

Use of Phenolic Profile and Fatty Acid Composition on Chemometric Discrimination of Turkish Virgin Olive Oils with Geographical Indication

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Received (Geliş Tarihi): 19.03.2021, Accepted (Kabul Tarihi): 28.05.2021

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ABSTRACT

A geographical indication (GI) of virgin olive oil (VOO) certifies the origin and production methodology within a determinative quality aspect. On the other hand, from the consumer perspective, VOO's with GI are expected to show a difference from other VOO's, even though it is not an official obligation. In this study, samples from seven different Turkish VOO's with GI were evaluated and possible discrimination among them was determined and justified using principal component analysis (PCA). Results revealed that saturated fatty acid (SAFA) content was a key factor for Nizip samples to discriminate from VOO's with GI. Samples of Southern Aegean (Güney Ege) and Ayvalık had the highest amount of secoiridoids content. When phenolic profile was considered alone, Mut and Nizip samples were clearly separated while groupings among other samples were somehow overlapped. PCA using fatty acid composition revealed that Nizip and Güney Ege samples were located on different sides of the score plot. Regional distances among sample sets greatly affected the discrimination of the PCA plot when their fatty acid composition was involved.

Keywords: Virgin olive oil, Geographical indication, Phenolic compounds, Fatty acid composition, Principal component analysis, Chemometric

Coğrafi İşaretli Türk Zeytinyağlarının Kemometrik Ayırımında Fenolik Profil ve Yağ Asidi Kompozisyonunun Kullanımı

ÖZ

Coğrafi işaret (Cİ), zeytinyağlarında menşei ve üretim metodolojisini belirli bir kalite kapsamı içinde onaylayan bir araçtır. Öte yandan, tüketici açısından bakıldığında, bir Cİ'li zeytinyağının Cİ olmayan zeytinyağı örneklerinden veya Cİ'li diğer zeytinyağlarından farklılık göstermesi beklenmektedir, ancak bu Cİ sertifikasyonu için gerekli bir resmi zorunluluk değildir. Bu çalışmada, Cİ'li yedi farklı Türk Zeytinyağından alınan numuneler değerlendirilmiş ve bunlar arasındaki olası farklılıklar, temel bileşen analizi (PCA) kullanılarak belirlenmiş ve yorumlanmıştır. Sonuçlar, doymuş yağ asidi içeriğinin (SAFA), Nizip örneklerinin diğer zeytinyağlarından ayırt edilmesinde önemli bir faktör olduğunu ortaya koymuştur. Güney Ege ve Ayvalık örneklerinin en yüksek sekoiridoid içeriğine sahip olduğu tespit edilmiştir. Yalnızca fenolik profil ile gerçekleştirilen temel bileşen analizinde (TBA), Mut ve Nizip numuneleri net bir şekilde diğer örnek gruplarından ayrılmışken, diğer örnek grupları örtüşmektedir. Yağ asidi bileşimi ile gerçekleştirilen TBA, Nizip ve Güney Ege örneklerinin skor grafiğinin farklı taraflarında bulunduğunu ortaya çıkarmıştır. Örnek grupları arasındaki

bölgesel mesafelerin, yağ asit kompozisyonu söz konusu olduğunda PCA'nın ayırt edici sonuçlar vermesinde büyük rol oynadığı düşünülmektedir.

Anahtar Kelimeler: Zeytinyağı, Coğrafi işaret, Fenolik profili, Yağ asidi kompozisyonu, Temel bileşen analizi, Kemometri

INTRODUCTION

Olive (*Olea europaea L.*) belongs to the *Oleaceae* plant family and it is an olive fruit that is an evergreen tree [1, 2]. Virgin olive oil (VOO) is defined as the oil of the olive fruit produced from the fruit of this tree by mechanical methods without any purification [3, 4]. Through centuries, olive oil has been an important part of Mediterranean cuisine as an icon representing the healthy diet, therefore the consumer demand has been increasing in parallel to its worldwide popularity [5, 6].

Most of the VOO content is triacylglycerol esterified with fatty acids, which are the major component (> 95%), and the remaining of this content consists of minor components, such as tocopherols, phenols, sterols, hydrocarbons, and volatile compounds [7-9]. The chemical and sensorial properties of VOO's fluctuate within limits since VOO's do not have a fixed chemical profile. The differences may derive from genetic, agronomic, and environmental factors such as olive variety, growing area, soil, age of trees, maturity index during harvest, pre-processing and post-processing strategies of VOO production [10]. The difference in variety that affects the main chemical and sensorial properties of VOO such as phenolic profile, fatty acid composition, sterol composition, cause significant differentiation between VOO's [11]. The precipitation or irrigation practices of the geographical region where olives are grown, as well as factors such as soil condition, altitude, and climate characteristics, create differences in the chemical composition of VOO. Even VOO's belonging to the same olive variety but grown in different regions were examined, a difference was observed in the sterol, phenolic, and volatile components [12-15].

To meet consumers' demands for originality and quality, producers tend to certify their VOO's based on geographical origin [16]. The geographical indication (GI) is a sign used on goods that have a specific geographical origin and possess qualities or a reputation that are due to that origin. Besides, geographical indication for VOO not only a useful tool to certify the origin to preserve the interests of rural producers but also helps to protect consumers and producers from economic frauds [17]. In addition, it has a role as a guide to express the identical standards of VOO with GI and support the development of the product quality from the perspective of producers and consumers [18]. Turkish Patent and Trademark Office and European Commission enforce three types of certification labels

for geographical indication (GI): protected designation of origin (PDO) and protected geographical indication (PGI). Besides, they may be certified as a traditional specialty guaranteed (TSG) product. For VOO's, PDO or PGI certifications are used in Turkey and European Union. Turkey has 10 different VOO with GI namely; "Ayvalık Zeytinyağı", "Edremit Zeytinyağı", "Güney Ege Zeytinyağı", "Kuzey Ege Zeytinyağı", "Milas Zeytinyağı" and "Mut Zeytinyağı", "Nizip Zeytinyağı", "Aydın Memecik Zeytinyağı", "Ödemiş Çekişte Zeytinyağı" and "Burhaniye Zeytinyağı" but only "Milas Zeytinyağı" was registered also the eAmbrosia EU GI registration system [19, 20]. The geographical regions determined for Turkish VOO's with GI are somehow close or overlapping in some places. Moreover, for some VOO's with GI is being produced using same variety, for instance, Ayvalık variety is main variety for Ayvalık Zeytinyağı", "Edremit Zeytinyağı" and "Kuzey Ege Zeytinyağı" while Memecik variety is used for "Güney Ege Zeytinyağı" and "Milas Zeytinyağı". In addition, VOO's with GI not necessarily be monovarietal olive oils, such as Mut VOO's which can be produced using both Gemlik or Ayvalık varieties. Also for most of the GI accepts extraction of VOO using different producing methods such as pressing or decantation. Moreover, not only extra virgin olive oil (EVOO), but also ordinary VOO's may be certified for same certain GI's [20]. Therefore, the variation in chemical composition emerged from diversity of variety, VOO extraction techniques and quality criterias make difficult to a reveal the difference in chemical composition of one VOO with GI to another in many cases. Moreover, there is not a study in the literature about the evaluation of possible differences in minor compositions of VOO's by taking GI difference as a factor.

Minor characteristics of VOO can be identified through several parameters such as fatty acid composition, phenolic profile, sterol composition, tocopherol composition. In general, chemometric techniques were used to determine possible classifications among VOO sample groups since the difference in multiple variables might be responsible for discrimination. For instance, chemometric techniques were found useful to differentiate EVOO samples according to their geographical origins or variety [14, 21-25].

In this study, seven Turkish VOO's with the geographical indication were studied. General quality characteristics such as free fatty acids (FFA), peroxide value (PV), p-anisidine value (p-anV), and specific absorption values (K232 and K270) were determined. Possible

discriminations among VOO samples were evaluated using Principal component analysis of fatty acid composition (%) and phenolic profile (mg/kg) of VOO samples. Moreover, total monounsaturated fatty acid (MUFA), total polyunsaturated fatty acid (PUFA) and total saturated fatty acid (SAFA) contents were calculated according to fatty acid composition. Total secoiridoids (in mg/kg) were calculated according to identified secoiridoid peaks of the sample phenolic profile. MUFA, SAFA, PUFA, and total secoiridoids were also used in PCA.

MATERIALS and METHODS

Materials

Seven different and 5-6 samples from each Turkish VOO's with GI as "Ayvalık Zeytinyağı" (VOO's with GI of Ayvalık region supplied from Ayvalık Chamber of Commerce), "Edremit Zeytinyağı" (VOO's with GI of Edremit region supplied from Edremit Chamber of Commerce), "Güney Ege Zeytinyağı", "Kuzey Ege Zeytinyağı" (VOO's with GI of South Aegean and North Aegean regions supplied from from Taris Olive and Olive Oil Agricultural Sales Cooperatives Union), "Milas Zeytinyağı" (VOO's with GI of Milas region supplied from Milas Chamber of Commerce and Industry), "Mut Zeytinyağı" (VOO's with GI of Mut region supplied from Mut Chamber of Commerce and Industry) and "Nizip Zeytinyağı" (VOO's with GI of Nizip region supplied from Nizip Chamber of Commerce) samples were requested and supplied. Since sample grouping factor is only GI in our study, additional differences emerged from side factors such as varietal and methodological variations are expected. All VOO samples with GI denoted with its regional name as Ayvalık, Edremit, Güney Ege, Kuzey Ege, Milas, Mut and Nizip in the text. The VOO samples were filtrated and filled into glass amber bottles. The headspace was stripped with a gentle stream of nitrogen prior to closing the lids. Samples were kept in in the freezer at -24°C until analyses.

Determination of Quality Characteristics

Free fatty acid content (FFA)

The samples were dissolved in diethyl ether and ethanolic solvent and the free fatty acids transferred to the solvent medium were titrated with ethanolic potassium hydroxide solution. The content of free fatty acids according to method COI/T.20/Doc. No 33/Rev.1. and expressed as the free fatty acid percent (%) in oleic acid [26].

Peroxide Value (PV)

The peroxide values (PV) of the samples were determined according to the method (PV), COI / T.20 / Doc. No 35 / Rev.1. Samples were dissolved in acetic

acid and chloroform and potassium iodide solution was added on them. Released iodine was titrated with sodium thiosulfate solution and results represented as milli-equivalent of active oxygen per kilogram [27].

p-Anisidine Value (p-anV)

The p-anisidine value was determined according to the AOCS-Cd-18 90 method. Briefly, isooctane was added to the samples and 5 mL of this solution was transferred to a separate glass tube prior to p-anisidine reactant was added. The absorbance of the sample solutions at 350nm measured against the isooctane and the p-anisidine values were calculated. Results were presented as mmol/kg oil [28].

Total Oxidation Value (TOTOX)

TOTOX measures oil deterioration and was calculated from the formula: $TOTOX = 2PV + p-anV$ [28].

Specific Absorptions at Ultraviolet Light

According to COI / T.20 / Doc. No 19 / Rev.5. samples were dissolved in cyclohexane at a concentration of 1% (w / v) and the specific absorption of the samples; It was measured in a 10 mm cell, at 232 and 270 nm wavelengths using UV-vis spectrophotometer (Optizen POP spectrometer, K-Lab, South Korea) [29].

Determination of Fatty Acid Composition

The fatty acid composition of the samples was determined according to the method given in Turkish Food Codex statement number 29181 [30]. 1 g of sample was weighed and mixed with 2 mL of heptane. 0.2 mL of methanolic potassium hydroxide solution was added to this mixture. The supernatant containing the methyl esters was transferred to vials for gas chromatography (GC) injection. The GC oven temperature was kept at the initial temperature of 165°C and then increased to 200°C. The injection block temperature was set to 250°C while the detector temperature was set to 280°C. The flow rate of the carrier gas was 1.2 mL/min and 1 µL of the sample was injected. After all the peaks were obtained and identified, the peak areas were presented as percentages.

Determination of Phenolic Profile

According to COI/T.20/Doc. No 29/Rev.1method, minor polar phenolic compounds were extracted and their quantities were determined by high-pressure liquid chromatography (HPLC) [31]. HPLC (Agilent 1260 model infinity II, USA) was equipped with a Spherisorb ODS-2 C18 reverse-phase column (4.6 mm x 25 cm), 100 Å° spectrophotometric UV detector, and integrator at 280 nm. According to the method, 2 g's of the sample

was weighed into glass tubes and 1 mL internal standard solution was added. After mixing with vortex for 30 seconds, 5 mL of methanol/water 80/20 (v/v) solution was added. The samples were kept in an ultrasonic bath for 15 minutes and centrifuged at 5000 revs/min for 25 minutes. For HPLC, the flow rate of mobile phases; methanol, acetonitrile, and orthophosphoric acid 99.8% (v/v) was 0.8 mL/min with a gradient flow composition. The injection volume was 40 µL and the peak areas were calculated with respect to the area of internal standard peak (syringic acid). The respective response factor (RRF value) was found to be 4.7 (the ratio of the response factor of syringic acid to tyrosol). The amounts of polar phenolic compounds are expressed in mg/kg oil as tyrosol equivalent.

Statistical Analysis and Chemometric Method

All analyses were duplicated, and measurements were triplicated to ensure the accuracy of the results. ANOVA and Tukey's post-hoc test at a significance level of %5 ($\alpha=0.05$) was applied using IBM SPSS 25 to evaluate differences in quality characteristics of VOO samples with GI. Three different principal component analyses were carried out using Unscrambler X (Camo Analytics, Norway) to visualize possible discriminations among VOO samples with GI. Fatty acid composition and phenolic profile data were mean-centered and randomized, leverage correction method was used for

internal validation. All values were weighted as reciprocal of the standard deviation to eliminate the size difference effect on the results. The singular value decomposition (SVD) algorithm was selected to build the PCA model. No rotation applied. The first two PC's were visualized on two-dimensional biplots to determine the discrimination on the score plots. PCA analysis of the fatty acid composition, phenolic profile, and fatty acid composition + phenolic profile data were presented separately.

RESULTS and DISCUSSION

The approximate regions of seven different Turkish VOO's with GI used in our study given in Figure 1. Within the process of our work, three new VOO's registered with GI by the Turkish Patent and Trademark Office, namely "Ödemiş Çekişte Zeytinyağı" (Ödemiş Çekişte VOO), "Aydın Memecik Zeytinyağı" (Aydın Memecik VOO) and "Burhaniye Zeytinyağı" (Burhaniye VOO) but not took a part in our study. Slight overlaps or close regions for Edremit Zeytinyağı, Ayvalık Zeytinyağı and Kuzey Ege Zeytinyağı in the northern Aegean region and Güney Ege Zeytinyağı and Milas Zeytinyağı may be mentioned. Since minor characteristics such as phenolic profile and fatty acid composition are generally affected by region and variety, among these samples tough discrimination may be expected at multivariate analysis.



Figure 1. The map of approximate geographical regions of Turkish virgin olive oils with geographical identification used in our study as declared official dossiers, coloring was matched with principal component analysis score plot groupings (Maps image of Turkey was used courtesy of Google LLC).

Table 1. Quality characteristics of virgin olive oils with geographical identification

Virgin olive oils with geographical identification	Free fatty acid content (% oleic acid) (P=0.076)	Peroxide value (meq O ₂ /kg) (P =0.641)	p-anisidine value (mmol/kg) (P <0.000)	Total oxidation value (P =0.024)	K232 (P <0.000)	K270 (P <0.000)
Ayvalık	0.19 (0.09)	10.65 (4.65)	17.54 ^{ab} (1.07)	38.84 ^{ab} (9.61)	1.566 ^a (0.260)	0.098 ^a (0.013)
Edremit	0.42 (0.20)	9.32 (4.08)	15.18 ^a (5.36)	33.82 ^{ab} (8.82)	2.017 ^{bc} (0.093)	0.106 ^{ab} (0.011)
Güney Ege	0.52 (0.29)	9.43 (3.12)	13.38 ^a (4.71)	32.24 ^{ab} (7.44)	1.889 ^b (0.181)	0.134 ^b (0.017)
Kuzey Ege	0.48 (0.20)	8.74 (2.47)	11.64 ^a (2.67)	29.11 ^a (5.08)	1.825 ^{ab} (0.195)	0.089 ^a (0.010)
Milas	0.35 (0.41)	10.98 (5.87)	15.99 ^a (7.92)	37.95 ^{ab} (8.27)	2.188 ^c (0.287)	0.133 ^b (0.051)
Mut	0.43 (0.12)	12.98 (9.08)	13.10 ^a (2.17)	39.06 ^{ab} (17.73)	1.803 ^{ab} (0.130)	0.093 ^a (0.017)
Nizip	0.43 (0.23)	11.10 (5.18)	22.66 ^b (5.29)	44.86 ^b (10.62)	1.771 ^{ab} (0.136)	0.117 ^{ab} (0.018)

The mean values were given with standard deviation (in parenthesis). Letters indicate the statistical grouping according to Tukey's post-hoc test along with columns. Significance (P) values of ANOVA for each variable were presented in the first row within parenthesis.

FFA content is an indication for hydrolysis reaction in oils, and during the progress of hydrolysis reaction, FFA content increases while quality decreases. According to the FFA contents given in Table 1, there was no statistical difference between samples (P=0.076). FFA values varied between 0.19 and 0.52. The PV and p-anV are quality parameters that express the oxidation degree while providing information about the oxidative deterioration and approximate storage time of the oil. According to the PV's, there was no statistical difference between samples, and the values varied between 9.74 and 12.98 (P=0.641). Worth to mention that the standard deviation is an expression of the variation in a value between different VOO samples with same GI. Therefore, if standard deviation is relatively high, the variation in that particular quality aspect is high between different VOO's produced within that geographical region. For instance, notable high standard deviations in PV implies that different VOO's with same GI has different oxidation reaction degrees. This may be emerged from variability in process methods practiced by VOO producers in that geographical region.

No statistical difference is observed between p-anV of Ayvalık, Edremit, Güney Ege, Kuzey Ege, Milas, Mut samples while p-anV of Nizip (22.661) was statistically higher than the others. TOTOX values of Ayvalık, Edremit, Güney Ege, Kuzey Ege, Milas, Mut VOO's showed no statistical difference. Specific absorption values are an indicator of the quality changes that may occur during the storage and processing stages of VOO. In general, specific absorption at 232 nm increases with diene conjugation and primary oxidation products, while absorption at 270 nm increases with triene conjugation and secondary oxidation products. K232 values of Ayvalık, Kuzey Ege, Mut, Nizip VOO's showed no statistical difference while K232 values of Edremit, Güney Ege, Kuzey Ege, Mut, Nizip VOO's were assigned in a different statistical group. Similar statistical Tukey groupings may be observed for K270 values.

Phenolic profile was identified by matching respective retention times with the standard IOC method and standardized chromatograms of a previous study [32].

Unidentified peaks did not included in the chemometric analysis. A sample RP-HPLC chromatogram can be seen in Figure 2 acquired in our study. From simple phenols, 3,4-DHPEA (tyrosol), p-HPEA (hydroxytyrosol), and p-coumaric acid were quantified. Pinoreosinol was only lignan and 3,4-DHPEA-EDA (oleacein), p-HPEA-EDA (oleocanthal), 3,4-DHPEA-EA (oleuropein aglycone monoaldehyde), and p-HPEA-EA (lignostride aglycone monoaldehyde) were determined as VOO secoiridoids as given in Table 2. Different phenolic compounds were identified in different studies with Turkish VOO's in the literature. According to a previous study, p-coumaric acid content of commercial VOO's from the Aegean region ranged between 0.10-0.69 mg/kg while samples obtained from the Çanakkale region and same season contain between 2.0-4.3 mg/kg p-coumaric acid. Pinoreosinol, which is a characteristic lignan in VOO's as reported in many studies, was not reported in some studies about the phenolic profile of VOO's [7]. This may be justified with two possible reasons. First; minor uncertainties in the chromatogram may be caused by some misidentifications. Second, the seasonal, regional, and varietal changes are greatly affective on the phenolic profile as suggested by Alkan et al. [13].

Amounts of 3,4-DHPEA in VOO samples with GI varied between 6.2 and 2.4 mg/kg. Güney Ege samples contained the highest p-HPEA value (6.9 mg/kg). p-coumaric acid varied between 0.5 and 4.8 mg/kg in our samples and Kuzey Ege samples have the highest p-coumaric acid content (4.8 mg/kg). Güney Ege samples contained the highest (83.9 mg/kg) pinoreosinol content. Amounts of 3,4-DHPEA-EDA were between 15.5 and 66.6 mg/kg, Ayvalık samples were richest in 3,4-DHPEA-EDA content. 3,4-DHPEA-EA was between 13.9 and 31.9 mg/kg in VOO samples. The amounts of p-HPEA-EA varied between 2.8 and 7.7 mg/kg. When the total amount of secoiridoids was considered, Ayvalık samples contain 111.4 mg/kg of secoiridoids while Kuzey Ege samples contained 38.6 mg/kg.

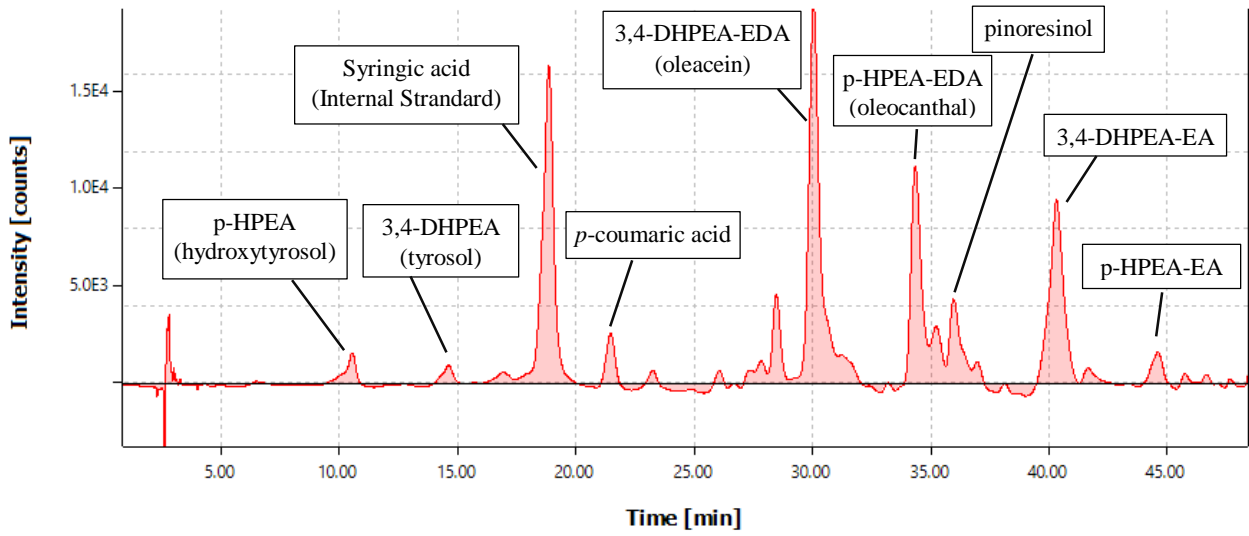


Figure 2. A sample chromatogram for phenolic profile of Edremit Zeytinyağı sample. 3,4-DHPEA-EA: oleuropein aglycone monoaldehyde, p-HPEA-EA: lignostride aglycone monoaldehyde

Table 2. Phenolic profile of Turkish virgin olive oils with geographical identification

		Turkish virgin olive oils with geographical identification *						
	Phenolics (mg/kg)	Ayvalık	Edremit	Güney Ege	Kuzey Ege	Milas	Mut	Nizip
Simple Phenols	3,4-DHPEA (tyrosol)	4.1 (2.5)	2.9 (2.3)	3.4 (1.4)	2.8 (1)	6.2 (3.8)	2.4 (0.4)	3.1 (1)
	p-HPEA (hydroxytyrosol)	2.7 (1.6)	2.8 (2.3)	6.9 (1.3)	4 (0.8)	5 (1.6)	3.3 (0.3)	3.4 (1.4)
	p-coumaric acid	3.9 (1.8)	3.9 (1.3)	2.2 (0.9)	4.8 (2.3)	2.9 (1.7)	0.9 (0)	0.5 (0.5)
Lignan	Pinoresinol	9.2 (4.8)	6.8 (0.7)	8.2 (0.6)	6.4 (2.3)	19.7 (6.1)	9.8 (1.8)	6.4 (2.5)
Secoiridoids	3,4-DHPEA-EDA (oleacein)	66.6 (6.9)	30.2 (12.2)	52.3 (20)	15.5 (4.6)	41.2 (2.1)	19.6 (1)	19 (10.6)
	p-HPEA-EDA (oleocanthal)	39.7 (13.8)	24.9 (5.6)	83.9 (5.2)	25.6 (2.3)	44.4 (17.5)	31.9 (1.6)	39.9 (7)
	3,4-DHPEA-EA (oleuropein aglycone monoaldehyde)	31.9 (6.8)	19.5 (4.5)	21.1 (3.8)	13.9 (3)	18.4 (8.9)	28.6 (1.8)	28 (2.4)
	p-HPEA-EA (lignostride aglycone monoaldehyde)	3.8 (0.7)	3.4 (0.5)	7.1 (1.9)	2.8 (0.5)	3.6 (0.9)	7.7 (0.9)	9.2 (2.9)
	Total secoiridoids	111.4 (16.1)	59.9 (16.6)	94.4 (20.4)	38.6 (8.3)	82.9 (7)	65.7 (2.3)	62.6 (12.5)

* The mean values were given with standard deviation (in parenthesis).

Table 3. Fatty acid composition of Turkish virgin olive oils with geographical identification

Fatty Acids (%)	Geographical Identification *						
	Ayvalık	Edremit	Güney Ege	Kuzey Ege	Milas	Mut	Nizip
C16:0 (palmitic acid)	14.3 (0.5)	15.5 (0.6)	13.4 (0.4)	13.9 (0.6)	14.6 (0.7)	15.2 (0.7)	15 (0.5)
C16:1 (palmitoleic acid)	0.9 (0.1)	0.9 (0.1)	1 (0)	1 (0.1)	1.3 (0.1)	1.2 (0.2)	1.1 (0.1)
C17:0 (margaric acid)	0.1 (0)	0.1 (0)	0 (0)	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)
C17:1 (margoleic acid)	0.2 (0)	0.2 (0)	0.1 (0)	0.2 (0)	0.2 (0.1)	0.1 (0.1)	0.2 (0)
C18:0 (stearic acid)	2.6 (0.2)	2.5 (0.1)	2.6 (0.1)	2.6 (0.1)	2.8 (0.2)	2.5 (0.4)	4.1 (0.4)
C18:1 (oleic acid)	70.3 (0.5)	69.5 (0.8)	71.3 (0.7)	70.2 (1.4)	70.1 (2.2)	69.4 (1.5)	68 (1.7)
C18:2 (linoleic acid)	10.1 (0.3)	9.6 (0.3)	10.1 (0.8)	10.4 (0.8)	9.5 (2.1)	9.9 (1.7)	9.7 (1.1)
C20:0 (arachidic acid)	0.4 (0)	0.4 (0)	0.4 (0)	0.4 (0)	0.4 (0)	0.4 (0)	0.6 (0)
C18:3 (linolenic acid)	0.6 (0)	0.6 (0)	0.7 (0)	0.6 (0)	0.7 (0.1)	0.7 (0.1)	0.7 (0)
C20:1 (gadoleic acid)	0.3 (0)	0.3 (0)	0.3 (0.1)	0.3 (0)	0.3 (0)	0.3 (0)	0.3 (0)
C22:0 (behenic acid)	0.1 (0)	0.1 (0)	0.1 (0.1)	0.1 (0)	0.1 (0)	0.1 (0)	0.2 (0)
C24:0 (lignoseriic acid)	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)	0.1 (0)
Σ MUFA (monounsaturated fatty acids)	71.7 (0.4)	71 (0.8)	73.4 (2.2)	71.7 (1.3)	71.8 (2.3)	71 (1.7)	69.5 (1.6)
Σ PUFA (polyunsaturated fatty acids)	10.7 (0.3)	10.2 (0.3)	10.8 (0.8)	11 (0.8)	10.1 (2.2)	10.6 (1.8)	10.4 (1.1)
Σ SAFA (saturated fatty acids)	17.6 (0.4)	18.8 (0.5)	16.7 (0.4)	17.3 (0.5)	18.1 (0.6)	18.4 (0.6)	20.1 (0.7)

* The mean values were given with standard deviation (in parenthesis).

The fatty acid composition values of VOO's with GI were given in Table 3. The major fatty acid in VOO is oleic acid (C18:1). According to the results, oleic acid varied between 68% and 71.3% in samples. It has been determined that the oleic acid percentage of Edremit, Nizip, and Mut VOO's is below 70% while the rest of the samples have oleic acid content above 70%. Güney Ege samples had the highest oleic acid content (71.3%). Another major fatty acid is palmitic acid (C16:0) for VOO's. Palmitic acid varied between 13.4% and 15.5% in whole VOO samples. Ayvalık, Edremit, Milas, Mut, Nizip samples contain more than 14% of palmitic acid according to the findings. Stearic acid (C18:0) content, which is another major fatty acid for VOO's varied between 2.5% and 4.1%. Nizip VOO's appear to have the highest stearic acid content (4.1%). When the linoleic acid values were examined, it was found that it varied between 9.5% and 10.4%. SAFA

(C16:0+C17:0+C18:0+C20:0+C22:0+C24:0), MUFA (C16:1+C17:1+C18:1+C20:1), PUFA (C18:2+C18:3) contents of the samples were determined according to the fatty acid compositions. MUFA values varied between 69.5% and 73.4% while SAFA content was between 16.7% and 20.1%. In a previous study; VOO's of Ayvalık variety produced from the north Aegean region were found to contain approximately 2.3% and 4.2% stearic acid (C18:0) likewise in our study [33]. Gurdeniz et al. found that virgin Memecik VOO of the İzmir region had approximately 71.2% oleic acid content which is a similar result to our study [23]. However, in another study about the characterization of the arbequina variety EVOO's grown in the Aegean region, it was indicated that oleic acid content was found to be lower, as 65% [34]. Therefore, not only the geographical region of the VOO but also variety a strong determinative factor for fatty acid composition. On the

other hand, linoleic acid and palmitic acid levels of Nizip Yağlık, Ayvalık, Kilis Yağlık, Halhalı, Karamani varieties from southeastern Anatolia were around 10.90% and

14.56%, respectively, which was similar to our results [35].

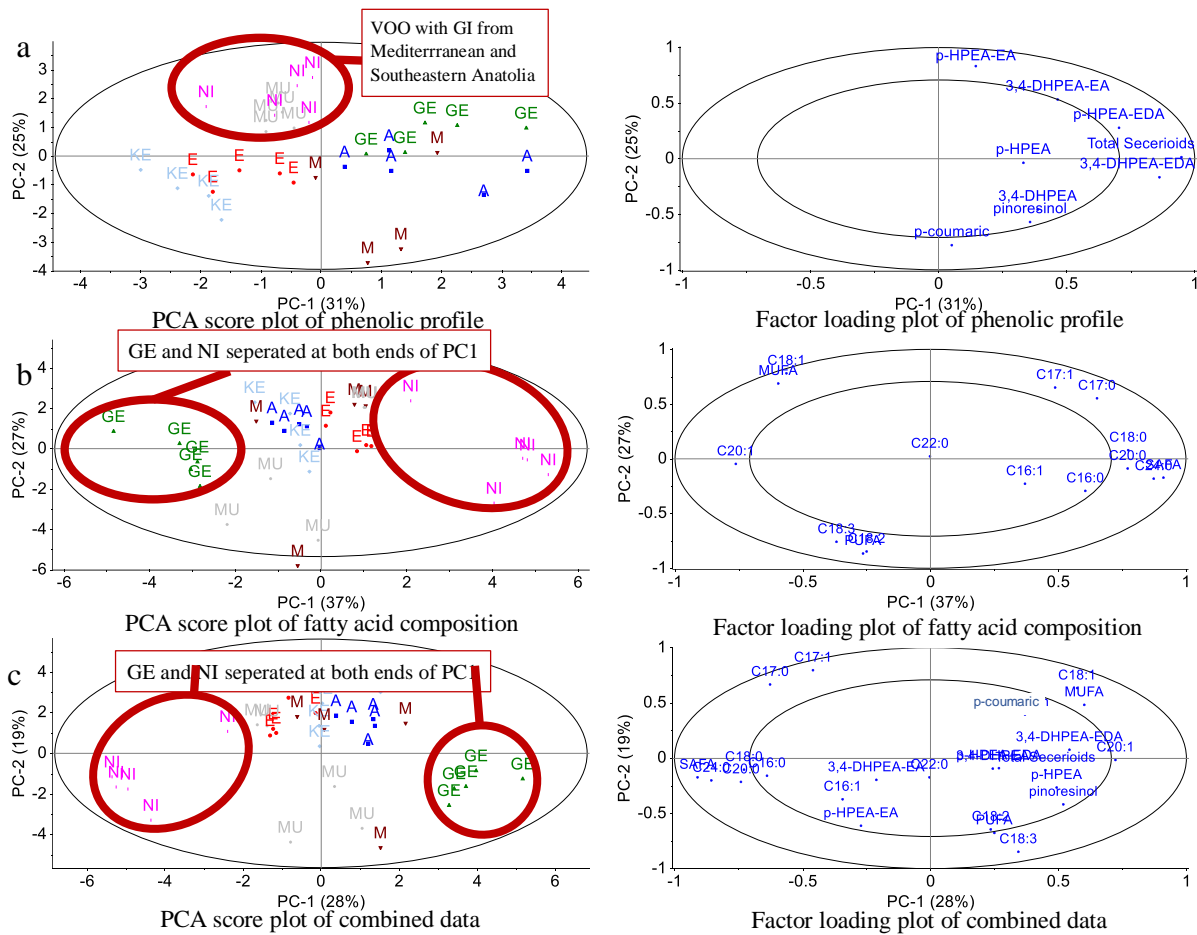


Figure 3. PCA score and loading plots for phenolic profile data (a), fatty acid composition data (b), and phenolic profile + fatty acid composition data combined (c). Ayvalık, Edremit, Güney Ege, Kuzey Ege, Milas Zeytinyağı, Mut and Nizip samples were abbreviated on plots as; A, E, GE, KE, M, MU, NI, respectively.

PCA is a non-biased multivariate analysis, that visualizes discrimination between samples on a reduced factor space. Labeling or coloring sample groups on PCA score plot gives explanative information about possible discrimination between sample groups. Figure 3 represents PCA score and loading plots for phenolic profile data, fatty acid composition, and phenolic profile + fatty acid composition (combined). MU and NI samples separated along PC2 of phenolic profile PCA score plot and located at positive region vertically (Figure 3a). The difference in the geographical region and possible climate conditions may be responsible for this separation from OO samples of the Aegean region. According to the official dossiers, NI was produced from "Nizip Yağlık" olives, while MU was produced from Gemlik and Ayvalık varieties. Therefore, this separation of NI and MU together from other samples, which are generally Ayvalık and Memecik olives, cannot be

justified by varietal difference. According to the absolute loadings of the variables, p-HPEA-EA has 0.8347 on PC2, which implies that p-HPEA-EA was a determinative factor for the separation of the samples along the PC2 axis. According to the Box-plots given in Figure 4b, p-HPEA-EA content of NI, MU, and GE samples were higher than other samples. In addition, the separation of GE and KE samples of phenolic profile PCA can be associated with the varietal and geographical differences in samples. Total secoiridoids, 3,4-DHPEA-EDA, p-HPEA-EDA were the main responsible variables for the separation of the samples along the PC1 axis according to their loadings (0.9485, 0.8632, 0.7044, respectively). Also, total secoiridoids content was low in KE samples but high in A and GE samples as seen in Figure 4. Phenolic composition determined as a discriminative factor for South Aegean and North Aegean VOO's in a previous study [13].

Although a good separation between sample groups was not noted on the score plot of fatty acid composition PCA, a separation between GE and NI samples along the PC1 axis may be observed. Both geographic and varietal differences may be stressed since NI and GE samples were produced from “Nizip Yağlık” and

“Memecik” varieties, respectively. Similar separation and allocation were observed on the score plot of combined data of fatty acid composition and phenolic profile, therefore fatty acid composition was more determinative for PCA separations than phenolic profile alone.

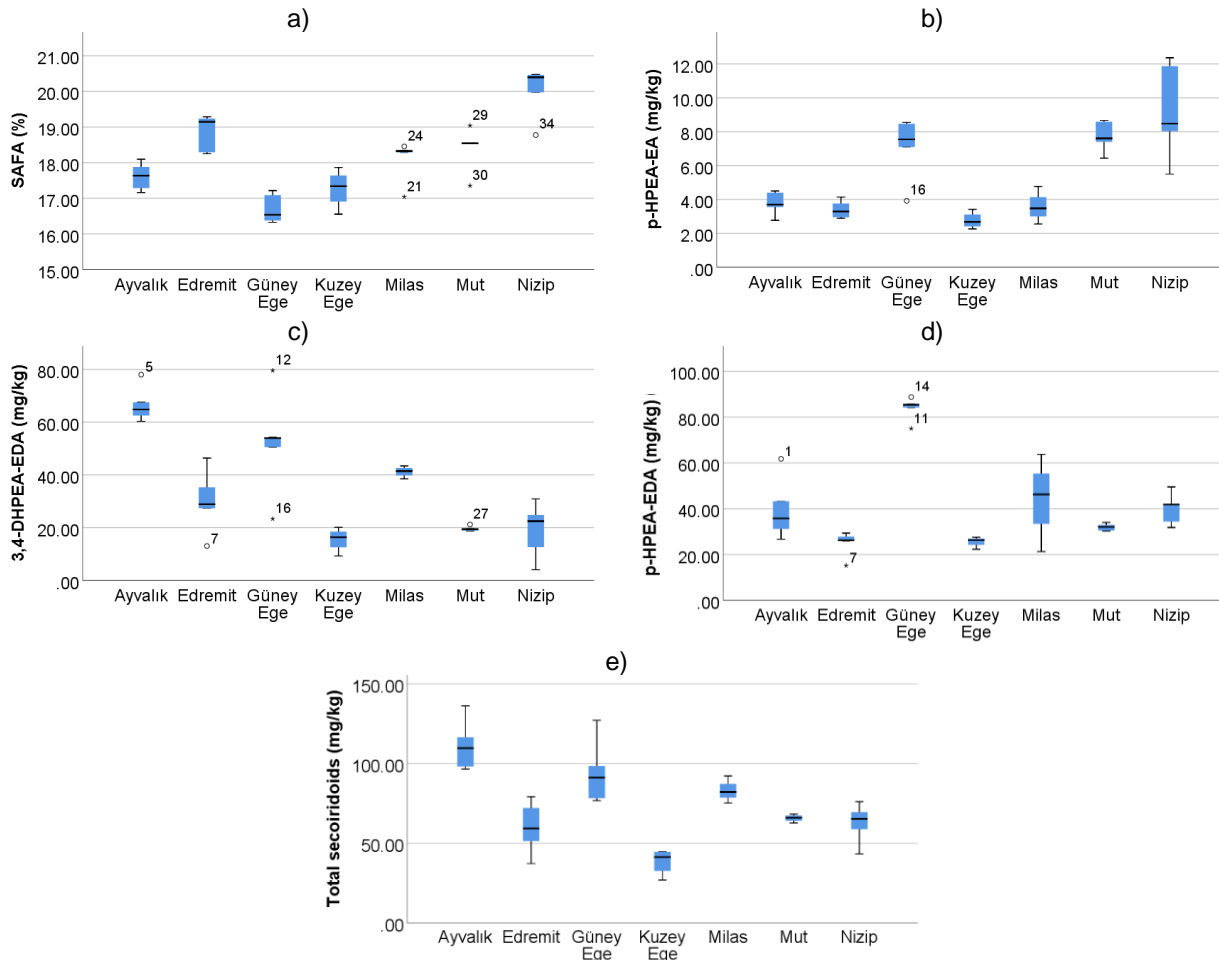


Figure 4. Box-plot graphs of main phenolics and fatty acids responsible discrimination on PCA's. Upper and lower limits of blue boxes represent ranges between first (25th percentile) and third (75th percentile) quartiles, whiskers represent the minimum and maximum values without outliers, and black lines located inside boxes represent medians. Outliers were presented with sample numbers on plots. SAFA: saturated fatty acids

Figure 4 represents main descriptive statistics such as min-max values, medians, and quartiles for factors most responsible for discriminations between VOO samples with GI according to PCA's. The highest and lowest SAFA content were measured in Nizip and Güney Ege samples respectively. Ayvalık and Güney Ege samples were contained the highest total secoiridoids which is a factor of 3,4-DHPEA-EDA, p-HPEA-EDA, and p-HPEA-EA. In a previous study, the fatty acid composition provided significant information to discriminate Nizip yağlık VOO's and Ayvalık samples using SIMCA, which is a multivariate method using PCA models [21]. In another study, the SIMCA, PLS-DA, and NPLS-DA models were evaluated to discriminate VOO's of

arsequina variety from non-arsequina samples among 64 VOO samples. Researchers indicate that LC-DAD chromatograms provide adequate information for discrimination unless the correct data pre-processing technique was selected [36].

CONCLUSION

Phenolic profile and fatty acid composition are two factors of minor properties of VOO's. The change in variety and geographical area of the olive are two main factors determinative on the content of the composition of these variables. p-HPEA-EA, p-HPEA-EDA, 3,4-DHPEA-EDA together with the total secoiridoids were

found to be effective factors for sample groupings on PCA score plots when phenolic profile involved. Nevertheless, the fatty acid composition was found more determinant on sample separation on PCA score plots. Higher SAFA contents than 20%, with one exception, in Nizip samples caused a separation from other OO's with GI. Consequently, a good separation was not achieved between Kuzey Ege, Milas, Edremit, and Ayvalık samples when fatty acid composition data involved. However, a tendency for minimal grouping, although not yet certain, maybe noted between all OO samples with GI when phenolic profile data was used alone. This result shows that when it comes to the authenticity of VOO, the profile of other minor constituents than commons such as fatty acid composition may provide more information.

ACKNOWLEDGMENTS

This study was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) under the project number of 120O872.

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