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Characterisation of Phenolic Compounds, Sterols and Geographical Fingerprint of Çekişte Extra-Virgin Olive Oils According to their Geographical Locations by Using LC IMS QTOF Mass Spectrometry

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Abstract

In this context, aim of this study is to determine the effect of Çekişte olive oils in different locations and show differences on geographical locations while taking geographical indication label. Çekişte olive oil variety which cultivated in six different locations (Birgi, Bademli, Beyazit, Yeniceköy, Zeytinlik, Uzumlu) 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 some locations differ significantly depending on olive growing area ($p < 0.05$), some of them not. 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, Çekişte variety, Geographical indication label.

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Ethical: This study follows all ethical practices during writing.

Contents

Contribution of this paper to the literature

There are a lot of studies carried out to differ EVOOs from geographical regions according to various chemical characterizations in Turkey. However, there is not any research on the effect of the geographical location on the chemical characterization of the Çekişte variety. In this study, 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.

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.). There are more factors affecting chemical composition, physico-chemical quality, sensory properties of olive oil. These are environmental factors (topography, ecological zone, humidity, altitude, climate), agronomic factors (irrigation, pruning, pesticide application, fertilization, harvesting time, ripening index) and post harvest factors (oil extraction system, oil storage conditions) [1].

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 [2]. 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 %. 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 these tools is LC IMS Qtof mass spectrometer and Progenesis QI software [3]. Çekişte 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.

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 charecterization of the Çekişte 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 affect 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 Çekişte olive oils in different locations (Birgi, Bademli, Beyazıt, Yeniceköy, Zeytinlik, Üzümlü) 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 Çekişte variety (~ 2,5 kg for each sample) were collected by hand randomly from 6 different locations (Birgi, Beyazıt, Bademli, Yeniceköy, Zeytinlik, Üzümlü) and 9 trees in each location. Olive fruits (2,04-4,05) were collected at the ripening index.

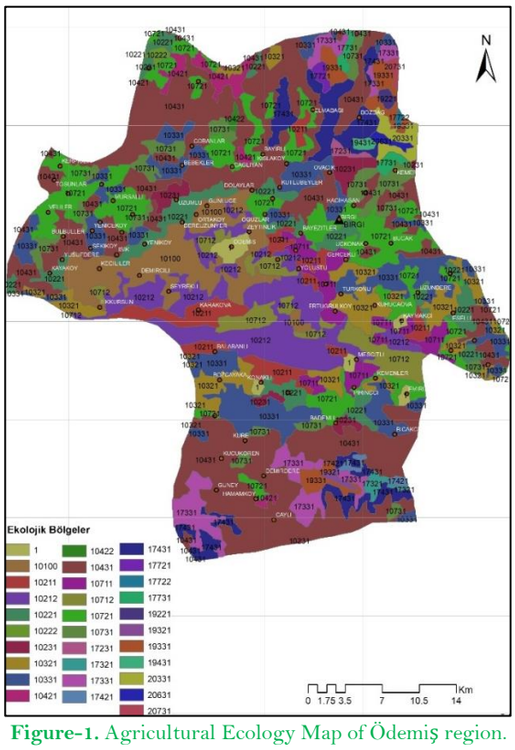


Figure-1. Agricultural Ecology Map of Ödemiş region.

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

Agricultural ecology maps of the locations were created with remote sensing methods (Figure 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 system.

Table-1. Agricultural ecological map information of Ödemiş Region.

Selçuk Region	Ecological Map	Altitude	Drought Index	Warmest Average	Coldest Average	Slope
Birgi location	10721	500-900 m	0.75-1 (humid)	20-30 °C	0-10 °C	%2-12
Bademli location	10731	300-500 m	0.5-0.75 (semi humid)	20-30 °C	0-10 °C	%12-30
Beyazit location	10721	500-900 m	0.75-1 (humid)	20-30 °C	0-10 °C	%2-12
Yeniceköy location	10331	300-500 m	0.5-0.75 (semi humid)	20-30 °C	0-10 °C	%2-12
Zeytinlik location	10100	100-300 m	0.5-0.75 (semi humid)	>20 °C	0-10 °C	%2-12
Uzumlu location	10221	100-300 m	0.5-0.75 (semi humid)	20-30 °C	0-10 °C	%2-12

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 (Birgi, Bademli, Beyazıt, Yeniceköy, Zeytinlik, Üzümlü) and olive oils were used with "Abencor" system for extracted. EVOO were stored in dark glass bottles at 4 °C.

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 [4].

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.

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.

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 aceate 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 % fort he 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 Tables 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.

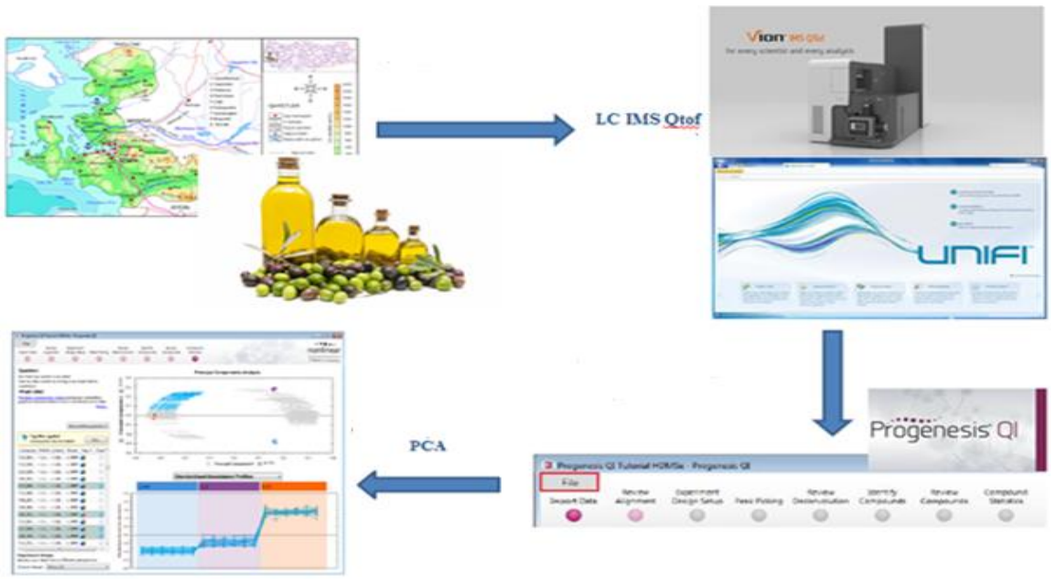


Figure-2. LC IMS Qtof Screening use phase.

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 °C	Analyzer mode: Sensitivity Capillary voltage: 3.0 kV Source temperature: 120 °C Desolvation temperature: 400 °C Cone gas: 50 L/h Desolvation gas: 1000 L/h	Temperature: 30 °C	Scan settings: 50 m/z – 1200m/z High Deffinition 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

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 sofware. 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 107.319 to 180.892 mg/kg^{-1} in olive oils.

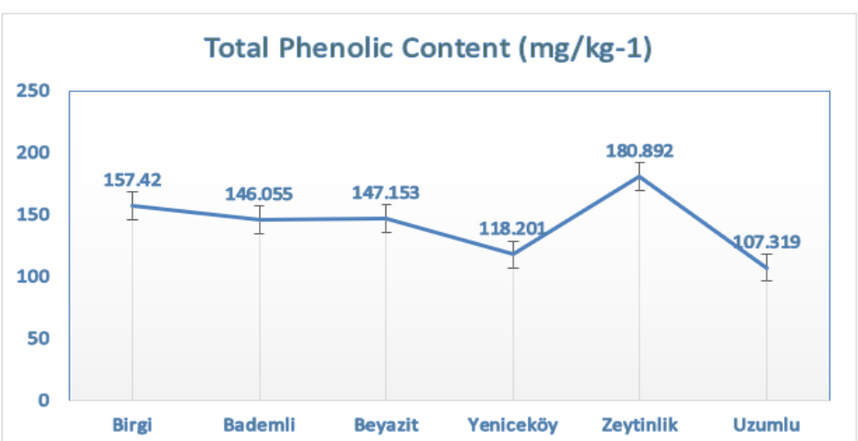


Figure-3. Total phenol contents in olive oil samples from Çekişte variety in six locations in the west of Turkey. Results are shown as means ± SD (n = 5). a-b Different letters indicate significantly different values at $p < 0.05$ according to Duncan test.

EVOOs of Zeytinlik locations have highest phenolic content (180.892 mg/kg-1). Zeytinlik location was showed that geographical location have a significant effect on the total phenolic content Figure 3. Del Monaco, et al. [5] 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.

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. There are significant differences among geographical regions in terms of different phenolic compounds in this study and also in previous studies. Bajoub, et al. [6] 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 Çekişte olive oil samples. As shown in Table 4, 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 79.09 % (Zeytinlik), 77.33 % (Bademli). Palmitic acid content is respectively 12.52 % (Bademli), 11.80 % (Üzümlü), 11.76% (Zeytinlik). The highest linoleic acid content was in Zeytinlik location (79.31 %). Fatty acid composition was found different among geographical locations (Table 4). Morelló, et al. [7] studied that fatty acid changes due to genetic factors as well as environmental conditions. Piravi-Vanak, et al. [8] found that fatty acid composition of EVOO is significantly affected by topographical and climatic conditions.

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

Ödemiş Region		Birgi	Bademli	Beyazıt	Yeniceköy	Zeytinlik	Üzümlü
1	Miristik Acid (C14:0)	0.01 ± 0.03^{a}	0.01 ± 0.04^{a}	0.01 ± 0.04^{a}	0.01 ± 0.05^{a}	0.01 ± 0.02^{a}	0.01 ± 0.04^{a}
2	Palmitik Acid (C16:0)	11.58 ± 0.12^{e}	12.52 ±0.08^{a}	11.64 ± 0.07^{d}	11.37 ± 0.05^{f}	11.76 ± 0.12^{c}	11.80 ± 0.06^{b}
3	Palmitoleik Acid (C16:1)	0.81 ± 0.06^{d}	0.87 ± 0.04^{b}	0.86± 0.13^{c}	1.01± 0.08^{a}	0.88± 0.05^{a}	0.78± 0.04^{e}
4	Heptadekanoik Acid (C17:0)	0.14± 0.07^{d}	0.18± 0.12^{b}	0.15± 0.11^{c}	0.13± 0.12^{e}	0.15± 0.13^{c}	0.19± 0.09^{a}
5	Heptadesenoik Acid (C17:1)	0.35± 0.07^{b}	0.35± 0.02^{b}	0.34± 0.04^{c}	0.33± 0.07^{d}	0.33± 0.06^{d}	0.39± 0.04^{a}
6	Stearik Acid (C18:0)	2.25± 0.11^{b}	2.56± 0.08^{a}	2.06± 0.13^{d}	1.95± 0.12^{e}	2.14± 0.09^{c}	2.21± 0.08^{b}
7	Oleik Acid (C18:1)	78.60± 0.06^{b}	77.33± 0.03^{d}	78.64± 0.03^{bc}	79.09± 0.06^{b}	79.31± 0.05^{a}	78.45± 0.08^{c}
8	Linoleik Acid (C18:2)	5.03± 0.04^{a}	4.83± 0.09^{b}	5.03± 0.09^{a}	4.83± 0.05^{b}	4.35± 0.04^{c}	4.83± 0.06^{b}
9	Linolenik Acid (C18:3)	0.69± 0.02^{d}	0.79± 0.05^{b}	0.79± 0.03^{b}	0.78± 0.07^{c}	0.62± 0.05^{e}	0.81± 0.03^{a}
10	Araşidik Acid (C20:0)	0.31± 0.12^{a}	0.29± 0.09^{b}	0.28± 0.07^{c}	0.26± 0.08^{e}	0.26± 0.05^{e}	0.27± 0.03^{d}
11	Gadoleik/eikosenoik Acid (C 20:1)	0.22± 0.04^{c}	0.25± 0.07^{b}	0.17± 0.12^{e}	0.22± 0.08^{c}	0.19± 0.06^{d}	0.26± 0.05^{a}
12	Behenik Acid (C 22:0)	ND	ND	ND	0.04± 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

Note: 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.

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-5-24-stigmastadienol in EVOO (Table 5). As shown in Table 5, phytosterol are mostly depending on the geographical location of EVOO. In the Çekişte 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 Üzümlü location (86.70 %), then Bademli (84.53 %) and Birgi location (84.33 %). Regarding the content of Δ-5-avenasterol, Zeytinlik location showed the highest value (10.87 %), while it was the lowest (7.83 %) in Üzümlü location.

Table-5. Phytosterol composition (%) of EVOOs in 6 different locations in Turkey (Birgi, Bademli, Beyazıt, Yeniceköy, Zeytinlik, Üzümlü).

Locations	Campesterol	Stigmasterol	Δ5-24 Stigmastadienol	β -Stosterol	Δ5-Avenasterol	Clerosterol	Apparent β -STEROL
Bademli	2.38± 0.02^{ef}	0.62± 0.00^{d}	0.70± 0.00^{b}	84.53± 0.05^{b}	10.00± 0.02^{d}	0.44± 0.00^{d}	94.46± 0.00^{b}
Beyazıt	2.39± 0.00^{e}	0.53± 0.02^{e}	0.52± 0.05^{e}	84.30± 0.02^{b}	10.54± 0.00^{b}	0.68± 0.05^{b}	93.26± 0.02^{c}
Birgi	2.65± 0.01^{c}	0.50± 0.00^{f}	0.66± 0.03^{c}	84.33± 0.00^{b}	9.79± 0.14^{e}	0.78± 0.03^{a}	94.92± 0.01^{b}
Üzümlü	2.85± 0.03^{a}	0.77± 0.08^{a}	0.43± 0.11^{f}	86.70± 0.01^{a}	7.83± 0.03^{f}	0.38± 0.11^{e}	93.43± 0.13^{c}
Yeniceköy	2.59± 0.00^{d}	0.64± 0.03^{c}	0.87± 0.06^{a}	83.82± 0.03^{c}	10.14± 0.00^{e}	0.55± 0.06^{c}	93.18± 0.07^{cd}
Zeytinlik	2.70± 0.04^{b}	0.69± 0.01^{b}	0.63± 0.02^{d}	83.24± 0.00^{c}	10.87± 0.05^{a}	0.30± 0.02^{e}	95.48± 0.03^{a}

Note: 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.

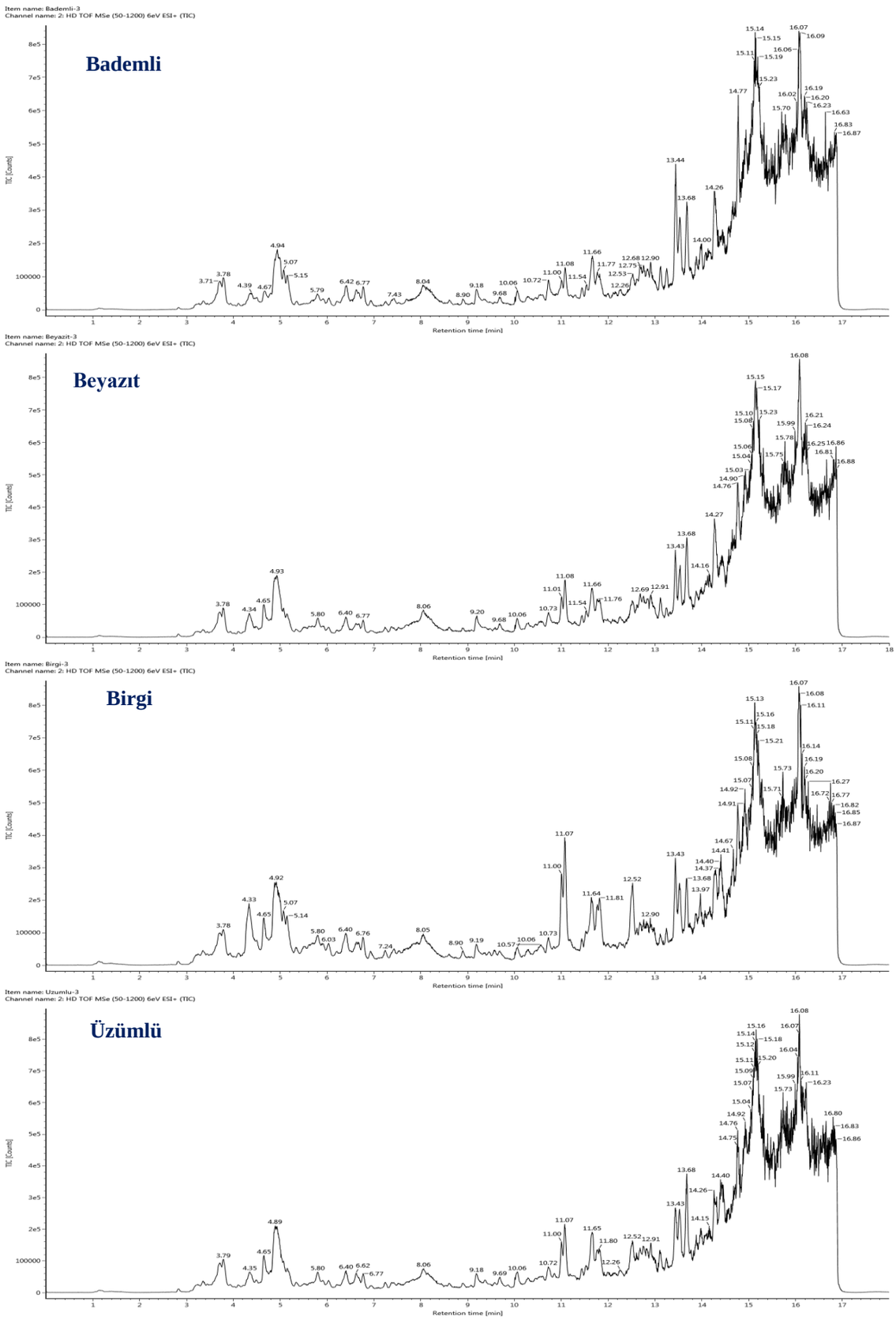
Çekişte EVOOs has low stigmastereol and campesterol content. In all EVOOs, the campesterol content was found below the limit between 2.85 % (Üzümlü) and 2.70 % (Zeytinlik) according to EU Regulations (4%).

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 (Δ5-24-stigmastadienol, clerosterol and Δ-5-avenasterol). Çekişte EVOO are found approximately the limit of 94 %. The highest apparent β-sitosterol (95.48 %) was detected in Zeytinlik location Table 5. In EVOOs, the content of stigmasterol is lower than campesterol as previous research [9].

In this study, different phytosterol content revealed in Çekişte 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 [10]. This result 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 Figure 4.



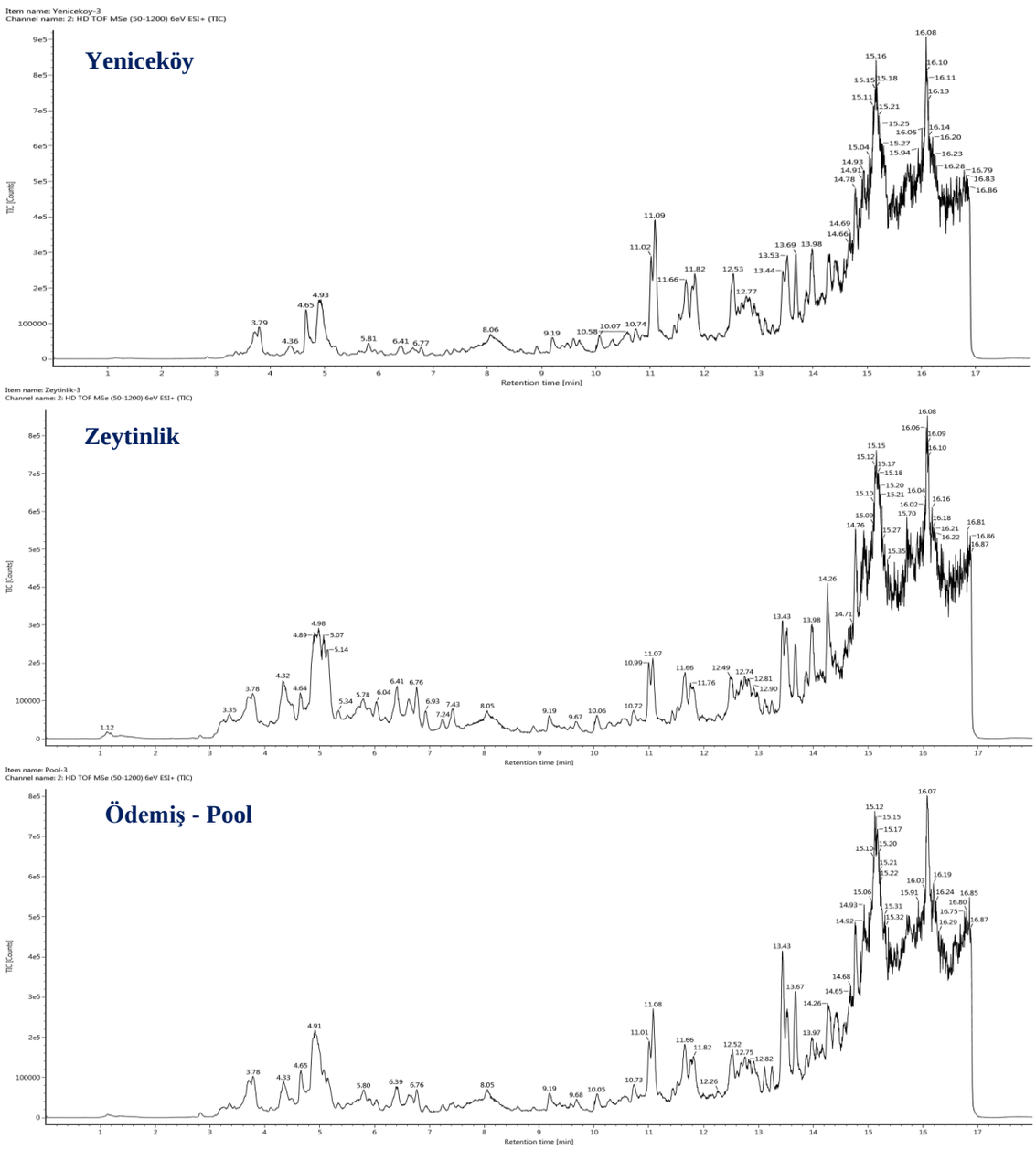
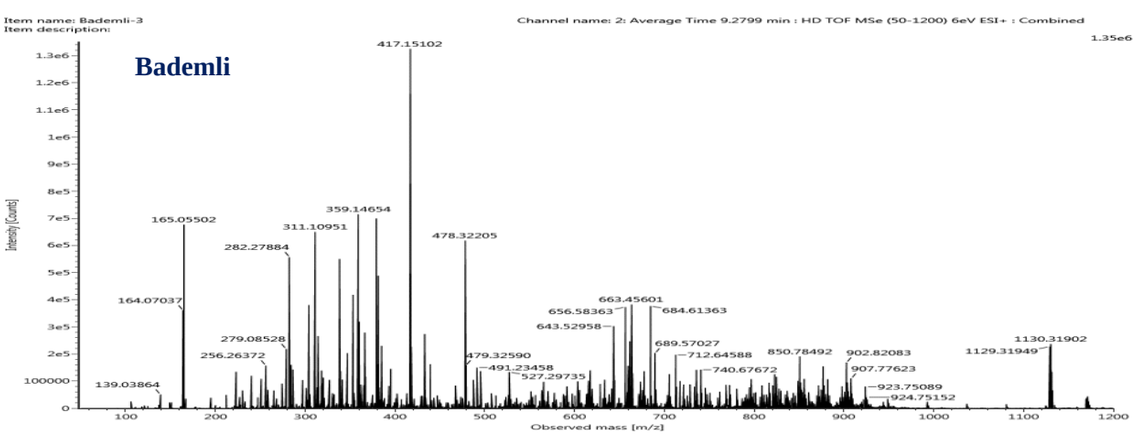


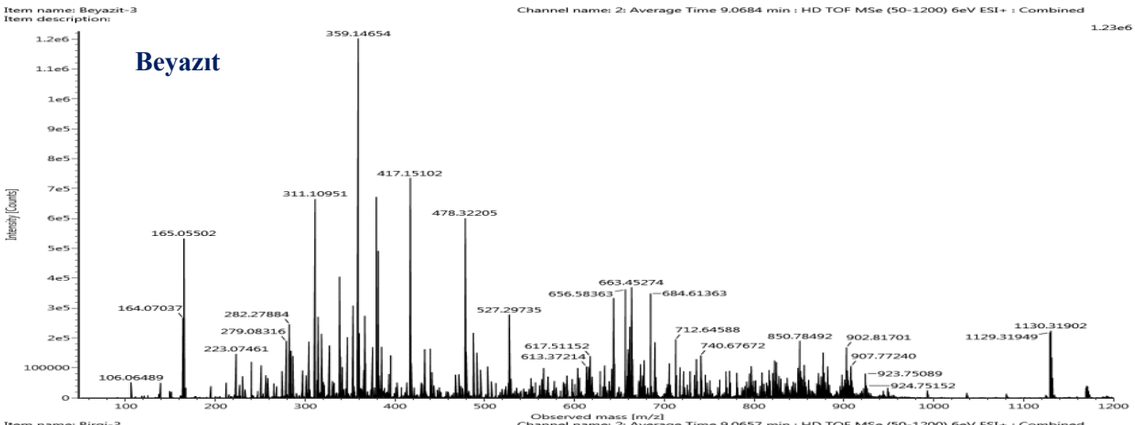
Figure-4. Peak intensity chromatograms of EVOOs (A-Acarlar, B-Gökçealan, C-Havutculu, D-Şirince, E-Sultaniye, F-Zeytindağ, G-Pool) from ultra-performance liquid chromatography–quadrupole time-of-flight MS in ESI+ ionization mode.

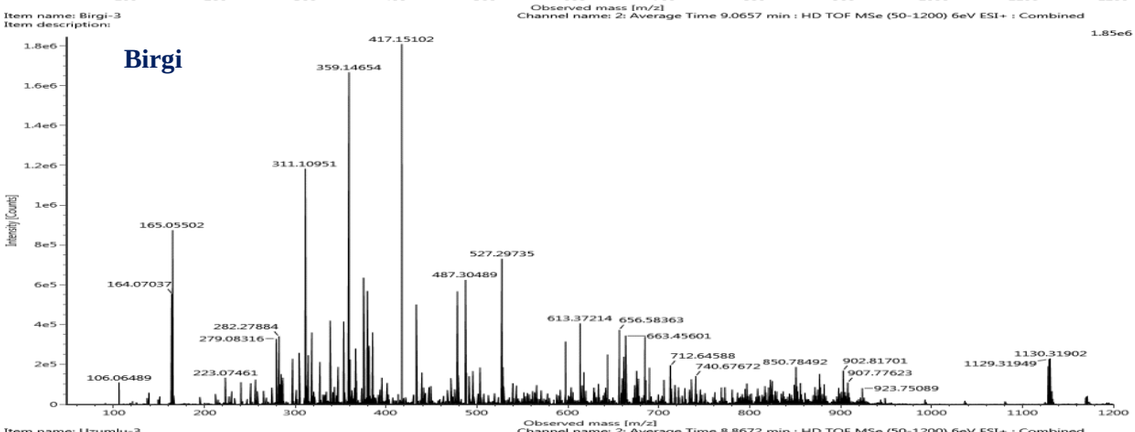
LC IMS Qtof system was used to determine the geographical indications of EVOOs. Primarily, all samples extracted were injected into the LC system under the conditions specified in the Method. The total ion chromatograms obtained in the positive ionization mode of Ödemiş region in Figure 4 are given.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

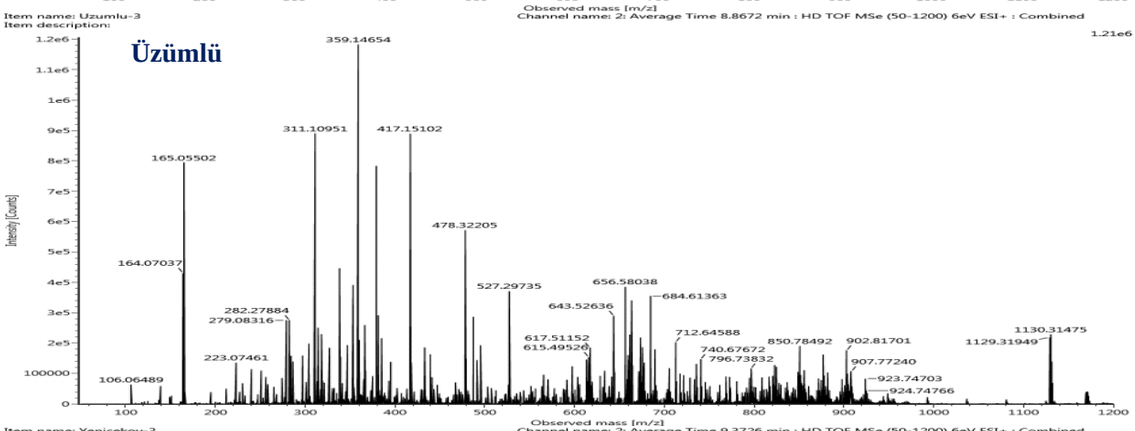
3.5. Statistical Analysis using Progenesis QI Software

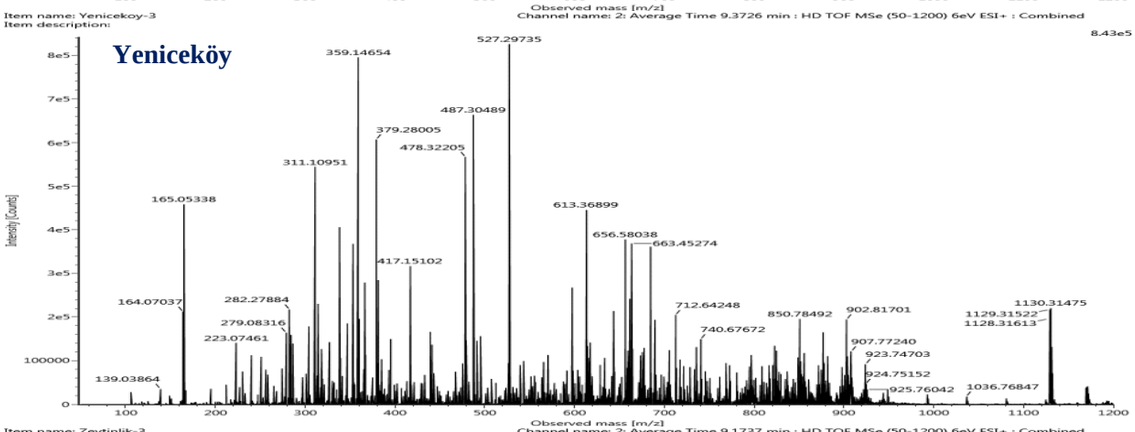
Progenesis QI software is widely used in metabolomics researches in recent years. In this study, Progenesis QI was used for multivariate statistical analysis. The spectral regions before 1.0 min and after 13 min of analysis were excluded from data evaluation.

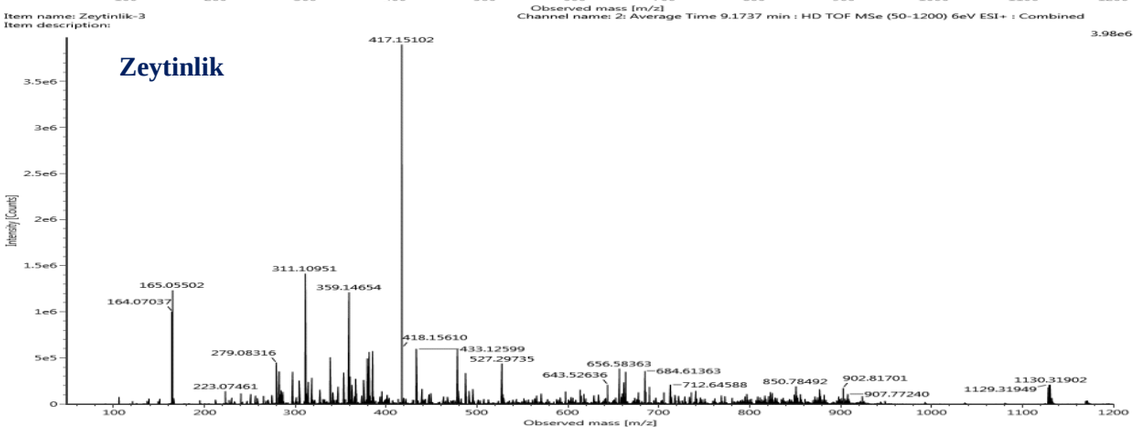












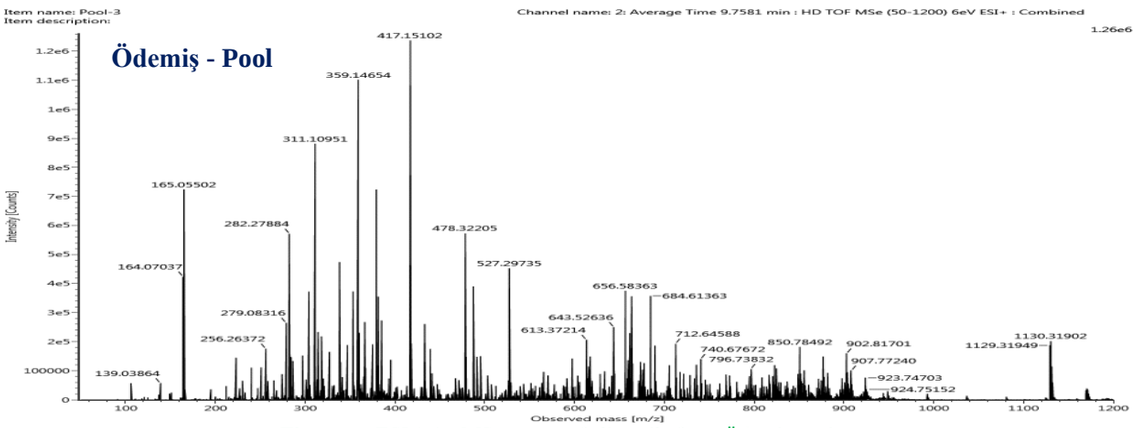


Figure-5. ESI (+) - MS spectra of olive oils from Ödemiş region.

ESI (+) MS spectra of Selçuk region are given in Figure 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 Figure 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 region.

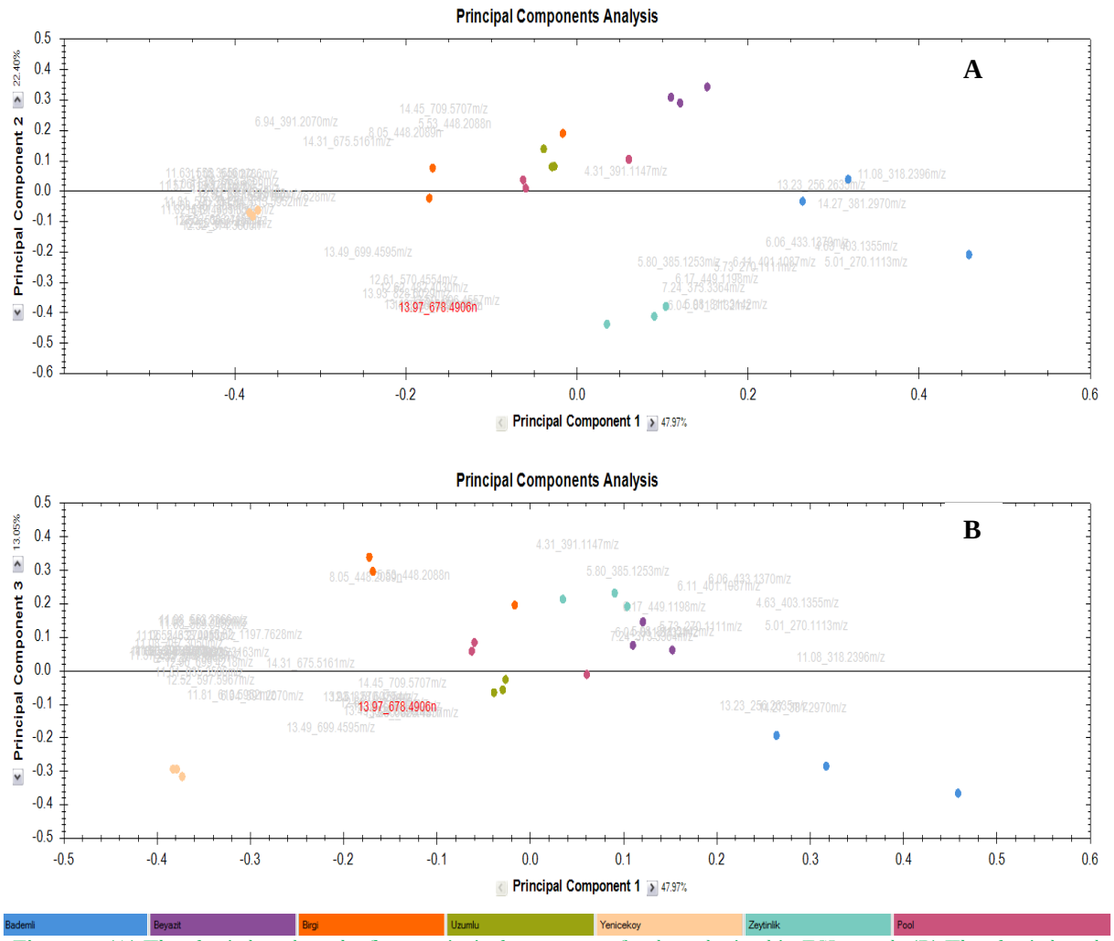


Figure-6. (A) The plot is based on the first 2 principal component for data obtained in ESI+ mode (B) The plot is based on the first and third principal component for data obtained in ESI+ mode.
Note: A: PCA1 x PCA2 B:PCA1 x PCA3.

3.6. Principal Component Analysis (PCA)

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 47.97 %, 22.40 % and 13.05 %, respectively. The total variance was determined as 83.42 in the 95 % confidence level. As can be seen in the PCA plot, it seems feasible to seperate the oil samples using this method (Figure 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, Lipidblast 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 Table 1. During the data processing and compounds identification, same compounds have different RT but have the same m/z ratio.

As a result, each olive oil samples belonging to the Ödemiş region is clustered in different regions. It has been determined that especially EVOOs belonging to Zeytinlik 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.

Abbreviatıons and Nomenclature

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

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