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Biochemical Characterization of Six Traditional Olive Cultivars: A Comparative Study

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Abstract: Olives are an essential ingredient in Turkish food culture. Turkey has grown to become one of the top five producers of olives and olive oils in the world. Olive trees in general are found in the coastal parts of the country. The fruits of six traditional cultivars of olive (Gemlik, Domat, Memecik, Ayvalik, Cilli, and Adana Topagi), grown in Adana, were characterized based on their fruit skin color, the amount of chlorophylls, fatty acids, antioxidant activity, and total phenolic compounds, as well as volatile compounds. The international cultivar Manzanilla, grown in the same orchards as the traditional cultivars, was also included in the study to make a comparison. Compared to the Manzanilla cultivar, Memecik showed the highest amount of total phenolic content and antioxidant activity with the highest level of lightness, blueness/yellowness, and color intensity. Ayvalik presented the highest level of greenness/redness. Although the highest fruit lightness and darkness were found in the Cilli cultivar, with the highest amount of chlorophyll a (2.63 mgL^{-1}), there was more chlorophyll b in Adana Topagi fruits (3.34 mgL^{-1}). The highest percentage of fatty acids was found in the Gemlik cultivar of 66.81%, among which oleic acid was the major component. The total aldehydes ranged between 33.43% and 50.60%. Compared to Manzanilla and the traditional cultivars, the Domat cultivar had the highest amount of hexanal of 44.42%. Adana Topagi had the highest amount of alcohols (61.34%) and acids (1.31%). Memecik registered the highest amount of ketones (17.86%) and terpenes (20.34%). Among all cultivars, Manzanilla displayed the highest amount of esters (2.30%). In this study, traditional cultivars were found to be the richest in health-promoting chemicals. Furthermore, a significant variability among the cultivars was revealed, implying that metabolic fingerprinting approaches could be used to differentiate cultivars once more research into the effects of the growing conditions and environmental factors on the chemical profiles of each cultivar is carried out.

Keywords: fatty acids; GC-FID; HS-SPME-GC-MS; olive; total phenol; volatile compounds

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1. Introduction

The botanical name of olive is *Olea europaea* L. It is a member of the *Olea* genus, which includes between 30 and 35 species belonging to the *Oleaceae* family. It is native to Central Asia, the Mediterranean region, and some parts of Africa. The two most important olive

species in the Mediterranean region are the native (wild) *Olea europea* L. spp. *(var. sylvestris)* and the cultivar *Olea europea* L. ssp. *europea* (var. sativa) [1]. Throughout history, this fruit has been an emblem of Mediterranean civilization, and it has been established in Turkey for long time. Subspecies of olive have been identified in a line spanning from Hatay to Kahramanmaraş and Mardin, supporting the hypothesis that southeast Anatolia is the cradle and gene hub of olive. This fruit moved from the southeast to the west of Anatolia, then across the Aegean Islands to Greece, Italy, and Spain [2,3].

The production of olives around the world is above 21,066.062 tons, and Turkey ranks fourth; it is among the biggest producers after Spain, Italy, and Morocco, with 1,500.467 tons [4]. The National Olive and Olive Oil Council of Turkey has registered approximately 119 olive cultivars [5].

One of the earliest cultivated fruits from which oil was extracted is olive. It provides a perfect balance of taste, flavor, aroma, and health benefits [6]. However, the mentioned parameters are strongly influenced by the method of extraction of the oil. Virgin olive oil obtained from olive fruit (*Olea europaea* L.) is extracted by physical methods under specific conditions, in particular thermal conditions. The recent development of a high-flow oil extraction plant, the Sono-Heat-Exchanger, combines ultrasound and a heat exchanger in order to change, through a radical innovative model in the oil mill, the historical paradigm, which views oil yield and the content of bio-phenols as inversely correlated [7]. The extraction of olive oil using a high temperature leads to its alteration and a decrease in its anti-oxidative activities [8].

Different factors such as harvesting time or fruit growth stage, cultivar, cultural practices, environmental factors, as well as oil processing technologies affect the chemical, physical, and physiological aspects of olive fruit and oil [9].

With the increasing interest in healthy living and nutrition in the world in recent years, the importance of olive oil and table olive production and consumption has also increased [10]. The draw of Turkey's production sectors is not only olive, which has increased, but is also the interest in the processing industry and trade [11].

Scientific studies on olives have revealed that these products have crucial functions in human health owing to their high levels of biologically active compounds. The diversity of olive cultivars offers great opportunities for researchers to find the best species for both human nutrition and the pharmaceutical industry, as well as for future crossbreeding processes [12–16].

The knowledge of the biochemical composition, in particular of specialized metabolites, is critical not only for cultivar categorization, but also for understanding the added value that these metabolites provide to cultivars producing them in terms of agronomic characteristics and nutraceutical potential [17–21]. Olive trees produce specific metabolites in each of their organs [22].

Phenolic compounds found in olive drupes are essential not only as possible biomarkers of distinct cultivars, but also as nutraceuticals. Table olives and olive oils can be thought of as pre-packaged functional foods [23].

The main classes of phenols, which are found in particular in the *Oleaceae* family, are phenolic alcohols, phenolic acids, lignans, flavonoids, and secoiridoids [23]. Olive oil has a characteristic aroma and flavor [24]. The main reasons for the increase in virgin olive oil consumption in recent years are these sensory properties, in combination with nutritional factors. The profile of the volatile chemicals in high-quality olive oil is a balanced flavor of green and fruity sensory characteristics, mostly consisting of aldehydes, ketones, esters, and alcohols [8].

Carotenoids and chlorophylls, in addition to phenolic compounds, are very common pigments in the plant kingdom and show a high antioxidant potential [25]. As crop ripeness progresses, both chlorophyll and carotenoid levels progressively decline [26]. The major chlorophyll pigments are chlorophylls a and b, differing in one of their side chains [27].

Several research works have been carried out to determine their distribution and characterization from the genetical, morphological, and phytochemical points of view

in order to expand knowledge about traditional olive cultivars, which is undoubtedly the first step toward their protection. The biochemical analysis is crucial because it can provide information on unique biomarkers for distinct cultivars while also providing a direct indication of nutraceutical value [15,28,29].

In this framework, the study was carried out on six traditional (Gemlik, Domat, Memecik, Ayvalik, Cilli, and Adana Topagi) compared with international cultivar Manzanilla grown in the same cultivation conditions in eastern Mediterranean, allowing the identification of the most biochemical-rich cultivars, additionally to the identification of specific metabolites using GC-FID and HS-SPME-GC-MS, respectively.

2. Materials and Methods

2.1. Plant Material

Six traditional olive cultivars of *Olea europaea*. L (Gemlik, Domat, Memecik, Ayvalik, Cilli, and Adana Topagi) have been harvested from the Implementation and Experimental research area at Çukurova University in October 2019. The international cultivar Manzanilla is also included and compared with other cultivars. The fruits were harvested at commercially maturity stage based on change skin color for each cultivar. For each cultivar approximately 2 kg of fruits were harvested from 3 trees per cultivar. The maturity index (MI) was assessed according to the method already defined by Cherubini et al. [30], which consists of the estimation of olive skin and pulp color.

2.2. Evaluation of the Fruit's Skin Color

The color of freshly harvested olive fruits was assessed on both sides of the fruits by using a handheld tri-stimulus colorimeter (Minolta Chroma Meter CR-300, CIE, 1976) and a CIE standard illuminant C to determine the CIE color space co-ordinates, L*, a*, b*, C, and hue° ($h°$) values. The fruits "a" has a negative value and "b" has a positive value, the hue angle formula is 180 + $\tan^{-1}$ (b/a) applied. The lightness value, L*, showed how dark/light the sample was (ranging from 0: black to 100: white), a* determined the measurement of greenness/redness (ranging from −60 to +60), while b* was indicated the grade of blueness/yellowness (also ranging from −60 to +60), C was the color intensity of the skin, and the $h°$ distinguished one color from another and is labeled using common color names like blue, yellow, red, green, etc. It defines a color in terms of how close it is to white or black. The colorimeter was calibrated against a standard [29] and the results are expressed as the mean of three replications.

2.3. Extraction and Quantification of Chlorophyll

In the study, 1 g homogenized olive sample was put into 50 mL tubes and later 5 mL solvent mixture (chloroform/methanol, 2:1) were added [31]. The resulting solid residue was filtered off. A 25 mL bottle was filled with the extraction solvent. The solid residue was extracted three times in succession until total discoloration was achieved. Finally, a volume of solvent mixture was added to make up the total volume of 25 mL. All these processes were carried out under dark conditions. The solution was investigated for chlorophyll a (Chl a) and chlorophyll b (Chl b) using a Thermo Multi Scan Go spectrophotometer at 645 nm and 663 nm, respectively. The total chlorophyll was calculated as the sum of chlorophyll a and b. The results were expressed as mg mL^{-1} and were calculated using McKinney equations (Equations (1)–(3)):

$$\text{Chl a} \left(\text{mg mL}^{-1}\right) = 12.7\, A_{663} - 2.69\, A_{645} \quad (1)$$

$$\text{Chl b} \left(\text{mg mL}^{-1}\right) = 22.9\, A_{645} - 4.68\, A_{663} \quad (2)$$

$$\text{Total} \left(\text{mg mL}^{-1}\right) = \text{Chl a} + \text{Chl b} \quad (3)$$

2.4. Oil Extraction

Oil extraction was performed based on the method of Bligh and Dyer [32]. A known weight of fresh olive fruits (15 g) was extracted using hexane solvent for 2 h using automatic Soxhlet equipment (Gerhardt Soxtherm, C Gerhardt GmbH & Co. Wiesbaden, Germany), and triplicate analysis was reported for each cultivar. The residue was dried to a constant weight. The fatty acid analysis was conducted according to method [33] by transforming the fatty acid to the corresponding methyl ester form.

2.5. Determination of Total Phenolic Compounds (TPC)

For TPC analysis phenolic compounds extraction was done. Their skin + flesh was homogenized using a kitchen blender and immediately stored at −80 °C until analysis. These triplicated homogenized materials were used for further TPC analysis. The determination of total phenolic compounds was made by the Folin–Ciocalteu method defined by Spanos and Wrolstad [34] with slight modifications. A volume of 50 µL from each sample was added to 100 µL Folin–Ciocalteu. Then, 1500 µL of distilled water was added and the mixture incubated in the dark for 2 h after the addition of 50 µL 20% Na_2CO_3. The total amount of phenolic compounds was read at 765 nm against a blank using a Thermo Multi Scan Go spectrophotometer (Long Beach, CA, USA). Each analysis was done three times and calculated based on the calibration curve of gallic acid and expressed as mg GAE 100 g^{-1} fresh weight (FW).

2.6. Determination of DPPH Scavenging Activity

The DPPH radical scavenging activity of olive was determined according to the assay method reported by Brand-Williams et al. [35]. The absorbance was measured at 515 nm after 30 min of incubation using a Thermo Multi Scan Go Spectrophotometer (Long Beach, CA, USA) against a blank, and the percentage was calculated according to the following formula (Equation (4)). The results were expressed as the mean of three replications (n = 3).

$$\text{DPPH } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

where

$A_{control}$: the absorbance of control
A_{sample}: the absorbance of the sample

2.7. Characterization of Fatty Acids and Volatile Compounds

2.7.1. Characterization of Fatty Acids by GC-FID

Fatty acids were analyzed using a Clarus 500 Gas Chromatograph equipped with an auto-sampler, a flame ionization detector (Perkin Elmer, Shelton, CT, USA), and a fused-silica capillary SGE column (ID 0.25 µm, BP20 0.25 UM, 100 m × 0.32 mm; Perkin Elmer, Austin, TX, USA). The oven temperature was held at 140 °C for 5 min, and then heightened to 200 °C during 4 °C min^{-1} and then to 220 °C for 1 °C min^{-1}, while the injector and the detector temperatures were set to 220 and 280 °C, respectively. The sample volume was 1 µL, and the carrier gas was controlled at 16 psi. The split ratio was 1:100. The retention indices of FAMEs were compared to a standard composing of FAME mixture of 37 chemicals to detect fatty acid (Supelco, Bellefonte, PA, USA).

2.7.2. Characterization of Volatile Compounds by HS-SPME-GC-MS

One gram of homogenized fruit was weighed and mixed to 1 mL $CaCl_2$ before incubating for 20 min at 30 °C. The grey SPME (85 µm Carboxen/Polydimethylsiloxane; Supelco Co., Sigma Aldrich, Spruce Street, Saint Louis, MO, USA) fiber was immersed in the vial headspace, and the volatiles was collected for 30 min at 40 °C. After sampling, the SPME fiber was inserted into the GC-MS injection port. Volatile compounds were analyzed on the GC-MS-QP2010 Shimadzu machine supplied with an Agilent (0.25 µm thickness,

30 m × 0.25 mm i.d.) CP Sil 5CB fused-silica capillary column. Helium (1 mL min^{-1} flow rate) was used as a carrier gas. The injector was set to spitless injection at a temperature of 250 °C. The oven temperature was held at 5 °C then 60 °C for 1 min and then raised to 260 °C for 20 min. MS was taken at 70 eV. The mass range was from 30 to 425 m/z. Using the NIST and Wiley library, and the in-house Libraries of Essential Oil Constituents the main components was identified by comparing their mass spectra and retention time data. Basing on the total ion chromatograms, relative percentage amounts of isolated molecules were calculated. The C7 to C24 alkanes were used.

2.8. Statistical Analysis

The experiments were conducted in three replicates and data were expressed as mean ± standard deviation. The data were statistically processed by one-way ANOVA and the mean separation was performed through Duncan's Multiple range test at 0.05 probability level, using the SPSS software version 21 (IBM Corp, Armonk, NY, USA).

3. Results and Discussion

3.1. Measurement of Olive's Skin Color

The four studied cultivars didn't have much different dynamics of fruit ripening. At the moment of sampling, Memecik, Cilli, Ayvalik, Manzanilla, Gemlik, Domat and Adana Topagi had comparable maturity indices (3.5, 3.6, 3.7, 3.9, 4.1, 4.2 and 4.3, respectively). Most of the fruits of cultivars had light violet epidermis.

Skin color for the seven cultivars is shown in Table 1 which lists the measurement of five parameters (L^*, a^*, b^*, C, and h°). Significant difference ($p < 0.05$) was recognized for all cultivars for color parameters.

Table 1. The characterization of the skin color ($n = 3$).

Cultivars	Parameters				
	L^*	a^*	b^*	C	h°
Manzanilla	50.59 ± 0.94 ^{d}	−8.53 ± 0.66 ^{bc}	33.68 ± 2.84 ^{ab}	34.75 ± 2.75 ^{ab}	104.29 ± 1.62 ^{ab}
Gemlik	53.54 ± 3.12 ^{b}	−7.29 ± 0.62 ^{b}	36.27 ± 3.28 ^{a}	37.01 ± 3.15 ^{a}	101.45 ± 1.73 ^{cd}
Domat	52.15 ± 2.59 ^{bc}	−8.94 ± 0.84 ^{c}	30.34 ± 1.58 ^{bc}	31.60 ± 1.59 b^{c}	106.45 ± 1.47 ^{a}
Memecik	58.60 ± 2.33 ^{a}	−7.34 ± 1.03 ^{b}	37.26 ± 4.16 ^{a}	37.99 ± 4.04 ^{a}	101.27 ± 2.24 ^{cd}
Ayvalik	57.30 ± 0.55 ^{a}	−6.03 ± 0.78 ^{a}	33.49 ± 0.65 ^{ab}	34.03 ± 0.76 ^{abc}	100.20 ± 1.14 ^{d}
Cilli	50.17 ± 1.25 ^{d}	−8.78 ± 0.83 ^{c}	29.14 ± 2.19 ^{c}	30.43 ± 2.25 ^{c}	106.77 ± 1.20 ^{a}
Adana Topagi	52.5 ± 0.56 ^{bc}	−8.15 ± 0.77 ^{bc}	34.44 ± 2.53 ^{ab}	35.41 ± 2.26 ^{ab}	103.43 ± 2.30 ^{bc}

L^*: Lightness; a^*: greenness/redness; b^*: blueness/yellowness of the fruit skin; C: color intensity an h°: distinguished one color from another and is labeled using common color names like blue, yellow, red, green. Different letters in the same column indicates there is a statistically significant difference ($p < 0.05$). Data are presented as means of triplicates.

The Memecik and Ayvalik cultivars showed the highest lightness values compared to the other investigated cultivars (Table 1). The highest green/red value was obtained for the Ayvalik cultivar while the lowest values were observed in Domat and Cilli cultivars (Table 1). Memecik and Gemlik had significantly higher fruit blue/yellow color ratio and fruit color intensity compared to Domat and Cilli and comparable values to Manzanilla, Ayvalik, and Adana Topagi cultivars (Table 1). Manzanilla, Domat, and Cilli had comparable fruit hue (average value from 104.29 to 106.77, respectively), as well as Ayvalik, Memecik, and Gemlik cultivars (average values from 100.20 to 101.45, respectively) (Table 1). The obtained hue for Adana Topagi was between the two groups with an average value of 103.43 (Table 1).

Our findings were in accordance with Kumcuoğlu et al. [36] who mentioned that the Memecik cultivar had a lightness level of around 51.46 with a blueness/yellowness value of about 31.65. While the Ayvalik cultivar showed the highest greenness/redness value of 6.03 followed by Gemlik, Memecik, Adana Topagi, Manzanilla, Cilli, and Domat. Comparatively to the studies of Kesen et al. [37] and Kaftan and Elmaci [38], our results

were different maybe because of the different cultivation area and conditions of each cultivar that influence the skin color and aspect.

3.2. Chlorophyll Content of Olives

The concentrations of pigments (chlorophyll a and b) were summarized in Table 2. The highest chlorophyll a were obtained from Cilli (2.63 mgL^{-1}) and Adana Topagi (2.48 mgL^{-1}) compared to the other investigated cultivars. Gemlik and Domat overall exhibited the lowest chlorophyll a values (2.63 and 2.48 mgL^{-1}, respectively) (Table 2). Adana Topagi had significantly higher fruit chlorophyll b values (3.34 mgL^{-1}) compared to rest of cultivars and followed by Cilli (2.74 mgL^{-1}) (Table 2). Among the cultivars Gemlik had the lowest chlorophyll b value (1.29 mgL^{-1}) (Table 2).

Table 2. Chlorophyll a, chlorophyll b and total chlorophyll content of cultivars (n = 3).

Cultivars	Concentration of Chlorophyll (mgL^{-1})		
	Chlorophyll a	**Chlorophyll b**	**Total**
Manzanilla	1.92 ± 0.10^{b}	2.18 ± 0.12^{cd}	4.10 ± 0.12^{b}
Gemlik	1.10 ± 0.08^{d}	1.29 ± 0.08^{e}	2.39 ± 0.06^{d}
Domat	1.19 ± 0.13^{d}	1.80 ± 0.11^{d}	2.99 ± 0.09^{cd}
Memecik	1.39 ± 0.14^{cd}	1.73 ± 0.09^{d}	3.12 ± 0.10^{c}
Ayvalik	1.60 ± 0.13^{bc}	2.27 ± 0.13^{c}	3.87 ± 0.09^{bc}
Cilli	2.63 ± 0.16^{a}	2.74 ± 0.14^{b}	5.37 ± 0.26^{a}
Adana Topagi	2.48 ± 0.13^{a}	3.34 ± 0.16^{a}	5.82 ± 0.29^{a}

Different letters in the same column indicate that there is a statistically significant difference ($p < 0.05$). Data are presented as means of triplicates.

The total amount of chlorophyll a and chlorophyll b was at the highest level of 5.82 and 5.37 mg L^{-1} for Adana Topagi and Cilli cultivars compared to the other cultivars (Table 2). Ayvalik, Memecik and Domat has the same total chlorophyll values (3.87, 3.12 and 2.99 mg L^{-1}, respectively). The obtained total chlorophyll for Manzanilla was between the two groups with an average value of 4.10 mg L^{-1} (Table 2). The cultivar Gemlik had the lowest total chlorophyll values (2.39 mg L^{-1}) (Table 2). Chlorophyll b was obtained by a direct transformation of chlorophyll a. As result, chlorophyll b was more accumulated in the fruits [39–41]. During the biosynthetic turnover of chlorophyll, the degradation pathway of chlorophyll a has been present potentially more than the anabolic one [41], which explains its low amounts in fruits.

3.3. TPC and DPPH Scavenging Activity

The TPC and DPPH scavenging activity were reported in Table 3. The Memecik cultivar exhibited the highest TPC as 762 mg GAE 100 g^{-1} and followed by Domat (669 mg GAE 100 g^{-1}) (Table 3) while the lowest TPC was obtained from Cilli and Adana Topagi (497 and 481 mg GAE 100 g^{-1}) (Table 3).

Memecik had significantly higher DPPH radical scavenging activity (83.58%) compared to all investigated cultivars, except Manzanilla (Table 3).

Some studies suggests that there is a relationship between the increase in total phenolic content provides more OH groups in the extract, which is considered a donner of proton and furnishes a potent scavenging activity of DPPH [42]. Although, in some cases the reagents added for the determination of TPC destroyed the structure of phenolic compounds [43]. Therefore, the OH groups will react with different reagents in the medium which gives a high antioxidant quantity, but since their structure is modified, this may give a low antioxidant activity [44].

Table 3. TPC content and DPPH scavenging activity of cultivar's olive fruit (n = 3).

Cultivars	TPC (mg GAE 100 g^{-1})	DPPH (%)
Manzanilla	592 ± 28 ^{c}	81.82 ± 3.2 ^{ab}
Gemlik	569 ± 21 ^{d}	80.21 ± 3.6 ^{b}
Domat	669 ± 28 ^{b}	74.00 ± 3.3 ^{bc}
Memecik	762 ± 32 ^{a}	83.58 ± 4.4 ^{a}
Ayvalik	526 ± 23 ^{e}	75.92 ± 4.2 ^{bc}
Cilli	497 ± 20 ^{f}	76.85 ± 3.4 ^{bc}
Adana Topagi	481 ± 19 ^{f}	76.35 ± 3.1 ^{c}

Different letters in the same column indicate that there is a statistically significant difference ($p < 0.05$). Data is presented as means of triplicates.

According to Cicerale et al. [45] phenolic compounds obtained from olives decrease atherosclerosis, cancer, cardiovascular diseases due to a synergic antioxidant and anti-inflammatory activity [46,47].

Brahmi et al. [48], proved that the concentration of phenols in *Olea europea* unripe fruits harvested in October exhibit a high amount of phenolic compounds around 802 mg GAE 100 g^{-1} FW with a potent DPPH scavenging activity range between 93.94% and 96.88% which are higher than our findings. In Greece, total phenolic content (TPC) ranged from 803–1796 mg 100 g^{-1} FW in green fruits of national olive cultivars that indicating higher values than our results. Valanolia, Pikrolia Kerkiras, Kalamon, and Romeiki presented the highest TPC concentrations, whereas Thassitiki, Megaritiki, Maronia' and 'Petrolia' the lowest ones. The remaining cultivars had medium TPC values [49]. Ozcan et al. [50] used a number of olive cultivars in Turkey to determine total phenolic content and DPPH radical scavenging capacity in different harvest periods. They reported that the highest total phenol (317.70 mg 100 g^{-1}) in Tavşan Yüreği olive fruit harvested in December. The highest antioxidant activities (83.84%) were determined in Edremit fruit harvested in August.

3.4. Characterization of Fatty Acids

The saturated, mono-unsaturated, and polyunsaturated fatty acids of the olive cultivars were presented in Table 4. The abundant fatty acid in olive is oleic acid (18:1), which is followed by palmitic acid (16:0), linoleic acid (18:2), palmitoleic acid (16:1), stearic acid (18:0), and linoleic acid (18:3), as well as other minor fatty acids whose abundance does not exceed 1% of overall fraction [51–53].

Manzanilla and Ayvalik cultivars showed the highest total amount of saturated fatty acids (SFA) (18.94 and 18.49, respectively), where the major compound was palmitic acid (18.7%). Our findings were in accordance with Xiang et al. [54], who proved that palmitic acid was the major saturated fatty acid in Manzanilla's cultivar.

For the mono-unsaturated amounts (MUFA), compared to all traditional cultivars, as well as, to Manzanilla, the Gemlik cultivar presents the highest oleic acid percentage (66.81%). Our finding was in accordance with Diraman and Dibeklioglu [47]. It was proved that during the postprandial state in healthy persons, palmitic acid has a regulatory effect on various fibrinolytic and thrombogenic indicators [55,56]. Indeed, oleic acid was a major compound in Turkish olive cultivars [47].

As for polyunsaturated acids (PUFA), Memecik cultivar displayed the highest amount of linoleic acid of 23.12%. On the other hand, Ayvalik olives exhibited 0.93% of linolenic acid, while Domat presents the lowest value of around 0.39%. Our results are in agreement with previous studies [48–51]. Memecik, Ayvalik, and Cilli cultivars presented a high amount in polyunsaturated acids compared with Manzanilla cultivar. The high amount in linoleic acid improved hypertension, heart diseases [57,58].

Table 4. Characterization of fatty acids by GC-FID (n = 3).

Fatty Acids (%)	Cultivars						
	Manzanilla	**Gemlik**	**Domat**	**Memecik**	**Ayvalik**	**Cilli**	**Adana Topagi**
Myristic	0.19 ± 0.01	0.48 ± 0.38	0.53 ± 0.50	0.51 ± 0.02	0.46 ± 0.57	0.26 ± 0.23	0.49 ± 0.09
Palmitic	18.7 ± 1.20 ^{a}	16.74 ± 0.02 ^{bc}	15.05 ± 0.09 ^{c}	13.09 ± 0.13 ^{d}	17.51 ± 0.19 ^{ab}	15.52 ± 0.58 ^{c}	11.99 ± 1.91 ^{d}
Stearic	0.06 ± 0.08 ^{b}	0.35 ± 0.04 ^{b}	0.48 ± 0.67 ^{a}	n.d.	0.53 ± 0.04 ^{a}	n.d.	n.d.
Total SFA	18.94 ± 1.29 ^{a}	17.51 ± 0.44 ^{b}	16.05 ± 1.27 ^{c}	13.59 ± 0.16 ^{d}	18.49 ± 0.79 ^{a}	15.78 ± 0.81 ^{c}	12.48 ± 2.00 ^{e}
Palmitoleic	0.44 ± 0.46 ^{cd}	0.23 ± 0.28 ^{d}	0.94 ± 0.41 ^{b}	0.73 ± 0.18 ^{bc}	0.47 ± 0.03 ^{cd}	1.46 ± 0.37 ^{a}	1.83 ± 0.13 ^{a}
Oleic	54.71 ± 3.95 ^{d}	66.81 ± 0.42 ^{a}	58.36 ± 0.38 ^{bc}	57.38 ± 0.51 ^{c}	53.41 ± 0.5 ^{d}	54.72 ± 1.87 ^{d}	60.38 ± 1.22 ^{b}
Total MUFAs	55.15 ± 4.41 ^{e}	67.04 ± 0.70 ^{a}	59.3 ± 0.79 ^{c}	58.11 ± 0.69 ^{cd}	53.88 ± 0.53 ^{e}	56.18 ± 2.23 ^{de}	62.21 ± 1.34 ^{b}
Linoleic	11.91 ± 0.74 ^{d}	8.18 ± 0.2 ^{e}	10.32 ± 0.18 ^{e}	23.12 ± 0.31 ^{a}	21.76 ± 0.27 ^{b}	16.65 ± 0.73 ^{c}	8.7 ± 0.33 ^{e}
Linolenic	0.59 ± 0.10 ^{bc}	0.66 ± 0.05 ^{b}	0.39 ± 0.02 ^{e}	0.49 ± 0.05 ^{d}	0.93 ± 0.04 ^{a}	0.67 ± 0.01 ^{b}	0.54 ± 0.15 ^{cd}
Total PUFA	12.5 ± 0.84 ^{d}	8.84 ± 0.26 ^{f}	10.71 ± 0.20 ^{e}	23.61 ± 0.36 ^{a}	22.69 ± 0.31 ^{b}	17.32 ± 0.74 ^{c}	9.24 ± 0.48 ^{f}

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; and PUFA: polyunsaturated fatty acid. Different letters in the same row indicate that there is a statistically significant difference ($p < 0.05$). Data is presented as means of triplicates. n.d.: non determined.

3.5. Characterization of Volatile Compounds

Quantitative data of volatile compounds in each cultivar of olives was reported in Table 5, 32 compounds were identified, namely aldehydes, alcohols, ketones, acids, esters, and terpenes. The sensory quality of olive oils is impacted by the diversity and concentration of volatile and non-volatile chemicals, which varies by climatic, edaphic, cultivation conditions, and cultivar, allowing the product's origin to be determined [59].

The total aldehydes amount ranged between 28.26% and 50.60%, in particular Domat had the highest amount of hexanal with 44.42%. Interestingly, the latter compound contributes to fruity, bitter, green grass, and astringent sensory features of the olive oil [60,61]. Alternative markers for oil oxidation could include volatile molecules like hexanal, which is directly linked to oxidative off-flavor [59].

In addition, compared with Manzanilla cultivar, Adana Topagi shows the highest level of alcohols around 61.34% in which the major compound was (*Z*)-3-hexen-1-ol at 54.35%, which is known to give banana note [58], as for three Dalmatian indigenous cultivars [55]. The major aromatic compounds in olives are *E*-2-hexenal (green leaf), (*Z*)-3-hexenol (green leaf, green banana), and hexanal (green leaf, apple) [62–65]. Luna et al. [66], Sánchez et al. [67], demonstrated that Manzanilla cultivar presented a mean content of alcohols.

Concerning ketones percentages, Memecik cultivar exhibited the highest level with a total of 17.86% (Table 5). Ethyl butyl ketone was the major compound registered in Manzanilla cultivar (Table 5). Paradoxically, ethyl butyl ketone was not detected in more than 70 cultivars analyzed by Luna et al. [66], Cecchi et al. [68] and Žanetic et al. [69].

On the other hand, Adana Topagi showed the highest amount of octadec-9-enoic acid with 1.63%. In the same context, three esters ranging between 0 and 2.65% were also characterized. The major compound was registered in Adana Topagi cultivar as isobutyl isobutyrate, associated to cheesy and fruity taste [70–72]. Compared with Manzanilla, there is no significant difference in ester concentration. Luna et al. [66], mentioned that Manzanilla's cultivar had a mean content of esters.

For the terpenes, the highest level was found in Memecik cultivar with an amount of 20.34%, in which the major compound was β-pinene, a bicyclic monoterpene with a wide array of biological activities [73]. The concentration of volatile compounds characterized the quality were strongly influenced by storage conditions, the extraction process, and the climate with the growing condition of the olive [66,69–71]. Previous studies showed that cultivars belong to different horticultural species have a significant effect on biochemical and aroma profile [74–81].

Table 5. Profile of volatile compounds for the seven olive cultivars (%).

RT (Min)	Compounds	Manzanilla	Gemlik	Domat	Memecik	Ayvalik	Cilli	Adana Topagi
				Aldehydes				
7.053	(*E*)-2-Pentenal	1.60 ± 0.08	0.73 ± 0.04	0.35 ± 0.03	0.31 ± 0.03	n.d.	0.45 ± 0.05	n.d.
8.064	Cis-3-Hexenal	0.92 ± 0.05	0.61 ± 0.01	0.27 ± 0.01	n.d.	2.88 ± 0.13	3.47 ± 0.19	3.23 ± 0.17
8.173	Hexanal	28.94 ± 1.12 ^{b}	27.59 ± 1.04 ^{b}	44.42 ± 2.20 ^{a}	16.90 ± 0.81 ^{c}	26.87 ± 1.21 ^{b}	15.82 ± 1.02 ^{c}	15.48 ± 1.07 ^{c}
8.958	2-Furancarboxaldehyde	n.d. ^{NS}	4.67 ± 0.30	n.d.	n.d.	n.d.	n.d.	2.01 ± 0.18
10.481	2-Hexenal	1.20 ± 0.01 ^{c}	3.82 ± 0.10 ^{b}	4.83 ± 0.18 ^{b}	5.17 ± 0.21 ^{b}	4.91 ± 0.19 ^{b}	15.36 ± 1.08 ^{a}	3.92 ± 0.20 ^{b}
12.471	2,4-Hexadienal	0.92 ± 0.07	n.d.	0.58 ± 0.06	2.07 ± 0.10	n.d.	n.d.	n.d.
16.15	Octanal	0.62 ± 0.04	1.19 ± 0.03	0.15 ± 0.00	1.97 ± 0.09	0.40 ± 0.01	n.d.	0.48 ± 0.01
19.727	Nonanal	1.10 ± 0.08	0.17 ± 0.00	n.d.	2.52 ± 0.11	2.07 ± 0.10	0.76 ± 0.08	0.72 ± 0.06
23.112	Decanal	1.17 ± 0.10	n.d.	n.d.	0.96 ± 0.07	1.32 ± 0.07	2.41 ± 0.11	2.78 ± 0.09
	Total aldehydes	36.47 ± 2.10 ^{b}	38.78 ± 2.43 ^{b}	50.60 ± 3.40 ^{a}	29.90 ± 2.80 ^{c}	38.45 ± 1.98 ^{b}	38.27 ± 2.24 ^{b}	28.62 ± 1.15 ^{c}
				Alcohols				
5.258	1-Penten-3-ol	1.05 ± 0.02	0.89 ± 0.01	n.d.	0.18 ± 0.01	n.d.	n.d.	n.d.
7.896	(*Z*)-2-Penten-1-ol	0.43 ± 0.02	1.23 ± 0.05	0.34 ± 0.01	2.21 ± 0.06	0.62 ± 0.00	1.59 ± 0.05	2.17 ± 0.09
10.578	(*Z*)-3-Hexen-1-ol	34.22 ± 1.80 ^{e}	34.37 ± 1.72 ^{e}	36.48 ± 1.87 ^{d}	15.40 ± 0.80 ^{f}	42.89 ± 2.14 ^{c}	45.16 ± 2.80 ^{b}	54.35 ± 2.96 ^{a}
11.157	1-Hexanol	6.98 ± 0.80 ^{a}	0.89 ± 0.03 ^{c}	2.88 ± 0.12 ^{b}	0.23 ± 0.03 ^{c}	6.76 ± 0.93 ^{a}	2.06 ± 0.18 ^{b}	n.d. ^{c}
18.69	1-Octanol	0.24 ± 0.02	n.d.	n.d.	0.39 ± 0.02	n.d.	n.d.	n.d.
29.049	4-Hydroxy-benzeneethanol	n.d. ^{NS}	n.d.	n.d.	7.34 ± 0.28	0.92 ± 0.02	n.d.	2.75 ± 0.06
48.996	Phytol	4.42 ± 0.15 ^{a}	n.d. ^{c}	n.d. ^{c}	5.82 ± 0.17 ^{a}	n.d. ^{c}	2.66 ± 0.09 ^{b}	2.07 ± 0.07 ^{b}
	Total alcohols	47.34 ± 2.21 ^{c}	37.38 ± 2.03 ^{e}	39.70 ± 1.96 ^{d}	31.57 ± 1.43 ^{f}	51.19 ± 2.43 ^{b}	51.47 ± 2.20 ^{b}	61.34 ± 3.01 ^{a}
				Ketones				
2.053	2-Propanone	n.d. ^{NS}	n.d.	0.87± 0.01	0.16± 0.01	n.d.	n.d.	n.d.
5.005	1-Penten-3-one	2.65 ± 0.10 ^{c}	7.76 ± 0.31 ^{a}	0.46 ± 0.02 ^{d}	5.38 ± 0.22 ^{b}	0.79 ± 0.02 ^{d}	0.75 ± 0.02 ^{d}	n.d.
13.595	Ethyl butyl ketone	8.17 ± 0.22 ^{a}	4.93 ± 0.12 ^{b}	2.31 ± 0.09 ^{c}	4.77 ± 0.14 ^{b}	n.d.	0.43 ± 0.02 ^{d}	n.d.

Table 5. *Cont.*

RT (Min)	Compounds	Manzanilla	Gemlik	Domat	Memecik	Ayvalik	Cilli	Adana Topagi
24.284	2-Methyl-5-(1-methylethenyl)-2-Cyclohexen-1-one	1.49 ± 0.04 ^{b}	n.d.	1.38 ± 0.03 ^{b}	7.55 ± 0.35 ^{a}	n.d.	1.85 ± 0.05 ^{b}	n.d.
	Total ketones	12.31 ± 0.48 ^{b}	12.69 ± 0.52 ^{b}	5.02 ± 0.12 ^{c}	17.86 ± 0.42 ^{a}	0.79 ± 0.02 ^{e}	2.99 ± 0.06 ^{d}	n.d.
				Acids				
2.845	Acetic acid	1.31± 0.02	n.d.	n.d.	n.d.	1.39± 0.02	n.d.	1.55± 0.01
21.785	Octanoic acid	n.d. ^{NS}	n.d.	n.d.	n.d.	0.64± 0.01	0.74	1.21± 0.01
42.410	Pentadecanoic acid	n.d. ^{NS}	n.d.	n.d.	n.d.	n.d.	n.d.	0.67± 0.01
46.221	Octadec-9-enoic acid	n.d. ^{NS}	n.d.	n.d.	n.d.	n.d.	n.d.	1.63± 0.03
	Total acids	1.31± 0.02 ^{bc}	n.d.	n.d.	n.d.	2.03 ± 0.04 ^{b}	0.74 ± 0.01 ^{c}	5.05± 0.05 ^{a}
				Esters				
14.706	Isobutyl isobutyrate	0.96 ± 0.03	0.57 ± 0.03	0.92 ± 0.04	n.d.	n.d.	n.d.	2.04 ± 0.07
15.088	Amyl acetate	1.14 ± 0.09	1.87 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.
34.399	2,2,4-Trimethyl-pentan-1,3-diol diisobutyrate	0.21 ± 0.03	0.21 ± 0.03	0.09 ± 0.00	n.d.	n.d.	n.d.	n.d.
	Total esters	2.30 ± 0.08	2.65 ± 0.07	1.01 ± 0.03	n.d.	n.d.	n.d.	2.04 ± 0.03
				Terpenes				
16.226	β-Myrcene	n.d. ^{NS}	0.92 ± 0.04	n.d.	1.80 ± 0.07	1.21 ± 0.08	n.d.	n.d.
17.372	β-Pinene	n.d. ^{b}	1.58 ± 0.08 ^{b}	n.d. ^{b}	12.38 ± 0.48 ^{a}	2.01 ± 0.09 ^{b}	1.05 ± 0.05 ^{b}	n.d. ^{b}
19.550	(1-Methylethenyl)-Benzene	0.27 ± 0.00	n.d.	0.51 ± 0.00	5.28 ± 0.28	2.17 ± 0.11	3.67 ± 0.13	n.d.
29.169	α-Copaene	n.d. ^{NS}	n.d.	0.11 ± 0.00	0.71 ± 0.02	1.96 ± 0.04	0.69 ± 0.03	n.d.
30.634	7-epi-sesquithujene	n.d. ^{NS}	n.d.	3.05 ± 0.14	0.18 ± 0.00	n.d.	n.d.	2.68 ± 0.12
	Total terpenes	0.27 ± 0.00 ^{f}	2.50 ± 0.12 ^{e}	3.67 ± 0.14 ^{d}	20.34 ± 1.08 ^{a}	7.35 ± 0.28 ^{b}	5.40 ± 0.22 ^{c}	2.69 ± 0.12 ^{d}

Different letters in the same row indicate that there is a statistically significant difference ($p < 0.05$). Data is presented as means of triplicates. n.d.: non determined. NS: Non-significant.

4. Conclusions

Even though more consideration has been consistently given to their beneficial and delicious oil as well as their food delights. Olive fruit remains one of the world's most widely savored foods. Their characterization was one of the important aspects that should have received great attention. In this context, our study gives importance to this aspect by characterizing six traditional olives compared by Manzanilla cultivar based on their fatty acids, volatile compounds, chlorophyll amount, phenolic compounds, their antioxidant activity, esters, and skin color. Compared to traditional olives and Manzanilla cultivar, Memecik showed the highest amount of total phenolic content, terpenes, ketones, and lightness. Meanwhile, Ayvalik showed the highest amount of greenness/redness. Chlorophyll b and alcohols found more in Adana Topagi olives. Although Cilli has the highest amount of total chlorophyll, Domat cultivar presented the highest amount of aldehydes, where the hexanal was the major compound, respectively. Thus, this characterization gives valuable information about each cultivar that can be relevant in the agriculture and industrial sectors at the same time and our local cultivars can be evaluated specifically for further breeding programs.

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