GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research/Araştırma
GIDA (2023) 48 (3) 510-525
doi: 10.15237/gida.GD23024

COMPARATIVE INVESTIGATION OF THE USE OF STEROL COMPOSITION, ECN42 DIFFERENCE AND FTIR SPECTROSCOPY IN THE DETERMINATION OF VIRGIN OLIVE OIL ADULTERATION

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Received /Geliş: 09.02.2023; *Accepted* / Kabul: 13.03.2023; *Published online* / Online baskı: 17.04.2023

Karacan, E., Özdikicierler, O., Yemişçioğlu, F. (2023). Comparative investigation of the use of Sterol composition, ECN42 difference and FTIR spectroscopy in the determination of virgin olive oil adulteration. GIDA (2023) 48 (3) 510-525 doi: 10.15237/ gida.GD23024

Karacan, E., Özdikicierler, O., Yemişçioğlu, F. (2023). Natürel zeytinyağı tağşişinin tespitinde sterol kompozisyonu, ECN42 farkı ve FTIR spektroskopisinin kullanımının karşılaştırmalı olarak incelenmesi. GIDA (2023) 48 (3) 510-525 doi: 10.15237/ gida.GD23024

ABSTRACT
Due to its high price and consumer demand, virgin olive oil is an essential product vulnerable to deception for unfair economic gain. In our research, sunflower, palm olein and cottonseed oil were used as adulterants in different amounts for the preparation of adulterated samples. Sterol composition, the difference in theoretical and actual equivalent carbon number 42 triglycerides (ΔECN42) value and FTIR spectra were used to classify the adulterated and virgin olive oil samples. Decision trees on Δ7-stigmastenol and campesterol allowed the detection of 10% and higher adulteration. ΔECN42 ad FTIR provided good detection of the adulterated samples, even for the mixed oils at 1% concentration. However, the detecting performance of the FTIR decreased as the virgin olive oil sample set expanded with different seasons and varieties. Correct multivariate approach and FTIR data selection significantly influence the performance of FTIR spectroscopy for detecting VOO adulteration.
Keywords: Virgin olive oil, adulteration, fatty acid composition, sterol composition, ΔECN42, FTIR, LDA

NATÜREL ZEYTİNYAĞI TAĞŞİŞİNİN TESPİTİNDE STEROL KOMPOZİSYONU, ECN42 FARKI VE FTIR SPEKTROSKOPİSİNİN KULLANIMININ KARŞILAŞTIRMALI OLARAK İNCELENMESİ

ÖZ
Natürel zeytinyağı, yüksek tüketici talebi ve fiyatı nedeniyle haksız ekonomik kazanç sağlamak için yapılan hileli uygulamalara açık bir üründür. Araştırmamızda ayçiçek yağı, palm olein ve pamuk yağı natürel zeytinyağına farklı oranlarda karıştırılmıştır. Hazırlanan karışım yağların natürel zeytinyağından ayrımının incelenmesi için örneklerin sterol kompozisyonu, ΔECN42 değeri ve FTIR spektrumları kullanılmış ve sonuçlar karşılaştırılmıştır. Mevzuatta belirtilen Δ7-stigmastenol ve kampesterol üzerindeki karar ağaçları, %10 ve daha yüksek tağşişin saptanmasını mümkün kılmıştır. ΔECN42 ve FTIR spektroskopisi ile %1 konsantrasyonda hazırlanan karışım yağlar bile, natürel

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zeytinyağı numunelerinden ayrılmıştır. Ancak, natürel zeytinyağı numune sayısı, farklı mevsimler ve çeşitlerle genişletildiğinde, FTIR spektroskopisi ile gerçekleştirilen ayrımın hassasiyeti azalmış ve özellikle düşük konsantrasyonlu karışım yağların ayrımı zorlaşmıştır. Doğru çok değişkenli yaklaşım ve FTIR veri seçimi, natürel zeytinyağı tağşişini saptamak için FTIR spektroskopisinin performansını önemli ölçüde etkilemektedir.
Anahtar kelimeler: Natürel zeytinyağı, tağşiş, yağ asidi kompozisyonu, sterol kompozisyonu, ΔECN42, FTIR, LDA

INTRODUCTION

Olive oil is a product having high economic importance for Mediterranean countries, obtained from the fruit of the olive tree (*Olea europaea* L.). Considering the costs of cultivation, production and storage periods, extra virgin olive oil, which is the highest quality class, is expected to have a higher price than virgin and lampante olive oils (Balkan and Meral, 2017; Anonymous, 2022; Filoda et al., 2019; Tsimidou et al., 2015; Uncu et al., 2017). In addition, consumer demand for extra virgin olive oil is increasing due to its unique flavor and high amount of natural antioxidants (Mariotti and Peri, 2014; Vitaglione et al., 2015). Because of its high price and increasing demand, extra virgin olive oil has become most vulnerable to fraudulent activities (Pan et al., 2018; Uncu et al., 2017). These fraudulent activities, generally called adulteration, are practiced to obtain unfair economic benefits by misleading the consumer about the origin, quality class or product content of virgin olive oil (VOO) by presenting false information on the label. The most common form of adulteration for VOO is mixing different vegetable oil with lower economic value into VOO (Tsimidou et al., 2015).

European Union legislation and Codex Alimentarius criteria set the VOO quality and purity limits to determine the quality classes and present the right product to be audited to the consumer (Anonymous, 2017; Anonymous, 2022). In particular, sterol composition and the difference between contents of theoretical and actual triglycerides having 42 equivalent carbon numbers (ECN) are the most examined purity criteria for adulterations made by mixing seed oils with VOO. On the other hand, sterol composition and ΔECN42 techniques have many disadvantages, such as requiring labor and chemicals and generating a significant amount of chemical waste in the analysis process. Applying rapid measurement techniques coupled with advanced statistical methods stands out in terms of efficient labor and time use by overcoming sample loss and the excessive use of chemicals recently (Uncu et al., 2019).

Chemometrics was defined as a multidisciplinary assessment technique in which mathematics, statistics and computer science are integrated to determine the most influential variables from extensive data obtained from chemical analysis to apply statistical evaluation (Massart et al., 1998). In this respect, chemometric techniques include using multivariate analysis methods to evaluate analytical or spectroscopic results and examine the possible differences and classifications between sample groups. Principal component analysis (PCA), linear discriminant analysis (LDA), hierarchical clustering analysis (HCA), and partial least squares (PLS) are widely used for chemometric assessment of VOO adulteration (Esteki et al., 2018; Gómez-Caravaca et al., 2016).

Various fast-measurement techniques have been used to detect VOO adulteration or predict adulterant concentration. UV-Visible (UV-Vis) spectroscopy, Near Infrared Spectroscopy (NIR), Raman Fluorescence Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) method and Differential Scanning Calorimetry (DSC) are the most used techniques in VOO adulteration studies (Aroca-Santos et al., 2016; Chiavaro, 2014; Lia et al., 2018; Milanez et al., 2017; Nigri and Oumeddour, 2013; Ordoudi et al., 2022; Öztürk et al., 2010; Zhang et al., 2011). Studies were mainly carried out with model samples prepared by mixing one or more selected VOO and adulterant oil. In most studies, VOO / adulteration oil ratios are generally designed between 0% and 100% to efficiently determine the mathematical relations between the

concentration of the adulteration and the spectroscopic responses. However, detection becomes more difficult as the proportion of adulteration oil in VOO decreases (<10%), which makes detecting adulterations in lower concentrations the main challenge. In addition, most of the purity criteria specified in the legislation can quickly reveal VOO adulteration containing 10% or more vegetable oil (Baeten et al., 2005; Green et al., 2020). In that manner, the detection performance of the spectroscopic and chromatographic methods became more significant when VOO adulteration in low concentrations was considered. Few studies compare the commonly used spectroscopic methods with traditional chromatographic techniques in detecting VOO adulteration.

In this study, the detection performances of the sterol composition, ΔECN42 values and FTIR spectral data were compared for the prepared adulterated VOO samples using sunflower oil, palm olein and cottonseed oil at low ratios (1-4-7-10-13%). Moreover, change in the detection performance of FTIR was evaluated by expanding the database size of VOO with different varieties and regions.

MATERIAL AND METHOD

Material

Thirty geographically indicated (GI) VOO samples were obtained from related Chamber of Commerce bureaus representing the 2019-2020 and 2020-2021 harvest seasons (at least five samples from each season) as VOO samples. GI VOO samples were, namely, Ayvalık Zeytinyağı, Edremit Zeytinyağı and Milas Zeytinyağı, registered by Turkish Patent and Trademark Office with dossier numbers C2004/003, C2017/048 and C2014/043, respectively. Among these, only VOO samples that fit "extra virgin olive oil" criteria according to FFA, PV and specific absorption values were used to reduce the variation due to the quality criteria and obtain comparable results. Olive oil samples were abbreviated as VOO as an indication of the main category name of the olive oils.

To prepare mixtures (will be mentioned as "adulterated samples" in the text), three different vegetable oil samples (will be mentioned as "adulterants" in the text) were used. For this purpose, as adulterant samples, refined sunflower oil, cottonseed oil, and palm olein were obtained from various companies in İzmir, Türkiye.

Adulterated samples were prepared by mixing a selected VOO sample (Ayvalık Zeytinyağı from the 2019-2020 season) and adulterants with the binary combination. The adulterant ratios were maintained as 1-4-7-10-13% in adulterated samples for each adulterant. In this way, 15 adulterated samples were prepared separately for each adulterant.

Samples were coded representing the adulterant used and its percentage in the mixture. The adulterated samples containing sunflower oil, palm olein, and cottonseed oil were coded as SOVO, POVO, and CSVO, respectively. The adulterant concentration in the mixture was indicated as a percentage right after the sample code.

VOO, adulterant and adulterated samples were placed in 150 ml brown bottles without leaving any headspace and stored at -40°C until the analyzes were carried out.

The chemicals and standards used in the analyzes were obtained directly from the local distributors of Merck and Sigma-Aldrich brands at the purity level specified in the analysis methods.

Methods

Determination of sterol composition

The sterol composition of the samples was determined according to the Turkish Food Codex Communiqué on Olive Oil and Olive Pomace Oil Analysis Methods (Communiqué No: 2014/53), and the peak areas were expressed as a percentage. This method refers to the thin layer chromatography method for determining the sterol fraction of animal and vegetable oils and fats composition. Analysis was carried out by saponification of the sample, extraction of the unsaponifiable matter and separation of the

sterols using thin layer chromatography, and determining sterol composition by gas chromatography. For this purpose, 0.5 ml of 0.1% concentration 5α-cholestan-3β-ol internal standard was added to 2-2.5 grams of oil sample. This sample was subjected to saponification using 20 ml of 2 N ethanolic potassium hydroxide (KOH) solution in a water bath at 98°C for 20 minutes. The saponification reaction was terminated with 20 ml of distilled water, the unsaponifiable fraction extracted from the soap fraction with 20 ml of diethyl ether, and the residual soap was removed with approximately 80 ml of distilled water. This unsaponifiable fraction was fractionated on Silica gel 20×20 cm (Sigma Aldrich, Germany) Thin Layer Chromatography (TLC) plates and with 110 ml of hexane and 65 ml of diethyl ether for 70 minutes. The sterol band marked with 0.2% 2',7' dichlorofluorescein dye, and sterol fraction were scraped from the plate. The sterols were derived for gas chromatography (GC) with a mixture of N,O-Bis(trimethylsilyl) trifluoroacetamide reagents containing Pyridine and trimethylchlorosilane at 1:1 ratio. A SE-54 column (5%-phenyl-1%-vinylmethylpolysiloxane, 30 m × 0.32 mm × 0.25 μm) was used for chromatographic analysis. GC conditions were selected as follows; carrier gas: helium, the flow rate of carrier gas: 0.9 ml/min., flame ionization detector (FID) temperature: 290°C, injector temperature: 280°C, furnace temperature: 260℃ 1:40 split ratio.

Determination of fatty acid composition

The fatty acid composition of samples was determined according to the International Olive Council (IOC) standard method (Anonymous, 2017b). For this analysis, 0.1 grams of sample was mixed with 2 ml of heptane, and the mixture was transesterified with 0.2 ml of 2N methanolic KOH solution. The upper phase containing methyl esters was injected into GC (Agilent 7820). The GC oven temperature was maintained at an initial temperature of 165 °C and then gradually increased to 200 °C. The injection block temperature was set at 250°C and the FID detector temperature at 280°C. The flow rate of the carrier gas will be 1.2 ml/min. The injection volume was 2 μl. The peak areas were determined, and fatty acid composition was expressed as percentages.

Determination of the difference between the actual and theoretical content of triacylglycerols with ECN42 (∠ECN42)

Triglyceride profile analysis and determination of the actual content of triglycerides with ECN42 (ΔECN42) od adulterated samples was carried out with HPLC Agilent 1200 (California, USA) utilized with Refractive Index Detector (RID) using the method specified by the IOC (Anonymous, 2017c). For this analysis, 0.05 grams of oil sample was dissolved in 1 ml of acetone. The mobile phase was prepared to contain 63.6% acetone and 36.4% acetonitrile, filtered and degassed in an ultrasonic bath. Analysis was conducted with a mobile phase flow rate of 1.5 ml/min and an RI detector temperature of 40°C. TG rates are given in percent (%). The ΔECN42 values were calculated via the excel file provided under the so-called method appendices of IOC.

Determination of Fourier Transform Infrared Spectroscopy (FTIR) spectra

FTIR spectra of the samples were measured on the Shimadzu (Kyoto, Japan) IRAffinity-1S spectrometer system. The detector consists of a DLATGS detector with a temperature control mechanism and a beam splitter germanium-coated KBr. Two drops of oil samples were placed directly on the ATR cell, and spectra were taken in the mid-infrared region (4000 – 600 cm^{-1}) at 2 cm^{-1} resolution with 32 interferograms. Before each measurement, the atmospheric measurement was taken and subtracted from each spectrum. After each reading, the ATR crystal was cleaned with hexane and acetone to ensure no residue from the previous sample remained on the crystal surface.

All spectra were imported to the IR Solution v1.5 software, and some pre-processing procedures were followed to minimize uninformative distribution among samples. First, spectra were manipulated with ATR Correction at 650 cm^{-1} reference value. Then, 11-point smoothing was applied according to the Savintzky-Golay

algorithm, and 2nd-degree derivatization was carried out. The spectral regions over 650 cm^{-1} and below 3100 cm^{-1} were eliminated with the cut function, and a new data set was obtained.

Statistical analysis and chemometric method

Pre-processed FTIR data were imported into SPSS 26 (IBM, USA) and then classified according to the purity level as VOO, SOVO, POVO, and CSVO with percentages. For the second LDA, in addition to the VOO sample used in the preparation of the adulterated samples, five 2020-2021 Ayvalık, five 2019-2020 Edremit and five 2019-2020 Milas samples were defined as VOO, and the LDA data set was expanded. The expanded VOO sample set was labeled as VOO-EXT for the repeated LDA.

RESULTS AND DISCUSSION

Chemical characteristics of VOO, sunflower oil, cottonseed oil and palm olein samples

The fatty acid, triglyceride, and sterol compositions of the VOO, refined sunflower oil, cottonseed oil, and palm olein samples used to prepare adulterated samples are given in Table 1, Table 2 and Table 3. The quality criteria and compositional properties of all oil samples (VOO, refined sunflower oil, cottonseed oil and palm olein oil) included in this study met the purity criteria defined in the related Codex Alimentarious standards (Anonymous, 1999, 2017).

According to the fatty acid compositions in Table 1, the C14:0 value of palm olein had a higher level (1.10%) than the other vegetable oils in our study. Also, it was observed that palm olein and cottonseed oil had a higher percentage of C16:0 than the other two oils, 41.47% and 22.93%, respectively. In contrast, sunflower oil had the lowest content, with 6.19% of C16:0. Çalışlar et al. (2018) similarly stated that palm olein is a high source of palmitic acid (C16:0) (Çalışlar et al., 2018). It can be noted that the margaric acid (C17:0) values are similar in all pure vegetable oils in this study. Still, VOO's heptadecenoic acid (C17:1) level stands out at 0.24%. The level of stearic acid (C18:0), a saturated fatty acid, in sunflower oil and palm olein was higher than in cottonseed oil. In parallel, Anushree et al. (2017) stated that sunflower and palm oil are high sources of stearic acid. It was claimed that some types of sunflower oil obtained from improved and/or biotechnologically treated sunflower seeds would be an alternative to palm oil (Anushree et al., 2017). VOO was characterized by high oleic acid (C18:1) content, as seen in Table 1. VOO had the highest oleic acid value at 69.38%, followed by palm olein oil at 41.68%. Sunflower oil has the highest linoleic acid (C18:2) content of 68.44%.

Table 1. Fatty acid compositions of VOO, sunflower oil, cottonseed oil and palm olein samples

	VOO	Sunflower oil	Cottonseed oil	Palm olein
C14:0	0.02±0.00	0.06±0.00	0.69±0.01	1.10±0.02
C16:0	13.78±0.27	6.19±0.21	22.93±0.57	41.47±1.12
C16:1	0.88±0.02	0.09±0.00	0.55±0.01	0.21±0.00
C17:0	0.16±0.00	0.03±0.00	0.08±0.00	0.08±0.00
C17:1	0.24±0.00	0.03±0.00	0.03±0.00	0.04±0.00
C18:0	2.84±0.05	4.01±0.08	2.42±0.05	3.89±0.10
C18:1	69.38±2.48	21.94±0.76	16.27±0.34	41.68±1.08
C18:2	11.29±0.26	68.44±1.54	57.67±1.07	10.78±0.22
C20:0	0.49±0.01	0.23±0.01	0.18±0.00	0.27±0.01
C18:3	0.68±0.02	0.09±0.00	0.13±0.00	0.21±0.00
C20:1	0.31±0.01	0.12±0.00	0.04±0.00	0.13±0.00
C22:0	0.14±0.00	0.05±0.00	0.08±0.00	0.06±0.00
C24:0	0.07±0.00	0.02±0.00	0.10±0.00	0.05±0.00

Table 2. Triglyceride compositions of VOO, sunflower oil, cottonseed oil and palm olein samples

	VOO	Sunflower oil	Cottonseed oil	Palm olein
LLL	0.16±0.00	0.13±0.00	0.54±0.01	0.49±0.02
OLLn+PoLL	0.23±0.01	0.03±0.00	0.40±0.01	2.41±0.04
PLLn	0.05±0.00	0.22±0.01	0.10±0.00	0.39±0.01
OLL+OLPo	2.91±0.09	35.79±0.73	20.67±0.33	0.34±0.01
OOLn	1.11±0.02	0.14±0.00	0.80±0.01	0.17±0.00
PLL	0.98±0.01	0.23±0.00	0.16±0.00	0.06±0.00
OOL+PoOO	0.56±0.02	0.17±0.00	0.19±0.00	0.03±0.00
OOL+LnPP	14.85±0.38	26.97±0.86	14.08±0.34	2.21±0.05
OOPo	0.82±0.03	0.36±0.01	0.00±0.00	12.43±0.32
PLO+SLL	8.13±0.28	10.92±0.19	26.97±0.81	0.54±0.02
PoOP	0.44±0.01	0.26±0.00	0.00±0.00	10.70±0.22
PPL	1.17±0.02	0.08±0.00	0.56±0.02	0.17±0.00
OOO	35.22±0.99	7.39±0.19	4.10±0.04	4.79±0.09
OOP	25.05±0.61	11.50±0.20	15.13±0.30	28.39±0.50
POP	4.31±0.07	1.03±0.02	8.96±0.15	33.28±0.71
SOO	4.81±0.13	3.85±0.11	3.29±0.13	0.49±0.01
POS	0.00±0.00	0.98±0.03	4.26±0.14	2.14±0.06
ΔECN42	0.06±0.02	30.57±1.05	16.31±0.18	3.12±0.02

Table 3. Sterol compositions of VOO, sunflower oil, cottonseed oil and palm olein samples

	VOO	Sunflower oil	Cottonseed oil	Palm olein
Cholesterol	0.05±0.00	0.13±0.00	0.35±0.01	3.38±0.11
brassicasterol	0.02±0.00	0.02±0.00	0.06±0.00	0.07±0.00
ergosterol	0.03±0.00	0.00±0.00	0.00±0.00	0.00±0.00
24-methylene-cholesterol	0.06±0.00	0.00±0.00	0.00±0.00	0.00±0.00
campesterol	3.29±0.05	10.25±0.29	7.71±0.19	21.70±0.27
campestanol	0.01±0.00	0.00±0.00	0.00±0.00	0.00±0.00
stigmasterol	0.35±0.00	7.15±0.03	1.01±0.02	12.61±0.16
Δ5.23-stigmastadienol	0.06±0.00	0.00±0.00	0.00±0.00	0.00±0.00
clerosterol	0.77±0.02	0.55±0.01	0.90±0.01	0.00±0.00
β-sitosterol	81.23±0.97	59.41±1.46	87.02±0.81	57.90±1.52
sitostanol	1.19±0.02	0.00±0.00	0.00±0.00	0.00±0.00
Δ5-avenasterol	10.10±0.36	1.39±0.02	1.08±0.02	0.91±0.02
Δ5.24-stigmastadienol	0.43±0.00	1.65±0.05	0.18±0.00	1.09±0.01
Apparent β-sitosterol	93.77±1.11	63.00±1.45	89.18±0.82	59.90±1.54
Δ7-stigmastenol	0.24±0.00	11.71±0.25	0.06±0.00	0.79±0.02
Δ7-avenasterol	0.33±0.01	6.68±0.13	0.25±0.01	0.96±0.02
eritrodiol	1.24±0.05	0.00±0.00	0.00±0.00	0.00±0.00
Uvaol	0.05±0.00	0.00±0.00	0.00±0.00	0.00±0.00

The characteristic triglyceride trilinolein (LLL) is as low as 0.16% and 0.13% in VOO and sunflower oil, respectively. The highest total value of OLL+OLPo triglycerides was measured in sunflower oil at 35.79%, followed by cotton oil at 20.67%. OOL+LnPP total has differed between 2.21% (palm olein) and 26.97% (sunflower oil). Palm olein has the highest OOPo triglyceride content at 12.43%. On the contrary, palm olein oil has the lowest content of PLO+SLLL triglycerides compared to other vegetable oils. The PoOP was not detected in cottonseed oil,

while palm olein had 10.70%. It is known that the major characteristic triglyceride of VOO is triolein (OOO) (Ben Hmida et al., 2022). Similarly, in our study, VOO had 35.22% of OOO. Again, OOP levels, one of the triglycerides rich in oleic acid, were high in VOO and palm olein oil.

Phytosterols constitute a significant part of the unsaponifiable fraction of oils and the sterol composition shapes during the maturation of the olive (Bozdogan Konuskan and Mungan, 2016). The hydrolytic and oxidative progress may affect the fatty acid composition. Therefore, the sterol composition of vegetable oil act as a fingerprint and is more reliable in the assessment of adulteration than fatty acid composition (Aloisi et al., 2020; Saygın Gümüşkesen and Yemişçioğlu, 2010). The sterol composition of VOO, sunflower oil, cottonseed oil and palm olein samples were given in Table 3. VOO has low content of campesterol and stigmasterol. The highest amount of sterol in VOO was β-sitosterol with 81.23%, while it was determined at 87.02% in cottonseed oil. The β-sitosterol level was relatively low in sunflower oil and palm olein (59.41% and 57.90%, respectively). Δ5-avenasterol was highest in VOO at 10.10%. According to the Codex Alimentarius, apparent β-sitosterol which is the sum of Δ5,23-stigmastadienol, clerosterol, β-sitosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol should be higher than 93% for VOO. The apparent β-sitosterol content of VOO was 93.77% in our study.

The differences in chemical properties of adulterated samples and detection using ΔECN42 and sterol composition

Table 4 depicts the fatty acid composition of the adulterated samples prepared by mixing refined sunflower oil, cottonseed oil and palm olein with 1%, 4%, 7%, 10% and 13%. Palm olein was richer in C16:0 fatty acid at 41.47%, compared to VOO at 13.78%. Therefore, the adulterated samples with palm olein had higher C16:0 content even at 1% concentration. It was determined that as the palm olein concentration of the adulterated sample increased, C16:0 fatty acid content gradually increased to 15.21%, 16.13%, 17.29% and 18.17%. Since the C16:0 content of sunflower oil was lower than the VOO, the C16:0 content of adulterated samples with sunflower oil decreased gradually to 14.17, 13.55, 13.50, 12.97 and 12.81%. Similarly, Dourtoglou et al. (2003) stated that the C16:0 content was reduced when VOO was adulterated with sunflower oil. This study also reported an increase in C16:0 content when VOO was adulterated with cottonseed oil. Another distinctive change was in C18:1, the major fatty acid of VOO (Dourtoglou et al., 2003). The percentages of C18:1 decreased as the amount of sunflower oil in adulterated samples increased. As the percentage of adulteration with sunflower oil increased, C18:2 content gradually increased to 12.34%, 13.64%, 15.69%, 17.54% and 19.69%. Similarly, C18:2 content increased as the percentage of adulteration with cottonseed oil increased. The sterol compositions of adulterated samples using cottonseed, palm olein, and sunflower oil are shown in Table 5. Stigmasterol levels of adulterated samples have increased as the percentage of adulteration has risen. Along with the increase in the adulteration proportion, the campesterol content also elevated. The campesterol contents of; SOVO10, SOVO13, CSVO10, CSVO13, and POVO13 were, respectively, 4.09%, 4.37%, 4.11%, 4.16%, and 4.22% were lied outside of the codex limits. The campesterol decision tree approach should be applied when the sample's campesterol percentage is between 4% and 4.5%, per European Union's olive oil requirements (Anonymous, 2022). According to this decision tree, Δ7-stigmastenol should be ≤ 0.3% and stigmasterol should be ≤ 1.4% to decide the sample is not adultered. The stigmasterol percentages of the samples were lower than 1.4%, but the Δ7-stigmastenol values of some samples were greater than 0.3% (Table 5). Therefore, only those with a 10% and 13% mixture ratio among the adulterated samples could be determined using the campesterol decision tree.

Table 4. Fatty acid compositions of adulterated samples

	SOVO					CSVO					POVO				
	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%
C14:0	0.04	0.02	0.02	0.03	0.03	0.04	0.05	0.07	0.09	0.12	0.03	0.07	0.10	0.14	0.17
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
C16:0	14.17	13.55	13.50	12.97	12.81	13.97	13.91	14.92	14.61	14.72	14.00	15.21	16.13	17.29	18.17
	(0.30)	(0.55)	(0.29)	(0.46)	(0.33)	(0.22)	(0.23	(0.30)	(0.24)	(0.30)	(0.27)	(0.20)	(0.41)	(0.38)	(0.56)
C16:1	0.81	0.87	0.86	0.81	0.78	0.91	0.90	0.88	0.88	0.85	0.84	0.87	0.85	0.86	0.82
	(0.02)	(0.02)	(0.03)	(0.01)	(0.03)	(0.02)	(0.02	(0.02)	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.02)	(0.02)
C17:0	0.16	0.15	0.16	0.16	0.14	0.17	0.15	0.15	0.16	0.15	0.16	0.16	0.16	0.16	0.14
	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01	(0.00)	(0.00)	(0.01)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)
C17:1	0.25	0.23	0.23	0.23	0.21	0.26	0.24	0.23	0.23	0.22	0.25	0.24	0.23	0.21	0.21
	(0.01)	(0.01)	(0.00)	(0.00)	(0.01)	(0.01)	(0.00	(0.01)	(0.00)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00)	(0.01)
C18:0	2.87	2.86	2.87	2.99	2.97	2.82	2.79	2.81	2.76	2.76	2.75	2.96	2.92	2.92	2.95
	(0.07)	(0.06)	(0.11)	(0.05)	(0.11)	(0.03)	(0.06	(0.05)	(0.11)	(0.10)	(0.04)	(0.07)	(0.09)	(0.07)	(0.08)
C18:1	68.39	66.79	64.65	62.59	61.73	67.86	67.36	64.91	63.86	61.96	69.09	66.81	69.05	66.60	64.51
	(0.84)	(1.66)	(1.38)	(1.81)	(2.11)	(1.88)	(0.44	(1.01)	(1.95)	(1.32)	(2.05)	(1.90)	(1.30)	(2.03)	(0.94)
C18:2	12.34	13.64	15.69	17.54	19.69	11.91	12.96	14.79	16.08	17.63	10.88	11.04	11.28	11.10	10.97
	(0.37)	(0.43)	(0.42)	(0.41)	(0.38)	(0.27)	(0.35	(0.47)	(0.42)	(0.59)	(0.21)	(0.27)	(0.23)	(0.39)	(0.27)
C20:0	0.41	0.42	0.42	0.39	0.39	0.40	0.42	0.41	0.39	0.36	0.36	0.43	0.43	0.41	0.43
	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.01)	(0.01)	(0.02)
C18:3	0.69	0.63	0.64	0.60	0.61	0.70	0.66	0.63	0.62	0.62	0.61	0.66	0.66	0.62	0.61
	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.02)	(0.02	(0.01)	(0.01)	(0.02)	(0.02)	(0.01)	(0.02)	(0.01)	(0.01)
C20:1	0.27	0.26	0.26	0.26	0.26	0.29	0.26	0.25	0.24	0.23	0.24	0.27	0.27	0.26	0.25
	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)	(0.01)	(0.00	(0.01)	(0.00)	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.01)
C22:0	0.12	0.12	0.14	0.16	0.17	0.10	0.11	0.10	0.09	0.10	0.09	0.10	0.10	0.10	0.09
	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)	(0.00	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
C24:0	0.05	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.06	0.04
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

Table 5. Sterol compositions of adulterated samples

	SOVO					CSVO					POVO				
	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%
k.terol	0.23	0.10	0.08	0.08	0.07	0.12	0.12	0.08	0.06	0.12	0.11	0.14	0.08	0.04	0.25
	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
b.sterol	0.01	0.01	0.01	0.01	0.02	0.02	0.05	0.09	0.02	0.03	0.00	0.01	0.01	0.00	0.01
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
e.sterol	0.04	0.04	0.04	0.05	0.06	0.05	0.05	0.03	0.04	0.06	0.89	0.05	0.06	1.08	0.06
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.04)	(0.00)
24m.k.strl	0.09	0.08	0.09	0.09	0.12	0.10	0.08	0.05	0.06	0.06	0.12	0.08	0.07	0.18	0.12
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
kmpsterol	3.62	4.00	3.98	4.09	4.37	3.55	3.79	3.81	4.11	4.16	2.53	3.74	3.95	3.14	4.22
	(0.05)	(0.03)	(0.02)	(0.09)	(0.09)	(0.08)	(0.05)	(0.04)	(0.09)	(0.10)	(0.06)	(0.16)	(0.06)	(0.04)	(0.11)
kmpstanol	0.01	0.01	0.07	0.03	0.03	0.02	0.01	0.02	0.05	0.03	0.00	0.02	0.00	0.00	0.12
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
stg.sterol	0.56	0.70	0.89	1.02	1.31	0.37	0.41	0.40	0.43	0.44	0.44	0.53	0.57	0.92	0.97
	(0.01)	(0.02)	(0.03)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.02)	(0.01)	(0.02)
Δ5.23	0.14	0.28	0.33	0.36	0.51	0.11	0.09	0.09	1.51	0.24	0.02	0.01	0.01	0.01	0.00
	(0.00)	(0.01)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
kleres	0.91	0.91	0.67	0.64	0.79	0.94	0.86	0.71	0.45	0.63	0.55	0.86	0.72	0.40	0.84
	(0.02)	(0.02)	(0.01)	(0.01)	(0.03)	(0.03)	(0.02)	(0.02)	(0.01)	(0.02)	(0.01)	(0.02)	(0.02)	(0.01)	(0.03)
βs.sterol	80.80	81.71	76.82	77.71	74.83	80.49	81.66	81.16	80.09	81.06	83.12	81.25	81.14	81.82	79.84
	(1.86)	(1.91)	(2.57)	(2.27)	(1.86)	(2.57)	(1.24)	(2.47)	(0.66)	(2.42)	(2.21)	(2.30)	(2.11)	(1.39)	(1.19)
s.stanol	1.34	1.02	1.42	1.42	1.61	1.39	1.29	1.28	1.40	1.99	0.53	0.95	0.72	0.84	1.39
	(0.02)	(0.01)	(0.07)	(0.05)	(0.05)	(0.05)	(0.04)	(0.03)	(0.03)	(0.06)	(0.01)	(0.02)	(0.00)	(0.03)	(0.03)
Δ5avena	9.08	9.39	11.28	10.96	11.22	9.42	9.07	9.72	8.92	8.97	6.87	8.68	8.72	6.99	8.83
	(0.23)	(0.18)	(0.30)	(0.33)	(0.31)	(0.16)	(0.21)	(0.06)	(0.12)	(0.08)	(0.16)	(0.25)	(0.22)	(0.07)	(0.19)
Δ5.24	0.86	0.79	0.88	0.72	0.85	0.89	0.76	0.68	0.65	0.64	0.59	0.82	0.96	0.62	0.91
	(0.02)	(0.03)	(0.02)	(0.02)	(0.01)	(0.03)	(0.019)	(0.01)	(0.01)	(0.02)	(0.01)	(0.00)	(0.01)	(0.01)	(0.02)
Σ.βs.sterol	93.13	94.09	91.41	91.81	89.81	93.24	93.72	93.64	93.02	93.52	91.67	92.58	92.26	90.67	91.81
	(1.65)	(1.95)	(2.35)	(2.08)	(2.13)	(2.65)	(1.35)	(2.54)	(0.58)	(2.33)	(2.33)	(2.54)	(2.19)	(1.41)	(1.06)
Δ7stig	0.38	0.44	0.48	0.43	0.56	0.47	0.49	0.54	0.45	0.57	0.29	0.47	0.42	0.34	0.55
	(0.01)	(0.01)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.00)	(0.02)	(0.02)	(0.01)	(0.01)
Δ7avena	0.68	0.86	1.34	1.58	2.35	0.38	0.35	0.32	0.32	0.31	0.32	0.41	0.40	0.35	0.37
	(0.02)	(0.01)	(0.05)	(0.04)	(0.05)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.01)	(0.00)	(0.01)
erit	1.45	1.34	1.20	1.22	1.28	1.39	1.42	1.68	1.63	1.67	1.63	1.65	1.66	1.44	1.54
	(0.03)	(0.03)	(0.03)	(0.02)	(0.03)	(0.02)	(0.05)	(0.05)	(0.04)	(0.02)	(0.06)	(0.01)	(0.04)	(0.02)	(0.04)
uva	0.02	0.01	0.12	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.01	0.03	0.02
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)

k.terol: kolesterol, b.sterol: brassikasterol, e.sterol: ergosterol, 24m.k.strl: 24-metilen-kolesterol, kmpsterol: kampesterol, kmpstanol: kampestanol, stg.sterol: stigmasterol, Δ5,23: delta-5.23-stigmastadienol, kleres: kleresterol, βs.sterol: beta-sitosterol, s.stanol: sitostanol, Δ5avena: delta-5-avenasterol, Δ5,24: delta-5.24-stigmastadienolΣp.βs.sterol: apparent b-sitosterol, Δ7stig: delta-7-stigmastenol, Δ7avena: delta-7-avenasterol, erit: eritrodiol, uva: uvaol.

Table 5 depicts that stigmasterol percentages increase as the percentage of adulteration increases for all adulterated samples. However, since the campesterol percentage of all adulterated samples is higher than the stigmasterol percentage, all adulterated samples comply with the purity criteria specified in the relevant codex. Similarly, it was observed that all adulterated samples' cholesterol and brassicasterol values were within the limits of VOO specified in the relevant regulation. When Δ7-stigmastenol amounts are considered, it is seen that only 13% of adulteration ratios were detected among all adulterated samples. Similarly, Jabeur et al. (2014) determined that a 10% sunflower oil mixture is required for the samples to be out of standard with the increase in Δ7-stigmastenol in VOO adulteration (Jabeur et al., 2014). Apparent β-sitosterol contents of the samples adulterated with sunflower oil were determined as 91.41, 91.81 and 89.81% at 7-10-13% adulteration rates, respectively. Thus, the adulterated samples containing 7% or more sunflower oil remained outside the limits. Apparent β-sitosterol contents of all samples adulterated with palm olein were found out of the limit according to the relevant communiqué. Still, it was observed that the apparent β-sitosterol contents of none of the adulterated samples containing cottonseed oil were out of this limit.

The ΔECN42 is an important parameter used to detect VOO adulteration. Actual ECN42 triglyceride content (LLL + OLnL + PLnL) was higher in all refined adulterants than in VOO samples. The limit in ΔECN42 is 0.20 for VOO and 0.30 for lampante and refined olive oil (Anonymous, 2017). The ΔECN42 value, characterized by the detection of the adulteration of seed oils in olive oil, was measured in olive oil with 0.06 in this study. The ΔECN42 value was determined as 30.57 in sunflower oil and 16.31 in cottonseed oil, and 3.12 in palm olein oil in this study. Table 6 shows the triglyceride composition of adulterated samples with sunflower oil, cottonseed oil, and palm olein. LLL values increased with the increase in adulteration rate for all samples. OLL+OLPo percentages increased as the adulteration rate of cotton oil and sunflower oil increased. OOO tended to decrease as the percentage of adulteration increased. The ΔECN42 value enabled the detection of adulteration in all samples except the one having 1% cottonseed oil. Jabeur et al. (2014) stated that adulterated VOO samples containing 1% sunflower oil, 3% soybean oil and 3% corn oil using ΔECN42 values could be detected (Jabeur et al., 2014).

Discrimination of adulterated samples according to LDA performed using FTIR data

Infrared spectroscopy exploits the fact that molecules absorb frequencies characteristic of their molecular structure. These absorptions are resonant frequencies, i.e., the absorbed radiation's frequency matches a molecule's vibrational frequency. The atoms in organic compounds can vibrate in nine ways; symmetric and antisymmetric stretching, scissoring, rocking, wagging and twisting. The range of 1500cm^{-1} to 650cm^{-1} is specified as the "fingerprint" region of the FTIR spectra of vegetable oils, where vibrational modes of the organic compounds of vegetable oil significantly affect the spectral shape in this region (Movasaghi et al., 2008; Squeo et al., 2019).

For instance, 1464–983cm^{-1} region is assigned to bending vibrations of -CH_2 and -CH_3 aliphatic groups and rocking vibrations. Symmetric H-C-H bending at 1377 cm^{-1} could be attributed to glycerol group O-CH_2 (mono-, di- and triglycerides). CH_2 scissoring are observed at 1462 cm^{-1} whereas band between 1125 and 1095 cm^{-1} depend on the stretching vibration of C=O ester groups and -CH_2 wagging. The last major peak located near 723 cm^{-1} could be associated with overlapping of the $(CH_2)_n$ rocking vibration and out-of-plane vibration (-CH wagging) of cis-di-substituted olefins (Uncu et al., 2019). In addition, as used in the AOCS Official Method 14-61, the trans-isomer content can be reached through the peak structure of 966cm^{-1}.

Figure 1 presents the FTIR spectra of adulteration samples prepared with sunflower, palm olein and cotton oils, pure oils and VOO, respectively, in

untreated form. In particular, the morphological similarity of the fingerprint regions in the FTIR spectra of palm olein and VOO given in Figure 1b is remarkable. Some deviations and differences can be seen in the fingerprint region in the FTIR spectra of sunflower oil and VOO given in Figure 1a and cotton oil and natural olive oil given in Figure 1c. LDA was applied after this spectrum data were pre-processed as explained under the title of material and method.

Figure 2 depicts the LDA results of adulterated olive oil samples obtained with sunflower, palm olein and cotton oils and their blends (1-4-7-10-13%) of the selected VOO sample. When the LDA graph of VOO and sunflower oil mixtures was examined (Figure 1a), it was seen that the first two functions obtained at the end of LDA explain the difference between the samples at a total rate of 92.1%. It has been determined that the VOO and sunflower oil are separated on the first function. The adulteration samples prepared with these two oils show a distribution between VOO and sunflower oil on the graph, depending on the sunflower oil content. The LDA graph shows that even the adulteration sample containing 1% of sunflower oil can be easily separated from the VOO sample.

The LDA graph showing the separation of adulterated samples prepared with palm olein from VOO is given in Figure 2b. According to the LDA results, the first two functions explained the difference between the sample groups at the rate of 94.9%. Although adulterated samples containing sunflower oil were effectively separated on the LDA graph, it was observed that VOO and palm olein samples overlapped on the LDA graph, especially adulteration samples containing 10% and 13% palm olein could not be separated from VOO. For this reason, it is thought that the differences seen in samples with lower palm olein content are not justifiable. This result means that the fingerprint region in the FTIR spectra does not provide sufficient information for the detection of olive oil adulteration with palm olein.

Table 6. Triglyceride compositions and ΔECN42 values of adulterated samples

	SOVO					CSVO					POVO				
	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%	1%	4%	7%	10%	13%
LLL	0.76 (0.01)	1.88 (0.05)	3.13 (0.09)	4.43 (0.08)	5.42 (0.14)	0.44 (0.01)	1.27 (0.02)	1.83 (0.06)	2.33 (0.04)	3.08 (0.05)	0.25 (0.01)	0.30 (0.01)	0.26 (0.01)	0.24 (0.00)	0.16 (0.01)
OLLn +PoLL	0.37 (0.00)	0.28 (0.01)	0.25 (0.01)	0.31 (0.00)	0.33 (0.01)	0.21 (0.01)	0.51 (0.02)	0.28 (0.00)	0.19 (0.00)	0.25 (0.01)	0.44 (0.02)	0.57 (0.01)	0.56 (0.01)	0.57 (0.01)	0.65 (0.00)
PLLn	0.11 (0.00)	0.09 (0.00)	0.07 (0.00)	0.09 (0.00)	0.09 (0.00)	0.09 (0.00)	0.29 (0.01)	0.04 (0.00)	0.13 (0.00)	0.36 (0.01)	0.12 (0.00)	0.23 (0.01)	0.07 (0.00)	0.10 (0.00)	0.23 (0.00)
OLL+ OLPo	3.22 (0.05)	4.12 (0.09)	5.07 (0.15)	5.94 (0.16)	6.71 (0.16)	3.07 (0.07)	3.61 (0.10)	4.13 (0.08)	4.41 (0.07)	4.84 (0.13)	2.95 (0.04)	2.97 (0.05)	3.03 (0.05)	2.79 (0.07)	2.56 (0.04)
OOLn	1.16 (0.03)	1.27 (0.03)	1.06 (0.02)	0.95 (0.02)	1.04 (0.03)	1.36 (0.05)	1.32 (0.04)	1.22 (0.02)	1.25 (0.03)	1.15 (0.03)	1.23 (0.04)	1.31 (0.03)	1.04 (0.02)	1.24 (0.04)	0.98 (0.02)
PLL	1.12 (0.02)	1.56 (0.04)	1.75 (0.03)	2.08 (0.04)	2.49 (0.06)	1.51 (0.05)	2.27 (0.04)	2.98 (0.13)	3.92 (0.13)	4.96 (0.10)	0.95 (0.03)	1.11 (0.03)	1.07 (0.02)	1.21 (0.03)	0.92 (0.00)
OOL+ PoOO	0.54 (0.01)	0.63 (0.02)	0.54 (0.02)	0.42 (0.01)	0.40 (0.01)	0.90 (0.01)	0.60 (0.01)	0.59 (0.01)	1.18 (0.03)	0.62 (0.02)	0.69 (0.01)	0.56 (0.01)	0.69 (0.02)	0.76 (0.02)	0.35 (0.00)
OOL+ LnPP	14.82 (0.23)	14.77 (0.29)	14.55 (0.24)	14.14 (0.37)	13.67 (0.28)	15.11 (0.53)	14.42 (0.19)	14.64 (0.47)	13.92 (0.36)	13.92 (0.45)	14.86 (0.39)	14.90 (0.37)	14.19 (0.26)	14.08 (0.35)	13.60 (0.29)
OOPo	1.16 (0.03)	0.77 (0.02)	0.85 (0.01)	0.64 (0.01)	0.82 (0.01)	0.85 (0.01)	0.73 (0.02)	0.75 (0.02)	0.74 (0.02)	0.82 (0.02)	0.91 (0.02)	0.83 (0.02)	0.76 (0.02)	0.61 (0.02)	0.84 (0.02)
PLO+ SLL	8.15 (0.18)	8.02 (0.27)	8.57 (0.14)	8.55 (0.03)	8.59 (0.11)	8.14 (0.19)	8.41 (0.24)	8.56 (0.16)	9.01 (0.18)	9.19 (0.16)	7.99 (0.19)	8.55 (0.21)	8.42 (0.10)	8.46 (0.16)	9.36 (0.17)
PoOP	0.72 (0.01)	0.42 (0.01)	0.48 (0.02)	0.33 (0.00)	0.40 (0.01)	0.44 (0.01)	0.48 (0.02)	0.30 (0.01)	0.41 (0.01)	0.42 (0.01)	0.50 (0.01)	0.57 (0.02)	0.41 (0.01)	0.57 (0.03)	0.44 (0.01)
PPL	1.08 (0.04)	0.93 (0.02)	0.95 (0.02)	0.96 (0.03)	0.99 (0.01)	0.98 (0.02)	1.36 (0.02)	1.45 (0.06)	1.78 (0.04)	2.02 (0.04)	1.09 (0.03)	1.10 (0.03)	1.36 (0.03)	2.02 (0.08)	2.03 (0.03)
OOO	33.96 (0.74)	33.04 (0.67)	31.87 (0.61)	31.78 (0.93)	29.59 (0.70)	34.17 (0.86)	33.27 (0.81)	32.85 (0.81)	31.01 (0.75)	30.64 (0.88)	34.36 (0.96)	34.12 (0.51)	34.53 (1.03)	32.31 (1.06)	31.60 (0.81)
OOP	23.35 (0.33)	22.94 (0.52)	22.98 (0.65)	22.06 (0.58)	21.09 (0.44)	23.99 (0.71)	23.24 (0.46)	23.37 (0.87)	22.14 (0.48)	21.59 (0.58)	24.55 (0.52)	25.41 (0.40)	25.14 (0.74)	24.70 (0.90)	25.63 (0.74)
POP	3.71 (0.10)	3.17 (0.13)	3.27 (0.09)	3.30 (0.08)	3.17 (0.03)	3.78 (0.06)	3.14 (0.06)	3.40 (0.07)	3.57 (0.04)	3.98 (0.10)	3.75 (0.10)	4.43 (0.08)	5.39 (0.11)	6.67 (0.07)	6.87 (0.25)
SOO	4.77 (0.18)	4.74 (0.14)	4.23 (0.08)	4.07 (0.07)	4.05 (0.11)	4.14 (0.11)	4.48 (0.05)	4.01 (0.06)	3.70 (0.07)	2.97 (0.10)	4.13 (0.11)	3.23 (0.13)	3.48 (0.09)	3.37 (0.02)	3.70 (0.06)
ΔECN 42	0.56 (0.03)	1.48 (0.05)	2.48 (0.10)	3.66 (0.09)	4.40 (0.06)	0.06 (0.00)	1.34 (0.00)	1.29 (0.06)	1.66 (0.03)	2.52 (0.10)	0.27 (0.01)	0.52 (0.01)	0.34 (0.01)	0.37 (0.03)	0.51 (0.01)

P: Palmitic acid, Po:Palmitoleic acid, S: Stearic acid, O: Oleic acid, L: Linoleic acid, Ln: Linolenic acid,

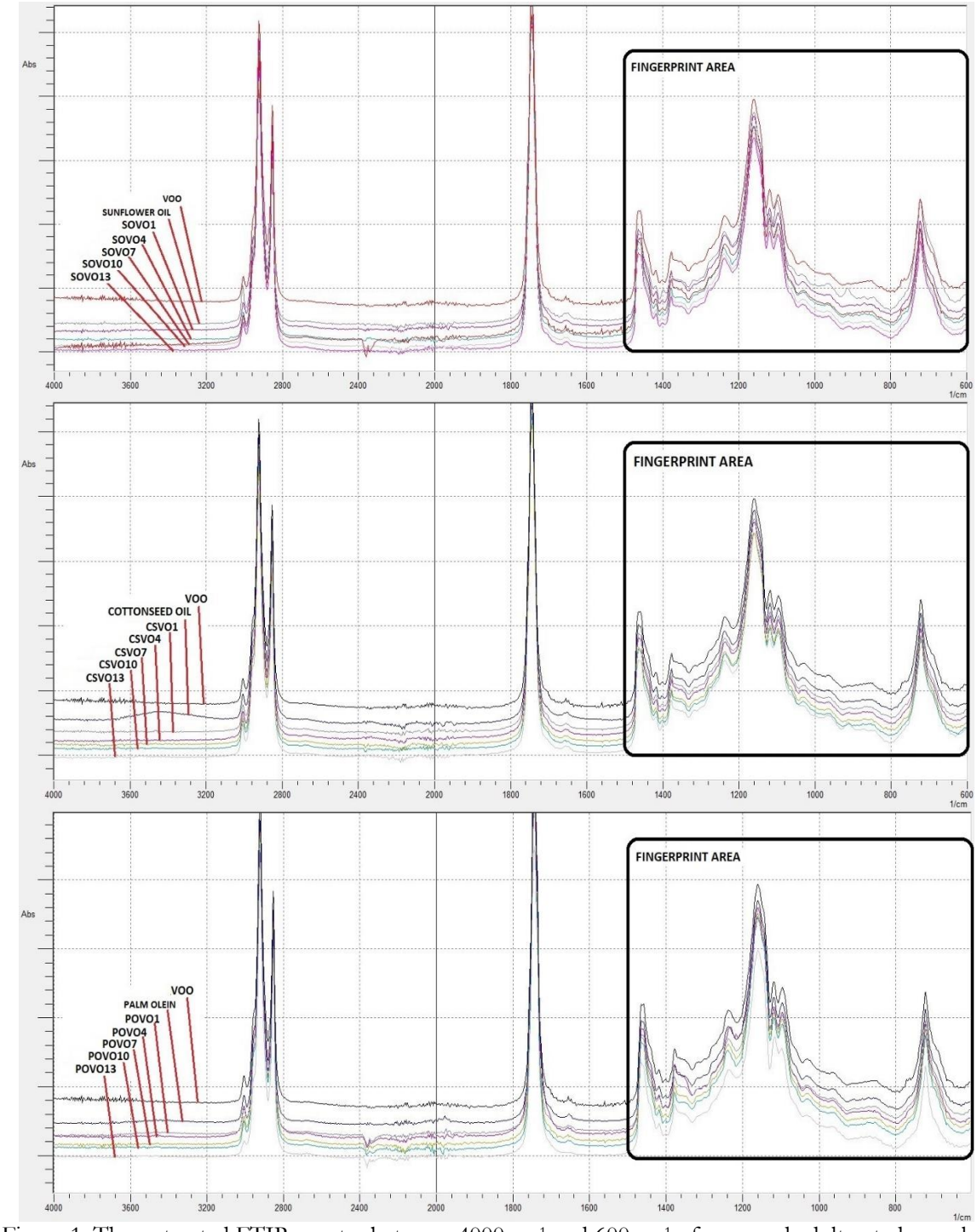

Figure 1. The untreated FTIR spectra between 4000cm^{-1} and 600cm^{-1} of pure and adulterated samples. VOO: Selected VOO for adulterated sample preparations.

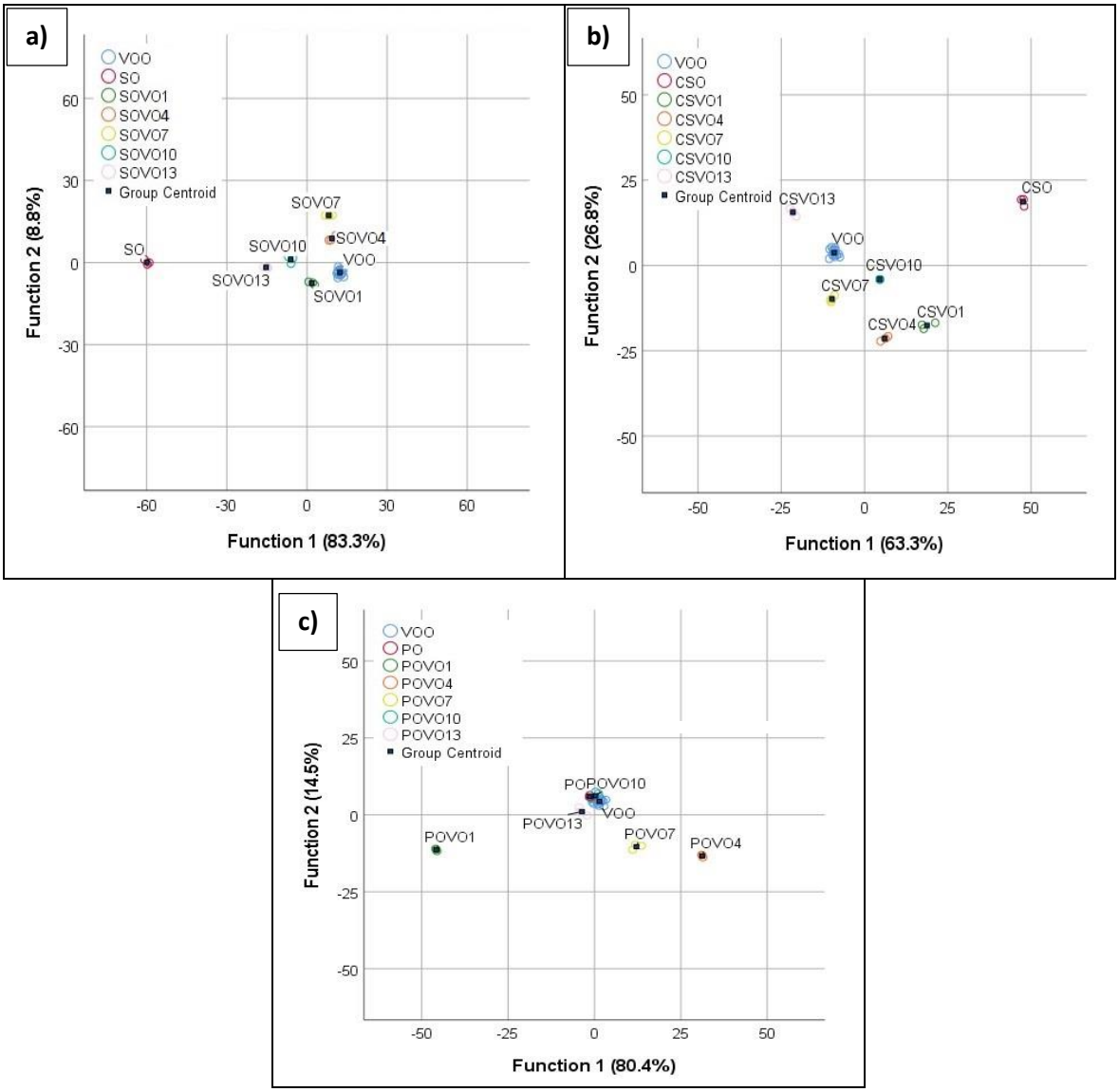

Figure 2. LDA results of adulterated oils and pure oils; a) VOO-sunflower oil, b) VOO-cottonseed oil, c) VOO-palm olein. Values in parentheses next to the functions are the percentage of explanation of the difference between the sample groups.

The LDA graph showing the separation of adulterated samples consisting of cottonseed oil, VOO and their mixtures is given in Figure 1c. It is seen that cottonseed oil and VOO samples are clearly separated, and the adulterated samples are also clustered in different locations on the LDA (Figure 3.) graph. Although no sequence was observed depending on the mixing ratio, it was determined that cottonseed oil and VOO mixture were separated from natural olive oil even at a rate of 1%.

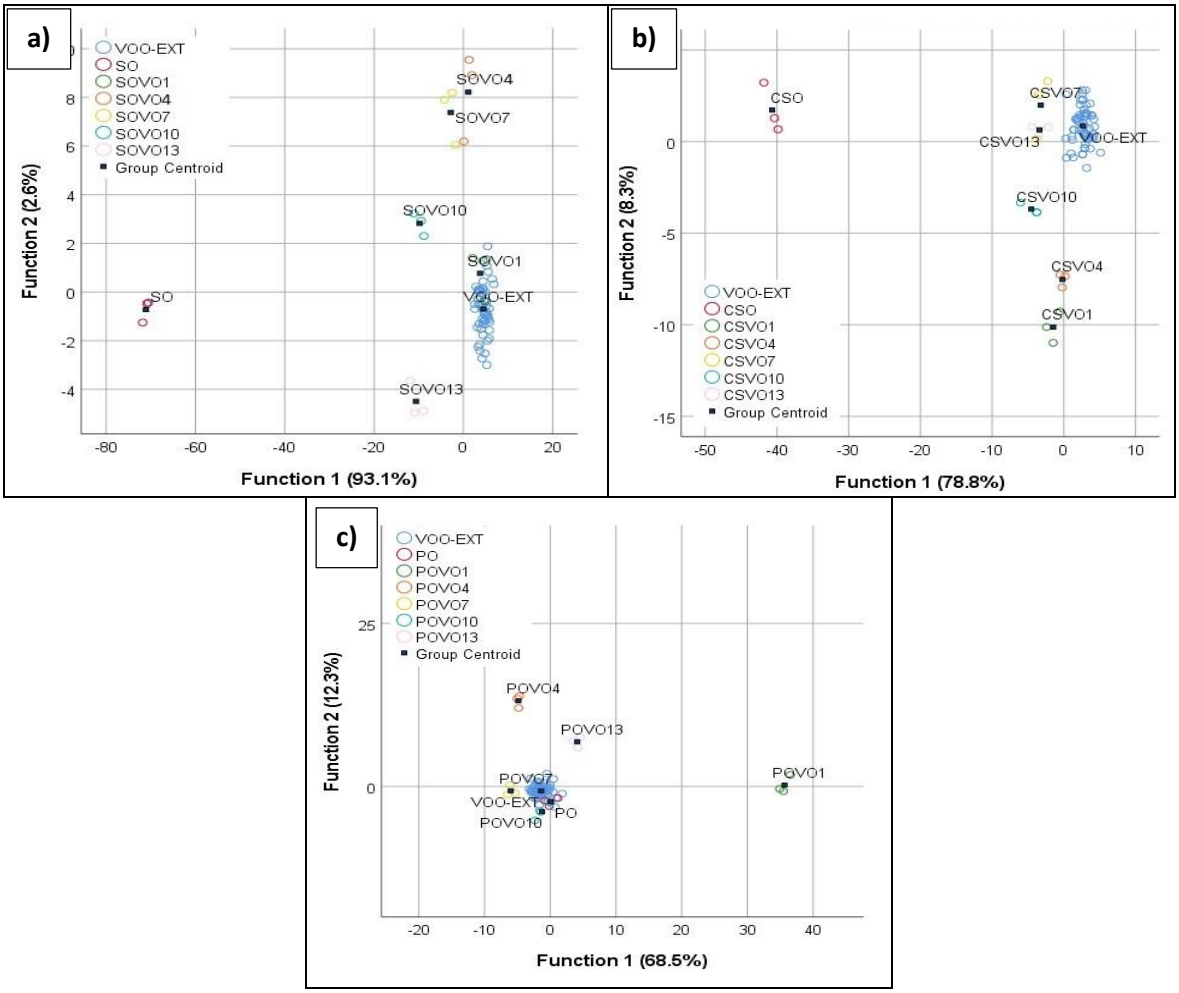

Figure 3. Extended olive oil sample set (VOO-EXT) and LDA results of adulteration samples; a) VOO-sunflower oil, b) VOO-cottonseed oil, c) VOO-palm olein. Values in parentheses next to the functions are the percentage of explanation of the difference between the sample groups.

As it is known, the chemical composition of VOOs can vary depending on many factors such as variety, climatic conditions, soil structure, agricultural practices and harvest season. This variability is likely to complicate the detection of adulterated samples and is assumed to be the major obstacle to standardizing spectroscopic techniques such as FTIR in detecting VOO adulteration. Therefore, in our study, the VOO sample set was expanded, and the LDA was repeated in order to evaluate the possible effects of the harvest season (year), variety and geographical region differences in VOOs on the detection of VOO adulteration over the FTIR spectrum - LDA. In addition to the VOO sample used in preparing the adulterated samples, five 2020-2021 Ayvalık, five 2019-2020 Edremit and five 2019-2020 Milas samples were defined as VOO, and the LDA data set was expanded. For the repeated LDA, the expanded VOO sample set was labeled VOO-EXT (Figure 3).

The LDA graph in Figure 3a, depicts the differentiation of adulteration samples prepared with sunflower oil from the expanded VOO set. The adulterated samples containing 1% sunflower oil overlapped with VOOs, while adulterated samples with higher sunflower oil concentrations were located away from the VOOs. According to the LDA results, it was observed that the samples

containing 1% sunflower oil could be separated only from the geographically indicated Ayvalık VOO samples of the 2019-2020 season. (Figure 1a). However, the chemical diversity due to the expansion of the VOO sample set made this discrimination impossible in the latter case. Similarly, the enlargement of the VOO sample set weakened the discrimination of cottonseed oil adulterated samples and especially the samples containing 7, 10 and 13% cottonseed oil located closer to the VOO samples. Nevertheless, adulterated samples prepared with cottonseed oil were close but did not overlap with VOOs.

CONCLUSION

Identifying frauds classified as adulteration is essential to preserve VOO's authenticity and actual economic value. The legislation establishes the standards for judging the purity of VOO, but chromatographic methods, which require substantial amounts of chemicals, time, and labor, are used to determine the chemical qualities. Numerous research has examined the possibility of detecting VOO adulteration using quick and non-destructive approaches like FTIR. However, the most significant barrier to the widespread use and standardization of spectroscopic methods has been the practicality issues related to varying validity performance at varied data sizes. The findings of our study showed that when sunflower oil, palm olein, and cottonseed oil were used as partial substitutes for VOO, the ΔECN42 value was a valid approach to detect adulteration. Using the ΔECN42 value, 1% palm oil, 1% sunflower oil and 4% cottonseed oil adulterated samples were discriminated from VOO. LDA results of FTIR data showed identical results to those of ΔECN42 except for palm olein adulterated samples. However, when VOO data was expanded to include VOO samples from a new variety, region, and season (Milas, Edremit samples, and 2020–2021 Ayvalık samples), the discrimination in LDA between VOO and adulterated samples prepared with cottonseed oil was diminished. Correct multivariate approach and FTIR data selection significantly influence the performance of FTIR spectroscopy for detecting VOO adulteration.

ACKNOWLEDGMENT

The authors thank the Ege University Office of Scientific Research Projects for funding this work under Project 22473. Also, the Geographically Indicated VOO samples used in this study were obtained via a previous scientific project (120O872) supported in the frame of the Short Term R&D Funding Program of The Scientific and Technological Research Council of Türkiye (TUBITAK).

CONFLICT OF INTEREST

There are no conflicts of interest or competing with the results of the presented article.

AUTHORS' CONTRIBUTIONS

Ms. Ebru Karacan conducted trials, carried out the experiments, and formatted the draft, Mr. Onur Özdikicierler conceived the idea, performed the statistical analysis, and carried out the correspondence of the MS, Mr. Fahri Yemişçioğlu was responsible for the supervision and maintaining the collaboration. All authors have participated in the writing of the MS.

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