# Identification of Turkish extra virgin olive oils produced in different regions with volatile compounds

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This study aims to characterize the composition of the volatile compounds in Turkish extra virgin olive oils (EVOOs) produced from three cultivars—Ayvalik, Gemlik, and Memecik—harvested in the South Marmara, South Aegean, and North Aegean regions during the 2014/15 and 2015/16 crop seasons. A total of 135 EVOO samples were obtained using industrial-scale 2-phase and 3-phase extraction systems. These samples were then analyzed using solid-phase microextraction (SPME) coupled with gas chromatography (GC). Among the twelve volatiles identified, trans-2-hexen-1-ol and cis-2-penten-1-ol exhibited the highest levels of abundance across all samples and seasons. Subsequently, 1-penten-3-one, hexanal, and cis-3-hexenyl acetate were identified, and it was determined that these contribute to the green and fruity sensory profile of high-quality olive oil. Two- and three-factor analyses of variance (ANOVA) revealed that volatile concentrations were significantly influenced by variety, harvest season, and extraction system. It is significant that 1-penten-3-one was found to be significantly influenced by both season and variety ($p < 0.05$), while 1-penten-3-ol exhibited a multifactorial dependency, with significant two-way interactions (season × variety, season × system, variety × system). Furthermore, PLS-DA-based classification successfully distinguished samples according to olive variety, indicating that volatile profiles could serve as reliable markers for authenticity and geographic origin. These findings underscore the potential of using volatile compounds as quality indicators and for geographic labelling in the olive oil industry.

**Keywords**: Volatile compounds, Ayvalık, Memecik, Gemlik, PCA, PLS-DA, Classification

## 1. INTRODUCTION

The olive tree has been cultivated since 3000 BC and is known as the oldest cultivated tree in the world. The native land of olive trees, which is a member of the Oleacea family (Olea europaea L.), is upper Mesopotamia and South Asia, including the Southeastern Anatolia Region. The olive tree is the symbol of Mediterranean civilization. Of the 900 million trees worldwide, 98% are located in the Mediterranean basin [1]. In recent years, the promotion of healthy and balanced eating habits as well as increasing interest in longevity, have boosted the consumption of olives and olive oil. In Turkey, olives and olive oil are one of the most economical and socially important products for the producer.

Olive oil (OO) is a vital product of the food industry. OOs obtained from different varieties are offered to the market as a blend or as a single variety. Therefore, products with different tastes and characteristics are marketed. This also enriches the production of regional varieties. Recently, olive oils with PDO (Protected Denominations of Origin) and PGI (Protected Geographical Indication) have started to appear. This requires a precise definition of many parameters such as variety, geographical origin, agronomic practices, production technology

and sensory quality. The quality of these monovarietal oils is related to the superior taste, consistency, colour and olive variety. Therefore, studies have focused on defining their origin [2].
National and international standards lay down that the authenticity of EVOO should be determined not only by its chemical composition, but also by considering its sensory properties. When the chemical components and sensory properties of OO are analyzed, fundamental differences emerge depending on the olive variety. The importance of olive variety is due to the amount of chemical compounds in OO produced by biochemical means. The olive variety is the basis of the sensory quality of OO and its characterization, so the sensory quality of OO reflects its authenticity [3].
The aromatic components of OO are made up of volatile and non-volatile components in the olive structure [4]. Volatile compounds play an important role in determining the quality of OO. The 5C and 6C volatile compounds are the most abundant classes in the VOO flavour, contributing to the "green" fruity scent [5]. Volatile compounds are formed as a result of the controlled oxidation of oils in the presence of various enzymes. Volatile formation in olive oil is explained by the lipoxygenase pathway. Aldehydes, alcohols, esters, aromatic and aliphatic hydrocarbons, ketones and furans are important volatile compounds for quality OOs. The composition of these compounds changes, depending on the enzyme activity [6]. Approximately 280 volatile compounds have been identified in OO.
The volatile composition of olive oil is affected by many parameters, the most important of which are olive variety, location (geographical region), harvest year, fruit maturity, climate, extraction systems and storage [3,4,6,7-12]. Volatile compounds accumulate differently according to the variety, and the accumulation of these metabolites is closely related to the genetically determined enzymatic storage (Kesen et al., 2014). Geographic region is highly influential on cis-3-hexenal, cis-3-hexenol, hexanal, hexanol, trans-2-hexenal, trans-3-hexenol, and trans-2-hexenol [12]. Under the same ecological conditions, the aroma compounds of olive oils obtained from different olive varieties may be different, and the aroma compounds of olive oils obtained from the same olive varieties grown in different ecologies may also be different [6].
The volatile compound profile of OOs changes according to the progress of maturation [13]. The content of volatile compounds and the stages at which they are maximized vary from variety to variety. Gómez-Rico et al. [14] found that C6 compounds increased in some Spanish cultivars in the early stages of maturation. Aparicio and Morales [15] described a steady decrease in the level of volatile compounds from the immature stages to the overripe stages, with the exception of oils from Coratina fruits, including trans-2-hexenal. When multiple regression is applied to the quantitative values of volatile compounds, the main contributions of trans-3-hexen-1-ol, cis-3-hexen-1-ol, trans-2-hexen-1-ol and hexanal and hexyl acetate compounds can be seen to the characterization of the maturation process [15]. A study conducted by Zunin et al [16] found that lipoxygenase products (six-carbon aldehydes, alcohols and acetates) vary depending on the olive variety. Cis-3-hexen-1-ol and cis-3-hexenyl acetate were found to be the most effective compounds in distinguishing EVOO samples. The results explain and underline that these parameters, which can be used to characterise OO, are significant. The link between the concentration of chemical compounds and the sensory properties is widely acknowledged.
Studies have shown that 70 of these compounds have an affect on odour [16]. Volatile compounds are considered to be the group responsible for flavour in OO and are extremely important to the food industry since they determine consumer preference. Flavour is one of the most important features that influence the quality of OO and is the most important criterion in distinguishing OO from other vegetable oils. All volatile compounds can be used to control the quality of OO and to determine the olive variety. Fresh, good quality EVOOs are appreciated by consumers and differentiated by their flavour and aroma [17].
Studies on the composition of the volatile compounds of the OO produced in South Marmara, South Aegean and North Aegean regions in Turkey were carried out for Ayvalık, Memecik and Gemlik OOs. For this purpose, OOs obtained from olive varieties widely produced in 3 different geographical regions of Turkey were collected from the regions under controlled conditions depending on their extraction systems (2 and 3 phases), during the 2014/15 and 2015/16 harvest years. As control samples, Memecik, Ayvalık and Gemlik fruit samples were harvested by the Olive Research Institute and extracted with the Abencor system.

## 2. MATERIAL AND METHODS

### 2.1. EVOO SAMPLES

Table I shows the EVOO samples collected in the South Marmara, South Aegean and North Aegean regions in Turkey with two- and three-phase extraction systems during the 2014/15 and 2015/16 crop seasons, between October and December. The majority of large olive oil millers, especially two- and three-phase extraction millers, are located in these regions. Memecik EVOOs were collected from the South Aegean region, Ayvalık EVOOs were collected from the North Aegean region and Gemlik EVOOs from the Marmara region in Turkey. For each sampling, 500 mL of EVOO was collected from the selected factories. A total of one hundred and twenty-nine EVOO samples were collected from these regions and 10 kg of control samples of Memecik, Ayvalık and Gemlik varieties were harvested in the Olive Research Institute and extracted with the Abencor system (MC2 Ingenieria y Sistemas, Sevilla, Spain). As a result, a total of one hundred thirty-five EVOO samples were used for both volatile composition and chemometrics multivariate data analysis.

## 2.2. EXTRACTION OF OLIVE SAMPLES

The olive samples were washed after being separated from their leaves, crushed in a crusher, then the olive paste was subjected to mixing (malaxation) at 25°C for 30 minutes. Separation of the oil phase from the olive paste was carried out by centrifuge. All OO samples were stored in amber-glass bottles, labelled with the code, without headspace and kept at +4°C in the dark until analysis.

## 2.3. METHODS

### 2.3.1 Volatıle compound analysıs

Quantitative analysis of volatile compounds in OO samples was carried out using the SPME technique [18]. Identification of aroma compounds in EVOO samples was accomplished using GC-7890 B coupled with an Agilent 5977E MSD system mass spectrometer detector (Agilent Technologies, Santa Clara, CA, USA). A high polarity DB-WAX column (polyethylene glycol) (Agilent J&W GC columns, 60m x 0.25 mm i.d. x 0.15 µm film thickness) was employed. The oven temperature was programmed to hold for 5 min at 50°C, then rise from 50°C to 220°C at a rate of 5°C/min, before being kept constant for 10 mins at the final temperature (total run time of 49 mins). Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The sample injections were conducted with the PAL RSI 85 automated sample preparation. System (CTC Analytics Switzerland) was used with a Splitless liner. The conditions for SPME injections were an incubation time of 5 min. at a temperature of 70°C. Fibre conditioning Saturation Temperature was used at 250°C. Sample extraction and desorption times were both set at 5 minutes. The conditioning time (post-desorption) was set at 5 minutes. The mass spectrometer conditions were as follows: ionization potential, 70 eV; ion source temperature, 150°C. Electron ionization mass spectra were recorded over the mass range 50-550 m/z, considering at least one precursor and two fragment ions for identification purposes (Table II). All the data were acquired in scan mode.

**Table I -** Distribution of collected EVOO samples by years and regions

| Regions | Varieties | 2014/15 | | | 2015/16 | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | 2 phase | 3 phase | Abencor | 2 phase | 3 phase | Abencor | |
| South Aegean | Memecik | 7 | 6 | - | 13 | 12 | - | 38 |
| North Aegean | Ayvalık | 17 | 20 | - | 11 | 14 | - | 62 |
| South Marmara | Gemlik | 1 | 16 | - | 1 | 11 | - | 29 |
| Total | | 25 | 42 | - | 25 | 37 | - | 129 |
| Olive Research Institute | Memecik | - | - | 1 | - | - | 1 | 2 |
| | Ayvalık | - | - | 1 | - | - | 1 | 2 |
| | Gemlik | - | - | 1 | - | - | 1 | 2 |
| Total | | - | - | 3 | - | - | 3 | 6 |
| Total Sample | | | | | | | | 135 |

**Table II -** GC-MSs working conditions

| | |
|---|---|
| Fiber: PDMS-DVB-CAR | Incubation time:5 min |
| Pulse Splitless Injection (HS-SPME) | Incubation temperature: 40°C |
| Sample Vial Penetration Dose: 40mm | Sample Vial Penetration Rate: 20 mm/sn |
| Agitotor Speed: 250 rpm | Transfer Line: 250°C |
| Adsorption Time: 30 min | Adsorption Temperature: 40°C |
| Column Flow: 0.5 | Desorption Time: 3 min |
| Column temperature program<br>35..........................6 min<br>240 °C in 4°C increments 10 min<br>Total time:67.25 min | Inlet Temperature: 250° C<br>Pulsed Splitless:<br>Injection Pulse Pressure:25psi .. 0.5 min<br>Septum Purge Flow: 15mL/min, 3 min |
| Carrier Gas | Helium |

Relative percentages of the separated components were calculated by integrating the normalized peak areas in the GC-MS chromatograms. Compounds in the oil samples were identified by comparison with reference standards and by matching their fragmentation patterns with those listed in commercial mass spectral libraries, such as Wiley.

### 2.4. STATISTICAL ANALYSIS

The statistical evaluation of the data obtained from the volatile compound analysis was conducted through the implementation of ANOVA (2 and 3 factor) and chemometrics models. Both unsupervised and supervised classification methods allow the development of mathematical and statistical models based on multivariate data. The purpose is to develop the best chemometrics model which successfully assigns new samples to the correct classes. In unsupervised classification, there is no prior information about which group each sample belongs to. Therefore, as a rule of thumb, the initial data is subjected to various data processing methods, such as principle component analysis (PCA) and hierarchical cluster analysis (HCA), in order to provide a general idea of how the samples are grouped. Unlike in unsupervised classification methods, to obtain a model with a supervised classification method a training data set must be provided, illustrating which sample belongs to which class. Then, in most cases, the classification problem becomes weighting the variables in order to provide best separation. Among these methods, Partial Least Squares Discriminant Analysis (PLS-DA) is one of the most common tools in chemometrics. PLS-DA is a projection-based classification method and may provide noise reduction of raw data, as well as significantly decreasing the number of variables to overcome the multicollinearity problem. The aim of this method is to find the best projection of the explanatory variables and the responses that maximizes their covariance. This approach also allows the identification of those variables which most contribute to classification. Currently, NIPALS and SIMPLS algorithms are the most common algorithms for PLS-DA. In both kinds of classifications (unsupervised and supervised), feature selection methods can also be employed to improve the models further since it is usually not known which combination of features carries the information that enables the best classification performance, especially for big data. One approach is genetic algorithms (GA) where the feature selection problem is converted to an evolutionary process. The GA picks random sets of variables and iterates them to provide the "fittest" variables, just as the nature does through natural selection.

In this study, both unsupervised and supervised chemometrics multivariate methods were evaluated in order to demonstrate possible classification and clustering of OO samples collected from three different regions of Turkey, based on volatile compounds. For this, principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were applied to the data set of quantitative composition of volatile compounds obtained from GC. Both PCA and PLS-DA algorithms were coded in MATLAB programming language using MATLAB 2018a (MathWorks Inc, Natick, MA, USA).

## 3. RESULTS AND DISCUSSION

In Turkey, Ayvalık, Memecik and Gemlik are the most prevalent and important olive varieties and their distribution is 20.66%, 19.11% and 48.71%, respectively. Ayvalık, Memecik and Gemlik olive varieties are economically important for Turkey [19]. The Ayvalık olive variety is consumed as a table olive and as olive oil and is usually processed as olive oil. The origin of the Ayvalık olive variety is Edremit province and it is one of the most popular olive oils among consumers because it has a medium fruitiness due to its sensory properties. The fruit ripens early. The origin of Memecik olive variety is Muğla province and a large part of the trees are in the Aegean region. Ayvalık and Memecik olive trees cover three-quarters of the vegetation of the South and North Aegean regions, respectively. Gemlik olive trees cover nearly half of the vegetation (48%) in the Marmara region. Approximately 40% of the total production of table olives comes from this region of Turkey [20]. The origin of the Gemlik olive variety is Gemlik province. The percentage of fat of these three varieties is more than 22%. These varieties are very important to our country so it is crucial to examine their volatile components, according to the regions and extraction methods.

C5 and C6 volatile compounds are the main, most powerful odorants found in EVOO. They contribute positively to the aroma and are also associated with sweetness and green notes. C6 aldehydes such as hexanal, cis-3-hexenal and trans-2-hexenal, alcohols such as hexanol, cis-3-hexenol, and trans-2-hexenol, and their acetyl esters such as hexylacetate and cis-3-hexenyl acetate, constitute 60–80% of the total volatile components. All of these compounds contribute to the EVOO with green notes [6,12,21]. The content of volatile components can be different and specific to an olive variety and can be used for the characterization of monovarietal VOOs [12,22].

The composition of 12 volatile compounds (μg/kg) of olive oil control samples obtained from the Olive Research Institute during the 2014/15 and 2015/16 harvest years are shown in Table III. As can be seen, cis-2-penten-1-ol was found to be the highest volatile compound, in both years. In the 2014/15 harvest year, hexanal in Ayvalık and Gemlik EVOOs and

**Table IV -** The average values of the samples subjected to ANOVA for the 2014/15 and 2015/16 seasons, samples (Ayvalık Memecik and Gemlik) and phase (2 phase and 3 phase)

| Season | Variety | Extraction System | 1-Penten-3-one | Hexanal | trans-2-pentanal | 1-penten-3-ol | 2-methyl-1-butanol | 1-pentanol | cis-3-hexenyl acetate | cis-2-penten-1-ol | 1-hexanol | trans-3-Hexen-1-ol | cis-3-hexen-1-ol | trans-2-hexen-1-ol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2014/15** | **Gemlik** | **3 Phase** | 277.72 | 226.81 | 50.14 | 74.87 | 3.46 | 18.98 | 410.14 | 873.29 | 139.66 | 16.37 | 96.36 | 1725 |
| **2014/15** | **Gemlik** | **2 Phase** | 279.60 | 180.87 | 89.45 | 371.34 | 1.27 | 1.71 | 296.46 | 1224 | 126.73 | 46.56 | 233.96 | 1012 |
| **2014/15** | **Ayvalık** | **3 Phase** | 372.71 | 317.26 | 118.19 | 63.87 | 8.21 | 25.52 | 223.81 | 1063 | 118.06 | 25.74 | 236.01 | 624 |
| **2014/15** | **Ayvalık** | **2 Phase** | 533.51 | 334.41 | 152.15 | 110.18 | 6.47 | 23.67 | 188.71 | 1362 | 123.93 | 21.36 | 270.82 | 674 |
| **2014/15** | **Memecik** | **3 Phase** | 255.71 | 265.20 | 76.22 | 119.87 | 1.60 | 6.55 | 440.09 | 656.39 | 125.95 | 27.73 | 124.38 | 1374 |
| **2014/15** | **Memecik** | **2 Phase** | 104.57 | 171.82 | 68.87 | 119.63 | 25.64 | 2.64 | 374.32 | 833.83 | 264.33 | 26.31 | 131.76 | 2926 |
| **2015/16** | **Gemlik** | **3 Phase** | 158.22 | 130.83 | 18.57 | 83.21 | 200.30 | 4.43 | 259.63 | 1034 | 230.61 | 24.37 | 112.86 | 2814 |
| **2015/16** | **Gemlik** | **2 Phase** | 125.78 | 1975 | 116.89 | 215.58 | 311.14 | 17.38 | 964.58 | 10169 | 690.48 | 31.23 | 871.68 | 2587 |
| **2015/16** | **Ayvalık** | **3 Phase** | 237.16 | 236.87 | 27.96 | 176.69 | 16.66 | 1.63 | 147.56 | 2227 | 90.87 | 17.42 | 192.76 | 911 |
| **2015/16** | **Ayvalık** | **2 Phase** | 315.80 | 230.11 | 48.98 | 69.87 | 6.03 | 3.12 | 200.74 | 1428 | 116.67 | 15.31 | 222.12 | 513 |
| **2015/16** | **Memecik** | **3 Phase** | 129.12 | 731.19 | 180.43 | 341.63 | 149.02 | 22.73 | 901.83 | 4889 | 499.51 | 49.23 | 385.96 | 3007 |
| **2015/16** | **Memecik** | **2 Phase** | 97.67 | 849.97 | 42.72 | 269.78 | 108.86 | 3.67 | 1132 | 5165 | 737.43 | 72.98 | 482.41 | 6148 |

**Table III -** Volatile compounds (µg/kg) of olive oil samples harvested from their own regions of origin and obtained with the Abencor system in the 2014/15 and 2015/16 harvest years

| Extraction System (Phases) | Year | Variety | Sample | 1-Penten-3-one | Hexanal | Trans-2-pentanal | 1-penten-3-ol | 2-methyl-1-butanol | 1-pentanol | Cis-3-hexenyl acetate | Cis-2-penten-1-ol | 1-hexanol | Trans-3-hexen-1-ol | Cis-3-hexen-1-ol | Trans-2-hexen-1-ol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Abencor system** | **2014/15** | **Gemlik** | 1 | 91.03 | 905.98 | 42.00 | 41.29 | 33.54 | 1.26 | 359.19 | 559.92 | 151.57 | 14.34 | 75.77 | 318.29 |
| | | **Ayvalık** | 2 | 47.44 | 2593 | 151.00 | 31.82 | 1.07 | 29.94 | 67.62 | 1425 | 975.19 | 34.19 | 551.47 | 600.60 |
| | | **Memecik** | 3 | 87.86 | 495.44 | 42.00 | 46.93 | 0.21 | 2.28 | 389.83 | 1123 | 24.16 | 14.34 | 15.61 | 21.69 |
| | **2015/16** | **Gemlik** | 1 | 61.49 | 154.44 | 2.84 | 3.57 | 3.00 | 0.70 | 159.23 | 871.09 | 12.00 | 4.00 | 39.51 | 6.99 |
| | | **Ayvalık** | 2 | 1020 | 118.92 | 161.38 | 44.89 | 0.34 | 0.55 | 330.08 | 1634 | 10.96 | 50.84 | 20.04 | 5.00 |
| | | **Memecik** | 3 | 107.47 | 141.18 | 9.01 | 33.69 | 2.19 | 15.46 | 288.28 | 1814 | 37.53 | 22.09 | 20.04 | 46.64 |

cis-2-penten-1-ol in Memecik EVOO were determined to be the highest volatile compounds. In the 2015/16, cis-2-penten-1-ol was found to be the highest volatile compound in Memecik, Ayvalık and Gemlik EVOOs. Cis-2-penten-1-ol is known to be a volatile compound found in green tea, EVOO and broccoli. It is associated with green as a sensory odour and taste type. The odour is sensually reminiscent of banana, green, grass, ethereal fruity cherry in EVOO [23]. Hexanal is a saturated fatty aldehyde and the major volatile compound in olive oil. The type of odour and taste is green. The odour description is associated with green, fruity, oily, leafy, herbal, fruity and woody, while the flavour is leafy, fruity, green apple, green, herbal, grassy. The high levels of hexanal described in EVOO often produce sensory odour notes of green, apple and sweetness [6,21,24]. In this study, cis-2-penten-1-ol and hexanal were evaluated as a descriptive volatile compound in determining varieties. The results are in accordance with Kalua et al. [6] (2007), Kesen et al. [17], Şişik Oğraş et al. [11], Mansouri et al. [24] and Psathas et al. [25].

As shown in Table I, of the 67 olive oil samples obtained in the 2014/15 harvest year, 37 were Ayvalık cultivars, 17 were Gemlik cultivars and the remaining 13 were Memecik cultivars. In the 2015/16 harvest season, on the other hand, a total of 62 olive oil samples were obtained, of which 25 were Ayvalık cultivars, 25 Gemlik cultivars and the remaining 12 Memecik cultivars.

A variance analysis (ANOVA) was carried for the 12 aroma compounds given in Table IV using a 3-factor (harvest season, variety and extraction system) and a 2-factor (harvest season and variety) mixed level (two levels for harvest season, three levels for variety and two levels for the extraction system). The data are derived from two different extraction systems (3-phase and 2-phase) used to obtain olive oils, and the average concentrations of their aroma components are reported. In the 2015/16 season, there was a notable increase in the concentrations of certain compounds, including hexanal, trans-2-pentanal, and cis-3-hexenyl acetate, when compared to the 2014/15 season. In particular, it has been determined that the 2015/16 season saw hexanal levels in EVOO from Gemlik reach a remarkably high value of 1975 mg/kg, as determined through two-phase extraction. As illustrated in Table 4, the presence of aldehyde compounds such as 1-penten-3-one and hexanal is typically observed at higher or variable levels in the oil derived from the Gemlik variety. Research findings have indicated the presence of cis-3-hexenyl acetate and trans-2-hexen-1-ol in Ayvalık olive oil, suggesting that harvesting season and extraction methods may be contributing factors to the observed variability. A study of the chemical composition of EVOO revealed that certain types of olive oil, specifically those produced in the 2015/16 season, exhibited significantly elevated levels of two specific compounds: trans-2-hexen-1-ol (6148 mg/kg) and cis-3-hexenyl acetate (1132 mg/kg). These compounds were identified as potential markers for distinguishing the quality and origin of olive oil. The concentration levels of specific components in the 2-phase extraction system are typically higher than those in the 3-phase extraction system. The 2015/16 season saw the detection of hexanal (1975 mg/kg) and cis-3-hexenyl acetate (964.58 mg/kg) in a substantial amount in the oil derived from the Gemlik variety. However, the hexanal levels were found to be considerably lower in the 3-phase system. In the 2015/16 season, a 2-phase extraction of the oil revealed elevated levels of numerous components. Hexanal, 1-penten-3-one and trans-2-pentanal, are green, fresh-smelling aldehydes that are particularly significant in contributing to the fresh and herbal notes of the aroma. Trans-3-hexen-1-ol and cis-3-hexenyl acetate, on the other hand, is an ester and alcohol compound that contributes to the fruity, green, and earthy aromas of various plants. The quantity of components can significantly impact the overall aroma profile and quality of the product.

In Table V, the results of the 2-factor ANOVA analysis are reported only for volatile components with P values below 0.05 (95% confidence level), that is, 1-Penten-3-one, 2-methyl-1-butanol cis-3-hexenyl acetate, 1-hexanol and trans-2-hexen-1-ol. It has been determined that both the season and the variety statistically significantly affect the amount of 1-Penten-3-one. For both factors, $p < 0.05$ has been determined, which shows that different seasons and varieties play an important role in the formation of 1-Penten-3-one. The impact of the season on 2-methyl-1-butanol has been determined to be statistically significant at the $p < 0.05$ level of confidence. The impact of variety on the cis-3-hexenyl acetate compound was found to have borderline significance ($p = 0.050$). The presence of cis-3-hexenyl acetate may vary among different varieties, exhibiting potential differences in potency. The effect of season was determined to be statistically significant ($p < 0.05$) for 1-hexanol. A substantial variation in the levels of 1-hexanol was observed during both seasons. A statistically significant effect of variety was found for the trans-2-hexen-1-ol compound. The different varieties noticeably affected the level of this compound. When viewed as a whole, the 1-Penten-3-one compound was most strongly affected by both season and variety. Seasonal changes were found to have a particularly significant effect on 1-hexanol and 1-Penten-3-one. Varietal differences exerted notable effects, particularly on trans-2-hexen-1-ol and 1-Penten-3-one. The effect of season on 2-methyl-1-butanol has been detected at the threshold level and requires further support with additional data.

In Table VI, the results of the 2-factor ANOVA analysis are only reported for volatile components with P values below 0.05 (95% confidence level), that is, 1-Penten-3-ol, 2-methyl-1-butanol and trans-2-hexen-1-ol. Many factors that statistically ($p < 0.05$) affect the 1-Penten-3-ol compound were identified. The varietal effect was found to be statistically important while two-way interactions were found to be highly significant. Important interactions were determined between season and variety, season and extraction system, and variety and extraction systems. The 2-methyl-1-butanol compound has been found to change noticeably depending on the season ($p < 0.05$). Seasonal changes have a significant effect on the formation of the 2-methyl-1-butanol compound. The trans-2-hexen-1-ol compound was found to be affected by both season and variety ($p < 0.05$) in a statistically important way. Considerable differences were also found between varieties, aggravated by seasonal conditions.

The residual (a) and main effect (b) plots of the 2-factor ANOVA for 1-Penten-3-one are shown in Figure 1. The $R^2$ value was determined to be 81.10%, indicating that 81% of the variation in the data can be attributed to seasonal and varietal factors. Both season and variety appear to have a noticeable effect on 1-Penten-3-one levels. Differences can also be seen in the main effect graph. Figure 2 shows the residual (a) and main effect (b) plots of the 2-factor ANOVA for 2-methyl-1-butanol. The $R^2$ value was 53.34%. The graph shows a significant seasonal difference for the methyl-1-butanol compound. Figure 3 displays the 2-factor ANOVA's residual (a) and main effect (b) plots for 2-cis-3-hexenyl acetate. The $R^2$ value was found to be 61.33%. The graph shows the variation in the effect of variety. Figure 4 displays the main effect (b) and residual (a) plots of the 2-factor ANOVA for 1-hexanol. The $R^2$ value was found to be 58.64%. The graph illustrates the variation in the seasonal effect.

The 3-factor ANOVA analysis demonstrates that the evaluated factors (variety, season, extraction system) on the 1-Penten-3-ol compound are statistically highly effective. The total variance explanation rate of the model is very high ($R^2$ = 99.01%) and the adjusted $R^2$ value is also quite strong (94.54%). This shows that the variables included in the model explain the variation of the compound not only by random variation but also with systematic and significant effects. The residual graph shows that the model assumptions are met; the main effect graph clearly shows the effect of each main factor on the compound. This situation shows that experimental factors are determinant in the production of 1-Penten-3-ol, especially with the evaluation of interactive effects (Figure 5a-b). The variance analysis results obtained for the 2-methyl-1-butanol compound (Figure 6a-b) show that the model is generally robust ($R^2$ = 96.38%) and that the effects of the factors are significant. Main effect plots are crucial for determining which factors play a dominant role. These findings indicate that the 2-methyl-1-butanol compound is significantly affected by season. Figure 7 displays the 3-factor ANOVA's residual (a) and main effect (b) plots for trans-2-hexen-1-ol. The trans-2-hexen-1-ol compound's high R2 value was found to be 98.18%. The directional effects of the factors are evident from the main effect and residual plots. Season and variety have a statistically important impact on the trans-2-hexen-1-ol compound, indicating that these factors have a considerable impact on volatile compound profiles.



**Table V -** Analysis of variance performed for 1-Penten-3-one, 2-methyl-1-butanol, cis-3-hexenyl acetate, 1-hexanol and trans-2-hexen-1-ol using ANOVA 2 factors results at 95% confidence level.

| Compunds | Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|---|
| 1-Penten-3-one | Season | 1 | 48142 | 48142 | 48142 | 11.11 | 0.01 |
| | Variety | 2 | 100586 | 100586 | 50293 | 11.61 | 0.004 |
| 2-methyl-1-butanol | Season | 1 | 46296 | 46296 | 46296 | 9.62 | 0.015 |
| cis-3-hexenyl acetate | Variety | 2 | 547477 | 547477 | 273739 | 4.45 | 0.050 |
| 1-hexanol | Season | 1 | 179321 | 179321 | 179321 | 5.71 | 0.044 |
| trans-2-hexen-1-ol | Variety | 2 | 14404572 | 14404572 | 7202286 | 6.20 | 0.024 |

**Table VI -** Analysis of variance performed for 1-Penten-3-ol, 2-methyl-1-butanol and trans-2-hexen-1-ol using ANOVA 3 factors results at 95% confidence level.

| Compunds | Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|---|
| 1-Penten-3-ol | Variety | 2 | 25132 | 25132 | 12566 | 19.69 | 0,048 |
| | Season*Variety | 2 | 33975 | 33975 | 16987 | 26.61 | 0.036 |
| | Season*Extraction System | 1 | 12600 | 12600 | 12600 | 19.74 | 0.047 |
| | Variety*Extraction System | 2 | 40878 | 40878 | 20439 | 32.02 | 0.03 |
| 2-methyl-1-butanol | Season | 1 | 46296 | 46296 | 46296 | 22.52 | 0.042 |
| trans-2-hexen-1-ol | Season | 1 | 4872819 | 4872819 | 4872819 | 18.73 | 0.049 |
| | Variety | 2 | 14404572 | 14404572 | 7202286 | 27.68 | 0.035 |

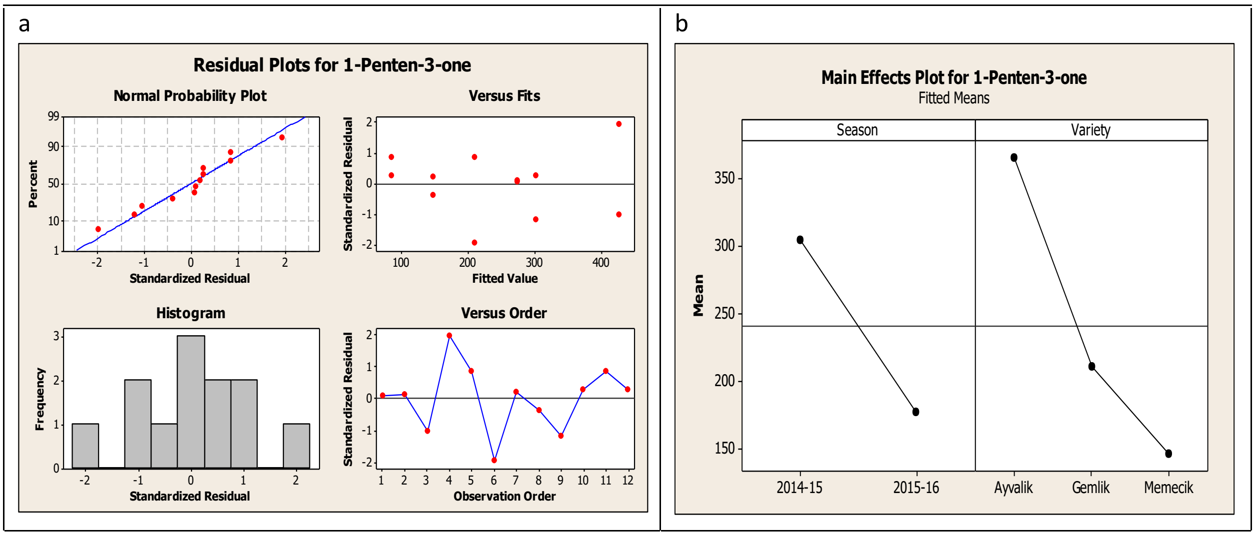


**Figure 1 -** Residual (a) and main effect (b) plots of 2 factors ANOVA for 1-Penten-3-one (S = 65.8129; R-Sq = 81.10%; R-Sq(adj) = 74.02%).

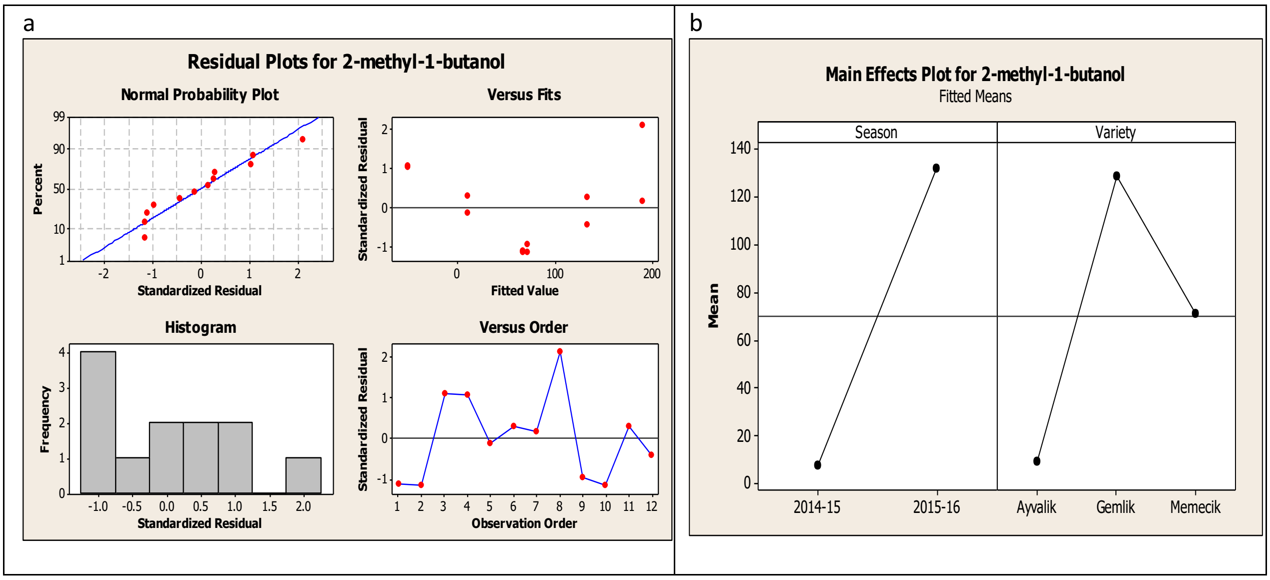


**Figure 2 -** Residual (a) and main effect (b) plots of 2 factors ANOVA for 2-methyl-1-butanol (S = 69.3780; R-Sq = 66.06%; R-Sq(adj) = 53.34%

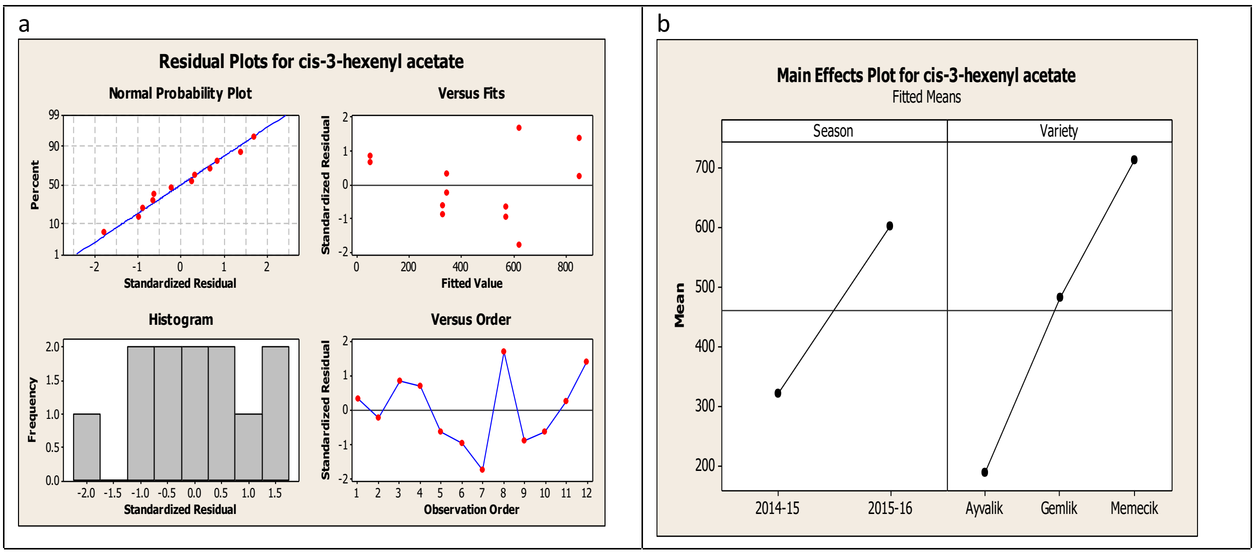


**Figure 3 -** Residual (a) and main effect (b) plots of 2 factors ANOVA for cis-3-hexenyl acetate (S = 248.081; R-Sq = 61.33%; R-Sq(adj) = 46.82%)

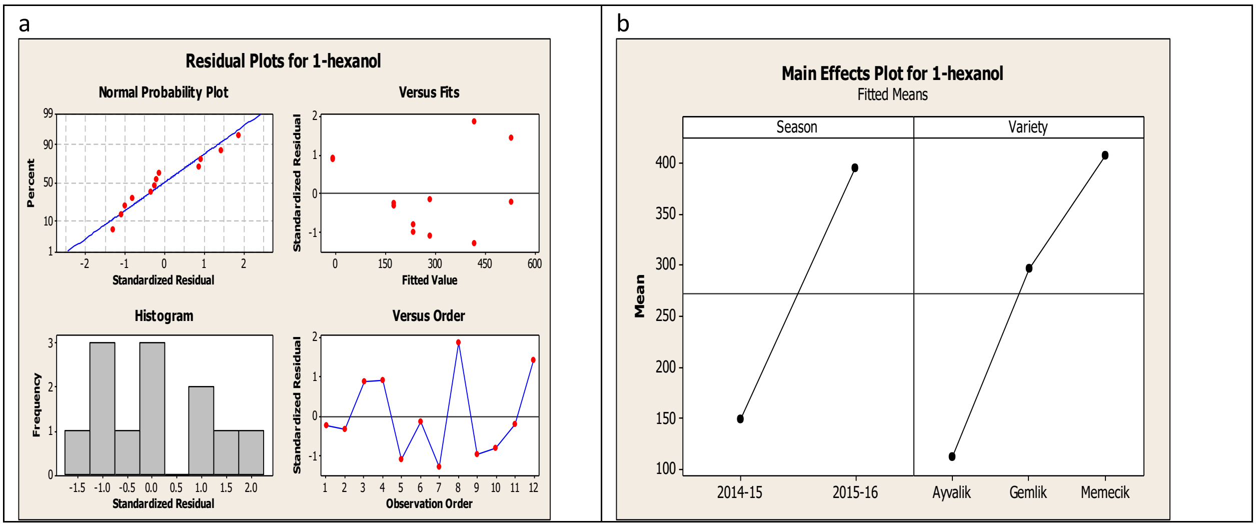


**Figure 4 -** Residual (a) and main effect (b) plots of 2 factors ANOVA for 1-hexanol (S = 177.268; R-Sq = 58.64%; R-Sq(adj) = 43.13%)

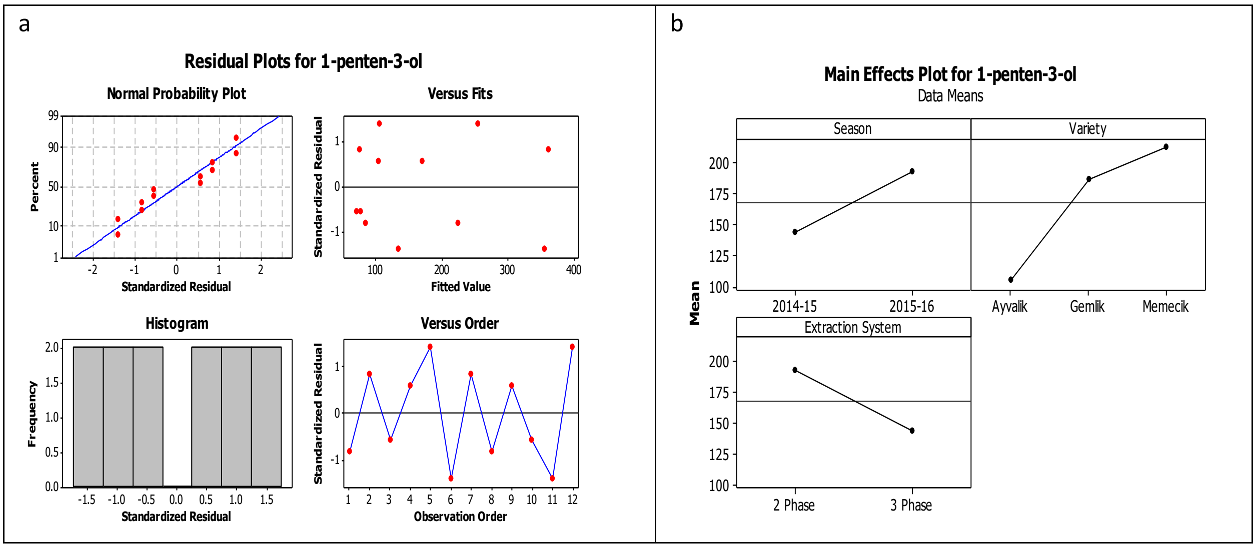


**Figure 5 -** Residual (a) and main effect (b) plots of 3 factors ANOVA for 1-Penten-3-ol (S = 25.2647; R-Sq = 99.01%; R-Sq(adj) = 94.54%)

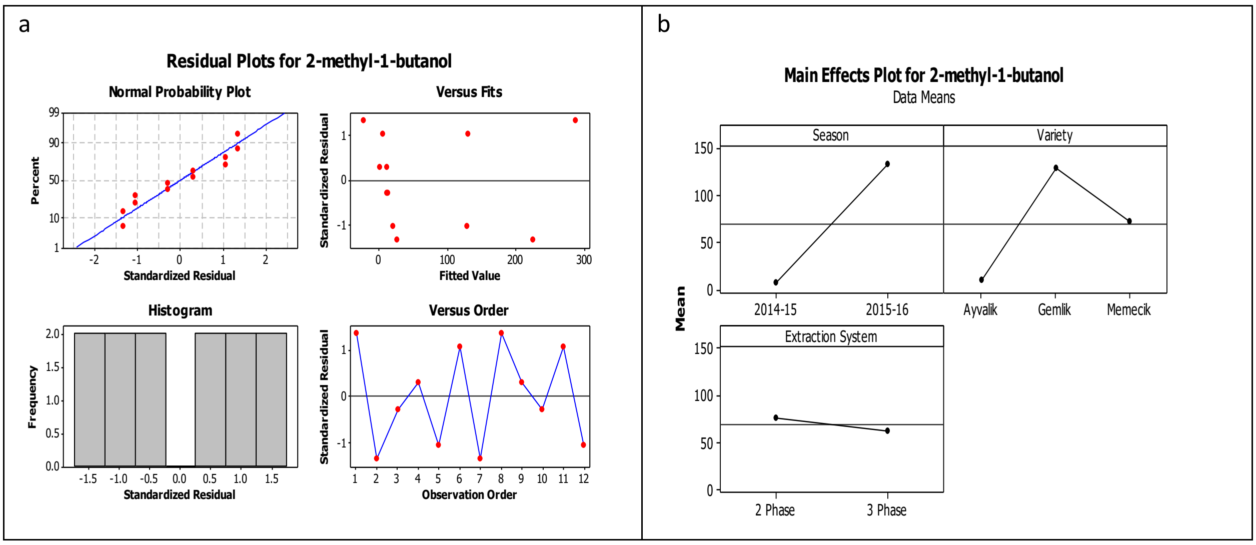


**Figure 6 -** Residual (a) and main effect (b) plots of 3 factors ANOVA for 2-methyl-1-butanol (S = 45.3365; R-Sq = 96.38%; R-Sq(adj) = 80.07%)

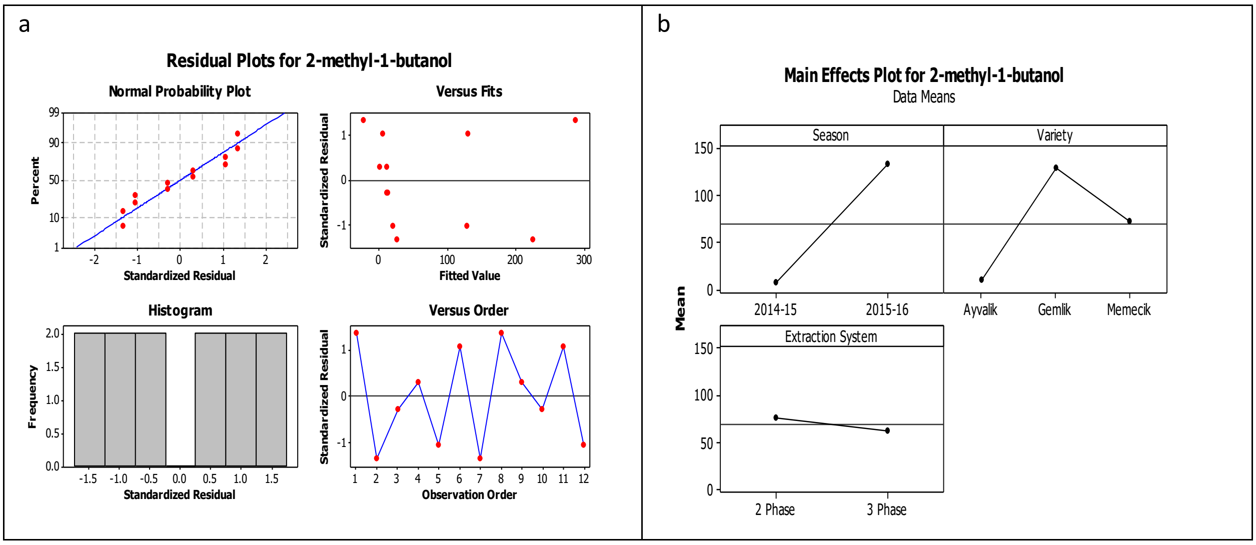


**Figure 6 -** Residual (a) and main effect (b) plots of 3 factors ANOVA for 2-methyl-1-butanol (S = 45.3365; R-Sq = 96.38%; R-Sq(adj) = 80.07%)

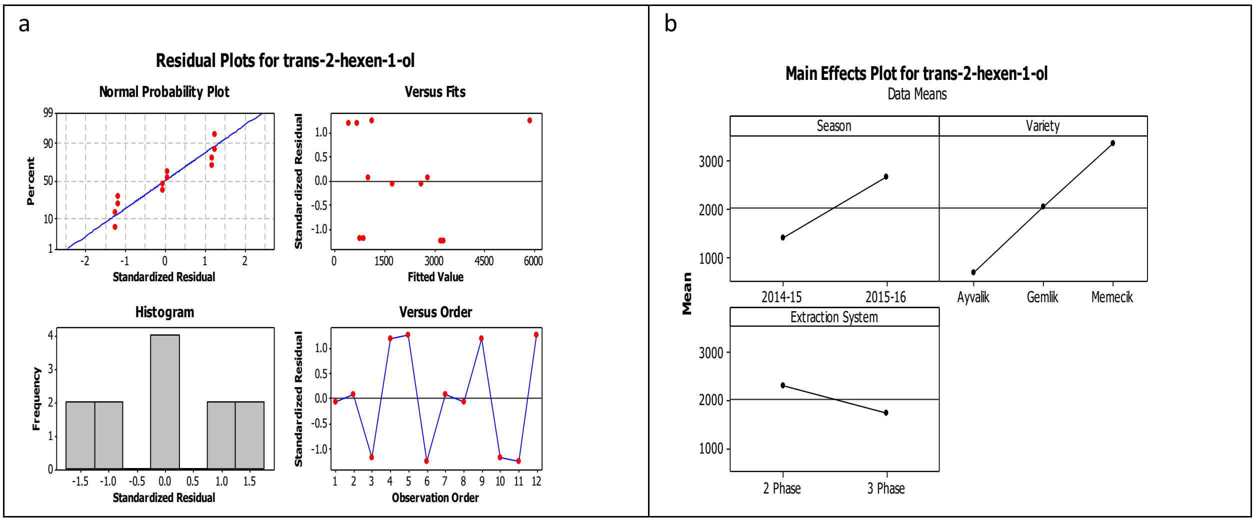


**Figure 7 -** Residual (a) and main effect (b) plots of 3 factors ANOVA for trans-2-hexen-1-ol (S = 510.116; R-Sq = 98.18%; R-Sq(adj) = 89.98%)



The quantitative compositions of volatile compounds of the cultivars are given in Table VII and Table VIII for the 2014/15 and 2015/16 crop season, respectively. As can be seen from Table 7, in the 2014/15 harvest year, trans-2-hexen-1-ol was detected as the highest volatile compound, followed by cis-2-penten-1-ol and cis-3-hexenyl acetate at 1725, 873.29 and 410.14 mg/kg, respectively, in EVOOs obtained from the Gemlik variety with the 3-phase system. Trans-2-hexen-1-ol is a primary allylic alcohol, the odour of which is described as fruity and the taste green. It is sensorially associated with the smell of sharp, green, leafy, fruity, unripe bananas and is described as sweet, fruity, slightly green and oily in taste [26]. Cis-3-hexenyl acetate is an acetate ester. Sensorially, it is a sharp fruity green in odour, sweet and associated with green top notes such as delicate natural floral freshness. The taste contains fruity flavours, apple, sweet, green, and all kinds of green fruit, especially tropical fruits including cherry, banana and peach. In the 2-phase system, cis-2-penten-1-ol was detected as the highest, followed by trans-2-hexen-1-ol and 1-penten-3-ol at 1224, 1012 and 371.34 mg/kg, respectively. 1-penten-3-ol is an alcohol, derived from the lipoxygenase pathway through the action of alkoxy radicals [27].

The type of odour and taste is green. The odour features sharp, green vegetable and tropical fruity nuances, and is associated with green vegetable that are fruity in taste. In 3-phase system trans-2-hexen-1-ol and cis-3-hexenyl acetate were found to be higher than in the 2-phase system. For Ayvalık EVOO, cis-2-penten-1-ol was determined to be the highest volatile compound at 1063 and 1362 mg/kg for 3 and 2-phase systems, respectively. It was followed by trans-2-hexen-1-ol and 1-penten-3-one for both 3 and 2-phase systems. The type of odour and taste is spicy. The odour is sharp, ethereal and peppery and the taste is sharp, ethereal, peppery and mustard. In the 2-phase system cis-2-penten-1-ol, trans-2-hexen-1-ol and 1-penten-3-one were found to be higher than in the 3-phase system. In Memecik EVOO, for both 3 and 2-phase systems, trans-2-hexen-1-ol was determined to be the highest, followed by cis-2-penten-1-ol and cis-3-hexenyl acetate, at 1374, 656.39, 440.09 mg/kg and 2926, 833.83, 374.32 mg/kg, respectively. In the 2-phase system, trans-2-hexen-1-ol and cis-3-hexenyl acetate were detected to be higher than in the 3-phase system. Trans-2-hexen-1-ol and 1-hexenol can be used for cultivar differentiation [27]. Trans-2-hexen-1-ol volatile compound can be used to distinguish Gemlik and Memecik EVOOs, and cis-2-penten-1-ol can be used to distinguish Ayvalık EVOO. Agronomic conditions and geographic location, in addition to genetic factors, influence volatile formation and therefore the organoleptic properties of VOO and so can be used to distinguish and characterize olive oils from each region [24]. These findings are consistent with those of Nigri et al. [28] in Algerian, Mansour et al. [24] and Chtourou et al. [29] in Tunisian olive oils.
Table 1 demonstrates that, of the 62 olive oil samples collected in the 2015/16 harvest year, 25 were the Ayvalık variety, 12 were Gemlik and 25 were from the Memecik variety. As shown in Table VIII, in the 2015/16 harvest year, trans-2-hexen-1-ol was found to be the highest volatile compound, followed by cis-2-penten-1-ol and cis-3-hexenyl acetate at 2814, 1034 and 259.63 mg/kg, respectively, in EVOOs obtained from the Gemlik variety with the 3-phase system. In the 2-phase system, cis-2-penten-1-ol was detected to be the highest, followed by trans-2-hexen-1-ol and hexanal at 10169, 2587 and 1975 mg/kg, respectively. For Ayvalık EVOO, cis-2-penten-1-ol was determined to be the highest volatile compound at 2227 and 1428 mg/kg for 3 and 2-phase systems, respectively. This was followed by trans-2-hexen-1-ol and 1-penten-3-one with 911.09 and 513.24 mg/kg and 237.16 and 315.80 mg/kg for 3 and 2-phase systems, respectively. In 3-phase system cis-2-penten-1-ol and trans-2-hexen-1-ol were higher than 2-phase system. In Memecik EVOO, for both 3 and 2-phase systems trans-2-hexen-1-ol was the highest, followed by cis-2-penten-1-ol and cis-3-hexenyl acetate, 3000, 4889, 901.83 mg/kg and 6148, 5165, 1132 mg/kg, respectively. In 2-phase system, trans-2-hexen-1-ol, cis-2-penten-1-ol and cis-3-hexenyl acetate were found to be higher than in the 3-phase system. For Gemlik EVOO, in the 2-phase system, cis-2-penten-1-ol can be used as a distinctive volatile compound since it is quite high. Trans-2-hexen-1-ol volatile compound can be used to distinguish Memecik EVOO, and cis-2-penten-1-ol can be used to distinguish Ayvalık OO. These results were in agreement with Krichene et al. (2010), who found that volatile compositions of olive oil varieties were affected by variety.
According to our findings, the volatile compounds C6 (hexanal, trans-2-hexenal, hexan-1-ol, cis-3-hexenal, trans-2-hexen-1-ol, hexyl acetate, and cis-3-hexenyl acetate) and C5 (1-penten-3-one, 1-penten-3-ol, 3-pentanone, trans-2-pentenal, and 3-ethyl 1,5-octadiene isomers) were generally identified in the samples of Turkish olive oils (Memecik, Gemlik, Domat, Uslu, and Ayvalık) [30,8]. At the low maturation index, trans-2-hexenal is usually present, and with advanced maturation, hexanal is mostly found to be the main volatile compound. In our research, cis-2-penten-1-ol and trans-2-hexen-1-ol were determined to be the highest in both years in all EVOO samples. In the 2014/15 harvest year, trans-2-hexen-1-ol was the highest in the 3-phase system for Gemlik and Memecik EVOOs, followed by cis-2-penten-1-ol. For Ayvalık EVOO, the opposite occurred. Cis-2-penten-1-ol was found to be the highest in Gemlik and Ayvalık EVOOs in the 2-phase system, followed by trans-2-hexen-1-ol. The opposite was true for Memecik EVOO. In the 2015/16 harvest year, trans-2-hexen-1-ol was detected to be the highest only in Gemlik EVOO in the 3-phase system, followed by cis-2-penten-1-ol. Conversely, in Ayvalık and Memecik EVOOs, cis-2-penten-1-ol was the highest for Gemlik and Ayvalık EVOOs in the 2-phase system, followed by trans-2-hexen-1-ol. The opposite was found for Memecik EVOO. Since cis-2-penten-1-ol was detected at the highest amount in both extraction systems in Ayvalık EVOO in both years, it can be used to distinguish Ayvalık EVOO. The volatile composition of the samples varies considerably over the years [8]. Şişik Oğraş et al. [12] found that the variety factor on the volatile profile is a more effective indicator than the harvest year, and that volatile compounds can play an important role in the separation of the regions with the principal component analysis. It is very important for the oil to be obtained from a single maturity index for better characterization [31]. The growing area has impact on cis-3-hexenal, cis-3-hexen-1-ol, hexanal, 1-hexanol, trans-2-hexenal, trans-3-hexen-1-ol and trans-2-hexen-1-ol [12].

**Table VII -** Volatile component results according to the extraction methods of the samples collected from the regions in 2014/15 (µg/kg)

| Variety | Extraction System (Phases) | Sample No | 1-Penten-3-one | Hexanal | Trans-2-pentanal | 1-penten-3-ol | 2-methyl-1-butanol | 1-pentanol | Cis-3-hexenyl acetate | Cis-2-penten-1-ol | 1-hexanol | Trans-3-hexen-1-ol | Cis-3-hexen-1-ol | Trans-2-hexen-1-ol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | 240.45 | 220.12 | 36.92 | 175.88 | 2.18 | 1.38 | 640.28 | 993.40 | 81.61 | 14.90 | 129.51 | 1120 |
| | | **2** | 425.22 | 218.44 | 22.01 | 113.70 | 2.24 | 1.22 | 641.86 | 1209.71 | 127.33 | 0.95 | 102.97 | 1337 |
| | | **3** | 518.74 | 184.43 | 23.72 | 123.57 | 0.97 | 1.09 | 570.71 | 861.33 | 55.42 | 12.47 | 112.97 | 870.24 |
| | | **4** | 105.03 | 261.50 | 4.81 | 16.90 | 2.79 | 1.89 | 368.71 | 1036.95 | 167.89 | 23.19 | 115.49 | 2762 |
| | | **5** | 576.27 | 228.16 | 8.00 | 159.96 | 2.11 | 1.82 | 24.98 | 1538.05 | 128.00 | 31.33 | 95.47 | 1077 |
| | | **6** | 234.82 | 290.68 | 70.94 | 171.88 | 3.39 | 1.67 | 708.65 | 621.08 | 150.13 | 13.74 | 92.63 | 1435 |
| | | **7** | 0.79 | 120.51 | 4.81 | 34.08 | 1.80 | 4.51 | 414.23 | 836.53 | 415.84 | 21.90 | 77.73 | 4923 |
| **Gemlik** | 3 | **8** | 528.15 | 225.10 | 70.75 | 27.56 | 25.02 | 1.37 | 78.94 | 967.20 | 117.82 | 20.90 | 112.02 | 1442 |
| | | **9** | 548.82 | 207.07 | 129.19 | 49.31 | 2.69 | 1.55 | 405.77 | 1172.18 | 108.91 | 16.88 | 116.14 | 1395 |
| | | **10** | 70.18 | 266.47 | 97.65 | 37.06 | 2.65 | 97.90 | 564.71 | 381.20 | 102.70 | 11.85 | 64.03 | 953.11 |
| | | **11** | 14.14 | 162.02 | 4.81 | 13.91 | 1.68 | 10.64 | 416.45 | 257.65 | 56.48 | 7.13 | 41.67 | 612.62 |
| | | **12** | 134.33 | 275.16 | 113.32 | 33.51 | 1.54 | 51.83 | 777.88 | 897.55 | 158.11 | 12.50 | 104.10 | 1190 |
| | | **13** | 65.84 | 191.27 | 16.02 | 82.00 | 1.93 | 48.49 | 306.07 | 657.99 | 139.16 | 16.02 | 107.93 | 1796 |
| | | **14** | 539.09 | 212.23 | 82.12 | 40.24 | 1.91 | 1.75 | 281.57 | 1150.70 | 165.96 | 22.60 | 83.80 | 2256 |
| | | **15** | 299.82 | 246.13 | 83.56 | 36.16 | 1.11 | 1.54 | 84.73 | 972.99 | 111.62 | 14.56 | 62.07 | 1402 |
| | | **16** | 141.90 | 319.74 | 33.58 | 82.13 | 1.29 | 74.96 | 276.69 | 418.15 | 147.66 | 20.97 | 123.30 | 3031 |
| | **Average** | | **277.72** | **226.81** | **50.14** | **74.87** | **3.46** | **18.98** | **410.14** | **873.29** | **139.66** | **16.37** | **96.36** | **1725** |
| | 2 | **17** | 279.60 | 180.87 | 89.45 | 371.34 | 1.27 | 1.71 | 296.46 | 1224.60 | 126.73 | 46.56 | 233.96 | 1012 |
| | **Average** | | **279.60** | **180.87** | **89.45** | **371.34** | **1.27** | **1.71** | **296.46** | **1224.60** | **126.73** | **46.56** | **233.96** | **1012** |
| | | **18** | 31.00 | 193.51 | 71.31 | 14.06 | 1.51 | 29.31 | 78.69 | 150.35 | 158.79 | 29.45 | 114.81 | 1276 |
| | | **19** | 399.89 | 210.91 | 148.05 | 55.45 | 32.31 | 1.21 | 167.07 | 1290 | 56.26 | 16.26 | 107.77 | 125.03 |
| | | **20** | 422.63 | 299.49 | 169.32 | 82.00 | 16.41 | 0.77 | 238.71 | 983.45 | 149.19 | 3.87 | 290.75 | 511.84 |
| | | **21** | 54.66 | 15.14 | 110.20 | 47.73 | 95.31 | 275.08 | 307.41 | 578.63 | 325.79 | 36.01 | 387.47 | 1661 |
| | | **22** | 392.34 | 212.64 | 155.40 | 82.64 | 1.42 | 45.85 | 122.56 | 914.52 | 73.83 | 6.15 | 185.40 | 666.80 |
| | | **23** | 175.65 | 445.78 | 65.00 | 43.44 | 0.49 | 1.16 | 278.58 | 669.79 | 183.44 | 46.40 | 360.12 | 944.88 |
| | | **24** | 247.00 | 469.45 | 112.00 | 107.02 | 1.08 | 58.81 | 261.41 | 1514 | 281.41 | 14.23 | 914.62 | 1656 |
| | | **25** | 49.90 | 332.37 | 119.00 | 34.83 | 2.69 | 1.11 | 212.89 | 1642 | 83.26 | 21.32 | 154.12 | 320.64 |
| | | **26** | 536.19 | 267.06 | 168.02 | 64.94 | 0.44 | 0.60 | 163.11 | 1755 | 73.28 | 16.00 | 119.92 | 38.98 |
| | 3 | **27** | 333.29 | 181.04 | 79.00 | 37.02 | 0.00 | 0.60 | 120.69 | 407.75 | 44.78 | 0.95 | 76.06 | 31.19 |
| | | **28** | 251.90 | 404.59 | 184.71 | 63.58 | 1.42 | 2.23 | 149.50 | 690.16 | 160.63 | 6.62 | 216.27 | 1208 |
| | | **29** | 517.02 | 301.91 | 156.92 | 63.94 | 0.79 | 17.77 | 82.02 | 693.55 | 71.49 | 41.70 | 139.63 | 464.02 |
| | | **30** | 644.54 | 397.48 | 112.27 | 82.54 | 0.68 | 1.85 | 258.56 | 1301 | 75.46 | 41.70 | 183.19 | 323.52 |
| | | **31** | 579.21 | 388.89 | 148.32 | 88.42 | 0.73 | 1.68 | 282.41 | 960.12 | 130.01 | 45.50 | 191.30 | 974.95 |
| | | **32** | 68.33 | 484.59 | 14.31 | 52.41 | 1.30 | 1.12 | 209.84 | 1756 | 72.79 | 24.78 | 238.98 | 438.05 |
| | | **33** | 323.41 | 246.15 | 104.72 | 70.78 | 1.23 | 0.60 | 127.32 | 1263 | 54.05 | 40.40 | 174.45 | 278.05 |
| | | **34** | 362.08 | 292.61 | 154.56 | 46.14 | 1.43 | 1.84 | 120.24 | 664.53 | 94.26 | 32.60 | 145.17 | 757.37 |
| **Ayvalık** | | **35** | 516.37 | 516.37 | 125.29 | 93.91 | 2.76 | 66.95 | 465.24 | 1055 | 119.33 | 46.40 | 308.36 | 490.58 |
| | | **36** | 834.88 | 383.45 | 116.19 | 78.39 | 0.69 | 0.87 | 494.91 | 1393 | 104.12 | 32.65 | 265.44 | 274.09 |
| | | **37** | 713.95 | 301.78 | 49.24 | 68.16 | 1.59 | 1.01 | 335.01 | 1578 | 48.98 | 11.80 | 146.35 | 31.19 |
| | **Average** | | **372.71** | **317.26** | **118.19** | **63.87** | **8.21** | **25.52** | **223.81** | **1063** | **118.06** | **25.74** | **236.01** | **623.64** |
| | | **38** | 362.10 | 315.48 | 124.00 | 109.27 | 2.59 | 92.50 | 301.65 | 1889 | 181.59 | 12.97 | 338.04 | 1145 |
| | | **39** | 321.17 | 336.93 | 87.00 | 94.78 | 1.52 | 6.57 | 211.92 | 1831 | 279.35 | 31.40 | 408.20 | 1662 |
| | | **40** | 676.24 | 308.83 | 185.13 | 92.32 | 55.14 | 63.34 | 198.21 | 1687 | 83.07 | 35.00 | 206.74 | 261.77 |
| | | **41** | 287.47 | 357.60 | 118.00 | 67.44 | 0.71 | 13.53 | 301.76 | 805.40 | 184.76 | 8.66 | 329.08 | 841.59 |
| | | **42** | 568.40 | 339.77 | 179.03 | 87.97 | 1.49 | 92.66 | 280.09 | 1737 | 134.31 | 2.60 | 252.44 | 887.94 |
| | | **43** | 494.26 | 262.66 | 145.00 | 84.44 | 1.73 | 1.48 | 159.77 | 1491 | 110.45 | 47.30 | 245.28 | 648.10 |
| | | **44** | 628.35 | 241.20 | 177.29 | 74.64 | 1.61 | 0.98 | 161.05 | 85.62 | 77.28 | 26.86 | 177.68 | 380.70 |
| | | **45** | 662.69 | 266.84 | 136.00 | 99.26 | 0.49 | 0.60 | 314.75 | 1630 | 88.81 | 29.70 | 211.00 | 275.76 |
| | 2 | **46** | 512.31 | 308.61 | 156.39 | 86.53 | 0.92 | 0.60 | 247.50 | 1349 | 112.53 | 47.90 | 326.45 | 549.07 |
| | | **47** | 407.43 | 311.49 | 135.05 | 92.35 | 36.02 | 0.60 | 180.18 | 1341 | 118.01 | 2.18 | 383.50 | 643.25 |
| | | **48** | 395.15 | 444.08 | 235.00 | 101.65 | 1.78 | 2.20 | 126.40 | 992.19 | 204.99 | 16.12 | 311.04 | 1132 |
| | | **49** | 727.00 | 402.73 | 169.00 | 90.80 | 1.06 | 47.64 | 113.75 | 925.59 | 125.50 | 8.83 | 228.18 | 787.46 |
| | | **50** | 399.09 | 411.30 | 136.00 | 73.19 | 1.69 | 1.36 | 188.79 | 660.87 | 87.20 | 25.30 | 224.66 | 344.41 |
| | | **51** | 533.13 | 375.71 | 152.69 | 76.30 | 1.44 | 1.72 | 124.67 | 1487 | 103.36 | 1.81 | 202.03 | 738.22 |
| | | **52** | 558.40 | 417.50 | 127.00 | 87.31 | 0.16 | 1.37 | 76.04 | 1246 | 90.71 | 27.90 | 225.03 | 582.71 |
| | | **53** | 548.71 | 294.22 | 140.79 | 87.65 | 0.83 | 27.48 | 102.26 | 1493 | 97.35 | 0.95 | 246.20 | 322.13 |
| | | **54** | 987.74 | 290.07 | 183.18 | 467.19 | 0.88 | 47.79 | 119.36 | 2503 | 27.44 | 37.59 | 288.43 | 253.54 |
| | **Average** | | **533.51** | **334.41** | **152.15** | **110.18** | **6.47** | **23.67** | **188.71** | **1362** | **123.93** | **21.36** | **270.82** | **673.97** |

Table VII - continue

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Memecik | 3 | **55** | 212.28 | 219.42 | 53.70 | 159.20 | 2.53 | 1.69 | 405.87 | 577.87 | 98.17 | 48.50 | 108.20 | 1113 |
| | | **56** | 27.83 | 224.71 | 45.20 | 23.54 | 0.76 | 1.64 | 372.38 | 62.55 | 102.42 | 13.28 | 56.49 | 695.26 |
| | | **57** | 439.67 | 421.47 | 24.89 | 293.79 | 1.25 | 5.12 | 688.60 | 1037 | 95.36 | 21.61 | 185.02 | 1070.76 |
| | | **58** | 490.76 | 119.31 | 178.10 | 77.53 | 0.84 | 26.83 | 225.31 | 1190 | 11.55 | 7.30 | 64.56 | 493.01 |
| | | **59** | 208.89 | 221.99 | 122.04 | 51.12 | 1.89 | 2.24 | 263.07 | 751.13 | 200.87 | 27.67 | 180.69 | 2650 |
| | | **60** | 154.81 | 384.28 | 33.42 | 114.01 | 2.35 | 1.79 | 685.30 | 318.21 | 247.29 | 48.00 | 151.33 | 2225 |
| | | **Average** | **255.71** | **265.20** | **76.22** | **119.87** | **1.60** | **6.55** | **440.09** | **656.39** | **125.95** | **27.73** | **124.38** | **1374.78** |
| | 2 | **61** | 578.26 | 269.72 | 104.51 | 534.24 | 3.04 | 1.83 | 877.85 | 2202 | 192.88 | 48.00 | 309.27 | 1664 |
| | | **62** | 0.79 | 11.21 | 10.80 | 122.19 | 18.31 | 3.83 | 360.90 | 167.73 | 308.56 | 23.23 | 95.22 | 2953 |
| | | **63** | 0.79 | 285.90 | 140.22 | 59.15 | 1.95 | 1.67 | 89.93 | 1410 | 183.41 | 31.35 | 190.75 | 2003 |
| | | **64** | 134.05 | 148.88 | 83.78 | 7.06 | 2.03 | 0.60 | 202.94 | 248.70 | 20.42 | 0.95 | 30.77 | 423.94 |
| | | **65** | 0.79 | 104.18 | 4.81 | 46.88 | 146.93 | 4.57 | 195.97 | 971.76 | 380.13 | 21.43 | 65.73 | 5870 |
| | | **66** | 0.79 | 189.93 | 36.31 | 37.78 | 4.16 | 3.74 | 343.32 | 336.09 | 402.58 | 30.30 | 108.85 | 4687 |
| | | **67** | 16.49 | 192.95 | 101.69 | 30.09 | 3.04 | 2.23 | 549.37 | 499.94 | 362.31 | 28.90 | 121.75 | 2879 |
| | | **Average** | **104.57** | **171.82** | **68.87** | **119.63** | **25.64** | **2.64** | **374.32** | **833.83** | **264.33** | **26.31** | **131.76** | **2926** |

**Table VIII -** Volatile component results according to the extraction methods of the samples collected from the regions in 2015/16 (mg/kg)

| Variety | Extraction System (Phases) | Sample No | 1-Penten-3-one | Hexanal | Trans-2-pentanal | 1-penten-3-ol | 2-methyl-1 butanol | 1-pentanol | Cis-3-hexenyl acetate | Cis-2 -penten-1-ol | 1-hexanol | Trans-3 -hexen-1-ol | Cis-3-hexen-1-ol | Trans-2-hexen-1-ol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gemlik | 3 | **1** | 0.85 | 36.77 | 6.70 | 43.74 | 952.00 | 4.93 | 245.35 | 1025 | 371.91 | 32.22 | 136.56 | 6177 |
| | | **2** | 0.39 | 73.63 | 4.14 | 46.94 | 174.00 | 5.43 | 286.53 | 958.00 | 390.77 | 27.46 | 114.34 | 3915 |
| | | **3** | 0.75 | 45.68 | 7.00 | 39.24 | 2.40 | 6.78 | 402.54 | 1313 | 441.57 | 49.99 | 183.21 | 7882 |
| | | **4** | 48.00 | 106.24 | 1.48 | 55.92 | 87.00 | 4.63 | 467.00 | 1321 | 248.42 | 25.36 | 119.53 | 2937 |
| | | **5** | 1051.00 | 365.81 | 13.48 | 107.51 | 365.52 | 1.87 | 28.00 | 2185 | 221.00 | 30.88 | 123.33 | 1331 |
| | | **6** | 0.39 | 16.00 | 4.80 | 70.59 | 130.00 | 6.65 | 154.54 | 1161 | 520.31 | 35.38 | 119.05 | 6423 |
| | | **7** | 100.00 | 78.52 | 19.00 | 43.00 | 1.24 | 5.50 | 504.88 | 427.84 | 47.78 | 0.27 | 67.55 | 755.15 |
| | | **8** | 0.46 | 67.44 | 7.20 | 67.40 | 2.00 | 4.60 | 107.70 | 227.28 | 111.67 | 18.17 | 15.00 | 881.90 |
| | | **9** | 56.48 | 301.44 | 0.89 | 14.35 | 0.45 | 0.49 | 403.09 | 856.51 | 59.07 | 0.27 | 49.44 | 200.45 |
| | | **10** | 269.86 | 151.82 | 36.90 | 406.27 | 1.20 | 1.95 | 238.42 | 1409 | 47.88 | 25.00 | 121.79 | 241.71 |
| | | **11** | 212.21 | 195.81 | 102.68 | 20.35 | 487.49 | 5.90 | 17.87 | 489.16 | 76.38 | 23.06 | 191.65 | 218.05 |
| | **Average** | | 158,22 | 130.83 | 18.57 | 83.21 | 200.30 | 4.43 | 259.63 | 1034 | 230.61 | 24.37 | 112.86 | 2814 |
| | **2** | **12** | 125.78 | 1975 | 116.89 | 215.58 | 311.14 | 17.38 | 964.58 | 10169 | 690.48 | 31.23 | 871.68 | 2587 |
| | **Average** | | 125,78 | 1975 | 116.89 | 215.58 | 311.14 | 17.38 | 964.58 | 10169 | 690.48 | 31.23 | 871.68 | 2587 |
| Ayvalik | 3 | **13** | 102.49 | 132.21 | 10.80 | 4.83 | 0.45 | 0.73 | 121.01 | 391.13 | 33.57 | 24.23 | 69.29 | 129.83 |
| | | **14** | 17.52 | 151.75 | 0.93 | 29.00 | 6.61 | 0.49 | 175.89 | 371.95 | 37.48 | 16.00 | 89.06 | 1029 |
| | | **15** | 154.29 | 194.32 | 36.70 | 133.92 | 51.96 | 3.54 | 179.64 | 2150 | 193.56 | 15.74 | 486.35 | 1331 |
| | | **16** | 511.64 | 459.83 | 91.86 | 111.20 | 31.00 | 1.91 | 90.41 | 16820 | 121.88 | 3.15 | 239.83 | 3320 |
| | | **17** | 78.11 | 201.20 | 11.30 | 95.34 | 54.03 | 1.79 | 93.25 | 1623 | 172.92 | 5.04 | 257.68 | 2499 |
| | | **18** | 993.05 | 243.00 | 84.06 | 128.40 | 23.00 | 1.23 | 147.74 | 1907 | 89.11 | 27.44 | 180.82 | 209.28 |
| | | **19** | 0.39 | 43.89 | 0.89 | 25.00 | 0.65 | 1.41 | 123.07 | 285.24 | 54.00 | 6.80 | 177.28 | 590.55 |
| | | **20** | 86.63 | 212.56 | 32.17 | 41.18 | 53.07 | 1.70 | 186.16 | 609.09 | 104.53 | 37.52 | 170.63 | 883.25 |
| | | **21** | 22.22 | 50.91 | 9.70 | 33.99 | 0.45 | 0.49 | 100.33 | 227.28 | 23.92 | 13.04 | 100.53 | 424.98 |
| | | **22** | 300.54 | 475.88 | 8.50 | 108.79 | 4.00 | 1.87 | 169.88 | 2139 | 154.00 | 8.74 | 321.35 | 1118 |
| | | **23** | 181.72 | 164.63 | 33.31 | 25.53 | 1.74 | 2.10 | 188.30 | 741.92 | 67.22 | 0.27 | 191.96 | 477.30 |
| | | **24** | 318.90 | 562.53 | 11.98 | 725.33 | 3.07 | 2.42 | 160.17 | 1294 | 77.38 | 21.48 | 116.53 | 243.47 |
| | | **25** | 479.37 | 357.84 | 49.00 | 1009 | 2.78 | 2.15 | 185.40 | 1748 | 105.21 | 32.00 | 187.18 | 314.92 |
| | | **26** | 73.41 | 65.69 | 10.27 | 1.33 | 0.45 | 1.02 | 144.62 | 870.95 | 37.46 | 32.44 | 110.15 | 183.78 |
| | **Average** | | 237.16 | 236.87 | 27.96 | 176.69 | 16.66 | 1.63 | 147.56 | 2227 | 90.87 | 17.42 | 192.76 | 911.09 |
| | 2 | **27** | 253.26 | 189.49 | 40.00 | 95.01 | 0.45 | 1.39 | 166.90 | 789.69 | 60.37 | 34.94 | 127.74 | 287.60 |
| | | **28** | 2.45 | 106.47 | 0.89 | 25.47 | 2.25 | 3.21 | 48.06 | 1275 | 87.12 | 3.87 | 132.75 | 1366 |
| | | **29** | 85.05 | 164.04 | 11.07 | 42.14 | 2.21 | 10.43 | 97.37 | 979.51 | 64.00 | 34.49 | 105.81 | 281.95 |
| | | **30** | 498.68 | 215.91 | 80.30 | 57.61 | 1.56 | 1.11 | 197.08 | 849.55 | 108.00 | 38.76 | 157.24 | 258.25 |
| | | **31** | 131.80 | 251.31 | 1.95 | 32.71 | 0.92 | 1.14 | 221.27 | 1603 | 103.79 | 6.70 | 270.57 | 415.43 |
| | | **32** | 365.27 | 321.55 | 109.00 | 111.85 | 20.00 | 1.36 | 101.79 | 1976 | 87.37 | 2.00 | 238.95 | 593.00 |
| | | **33** | 187.11 | 406.58 | 58.00 | 163.00 | 26.00 | 9.59 | 170.64 | 2611 | 228.98 | 20.82 | 564.53 | 864.00 |
| | | **34** | 90.33 | 142.30 | 75.26 | 13.88 | 7.12 | 1.96 | 199.33 | 394.79 | 115.00 | 10.54 | 180.93 | 639.34 |
| | | **35** | 214.60 | 166.03 | 9.70 | 42.74 | 1.03 | 1.42 | 243.04 | 1520 | 80.26 | 0.50 | 213.18 | 208.00 |
| | | **36** | 1072.14 | 160.28 | 14.56 | 63.74 | 2.20 | 0.52 | 643.00 | 1563 | 213.42 | 7.74 | 203.32 | 225.61 |
| | | **37** | 573.16 | 407.23 | 138.00 | 120.40 | 2.57 | 2.20 | 119.63 | 2147 | 135.03 | 8.01 | 248.34 | 505.79 |
| | **Average** | | 315.80 | 230.11 | 48.98 | 69.87 | 6.03 | 3.12 | 200.74 | 1428 | 116.67 | 15.31 | 222.12 | 513.24 |

Table VIII - continue

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Memecik | 3 | **38** | 1.90 | 197.72 | 3.00 | 10.56 | 1.63 | 1.14 | 386.15 | 499.25 | 92.33 | 128.00 | 46.00 | 338.91 |
| | | **39** | 46.03 | 173.18 | 28.88 | 26.93 | 8.08 | 1.66 | 946.67 | 766.22 | 308.39 | 25.00 | 119.45 | 605.58 |
| | | **40** | 0.48 | 122.08 | 3.00 | 19.00 | 2.62 | 1.66 | 765.42 | 227.28 | 176.14 | 21.45 | 74.69 | 1108 |
| | | **41** | 24.76 | 196.08 | 5.48 | 10.95 | 3.35 | 1.29 | 1797 | 376.33 | 217.50 | 31.00 | 141.43 | 448.21 |
| | | **42** | 44.35 | 2812.69 | 7.39 | 460.06 | 1.03 | 0.60 | 3002 | 21088 | 1324.36 | 52.44 | 1510 | 4676 |
| | | **43** | 80.85 | 224.85 | 2.48 | 11.28 | 1.91 | 1.04 | 800.88 | 553.73 | 173.54 | 125.99 | 129.00 | 561.53 |
| | | **44** | 122.48 | 110.70 | 8.24 | 166.45 | 444.00 | 25.02 | 164.07 | 4418 | 386.07 | 24.82 | 270.59 | 5890 |
| | | **45** | 39.61 | 225.58 | 96.31 | 1359 | 208.00 | 31.40 | 205.36 | 2781 | 457.66 | 11.09 | 252.35 | 3702 |
| | | **46** | 178.44 | 208.98 | 126.00 | 1319 | 342.00 | 4.00 | 122.45 | 2722 | 252.75 | 50.19 | 103.05 | 3401 |
| | | **47** | 165.16 | 268.79 | 48.00 | 43.70 | 0.45 | 2.63 | 427.93 | 726.28 | 106.00 | 29.51 | 100.93 | 1384 |
| | | **48** | 96.35 | 459.04 | 10.35 | 123.96 | 304.80 | 8.89 | 343.13 | 3659 | 582.43 | 42.44 | 335.91 | 1559 |
| | | **49** | 748.98 | 3774 | 1826 | 547.32 | 470.38 | 193.46 | 1859 | 20860 | 1916 | 48.88 | 1547 | 12409 |
| | **Average** | | 129,12 | 731.19 | 180.43 | 341.63 | 149.02 | 22.73 | 901.83 | 4889 | 499.51 | 49.23 | 385.96 | 3007 |
| | 2 | **50** | 228.21 | 339.57 | 6.74 | 78.70 | 27.00 | 5.27 | 129.51 | 1515 | 192.93 | 22.73 | 310.32 | 29.00 |
| | | **51** | 1.97 | 84.07 | 9.00 | 13.76 | 5.00 | 2.05 | 743.75 | 497.68 | 131.13 | 32.00 | 106.48 | 1825 |
| | | **52** | 5.58 | 1291 | 37.43 | 266.58 | 2.33 | 3.41 | 634.30 | 10148 | 1066 | 136.00 | 981.07 | 10612 |
| | | **53** | 1.11 | 114.19 | 0.89 | 29.00 | 0.99 | 0.69 | 1127 | 360.76 | 179.00 | 148.14 | 153.00 | 544.12 |
| | | **54** | 60.56 | 326.42 | 10.00 | 19.03 | 2.76 | 1.47 | 1333 | 1147 | 216.05 | 40.00 | 187.03 | 1020 |
| | | **55** | 0.73 | 138.95 | 5.20 | 4.69 | 3.33 | 2.06 | 934.64 | 343.44 | 289.78 | 151.13 | 156.00 | 2718 |
| | | **56** | 0.87 | 172.49 | 2.94 | 3.86 | 8.00 | 2.90 | 662.61 | 227.28 | 343.04 | 33.00 | 95.43 | 2578 |
| | | **57** | 1.10 | 4012 | 0.89 | 484.66 | 0.45 | 0.49 | 1394 | 15528 | 1305 | 9.07 | 497.63 | 7828 |
| | | **58** | 214.06 | 1712 | 75.18 | 167.56 | 0.45 | 0.49 | 3127 | 9085 | 1233 | 20.45 | 835.72 | 29.00 |
| | | **59** | 399.32 | 168.06 | 67.00 | 36.46 | 458.53 | 0.49 | 499.03 | 819.73 | 79.31 | 0.27 | 93.04 | 338.24 |
| | | **60** | 68.11 | 148.35 | 60.75 | 42.38 | 2.44 | 0.49 | 450.70 | 877.53 | 204.17 | 40.60 | 177.33 | 4264 |
| | | **61** | 285.13 | 188.58 | 132.96 | 1721 | 163.00 | 19.60 | 309.05 | 3123 | 351.63 | 146.92 | 289.31 | 4553 |
| | | **62** | 2.90 | 2352 | 146.36 | 639.35 | 740.86 | 8.35 | 3373 | 23478 | 3993 | 168.38 | 2389 | 43587 |
| | **Average** | | 97.67 | 849.97 | 42.72 | 269.78 | 108.86 | 3.67 | 1132 | 5165 | 737.43 | 72.98 | 482.41 | 6148 |

The PCA was applied to the EVOO samples together in Table VII and Table VIII to see if it provides a meaningful classification and grouping based on harvest year, production method, and variety based on the volatile compounds. Figure 8 shows two- and three-dimensional PCA scores plots (a and b) and PCA scores and loading bi-plots (c and d) from standardized data of volatile compounds of Ayvalık, Memecik and Gemlik EVOOs obtained by two- and three- phase extraction systems in the 2014/15 and 2015/16 harvest seasons. As can be seen from both two- (PC1 vs PC2) and three- (PC1, PC2 and PC3) dimensional score plots, Memecik samples are more scattered compared to the Ayvalık and Gemlik varieties. While there is a strong overlap of the Ayvalık and Gemlik samples, the Memecik samples are partially separated from others. However, it is evident that PCA as an unsupervised method is not very successful in demonstrating a clear classification of these EVOO samples based only on 12 volatile compounds. However, it possible to make some generalizations based on the two- and three-dimensional bi-plots of scores and loadings. Among the oils obtained with the 2-phase system in the 2014/15 harvest year, Ayvalık EVOO was distinguished by hexanal, trans-2-pentanal and 1-hexanol, while Memecik EVOO was characterized by 2-methyl-1-butanol, 1-hexanol and trans-2-hexen-1-ol. Among the oils obtained with the 3-phase system, 1-penten-3-one, hexanal and cis-2-penten-1-ol separation from other varieties were detected only in Ayvalık EVOO. In addition, while 23.5% of the total variability in the data was explained with PC1, approximately 20% was expressed by PC2 and 12% by PC3. After observing the results of the PCA, it was decided to apply supervised classification by using PLS-DA in order to see a better separation of the three varieties from the three different geographical regions. The same data set was used to construct a training set (43 Ayvalık, 22 Gemlik and 27 Memcik samples) and an independent validation set (21 Ayvalık, 9 Gemlik and 13 Memcik samples). While constructing the training and independent validation samples, about two third of the samples were put into the training set and the remaining one third were reserved for the independent validation set, taking into consideration that approximately half of the samples in both training and validation sets were from the first and the other half from the second harvest year. In addition, the PLS-DA algorithm was performed with standardized data by applying leave-one-out cross-validation. Figure 9 shows PLS-DA 2D scores plot (a), the number of latent variables plot (b), PLS-DA 3D scores plot (c) and similarity plot (d) from standardized data of volatile compounds of the Ayvalık, Memecik and Gemlik EVOOs obtained by two- and three-phase extraction systems in the 2014/15 and 2015/16 harvest seasons. Compared to the score plots given in Figure 8 from PCA, relatively better separation of the Memecik samples is seen in the PLS-DA two- and three-dimensional score plots. However there is still a strong overlap of the Alvalık and Gemlik varieties, especially

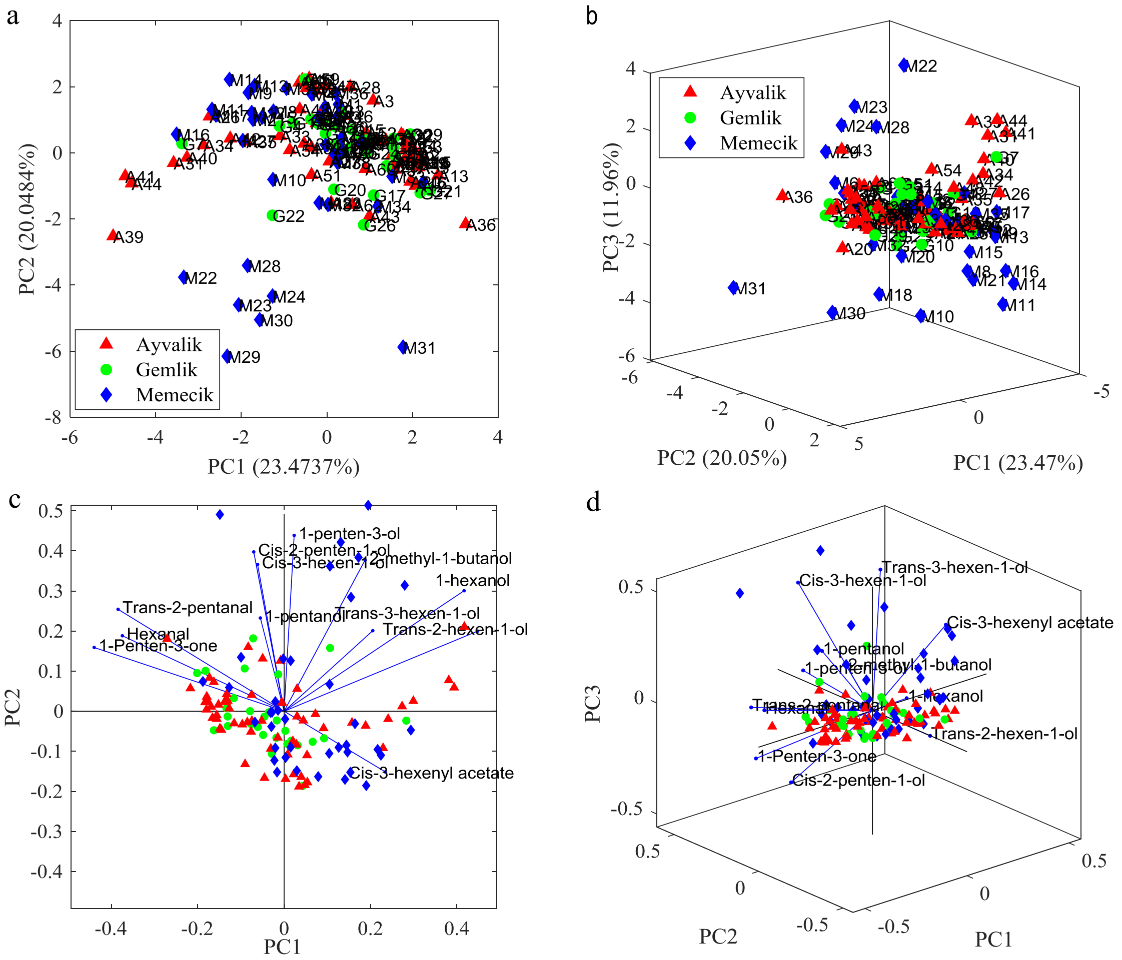


**Figure 8 -** PCA scores plots (a and b) and PCA scores and loading biplots (c and d) from standardized data of volatile compounds of Ayvalık, Memecik and Gemlik EVOOs obtained by two and three phase extraction system on 2014/15 and 2015/16 harvest seasons.



for the independent validation set samples. The final PLS-DA similarity plot was constructed with five latent variable based on leave-one-out cross-validation, indicating that there is no strong evidence for overfitting of the model since the validation samples of the Memcik EVOO were also located away from the cluster of overlapped Ayvalık and Gemlik samples in the similarity plot (Figure 9d). On the other hand, overall correct classification rates for both training and independent test sets (65.2% and 57.55 for training and independent test sets, respectively) were relatively low.

Bar graphs of the PLSD-DA group membership plots for the training set and the independent validation set samples from the standardized data of volatile compounds of the Ayvalık, Memecik and Gemlik EVOOs obtained by the two- and three-phase extraction systems in the 2014/15 and 2015/16 harvest seasons are given in Figure 10 and Figure 11, respectively. As can be seen from Figure 10, there are three bars (representing Ayvalık, Memecik and Gemlik EVOOs) for each sample, the varying length of which should ideally be between 0 and 1. However, it is clear that the length of the bars fluctuates between some negative and positive values, resulting in overlaps of the group membership of the samples in both the training and independent validation sets. Among the three varieties, it is clear that the Memecik samples in the training set are best separated from the other two varieties based on the group membership plot as seen from the gray bars, which are mostly positive and longer than the blue and red bars on the bottom chart of Figure 10. On the other hand, the same success is not seen in Figure 11 for all the validation sets of samples of the Memecik variety. Here, only M4, M15 and M40 are clearly represented with predominant gray bars, indicating strong overlaps. In summary, it can be said that classification and clustering of the Ayvalık, Memecik and Gemlik EVOOs based on volatile compounds has resulted in a relative lack of success in both training and independent validation sets. One possible reason for this could be the size of the variables (volatile compounds) of which there were only 12. For a supervised multivariate evaluation of about 130 samples, only 12 variables are probably not sufficient for this particular case.

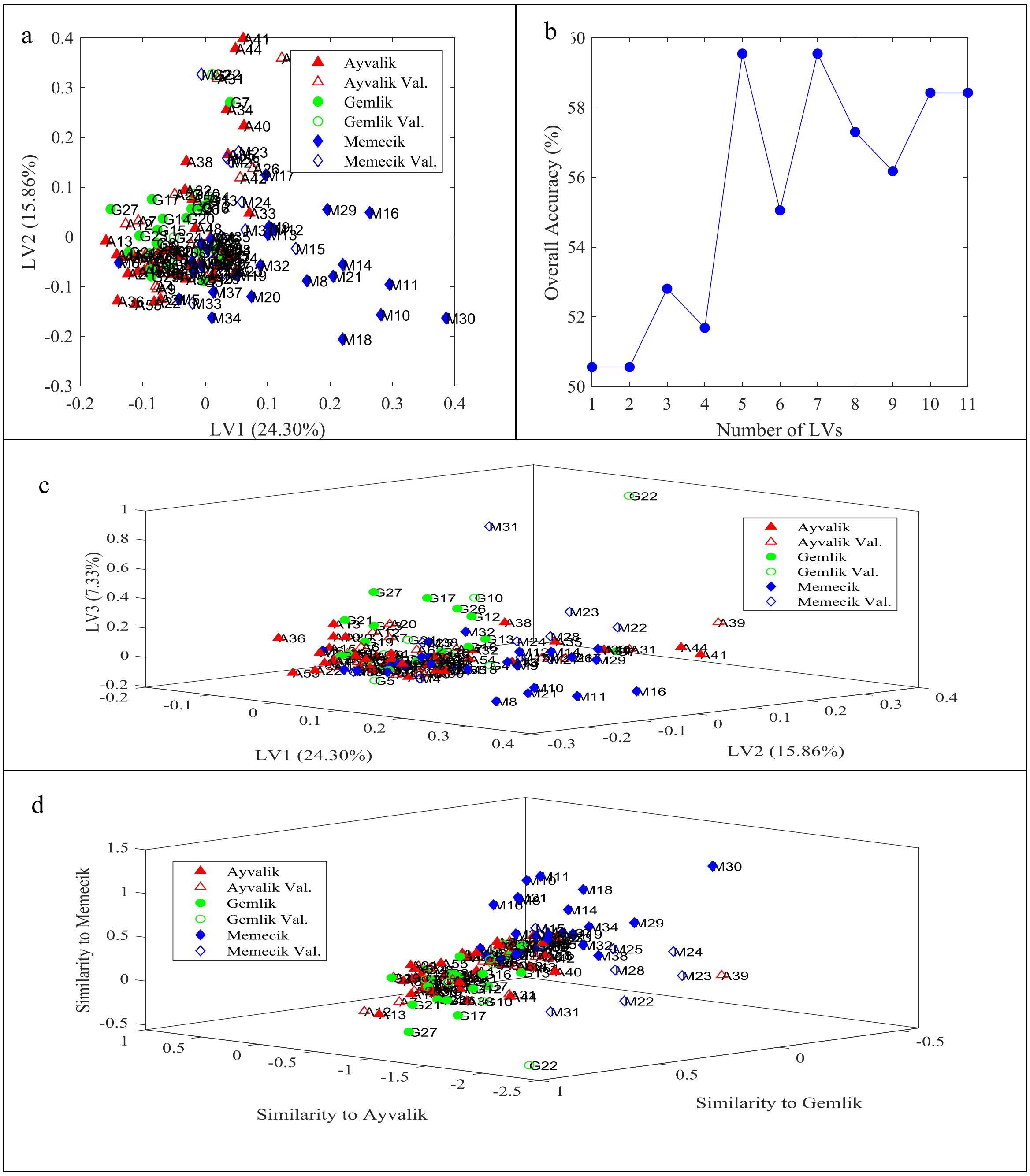


**Figure 9 -** PLS-DA 2D scores plot (a), number of latent variable plot (b), PLS-DA 3D scores plot (c) and similarity plot (d) from standardized data of volatile compounds of Ayvalık, Memecik and Gemlik EVOOs obtained by two and three phase extraction system on 2014/15 and 2015/16 harvest seasons.

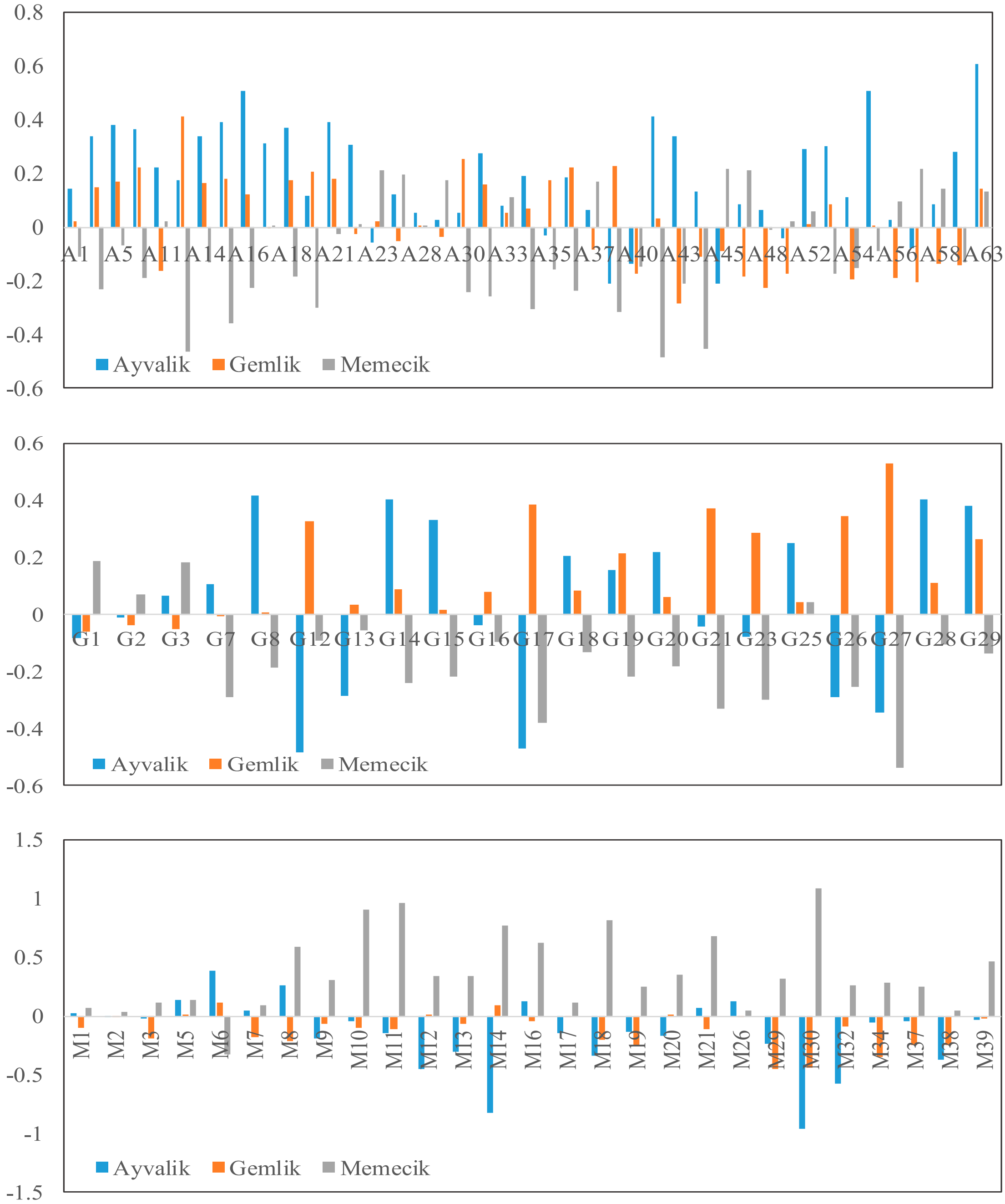


**Figure 10 -** PLSD-DA group membership plots for training set samples from standardized data of volatile compounds of Ayvalık, Memecik and Gemlik EVOOs obtained by two and three phase extraction system on 2014/15 and 2015/16 harvest seasons.

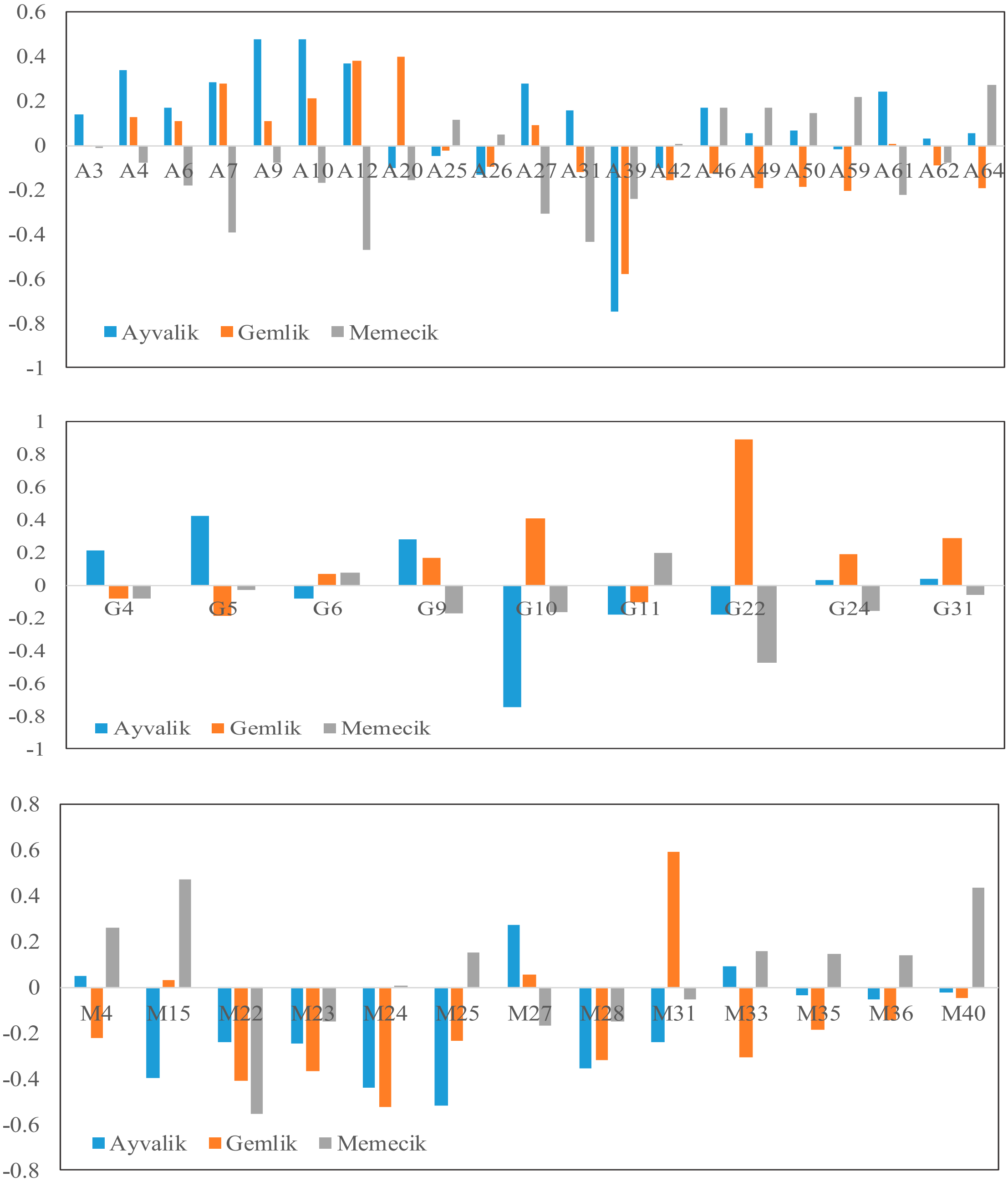


**Figure 11 -** PLSD-DA group membership plots for independent validation set samples from standardized data of volatile compounds of Ayvalık, Memecik and Gemlik EVOOs obtained by two and three phase extraction system on 2014/15 and 2015/16 harvest seasons

## 4. CONCLUSION

During the 2014/15 and 2015/16 crop seasons, volatile compounds of EVOOs (Ayvalk, Memecik, and Gemlik cv.) obtained from two- and three-phase continuous systems from different regions (North Aegean, South Aegean, and South Marmara) of Turkey were analyzed. The highest volatile compounds in the olive oils studied for both years were determined to be trans-2-hexen-1-ol and cis-2-penten-1-ol. These were followed by 1-penten-3-one, hexanal, and acetic acid hexylester/cis-3-hexenil acetate. The results of two- and three-factor ANOVA analyses showed that a combination of variety, harvest season, and extraction method significantly affects volatile compound concentrations. One notable difference was that 1-penten-3-one responded very well to variety and seasonal variation, while 1-penten-3-ol displayed a multifactorial dependency with significant interactions between all three factors assessed. These results emphasize how processing technology, genetics, and the environment all play significant roles in determining the sensory and chemical properties of EVOOs. The results demonstrate that geographic marking and labelling of these oils can be accomplished in one year or with a single production system (two/three phase continuous system) in addition to volatile compounds by using classification and clustering models. Finally, the results obtained using supervised classification models created with PLS-DA showed that the olive oils were divided into three subgroups according to varieties (Ayvalık, Gemlik and Memecik); This shows that geographical indication and labelling of these oils can be done with volatile compounds.

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