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## ORIGINAL ARTICLE

# Determination of the quality and purity characteristics of olive oils obtained from different regions of Turkey, depending on climatic changes

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## Abstract

Virgin olive oils (VOOs) obtained from olives grown in different regions of Turkey under changing climatic conditions sometimes show different sensory and chemical properties. This study was planned to determine whether these deviations are due to climatic changes or not. For this purpose, five different olive varieties (Ayvalık, Memecik, Gemlik, Nizip Yağlık, Kilis Yağlık) of commercial importance were harvested from the provinces/districts (four different region) where cultivation is intense during the 2017/2018–2020/2021 harvest years. Every year, olive samples were collected from 3 orchards from 13 provinces/districts. One hundred and fifty-six samples were subjected to the purity, quality and sensory analysis. Basic climatic values (average, minimum and maximum temperature, humidity and precipitation) were examined for four consecutive years. All of the examined olive oil samples were determined within the legal limits in terms of fatty acid composition and fatty acid ethyl ester values. However, delta-7-stigmastenol value from the sterol composition was found to be above 0.5% in some samples in all the years studied (total 21 samples). Delta-7-stigmastenol values of olive oil samples varied between 0.16% and 1.14%. Multiple linear regression analysis was applied using a genetic algorithm-based inverse least squares method to determine whether there is a relationship between climate data and delta-7-stigmastenol values. According to this result, it has been determined that the delta-7-stigmastenol value is high when the annual average relative humidity is low and the annual average temperature is high. There is an urgent need to make forward-looking plans due to climate change.

## KEYWORDS

climatic conditions, olive oil, PCA, purity, quality

## INTRODUCTION

The olive tree (*Olea europaea* L) is largely grown in Mediterranean countries and is one of the most important crops grown in this region. In Turkey, olive cultivation is carried out in five region, namely Aegean, Marmara, Mediterranean, Southeastern Anatolia and

Black Sea regions. Olive cultivated areas in Turkey tend to increase over the years. In 2019, the total olive grove area was determined as 8.79 million decares. As of 2019, the provinces where olives are grown (especially for olive oil) and production is intense can be listed as Aydın (20%), Muğla (15.2%), İzmir (13.4), Balıkesir (11.3%), Manisa (7.2%), Hatay (6.8%), Gaziantep

(6.6%), Çanakkale (4.8%) and Kilis (4.2%) (Özkan, 2021). Olive varieties that have commercial importance and are cultivated intensively are distributed as 48.71% Gemlik, 20.66% Ayvalık, 19.11% Memecik, 7.56% Domat and 3.73% other varieties (Özaltas et al., 2016).

Climate is one of the most influential physical geography factors influencing plant growth and development. According to the Intergovernmental Panel on Climate Change report, it is estimated that the increase in global temperature will reach 1.5°C in 2030, 2.5°C in 2050 and 4°C in 2100 (Kaya, 2021). In the RCP8.5 scenario, it is estimated that seasonal and annual average air temperatures in Turkey, especially in the Mediterranean, Aegean and Southeastern Anatolia regions, where olive cultivation is intense, will increase significantly in the range of 4–6°C and will become more intense towards the end of 2100. Similarly, between the years 2015–2100; decreases of up to 250–300 mm are foreseen in the total precipitation amounts throughout Turkey, with the exception of the Eastern Black Sea region, which will be more pronounced as of 2050 (Kadioğlu et al., 2017).

It is known that one of the most important factors affecting the spread of the olive tree is temperature. Generally, it is desired that the annual average temperature is between 15 and 20°C in olive growing regions. When the maximum temperature rises to 40°C and/or when the minimum temperature is lower than –7°C, olive trees might be damaged. One of the important factors in olive cultivation is precipitation. Although the olive tree is said to be drought resistant, the annual precipitation demand is 600–800 mm (Özaltas et al., 2016; Ozturk et al., 2021).

Unfortunately, the increase in drought conditions in Southern Europe in recent years (especially the extreme changes observed in temperature and precipitation in spring and summer) has had negative consequences for olive yield. Even the most optimistic future scenarios show that there might be a decrease in fruit production in most olive growing areas (Orlandi et al., 2020). Future climate forecasts point to significant warming and drought trends, and especially the Mediterranean Basin is considered a “hot spot” in terms of climate change. These changes are of great importance for the agricultural sector and especially the olive tree sector (Fraga et al., 2021). The increase in drought conditions observed in some parts of Italy in summer will pose a significant risk in terms of reduced olive production (Orlandi et al., 2020). Global warming will threaten olive groves so that insect and pest populations will increase, in parallel with climate change, olive harvest time will need to be redefined to achieve a balance between high yield and quality of the final product (Algataa, 2020). The predicted changes in terms of annual temperature increases and precipitation decreases will have an impact on many parameters such as the potential distribution area and phenological cycle of olive cultivation (Rodriguez Sousa et al., 2020).

The quality of olive oil in National and International Standards; is evaluated on the basis of free fatty acidity (FFA), peroxide value, UV-specific absorbance values ( $K_{232}$  and  $K_{270}$ ), fatty acid ethyl ester (FAEE) and sensory properties. The chemical composition of olive oil consists of triacylglycerol (~99%) and FFAs, mono and diacylglycerols (Sevim et al., 2019). The fatty acid composition of olive oil is affected by many factors such as variety, maturity and climate (Köseoglu et al., 2016). For this reason, the limits given in both International and National Standards are quite wide. Olive oil contains higher oleic acid, less linoleic and linolenic acid than other vegetable oils. With this feature, it is more resistant to oxidation than other vegetable oils (Papadimitriou et al., 2006). It has been reported that high temperatures change the fatty acid composition and decrease the oil quality by causing a decrease in oleic acid (Nissim et al., 2020). The most important part of the unsaponifiable matter of olive oil is sterols. The composition and content of sterols, vary depending on the agronomic and climatic conditions, the quality of the fruit, the extraction and refining technique applied, and the storage conditions. The main sterols of olive oil are  $\beta$ -sitosterol, delta-5-avenasterol and campesterol (İlyasoğlu, 2009; Lukić et al., 2021). High levels of stigmasterol are associated with high acidity and low organoleptic olive oil quality (Hmida et al., 2022).

Esters of fatty acids and short-chain alcohols in olive oil have been known for more than 30 years. On April 1, 2011, the EU commission made a regulation 61/2011 and established the limits for FAEE parameters for the evaluation of the quality of virgin olive oil. In this way, it is aimed to detect the mixture of natural extra virgin olive oil and low quality oils such as lampant or some deodorized oils. These esters are an indicator of the presence of lampant oil in virgin olive oil (Mariani & Bellan, 2008). FAEE and sensory data are complementary criteria in the classification of olive oil (Gomez-Coca et al., 2012).

The sensory properties of virgin olive oils (VOOs) are affected by several factors, including production area, degree of fruit ripening and olive quality, cultivar, regional climate conditions, and process systems (Perestrelo et al., 2017). Quality extra virgin olive oil is distinguished by its flavor and aroma.

During the development of the olive fruit, there are chemical, physical and physiological changes in the olive fruit, and these changes directly affect the table olive and oil quality. It is critical to investigate the effects of recent changes in climatic events on the chemical properties of olive oil, particularly on purity criteria. According to literatures, no research on the effect of climate change on the quality and purity criteria of olive oils in Turkey has been found so far. For this purpose, 13 olive samples were collected from different provinces/districts during the 2017/2018–2020/2021 harvest years. Olives were harvested from three orchards for

each variety, and the analysis results of the olive oils obtained were evaluated as the average of the orchards. Maturity index (MI) of olive fruits, FFA,  $K_{232}$  and  $K_{270}$ , sterol composition, fatty acid composition, sensory properties and FAEE analysis of olive oils were performed.

## MATERIALS AND METHODS

### Olive samples

In the research, olive samples were harvested from different provinces/districts (the Marmara, Aegean, Mediterranean and Southeastern Anatolia regions) in Turkey, where olive oil production is intense. It was aimed to collect olive varieties with high economic value and intensive cultivation from each region to represent that region.

Care was taken to ensure that the selected gardens were in loamy, clayey-loamy textured, slightly calcareous soils. Since the selected varieties are economically grown at altitudes of 100–600 m, the gardens at these altitudes were chosen. For the Gemlik, Ayvalık and Memecik cultivars, the orchards with 5\*5 m planting range, 12\*12 m for Kilis Yağlık and 10\*10 m planting range for the Nizip Yağlık olive cultivar were determined. In the gardens where the study was carried out, it was tried to be similar to the minimum level of cultural processes. Except for the samples on the trees that were damaged by the disease, all olive fruits were included in the sample to represent the provinces/districts. In this context, five important olive varieties with commercial value in Turkey were harvested. The samples were collected from 13 different provinces/districts from 3 orchards in October–December according to MI.

Figure S1 shows the provinces/districts where olives are harvested. Gemlik samples were harvested from Antalya, Bursa, Hatay, Manisa and Şanlıurfa, Memecik samples were harvested from İzmir, Aydın and Muğla, Ayvalık samples were harvested from Balıkesir, Çanakkale and Mersin, Nizip Yağlık sample was harvested from Gaziantep, Kilis Yağlık sample was harvested from Kilis.

### Climate data

Turkey lies between the temperate and subtropical climate zones. Because of our country's geographical location and landforms, its climate has resulted in the formation of various climate types. Climate data (temperature, min. temperature, max. temperature, relative humidity, rainfall) in the provinces/districts where olive orchards are located were obtained monthly at the end of each year (during 2017/2018–2020/2021 harvest years) from the General Directorate of Meteorology of the Ministry of Environment, Urbanization and climate change at the end of each year (Figure 1).

### Oil extraction

Olive oils were obtained from 10 kg olive fruits with the Abencor system (MC2 Ingenieria y Sistemas, Sevilla, Spain). Olive samples were washed after being separated from their leaves and crushed in a crusher. It was subjected to mixing (malaxation) at 25°C for 30 min. After malaxation, a centrifuge was used to separate the oil from the olive paste. The obtained olive oils were stored under controlled conditions in amber colored bottles at +4°C until the analyses were done after filtering. The total number of samples analyzed was 156 (3 orchard × 13 province/district × 4 crop season).

### Reagents

Chemicals from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), Carlo Erba Reagents (Peypin, France), CHEM-LAB (Zedelgem, Belgium), ISOLAB (Eschau, Germany) and Sigma-Aldrich (Steinheim, Germany) were used in the analyses.

### Determination of maturity index

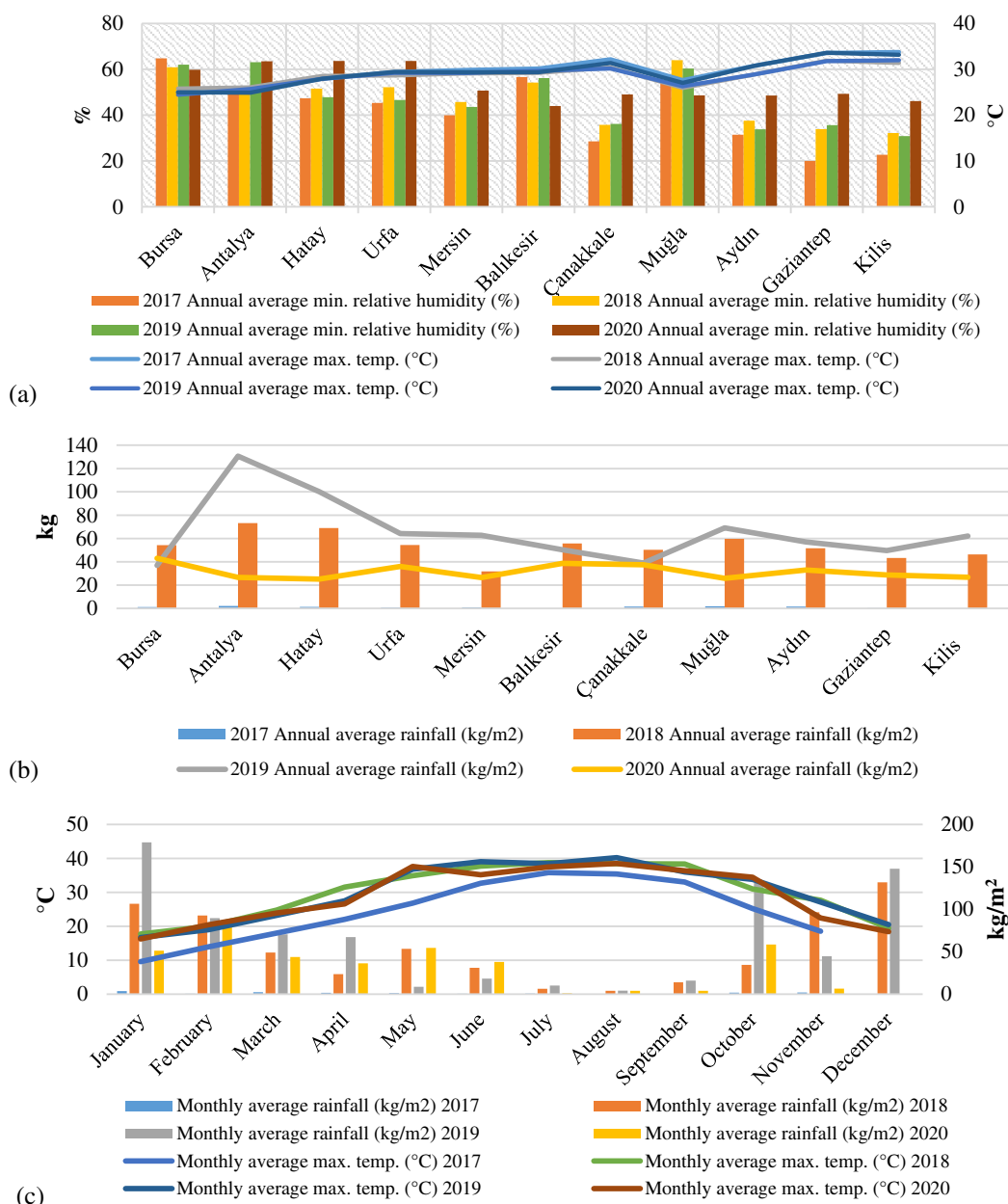
To determine the maturity index (MI) of the olives used in the research, a calculation based on the evaluation of olive skin and pulp colors using 100 olives randomly taken from a 1 kg sample recommended by the International Olive Council (IOC) (IOC, 1991) was used.

### Determination of quality parameters

The FFA (in oleic acid %) (IOC, 2017a) and UV-spectrophotometric indices ( $K_{232}$  and  $K_{270}$  measurements) (IOC, 2019) were measured according to the methods given by the IOC.

### Determination of methyl esters of fatty acids by gas chromatography

The analysis of fatty acid composition of samples was performed using gas chromatography system (HP 6890, Agilent Technologies) equipped with flame ionization detector (FID) described by IOC Methods (modified)—COI/T.20/ Doc. No.332015 (IOC, 2017b). Fatty acid methyl esters were prepared by dissolving 0.1 g of oil sample in 5 ml of n-hexane and 1 ml of potassium hydroxide with methanol. DB 23.30 m × 0.25 mm × 0.250 µm capillary column (AgilentJ & WGC Columns) was used for analyses. The injection volume was 1 µl and the temperature of the detector and injector was 250°C. The temperature of the oven was programmed from 170 to 210°C in increments of 2°C/min. The analysis was terminated by



**FIGURE 1** Climate data of provinces/districts during 2017/2018–2020/2021 harvest years (a) annual average max. Temperature (°C) and annual average min. Relative humidity (%), (b) annual average rainfall (kg/m<sup>2</sup>), (c) monthly average rainfall (kg/m<sup>2</sup>) and monthly average max. Temperature (°C)

keeping the temperature at 210°C for 10 min. The Supelco FAME mix were used as reference standards to identify the fatty acids of the olive oil samples. All fatty acid peak areas were calculated by the HP 3365 Chemstation program and recorded as the peak area percentage.

### Determination of fatty acids ethyl esters by capillary column gas chromatography

The analysis of FAEE contents was made according to the methods in the IOC COI/T.20/Doc. No 28/Rev.2

(IOC, 2017c). Analysis was performed using Gas Chromatography (Agilent Technologies 6850) using a 15 m × 0.32 mm ID × 0.25 μm SPB-5 fused silica capillary column. Fifteen grams of silica gel was suspended in n-hexane and added to the column. Using the analytical balance, exactly about 500 mg of the sample was weighed into a 25 ml bottle and the appropriate amount of internal standard, 0.05 mg of methyl heptadecanoate for olive oil was added. The prepared sample was transferred to the chromatography column with the help of two 2 ml n-hexane fragments. A chromatographic elution of n-hexane/ethyl ether (99:1) was then started and



220 ml was collected every 10 s in a flow of about 15 drops. The resulting fractions were evaporated on a rotary evaporator until the solvent was almost gone. The last 2 ml was removed under a weak stream of nitrogen. The fraction containing methyl and ethyl esters was collected, diluted with 2 ml of n-heptane; 1  $\mu$ l of n-heptane solution with helium was used as carrier gas at an injection volume of 0.5 ml/min. The column temperature was adjusted to 80°C, increased at 20 to 140°C/min, then increased to 5 to 335°C/min and waited for 20 min. The detector temperature was set to 350°C.

### Determination of sterol composition and amount by capillary column gas chromatography

The analysis of sterol composition was made according to the methods in the IOC COI/T.20/Doc. No 26/Rev. 5 (IOC, 2020). Diethyl ether was used to extract the unsaponifiable fraction of olive oil. Thin layer chromatography was used to separate the unsaponifiable sterol fraction. Analysis was performed using Gas Chromatography (Agilent Technologies 6850) equipped with flame ionization detector (FID) using a 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m Supelco 24,034 column. The injection volume was 1  $\mu$ l and the temperature of the detector was 290°C and injector was 280°C. The temperature of the oven was programmed from 260°C. Hydrogen was used as carrier gas at an injection volume of 0.5 ml/min. The flow rate was 0.7/0.8 ml/min. The split ratio was 1:50. It was silylated using pyridine and BSTFA+TMCS as the silylation reagent. 5 $\alpha$ -cholestan-3 $\beta$ -ol was used as internal standard in the analysis. Results were given in both % and mg/kg.

### Evaluation of sensory analysis in virgin olive oil

A sensory evaluation was carried out by the panel, which is recognized by the IOC and has an ISO 17025 accreditation certificate. The sensory profile was conducted by the Turkey Olive Research Institute olive oil panel according to the official methods of IOC-COI/T.20/Doc. No 15/Rev. 10 (IOC, 2018) and the guidelines for the accomplishment of requirements of standard ISO 17025 of sensory testing laboratories with particular reference to virgin olive oil COI/T.28/Doc. No 1/Rev. 6 (IOC, 2021a). Sensory analysis of olive oils was carried out according to the ISO 17025 accreditation procedures of the Institution after the results of the quality and purity analysis. If the results of quality and purity analyzes were appropriate, sensory analysis was performed. Eight panelists (four woman, four man) ranging in age from 33 to 58 years old, were fully trained in the evaluation of virgin olive

oil. Sensory analysis was performed at 20–25°C in a well-lit, neutral, noise-free, and ventilated room. At 10:00 a.m., each panelist tasted in separate booths. A tasting glass was filled with 15 ml of each olive oil sample. The samples were kept at a constant temperature of  $28 \pm 2^\circ\text{C}$ . Each taster in the panel first smelled and then tasted the oil in the tasting glass. Oils were evaluated according to positive properties (fruity, bitter, pungent) and negative properties (heating-muddy residue, moldy-moist, vinous-vinegar, metallic, rancid (obsolete-stale), heated or burnt, straw-woody, coarse, machine oil, black water, brine, whitish, earthy, wormy, cucumber, wet wood). Profile paper was used in the evaluation. Scoring was made on the profile paper with a scale of 10 cm. The results were evaluated by a computer program for obtaining statistical calculations (median).

### Statistical analysis

Significance of the differences among the means of olive oils was determined by ANOVA using the Fisher's least significant difference test at  $p < 0.0001$  significance level. Among the chemometrics methods in the classification of olive oils; principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied and the obtained data were evaluated with Minitab (Minitab 16 Statistical Software, Minitab, Inc., [www.minitab.com](http://www.minitab.com)) statistical data analysis program. For the relationship between climate data and delta-7-stigmastanol values, multiple linear regression analysis was applied by using a genetic algorithm based inverse least squares (GILS) method. The GILS method was written in MATLAB programming language using Matlab R18 (MathWorks Inc.).

## RESULTS AND DISCUSSION

### Climate assessment

Figure 1 shows the climate data of provinces/districts during 2017/2018–2020/2021 harvest years (a) annual average max. temperature ( $^\circ\text{C}$ ) and annual average min. relative humidity (%), (b) annual average rainfall ( $\text{kg}/\text{m}^2$ ), (c) monthly average rainfall ( $\text{kg}/\text{m}^2$ ) and monthly average max. temperature ( $^\circ\text{C}$ ). The annual average max. temperatures were determined from Kilis in 2017 and 2019, from Gaziantep in 2018 and 2020 (Figure 1a). The annual average min. relative humidity was detected from Gaziantep in 2017, from Kilis in 2018 and 2019 and from Balıkesir in 2020. As can be seen in Figure 1c, Turkey is characterized by a very hot climate between June and September and a rather rainy climate between October and June. The temperatures in 2018, 2019 and 2020 were higher than in 2017, the rainfall was at the lowest  $\text{kg}/\text{m}^2$  in 2017, reaching the highest  $\text{kg}/\text{m}^2$  in 2019 and then decreasing again.

The data of 4 years shown that there is an increase in temperatures and fluctuations in precipitation.

## Olive oil analysis

Harvest time is very important parameter on the quality of olive oil. Olives for oil extraction are generally harvested during the greenish/pink/black period, while olives to be processed into table olives are harvested at different maturity levels depending on the processing method (Emmanouilidou et al., 2020). Table 1 shows the MI of olives, FFA (in oleic acid %),  $K_{232}$  and  $K_{270}$ , FAEE (mg/kg) values along with sterol composition (%) of olive oils.

As seen in Table 1, the lowest MI values was determined from Kilis (KY) with 0.94 in 2018, and the highest was determined from Bursa (G) with 4.15 in 2018 ( $p < 0.0001$ ). In general, it was determined that the MI values of the G were higher, and the A and M were lower. Maturation time may vary depending on climatic conditions, irrigation, amount of product and variety characteristics (Roshani et al., 2016). In the same garden and sometimes even on the same tree, the maturation and coloration of olives do not occur at the same time. Considering that low temperatures and high precipitation delay fruit growth and ripening climate is very important for MI and fruit quality (Mafrica et al., 2021).

The FFA is often the first parameter discussed to assess the quality of olive oil production. It is well known that FFA is affected by many factors, including fruit quality, harvest time, storage and production conditions (Piscopo et al., 2021). According to the years, the lowest FFA was found in Bursa (G) with 0.07 (in oleic acid %) in 2017, and the highest in Çanakkale (A) with 1.27 (in oleic acid %) in 2019. The FFA values of only two (Balıkesir [A] and Çanakkale [A]) samples were exceeded the limit of 0.8 (in oleic acid %) for extra virgin olive oil. For that reason, they were categorized as VOO (IOC, 2021b) (Table 1). This increase can be explained by experiencing higher temperatures in the summer and autumn compared to previous years. The above-normal temperatures (37°C) in Balıkesir in September 2018/2019 may have caused the FFA to increase in sample A. Similarly, the FFA of the A sample may have increased due to the high temperatures (36–32°C) especially in July, August and September in Çanakkale in 2019/2020. FFA of olive oil obtained from warmer regions is higher than that of cooler regions (Issaoui et al., 2010). UV-spectrophotometric indices ( $K_{232}$  and  $K_{270}$  values) of olive oils did not exceed the limit that determined by IOC (2021b; Table 1).

The presence of methyl and ethyl esters in olive oil is due to the fermentation that occurs in the olive fruit. Therefore, it is the formation of methyl and ethyl alcohols esterified with FFAs. Methyl and ethyl alcohols are higher in lampant oils. The FAEE value is both a quality

criterion and a purity criterion. The FAEE values of samples were found to be statistically significant. According to the years, the lowest FAEE value was determined in Bursa (G) in 2017 with 1.56 mg/kg, and the highest in Balıkesir (A) in 2018 with 27.32 mg/kg (Table 1). Since the FAEE values of all olive oil samples did not exceed the limit of 35 mg/kg, they were classified as extra virgin olive oil in accordance with the IOC standard (IOC, 2021b). The FAEE values of Balıkesir (A) and Çanakkale (A) samples were found to be quite high compared with the others. High values may be related to high FFA values of the samples. It has been determined in some studies that FAEE is 30–90 mg/kg in Spanish oils and 1–11 mg/kg in Italian oils. It is generally stated that it is higher in Spanish oils and lower in Italian oils (Garcia-Oliveira et al., 2021). Depending on the olive quality, methanol and ethanol formed by the developing fermentation process are transformed into FAEEs by transesterification (Mariani & Bellan, 2008). As previously investigated by Mafrica et al. (2021) the FAEE content was not affected from the climate of the growing environment or harvesting time, it is linked more to processing conditions. Low temperatures and high precipitation slow down fruit growth and development and delay ripening (Mafrica et al., 2021). Therefore, low temperatures, high precipitation and maturity index are very important parameters for olive oil quality.

Sterol content and composition are crucial for many aspects of extra virgin olive oil quality. These compounds are among the key parameters for trade standards requirements to verify olive oil authenticity and detect counterfeiting. Furthermore, sterols have significant potential as useful chemical markers for ensuring traceability and the diversity and geographical origin of olive oil (Lukić et al., 2021). It is critical to detect and prevent changes in the sterol composition of olive oil caused by climatic changes. The total beta-sitosterol, delta-7-stigmastenol, campesterol, stigmasterol and cholesterol values can see at Table 1. All the results were within the limits of IOC (2021b) except for delta-7-stigmastenol. The delta-7-stigmastenol value varied between 0.50 and 1.14% in 21 olive oil samples according to years. The other 18 samples were determined below the value of  $\leq 0.5\%$ . Deviations occurred in 5 samples in 2017, 5 samples in 2018, 2 samples in 2019 and 9 samples in 2020. Considering that the delta-7-stigmastenol value generally deviates more in G variety, it can be thought that it may be due to the variety characteristics rather than regional and climatic changes. However, the deviation in the samples obtained from the Mediterranean region and Southeastern Anatolia region may be associated with the high temperatures in these regions. When we look at the maximum temperatures, it is seen that the temperatures increased in 2018/2019, 2019/2020 and 2020/2021 compared with the previous year.

TABLE 1 MI of olives, FFA (in oleic acid %), K<sub>232</sub> and K<sub>270</sub>, FFAE (mg/kg) values and sterol composition (%) of olive oils

Years	Provinces/districts	MI	FFA	K <sub>232</sub>	K <sub>270</sub>	FAEE	Total Beta sitosterol	Delta-7-stigmastenol	Campesterol	Stigmasterol	Cholesterol
2017	Antalya (G)	1.62 <sup>o-u</sup>	0.21 <sup>-p</sup>	1.50 <sup>-o</sup>	0.09 <sup>-i</sup>	2.47 <sup>jk</sup>	93.88 <sup>tu</sup>	0.68 <sup>-f</sup>	2.78 <sup>-n</sup>	1.71 <sup>a-e</sup>	0.95 <sup>b</sup>
	Aydın (M)	3.31 <sup>b-f</sup>	0.16 <sup>m-p</sup>	1.42 <sup>-o</sup>	0.11 <sup>-i</sup>	5.13 <sup>-k</sup>	95.28 <sup>f-p</sup>	0.32 <sup>-l</sup>	3.75 <sup>a</sup>	0.51 <sup>p-s</sup>	0.14 <sup>f-m</sup>
	Balıkesir(A)	2.03 <sup>m-s</sup>	0.52 <sup>d-h</sup>	1.53 <sup>-e-n</sup>	0.09 <sup>-l</sup>	2.66 <sup>h-k</sup>	96.01 <sup>a-f</sup>	0.33 <sup>-l</sup>	3.19 <sup>b-j</sup>	0.37 <sup>st</sup>	0.10 <sup>-m</sup>
	Bursa(G)	2.96 <sup>e-k</sup>	0.07 <sup>p</sup>	1.42 <sup>-o</sup>	0.09 <sup>-l</sup>	1.56 <sup>k</sup>	94.57 <sup>n-u</sup>	0.50 <sup>-l</sup>	2.03 <sup>q-t</sup>	1.13 <sup>f-n</sup>	1.77 <sup>a</sup>
	Çanakkale(A)	2.55 <sup>-g-m</sup>	0.41 <sup>f-m</sup>	1.40 <sup>-k-o</sup>	0.06 <sup>i</sup>	4.06 <sup>-g-k</sup>	96.02 <sup>a-f</sup>	0.38 <sup>-l</sup>	3.15 <sup>-o-j</sup>	0.26 <sup>i</sup>	0.18 <sup>e-l</sup>
	Hatay (G)	2.27 <sup>-q</sup>	0.25 <sup>-p</sup>	1.61 <sup>-c-k</sup>	0.12 <sup>-d-h</sup>	3.04 <sup>h-k</sup>	95.48 <sup>e-m</sup>	0.61 <sup>-d-h</sup>	2.38 <sup>m-r</sup>	1.17 <sup>e-m</sup>	0.35 <sup>de</sup>
	İzmir (M)	2.65 <sup>f-m</sup>	0.11 <sup>op</sup>	1.61 <sup>-c-k</sup>	0.13 <sup>-c-g</sup>	3.34 <sup>h-k</sup>	94.08 <sup>q-u</sup>	1.14 <sup>a</sup>	3.20 <sup>b-j</sup>	1.18 <sup>e-m</sup>	0.41 <sup>d</sup>
	Kilis (KY)	1.60 <sup>p-u</sup>	0.42 <sup>-l</sup>	1.53 <sup>-d-n</sup>	0.08 <sup>-l</sup>	20.93 <sup>abc</sup>	94.01 <sup>f-u</sup>	0.50 <sup>-l</sup>	3.40 <sup>a-g</sup>	1.25 <sup>d-m</sup>	0.63 <sup>c</sup>
	Manisa (G)	2.22 <sup>k-q</sup>	0.10 <sup>op</sup>	1.50 <sup>-g-o</sup>	0.10 <sup>-i</sup>	2.03 <sup>jk</sup>	95.79 <sup>a-i</sup>	0.45 <sup>-l</sup>	2.32 <sup>n-r</sup>	1.43 <sup>-c-i</sup>	0.40 <sup>d</sup>
	Muğla (M)	2.12 <sup>k-r</sup>	0.22 <sup>k-p</sup>	1.30 <sup>-o</sup>	0.06 <sup>i</sup>	5.34 <sup>-g-k</sup>	95.41 <sup>f-o</sup>	0.42 <sup>-l</sup>	3.09 <sup>-e-j</sup>	0.82 <sup>-l-s</sup>	0.27 <sup>d-i</sup>
	Mersin (A)	2.58 <sup>f-m</sup>	0.76 <sup>bcd</sup>	1.57 <sup>-d-m</sup>	0.10 <sup>-e-i</sup>	2.92 <sup>h-k</sup>	95.22 <sup>f-p</sup>	0.51 <sup>d-k</sup>	3.44 <sup>a-f</sup>	0.51 <sup>p-s</sup>	0.31 <sup>def</sup>
	Gaziantep (NY)	2.35 <sup>-o</sup>	0.25 <sup>h-p</sup>	1.84 <sup>ab</sup>	0.17 <sup>ab</sup>	5.59 <sup>f-k</sup>	93.98 <sup>stu</sup>	0.56 <sup>-d-k</sup>	3.66 <sup>ab</sup>	1.55 <sup>b-f</sup>	0.25 <sup>d-j</sup>
	Şanlıurfa (G)	3.42 <sup>a-e</sup>	0.26 <sup>h-p</sup>	1.38 <sup>mno</sup>	0.06 <sup>i</sup>	7.48 <sup>d-k</sup>	95.84 <sup>a-h</sup>	0.43 <sup>-l</sup>	2.43 <sup>-q</sup>	1.01 <sup>f-q</sup>	0.29 <sup>d-g</sup>
	Antalya (G)	1.45 <sup>-u</sup>	0.28 <sup>h-p</sup>	1.51 <sup>-f-o</sup>	0.08 <sup>-l</sup>	4.15 <sup>-g-k</sup>	95.28 <sup>f-p</sup>	0.41 <sup>-e-l</sup>	2.04 <sup>q-t</sup>	2.04 <sup>ab</sup>	0.24 <sup>d-k</sup>
	Aydın (M)	1.38 <sup>f-u</sup>	0.24 <sup>-p</sup>	1.72 <sup>a-f</sup>	0.11 <sup>-e-i</sup>	4.81 <sup>-g-k</sup>	94.80 <sup>-s</sup>	0.39 <sup>-e-l</sup>	2.90 <sup>h-l</sup>	1.70 <sup>a-e</sup>	0.21 <sup>e-k</sup>
	Balıkesir(A)	2.59 <sup>f-m</sup>	0.86 <sup>b</sup>	1.62 <sup>b-k</sup>	0.09 <sup>-l</sup>	27.32 <sup>a</sup>	95.94 <sup>a-g</sup>	0.34 <sup>-l</sup>	3.00 <sup>-e-k</sup>	0.59 <sup>n-s</sup>	0.13 <sup>-g-m</sup>
2018	Bursa(G)	4.15 <sup>a</sup>	0.32 <sup>h-p</sup>	1.91 <sup>a</sup>	0.09 <sup>-l</sup>	5.11 <sup>-g-k</sup>	96.68 <sup>a</sup>	0.58 <sup>-d-k</sup>	1.60 <sup>t</sup>	0.84 <sup>-s</sup>	0.28 <sup>d-h</sup>
	Çanakkale(A)	2.34 <sup>-o</sup>	0.79 <sup>bc</sup>	1.70 <sup>a-g</sup>	0.09 <sup>-l</sup>	8.68 <sup>d-k</sup>	96.34 <sup>a-e</sup>	0.24 <sup>kl</sup>	2.79 <sup>-n</sup>	0.51 <sup>p-s</sup>	0.12 <sup>-g-m</sup>
	Hatay (G)	2.43 <sup>-o</sup>	0.43 <sup>-l</sup>	1.47 <sup>h-o</sup>	0.08 <sup>-l</sup>	13.33 <sup>-c-i</sup>	96.38 <sup>a-d</sup>	0.51 <sup>d-k</sup>	1.92 <sup>rst</sup>	0.97 <sup>-g-r</sup>	0.22 <sup>e-k</sup>
	İzmir (M)	1.81 <sup>n-t</sup>	0.49 <sup>-i</sup>	1.62 <sup>b-k</sup>	0.11 <sup>-e-i</sup>	5.43 <sup>-g-k</sup>	94.65 <sup>m-u</sup>	0.25 <sup>kl</sup>	2.81 <sup>-m</sup>	2.05 <sup>ab</sup>	0.24 <sup>d-k</sup>
	Kilis (KY)	0.94 <sup>u</sup>	0.24 <sup>-p</sup>	1.55 <sup>-d-n</sup>	0.09 <sup>-l</sup>	3.15 <sup>h-k</sup>	94.43 <sup>p-u</sup>	0.36 <sup>-l</sup>	3.62 <sup>abc</sup>	1.45 <sup>-c-h</sup>	0.15 <sup>f-m</sup>
	Manisa (G)	2.09 <sup>m-r</sup>	0.22 <sup>-p</sup>	1.46 <sup>-o</sup>	0.08 <sup>-l</sup>	7.26 <sup>d-k</sup>	96.47 <sup>abc</sup>	0.27 <sup>h-l</sup>	1.68 <sup>st</sup>	1.39 <sup>-c-j</sup>	0.18 <sup>e-l</sup>
	Muğla (M)	1.95 <sup>m-s</sup>	0.37 <sup>-n</sup>	1.79 <sup>abc</sup>	0.11 <sup>-e-i</sup>	6.68 <sup>d-k</sup>	94.80 <sup>-s</sup>	0.25 <sup>kl</sup>	2.99 <sup>-f-k</sup>	1.79 <sup>a-d</sup>	0.17 <sup>f-m</sup>
	Mersin (A)	2.47 <sup>h-n</sup>	0.71 <sup>b-e</sup>	1.63 <sup>-b-j</sup>	0.09 <sup>-l</sup>	6.51 <sup>-e-k</sup>	94.90 <sup>-r</sup>	0.53 <sup>-d-k</sup>	3.67 <sup>ab</sup>	0.83 <sup>-k-s</sup>	0.08 <sup>-m</sup>
	Gaziantep (NY)	1.32 <sup>stu</sup>	0.34 <sup>-o</sup>	1.75 <sup>a-d</sup>	0.14 <sup>a-f</sup>	6.43 <sup>-e-k</sup>	94.52 <sup>o-u</sup>	0.77 <sup>bcd</sup>	3.22 <sup>-b-j</sup>	1.35 <sup>-c-l</sup>	0.14 <sup>f-m</sup>
	Şanlıurfa (G)	2.23 <sup>-q</sup>	0.25 <sup>h-p</sup>	1.57 <sup>-d-m</sup>	0.11 <sup>-e-i</sup>	8.03 <sup>-d-k</sup>	95.69 <sup>-c-l</sup>	0.61 <sup>-d-i</sup>	2.04 <sup>p-t</sup>	1.54 <sup>-b-f</sup>	0.12 <sup>-g-m</sup>
	Antalya (G)	1.42 <sup>-u</sup>	0.16 <sup>m-p</sup>	1.53 <sup>-e-n</sup>	0.09 <sup>-l</sup>	10.79 <sup>-c-k</sup>	94.57 <sup>n-u</sup>	0.42 <sup>-l</sup>	2.80 <sup>-n</sup>	2.22 <sup>a</sup>	0.00 <sup>m</sup>
	Aydın (M)	1.16 <sup>tu</sup>	0.13 <sup>nop</sup>	1.84 <sup>ab</sup>	0.17 <sup>ab</sup>	10.04 <sup>-d-k</sup>	94.87 <sup>-k-s</sup>	0.33 <sup>-l</sup>	3.44 <sup>a-f</sup>	1.29 <sup>d-m</sup>	0.07 <sup>-m</sup>
	Balıkesir(A)	2.11 <sup>-r</sup>	0.79 <sup>bc</sup>	1.30 <sup>-o</sup>	0.06 <sup>kl</sup>	13.35 <sup>-c-h</sup>	96.33 <sup>a-e</sup>	0.32 <sup>-l</sup>	2.78 <sup>-n</sup>	0.46 <sup>qrs</sup>	0.11 <sup>h-m</sup>
	Bursa(G)	3.94 <sup>abc</sup>	0.30 <sup>h-p</sup>	1.48 <sup>h-o</sup>	0.10 <sup>-e-i</sup>	10.19 <sup>-d-k</sup>	96.55 <sup>ab</sup>	0.50 <sup>-d-k</sup>	1.95 <sup>rst</sup>	0.81 <sup>-u,kl</sup>	0.20 <sup>e-k</sup>
	Çanakkale(A)	2.59 <sup>fm</sup>	1.27 <sup>a</sup>	1.40 <sup>-k-o</sup>	0.06 <sup>i</sup>	24.33 <sup>ab</sup>	96.43 <sup>abc</sup>	0.43 <sup>-l</sup>	2.47 <sup>-q</sup>	0.59 <sup>n-s</sup>	0.09 <sup>-m</sup>
	Hatay (G)	3.24 <sup>-o-g</sup>	0.57 <sup>-g</sup>	1.50 <sup>-g-o</sup>	0.10 <sup>-e-i</sup>	12.54 <sup>-c-j</sup>	96.34 <sup>a-e</sup>	0.38 <sup>-l</sup>	2.13 <sup>-o-s</sup>	1.15 <sup>-e-m</sup>	0.00 <sup>m</sup>

(Continues)



TABLE 1 (Continued)

Years	Provinces/districts	MI	FFA	K <sub>232</sub>	K <sub>270</sub>	FAEE	Total Beta sitosterol	Delta-7-stigmastenol	Campesterol	Stigmasterol	Cholesterol
2020	İzmir (M)	2.38 <sup>e-n</sup>	0.21 <sup>-p</sup>	1.53 <sup>d-n</sup>	0.08 <sup>-l</sup>	6.39 <sup>e-k</sup>	95.32 <sup>f-p</sup>	0.16 <sup>i</sup>	3.32 <sup>a-h</sup>	1.08 <sup>f-o</sup>	0.13 <sup>g-m</sup>
	Kilis (KY)	1.57 <sup>q-u</sup>	0.47 <sup>e-k</sup>	1.42 <sup>-o</sup>	0.09 <sup>-l</sup>	7.50 <sup>d-k</sup>	95.46 <sup>e-n</sup>	0.32 <sup>g-l</sup>	3.28 <sup>a-i</sup>	0.88 <sup>-s</sup>	0.06 <sup>k-lm</sup>
	Manisa (G)	2.38 <sup>-n</sup>	0.14 <sup>no</sup>	1.61 <sup>c-k</sup>	0.12 <sup>d-h</sup>	8.35 <sup>d-k</sup>	95.79 <sup>a-j</sup>	0.56 <sup>d-k</sup>	2.53 <sup>k-p</sup>	1.12 <sup>f-n</sup>	0.00 <sup>m</sup>
	Muğla (M)	2.99 <sup>e-i</sup>	0.28 <sup>h-p</sup>	1.61 <sup>c-k</sup>	0.13 <sup>-g</sup>	7.05 <sup>d-k</sup>	94.88 <sup>k-s</sup>	0.26 <sup>kl</sup>	3.31 <sup>a-h</sup>	1.47 <sup>c-h</sup>	0.09 <sup>-m</sup>
	Mersin (A)	1.43 <sup>-u</sup>	0.69 <sup>b-e</sup>	1.50 <sup>g-o</sup>	0.09 <sup>-l</sup>	6.13 <sup>e-k</sup>	95.53 <sup>d-m</sup>	0.63 <sup>d-g</sup>	3.39 <sup>a-g</sup>	0.44 <sup>rs</sup>	0.00 <sup>m</sup>
	Gaziantep (NY)	3.74 <sup>a-d</sup>	0.62 <sup>b-f</sup>	1.42 <sup>-o</sup>	0.11 <sup>e-i</sup>	17.51 <sup>a-d</sup>	95.00 <sup>h-p</sup>	0.41 <sup>e-l</sup>	3.48 <sup>a-e</sup>	0.97 <sup>a-r</sup>	0.14 <sup>f-m</sup>
	Şanlıurfa (G)	3.86 <sup>a-d</sup>	0.29 <sup>h-p</sup>	1.57 <sup>d-m</sup>	0.10 <sup>-i</sup>	5.82 <sup>f-k</sup>	95.73 <sup>b-k</sup>	0.26 <sup>kl</sup>	2.57 <sup>k-o</sup>	1.39 <sup>c-k</sup>	0.06 <sup>k-lm</sup>
	Antalya (G)	2.08 <sup>m-r</sup>	0.34 <sup>g-o</sup>	1.46 <sup>-o</sup>	0.10 <sup>-i</sup>	10.69 <sup>c-k</sup>	93.79 <sup>u</sup>	1.08 <sup>ab</sup>	2.92 <sup>g-l</sup>	2.21 <sup>a</sup>	0.00 <sup>m</sup>
	Aydın (M)	1.76 <sup>n-t</sup>	0.28 <sup>h-p</sup>	1.70 <sup>a-g</sup>	0.15 <sup>a-d</sup>	11.20 <sup>c-k</sup>	95.52 <sup>d-m</sup>	0.55 <sup>d-k</sup>	2.55 <sup>k-o</sup>	1.38 <sup>c-l</sup>	0.00 <sup>m</sup>
	Balıkesir (A)	2.59 <sup>f-m</sup>	0.30 <sup>h-p</sup>	1.49 <sup>g-o</sup>	0.10 <sup>-i</sup>	16.30 <sup>b-f</sup>	95.46 <sup>e-n</sup>	0.39 <sup>e-l</sup>	3.22 <sup>b-j</sup>	0.53 <sup>o-s</sup>	0.00 <sup>m</sup>
	Bursa (G)	4.04 <sup>abc</sup>	0.25 <sup>h-p</sup>	1.62 <sup>b-k</sup>	0.14 <sup>a-f</sup>	8.46 <sup>d-k</sup>	96.07 <sup>a-f</sup>	0.68 <sup>c-g</sup>	1.59 <sup>t</sup>	0.91 <sup>h-s</sup>	0.00 <sup>m</sup>
	Çanakkale (A)	2.84 <sup>e-l</sup>	0.37 <sup>f-n</sup>	1.51 <sup>f-o</sup>	0.10 <sup>-i</sup>	13.37 <sup>c-h</sup>	95.50 <sup>d-m</sup>	0.44 <sup>d-l</sup>	2.76 <sup>-n</sup>	0.59 <sup>n-s</sup>	0.00 <sup>m</sup>
	Hatay (G)	2.29 <sup>-q</sup>	0.27 <sup>h-p</sup>	1.39 <sup>-o</sup>	0.10 <sup>-i</sup>	7.39 <sup>d-k</sup>	94.72 <sup>m-t</sup>	1.01 <sup>abc</sup>	2.47 <sup>-q</sup>	1.80 <sup>a-d</sup>	0.00 <sup>m</sup>
	İzmir (M)	3.88 <sup>a-d</sup>	0.41 <sup>-m</sup>	1.75 <sup>a-e</sup>	0.18 <sup>a</sup>	11.71 <sup>c-k</sup>	95.32 <sup>f-p</sup>	0.41 <sup>e-l</sup>	3.10 <sup>d-j</sup>	0.95 <sup>h-r</sup>	0.00 <sup>m</sup>
	Kilis (KY)	2.97 <sup>e-j</sup>	0.52 <sup>d-h</sup>	1.67 <sup>b-i</sup>	0.13 <sup>-g</sup>	14.49 <sup>b-g</sup>	95.09 <sup>g-p</sup>	0.49 <sup>d-l</sup>	3.38 <sup>a-h</sup>	0.94 <sup>h-r</sup>	0.11 <sup>h-m</sup>
	Manisa (G)	3.80 <sup>a-d</sup>	0.12 <sup>op</sup>	1.41 <sup>-o</sup>	0.14 <sup>a-f</sup>	7.83 <sup>d-k</sup>	95.93 <sup>a-g</sup>	0.60 <sup>d-j</sup>	2.03 <sup>q-t</sup>	1.46 <sup>c-h</sup>	0.00 <sup>m</sup>

Note: a-u: Different letters in the same column concerning all samples significantly different values ( $p < 0.0001$ ), A, Ayvalık; G, Gemlik; KY, Kilis Yağlık; M, Memecik; NY, Nizip Yağlık.

**TABLE 2** The fatty acid composition (%) values of olive oils

Years	Provinces/ districts	Palmitic acid (16:0)	Margaroleic acid (17:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)
2017	Antalya (G)	14.65 <sup>j-r</sup>	0.19	2.74 <sup>e-i</sup>	71.74 <sup>b-m</sup>	6.24 <sup>n-q</sup>	0.65
	Aydın (M)	11.96 <sup>v</sup>	0.05	2.91 <sup>ef</sup>	72.35 <sup>b-j</sup>	9.14 <sup>h-k</sup>	0.74
	Balıkesir (A)	13.69 <sup>q-t</sup>	0.21	2.39 <sup>i-s</sup>	70.57 <sup>c-p</sup>	10.09 <sup>f-i</sup>	0.51
	Bursa (G)	12.24 <sup>uv</sup>	0.19	2.87 <sup>efg</sup>	75.79 <sup>a</sup>	5.42 <sup>pq</sup>	0.49
	Çanakkale (A)	12.91 <sup>s-v</sup>	0.18	2.20 <sup>n-v</sup>	71.92 <sup>b-l</sup>	9.59 <sup>hij</sup>	0.47
	Hatay (G)	14.78 <sup>i-q</sup>	0.20	2.85 <sup>e-h</sup>	72.02 <sup>b-k</sup>	6.30 <sup>n-q</sup>	0.59
	İzmir (M)	12.8 <sup>tuv</sup>	0.06	2.11 <sup>q-v</sup>	72.88 <sup>a-g</sup>	8.75 <sup>h-m</sup>	0.72
	Kilis (KY)	14.88 <sup>g-p</sup>	0.16	3.59 <sup>bc</sup>	67.97 <sup>o-t</sup>	9.45 <sup>hij</sup>	0.61
	Manisa (G)	13.67 <sup>q-t</sup>	0.22	2.52 <sup>g-p</sup>	73.91 <sup>ab</sup>	5.83 <sup>opq</sup>	0.58
	Muğla (M)	12.96 <sup>s-v</sup>	0.12	2.34 <sup>j-s</sup>	70.24 <sup>e-q</sup>	10.85 <sup>c-h</sup>	0.72
	Mersin (A)	14.23 <sup>l-r</sup>	0.21	2.29 <sup>l-t</sup>	69.27 <sup>i-s</sup>	10.74 <sup>c-h</sup>	0.54
	Gaziantep (NY)	15.41 <sup>d-l</sup>	0.18	3.83 <sup>ab</sup>	66.44 <sup>f-u</sup>	10.25 <sup>e-i</sup>	0.6
	Şanlıurfa (G)	13.98 <sup>n-s</sup>	0.24	2.97 <sup>de</sup>	71.85 <sup>b-l</sup>	6.59 <sup>m-q</sup>	0.74
	Antalya (G)	15.76 <sup>d-j</sup>	0.17	2.50 <sup>g-p</sup>	71.95 <sup>b-k</sup>	6.89 <sup>k-q</sup>	0.65
2018	Aydın (M)	15.90 <sup>c-i</sup>	0.06	2.02 <sup>s-v</sup>	66.47 <sup>f-u</sup>	12.95 <sup>bc</sup>	0.79
	Balıkesir (A)	15.17 <sup>d-m</sup>	0.19	2.15 <sup>p-v</sup>	67.44 <sup>p-t</sup>	12.61 <sup>b-e</sup>	0.63
	Bursa (G)	13.75 <sup>p-t</sup>	0.18	2.46 <sup>i-q</sup>	71.21 <sup>b-o</sup>	9.67 <sup>g-i</sup>	0.65
	Çanakkale (A)	14.64 <sup>j-r</sup>	0.16	2.03 <sup>r-v</sup>	68.62 <sup>l-s</sup>	12.20 <sup>b-f</sup>	0.56
	Hatay (G)	15.73 <sup>d-j</sup>	0.16	2.62 <sup>e-m</sup>	72.12 <sup>b-k</sup>	6.45 <sup>m-q</sup>	0.66
	İzmir (M)	16.01 <sup>c-g</sup>	0.05	1.95 <sup>tuv</sup>	66.11 <sup>stu</sup>	13.33 <sup>ab</sup>	0.83
	Kilis (KY)	17.22 <sup>ab</sup>	0.17	2.53 <sup>f-o</sup>	70.16 <sup>f-q</sup>	6.67 <sup>l-q</sup>	0.88
	Manisa (G)	14.82 <sup>h-q</sup>	0.21	2.44 <sup>i-q</sup>	73.08 <sup>a-f</sup>	6.54 <sup>m-q</sup>	0.69
	Muğla (M)	16.22 <sup>b-e</sup>	0.09	1.90 <sup>uv</sup>	63.39 <sup>u</sup>	15.56 <sup>a</sup>	0.9
	Mersin (A)	16.10 <sup>b-f</sup>	0.15	2.06 <sup>r-v</sup>	66.64 <sup>f-u</sup>	12.29 <sup>b-f</sup>	0.61
	Gaziantep (NY)	17.88 <sup>a</sup>	0.15	2.76 <sup>e-i</sup>	68.52 <sup>m-t</sup>	7.54 <sup>j-p</sup>	0.75
	Şanlıurfa (G)	14.42 <sup>k-r</sup>	0.20	2.87 <sup>efg</sup>	73.63 <sup>a-d</sup>	6.10 <sup>opq</sup>	0.82
	Antalya (G)	14.95 <sup>f-o</sup>	0.21	2.64 <sup>e-l</sup>	73.53 <sup>a-e</sup>	5.77 <sup>opq</sup>	0.75
	Aydın (M)	13.89 <sup>n-t</sup>	0.07	1.94 <sup>tuv</sup>	72.51 <sup>a-i</sup>	9.09 <sup>h-l</sup>	0.72
	Balıkesir (A)	14.03 <sup>m-s</sup>	0.19	2.41 <sup>i-r</sup>	70.22 <sup>f-q</sup>	10.87 <sup>c-h</sup>	0.52
2019	Bursa (G)	13.06 <sup>s-v</sup>	0.20	2.83 <sup>e-h</sup>	72.36 <sup>b-i</sup>	9.12 <sup>h-k</sup>	0.58
	Çanakkale (A)	13.98 <sup>n-s</sup>	0.20	2.48 <sup>h-q</sup>	70.22 <sup>f-q</sup>	11.02 <sup>b-h</sup>	0.46
	Hatay (G)	15.48 <sup>d-k</sup>	0.14	2.54 <sup>f-n</sup>	71.40 <sup>b-n</sup>	7.45 <sup>j-p</sup>	0.43
	İzmir (M)	13.98 <sup>n-s</sup>	0.06	1.83 <sup>v</sup>	70.88 <sup>b-o</sup>	10.63 <sup>c-h</sup>	0.81
	Kilis (KY)	16.27 <sup>bcd</sup>	0.15	2.87 <sup>efg</sup>	69.13 <sup>j-s</sup>	8.66 <sup>h-n</sup>	0.68
	Manisa (G)	13.96 <sup>n-t</sup>	0.19	2.51 <sup>g-p</sup>	74.18 <sup>ab</sup>	6.37 <sup>m-q</sup>	0.64
	Muğla (M)	14.31 <sup>k-r</sup>	0.06	2.16 <sup>o-v</sup>	68.91 <sup>k-s</sup>	12.20 <sup>b-f</sup>	0.73
	Mersin (A)	15.07 <sup>e-n</sup>	0.18	2.46 <sup>i-q</sup>	69.40 <sup>h-s</sup>	10.47 <sup>d-i</sup>	0.56
	Gaziantep (NY)	14.01 <sup>m-s</sup>	0.18	4.01 <sup>a</sup>	69.63 <sup>g-r</sup>	9.67 <sup>g-j</sup>	0.57
	Şanlıurfa (G)	15.36 <sup>d-l</sup>	0.22	2.48 <sup>h-q</sup>	71.03 <sup>b-o</sup>	7.42 <sup>j-p</sup>	0.91
	Antalya (G)	16.00 <sup>c-h</sup>	0.19	2.71 <sup>e-j</sup>	69.63 <sup>g-r</sup>	8.13 <sup>i-o</sup>	0.85
	Aydın (M)	15.81 <sup>d-j</sup>	0.08	2.33 <sup>k-s</sup>	67.09 <sup>q-t</sup>	12.10 <sup>b-g</sup>	0.71
	Balıkesir (A)	13.79 <sup>o-t</sup>	0.22	2.29 <sup>l-t</sup>	71.67 <sup>b-m</sup>	9.69 <sup>g-j</sup>	0.58
	Bursa (G)	13.44 <sup>r-u</sup>	0.19	3.35 <sup>cd</sup>	69.96 <sup>f-r</sup>	10.63 <sup>c-i</sup>	0.58
	Çanakkale (A)	13.80 <sup>o-t</sup>	0.21	2.26 <sup>m-u</sup>	71.42 <sup>b-n</sup>	10.07 <sup>f-i</sup>	0.59
2020	Hatay (G)	15.70 <sup>d-j</sup>	0.18	2.69 <sup>e-k</sup>	70.47 <sup>d-p</sup>	8.08 <sup>i-o</sup>	0.71
	İzmir (M)	14.35 <sup>k-r</sup>	0.06	2.71 <sup>e-k</sup>	68.20 <sup>n-t</sup>	12.23 <sup>b-f</sup>	0.85

(Continues)

TABLE 2 (Continued)

Years	Provinces/ districts	Palmitic acid (16:0)	Margaroleic acid (17:1)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)
	Kilis (KY)	15.24 <sup>d-l</sup>	0.20	3.77 <sup>abc</sup>	68.86 <sup>k-s</sup>	9.08 <sup>h-l</sup>	0.75
	Manisa (G)	14.01 <sup>m-s</sup>	0.23	2.85 <sup>e-h</sup>	73.93 <sup>ab</sup>	6.05 <sup>opq</sup>	0.69
	Muğla (M)	15.73 <sup>d-j</sup>	0.08	2.45 <sup>t-q</sup>	66.48 <sup>t-u</sup>	12.72 <sup>bcd</sup>	0.72
	Mersin (A)	15.71 <sup>d-j</sup>	0.21	2.46 <sup>t-q</sup>	68.87 <sup>k-s</sup>	10.16 <sup>f-i</sup>	0.66
	Gaziantep (NY)	17.02 <sup>abc</sup>	0.19	4.03 <sup>a</sup>	65.29 <sup>tu</sup>	10.44 <sup>d-i</sup>	0.8
	Şanlıurfa (G)	15.72 <sup>d-j</sup>	0.27	2.85 <sup>e-h</sup>	73.82 <sup>abc</sup>	4.47 <sup>q</sup>	0.75

Note: a–q: Different letters in the same column concerning all samples significantly different values ( $p < 0.0001$ ), A, Ayvalık; G, Gemlik; KY, Kilis Yağlık; M, Memecik; NY, Nizip Yağlık.

Especially in May 2020/21, temperatures were higher than other years. The deviations in nine samples in 2020/21 may be due to the temperature increase in May. Detection of deviation in only two samples in 2019/2020 may be due to the highest rainfall this year. It may be due to the heavy rainfall, especially in October, November and December. In a study conducted by Yorulmaz and Bozdoğan (2017) in the Mediterranean region of Turkey in the 2013 harvest year, they found the delta-7-stigmastanol value in the range of 0.64%–0.88% and stated that this may be a regional problem. Characterization and differentiation studies of extra virgin olive oil varieties based on the content and composition of sterols have been carried out by many researchers and deviations in delta-7-stigmastanol values according to European Commission regulations have been determined in Spain (Cornicabra, Arbequina, Picual, Hojiblanca varieties) (Rivera del Álamo et al., 2004), in Spain (43 world varieties) (Kyçyk et al., 2016), in Turkey (Gemlik, Halhalı varieties) (Bozdoğan Konuskan & Mungan, 2016), in Turkey (San Hasebi, Gemlik, Halhalı varieties) (Yorulmaz & Bozdoğan, 2017), in Tunisia (Semni, Jdallou, Chemlali Sfax, Swabâa Algia, Oueslati, El Hor varieties) (Krichène et al., 2010), in Argentina (Arbequina, Barnea, Picual, Frantoio, Empeltre, Manzanilla, Arauco, Coratina varieties) (Ceci & Carelli, 2007) and in Iran (Beleydi, Mission, Koroneiki varieties) (Noorali et al., 2014). Results are in accordance with Yorulmaz and Bozdoğan (2017), İlyasoğlu (2009), Ben Temime et al. (2008), Hannachi et al. (2013), Fernández-Cuesta et al. (2013). Most compounds in the sterol composition of olive oil are affected not only by geographical origin but also by environmental conditions (Ben Temime et al., 2008). In some research, it seen that total  $\beta$ -sitosterol concentration values in olive oil increased in the colder region (Piravi-Vanak et al., 2012).

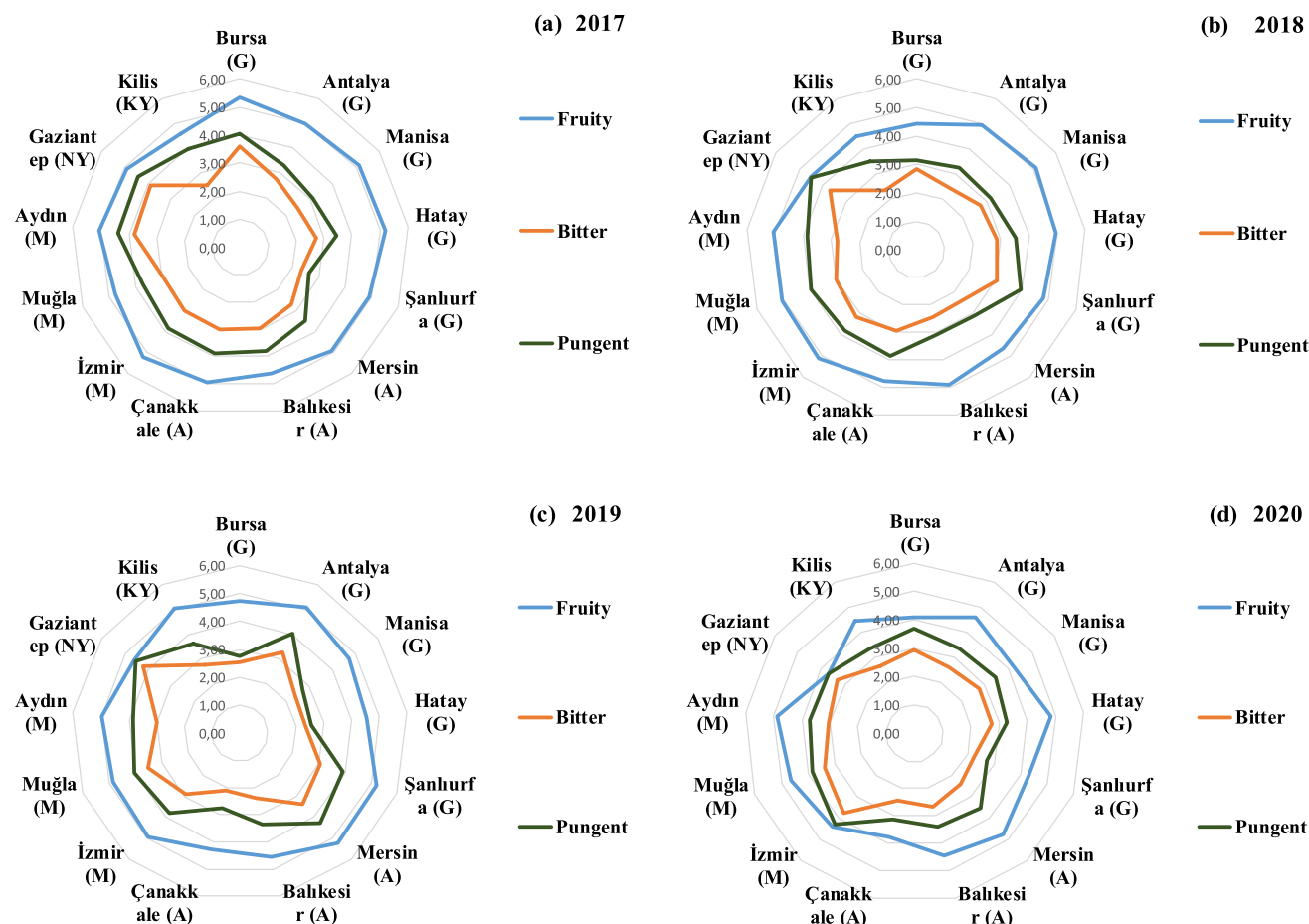
The fatty acid composition (%) of olive oils by years 2017, 2018, 2019 and 2020 are given in Table 2.

The fatty acids results seen in Table 2 are in accordance with researchers of Yorulmaz and Bozdoğan (2017), Köseoğlu et al. (2016), Aparicio and Luna (2002), Mafrica et al. (2021) and Rodrigues et al. (2021).

The olive variety, harvest year and climatic conditions have significant effects on the fatty acid composition of olive oils. The environmental conditions also affect oleic acid and linoleic acid content (Rodrigues et al., 2021). İlyasoğlu (2009) stated that the chemical composition of A and M olive oils varies according to the harvest season, and these changes may be due to changes in climatic conditions (temperature and precipitation amount). It is stated that the irrigation regime positively affects the oil content (%) and fatty acid composition of olive oil. In recent studies, it has been reported that there are differences in the sensory and chemical characteristics of olive oil obtained from trees that are irrigated and grown depending on precipitation (Aparicio & Luna, 2002).

The chemical composition and sensory properties of extra virgin olive oil are affected by many different variables (Kalua et al., 2007). High temperatures cause losses in some sensory properties and total phenol content of virgin olive oil (Servili & Montedoro, 2002). According to the sensory analysis results of fruitiness, bitterness and pungency were found to be statistically significant (Figure 2).

As seen from Figure 2, no negative properties were detected in any sample. The lowest fruitiness was determined in Gaziantep (NY) olive oil with 3.68 in 2020 and the highest in Bursa (G) olive oil with 5.33 in 2017. It has been determined that samples are in the “Medium Fruity” group ( $3.0 < \text{median} \leq 6$ ) according to the IOC (2021b) (Figure 2). The bitterness of olive oils by years were not determined statistically significant. The bitterness ranged between 2.10 and 4.20. The lowest pungency in 2019 was obtained from Hatay (G) olive oil with 2.57, and the highest from Gaziantep (NY) olive oil with 4.53. The samples were in the “Medium Bitter” and “Medium Pungent” group ( $3.0 < \text{median} \leq 6$ ) and “Light Bitter” and group “Light Pungent” ( $\text{median} \leq 3$ ) in terms of bitterness and pungency according to the IOC (2021b). Origin, variety and fruit maturity have a great influence on sensory characteristics of olive oils (Delgado & Guinard, 2011). Results are in accordance with Büyükgök and Saygın (2015).



**FIGURE 2** Sensory analysis spider web graph of olive oils of the harvest years (a) 2017, (b) 2018, (c) 2019 and (d) 2020

## Climate data and delta-7-stigmastanol value

Correlation analysis was performed on annual average climate data (Figure 1) with respect to delta-7-stigmastanol values for each year. Table 3 gives the correlation analysis results of annual average climate data by years as a function of delta-7-stigmastanol.

As can be seen from each year results, it is demonstrated that annual average relative humidity and annual average temperature had the highest correlation with delta-7-stigmastanol. Figure 3 shows three-dimensional scatter plots of delta-7-stigmastanol versus annual average relative humidity and annual average temperature for the years (a) 2017, (b) 2018, (c) 2019 and (d) 2020.

It is evident that Figure 3 shows quite similar grouping of the varieties in terms of annual average relative humidity and annual average temperature for the years as a function of measured delta-7-stigmastanol values. As it can also be seen from Table 1 and Figure 3, in 2017/2018 five samples are determined above the limit value of 0.5%, these are 2 Gemlik (G), 1 Memecik (M), 1 Ayvalık (A) and 1 Nizip Yağlık (NY). In 2018/2019,

again, it was determined that five samples were out of limits. These are three Gemlik (G), 1 Ayvalık (A) and 1 Nizip Yağlık (NY). When looked at in 2019/2020, only two samples that are 1 Gemlik (G) and 1 Ayvalık (A) were detected outside the limit. In 2020/2021 the highest deviation was determined; a total of nine samples, including five Gemlik (G), two Memecik (M), one Ayvalık (A) and one Nizip Yağlık (NY). At Figure 3 it is observed that the delta-7-stigmastanol values are high when the annual average relative humidity are low and the annual average temperatures are high. Efe et al. (2009) found that extremely high and low temperatures had negative effects on olive growth, quality and yield. High-temperature stress, especially high temperatures exceeding 35 to 40°C, affects olive leaf cell membranes and adversely affects olive plants by reducing the leaf water content (Ozturk et al., 2021).

Multivariate calibration models were generated using GILS method on monthly climate data given in Figure 1 for year 2017, 2018, 2019 and 2020. GILS is modified versions of original inverse least squares (ILS) method in which a small set of variables are selected from a full range data matrix and evolved to an optimum solution using a genetic algorithm (GA), and has been

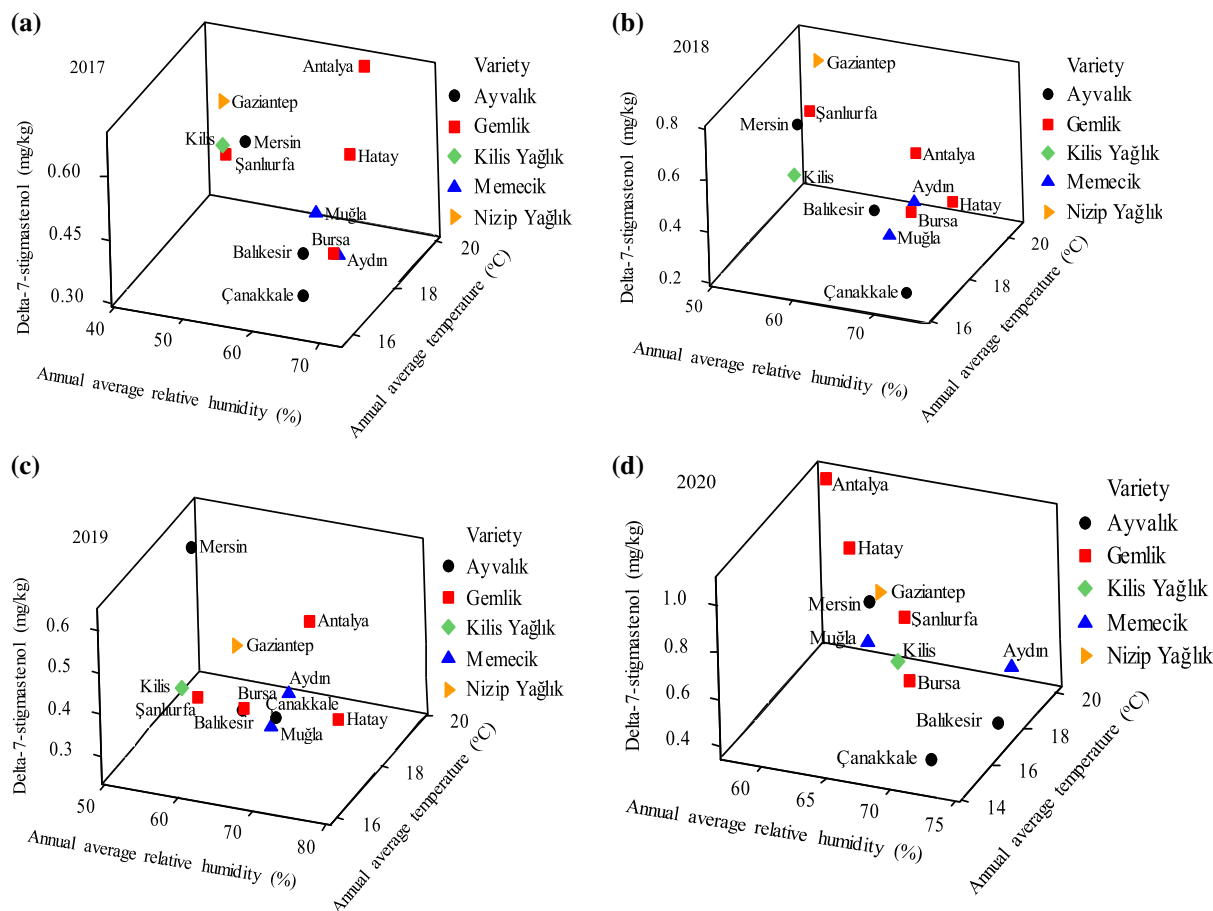
**TABLE 3** Correlation analysis results of annual average climate data by years as a function of delta-7-stigmastenol

		Delta-7-stigmastenol	Annual average temperature (°C)	Annual minimum temperature (°C)	Annual maximum temperature (°C)	Annual average relative humidity (%)	Annual average rainfall (kg/m <sup>2</sup> )
2017	Delta-7-stigmastenol	1					
	Annual average temperature (°C)	0.328766967	1				
	Annual minimum temperature (°C)	0.561562395	0.801143252	1			
	Annual maximum temperature (°C)	0.102315616	0.919383706	0.516145398	1		
	Annual average relative humidity (%)	−0.045501614	−0.716127957	−0.50204293	−0.67830358	1	
	Annual average rainfall (kg/m <sup>2</sup> )	0.178942465	−0.19510878	−0.336498909	−0.027112148	0.633680948	1
2018	Delta-7-stigmastenol	1					
	Annual average temperature (°C)	0.253174707	1				
	Annual minimum temperature (°C)	0.16227683	0.883729379	1			
	Annual maximum temperature (°C)	0.228756878	0.918563601	0.716509979	1		
	Annual average relative humidity (%)	−0.344787716	−0.74891546	−0.60827762	−0.689008247	1	
	Annual average rainfall (kg/m <sup>2</sup> )	−0.254853513	−0.053251069	0.120338738	−0.079434009	0.584947097	1
2019	Delta-7-stigmastenol	1					
	Annual average temperature (°C)	−0.126184586	1				
	Annual minimum temperature (°C)	0.03868113	0.701832314	1			
	Annual maximum temperature (°C)	−0.080617798	0.871455049	0.447802122	1		
	Annual average relative humidity (%)	−0.025508833	−0.536486555	−0.419111417	−0.546431098	1	
	Annual average rainfall (kg/m <sup>2</sup> )	−0.027251314	0.574927468	0.715392917	0.340527439	0.180455041	1
2020	Delta-7-stigmastenol	1					
	Annual average temperature (°C)	0.40245338	1				
	Annual minimum temperature (°C)	0.25253821	0.640012397	1			
	Annual maximum temperature (°C)	0.234542956	0.337055523	0.682519964	1		
	Annual average relative humidity (%)	−0.73333592	−0.639959532	−0.584477005	−0.5034633	1	
	Annual average rainfall (kg/m <sup>2</sup> )	−0.480096112	−0.643604091	−0.547607544	−0.249775614	0.743639511	1

applied to a number of variable selection problems (Özdemir & Dinç, 2004). Figure 4 shows measured versus GILS predicted delta-7-stigmasterol plots of the samples in each year along with  $R^2$ , regression equation and standard error of cross-validation (SECV) values.

Although relatively good regression coefficients were obtained for each year data, it should be mentioned that the low number of samples is the main concern about the reliability of the models for independent validation. Therefore, reliability of the models should be treated with caution since the iterative nature of the





**FIGURE 3** Three-dimensional scatter plots of delta-7-stigmastanol versus annual average relative humidity and annual average temperature for the years (a) 2017, (b) 2018, (c) 2019 and (d) 2020

GILS with such a low number of samples always tends to over fit models.

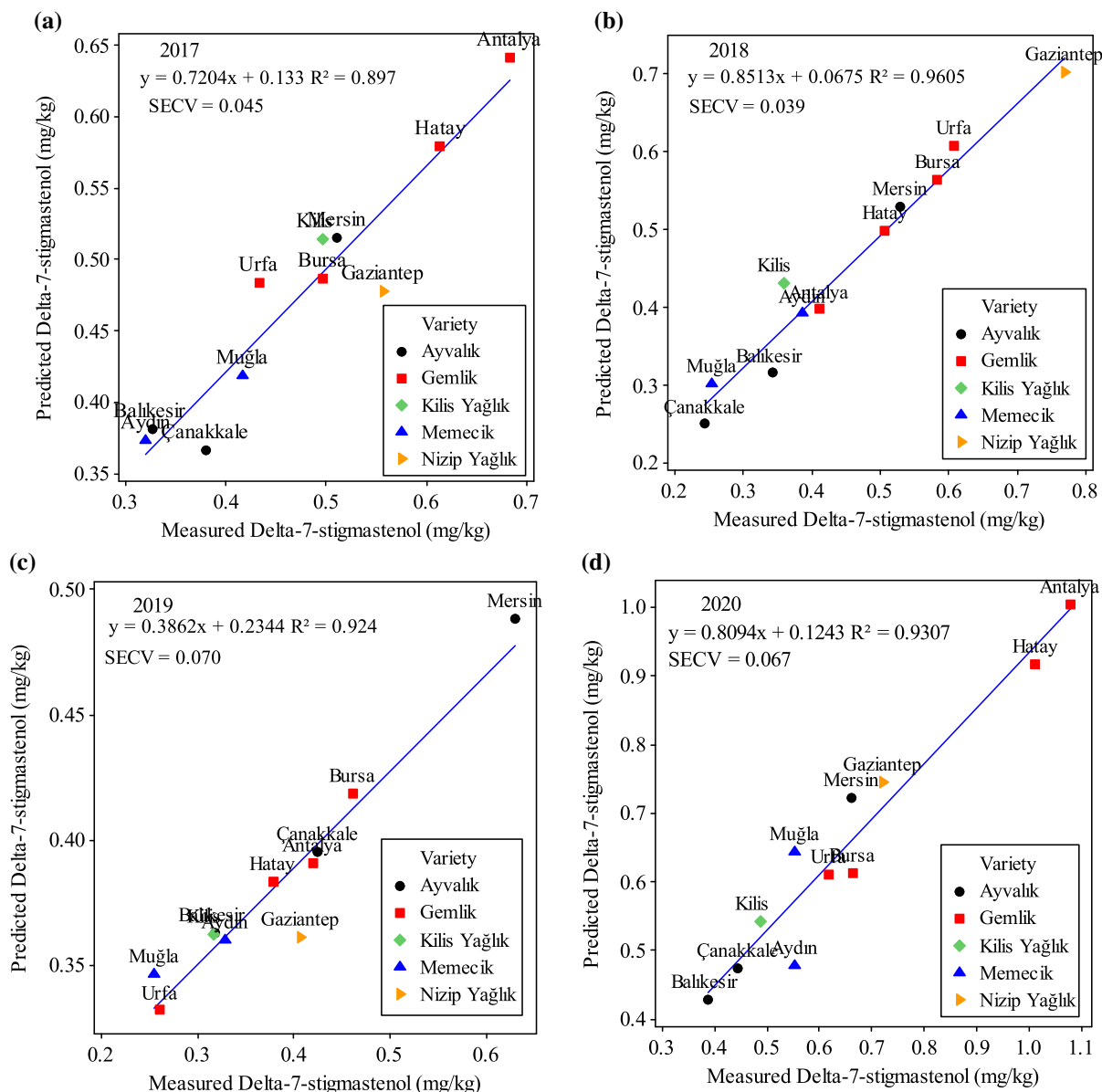
## PCA and HCA

The score plot obtained from the PCA analysis of olive oils obtained from the provinces in 2017 according to the variety is shown in Figure S2a.

As can be seen from score plot of PC1 versus PC2, olive oil samples were classified into three distinct groups based on varieties. Among the five varieties given in the legend of the score plot, all of the Gemlik samples were separated on the lower left corner of the plot regardless of the such a large geographical differences from Southern Marmara (Bursa) to Mediterranean (Antalya) regions. On the other hand, samples coming from Southeastern Anatolian region (Kilis Yağlık and Nizip Yağlık) were classified on the lower right part of the score plot indicating that PC1 scores are sufficient to distinguish these samples from the other varieties. When it comes to Ayvalık and Memecik varieties, all the samples are classified based on their positive PC2 scores. It is interesting to note that these

two varieties are mainly cultivated in Aegean region of Türkiye where Ayvalık is the dominating variety in the northern part of the Aegean region and Memecik is in the Southern Aegean region but the samples labeled as Ayvalık were taken from Eastern Mediterranean (Mersin) regions which is geographically quite far from Aegean region yet they are classified with all the Ayvalık varieties. As a result, it can be said that regardless of geographical and climate differences, the variety is taking the dominating role in the classification of the samples. In summary, the PC1 and PC2 results demonstrated that the orchards were divided into four main groups: one group containing the Gemlik (G) variety, one group for the Nizip Yağlık (NY) and Kilis Yağlık (KY) varieties, one group including the Ayvalık (A) variety and the last group for the Memecik (M) variety. As a result, the cultivars were successfully clustered into subgroups on the basis of cultivars according to the orchard from which they were harvested in year 2017.

When it comes to the PCA loading plot, Gadoleic acid is responsible for the classification of the Ayvalık and Memecik varieties whereas Margaroleic and Palmitoleic acid are the leading variables for the classification and characterization of the Gemlik samples.



**FIGURE 4** Measured versus predicted delta-7-stigmasterol plots of the olive oil samples obtained with GILS for the years 2017 (a), 2018 (b), 2019 (c) and 2020 (d)

In addition, the variables on the lower right part of the loadings plot are responsible for the classification of the Kilis Yağlık and Nizip Yağlık samples. Margaroleic acid values were determined as 0.18%–0.21%, 0.05%–0.12%, 0.19%–0.24%, 0.16% and 0.18% for A, M, G, KY and NY olive oils, respectively (Table 2). It is seen that one of the most prominent variables in the characterization of the KY and NY samples were arachidic, palmitic and stearic acid. The scores and loadings plots obtained from the PCA analysis of olive oils obtained from the provinces in 2018 according to the variety is shown in Figure S3.

As in year 2017, the samples from year 2018 were also classified in to four subclusters. According to the score plot of PC1 and, obtained from PCA, the

orchards were divided into the main groups as: one on the lower left part of Figure S3a for the A variety, one on the upper left part for, the M variety, one group, the G variety and the last group on the upper right part for, the NY–KY varieties. Overall, the samples were successfully clustered into subgroups on the basis of variety in 2018 according to the orchards from which they were harvested. It is seen in Figure S3b that margaroleic acid, oleic acid and palmitoleic acid contents are quite decisive in the classification and characterization of the G samples. Margaroleic acid values were determined as 0.15%–0.19%, 0.05%–0.09%, 0.16%–0.20%, 0.17% and 0.15% for A, M, G, KY and NY olive oils, respectively (Table 2). In addition, one of the most prominent variables in the characterization of the KY

and NY samples was arachidic acid. The linoleic acid variant also comes to the fore in the characterization of the M cultivars.

Figure S4 shows the scores and loadings plot of PC1 and PC2 obtained from PCA analysis of olive oils obtained from provinces/districts in 2019 according to varieties.

In 2019, the orchards where the M variety was harvested were clearly classified into a unique group in the lower right part of the score plot with positive PC1 and negative PC2 scores (Figure S4a). On the other hand, A and G varieties were classified on the left side of the score plot in parallel directing on diagonal shape but with a sufficient separation between the two varieties. In addition to the results of the PCA showed that the orchards where the NY variety was harvested are separated clearly as a group on the upper central part of the score plot. However, when it comes to the three KY variety samples, two of them were seen in the class of G variety samples. When the loading graph was examined closely, it was determined that the linoleic acid, linolenic acid and gadoleic acid contents were quite determinative in the classification and characterization of the M variety samples (Figure S4b). It was determined that one of the most significant variables in the characterization of the NY samples was stearic and arachidic acid. On the other hand, variables such as miristic, gadoleic and linolenic acid were dominating the M variety samples.

Figure S5 shows the scores and loadings plot of PC1 and PC2 obtained from PCA analysis of olive oils obtained from provinces/districts in 2020 according to varieties.

As can be seen from Figure S5a, the G variety samples were successfully separated from the rest of the other varieties on the left side of the score plot whereas the A variety samples were located on the center of the plot. On the other hand, the KY and NY varieties were grouped together on the lower right side of the score plot while M variety samples located on the upper right side of the plot. When it comes to the pCA loading plot, it has been determined that linoleic acid contents one of the main variables in the classification and characterization of the M samples. It is also seen that one of the most prominent variables in the characterization of NY and KY samples is arachidic acid whereas stigmastanol and oleic acid can be attributed to the G variety samples.

Hierarchical cluster analysis (HCA) was also applied to the PCA scores (PC1 and PC2) in order to evaluate the olive oil samples obtained in four consecutive harvest years. Figure S6 shows HCA dendrogram of olive oils obtained from the first two score vectors (PC1 and PC2) of PCA analysis for the years 2017 (a), 2018 (b), 2019 (c) and 2020 (d).

As can be seen from the 4 years HCA dendrograms, almost of the varieties were correctly clustered in their own classes. In years 2017, 2018 and 2020, the varieties were clustered into their main sub clustered

where Gemlik variety was located on the left side and both NY and KY varieties on the right side of the dendrogram. On the other hand, samples were first clustered into two main clusters where Gemlik and Ayvalık varieties were on the left side of the dendrogram where as Memecik and both NY and KY samples on the right side of the dendrogram. As a result, it can be said that HCA applied to the PC1 and PC2 score vectors coming from PCA analysis resulted in very successful clustering of the olive oil samples based on the variety.

## CONCLUSIONS

The Mediterranean Basin is considered as a “hot spot” in climate change. This situation indicates a very difficult process especially for olive growers. This research was aimed to determine some deviations in olive oil chemical composition are due to climatic changes or not. The fatty acid composition, the FAEE values of all olive oils have been determined within the national and international limits. In the sterol composition, only deviations have been detected in the delta-7-stigmastanol values. The delta-7-stigmastanol values ranged from 0.16 to 1.14%. In 21 samples, the delta-7-stigmastanol values have been found to be higher than the legal limit (0.51%–1.14%). According to GILS method result, it has been determined that the delta-7-stigmastanol value is high when the annual average relative humidity is low and the annual average temperature is high. The results demonstrated that there is an urgent need to make forward-looking plans due to climate change. The results demonstrated that there is an urgent need to make forward-looking plans due to climate change.

Future climatic predictions reveal that olive trees, can have serious adverse effects on final yield and quality characteristics. It has been clearly shown that unsupervised classification methods such as PCA and HCA could be used for the classification and clustering the olive oil varieties based on their sterol and fatty acid composition regardless of such a large difference in geographical regions where the samples come from.

## AUTHOR CONTRIBUTIONS

**Didar Sevim:** Methodology, investigation, oil extraction, analysis, writing, editing, supervision. **Oya Köseoğlu:** Investigation, oil extraction, formal analysis. **Durmuş Özdemir:** Statistic, software. **Mehmet Hakan:** Field observation, sample collection, statistic, software. **Elif B. Büyükgök:** Oil extraction, formal analysis. **Hatice Uslu:** Data collecting, analysis. **Özgür Dursun:** Field observation, sample collection. **M. Kerem Savran:** Field observation, sample collection. **Önder Eralp:** Field observation, sample collection. **Serkan Kaptan:** Field observation, sample collection. **Halil Köktürk:** Field observation, sample collection. **Özlem Asker:** Oil extraction, analysis. **Sibel Pazarlı:** Formal analysis. **Melike Ayaztek:** Formal

analysis. **Nurdan Akbaş:** Formal analysis. **Serkan Yalçın:** Formal analysis. **Pınar Çakır Topdemir:** Formal analysis.

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## CONFLICT OF INTEREST


The authors declare that they have no conflict of interest.

## ETHICS STATEMENT

This research did not involve any human or animal study and institutional ethical approval was not required.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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