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Characterization of phenolic compounds, sterols and geographical fingerprint of Memecik extra-virgin olive oils according to their geographical locations by using LC IMS QTOF Mass Spectrometry

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Abstract

Memecik olive oil variety which cultivated in six different locations were evaluated the effects of geographical locations on the chemical characterization of in the southwest of Turkey. The agricultural ecological map of each location was created using GIS. Olive oil samples were analyzed fatty acid, sterol and phenolic. Moreover, LC IMS QTOF spectrometer and Progenesis QI software were used to determine the geographical fingerprints of olive oil samples in different locations. Results showed that oil qualities of all locations differ significantly depending on olive growing area ($p < 0.05$). The Principal Component Analysis of the different locations analyzed revealed that "geographical location" factor significantly affects the olive oil quality.

Keywords: Olive oil; Sterols; Phenolic compounds; LC IMS QTOF spectrometer

1. Introduction

Extra virgin olive oil (EVOO) is considered the healing oil due to its essential nutrients. EVOO is unique compared to other oils (vegetable, animal) with its chemical composition (antioxidants, phenols, fatty acid, vitamins, etc.) [4].

There are more factors affecting chemical composition, physico-chemical quality, and sensory properties of olive oil. These are environmental factors (topography, ecological zone, humidity, altitude, and climate), agronomic factors (irrigation, pruning, pesticide application, fertilization, harvesting time, and ripening index) and post-harvest factors (oil extraction system, oil storage conditions) [2].

In addition, it is argued that quality of olive oil is effected by olive variety and geographical regions. Polyphenols and antioxidant are the main parameters to be considered in geographic fingerprint according to geographical region and olive variety in EVOO [7]. Also, fatty acid plays important parameter in the chemical of EVOO which has a high content of fatty acids, for example oleic acid ranging from 56 % to 84 % [9]. Another important parameter is the sterol profile which is considered as a fingerprint to examine its geographic originality in the EVOO. Olive oil leaders such as Spain, Italy and Greece use some tools to separate EVOOs by geographical location. One of the tools is LC IMS QTOF mass spectrometer and Progenesis QI software [14]. Memecik olive variety, dominated by the southwestern part of Turkey and the coastal part, has a long history. The oil properties of the Memecik olive variety are quite high [6].

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Various studies are carried out to differ EVOOs from geographical regions according to various chemical characterization in Turkey. However, there is not any research on the effect of the geographical location on the chemical characterization of the Memecik variety which cultivated in the southwest of Turkey and has a high oil content. In addition, the analysis of sterol and polyphenolic compounds of EVOOs in the same region but under different ecological zone (climatic and topographic conditions) was performed for the first time using LC IMS QTOF mass spectrometry via appropriate extraction method. Only a few researchers have focused their attention on geographical location of olive tree crops and how it may reveal its effect on physical, chemical properties of EVOOs. The characterization of geographical indication studies related to EVOOs have not been published on literature in Turkey.

In this context, propose of this study is to determine the effect of Memecik olive oils in different locations (Acarlar, Gökçealan, Sultaniye, Şirince, Zeytindağ, Havutçulu) and show differences on geographical locations while taking geographical indication label.

2. Material and methods

2.1. Fruit Samples

The research was conducted throughout 2020/2021 olive season. Olive fruit samples of the Memecik variety (~ 2,5 kg for each sample) were collected by hand randomly from 6 different locations (Acarlar, Gökçealan, Sultaniye, Şirince, Zeytindağ, Havutçulu) and 9 trees in each location. Olive fruits (2, 04-4, 05) were collected at the ripening index.

2.2. Remote Sensing Methods (GIS) and Agricultural Ecology Map

Agricultural ecology maps of the locations were created with remote sensing methods (fig 1). Agricultural Ecoregion Maps which is the creation of similar homogeneous areas by bringing together the climate, topographic, soil parameters that make up the land features by means of GIS. The classification system is based on the UNESCO (1979) system.

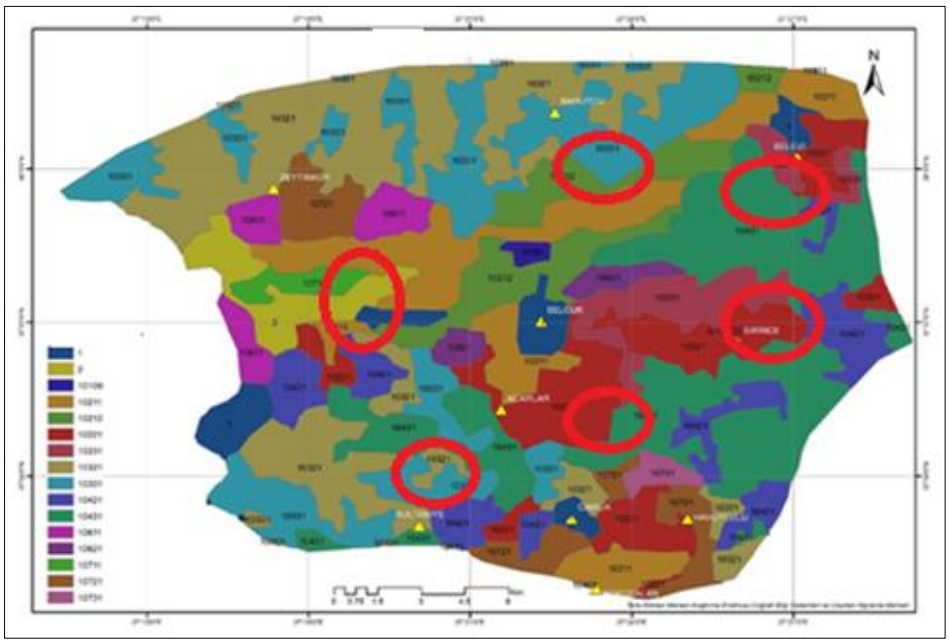

Figure 1 Agricultural Ecology Map of Selçuk region

2.3. Oil extraction

Following the harvest, olive fruites were brought in laboratory and olive oil was extracted within 24 hours. Fruits were taken from 6 different locations (Kösedere, Eğlenhoca, Tepebozköy, Haseki, Mordoğan, Ambarseki) and olive oils were used with "Abencor" system for extracted. EVOO were stored in dark glass bottles at 4 ℃.

2.4. Determination of sterol composition and amount by capillary column gas chromatography

Sterol composition and amount of EVOOs were determined by capillary column gas chromatography and erythrodiol and uvaol of total sterols were made by using Turkish Food Codex related to olive oil sampling and analysis methods communiqué 2014/53 [1].

Table 1 Agricultural ecological map information of Selçuk Region

Selçuk Region	Ecological Map	Altitude	Drought Index	Warmest Average	Coldest Average	Slope
Şirince	10221	300-500 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	2-12%
Sultaniye	10431	300-500 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	12-30%
Acarlar	10221	0-100 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	2-12%
Havutçulu	10721	100-300 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	2-12%
Zeytinköy	10321	0-100 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	2-12%
Gökçealan	10421	100-300 m	0.5-0.75 (semi humid)	>20 ˚C	0-10 ℃	2-12%

2.5. Chemicals and extraction of sterols and phenolic compounds

Methanol of LC grade used for the extraction of sterols and phenolics from samples and preparing the mobile phase were supplied from Isolab. Deionized water was obtained by filtration using a Milli-Q-system (Millipore, Bedford, MS, USA). Ammonium acetate used for preparing the mobile phase was purchased from Sigma Aldrich (St. Louis, MO, USA).

Extraction of sterols and phenolic compounds from EVOOs was carried out using liquid extraction method. MeOH: H2O (80:20, v/v) was used as the extraction solvent. Three grams of olive oil samples weighted into centrifuge tube, and added with 3 mL of 80 % MeOH solution. After homogenization via vortex, samples were centrifuged for 10 min at 7500 rpm. The supernatant was collected, the pellet was used again. The same procedure was repeated 3 times. All supernatant fractions were collected, combined and filtered through 0, 22 µm PTFE syringe filters and stored in vial until LC IMS QTOF analysis. The extracts were mixed with an equal volume of water prior to analysis. Also, a pool sample consisting of all oil samples was prepared in the same way to check the accuracy of our research.

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2.6. Fatty Acid Composition

The fatty acid composition was determined by gas chromatography (GC) after saponification/methylation with methanolic KOH via the official method (EEC Reg.2568/91). The fatty acids was detected by the comparison of retention time in standard compounds.

2.7. LC IMS QTOF Screening

The LC IMS QTOF system (Ultra-high performance liquid chromatography with a ACQUITY UHPLC I-Class system (Waters, Milford, MA, USA) was coupled to a VION® IMS QTOF (Waters, Manchester, UK), ion mobility Quadrupole Time-of-Flight) was used. The LC separation was performed using an Acquity UPLC BEH C18 (100x2, 1 mm, id. 1, 7 µm particle size, Waters) analytical column.

The oven was set at 30 °C. The solvents used consisted of (A) 90% H2O, 10% MeOH, and 5 mM ammonium acetate and (B) 100% MeOH and 5 mM ammonium aceate.

The used flow gradient started with 1% solvent B with flow rate 0.35 mL/min during 1 min, increasing to 39 % for the following two min and then incereasing to 99 % fort the next 11 min. Organic conditions were kept for 2 min and then initial conditions were restored within 0.1 min and the column re-equilibrated for 2.5 min. The total elution programme was 18 min. The injection volume was 3 µL. The QTOF system was operated in positive ionization mode, capillary voltage of 3.0 kV, mass range 50-1200 m/z. source temperature of 120 °C, desolvation temperature of 400 °C. External calibration was performed using a Leu-enkephalin solution injected during the run 1 min intervals. Data were collected

under low collision energy of 6.0 eV and high collision energy of 15 to 45 eV (Table 2). Olive oil samples were analyzed in Waters brand Vion LC IMS QTOF system (Waters, Milford, MA, USA) in order to determine the origin (Table 2 and 3).

All samples were analyzed in the same batch without any stopping. Data acquisition and data analysis were carried out by UNIFI (Waters, USA) software. Then, the raw datas were subjected to principal component analysis (PCA) using Progenesis QI (Nonlinear Dynamics, Waters, USA) software. UNIFI data format were converted to .uep format using the peak picking options.

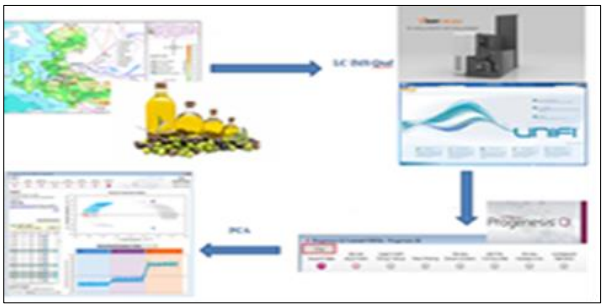


Figure 2 Flow chart of this study conducted to determine marker ions in olive oil

2.8. Statistical Analysis

Social Sciences (SPSS) program release 16.0 was made for statistical analyses. The results are determined as mean ± standard deviation (SD) of five measurements for each analytical data. Significant differences were determined at $p < 0.05$ between the values of all parameters according to the one-way ANOVA: Post Hoc Comparisons (Duncan test). The Principal Component Analysis (PCA) was made using Progenesis QI software. It was applied to separate EVOOs each geographical locations according to all parameters.

3. Results and discussion

3.1. Total Phenolic Content

The total amount of phenolic was determined in EVOOs. Statistical analysis are found significant differences ($p < 0.05$) in total phenol contents among oil samples. Total phenol content ranges from 125.861 to 263.89 mg/kg-1 in olive oils

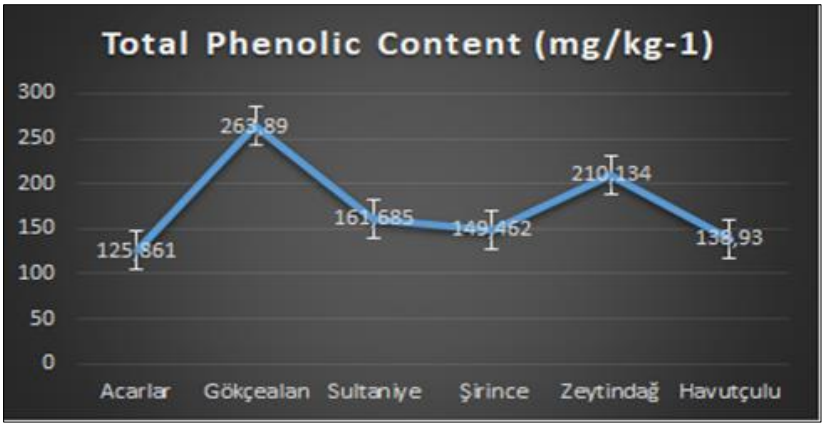


Figure 3 Total phenol contents in olive oil samples from Memecik variety in six locations in the west of Turkey. Results are shown as means ± SD (n = 5). A-b Different letters indicate significantly different values at $p < 0.05$ according to Duncan test

EVOOs of Gökçealan locations have highest phenolic content (263.89 mg/kg-1, respectively). Gökçealan location was showed that geographical location have a significant effect on the total phenolic content (fig 3). Del Monaco et al. (2015) examined some of EVOOs from different locations and varieties in a study. He reported that characterization of EVOOs according to geographical regions and total phenol content differs greatly according to geographical location in Italy. This study reveals that it is in line with our results.

Table 2 The operating conditions of the Vion LC IMS QTOF system

Sample manager	IMS QTOF	Column manager	Scan settings
Wash solvent: Metanol:Su (70:30, v/v) Sample temperature: 8.0 ºC	Analyzer mode: Sensitivity Capillary voltage: 3.0 kV Source temperature: 120 ºC Desolvation temperature: 400 ºC Cone gas: 50 L/h Desolvation gas: 1000 L/h	Temperature: 30 ºC	Scan settings: 50 m/z–1200m/z High Definition MS^E Low energy: 6.00Ev High energy ramp: 15-15 eV

Table 3 Flow gradient of Vion LC IMS QTOF system

Time (dk)	Flow (mL/dk)	% A	% B	Curve
0.00	0.350	99.0	1.0	6
1.00	0.350	99.0	1.0	6
3.00	0.350	61.0	39.0	6
14.00	0.350	1.0	99.0	6
16.00	0.350	1.0	99.0	6
16.01	0.350	99.0	1.0	6
18.00	0.350	99.0	1.0	6

Another study related to Zarazi varieties in Tunisia, higher phenolic content was detected in olive oil samples in the south of Tunisia. Result of study, it was stated that water scarcity increases the phenolic content due to drought creates a stress condition that triggers phenolic synthesis in olive fruits (Arslan, 2013). There are significant differences among geographical regions in terms of different phenolic compounds in this study and also in previous studies. Bajoub et al. (2015) found that qualitative and quantitative phenolic composition of EVOOs is strongly influenced by agricultural parameters, but effected by the genetic factors and environmental conditions particularly climate and topography.

Our datas showed that there are different phenolic contents of olive trees in same altitude due to soil structure, nutritional status. Altitude effects phenolic content as positive in EVOO.

3.2. Fatty Acid Composition

Important fatty acid compositions were determined in Memecik olive oil samples. As shown in Table 5, oleic (C 18: 1), palmitic (C 16: 0), linoleic (C 18: 2) and stearic acids (C 18: 0) are the main fatty acids in EVOO.

The contents of oleic acid is between 72.00 % (Gökçealan), 67.17 % (Şirince). Palmitic acid content is respectively 14.97 % (Zeytindağ), 14.80 % (Şirince), and 14.57 % (Sultaniye). The highest linoleic acid content was in Şirince location (13.19 %). Fatty acid composition was found different among geographical locations (Table 5). Morelló (2004) studied that fatty acid changes due to genetic factors as well as environmental conditions. Piravi-Vanak et al. (2012) found that fatty acid composition of EVOO is significantly affected by topographical and climatic conditions.

3.3. Effects of geographical location on phytosterol contents

Some sterols are the main sterols such as β-sitosterol, campesterol and Δ-5-avenasterol. Other sterols are minor sterols such as stigmasterol, clerosterol and-5, and 24-stigmastadienol in EVOO (Table 6). As shown in Table 6, phytosterol are mostly depending on the geographical location of EVOO. In the Memecik variety, the highest phytosterol content is β-sitosterol, followed by Δ-5-avenasterol. Significant differences were found in the contents of β-sitosterol and Δ-5-avenasterol according to different geographical locations. ($p < 0.01$).

The highest β-sitosterol content was found in Zeytindağ location (88.98 %), then Acarlar (89.22 %) and Havutçulu location (87.56 %). Regarding the content of Δ-5-avenasterol, Şirince location showed the highest value (7.36 %), while it was the lowest (5.19 %) in Zeytindağ location.

Table 4 Fatty acid composition (%) of EVOOs in six different geographic locations in the southwest of Turkey

	Selçuk Region	Acarlar	Gökçealan	Havutçulu	Şirince	Sultaniye	Zeytindağ
1	Miristik Acid (C14:0)	0.02 ± 0.03^{b}	0.07 ± 0.04^{a}	0.01 ± 0.04^{c}	0.02 ± 0.05^{b}	0.01 ± 0.02^{c}	0.01 ± 0.04^{c}
2	Palmitik Acid (C16:0)	14.18 ± 0.12^{d}	14.18 ±0.08^{d}	14.02 ± 0.07^{e}	14.80 ± 0.05^{b}	14.57 ± 0.12^{c}	14.97 ± 0.06^{a}
3	Palmitoleik Acid (C16:1)	0.82 ± 0.06^{e}	0.89 ± 0.04^{b}	0.86± 0.13^{d}	0.89± 0.08^{b}	0.92± 0.05^{a}	0.88± 0.04^{c}
4	Heptadekanoik Acid (C17:0)	0.03± 0.07^{a}	0.02± 0.12^{b}	0.03± 0.11^{a}	0.03± 0.12^{a}	0.02± 0.13^{b}	0.02± 0.09^{b}
5	Heptadesenoik Acid (C17:1)	0.03± 0.07^{c}	0.04± 0.02^{b}	0.05± 0.04^{a}	0.04± 0.07^{b}	0.03± 0.06^{c}	0.04± 0.04^{b}
6	Stearik Acid (C18:0)	2.78± 0.11^{a}	2.35± 0.08^{d}	2.34± 0.13^{de}	2.49± 0.12^{c}	2.63± 0.09^{b}	2.09± 0.08^{e}
7	Oleik Acid (C18:1)	71.13± 0.06^{b}	72.00± 0.03^{a}	71.50± 0.03^{bc}	67.17± 0.06^{e}	68.34± 0.05^{d}	70.78± 0.08^{c}
8	Linoleik Acid (C18:2)	9.77± 0.04^{d}	9.62± 0.09^{de}	10.09± 0.09^{cd}	13.19± 0.05^{a}	12.36± 0.04^{b}	10.13± 0.06^{c}
9	Linolenik Acid (C18:3)	0.71± 0.02^{b}	0.43± 0.05^{e}	0.55± 0.03^{d}	0.76± 0.07^{a}	0.55± 0.05^{d}	0.60± 0.03^{c}
10	Araşidik Acid (C20:0)	0.36± 0.12^{a}	0.28± 0.09^{d}	0.33± 0.07^{c}	0.34± 0.08^{b}	0.34± 0.05^{b}	0.26± 0.03^{e}
11	Gadoleik/eikosenoik Acid (C 20:1)	0.15± 0.04^{d}	0.17± 0.07^{c}	0.23± 0.12^{a}	0.21± 0.08^{b}	0.23± 0.06^{a}	0.15± 0.05^{d}
12	Behenik Acid (C 22:0)	ND	ND	ND	0.07± 0.06^{a}	ND	ND
13	Lignoserik Acid (C24:0)	ND	ND	ND	ND	ND	ND
14	Trans Oleik Acid (C18:1T)	ND	ND	ND	ND	ND	ND
15	Trans Linoleik Acid +Trans Linolenik Acid (C18:2 T+C18:3 T)	ND	ND	ND	ND	ND	ND

Each value represents the mean of five determinations (n = 5) ± standard deviation. ND not determined. a-f Different letters in the same row indicate significantly different values ($p < 0.05$) according to Duncan test.

Table 5 Phytosterol composition (%) of EVOOs in 6 different locations in Turkey (Acarlar, Gökçealan, Havutçulu, Şirince, Sultaniye, Zeytindağ)

Locations	Campe-sterol	Stigmasterol	Δ5-24 Stigmastadienol	β -Stosterol	Δ5-Avenasterol	Clerosterol	Apparent β -STEROL
Acarlar	3.25± 0.02^{b}	0.98± 0.00^{c}	0.30± 0.00^{e}	89.22± 0.05^{a}	3.96± 0.02^{f}	0.98± 0.00^{a}	94.46± 0.00^{b}
Gökçealan	2.93± 0.00^{d}	0.89± 0.02^{d}	0.62± 0.05^{a}	87.37± 0.02^{b}	5.25± 0.00^{d}	0.72± 0.05^{d}	93.26± 0.02^{c}
Havutçulu	3.12± 0.01^{c}	1.19± 0.00^{f}	0.27± 0.03^{f}	87.56± 0.00^{b}	6.34± 0.14^{b}	0.74± 0.03^{e}	94.92± 0.01^{b}
Şirince	3.43± 0.03^{a}	1.58± 0.08^{a}	0.37± 0.11^{b}	85.04± 0.01^{d}	7.36± 0.03^{a}	0.66± 0.11^{e}	93.43± 0.13^{c}
Sultaniye	2.93± 0.00^{d}	1.44± 0.03^{b}	0.34± 0.06^{c}	86.38± 0.03^{c}	5.72± 0.00^{e}	0.74± 0.06^{b}	93.18± 0.07^{cd}
Zeytindağ	2.68± 0.04^{e}	0.72± 0.01^{e}	0.32± 0.02^{d}	89.38± 0.00^{a}	5.19± 0.05^{e}	0.59± 0.02^{f}	95.48± 0.03^{a}

Apparent β-sitosterol (sum of clerosterol + β-sitosterol + Δ-5-avenasterol + Δ-5, 24-stigmastadienol). Each value represents the mean of five determinations (n = 5) ± standard deviation. a-f Different letters indicate significantly different values ($p < 0.05$) in the same row according to Duncan test.

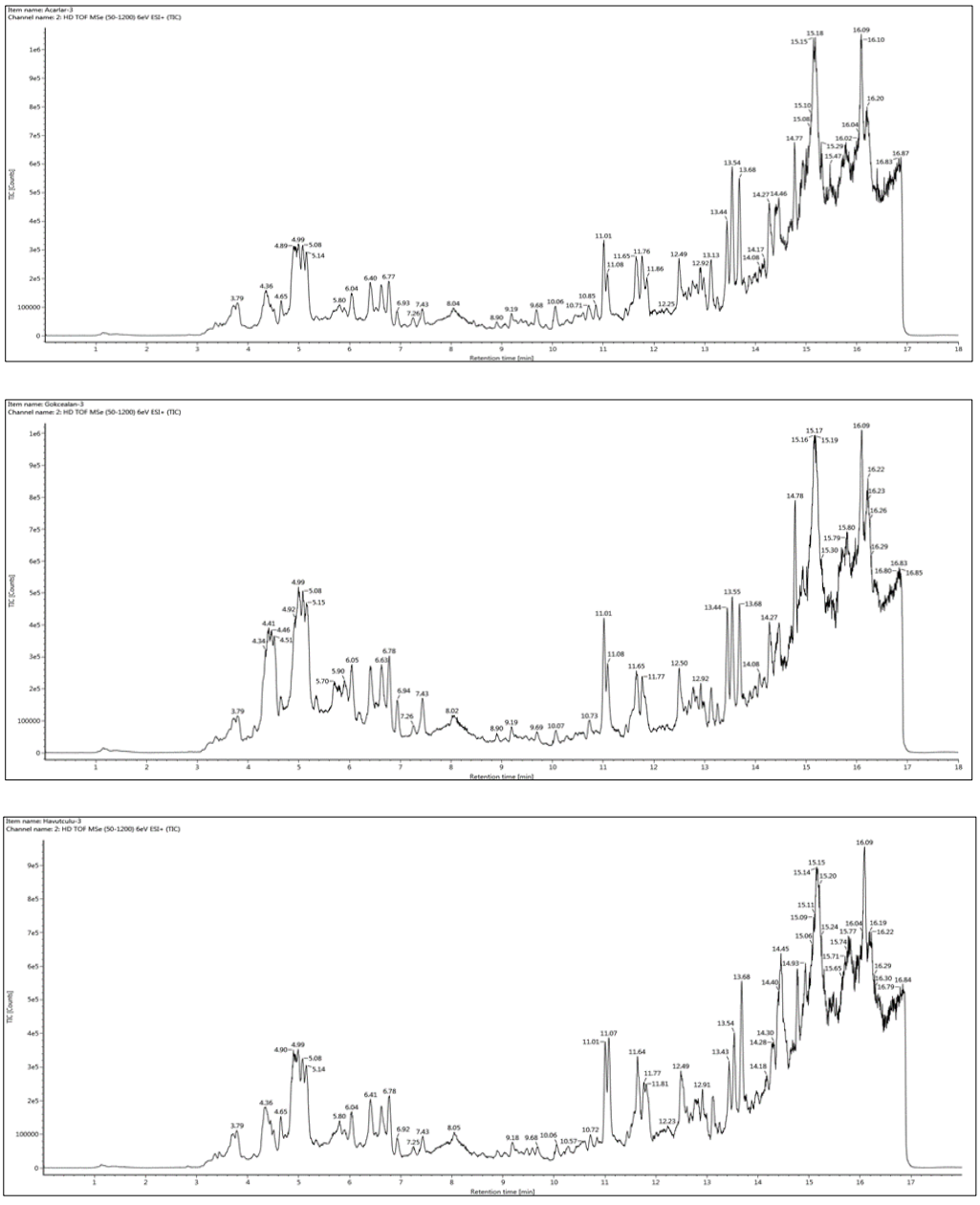
Memecik EVOOs has low stigmastereol and campesterol content. In all EVOOs, the campesterol content was found below the limit between 3.43 % (Şirince) and 2.68 % (Zeytindağ) according to EU Regulations (4%)

There is significant difference in campesterol content according to the geographical locations. In addition to apparent β sitosterol, it was detected by the sum of β-sitosterol and other three sterols (5, 24-stigmastadienol, clerosterol and Δ-5-avenasterol). Memecik EVOO are found approximately the limit of 94 %. The highest apparent β-sitosterol (95.48 %) was detected in Zeytindağ location (Table 6). In EVOOs, the content of stigmasterol is lower than campesterol as previous research (Chtourou et al., 2013).

In this study, different phytosterol content revealed in Memecik EVOOs were found to be similar as Chemlali varieties. Many studies showed that various factors affect the sterol content such as olive variety, ecological zone, soil structure and harvest time. (Pardo et al., 2012). This results of study is parallel with previous studies.

3.4. Determination of marker ions in EVOOs by using LC IMS QTOF Screening

The determination of marker ions in olive oils carried out using LC IMS QTOF mass spectrometry system (Fig 4)



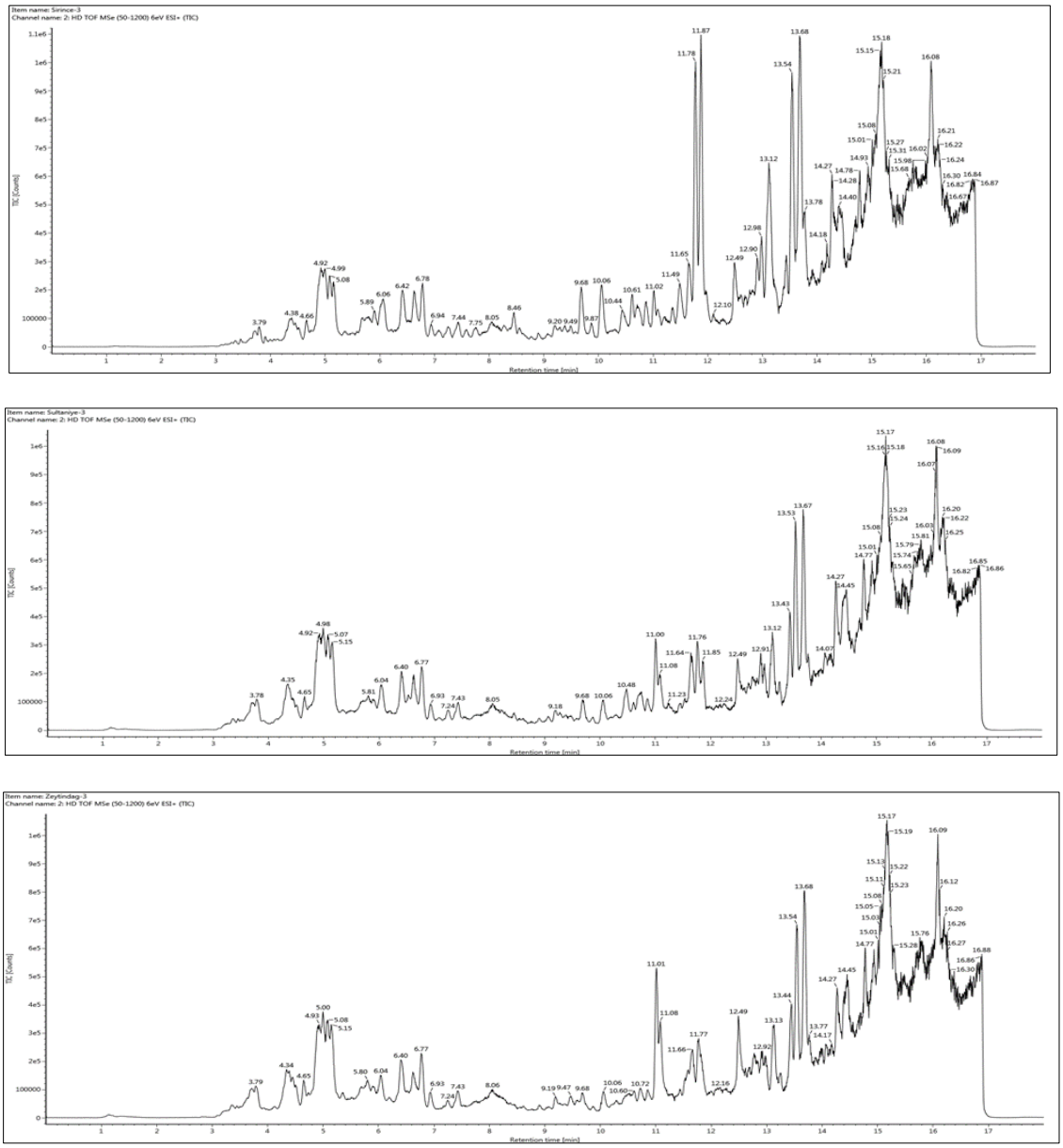
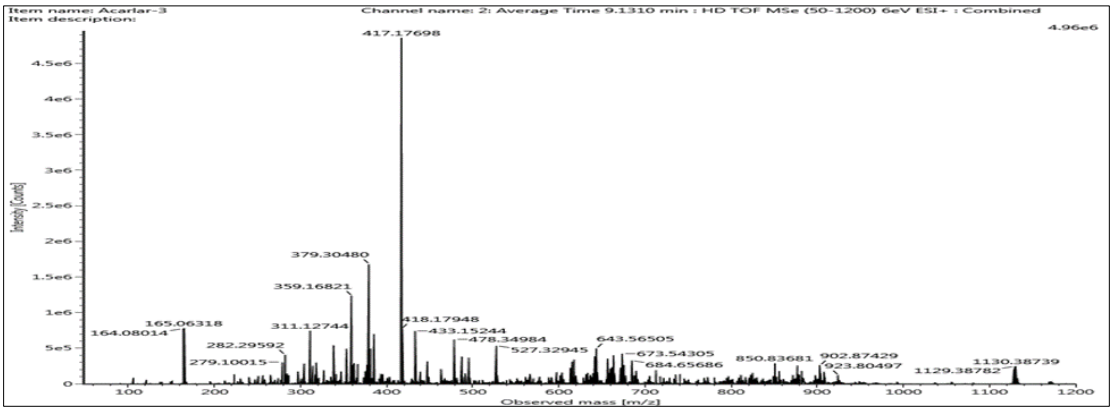


Figure 4 Peak intensity chromatograms of EVOOs (A-Acarlar, B-Gökçealan, C-Havutculu, D-Şirince, E-Sultaniye, F-Zeytindağ, G-Pool) from ultra-performance liquid chromatography–quadrupole time-of-flight MS in ESI+ ionization mode

LC IMS QTOF system was used to determine the geographical indications of EVOOs. Primarily, all samples extracted were injected into the LC system under the conditions specified in the Method. The total ion chromatograms obtained in the positive ionization mode of Selçuk region in Fig 4 are given. As can be seen in the total ion chromatograms, there are differences between the olive oil methanols: water extracts studied in the study.

3.5. Statistical analysis using Progenesis QI software

Progenesis QI software is widely used in metabolomics researches in recent years. In this study, Progenesis QI was used for multivariate statistical analysis. The spectral regions before 1.0 min and after 13 min of analysis were excluded from data evaluation.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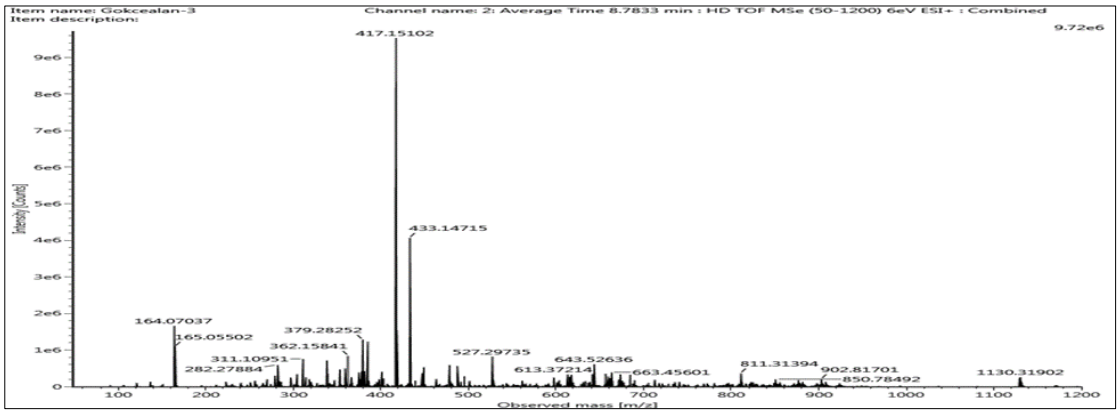
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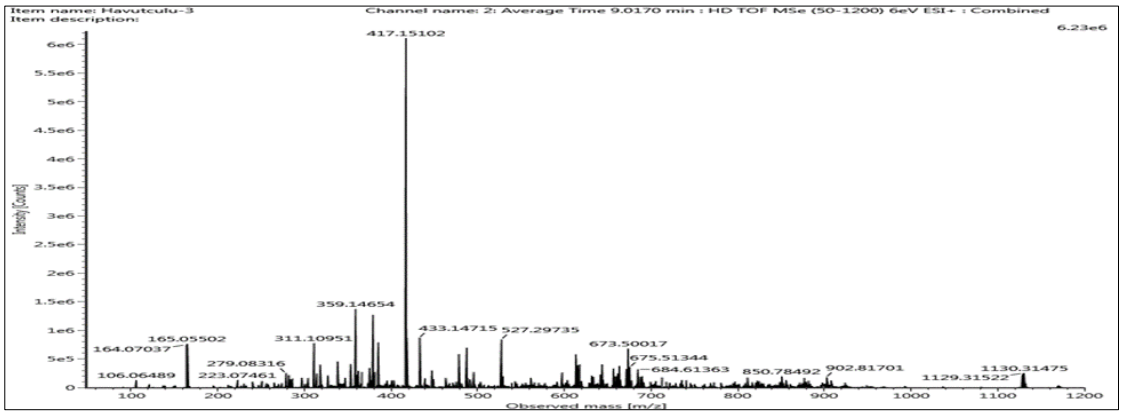
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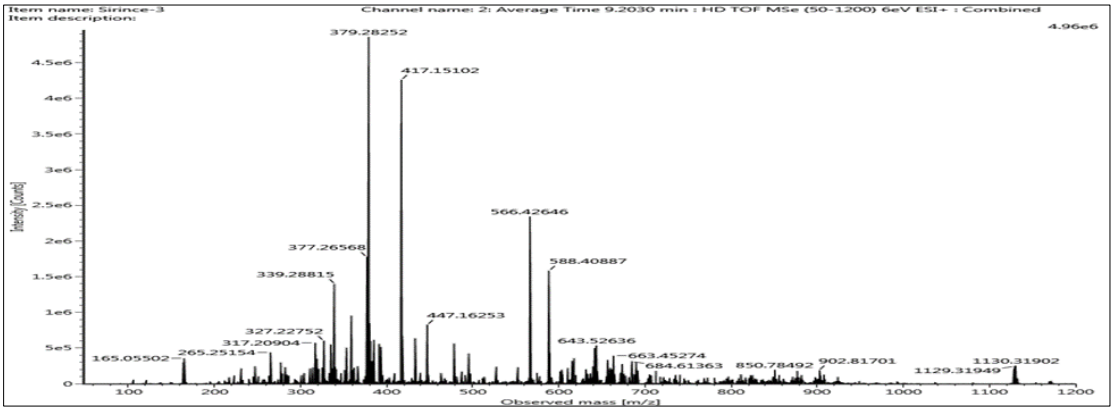
Observed mass [m/z]


Item name: Sirince-3
Item description:
Channel name: 2: Average Time 9.2030 min : HD TOF MSe (50-1200) 6eV ESI+ : Combined
4.96e6
379.28252
417.15102
566.42646
377.26568
588.40887
339.28815
447.16253
327.22752
317.20904
643.52636
265.25154
165.05502
663.45274
684.61363
850.78492
902.81701
1129.31949
1130.31902
Intensity [Counts]
Observed mass [m/z]

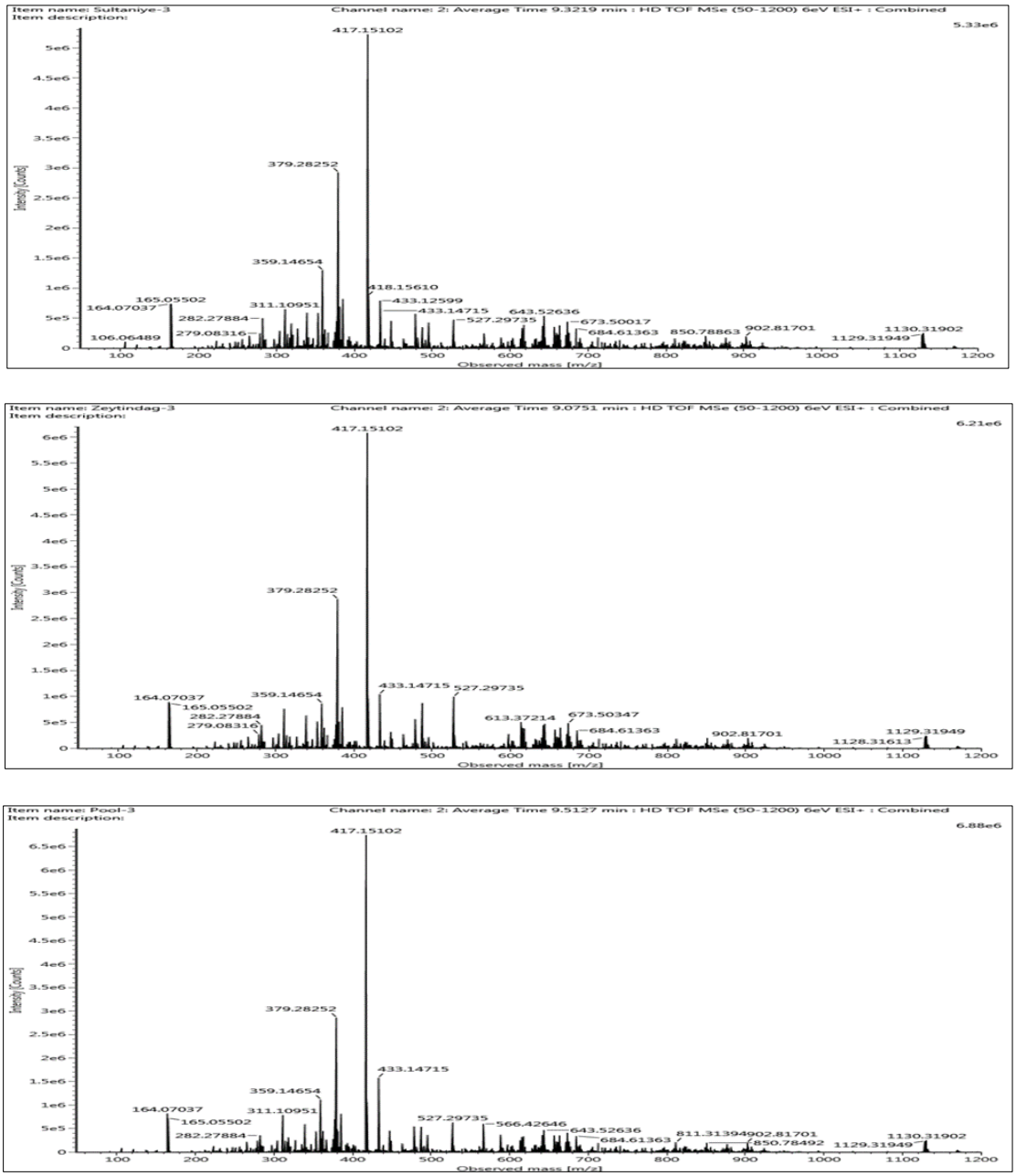


Figure 5 ESI (+) - MS spectra of olive oils from Selçuk region

ESI (+) MS spectra of Selçuk region are given in Fig 5. The MS spectrum of each olive oil sample is different from each other. While some masses gave higher intensity in some EVOOs, some showed lower intensity. In addition, some masses are found in some EVOOs but not in others. Progenesis QI software was used to reveal these differences statistically.

As can be seen in Fig 5, there are some differences among the olive oil extracts. A distinct clustering among the EVOOs was detected, which suggest that the metabolites significantly changed between different regions.

3.6. Principal Component Analysis (PCA)

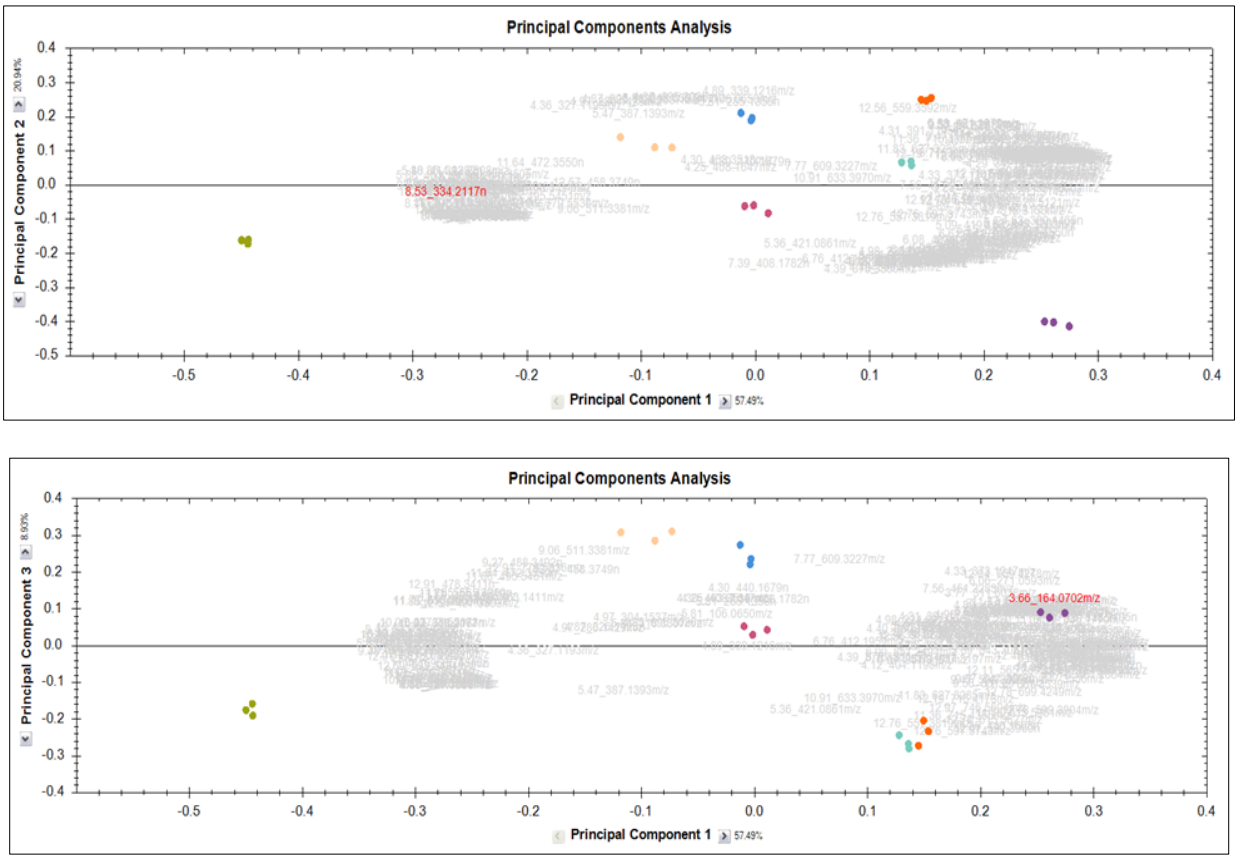


Figure 6 (A) The plot is based on the first 2 principal component for data obtained in ESI+ mode (B) The plot is based on the first and third principal component for data obtained in ESI+ mode

A: PCA1 x PCA2B: PCA1 x PCA3

Pool samples were located in the centre of the PCA pilot shows that the analytical system is reliable. The samples in the same group are clustered together.

PCA1, PCA2, PCA3 were used to show the clustering of the EVOOs. PCA1, PCA2 and PCA3 were 57.49 %, 20.94 % and 8.93 %, respectively. The total variance was determined as 87.26 % at 95 % confidence level. As can be seen in the PCA plot, it seems feasible to separate the oil samples using this method (Fig 6). The obtained data were filtered with anova p (p value≤0.05), not fragmented and max-fold change. The markers in each sample were identified using EZ Info software. In this study, we used a method for untargeted metabolomics in EVOOs. The detected masses were then subjected to library scanning. Chemspider Library, Lipid blast Library, and elemental composition (H, C, N, O, P and H, C, N, O, separately) were used for scanning. The compounds were summarized along peak number, retention time, observed m/z, empirical formula, adduct, mass error, and mSigma, isotope similarity ratio (%) score and proposed compounds in Table1. During the data processing and compounds identification, same compounds have different RT but have the same m/z ratio. These results inferred that these compounds might be isomers. As a result of the library scanning, only one isomer with the highest isotope similarity ratio is shown in the table 6.

As a result, each olive oil samples belonging to the Selçuk region is clustered in different regions. It has been determined that especially EVOOs belonging to Zeytindağ and Şirince are clustered in quite different regions from the others, and a successful distinction can be made with this research. It has been determined that different climatic and topographical conditions cause differences in the geographical origin of EVOOs. In this respect, it is recommended that EVOOs to be labeled as geographical indication should be labeled on the basis of small local region.

Table 6 Proposed marker ions identified in different regions using UPLC-ESI-Q-TOF-MS and Progenesisi-QI software

No	Regions	RT (min)	m/z	Formula	Adduct	Mass error (ppm)	mSigma Score	Isotope Similarity (%)	Proposed Compound
1	Zeytindağ	11.91	633.3970	$C_{35}H_{52}O_8$	M+CH3OH+H	-4.44	32.4	66.91	a-yldeconoate
2	Şirince	11.75	565.4201n	$C_{30}H_{55}N_5O_5$	M+H	-0.30	38.9	95.09	$C_{30}H_{55}N_5O_5$
3		10.43	368.2558n	$C_{15}H_{37}N_4O_4P$	M+Na	1.65	39.1	97.28	$C_{15}H_{37}N_4O_4P$
4		8.44	335.3845						
5		12.49	325.2109	$C_{13}H_{30}N_6O$	M+K	-1.16	39.5	98.75	$C_{13}H_{30}N_6O$
6		12.48	643.4076	$C_{33}H_{56}NO_8P$	M+NH4	-0.98	35.0	76.10	$C_{33}H_{56}NO_8P$
7		11.86	627.4332	$C_{37}H_{64}O_5$	M+K	-9.01	36.4	91.87	DG(10:0/24:4/0:0)
8		11.85	538.4307						
9		11.77	656.3941	$C_{33}H_{54}NO_8P$	M+CH3OH+H	3.00	33.5	71.17	GPCho(22:6/3:0)
10		11.76	624.4782						
11		11.76	670.4117	$C_{34}H_{56}NO_8P$	M+CH3OH+H	6.03	32.1	67.36	GPCho(22:6/4:0)
12		11.76	566.7055						
13		12.59	593.3424	$C_{34}H_{50}O_7$	M+Na	-4.37	33.4	72.04	Carbenoxolone
14		11.75	626.3641	$C_{28}H_{52}NO_{10}P$	M+CH3OH+H	-3.85	33.4	71.53	GPSer(14:1/8:0)
15		11.63	610.3879						
16		11.49	590.3663	$C_{33}H_{48}O_8$	M+NH4	-4.26	34.1	75.26	$C_{33}H_{48}O_8$
17		11.48	596.3745						
18		10.86	393.4401						
19		10.41	391.4243						
20		10.06	277.2155	$C_{12}H_{29}N_4OP$	M+H	1.19	39.1	96.81	$C_{12}H_{29}N_4OP$
21		10.03	637.4403	$C_{32}H_{61}O_8P$	M+CH3OH+H	-6.0	33.8	75.67	$C_{32}H_{61}O_8P$
22		9.48	409.2554	$C_{15}H_{34}N_{10}O$	M+K	1.49	38.8	95.59	$C_{15}H_{34}N_{10}O$
23		12.91	301.2110	$C_{14}H_{26}N_6$	M+Na	-0.57	39.5	98.43	$C_{14}H_{26}N_6$

24		4.11	396.1412n						
25		3.99	419.1303	$C_{13}H_{20}N_{10}O_4$	M+K	0.71	39.4	98.03	$C_{13}H_{20}N_{10}O_4$
26		5.43	398.1565	$C_{22}H_{20}O_6$	$M+NH_4$	-1.02	37.4	88.44	Rubrophen
27		6.17	827.3082						
28		6.17	434.1447	$C_{21}H_{20}O_9$	$M+NH_4$	0.41	55.6	80.90	$C_{21}H_{20}O_9$
29		5.89	843.3037						
30		5.89	857.3197						
31		5.46	270.1115	$C_{16}H_{12}O_{83}$	$M+NH_4$	-3.69	55.7	87.86	7-methoxy-3-phenyl-4H-chromen-4-one
32		5.43	164.0699	$C_3H_{10}N_5OP$	M+H	2.24	39.3	98.94	$C_3H_{10}N_5OP$
33	Gökçealan	4.98	362.1592	$C_{12}H_{20}N_5O_4P$	$M+CH_3OH+H$	1.36	39.2	97.82	$C_{12}H_{20}N_5O_4P$
34		4.75	392.1692	$C_{20}H_{22}O_7$	$M+NH_4$	-3.14	55.2	85.39	$C_{20}H_{22}O_7$
35		4.51	423.1012						
36		4.48	431.1302	$C_{20}H_{24}O_9$	M+Na				
37		4.40	360.1429	$C_{19}H_{18}O_6$	$M+NH_4$	-3.74	53.9	84.20	$C_{19}H_{18}O_6$
38		4.39	378.3300						
39		4.39	224.0910	$C_{11}H_{10}O_4$	$M+NH_4$	-3.51	55.6	88.16	$C_{11}H_{10}O_4$
40		4.39	286.1062	$C_{16}H_{12}O_4$	$M+NH_4$	-4.40	55.1	85.54	İsoformonenetin
41		4.12	401.1198	$C_{19}H_{22}O_8$	M+Na	-2.47	36.7	86.53	Hydroxyvernolide
42		6.93	811.3148	$C_{40}H_{52}O_{16}$	M+Na	0.06	56.4	83.95	Correolide

4. Conclusion

Different climatic and topographical conditions cause differences in the geographical origin of EVOOs. In this respect, it is recommended that EVOOs to be labeled as geographical indication should be labeled on the basis of small local region.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

Abbreviations and nomenclature

GI :Geographical Indication; PCA :Principal Component Analysis; EVOO : Extra Virgin Olive Oil; RT : Retention Time; LC: Liquid Cromatography; IMS: Ion Mobility Spectrometry.

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References

[1] Anonymous. Trade Standard Applying to Olive Oil and Olive-Pomace Oil. 2014; 1-17.

[2] Arslan D, Karabekir Y, Schreiner M. Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sariulak olive oil as induced by growing area. Food Research International. 2013; 54: 1897-1906.

[3] Bajoub A, Carrasco-Pancorbo A, Ajal EA, et al. Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of north Moroccan virgin olive oils. Food Chemistry. 2015; 166: 292-300.

[4] Bozdogan Konuskan D, Mungan B. Effects of Variety, Maturation and Growing Region on Chemical Properties, Fatty Acid and Sterol Compositions of Virgin Olive Oils. JAOCS, Journal of the American Oil Chemists' Society. 2016; 93: 1499-1508.

[5] Canözer Ö. Standart zeytin çeşitleri katoloğu. T.C. Tarım ve Köyişleri Bakanlığı. TÜGEM. Mesleki Yayınlar Genel. 1991; 334.

[6] Chtourou M, Gargouri B, Jaber H, et al. Comparative study of olive oil quality from Chemlali Sfax versus Arbequina cultivated in Tunisia. European Journal of Lipid Science and Technology. 2013; 115: 631-640.

[7] Del Monaco G, Officioso A, D'Angelo S, et al. Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile. Food Chemistry. 2015; 184: 220-228.

[8] Ouni Y, Taamalli A, Gómez-Caravaca AM, et al. Characterisation and quantification of phenolic compounds of extra-virgin olive oils according to their geographical origin by a rapid and resolutive LC-ESI-TOF MS method. Food Chemistry. 2011; 127: 1263-1267.

[9] Manai-Djebali H, Krichène D, Ouni Y, et al. Chemical profiles of five minor olive oil varieties grown in central Tunisia. Journal of Food Composition and Analysis. 2012; 27: 109-119.

[10] Morelló JR, Motilva MJ, Tovar MJ, Romero MP. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry. 2004; 85: 357-364.

[11] Sevim D. Erkence Zeytin Çeşidinden Elde Edilen Yağların (Hurmalaşmış Ve Hurmalaşmamış) Karşılaştırılması, Gıda Dergisi, Yıl 2013, Cilt 38 , Sayı 3, Unesco, (1979) New Inscribed Properties Luxor, Arab Republic of Egypt, 23-27 October, s. 2013; 159 – 166.

[12] Pardo JE, Cuesta MA, Gómez R, et al. Quality of virgin olive oil from the Designation of Origin (D.O.) Aceite Campo de Montiel (Ciudad Real, Spain) from two crop seasons. Rivista Italiana delle Sostanze Grasse. 2012; 89: 103-111.

[13] Piravi-Vanak Z, Ghasemi JB, Ghavami M, et al. The Influence of Growing Region on Fatty Acids and Sterol Composition of Iranian Olive Oils by Unsupervised Clustering Methods. Journal of the American Oil Chemists Society. 2012; 89: 371-378.

[14] Portarena S., Gavrichkova O., Lauteri M., Brugnoli E. Authentication and traceability of Italian extra-virgin olive oils by means of stable isotopes techniques. Food Chem. 2014; 164: 12–16.