



# Using chromatographic methods in detection of olive oil adulteration

## Zeytinyağı tağışının belirlenmesinde kromatografik yöntemlerin kullanılması

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### ABSTRACT

In research study, olive oil adulteration with olive pomace oil was monitored by fatty acids,  $\Delta$ ECN42 values and sterol analysis. To this end, virgin olive oil obtained from cv. Kilis Yağlık (KY) was mixed with olive pomace oil at different proportion (1, 5 and 10 %). Gas Chromatography (GC) was used to analyse fatty acid and sterol compositions. The fatty acids with Equivalent Carbon Number 42 (ECN42) and  $\Delta$ ECN42 values of pure and adulterated oils were also used to determine adulteration. Considering the results of fatty acids analysis, when olive pomace oil was mixed, the ratios of oleic acid and palmitic acid in olive oil, was decreased. The difference of theoretical and experimental ECN42 values ( $\Delta$ ECN42) were increased in adulterated oils. Beta-sitosterol which is important compound in the sterol composition, increased up to 81.42 % when mixed with 10 % olive pomace oil. Taking into account the  $R_{mar}$  values of the oil samples, adulterated oils displayed higher value than of pure oil. According to PCA analyses, oil samples took placed in three different groups according to fatty acids and TAGs profile, while in four different groups due to sterol composition. In all of the PCA analyzes, pure KY oil was clearly separated from the adulterated oils.

**Key Words:** Adulteration, Olive oil, Fatty acids, ECN42 value, Sterols

### Öz

Bu araştırma çalışmasında, zeytin pirina yağı karıştırılarak yapılan zeytinyağı tağışı, yağ asitleri, ECN42 değerleri ve sterol analizleri ile izlenmiştir. Bu amaçla, Kilis yağlık (KY) çeşidinden elde edilen yağlar farklı oranlarda (% 1, 5 ve 10) pirina yağı ile karıştırılmıştır. Yağ asidi ve sterol bileşimlerini analiz etmek için Gaz Kromatografisi (GC) kullanılmıştır. Tağışı belirlemek için, saf ve katkılı yağların Eşdeğer Karbon Sayısı 42 (ECN42) ve deltaECN42 değerleri de kullanılmıştır. Yağ asidi analizinin sonuçları dikkate alındığında, pirina yağı karıştırılan zeytinyağlarında oleik asit ve palmitik asit oranları azalmıştır. Hileli yağlarda teorik ve deneysel ECN42 değerlerinin farkı ( $\Delta$ ECN42) artmıştır. Sterol bileşimi içinde önemli bir bileşik olan beta-sitosterol oranı, % 10 pirina yağı karıştırıldığında % 81.42'ye kadar yükselmiştir. Yağ örneklerinin  $R_{mar}$  değerleri dikkate alındığında, katkılı yağlar saf yağdan daha yüksek bir değer sergilemiştir. PCA analizlerine göre, yağ numuneleri yağ asitlerine ve TAG profiline göre üç farklı gruba ayrılırken, sterol bileşimine göre dört farklı grupta yer almıştır. Tüm PCA analizlerinde saf KY yağı, tağışlı yağlardan belirgin bir şekilde ayrılmıştır.

**Anahtar Kelimeler:** Tağış, Zeytinyağı, Yağ asitleri, ECN42 değeri, Steroller

### Introduction

Adulteration is a process of making a cheaper product, disrupting the purity of something, replacing the precious component with the not

worth one, or adding another cheap addition. Food adulteration have been alarming in recent years for food technology, specialists, producers and regulatory authorities because of food-borne illnesses or economic reasons. Therefore, fast and

simple detection of food fraud is very important in the food industry. Many foods are exposed to fraud during production or in daily life. One of the foods the most exposed to adulteration is olive oil. (Jha et al., 2016).

Olive oil is obtained by squeezing from directly the fruit of olive tree (*Olea europea* L.) without any chemical treatment and additive material. It is a greenish-yellowish colored liquid oil that can be consumed as liquid at room temperature especially in Mediterranean diet. Olive oil is widely appreciated for its nutritional, health and sensory properties and it constitutes one of the principal ingredients of the nutrition (Türkoğlu et al., 2012; Tsopeles et al., 2018). Because of these superior properties, olive oil is one of the most exposed agricultural products for adulteration. Since natural olive oil is a herbal source oil, the fraud is mostly vegetable origin. The most important economic source of adulteration to profit is addition of different cheaper oil sources in various rates such as soya, sunflower, cotton, hazelnut, olive pomace, corn, canola, etc. Therefore, detection of adulteration is important in order to maintain the quality of the oils and relieve the health concerns. In the literature, different methods have been examined to detect the fraud of olive oil with other vegetable oils. In previous studies, chromatographic (Jabeur et al., 2014; Shi et al., 2018) and spectroscopic (Jimenez-Carvelo et al., 2017; Li et al., 2018; Meras et al., 2018) methods were used to identify adulteration of olive oil.

Methyl esters of fatty acids normally determine the limits on the content of fatty acids in olive oil and are also chemical compounds used in the differentiation between the original olive oil and other vegetable oils such as sunflower, soybean and corn (Jabeur et al., 2014). In the analysis of the fatty acid composition, cold transmethylation method was used to obtain methyl esters and they were characterized by gas chromatography (GC) (Kelebek et al., 2014).

Triacylglycerol's (TAGs) are the principal component of oils used in the detection of olive oil adulteration. They were analyzed by high

performance liquid chromatography (HPLC). TAGs are separated according to the equivalent carbon number (ECN) and double bond(s) position. The triglycerides in oils starts from 42 carbons and ends with 52 carbons. The ECN value is an important parameter used to detect the adulteration of oils. Olive oil contains a total of nine triglycerides with an ECN value of 42. Trilinolein is the most noteworthy because of its abundance. To determine the presence of other oils mixed with olive oil, the difference between the experimental and theoretical ECN 42 triacylglycerol's content is calculated. For this purpose, the difference between the experimental value of TAGs with the equivalent carbon number 42 (ECN<sub>42</sub><sup>HPLC</sup>) determined by high performance liquid chromatography and the theoretical value of the TAGs with the equivalent carbon number of 42 (ECN<sub>42</sub><sup>theoretical</sup>) obtained from the fatty acid composition by GC are calculated and expressed as  $\Delta$ ECN42 value.

Sterols form a major part of unsaponifiable substances and are found in almost all fats and oils. Sterols are also characteristics of the originality of vegetable oils (Jabeur et al., 2014). They are major to quality regulation of virgin olive oil (VOO). Owing to the useful effects on health, the concern in sterols has increased in recent years. In sterol analysis, it is understood that even vegetable oil and other oils are added to olive oil in very small proportions.

Thus, the main purpose of this study was to identify adulteration of olive oil mixed with olive pomace oil by using gas chromatography and high-performance liquid chromatography. To this aim, analyses of fatty acids,  $\Delta$ ECN42 values and sterols were applied and compared pure and adulterated oils.

## Materials and Methods

### Reagents

The purified water was achieved by using a Millipore-Q system (Millipore Corp., Saint Quentin, France). The standards of fatty acids (oleic, palmitic, palmitoleic, myristic, margaric,

stearic, linoleic, arashidic, margoleic, gadoleic, linolenic, lignoceric and behenic acids) were obtained from Merck (Darmstadt, Germany). All chemicals used in sterol analysis were also obtained from Merck except for internal standards (3- $\alpha$ -cholestenol) from Sigma-Aldrich, Supelco (Bellefonte, PA, USA).

#### *Oil Samples*

Olive oil sample was obtained from Kilis Yağlık (KY) variety got from Kilis province which is a natural geographical region of Southern Anatolian Region of Turkey in 2017-2018 harvest year. The olive pomace oil used as adulterant were obtained from the local producer in Gaziantep province.

Adulterated oils were prepared by mixing different proportions (1, 5 and 10 % v/v) of olive pomace oil to virgin olive oil. These proportions were prepared as follow; for ratios of 1%, 5% and 10% were used 99 virgin olive oil:1 ml olive pomace oil, 95 virgin olive oil:5 ml olive pomace oil and 90 virgin olive oil:10 ml olive pomace oil, respectively. Oil samples were abbreviated as follows; Virgin olive oil of Kilis Yağlık cultivar: KY, olive pomace oil: OPO.

#### *Analysis of Fatty Acids (FA)*

The FA methyl esters of oil samples were analyzed by capillary column gas chromatography equipped with split injection (1:50) and a flame ionization detector (FID) (Agilent 7890 A, CA, USA). Fatty acid methyl esters were isolated by cold trans methylation method (IOOC, 2001a). Separation was executed by a capillary column that had a 60 m length, 0.25 mm I.D. and 0.20  $\mu$ m film thickness (HP-88, Agilent Inc., USA) for isolation of cis-trans fatty acid methyl esters (FAMES). To obtain methyl esters, 0.1 g of oil sample was weighed in a 5 ml screw-top test tube and added 0.2 ml of 2 N methanolic potassium hydroxide solution. It was capped by fitted with a PTFE joint, tightened, and shaken vigorously for 30 sec. Clear upper solution was decanted and upper phase containing methyl esters was injected to GC (1  $\mu$ l of injection volume). Hydrogen was used as a carrier gas. Detector and

injector temperatures were calibrated to 280 and 250°C, respectively. Oven temperature was adjusted at 165°C for 15 min, then raising the temperature by 5°C min<sup>-1</sup> to 200°C, then fixed at this temperature for 15 min (IOOC, 2001b). The qualification of specific FAs (oleic, palmitic, palmitoleic, myristic, margoric, stearic, linoleic, arashidic, margoleic, gadoleic, linolenic, lignoceric and behenic acids) was characterized from their retention times of known standards and quantified by determination of the area under the about the topic peak based on the sum of the areas under all peaks of fatty acids. Findings were finally expressed as a percentage of the total. Fatty acid analysis was performed in triplicate.

#### *Determination of Delta ECN42 Values*

International olive oil council method (IOOC, 2017a) was used to detect TAGs with equivalent carbon number 42 as experimentally and theoretically. Delta ECN42 values were calculated using ECN42 values obtained from experimentally and theoretically results.

#### *TAGs with equivalent carbon number 42 as experimentally (ECN42<sub>HPLC</sub>)*

The 5 % sample solution is prepared by weighing 0.5 g of the oil into a 10 ml graduated flask and dissolved in 10 ml acetone. The analysis of ECN<sub>42HPLC</sub> was performed on a HPLC equipped with a Refractive Index Detectors (RID) (Agilent 1200, CA, USA). A Lichrospher 18-250AF column (250x4.0 mm from Merck, Darmstadt, Germany) was used to separation. Separation conditions were as follows: mobile phase was acetone/acetonitrile mixture with ratio of 636:364 and temperature of column, 35 °C; flow rate of 1.0 ml min<sup>-1</sup>; and 10  $\mu$ l injection volume of samples. At the end of the analysis, the percent areas of the three peaks determined according to the peak order in the chromatogram were summed and specified as value of ECN<sub>42HPLC</sub>.

#### *TAGs with equivalent carbon number 42 as theoretically (ECN42<sub>theoretical</sub>)*

This value was calculated by using fatty acid profile. But, at the end of the analysis, only the

percent peak areas of the fatty acids listed below (Table 1) were included in the calculation of this value. Calculation details are also explained in the international olive oil council method (IOOC, 2017a).

Table 1. Fatty acids used in ECN<sub>42</sub><sup>theoretical</sup> calculations  
Çizelge 1. ECN<sub>42</sub><sup>theoretical</sup> hesaplamalarında kullanılan yağ asitleri

Fatty acid (FA) Yağ asidi (YA)	Abbreviation Kısa Yazılışı	Molecular weight Molekül ağırlığı	ECN EKS
Palmitic acid	P	256.4	16
Palmitoleic acid	Po	254.4	14
Stearic acid	S	284.5	18
Oleic acid	O	282.5	16
Linoleic acid	L	280.4	14
Linolenic	Ln	278.4	12

### Calculation of Delta ECN42 ( $\Delta$ ECN42) Values

Delta ECN 42 values were calculated by subtracting experimental and theoretical ECN 42 values from each other.

### Analysis of Sterol Composition

Sterol composition was determined by method of International Olive Oil Council (IOOC, 2017b). The internal standard ( $\alpha$ -cholestanol, 1 ml) was added into pure olive oil samples (KY) and adulterated oils (KY+OPO). Then, mixtures were saponified by potassium hydroxide solution with ethanol (50 ml, 2 N) using the reflux condenser, and then extracted with diethyl ether. The thin-layer chromatography on a basic silica gel plate was used to separate sterols and triterpene dialcohols fraction from the unsaponifiable matter. The 2,7-dichlorofluorescein in 0.2 % ethanolic solution was sprayed onto the sterol band, scraped off with a spatula and extracted with chloroform, visualized under UV light. The sterol and diol fractions collected from the silica gel is converted into trimethylsilyl ethers by the addition of silylation reagent mixture of pyridine/hexamethyldisilazane/trimethylchlorosilane (9:3:1, v/v/v) left for 15 min, and then centrifuged. The sterol analysis was conducted on a gas chromatography coupled with a flame ionization detector (Agilent 7890 A, CA, USA).

Separation of the sterols was carried out using capillary column with stationary phase SE-54 ((5 % -Phenyl)(1 % -Vinyl)-methylpolysiloxane-30 m length  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness) from Agilent (J&W Sci., Santa Clara, USA). The conditions in GC were as follows: Helium was used as a carrier gas with a flow rate of 1.2 ml min<sup>-1</sup>; inlet temperature, 280 °C; detector temperature, 290 °C; oven temperature, 260 °C held for 45 min. Identification of sterols was completed by comparing the retention times of peaks with those of the standards. The result of each sterol was expressed as percent concentration and total amount was stated as ppm.

At the same time, Mariani Ratio ( $R_{MAR}$ ) (Equation 1) as follows was used to detect the adulteration of some oils to olive oils. This value was observed lower or equal to 1 for pure olive oils (Mariani et al., 1999).

$$R_{MAR} = \% \text{ campesterol} \times \frac{(\% \text{ 7 stigmastenol})^2}{\% \text{ 7 avenasterol}} \quad (\text{Eq. 1})$$

### Statistical Analysis

The results were specified as the average of three replication and standard deviation. All findings were subjected to variance analysis using SPSS 21 software package and also Duncan's multiple comparison test was applied to determine significant differences at 0.05 level ( $p < 0.05$ ). Principal component analysis (PCA) was also applied using XLStat-Premium (2018) for Windows (Addinsoft, NY, USA).

## **Results and Discussion**

### Results of Fatty Acid Profile

Table 2 indicates the fatty acid profile of pure KY oil and its adulterations created by mixing 1, 5 and 10 % (v/v) quantities of olive pomace oil. As shown in Table 2, the ratios of fatty acids located in the first three ranks of KY oil were 74.47, 12.87, and 5.86 % for oleic, palmitic, and linoleic acids, respectively. Due to the results, the percentage of oleic acid was decreased when OPO were added.

As in oleic acid, palmitic acid was also decreased after 5 % mixing of OPO (12.47 and 12.62 % for adulterated oils with 5 and 10 % ratios). Linoleic acid and stearic acid were increased when OPO was added. While the ratio of miristic and trans linoleic acids did not show any change, the ratio of trans linolenic acid was increased when OPO was mixed. According to the fatty acid profile of adulterated oils, the ratio of all acids was in the range of official limits (IOOC, 2016). Besides, all acid ratios between oil samples did not found different as statistically ( $P < 0.05$ ). Based on these results, it can be said that fatty acids alone are not satisfactory to detect adulteration. In previous studies, adulteration was investigated related to various oils. Jabeur et al. (2014) was made an effort to detect adulteration of olive oil

with 1–10 % proportions of soybean, corn, and sunflower oils. They pointed out that the results of fatty acids did not give sufficient information owing to the levels of adulteration. In another study, the composition of fatty acids of camellia oil by mixing corn, sunflower and canola oil at different ratios was examined. They stated that pure and adulterated oils were chemometrically different due to profile of fatty acids (Shi et al., 2018). Fatty acids of pure sesame oil and adulterated with hazelnut, canola and sunflower oils in different proportions (1-50 %) were examined by Ozulku et al. (2017). They compared fatty acids of pure and adulterated oils with chemometrics methods. They denoted that fatty acid composition could be used for the detection of sesame oil adulteration.

Table 2. Fatty acid composition of pure and adulterated oils (%)  
*Çizelge 2. Saf ve tağışlı yağların yağ asitleri bileşimi (%)*

Fatty acids <i>Yağ asitleri</i>	Official Limit <sup>b</sup> , <i>Resmi limit,</i> %	Fatty acids percentage (%) <sup>a</sup> <i>Yağ asitleri yüzdesi</i>				
		Adulterated oils <i>Tağışlı yağlar</i>				
		KY	KY+1% OPO	KY+5% OPO	KY+10% OPO	
1	Miristic acid (C14:0)	≤0.03	0.01	0.01	0.01	0.01
2	Palmitic acid (C16:0)	7.5-20.0	12.87	12.87	12.47	12.62
3	Palmitoleic acid (C16:1)	0.3-3.5	1.21	1.20	1.14	1.15
4	Margaric acid (C17:0)	≤0.4	0.17	0.21	0.20	0.19
5	Margoleic acid (C17:1)	≤0.6	0.27	0.27	0.26	0.25
6	Stearic acid (C18:0)	0.5-5.0	3.53	3.57	3.71	3.67
7	Trans oleic acid (C18:1T)		0.01	0.04	0.03	0.02
8	Oleic acid (C18:1)	55.0-83.0	74.47	74.13	74.31	74.15
9	Trans linoleic acid (C18:2T)		0.02	0.02	0.02	0.02
10	Linoleic acid (C18:2)	2.5-21.0	5.86	5.85	5.93	6.04
11	Arachidic acid (C20:0)	≤0.6	0.49	0.50	0.54	0.53
12	Trans Linolenic acid (C18:3T)		0.67	0.88	0.88	0.85
13	Linolenic acid (C18:3)	≤1.0	0.25	0.26	0.28	0.28
14	Gadoleic acid (C20:1)	≤0.5	0.12	0.13	0.14	0.14
15	Behenic acid (C22:0)	≤0.2	0.07	0.07	0.08	0.07
16	Lignoseriac acid (C24:0)	≤0.2	0.11	0.10	0.08	0.09

<sup>a</sup>Results are the means of three replications as percentage. <sup>b</sup>Official limits of extra virgin olive oil (IOOC, 2016). Values are not significant statistically ( $p < 0.05$ )

The pure and adulterated oils was subjected to principal component analysis to find the difference as chemometrically. Sixteen fatty acids quantified were identified by two principal factors (F1 x F2). The scores of the oil samples and the

loadings of the variables and observations on the two principal components are plotted in Figure 1. The first two principal components, explained as 86.23 % of the total variance (66.05 % and 20.19 % for F1 and F2, respectively). As seen in score

plot, pure oil was clearly separated from the adulterated oils. While oils adulterated with the

ratios of 5 and 10 % were in the same group, others settled in different groups.

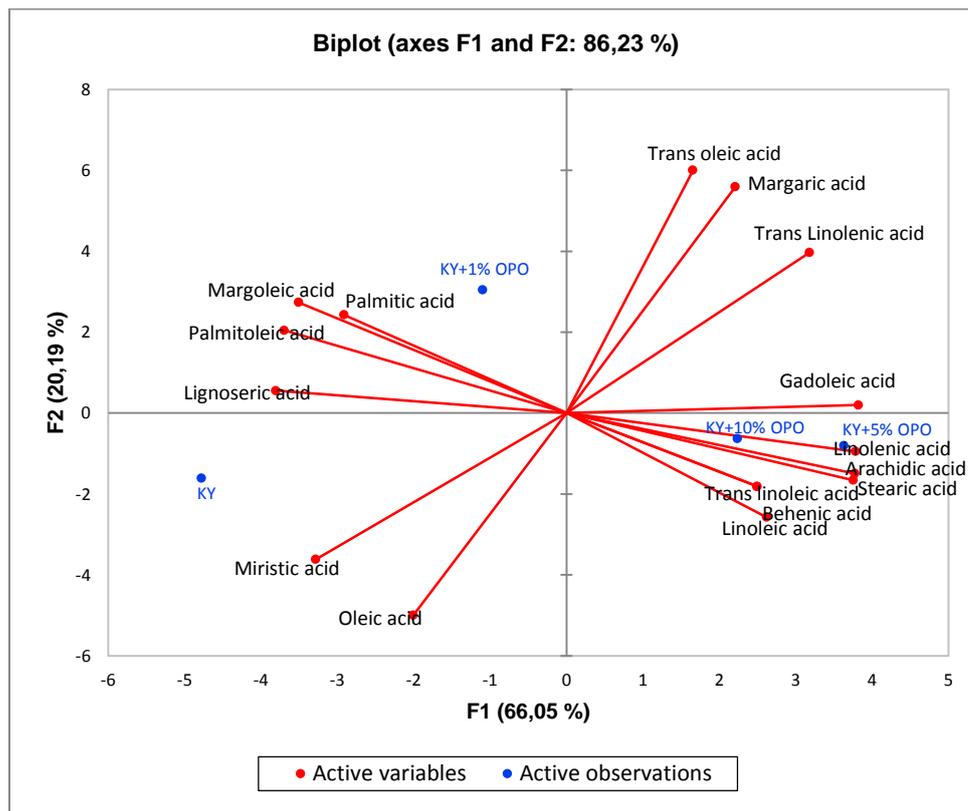


Figure 1. Projection of the variables and observations on the factor plane (F1  $\times$  F2) according to fatty acid profile

Şekil 1. Yağ asidi profiline göre faktör düzleminde (F1  $\times$  F2) gözlemler ve değişkenlerin yansımaları

### Results of Delta ECN42 Values

The TAGs with equivalent carbon number 42, theoretical and experimental ECN42, and  $\Delta$ ECN42 values were given in Table 3. As shown in table, the difference of theoretical and experimental ECN42 values ( $\Delta$ ECN42) in adulterated oils was higher than that in the pure KY olive oil. This value is 0.04 in pure olive oil, while it is 0.12, 0.13, and 0.11 in oils adulterated with 1 %, 5 %, and 10 % OPO, respectively. According to official standards, this value should not be greater than 0.2 for virgin olive oil. As a result, it can be said that when OPO was added to olive oil, the adulteration could not be detected by the  $\Delta$ ECN42 value. However, only  $\Delta$ ECN42 value of pure KY oil was found different as statistically ( $P < 0.05$ ) from adulterated oils.

In similar study, adulterations of extra virgin olive oil with sunflower, soybean and olive pomace oils using  $\Delta$ ECN42 values was determined by Continas et al. (2008). They clearly differentiated the olive oils adulterated with 1 %, 2 % and 10 % sunflower oil, soybean oil and

refined olive pomace oil, respectively, by applying discriminant analysis techniques using  $\Delta$ ECN42 values. Christopoulou et al., (2004), the  $\Delta$ ECN42 values were used to detect the olive oil adulteration with the other vegetable oils. They found that the values of  $\Delta$ ECN42 were effective in detecting the adulteration of olive oils even at very low levels of vegetable oils.

PCA was applied to determine the differentiation of pure and adulterated oils by considering TAGs profile, ECN42, and  $\Delta$ ECN42 values. The scores of observations and variables are plotted in Fig. 2. The variables were selected for the PCA and the first two principal components were explained as 94.16 % of the total variance (62.43 % and 31.73 % for F1 and F2, respectively). As seen in score plot, pure oil was clearly separated from the adulterated oils. The pure and adulterated oils were separated clearly in three different groups. While oils adulterated with the ratios of 1 and 5 % were in the same group, others settled in different groups.

Table 3. Triglyceride compositions and  $\Delta$ ECN42 values of pure and adulterated olive oils  
 Çizelge 3. Saf ve tağışli yağların trigliserid kompozisyonları ve  $\Delta$ ECN42 değerleri

Theoretical composition of TAGs with ECN42 EKS42 olan yağ asitlerinin teorik kompozisyonu				
TAGs (ECN42) Triaçilgliseroller (EKS42)	KY	KY+1% OPO	KY+5% OPO	KY+10% OPO
LLL	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>
PoLL	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>
PoPoL	0.00	0.00	0.00	0.00
PoPoPo	0.00	0.00	0.00	0.00
OLnL	0.17 <sup>a</sup>	0.23 <sup>a</sup>	0.23 <sup>a</sup>	0.23 <sup>a</sup>
PoOLn	0.04 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>
PLnL	0.04 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.05 <sup>a</sup>
PPoLn	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>	0.01 <sup>a</sup>
SLnLn	0.00	0.00	0.00	0.00
ECN42 <sub>theoretical</sub>	0.29	0.38	0.38	0.37
Experimental composition of TAGs with ECN42 EKS42 olan yağ asitlerinin deneysel kompozisyonu				
	KY	KY+1% OPO	KY+5% OPO	KY+10% OPO
LLL+PoLL	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.06 <sup>a</sup>	0.08 <sup>a</sup>
OLLn+PoOLn	0.17 <sup>a</sup>	0.16 <sup>a</sup>	0.15 <sup>a</sup>	0.15 <sup>a</sup>
PLLn	0.04 <sup>a</sup>	0.05 <sup>b</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>
ECN42 <sub>experimental</sub>	0.25 <sup>a</sup>	0.25 <sup>a</sup>	0.24 <sup>a</sup>	0.26 <sup>a</sup>
Difference ECN42 EKS42 farkı ( $\Delta$ ECN42)	0.04 <sup>a</sup>	0.12 <sup>b</sup>	0.13 <sup>b</sup>	0.11 <sup>b</sup>

Results are the means of three replications as percentage. Values with different letters in the same row are significant statistically ( $p < 0.05$ )

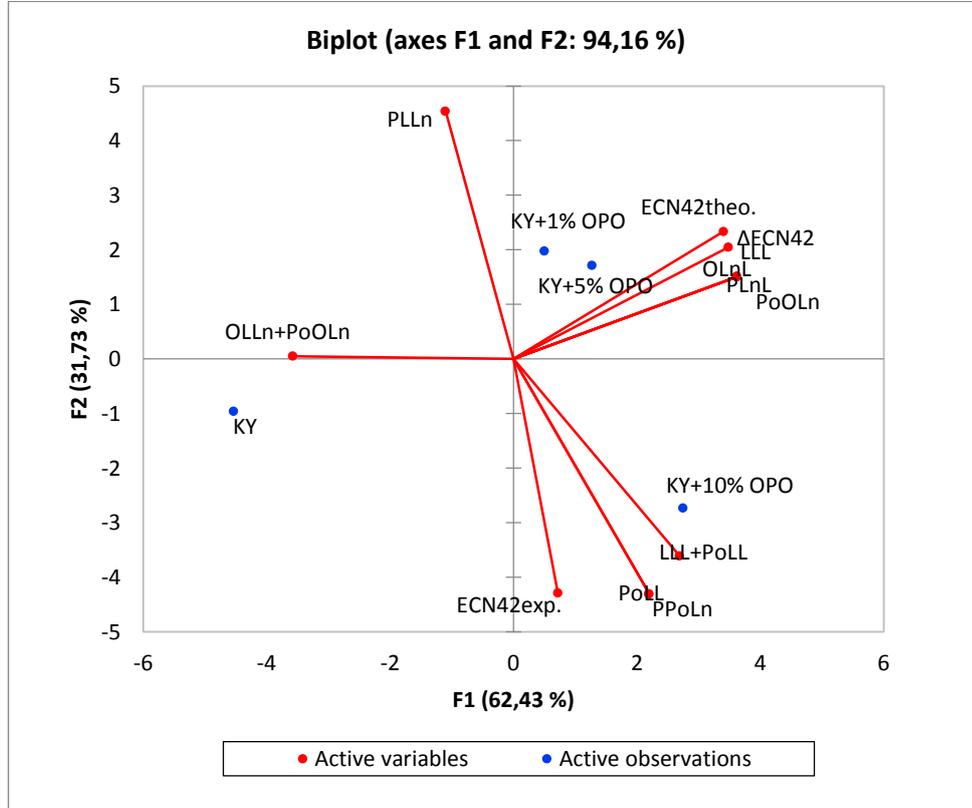


Figure 2. Projection of the variables and observations on the factor plane (F1  $\times$  F2) according to TAG profile and ECN42 values

Şekil 2. Triaçilgliserol profili ve EKS42 değerlerine göre faktör düzleminde (F1  $\times$  F2) gözlemler ve değişkenlerin yansımaları

### Results of Sterol Profile

The sterol profiles of pure oil and adulterated oils were shown in Table 4. According to results, the percent of some sterol compounds increased or some of decreased when OPO was mixed. It has been found that the amount of some sterols were not in the range of official limit. The amount brassicasterol should be below 0.1 % according to the IOOC limits. In pure Kilis yaglik olive oil, the ratio of this compound was 0.02 %. However, in olive oil adulterated with OPO, this value was 0.17 for adulterated oil with 5 % OPO and 0.22 for adulterated oil with 10 % OPO. It can be seen that the percentage of beta-sitosterols decreased to lower limit (93 %) when mixed with 5 % and 10 % olive pomace oil. On the other hand,  $\Delta$ -7-Stigmastenol was also increased when

KY oil was adulterated. It was seen that if the rate of mixing of the olive pomace oil increased, its rates exceeded the upper limit. Another important finding was observed in erythrodiol+uvaol compound. Due to official limit its content should be less than or equal to 4.5 %. However, its percentage increased to 6.40 for adulterated oil with 5 % OPO and 10.14 for adulterated oil with 10 % OPO. According to results of statistical analysis, there were significant difference in some compounds between pure and adulterated oils. For example, brassicasterol,  $\Delta$ -7-campesterol, erythrodiol+uvaol compounds and esterified sterol fraction (Rmar) value and total sterol content showed statistically differences ( $p < 0.05$ )

Table 4. Sterol composition of pure and adulterated oils (%)  
Çizelge 4. Saf ve tağışışli yağların sterol kompozisyonu

Sterols Steroller	Official Limit <sup>b</sup> Resmi limit, %	Sterol percentage (%) <sup>a</sup> Sterol yüzdesi			
		KY	KY+1% OPO	KY+5% OPO	KY+10% OPO
1 Cholesterol	≤0.5	0.62 <sup>a</sup>	0.66 <sup>a</sup>	0.66 <sup>a</sup>	0.53 <sup>a</sup>
2 Brassicasterol	≤0.1	0.02 <sup>a</sup>	0.04 <sup>a</sup>	0.17 <sup>b</sup>	0.22 <sup>b</sup>
3 24-Methylen cholesterol		0.09 <sup>a</sup>	0.09 <sup>a</sup>	0.12 <sup>a</sup>	0.08 <sup>a</sup>
4 Campesterol	≤4.0	1.90 <sup>a</sup>	1.92 <sup>a</sup>	2.04 <sup>a</sup>	2.11 <sup>a</sup>
5 Campestanol		0.08 <sup>a</sup>	0.09 <sup>a</sup>	0.10 <sup>a</sup>	0.11 <sup>a</sup>
6 Stigmasterol	<campe	1.39 <sup>a</sup>	1.41 <sup>a</sup>	1.69 <sup>a</sup>	1.76 <sup>a</sup>
7 $\Delta$ -7-Campesterol		0.04 <sup>a</sup>	0.08 <sup>a</sup>	0.17 <sup>b</sup>	0.19 <sup>b</sup>
8 Beta-sitosterols	≥93	94.41 <sup>a</sup>	94.15 <sup>a</sup>	93.56 <sup>a</sup>	93.59 <sup>a</sup>
$\Delta$ -5.23-Stigmastadienol		0.10 <sup>a</sup>	0.14 <sup>a</sup>	0.11 <sup>a</sup>	0.10 <sup>a</sup>
Clerosterol		0.98 <sup>a</sup>	0.90 <sup>a</sup>	0.83 <sup>a</sup>	0.85 <sup>a</sup>
Beta-sitosterol		79.10 <sup>a</sup>	79.03 <sup>a</sup>	80.25 <sup>a</sup>	81.42 <sup>a</sup>
Sitostanol		1.53 <sup>a</sup>	1.62 <sup>a</sup>	1.72 <sup>a</sup>	1.95 <sup>a</sup>
$\Delta$ -5-Avenasterol		12.15 <sup>a</sup>	11.88 <sup>a</sup>	10.11 <sup>a</sup>	8.74 <sup>a</sup>
$\Delta$ -5.24-Stigmastadienol		0.56 <sup>a</sup>	0.59 <sup>a</sup>	0.53 <sup>a</sup>	0.53 <sup>a</sup>
9 $\Delta$ -7-Stigmastenol	≤0.5	0.39 <sup>a</sup>	0.46 <sup>a</sup>	0.53 <sup>a</sup>	0.53 <sup>a</sup>
10 $\Delta$ -7-Avenasterol		1.05 <sup>a</sup>	1.10 <sup>a</sup>	0.96 <sup>a</sup>	0.89 <sup>a</sup>
11 Erythrodiol+Uvaol	≤4.5	2.65 <sup>a</sup>	3.68 <sup>a</sup>	6.40 <sup>b</sup>	10.14 <sup>c</sup>
12 Esterified sterol fraction (Rmar)		0.27 <sup>a</sup>	0.38 <sup>a</sup>	0.60 <sup>b</sup>	0.66 <sup>c</sup>
13 Total sterol (ppm)	≥1000	1449.38 <sup>a</sup>	1545.60 <sup>ab</sup>	1824.32 <sup>ab</sup>	2202.77 <sup>b</sup>

<sup>a</sup>Results are the means of three repetitions as %. Values with different letters in the same row are significant statistically ( $p < 0.05$ )

<sup>b</sup>International Olive Oil Council, 08/11/2001, COI/T.15/NC.no. 2/Rev. 10 Trade Standard Applying to Olive Oil and Olive-Pomace Oil.

In previous studies, sterol profile was used to detect adulteration. Jabeur et al. (2014) investigated sterol composition by mixing soya, corn and sunflower oils at various rates (1, 2, 3, 4, 5, and 10 %) to extra virgin olive oil. They found that the amounts of campesterol and  $\Delta 7$ -stigmastenol in olive oil mixed with 10 % soybean oil increased above the limit values ( $6.17 > 4.0$  % and  $0.59 > 0.5$  %) and the amount of  $\beta$ -sitosterol decreased ( $89.21 < 93$  %). They said that the sterol profile is almost determinative in declaring the adulteration of olive oils with other vegetable oils: 1 % of sunflower oil could be detected by the rising of  $\Delta 7$ -stigmastenol and 4 % of corn oil by the enhancing of campesterol.

Another important finding that gives information about the adulteration was the  $R_{mar}$  values. In previous studies, it was stated that  $R_{mar}$  is not more than 1 for non-adulterated olive oil, (Mariani et al., 1999; Azadmard-Damirchi, 2010). Considering the  $R_{mar}$  of the oil samples, the pure olive oil and adulterated oils displayed a value lower than 1. However, this value increased as the rate of addition of OPO increased. Consequently, it can be said that when higher amounts of olive pomace oil are mixed, this value could be used for detection of adulteration.

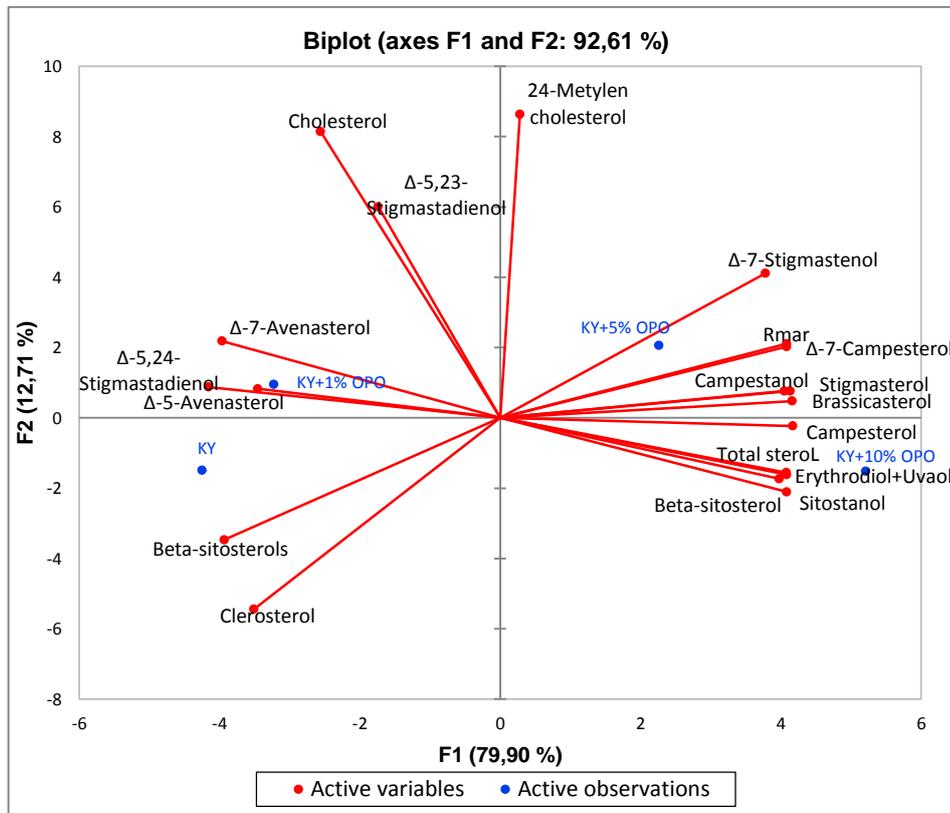


Figure 3. Projection of the variables and observations on the factor plane (F1  $\times$  F2) according to sterol profile.

Şekil 3. Sterol profiline göre faktör düzleminde (F1  $\times$  F2) gözlemler ve değişkenlerin yansımaları

PCA biplot including variables and observations of pure and adulterated olive oils according to sterols are illustrated in Figure 3. As can be seen in figure, oil samples demonstrated four separate groups. The total variance was reported as 92.61 % (F1: 79.90 %; F2: 12.71 %). The score plot in the PCA analysis showed that KY oil was clearly separated from the adulterated oils due to content of beta-sitosterols and clerosterol. Total sterol content was effective on discrimination of

adulterated oils with 10 % OPO. Considering the value of  $R_{mar}$ , it was determined that the oils mixed with 5 % and 10% OPO were distinctive.

## Conclusion

Olive oil is one of the most food products exposed to adulteration in the world due to its relatively low production and high cost compared to other vegetable oils. Therefore, identification

of adulteration has been an important issue in recent years. This research was focused on detection of olive oil adulteration with olive pomace oil by using fatty acid profile,  $\Delta$ ECN42 values and sterol composition. When olive oil is mixed with other vegetable oils or pomace oils, these profiles changes. According to the our results, it can be said that fatty acids and  $\Delta$ ECN42 values are not very effective in detecting adulteration, but sterols can be used to detect adulteration of olive oil with olive pomace oil. On the other hand, PCA analysis gave good separation of pure and adulterated oils in different groups.

## References

- Azadmard-Damirchi, S. (2010). Review of the use of phytosterols as a detection tool for adulteration of olive oil with hazelnut oil. *Food Additives & Contaminants*, 27(1), 1–10.
- Christopoulou, E., Lazaraki, M., Komaitis, M., & Kaselimis, K. (2004). Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. *Food Chemistry*, 84(3), 463–474.
- Continas, A., Martinez, S., Carballo, J., & Franco, I. (2008). Detection of contaminations and/or adulterations of the extra virgin olive oil with seeds oils (sunflower and soybean) and olive pomace oil. *Grasas y Aceites*, 59(2), 97–103.
- IOOC, International Olive Oil Council, method of analysis, preparation of the fatty acid methyl esters from olive oil and olive pomace oil, COI/T.20/Doc.no. 24. (2001a). <http://www.internationaloliveoil.org>.
- IOOC, International Olive Oil Council, method of analysis, determination of the *trans* unsaturated fatty acids by capillary column gas chromatography, COI/T.20/Doc.no. 17. (2001b). <http://www.internationaloliveoil.org>
- IOOC, International Olive Oil Council, trade standard applying to olive oil and olive pomace oil, COI/T.15/NC No 3/Rev. 11. (2016). <http://www.internationaloliveoil.org>.
- IOOC, International Olive Oil Council, method of analysis, determination of the difference between actual and theoretical content of triacylglycerols with ECN 42, COI/T.20/Doc. No 20 /Rev. 4. (2017a) <http://www.internationaloliveoil.org>
- IOOC, International Olive Oil Council, method of analysis, determination of the composition and content of sterols and triterpene dialcohols by capillary column gas chromatography, COI/T.20/Doc. No 30/Rev. 2. (2017b). <http://www.internationaloliveoil.org>
- Jabeur, H., Zribi, A., Makni, J., Rebai, A., Abdelhedi, R., & Bouaziz, M. (2014). Detection of Chemlali extra-virgin olive oil adulteration mixed with soybean oil, corn oil, and sunflower oil by using GC and HPLC. *Journal of Agricultural and Food Chemistry*, 62(21), 4893–4904.
- Jha, S.N., Jaiswal, P., Grewal, M.K., Gupta, M., & Bhardwaj, R. (2016). Detection of adulterants and contaminants in liquid foods—A Review. *Critical Reviews in Food Science and Nutrition*, 56(10) 1662–1684.
- Jimenez-Carvelo, A.M., Osorio, M.T., Koidis, A., Gonzalez-Casado, A., & Cuadros-Rodríguez, L. (2017). Chemometric classification and quantification of olive oil in blends with any edible vegetable oils using FTIR-ATR and Raman spectroscopy. *LWT - Food Science and Technology*, 86, 174–184.
- Kelebek, H., Kesen, S., & Selli, S. (2014). Comparative study of bioactive constituents in Turkish olive oils by LC-ESI/MS/MS. *International Journal of Food Properties*, 18(10), 2231–2245.
- Li, Y., Fang, T., Zhu, S., Huang, F., Chen, Z., & Wang, Y. (2018). Detection of olive oil adulteration with waste cooking oil via Raman spectroscopy combined with iPLS and SiPLS. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, 189, 37–43.
- Mariani, C., Bellan, G., Morchio, G., & Pellegrino, A. (1999). Free and esterified minor components of olive and hazelnut oils: Their potential utilisation in checking oil blend. *Rivista Italiana Delle Sostanze Grasse*, 76, 297–305.
- Meras, I.D., Manzano, J.D., Rodriguez, D.A., & De la Pena, A.M. (2018). Detection and quantification of extra virgin olive oil adulteration by means of autofluorescence excitation-emission profiles combined with multi-way classification. *Talanta*, 178, 751–762.
- Ozulku, G., Yildirim, R.M., Toker, O.S., Karasu, S., & Durak, M.Z. (2017). Rapid detection of adulteration of cold pressed sesame oil adulterated with hazelnut, canola, and sunflower oils using ATR-FTIR spectroscopy combined with chemometric. *Food Control*, 82, 212–216.
- Shi, T., Zhu, M.T., Chen, Y., Yan, X.L., Chen, Q., Wu, X.L., Lin, J., & Xie, M. (2018).  $^1\text{H}$  NMR combined with chemometrics for the rapid detection of adulteration in camellia oils. *Food Chemistry*, 242, 308–315.
- Tsopelas, F., Konstantopoulos, D., & Kakoulidou, A.T. (2018). Voltammetric fingerprinting of oils and its combination with chemometrics for the detection of extra virgin olive oil adulteration. *Analytica Chimica Acta*, 1015, 8–19.
- Türkoğlu, H., Kanik, Z., Yakut, A., Güneri, A., & Akın, M. (2012). Nizip ve çevresinde satışı sunulan zeytinyağı örneklerinin bazı özellikleri. *Harran Tarım ve Gıda Bilimleri Dergisi*, 16(3), 1–8.