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ORIGINAL ARTICLE

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Discrimination of Turkish Gemlik virgin olive oils by growing regions and environmental conditions

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Abstract

Geographic origins together with environmental conditions (temperature, rainfall, humidity, altitude) during harvest, could alter the olive oil quality, nutritional value, and traceability. The impact of geographic region (Bursa PDO, Hatay, Mersin, and Izmir NPDO) and conditions at harvest years (2016 and 2017) on the quality and traceability of Gemlik virgin olive oils were investigated. Environmental conditions (high temperature and relative humidity, low rainfall) resulted in low quality due to increase in FFA, linoleic acid, PUFA, Δ7-stigmastenol, stigmasterol and total sterols in oils obtained from southeast (Hatay and Mersin) NPDO regions. Bursa PDO and Izmir NPDO Gemlik virgin olive oils were high in oleic acid, MUFA, hydroxytyrosol, tyrosol, antioxidant activity and, Δ5-avenesterol and discriminated by 99% accuracy with discriminant analysis. PCA and DA can easily detect and distinguish differences in virgin olive oils quality and purity coming from different growing regions and subjected to inverse environmental conditions. Bursa PDO and Izmir NPDO growing regions have better conditions for Gemlik olives cultivation to obtain high quality and oxidative resistant Gemlik virgin olive oils.

KEYWORDS
antioxidant activity, discriminant analysis, fatty acids, Gemlik virgin olive oil, geographical origin, PCA, phenolic compounds, sterols

INTRODUCTION

Olive trees are demonstrated to be more challenging and can flourish on numerous distinctive territories and beneath different climatic conditions (Hmida et al., 2022). According to the International Olive Council, olive cultivation has the ability to fix carbon, and one hectare of average olive grove neutralizes one person's carbon footprint in the atmosphere, so potential olive cultivation areas must be protected. Olive oil is mainly composed of glycerides (98% to 99%), and the remaining part is called unsaponifiable compounds (1%–2%), including phenols, tocopherols, sterols, pigments, phospholipids, fatty alcohols, waxes, squalene, and other hydrocarbons (Kiritsakis et al., 2020; Kiritsakis & Keceli, 2023). Oleic acid, phenolic compounds, tocopherols, and sterols contribute to the multiple health benefits associated with the consumption of virgin olive oil (Dugo et al., 2020; Keceli et al., 2017). The determination of the authenticity of virgin olive oil is important in geographical labeling, as the protected designation of origin (PDO) guarantees the geographical origin and quality of the virgin olive oil set by official European regulations. The geographical origin of olive oil sold in the European Union must also be declared on the label (El-Gharbi et al., 2018; Keceli et al., 2017; Polari et al., 2021). Sensory properties, health benefits, and nutritional value of virgin olive oils are closely associated with the geographic region (Špika et al., 2021). Gemlik olives are produced in Turkiye's Marmara region. Gemlik is a town and district in the province of Bursa. The European Union granted the Protected Designation of Origin (PDO) status to Gemlik olives from Türkiye in June 2023. Gemlik olives are cultivated Bursa as PDO region and in NPDO regions, where they may have their own specific identities in the country (Dıraman et al., 2020; Gamlı & Eker, 2017). Planting

Abbreviations: IOC, International Olive Council; NPDO, Non Protected Designated Origin; PDO, Protected Designated Origin; VOO, virgin olive oil.

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Gemlik olives outside of its geographic origin may result in changes in olive oil quality and composition and this may result in a non-profitable generation (Ozturk et al., 2021). Research has been conducted on the composition of Gemlik virgin olive oil, but to the best of our knowledge, no study has shown the possibility of geographical traceability for Gemlik cultivar. Thus, this study aimed to distinguish Gemlik virgin olive oils from Bursa PDO and Hatay, Izmir, and Mersin NPDO growing areas over two consecutive harvest years in 2016 and 2017 by using multivariate analysis.

MATERIALS AND METHODS

Gemlik olives

Gemlik Olives produced in four recognized geographical areas (“Marmara”- Bursa, “Agean” -Izmir, “Mediterranean,” -Mersin, and Hatay) regions of Turkiye were evaluated. The study was conducted over two consecutive crop seasons in 2016, and 2017 to evaluate the effects of annual variability, which is mainly associated with climatological variables and periodicity. Fertilization, agricultural practices, and cultivars are mainly rain fed and habitually applied in the production areas. Gemlik olives produced in the “Bursa” can be placed under PDO, while those from the “Izmir,” and “Hatay and Mersin” are considered as NPDO areas.

Determination of maturity index

Gemlik olives from each growing area Bursa (PDO), Izmir, Hatay and Mersin (NPDO) region in each crop seasons (2016 and 2017) were harvested by hand in December 25th and 100 olives were taken from 1 kg of olives were subjected to maturity index analysis according to the method of the IOC (International Olive Council). The method was based on the coloring of the epicarp and the mesocarp of the olive fruit during the maturity process.

Gemlik olive oil samples

The Gemlik virgin olive oils were extracted using an Abencor laboratory oil mill (MC2 Ingenieria y Sistemas, Sevilla, Spain) containing fruit crushing, malaxation, and centrifuge parts. Gemlik virgin olive oils obtained a malaxation temperature of 30°C for 30 min. After extraction, Gemlik virgin olive oils were filtered using a filter made of hydrophilic cotton, placed in dark bottles, and stored at +4°C until their analyses. Gemlik virgin olive yield ranged between 10% to 12% for both harvest years for each PDO and NPDO regions.

Reagents and reference compounds

Folin–Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), caffeic acid, p-HPEA (tyrosol), 3,4-DHPEA (hydroxytyrosol), oleuropein, apigenin-7 glucoside, luteolin-7-glucoside, p-coumaric, vanillic acid, and gallic acids were purchased from Sigma Aldrich (Steinheim, Germany). The purities of all standards were stated as 98%, except for apigenin-7-glucoside and vanillic acid (97%) and ligstroside (95%). A fatty acid methyl ester (FAME) mixture and a sterol mixture were purchased from Supelco (Bellefonte, PA). The rest of the chemicals and solvents (ethanol, diethyl ether, acetic acid, chloroform, acetonitrile, hexane, cyclohexane, and methanol) used throughout the study were HPLC or analytical grade obtained from Merck (Darmstadt, Steinheim, Germany).

Determination of olive oil quality indices and other properties

The determinations of free fatty acidity, peroxide value, and UV coefficients (K232 and K270), ΔECN42, and the waxes were conducted according to the procedures of the International Olive Council (IOC, 2022, 2017). The analyses of chlorophyll and carotenoid contents were determined by the method of Minguez-Mosquera et al. (1991). The color was measured with a Color Flex calorimeter (Hunter Lab, Reston, VA, USA) using a 6 × 2 cm sample cell. The total phenols and the total tocopherols were determined calorimetrically according to the methods of Keceli (2013a), and Rutkowski & Grzegorczyk (2007), with a spectrophotometer (Shimadzu 1200 UV/Vis, Japan) at a wavelength of 725 and 539 nm, respectively. Radical scavenging activity (RSA) was determined by using the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) described by Keceli & Gordon (2001).

Determination of fatty acids

The FA composition was determined using the method described in International Olive Council T.20/Doc. No. 332015 (IOC, 2017). Fatty acid methyl esters were analyzed using an HP 6890 gas chromatograph equipped with an ionization detector (FID). To prepare fatty acid methyl esters, 0.1 g of the oil sample was dissolved in 5 mL of n-hexane and 1 mL of potassium hydroxide in methanol. A split GC mode was used to inject approximately 1 L through a DB-23 capillary column (30 m × 0.25 mm i.d) with a 0.25 μm film thickness. Oven, injector, and FID temperatures varied from 170 to 210°C in 2°C/min increments, with a 10-min hold at 210°C. The carrier gas was helium, which flowed at

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a rate of 1 mL/min. The Supelco FAME mix was used as a reference standard to identify the fatty acids in Gemlik virgin olive oils. The results were expressed as a relative area percentage of total fatty acid methyl esters determined by comparing their retention times with those of reference compounds.

Determination of phenolic compounds

Extraction with aqueous methanol and purification of the polar extracts from Gemlik virgin olive oils were performed according to (Keceli & Gordon, 2001). The phenolic composition of olive oil samples was determined using an HPLC system (Agilent 1100 Technologies, DE, USA) equipped with a DAD detector (Agilent Technologies, Palo Alto, CA, USA) operated by Windows with Chem Station software. The quantification of each identified compound was performed with an autosampler (SIL-10 AD vp), the system controller (SCL-10 Avp), pump (LC-10 AD vp), degasser (DGU-14A), column oven (CTO-10 Avp). Agilent Eclipse XDB-C18 (250 × 4.6 mm i.d. with a particle size of 5, USA column was employed for separation, and the injection volume was 20 μL. Gradient elution was used with a binary solvent system comprising water, methanol, acetic acid (3%) (solvent A), and acetonitrile (solvent B) at a flow rate of 1 mL/min. Methanol (solvent C) was used for column washing. The gradient composition was 95% A (0 to 10 min), 50% A (10 to 45 min), 95% A (45 to 64 min), and 100% C for washing (65 to 75 min) until the end of the run, with a flow rate of 0.8 mL/min and an oven temperature of 30°C. The phenolic compounds' peak areas were calculated by the HP 3365 Chemstation program and recorded as the peak area percentage.

The identification and quantification of phenolic compounds was confirmed by using LC/MS-MS mass spectrometry (Agilent 6410 Triple Quad LC/MS-MS) connected with the software system Windows NT-based Chem Station. Gradient elution was used with a binary solvent system comprising water, formic acid (0.1%) (solvent A), and acetonitrile (solvent B) at a flow rate of 0.7 μL/min. Agilent Zorbax-1,8 μm SB C18 (50 × 4.6 mm i.d. column was employed for quantification and the injection volume was 3 μL. The electrospray ionization (ESI) mass spectrometry was operated in negative ion mode scanned with MRM (double ion) mode with 400 Delta EMV, 350°C capillary temperature, −4 V capillary voltage, 12 L min^{-1} nebulizer gas flow, 1 L min^{-1} gas flow and 4 kV spray voltage, 40 psi nebulizer].

Determination of sterols

Sterol compositions were determined according to the IOC method COI/T.20/Doc. no. 26/Rev. 5/2020 (IOC, 2020). The oil samples were saponified with a 2 M ethanolic potassium hydroxide solution, and the unsaponifiable fraction was extracted with diethyl ether. Thin layer chromatography was used to separate the unsaponifiable sterol fraction. Separation and quantification of the silylated sterol fraction was performed by capillary gas chromatography (HP 6850, GC System Agilent Technologies, USA) equipped with FID detector with Supelco 24,034 column (30 mm × 0.25 mm × 0.25 m film thickness). The GC-FID with an injector temperature of 280°C, column and detector temperatures were 260 and 290°C, respectively with the 1 μL injection volume, using hydrogen as carrier gas at a flow rate of 0.5 mL/min. The split ratio was 1:50, silylated by using pyridine, bistrifluoroacetamide with trimethylchlorosilane (BSTFA+TMCS). Sterols and erythrodiol and uvaol) in Gemlik virgin olive oils were determined according to their relative retention times by using cholestanol5 α-cholestanol-3β-ol as an internal standard.

Statistical analysis

The results obtained from the study were given as the mean and standard deviation of triplicates of quality parameters including chemical indices, phenolic compounds, fatty acids, and sterols of Gemlik virgin olive oils. An ANOVA test ($p < 0.05$) was used to determine the existence of significant pairwise differences between the groups. PCA (correlation, varimax rotation, and Kaiser normalization) is used to determine variables. Geographic traceability was studied using a statistical procedure based on discriminant analysis (DA) built using the backward stepwise analysis option (within-class covariance matrices are assumed to be equal and prior probabilities are not taken into account) to discriminate Gemlik virgin olive oils according to their growing region and harvest date by using the XLSTAT 2022.1.1.1251, Addinsoft, New York, NY, USA software package.

RESULTS AND DISCUSSION

Gemlik virgin olive oils quality parameters

The climate is categorized as continental Mediterranean, with warm summers and cool winters (CSa) in both PDO (Bursa) and NPDO (Izmir, Hatay, and Mersin) geographical areas (Ozturk et al., 2017). Table 1 displays geographical and climatic information for the chosen region obtained from the General Directorate of Meteorology of the Ministry of Environment, Urbanization, and climate change database.

Average temperatures are projected to rise due to global warming. Gemlik olives growing regions of PDO and NPDO are affected significant increases in temperature, may alter the quality parameters in Gemlik virgin olive oils (Table 1). The temperature expanded 6°C in

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TABLE 1 Sampling and geographic and climatic information of Gemlik olives in 2016 and 2017 harvest years.

Gemlik olives growing regions (GR)	Geographic and climatic information			Temperature (°C) (Harvest Year (HY) (2016–2017)	Rainfall (kg/m^2) (HY (2016–2017)	Relative Humidity (%) (HY) (2016–2017)	Crop system
	Altitude (m)	Latitude	Location				
Bursa (Gemlik) PDO	85 m	40° 12 N	Northwest	4.7–10.7	4.8–3.6	74.7–69.5	Traditional rain-fed system
Izmir (Bornova) NPDO	14 m	38° 45 N	West	6.8–11.8	0.5–2.6	63.2–76.4	
Hatay (Altinozu) NPDO	341 m	36° 57 N	Southeast	5.8–10.5	2.4–1.3	51.1–78.2	
Mersin (Tarsus) NPDO	12 m	36° 48 N	Southeast	7.6–12.3	8.8–0.7	63.4–69.5	

PDO: Protected Designated Origin, NPDO: Non Protected Designated Origin.

Bursa-PDO and 5°C in Izmir, Hatay and Mersin NPDO due to warming in 2017 harvest year. Relative humidity expanded in all NPDO regions. Global climate change is a complex system (changes heat waves, precipitation, carbon dioxide levels, and temperature) adversely affecting olive growth and productivity, leading to yield loss and altered quality in olive oil.

Table 2 displays the quality characteristics of Gemlik virgin olive oils from PDO and NPDO regions, harvest years in 2016 and 2017.

The harvest year (environmental conditions) factor was significant for chlorophyll content, a and b values, and radical scavenging activity. Bursa PDO and Izmir NPDO Gemlik virgin olive oils had low PV, FFA, K_{270} values (Table 2). Gemlik olives have a ripening index of 4.9 (Hatay NPDO) to 5.6 (Bursa PDO) in 2016 and 2017 harvest years, respectively (Table 2) are at the optimal value for oil extraction. The quality and classification of olive oil are determined by free fatty acidity may change for a number of reasons (Polari et al., 2021). The free fatty acid content of Gemlik virgin olive oils were between 0.8% to 1.3% as oleic acid are in the limits of extra virgin (≤0.8) and virgin olive oil (≤2) class, respectively established by IOC (2022) (Table 2). FFA is higher in warmer regions including Hatay and Mersin NPDO. This could be due to increased temperature stress and less rain in 2017 harvest year (0.7 and 1.3 kg/m^2) and in Hatay and Mersin NPDO growing regions, respectively (Table 1). Our findings were consistent with reports that the FFA content of olive oils ranged from 0.4% to 2.3% as oleic acid (Arslan & Ozcan, 2011; Dıraman et al., 2020; Keceli, 2013a, 2013b; Sevim et al., 2023; Uluata et al., 2021). Oil's green color comes from chlorophylls (Minguez-Mosquera et al., 1991). The chlorophyll contents of Gemlik virgin olive oils significantly increased from 10.2 to 26 mg/kg ($p < 0.05$) in two harvest years, respectively (Table 2). This could be due to impact of environmental conditions (increase in temperature and the relative humidity especially in 2017 harvest year) (Tables 1 and 2). Similarly, the chlorophyll expanded depending on amid the blustery season (Hmida et al., 2022) and (El-Gharbi et al., 2018) for Zalmati and Zarazi cultivar. The chlorophyll contents of Gemlik olive oils were in the range reported by (Arslan & Ozcan, 2011; Dıraman & Dibeklioglu, 2014; Keceli, 2013a, 2013b; Uluata et al., 2021) for Gemlik, Ayvalik, Halhalı, and Hasebi olive varieties. The minor polar compounds known as antioxidants significantly improve the virgin olive oil's resistance to oxidation. The radical scavenging capacity of Gemlik virgin olive oils ranged from 25.20% (Bursa, PDO) to 30.22% (Izmir, NPDO) and significantly changed from 24.6% to 31.7% in 2017 harvest year ($p < 0.05$), respectively, (Table 2). High antioxidant activity of Izmir NPDO (30%) Gemlik virgin olive oils could be caused by the high concentration of total phenols and tocopherol (190 and 200 mg/kg) contents, respectively (Table 2). Sicari (2017), who found a positive correlation ($r = 0.96$)

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TABLE 2 Analysis of variance (2-way ANOVA) with significance levels and *F*-values of general properties of Gemlik olive oils by growing regions (GR) and harvest years (HY).

	Growing region (GR)				Significance of GR		Harvest year (HY)		Significance of HY	
Parameters	**Bursa-PDO**	**Izmir-NPDO**	**Hatay-NPDO**	**Mersin-NPDO**	**F (GR)**	**Significant**	**2016**	**2017**	**F (HY)**	**Significant**
Maturity index	5.59[a]	4.70[a]	4.87[a]	5.56[a]	0.38	ns	5.01[a]	5.35[a]	0.55	ns
FFA	0.77[a]	0.78[a]	1.26[a]	0.90[a]	0.30	ns	0.87[a]	0.97[a]	0.59	ns
PV	4.68[a]	5.17[a]	8.16[a]	9.12[a]	0.45	ns	7.45[a]	6.11[a]	0.56	ns
K232	1.69[a]	1.59[a]	1.75[a]	1.83[a]	0.60	ns	1.66[a]	1.77[a]	0.41	ns
K270	0.13[a]	0.14[a]	0.16[a]	0.18[a]	0.31	ns	0.13[a]	0.17[a]	0.07	ns
Chlorophyll	19.20[a]	15.43[a]	19.51[a]	18.19[a]	0.68	ns	10.21[b]	25.95[a]	0.01	**
Carotenoid	1.46[a]	1.73[a]	3.12[a]	1.16[a]	0.35	ns	1.45[a]	2.28[a]	0.30	ns
L	47.88[a]	47.43[a]	42.89[a]	42.77[a]	0.75	ns	47.05[a]	43.43[a]	0.46	ns
a	−3.40[a]	−2.80[a]	−3.04[a]	−2.98[a]	0.85	ns	−3.92[b]	−2.19[a]	0.04	**
b	28.68[a]	27.12[a]	22.54[a]	23.89[a]	0.13	ns	27.85[a]	23.27[b]	0.04	**
Total phenol content	120.06[a]	189.15[a]	121.99[a]	186.39[a]	0.40	ns	142.97[a]	165.82[a]	0.54	ns
Total tocopherol content	80.80[a]	197.53[a]	98.62[a]	91.14[a]	0.16	ns	98.92[a]	135.12[a]	0.29	ns
Antioxidant activity	25.20[a]	30.22[a]	29.98[a]	27.27[a]	0.43	ns	24.63[b]	31.69[a]	0.05	**
ΔECN42	0.11[a]	0.19[a]	0.06[a]	0.12[a]	0.21	ns	0.09[a]	0.15[a]	0.19	ns
Wax	72.25[a]	61.25[a]	89.75[a]	84.25[a]	0.73	ns	82.50[a]	71.25[a]	0.59	ns

Note: Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$), *ns* indicates no significance ($p \geq 0.05$).

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TABLE 3 Analysis of variance (2-way ANOVA) with significance levels and *F*-values of fatty acids of Gemlik olive oils by growing regions (GR) and harvest years (HY).

	Growing region (GR)				Significance of GR		Harvest year (HY)		Significance of HY	
Fatty acids (%)	Bursa-PDO	Izmir-NPDO	Hatay-NPDO	Mersin-NPDO	F (GR)	Significant	2016	2017	F (HY)	Significant
Palmitic	10.95[a]	12.93[a]	13.35[a]	13.65[a]	0.22	ns	13.33[a]	12.11[a]	0.20	ns
Palmiteloic	1.14[a]	0.98[a]	1.42[a]	1.43[a]	0.20	ns	1.30[a]	1.18[a]	0.44	ns
Stearic	3.06[a]	3.40[a]	3.35[a]	3.26[a]	0.84	ns	3.34[a]	3.20[a]	0.64	ns
Oleic	73.98[a]	72.58[a]	68.19[a]	67.92[a]	0.17	ns	70.58[a]	70.76[a]	0.92	ns
Linoleic	8.96[a]	7.93[a]	11.67[a]	11.64[a]	0.25	ns	9.56[a]	10.54[a]	0.49	ns
Linolenic	0.55[a]	0.83[a]	0.68[a]	0.70[a]	0.39	ns	0.67[a]	0.70[a]	0.76	ns
∑SFA	17.03[a]	14.68[a]	17.45[a]	17.63[a]	0.24	ns	17.39[a]	16.00[a]	0.21	ns
∑MUFA	74.05[a]	75.60[a]	70.08[a]	69.85[a]	0.21	ns	72.35[a]	72.44[a]	0.96	ns
∑PUFA	9.51[a]	8.74[a]	12.35[a]	12.33[a]	0.29	ns	10.22[a]	11.24[a]	0.50	ns
∑MUFA/∑PUFA	7.80[a]	8.88[a]	5.59[a]	5.77[a]	0.29	ns	7.12[a]	6.90[a]	0.86	ns
Oleic/Linoleic	8.26[a]	9.41[a]	5.96[a]	5.94[a]	0.27	ns	7.43[a]	7.36[a]	0.95	ns
Palmitic/Linoleic	1.23[a]	1.66[a]	1.16[a]	1.19[a]	0.37	ns	1.40[a]	1.22[a]	0.42	ns

Note: Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$), *ns* indicates no significance ($p \geq 0.05$).

between antioxidant activity and α-tocopherol content of Roggianella, Ottobratica, and Sinopolese virgin olive oils than found here ($r = 0.66$). The findings of this study related to antioxidant activity are line with some Turkish virgin olive oils ranged between 15.4 and 95% (Gamlı & Eker, 2017; Keceli, 2013a, 2013b).

Gemlik virgin olive oils fatty acids

Olive oil's fatty acid composition is used for quality assessment, shelf life, characterization, authenticity, and health benefits (Arslan & Ok, 2020; Špika et al., 2021).

Table 3 shows the composition of the major fatty acids found in Gemlik virgin olive oils from PDO and NPDO regions, harvest years in 2016 and 2017.

The major fatty acids were oleic, palmitic, linoleic, and stearic acids and palmitoleic and linolenic acids were minor fatty acids (Table 3). Lignoseric, miristic, margaric, margoleic, arachidic, gadoleic, behenic, and lignoseric acids are also determined under 0.5% not shown at Table 3. The harvest year and growing region factors were null for fatty acids $p > 0.05$ (Table 3). Bursa PDO and Izmir NPDO Gemlik virgin olive oils had high oleic acid (73%–74%) and MUFA contents (74%–75.6%), MUFA/PUFA ratio (8%–9%), and oleic/linoleic ratios (8.2–9.4) (Table 3). Higher linoleic (12%) and lower oleic (68%), higher palmitic (14%), PUFA (12.3%) contents of Hatay and Mersin NPDO Gemlik virgin olive oils could be caused by environmental variables (especially less rain (0.7 and 1.3 kg/m^2) and high temperature stress (10.5 and 12.3°C) during oil biosynthesis in 2017 harvest year (Table 1). This finding is consistent with those of (Hmida et al., 2022; Špika et al., 2021) who reported that a decrease in oleic acid and an increase in the levels of stearic, palmitic acid and linoleic acid in olive oil extracted from high temperature and less rainy areas. Gemlik virgin olive oils fatty acid composition is consistent with that described by other authors for Turkish olive varieties such as Gemlik (Arslan & Ok, 2020; Keceli, 2013b; Korkmaz, 2023; Özcan et al., 2019; Sevim et al., 2023; Uluata et al., 2021) and some Tunisian and Spanish olives (El-Gharbi et al., 2018; Hannachi et al., 2013; Hmida et al., 2022; Polari et al., 2021).

Gemlik virgin olive oils phenolic compounds

Phenolic compounds have important functional properties because they inhibit oxidation, increase shelf life, add bitterness and astringency, and stimulate sensory properties (Kiritsakis & Keceli, 2023). Table 4 lists the major phenolics Gemlik virgin olive oils from PDO and NPDO regions, harvest years in 2016 and 2017.

Growing regions had a significant impact on tyrosol levels while harvest year had a significant impact tyrosol and p-coumaric acid levels ($p < 0.05$) (Table 4). Tyrosol contents ranged from 17.5 to 68 mg/kg (Hatay and Izmir NPDO). Izmir NPDO Gemlik virgin olive oils had higher tyrosol and hydroxytyrosol concentrations (Table 4). A daily dose of 2–15 mg of hydroxytyrosol or oleuropein-related compounds is required for health claims made by EFSA (European Commission, 2012).

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TABLE 4 Analysis of variance (2-way ANOVA) with significance levels and *F*-values of phenolic compounds of Gemlik olive oils by growing regions (GR) and harvest years (HY).

	Growing region (GR)				Significance of GR		Harvest year (HY)		Significance of HY	
Phenols (mg/kg)	Bursa-PDO	Izmir-NPDO	Hatay-NPDO	Mersin-NPDO	F (GR)	Significant	2016	2017	F (HY)	Significant
Hydroxytyrosol	4.53[a]	42.26[a]	3.12[a]	2.52[a]	0.06	ns	9.02[a]	17.01[a]	0.32	ns
Tyrosol	25.54[b]	68.03[a]	17.52[b]	27.04[b]	0.02	**	23.01[b]	46.02[a]	0.03	**
p-coumaric	4.52[a]	7.53[a]	2.51[a]	4.52[a]	0.13	ns	2.75[b]	6.75[a]	0.03	**
Vanilic acid	0.72[a]	2.02[a]	2.04[a]	3.52[a]	0.21	ns	1.35[a]	2.75[a]	0.13	ns
Hydroxytyrosol acetate	0.42[a]	1.05[a]	1.52[a]	3.02[a]	0.19	ns	1.35[a]	1.60[a]	0.72	ns
Oleacein	9.50[a]	6.00[a]	12.00[a]	4.50[a]	0.61	ns	8.03[a]	8.04[a]	1.00	ns
Oleocanthal	4.00[a]	8.00[a]	5.00[a]	8.00[a]	0.28	ns	5.25[a]	7.25[a]	0.25	ns
Luteolin	8.00[a]	22.00[a]	10.00[a]	13.00[a]	0.05	ns	14.01[a]	12.50[a]	0.52	ns
Apigenin	9.00[a]	16.00[a]	15.00[a]	18.50[a]	0.12	ns	17.03[a]	12.25[a]	0.09	ns
Total phenol content	120.06[a]	189.15[a]	122.00[a]	186.35[a]	0.40	ns	142.97[a]	165.81[a]	0.54	ns

Note: Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$), *ns* indicates no significance ($p \geq 0.05$).

Our findings are consistent with the hydroxtyrosol and tyrosol contents of Turkish cultivars (Al-Juhaimi et al., 2017; Arslan & Ozcan, 2011; Korkmaz, 2023; Uluata et al., 2021) and some Spanish and Tunisian cultivars reported by (El-Gharbi et al., 2018; Polari et al., 2021). On the other hand, changes in environmental conditions (increase in temperature and relative humidity and decrease in rainfall see Table 1) may have increased both tyrosol and p-coumaric acid levels significantly in Gemlik virgin olive oils in 2017 harvest year (Table 4). The higher amount of total phenolic compounds (190 mgkg) from the NPDO producing regions (Izmir and Mersin) could be attributed to their lower altitude (12 and 14 m) as well as at the highest temperature (11.8 and 12.3°C), especially in harvest year 2017 (Table 1). Thus, phenolic compounds in the oils obtained from low altitude and high temperature areas (Izmir and Mersin NPDO) is higher than in oils obtained from high altitude and low temperature area (Bursa PDO) which is consistent with findings of (Hmida et al., 2022; Mailer et al., 2010). The total phenol value determined by spectrophotometer lower than the sum of the individual phenol compounds obtained by HPLC (Dugo et al., 2020; Korkmaz, 2023). This clearly explains the difference between HPLC results and TPC results at Table 4. Our results are consistent with TPCs in Turkish olive oils ranging between 12 and 990 mg/kg of oil, as reported by (Keceli, 2013a; 2013b; Arslan & Ok, 2020; Korkmaz, 2023; Uluata et al., 2021).

Gemlik virgin olive oils sterols

Sterols are major constituents of the unsaponifiable fraction, and their determination is an important tool to check the genuineness of an oil (Hannachi et al., 2013; Noorali et al., 2014). Table 5 depicts the variation in sterol composition of Gemlik virgin olive oils produced in four selected olive-growing areas over two consecutive crop years.

The results showed that the major sterols in Gemlik PDO and NPDO virgin olive oils were β-sitosterol (77.8% to 83.1%), Δ5-avenasterol (9.7% to 15.4%), and campesterol (1.8% to 2.2%) (Table 5). Table 5 clearly showed that while growing region had no effect on sterol composition, harvest year had a significant effect on reduction in cholesterol, campestanol levels, and increase in Δ7-stigmastenol amounts ($p < 0.05$) (Table 5). This result is consistent with previous findings that the sterol composition of olive oil is affected not only by geographical origin (Hannachi et al., 2013; Sevim et al., 2023) and also by environmental conditions (temperature, rainfall, humidity) (Lukić et al., 2021). The IOC set the level of Δ7-stigmastenol in olive oil at 0.5% as a criterion for oil purity. Gemlik virgin olive oils had Δ7-stigmastenol contents increases significantly from 0.26 to 0.41 in 2017 harvest year ($p < 0.05$) (Table 5). This value slightly higher for southeast Hatay and Mersin NPDO Gemlik virgin olive oils (0.35%) ($p > 0.05$). This could be due to higher temperatures (12.3°C) and the lowest level of rain (0.7 and 1.3 kg/m^2) of Mersin and Hatay NPDO regions (Table 1). This finding supports the findings of Sevim et al. (2023), who found that high Δ7-stigmastenol contents in virgin olive oils obtained from Turkiye's southeast region. Warmer environmental conditions tend to increase total sterol content as well as β-sitosterol contents (Mailer et al., 2010; Sevim et al., 2023). Thus, "southeast" oils from Hatay and Mersin NPDO had higher total sterol and β-sitosterol concentrations than Gemlik virgin olive oils from Bursa and Izmir (Table 5).

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TABLE 5 Analysis of variance (2-way ANOVA) with significance levels and *F*-values of sterols of Gemlik olive oils by growing regions (GR) and harvest years (HY).

	Growing region (GR)				Significance of GR		Harvest year (HY)		Significance of HY	
Sterols (%)	**Bursa-PDO**	**Izmir-NPDO**	**Hatay-NPDO**	**Mersin-NPDO**	**F (GR)**	**Significant**	**2016**	**2017**	**F (HY)**	**Significant**
Cholesterol	0.24[a]	0.12[a]	0.19[a]	0.20[a]	0.35	ns	0.33[a]	0.07[b]	0.00	**
Brassicasterol	0.06[a]	0.07[a]	0.08[a]	0.06[a]	0.78	ns	0.06[a]	0.07[a]	0.44	ns
24-methylen cholesterol	0.16[a]	0.20[a]	0.15[a]	0.18[a]	0.88	ns	0.21[a]	0.13[a]	0.20	ns
Campesterol	1.88[a]	2.18[a]	1.84[a]	1.84[a]	0.75	ns	1.83[a]	2.04[a]	0.47	ns
Campestanol	0.18[a]	0.18[a]	0.20[a]	0.20[a]	0.58	ns	0.32[a]	0.06[b]	0.00	**
Stigmasterol	0.53[a]	0.89[a]	1.42[a]	1.76[a]	0.25	ns	1.37[a]	0.92[a]	0.29	ns
Clerosterol	1.14[a]	1.13[a]	0.89[a]	1.12[a]	0.51	ns	1.20[a]	0.94[a]	0.12	ns
β-sitosterol	77.8[a]	81.4[a]	83.1[a]	82.6[a]	0.49	ns	77.53[a]	84.93[a]	0.05	ns
Apparent (total) β-sitosterol	95.97[a]	95.43[a]	95.19[a]	94.49[a]	0.38	ns	94.92[a]	95.62[a]	0,26	ns
Sitostanol	0.82[a]	0.42[a]	0.87[a]	0.77[a]	0.31	ns	0.83[a]	0.60[a]	0.22	ns
Δ5-avenasterol	15.43[a]	11.89[a]	9.71[a]	9.90[a]	0.44	ns	14.84[a]	8.62[a]	0.08	ns
Δ5,24 stigmastadional	0.78[a]	0.57[a]	0.63[a]	0.51[a]	0.24	ns	0.70[a]	0.55[a]	0.13	ns
Δ7-stigmastenol	0.31[a]	0.32[a]	0.35[a]	0.35[a]	0.78	ns	0.26[b]	0.41[a]	0.02	**
Δ7-avenasterol	0.84[a]	0.74[a]	0.76[a]	0.80[a]	0.97	ns	0.80[a]	0.77[a]	0.87	ns
Erythrodiol + uvaol	1.45[a]	1.40[a]	1.42[a]	1.61[a]	0.83	ns	1.45[a]	1.49[a]	0.83	ns
Total sterol (mg/kg)	1146[a]	1435[a]	1679[a]	1692[a]	0.19	ns	1333[a]	1643[a]	0.13	ns

Note: Different letters in the same row denote a statistically significant difference ($p < 0.05$). **indicates significance ($p \leq 0.05$), *ns* indicates no significance ($p \geq 0.05$).

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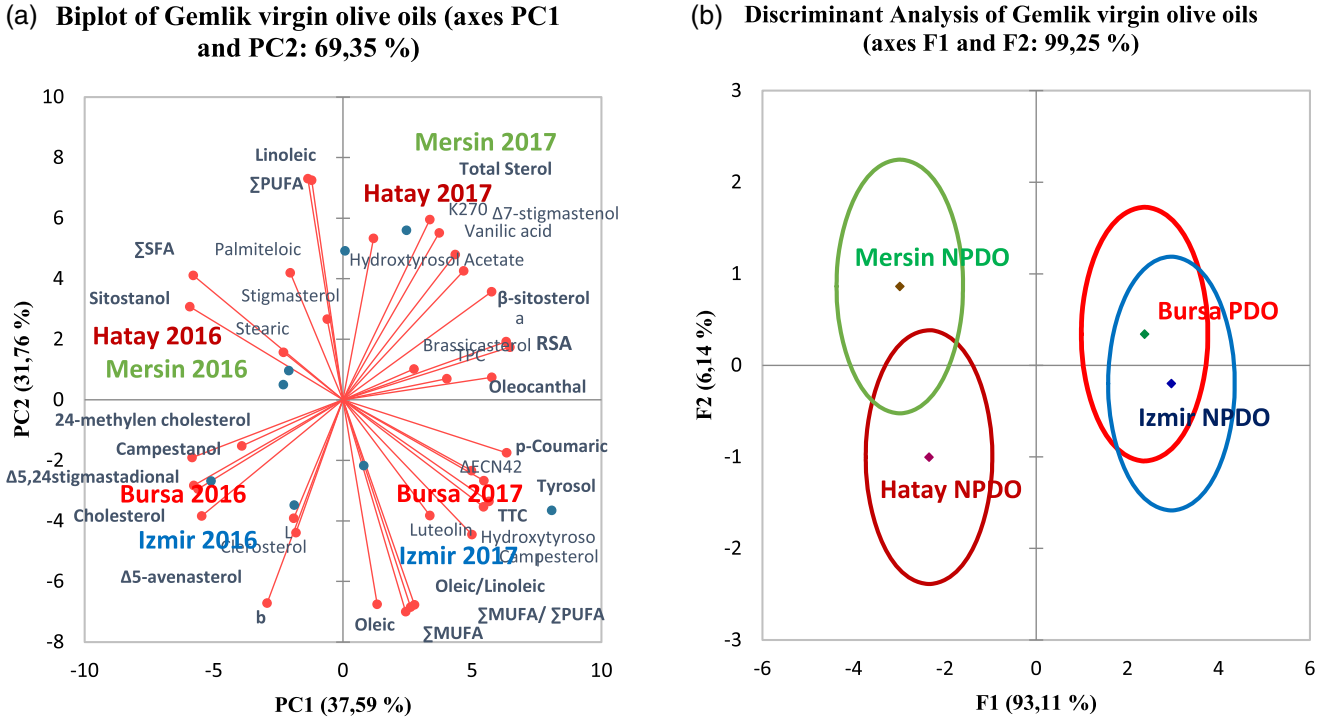


FIGURE 1 (a) PCA bioplot, (b) Discriminant analysis (DA) of Bursa (PDO) and Izmir, Hatay and Mersin (NPDO) Gemlik virgin olive oils in harvest years 2016 and 2017.

Δ5-avenasterol is strongly associated with the antioxidant activity of virgin olive oil (Hannachi et al., 2013) and negatively correlated with β-sitosterol (Noorali et al., 2014). The Δ5-avenasterol contents of Gemlik virgin olive oils from Bursa PDO and Izmir NPDO (15.4 and 11.9%) were found to be the higher than that of Hatay and Mersin NPDO (10%), respectively (Table 5). Stigmasterol is associated with lower quality virgin olive oils (Noorali et al., 2014). High levels are linked to high acidity and poor organoleptic quality. Bursa-PDO and Izmir NPDO have lower stigmasterol contents (0.5% to 0.9%) indicating that they are derived from healthy olives, than Hatay and Mersin NPDO (1.4% and 1.8%) (Table 5). This is also supported by high FFA values of Hatay and Mersin non PDO Gemlik virgin olive oils (1.26% and 0.9%, respectively) shown at Table 2. Sevim et al. (2023) recently reported higher stigmasterol contents (2.22%) for Antalya Gemlik virgin olive oils.

Gemlik PDO and NPDO virgin olive oils discrimination

According to ANOVA results, environmental conditions at harvest years was effective on sterols (cholesterol, campestenol, and Δ7-stigmastenol) and quality parameters (chlorophyll content, a and b values, and radical scavenging activity and phenolic compounds (tyrosol and p-coumaric acid), while the growing region was primarily effective tyrosol. This results were not sufficient to differentiate Gemlik PDO and NPDO virgin olive oils according to their geographical origin. As a result, chemometric analyses such as PCA is used to highlight differences and similarities, and DA is used to differentiate between Gemlik PDO and NPDO virgin olive oils. PCA was performed on 51 variables (four regions and two harvest years) of Gemlik virgin olive oils. Kaiser–Meyer–Olkin measure of sampling adequacy index were lower than 0.5 were eliminated (37 variables remained). Figure 1a,b display the results of the statistical analysis by PCA and DA.

Figure 1a shows PCA was effective differentiating Gemlik PDO and NPDO virgin olive oils with PC1 (37.59%) and PC2 (31.76%) and total variance (69.35%). PC1 and PC2 differentiated the oils in 2016 2017 harvest year. PC1 is primarily related to variables with ≥0.8 correlations, such as a values, total tocopherol and phenol and antioxidant activity, phenolic compounds (tyrosol, p-coumaric acid, and oleocanthal), and sterols (cholesterol, campestenol, β-sitosterol, sitostenol, Δ5-Avenestrol, Δ5,24stigmastadional). PC2 was primarily associated with b values, fatty acids (oleic and linoleic acids), and total sterols (Figure 1). PCA clearly explained total variance up to 69% and showed BursaPDO and Izmir NPDO Gemlik virgin olive oils had high contents of oleic acid, total tocopherols and antioxidant activity, oleocanthal, p-coumaric acid, and tyrosol, while the Hatay and Mersin NPDOs had the high contents of linolenic acid, PUFA, and total

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sterols (Figure 1a). Our findings were consistent with previous studies that used PCA to differentiate between virgin olive oils, with total variance explained ranging from 65.2% to 90% for Turkish virgin olive oils by (Dıraman et al., 2020; Dıraman & Dibeklioglu, 2014; Uluata et al., 2021) 53% to 92% for Tunisian, Spanish, Iranian and Italian virgin olive oils by (El-Gharbi et al., 2018; Hmida et al., 2022; Manai-Djebali et al., 2018; Noorali et al., 2014). According to the discriminant analysis depicted in Figure 1b, the groupings in the score plot are based on both geographic origin and harvest year.

The discriminant analysis clearly discriminated Gemlik PDO and NPDO virgin olive oils with 99.25% the total variance explained (Figure 1b). Twenty-four variables with correlations of ≥0.8 including oleic, linoleic acids, ∑SFA, ∑PUFA, ∑MUFA, Oleic/Linoleic, ∑MUFA/PUFA ratios and hydroxytyrosol on t1, Δ5,24stigmastadional, Δ5-Avenestrol, TPC, a value, β-sitosterol, RSA, vanillic acid and, oleocanthal on t2 were determined. DA exaplianed toatal variance up to 99% and clearly showed Bursa PDO and Izmir NPDO Gemlik virgin olive oils strongly separated from other Hatay and Mersin NPDO Gemlik olive oils.

CONCLUSIONS

The findings revealed that geographical area and environmental conditions during harvest year play significant roles in the characteristics of Gemlik PDO and NPDO virgin olive oils. High temperature, less rain high relative humidity in south region increased FFA, chlorophyll, linoleic acid, PUFA, stigmasterol, Δ7-stigmastenol, β-sitosterol, and total sterol content of Gemlik virgin olive oils obtained from Hatay and Mersin NPDO regions. Thus inverse conditions during oil biosynthesis degrade oil quality by changing the fatty acid, sterol and phenolic composition, resulting in lower polyphenol and oleic acid concentrations. Bursa PDO and Izmir NPDO virgin olive oils had high total tocopherol, total phenol, radical scavenging activity, oleic acid, MUFA, hydroxytyrosol, tyrosol p-coumaric acid, Δ5,24-stigmastadional, and low linoleic acid, PUFA and stigmasterol contents. This study clearly showed that Bursa-PDO and Izmir NPDO have better quality, purity and oxidative resistance properties distinguished from Hatay and Mersin NPDO Gemlik virgin olive oils. Marmara (Bursa PDO) and north Aegean (Izmir NPDO) regions appear to have more favorable environmental conditions for the development of the Gemlik olives than the eastern Mediterranean (Hatay and Mersin non-PDO regions). Olive cultivation in the Mediterranean is affected by environmental conditions during harvest times but sustainable production requires research regarding geographic traceability of olive oils. The chemometric analysis such as PCA and DA can be used to distinguish virgin olive oils from different geographic origins and environmental conditions to ensure their authenticity, save consumers, and stop unfair competition between the producers for marketing, labeling, and pricing.

AUTHOR CONTRIBUTIONS

Turkan Mutlu Keceli: Methodology, investigation, analysis, statistic, software, writing, editing, supervision. **Fulya Harp Celik:** Investigation, sample collection, oil extraction, analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No human or animal subjects were used in this research.

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