lukic and coworkers [23] also observed an increasing trend in the C6 alcohol levels of Oblica cultivar VOOs during ripening. Such increase was related to increase alcohol dehydrogenase activity throughout harvest. 1-hexanol, linked with flowery, fruity, and green odors [6, 71], gradually increased from 0 to 20.6 mg/L and (E)-2-hexen-1-ol, also related to green aromas, performed similarly, reaching its maximum concentration (6.6 mg/L) at H5. Still, once again reported findings are variable [39].

Copaene related with fruity, sweet, wood, and spice and α -farnesene with floral, herb, wood, and sweet odors [71]

are main terpene VOO VOCs [15, 70, 72]. In the examined Ch VOOs α -farnesene concentrations increased during fruit maturation, when those of copaene decreased, in line with the published findings [15, 67].

Two esters, namely the ethyl acetate and the (Z)-3-hexenyl acetate, were present in relatively low amounts (0.067–0.101 and 0.103–0.170 mg/L, respectively) and did not follow clear trend throughout ripening. The C6 (Z)-3-hexenyl acetate is considered a significant positive fruity/green contributor and its presence has been reported, among others, in several Greek VOOs [2, 39, 63, 73]. Low levels of esters relate, among others, to lower content and activity of alcohol acetyltransferase enzyme [63, 74].

The co-presence of VOCs with variable OTV, and their synergistic and antagonistic effects make conclusion to optimal harvest times an extremely difficult task. It could be said that intermediate examined harvest times were considered better than H1 and H6, since in the former harvest times, most VOCs related to positive attributes are shown to evolve. Combination of information driven from chemical composition along with sensory analysis would be useful.

Conclusions

All Ch VOOs examined showed interesting quality characteristics. Harvest time did not have a significant impact on FFAs, PV, K values, and fatty acid composition. Content in total phenols, chlorophylls and carotenoids, as well as oxidative stability decreased with ripening. Optimal aroma profiles of olive oils examined were related to olive fruits of intermediate ripening stage. Obtained outcomes may build scientific knowledge on quality attributes of Ch VOOs, and may function as a marketing lever for advantage of local producers, ultimately boosting products' competitiveness in the global olive oil market. This cultivar, mainly exploited for table olives, may serve as a representative for similar cultivars worldwide that could switch or in parallel invest to high-quality VOOs' production.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with humans or animal studies.

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