EFFECTS OF OLIVE VARIETY, HARVEST TIME, AND MALAXATION TEMPERATURE ON VOLATILE COMPOUNDS AND SENSORIAL CHARACTERISTICS OF OLIVE OILS

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ABSTRACT

EFFECTS OF OLIVE VARIETY, HARVEST TIME, AND MALAXATION TEMPERATURE ON VOLATILE COMPOUNDS AND SENSORIAL CHARACTERISTICS OF OLIVE OILS

In this study, it was aimed to determine the differences between sensory properties of olive oils based on types of olives, malaxation temperature and harvest time. Olive oil samples were extracted from two different Turkish olive cultivars (Ayvalık and Memecik), provided by Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union. Olives which were harvested at three different stages of ripening (early (first harvest time), mid (second harvest time) and late (third harvest time)) were extracted at three different malaxation temperatures (27°C, 37°C, 47°C). Samples were classified into eighteen groups by two olive varieties, by three harvest time and malaxation temperatures. Totally, 36 samples (18 treatments × 2 replicates) were obtained from two parallel extraction processes. The effects of cultivar, malaxation temperature and harvest time on volatile composition, sensory properties and color of olive oils were investigated. SIMCA 13.0.3 (Umetrics AB, Umeå, Sweden) and multilevel factorial experimental design were used for data analysis and result interpretation.

As a conclusion, it was found that cultivar and harvest time of olives had a significant effect on sensorial characteristics of olive oils. However, no obvious effect of malaxation temperature was observed.

ÖZET

ZEYTİN ÇEŞİDİNİN, HASAT ZAMANIN VE MALAKSASYON SICAKLIĞININ ZEYTİNYAĞLARIN UÇUCU BİLEŞİKLERİNE VE DUYUSAL ÖZELLİKLERİNE ETKİLERİ

Bu çalışmada, zeytin çeşidine, malaksasyon sıcaklığına ve hasat zamanına bağlı olarak zeytinyağların duyusal özellikleri arasındaki farklılıkları belirlemek amaçlanmıştır. Zeytinyağı örnekleri Tariş Zeytin ve Zeytinyağı Tarım Satış Kooperatifleri Birliği tarafından sağlanan iki farklı Türk zeytin çeşidinden (Ayvalık ve Memecik) elde edilmiştir. Üç farklı olgunluk dönemine (erken (birinci hasat zamanı), orta (ikinci hasat zamanı) ve geç (üçüncü hasat zamanı)) göre hasat edilen zeytinler, üç farklı malaksasyon sıcaklığında (27°C, 37°C, 47°C) sıkılmıştır. Örnekler iki zeytin çeşidi ile üç hasat zamanı ve üç malaksasyon sıcaklığında onsekiz gruba ayrılmıştır. Toplamda 36 numune (18 uygulama × 2 paralel), iki paralel ekstraksiyon işleminden edilmiştir. Zeytin çeşidinin, malaksasyon sıcaklığının ve hasat zamanının zeytinyağların uçucu bileşenleri, duyusal özellikleri ve rengi üzerindeki etkisi araştırılmıştır. Veri analizi ve sonuçları yorumlamada SIMCA 13.0.3 (Umetrics AB, Umeå, Sweden) ve çok seviyeli faktöriyel tasarım kullanılmıştır.

Sonuç olarak, zeytinin hasat zamanının ve çeşidinin, zeytinyağın duyusal özellikleri üzerinde önemli bir etkisi vardır. Bununla birlikte, malaksasyon sıcaklığının belirgin bir etkisi gözlemlenmemiştir.

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LIST OF ABBREVIATIONS

IOC	International Olive Council
FFA	Free Fatty Acids
LOX	Lipoxygenase pathway
HPLC	High performance liquid chromatography
GC	Gas chromatography
GC/MS	Gas chromatography/Mass spectrometry
SPME	Solid Phase Microextraction
HS	Headspace
eNose	Electronic Nose
FGC-SAW	Fast Gas Chromatography−Surface Acoustic Wave
SAW	Surface Acoustic Wave
DOE	Design of Experiment
PCA	Principle Component Analysis
PLS-DA	Partial Least Square Discriminant Analysis
HDs	Harvest Dates
IZTECH	İzmir Institute of Technology
UZZK	National Olive and Olive Oil Council
DVB/CAR/PDMS	A divinylbenzene / Carboxene / Polydimethylsiloxane
SIM	Selected Ion Monitoring
KIs	Kovats Indices

CHAPTER 1

INTRODUCTION

Olive oil is extracted from fresh and healthy olive fruits by using only mechanical or physical methods (Baccouri et al., 2008). Olive oil differs from other vegetable oils, because it can be used as natural forms and it provides health benefits, basically it has own unique flavor and taste characteristics. Recently, it is becoming very popular all over the world (Boskou, 2007).

The quality of olive oils is determined primarily by its sensory characteristics. It plays an important role for the preference of consumers (Dierkes et al., 2011 and Escuderos et al, 2007). The sensory properties of olive oils (aroma and taste (flavor)) are characterised by mainly its minor components, phenolic and volatile compounds (such as carbonyl compounds, alcohols, esters and hydrocarbons) (Fregapane and Salvador, 2013 and Sonia et al., 2009). Aroma and phenolic composition of olive oils are affected by a lot of factors such as olive cultivar, the degree of fruit ripening, the environment, the growing season, the extraction process, the storage conditions in particular the milling and malaxation conditions (Kesen et al., 2013a). Particularly, ripening and cultivar play a fundamental roles for the olive oil final composition. Cultivar has a genetic characteristic and effects of cultivar change according to activity of enzymes (Gomes da Silva et al., 2012). During ripening process, physical and chemical composition of oil and enzyme activities change in the fruits (Dag et al., 2011). In order to maximize the amount of oil, malaxation is performed by forming larger oil droplets. Time and temperature can affect the efficiency of malaxation. Consequently, sensory and healthy benefits of olive oils can vary according to such factors (Gomes da Silva et al., 2012).

The aim of this study was therefore to investigate about the effects of cultivar, malaxation temperature and harvest time on volatile composition, sensory properties and the color of olive oils. The combination of temperature and harvest time with types of olive oils were done to improve the knowledge of the variables and to enhance the sensory characteristics and hence consumer acceptance of the oil.

CHAPTER 2

OLIVE OIL

2.1. Olive Fruit and Olive Oil

Olea genus belongs to the family *Oleacea*, comprises about 35 species of evergreen tree and shrubs (Boskou, 2006). It is one of the oldest domesticated species that can adapt to humidity and soil properties under Mediterranean climate (Tanasijevic et al., 2014). The cultivation of olive tree in Mediterranean basin countries (including Spain, Portugal, Italy, Greece, Turkey, Tunisia, Morocco and Syria) from ancient times up to present time, is very important for this region in terms of culture, nutrition, and economy (Loumou and Giourga, 2003, Wiesman, 2009). Turkey is the fifth largest producer of olive oil in the world with olive growing region such as Aegean Region, Marmara Region, Mediterranean Region and Anatolia Region. North and South Aegean are the major olive growing regions in Turkey, where Ayvalık and Memecik are major cultivars for economy respectively (Dıraman and Dibeklioğlu, 2009). Approximately, 75–80% of total olive oil production is provided by Aegean region (Ilyasoglu et al., 2011). However, the olive oil industry is also important for other regions in the world which have same climatic conditions with Mediterranean basin. About 3 million tonnes of annual world oil production can not be ignored (Fernández-Hernández et al., 2014). Today the production and consumption of olive oil are increasing slowly beyond the Mediterranean countries. In order to enhance olive oil yield with no loss of sensory and nutritional properties, new agricultural practices are developed (García-González and Aparicio, 2010).

Virgin olive oil (VOO) is extracted from the olive fruit by using only mechanical methods. For this reason, it is a 'fruit juice' oil and it is ready for direct human consumption in the crude form (Fregapane and Salvador, 2013). Olive oil is a very versatile product. It is highly appreciated by many consumers around the world for its nutritional, health and sensory properties (Haddada et al., 2007). Sensory characteristics are used to describe virgin olive oil quality and volatile chemical compounds are responsible for sensory attributes (Haddada et al., 2007; López-Feria et

al., 2008). Several factors affects volatile composition of olive oil such as: cultivar, the degree of fruit ripening, the environmental factors, the growing season, the extraction process, particularly malaxing conditions and the storage conditions (Kesen et al., 2013b).

2.2. Definitions and Classification of Olive Oils

The International Olive Council (IOC) is world's only international intergovernmental organisation in the field of olive oil and table olives. Members of council that produce and export olive oil and table olive. IOC producer members produce 98% of world production of olive oil. Therefore IOC sets the standards of quality used by the major olive oil producing countries. According to the International Olive Council olive oil is the oil obtained solely from the fruit of the olive tree (Olea europaea L.) and it does not contain oils obtained by using solvents or by using a re-esterification processes or any other mixture with oils of different characteristics. Olive oil can be classified by production methods as follows:

Virgin olive oil is produced from the fruit of the olive tree just using mechanical and other physical processes. It is obtained under particularly thermal conditions that do not generate any alterations in the oil. In this way, it has not undergone any treatment other than washing, decantation, centrifugation and filtration (IOC 2015b).

Refined olive oil is the olive oil obtained from virgin olive oils by refining steps (settling, neutralizing, bleaching and deodorizing) which do not alter in the initial glyceridic structure (Antonopoulos et al., 2007, IOC 2015b).

Olive-pomace oil is the oil extracted from the olive pomace which is the solid residue obtained from the olive oil production process using chemical solvents or other physical treatments, not including oils obtained by re-esterification processes and of any mixture with oils of other kinds (Antonopoulos et al., 2007, IOC 2015b). Olive pomace oil can be classified in three groups: crude olive-pomace oil, refined olive pomace oil and olive pomace oil. Crude olive-pomace oil is intended for refining for use for human consumption, or it is intended for technical use. Refined olive pomace oil is the oil obtained from crude olive pomace oil by refining methods. Olive pomace oil consists of a mixture of refined olive-pomace oil and virgin olive oils.

In order to classify olive oil into commercial grades, the quantity of free fatty acids (FFA) is an important factor (Kalua et al., 2007). According to Turkish Food Codex (Communication 2014/54) classification of olive oil and olive pomace oils by grades is given in Table 2.1.

Table 2.1. General classification of olive oils and olive pomace oils based on FFA

Olive oil classification	**FFA limit (as oleic acid)%**
Extra-virgin olive oil	0.8 (max)
Virgin olive oil	2.0 (max)
Lampante olive oil	2.0 (min)
Refined olive oil	0.3 (max)
Riviera olive oil	1.0 (max)
Refined olive pomace oil	0.3 (max)
Olive pomace oil	1.0 (max)

Extra virgin olive oil whose free acidity, expressed as oleic acid, is not more than 0.8 gram per 100 grams. It is ready for direct human consumption (IOC 2015b).

Virgin olive oil has a free acidity, expressed as oleic acid, is not more than 2 gram per 100 grams. It is ready for direct human consumption (IOC 2015b).

Lampante olive oil which has a free acidity, expressed as oleic acid, of more than 2.0 grams per 100 grams. It is intended for refining or for technical use. It is not fit for human consumption and must be refined (IOC 2015b).

Refined olive oil obtained by refining virgin olive oils, whose free acidity, expressed as oleic acid, cannot be more than 0.3 grams per 100 grams (IOC 2015b).

Riviera olive oil is the oil which is a mixture of refined olive oil and virgin olive oils. It has a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams (IOC 2015b).

Refined olive pomace oil whose free acidity, expressed as oleic acid, cannot be more than 0.3 grams per 100 grams (IOC 2015b).

Olive pomace oil whose free acidity, expressed as oleic acid, cannot be more than 1 gram per 100 grams (IOC 2015b).

2.3. Olive Oils Extraction

Olive oil is fruit juice oil, extracted from fresh, ripe, quality olive and the oil separated from the other components of the fruit during extraction methods. The aim of any extraction method is to obtain the huge amount of oil whenever possible without organoleptic alteration (Petrakis, 2006). Since ancient times, olive oil has been extracted with evolving methods. The first manufacturing equipment consisted of stone mortars (for squeezing) and the atmosphere decantation. In order to respond to the increased demand for olive oil, modern and more productive, developed technology needed to be applied. Therefore this technology was developed with electrical power, the hydraulic press, rolling wheels and centrifuges (Torrecilla, 2010).

There are four most advanced techniques to produce extra virgin olive oil: the pressure method as discontinuous process, the two-phase and three-phase techniques and percolation/centrifugation as continuous process. Two-phase and three-phase techniques are separated from each other in terms of properties of centrifuge system known as decanter (Torrecilla, 2010).

Olive oil extraction process comprises of four main steps. Extraction process begins with fruit cleaning (leaf removal and olive washing). The second step consists of preparation of the paste (crushing and malaxation) and third step consists of separation of the solid (pomace) and liquid phases (oily must and wastewater). Extraction process ends with separation of the liquid phases (oil and wastewater) (Petrakis, 2006).

2.3.1. Cleaning of Olive Fruit

Fruit cleaning step is very important. If this step is not carried out correctly, unpleasant taste can be detected in final olive oil (Petrakis, 2006). Therefore, the aim of washing is to remove any foreign material that could damage or contaminate the olive oil. The olives are washed in water and leaves, light materials are removed by a powerful flow air (Torrecilla, 2010).

2.3.2. Crushing

Olive fruit is composed of approximately 1/3 solid material, 1/3 water, and 1/3 oil (Vossen 2005). The main objective of this step is to tear the fruit cells to release the droplets of oil (Petrakis, 2006). Mainly, two types of machines are used for crushing the olives: stone mills and stainless steel hammer mills.

Stone crushers comprise of a stone base and upright millstones covered in a metal basin. It crushes olives and mixes the paste. The major disadvantages of stone mills are that they are slow and expensive, crushing is not continuous and cleaning of the stones is more difficult. Furthermore the slow milling time can cause paste fermentation with more oxygen exposure. Therefore hammer mills have replaced stone mills. Hammer mills comprise of a metal body that rotates at high speed, throwing the olives against a metal screen. Hammer crushers are widespreadly used. Because they can operate fast and continuously. Moreover, they are cheap and easy to clean. The drawback of method is that the oil is more emulsified. Therefore a longer malaxation period is required (Petrakis, 2006, Vossen 2005).

2.3.3. Malaxation

Malaxation is the main step for easier extraction and increasing the amount of available olive oil. The prime aim of malaxation is to break up the oil/water emulsion. The paste slowly mixed (less than 30 rpm) by malaxator which consist of cylindrical mixing vats with rotating blades and the smaller droplets can merge into larger drops. In this way, the extractability of the oil is increased. Malaxation usually takes from 45 to 60 minutes and temperature is very important parameter for malaxation. Usually, temperatures above 30°C can cause sensory problems. For this reason, the movement of the blades, the temperature and times are need to be properly adjusted to rheological characteristics of paste (Petrakis, 2006, Torrecilla, 2010, Vossen, 2005).

2.3.4. Oil Extraction from the Paste

The main aim of this step is to separate the oily juice of the olive from the olive paste. Pressing, centrifugation, percolation, or other combinations of the different methods can be used for extraction of oil (Vossen, 2005). The flow diagram of the olive oil extraction from the tree to the bottle by pressing, percolation and decanter (centrifugation) methods are given in Figure 2.1.

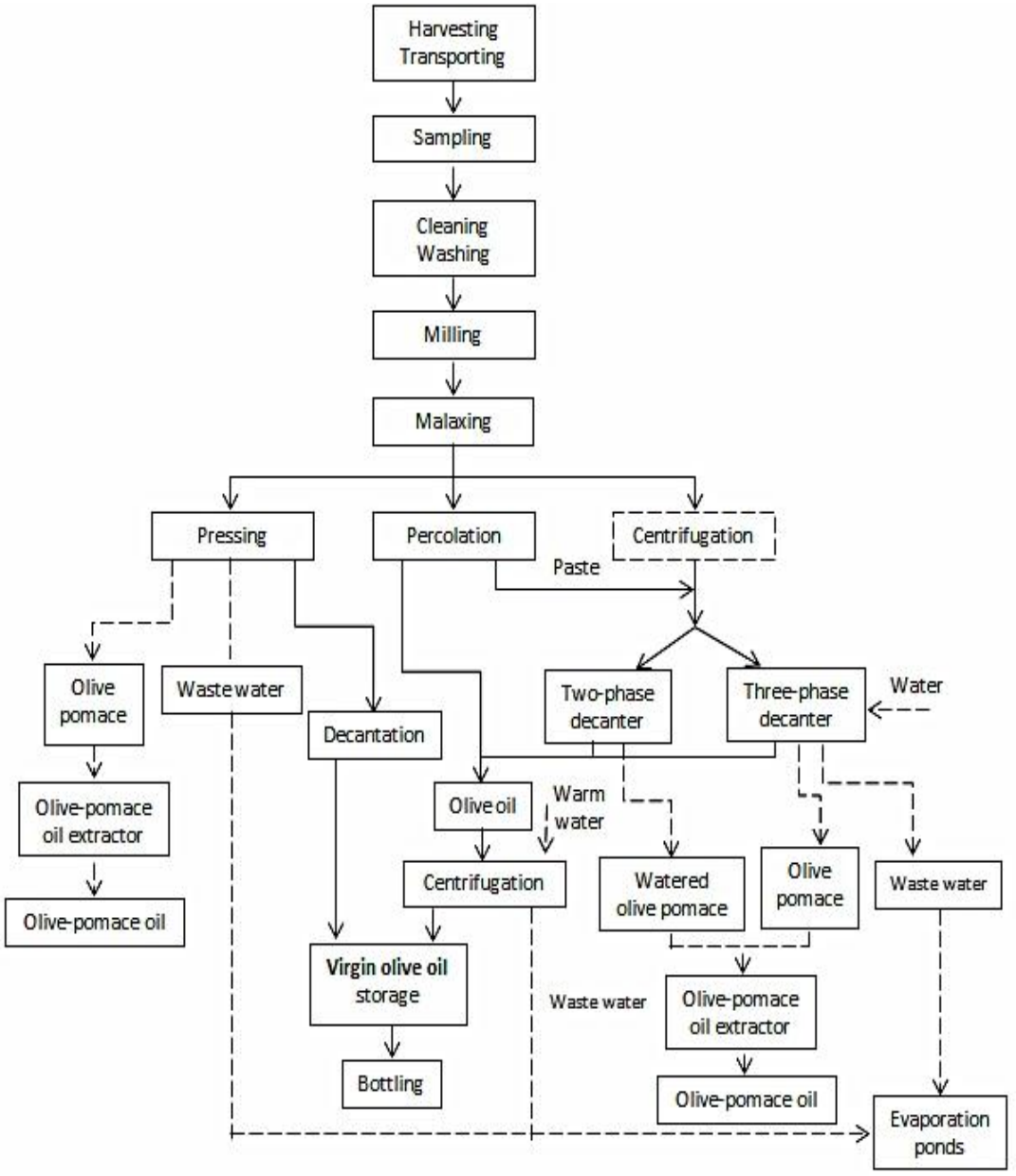

Figure 2.1. The steps of olive oil production by pressing, percolation and decanter (centrifugation) methods (Source: The AOCS Lipid Library, 2011)

Each process has its own advantages and disadvantages (Table 2.2). The main advantages of pressure process are less energy and water consumption. On the other hand they are non continuous process. Additionally, the disadvantages include more labor intensive, less capacity, difficulties of cleaning and maintenance. Both 3-phase and 2-phase systems are continuous and automated process. They reduce labor requirements and increases yield performance. Moreover, three phase decanter produces relatively dry pomace while two phase decanter produces a wet pomace. On the other hand, two phase system reduces water consumption and produces oil that contains more phenols and aroma while three phase system reduces antioxidant concentration. Both systems are expensive and consume more energy. The last one percolation/centrifugation process produces olive oil with higher polyphenol content like two phase decanter. They reduces labor and energy requirement. Otherwise, their large surface areas can cause to rapid oxidation (Torrecilla, 2010).

Table 2.2. Advantages and disadvantages: comparison of main properties of olive oil extraction processes (Source: Torrecilla, 2010)

	Pressing	**Three-phase**	**Two-phase**	**Percolation**
Process	Discontinuous	Continuous	Continuous	Continuous
Capacity	Small	Medium High	Medium High	Medium High
Labor cost	High	Low	Low	Low
Energy consumption	Low	High	High	High
Water consumption	Low	High	Low	High
Polyphenol content	High	Low	High	High
Contamination risk	High	Low	Low	Low

CHAPTER 3

CHEMICAL AND ORGANOLEPTIC COMPOSITION OF OLIVE OIL

3.1. The Chemical Composition of Olive Oil

Olive oil has complex chemical composition and it is influenced by a lot of factors such as variety, ripeness and the extraction process. Chemical composition of olive oil can be classified into major and minor components based on their content. Basically, it is formed by two fractions: the saponifiable and unsaponifiable fractions (Azadmard-Damirchi, 2011).

The saponifiable compounds represent approximately 98% of the chemical composition, consisting mainly of triglycerides. The other parts mainly composed of free fatty acids and also fatty acids derivatives such as mono and diacylglycerols, phospholipids, waxes and sterol esters (Youssef et al., 2011).

Unsaponifiable fractions represent about 2% of total content and consist of phytosterols, tocopherols, hydrocarbons, pigments, phenols, flavonoids or volatile compounds (Azadmard-Damirchi, 2011). If these unsaponifiable components are compared with saponifiables, they are very small portion of the olive oil. On the other hand, they have very significant roles (Wiesman, 2009). They are very important for oxidative stability of the olive oil and for its peculiar flavor. Aroma and phenols are evaluated directly by consumers (Youssef et al., 2011).

Hydrocarbons that include squalene and carotenoids (the main pigment of olive oil), are important parts of the unsaponifiable fraction. They represent 30 to 50 percent of the total amount. The concentration of chlorophyll fraction ranges between 1 and 20 ppm (La Lastra et al., 2001). Olive oil green color is a consequence of the presence chlorophylls and carotenoids (Moyano et al., 2011). Variety of olive affects the total content of tocopherols and its concentration ranges from 5 to 300 ppm. The tocopherol concentration is high in good quality virgin olive oils. Because they are natural antioxidant agents and increase stability of virgin olive oil. Linear aliphatic alcohols

with even-numbered carbon atoms (C18 to C28) are found in olive oils. Concentration of sterols ranges from 100 to 220 mg/100 g of oil (La Lastra et al., 2001). The main phenolic compounds in extra virgin olive oil are oleuropein, hydroxytyrosol and tyrosol (Rodríguez-Gutiérrez and Fernández-Bolaños, 2011). Several factors such as cultivar, degree of maturation, climate, other agronomic and technological factors, affect the absolute concentration of phenols. Phenolic compounds contribute to organoleptic properties (flavor and aroma) of olive oil. In this way, most phenols give a very bitter and pungent taste to the oil. They are also antioxidative compound and they prevent rancidity of olive oil. Aroma and flavor are constituted by a number of volatile compounds that are present at extremely low concentrations.

3.2. Volatile Compounds

3.2.1. Formation of the Volatile Compounds in Olive Oil

Volatile compounds are not generated in remarkable amounts during fruit growth but rise during the climacteric stage of ripening. During this period there is a dramatic increase in ethylene production that induce biochemical, physical and chemical changes and an increase in some protein and enzyme activities (Kalua et al., 2007). Enzymatic reactions and auto-oxidation play important roles for creation of aroma compounds. Most of aromatic compounds are formed at the moment of cell disruption during the crushing of the olives and continues during the extraction process (Kesen et al., 2014).

Various types of volatile compounds are produced through lipoxygenase (LOX) pathways (biogenic pathways of the olive fruit), fatty acid or aminoacid metabolism (Figure 3.1). Not only several compounds namely carbonyl compounds, alcohols, esters and hydrocarbons contribute to the aroma profile of olive oil, but also aldehydes derived from auto-oxidation processes produce volatile compounds. Various off-flavor compounds of virgin olive oil are formed by fermentations, conversion of some amino acids, enzymatic activities of moulds or oxidative processes (Da Silva et al., 2012).

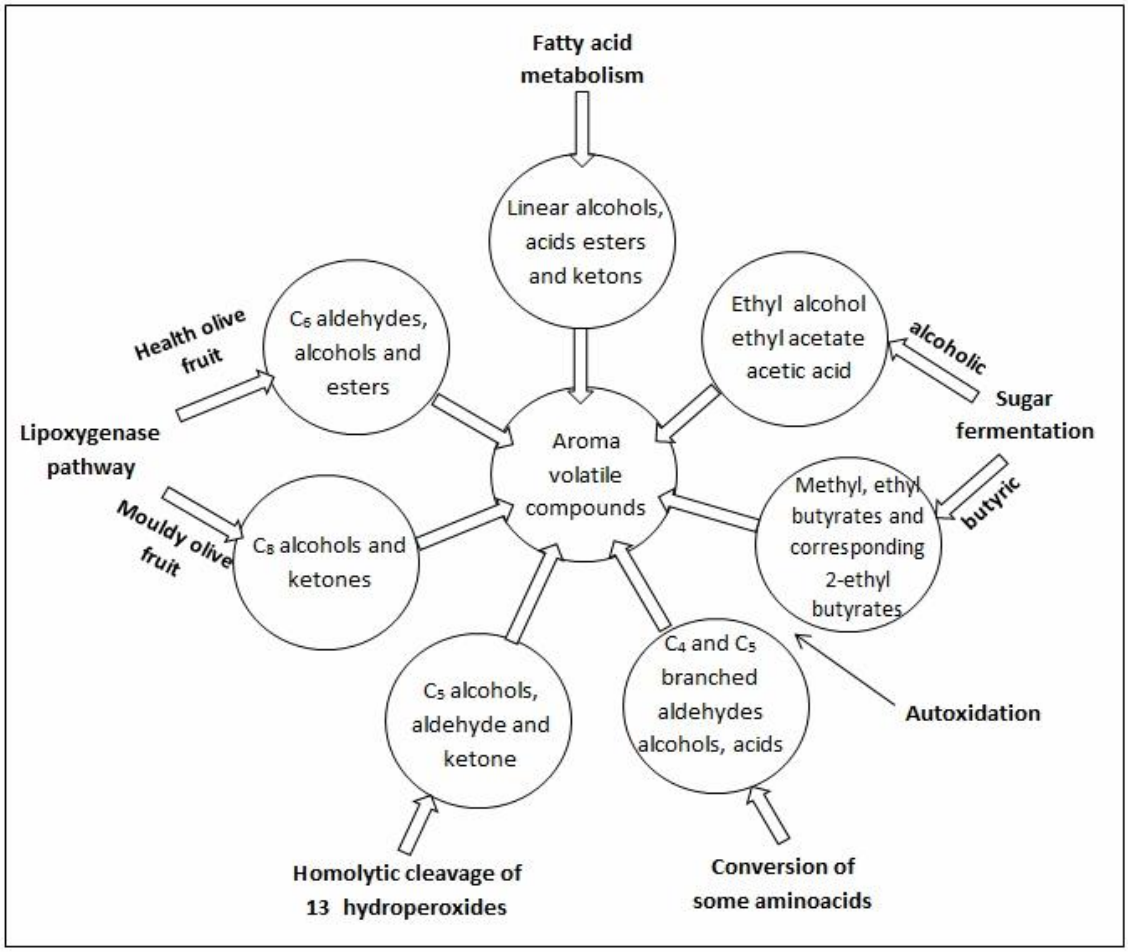

Figure 3.1. Formation of volatile aroma compounds
(Source: Da Silva et al., 2012)

Mainly, olive oil volatile compounds are formed by chemical and enzymatic oxidation. While chemical oxidation is responsible for the off-flavor, enzymatic oxidation is responsible for the aroma of the oil (Kalua et al., 2007). As depicted in Figure 3.2, the enzyme action starts with hydrolization of triglycerides and phospholipids by acyl hydrolases to release free fatty acids. After the occurrence of enzymes, the lipoxygenase oxidises the fatty acids to form hydroperoxides and the hydroperoxide lyase cleaves them to yield aldehydes. Then, they are reduced to alcohols by alcohol dehydrogenase, and esterified to produce esters by alcohol acyltransferase (Cecchi and Alfei, 2013).

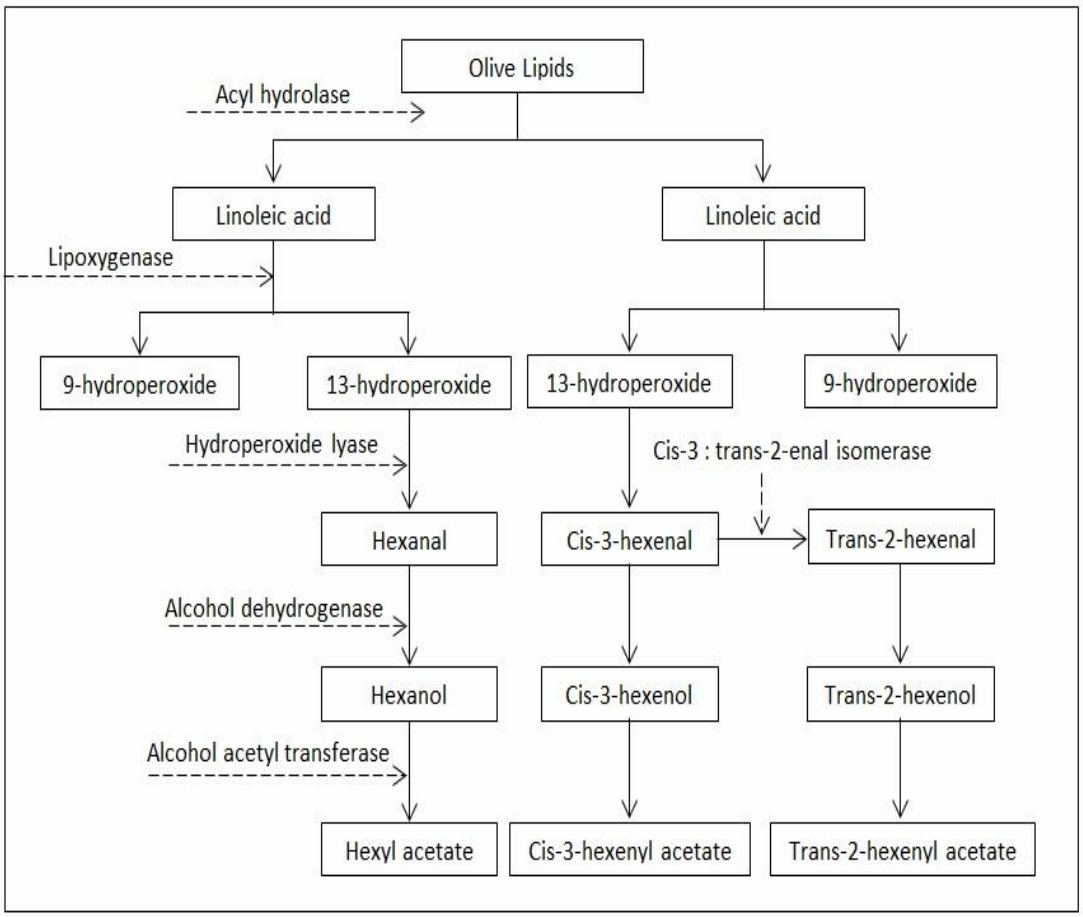

Figure 3.2. Pathway for the formation of major volatile compounds in virgin olive oils (Source: Kalua et al., 2007)

3.2.2. The Role of Volatile Compounds in Olive Oil Sensorial and Nutritional Quality

Sensory characteristics of virgin olive oil have a unique aroma and taste (flavor) with remarkable nutritional and biological properties. Sensory properties are strictly related to the phenols and volatile compounds. As shown in Figure 3.3 phenolic compounds affect the taste, the positive bitter and pungent sensory attributes, and the oxidative stability, the shelf life, and the nutritional and bioactive properties of the olive oils. Volatile compounds are mainly responsible for fruity and green aroma of high quality virgin olive oils. In this reason, phenolics and volatiles play a key role for the degree of consumer's preference for virgin olive oil (Fregapane and Salvador, 2013).

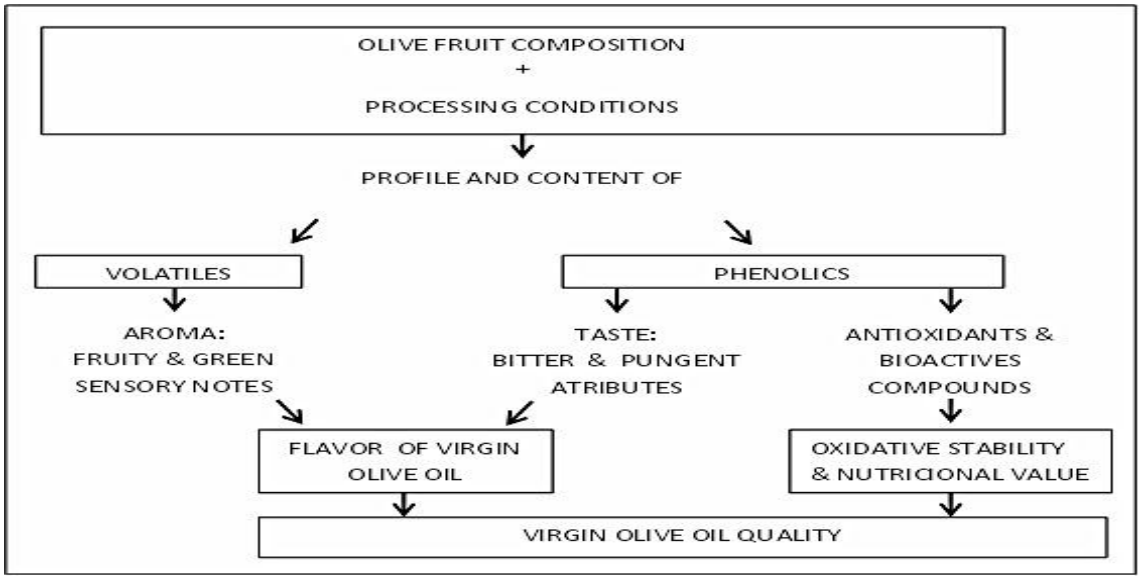

Figure 3.3. Virgin olive oil quality is related to its minor components
(Source: Fregapane and Salvador, 2013)

Volatile compounds are low molecular weight compounds (less than 300 Da). They can vaporize at room temperature can reach the olfactory epithelium as molecular dispersion, transported by the air streams. When they reach the olfactory receptors they give an odor sensation. They have sufficient hydrosolubility to diffuse into the mucus that covers the sensitive olfactory cells. They are often liposoluble and easily bind to proteins (membrane receptors) (Angerosa, 2002).

Approximately 280 compounds have been detected and identified in the volatile fraction of virgin olive oils. These compounds are hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, furan derivatives, thiophene derivatives, pyranones, thiols, and pyrazines (Boskou et al., 2006). Hexanal, trans-2-hexenal, 1-hexanol, and 3-methylbutan-1-ol are the major volatile compounds of olive oil. Octanal, nonanal, and 2-hexenal, propanol, amyl alcohols, 2-hexenol, 2-hexanol, and heptanol, characterize the olive cultivar (Kiritsakis, 1998).

3.2.3. The Factors Affecting the Volatile Composition of Olive Oil

Various factors affecting the composition of volatile compounds can be classified into four main groups: environmental (soil and climate); agronomic (irrigation, fertilization); cultivation (harvesting, ripeness) and technological procedures (post-harvest storage and extraction systems) (Da Silva et al., 2012).

The quality and incomparability of extra virgin olive oils are mainly determined by principal factors, such as genetic and pedoclimatic factors. The impact of the cultivar on the olive oil profile depends on the activity of enzymes (D'Imperio et al., 2010). The level and the activity of the enzymes involved in LOX pathway are very important for volatile profile of virgin olive oils. The enzymatic levels differ from cultivar to cultivar. On the other hand, enzymatic activity is affected by all factors (environmental, agronomic, cultivation and technological procedures). Not only endogenous plant enzymes, responsible for the positive aroma perception in olive oils but also chemical oxidation and microbial activity (led to sensory defects) should be considered. Pedoclimatic factors such as soil composition, climate, temperature, rainfall, altitude and latitude have an impact on chemical and sensory profiles of olive oil. Because, olive grove regions are spread over a wide range of altitudes, where climatic conditions can be quite different (Da Silva et al., 2012). In addition to the principal factors, olive oil composition is affected by secondary factors, governed by farmers. While agronomic practices such as irrigation, fertilization, and harvest method might affect the fruit physiology, technological procedures such as processing and storage might alter the olive oil composition (D'Imperio et al., 2010).

3.2.3.1. Cultivar (Types of Olives)

Cultivar is one of the most important factors that markedly affects volatile composition and sensorial properties of virgin olive oil. VOO produced from different cultivars, under identical growth conditions, harvested at almost the same ripeness degree and processed in the same way, is characterized by more or less different composition and concentration of minor compounds (Kesen et al., 2014). The levels and activity of the enzymes involved in the lipoxygenase pathway really affects the type and the amount of the accumulation products. The levels of enzymes can be different for the various cultivars. For this reason, different accumulation of the main volatile compounds at the end of the enzymatic oxidation gave rise to the varietal differentiation (Angerosa and Basti, 2003). Accumulation of aroma compounds in olive oil depends on the enzymatic store that is genetically determined according to the cultivar (Shaker and Azza, 2013). The effect of the cultivar can be demonstrated by the different amounts of C6 compounds arising from the enzymatic oxidation of linolenic acid of oils. On the

other hand, the different concentration of trans 2-hexenal represents an effective tool for differentiating monovarietal oils from different cultivars (Angerosa et al., 2004).

3.2.3.2. Harvest Time (Degree of Fruit Maturation)

The maturation of the olive fruit is a slow and long process which continues several months and varies according to the properties of the growing area, cultivar, water availability, temperature, and cultural practices. Olives must be properly extracted from mature, undamaged and healthy ones to obtain characteristically fragrant but delicately flavored oil. For this reason, the degree of ripeness is an important quality factor (Boskou, 2006).

The influence of fruit ripening on olive oil composition and quality is investigated mostly (D'Imperio et al., 2010). During the ripening process, the weight, pulp-to-stone ratio, color, oil accumulation, chemical composition of the oil and enzyme activities change significantly in the fruits. All of these parameters affect ease of oil extraction, chemical and sensory properties of fruit (Dag et al., 2011).

"Green" stage is known as the first stage of olive ripening. This corresponds to green mature fruits which have reached their final proportions. Then, chlorophylls in the skin are slowly replaced by anthocyanins. This is the passing to a "spotted", "purple" and "black" stage. Between the yellow green and purple skin stage the olives have the highest phenolic compounds content (Boskou, 2006).

Economic factors and quantity of yield are important for grower of olive. For this reason quantity and quality of olive oil should be considered together (Dag et al., 2011). Decision of the harvest time affects the quality and quantity of olive oil, the production in the following year, and the economic return (D'Imperio et al., 2010). However, in Turkey olives are harvested late after they are fully ripened. Because olives are collected at once and harvesting become more economical (Dıraman and Dibeklioğlu, 2009).

At the beginning of the season, early harvest olive oil is produced from younger and greener olives. They have a more bitter flavor based on their higher polyphenol content and less oil than black olives. They have pungent, astringent, grassy and green leaf flavors. Olives are usually harvested when they are purple-black, fully ripe, and contain the optimum level of oil. They have a light mellow taste with little bitterness

and more floral flavors. Peach, melon, apple and banana flavor are recognized. So that, the terms are used to describe the oil such as perfumy, buttery, fruity, rotund, soave and sweet (Wiesman, 2009).

3.2.3.3. Malaxation Temperature

Malaxation of the olive paste is the main phase of the extraction process. In order to increase the yield of the oil extraction, the olive paste has to be malaxed (Boselli et al., 2009). The malaxation step is formed by a low and continuous kneading of olive pastes. This step is particularly useful to obtain high and satisfactory yields of extraction. Actually, this essential technological process can break up the oil/water emulsions and help the small droplets to merge into larger drops. By this way larger drops of oils can be separated easily (Angerosa et al., 2001).

The composition of the oil is modified by chemical and enzymatic reactions during malaxation. Malaxation temperature and time are the technological parameters that have very significant roles in determining the yield of oil and chemical and sensory properties of the final product. The rate and extent of these reactions are most affected by these parameters (Boselli et al., 2009). VOO quality are affected both malaxing time and temperature. The malaxing temperature has a great effect on the process yield due to a reduction in the oil viscosity. On the other hand, excessive heating can cause undesirable effects, such as loss of phenolic compounds, loss of volatile compounds responsible for oil flavor and fragrance and accelerates its oxidative process (Clodoveo, 2012). On the other hand, malaxation temperature is one of the most important parameter with regard to possible manipulation in the process (Salas and Sánchez, 1999).

3.2.4. Detection of Aromatic Profile

All volatile components can be used to control the quality of an olive oil, to determine adulteration, possible rancidity (off-flavors) and the variety of olive used (Kesen et al., 2013a). In order to isolate, identify and quantify the volatile components that characterize olive oil aroma, many analytical procedures have been used (Baccouri et al., 2007). Traditional analytical and quantitative techniques for flavor analysis

involve high-performance liquid chromatography (HPLC), gas chromatography (GC) with headspace sampling and gas chromatography–mass spectrometry (GC-MS) analysis with solid phase microextraction (SPME) (Lammertyn et al., 2004).

Direct analysis of olive oil aims at determining the volatile compounds present in the sample. These analyses can be performed by these methodology: headspace with chromatographic separation (HS-GC/HS-GC-MS), headspace without chromatographic separation (chemical sensor), electronic olfactometry (e-noses) and expert panels as shown in Figure 3.4 (Valcárcel et al., 2007, Source López-Feria et al., 2011). Polarity and volatility of the analytes have an effect on the choice among sampling and chromatography. Volatiles are most properly studied by headspace (HS) analysis followed by GC (GC/MS) (Vas and Vékey, 2004). The headspace technique was used for determining and detecting volatile compounds present in headspace of sample. Therefore chromatographic separation using different detectors or by direct introduction of the vapor phase into a mass spectrometer were used (Valcárcel et al., 2007).

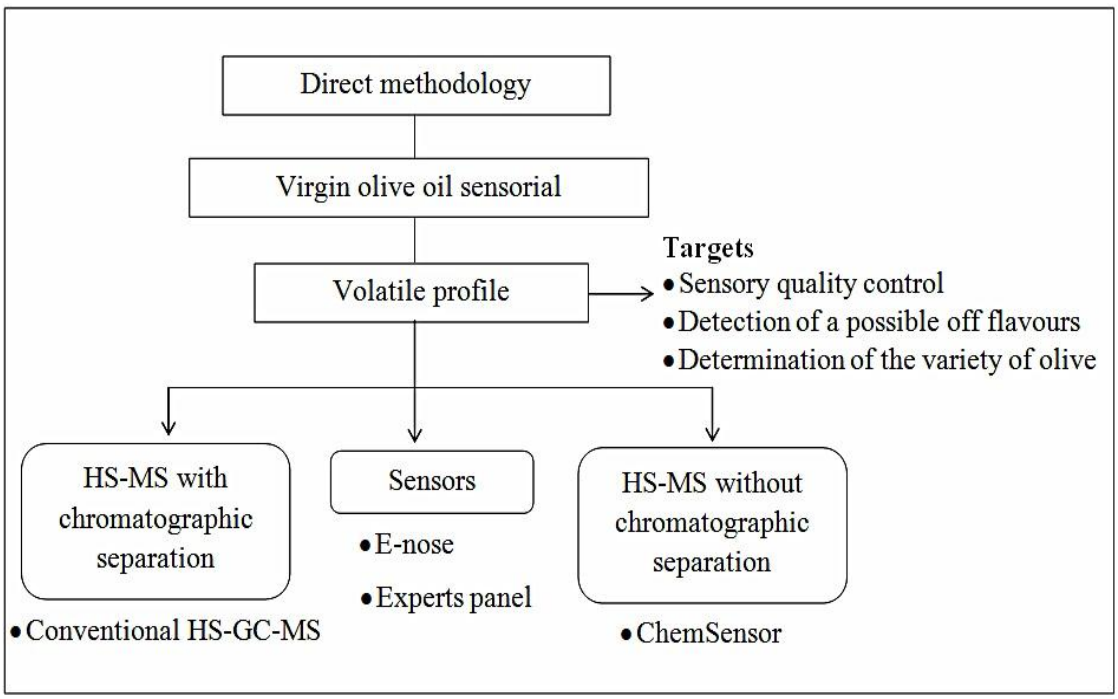

Figure 3.4. Direct analytical methodologies used for the sensorial characterization of the olive oil samples (Source: Valcárcel et al., 2007 and López-Feria et al., 2011).

3.2.4.1. HS-SPME/GC-MS Analysis

The composition of the headspace of an extra virgin olive oil is extremely complex. On the other hand, components of headspace are present in very low concentrations, just a few ppms or even less. For this reason, before GC analysis, extraction–concentration of volatiles is necessary such as using dynamic headspace sampling techniques. On the other side, headspace solid-phase microextraction (HS-SPME) is used as an alternative to dynamic headspace analysis (Contini and Esti, 2006).

Solid phase microextraction is a sorbent-based method. It was invented by Pawliszyn and co-workers. SPME, which is usually used in combination with gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), also is used for extraction of volatile and semi-volatile organic compounds from environmental, biological and food samples (Vas and Vékey, 2004). It was applied to the analysis of flavors and off-flavors in virgin olive oil widely (Peres et al., 2013). It is an impressive adsorption/desorption technique that successfully employed for analysing flavor compounds. It is simple because sampling, extraction, concentration, and sample introduction steps are integrated in a single step without the use of solvents (Contini and Esti, 2006). After extraction and concentration of volatile compounds on absorbing fibers, direct desorption into the gas chromatograph injector is carried out (Sonia et al., 2009).

Optimization of the equilibration time, sample, temperature and duration of the extraction are the main parameters of this method (Peris and Escuder-Gilabert, 2009). Although based on fiber, it shows acceptable repeatability and a good linearity. On the other hand, SPME has some disadvantages dealing with fiber performance and death. In long-term experiments, the fiber need to be changed with the other new one (Escuderos et al., 2007). In addition to this, GC-MS is very expensive with high maintenance costs and trained personnel requirement (Haddi et al., 2013).

3.2.4.2. Basics in Solid Phase Microextraction (SPME)

The SPME device, which looks like syringe in Figure 3.5, is simple. It consists of a fiber holder and retractable fibre. The fibre is a thin fused silica optical fibre that coated with a thin polymer film (Vas and Vékey, 2004).

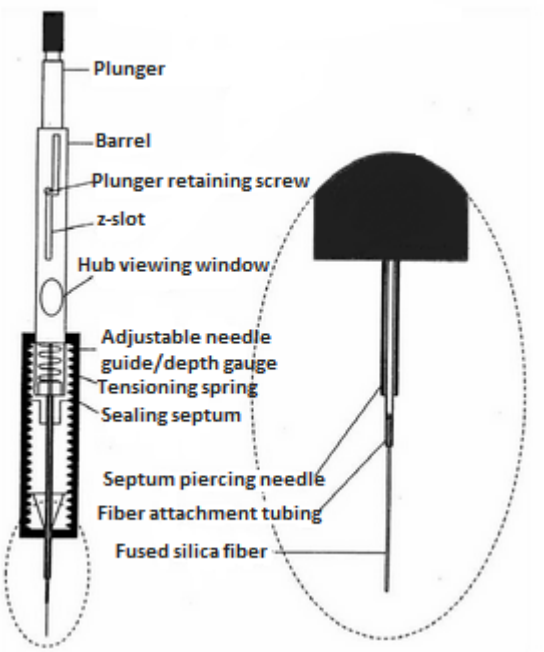

Figure 3.5. Components of manual SPME holder
(Source: Vas and Vékey, 2004)

SPME applications have two different sampling systems: sampling from gases (headspace) or sampling from solutions. In both application, in order to concentrate the analytes by absorption/adsorption processes the SPME needle is inserted into the appropriate position through a septum into the headspace or into the solution (Vas and Vékey, 2004). The main steps of HS-SPME techniques with adsorption/desorption processes are shown in Figure 3.6.

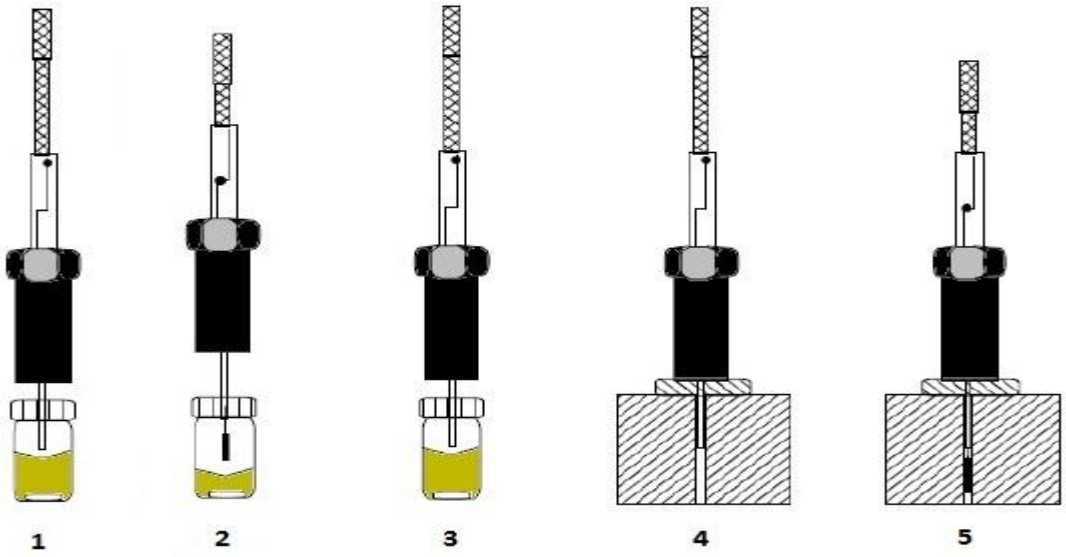

Figure 3.6. The main steps of headspace solid-phase microextraction techniques
(Source: Stien, 2001)

1. After incubation and equilibration, sample vial is pierced through the septum of headspace vial.
2. Fiber absorbs the volatile compounds from headspace for certain period of time.
3. After sampling, the fibre is retracted into the metal needle.
4. The needle is inserted into the the injector.
5. The analyte is transferred from the fibre into the chromatograph immediately. Thermal desorption of the analyte is occured in the hot GC injector for a while. After that, the fibre is pushed outside the metal needle (Vas and Vékey, 2004).

3.2.4.3. Electronic Nose (eNose) and Fast Gas Chromatography-Surface Acoustic Wave (FGC-SAW/zNose)

The `electronic nose' term, came out the late 1980s, when it was especially used at a conference in 1987 (Gan et al., 2005). Gardner and Bartlett (1994) defined an 'electronic nose' as 'an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odor'.

Electronic nose (eNose) is an instrument which mimics the human sense of smell. Electronic noses automatically detect, recognize and distinguish odors, vapors and gases precisely in complex samples and at low cost. These properties make eNoses very useful not only in the food, cosmetic and pharmaceutical industry but also in environmental control or clinical diagnostics. On the other hand, eNoses is used mostly in the field of food control (Peris and Escuder-Gilabert, 2009). In recent years, a lot of implementations have been developed for the quality analysis of olive oil. Electronic nose have been applied to detect of rancidity, adulterants and determination of geographical origin, characterisation of olive oil and VOO sensory evaluation (Haddi et al., 2013; Mildner-Szkudlarz and Jeleń, 2008).

Electronic nose technology is a fast and non-destructive alternative for aroma analysis. It measures the change in piezoelectric properties of a sensor array in the presence of volatile components in the sample headspace (Lammertyn et al., 2004). Unfortunately, the eNose has been sometimes unsuccessful. It is not sensitive enough and major compounds that are not relevant for aroma can be dominant in the headspace

(Haddi et al., 2013). On the other hand, eNoses using uncorrelated sensor arrays produce confusing patterns that cannot be recognized except with advanced computer software. In addition to this, impossibility of calibration with chemical standards and therefore lack of quantitative scientific methods of measurement make eNoses difficult to use (Staples, 2000).

Every units of eNoses are equivalent to every part of mammalian nose (Ramgir, 2013). In human sensory system the sense of smell (olfaction) plays a key role. For this reason, it is used alone to develop various products in many commercial industries for improving product attraction and quality (Gardner and Bartlett, 1994, Wilson and Baietto, 2009). Basically, the eNoses try to emulate the mammalian nose by using sensory arrays that can simulate mammalian olfactory responses to aroma (Arshak et al., 2004) as shown in Figure 3.7. Simply, the main components of eNoses, sample handling mechanisms, chemical sensor array, signal preprocessing and conditioning, and pattern recognition (Korel and Balaban, 2008). The odor molecules slide into the e-nose using sampling methods such as headspace sampling. When the odor sample land on the sensor array a reversible change in a chemical or physical property is induced. The cells of sensory array behave like a receptor to distinguish them according to different degrees. These changes are transduced into electrical signals that are processed and recorded. Pattern recognition methods are applied to analyze the sensor data to detect, classify, or identify the analytes (Arshak et al., 2004, Li, 2014).

In order to detect odor of sample conducting polymer, metal oxides, lipid layer, piezoelectric materials are being used to manufacture sensors. Sensor technology is changing rapidly to produce more sensitive, stable and fast sensors. Different types of sensors such as conducting polymers, semiconductor metal oxide, chemoresistive sensors, quartz resonator sensors and surface acoustic wave sensors are used commercially (Korel and Balaban, 2008).

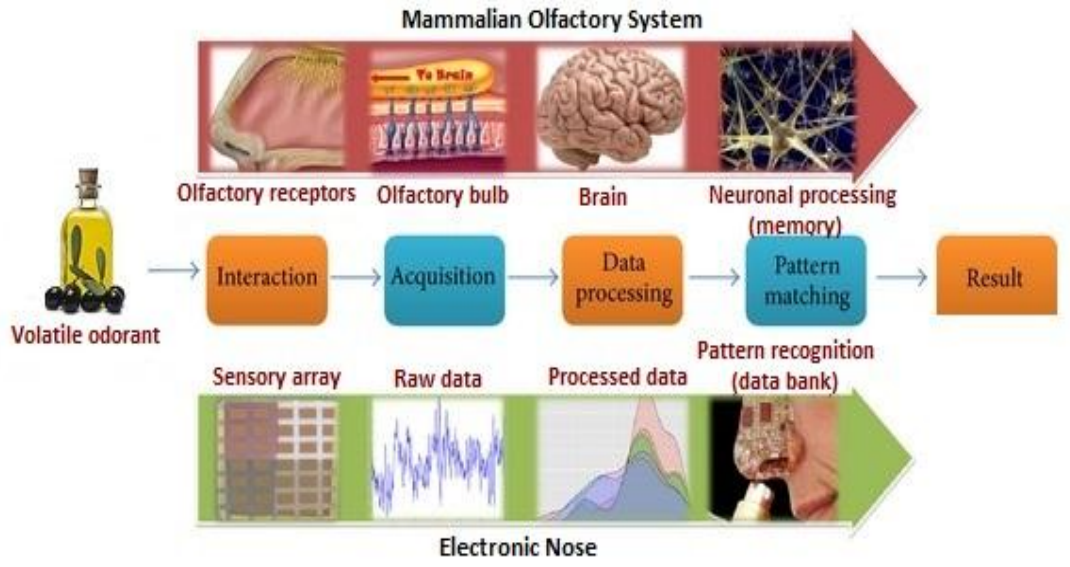

Figure 3.7. Comparison between mammalian olfactory system and an electronic nose (Source: Ramgir, 2013)

The fast gas chromatography−surface acoustic wave (FGC-SAW) instrument, which is sold commercially under the name zNose™, is a kind of electronic nose. An electronic nose uses multiple sensors (which are specific for certain volatiles) for sampling the unconcentrated and unseparated volatiles (Du et al., 2012). zNose™ is fast, non-destructive, low-cost, and sensitive another instrument for food aroma analysis. The zNose™ is able to identify aroma as GC and operate at the speed of the eNose. It uses a fast GC technique (Lammertyn et al., 2004). In addition, FGC-SAW traps and concentrates volatiles, separates on a short chromatographic column and detects them with a single quartz surface acoustic wave (SAW) detector. Generally, it can separate and analyze headspace volatiles within few seconds. The dynamic headspace trapping and concentrating are easy and relatively short (typically 5−20 s) (Du et al., 2012).

zNose™ and eNose are separated from each other according to the following properties as shown in Table 3.1 briefly. The detector of this zNose™ is produced from single quartz crystal without polymer coatings; therefore long term stability is succeed over a wide temperature range. The specific property of the SAW detector is based upon the temperature of the crystal surface and the vapor pressure characteristics of the condensate itself. At a certain crystal temperature, only analytes with dew points below the crystal temperature can be detected by transferring the analytes to condensed phase.

Volatiles are separated from non-volatile vapors by operating temperature of the SAW crystal (Gan et al., 2005).

Table 3.1. Comparison of zNose™ and eNose technology characteristics (Source: Staples, 2000)

Properties	**zNose™**	**eNose**
Sensitivity	Ppb	Ppm
Speed	Seconds	Minutes
Intelligence	Human	Artificial
Accuracy	High	Low
Stability	Months	Hours
Calibration Standard	Yes	No

3.2.4.4. Sensory Analysis

Sensory quality plays an important role in the whole quality of olive oil (María Elena Escuderos et al., 2007). It affects the food acceptability and consumer's preference mostly. Volatile and some nonvolatile aromatic compounds, give rise to the different sensory receptors that can be detected by consumers (Angerosa, 2002). However, volatile compounds are the most important factors for defining olive oil sensory quality (Purcaro et al., 2014). Sensory quality analysis is important improving volatile and phenol analysis, identifying the part of flavor compounds during consumption, getting information about physiological process implied in sensory perception, developing an objective and non-destructive methodology for sensory evaluation (García-González and Aparicio, 2010).

The volatile compounds contribute to the flavor (the combination of smell and taste). In order to evaluate the sensory quality of virgin olive oils, both favourable and unfavourable sensory attributes are considered. Evaluation of sensory defects are used to classify oils into various grades (Kalua et al., 2007).

A basic vocabulary has been developed for virgin oil sensory analysis (IOC, 2013). The positive attributes of virgin olive oil are explained as follows.

- **Fruity:** the main positive attribute of virgin olive oil, characteristic of oil from healthy, fresh fruits, either ripe or unripe. Flavor of olive oil obtained from unripe

fruits is characterised by grassy or leafy attributes. However olive oil from ripe fruits is characterised by aromatic flavors (IOC, 2013, Kalua et al., 2007).

- **Bitter:** taste characteristic of oil obtained from green olives or from olives which are turning in color. While there is a positive correlation with 1-penten-3-one, there is a negative correlation with cis-3-hexen-1-ol and hexanal. Various substances such as quinine, caffeine and many alkaloids give rise to bitter taste (IOC, 2013, Kalua et al., 2007).
- **Pungent:** the biting tactile sensation characteristic of oils produced at the start of the crop year, primarily from olives that are unripe (IOC, 2013). The occurrence of 1-penten-3-one is positively correlated with pungent taste, whereas trans-2-hexenal and hexanal are negatively correlated (Kalua et al., 2007).

Some volatiles cause sensory defects when they are present at high levels. They are produced by over-ripening of the fruit, when they are injured or attacked by molds and bacteria or stored for a long period prior to oil extraction, and also by advanced autoxidation of the unsaturated fatty acids because of adverse storage conditions (Boskou et al., 2006). The main negative attributes (defects) of virgin olive oil are summarized in Table 3.2 and explained as follows.

Table 3.2. Source of defects perceived in olive oils and characterization of flavor (Source: Angerosa, 2002).

Source of defects	**Defects**	**Flavor Characterization**
Bad sanitary conditions of fruits	• grubby	• olives which have suffered a *Dacus* of fruits *oleae* infestation
Wrong harvesting process	• ground picked olives	• olives, spontaneously fallen from trees and remained on the ground for several days
Fruit storage conditions and time	• fusty • winey • musty	• result of degradation phenomena • result of some fermentation • result of fungal invasion

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Table 3.2. (Cont.)

Unsuitable extraction technology	• earth • heated • metallic	• bespattered technology with mud and processed without washing • heating too long times or too high temperatures in the malaxation step • extraction with both new processing plants and/or used the first time during the crop year
Unsuitable oil storage conditions	• rancid • muddy sediment • cucumber	• strongly oxidized • stored for a long time on their sediment • stored for a long time during the hermetic bottling

Fusty: characteristic for oils obtained from olive fruits stored in piles for long periods before extraction and undergoing an advanced stage of anaerobic fermentation. Generally, insufficient space of fruit storage gives rise to kind of defects (IOC, 2013, Kalua et al., 2007).

Muddy sediment: characteristic defect of oil that has been left in contact with the sediment for a long time (IOC, 2013, Kalua et al., 2007).

Musty-humid: characteristic moldy flavor of oils obtained from fruit in which large numbers of fungi and yeast have developed as a result of storage at low temperatures and high humidity. Fungi are able to oxidize free fatty acids to volatile compounds, whereas yeasts readily reduce carbonyl compounds (IOC, 2013, Kalua et al., 2007).

Winey-vinegary acid-sour: the process of fermentation in the olives, leading to the formation of acetic acid, ethyl acetate and ethanol give rise to this flavor of olive oils. It is a characteristic flavor of oils remembering that one of wine or vinegar (IOC, 2013, Kalua et al., 2007).

Rancid: a flavor of oils that have undergone oxidation. The main contributors are unsaturated aldehydes (IOC, 2013, Kalua et al., 2007).

Metallic: a flavor, reminiscent of metals that occur in oil that has been kept for a long time in contact with metallic surfaces during the procedures of crushing, mixing, pressing or storage. 1-penten-3-one could be useful markers for metallic off-flavors (IOC, 2013, Kalua et al., 2007).

3.3. External Appearance of Olive Oils

External appearance (color, texture, and so on) plays an important role and influences consumer choices. Because color of foods gives an idea about their stage of maturity, the presence of contaminants or microorganisms, the conditions of the industrial processing, and more (Heredia and Antonio, 2010). For this reason color is the main aspect that defines olive oil's quality. The pigment dispersion of the olive paste between the solid (pomace) and the liquid phases (oil and wastewater) is determined by mass partitioning phenomena during oil extraction. The lipophilic property of chloroplast pigments affects their affinity for the oily phase, and the more hydrophilic nature of anthocyanins affects their retention in the pomace and the wastewater. Therefore, chlorophyll and carotenoids (chloroplast pigments) are responsible for the color of virgin olive oil, ranging from yellow–green to greenish gold (Criado et al., 2008).

CHAPTER 4

MATERIAL AND METHODS

4.1. Materials

4.1.1. Olive Oil Samples

In this study, healthy olive fruits from the 2012/2013 crop season were used. All the olives were provided by Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union. Samples were classified into eighteen groups by two olive varieties, by three harvest times and three malaxation temperatures (Table 4.1). There were totally 36 samples (18 treatments × 2 replicates) with two parallel extractions. Ayvalık (Burhaniye, Havran, Ayvalık, Ezine, Edremit, Küçükkuyu, Altınoluk) and Memecik (Bozdoğan, Horsunlu, Bayındır, Selçuk, Erbeyli, Ortaklar) were chosen as olive cultivars. Olives were harvested according to different stages of ripening: early (first harvest time), mid (second harvest time) and late (third harvest time). They were picked up at three different harvest dates (HDs), corresponding to between the 15th of November–6th of December (1st HD), the 21th December–10th of January (2nd HD) and the 7th of February–28th of February (3rd HD).

Refined olive oil, which was used for calibration curves, was provided by Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union.

Table 4.1. Classification of olive oils with sample codes according to olive variety, harvest time and malaxing temperature

Olive Varieties	Harvest Time	Malaxing Temperature	Sample Codes
Ayvalık	Early	27°C	AE27*
Ayvalık	Early	37°C	AE37
Ayvalık	Early	47°C	AE47
Ayvalık	Mid	27°C	AM27
Ayvalık	Mid	37°C	AM37
Ayvalık	Mid	47°C	AM47

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Table 4.1. (Cont.)

Ayvalık	Late	27°C	AL27
Ayvalık	Late	37°C	AL37
Ayvalık	Late	47°C	AL47
Memecik	Early	27°C	ME27
Memecik	Early	37°C	ME37
Memecik	Early	47°C	ME47
Memecik	Mid	27°C	MM27
Memecik	Mid	37°C	MM37
Memecik	Mid	47°C	MM47
Memecik	Late	27°C	ML27
Memecik	Late	37°C	ML37
Memecik	Late	47°C	ML47

* 2 is used with sample codes for second parallel extraction of olive oils.
For example AE27-2.

4.1.2. Extraction of Olive Oils

Olives, provided by Tariş were extracted at İzmir Institute of Technology (IZTECH Olive and Olive Oil Processing Center). Continuous two-phase industrial system (Polat machinery Inc. (Turkey)) was used for olive oil extraction. Olives, organized according to harvest times and olive varieties extracted at three different malaxation temperature (27°C, 37°C, 47°C) for 60 min. Each extraction was performed on a sample of approximately 200 kg of olives. Lastly, each oil sample was transferred into two glass bottles (500 ml×2) and stored at ± 4°C until analysis. One of the bottles was used for only sensory analysis and the other one was used for other analyses.

4.1.3. Reagents

The standard compounds were used for volatile identification and quantification. Thirty standard compounds were selected from those typically found in extra virgin olive oil Ethanol, 2-butanone, ethyl acetate, 1-penten-3-ol, isoamylalcohol, trans-2-pentenal, n-octane, hexanal, trans-2-hexen-1-ol, 1-hexanol, 2-heptanone, heptanal, octanal, hexyl acetate, nonanal, 2-nonanon3 were purchased from Merck (Germany); 1-

penten-3-one, 3-pentanone, trans-2-hexenal, cis-3-hexen-1-ol, trans-2-heptenal, (R)-(+)-limonene, 1-octanol, terpinolene, trans-2-decenal were purchased from Fluka (Switzerland) and trans, trans 2,4 nonedienal, trans-2-nonenal, cis-3-hexenyl-acetate were purchased from Sigma-Aldrich (USA); butyl acetate was purchased from Riedel de Haen (Germany). All standards had a gas chromatography (GC) purity of 95% or higher (except trans, trans-2,4-Nonadienal, see preparation calibration solution section for more details).

4.2. Methods

4.2.1. Color Measurement

Olive oil samples were measured for color characterization by using a Minolta CR-400 chroma meter (Konica Minolta Sensing Inc, Osaka, Japan). Prior to analysis, the chroma meter was calibrated on a white standard plate (with reference to illuminant D_{65} (natural daylight), Y=93.58, x=0.3159, y=0.3322). Then L* a* b*, Hunter Lab, L* C* h were selected as the color space. Approximately 20-25 mL of oil sample was placed in CR-A502 tube cell (Ø 60 / 40 mm depth) to perform color measurements. Three different readings were taken at three different points. Measurements were performed under the same conditions to prevent differences of external conditions.

4.2.2. GC Analysis

4.2.2.1. HS-SPME Conditions and GC/MS Analysis

Volatile compounds were identified and quantified by GC/MS (Trace GC Ultra/ISQ, Thermo Scientific, U.S.A.) and TR-5MS capillary column (30m×0.25 mm internal diameter, 0.25 μM film thickness, Thermo Scientific, U.S.A.) was employed. Solid phase microextraction (SPME) was used as a technique for the extraction of volatile compounds. A divinylbenzene / carboxene / polydimethylsiloxane (DVB / CAR / PDMS) fiber and SPME device from Supelco (50 / 30 μm, 2 cm long, Bellefonte, PA) was used for headspace sampling and the fiber conditioned according to the

manufacturer's instructions prior to use in the gas chromatograph injection port. A DVB/CAR/PDMS fiber coating, which is the most suitable one, was chosen for the analysis of virgin olive oil volatiles (Vichi et al., 2003).

Prior to GC analysis, olive oils stored at +4°C were conditioned to room temperature. Then, 10 ml of oil sample were placed into a 20 ml headspace vial containing a microstirring bar, and a PTFE/silicone septa (Supelco) was sealed with an aluminum crimp seal (supelco). All samples were left overnight at room temperature for more efficient analysis. They were allowed to equilibrate for 30 min in a water bath at 40°C. After equilibration time, the septa was pierced with the SPME needle and the fibre was exposed to the headspace for 30 min at 40 °C with magnetic stirring (300 rpm). In this way, preconcentration of volatiles were done by the fiber and it was ready for injection. At the end of the preconcentration time, the fiber was withdrew and inserted into the GC injector at 250 °C (5 min) to desorb the adsorbed volatile compounds. The GC oven temperature was initially held at 40 °C for 5 min, increased to 100 °C at a rate of 5 °C/min and then to 300 °C at a rate of 25 °C/min. Helium was used as a carrier gas, with a flowrate of 1.0 mL/min. The MS was operated in electron ionization mode (70 eV) and the dwell time was set to 100 ms. The ion source temperature was set at 230 °C. Two replicates were performed for each olive oil sample. Blank runs were done periodically before starting the study to remove the pollution peaks of the matrix.

The calibration standards were analyzed first in GC/MS in full scan mode. All samples were analyzed by GC/MS in selected ion monitoring (SIM) mode. Data analysis, integration and calibration were done by using Xcalibur Quan Browser software (Thermo Scientific). The identification of volatile compounds was performed by comparing their mass spectra with standards and to mass spectra from Wiley and NIST library database.

Effects of factors (harvest time, temperature and olive variety) on volatile compounds taken from HS-SPME method were determined by using a multilevel full factorial design of experiment method by using MINITAB. Firstly, volatile compounds were classified according to lipoxygenase pathway and they were interpreted accordingly. After that, volatile compounds, which were affected by harvest time, temperature and/or olive variety, were analysed by using SIMCA.

Basically, carbonyl compounds, alcohols, esters and hydrocarbons are found in volatile fraction of olive oil. C6 and C5 volatile compounds are produced from enzymatically polyunsaturated fatty acids through lipoxygenase (LOX) pathway (Angerosa et al., 2004). For this reason olive oils classified as, C6 volatile compounds produced from lipoxygenase pathway, C5 volatile compounds produced from lipoxygenase pathway and volatile compounds produced from other ways exclusive of lipoxygenase pathway.

4.2.2.2. Preparation of Calibration Solutions

Refined olive oil, provided by Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union was used for calibration curves as oil medium. The refined olive oil was used for preparing standard solutions in a concentration range from 0.1 to 20 mg/kg (0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 mg/kg). Standarts were listed in Table 4.2 with correlation coeffient.

Table 4.2. Standards with correlation coeffient (R^2)

Standard Name	Density (mg/ml) 25°C	Purity	Volume of Standard (µl)	Correlation coefficent (R^2)
1. Ethanol	790	0.99	12.8	0.99
2. 2-Butanone	805	0.99	12.5	0.96
3. Ethyl acetate	898	0.99	11.2	0.98
4. 1-Penten-3-ol	838	0.99	12.1	0.98
5. 1-Penten-3-one	851	0.97	12.1	0.99
6. 3-Pentanone	813	0.99	12.4	0.90
7. Isoamyl alcohol	810	0.98	12.6	0.94
8. Trans-2-Pentenal	860	0.97	12.0	0.99
9. N-octane	702	0.99	14.4	0.98
10. Hexanal	816	0.98	12.5	0.90
11. Butyl acetate	880	0.99	11.5	0.99
12. Cis-3-Hexen-1-ol	850	0.98	12.0	0.99
13. Trans -2-Hexen-1-al	846	0.97	12.2	0.99
14. Trans -2-Hexen-1-ol	842	0.97	12.2	0.99
15. 1-Hexanol	818	0.98	12.5	0.90
16. 2-Heptanone	814	0.98	12.5	0.99
17. Heptanal	820	0.97	12.6	0.99
18. Trans-2-Heptenal	847	0.98	12.0	0.99
19. Octanal	822	0.98	12.4	0.99
20. Cis -3-Hexenyl acetate	897	0.98	11.4	0.99
21. Hexyl acetate	872	0.98	11.7	0.90

(Cont. on next page)

Table 4.2. (Cont.)

22. (R)-(+)-Limonene	842	0.96	12.4	0.99
23. 1-Octanol	825	0.99	12.2	0.99
24. Terpinolene	860	0.97	12.0	0.99
25. 2-Nonanone	820	0.98	12.4	0.99
26. Nonanal	827	0.98	12.3	0.99
27. Trans -2-nonenal	846	0.97	12.2	0.98
28. Trans, trans-2,4-Nonadienal	862	0.85	13.6	0.98
29. Trans -2-Decenal	849	0.95	12.4	0.98
Total Standard Volume			357.1	
Required refined olive oil			9642.9	

*Standard solution was prepared firstly as a 1000 ppm, 10 mL stock solution

In order to prepare 1000 ppm (mg/L) stock standard solution, all kinds of standards were put respectively (totally 357.1 µl) in refined olive oil (9642.9 µl). As shown in the Table 4.2. Volumes of the standard contents were calculated according the following calculation:

- Density x purity of Standard
- C_1 x V_1= C_2 x V_2 (Standard dilution formula in general chemistry)
  - V_1 = Volume of standard
  - C_1 = Concentration of standard
  - V_2 = Final volume of stock solution
  - C_2 = Final concentration of stock solution

For example, calculation of isoamyl alcohol volume for 10 mL standard solution:

- 810 mg/mL x 0.98 =793.8 mg/mL x 1000 mL/L = 793.8 x 10^3ppm
- 793.8 x 10^3 ppm x V_1 = 1000 ppm x 10 mL V_1=12.6 µl

The amount of volatile components in the samples was determined by the external standard method. For this reason, 8 different calibration points were prepared (0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 mg/kg) as follows:

- 1000 ppm (The main stock solution)
- 1000 ppm x V_1= 100 ppm x 10 mL(100 ppm new stock solution)
- V_1=1 mL (1 mL 1000 ppm main stock + 9 mL refined olive oil)

1. 20 ppm-100 ppm xV_1 = 20 ppm x 10 mLV_1= 2 mL(2 mL + 8 mL refined olive oil)
2. 10 ppm-100 ppm xV_1 = 10 ppm x 10 mLV_1= 1 mL(1 mL + 9 mL refined olive oil)
3. 5 ppm-100 ppm xV_1 =5 ppm x 10 mLV_1= 0.5 mL(0.5 mL + 9.5 mL refined olive oil)
4. 2 ppm-100 ppm xV_1 =2 ppm x 10 mLV_1= 0.2 mL(0.2 mL + 9.8 mL refined olive oil)
5. 1 ppm-100 ppm xV_1 =1 ppm x 10 mLV_1= 0.1 mL(0.1 mL + 9.9 mL refined olive oil)

6. 0.5 ppm-100 ppm xV_1 =0.5 ppm x 10 mL V_1= 50 µL(50 µL + 950 µL refined olive oil)
7. 0.2 ppm-100 ppm xV_1 =0.2 ppm x 10 mL V_1= 20 µL(20 µL + 980 µL refined olive oil)
8. 0.1 ppm-100 ppm xV_1 =0.1 ppm x 10 mL V_1= 10 µL(20 µL + 990 µL refined olive oil)

Two replicates were performed for each standard solution. For all kind of standard samples, 5 calibration points (0.1, 0.2, 0.5, 1, and 2 ppm) were used, except Trans -2-Decenal. 0.1, 1, 2, 5 and 10 ppm standard solution were used as calibration points for Trans-2-Decenal.

Peaks corresponding to external standards were integrated using the Xcalibur™ software system. An external standard is a separate sample that contains a known amount of the target compound. In order to use external standard calibration, a set of standard solutions containing a known amount of the target compounds were prepared as calculated above. After injection of these solutions, the resulting chromatograms analyzed by the Xcalibur™ software and a calibration curve for each target compound by plotting the magnitude of the detector's response as a function of the amount of the target compound was constructed according to Thermo Xcalibur user guide. In Figure 4.1 calibration curve of 1-Penten-3-one is shown as an example from Xcalibur™ software.

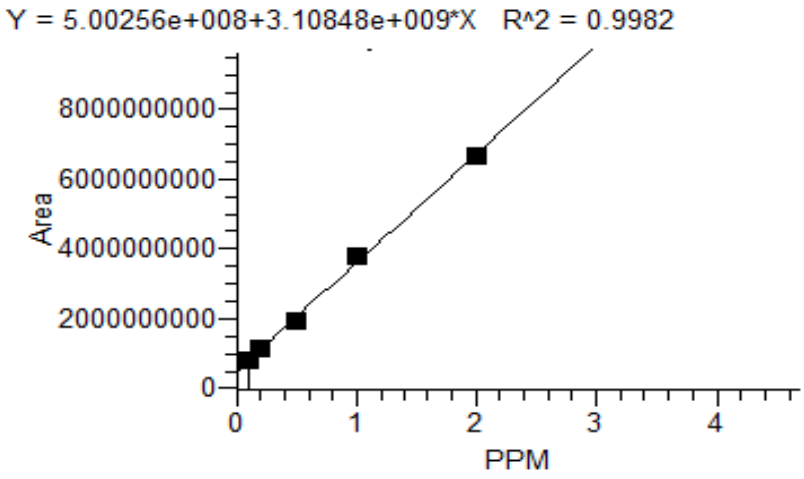


Figure 4.1. Calibration curve of 1-Penten-3-one from Xcalibur™ software

4.2.2.3. Selected Ion Monitoring

Twenty nine target compounds are given with retention times and selected ions in Table 4.3 including groups and group start times. In order to gain the benefits of SIM, the numbers of ions were limited at a time segment. They were independent groups and changes in one group didn't affect any other group (Thermo Xcalibur user guide).

Table 4.3. SIM parameters

	Standard Name	**Retention Time (min)**	**Selected Ions (m/z)**
SIM Group 1 1-4 min.	Ethanol	1.69	31, 45
	2-Butanone	2.22	42, 43, 72
	Ethyl acetate	2.36	42, 43, 70
	1-Penten-3-ol	3.26	29, 56, 57
	1-Penten-3-one	3.34	54, 55, 84
SIM Group 2 4-8 min.	3-Pentanone	3.41	29, 57, 86
	Isoamyl alcohol	4.37	41, 42, 70
	Trans-2-Pentenal	5.07	54, 55, 83, 84
	N-octane	5.99	43, 57, 85
	Hexanal	6.45	43, 44, 56
	Butyl acetate	6.84	43, 56, 61, 73
SIM Group 3 8-11 min.	Cis-3-Hexen-1-ol	8.42	41, 66, 67, 81
	Trans -2-Hexenal	8.49	41, 55, 69
	Trans -2-Hexen-1-ol	8.83	41, 56, 57, 82
	1-Hexanol	8.95	43, 55, 56
	2-Heptanone	9.68	43, 58
	Heptanal	10.17	43, 44, 69, 70
SIM Group 4 11-15.50 min.	Trans-2-Heptenal	12.28	55, 56, 68, 83
	Octanal	13.84	41, 43, 56, 57
	Cis -3-Hexenyl acetate	13.91	43, 67, 81
	Hexyl acetate	14.07	42, 43, 55, 56
	(R)-(+)-Limonene	14.54	67, 68, 93
SIM Group 5 15.50-end	1-Octanol	16.07	55, 56, 69
	Terpinolene	16.43	93, 121, 136
	2-Nonanone	16.72	43, 57, 58, 59
	Nonanal	17.16	43, 56, 57
	Trans -2-nonenal	18.28	43, 55, 70
	Trans, trans-2,4 Nonadienal	19.05	41, 67, 80, 81
	Trans -2-Decenal	19.49	55, 69, 70, 73

Figure 4.2. is given as an example chromatogram of calibration standards. As seen in the chromatogram there were 29 peaks.

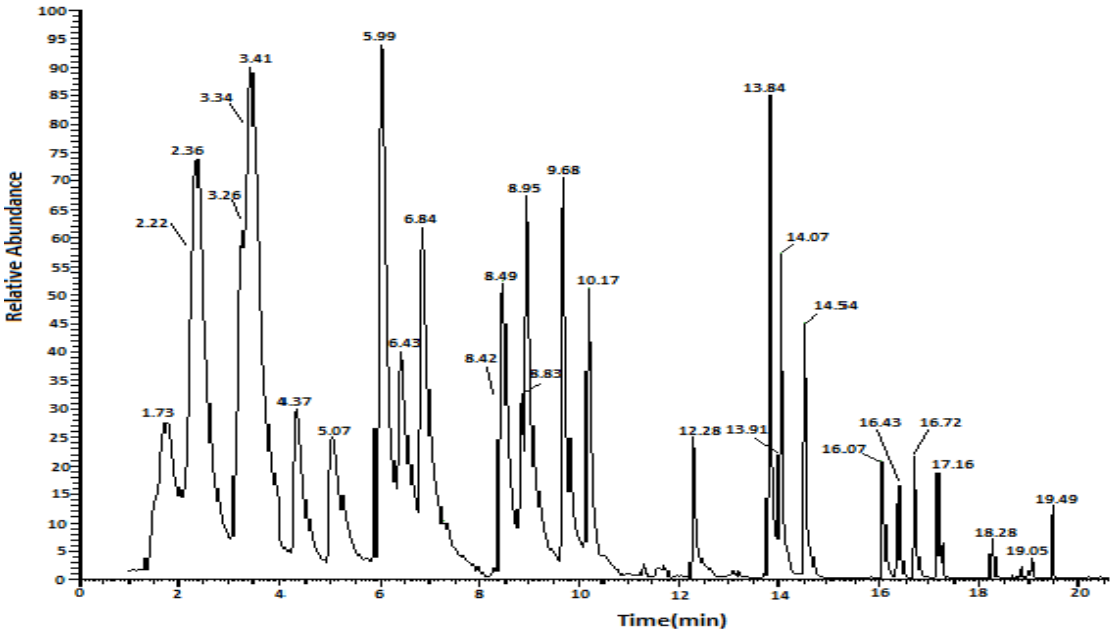


Figure 4.2. Chromatogram of 0.5 ppm standard solution in SIM mode

4.2.3. Electronic Nose Analysis

The aroma measurements of olive oils were determined by electronic nose (zNose™ model 7100 Fast GC Analyzer, Electronic Sensor Technology-EST, New Bury Park, CA, USA) (Figure 4.3). The zNose™ incorporates a fast chromatography column and a (surface acoustic wave detector) SAW crystal detector to separate and identify chemical odor components, respectively.

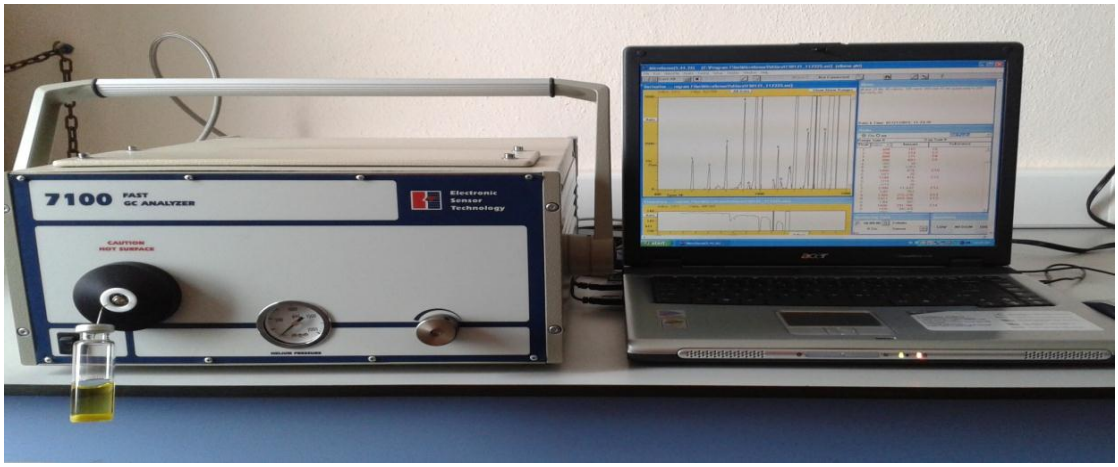


Figure 4.3. zNose™ model 7100 Fast GC Analyzer

Before zNose™ measurements, 10 mL of olive oil was taken into a 20 mL headspace vial and a PTFE/silicone septa (Supelco) was sealed with an aluminum crimp seal (Supelco). Prepared samples were kept at room temperature before analysis. After that, the samples were incubated at 40 °C for 15 min for headspace equlibration above the sample.

The measurement sequence was started with the loading the appropriate analysis method on the zNose™. The major operating parameters of olive oil odor analysis in the MicroSense version 5.44.26 software (Newbury Park, CA, USA) of the electronic nose are shown in Table 4.4 with value and units. A stainless steel needle was connected to the zNose™ inlet and purged the system several times with ambient air until the baseline was steady and no peaks larger than approximately 200 counts (Ct) were detected. After that, the instrument was tuned with an alkane standard solution (C6-C14) which was prepared in methanol. Then, the retention times were automatically converted into Kovats Indices (KIs). After tuning, the instrument was ready for analysis. The chromatogram of alkane standard solution (C6-C14) can be seen in Figure 4.4. In order to analyze equilibrated olive oil samples, vial's septum was inserted by the needle to initiate headspace sampling. For each oil sample at least 4 vials were prepared and 4-5 readings were taken from each vial (Vallone et al., 2012).

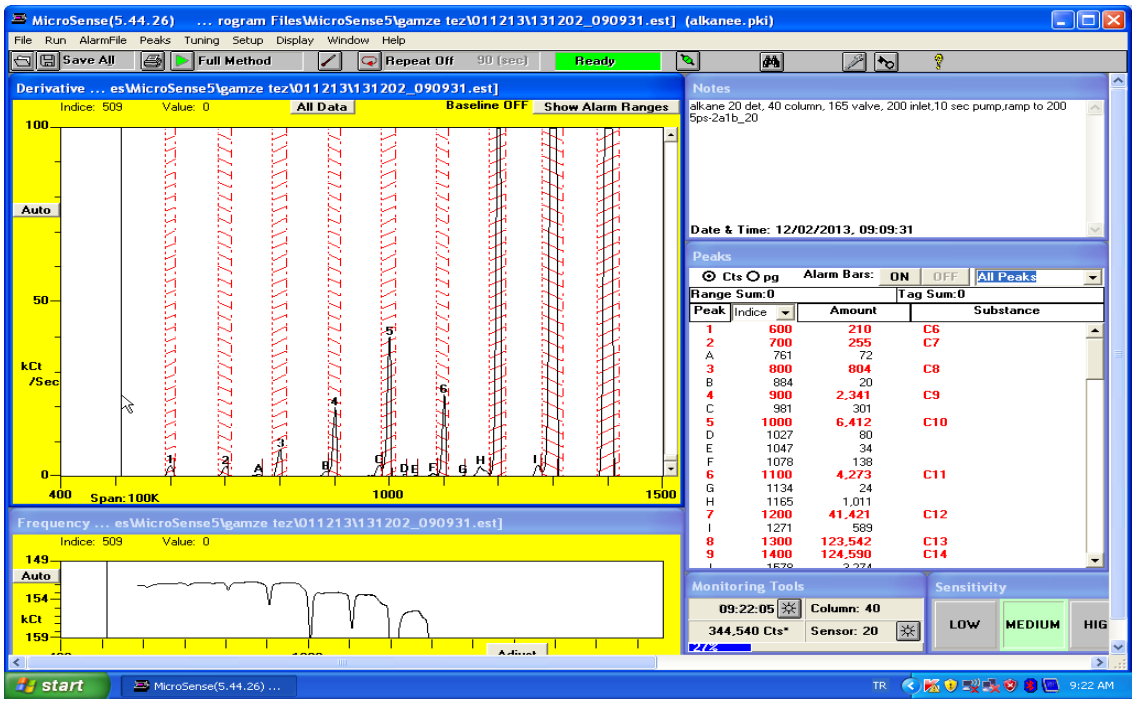


Figure 4.4. Chromatogram of alkane standard solution (C6-C14) after tuning

At the end of the analysis, a chromatogram appears on the screen shown in Figure 4.4, and the sensor was automatically heated to 150 °C for 30 seconds to clean it. During this cleaning period (baking period), the temperature of the inlet, column, and sensor were reset to the initial conditions. In order to ensure a stable baseline and proper system cleaning, air blanks were run between parallel olive oil samples. And also, the n-alkane solution and air blanks were run between different olive oil samples (Vallone et al., 2012).

Table 4.4. Operation parameters of the zNose™

Parameters	**Value**	***Units***
Sensor (Temperature of the SAW detector)	20	*ºC*
Column (Temperature of the column GC)	40	*ºC*
Valve (Temperature of valve)	165	*ºC*
Inlet (Temperature of the input gas)	200	*ºC*
Trap (Temperature of the trap)	280	*ºC*
Ramp (Value of the temperature ramp)	5	*ºC/s*
Acquisition time (Data acquisition time)	30	*s*
Sampling rate (Rate at which the information is registered)	0.02	*s*

In this research, the zNose™ consists of a surface acoustic wave detector (SAW). The main material for the SAW detector is a piezo-electric quartz crystal that operates at a resonant surfaceacoustic wave of 500MHz on its surface. When volatiles adsorb on the surface of the sensor, the frequency of sensor is altered. Adsorbed volatiles changes the detection signal and allows identification of the volatiles (Li and Heinemann, 2007). The temperature of SAW detector was chosen as 20 ºC. According to the users manual the suggested temperature of sensor must be between 20-60 ºC. Temperature of the SAW influences the sensitivity. Lower temperatures give higher sensitivity. But the decrease in temperature within the sensor can cause overloading the sensor. For this reason, 20 ºC was used for olive oil samples and 40 ºC was used for needle readings and blank analysis.

A DB-5 capillary column (1 m length) was used in the zNose™ and the column was heated from 40 °C to 200 °C at a rate of 5 °C/s and the compounds were separated. According to the users manual DB-5 column is appropriate for food and beverages analysis. For this reason this type of column was chosen. If column ramp rate decrease,

peak separation will increase. For this reason, 5 °C/s, 7 °C/s, 8 °C/s and 10 °C/s were tried respectively for taking acceptable peak separation. At the end of the trials, 5 °C/s was chosen because peak separation was seen more clear.

According to the users manual, the recommended valve temperaure is between 30 °C -190 °C and the standard method for DB-5 column is set up valve temperature at 165 °C. This valve temperature is appropriate for volatiles and semi-volatiles. The trap temperature should be in the range of 150 to 300 °C. For this reason 280 °C was chosen as appropriate temperature. Inlet temperature was set up 200 °C. The carrier gas (helium) flow was adjusted to 3 cm^3. According to users manual if helium flow decrease, peak separation will increase and it should be in the range of 1.0-10.0 cm^3. In order to separate peaks more clearly, trials was done with helium flow rate (2 cm^3 and 3 cm^3). Finally 3 cm^3 helium flow was chosen, due to clear peak separation.

zNose™ can perform three main steps of aroma analysis: headspace sampling phase, (injection phase) separation of volatile compounds, and detection. Basically, in the headspace sampling phase, headspace vapor was swept into the inlet via a pump, and then the vapor passes through the heated valve where the compounds were adsorbed onto the trap inside the system (Figure 4.5 (A)). Then, the valve was rotated to put the trap in line with the GC column to prepare for injection (Figure 4.5 (B)). The Tenax trap was quickly heated to release absorbed volatiles. The helium carrier gas transport the desorbed volatiles to a capillary column. A SAW crystal detects the volatiles exiting from the column. The crystal was mounted internally with a small thermoelectric cooler, which can provide cooling needed during vapor adsorption and heating needed to clean the crystal when required (Du et al., 2012).

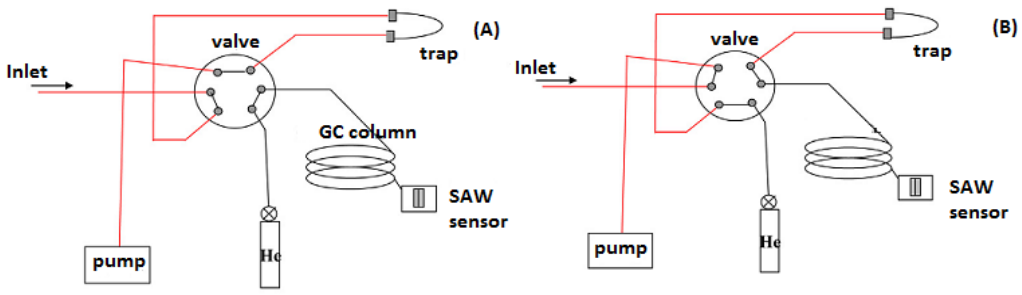


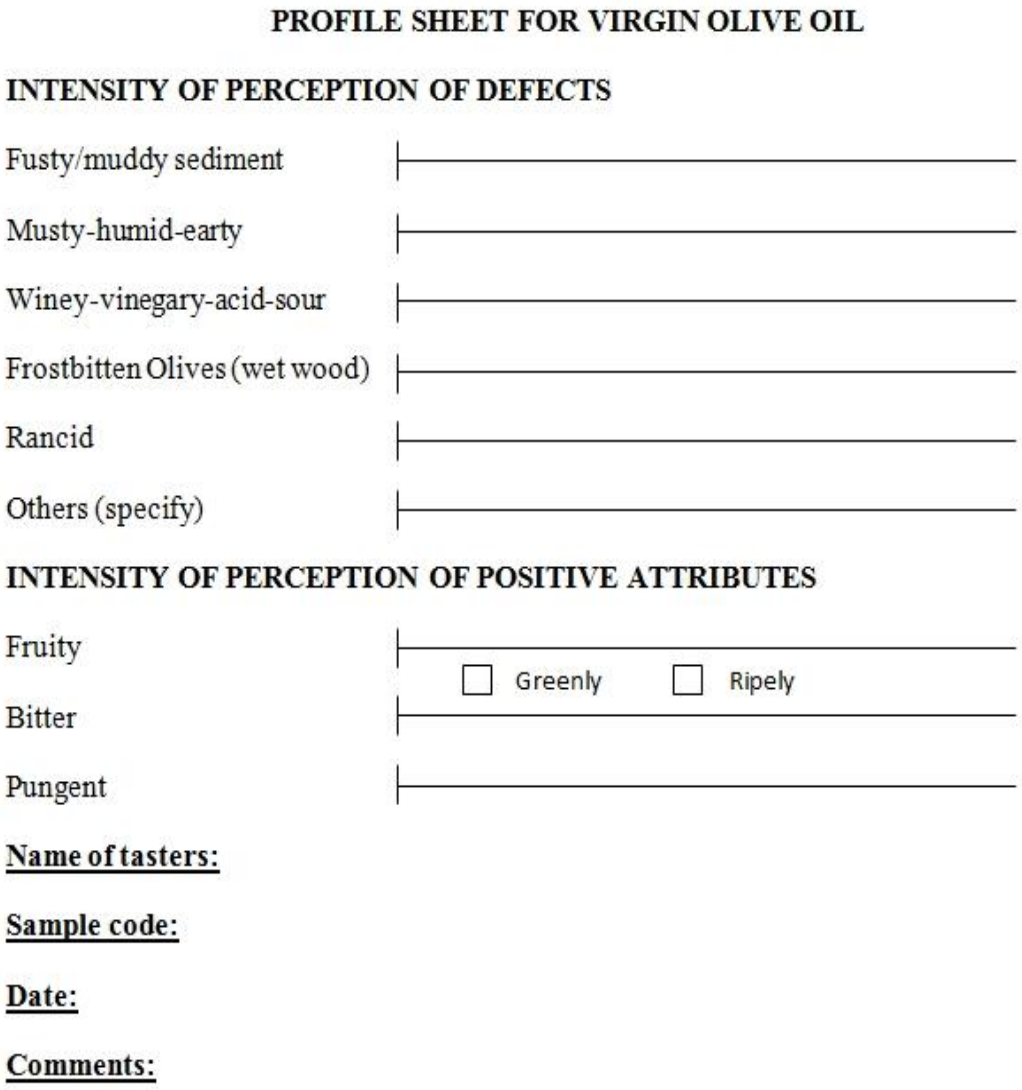
Figure 4.5. Shematic diagram of headspace sampling (A) and injection phase (B) of zNose™ (Source: Oh, 2013).

4.2.4. Sensory Analysis

Sensory analysis was carried out by the official procedure. It was regulated by National Olive and Olive Oil Council (UZZK). Total of 36 olive oil samples were evaluated under the conditions described in European Commission Regulation n. 1640/2008. Under the leadership of Ümmühan Tibet (UZZK Panel Leader), all olive oil samples were analyzed. Four different tasting sessions were conducted by trained panelists throughout three days. Most of the sessions were held during the morning hours and there were eight or more trained panelists at each session (Figure 4.6). Each taster smelled and tasted the oils in order to analyze the positive and negative characteristics. In order to establish the sensory profile of virgin olive oil, the official evaluation sheet (with six standard defects (fusty-muddy sediment, musty, winey, metallic, rancid and "other") and three positive attributes (fruity, bitter and pungent) were used (Figure 4.7). Panelists quantified the intensity of perception of defects and positive attributes into a scale from 0 to 10.

Figure 4.6. Trained panelists during tasting sessions in UZZK sensory room

Before starting sensory analysis, special tasting glasses were coded and approximately 15 mL olive oils were poured into the coded glasses. Samples were randomly presented to panelists. The oil samples were kept at 28 °C ± 2 °C (optimum temperature for observation of organoleptic differences) throughout the test by using a heating instrument (Tefal yoghurt machine). After removing the cover of glass, firstly panelists smelled the oil and they took approximately 3 mL olive oil to their mouth to assess its flavor (Figure 4.6). Olive oil was distributed on tongue up to palate and they took breaths short and repeatedly. Before tasting another kind of olive oil they ate sliced green apples and drank water.


PROFILE SHEET FOR VIRGIN OLIVE OIL

INTENSITY OF PERCEPTION OF DEFECTS

Fusty/muddy sediment

Musty-humid-earty

Winey-vinegary-acid-sour

Frostbitten Olives (wet wood)

Rancid

Others (specify)

INTENSITY OF PERCEPTION OF POSITIVE ATTRIBUTES

Fruity

☐ Greenly ☐ Ripely

Bitter

Pungent

Name of tasters:

Sample code:

Date:

Comments:

Figure 4.7. Sheet used for sensory analysis

4.2.5. Statistical Data Analyses

Statistical analyses were performed by using Microsoft Excel 2007 (Redmond, WA, USA), Minitab 16 (Minitab Inc., State College, PA, USA) and Soft Indipendent Multivariate Class Analogy (SIMCA) 13.0.3 (Umetrics AB, Umeå, Sweden).

Firstly, design of experiment (DOE) was set up to find out significance level of multi level factors. After that, in order to emphasize the similarities and differences between samples and characterize samples with targeted compounds, principle component analysis (PCA) and partial least square discriminant analysis (PLS-DA) was applied to results of HS-SPME, zNose^{TM} and color respectively.

4.2.5.1. Design of Experiments (DOE) Set-up

Design of experiments is essential to verify that data obtained are valid. Especially, when there are many variables in data analysis, the use of the DOE is highly important. By means of DOE, the experimenter can make the identification of the input variables which affect the output of any process. DOE allows the user to reduce the amount of effort needed in an experiment by eliminating unnecessary observations (Peris and Escuder-Gilabert, 2009).

The process parameters were studied by using the design of experiment method. Full factorial design of experiment (DOE) method was chosen in order to determine the effects of different parameters (harvest time, malaxation temperature and olive variety) on volatile compounds by using Minitab 16. As shown in Table 4.5, the DOE was configured as a Multi-level Full Factorial Design, because of the different levels within the parameters. Table 4.6 summarizes the DOE for each parameter levels and all parameter combinations which was taken from Minitab.

Table 4.5. Experimental Design Parameters

Parameters	**Levels**	**Values**
Olive	2	A (Ayvalık), M (Memecik)
Harvest Time	3	E (Early), M (Middle), L (Late)
Temperature	3	27, 37, 47 (°C)

Table 4.6. Design of Experiment (DOE) set-up

Run	Block	A	B	C	Run	Block	A	B	C	
1	1	1	1	1	19	1	1	1	1	
2	1	1	1	2	20	1	1	1	2	
3	1	1	1	3	21	1	1	1	3	**Factors=3**
4	1	1	2	1	22	1	1	2	1	**Replicates=2**
5	1	1	2	2	23	1	1	2	2	
6	1	1	2	3	24	1	1	2	3	**Base Runs=18**
7	1	1	3	1	25	1	1	3	1	**Total Runs=36**
8	1	1	3	2	26	1	1	3	2	
9	1	1	3	3	27	1	1	3	3	**Base Blocks=1**
10	1	2	1	1	28	1	2	1	1	**Total Blocks=1**
11	1	2	1	2	29	1	2	1	2	
12	1	2	1	3	30	1	2	1	3	**Number of levels=2, 3, 3**
13	1	2	2	1	31	1	2	2	1	
14	1	2	2	2	32	1	2	2	2	**A=Olive Variety (A, M)**
15	1	2	2	3	33	1	2	2	3	**B=Harvest Time (E, M, L)**
16	1	2	3	1	34	1	2	3	1	**C=Temperature (27, 37, 47)**
17	1	2	3	2	35	1	2	3	2	
18	1	2	3	3	36	1	2	3	3	

4.2.5.2. Multivariate Data Analyses

Principal Component Analysis (PCA) is a chemometric linear, unsupervised and pattern recognition technique. It gives natural pattern of all the observations. This technique is usually used for classification and reduction of the dimension of numerical data in a multivariate problems (Escuderos et al., 2011). This methodology is also known as “parsimonious summarization” (Maitra and Yan, 2008). It provides graphical representation related with the dependent variable and the independent variables. This method makes data analysis more comprehensible (Haddada et al., 2007). Partial least squares regression analysis (PLS-DA) is a supervised reduction of dimension methodology (Maitra and Yan, 2008). It provides both classification and discrimination (Purcaro et al., 2014). In order to obtain new variables, PLS-DA performs reduction of

the dimension of the variables. Therefore, it provides maximum separation between classes and correlation with the dependent variable.

PCA and PLS-DA models were built to analyze the influence of the cultivar, malaxation temperature and harvest time. Basically, principal component analysis (PCA- unsupervised pattern recognition technique) is a tool for preliminary inspection of the data structure (Cajka et al., 2010). Application of multivariate statistical analysis including principal component analysis (PCA) allows differentiation between samples (Mildner-Szkudlarz and Jeleń, 2008). For this reason, firstly, a PCA model was built to analyze the influence of the processing parameters (harvest time, malaxation temperature and cultivar) on the volatile compounds and electronic nose data.

The aims of two dimensional principle component analysis were to reduce the number of the variables and eliminate the redundancy. While PC1 was the x-axis, PC2 was perpendicular to PC1. The PC1 contained the largest amount of information (Mildner-Szkudlarz and Jeleń, 2008). The summary of the fit of the PCA model was displayed with R^2X, $R^2X(cum)$, Q^2 and $Q^2(cum)$ and eigenvalues. While R^2X represents fraction of the variation of the data explained by each component and Q^2 represents prediction properties of the model (Umetrics, 2005).

After that the data taken from e-nose and GC-MS were analyzed via partial least square-discriminant analysis (PLS-DA). The summary of the fit of the PCA model was displayed with R^2X, $R^2X(cum)$, R^2Y, $R^2Y(cum)$, Q^2 and $Q^2(cum)$ and eigenvalues. $R^2Y(cum)$, the fraction of the variation of Y (all the responses) explained by the model after each components, and $Q^2(cum)$, the fraction of the variation of Y that can be predicted by the model according to the cross validation. Values of $R^2Y(cum)$ and Q2Y(cum) close to 1.0 indicate an excellent model (Umetrics, 2005).

CHAPTER 5

RESULT AND DISCUSSION

5.1. GC/MS Analysis and Volatile Compounds

5.1.1. C6 Volatile Compounds Produced from Lipoxygenase Pathway

The results of the quantitative C6-aldehydes, alcohols and esters coming from linolenic acids (trans-2-hexenal, trans-2-hexenol, cis-3-hexen-1-ol, cis-3-hexenyl acetate) and linoleic acids (hexanal, 1-hexanol and hexyl acetate) are given in Table 5.1. The data were reported as mean values of two independent experiments conducted consecutively.

Table 5.1. C6 Volatile compounds content (ppm) of olive oils

CODE	Hexanal	1-Hexanol	Hexyl acetate	Trans-2-hexenal	Trans-2-hexen-1-ol	Cis-3-Hexen-1-ol	Cis -3-Hexenyl acetate
AE27	2.288	1.773	0.109	1.684	2.459	2.187	0.249
AE37	0.009	2.213	0.081	2.153	4.104	4.255	0.166
AE47	0.271	3.084	0.098	2.770	5.951	5.110	0.168
AM27	4.550	1.526	0.046	3.083	2.277	2.198	0.082
AM37	4.603	1.503	0.030	3.952	1.727	3.345	0.071
AM47	1.593	1.708	0.034	2.578	3.782	3.000	0.074
AL27	0.947	1.169	0.124	2.346	0.623	2.740	0.505
AL37	0.754	1.448	0.052	2.205	1.395	3.788	0.214
AL47	0.493	1.157	0.031	1.609	1.493	2.324	0.176
ME27	1.492	4.076	0.034	1.069	9.647	0.730	0.029
ME37	3.182	2.100	0.065	3.043	10.707	0.842	0.101
ME47	4.567	2.995	0.130	4.711	12.295	1.790	0.293
MM27	1.778	4.953	0.384	3.084	16.491	1.153	1.426
MM37	2.263	2.515	0.111	3.359	12.250	0.430	0.317
MM47	3.037	3.740	0.519	6.930	9.225	1.463	1.971
ML27	0.831	0.700	0.156	4.227	2.332	1.032	0.405
ML37	0.386	1.374	0.059	3.732	3.683	0.765	0.267
ML47	1.419	1.196	0.067	3.593	3.330	0.661	0.240
AE27-2	1.539	2.513	0.136	2.399	2.975	4.447	0.229
AE37-2	0.000	2.350	0.063	1.512	1.932	2.862	0.102
AE47-2	1.905	2.698	0.074	2.231	3.865	4.396	0.150
AM27-2	3.786	1.137	0.039	3.048	1.621	3.027	0.095
AM37-2	2.702	1.139	0.018	3.119	2.026	2.757	0.050
AM47-2	2.193	0.856	0.028	3.322	2.534	1.735	0.091
AL27-2	0.884	0.930	0.078	2.121	0.801	2.220	0.366
AL37-2	2.146	1.052	0.096	2.231	3.222	1.021	0.424

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Table 5.1. (Cont.)

AL47-2	0.530	0.976	0.030	1.680	1.188	2.390	0.187
ME27-2	2.396	3.134	0.022	4.367	9.505	0.909	0.025
ME37-2	1.603	3.980	0.057	4.298	11.133	0.857	0.115
ME47-2	1.360	2.805	0.029	5.422	9.804	0.806	0.071
MM27-2	2.588	2.289	0.394	6.675	6.582	1.247	1.778
MM37-2	3.102	1.076	0.646	8.206	3.847	1.420	2.667
MM47-2	1.887	2.444	0.528	6.507	7.056	1.081	1.952
ML27-2	0.299	1.118	0.076	3.907	6.250	0.524	0.221
ML37-2	0.279	0.933	0.050	5.100	5.207	0.261	0.272
ML47-2	0.320	1.632	0.129	5.337	4.793	0.901	0.454

'Harvest time' and 'olive-harvest time' interactions were statistically significant ($p<0.05$) for hexanal contents. When harvest time was changed, hexanal contents was undergone statistically significant variations. On the other hand, there was no evidence to suggest that olive and temperature variables or/and the other interactions have any statistical significance for the experiment.

As shown in Figure 5.1 the maximum value of hexanal content was found in AM37. Generally for olive cultivars, the content of hexanal increased initially when the ripening stages increased. At the last stage of ripening, hexanal content decreased. Moreover, hexanal was reported that as indicator for ripeness in olive oil (Kalua et al., 2007).Vekiari et al. (2010) reported that the olive oils had the maximum value of hexanal at purple stage (samples were collected green, purple and black stages). Gómez-Rico et al. (2008) stated that, when the growing stages was increased, the hexanal value of olive oils was decreased. Baccouri et al. (2008) reported that when ripening stage was increased, some of olive cultivars' hexanal content initially was increased and then decreased. These studies were in agreement with the present study.

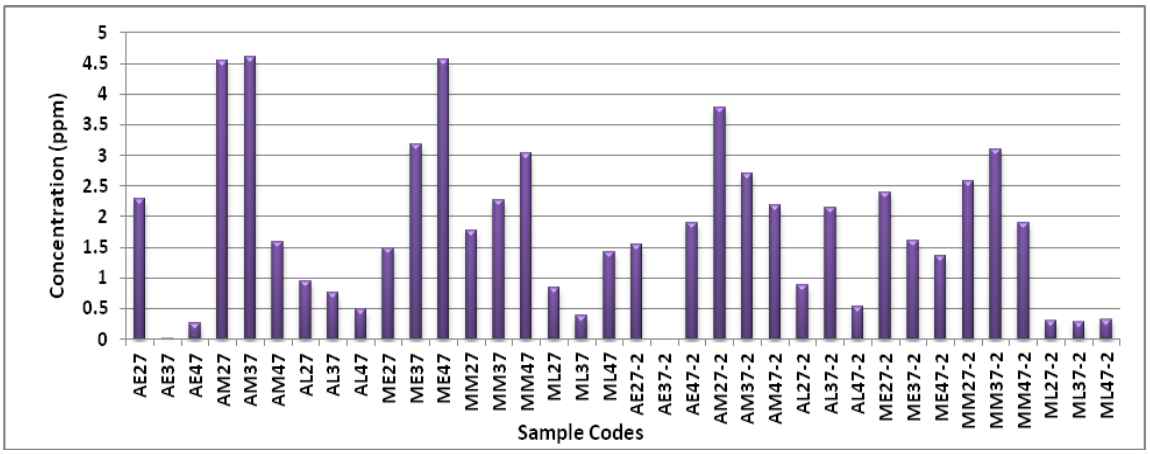

Figure 5.1. Changes in the contents of hexanal

'Olive', 'harvest time' and 'olive-harvest time' interactions were statistically significant ($p<0.05$) for 1-hexanol contents. Kalua et al. 2005 reported that 1-hexanol was responsible for the separation of both cultivar and maturity stages as a supporting result. The other parameter and interactions were not significant ($p>0.05$). While Ayvalık cultivars' 1-hexanol level showed a decrease throughout the ripening, the highest level was recorded in MM27 as shown in Figure 5.2. Generally, at the end of the ripening stages all types of olives had minimum 1-hexanol content. However, Memecik cultivar had more content compared to Ayvalık types of olives.

Vekiari et al. (2010) found the highest 1-hexanol content at the second stage of ripening for one type of olive and this type of olive showed a decrease at the third stage of ripening. Muzzalupo et al. (2012) evaluated olive samples (Rende and Mirto-Crosia) at three ripening stages. As a result, the last stage of maturation was found that this may cause the 1-hexanol levels' reduction and its levels reached the maximum level at the second stage of ripening for Mirto-Crosia olives. In this experiment, while Memecik cultivar showed similar variations for all stages, Ayvalık cultivar showed similarity only the last stage of ripening.

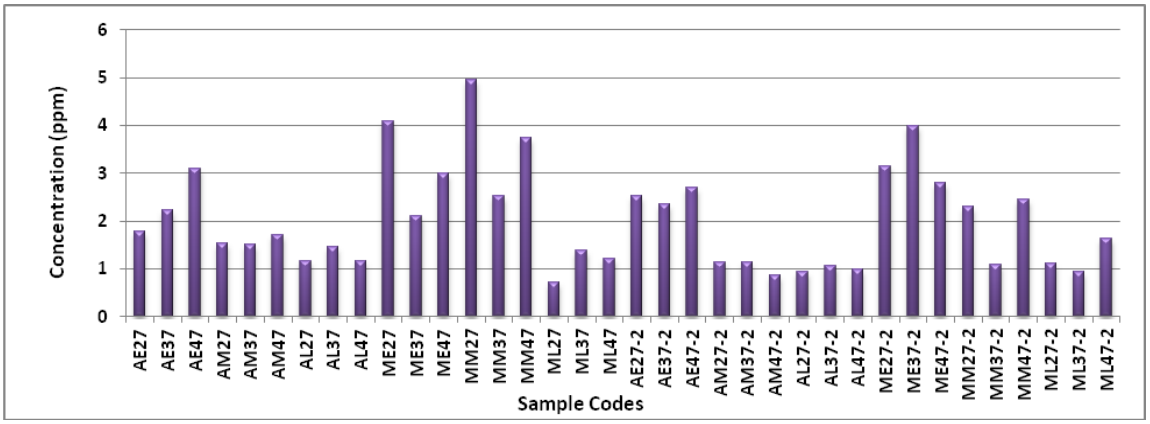

Figure 5.2. Changes in the contents of 1-hexanol

'Olive', 'harvest time' and 'olive-harvest time' interactions were statistically significant ($p<0.05$) for hexyl acetate and cis-3-hexenyl acetate contents. The other parameter and interactions were not significant ($p>0.05$). As shown in Figures 5.3 and 5.4, the maximum levels of both hexyl acetate and cis-3-hexenyl acetate were found in MM37-2 sample. Generally, Memecik types of olives had higher values of this volatiles at the middle stage of ripening. Furthermore, hexyl acetate was reported as indicator for ripeness in olive oil (Kalua et al., 2007).

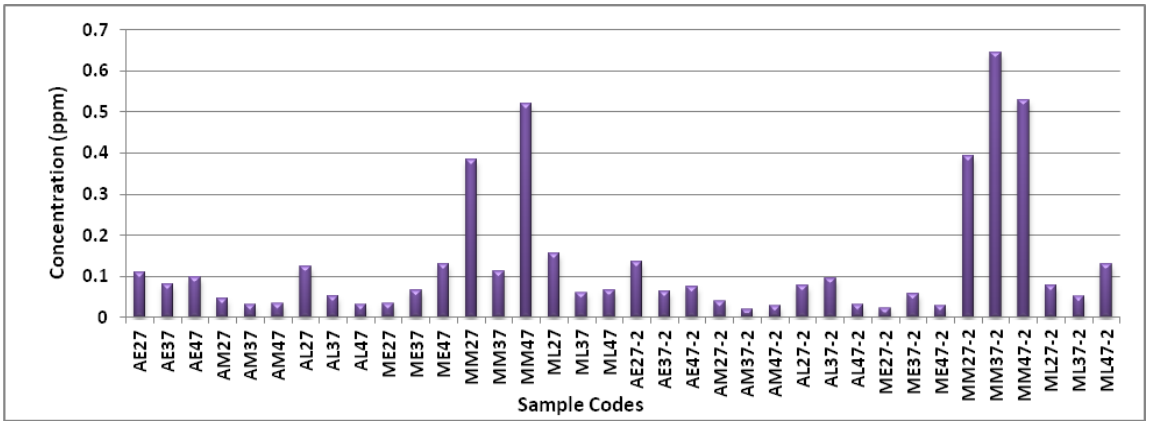

Figure 5.3. Changes in the contents of hexyl acetate

Hexyl acetate and cis-3-hexenyl acetate were minor components compared with aldehydes or alcohols. On the other hand, these esters contribute to fruity sensory notes (Baccouri et al., 2008). These esters were produced by alcohol acyltransferase in the LOX pathway. Furthermore, the low level of esters in the cultivars also demonstrate that a lower content of alcohol acyltransferase (Youssef et al., 2011).

In this study the amounts of hexyl acetate were lower than cis-3-hexenyl acetate. Additionally, Baccouri et al. (2008) reported similar trends, too. Moreover, there was not linear increase with the harvest periods. On the other hand, Vekiari et al. (2010) found that hexyl acetate and cis-3-hexenyl acetate contents increased during the three stages of ripening period for two types of olives. These findings were not with agreement with this study.

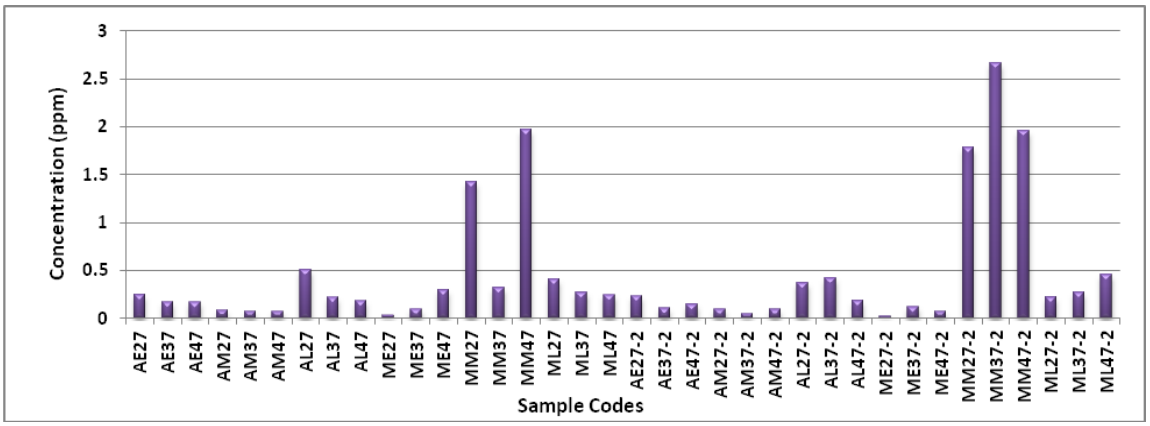

Figure 5.4. Changes in the contents of cis-3-hexenyl acetate

'Olive' and 'harvest time' were statistically significant ($p<0.05$) for trans-2-hexenal, trans-2-hexen-1-ol and cis-3-hexen-1-ol contents. On the other hand, there was no evidence to suggest that olive and temperature variables or/and the other interactions have any statistical significance for the experiment. Cis-3-hexen-1-ol and trans-2-hexen-1-ol were reported as indicators for ripeness in olive oil (Kalua et al., 2007).

As shown in Figure 5.5. the maximum level of trans-2-hexenal was detected in Memecik type of olive at middle stage of ripening time (MM37-2). Generally the content of trans-2-hexenal initially increased, then it decreased throughout the harvest time. Trans-2-hexenal concentration has been used to discriminate olive oils between different types of olives (Ruiz-Samblás et al., 2012). In this study, Memecik variety had the highest trans-2-hexenal levels. It was separated from Ayvalık cultivar by the contribution of trans-2-hexenal and the other parameters.

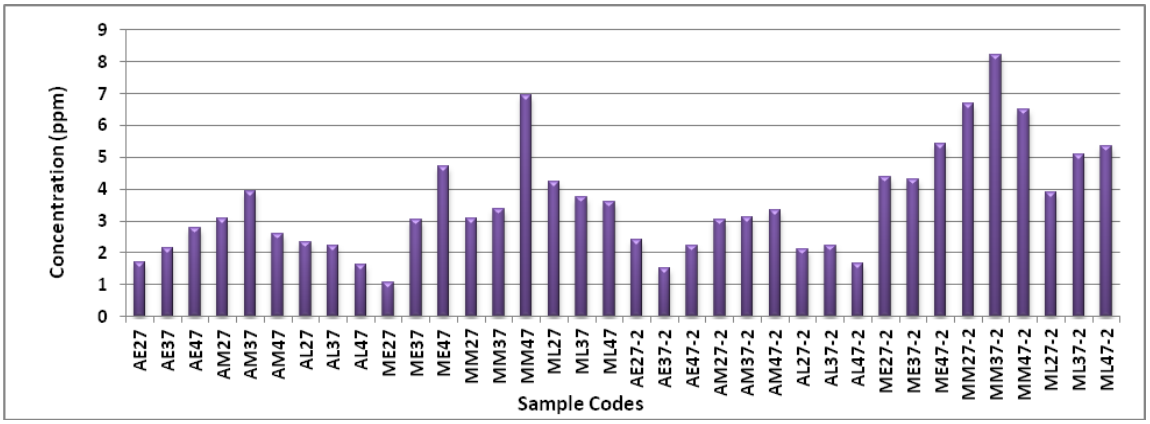

Figure 5.5. Changes in the contents of trans-2-hexenal

Baccouri et al. (2008) reported that the highest trans-2-hexenal content at the second stage of ripening for two types of olives and olive oils extracted from this olives showed a decrease at the third stage of ripening. Gómez-Rico et al. (2008) stated that trans-2-hexenal levels decreased for some types of olives during ripening periods and increased for other types. According to Angerosa (2002), quantity of volatiles increased till a maximum value was reached. When fruits changed skin color from yellow-green to purple, the amount of volatile composition decreased. This action was mainly associated with the changes in the concentration of trans-2-hexenal (Angerosa, 2002). In this study, the results were in agreement with the Baccouri et al. (2008) and Angerosa (2002).

As shown in Figure 5.6 the maximum level of trans-2-hexen-1-ol was detected in Memecik type of olive at the middle stage of ripening time (MM27). In this study Memecik cultivar had the higher trans-2-hexen-1-ol content than Ayvalık cutivar. Furthermore, trans-2-hexen-1-ol contents was the highest one within the C6 volatile compounds produced from lipoxygenase pathway. According to Kesen et al. 2013b, Memecik types of olives had the higher content of trans-2-hexen-1-ol, if it was compared with the Ayvalık and Gemlik cultivars. This findings were in agreement with this study.

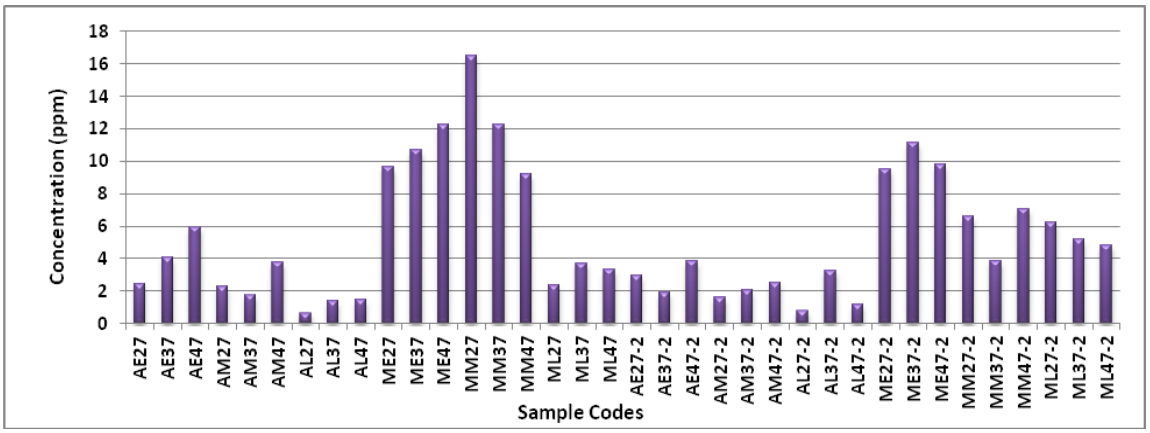

Figure 5.6. Changes in the contents of trans-2-hexen-1-ol

Arafat and Ahmed 2011, trans-2-hexen-1-ol content decreased in three different olive cultivar while ripening period progress. Baccouri et al. (2008) found that for one type olive oil, trans-2-hexen-1-ol content initially increased and then decreased during olive maturation process. In this study, results for trans-2-hexen-1-ol showed similarity with Baccouri et al. (2008). On the other hand, the content of trans-2-hexen-1-ol did not decrease linearly.

As shown in Figure 5.7 the maximum value of cis-3-hexen-1-ol content was found in AE47. Generally Ayvalık cultivar had the higher contents than Memecik. Baccouri et al. (2008) reported that a decrease in cis-3-hexen-1-ol content from unripe to over-ripe was found in all kinds of VOO (Chétoui and Chemlali, Tunisia; Nocellara del Belice, Biancolilla and Cerasuola, Sicily- Italy). Gómez-Rico et al. (2008) stated that cis-3-hexen-1-ol levels decreased some types of olives during ripening period. When compared to literature, similar pattern was determined for Ayvalık cultivar. In this study, generally the content of cis-3-hexen-1-ol of Ayvalık was high until the second stage of ripening.

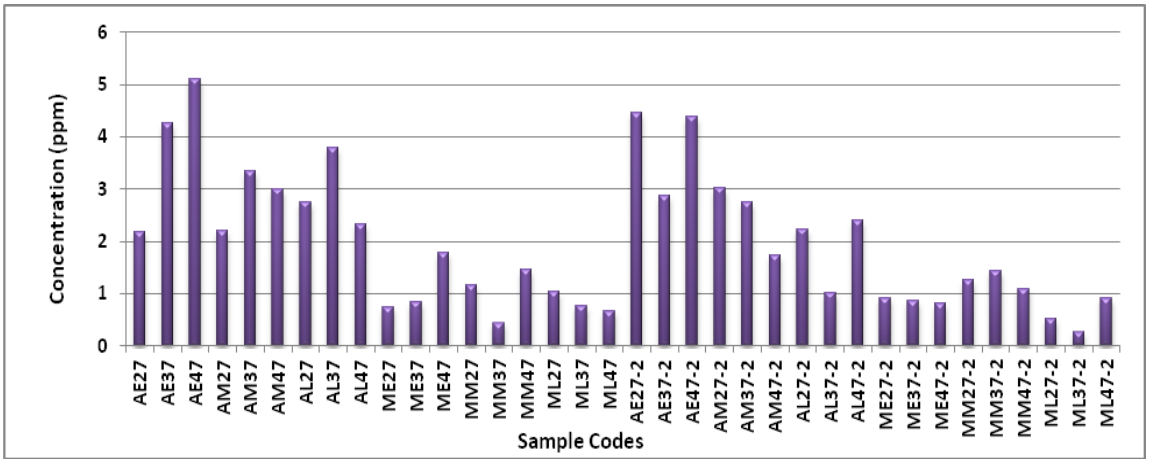

Figure 5.7. Changes in the contents of cis-3-hexen-1-ol

5.1.2. C5 Volatile Compounds Produced from Lipoxygenase Pathway

The results of the quantitative C5-aldehydes, alcohols and ketones coming from linolenic acids (1-Penten-3-ol, 1-Penten-3-one, trans-2-pentenal) and linoleic acids (3-Pentanone) were given in Table 5.2. The data were reported as mean values of two independent experiments conducted consecutively.

Table 5.2. C5 Volatile compounds content (ppm) of olive oils

CODE	1-Penten-3-ol	1-Penten-3-one	3-Pentanone	Trans-2-pentenal
AE27	0.463	0.000	0.126	0.012
AE37	0.801	0.000	0.083	0.015
AE47	0.247	0.000	0.000	0.011
AM27	0.024	0.000	0.000	0.014
AM37	0.217	0.000	0.000	0.017
AM47	0.264	0.000	0.000	0.007
AL27	0.394	0.000	0.017	0.013
AL37	0.255	0.000	0.000	0.009
AL47	0.196	0.000	0.019	0.007
ME27	0.792	0.000	0.000	0.001
ME37	0.395	0.000	0.000	0.004
ME47	0.227	0.000	0.000	0.031
MM27	0.120	0.000	0.000	0.005
MM37	0.082	0.000	0.000	0.006
MM47	0.177	0.000	0.000	0.014
ML27	0.000	0.000	0.000	0.008
ML37	0.028	0.000	0.000	0.004
ML47	0.000	0.000	0.000	0.004
AE27-2	0.407	0.000	0.166	0.010
AE37-2	1.424	0.018	0.403	0.004
AE47-2	0.654	0.385	0.419	0.029
AM27-2	0.083	0.000	0.000	0.012

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Table 5.2. (Cont.)

AM37-2	0.264	0.000	0.000	0.011
AM47-2	0.178	0.000	0.000	0.013
AL27-2	0.161	0.000	0.000	0.010
AL37-2	0.117	0.000	0.000	0.016
AL47-2	0.251	0.000	0.000	0.009
ME27-2	0.000	0.000	0.000	0.013
ME37-2	0.000	0.000	0.000	0.005
ME47-2	0.002	0.000	0.000	0.014
MM27-2	0.023	0.000	0.000	0.015
MM37-2	0.118	0.000	0.000	0.026
MM47-2	0.163	0.000	0.000	0.012
ML27-2	0.119	0.000	0.000	0.001
ML37-2	0.212	0.000	0.000	0.011
ML47-2	0.001	0.000	0.000	0.008

'Olive' and 'harvest time' were statistically significant ($p<0.05$) for 1-penten-3-ol and 3-pentanone. In addition to this 'olive and harvest time' interactions were significant for 3-pentanone. The other parameters and interactions were not significiant ($p>0.05$) for two types of volatile compounds. All parameters, which were investigated, were not statistically significant ($p>0.05$) for 1-penten-3-one and trans-2-pentenal. As shown in Table 5.2, trans-2-pentenal was found in all types of olive oils in this study, but it did not contribute to the classification of olive oils. Trans-2-pentenal contents were very close to each other and no comparison could be done.

As shown in the Table 5.2 3-pentanone and 1-penten-3-one were only found in some Ayvalık cultivar. Gómez-Rico et al. (2008) conducted research on the effect of cultivar and ripening on minor components in olive fruits and Vekiari et al. (2010) investigated that characterization and seasonal variation of the quality of olive oil. Nevertheless, they did not mention about 3-pentanone. Ben-Hassine et al. (2013) reported that 3-pentanone was important for characterization of cultivar. In this study, 3-pentanone was found in higher content in early stages of Ayvalık cultivar. For his reason it affected the type and cultivar. Tena et al. (2007) stated that 1-penten-3-one contributed to classification of compounds by ripeness. They found that 1-penten-3-one composition diminished with ripeness. Gómez-Rico et al. (2008) stated that 1-penten-3-one composition changed according to cultivar and ripeness. But there was no linearity. Youssef et al. (2011) investigated that the effect of cultivar on minor components in Tunisia olives and Ben-Hassine et al. (2013) determined physicochemical and sensory characteristics of virgin olive oils in relation to cultivar. On the other hand, they did not discuss about 1-penten-3-one composition. Therefore, in this study 1-penten-3-one values were in agreement with Youssef et al. (2011) and Ben-Hassine et al. (2013).

As shown in Figure 5.8. the maximum value of 1-penten-3-ol content was found in AE37-2. Ayvalık cultivar had higher levels of 1-penten-3-ol compared to Memecik cultivar. Kalua et al. 2005 reported that 1-penten-3-ol was responsible for the separation of both cultivars and maturity stages. Gómez-Rico et al. (2008) found that generally 1-penten-3-ol contents of olive oils decreased throughout the ripening periods. Conversely, according to Vekiari et al. 2010 , 1-penten-3-ol levels of two types of olives increased during growing stages. In this study, data were similar with Gómez-Rico et al. (2008). Because 1-penten-3-ol levels decreased generally, when the ripening stages increased.

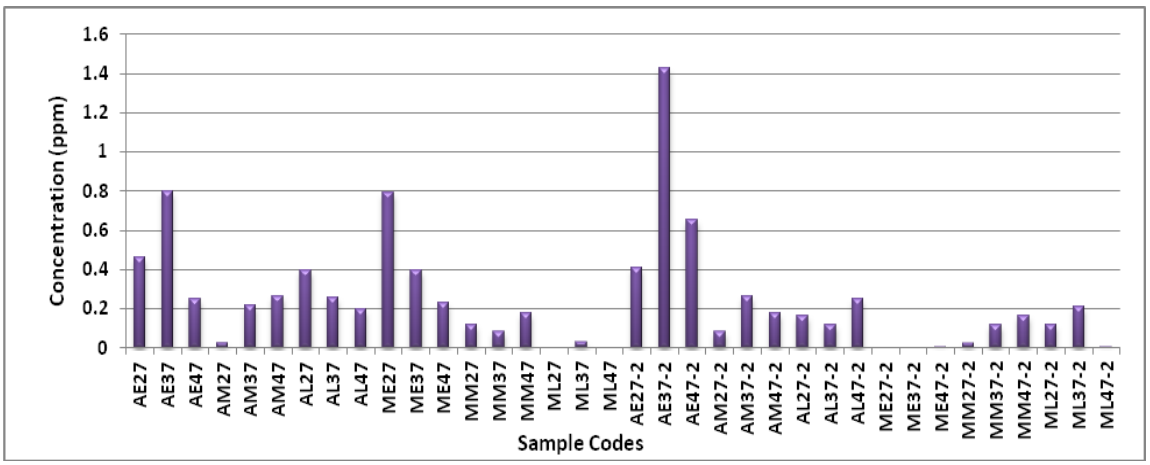


Figure 5.8. Changes in the contents of 1-penten-3-ol

5.1.3. Volatile Compounds Produced from Other Ways Exclusive of Lipoxygenase Pathway

The multilevel full factorial design was applied to obtaine quantitative volatile values to observe the significant differences ($p<0.05$) based on cultivar, malaxation temperature and harvest time. Ethanol, 2-butanone, ethyl acetate, isoamylalcohol, n-octane, 2-heptanone, heptanal, octanal, nonanal, 2-nonanone, trans-2-heptenal, (R)-(+)-limonene, 1-octanol, terpinolene, trans-2-decenal, trans, trans 2,4 nonedienal, trans-2-nonenal, butyl acetate were analyzed one by one.

2-heptanone, heptanal, terpinolene, trans, trans 2,4 nonedienal, 2-butanone and ethyl acetate were not detected in this study. Isoamyl alcohol, n-octane, butyl acetate, ethanol and trans-2-decenal were not significant in discriminating samples based on the

selected parameters ($p>0.05$). For this reason, volatiles which were effective, were selected based on statistical analysis as shown in Table 5.3.

Table 5.3. Volatile contents (ppm) produced from other ways exclusive of lipoxygenase pathway

CODE	(R)-(+)-limonene	Octanal	Nonanal	1-Octanol	Trans-2-heptenal	2-Nonanone	Trans-2-nonenal
AE27	0.000	0.219	1.520	0.070	0.058	0.032	0.030
AE37	0.378	0.090	0.739	0.035	0.000	0.040	0.002
AE47	0.133	0.080	0.485	0.045	0.027	0.065	0.009
AM27	0.000	0.068	1.525	0.045	0.097	0.029	0.056
AM37	0.000	0.034	0.974	0.013	0.005	0.003	0.023
AM47	0.000	0.059	1.075	0.050	0.035	0.014	0.033
AL27	0.000	0.311	1.376	0.050	0.014	0.011	0.011
AL37	0.000	0.136	1.499	0.040	0.002	0.007	0.025
AL47	0.000	0.106	1.391	0.034	0.002	0.006	0.033
ME27	0.000	0.023	2.694	0.097	0.064	0.063	0.115
ME37	0.198	0.041	1.926	0.045	0.049	0.013	0.060
ME47	0.034	0.204	2.141	0.088	0.146	0.019	0.149
MM27	0.000	0.853	2.558	0.122	0.070	0.028	0.073
MM37	0.000	0.927	1.892	0.166	0.138	0.129	0.178
MM47	0.000	1.153	4.060	0.130	0.098	0.029	0.085
ML27	0.000	0.261	1.909	0.106	0.140	0.034	0.046
ML37	0.000	0.154	0.710	0.025	0.057	0.001	0.006
ML47	0.000	0.136	1.380	0.061	0.069	0.006	0.041
AE27-2	0.020	0.151	1.065	0.051	0.028	0.076	0.022
AE37-2	0.331	0.042	0.525	0.037	0.000	0.021	0.000
AE47-2	0.004	0.088	0.894	0.027	0.055	0.024	0.027
AM27-2	0.000	0.044	0.636	0.009	0.000	0.003	0.008
AM37-2	0.000	0.005	0.722	0.006	0.000	0.000	0.007
AM47-2	0.000	0.038	0.912	0.029	0.038	0.008	0.023
AL27-2	0.000	0.209	0.846	0.032	0.007	0.007	0.018
AL37-2	0.000	0.262	1.205	0.056	0.065	0.010	0.038
AL47-2	0.000	0.102	1.213	0.028	0.002	0.003	0.010
ME27-2	1.019	0.028	1.404	0.053	0.067	0.012	0.077
ME37-2	0.000	0.070	1.513	0.043	0.032	0.014	0.076
ME47-2	0.000	0.049	1.696	0.051	0.063	0.008	0.087
MM27-2	0.000	1.040	2.463	0.090	0.063	0.016	0.063
MM37-2	0.000	1.462	3.540	0.122	0.090	0.026	0.083
MM47-2	0.000	1.172	3.454	0.108	0.074	0.021	0.091
ML27-2	0.000	0.110	0.956	0.117	0.000	0.025	0.071
ML37-2	0.000	0.159	1.438	0.030	0.002	0.003	0.021
ML47-2	0.000	0.305	2.330	0.092	0.113	0.014	0.072

The results, as indicated in Table 5.3, (R)-(+)-limonene was found in only early stages of olive oils. It was not found in other harvest time of olive. The highest levels were determined in ME37-2 samples. Based on statistical analysis (R)-(+)-limonene contents were affected by the harvest time of olive. For this reason 'harvest time' was statistically significant ($p<0.05$). Baccouri et al. (2008) reported that (R)-(+)-limonene

had a role in the fragrance of food and their response to maturity was very low. Furthermore, the results obtained by this study were supported by this information. Conversely, Vekiari et al. (2010) found limonene at second stage and third stage of growth of olives. There was no (R)-(+)-limonene compound in early stages of olives.

The hydrocarbons n-octane and the aldehydes, heptanal, trans-2-heptenal, octanal and nonanal were produced from autoxidation reactions after extraction of olive oil and almost all of these volatiles are responsible for olive oil off-flavors (Baccouri et al., 2008). As it can be seen in the Figure 5.9, the maximum value of octanal content was found in MM37-2. And also, the higher content of this compound was found in middle stage of growth. 'Olive' and 'harvest time' and 'olive- harvest time interaction' were statistically significant ($p<0.05$) for this volatile compound. Vekiari et al. (2010) reported that throumbolia type of olive had octanal in the second stage of ripening time and koroneiki type of olive had octanal in the third stage of ripening time. This result was similar with the present study. Because, Memecik cultivar had high content of octanal at middle stage while Ayvalık cultivar had it of the last stage.

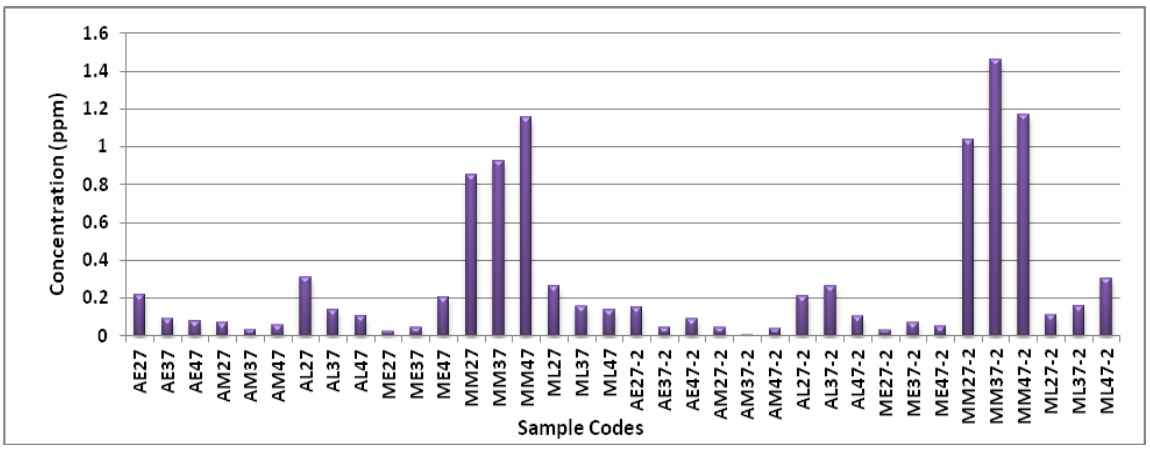


Figure 5.9. Changes in the contents of octanal

As shown in Figure 5.10, the maximum nonanal content was found in MM47.'Olive' and 'harvest time' and 'olive- harvest time interaction' were statistically significant ($p<0.05$) for nonanal compound and the higher contents were found in middle stage of growth time for Memecik cultivar such as octanol compound. Furthermore, Ayvalık cultivar had more nonanal content at the third stage. Vekiari et al. (2010) indicated that throumbolia type of olive had nonanal at the second stage of

ripening time and koroneiki type of olive had it at the third stage of ripening time. For this reason, cultivar and harvest time affect the contents of this compound.

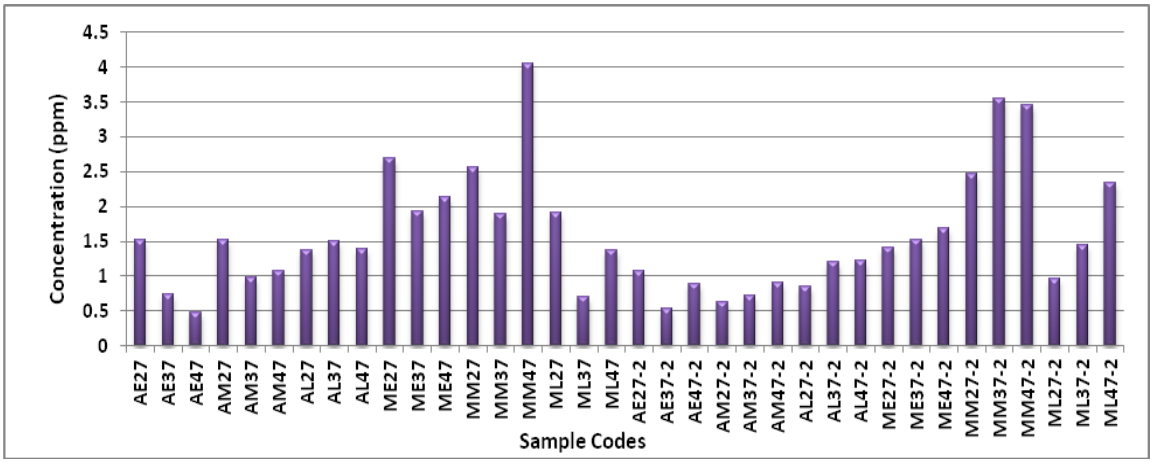


Figure 5.10. Changes in the contents of nonanal

As shown in Figure 5.11, the maximum 1-octanol content was found in MM37.'Olive' and 'harvest time' and 'olive- harvest time-temperature interaction' were statistically significant ($p<0.05$). Memecik type of olive, especially middle stage, had higher content. Kalua et al. (2005) reported that 1-octanol was predominant volatile for manzanilla type of olive. Additionally, Vekiari et al. (2010) indicated that throumbolia cultivar had higher content at the middle stage of ripening. For this reason, cultivar and harvest time results were similar with these reports.

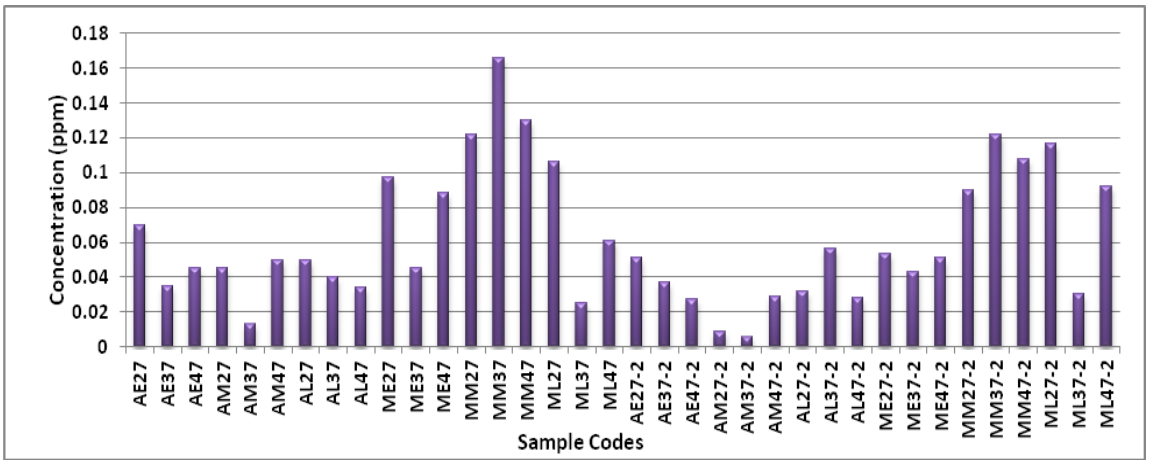


Figure 5.11. Changes in the contents of 1-octanol

The other volatile compounds, which were shown in Table 5.3, were statistically significant. Namely, ‘olive’, ‘olive-harvest time’, ‘olive, harvest time and olive–harvest time interaction’ were statistically significant for trans-2-heptenal, 2-nonanone, trans-2-nonenal, respectively. The highest levels were calculated for trans-2-heptenal in ME37-2 for 2- nonanone and trans-2-nonenal in MM37. The similarity between them was all these volatile compounds were high in Memecik type of olives. Trans-2-nonenal, which was formed during the early stages of autoxidation process of linoleic acid, was found in Memecik cultivar higher than Ayvalık cultivar.

5.1.4. Multivariate Data Analyses

Data obtained as a result of HS-SPME-GC/MS were analyzed using statistical programme Minitab firstly. After comparing results based on the level of statistical significance of the data set ($p<0.05$) were subjected to PCA and PLS-DA. It was possible to distinguish between samples by using volatile compounds.

The results of PCA model with $R^2 = 0.83$ and $Q^2 = 0.43$ were reported in score and loading PCA plots (Figures 5.12). PCA obtained from volatile data sets resulted in five PCs explaining 83.2% of the total variation. The two dimensional score plot of the first two components (PC1 and PC2) account for 55% of total variance.

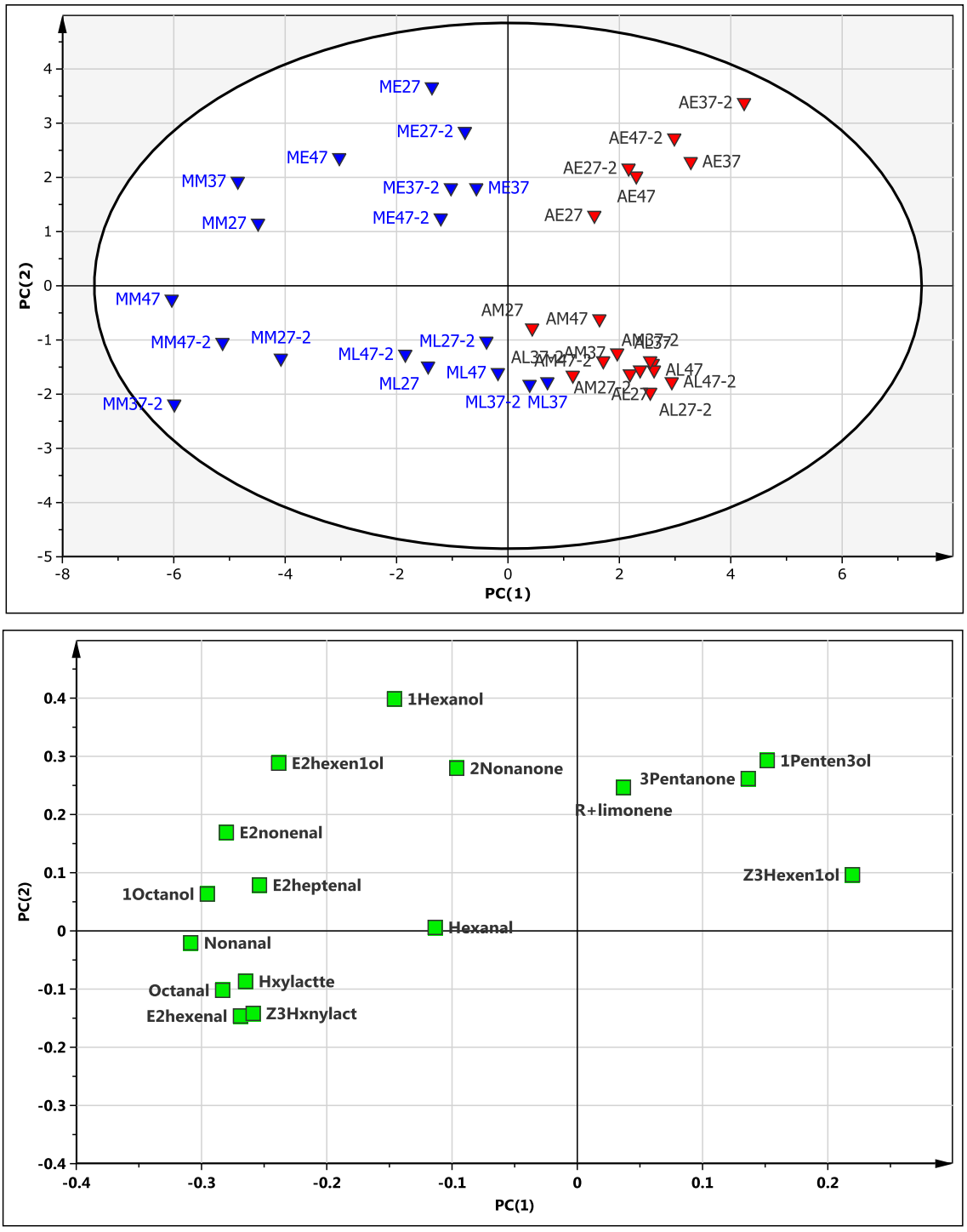


Figure 5.12. PCA score plot (a) and loading plot (b) based on SPME-GC/MS analysis

The PCA results were graphically displayed using two plots; the score and loading PCA plot. They were structured by using the volatile data sets. PCA score plot shows which samples were similar or different. Score plot was colored based on variety discrimination. While similar samples were close to each other different ones were far away. The Ayvalık and Memecik samples were separated in the right and left direction.

Loading plot and shows which variables that are important for the positions of the samples. The volatile compounds shown in loading plot was responsible for the discrimination of the samples. According to this plot the number of volatile compounds of Memecik olive oils, which play a role for discrimination of olive oils, were higher than Ayvalık olive oils. As shown in Figure 5.12 (b), especially, 1-hexenol, trans-2-hexen-1-ol, 2-nonanone, trans-2-nonenal, 1-octanol, trans-2-heptenal, nonanal, hexanal, hexyl acetate, octanal, trans-2-hexenal, cis-3-hexenyl acetate were effective for discrimination of Memecik olive oils. Furthermore, (R)-(+)-limonene, 1-penten-3-ol, 3-pentanone, and cis-3-hexen-1-ol contributed to separation of Ayvalık olive oils. Totally number of volatile compounds of Memecik olive oils were determined higher than Ayvalık olive oils. Furthermore, Kesen et al., (2013b) reported that the highest volatile compounds were found in Memecik cultivar, than Gemlik and finally Ayvalık. Additionally, they found that the powerful aroma active compounds were guaiacol (soapy) for Ayvalık and hexanal (cut grass), octanal (citrus, lemon) and cis-3-hexenyl acetate (fruity) for Memecik. Mainly, these results were supported by this information.

Memecik cultivar and early harvest olive oils were characterized by 1-hexanol, trans-2-hexen-1-ol and 2-nonanone. Memecik and middle harvest olive oils were affected by trans-2-nonenal, trans-2-heptenal, 1-octanol, nonanal, octanal, trans-2-hexenal, cis-3-hexenyl acetate and hexyl acetate. Ayvalık and early harvest olive oils were separeted from other types of olive oils through these volatiles: (R)-(+)-limonene, 1-penten-3-ol, 3-pentanone, and cis-3-hexen-1-ol. On the other hand, late harvest olive oils gave less of hexanal compounds compared to early and middle harvest time.

D'imperio et al., (2010) illustrated that hexanal and trans-2-hexenal decreased during ripening process. On the other hand, only green and ripe harvest period were studied. Formation of hexanal and trans-2-hexenal can be affected by many factors or substrates, which were responsible for inhibition during lipoxygenase pathway. In this study hexanal decreased throughout the ripening process, but trans-2-hexenal was high at the middle stage of ripening for Memecik cultivar. After this stage it decreased.

Angerosa et al. (2001) indicated that C_5 and C_6 components were responsible for green odor notes. In this study, while 1-hexanol and trans-2-hexenol from C6 component contribute to discrimination of early harvest Memecik type of olive oil, 1-penten-3-ol and 3-pentanone from C5 component with cis-3-hexen-1-ol from C6 compounds contribute to discrimination of the early harvest Ayvalık types of olive oil.

García-González et al. (2008) stated that sensory perception of 2-nonanone and 1-hexanol were green tomato. Furthermore, these compounds were found as a distinction factor for early harvest and Ayvalık type of olive oils.

From this analysis, it appeared that the volatile compounds were good candidates for the classification of olive oils according to cultivar and harvest time. As shown in Figure 5.12 (a) and (b) temperature was not really effective. The samples were not clustered according to values of temperatures.

According to the PCA model, the samples of different harvest time and cultivar formed groups. Furthermore, a two-component PLS-DA model with $R^2X = 0.88$, $R^2Y = 0.99$, $Q^2 = 0.97$ was built to further resolve the effect of the harvest time. As shown in Figure 5.13 (a) PLS-DA model shows that harvest time was a strong discriminating component especially for early and late harvest times.

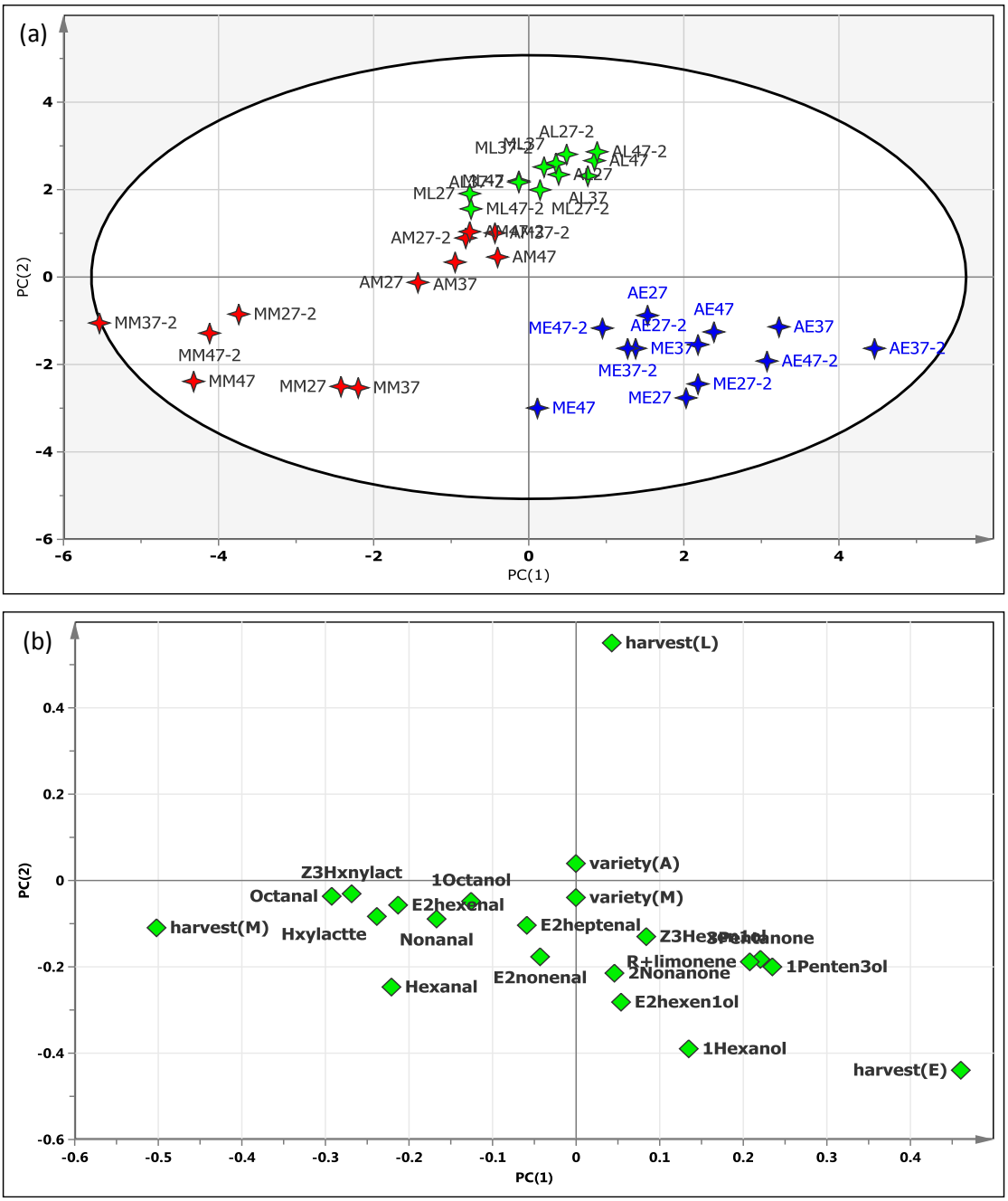


Figure 5.13. PLS-DA score plot (a) and loading plot (b) based on SPME-GC/MS analysis

5.2. Electronic Nose Analysis and Volatile Compounds

Nineteen obvious peaks were chosen from the first derivative plot of each aroma profile of olive oil samples, respectively. Corresponding peak areas which represents the amount of different volatile compounds, were calculated using the system of zNose™ software MicroSense 5.44.26 version and these values were used in data

analysis. As shown in the Table 5.4, firstly effects of factors (harvest time, temperature and olive variety) on volatile compounds taken from zNose^{TM} were determined by using a multilevel full factorial design of experiment method by using MINITAB according to significance level (at P-value<0.05). After that, volatile compounds, which were affected by harvest time, temperature and/or olive variety, were analysed by using SIMCA.

As can be seen in Table 5.4, response factors (peaks) particularly were affected by 'cultivar (olive), harvest time and olive*harvest time interactions'.

Table 5.4. The effects of input variables (factors) on output variables (peaks)

Peaks	**Significant (p<0.05) Factors**
P1	Olive/harvest time/olive*harvest time
P2	Olive/harvest time/olive*harvest time
P3	Olive/harvest time/olive*harvest time/olive*temperature/harvest time*temperature
P4	Olive/harvest time/temperature /olive*harvest time
P5	Olive/harvest time/olive*harvest time
P6	Harvest time/temperature/olive*harvest time/olive*temperature/harvest time*temperature/olive*harvest time*temperature
P7	Harvest time/olive*harvest time
P8	Olive/harvest time/temperature/olive*harvest time/ harvest time*temperature/olive*harvest time*temperature
P9	Olive/harvest time/olive*harvest time/ harvest time*temperature/olive*harvest time*temperature
P10	Olive/harvest time/olive*harvest time
P11	Olive/ harvest time/olive*harvest time
P12	Olive/ harvest time
P13	Olive/ harvest time/olive*harvest time
P14	Harvest time
P15	Harvest time/temperature/olive*harvest time/olive*temperature/olive*harvest time*temperature
P16	Olive/ harvest time/olive*harvest time
P17	Harvest time/olive*harvest time
P18	Harvest time
P19	Olive/harvest time

Figure 5.14 (a) shows the PCA score plot results from a model containing the 36 olive oil samples characterised by nineteen peaks. The result of PCA model with R^2 = 0.83 and Q^2 = 0.44 was reported in score and loading PCA plot. PCA obtained from enose data sets resulted in five PCs explaining 82.9% of the total variation. The two dimensional score plot of the first two components (PC1 and PC2) account for 54.3% of total variance, 30.1% and 24.1%, respectively.

Middle and early harvest Ayvalık olive oil appeared in the right part of the graph and middle and late harvested Memecik olive oil appeared in the left part of the graph. Oil samples from different cultivars were distinguished by PC1. Early and middle harvest Ayvalık type of olive oils presented in positive score values according to PC1 and late and middle harvest Memecik type of olive oils presented in negative score values according to PC1. This showed that early and middle harvest Ayvalık type of olive oils had similar aroma profiles. Furthermore, late and middle harvest Memecik type of olive oils had similar aroma profiles, too. On the other hand, some of late harvest Ayvalık cultivar and early harvest Memecik cultivar presented in both positive score values and negative score values.

Figure 5.14 (b) shows the loading plot of the PCA and the high positive correlation between peaks (P1, P5, P10, P12, P13, P6, P7 and P14) and PC1 indicated that the volatile profile of early harvest Ayvalık olive oil had a higher proportion of these peaks. The high negative score of middle harvest Memecik olive oil was determined by the highest amount of the P19, P2 and P3. Furthermore, while P15 was effective for discriminating late harvest Memecik olive oil with late harvest Ayvalık olive oil, P4 was effective for discriminating late harvest Ayvalık olive oil.

According to data obtained from HS-SPME previously; early harvest Ayvalık olive oils clustered from other types of olive oils through these volatiles: (R)-(+)-limonene, 1-penten-3-ol, 3-pentanone, and cis-3-hexen-1-ol. Middle harvest olive oil were affected by trans-2-nonenal, trans-2-heptenal, 1-octanol, nonanal, octanal, trans-2-hexenal, cis-3-hexenyl acetate and hexyl acetate as previously described while interpreting the volatile compounds. As found in results from HS-SPME and zNose^{TM}, especially, early harvest Ayvalık olive oil and middle harvest Memecik olive oil had several volatile compounds. These results supported each other. Volatile compounds taken from HS-SPME may have been the same volatile compounds which caused a separation of the olive oils in the graph of zNose^{TM}.

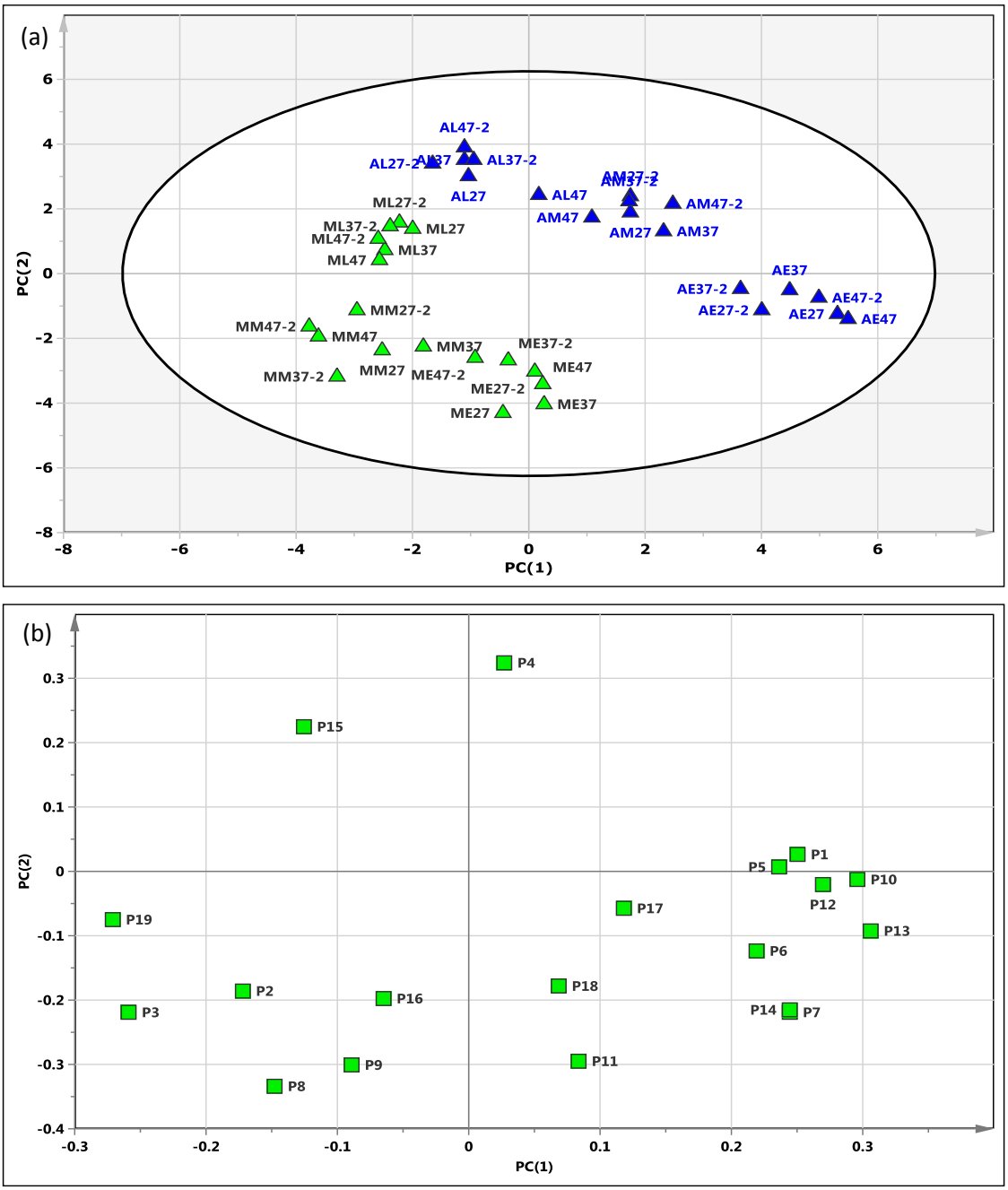


Figure 5.14. PCA score plot (a) and loading plot (b) based on results of zNose^{TM}

According to the PCA model, especially, the samples of different harvesting times and cultivar formed groups. Furthermore, a two-component PLS-DA model with $R^2X = 0.74$, $R^2Y = 0.97$, $Q^2 = 0.95$ was built to further resolve the effect of the harvest times. As shown in Figure 5.15, these plots show that the effects of the harvest times were predominant in the discrimination of oil samples according to volatile compositions, which were taken from zNose^{TM}.

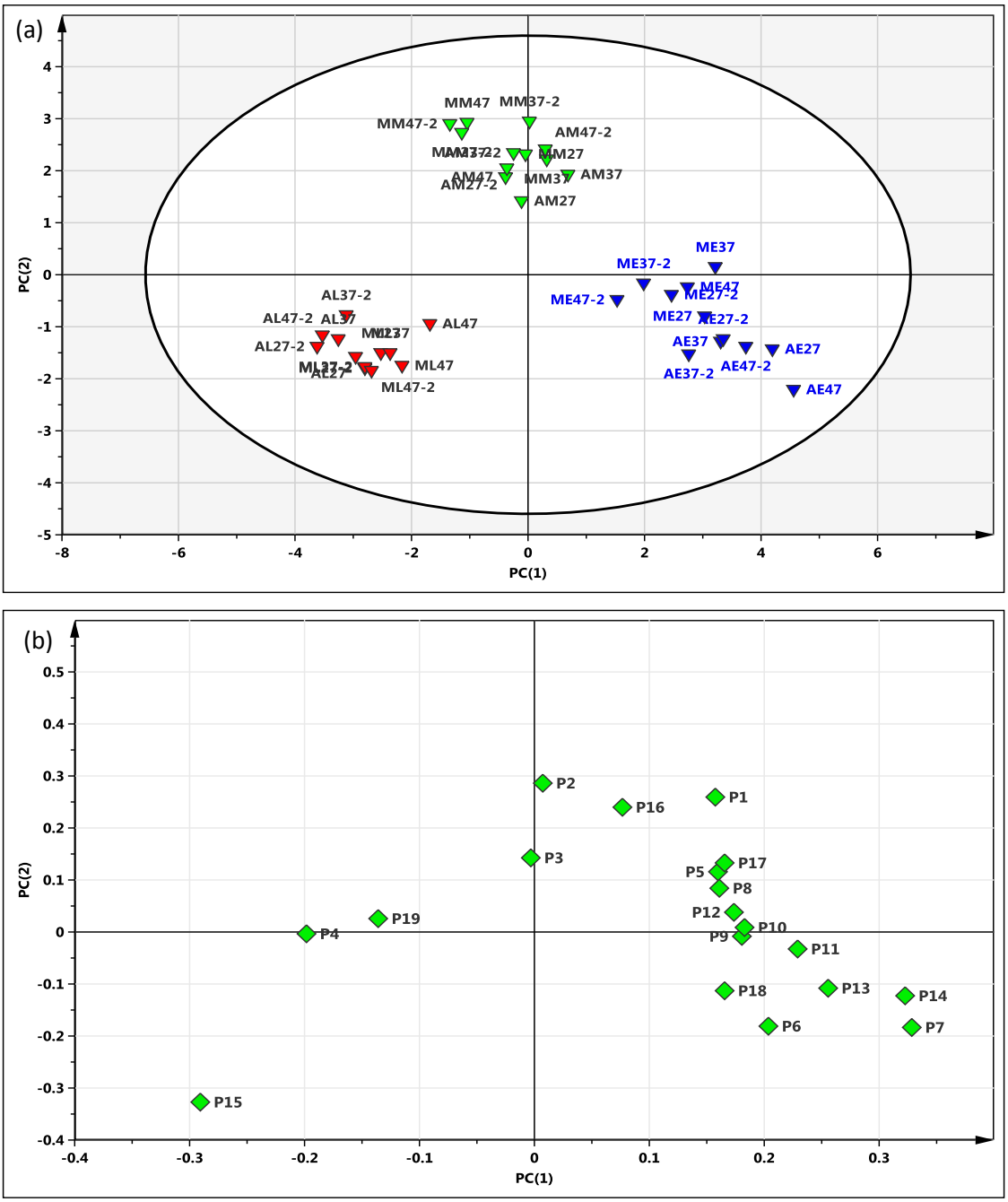


Figure 5.15. PLS-DA score plot (a) and loading plot (b) based on results of zNose™

5.3. Sensory Analysis

Thirtysix samples of olive oils were assessed by the taste panel, in order to determine the positive and negative characteristics of olive oils. After sensory evaluation of the samples, the statistical processing was done according to the testing methods which was downloaded from the website of IOC with panel leader (IOC,

2015a). By using these testing method the median of the defects (each negative characteristic) and the median of the fruity attribute (each positive characteristic) were calculated with robust coefficient variation. This coefficient variation was used to measure the efficiency of the panelists. It must be lower than 20%. When it was higher than 20%, the panelist who caused increment of the variation was excluded from the calculation.

According to intensity of perception; the term "high" can be used when the median of the attribute concerned was higher than 6, the term "medium " can be used when the median of the attribute concerned was between 3 and 6, the term "light " can be used when the median of attribute was less than 3 (Santis and Frangipane, 2015).

According to European Regulation EC 1989/03 the oil is graded by comparing the median value of the defects and the median value of the fruity attribute (Table 5.5). After the statistical calculation, each sample was evaluated one by one, whether they were in this range or not. In order to compare HS-SPME results with sensory analysis, olive oils which had the maximum levels of these volatile compounds was indicated in Table 5.5.

Table 5.5 Median values of defects and fruity aroma
(Source: Santis and Frangipane, 2015)

Median of defects	**Median of fruity aroma**	**Olive oil grade**
0	>0	Extra Virgin
>0≤3.5	>0	Virgin
>3.5	>0	Lampante
≤3.5	0	Lampante

Sensory description of volatile compounds including positive and negative attributes were listed in Table 5.6 (Luna et al., 2006, Temime et al., 2006, Serio et al., 2014, Kesen et al., 2014, Kesen et al., 2013a, García-González et al., 2008,Vossen, 2007).

Table 5.6. Sensory description of volatile compounds and maximum levels for olive oils

Volatile compounds	Sensory description	Max Level
Ethanol	alcoholic, winey-vinegary	ML47-2
2-Butanone	tomato, apple	nd*
Ethyl acetate	winey-vinegary	nd
1-Penten-3-ol	butter, soft green	AE37-2
1-Penten-3-one	sweet tomato, green, pungent, mustard	AE47-2
3-Pentanone	sweet tomato, fruity	AE47-2
Isoamyl alcohol	fusty	AL27
Trans-2-Pentenal	green apple-tomato, pungent	ME47
n-octane	solvent, rancid, winey-vinegary	AE27
Hexanal	green fruity- apple, grass	AM37
Butyl acetate	green, fruity, pungent, sweet	ME47
Cis-3-Hexen-1-ol	green fruity- grassy, banana, fresh	AE47
Trans -2-Hexen-1-al	apple, green, almond, bitter, astringent	MM37-2
Trans -2-Hexen-1-ol	green, grassy, astringent	MM27
1-Hexanol	green tomato, soft, aromatic, alcoholic	MM27
2-Heptanone	green tomato	nd
Heptanal	fatty, citrus, rancid	nd
Trans-2-Heptenal	chemical, fatty	ME37-2
Octanal	fatty, sharp, citrus-like	MM37-2
Cis -3-Hexenyl acetate	green, banana like	MM37-2
Hexyl acetate	fruit, herb, green fruity-grassy	MM37-2
(R)-(+)-Limonene	citrus, lemon, orange	ME37-2
1-Octanol	floral, grassy	MM37
Terpinolene	floral, light	nd
2-Nonanone	fruity, floral	MM37
Nonanal	soapy, fatty, citrus, green	MM47
Trans -2-nonenal	paper like, fatty, undesirable	MM37
Trans, trans-2,4-Nonadienal	fatty	nd
Trans -2-Decenal	rancid	MM37

*nd; not detected

As can be seen in the Table 5.7, according to median of attributes early harvest Ayvalık olive oils (samples of AE and AE-2) were classified in two categories: extra virgin olive oil and virgin olive oil. Their median of defect was equal to 0, except AE27 sample. These defects of this olive oil was described as musty-humid-earthy. This defects are related with unsuitable olive storage conditions and storage time (Angerosa, 2002). For this reason, this defect, makes hard to comment on the effects of olive variety, harvest time and malaxation temperature on sensorial characteristics. The aroma compounds of olive oils from unripe fruits usually are characterised by grassy or

leafy attributes (Kalua et al., 2007). As a result of HS-SPME analysis, (R)-(+)-limonene, 1-penten-3-ol, 3-pentanone, and cis-3-hexen-1-ol were effective for the separation of early-harvested Ayvalık olive oil. AE27, which had defect, did not have (R)-(+)-limonene compounds. As shown in Table 5.7, volatile compounds contribute to positive attributes and findings support each other.

Table 5.7. Grade assessment with median of positive and negative attributes

Sample	**Fruitiness Median (Mf)**	**Defects Median (Md)**	**Oil Grade**
AE27	1.5	2	Virgin
AE37	3	0	Extra Virgin
AE47	2	0	Extra Virgin
AM27	1.5	0	Extra Virgin
AM37	2.5	0	Extra Virgin
AM47	2	0	Extra Virgin
AL27	0	3.5	Lampante
AL37	1	2	Virgin
AL47	0	2.5	Lampante
ME27	3	2	Virgin
ME37	3.5	1.5	Virgin
ME47	4	0	Extra virgin
MM27	3	0	Extra virgin
MM37	3	1.75	Virgin
MM47	3.4	0	Extra virgin
ML27	0	3.5	Lampante
ML37	1.75	2.5	Virgin
ML47	1	3	Virgin
AE27-2	2.5	0	Extra Virgin
AE37-2	2.1	0	Extra Virgin
AE47-2	2.5	0	Extra Virgin
AM27-2	2	0	Extra Virgin
AM37-2	2.75	0	Extra Virgin
AM47-2	2	2	Virgin
AL27-2	2	2	Virgin
AL37-2	0	3	Lampante
AL47-2	1.25	2.5	Virgin
ME27-2	3	0	Extra virgin
ME37-2	2.5	0	Extra virgin
ME47-2	3.5	0	Extra virgin
MM27-2	3.5	0.75	Virgin
MM37-2	3	2.5	Virgin
MM47-2	3	0	Extra virgin
ML27-2	1.5	2	Virgin
ML37-2	2.2	2	Virgin
ML47-2	0	2.5	Lampante

As can be seen in the Table 5.7, according to median of attributes middle harvest olive oils (samples of AM and AM-2) were classified in two categories: extra virgin olive oil and virgin olive oil. Their median of defect was equal to 0, except AM47-2 sample. The defect of this olive oil was described as frostbitten olives (wet wood). This defect is characteristic flavor of oils extracted from olives which have been injured by frost while on the tree (IOC 2015c).. As a result of HS-SPME analysis, hexanal (green fruity- apple, grass) and trans-2-hexenal (apple, green, almond, bitter, astringent) contents of Ayvalık olive oils were found at the highest level at the stage of mid-harvest time. Furthermore, 3-pentanone (Sweet tomato, fruity) and (R)-(+)-limonene (citrus, lemon, orange) were not found in middle harvest olive oils for Ayvalık cultivar. For this reason, these compounds did not contribute to fruity attributes. Cis-3-hexen-1-ol, hexyl acetate and cis-3-hexenyl acetate, that contribute to green attributes, decreased at the stage of middle harvest time.

As shown in the Table 5.7, according to median of attributes for late harvest olive oils (samples of AL and AL-2) were classified in two categories: lampante olive oil and virgin olive oil. Their median of defects were between 2-3.5. According to sensory description, the common traits of all late harvest olive oils were defects. The defect of this olive oil was described as fusty-muddy sediment. Fusty were related with unsuitable olive storage conditions and time and muddy sediment were related with unsuitable oil storage conditions (Angerosa, 2002). However, all kinds of olive oils were stored under the same conditions and the same time. For this reason, it may be considered that these defects have revealed, as a result of harvest time. According to results of HS-SPME analysis, octanal (fatty, sharp, citrus-like) and nonanal (soapy, fatty, citrus, green) were high at the last stage of ripening time for Ayvalık olive oil. While (R)-(+)-limonene (citrus, lemon, orange) was not detected, hexanal and trans-2-hexenal decreased at this stage.

In this research Ayvalık olive oil samples were characterized as medium or light for all positive attributes. In Figure 5.16, the effects of ripening stages and malaxation temperature on the sensory characteristics of Ayvalık olive oil were demonstrated according to predominant positive attributes and negative attributes. As can be seen in Figure 5.16, defects were higher at the last stage of ripening time. Fruity attributes were really lower when compared with other harvest times. Otherwise, Ayvalık olive oils were not characterized by different malaxation temperatures.

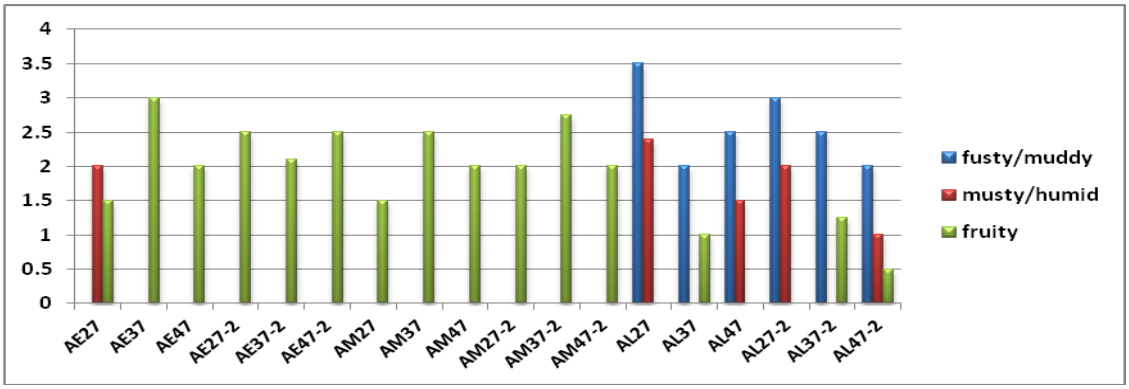


Figure 5.16. Effect of ripening stages and malaxation temperature on the sensory characteristics of Ayvalık olive oil

As indicated in Table 5.7, according to median of attributes early harvest Memecik olive oils (samples of ME and ME-2) were classified in two categories: extra virgin olive oil and virgin olive oil. Their median of defect was equal to 0, except ME27 and ME37 samples. The defects of this olive oils were described as fusty-muddy sediment for ME27 and musty-humid-earthy for ME37. As a result of HS-SPME analysis, Memecik early harvest olive oils were characterized by 1-hexanol (green tomato, soft, aromatic), trans-2-hexen-1-ol (green, grassy, astringent) and 2-nonanone (fruity, floral). 1-hexanol, trans-2-hexen-1-ol and trans-2-hexenal (apple, green, almond, bitter, astringent) contents were high when compared to Ayvalık type of olive oil. Furthermore, median of fruity attributes of Memecik type of olive oils were higher. Besides that, bitter and pungent attributes were high in Memecik type of olive oil according to sensory analysis, when compared to Ayvalık types. But the main and high positive attributes were shown in Table 5.7.

According to median of attributes middle harvest olive oils (samples of MM and MM-2) were classified in two categories: extra virgin olive oil and virgin olive oil (Table 5.7). MM37, MM37-2 and MM27-2 had defects. Their defects were related with both fusty-muddy sediment and musty-humid-earthy. As a result of HS-SPME analysis, Memecik middle harvest olive oils were characterized by many volatile compounds. The content of 1-octanol (floral, grassy), nonanal (soapy, fatty, citrus, green), octanal (fatty, sharp, citrus-like), trans-2-hexenal (apple, green, almond, bitter, astringent), hexyl acetate (fruit, herb, green fruity-grassy) and cis-3-hexenyl acetate (green, banana like) increased when the ripening period was middle harvest time. As shown in Table 5.7, volatile compounds contribute to positive attributes and findings support each

other. On the other hand, sensory analysis results of Memecik early and middle harvest times were compared to each other, there were no differences between them.

According to median of attributes late harvest olive oils (samples of ML and ML-2) were classified in two categories: lampante olive oil and virgin olive oil (Table 5.7). Their median of defects were between 2-3.5. The common traits of all late-harvest Memecik olive oils had high defects. These defects of this olive oil were described as fusty-muddy sediment and musty-humid-earty. This defects were related with unsuitable olive and olive oil storage conditions and storage time (Angerosa, 2002). However, as previously described, all kinds of olive oils were stored under the same conditions and the same period of storage time. So these defects may be related with the harvest time. As a result of HS-SPME analysis, many volatile compounds decreased at the last stage of ripening time, especially hexanal (green fruity- apple, grass). Depending on the reduction of the this compounds, defects may become more predominant.

In this study Memecik olive oil samples were characterized as medium or light for all positive attributes, such as Ayvalık olive oil. In Figure 5.17, the effects of ripening stages and malaxation temperatures on the sensory characteristics of Memecik olive oil were shown in radar chart according to predominant positive attributes and negative attributes. As can be seen in Figure 5.17, defects were higher at the last stage of ripening time and black color was dominant. Fruity attributes were higher at middle and early stages and green color was noticeable. On the other hand, there was no linear interaction between temperature variables.

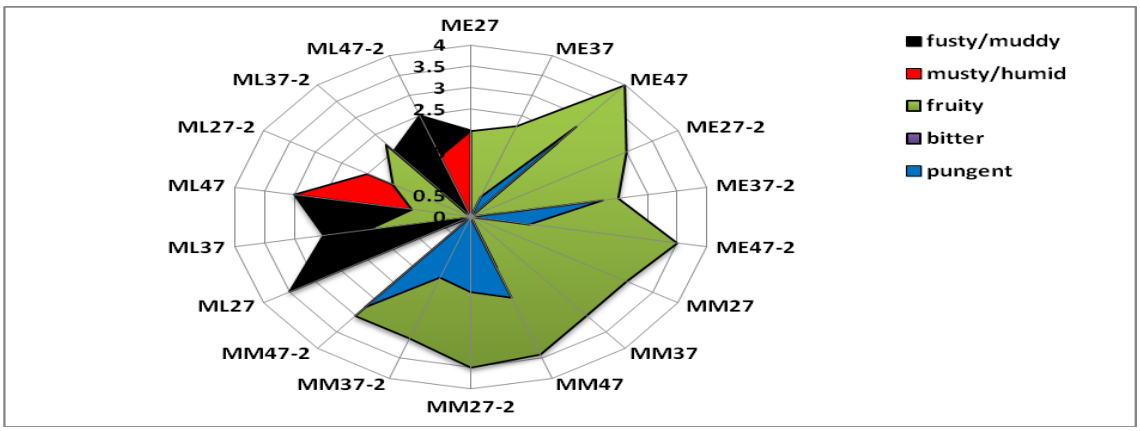


Figure 5.17. Radar plot showing effect of ripening stages and malaxation temperature on the sensory characteristics of Memecik olive oil

5.4. Color Analysis

The results of olive oil colors were expressed as L*, a*, b*, c* and h values (Table 5.8). The actual color is represented by hue angle and c* values (color saturation) (Seleem, 2015, Suseno, et al., 2012).

- L* values measure for lightness; L = 100 for lightness, and L = 0 for darkness
- a* values measure for greenness (−) and redness (+)
- b* values measure for blue (−) and yellow (+)
- h; hue angle were 0˚ = red to purple, 90˚ = yellow, 180˚ = bluish to green and 270˚ = blue
- c* values for color saturation- the intensity of the color

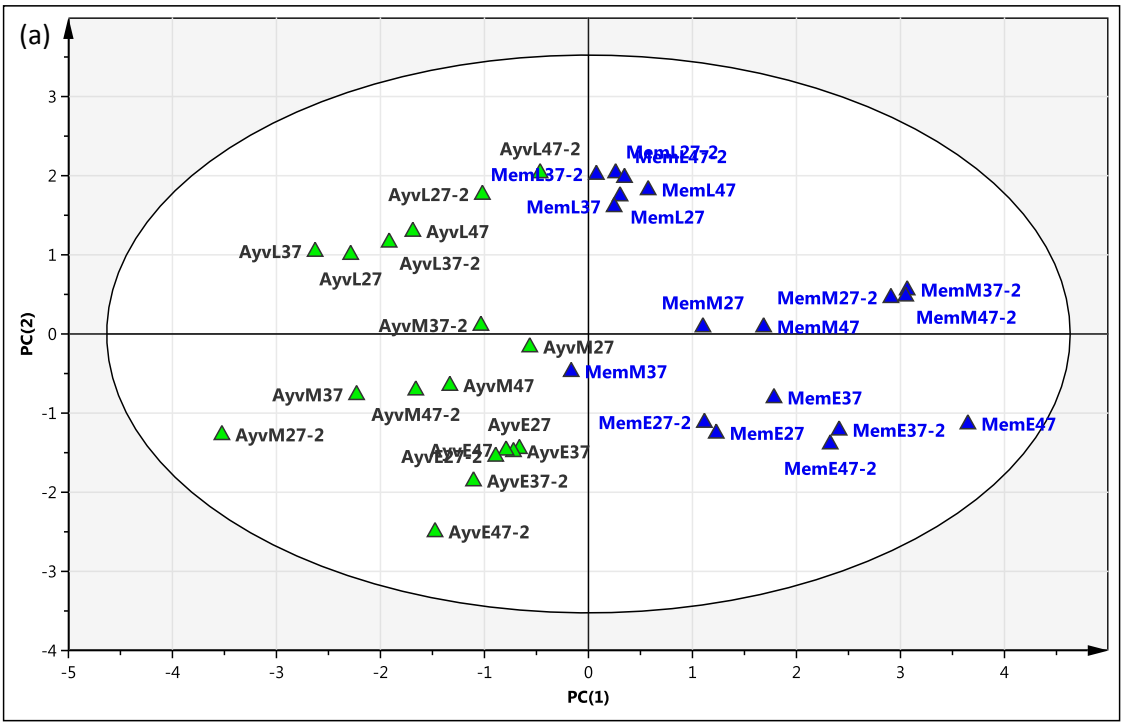
The highest L* values were achieved between 46.10-49.76 in Ayvalık olive oils. The lowest L* values were achieved between 43.16-48.19 in Memecik olive oils. For this reason, Memecik olive oils were darker (lower L values) than Ayvalık olive oils. Furthermore L* values usually increased when the ripening periods increased, especially for the last stage of olive oils. The c* values (the intensity of the color) were obtained between 23.79-36.17 for Ayvalık olive oils and 29.92-46.24 for Memecik olive oils. Therefore, color of Memecik olive oils were more saturated. The highest h values were taken between 100.33-102.66 in Ayvalık olive oils. The lowest h values were taken between 96.73-99.99 in Memecik olive oils. All olive oils had a hue angle higher than 90˚. Furthermore, with regard to negative a* values were similar for Ayvalık and Memecik type of olive oils. The highest b* values (yellowness) were achieved between 36.31-47.11 in Memecik olive oils. The lowest b* values were achieved between 25.03-35.57 in Ayvalık olive oil.

Table 5.8. Color measurement of olive oils

Cultivar	Parameters				
	L*	c*	h	a*	b*
AE27	46.10	31.33	100.35	-5.91	32.85
AE37	46.36	33.44	100.97	-5.79	32.93
AE47	46.96	32.39	100.30	-5.79	31.87
AM27	47.15	34.04	100.33	-6.10	33.48
AM37	48.12	28.12	101.57	-5.64	27.55
AM47	47.16	30.36	100.71	-5.64	29.84
AL27	47.79	27.97	101.81	-5.72	27.37
AL37	48.87	27.64	102.02	-5.75	27.03
AL47	47.57	30.34	101.40	-6.00	29.74
ME27	43.63	37.49	98.95	-5.83	37.03
ME37	46.04	41.53	98.19	-5.91	41.11
ME47	43.54	45.28	96.29	-4.96	45.01
MM27	44.94	38.61	99.18	-6.16	38.12
MM37	45.57	29.92	99.85	-5.63	36.39
MM47	45.42	40.55	98.28	-5.84	40.12
ML27	47.20	37.67	99.76	-5.91	37.12
ML37	46.71	36.92	99.80	-5.78	36.31
ML47	47.78	37.76	98.69	-5.89	37.20
AE27-2	46.81	31.86	100.40	-5.75	31.34
AE37-2	46.24	30.01	100.39	-5.41	29.52
AE47-2	44.65	26.38	100.53	-4.82	25.94
AM27-2	48.91	23.79	102.66	-5.21	23.21
AM37-2	49.76	34.55	100.52	-6.31	33.96
AM47-2	47.24	29.39	101.09	-5.65	28.84
AL27-2	48.35	33.80	100.84	-6.35	33.20
AL37-2	47.64	29.33	101.52	-5.86	28.74
AL47-2	48.44	36.17	100.42	-6.55	35.57
ME27-2	45.63	38.34	98.67	-5.78	37.91
ME37-2	44.74	41.56	97.19	-5.20	41.23
ME47-2	43.16	40.06	97.50	-5.23	39.72
MM27-2	46.34	46.06	97.18	-5.73	45.70
MM37-2	47.74	47.43	96.73	-5.56	47.11
MM47-2	45.69	46.24	97.22	-5.81	45.87
ML27-2	48.69	39.43	99.37	-6.20	36.82
ML37-2	48.63	38.44	99.99	-6.19	37.84
ML47-2	47.39	37.85	99.56	-6.28	37.32

A two-component PLS-DA model with R2X = 0.68, R2Y = 0.72, Q2 = 0.68 was built by color data to see discrimination of olive oils according to cultivar and harves time. As shown in Figure 5.18, these plots show that the effect of the cultivar were predominant in the discrimination of oil samples. Oil samples from different cultivars were distinguished by PC1. PC1 indicated that, while the color of Ayvalık olive oil had a higher proportion of L* and hue vales, the color of Memecik olive oil had a higher

proportion of c* and b* values. Furthermore, Memecik olive oils were discriminated by harvest time. While late harvested Ayvalık olive oils distinguished from the other types of Ayvalık olive oils, there was no a clear separation in the other harvest times.



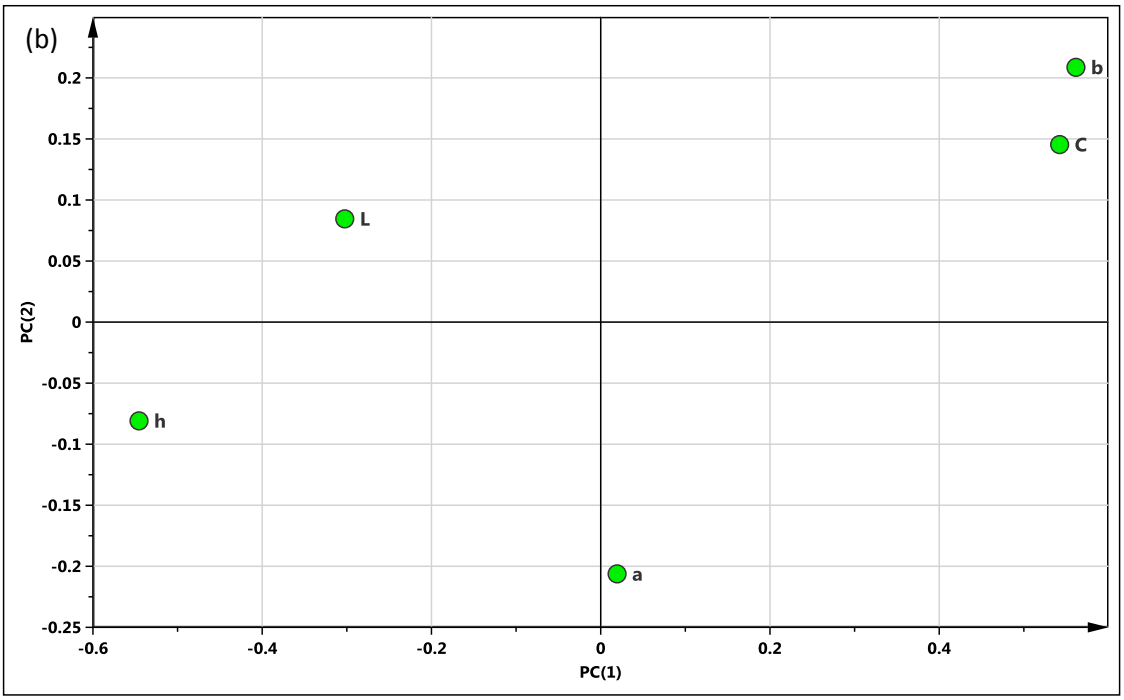


Figure 5.18. PLS-DA score plot (a) and loading plot (b) based on results of color

CHAPTER 6

CONCLUSION

In this study, volatile compounds, sensory properties and color of extracted olive oils obtained from different olive varieties, at different harvest times and malaxation temperature were investigated by using HS-SPME, zNose^{TM}, taste panel and colorimeter. Consequently, the results taken from HS-SPME and zNose^{TM}, indicate that both harvest time and types of olives strongly influenced the volatile production of olive oils. Furthermore, the electronic nose has a potential to be used as a rapid technique to detect harvest time and cultivar of olives. According to results of sensory analysis, properties of olive oils were mostly affected by harvest time. According to color analysis, olive oils were affected by cultivar as far as harvest time. Amongst the various factors that influenced the olive oil volatile composition, the harvest time was found to be the most effective. On the other hand, there was no evidence to suggest that malaxation temperatures could affect the investigated properties of olive oils.

According to results of HS-SPME and zNose^{TM}, various olive oil aroma compounds accumulated differently based on the cultivar and harvest time of olive oils. 1-hexanol, trans-2-hexen-1-ol and 2-nonanone were effective for discrimination of early harvest Memecik olive oils. Middle-harvested Memecik olive oils clustered together from other types of olive oils through these volatiles; trans-2-nonenal, trans-2-heptenal, 1-octanol, nonanal, octanal, trans-2-hexenal, cis-3-hexenyl acetate and hexyl acetate. (R)-(+)-limonene, 1-penten-3-ol, 3-pentanone, and cis-3-hexen-1-ol were effective for discrimination of early harvest Ayvalık olive oils. On the other hand, late harvest olive oils gave less of volatile compounds compared to early and middle harvest olive oils. Totally, volatile compounds of Memecik olive oils were determined higher than volatile compounds of Ayvalık olive oils.

According to the results of sensory attributes, Ayvalık and Memecik types of olive oils were characterized as medium or light for all positive attributes. Defects of olive oils were higher at the last stage of ripening time for two cultivars. Generally, fruity attributes were higher at middle and early stages. The color measurements showed that, cultivar was an effective tool for discrimination oil samples. While the

color of Ayvalık olive oils had higher proportion values of lightness and hue values, the color of Memecik olive oils had higher proportion of values of color saturation and yellowness.

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APPENDIX A

LIST OF DATAS TAKEN FROM GC/MS AND ELECTRONIC NOSE ANALYSIS

Table A.1. Volatile contents (ppm) produced from other ways exclusive of lipoxygenase pathway

CODE	Isoamyl alcohol	n-octane	Butyl acetate	Ethanol	Trans -2-Decenal
AE27	0.000	0.568	0.000	0.155	1.153
AE37	0.000	0.012	0.000	0.131	0.862
AE47	0.001	0.065	0.000	0.119	0.687
AM27	0.073	0.064	0.000	0.453	0.785
AM37	0.000	0.000	0.000	0.359	0.000
AM47	0.273	0.017	0.000	0.151	0.682
AL27	0.570	0.012	0.000	0.667	0.478
AL37	0.075	0.029	0.000	0.988	0.000
AL47	0.016	0.018	0.000	1.813	0.079
ME27	0.474	0.274	0.000	0.558	1.415
ME37	0.043	0.051	0.000	0.978	0.000
ME47	0.003	0.163	0.171	0.266	0.000
MM27	0.000	0.168	0.000	0.473	1.101
MM37	0.184	0.098	0.000	0.121	1.815
MM47	0.180	0.147	0.053	0.875	0.042
ML27	0,413	0,474	0,017	1,354	0,270
ML37	0,100	0,063	0,000	0,549	0,000
ML47	0,113	0,068	0,000	0,527	0,000
AE27-2	0,000	0,048	0,000	0,563	0,906
AE37-2	0,000	0,022	0,000	1,356	0,832
AE47-2	0,043	0,036	0,000	0,855	0,879
AM27-2	0,000	0,000	0,000	0,495	0,199
AM37-2	0,000	0,000	0,000	0,445	0,875
AM47-2	0,037	0,106	0,000	0,481	0,391
AL27-2	0,034	0.046	0.000	0.315	0.000
AL37-2	0.520	0.118	0.015	0.305	1.151
AL47-2	0.000	0.001	0.000	0.245	0.072
ME27-2	0.007	0.112	0.000	0.000	0.000
ME37-2	0.000	0.050	0.000	0.808	0.000
ME47-2	0.000	0.000	0.000	0.181	0.000
MM27-2	0.000	0.008	0.000	0.261	1.152
MM37-2	0.166	0.101	0.034	0.371	0.274
MM47-2	0.082	0.031	0.000	0.620	0.000
ML27-2	0.000	0.033	0.000	0.250	0.000
ML37-2	0.118	0.000	0.000	0.497	0.000
ML47-2	0.138	0.121	0.007	1.976	0.029

Table A.2. The electronic nose data of the 36 type of olive oils based on peaks of zNose™

Sample Code	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19
AE27	221.250	29.025	41.600	83.863	40.571	61.983	32.363	354.250	49.600	269.850	70.125	141.550	125.788	33.150	93.963	95.050	113.888	39.208	0.000
AE37	253.850	27.208	38.004	68.575	38.450	73.858	38.400	528.750	39.013	89.700	38.863	133.250	159.500	25.250	62.063	62.500	146.750	28.208	0.000
AE47	173.650	27.104	35.438	57.317	45.292	132.271	69.000	484.500	37.350	95.500	40.271	120.450	151.500	23.222	52.583	69.354	165.129	33.375	0.000
AM27	236.133	0.000	48.200	200.533	45.333	53.889	0.000	405.800	109.000	55.067	28.900	117.667	70.233	0.000	76.833	36.667	245.600	25.389	42.944
AM37	359.050	20.000	58.208	224.750	37.821	53.375	0.000	480.200	32.392	81.100	33.625	138.900	47.792	24.333	46.583	51.938	214.350	24.222	30.438
AM47	262.800	22.500	45.300	179.450	43.792	34.938	0.000	368.000	97.900	48.613	25.333	80.613	31.563	0.000	26.500	33.938	227.517	32.667	29.667
AL27	61.200	27.000	58.063	324.800	28.000	30.896	0.000	351.750	0.000	0.000	25.306	81.850	31.500	0.000	223.450	68.833	182.229	38.208	39.625
AL37	62.600	22.333	59.425	443.850	30.208	26.771	0.000	377.000	47.750	0.000	29.500	45.050	25.500	0.000	113.125	34.667	117.979	27.000	0.000
AL47	64.925	30.917	58.100	279.600	37.063	30.833	0.000	235.800	32.550	0.000	23.729	129.950	43.125	21.500	124.025	87.896	147.075	29.333	32.938
ME27	133.050	27.833	95.450	45.104	28.883	59.063	20.000	1155.100	726.350	0.000	45.504	77.083	33.983	25.000	27.375	52.267	227.500	48.250	22.000
ME37	109.950	33.292	70.900	44.688	43.513	58.633	20.000	1675.550	408.800	22.500	74.163	121.150	51.050	21.833	31.250	96.325	270.925	27.375	93.375
ME47	137.658	28.375	74.408	35.567	47.000	46.775	22.000	1570.650	263.717	0.000	46.104	130.800	54.125	24.500	41.004	68.083	202.575	38.875	115.229
MM27	64.450	91.650	81.100	59.888	32.158	30.304	0.000	1417.300	114.850	0.000	43.667	82.538	29.292	24.000	55.313	146.042	103.900	33.833	72.688
MM37	93.367	84.000	79.500	84.833	24.361	36.292	0.000	1147.200	298.375	22.000	58.000	136.708	59.833	0.000	43.833	124.642	126.175	34.750	31.708
MM47	72.917	118.250	104.058	26.563	29.521	34.438	0.000	814.100	202.967	0.000	34.625	62.117	28.833	0.000	37.833	137.417	120.729	29.667	91.242
ML27	126.800	37.854	51.725	35.583	27.875	60.217	0.000	504.050	42.550	21.000	26.875	81.288	28.917	0.000	187.150	31.375	115.000	25.604	74.796
ML37	81.000	25.250	52.500	44.838	27.854	49.229	0.000	857.550	141.450	23.125	45.146	87.050	40.125	0.000	207.388	35.542	163.246	22.375	139.200
ML47	133.100	0.000	78.100	34.750	32.958	49.438	0.000	872.200	210.900	31.000	42.233	71.100	22.500	0.000	262.200	30.000	181.500	32.375	134.775
AE27-2	235.300	28.958	39.213	72.450	30.729	59.383	40.975	443.650	40.125	119.550	72.167	113.150	132.792	25.000	55.321	92.750	131.600	30.992	0.000
AE37-2	208.000	25.250	39.713	50.583	34.229	59.792	34.000	371.000	70.550	96.663	31.333	96.350	135.317	24.667	69.167	65.833	140.917	38.583	0.000
AE47-2	225.800	23.667	35.396	65.625	44.313	108.188	27.396	510.600	68.750	88.950	47.875	152.000	154.717	25.500	82.800	79.400	182.483	27.333	0.000
AM27-2	422.900	0.000	32.871	248.150	32.333	38.229	0.000	557.400	82.775	55.067	23.667	123.025	32.542	0.000	29.500	23.667	243.063	24.000	32.313
AM37-2	285.650	24.000	43.775	265.700	50.833	39.492	0.000	487.250	51.700	81.100	22.000	94.400	28.833	0.000	26.750	27.750	288.300	23.750	25.167
AM47-2	350.350	19.325	49.650	228.692	55.583	34.821	0.000	471.208	53.042	48.613	0.000	164.700	47.208	21.000	51.213	36.200	260.050	21.667	60.542
AL27-2	58.050	30.550	53.200	339.108	34.750	30.063	0.000	534.450	68.050	0.000	0.000	96.488	33.958	0.000	232.400	69.479	121.800	30.750	130.796
AL37-2	36.950	29.675	63.450	389.300	31.038	27.667	0.000	410.000	24.500	0.000	0.000	112.425	31.708	0.000	97.183	55.063	142.350	25.833	37.667
AL47-2	48.875	30.583	56.638	385.383	29.792	27.667	0.000	280.050	37.613	0.000	0.000	87.888	26.778	0.000	140.083	40.063	130.979	25.667	29.167
ME27-2	141.150	27.333	75.300	31.333	41.542	56.375	23.500	1148.200	499.800	0.000	38.250	119.650	39.438	22.000	28.292	42.750	211.013	30.521	80.117
ME37-2	142.850	28.000	88.063	29.375	32.979	48.717	23.250	1495.300	264.000	0.000	36.044	87.338	35.167	23.000	29.583	29.500	279.300	25.667	44.154
ME47-2	126.150	28.000	69.313	28.125	27.333	42.542	26.000	1537.450	322.350	0.000	30.667	72.979	32.625	21.000	27.313	25.375	242.563	28.000	92.100
MM27-2	103.675	78.975	75.900	37.350	33.875	32.292	0.000	1145.717	136.550	0.000	20.000	64.717	27.750	0.000	48.479	141.250	139.700	25.667	83.183
MM37-2	133.250	137.700	92.250	60.688	27.917	38.713	22.000	1543.100	236.500	0.000	52.263	75.900	48.417	0.000	49.438	134.667	135.000	29.833	139.450
MM47-2	104.950	92.388	107.150	28.125	26.275	37.692	0.000	954.200	147.800	0.000	31.938	52.075	26.167	0.000	36.692	141.083	117.625	23.500	100.238
ML27-2	96.800	0.000	53.650	41.729	25.750	52.000	0.000	519.400	58.600	0.000	31.079	70.413	36.000	0.000	165.550	27.125	104.542	22.000	79.538
ML37-2	86.600	25.000	42.733	32.667	30.313	53.833	0.000	614.000	68.900	0.000	26.771	59.879	23.333	0.000	191.404	25.000	111.850	27.000	91.413
ML47-2	88.750	22.000	68.413	29.396	27.208	71.542	0.000	631.033	118.175	0.000	29.250	59.117	24.556	0.000	199.242	25.500	96.246	24.333	72.050