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Original Research Article

Effects of Geographical Location on Chemical Characterization of Erkence Ekstra Virgin Olive Oil in the West of Turkey

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Abstract: Erkence olive oil variety which cultivated in six different locations (Gödence, Orhanlı, Kavakdere, Gölcük, Ulamış, Beyler) were evaluated the effects of geographical locations on the chemical characterization of in the Seferihisar region in the west of Turkey**.** The agricultural ecological map of each location was created using GIS (Geographic İnformation System). Olive oil samples were analyzed fatty acid, sterol and phenolic. In addition, LC IMS Qtof spectrometer and Progenesis QI software were used to determine the geographical fingerprints of olive oil samples in different locations. In general, oil qualities of all locations differ significantly depending on olive growing area ($p < 0.05$). Statistical analysis show that olive oil compositions vary significantly by region. The Principal Component Analysis of the different locations analyzed revealed that "geographical location" factor significantly affects the olive oil quality.

Keyword: Geographical location, olive oil, sterols, phenolic compounds, LC IMS Qtof spectometer.

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.) [1].

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) [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 [3]. 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 % [4]. 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 these tools is LC IMS Qtof mass spectrometer and Progenesis QI software [5].

Erkence olive variety, dominated by the western part of Turkey and the coastal part, has a long history. The oil properties of the Erkence olive variety are quite high [6].

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 Erkence variety which cultivated in the west 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

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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 Erkence olive oils in different locations (Ulamış, Gölcük, Gödence, Kavakdere, Orhanlı, Beyler) and show differences on geographical locations while taking geographical indication label.

2. MATERİAL AND METHODS

2.1. Fruit Samples

The research was conducted throughout 2020/2021 olive season. Olive fruit samples of the Erkence variety (~ 2,5 kg for each sample) were collected by hand randomly from 6 different locations (Ulamış, Gölcük, Gödence, Kavakdere, Orhanlı, Beyler) 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 (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 UNESCO (1979) (7) system.

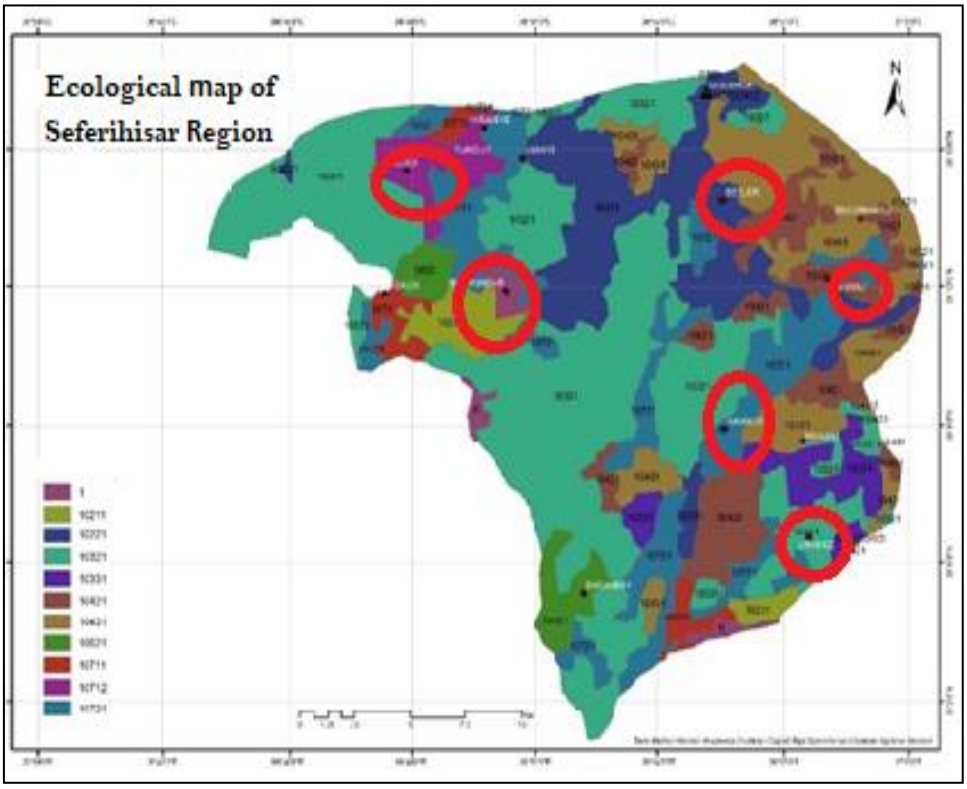


Fig-1: Agricultural Ecology Map of Seferihisar region

Table-1: Agricultural ecological map information of Seferihisar Region

Location (Seferihisar)	Altitute	Ecologic Zone	Drought Index	Warmest average	Coldest average	Slope
Gödence	300-500 m	10221	0,5-0,75(semi humid)	>20 ^{o}C	0-10 ^{o}C	%2-12
Beyler	300-500 m	10221	0,5-0,75(semi humid)	>20 ^{o}C	0-10 ^{o}C	%2-12
Gölcük	300-500 m	10321	0,5-0,75(semi humid)	>20 ^{o}C	0-10 ^{o}C	%2-12
Orhanlı	300-500 m	10421	0,5-0,75(semi humid)	>20 ^{o}C	0-10 ^{o}C	%2-12
Kavakdere	0-100 m	10721	0,75-1(humid)	20-30 ^{o}C	0-10 ^{o}C	%2-12
Ulamış	0-100 m	10721	0,75-1 (humid)	20-30 ^{o}C	0-10 ^{o}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 (Ulamış, Gölcük, Gödence, Kavakdere, Orhanlı, Beyler) and olive oils were used with "Abencor" system for extracted. EVOO were stored in dark glass bottles at 4 ^{o}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 (Anonymous, 2014) [8].

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.

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 (Table 2 and 3).

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 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

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.

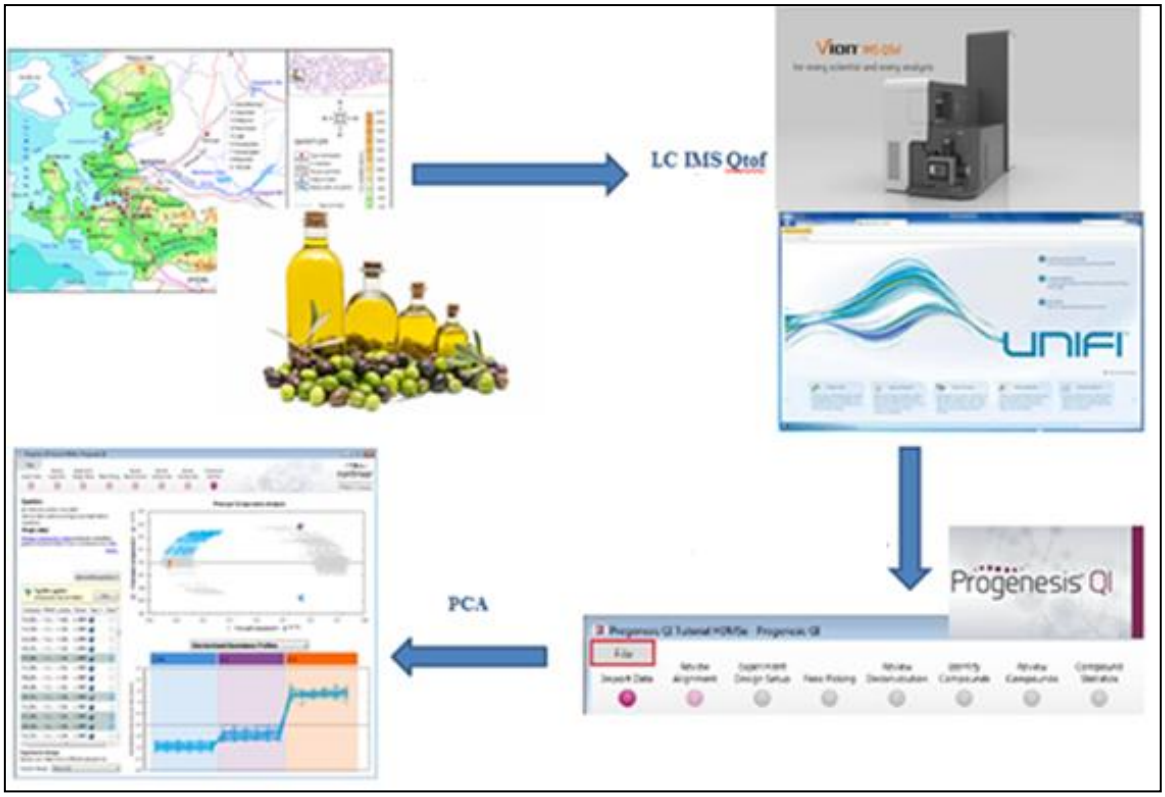


Fig-2: Flow chart of this study conducted to determine marker ions in olive oils

2.8. STATİSTİCAL ANALYSİS

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 DİSCUSSİON

3.1. Total Phenolic Content

The total amount of phenolic matter was determined in EVOO. Statistical analysis are found significant differences ($p < 0.05$) in total phenol contents among oil samples. Total phenol content ranges from 138.539 to 379.943 mg / kg in olive oils.

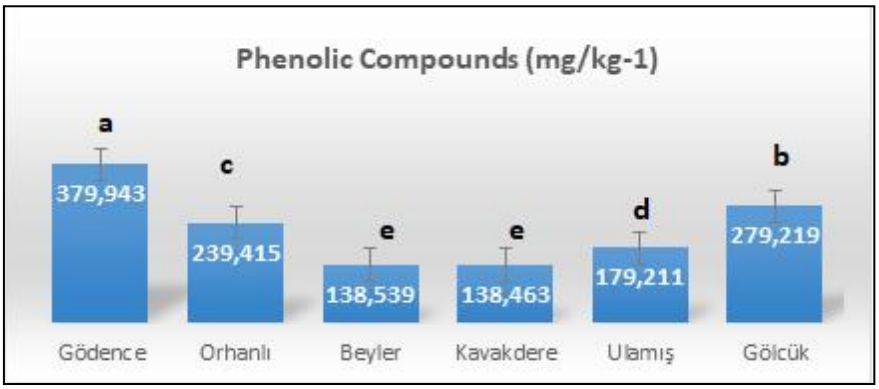


Fig-3: Total phenol contents in olive oil samples from Erkence variety in six locations in the west of Turkey. Results are shown as means ± SD (n = 5). a-b Different letters indicate significantly different values at $p < 0.05$ according to Duncan test

EVOOs of Gödence and Gölcük locations have highest phenolic content (379.943 and 279.219 mg/kg-1, respectively). Gödence location was showed that geographical location have a significant effect on the total phenolic content (figure 3). Del Monaco et al. (2015) [4] 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 [2]. There are significant differences among geographical regions in terms of different phenolic compounds in this study and also in previous studies [9]. Bajoub *et al*. (2015) [10] 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 Erkence 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.

Table-5: Fatty acid composition (%) of EVOOs in six different geographic locations in the west of Turkey

Seferihisar Region Locations		Gödence	Orhanlı	Beyler	Kavakdere	Ulamış	Gölcük
1	Miristik Asit (C14:0)	0,01 ± 0.05^{a}	0,02 ± 0.03^{a}	0,01 ± 0.04^{a}	0,01 ± 0.05^{a}	0,02 ± 0.03^{a}	0,01 ± 0.04^{a}
2	Palmitik Asit (C16:0)	12,66 ± 0.13^{d}	13,54 ±0.09^{c}	14,47 ± 0.08^{b}	14,76 ± 0.05^{b}	15,56 ± 0.12^{a}	12,29 ± 0.06^{d}
3	Palmitoleik Asit (C16:1)	0.74 ± 0.08^{c}	0,76 ± 0.05^{c}	1,15± 0.03^{b}	1,38± 0.07^{a}	1,02± 0.05^{b}	0,55± 0.03^{d}
4	Heptadekanoik Asit(C17:0)	0,13± 0.09^{a}	0,10± 0.13^{b}	0,08± 0.11^{c}	0,11± 0.12^{b}	0,11± 0.13^{b}	0,07± 0.09^{c}
5	Heptadesenoik Asit (C17:1)	0,23± 0.08^{b}	0,19± 0.05^{c}	0,26± 0.05^{a}	0,20± 0.08^{c}	0,14± 0.09^{d}	0,12± 0.04^{d}
6	Stearik Asit (C18:0)	2,39± 0.11^{b}	1,86± 0.09^{d}	2,57± 0.08^{a}	2,32± 0.13^{c}	2,43± 0.13^{a}	2,38± 0.08^{b}
7	Oleik Asit (C18:1)	70,76± 0.09^{c}	68.07± 0.04^{d}	74,79± 0.03^{a}	74,68± 0.07^{a}	67,71± 0.05^{d}	73,26± 0.06^{b}
8	Linoleik Asit (C18:2)	11,68± 0.12^{b}	14,08± 0.11^{a}	5,87± 0.09^{d}	5,40± 0.05^{d}	11,92± 0.04^{b}	10,01± 0.07^{c}
9	Linolenik Asit (C18:3)	0,88± 0.05^{b}	0,90± 0.06^{a}	0,70± 0.03^{d}	0,68± 0.08^{e}	0,54± 0.05^{f}	0,82± 0.03^{c}
10	Araşidik Asit (C20:0)	0,28± 0.12^{b}	0,24± 0.09^{c}	0,06± 0.07^{d}	0,32± 0.08^{a}	0,34± 0.05^{a}	0,29± 0.03^{b}
11	Gadoleik/eikosenoik Asit (C 20:1)	0,19± 0.05^{a}	0,19± 0.07^{a}	0,02± 0.13^{e}	0,12± 0.08^{d}	0,15± 0.06^{c}	0,18± 0.05^{b}
12	Behenik Asit /C 22:0)	0,03± 0.08^{c}	0,04± 0.13^{b}	ND	ND	0,07± 0.09^{a}	0,03± 0.11^{c}
13	Lignoserik Asit (C24:0)	ND	ND	ND	ND	ND	ND
14	Trans Oleik Asit (C18:1T)	ND	ND	ND	ND	ND	ND
15	Trans Linoleik Asit +Trans Linolenik Asit (C18:2 T+C18:3 T)	0,008± 0.08^{c}	0,01± 0.13^{a}	0,004± 0.11^{b}	ND	0,01± 0.02^{a}	0,004± 0.03^{b}

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

The contents of oleic acid is between 68.07% (Orhanlı), 74.79% (Beyler). Palmitic acid content is respectively 12.29% (Gölcük), 14.76% (Kavakdere), 14.47% (Beyler). The highest linoleic acid content was in Orhanlı location (14.08 %). Fatty acid composition was found different among geographical locations (Table 5).

Morelló (2004) [11] studied that fatty acid changes due to genetic factors as well as environmental conditions. Piravi-Vanak *et al*. (2012) [12] 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-5,24-stigmastadienol in EVOO (Table 6). As shown in Table 6, phytosterol are mostly depending on the geographical location of EVOO. In the Erkence 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 Beyler location (86.7 %), then Kavakdere (86.3%) and Orhanlı location (85.6%). Regarding the content of-5-avenasterol, Gölcük location showed the highest value (19.1 %), while it was the lowest (5.3%) in Beyler location.

Table-6: Phytosterol composition (%) of EVOOs in 6 different locations in Turkey (Ulamış, Gölcük, Gödence, Kavakdere, Orhanlı, Beyler)

Locations	Campesterol	Stigmasterol	Δ5-24 Stigmastadienol	β -stosterol	Δ5-Avenasterol	Clerosterol	Apparent β -STEROL
Beyler	2,34± 0.02^{c}	1,38± 0.00^{a}	0,62± 0.00^{f}	86,70± 0.05^{a}	5,27± 0.02^{e}	1,08± 0.00^{a}	93,68± 0.00^{c}
Gödence	2,63± 0.00^{b}	0,46± 0.02^{d}	0,86± 0.05^{b}	84,33± 0.02^{d}	7,57± 0.00^{b}	0,76± 0.03^{d}	93,53± 0.02^{c}
Gölcük	1,96± 0.01^{de}	0,55± 0.00^{c}	0,80± 0.03^{c}	73,74± 0.00^{f}	19,15± 0.14^{a}	0,68± 0.05^{e}	94,36± 0.01^{a}
Kavakdere	2,11± 0.03^{d}	1,25± 0.08^{b}	0,72± 0.11^{d}	86,29± 0.01^{ab}	6,13± 0.03^{d}	0,99± 0.09^{b}	94,13± 0.12^{b}
Orhanlı	2,34± 0.00^{c}	0,58± 0.03^{c}	0,66± 0.06^{e}	85,63± 0.03^{c}	7,11± 0.00^{bc}	0,81± 0.02^{c}	94,21± 0.07^{ab}
Ulamış	3,13± 0.04^{a}	0,41± 0.01^{d}	1,47± 0.02^{a}	85,13± 0.00^{cd}	7,03± 0.05^{c}	0,49± 0.00^{f}	94,12± 0.03^{b}

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-c Different letters indicate significantly different values ($p < 0.05$) in the same row according to Duncan test.

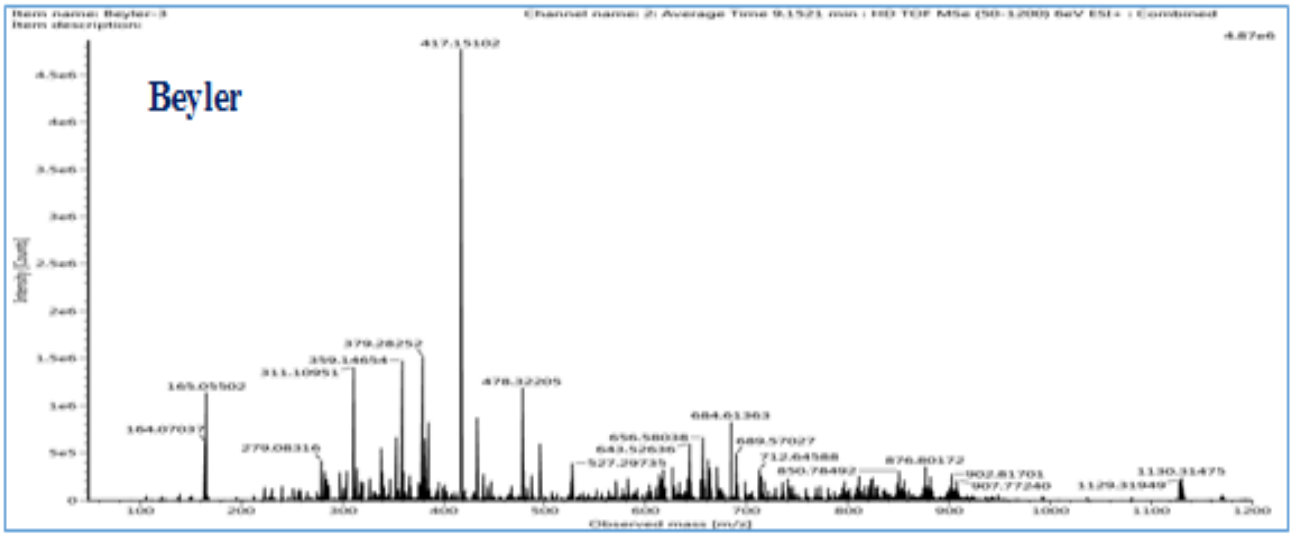
Erkence EVOOs has low stigmastereol and campesterol content. In all EVOOs, the campesterol content was found below the limit between 3.1% (Ulamış) and 2.0% (Gölcük) according to EU Regulations (4%).

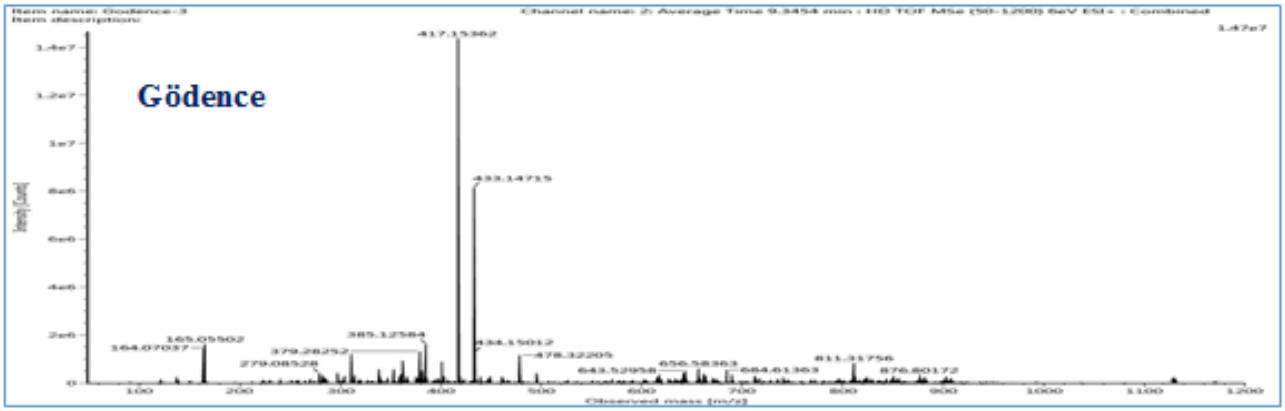
There is significant difference in campesterol content according to the geographical locations. In addition to apparent β sitosterol, it was detected by the sum of β-sitosterol and other three sterols (5, 24-stigmastadienol, clerosterol and Δ-5-avenasterol). Erkence EVOO are found approximately the limit of 93 %, except Gödence and Beyler (92.7% and 92.8%). The highest apparent β-sitosterol (93.8%) was detected in Gölcük location (Table 6). In EVOOs, the content of stigmasterol is lower than campesterol as previous research [13]. In this study, different phytosterol content revealed in Erkence EVOOs were found to be similar as Chemlali varieties [14].

Many studies showed that various factors affect the sterol content such as olive variety, ecological zone, soil structure and harvest time [14]. This results of study is parallel with previous studies.

3.4. Principal Component Analysis (PCA)

Figure 4. ESI (+) MS spectra of Seferihisar region are given. 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.




Gödence
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165.05502
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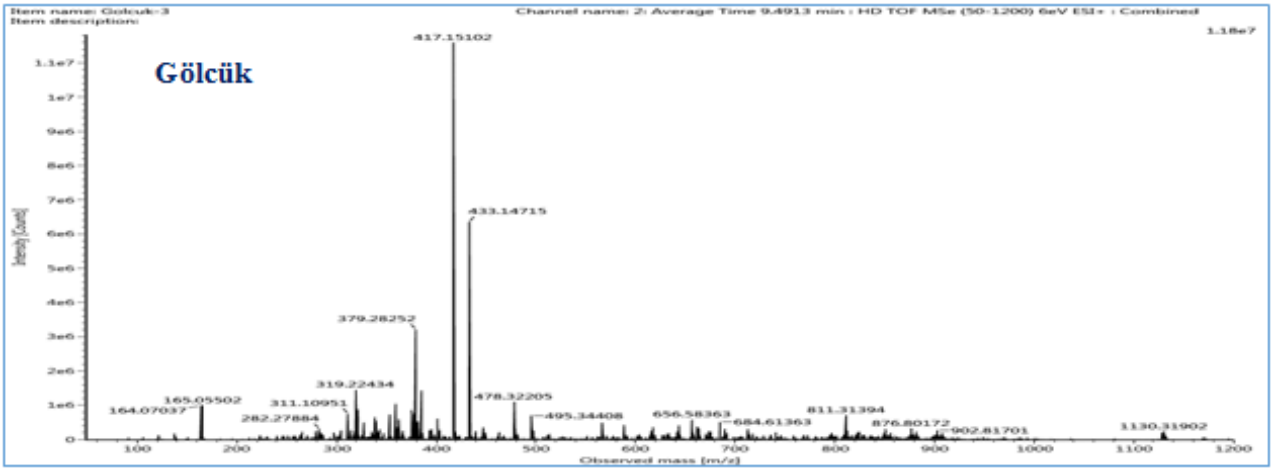
Observed mass [m/z]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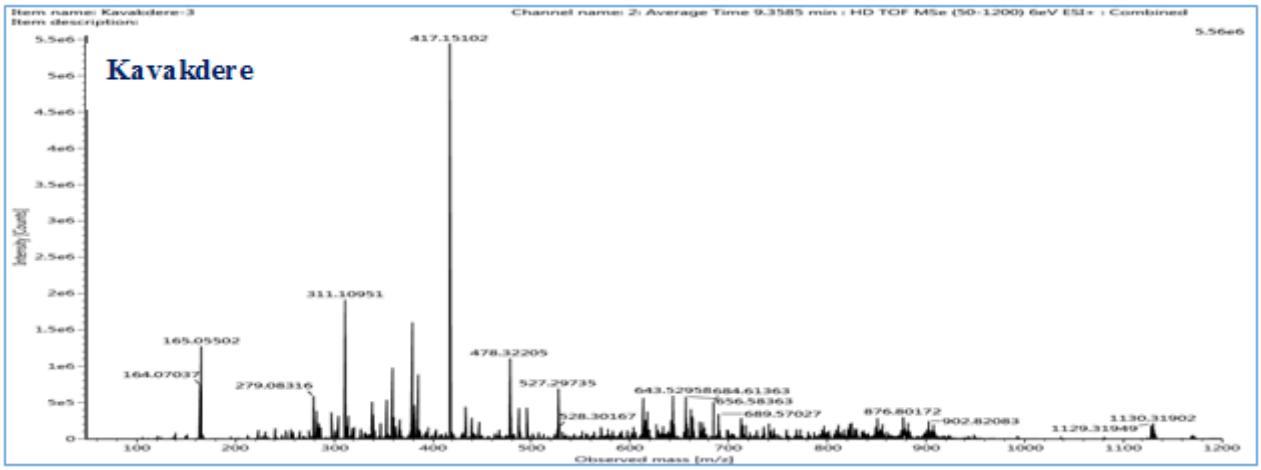
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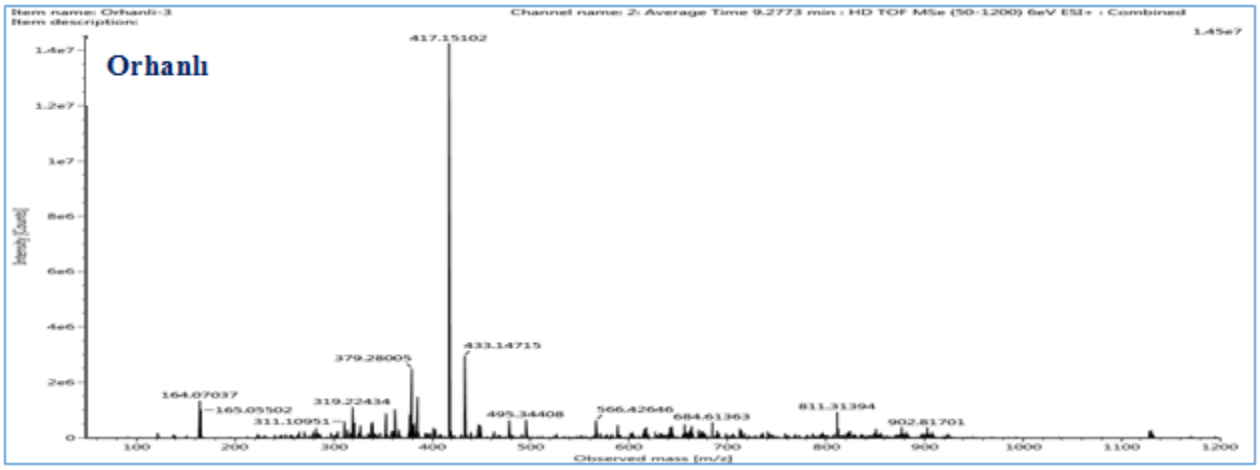
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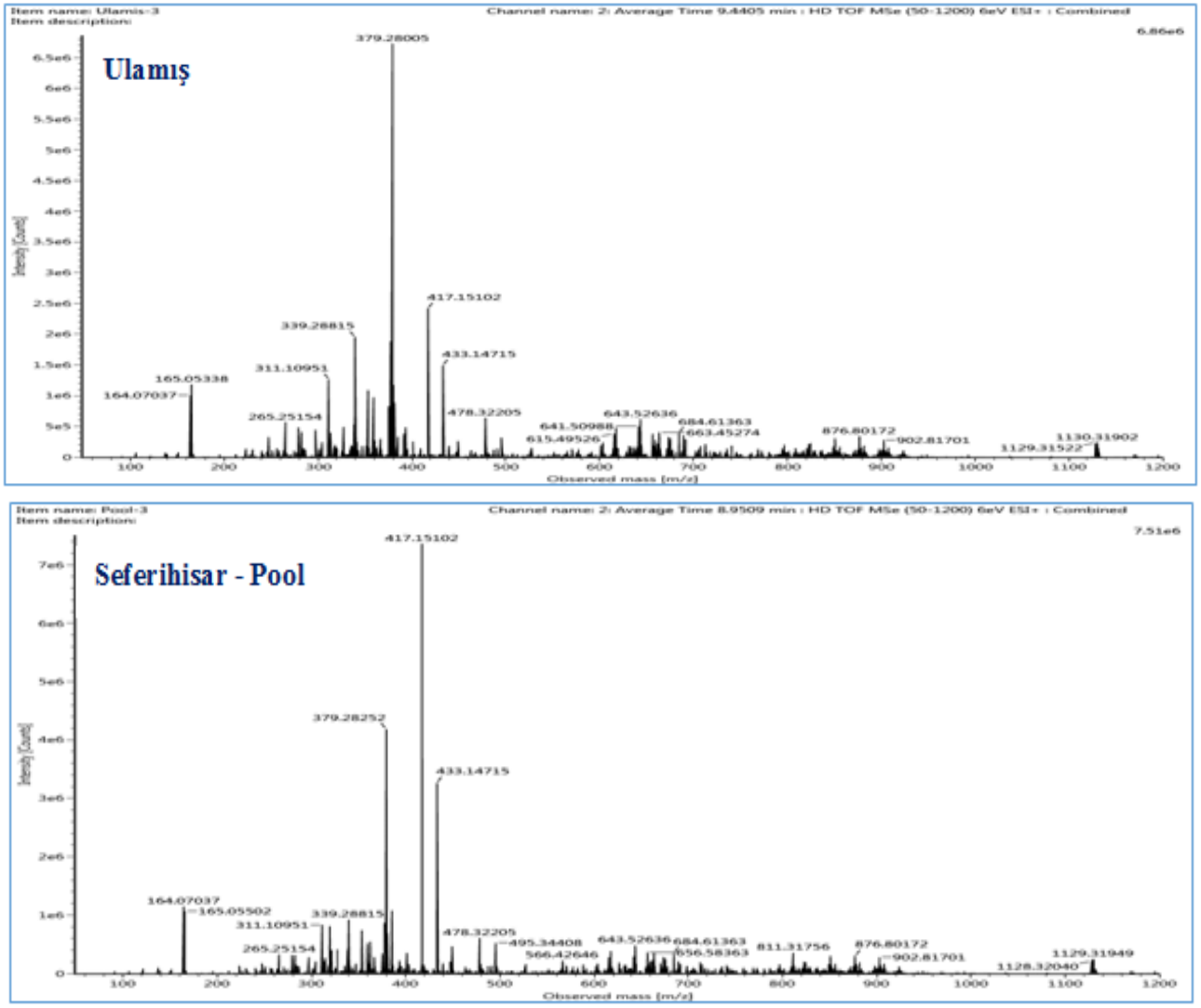
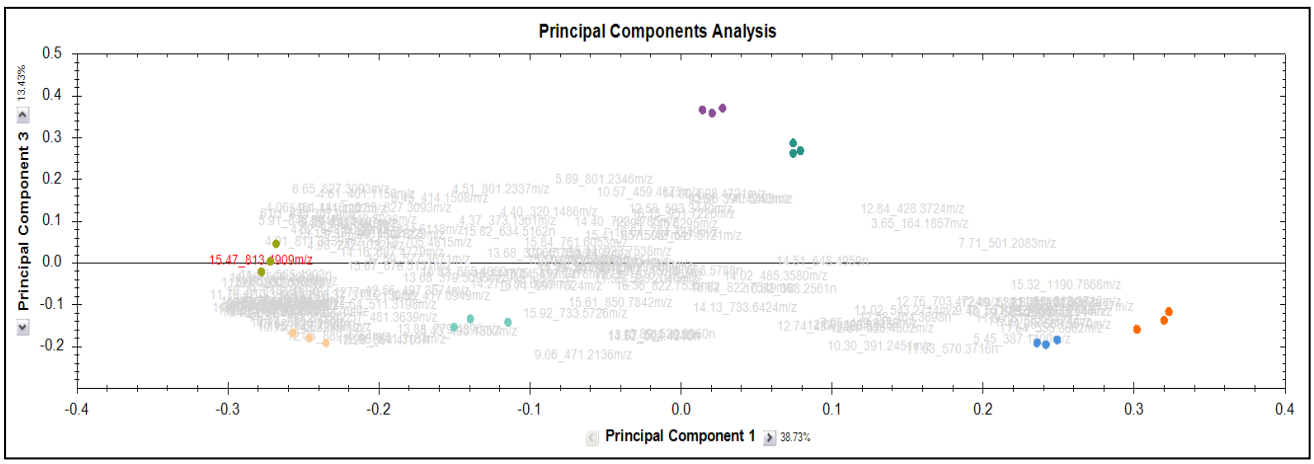


Fig-4: ESI (+) - MS spectra of EVOOs belonging to Seferihisar region

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 Seferihisar in Figure 4 are given. As can be seen in the total ion chromatograms, there are differences between the olive oil methanol: water extracts studied in the study.



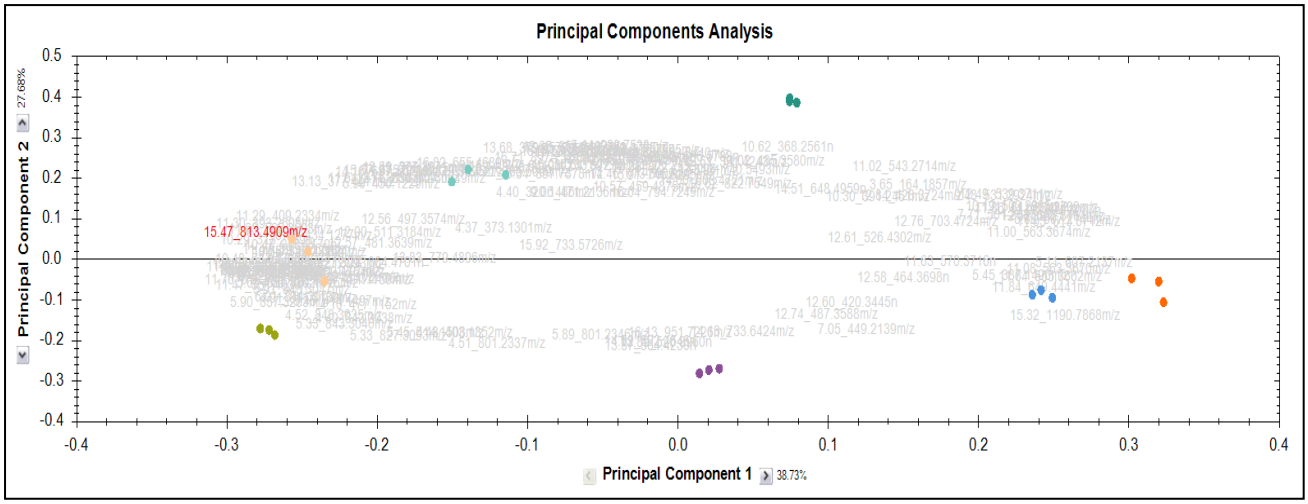


Fig-5: Results of the PCA principal component analysis of the phenolic and sterolic profiles of VOOs obtained from the Seferihisar region, depending on their geographical origins.

A: PCA1 x PCA2 B: PCA1 x PCA3

PCA1, PCA2 and PCA3 were found to be 38.73, 27.68 and 13.43, respectively for Seferihisar region. According to the results of principal component analysis of EVOOs from Seferihisar region, the total variance is 79.84 at 95% confidence interval (figure 5). As can be seen in the figures, it has been determined that each EVOO belonging to Seferihisar region is clustered in different regions.

4. RESULT

This study show that all parameters, especially total ion via LC IMS QTof system, phenolic compounds and fatty acid content and phytosterol content showed variation. Even if Erkence olive variety of Turkey was grown in the same region but in different locations, it revealed that EVOOs have different qualities. As a result, the geographical conditions have important effect on the chemical characterization of EVOO. This situation can be explained by ecological zone, topography and climatic conditions among different locations This study show that olive variety and geographical location are important parameters in determining oil quality characteristics. The Erkence variety of Seferihisar region have very different olive oil qualities depending on different locations.

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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; QTof: Quadropole Time-of-Flight

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