CLASSIFICATION OF TURKISH VIRGIN OLIVE OILS BASED ON THEIR PHENOLIC PROFILES

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ABSTRACT

CLASSIFICATION OF TURKISH VIRGIN OLIVE OILS BASED ON THEIR PHENOLIC PROFILES

Virgin olive oil is different from other plant oils with its high phenolic content. The resistance to oxidation and the protection against some diseases has been linked to these components of olive oil. The sensorial characteristic of extra virgin olive oil is also related to its phenolic compounds.

In this work, it is aimed to determine the phenolic profiles of Turkish olive oils, which have high economic value for Turkey. Phenolic profiles of monovarietal extra virgin olive oil samples extracted from six dominant and economically important Turkish olive cultivars (memecik, erkence, domat, nizip-yaglik, gemlik, ayvalik) and commercial extra virgin olive oil samples from two different areas (south and north) of the Aegean coast were determined for 2005 and 2006 harvest years. Total phenol contents, oxidative stabilities and chromatic ordinates as colour parameters were also measured. The effect of cultivar, geographical area and harvest year on phenolic profiles of olive oils was investigated. Multivariate data were subjected to principal component and partial least square-discriminant analyses.

Typical phenolic substances of extra virgin olive oils from different variety and regions are; *p*-coumaric acid, cinnamic acid & apigenin for memecik, erkence oils and also for oils of south Aegean; vanillin & syringic acid for ayvalik, gemlik and also for oils of north Aegean. Domat oils were characterized by their relatively high content of oleuropein aglycon. Nizip oils were separated by their 4-hydroxyphenyl acetic acid content, which was determined in very low amounts or none in other olive oils. It was observed that harvest year strongly affected the phenolic profiles of olive oils. In addition, phenolic composition was found to be useful in discriminating the olive oils from different variety and geographical area.

ÖZET

TÜRK SIZMA ZEYTİNYAĞLARININ FENOLİK MADDELERİNE GÖRE SINIFLANDIRILMASI

Sızma zeytinyağı, içerdiği fenolik bileşikler açısından diğer bitkisel yağlardan ayrılır. Oksidasyona karşı kararlılığı ve bazı hastalıklardaki koruyucu etkileri zeytinyağının bu özelliği ile ilişkilendirilmiştir. Zeytinyağının duyusal özellikleri de fenolik yapısı ile ilgilidir. Bu çalışmada, Türkiye'nin ekonomisinde önemli bir yeri olan zeytinyağının detaylı fenolik profillerinin iki hasat sezonu için tespiti amaçlanmıştır.

Ekonomik değeri yüksek altı çeşit zeytinden (memecik, erkence, domat, nizip-yağlık, gemlik, ayvalık) elde edilen zeytinyağlarının ve aynı zamanda Tariş Zeytin ve Zeytinyağı Tarım Satış Kooperatifleri Birliği'nden sağlanan kuzey ve güney Ege bölgelerinin zeytinyağlarının 2005 ve 2006 hasat sezonları için fenolik profilleri elde edilmiştir. Aynı zamanda toplam fenol içeriği, oksidatif stabilite (peroksit değerleri) ve renk ölçümleri de yapılmıştır. Zeytin tipi, coğrafi bölge ve hasat sezonunun fenolik profil üzerine etkisi çok değişkenli istatistiksel yöntemler olan temel bileşenler analizi ve kısmi en düşük kareler-ayırtaç analizi ile incelenmiştir.

Değişik zeytinlerden ve coğrafi bölgelerden elde edilen zeytinyağlarının tipik fenolik bileşikleri şu şekilde bulunmuştur; *p*-kumarik asit, sinamik asit ve apigenin, memecik, erkence ve aynı zamanda güney Ege yağlarında; vanilin ve syringic asit, ayvalık, gemlik ve aynı zamanda kuzey Ege yağlarında daha fazla bulunmuştur. Domat yağları yüksek oleuropein aglycon içerikleri ile karakterize edilebilirler. Nizip yağları ise diğerlerine oranla daha yüksek 4-hidroksifenilasetik asit içeriği ile ayrılmaktadır. İstatistiksel analizler sonucunda hasat sezonun en etkili ayırtaç olduğu görülmüştür. Aynı zamanda değişik zeytin tiplerinden ve coğrafi bölgelerden elde edilen zeytinyağlarının fenolik bileşiklerine göre farklılıklar gösterdikleri de istatistiksel modellerle gösterilmiştir.

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF ABBREVIATIONS

ANN	Artificial neural network
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CA	Cluster analysis
CHD	Coronary hearth diseases
CIE	International Commission on Illumination
DAG	Diacylglycerols
DNA	Deoxyribonucleic acid
EVOO	Extra virgin olive oil
FFA	Free fatty acids
GA	Gallic acid
GI	Geographical indication
HCA	Hierarchical cluster analysis
HDL	High density lipoprotein
HPLC-DAD	High Performance Liquid Chromatography-Diode Array Detector
LC-MS	Liquid Chromatography-Mass Spectroscopy
LDL	Low density lipoprotein
MAG	Monoacylglycerols
MI	Maturation index
OMW	Olive mill waste
PCA	Principal component analysis
PDO	Protected Designation of Origin
PGI	Protected Geographical Indication
PLS-DA	Partial least squares-discriminant analysis

PV	Peroxide value
RSD	Relative standard deviation
SIMCA	Soft Independent Modelling Class Analogy
TAG	Triacylglycerols OPO
TPC	Total phenol content
TSG	Traditional speciality guaranteed
VIP	Variable importance
VOO	Virgin olive oil
g_a	The a th eigenvalue
Q^2	The goodness of prediction parameter
R^2	The goodness of fit parameter
t_{ia}	Score vectors

CHAPTER 1

INTRODUCTION

Olive oil is the momentous edible vegetable oil which is derived from olive fruit (*Olean europaea L.*). Olive tree is mostly cultivated in the Mediterranean region by reason of climatic necessities of olive tree. Spain, Italy, Greece, Tunisia, Turkey and Morocco are the most considerable olive producer countries. Australia, Japan, The United States, South Africa, Canada, Soviet Union and China can be counted as other countries where olive oil production has been recently increased. In recent years, olive oil has been the indispensable commodity of the Mediterranean diet and increasing popularity of olive oil has been related to its high content of mono-unsaturated fatty acids and its minor components (Tuck, et al. 2002, Visioli, et al. 2002).

The chemical composition of olive oil is composed of major and minor components. Almost 98% of the total oil weight is constituted by major components that enclose glycerols while minor components such as aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants represent 2% of the total oil weight. The fundamental antioxidants of virgin olive oil (VOO) are carotenoids and phenolic compounds, which have both lipophilic and hydrophilic properties. Tocopherols are known as lipophilics, while phenolic alcohols and acids, hydroxy-isochromans, flavonoids, secoiridoids, and lignans constitute the hydrophilic compounds (Servili, et al. 2002).

Phenolic acids with the basic chemical structure of C_6-C_1 (benzoic acids) and C_6-C_3 (cinnamic acid) are found in olive fruit. The compounds, such as caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic and *p*-hydroxybenzoic acid are the first group of phenols observed in VOO (Brenes et al., 1999, Servili et al., 2004). Hydroxytyrosol (3,4-dihydroxyphenyl-ethanol) and tyrosol (*p*-hydroxyphenyl-ethanol) are the most abundant phenolic alcohols in olives. The secoiridoids (oleuropein, demethyloleuropein, ligstroside) and the lignans (1-acetoxypinoresinol, pinoresinol) have also been isolated and characterized (Brenes, et al. 2000, Bendini, et al. 2007). Luteolin and apigenin are the flavonoid compounds of olive oil.

Phenolic compounds make important contributions to the nutritional properties, sensory characteristics, and the shelf life of olive oil. Those derived from the hydrolysis of oleuropein contribute to the intensity of the bitterness of VOO, and especially hydroxytyrosol, tyrosol, caffeic acid, coumaric acids, and *p*-hydroxybenzoic acid influence the sensory characteristics of VOO (Kiritsakis 1998). Phenolic compounds play an important role in human health because of their anti-inflammatory, antiallergic, antimicrobial, anticarcinogenic, and antiviral activities (Tripoli, et al. 2005). They prevent lipid peroxidation and oxidative modification of low density lipoprotein (LDL) by means of their antioxidant activities (Servili, et al. 2004, Ryan, et al. 1998).

The concentration and composition of phenolic compounds in VOO is strongly affected by many agronomical and technological factors, such as olive cultivar (Tura, et al. 2007), the place of cultivation (Vinha, et al. 2005), the climate, degree of maturation (Kalua, et al. 2005), crop season (Gomez-Alonso, et al. 2002), irrigation (Tovar, et al. 2001) and the production process (Ranalli, et al. 2001).

Recently, several studies have been conducted in order to emphasize the certification of the geographical origin of food products, since authenticity and quality issues can be often associated with a given geographical origin. The protected designation of origin (PDO) and Protected Geographical Indication (PGI) for agricultural products has been introduced with official European regulations, which allow the labelling of some products with the names of the geographical area of production. This designation guarantees that the quality of the product is apparently engaged to its geographical origin.

Many studies have been reported on the classification of olive oils according to their cultivars or geographical origins by means of statistical analysis applied to fatty acids and triacylglycerols (Stefanoudaki, et al. 1997), sterol compositions (Alves, et al. 2005), sensory attributes (Haddada, et al. 2007), volatile compositions (Araghipour, et al. 2008), trace elements (Benincasa, et al. 2007) and also minor components (Cerretani, et al. 2006). Olive varieties from the same geographical regions and same varieties from different geographical regions have been well classified by models based on principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Japon-Lujan, et al. 2006). Phenolic acids, hydroxytyrosol and tyrosol have been found more suitable variables than other phenolics for classification of VOO varieties by means of PCA and stepwise discriminant analyses (Gomez-Alonso, et al. 2002). In addition to this, the

effect of growing area on phenolic fractions of VOOs was studied and it was found that phenolic fractions of oils changed quantitatively with growing area and environmental conditions (Criado, et al. 2004). In a study of the influence of the extraction system, crop season and production area on the chemical composition and quality of Cornicabra VOO, the production area affected the concentrations of phenols and tocopherols (Salvador, et al. 2003). However, some authors have encountered some problems to use of phenolic compounds for classification of olive oils obtained from different cultivars because these minor components are also affected by climatic and environmental conditions, and technological process (Cerretani, et al. 2006).

To best of our knowledge, little has been published about olive oils produced in Turkey, which is in the fifth place in the olive oil production (5%) in the world and contributes to 11.3% of the world export (International Olive Council). The aim of this study was to evaluate the phenolic profiles of Turkish extra VOOs obtained from six olive varieties, which were chosen among the most dominant and economically important types for two harvest years. Moreover, to examine the influence of the geographical area, commercial extra virgin olive oils (EVOOs) from different growing areas of the Aegean coast of Turkey, namely north and south Aegean were chosen. Quantitative parameters including peroxide value (PV), total phenol content (TPC), colour, and also individual phenolic compounds of oil samples were determined, and the influence of the cultivar, geographical origin and harvest year on these parameters was studied. The relationship of phenolic profile in olive oil with the oxidative stability, TPC and colour was also examined. The classification of olive oil samples according to their phenolic profiles was performed by PCA and partial least squares-discriminant analysis (PLS-DA). The findings of this study can provide ways for the varietal authenticity of Turkish olive oil according to their phenolic profiles as the geographical indicators, therefore can be used in PDO or PGI labelling of Turkish EVOOs.

CHAPTER 2

OLIVE OIL AND PHENOLS

2.1. The Olive Plant and Olive Oil

2.1.1. History of Olive and Olive Oil

Olive oil is the major edible vegetable oil of the Mediterranean countries. Olive oil is obtained by milling and pressing the fruits of the cultivated olive tree, which was domesticated approximately 6,000 years ago in the east Mediterranean area. By late Roman times the olive cultivation and the techniques of olive oil production had spread to all parts of the Mediterranean basin, but did not expand, except in parts of Spain and North Africa (Grigg 2001).

The origin of the olive tree dates back to ancient times. Its expansion encounters with the civilizations that developed in the Mediterranean from east to west. Most fossilized olive tree leaves and remainders which are relating to Eneolithic and Bronze Age demonstrate that there were olive trees in the XII millennium B.C (Vossen 2007).

Some researchers declared that the cultivated olive tree originated in Asia Minor, between present Syria, Lebanon and Israel. Its cultivation may have started in the Phoenician colonies of the present territories of Palestine and Lebanon, much nearer to the Mediterranean, at the beginning of the Neolithic period, i.e. around the year 6000 B.C. From this origin, the olive tree outspreaded towards the West. Firstly, spread to the coasts of Egypt and the island of Crete; then, to Lybia, Greece, Sicily and southern Italy in the fourth millennium BC. Greeks and Romans extended its cultivation in the Northern Mediterranean coasts. The Phoenicians from Lebanon improved the cultivation in the South, from Libya and Tunisia to Algeria, Morocco and Spain (Harwood and Aparicio 2000, Vossen 2007).

The Romans may have introduced the tree to Provence; certainly the demand for olive oil in Italy prompted the expansion of production in the west Mediterranean,

particularly North Africa. The olive tree was transferred from Asia Minor to Greece in the year 1582 B.C. Its cultivation in Italy started in the seventh century B.C. during the kingship of Lucius Tarquinius Priscus, called "the Old", the fifth legendary king of Rome. The olive tree continued its expansion towards the Gallia (France), where it was brought by the founders of Marseille, called Phocenses, around 600 years B. C (Luchetti 2002, Grigg 2001).

The expansion of the olive tree in the New World was undertaken by the Spanish Conquistadors from the beginning of the Sixteenth Century. Firstly, the planting of this tree started in the Antilles, and afterwards in the American continent. Mexico had olive trees towards the end of the Sixteenth Century. From here, they expanded to Peru and then to Chile. Concurrently with these countries, the plant was introduced in Argentina where it adapted perfectly well in the provinces of La Rioja and Catamarca. The olive tree reached the United States, especially California, in the Eighteenth Century, when it was introduced by Fray Junípero Serra, founder of the San Diego de Alcalá mission (Vossen 2007, Kapellakis, et al. 2008).

2.1.2. World Production and Consumption of Olive Oil

Economic significance of olive oil on the world sector is considered in the light of the positive contribution on health associated with olive oil consumption. In terms of product value, production and pricing of olive oil on the world market is significantly higher than other vegetable fats and oils. In fact, price might differ depending on the country, category of oil and year. While olive oils are responsible for a great percentage of the agricultural export of Tunusia (38 percent), this percentage is 5.5 and 4.4 for Spain and Italy, respectively.

Olive oil production has been intensified in the Mediterranean basin countries: Spain, Italy, Greece, Portugal, Tunusia, Turkey, and Morocco. These seven countries alone represent 90 percent of world production. Production trend by country is increasing but especially two major producing countries are the leading players for olive oil production. Actually, the levels of yields in Italy (25%) and Spain (36%) are higher than the other producing countries. Greece, Tunusia and Turkey have 18, 8% and 5% of world production, respectively (International Olive Oil Council).

This heavy concentration of olive oil production in these countries is explained by the very demanding climatic requirements of the olive tree and the fact that virtually all olive trees are grown in a Mediterranean-type climate (Grigg 2001). It should be also mentioned that the production of olive oil in other countries, such as Australia and United States, is ascending in recent years.

The main producer countries are also the main consuming countries, such as Spain, Italy and Greece. 71 percent of world consumption is concentrated in European Union countries. Mediterranean basin countries represent 77% of world consumption. United States, Canada, Australia and Japan can also be counted among the other consuming countries (Luchetti 2002, Visioli, et al. 2002). Tunisia and Turkey which have important percentage in the world olive oil production consume less olive oil than other countries (2.7, 3.2% of the world olive oil consumption). This is related to domestic economic policies of these countries. In the mid 1990s there was a strong increase both in production and consumption. This expanding consumption of olive oil is associated with its nutritional and health properties (Harwood and Aparicio 2000).

2.1.3. Olive Processing

Olive picking, harvesting time, storage and olive processing steps are important parameters that affect significantly the sensory quality and cost of VOO. The picking carried out at the beginning of harvesting time causes bitter and pungent taste for EVOO. On the contrary, if the picking is done at the late harvesting period then olive oil will have ripe flavour and sweet taste. Generally, olive fruits are picked from the tree by hand or mechanical devices. It is recommended that olives should be picked by hand from the trees for good quality and the olives should be taken to the oil mill for processing without delay. In order to keep away olives from any contamination and damages because of the foreign material during the extraction process, leaf and peduncle of fruit should be removed and washing is necessary (Di Giovacchino, et al.2002, Harwood and Aparicio 2000).

Both the traditional discontinuous pressing and the continuous centrifuging processes and percolation system in traditional mills or in modern units are used in order to obtain EVOO from the olives.

In the pressing method, pressure is applied to olives so that the separation of the liquid phases from the solid phase is confirmed. The first step of the pressing operation is crushing of olives by a millstone. Crushing provides separation of the greatest part of the oil content from the vacuoles of the olive mesocarp cells. Following the crushing operation, the mixing of the olive paste is carried out in semi-spherical or semi-cylindrical mixers (made of stainless steel) at ambient temperature. The mixing time and the temperature of olive paste should be 20-30 minutes and 22-25 $°C$, respectively. Because of the fact that the natural volatile compounds are produced during the crushing and malaxation steps, these operations have great importance related to priced aroma of olive oil (Angerosa, et al. 2004). On the other hand, an increase of the malaxing time is an effective reason for a decrease in the total polyphenol content of oils because of the oxidation during the mixing step (Di Giovacchino, et al. 2002). Next step to extract olive oil is paste application on mats. Three or five mats with olive paste are placed between two metallic discs. This operation permits the separation of olive oil and vegetation water from the pomace. At the end of the pressing method, VOO separate from the other phases of the olive paste, either vegetable water or pomace, by means of the centrifugal force. The advantages of this method include the use of simple, reliable machinery and little initial investment; the low energy requirement; a resulting pomace that is low in moisture/liquid content and precious little oil is lost to the water component. The disadvantages include a high labour intensity and the production is not continuous (Harwood and Aparicio, 2000).

The centrifugation method is a continuous or on-line process that is able to separate olive oil from the other phases of the olive paste, either liquid or solid, by means of the centrifugal force. Centrifugal force moves the solid materials to the outside. Water layer is formed in the middle whereas oil layer on the inside. For centrifugation method, crushing operation is carried out generally by a metallic crusher instead of a millstone crusher. This crushing method produces VOOs with a higher content of polyphenols and more bitter taste when it is compared with millstone crushing method. On the contrary, higher content of volatile compounds are obtained by millstone crushing method (Di Giovacchino, et al. 2002). Extraction can be carried out by two- and three-phase decanter. In a three-phase system, process requires the addition of warm water in order to get the paste to flow through the decanter. This washing causes loss of some of the flavour and polyphenols. This process is able to separate

olive oil from the other phases of the olive paste, vegetable water and pomace. Two-phase differs by operating without adding any water, so there is better retention of polyphenols. Two-phase extracted oils have green flavour, bitterness, pungency, and higher levels of fruitiness. This process produces a semi-solid cake of pressed olive fruits and no wastewater compared with the three-phase system (Vossen 2007).

The main advantages of centrifugal processing systems are:

1. Limited labour is needed, since the process is continuous and automated;
2. Stainless steel materials are always used and thus the oil is well protected from contamination;
3. Since no diaphragms are used, the risk of contaminating the oil is eliminated; and
4. Better yield performance, as most of the oil is collected.

The main disadvantages of centrifugal processing systems are:

1. Water and energy demanding, while a significant amount of phenols (natural antioxidants) are lost during the centrifuge process in olive mill waste (OMW).
2. The olive pomace contains a high percentage of moisture
3. Increased production of OMW, which is approximately 50% more than the pressure process (Kapellakis, et al. 2007, Harwood and Aparicio 2000).

Alternatively, the other extraction method is percolation method. Percolation method is based on the difference of the surface tension between oil and vegetation water. Percolation is carried out at ambient temperature and diluting water and mats are not used. Percolation incorporates the use of a metal plate dipped into the mixed paste which in theory becomes wetted with oil, and not with oil mixed with water, when withdrawn. The oil then drips off the plate. The disadvantage of this process is that it is inefficient because the wet pomace contains a great deal of olive oil. That is why the percolation process is usually combined with another process such as pressing or centrifugation. The high initial cost and energy requirements, the resulting wet pomace and a high amount of remaining olive oil still attached to water make this procedure less than ideal (Ranalli 2001, Harwood and Aparicio 2000).

The phenol content of oils is significantly affected by the extraction systems. Phenolic contents of VOO extracted by the three-phase centrifugation are lower than that of oil extracted with either pressure or percolation systems. This occurs because the

centrifugation system requires the addition of warm water to the olive paste. Therefore, larger amounts of phenols are eliminated with water wastes (Di Giovacchino, et al. 2002).

2.1.4. Regulations and Definition of Olive Oils

Protected designation of origin (PDO), protected geographical indication (PGI) and traditional speciality guaranteed (TSG) are geographical indications (GIs) defined in European Union Law to protect the names of regional foods. This law provides the protection of the reputation of the regional foods and prevention from the mean competition and misleading of consumers by virtual products which can be poor quality or different flavour. A geographical indication is a name or sign that reflects the certain products in a specific geographical location or origin (town, region, or country). The labelling of VOOs with their geographical area of production is provided by European legislation. These indications ensure that high quality parameters of olive oil are apparently engaged to its geographical origin (E.C. European Community, Regulation 2081, 1992).

According to the International Olive Oil Council and Turkish Food Codex, the designation and categorization of olive oils and olive–pomace oils are explained below. Free acidity is expressed as % oleic acid.

Extra virgin olive oil: virgin olive oil which has a free acidity, of not more than 0.8 grams per 100 grams.

Virgin olive oil: virgin olive oil which has a free acidity, of not more than 2 grams per 100 grams.

Ordinary virgin olive oil: virgin olive oil which has a free acidity, of not more than 3.3 grams per 100 grams.

Lampante is olive oil not used for consumption which has a free acidity, of more than 3.3 grams per 100. It is intended for refining or for technical use.

Refined olive oil is the olive oil obtained from virgin olive oils by refining methods which do not lead to alterations in the initial glyceridic structure. It has a free acidity, of not more than 0.3 grams per 100 grams.

Riviera olive oil is the oil which is a blend of refined olive oil and virgin olive oils. It has a free acidity, of not more than 1 gram per 100 grams.

Olive-pomace oil is the oil obtained by treating olive pomace with solvents or other physical treatments, to the exclusion of oils obtained by reesterification processes and of any mixture with oils of other kinds. It is marketed in accordance with the following designations and definitions:

Crude olive-pomace oil is olive pomace oil that 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 which do not lead to alterations in the initial glyceridic structure. It has a free acidity, of not more than 0.3 grams per 100 grams.

Olive pomace oil is the oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity of not more than 1 gram per 100 grams.

EVOO oxidative deterioration is supported by exposure to light, contact with air, high temperature (more than $30\,°C$) and high contents of metals. In order to avoid oxidation, containers should be filled to the brim, hermetically closed and stored in the darkness.

2.1.5. Chemical and Organoleptic Composition of Olive and Olive Oil

The olive fruit is a drupe, oval in shape and composed of two basic parts; the pericarp and the endocarp (the pit or kernel). The pericarp is composed of the epicarp (skin) and the mesocarp (pulp). The pericarp contains 96% to 98% of the total amount of oil, with the remaining 2% to 4% in the kernel (Hashim, et al. 2005).

Olive oil can be divided into major and minor fractions with regard to its chemical composition. The major components that include triacylglycerols (TAG) and the group of glyceridic compounds made up of free fatty acids (FFA) and mono- (MAG) and diacylglycerols (DAG), represent more than 98% of the total oil weight. Minor components, that amount to about 2% of the total oil weight, include more than 230 chemical compounds such as phospholipids, waxes, aliphatic and triterpenic

alcohols, esters of sterols, hydrocarbons, volatile compounds, carotenoids, chlorophylls and antioxidants (Servili, et al. 2004).

Major components;

This fraction is also known as the saponifiable fraction or glyceride fraction. It constitues about 98% of the oil weight and is composed mainly of triacylglycerols. The oil fraction consists of six main fatty acids; oleic and palmitoleic, which are mono-unsaturated; palmitic and stearic, which are saturated; and linoleic and linolenic, which are poly-unsaturated fatty acids. Oleic acid (a mono-unsaturated fatty acid) is represented in much higher concentration (55.23-86.64%) than the other fatty acids; linoleic (2.7-20.24%), palmitic (6.30-20.93%), stearic (0.32-5.33%), palmitoleic (0.32-3.52%) and linolenic acids (0.11-1.52%). Oleic acid (18:1n-9) and palmitoleic acid (16:1n-7) have one double bond in their structure, linoleic acid (18:2n-6) two double bonds and linolenic acid (18:3n-3) three double bonds. Because of the fact that oleic acid is predominant in olive oil, classification of olive oil is achieved by mono-unsaturated fat. Other fatty acids found in olive oil at low concentrations are myristic, margaric, heptadecanoic, arachidic, behenic and lignoceric acids (Quiles 2006, Garcia-Gonzalez, et al. 2008).

Minor components;

This fraction includes compounds from the unsaponifiable matter, derived from lipids such as phospholipids, waxes, and compounds which are not related to lipids such as phenols, pigments and carotenoids (Hashim, et al. 2005).

Sterols, or 4-demethylsterols, make up an extensive series of compounds that are commonly called phytosterols, while 4,4-dimethylsterols are called triterpenic alcohols and 4-monomethylsterols are named methylsterols. The composition and concentrations of sterols in olive oil are used to determine genuiness or authenticity of olive oil so that it is labelled correctly in the marketplace. Waxes are esters of long chain aliphatic alcohols (C27-C32). Waxes are mainly located on the skin of the fruit and prevent water loss. Squalene is the main hydrocarbon of olive oil and constitutes around 50% of the unsaponifiable matter. Other hydrocarbons present as volatiles in olive oil are phenanthrene, pyrene, fluoranthrene, 1,2ben-zanthracene, chrysene, and perilene. Tocopherols are heteroacid compunds which have high molecular weight and they are designated as $\alpha, \beta, \gamma, \delta$-tocopherols. Tocopherols contribute to the antioxidant

properties of olive oil. The most important carotenoids present in olive oil are β-carotene and lycopene. Chlorophyll and carotenoid pigments are responsible for the colour of VOO, ranging from yellow-green to greenish gold. Volatile compounds are retained by virgin olive oils during their mechanical extraction process, and they are responsible for the whole aroma of the virgin olive oil (Angerosa, et al. 2004, Garcia-Gonzalez, et al. 2008).

Phenolic compounds that include hydrophilic and lipophilic phenols are the most important components of the polar fraction of olive oil owing to their sensory and health properties as natural antioxidants. Moreover, these components have important effect on the evaluation of the quality of an EVOO due to their role in oxidation stability, nutritional value, flavour (bitterness and astringency), and organoleptic characteristics in general (Servili, et al. 2004, Carrasco-Pancorbo, et al. 2005). Phenolic compounds of olive oil are discussed extensively in section 2.2.

2.1.6. The Role of Olive Oil in Human Health

Olive oil is a good source of mono-unsaturated fat and is a prime component of the Mediterranean Diet. Olive oil is considered as a natural juice which contributes to the taste, aroma, and vitamins. The role of olive oil in human health is related to its high content of mono-unsaturated fatty acids and its high content of antioxidative compounds. Olive oil provides preservation against heart disease by controlling low density lipoprotein (LDL) cholesterol levels while raising high density lipoprotein (HDL) cholesterol levels. Vitamin E (alpha-tocopherol), carotenoids and phenolic compounds such as hydroxytyrosol and oleuropein are all antioxidants which demonstrate some health effects in the prevention of certain diseases and ageing. Olive oil reduces the risk of breast cancer, certain malignant tumours (prostate, endometrium, digestive tract). It was reported that consumption of olive oil as part of Mediterranean Diet decreases systolic and diastolic blood pressure. It has also been demonstrated that a diet that is rich in olive oil, low in saturated fats, moderately rich in carbohydrates and soluble fibre from fruit, vegetables, pulses and grains is the most effective approach for diabetics. Besides lowering the "bad" low-density lipoproteins, this type of diet improves blood sugar control and enhances insulin sensitivity. Moreover, it has been

determined that longer-lasting weight loss could be achieved with this type of diet. Olive oil also inhibits gastric motility. As a result, the gastric content of the stomach is released more slowly. Olive oil partially prevents cholesterol absorption by the small intestine because of the presence of sitosterol in olive oil. It also mobilizes the absorption of various nutrients such as calcium, iron, and magnesium (Perez-Jimenez, et al. 2007, Visioli, et al. 2002, Owen, et al. 2000).

2.2. Phenolic Compounds

2.2.1. Chemistry of Phenolics

Phenolic compounds are complex class of chemicals including a hydroxyl group on a benzene ring. The plant phenols are aromatic secondary metabolites that contain a fundamental range of substances having an aromatic ring bearing one or more hydroxyl compounds. Plant phenols are defined based on metabolic origin and these substances derived from the shikimate pathway and phenylpropanoid metabolism (Ryan, et al. 1998). Although the presence of phenolic compounds is expansive along nature, respectable variation occurs between plant species. Phenolic compounds can be separated into different component classes listed in Table 2.1.

The term phenolic acids represent the seven carbon benzoic acids (C_6-C_1) and nine carbon cinnamic acids (C_6-C_3). Hydroxycinnamic acid compounds occur most frequently as simple esters with hydroxy carboxylic acids or glucose. Hydroxybenzoic acid compounds are present mainly in the form of glucosides. *p*- hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid and syringic acid are the major benzoic acids. Salicyclic acid and gentisic acid have an OH group ortho to the carboxylic acid function and gallic acid occurs as quinic acid esters in plants. *p*-coumaric, caffeic, ferulic, and sinapic acids are also the most important cinnamic acids. Cinnamic acids can be found in two isomeric forms, cis- and trans-cinnamic acid, because of the fact that they possess a double bond. Phenolic acids may be conjugated with organic acids, sugars, amino compounds, lipids, terpenoids, or other phenolics.

Many phenolic compounds are attached to sugar molecules and are called glucosides or glycosides, depending on the type of sugar. Vanillin is a single-ring phenolic compound derived from the breakdown of lignin. The coumarins contain an oxygen heterocyclic of six atoms fused with a benzene ring. Because they also possess the (C_6-C_3) configuration, they can be considered in same class with the cinnamic acids. Coumarins are lactones of O- hydroxycinnamic acid.

Table 2.1. Phenolic classes in plants

(Source: Shahidi 2004)

Phenolic classes	**Chemical Structure**
Simple phenols, benzoquinones	C_6
Phenolic acids	$C_6 - C_1$
Acetophenones, phenylacetic acids	$C_6 - C_2$
Hydroxycinnamic, phenylpropenes, coumarins, isocoumarins, chromones	$C_6 - C_3$
Naphthoquinones	$C_6 - C_4$
Xanthones	$C_6 - C_1 - C_6$
Stilbenes, anthraquinones	$C_6 - C_2 - C_6$
Flavonoids, isoflavonoids	$C_6 - C_3 - C_6$
Lignans, neolignans	$\left(C_6 - C_3 \right)_2$
Bioflavonoids	$\left(C_6 - C_3 - C_6\right)_2$
Lignins	$\left(C_6 - C_3 \right)_n$
Condensed tannins	$\left(C_6 - C_3 - C_6\right)_n$

Some phenolic compounds occur as polymers (often combined with glucose). Tannins are phenolic polymers that combine with the protein of animal skins (collagen) forming leather. Flavonoids are 3-ring phenolic compounds consisting of a double ring attached by a single bond to a third ring. These components enclose the flavones, flavonols, flavanones, dihydroflavonols, anthocyanins, chalcones, and iso-flavonoids (Ryan, 1998).

2.2.2. Occurrence of Hydrophilic Phenols in Olive Oil

Phenolic compounds which often observed in the lists of olive oil polyphenols are: 4-acetoxy-ethyl- 1, 2-dihydroxybenzene, 1-acetoxy-pinoresinol, apigenin, caffeic acid, cinnamic acid, *o*- and *p*-coumaric acids, elenolic acid, ferulic acid, gallic acid, homovanillic acid, *p*-hydroxybenzoic acid, hydroxytyrosol and derivatives, luteolin, oleuropein, pinoresinol, protocatechuic acid, sinapic acid, syringic acid, tyrosol and derivatives (Dimitrios 2006).

The hydrophilic phenols in VOO are the most fundamental class of minor constituents and they are associated with the stability of the oil in addition to its biological properties. VOO is composed of various classes of phenolic compounds such as phenolic acids, phenolic alcohols, hydroxy-isocromans, flavonoids, secoiridoids and lignans (Table 2.2). Chemical structures of phenols are given in Figure 2.1. The phenolic acids are the first group of phenolic compounds observed in VOO and these compounds together with phenyl-alcohols, hydroxy-isochromans and flavonoids are found in small amounts in VOO. Phenolic acids with the basic chemical structure of C_6-C_1 (benzoic acids) and C_6-C_3 (cinnamic acid) are found in olive fruit. These compounds, such as caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic and *p*-hydroxybenzoic acid are also the first group of phenols observed in VOO (Servili, et al. 2004; Brenes, et al. 1999).

Hydroxytyrosol and tyrosol are the major phenolic alcohols of VOO; their concentration increases during oil storage because of the hydrolysis of VOO secoiridoids such as dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3, 4-DHPEA-EDA), dialdehydic form of decarboxymethyl elenolic linked to tyrosol (*p*-HPEA-EDA) and aldehydic form of oleuropein aglycone (3,4-DHPEA-EA) that contain 3,4-DHPEA and *p*-HPEA in their molecular structure.

Secoiridoids, oleuropein, demethyloleuropein, and ligstroside are the main phenolic glucosides and verbascoside (caffeoylrhamnosylglucoside of hydroxytyrosol) is the main hydroxycinnamic acid derivative of olive fruit. During crushing and malaxing processes, oleuropein and demethyloleuropein are hydrolyzed by endogenous β-glycosidases to 3, 4-DHPEA-EDA and 3, 4-DHPEA-EA. These newly formed substances are the most abundant secoiridoids in VOO (Bendini, et al. 2007).

Flavonoids such as luteolin and apigenin have been also reported as phenolic component of VOO by Rovelli et al. (1997). The last group of phenols found in VOO are the lignans and (+)-1-acetoxypinoresinol and (+)-1-pinoresinol and they have been recently isolated and characterized as the most concentrated lignans in VOO (Owen, et al. 2000, Brenes, et al. 2000).

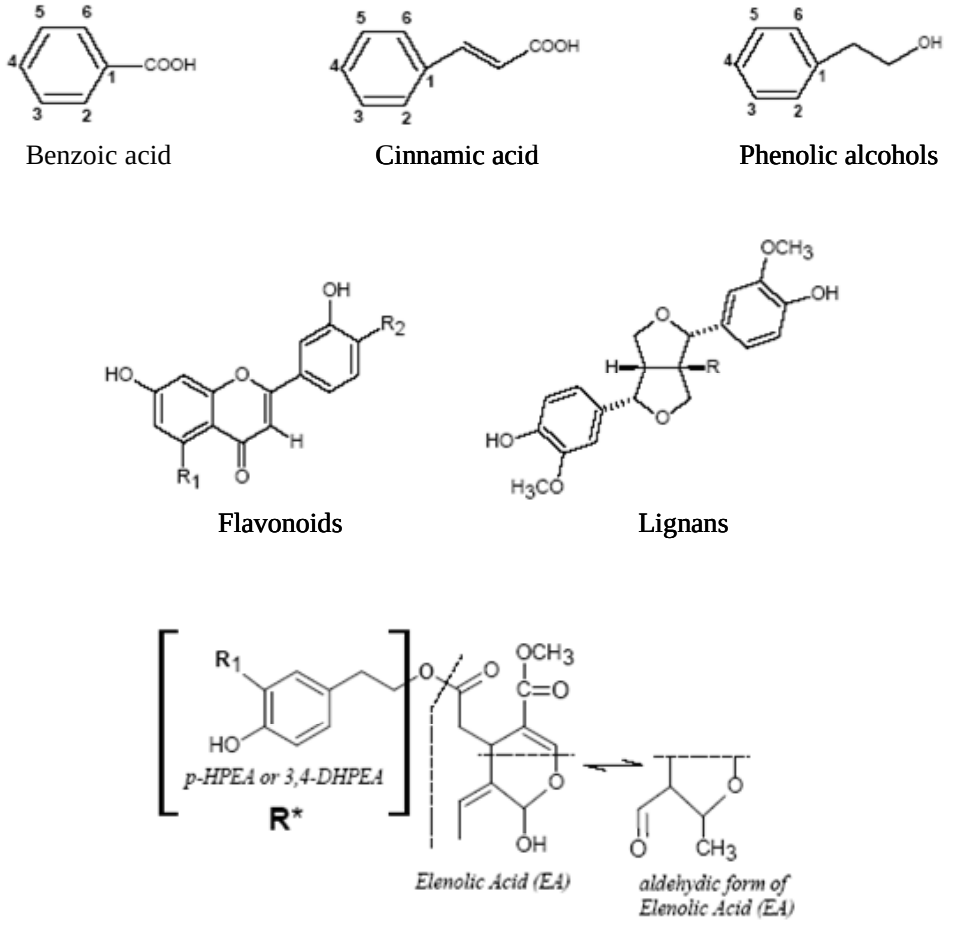


Figure 2.1. Chemical structures of phenolic compounds
(Source: Bendini, et al. 2007)

Table 2.2. Major classes of phenolic compounds in VOO

(Source: Servili, et al. 2004)

Major classes of phenolic compounds in VOO
Phenolic acids and derivatives
Vanillic acid
Syringic acid
p-coumaric acid
o-coumaric acid
Gallic acid
Caffeic acid
Protocatechuic acid
p-hydroxybenzoic acid
Ferulic acid
Cinnamic acid
4-(Acetoxyethyl)-1, 2-dihydroxybenzene
Benzoic acid
Hydroxy-isochromans
Phenolic alcohols
Hydroxytyrosol
Tyrosol
(3,4-Dihdroxyphenyl)ethanol-glucoside
Secoiridoids
3, 4-DHPEA (3, 4-DHPEA-EDA)
(*p*-HPEA-EDA)
(3, 4-DHPEA-EA)
Ligstroside aglycon
Oleuropein
p-HPEA-derivative
Dialdehydic form of oleuropein aglycon
Dialdehydic form of ligstroside aglycon
Lignans
(+)-1-Acetoxypinoresinol
(+)-Pinoresinol
Flavones
Apigenin
Luteolin

2.2.3. Role of Phenolics in Olive Oil

Phenolic compounds are secondary plant metabolites which are the products of complex metabolic pathways. Their occurrence and concentration may vary markedly from tissue to tissue, and depend on growth condition. Owing to this variation, determination of the biological function for these compounds is fairly difficult. Nonetheless, almost all of the phenolic compounds have been associated with several common biological and chemical properties; antioxidant activity, the ability to scavenge both active oxygen species and electrophiles, the ability to inhibit nitrosation and to chelate metal ions, the potential for autoxidation, and the capability to modulate certain cellular enzyme activities (Visioli, et al. 2002, Ryan, et al. 1998). They even have nutritional and health related properties. For example, hydroxytyrosol showed an interesting activity *in vitro* as an inhibitor of blood platelet aggregation and synthesis of tromboxane in human cells (Visioli, et al. 1998). Phenols also inhibited the oxidation of phospholipids and they are also important due to their contribution to the sensory quality of fresh fruits and processed products including colour, astringency, bitterness, and flavour.

2.2.3.1. Antioxidant Activities

The antioxidant activity of hydrophilic phenols of VOO has been studied by several researchers. The antioxidant activity of phenolic compounds in olive oil is an attractive topic because of not only its chemoprotective effect in human health but also its contribution to oxidative stability of olive oil. It has been demonstrated by different authors that the concentration of phenolic compounds, determined colorimetrically and expressed as total phenols, is associated with the stability of VOO (Aparicio et al., 1999, Blekas, et al. 2002, Keceli, et al. 2001). Gorinstein et al. (2003) compared, the contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils and found that the correlation of TPC and the radical scavenging capacity was very high (R^2 = 0.9197-0.9958).

There have been several studies which have investigated the scavenging effects of hydroxytyrosol and oleuropein with 2, 2-diphenyl-1-picrylhydrazyl radical scavenger

(DPPH). These studies determined that hydroxytyrosol and oleuropein which are the main phenolic compounds in olive oil possess greater antioxidant capacity and the antioxidant activity of hydroxytyrosol acetate is higher than that of oleuropein and oleuropein aglycone (Tuck, et al. 2002). According to an earlier study, antioxidant activity in refined olive oil decreases in the series hydroxytyrosol, caffeic acid, butylated hydroxytoluene (BHT), protocatechuic acid, syringic acid. Tyrosol, *p*-hydroxyphenylacetic acid, *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and vanillic acid have very little or no antioxidant activity (Ryan, et al. 1998).

Antioxidative and free radical-scavenging activity of phenolic compounds is related to their chemical structure that includes the phenolic hydroxyl group. Hydrophilic phenols prevent the propagation reactions during the oxidation process by means of their ability of donating the hydrogen atom of the phenolic hydroxyl group to the free radicals. Moreover, the occurrence of a second hydroxyl group at the ortho-position precipitates H-atom transfer to peroxyl radicals because of the decline of O-H bond dissociation enthalpy. A third hydroxyl group in the phenolic ring increases the antioxidant capacity further (Pinedo, et al. 2007, Lucarini, et al. 2002). The primary hydroxyl group on the alkyl chain of tyrosol and hydroxytyrosol has positive effect on the antioxidant capacity of these antioxidants and it has been demonstrated that hydroxytyrosol is much better antioxidant than caffeic acid and homoprotocatechuic acid (Ranalli, et al. 2003). In addition to this, some phenolic antioxidants such as caffeic acid and sinapic acid contain an alkyl chain connecting the phenolic ring and the carboxylic or alcohol group and this efficiency provides the stabilization of the radical formed (Silva, et al. 2000).

Gorinstein et al. (2003) discussed that there was a positive correlation between TPC and free radical scavenging ability. They reported that increasing total polyphenol content provided high antioxidant activity. Especially specific phenolic compounds such as hydroxytyrosol, tyrosol and phenolic acids (caffeic acid, ferulic acid, *p*-coumaric acid, syringic acid and vanilic acid) are accepted as highly antioxidant substances (Servili, et al. 2002). In opposition to these studies, some researchers have declared that a relationship is not available between TPC and free radical scavenging (Yu, et al. 2002).

2.2.3.2. Antimicrobial Activities

The other beneficial effect, of phenolic compounds is related to their anti-imflammatory and antimicrobial activity. Some of the phenolic compounds have antimicrobial activity and inhibit the growth of some bacteria species, fungi and viruses.

Aziz et al. (1998) have reported that caffeic and protocatechuic acids (0.3 mg/mL) inhibited the growth of *Escherichia coli* and *Klebsiella pneumoniae*. *p*-hydroxy benzoic, vanillic, caffeic, protocatechuic, and *p*-coumaric acids, oleuropein and quercetin (0.5 mg/mL) completely inhibited the growth of *Bacillus cereus*. Oleuropein, and *p*-hydroxy benzoic, vanillic and *p*-coumaric acids (0.4 mg/mL) were effective on *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus*. Most studies are concerned with the antimicrobial activity of hydroxytyrosol and oleuropein against ATCC bacterial strains. The bacteriostatic and bactericidal activities of oleuropein and the hydrolysis products, hydroxytyrosol and tyrosol, have been investigated in vitro against many pathogenic micro-organisms: bacteria, fungi, viruses and protozoa. Especially, oleuropein is effective phenol against gram-positive and gram-negative human pathogenic bacterial strains (Bisignano, et al. 1999). Futhermore, it is proposed that oleuropein and derivatives can prevent the development of *enterotoxin B* by *Staphylococcus aureus*, Salmonella species and spores of *Bacillus cereus*. Other phenolic compound, verbascoside has antibacterial attribution against *Staphylococcus aureus, Escherichia coli* and other bacteria and antiviral activity against the syncytial virus (Tripoli, et al. 2005).

2.2.3.3. Health Properties

The health properties of VOO hydrophilic phenols are associated with antioxidant activity which is related to the prevention for chronic and degenerative diseases as coronary hearth diseases (CHD), ageing neuro-degenerative diseases and tumours of different localizations. Especially, the protection of LDL oxidation; the reduced oxidative damage of the human erythrocytes by hydroxytyrosol and the reduction of free radical production in the faecal matrix are the most important effects

(Carrasco-Pancorbo, et al. 2005). Inhibition of LDL oxidation prevents the formation of atherosclerotic plaques, which in turn contribute to the development of CHD (Edgecombe, et al. 2000).

In particular hydroxytyrosol has protective effect against the chronic degenerative diseases and reduce the risk of CHD and atheroscelosis. In addition to this, hydroxytyrosol inhibits arachidonic acid lipoxygenase or inhibits platelet aggregation (Tuck, et al. 2002). It has been demonstrated that oleuropein and derivatives are possible therapeutic tools for the pharmacological treatment of CHD as well as in the case of cardiac surgery, including transplantation because of their antithrombotic and antiatherogenic activity (Manna, et al. 2002).

Phenolic compounds can prevent lipid peroxidation and oxidative modification of LDL by means of their antioxidant activities (Servili, et al. 2004). Peroxynitrites ($ONOO^-$) are highly reactive compounds capable of inducing peroxidation in lipids, oxidising methionine and damaging the deoxyribonucleic acid (DNA) by deamination and nitration. Peroxynitrites are formed by reaction between NO and O_2^- (superoxide radical). The deamination of guanine and adenine causes breaks in the DNA chain, with consequent mutations; DNA oxidation is also potentially mutagenic. In vitro, the presence of hydroxytyrosol reduces the biochemical effects of peroxynitrites, such as the deamination of adenine and guanine in some cell lines. The lignans are the most important substances possessing anticancer activity and they prevent the development of various tumours: cutaneous, mammary, colonic, and pulmonary. The antitumoral effect of the lignans is associated with their antioxidant activity and their antiviral activity. Caffeic acid is a simple polyphenol with an ortho-diphenolic structure and presents pro-oxidant activity in the propagation phase of LDL oxidation induced by Cu^{2+}. Caffeic acid could have cytoprotective (protecting cells from destructive chemicals or other stimuli) effects on endothelial cells related to its ability to block the concentration increase of intracellular Ca^{2+} in response to lipoprotein oxidation. The ability of polyphenolic compounds to react with metal ions could make them pro-oxidant (Tripoli, et al. 2005).

2.2.3.4. Sensory Properties

The phenolic constituents mainly affect the sensory properties of VOO. These phenols are responsible for the key sensory characteristics of bitterness, pungency, and astringency. Kiritsakis (1998) reported that there is a good correlation between aroma and flavour of olive oil and its polyphenol content. Hydroxytyrosol, tyrosol, caffeic acid, coumaric acid, and *p*-hydroxybenzoic acid influence mostly the sensory characteristics of olive oil. Hydroxytyrosol is present in good-quality olive oil, while tyrosol and some phenolic acids are found in olive oil of poor quality.

Previous studies indicated that the intensity of bitterness for olive oil is highly correlated with the content of the dialdehydic forms of the aglycones of oleuropein, the aldehydic forms of the aglycones of oleuropein and ligstroside (Gutierrez-Rosales, et al. 2003). Phenolic compounds derived from the hydrolysis of oleuropein, a secoiridoid glucoside characteristic of the Oleaceae contribute to the intensity of bitterness of VOO (Kiritsakis 1998).

In another study, the relationship between polyphenols and olive oil pungency was investigated (Andrewes, et al. 2003). Most polyphenol fractions were described as bitter and astringent. Especially one polyphenol fraction was different from other phenols because of its strong pungent (burning) sensation at the back of the throat. This study showed that deacetoxy-ligstroside aglycon is responsible for the burning sensation perceived in many olive oils. On the contrary, deacetoxy-oleuropein aglycon caused very little burning sensation.

In addition to antioxidant activities of phenolic compounds, it is supported that the non-volatile phenolic compounds contribute to organoleptic properties of VOOs which are characterized with bitter, pungent and leafy attributes (Romero, et al. 2002). For instance, Picual variety that is described by a very low content of phenolic components has low oxidative stability and bitter attributes (Stefanoudaki, et al. 2000). Likewise, oleuropein and its aglycon form that are the most important secoridoids found in olive oil are especially responsible for the bitterness of VOO and the amount of these phenolics decreases during the maturation of the olives.

2.2.4. Factors Affecting the Phenolic Profile of Olive Oil

Factors affecting the phenolic distribution of olive oil include agronomic aspects that are cultivar and genetics, maturity, climate, position on the tree, agricultural practices and technological aspects.

2.2.4.1. Agronomic Aspects

The most important agronomic parameters such as cultivar, fruit ripening, pedo-climatic conditions of production and the irrigation can strongly affect the phenolic profile and concentration of VOO (Tovar, et al. 2001, Romero, et al. 2002, Garcia, et al. 2003, Vinha, et al. 2005, Cerretani, et al. 2004).

Phenolic composition of olive fruits is influenced by the cultivar. The main phenolic compounds depend on the cultivar are oleuropein, verbacoside, apigenin-7-glucoside, and luteolin-7- glucoside (Japon-Lujan, et al. 2006, Servili, et al. 2002).

As reported by different researchers changes of phenols in VOO with maturation are consequential. The concentration of hydroxytyrosol, tyrosol, and luteolin increased in oils with maturation of fruits whereas the concentration of glucoside aglycons declined with maturation (Brenes, et al. 1999, Ryan, et al. 1999, Bonoli, et al. 2004). Caponio et al. (2001) showed that the amount of oleuropein decreased during maturation while that of demethyloleuropein increased. Oleuropein and its aglycon form also decreased as ripening of the olives progressed.

Romero et al. (2002) concluded that the concentration of lignans, vanillic acid, and vanillin increased in the oils from the most irrigated treatments while the secoiridoid derivatives increased in the oils from the most stressed irrigation treatments. As a result of this study, it was found that water stress during a determined period of the olive cycle (pit hardening and fruit growth) influenced not only the total amount of phenolic compounds in the oil but also their profile.

Harvesting time of olive oil has significant impact on the organoleptic properties and shelf life of olive oil. For example, it is advisable to wait for harvesting of olive fruits yielding bitter to pungent oils and occupying sweet tasting oils to obtain more abditive oils (Caponio, et al. 2001, Ryan, et al. 1999). And also decreasing of some

phenolic compounds especially secoridoids is observed during the storage period with changing storage conditions (Morello, et al. 2004).

2.2.4.2. Technological Aspects

Olive oil involves extraction and/or chemical treatment of the olive fruit and these technological treatments fairly affect the phenolic content of the olive oil and hence oil stability and quality. The composition of phenolic substances in VOO represents main dissimilarities which are affected by some chemical and enzymatic reactions of various endogenous enzymes of olive fruit during oil extraction. Crushing and malaxation are the most significant steps of the oil mechanical extraction process. Storage conditions of olives can also cause important reduction in the content of phenols and other quality parameters (Servili, et al. 2004, Ryan, et al. 1998).

During crushing, secoiridoid aglycons such as 3, 4-DHPEA-EDA, *p*-HPEA-EDA and 3, 4-DHPEA-EA can be generated by means of the hydrolysis of oleuropein, demethyloleuropein and ligstroside which is catalyzed by the endogenous β-glucosidases. Application of blanching before crushing causes inactivation of endogenous glycosidases. The concentration of oleuropein and demethyloleuropein is not significantly modified and the aglycon derivatives of these substances are not observed in oils (Servili, et al. 2004).

The concentration of secoiridoid aglycons and phenolic alcohols is negatively correlated with time and temperature of processing during malaxation. The reduction of the oil phenolic concentration during malaxation is related to oxidative reactions catalysed by endogenous oxidoreductases such as polyphenoloxidase and peroxidase which induce the phenolic oxidation. As reported by different authors control of O_2 concentration in the paste during processing prevents the activation of polyphenoloxidase and peroxidase, hence promotes the concentration of hydrophilic phenols in olive paste and VOO (Servili, et al. 2003, Garcia, et al. 2002). The findings of Ranalli et al. (2001) who investigated the effects of malaxation temperature on the phenolic composition of VOO reported that the concentration in total phenols of the oils increased with increasing levels of olive paste kneading temperature. The increase in phenol concentration was more significant when the paste temperature increased from

25 to 30°C whereas phenol content did not increase when the paste temperature increased from 30 to 35°C.

The method of oil extraction has a significant effect on the content of phenols. The physical forces used for oil separation and the amount of water added to the olive paste during extraction are important parameters. The study of Di Giovacchino et al. (2002) mentioned that addition of water to the olive paste effectively reduced the phenolic content of the oil. It was also shown that the total phenol and *o*-diphenol content of oils obtained by pressing and percolation were significantly greater than that of the centrifugally extracted oils. However, phenolic concentration of olive oil obtained by the pressure system was higher than one obtained by the traditional centrifugation process because of the low addition of water to the olive paste in pressure system.

The main changes in the phenolic composition of VOOs during storage period have been observed by different authors. Considerable decreases were observed in secoiridoid derivatives and 3, 4-DHPEA-AC after the storage period whereas lignans were the more stable phenolic compounds (Morello, et al. 2004). Brenes et al. (2001) concluded that the hydrolysis of the secoiridoid aglycons in olive oil during storage in darkness at 30°C occured and this reaction gave rise to an increase in the free phenolics hydroxytyrosol and tyrosol in the oil. In opposition to this, the concentration of lignans, 1-acetoxypinoresinol and pinoresinol remained constant during storage.

CHAPTER 3

MULTIVARIATE STATISTICAL ANALYSIS

Analytical data are used in order to describe objects (meteorites, olive oil samples, blood samples from patients, etc.). Description of these objects is relatively easy in case of a few analytical data (up to three) for each object. On the other hand, most chemical measurements are inherently multivariate. This means that more than one measurement can be made on a single sample. For instance, a spectrum at hundreds of wavelengths on a single sample can be recorded in spectroscopy, or in a chromatogram in which a number of compounds are detected with different elution times are recorded. High performance liquid chromatography-diode array detector (HPLC-DAD) and liquid chromatography-mass spectroscopy (LC-MS) are increasingly common in modern laboratories, and present a rich source of multivariate data (Brereton 2003).

Multivariate statistical analysis describes a collection of procedures which involve observation and analysis of more than one statistical variable at a time and provides separating the signal from the noise in data with many variables and presenting the results as easily interpretable plots. Rather than investigating the variable effect on the samples individually, a multivariate data matrix, X, is formed by putting together all variables observed for the samples. X is subjected to statistical analyses to use the information coming from all measurements at once and extract the most relevant.

Any large complex table of data can easily be transformed into intuitive plots summarizing the essential information. Multivariate approach can be described based on some projections methods. This approach explains the samples as a swarm of points in a K-dimensional space (K = number of variables), and presents the point swarm down onto a lower-dimensional plane or hyper-plane. The coordinates of the points on this hyper-plane provide a compressed representation of the observations, and the direction vectors of the hyper-plane provide a corresponding representation of the variables (Eriksson, et al. 2001).

Multivariate statistical analysis is applied in many instances, such as monitoring and controlling processes, determinations of geographical origin and sources of food

and detection of fraudulent practices (Massart 1988). Classification is one of the major techniques in multivariate statistical analysis and includes a mathematical model able to evaluate the membership of a sample to its class. This classification model provides the prediction of the membership of new samples. Vegetable oil classifications have been performed by multivariate data analysis of chromatographic profiles, headspace-mass spectrometry, metal-oxide sensors and near-infrared spectroscopy (Bortoleto, et al. 2005). In recent years, many studies have been made to classify olive oils according to their geographical origin or variety by means of multivariate statistical analysis with different chemical and physical parameters. Common projection methods used in multivariate analysis are PCA for projecting X down onto a few latent variables, Soft Independent Modelling Class Analogy (SIMCA) and PLS-DA for classification.

3.1. Principal Component Analysis (PCA)

In some situations the class membership of the samples is unknown and the analyst can trend to identify and show natural aggregation in the data irrespective of class membership on the samples. In such a case, the technique applied is termed unsupervised pattern recognition. The aim of this technique is to develop the understanding of the data set by means of examining the natural clustering of the sample. PCA is a very common, unsupervised multivariate technique and it helps us to interpret in what aspect a sample is different from another (Beebe 1998). PCA represents the relationship among the observations and reveals any deviating observations or groups of observations in the data.

PCA acts on a single data matrix X of size (n x k) and reduces a large number of original measurement variables, k, to a much smaller number of new, uncorrelated p variables (principal components), which are derived from the correlation matrix of X. Mathematical transformations of the original data matrix can be represented as;

$$X = TP + E \qquad (3.1)$$

where

- **T** are called score matrix that have as many rows as the original data matrix;
- **P** is the loadings and has as many columns as the original data matrix; Columns (rows) of P are the eigen-vectors of correlation matrix
- **E** is the residual matrix
- The number of columns in the matrix T and the number of rows in the matrix P are equal.

PCA starts with the determination of the number of principal components by the percentage of explained variance, eigenvalues, and cross-validation. Eigenvalue is called as the size of each component. The most significant component has the largest size. Simple definition of eigenvalue of a principal component is the sum of squares of the scores, so that

$$g_a = \sum_{i=1}^{I} t_{ia}^2 \qquad (3.2)$$

where g_a is the a^{th} eigenvalue and t_{ia}: score vectors. Note that if the data are preprocessed prior to PCA, x must likewise be preprocessed for this property to hold; if mean centering has been performed, K cannot be larger than I-1, where I equals the number of samples.

The sum of all nonzero eigenvalues for a data matrix equals the sum of squares of the entire data matrix, so that

$$\sum_{a=1}^{K} g_a = \sum_{i=1}^{I} \sum_{j=1}^{J} x_{ij}^2 \qquad (3.3)$$

where

x_{ij}: each element, K is the smaller of I or J. Then, eigenvalues are presented as percentages.

$$V_a = 100 \frac{g_a}{\sum_{i=1}^{I} \sum_{j=1}^{J} x_{ij}^2} \qquad (3.4)$$

The cumulative percentage eigenvalue explains the proportion of the data which has been modelled using PCA and is given by $\sum_{a=1}^{A} g_a$. The model is faithful if this value is close to 100%. Using the size of eigenvalues, estimation of the number of significant components in the dataset is carried out.

The significance of the each principal component can be tested by cross-validation. In cross-validation, each sample is removed once from the dataset and PCA is performed on the remaining samples. Different scores and loadings matrices are obtained depending on removed sample. In this way, all samples are removed once and the remaining sample is predicted.

Each principal component can be expressed as a linear combination of the original variables that contribute to making the samples different from each other. The first principal component includes most explained information (variance); the second principal component carries the next maximum explained information and so on. In this way, PCA creates an alternative set of coordinate axes, principal components that are orthogonal to each other. After the determination of significant components, the possible natural groupings within data are visualized by plotting the first two or three latent variables, which are also called score plots. In the score plot, the horizontal axis shows the scores for the first PC and the vertical axis those for the second PC (Brereton 2003).

In the study of Penza et al. (2001), PCA and cluster analysis (CA) have been used in order to classify and identify different classes of flavour samples such as olive

oils and seed oils, fruit juices, tomato sauces, and perfumes. In the case of oil samples, PCA results showed that six oils (two seed oils and four olive oils) can be classified in separated clusters and the type of olive cultivar can be identified for the examined cases.

Brodnjack-Voncina et al. (2005) distinguished different edible vegetable oils using fatty acid composition in combination with multivariate analyses such as PCA. PCA was used for screening of the data and 97.8% variance was explained in the first two principal components. The analysis showed that the variables with the greatest discriminating power were the percentage levels of the oleic and the linoleic acids. A high correlation between these two variables was found for all oil samples.

Multivariate analysis, including PCA was used to characterize the oils according to cultivar, location and sampling date by Stefanoudaki et al. (1997) and classification of olive oil samples according to cultivar and geographic origin was achieved using the triglyceride compositional data.

Poulli et al. (2005) studied the classification of VOOs based on their synchronous fluorescence spectra by hierarchical cluster analysis (HCA) and PCA using the spectral range of 429–545 nm. As a result of this study, PCA provided better discrimination between the two classes, without any classification error, while HCA allowed 97.3% correct classification.

Diaz et al. (2005) examined the characterization of VOOs according to its triglycerides and sterols composition by multivariate techniques. This study demonstrated that it was possible to characterize the oils obtained from a specific type of olives (‘‘Manzanilla Cacerena’’ of North of Caceres (Extremadura—Spain)) according to their chemical composition using the PCA, and soft independent modelling class analogy (SIMCA).

3.2. Soft Independent Modelling Class Analogy (SIMCA)

The classification method, SIMCA, is used to determine the class membership of the samples and to form the known classes. SIMCA develops principal component models for each training class separately and provides information including critical distances which can be calculated as the geometric distance of each object from the principal component models. Following the modelling for classes, each sample is fitted

to each model and classification of the sample with corresponding class is achieved. SIMCA results can be visualized by Cooman's plot, which shows the discrimination of two classes. In Cooman's plot, the distance from the model for class 1 is plotted against that from model 2 and both axes indicate the critical distances. Four zones are defined on the plot: class 1, class 2 (the object is situated within the boundaries of only one class), overlap of classes 1 and 2 (the object is situated inside the boundaries of more than one class), and outlier zone (far from both classes). By plotting objects in this plot it is easy to visualize how certain a classification (Berrueta, et al. 2007).

3.3. Partial Least Squares-Discriminant Analysis (PLS DA)

In opposition to unsupervised methods, supervised pattern recognition methods benefit by class membership information in the calculations. The purpose of these kinds of methods is to compose models using analytical measurements in order to predict class membership of future samples (Beebe 1998).

When a single PCA is used with a set of observations representing one or several classes, the location of the principal components is obtained without information related to class membership. PCA gives the information about the directions in multivariate space that represent the largest sources of variation, the so called principal components. On the other hand, it is unnecessary in case of the maximum variation directions encounter with the maximum separation directions among the classes. In other words, other directions can be more relevant for discriminating among classes of observations. At this point, PLS based technique, called PLS discriminant analysis, can be seen more useful.

PLS, as a regression method, connects the information in two blocks of variables X and Y (n x q) by maximizing the correlation between them. PLS-DA is an extension of PLS analysis. In PLS-DA, there is actually no response (quality) matrix Y. A dummy y variable vector, expressing different values for each class, such as 0, 1 or 2 is created and processed with X matrix. The principle of PLS-DA is to find a model that separates classes of observations on the basis of their X-variables. This model is developed from a training set of observations of known class memberships (y). The variable influence on the projection (VIP) of X into artificial Y can be demonstrated by the weighted sum of

squares of PLS weights, w, taking into account the explained Y-variance for a given PLS-DA model (Eriksson, et al. 2001).

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

4.1.1. Olive Oil Samples

Two sets of EVOO samples were used in this study. The first set of samples were obtained from erkence (E), memecik (M), domat (D), nizip-yaglik (N), gemlik (G) and ayvalik (A) varieties. Ayvalik variety is also known as edremit-yaglik. Nizip is a variety cultivated in the south-east part of the country and has very high oil productivity. Other cultivars are indigenous to the west cost. All the olive varieties are used mainly in oil production except domat, which is an important variety in table olive production. The olives were obtained from a nursery in Izmir, a city in the Aegean cost of Turkey (Research Institute of Olive, Izmir, Turkey). Gemlik & ayvalik varieties (GE & AE) were also obtained from an olive grove, which is about 150 kilometres north of Izmir (Edremit Olive Nursery, Balikesir, Turkey) in order to study the possible geographical differences among the same cultivars. Olive varieties and codes are listed in Table 4.1. Olive fruit samples were hand-picked randomly from olive trees at the beginning of November in 2005 and 2006 harvest years, at the same maturity level. Only healthy fruits, without any kind of infection or physical damage, were used. Olive fruits of each variety were randomly distributed in 5 kilogram batches for the extraction processes. Olive oils were produced in a 5-kilogram capacity laboratory scale olive mill (Spremoliva, Italy) in the Department of Food Engineering at Izmir Institute of Technology. The extraction of each variety was replicated minimum twice, and maximum five times in both years. The chemical analyses were performed after the extraction process in each particular year. Total of 48 samples were analysed in two years. The numbers beside each letter designated for oil samples represent the extraction batch and 05 and 06 represent the harvest years.

Table 4.1. First set of samples (Extracted EVOO samples)

Olives obtained from the Research Institute of Olive & Olive oil (Bornova, Izmir)	
Sample	**Sample code**
Memecik	M
Erkence	E
Gemlik	G
Ayvalik	A
Domat	D
Nizip	N
Olives obtained from the Olive Nursery (Edremit, Balıkesir)	
Sample	**Sample code**
Gemlik Edremit	GE
Ayvalik Edremit	AE

Second set of samples analyzed in this study were supplied by the Union of Taris Olive and Olive Oil Agricultural Sales Co-operatives in Izmir, Turkey. Olive oil samples came from two different areas (south and north) of the Aegean coast of the Turkey (between 36-40 north parallels and 26-29 east meridians) for two successive harvest years (2005/2006 and 2006/2007). The sampling has included 22 commercial EVOO samples for 2005 harvest year: 13 samples obtained from cooperatives in north Aegean and 9 samples from cooperatives in south Aegean. 25 commercial EVOO samples obtained in 2006 harvest year: 10 samples from north Aegean and 15 samples from south Aegean. Oil samples, codes and their geographical origins are given in Table 4.2. For geographical classification, all the samples for two harvest years were divided into two classes: south Aegean comprises the coastal region from Izmir to Milas and north Aegean comprises Edremit Gulf Region & Ezine (Figure 4.1). Olive oil samples were stored at 9°C in dark glass bottles and the headspaces were replaced by nitrogen during storage. The numbers 05 and 06 beside each letter designated for oil samples represent the harvest years.

Table 4.2. Second set of samples (Commercial EVOO samples)

2005 Olive oils			
North part	**Sample code**	**South part**	**Sample code**
Ezine	Ez	Akhisar	Ak
Ezine Gulpinar Organik	Ez-or	Menemen	Me
Kucukkuyu	Kk1	Tepekoy	Te
Kkuyu	Kk2	Bayindir	Ba
Altinoluk	Aol	Selcuk	Se
Altinoluk-sulubaski	Aol-su	Aydin	Ayd
Edremit	Ed	Ortaklar	Or
Havran	Ha	Kocarli	Koc
Burhaniye	Bu	Milas	Mi
Gomec	Go		
Ayvalik	Ay		
Altinova	Aov		
Zeytindag	Ze		
2006 Olive oils			
North part	**Sample code**	**South part**	**Sample code**
Ezine	Ez	Tepekoy	Te
Kucukkuyu	Kk	Bayindir	Ba
Altinoluk	Aol	Odemis	Od
Edremit	Ed	Tire	Ti
Havran	Ha	Selcuk	Se
Burhaniye	Bu	Kusadasi	Ku
Gomec	Go	Germencik	Ge
Ayvalik	Ay	Aydin	Ayd
Altinova	Aov	Ortaklar	Or
Zeytindag	Ze	Kosk	Kos
		Dalama	Da
		Kocarli	Koc
		Erbeyli	Er
		Cine	Ci
		Milas	Mi

Figure 4.1. Commercial EVOO samples from Aegean region
(Source: Tariş Zeytinyağı 2008)

4.1.2. Chemicals

Reference compounds used for quantitative determination of phenolic compounds and chemicals used for determination of PV, TPC and HPLC analysis of phenolic compounds are given in Tables 4.3 and 4.4.

Table 4.3. Chemicals used in the analysis

NO	CHEMICAL	CODE
	Peroxide value	
1	Acetic acid	Riedel-deHaen 27225
2	Chloroform	Riedel-deHaen 24216
3	Potassium iodate KIO_3	Fluka 60390
4	Potassium iodide KI	Riedel-deHaen 03124
5	Sodium thiosulphate $Na_2O_3S_2$	Fluka 72049
6	Starch	Carlo Erba 417587
7	Sulfuric acid H_2SO_4	Merck 1.00713.2500-UN1830
	Total Phenol Content	
8	Gallic acid	Fluka 48630
9	Folin–Ciocalteau reagent	Fluka 47641
10	Methanol	Sigma-Aldrich 34885
11	Sodium carbonate Na_2CO_3	Riedel- deHaen 13418
12	Tween 20	Sigma-Aldrich P1379
	HPLC Analysis of phenolic compounds	
13	Acetonitrile	Sigma-Aldrich 34888
14	Gallic acid	Fluka 48630
15	Hexane	Sigma-Aldrich 34859
16	Methanol	Sigma-Aldrich 34885

Table 4.4. Standard phenolic compounds

NO	CHEMICAL	CODE
	Standard phenolic compounds	
17	Apigenin	Fluka 10798
18	Caffeic acid	Fluka 60020
19	Chlorogenic acid	Fluka 25700
20	Cinnamic acid	Fluka 96340
21	2,3 dihydroxybenzoic acid	Fluka 37528
22	Ferulic acid	Fluka 46278
23	Hydroxytyrosol	Extrasynthese 4986
24	3 hydroxyphenylacetic acid	Fluka 56130
25	Luteolin	Fluka 62696
26	*m* -coumaric acid	Fluka 28180
27	*o* -coumaric acid	Fluka 28170
28	Oleuropein	Extrasynthese 0204
29	*p* -coumaric acid	Fluka 28200
30	*p* -hydroxybenzoic acid	Fluka 54630
31	*p* - hydroxyphenylacetic acid	Fluka 56140
32	Syringic acid	Fluka 86230
33	Tyrosol	Fluka 56105
34	Vanillic acid	Fluka 94770
35	Vanilin	Fluka 94750

4.2. Methods

4.2.1. Maturation Index

The maturation index (MI) of olive fruits was determined according to the method given in Vinha et al. (2005). Olive fruits, 100 for each sample, were randomly taken, classified into the categories below. The categories were: 0 – olives with intense green or dark green epidermis; 1 – olives with yellow or yellowish green epidermis; 2 – olives with yellowish epidermis but with reddish spots or areas over less than half of the

fruit; 3 – olives with reddish or light violet epidermis over more than half of the fruit; 4 – olives with black epidermis and totally white pulp; 5 – olives with black epidermis and less than 50% purple pulp; 6 – olives with black epidermis and violet (more than 50%) or purple pulp; 7 – olives with black epidermis and totally dark pulp.

With *a* to *h* being the number of fruits in each category, the MI is

$$MI = (a*0 + b*1 + c*2 + d*3 + e*4 + f*5 + g*6 + h*7)/100 \quad (4.1)$$

Green olives MI = 1.48 - 2.56

Mature olives MI = 3.10 - 4.65

4.2.2. Oxidative Stability

Peroxide value (PV) were determined according to the analytical method described in European Official Method of Analysis (Commission Regulation EEC N-2568/91) and expressed as meq O_2/kg. For the evaluation of oxidative stability of oils, samples were subjected to oxidative conditions in dark at 60°C and oxidation of oil samples was monitored for eleven days in terms of PV. In the text, the number beside 'PV' term represents the day when the observation was taken during the oxidation test. For the replicated samples, the relative standard deviation (RSD) was found in a range 3% and 11%. It is calculated as follows:

$$\text{Relative standard deviation, } RSD = \frac{100 \times S}{\overline{X}} \quad (4.2)$$

where

S = Standard deviation

$\overline{X}$ = Average

4.2.2.1. Standardization of 0.01 M Sodium Thiosulphate

2 g of KIO_3 (potassium iodate) was dried in an incubator (Memmert) at 90-100 $°C$ for 1-2 hours. After 1-2 hours, 0.001 mol/L KIO_3 solution (≈ 0.1070 gr KIO_3/500 mL deionised water) was prepared with dried potassium iodate. Exact weight of KIO_3 was recorded. In order to prepare 0.5 M H_2SO_4 solution, 2.8 mL of H_2SO_4 (96% purity) was diluted to 100 mL with deionised water. For preparation of starch solution; 1 g of starch was weighed and dissolved in 10 mL of deionised water. 90 mL of boiling deionised water was added to starch solution and boiling continued for 2-3 minutes.

Before titration, 0.2 g of KI (potassium iodine) was weighed and 1 mL of H_2SO_4 (0.5 M), and 50 mL of KIO_3 (0.001 M) solution were added. Reddish brown solution was titrated with sodium thiosulphate (0.01 mol/L) until the solution has lost its initial reddish brown colour and has become pale yellow. Starch indicator (2 mL) was added into pale yellow solution and titration was continued until the solution become colourless. After the titration was completed, sodium thiosulphate spent during titration was recorded.

Molarity of standardized sodium thiosulphate was calculated by means of the following equations.

$$M_{KIO_3} = \frac{m_{KIO_3}(g) / MW_{KIO_3}(g/mol)}{V_{KIO_3}(mL) solution} \qquad (4.3)$$

$$M_{sodiumthiosülphate} = \frac{6 \times M_{KIO_3}(mol/L) \times V_{KIO_3}(mL)}{V_{sodiumthiosülphate}(mL) spent} \qquad (4.4)$$

where

m_{KIO_3} = weight of KIO_3 (0.1070 g)

MW_{KIO_3} = molecular weight of KIO_3 (214 g/mol)

$V_{KIO_3}solution =$ Total volume of KIO_3 solution (500 mL)

$V_{KIO_3} =$ Volume of KIO_3 solution (50 mL)

$V_{sodiumthiosülfate} =$ Amount of sodium thiosulphate used in titration

4.2.2.2. Determination of Peroxide Value

10 mL of chloroform, 15 mL of acetic acid and 1 mL of potassium iodide solution (recently prepared saturated aqueous solution) were added into 3 g of olive oil samples and mixed rapidly for 1 min. After that, sample was kept away from the light at the room temperature (15-25 $°C$) for exactly five minutes. Finally, 75 mL of deionised water and 0.5 mL of starch solution were added.

Titration was carried out with 0.002 M sodium thiosulphate solution until the blue colour of solution become colourless and total sodium thiosulphate volume spent during titration was recorded. At the measurements carried out at first day, 0.002 M sodium thiosulphate was used without standardization and as a result of this measure, peroxide values of some olive oil samples were found over 12. Therefore, standardized 0.01 M sodium thiosulphate was used at the analysis of these samples for following days and 1-2 g of oil sample was weighted instead of 3 g.

The method used for calculation of peroxide values in terms of meq O_2/kg oil;

$$PV = \frac{V(mL) \times M(mol/L) \times 1000}{m(g)} \qquad (4.5)$$

where

V: mL of sodium thiosulphate solution required to titrate the sample

M: molarity of sodium thiosulphate solution

m: weight in g of the sample

4.2.3. Colour

A colorimeter (chromometer type CR-400, Minolta Sensing, Osaka, Japan) was used to assess the oil colour. Colour coordinates were measured following the white calibration (For illuminants D_{65}, Y=93.5, x=0.3140, y=0.3318). Before measurement, the colour spaces were selected. For absolute measurement, the specimen (approximately 20 mL of oil sample) was placed on the measuring head of instrument while in the measurement screen and three readings were taken at three different positions. Measurements were carried out under the same temperature conditions as calibration. For samples, reflected object colour with the colour spaces, L* a* b*, Hunter Lab, L* C* h* were measured by chromometer. Following the measurements, the data including L* a* b*, Hunter Lab, L* C* h* colour spaces was displayed in the measurement screen. The oil colour was reported as the average of three readings for L*, a*, b*.

Hunter 1948 (Lab), CIE 1976 (L* a* b*), L* C* h* colour spaces are colour-opponent space with dimension ***L*** for luminance and ***a*** and ***b*** for the colour-opponent dimensions, based on nonlinearly-compressed CIE XYZ colour space coordinates created by the International Commission on Illumination (CIE) in 1931. The opponent colour theory suggests that there are three opponent channels: black versus white, red versus green and blue versus yellow. The three basic coordinates represent the lightness of the colour (L*, L*=0 indicates black and L*=100 indicates white), its position between red/magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow), ranging from – 120 to 120.

4.2.4. Total Phenol Content

TPCs of the olive oil extracts were determined by the Folin–Ciocalteau spectrophotometric method at 765 nm, in terms of gallic acid as mg GA/kg oil (Montedoro, et al. 1992). The measurements were repeated three times. For the replicated samples, RSD was found in a range 0.01% and 12%. GA calibration curves were obtained each year (R^2 = 0.99 and 0.97).

4.2.4.1. Extraction Procedure

10 mL of methanol/water mixture (80:20 v/v) plus Tween 20 was added to 2 g of olive oil sample and mixed with a homogenizer (Heidolph–SilentCrusher M, Germany) at 25000 rpm for 1 min and centrifuged at 5000 rpm for 10 min (Nüve NF 615, Ankara, Turkey). After the centrifugation, supernatant (methanolic extract) was collected in a clean tube. The extraction was repeated two times (only with addition of 10 mL methanol/water) and each time, supernatant was collected in the same tube. Methanolic extract was recorded as total volume.

4.2.4.2. Folin_Ciocalteu Method

Immediately following the extraction, 1 mol of aliquot of the aqueous- methanol solution of phenolic compounds extracted from olive oil was diluted to 6 mL with deionised water. 0.5 mL of Folin_Ciocalteau reagent was added and waited for 1 min. Then, 2 mL of Na_2CO_3 solution (15% g/mL) was added and diluted with 1.5 mL of deionised water and mixed with a vortex (Velp Scientifika, Europe) for 30 second. The same protocol was repeated for blank samples prepared as parallel to olive oil samples by using of 1 mL of methanol/water mixture instead of phenolic extract. After the samples were mixed with a vortex, they were left in a dark place for 2 hours and then total phenol content of extract was determined by spectrophotometric method at 765 nm, using a GA calibration curve.

GA calibration curve was constructed by means of the standard solution of GA that was prepared with different concentrations changing from 0.01 mg/mL to 1 mg/mL. Three parallel analyses were prepared for standard solution of GA (0.01 mg/mol–1 mg/mL) obtained from mother solution of GA (25 mg GA/250 mL deionised water) and blank sample. GA calibration curve was obtained with a spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Japan) using the absorbance values at 765 nm. Absorbance values were converted to concentration by means of the GA calibration curve and TPC was determined in terms of GA as mg GA/kg oil.

4.2.5. HPLC Analysis of Phenolic Compounds

4.2.5.1. Phenolic Extraction

The phenolic extracts were obtained following the procedure of Brenes et al. (1999). Briefly, a sample of olive oil (14 g) was extracted by using 4x14 mL of methanol/water (80:20 v/v), 0.01 mol of GA solution (0.05g GA/25 mL methanol-water) as the internal standard was added to sample at the beginning of analysis and mixed with a homogenizer, then centrifuged to separate the phases. Supernatant (phenolic extract) was collected in a clean tube. Methanol was removed with a rotary evaporator (Heidolph Laborota-4000, Germany) for 22 minutes at 35°C under vacuum, and then 15 mL of acetonitrile was added to the residue and washed with (3×20 mL) of hexane. The resulting acetonitrile solution was evaporated under vacuum for 37 minutes, at 35°C. Residue was flushed with nitrogen for approximately 10 minutes and dissolved in 1 mL of methanol/water. Final extract was filtered through a 0.45 μm pore-size membrane filter (Minisart, Sartorious, Goettingen, Germany) and transferred into a tube. 20 μL of extract was immediately injected to HPLC.

4.2.5.2. HPLC Analysis

HPLC system with a Perkin Elmer (PE) series 200 pump (Norwalk CT 06859 , USA) , PE series 200 diode array detector, PE-Nelson 900 series interface, Meta Therm HPLC column heater (series no:9540, Torrance) and a 5 μm, 25 cm×4.6 mm, C18 column (Ace, Aberdeen, Scotland) was used to analyse phenolic compounds. Separation was achieved by elution gradient using an initial composition of 90% water (pH adjusted to 3.1 with 0.2% acetic acid) and 10% methanol. The concentration of the methanol was increased to 30% in 10 min and maintained for 20 minutes. Subsequently, the methanol percentage was raised to 40% in 10 min, maintained for 5 min, increased to 50% in 5 min, and maintained another 5 min. Finally, methanol percentage was increased to 60, 70, and 100% in 5 min periods. Initial conditions were reached in 15 min. The flow rate was 1 mL/min. Column temperature was kept at 35°C. In order to

obtain effective separation of individual phenolic compounds, degassing of mobile phase was provided by helium gas during the HPLC analysis.

Identification and quantification of phenolic compounds;

Phenolic compounds were identified by comparing retention times with those of commercial standards at 280 and 320 nm. Phenolic compounds were quantified by using internal standard method. Internal standard added to the oil sample in known concentration to enable the qualitative identification and quantitative determination of the phenolic compounds. Concentration ratio was found as the ratio between the amount of component in the sample and internal standard component in the same sample. This ratio for the samples was then used to quantify phenolic compounds from 4-point calibration curves (R^2 ranges between 0.965 and 0.999). The internal standard was also used for the calibration by plotting the ratio of the reference component signal to the internal standard signal as a function of the concentration of the standards. Internal standard method was preferred in order to correct any loss of phenolic compounds during sample preparation.

4.2.6. Data Analysis

4.2.6.1. Univariate Statistical Analysis

Chemical data including TPC, PV, and colour measurements of extracted and commercial EVOO samples were analyzed by analysis of variance (ANOVA). Tukey's test at 5% significance level was used for pairwise comparison of means (Minitab 14, Minitab Inc., State College, USA).

4.2.6.2. Multivariate Statistical Analysis

PCA and PLS-DA models were built to analyze the influence of the cultivar, geographical origin, and harvest year. The multivariate analyses were performed by SIMCA-P v.10.5 (Umetrics, Umea, Sweden). Multivariate data of all measurements

obtained over two years were evaluated to investigate the effect of harvest year, effect of cultivar, and growing region. Data obtained from analyses were put in a matrix with the rows relating to the olive varieties and geographical origins for classification based on cultivar and geographical areas (*n* observations) and the columns relating to the individual phenolic compounds and chemical measurements (*k* variables). The multivariate data matrix **X** of size (48x31) represents 48 extracted EVOO samples analyzed for two years, with 18 phenolic compounds determined by HPLC, TPC, 9 PV measurements, and 3 colour parameters. With regard to commercial EVOO samples, the multivariate data matrix **X** composed of 47x25 elements. 47 rows represent commercial EVOO samples analyzed for two years and 25 columns represent 20 phenolic compounds determined by HPLC, TPC, PV0, and 3 colour parameters.

Prior to multivariate analysis, the data were pre-processed by the standard procedure. This procedure includes mean-centering (the mean value of each variable is calculated and subtracted from the data), and transformations for the variables. Simca-P software summarized the *goodness of fit* parameter R^2 and the *goodness of prediction* parameter Q^2. The *goodness of prediction* parameter Q^2 is calculated by leave-one-out cross validation and indicates the predictive power of the model. PCA results were summarized in the plots of scores, showing the patterns present among the observations and loadings, showing which variables are responsible for the similarity and dissimilarity between the samples, and also how the variables are correlated.

Simca models on principal components were developed for classification of oil samples according to geographical origin and cultivar. The distance from the model for class 1 was plotted against that from model 2. The discrimination of each class was shown in the Cooman's plots of the class models.

PLS-DA analyses were performed after a general PCA model of data set. Result of PLS-DA gives R^2Y (cum), the fraction of the variation of Y 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. As a result of PLS-DA, each variable can have different importance in describing one or more classes. Importance of all variables was given in variable importance (VIP) list. VIP values indicate the most important variables that provide discrimination of samples.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. Extracted Extra Virgin Olive Oils

5.1.1. Maturation Index

Maturation index (MI) of olive fruits was determined only in 2006 harvest year. Since the harvesting was done at the same times of the year (the first and second weeks of November in Bornova, Izmir and the third week of November in Edremit, Balıkesir), the olives of both years were considered to be at the same maturity level. Maturation index (MI) of all olive examined in this study varied between 2.85 and 4.51 (Table 5.1). Erkence and domat olives had low maturation indices whereas other olive varieties had high maturation indices.

Table 5.1. Average MI of olives

Olive varieties	M.I.
M	4.27
E	2.85
G	4.36
A	3.88
D	2.97
N	4.11
GE	4.51
AE	3.72

ANOVA were performed for PV, colour and TPC on the basis of olive oil types.

5.1.2. Peroxide Value

5.1.2.1. 2005 Harvest Year

Peroxide value is used as an indicator of the initial oxidation because it measures the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation.

The autoxidation reaction of oils, free radical chain reaction, includes three steps; initiation, propagation and termination. At the initiation step of oxidation, lipid free radical $R^{\cdot}$, alkoxy radical $RO^{\cdot}$, peroxy radical $ROO^{\cdot}$, and hydrogen radical $H^{\cdot}$ are formed by hydroperoxide decomposition, by metal catalysis, heating or by exposure to light. After initiation, oxidation is propagated by abstraction of hydrogen atoms at positions α to fatty acid double bonds, producing free radical species $R^{\cdot}$. This free radical combines with oxygen to form peroxy radicals $ROO^{\cdot}$, which can in turn abstract hydrogen from another unsaturated molecule to yield hydoperoxides ($ROOH$) and new free radicals ($R^{\cdot}$). This reaction initiates the propagation step. The new $R^{\cdot}$ groups react with oxygen, and the sequence of reactions just described is repeated. At the end of the propagation step, $R^{\cdot}$ groups interact with each other and neutralized and $ROO^{\cdot}$ groups produce nonradical compounds $ROOR$. This formation is the main reaction of termination step. Hydroperoxides, the primary initial products of lipid oxidation, are relatively unstable and decompose into secondary oxidation products, such as aldehydes, ketones, hydrocarbons, and some acids, furans that decrease the nutritional quality of oil (Fennema 1996).

Initial PVs of all oil samples except nizip oil were below the upper legal limit values established by EU regulations (Commission Regulation EEC No 2568/91) and Turkish Food Codex (Communication No 98/7) for the EVOO category (PV<20 meq/kg). Considering the initial PV, erkence and nizip oils had higher concentration of peroxide whereas gemlik-edremit oil exhibited lower concentration of peroxide. Changes in PV during the oxidation for each variety are given in Figure 5.1 and Figure 5.2. According to the Figure 5.1 and 5.2, PVs of oil samples were significantly increased during the oxidation (for eleven days). When the stability of olive oil related

to olive variety was examined, it was observed that gemlik and ayvalik variety (from Bornova and Edremit) were more stable against oxidation. During the oxidation period, peroxide content of gemlik and ayvalik oils increased slightly. It was clear that gemlik and ayvalik oils showed small changes in PV and oxidation of these oil samples progressed slowly within 4 days. On the contrary, memecik, erkence, domat and nizip oils were relatively sensitive to oxidation during 8 days at $60\,°C$ and reached up to highest concentration of peroxides at the end of the oxidation (Figure 5.1).

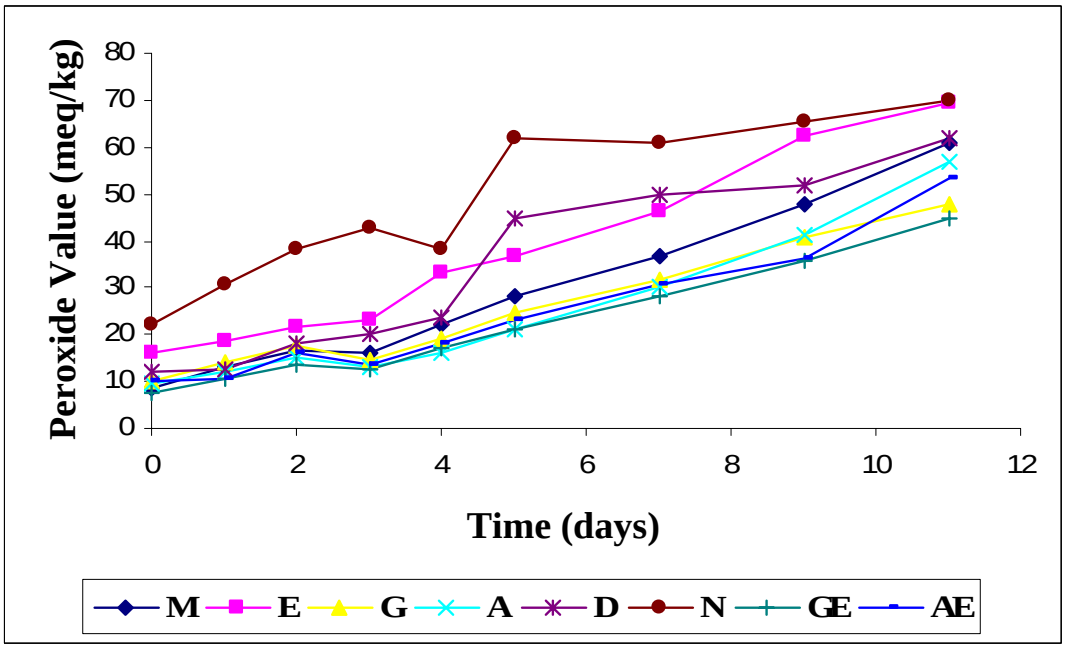


Figure 5.1. Changes in PVs of oils during oxidation (2005 harvest year)

In order to determine the differences among the EVOO samples in terms of initial PV, ANOVA was applied with respect to $\alpha = 0.05$ significance level. There was sufficient evidence to conclude that mean initial PVs of oil samples were different (p-value $\cong$ 0 << 0.05). According to Tukey's multiple comparison tests initial PVs of erkence and nizip oils were significantly higher and initial PV of gemlik edremit oil was significantly lower. There were no differences among olive varieties according to ΔPV (Table 5.2).

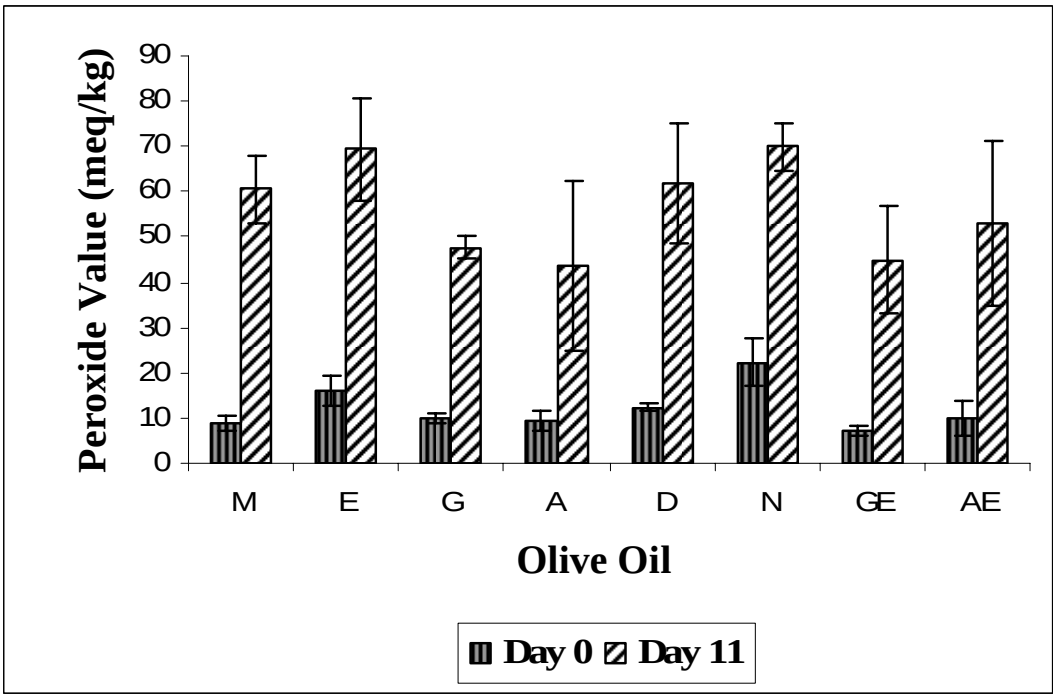


Figure 5.2. Changes in PVs of oils between Day 0 and Day 11 (2005 harvest year)

Table 5.2. Initial PV and ΔPV of olive oil samples in 2005 harvest year (mean ± SD)

Olive Oil	PVs	ΔPV
M	8.68 ∓ 1.58^{b}	51.95±9.05
E	16.08 ∓ 3.35^{cd}	53.42±14.62
G	9.93 ∓ 0.86^{bc}	37.73±2.58
A	9.40 ∓ 1.37^{bc}	34.14±4.44
D	12.28 ∓ 0.852^{bc}	49.6±14.12
N	22.30 ∓ 5.36^{d}	47.56±0.37
GE	7.37 ∓ 1.1^{ab}	37.58±12.7
AE	9.98 ∓ 3.94^{bc}	43.1±22.14

a-d:Different letters within a column indicate samples that were significantly different ($p < 0.05$).

5.1.2.2. 2006 Harvest Year

Initial PVs of olive oil samples were all below 20 meq/kg (Figure 5.3 and Figure 5.4). The peroxide contents of memecik and erkence oils were higher and the peroxide content of ayvalik oil was lower. In order to examine the oxidation of each oil sample during 11 days, the changes in PVs of oil samples versus oxidation time are exhibited in Figure 5.3. Oxidation of all oil samples progressed slowly at the beginning of oxidation (within 4 days) but a significant increase was observed in peroxide content for all samples after 5th day of oxidation. When the behaviour of each oil samples during the oxidation period was evaluated, it was noticed that gemlik oil (Bornova and Edremit) was relatively stable whereas domat, erkence and ayvalik (Bornova and Edremit) oils were sensitive (Figure 5.4).

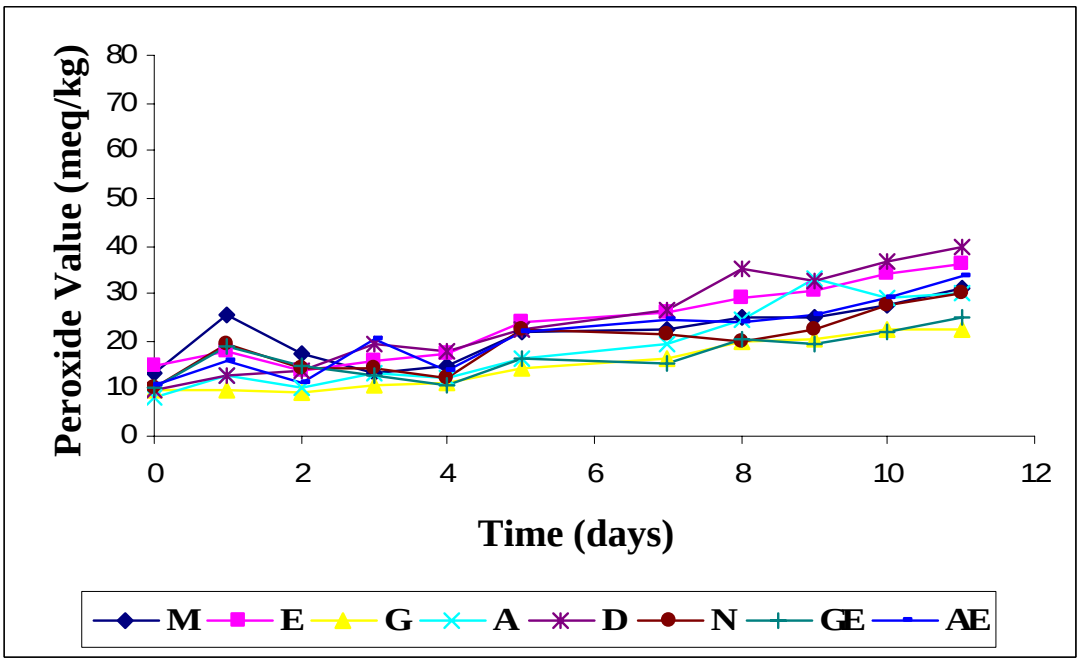


Figure 5.3. Changes in PVs of oils during oxidation (2006 harvest year)

ANOVA was also applied to data in order to determine the differences among the EVOO obtained from different olive varieties harvested in 2006. Initial PVs of the oils varied from 8.21 to 14.548 for ayvalik and erkence oils, respectively (Table 5.3).

However, these differences among the oil samples were not statistically significant ($p>0.05$). ΔPVs of oil samples were not statistically different.

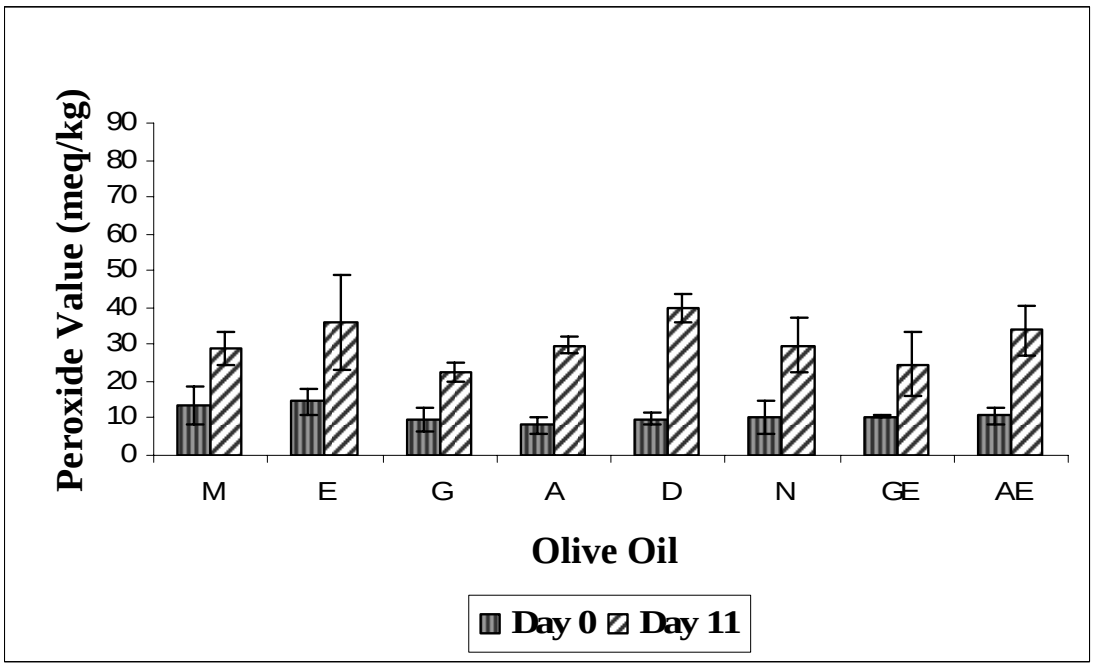


Figure 5.4. Changes in PVs of oils between Day 0 and Day 11 (2006 harvest year)

Table 5.3. Initial PV and ΔPV of olive oil samples in 2006 harvest year (mean ± SD)

Olive Oil	PVs	ΔPV
M	13.45∓5.22	15.41±0.98
E	14.55∓3.54	21.65±9.46
G	9.57∓3.15	12.79±1.10
A	8.21∓2.27	21.66±4.43
D	9.84∓1.52	29.99±5.09
N	10.31∓4.39	19.51±3.24
GE	10.37∓0.38	14.37±8.91
AE	10.64∓2.44	23.16±9.04

5.1.3. Colour

5.1.3.1. 2005 Harvest Year

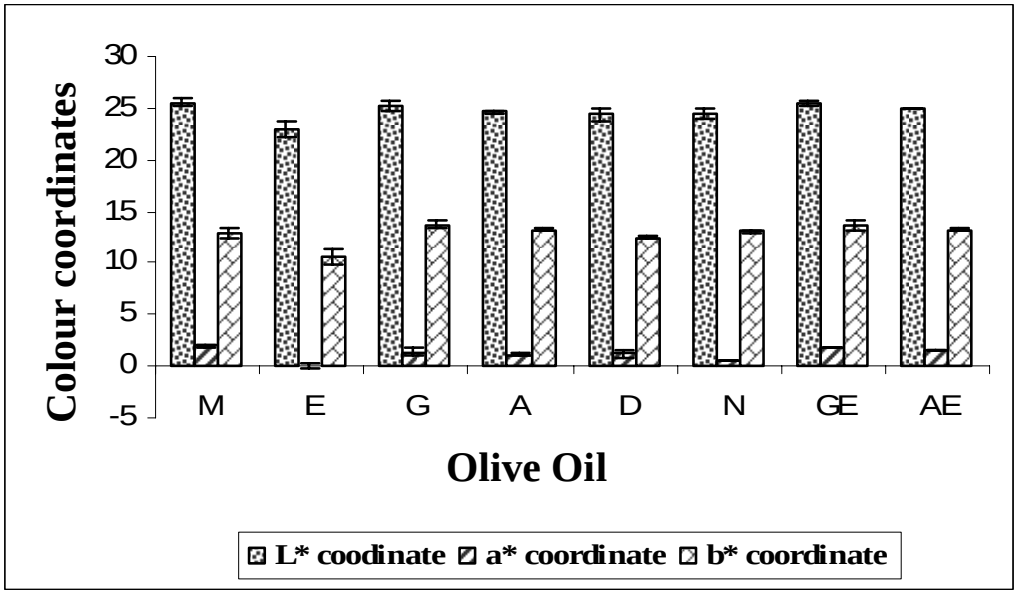
The colour of oil affects the consumer's perception of quality. This property can also be used as an estimate of pigment content. It has been mentioned that olive oil colour demonstrates the variability (from green to yellow) depending on several factors, such as olive variety, olive maturation index, oil extraction methods, harvest year, and conservation conditions (Moyano, et al. 2001, Romero, et al. 2003). ANOVA was applied to the CIE- L*, a*, b* colour coordinates. The results indicated that there were significant differences among the EVOO samples based on olive varieties.

The results of the multiple comparison test at the 5% level and colour coordinates as the means ± SD of measurements are given in Table 5.4. The results showed that erkence oils had different colour parameters from other oils (Figure 5.5). Luminosity value (L*) varied from 22.92 to 25.59 for erkence and memecik oils, respectively. Erkence oil was different from other oils in low L*. With regard to a* values, the highest negative a* value and lowest negative a* values were -1.97 and -0.07, for memecik and erkence oils. Erkence oil had lower b* value of 10.65. Also in other studies, oil colour has been assessed by a colorimeter using the CIELAB colorimetric system and expressed as chromatic ordinates L*, a*, and b*, which were reported as [76.4-84.79], [(-1.59)-(-1.03)] and [95.8-105.9] (Romero, et al. 2003). Olive oil colour was also determined with a visible spectrophotometer and an artificial neural network (ANN) for VOO and refined olive oil mixture and the L*, a*, b* values were found as 36.5, 0.9, and 21.2, respectively (Kılıc, et al. 2007). Our results except for a* colour coordinate were not similar to those of found in the studies of Romero et al. and Kılıc et al. It has been expressed that the main carotenoids of VOO are lutein and β-carotene that are responsible for the yellow colour. Chlorophyllic compounds, such as chlorophylls (a) and (b), and pheophytins (a) and (b) are responsible for the green colour of VOO (Luaces, et al. 2005). Colour differences in olive oils can be attributed to the differences in concentrations of these pigments; chlorophylls and carotenoids.

Table 5.4. Colour coordinates of olive oil samples in 2005 harvest year (mean ± SD)

	Colour Coordinates		
Olive Oil	**L***	**a***	**b***
M	25.59 ∓ 0.35^{c}	-1.97 ∓ 0.07^{a}	12.95 ∓ 0.51^{b}
E	22.92 ∓ 0.70^{a}	-0.07 ∓ 0.25^{ef}	10.65 ∓ 0.77^{a}
G	25.21 ∓ 0.45^{bc}	-1.38 ∓ 0.38^{ac}	13.76 ∓ 0.46^{b}
A	24.61 ∓ 0.11^{b}	-1.15 ∓ 0.1^{c}	13.22 ∓ 0.12^{b}
D	24.39 ∓ 0.62^{b}	-1.18 ∓ 0.42^{bcd}	12.4 ∓ 0.12^{b}
N	24.52 ∓ 0.44^{b}	-0.48 ∓ 0.05^{de}	13.06 ∓ 0.09^{b}
GE	25.40 ∓ 0.22^{bc}	-1.80 ∓ 0.06^{ab}	13.67 ∓ 0.43^{b}
AE	24.94 ∓ 0.06^{bc}	-1.62 ∓ 0.04^{ac}	13.23 ∓ 0.12^{b}

a-d:Different letters within a column indicate samples that were significantly different ($p < 0.05$).



(Note: The value of a* coordinate is given as absolute value in the graph.)

Figure 5.5. Colour coordinates of EVOOs in 2005 harvest year

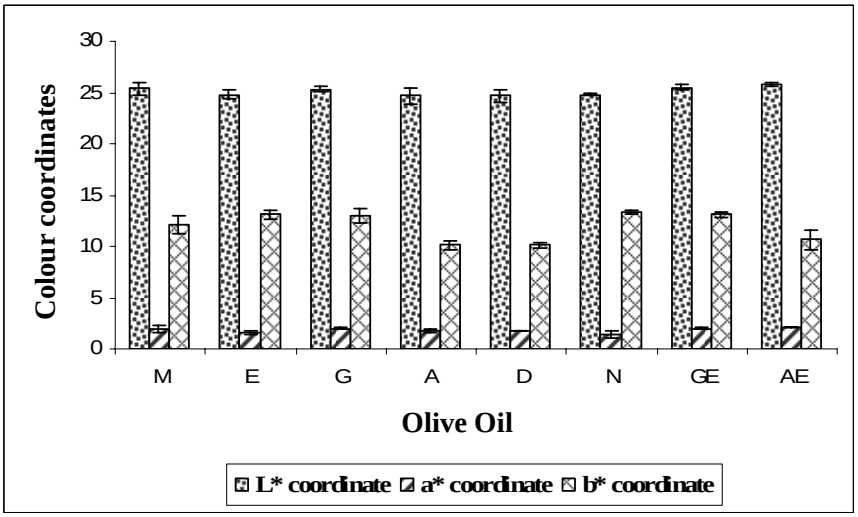
5.1.3.2. 2006 Harvest Year

Significant differences were determined among the colour coordinates of EVOOs of 2006 season. The results of the multiple comparison test and colour coordinates as the means ± SD of measurements are given in Table 5.5. In terms of a* value, erkence and nizip oils are significantly different than other oils. In terms of b* value, ayvalik (Bornova and Edremit) and domat oils possess lower b* values than other oils (Figure 5.6).

Table 5.5. Colour coordinates of olive oil samples in 2006 harvest year (mean ± SD)

	Colour Coordinates		
Olive Oil	**L***	**a***	**b***
M	25.37∓0.65^{ab}	-1.93∓0.32 ^{ab}	12.12∓0.88 ^{ba}
E	24.76∓0.42^{a}	-1.52∓0.17 ^{b}	13.08∓0.45 ^{b}
G	25.32∓0.20 ^{ab}	-2.00∓0.12 ^{a}	12.95∓0.76 ^{b}
A	24.66∓0.74 ^{ab}	-1.79∓0.13 ^{ab}	10.14∓0.44 ^{a}
D	24.67∓0.61^{ab}	-1.74∓0.07 ^{ab}	10.11∓0.3 ^{a}
N	24.81∓0.15 ^{ab}	-1.41∓0.27 ^{b}	13.34∓0.13 ^{b}
GE	25.52∓0.32 ^{ab}	-2.00∓0.11 ^{a}	13.07∓0.32 ^{b}
AE	25.84∓0.17 ^{b}	-2.10∓0.08 ^{a}	10.66∓0.93^{a}

a-b:Different letters within a column indicate samples that were significantly different ($p < 0.05$).



(Note: The value of a* coordinate is given as absolute value in the graph.)

Figure 5.6. Colour coordinates of EVOOs in 2006 harvest year

5.1.4. Total Phenol Content

5.1.4.1. 2005 Harvest Year

The significance of differences at a 5% level among TPC averages of oils was determined by one-way ANOVA and Tukey's test. Significant differences were observed among olive oil samples. The mean values and standard deviations of TPCs for the EVOO samples are presented in Table 5.6. TPCs of the samples can be considered medium-high levels in accordance with previous reports (Aparicio, et al. 1999, Cerretani, et al. 2006, Psomiadou, et al. 2002). It was reported that the TPC of Turkish olive oils ranged from 22.5 to 97.1 mg of GA/kg of oil in 2003 season (Tanilgan, et al. 2007). However, it is difficult to reach a general conclusion about TPC if it is not for the same harvest year.

Erkence oils had the highest TPC (356.65 ± 59.2 mg GA/kg of oil), while nizip had the lowest (102.4±32.68 mg GA/kg of oil). TPC of memecik, ayvalik and domat oils were close to that of erkence oil (Figure 5.7). It was found that there were no differences between TPCs of gemlik olives grown in south of Aegean region (Bornova)

and north of Aegean region (Edremit) whereas TPCs of EVOOs produced from ayvalik olives grown in Bornova and Edremit were relatively different.

Table 5.6. TPC of olive oil samples in 2005 harvest year (mean ± SD)

Olive Oil	TPC
M	330.92 ∓ 35.69^{c}
E	356.65 ∓ 59.2^{c}
G	274.09 ∓ 21.61^{bc}
A	329.75 ∓ 20.21^{c}
D	301.99 ∓ 83.4^{bc}
N	102.4 ∓ 32.68^{a}
GE	245.21 ∓ 36.98^{bc}
AE	186.25 ∓ 5.82^{ab}

a-c:Different letters within a column indicate samples that were significantly different ($p < 0.05$).

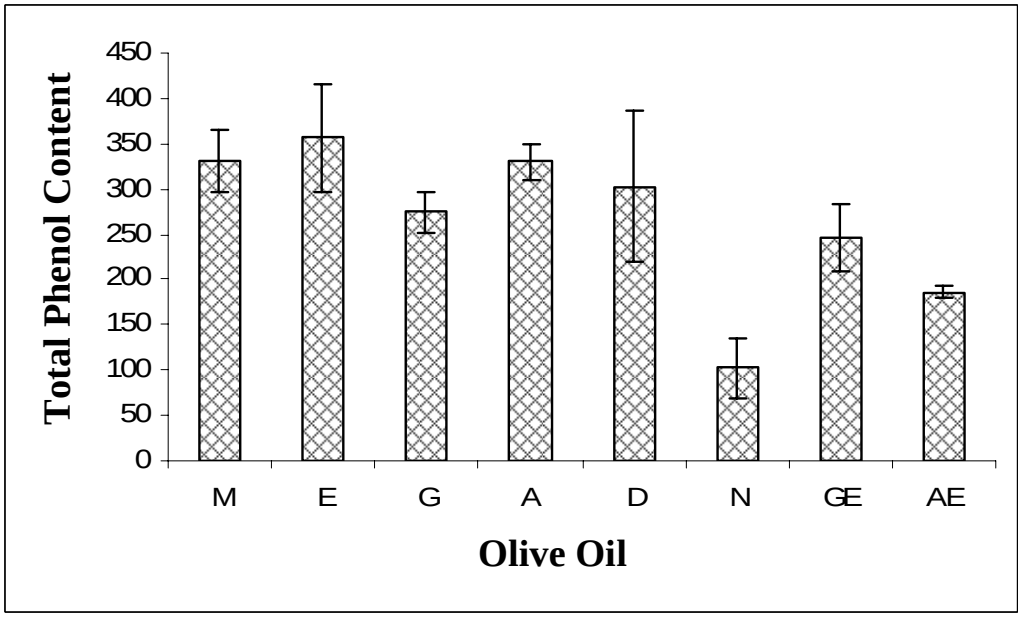


Figure 5.7. TPCs of EVOOs in 2005 harvest year

5.1.4.2. 2006 Harvest Year

Lower phenolic contents were observed in the second year. ANOVA showed significant differences among TPCs of olive oils of 2006 season. TPCs as the means ± SD of measurements are presented in Table 5.7. Mean TPC of 2006 season varied from 67.04±33.05 (ayvalik oil) to 333.37± 43.89 (erkence oil). Erkence oil was different from other oils with higher TPC (Figure 5.8).

Table 5.7. TPC of olive oil samples in 2006 harvest year (mean ± SD)

Olive Oil	TPC
M	137.15∓19.92[a]
E	333.37∓43.89[b]
G	91.57∓49.41[a]
A	67.04∓33.05[a]
D	143.8∓5.44[a]
N	112.7∓17.82[a]
GE	69.03∓21.09[a]
AE	75.46∓22.33[a]

a-b:Different letters within a column indicate samples that were significantly different ($p < 0.05$).

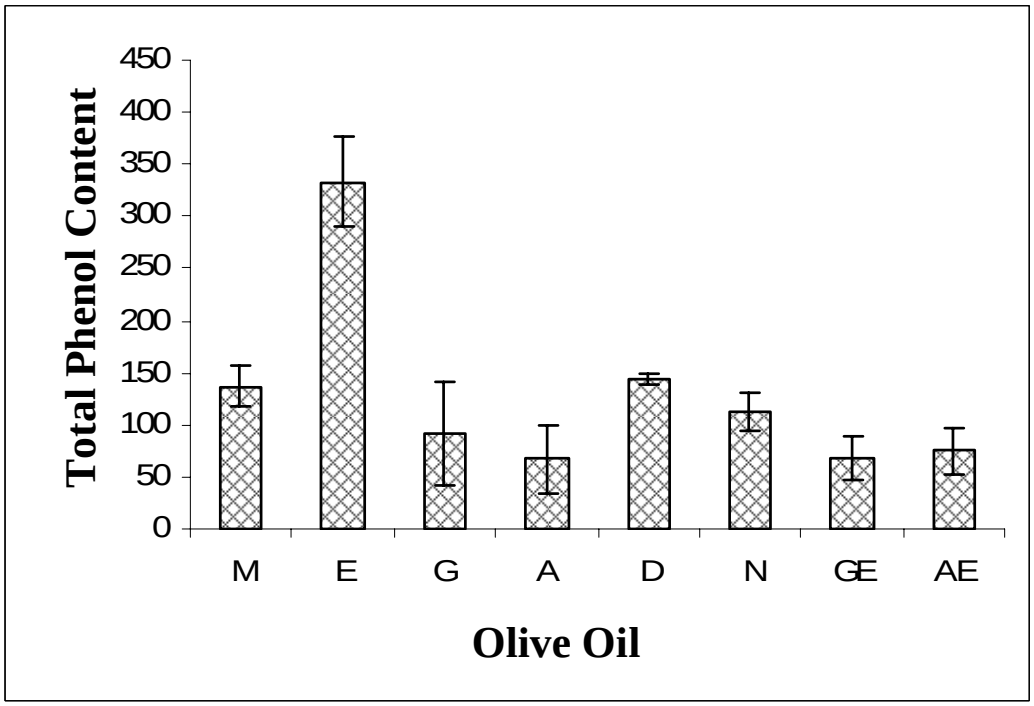


Figure 5.8. TPCs of EVOOs of 2006 harvest year

5.1.5. Influence of Harvest Year on Quality Parameters

The comparison of the oxidative stability of EVOOs associated with harvest year is shown in Figure 5.9. As shown in these figures, the oxidative stabilities of oils were affected by harvest year. All oil samples belonging to 2006 harvest year showed more resistance to oxidation as compared to previous year. This suggested that climatic conditions and crop season were important parameter for stability of olive oil. When ΔPV was considered, it was observed that gemlik olive oils showed more stable profile against oxidation whereas erkence and domat olive oils were sensitive to oxidation. The influence of harvest year on PV of olive oil has also been studied by others. It was concluded that the effect of harvest year was significant for PV ($p < 0.001$, Romero, et al. 2003) and induction time showed a significant year effect ($p < 0.002$, Ayton et al. 2007).

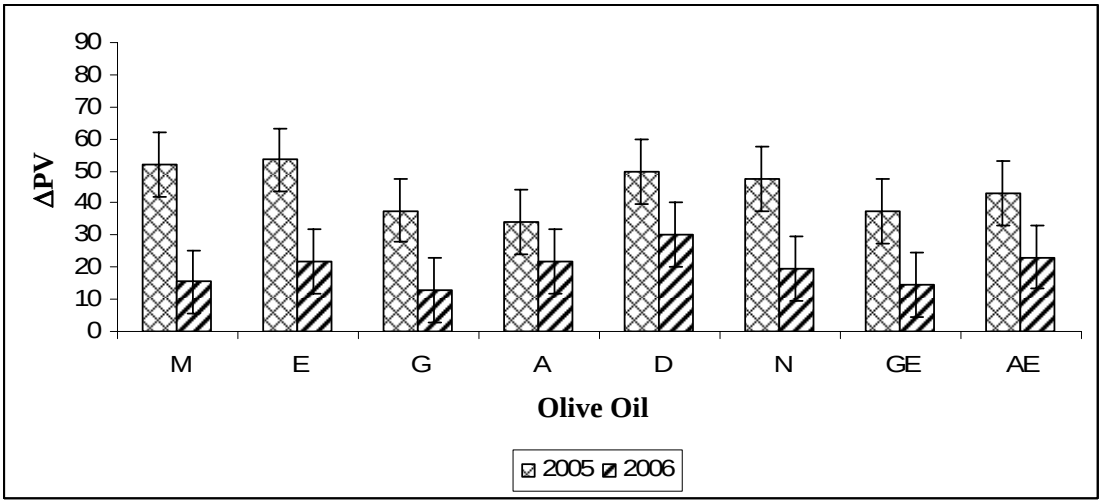
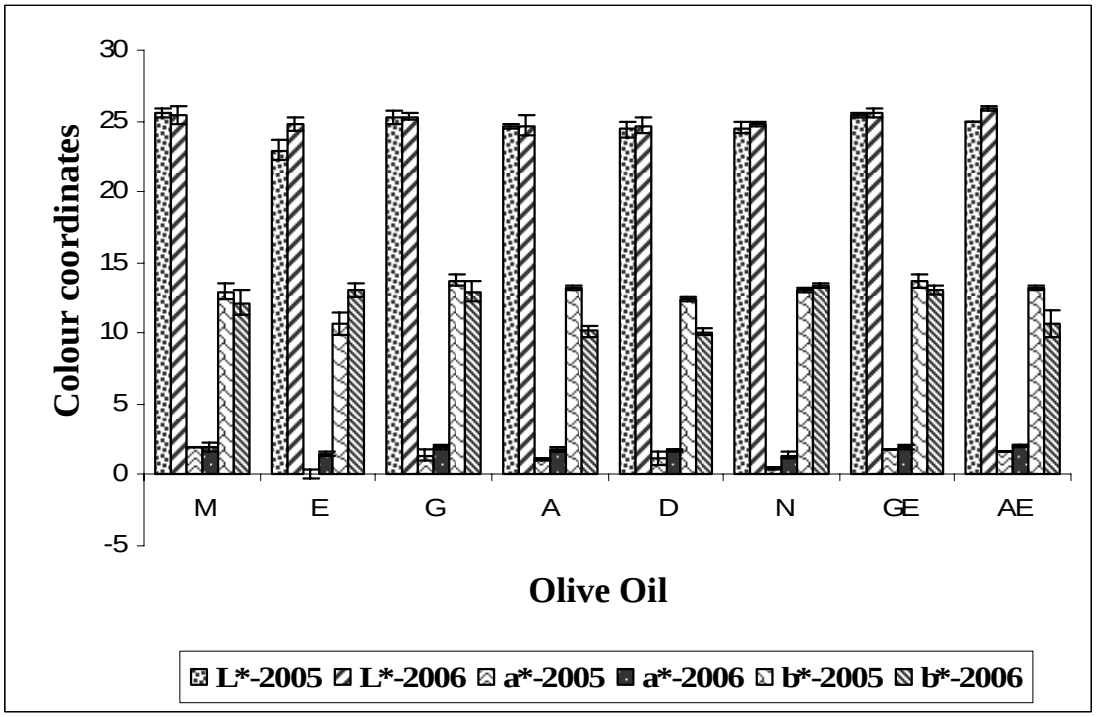


Figure 5.9. Comparison of ΔPV of oil samples for two harvest years

The comparison of colour coordinates of oils for both harvest years is shown in Figure 5.10. Erkence had the lowest L^* and less negative a^* values, while memecik, gemlik and ayvalik oils consistently showed higher L^* and more negative a^* in both years. Erkence oils were observed as the darkest of all oils. Nizip oils showed similarities to erkence oils in a^*.

Ayvalik-edremit and erkence oils of 2006 season were different for high L* value in comparison to the year before. Oils from the 2006 season showed the highest a* values indicating green colour. The b* values corresponding to the yellow colour of oils showed differences. Except for erkence oil, EVOOs of 2006 season were different from those of previous year according to their low b* values. This result supported the study of Romero et al. (2003), who found the significant differences in the pigment content and colour parameters of the oils in relation to the year. The main effect of this variation can be climatic conditions, such as temperature and rainfall regime.

There were significant differences in TPC of the oils in relation to harvest year. Except for nizip oil, TPCs of all other samples were found less than those of 2005 season (Figure 5.11). Erkence oil has the highest TPC among all oil samples for both seasons. A significant effect of the year on TPCs of the oils ($p< 0.001$) has been found by other researchers (Romero, et al. 2003, Ayton, et al. 2007).



(Note: The value of a* coordinate is given as absolute value in the graph.)

Figure 5.10. Comparison of colour coordinates of oil samples for two harvest years

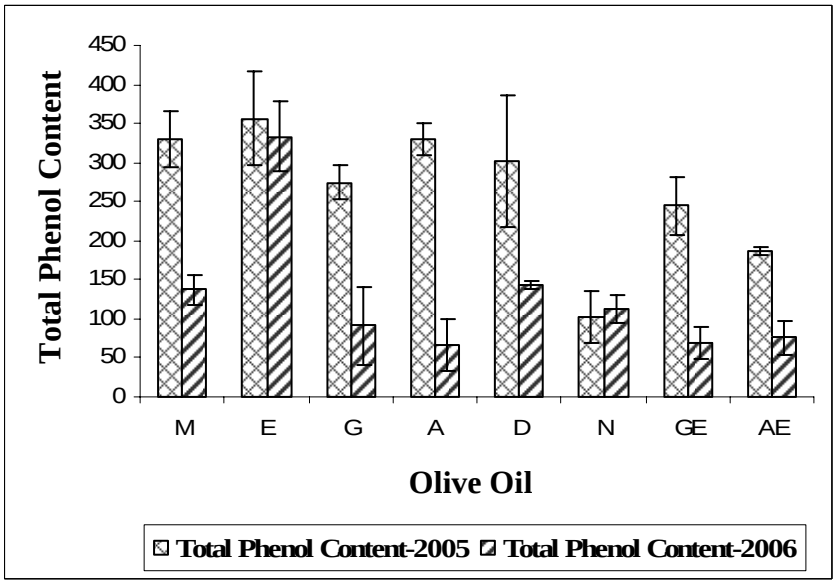


Figure 5.11. Comparison of TPCs of oil samples for two harvest years

5.1.6. Phenol Composition

Typical HPLC chromatograms of olive oils in 2005 and 2006 year are given in Figure 5.12 and Figure 5.13. All oil samples contained similar chromatographic peaks, while the quantitative amounts of phenolic compounds showed differences depending on the variety and harvest year. In all cases, the major phenolic compounds identified were hydroxytyrosol, tyrosol, vanillic acid, *p*-coumaric acid, vanillin, cinnamic acid, luteolin, and apigenin. The data (expressed in mg/kg olive oil) as the average of different batches of the same cultivar (2 to 5 in each year) were given in Table 5.8. Simple phenols such as hydroxytyrosol and tyrosol were present in all olive oils studied. The concentration of tyrosol in oils was greater than that of hydroxytyrosol for two years. The main phenolic acids identified in this study; such as vanillic acid, syringic acid and *p*-coumaric acid were also determined previously in Turkish olive oils as 0.33-0.83 mg/kg, 0.49-1.46 mg/kg, and 0.5-10.37 mg/kg, respectively (Nergiz, et al. 1991). Memecik and erkence oils contain higher levels of luteolin and apigenin for two years. These flavonoid compounds were characterized in most of the Spanish, Italian and Portuguese virgin olive oils (Vinha, et al. 2005). Several phenolic compounds, such as 4-hydroxybenzoic acid, 4-hydroxyphenylacetic, and 2,3-dihydoxybenzoic acids, were present in very low concentrations. Cinnamic acid was found in low amount in all oils for the first year, its concentration increased in the following year. This phenolic acid was identified and quantified in high levels in olive oils previously by Montedoro et al. (1992). Among the oil samples, nizip oil had the lowest contents of phenolic compounds for two years.

HPLC profiles of Turkish EVOOs in Figure 5.12 and 5.13 were compared with those given in the studies of Brenes and co-workers to identify some of the secoiridoids and lignans qualitatively since the method for phenolic identification was adopted from their studies (Brenes, et al. 2000). The unidentified peaks (number 13 and 14 in Figure 5.12, number 14 and 15 in Figure 5.13) appeared before cinnamic acid can be considered as oleuropein and oleuropein aglycon and unidentified peak (number 17 in Figure 5.12, number 18 in Figure 5.13) between luteolin and apigenin might be identified as ligstroside aglycon. Similarly, the peaks between 41–45 minutes can be attributed to dialdehydic forms of elenolic acid and lignans (1-acetoxypinoresinol and pinoresinol).

The relationship between TPC and oxidative stability has been discussed by others in terms of correlation coefficient (r = 0.72 in Blekas, et al. 2002; r = 0.87 in Aparicio, et al. 1999). In this study, a positive relation between ΔPV and TPC was observed (r = 0.56). High total phenolic concentration does not always mean 'protection against oxidation'. Phenolic compounds might contribute to the oxidative stability individually or through synergic effects. Small contribution of the minor components to the stability of oil was reported by Mateos et al. (2003). Tura et al. (2007) found that hydroxytyrosol had correlation coefficient r = 0.397 and total polyphenols had correlation coefficient r ranged 0.338 to 0.669 with oxidative stability. The dialdehydic form of elenolic acid linked to hydroxytyrosol and to tyrosol, and aglycon derivatives of oleuropein were shown to be positively correlated to the induction period (hours) of olive oil by De Stefano et al. (1999). In this study, when individual phenolic compound and ΔPV were compared, weak correlations were found with vanillin, syringic acid, and colour parameter a*, as 0.55, -0.42, 0.51, respectively, in terms of correlation coefficient r.

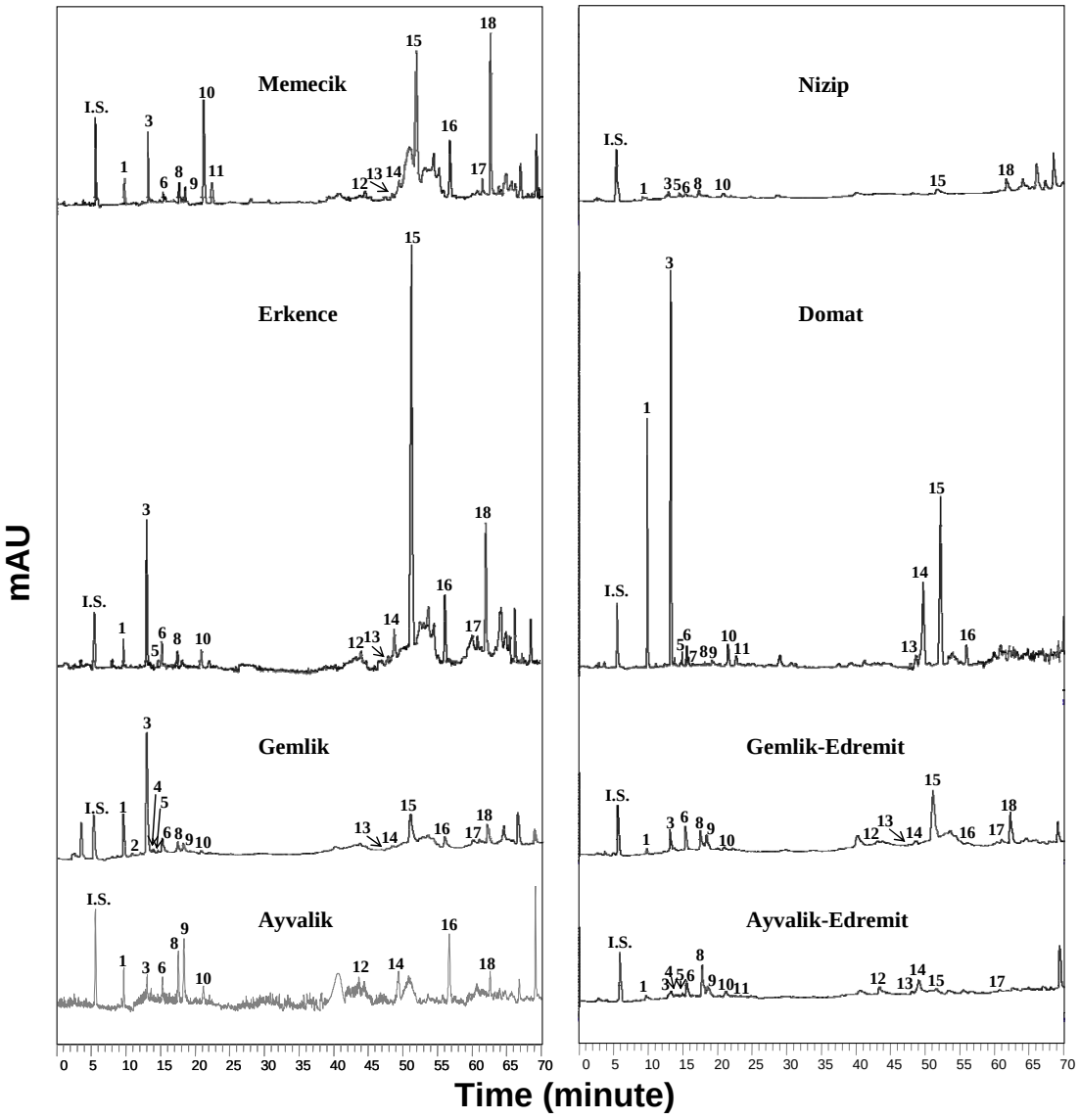


Figure 5.12. HPLC chromatograms of the phenolic extract of EVOOs in 2005 at 280 nm: (IS) gallic acid; (1) hydroxytyrosol (Hyt); (2) 2,3dihydroxybenzoic acid (Dba); (3) tyrosol (Tyr); (4) 4hydroxybenzoic acid (Hdba); (5) 4hydroxyphenylacetic acid (Hpha); (6) vanillic acid (Va); (7) caffeic acid (Ca); (8) vanillin (Val); (9) unidentified; (10) *p*-coumaric acid (Pcoa); (11) ferulic acid (Fa); (12) unidentified; (13) unidentified; (14) unidentified; (15) cinnamic acid (Cina); (16) luteolin (Lut); (17) unidentified; (18) apigenin (Apg).

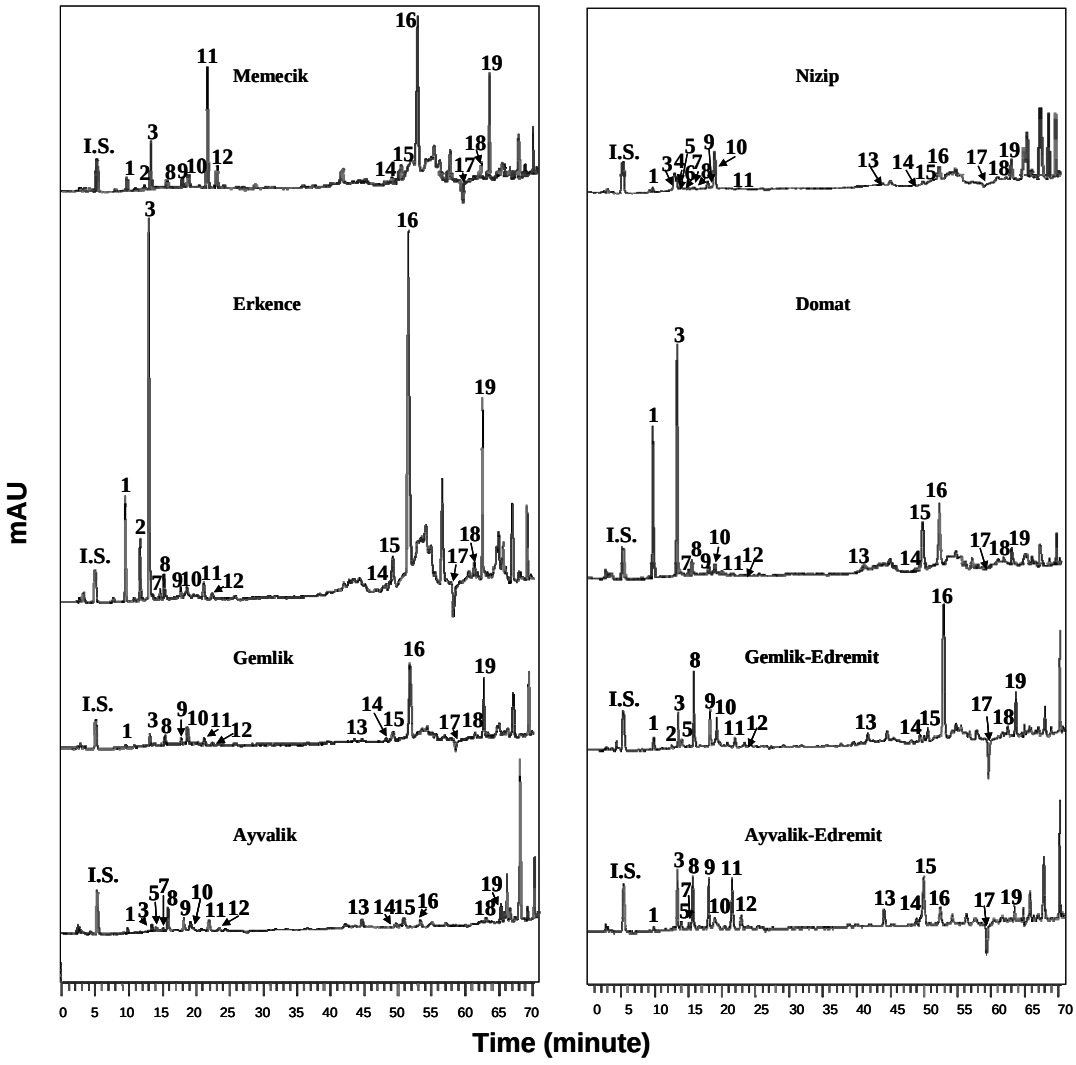


Figure 5.13.HPLC chromatograms of the phenolic extract of EVOOs in 2006 at 280 nm: (IS) gallic acid; (1) hydroxytyrosol (Hyt); (2) 4hydroxybenzoic acid (Hdba); (3) tyrosol (Tyr); (4) chlorogenic acid; (5) 2,3dihydroxybenzoic acid (Dba); (6) 4hydroxyphenylacetic acid (Hpha); (7) caffeic acid (Ca); (8) vanillic acid (Va); (9) syringic acid; (10); unidentified; (11) *p*-coumaric acid (Pcoa); (12) ferulic acid (Fa); (13) unidentified; (14) unidentified; (15) unidentified; (16) cinnamic acid (Cina); (17) luteolin (Lut); (18) unidentified; (19) apigenin (Apg).

Table 5.8. Individual phenolic compounds of EVOOs of 2005 and 2006 harvest years±

2005	Phenols	M	E	G	A	D	N	GE	AE
	Hyt[1]	2.32±2.36	0.98±0.4	3.02±1.96	1.1±0.4	4.25±6.01	0.07±0.09	1.03±1.3	0.26±0.03
	Hydba[2]	nd	nd	0.18±0.25	0.02±0.03	nd	nd	nd	0.03±0.02
	Tyr[3]	14.17±19.8	4.3±3.21	9.42±6.84	0.67±0.23	10.51±5.98	0.25±0.05	4.02±4.25	0.45±0.21
	dba[4]	nd	nd	0.11±0.19	nd	nd	nd	nd	nd
	Hypha[5]	nd	0.09±0.15	0.09±0.07	0.03±0.05	0.15±0.21	0.24±0.02	nd	0.16±0.07
	Ca[6]	nd	nd	nd	nd	0.03±0.04	nd	nd	nd
	Va[7]	0.07±0.07^{a}	0.28±0.08^{ab}	0.41±0.24^{ab}	0.13±0.03^{a}	0.47±0.42^{ab}	0.03±0.01^{a}	0.6±0.17^{b}	0.43±0.01^{ab}
	Val[8]	0.12±0.1^{a}	0.19±0.02^{a}	0.13±0.03^{a}	0.32±0.13^{ab}	0.12±0.13^{a}	0.05±0.07^{a}	0.29±0.07^{a}	0.59±0.11^{b}
	Sya[9]	nd	0.14±0.13	nd	nd	nd	nd	nd	nd
	Pcoa[10]	0.8±0.06^{b}	0.12±0.05^{a}	0.03±0.02^{a}	0.04±0.04^{a}	0.06±0.09^{a}	0.04±0.01^{a}	0.01±0.01^{a}	0.06±0.001^{a}
	Fa[11]	0.27±0.16	nd	nd	nd	0.05±0.06	nd	nd	0.02±0.02
	Peak12[12]	0.34±0.3	0.58±0.54	nd	0.16±0.28	nd	nd	0.14±0.13	1.00±0.03
	Peak13[13]	0.37±0.65^{a}	1.62±1.4^{ab}	0.13±0.12^{a}	0.05±0.09^{a}	2.84±0.22^{b}	nd	0.11±0.1^{a}	0.26±0.08^{a}
	Peak14[14]	1.88±1.8^{ab}	6.7±3.04^{b}	nd	3.33±0.94^{ab}	19.52±1.6^{c}	0.43±0.61^{a}	nd	6.36±0.97^{ab}
	Cina[15]	0.53±0.19^{a}	1.83±0.61^{b}	0.36±0.12^{a}	nd	0.71±0.08^{a}	0.04±0.003^{a}	0.44±0.12^{a}	0.02±0.03^{a}
	Lut[16]	2.4±0.27^{b}	2.74±1.24^{b}	1.38±0.6^{ab}	2.27±0.74^{b}	0.4±0.56^{a}	nd	0.31±0.39^{a}	nd
	Peak17[17]	0.87±0.17	0.70±0.61	0.32±0.29	nd	nd	nd	0.18±0.06	nd
	Apig[18]	10.66±1.57^{b}	8.68±4.58^{b}	4.83±1.14^{ab}	0.84±0.3^{a}	0.92±1.3^{a}	1.46±1.48^{a}	4.6±1.14^{ab}	nd

(cont. on next page)

Table 5.8. (cont.) Individual phenolic compounds of EVOOs of 2005 and 2006 harvest years±

2006	Phenols	M	E	G	A	D	N	GE	AE
	Hyt[1]	0.25±0.31	5.78±5.53	0.07±0.08	0.21±0.05	1.97±3.14	0.43±0.33	0.24±0.23	0.23±0.06
	Hydba[2]	0.03±0.06	0.69±0.93	0.06±0.07	nd	0.12±0.19	0.03±0.04	0.07±0.12	nd
	Tyr[3]	3.31±0.42^{ab}	19.3±16.39^{b}	0.64±0.07^{a}	0.4±0.05^{a}	4.33±4.73^{ab}	2.15±1.02^{ab}	0.94±0.64^{a}	1.11±0.58^{a}
	dba[4]	nd	0.48±1.06	nd	0.32±0.45	nd	0.99±1.41	0.18±0.35	0.41±0.39
	Hypha[5]	nd	0.04±0.05	0.03±0.05	nd	0.04±0.06	0.1±0.04	0.08±0.12	nd
	Ca[6]	0.006±0.01	0.05±0.04	nd	0.02±0.03	0.01±0.01	0.03±0.03	nd	0.04±0.07
	Va[7]	0.12±0.09^{a}	0.3±0.15^{ab}	0.38±0.19^{ab}	0.72±0.27^{b}	0.41±0.23^{ab}	0.14±0.14^{ab}	0.86±0.48^{b}	0.59±0.25^{ab}
	Val[8]	nd	nd	nd	nd	nd	0.02±0.02	nd	nd
	Sya[9]	0.09±0.04^{a}	0.07±0.02^{a}	0.1±0.05^{a}	0.09±0.04^{a}	0.09±0.05^{a}	0.06±0.02^{a}	0.22±0.14^{a}	0.4±0.09^{b}
	Pcoa[10]	0.96±0.77^{b}	0.2±0.06^{a}	0.08±0.04^{a}	0.19±0.01^{ab}	0.02±0.01^{a}	0.05±0.05^{a}	0.08±0.04^{a}	0.48±0.21^{ab}
	Fa[11]	0.28±0.18^{b}	0.09±0.05^{a}	0.02±0.02^{a}	0.05±0.004^{a}	0.0003±0.0006^{a}	nd	0.03±0.02^{a}	0.14±0.06^{ab}
	Peak12[12]	0.09±0.16^{a}	0.73±0.89^{ab}	0.33±0.14^{ab}	0.65±0.17^{ab}	0.62±0.11^{ab}	0.13±0.18^{ab}	0.54±0.34^{ab}	1.33±0.24^{b}
	Peak13[13]	0.92±0.12	0.64±0.64	0.63±0.19	1.04±0.47	1.31±0.85	0.32±0.39	0.84±0.44	0.96±0.21
	Peak14[14]	2.5±0.52^{a}	3.26±1.24^{a}	1.2±0.4^{a}	4.04±3.4^{a}	14.15±7.33^{b}	1.23±1.03^{a}	1.62±1.11^{a}	7.12±2.53^{ab}
	Cina[15]	0.97±0.22^{a}	2.55±0.93^{b}	0.63±0.08^{a}	0.06±0.02^{a}	0.65±0.29^{a}	0.38±0.41^{a}	0.67±0.34^{a}	0.13±0.04^{a}
	Lut[16]	1.91±0.4	1.57±1.19	0.56±0.35	0.67±0.94	0.07±0.06	0.5±0.35	1.43±0.91	1.67±1.71
	Peak17[17]	0.56±0.59	1.11±0.5	0.22±0.20	1.02±0.97	0.32±0.08	0.60±0.60	0.28±0.24	0.22±0.31
	Apig[18]	11.19±5.7^{a}	24.06±7.48^{b}	4.98±2.49^{a}	2.52±0.11^{a}	1.78±0.37^{a}	1.85±0.51^{a}	3.56±2.01^{a}	1.66±0.53^{a}

[1] hydroxytyrosol, [2] 4hydroxybenzoic acid,[3] tyrosol, [4] 2,3dihydroxybenzoic acid, [5] 4hydroxyphenylacetic acid,[6] caffeic acid, [7] vanillic acid, [8]vanillin, [9] syringic acid, [10] *p*-coumaric acid, [11] ferulic acid, [12] peak12 (RT:44), [13] peak13 (RT:47.5), [14] peak14 (RT:49.5), [15] cinnamic acid, [16] luteolin, [17] peak17 (RT:61.5), [18]apigenin, nd: not detected

a-b:Different letters within a row indicate samples that were significantly different ($p < 0.05$)

Individual phenolic substances of olive oil samples were investigated by multivariate techniques to see their effect on the classification of oils according to cultivar, geographical origin, and harvest year. Data set includes chemical measurements, identified phenolic compounds and some unidentified peaks. The unidentified peaks appeared before cinnamic acid were expressed as mg oleuropein/kg oil and unidentified peak between luteolin and apigenin were expressed as mg tyrosol/kg oil. Similarly, the peak appeared after ferulic acid (between 43–45 minutes) was expressed as mg tyrosol/kg oil.

5.1.7. Influence of Olive Variety

5.1.7.1. 2005 Harvest Year

In order to examine the cultivar effect on the phenolic composition, PCA was performed on phenolic compounds, TPC, PV, and colour coordinates. The data matrix with 21 EVOO samples and selected 28 variables was built to classify the EVOOs. The result of the two-component PCA model with $R^2 = 0.52$ and $Q^2 = 0.26$ is reported in Figure 5.14 (a). The first two components (PC1 and PC2) account for 53% of total variance. Erkence, domat and nizip oils separated from all other samples while gemlik and ayvalik oils cluster closely on the other half of the control ellipse. Memecik oils are between these groupings, but more close to gemlik and ayvalik oils.

Oliveras-Lopez et al. (2007) has illustrated that the phenolic compounds can be employed, together with other chemical parameters, to classify Spanish and Italian oils in accordance with their cultivar. Garcia et al. (2003) and Vinha et al. (2005) demonstrated that differentiation among olive oil samples with the same geographical origin and different variety was possible. In another study, the phenolic composition was found to be not useful in discriminating the olive oil samples due to the fact that the phenolic content of oils was affected not only by the olive cultivars, but also climatic and environmental conditions, agronomic practice and the technological process (Cerretani, et al. 2006). In our work, olive fruits were supplied about at the same time in two consecutive years from the same nurseries where the trees were subjected to the

similar agronomic procedures. Olive oils were extracted by the same process. In order to average out the climatic conditions, it would obviously be more informative to monitor the oils over more than two years. On the other hand, even the two-year study in our case provided an information depicting discrimination among olive oils of different cultivars.

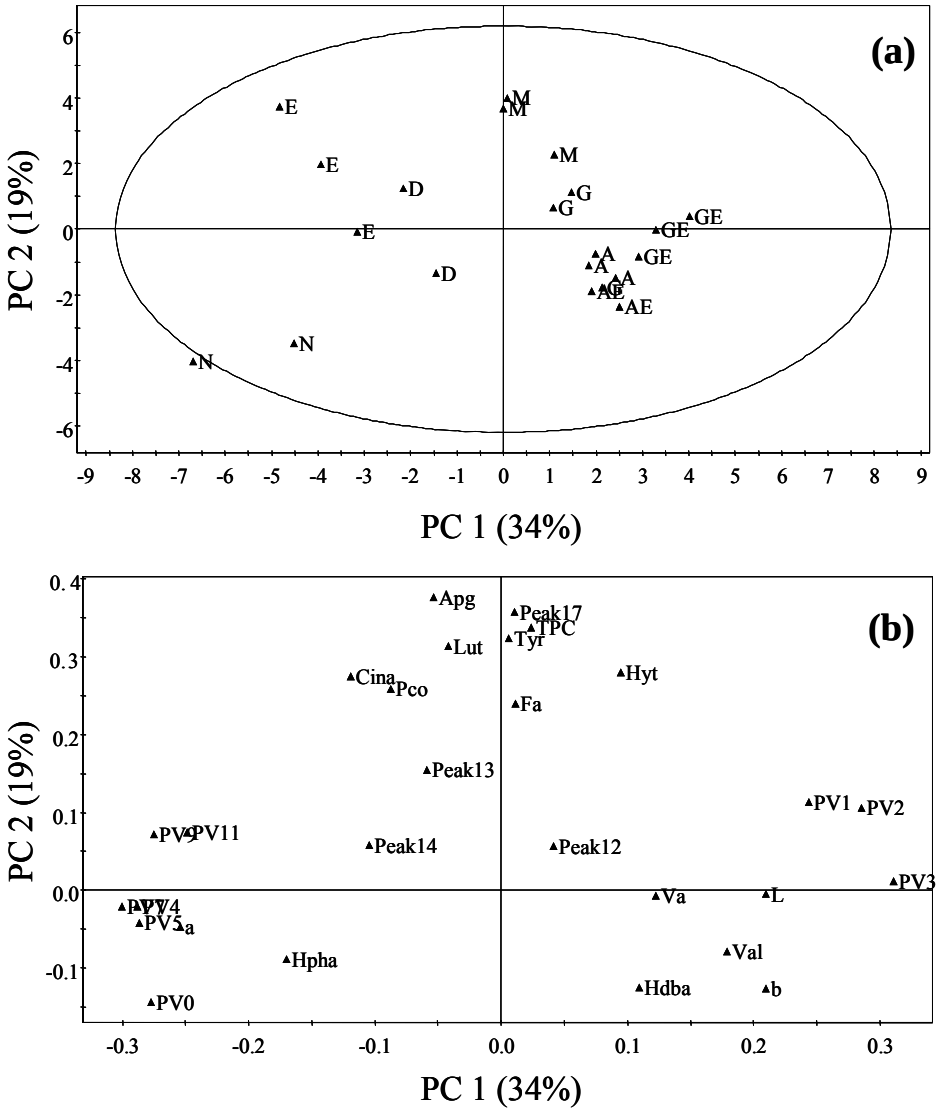


Figure 5.14. PCA of EVOOs in 2005 harvest year (a) score plot (b) loadings plot

Figure 5.14 (b) shows the loadings scatter plot (PC 1 vs. PC 2) obtained from PCA of the oil samples represented with 28 variables. The position of the cluster formed by three erkence oils depends mainly on unidentified peaks (number 13 and 14), cinnamic acid, apigenin, and TPC, whereas *p*-coumaric acid, ferulic acid, luteolin and apigenin contribute to the memecik oils. Domat oils and nizip oils can be grouped by the unidentified peaks (number 13 and 14) and 4-hydroxyphenylacetic acid information, respectively. Other oil samples are differentiated by the unidentified peak (number 12), vanillin, vanilic acid, colour coordinates (L* and b*), and PVs.

5.1.7.2. 2006 Harvest Year

For 2006 harvest season, influence of the cultivar on the phenolic composition was investigated using PCA. Classification of the 27 olive oil samples is performed by PCA using all quality parameters. A three-component PCA model with $R^2 = 0.57$ and $Q^2 = 0.17$ was built (Figure 5.15 (a)). PC1 and PC2 explained 28 and 16% of the total variance. A similar pattern to 2005 year was observed in groups. Erkence, domat and nizip oils grouped separately from gemlik and ayvalik oils. Memecik oils again appeared in the middle of the plot.

Loadings scatter plot obtained from PCA of oils is shown in Figure 5.15 (b). In particular, erkence, domat and memecik oils are differentiated by the unidentified peaks (number 13, 14 and 17), hydroxytyrosol, caffeic acid, oleuropein, cinnamic acid, luteolin, TPC and PVs. For gemlik and ayvalık oils, vanillic acid, syringic acid, and tyrosol are the effective parameters. Nizip oils can be characterized with the information of 4-hydoxyphenylacetic acid.

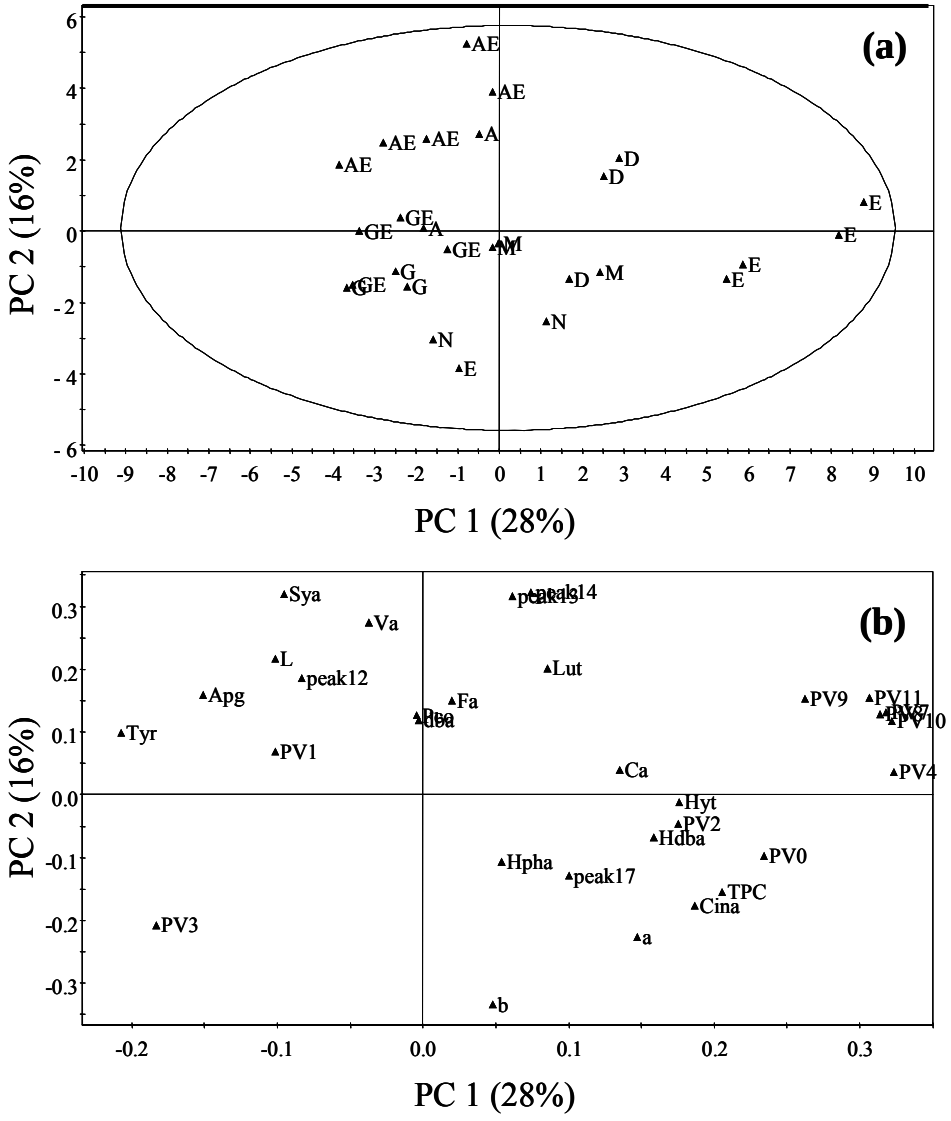


Figure 5.15. PCA of EVOOs in 2006 harvest year (a) score plot (b) loadings plot

5.1.8. Influence of Harvest Year

According to the PCA model, the samples of different years formed groups. Then, a two-component PLS-DA model with $R^2_X = 0.37$, $R^2_Y = 0.9$, $Q^2 = 0.84$ was built to further resolve the effect of the harvest year by using all observations over two years. Score plot of PLS-DA model shows that harvest year is a strong discriminating component (Figure 5.16). The samples of 2006 year were clustered together in the same area of the plot and separated from the samples of 2005 year. The model VIP values

show that the most influential variables in the group separation in descending order are vanillin, syringic, and PV11. Other variables are shown in Table 5.9. The reason of high discriminating power of these phenols is the absence or trace presence of the compound in one particular year and the presence of that in higher concentrations in the other harvest year. Different phenolic compositions with respect to harvest year have been also reported by other authors. Romero et al. (2003) investigated the composition of VOOs produced over four consecutive crop seasons in the region of the protected designation of origin "Les Garrigues" (Catalonia, Spain), taking the harvest period and the climatic conditions into consideration and found that phenolic profiles were influenced mainly by the cumulative rainfall. Effect of crop season on the composition of olive oils with special emphasis on the phenolic fraction was also studied by Morello et al. (2006). Their study indicated that the main differences between crop seasons were observed in secoiridoid derivatives, vanillin, tyrosol, apigenin, luteolin, and lignans.

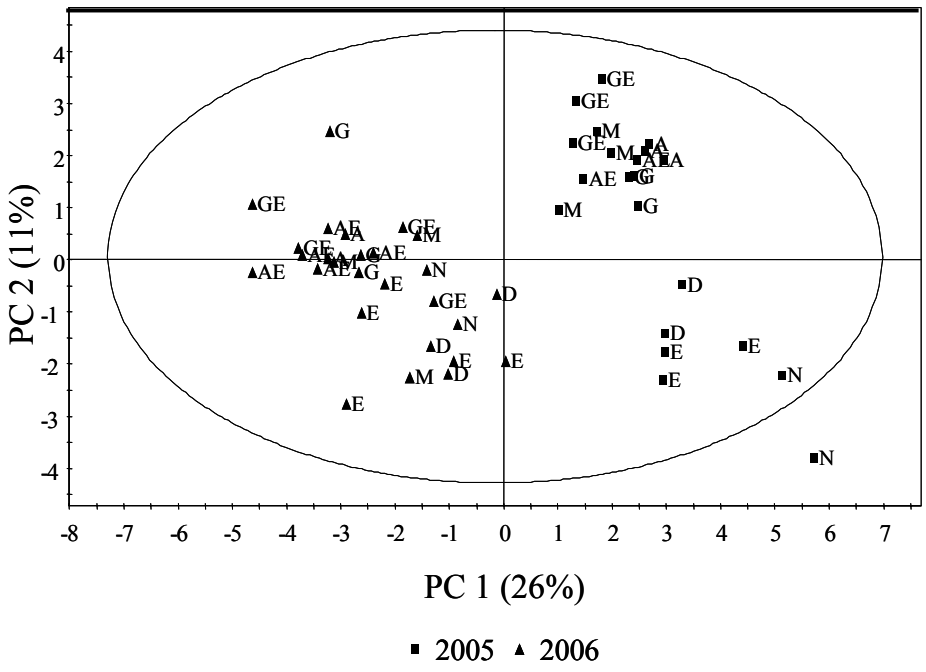


Figure 5.16. Score plot of PLS-DA of olive oils from both harvests

Table 5.9. Model VIP values of PLS-DA model for extracted EVOOs

Variables	VIP values
Val	1.965
Sya	1.916
PV11	1.673
PV9	1.649
PV7	1.602
PV4	1.426
a	1.196
TPC	1.195
PV2	1.105
Ca	1.069
PV5	1.035
Fa	1.013
Peak12	0.915
Peak13	0.799
Pco	0.687

In order to show the separation of olive oils of different cultivars, ayvalik versus memecik oils, ayvalik versus gemlik oils and ayvalik versus erkence oils were plotted and shown in Figure 5.17, 5.18 and 5.19. Ayvalik and gemlik are the most common olive varieties in the north side of the west (Aegean) cost of Turkey, while memecik is the dominant cultivar in the south side of the west cost. Erkence variety is cultivated only in a very narrow area (Karaburun, Cesme and Urla regions of city of Izmir). Phenolic content of erkence oil was found consistently and significantly higher than the other EVOOs over two harvest years studied, besides its high oil productivity. SIMCA models were created for ayvalik, gemlik, memecik and erkence oils of two seasons. Model parameters are given in Table 5.10. According to the Cooman's plots of the models (Figure 5.17, 5.18 and 5.19), these olive oil types have different phenolic and chemical compositions that could lead to differentiation. Samples did not exceed their

limits and were correctly classified into their classes. Separation of ayvalik oils from other varieties was also investigated and it was observed that ayvalik oils differ from all varieties according to Cooman's plots.

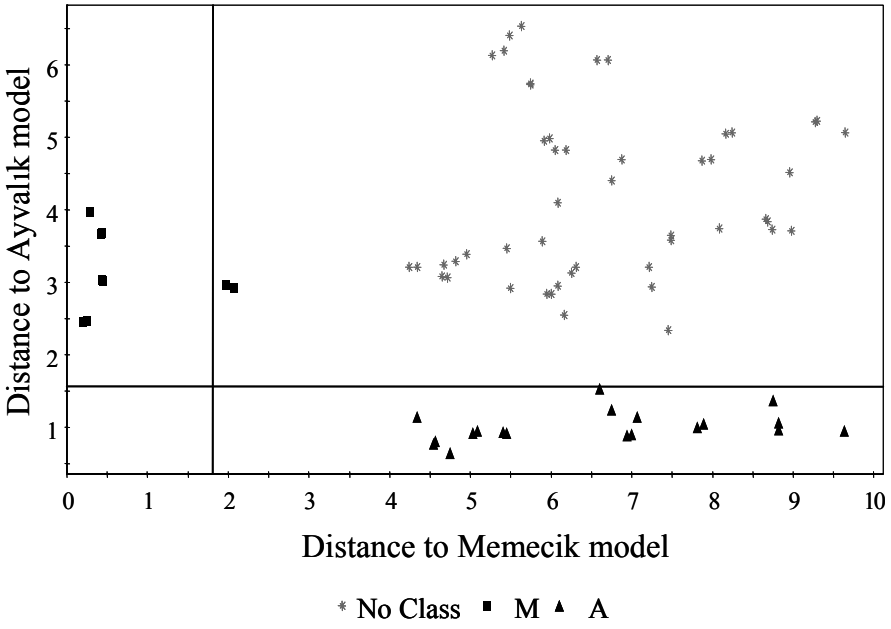


Figure 5.17. Cooman's plots of M versus A (for two harvest years)

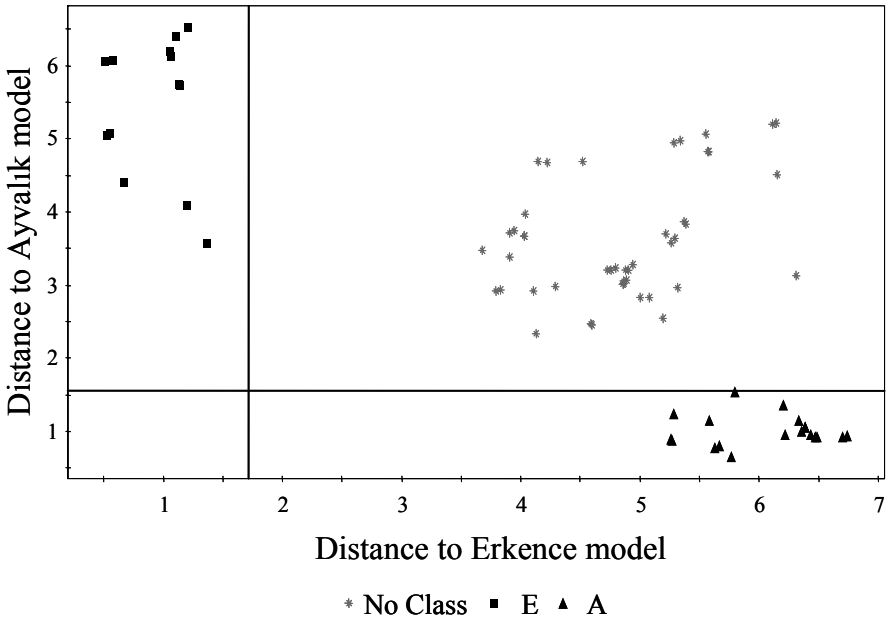


Figure 5.18. Cooman's plots of E versus A (for two harvest years)

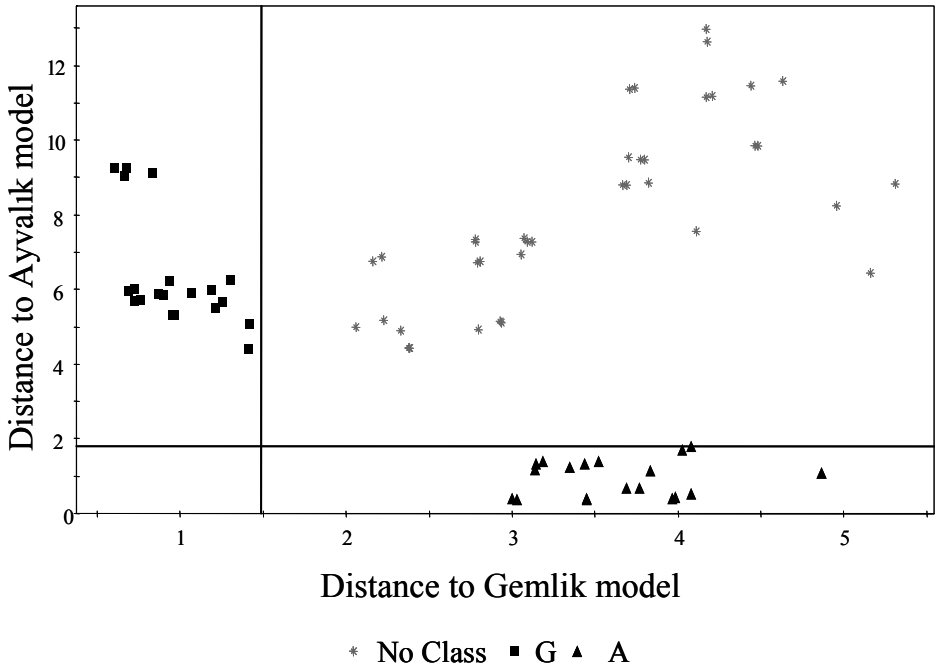


Figure 5.19. Cooman's plots of G versus A (for two harvest years)

Table 5.10. Model parameters of PCA class model for A, G, E and M

Olive oil	Number of PCs	R^2X	Q^2X(cum)
A	5	0.88	0.53
G	4	0.83	0.54
E	5	0.93	0.48
M	4	0.94	0.57

Influence of geographical origin;

The effect of geographical origin was investigated by the differences in the oils of ayvalik and gemlik varieties harvested in two different regions. The ayvalik and gemlik oils from different growing regions could be differentiated based on their phenolic profiles with PCA class models. Model parameters of this class model are presented in Table 5.11. Cooman's plots for two oils are shown in Figure 5.20 and 5.21. Ayvalik oils from Izmir region were separated from those of Edremit area, while no clear separation was observed between gemlik and gemlik-edremit oils.

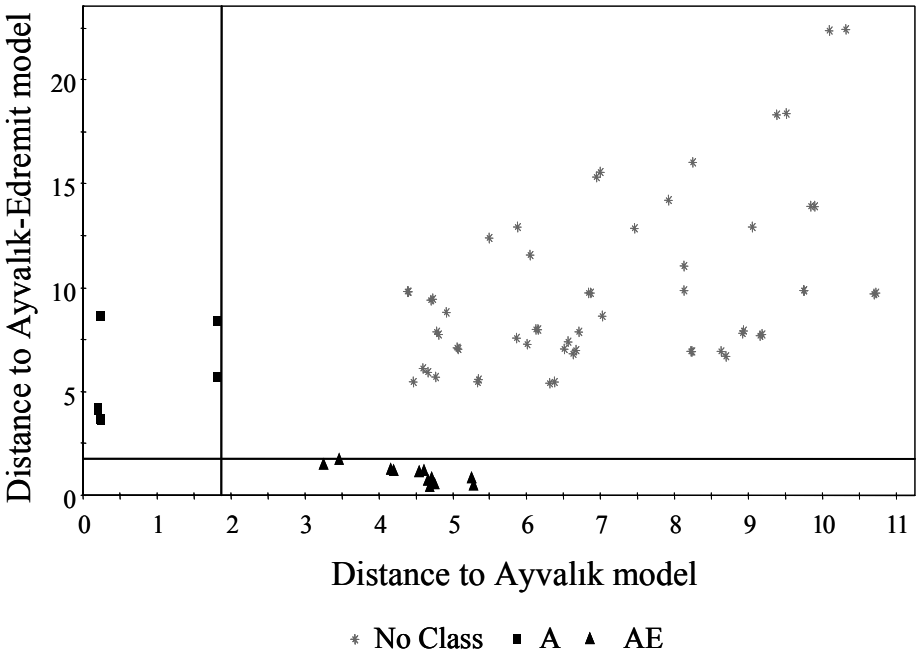


Figure 5.20. Cooman's plots of A versus AE (for two harvest years)

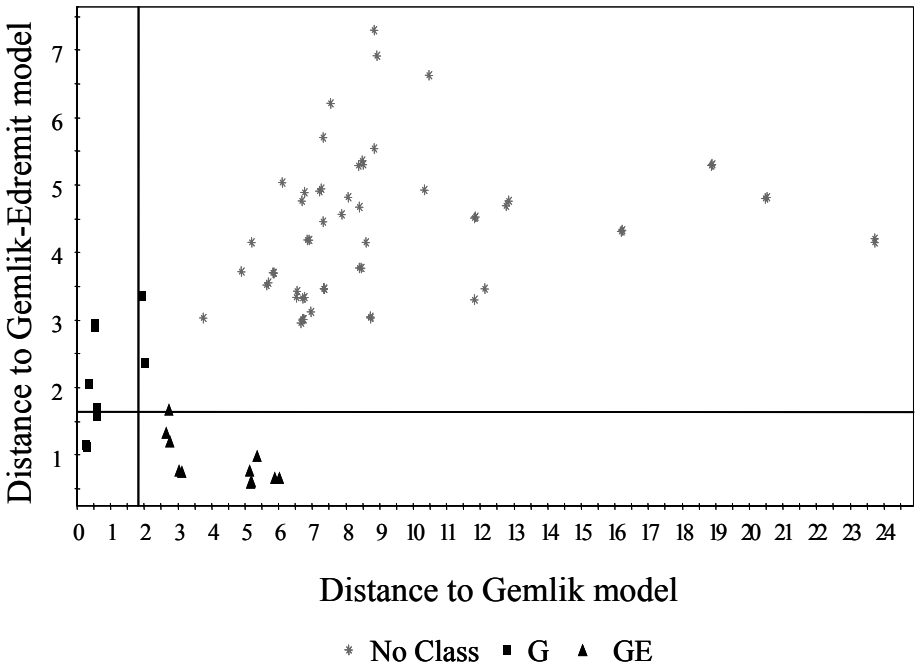


Figure 5.21. Cooman's plots of G versus GE (for two harvest years)

Table 5.11. Model parameters of PCA class model for A, G, AE and GE

Olive oil samples	Number of PCs	R^2X	Q^2X(cum)
A	3	0.97	0.88
G	4	0.97	0.78
AE	5	0.96	0.73
GE	3	0.89	0.67

5.2. Commercial Extra Virgin Olive Oils

5.2.1. Influence of Geographical Origin on Quality Parameters

ANOVA were performed for PV, colour, and TPC on the basis of geographical origin. Table 5.12 shows the mean values and standard deviations of the quality parameters of commercial EVOOs from different geographical areas in the Aegean coast of country. TPC significantly differs in 2006 with respect to growing region. Mean TPC of 2005 and 2006 season varied from 199 to 204 and from 231 to 287, respectively. In the study of Öğütçü et al., 2008, physico-chemical characterization of VOOs (2005-2006 seasons) produced in the Çanakkale region was carried out. TPC of the samples in this study ranged from 34.60 to 162.61 mg gallic acid/kg and PVs ranged from 7.86 to 29.751 (meq/kg). They state that chemical parameters did not show significant differences based on geographical origin.

Table 5.12. Chemical parameters of commercial EVOOs according to geographical origin (mean ± SD)

	North-2005	**South-2005**
PV	17.07±6.4	17.88±7.32
L*	23.45±1.11	24.33±0.93
a*	-0.22±0.84	-0.81±0.85
b*	11.39±1.46	12.53±1.01
TPC	203.93±65.36	199.27±72.90
	North-2006	**South-2006**
PV	11.44±2.82	11.88±2.19
L*	23.81±0.81	23.72±1.03
a*	-0.69±0.6	-0.54±0.73
b*	11.48±1.04	11.29±1.54
TPC	230.71±55.3[a]	287.35±58.2[b]

a-b:Different letters within the same row indicate a significant difference ($p < 0.05$)
If there is no letter, this indicates that there is no difference

5.2.2. Influence of Harvest Year on Quality Parameters

ANOVA was performed for PV, colour, and TPC on the basis of harvest year. Important difference among PVs of EVOOs from north area based on harvest year was found whereas there was no evidence of a difference in colour and TPC. EVOOs of the south area showed significant differences in some quality parameters such as PV, TPC and b* value (Table 5.13).

Table 5.13.Chemical parameters of commercial EVOOs according to harvest year (mean ± SD)

	North-2005	North-2006
PV	17.07±6.4[b]	11.44±2.82[a]
L*	23.45±1.11	23.81±0.81
a*	-0.22±0.84	-0.69±0.6
b*	11.39±1.46	11.48±1.04
TPC	203.93±65.36	230.71±55.3
	South-2005	**South-2006**
PV	17.88±7.32[b]	11.88±2.19[a]
L*	24.33±0.93	23.72±1.03
a*	-0.81±0.85	-0.54±0.73
b*	12.53±1.01[b]	11.29±1.54[a]
TPC	199.27±72.9[a]	287.35±58.2[b]

a-b:Different letters within the same row indicate a significant difference ($p < 0.05$)
If there is no letter, this indicates that there is no difference

5.2.3. Phenol Composition

Among several factors that affect the pattern of phenolic profiles of olive oils, geographical origin plays an important role (Vinha, et al. 2005, Garcia, et al. 2002, Japon-Lujan, et al. 2006).

HPLC analysis of phenolic compounds allowed the quantification of 17 phenols. Typical HPLC chromatograms of the commercial EVOOs in 2005 and 2006 harvest year are given in Figure 5.22 and 5.23. Table 5.14 presents the mean values and standard deviations of phenol contents of commercial EVOOs. Individual phenols varied depending on the geographical origin for two harvest years, with statistically significant differences in some compounds. For the first harvest year, the main differences in the phenolic fraction among oils of two growing areas were different

contents of tyrosol, vanillin, and luteolin. Actually, tyrosol were higher in olive oils from south Aegean than those from north Aegean, which had lower vanillin and luteolin contents. For the second harvest year, no qualitative differences were observed in the HPLC phenolic fraction profile among olive oils from two growing regions. However, significant quantitative differences were observed in a wide number of phenolic compounds (hydroxytrosol, 4-hydroxybenzoic acid, tyrosol, syringic acid, *p*-coumaric acid, *m*-coumaric acid, cinnamic acid and apigenin). Concentrations of phenolic compounds (expressed in mg/kg olive oil) found in olive oils were given in Table 5.15.

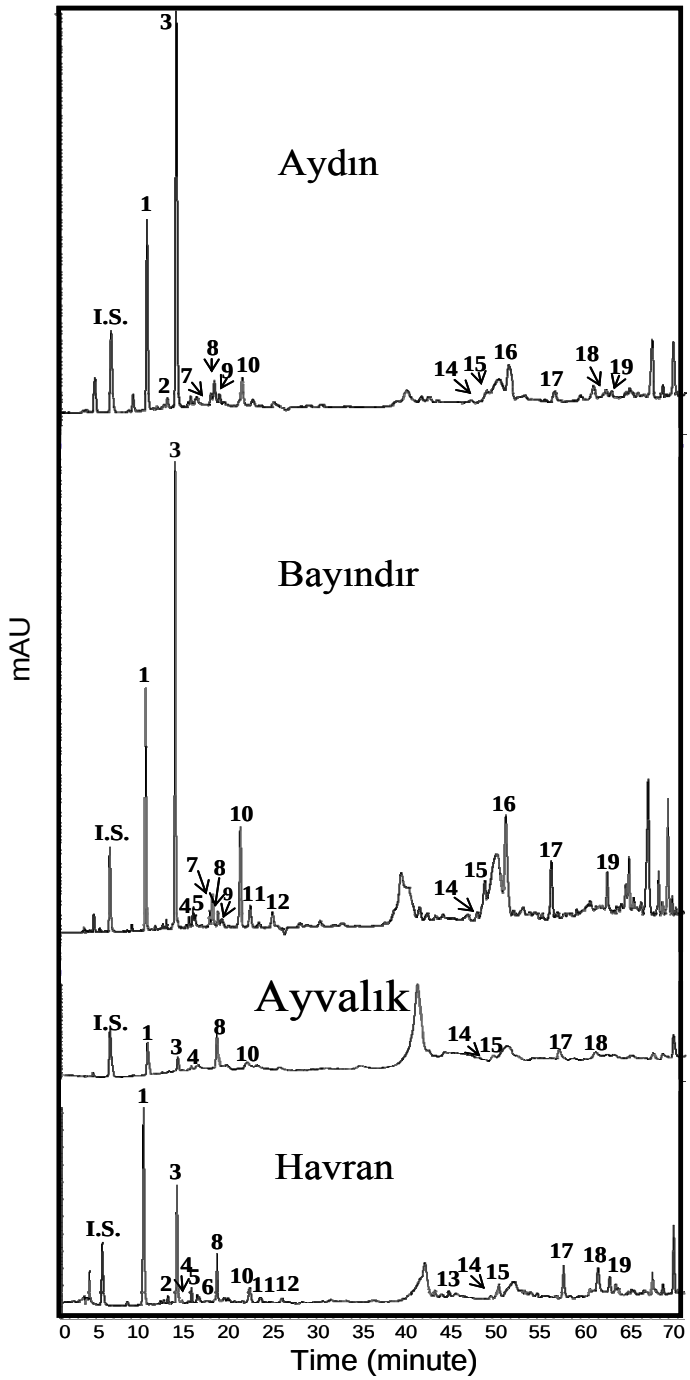


Figure 5.22. HPLC chromatograms of the phenolic extract of EVOOs of 2005 year at 280 nm: (IS) gallic acid; (1) hydroxytyrosol (Hyt); (2) 2,3dihydroxybenzoic acid (Dba); (3) tyrosol (Tyr); (4) 4hydroxyphenylacetic acid (Hpha); (5) vanillic acid (Va); (6) 3hydroxyphenylacetic acid (3hpha); (7) unidentified; (8) vanillin (Val); (9) unidentified; (10) *p*-coumaric acid (Pcoa); (11) ferulic acid (Fa); (12) unidentified; (13) unidentified; (14) unidentified; (15) unidentified; (16) cinnamic acid (Cina); (17) luteolin (Lut); (18) unidentified; (19) apigenin (Apg).

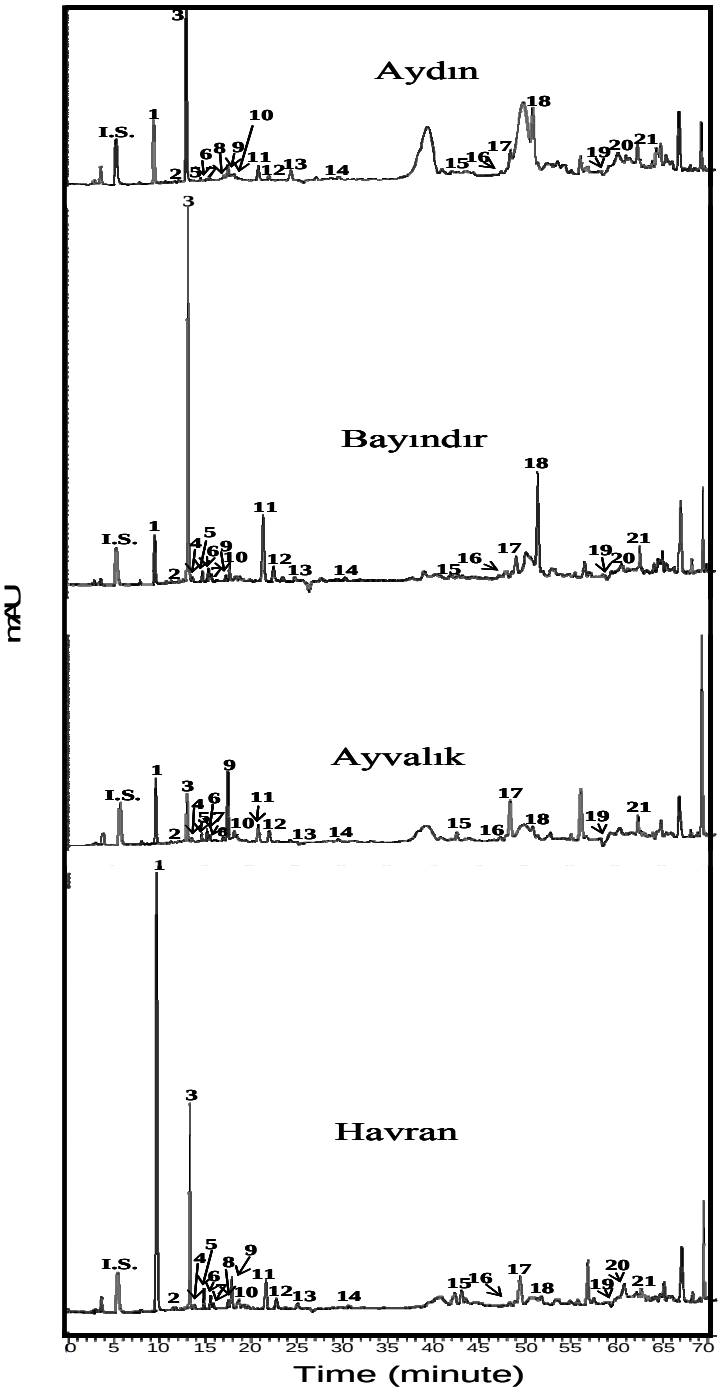


Figure 5.23. HPLC chromatograms of the phenolic extract of EVOOs of 2006 year at 280 nm: (IS) gallic acid; (1) hydroxytyrosol (Hyt); (2) 4hydroxybenzoic acid (Hdba); (3) tyrosol (Tyr); (4) 2,3dihydroxybenzoic acid (Dba); (5) 4hydroxyphenylacetic acid (Hpha); (6) Caffeic acid (Ca); (7) vanillic acid (Va); (8) vanillin (Val); (9) syringic acid (Sya); (10) unidentified; (11) *p*-coumaric acid (Pcoa); (12) ferulic acid (Fa); (13) unidentified; (14) *m*-coumaric acid; (15) unidentified; (16) unidentified; (17) unidentified; (18) cinnamic acid (Cina); (19) luteolin (Lut); (20) unidentified; (21) apigenin (Apg).

Table 5.14. Comparison of phenolic contents of commercial EVOOs with respect to geographical origin (mean ± SD) (For the abbreviations, see Figure 5.22 and 5.23)

	North-2005	**South-2005**	**North-2006**	**South-2006**
Hyt	3.16±1.58	4.27±2.65	7.36±6.7[b]	3.89±2.57[a]
Hdba	0.017±0.047	0.0062±0.019	0.02±0.02[a]	0.06±0.05[b]
Tyr	1.70±0.91[a]	6.96±4.37[b]	4.92±5.05[a]	10.67±7.44[b]
dba	0.07±0.15	0.052±0.16	0.34±0.38	0.19±0.23
Hpha	0.17±0.097	0.12±0.18	0.22±0.19	0.11±0.10
3 hpha	0.0063±0.023	nd	0.13±0.23	0.01±0.03
Ca	0.003±0.01	nd	0.04±0.04	0.05±0.04
Va	0.06±0.05	0.07±0.07	0.17±0.15	0.10±0.10
Val	0.35±0.12[b]	0.16±0.06[a]	0.02±0.02	0.01±0.02
Sya	nd	nd	0.36±0.2[b]	0.16±0.1[a]
Pco	0.1±0.08	0.14±0.16	0.32±0.21[a]	0.69±0.46[b]
Fa	0.03±0.03	0.06±0.09	0.15±0.14	0.26±0.14
Mco	nd	nd	0.02±0.01[a]	0.04±0.02[b]
Cina	nd	0.13±0.09	0.06±0.05[a]	0.66±0.21[b]
Lut	1.66±0.63[b]	0.82±0.75[a]	1.13±1.05	1.26±0.9
Apg	0.77±0.85	1.1±0.8	1.56±0.83[a]	2.64±1.29[b]

a-b:Different letters within the same row indicate a significant difference ($p < 0.05$)

Table 5.15. Individual phenolic compounds of commercial EVOOs of 2005 and 2006 harvest years

2005	Phenols	Hyt[1]	Hdba[2]	Tyr[3]	dba[4]	Hpha[5]	3 hpha[6]	Ca[7]	Va[8]	Val[9]	Sya[10]	Pco[11]	Fa[12]	Mco[13]	Peak14[14]	Peak15[15]	Peak16[16]	Cina[17]	Lut[18]	Peak19[19]	Apg[20]
	Ez	2.57	0.03	1.47	nd	0.18	nd	nd	0.05	0.33	nd	0.07	0.03	nd	0.3	0.42	5.9	nd	1.54	0.28	0.39
	Ez-or	1.11	nd	0.33	nd	nd	nd	nd	0.09	0.23	nd	0.02	nd	nd	0.11	0.13	2.07	nd	2.01	0.63	0.29
	Kk1	3.02	nd	1.83	nd	0.2	nd	nd	0.14	0.45	nd	0.1	0.05	nd	0.28	0.2	4.43	nd	1.93	0.28	0.83
	Kk2	3.25	0.02	1.39	nd	0.1	nd	nd	0.09	0.26	nd	0.06	nd	nd	0.06	0.08	1.89	nd	2.55	1.24	0.83
	Aol	2.48	nd	1.27	0.15	0.19	nd	nd	0.07	0.32	nd	0.12	0.02	nd	0.17	0.31	7.05	nd	1.85	0.89	0.61
	Aol-su	1.66	0.17	2.62	0.3	0.19	nd	nd	0.12	0.13	nd	0.34	0.1	nd	0.36	0.16	3.18	nd	2.82	nd	3.39
	Ed	3.5	nd	1.77	nd	0.22	nd	nd	0.04	0.42	nd	0.13	0.04	nd	0.17	0.26	6.42	nd	1.96	0.35	0.81
	Ha	7.67	nd	3.93	0.46	0.37	0.08	nd	0.05	0.33	nd	0.08	0.04	nd	0.29	0.31	2.01	nd	1.2	1.49	1.13
	Bu	3.33	nd	1.34	nd	0.21	nd	nd	nd	0.46	nd	0.08	0.07	nd	nd	0.57	2.95	nd	1.34	0.79	0.34
	Go	3.28	nd	1.32	nd	0.19	nd	0.04	0.05	0.46	nd	0.04	0.03	nd	0.24	0.2	1.64	nd	0.78	0.84	0.23
	Ay	2.8	nd	1.32	nd	0.17	nd	nd	nd	0.51	nd	0.068	nd	nd	nd	0.35	1.53	nd	0.79	0.92	nd
	Aov	4.26	nd	2.63	nd	0.21	nd	nd	0.15	0.51	nd	0.11	0.05	nd	nd	nd	1.7	nd	1.04	1.42	0.48
	Ze	2.17	nd	0.92	nd	nd	nd	nd	0.02	0.19	nd	0.03	nd	nd	0.13	0.16	1.41	nd	1.79	0.34	0.66
	Ak	3.53	0.06	6.63	nd	0.1	nd	nd	0.19	0.28	nd	0.09	nd	nd	nd	nd	1.85	0.18	0.82	1.75	1.59
	Me	6.25	nd	3.39	nd	0.54	nd	nd	0.15	0.08	nd	0.02	nd	nd	0.4	0.42	0.84	0.04	1.65	1.81	2.21
	Te	8.51	nd	6.2	nd	nd	nd	nd	nd	0.13	nd	0.14	0.07	nd	0.26	nd	nd	nd	0.47	nd	1.06
	Ba	6.99	nd	15.3	nd	0.29	nd	nd	0.09	0.2	nd	0.55	0.17	nd	nd	1.03	4.25	0.15	2.39	nd	2.33
	Se	0.69	nd	3.89	nd	0.07	nd	nd	0.02	0.16	nd	0.05	nd	nd	0.09	nd	nd	0.16	nd	nd	0.41
	Ayd	4.85	nd	11.5	0.47	nd	nd	nd	nd	0.11	nd	0.12	nd	nd	nd	0.13	nd	0.1	0.31	0.27	0.31
	Or	0.99	nd	1.8	nd	0.07	nd	nd	0.04	0.14	nd	0.04	0.03	nd	0.11	0.47	1.7	0.15	0.33	0.39	0.22
	Koc	2.88	nd	4.45	nd	nd	nd	nd	0.1	0.14	nd	0.09	0.04	nd	nd	0.08	0.58	0.08	0.56	0.77	0.63
	Mi	3.69	nd	9.48	nd	nd	nd	nd	nd	0.23	nd	0.2	0.26	nd	nd	0.61	nd	0.15	0.89	nd	1.14

(cont. on next page)

Table 5.15. (cont.) Individual phenolic compounds of commercial EVOOs of 2005 and 2006 harvest years

2006	Phenols	Hyt[1]	Hdba[2]	Tyr[3]	dba[4]	Hpha[5]	3 hpha[6]	Ca[7]	Va[8]	Val[9]	Sya[10]	Pco[11]	Fa[12]	Mco[13]	Peak14[14]	Peak15[15]	Peak16[16]	Cina[17]	Lut[18]	Peak19[19]	Apg[20]
	Ez	18	nd	16.5	nd	0.55	nd	nd	0.6	nd	0.45	0.41	0.15	nd	0.45	0.47	12.07	0.09	3.75	nd	1.7
	Kk	1.72	nd	1.98	0.44	0.13	0.28	nd	0.11	0.02	0.29	0.23	0.12	nd	0.15	0.63	8.98	0.04	1.35	0.23	3.67
	Aol	1.68	nd	0.83	nd	0.05	0.11	nd	0.13	0.02	0.3	0.2	0.05	0.01	0.33	0.34	3.65	0.02	0.18	0.06	1.07
	Ed	2.37	0.03	1.88	nd	0.09	nd	0.03	0.17	nd	0.02	0.24	0.09	0.02	0.26	0.31	4.99	0.02	0.71	0.63	1.23
	Ha	18.6	0.053	10.6	0.56	0.47	nd	0.1	0.09	0.05	0.21	0.42	0.18	0.02	0.16	0.55	4.93	0.03	0.23	0.21	0.77
	Bu	10.4	0.07	4.34	1.2	0.41	0.73	nd	0.18	0.05	0.69	0.86	0.52	0.05	0.6	1.02	9.55	0.05	1.76	1.06	1.67
	Go	6.27	0.04	2.57	0.53	0.04	0.18	0.09	0.13	0.04	0.64	0.28	0.07	0.02	0.48	0.67	6.63	0.04	1.2	1.26	1.49
	Ay	2.4	0.03	2.09	0.43	0.17	nd	0.09	0.11	0.02	0.46	0.22	0.19	0.02	0.27	0.67	5.75	0.04	0.5	nd	1.92
	Aov	10.4	nd	6.79	0.27	0.24	nd	0.06	0.07	0.03	0.24	0.079	0.03	0.02	0.34	0.54	3.98	0.17	0.5	0.15	1.12
	Ze	1.74	nd	1.6	nd	0.06	nd	0.02	0.16	0.02	0.3	0.27	0.08	0.02	0.26	0.62	5.77	0.08	1.07	nd	0.92
	Te	2.26	nd	2.97	nd	nd	0.03	0.04	0.05	nd	0.18	0.56	0.09	nd	0.11	0.56	1.37	0.48	0.68	0.13	1.1
	Ba	2.53	0.05	24.7	0.63	0.31	nd	0.14	0.17	nd	0.04	1.29	0.37	0.04	0.13	1.22	3.75	0.88	0.35	0.14	3.31
	Öd	4.96	0.16	7.4	0.42	0.23	nd	nd	0.08	0.02	0.21	0.62	0.16	0.05	0.14	1.22	5.27	1.06	2.07	0.45	3.81
	Ti	1.52	nd	5.03	0.49	0.09	nd	0.13	0.06	nd	0.12	0.98	0.44	0.05	0.16	1.31	3.63	0.86	0.76	0.33	1.18
	Se	8.07	0.06	7.77	0.32	0.09	nd	0.05	0.07	0.03	0.31	0.85	0.28	0.06	0.13	0.77	3.6	0.65	0.93	0.27	1.85
	Ku	3.32	0.06	6.05	nd	0.06	nd	0.05	0.11	nd	0.18	0.43	0.31	0.03	0.12	0.35	1.67	0.54	1.87	0.7	2.49
	Ge	6.02	0.05	4.28	nd	0.04	0.06	0.04	0.07	nd	0.17	0.81	0.21	0.05	nd	0.93	2.99	0.58	1.02	0.28	1.21
	Ayd	2.23	0.04	7.44	nd	0.06	nd	0.01	0.03	0.01	0.07	0.17	0.06	0.02	0.06	0.32	1.74	0.24	0.22	0.21	1.66
	Or	7.6	nd	16.1	0.53	0.2	nd	nd	0.44	0.03	0.26	1.95	0.51	0.05	0.81	1.61	4.46	0.89	2.73	0.39	1.57
	Kos	4.64	0.1	6.63	0.19	0.09	nd	0.02	0.04	nd	0.02	0.42	0.12	0.03	0.07	0.63	2.23	0.6	nd	1.49	1.61
	Da	2.07	0.09	4.77	0.28	0.06	nd	0.03	0.08	nd	0.24	0.36	0.24	0.04	0.12	1.23	4.72	0.75	2.29	0.23	4.23
	Koc	3.54	0.12	8.59	nd	0.06	nd	0.04	0.12	0.02	0.13	0.39	0.17	0.04	0.29	0.53	2.68	0.47	1.89	0.59	5.06
	Er	0.43	nd	26.7	nd	0.31	nd	0.04	0.07	nd	0.05	0.43	0.2	0.03	nd	0.63	2.9	0.61	0.81	0.14	3.07
	Ci	1.03	0.04	16	nd	0.12	nd	0.03	0.03	0.04	0.07	0.26	0.31	0.02	0.07	0.78	2.92	0.47	0.63	0.37	3.8
	Mi	8.18	0.08	15.6	nd	nd	0.12	0.07	0.12	0.06	0.34	0.84	0.45	0.08	0.16	1.71	4.23	0.82	2.6	0.31	3.71

[1] hydroxytyrosol, [2]4hydroxybenzoic acid, [3] tyrosol, [4] 2,3dihydroxybenzoic acid, [5] 4hydroxyphenylacetic acid, [6] 3hydroxyphenylaceticacid, [7] caffeic acid, [8] vanillic acid, [9]vanillin, [10] syringic acid, [11] *p*-coumaric acid, [12] ferulic acid, [13] *m*-coumaric acid, [14] peak14 (RT:44), [15] peak15 (RT:47.5), [16] peak16 (RT:49.5), [17] cinnamic acid, [18] luteolin, [19] peak19 (RT:61.5), [20] apigenin, nd: not detected

Different letters within a row indicate samples that are significantly different ($p<0.05$)

5.2.4. Influence of Geographical Origin

5.2.4.1. 2005 Harvest Year

In order to achieve the geographic characterization of commercial EVOO samples, a three-component PCA model with R^2 = 0.59, Q^2 = 0.14 was built. Differentiation of olive oil samples as a function of their geographical origin was achieved (Figure 5.24 (a)). Olive oils belonging to south & north Aegean are grouped separately except for Akhisar, Menemen and Zeytindag oils.

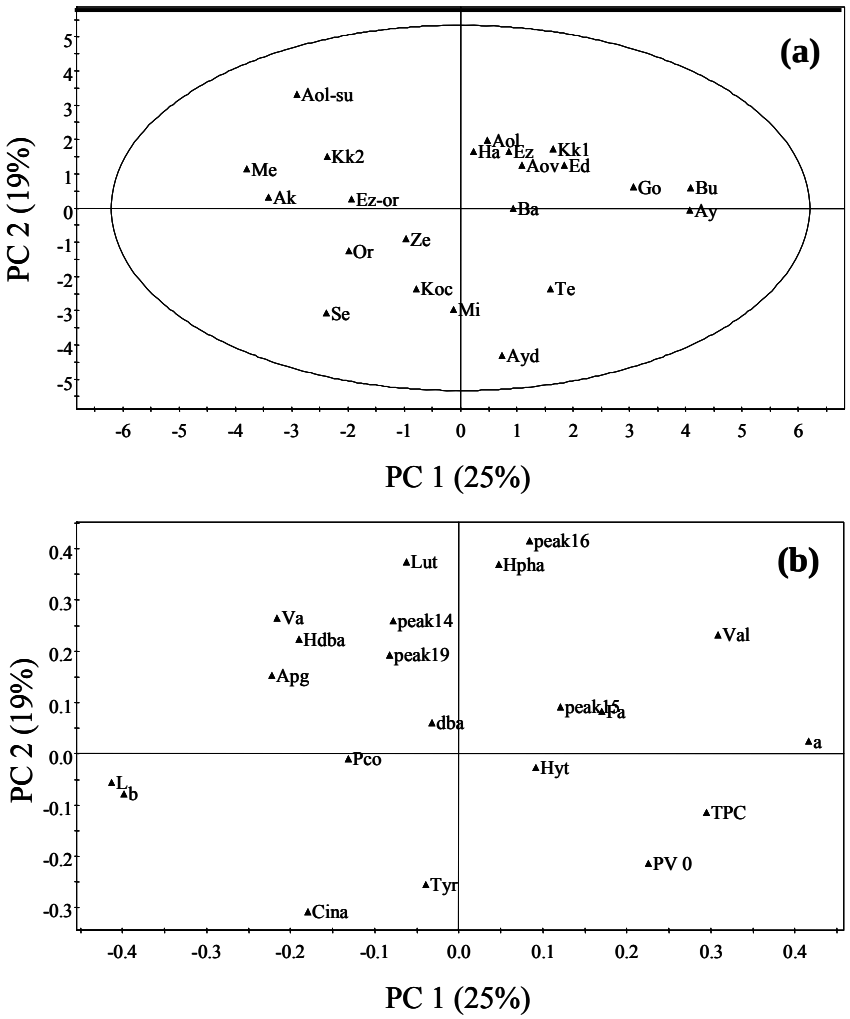


Figure 5.24. PCA of commercial EVOOs in 2005 harvest year (a) score plot (b) loadings plot

As shown in the loading plot of PCA obtained from 2005 harvest year (Figure 5.24 (b)), colour parameters, TPC, PV0, tyrosol, vanillin, cinnamic acid, luteolin and apigenin were the variables which were effective in groupings in the score plot.

5.2.4.2. 2006 Harvest Year

Seperation of olive oil samples from north & south regions in 2006 harvest year was achieved by a four-component PCA model with R^2 = 0.65, Q^2 = 0.12. The application of the PCA to all chemical data showed two distinctive groups (Figure 5.25 (a)). The samples of north Aegean & south Aegean are located in different halves of the control ellipse (north on the upper). Olive oils belonging to north Aegean region are grouped separately from other oils. Tepeköy and Ortaklar oils from south Aegean are located into the group of oils from north Aegean.

From loading plot, the most important variables to characterize olive oils from 2006 harvest year are unidentified peak (number 15), colour parameters, vanillic acid, *p*-coumaric acid, syringic acid, *m*-coumaric acid and ferulic acid (Figure 5.25 (b)). Distribution of oil samples of the south region in the score plot was affected by the high level of the unidentified peak (number 15), tyrosol, ferulic acid, *p*-coumaric acid, *m*-coumaric acid, oleuropein, cinnamic acid, apigenin and low level of syringic acid.

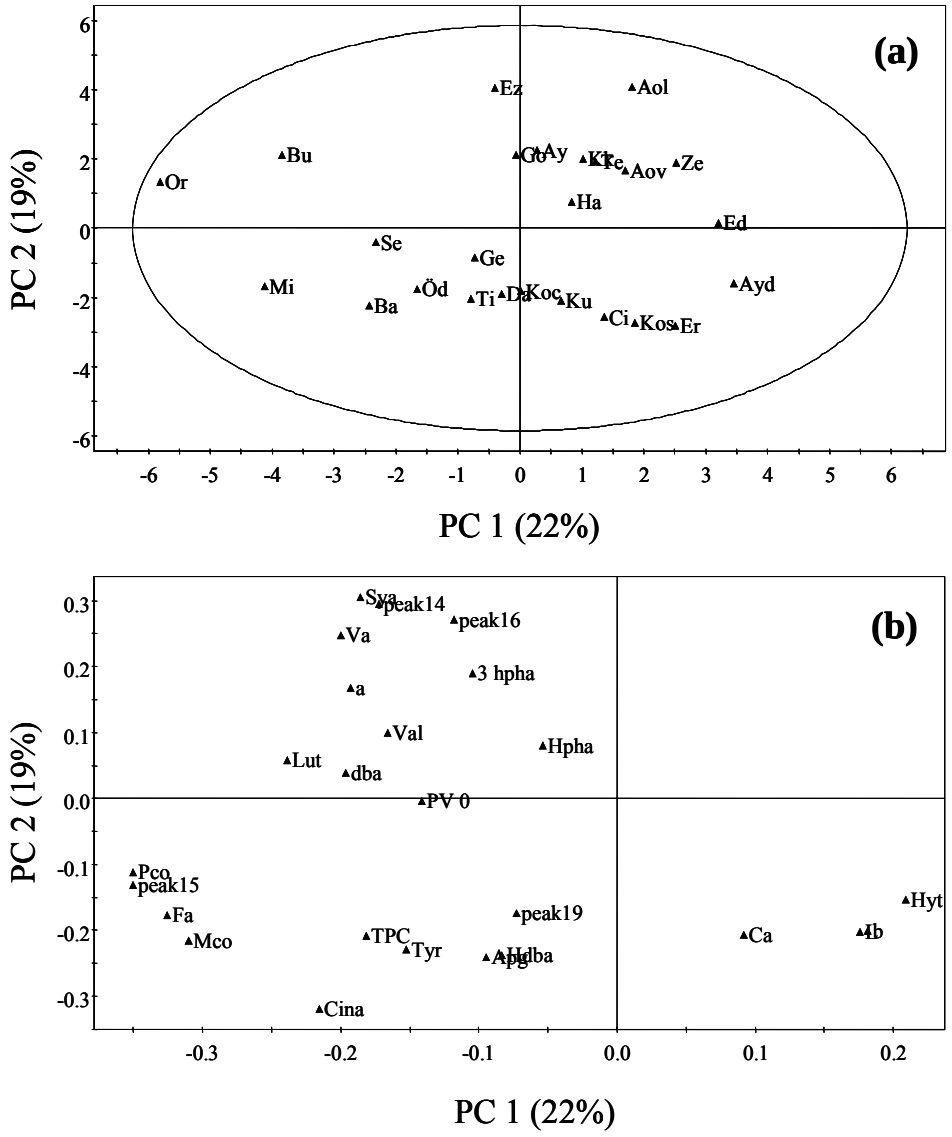


Figure 5.25. PCA of commercial EVOOs in 2006 harvest year (a) score plot (b) loadings plot

5.2.5. Influence of Harvest Year

To clarify influence of harvest year, all chemical parameters measured over two consecutive harvest seasons were studied. A two-component PLS-DA model with R^2_X = 0.39, R^2_Y = 0.94, Q^2 = 0.89 was built. A clear separation between groups of 2005 and 2006 olive oils can be seen in Figure 5.26. Olive oils from 2005 year were grouped together in the same area of the plot and were not similar to olive oils from 2006 year. This plot shows that the effect of the harvest year is predominant in the discrimination

of oil samples according to phenolic composition and quality characteristics. The model VIP values indicated that the variables with the highest discriminating power for year effect are syringic acid, m-coumaric acid, vanillin and p-coumaric acid (Table 5.16). The strong effect of these phenols on the discrimination of oil samples is related to their different concentrations in two successive years. Actually, syringic acid and *m*-coumaric acid were not found in olive oils from 2005 year whereas these phenols were quantified in olive oils of 2006 year. Likewise, the amounts of vanillin and *p*-coumaric acid considerably changed with harvest years. In an earlier study, Ninfali et al. (2008) compared the quality of EVOOs from organic and conventional farming during 3-year period. These researchers found that the concentrations of phenols, o-diphenols, and tocopherols showed differences in some years. Genotype and year-to-year changes in climate had more marked effects than cultivation. In the other study, Salvador et al. (2003) indicated that the chemical composition (such as phenolic, sterol, fatty acid composition and PV, TPC) of olive oil, varied considerably from one crop season to the next one.

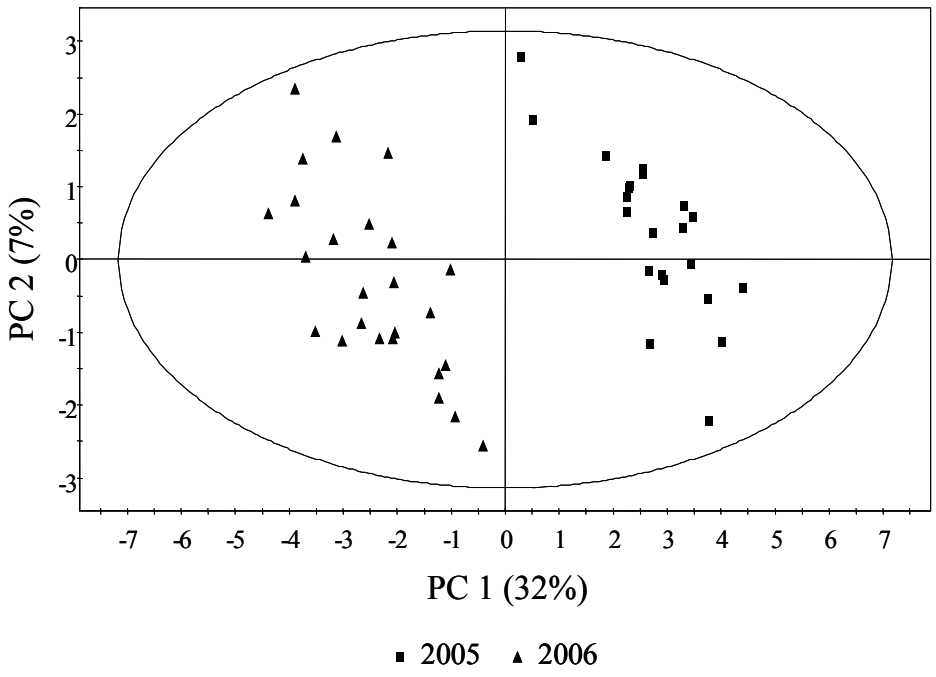


Figure 5.26. Score plot of PLS-DA of commercial olive oils from both harvest years

Table 5.16. Model VIP values of PLS-DA model for commercial EVOOs

Variables	VIP values
Sya	1.832
Mco	1.671
Val	1.574
Pco	1.387
Ca	1.369
Cina	1.338
Fa	1.287
Peak13	1.264
Apg	1.125
PV0	0.957
Peak14	0.947
Hdba	0.889
TPC	0.852
Va	0.831
Tyr	0.795

In order to investigate the effect of harvest year together with geographical origin, commercial oil samples were grouped into two different classes; north 2005 and 2006, south 2005 and 2006. A two-component PLS-DA model with $R^2_X = 0.43$, $R^2_Y = 0.78$, and $Q^2 = 0.67$ was built. Except Bayındır and Altınoluk-sulubaskı samples of 2005, the differentiation of oil samples was achieved according to both factors includes harvest year and geographical origin (Figure 5.27). Although geographical origin affected the separation of olive oils, harvest year could be considered as more effective parameter for the classification of oils.

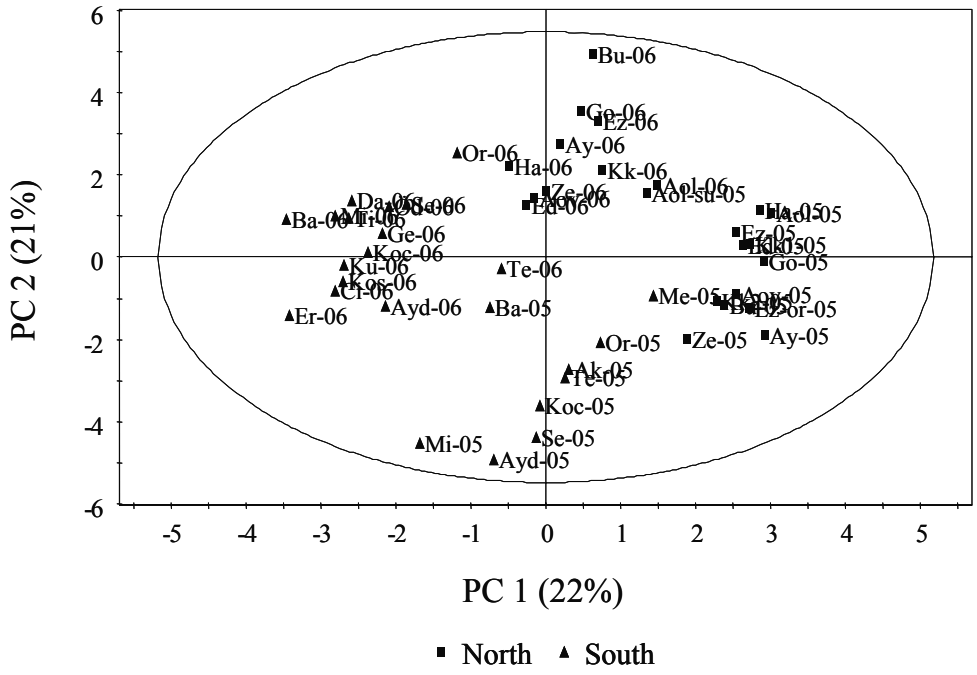


Figure 5.27. Score plot of PLS-DA of commercial olive oils of different geographical origins from both harvest years

5.3. Overall

The main findings from this work evidenced important differences with regard to quality parameters among eight EVOOs. It was found that erkence and memecik oils show high amounts of TPC for two harvest years. Besides, erkence oil can be seperated from other oils by high initial PV, lower L, and higher a value.

Regarding the individual phenol content, following consistent patterns can be observed for extracted EVOOs in both years: **1**. Memecik oils separated from others by high content of *p*-coumaric acid, ferulic acid, and apigenin. **2**. Erkence oils have high cinnamic acid, apigenin and TPC for both years. **3**. Domat oils were different from other oils by high concentrations of the unidentified peaks which can be considered as oleuropein and oleuropein aglycon. Memecik, erkence, gemlik, gemlik-edremit, ayvalik and ayvalik–edremit oils have moderate amount of these unidentified peaks compared to domat oils. **4**. Generally, ayvalik and gemlik oils (from Bornova and Edremit groves) have high vanillic acid and vanillin. **5**. All ayvalik oils have very poor cinnamic acid

content unlike erkence oils. **6**. 4-hydroxyphenylacetic acid contributes to the separation of Nizip oils in both years. Consequently, considerable differences were observed in the phenol profile of oils from six Turkish varieties studied. Effect of cultivar on phenolic components in VOOs from Spanish olive fruits was investigated by Gomez-Rico et al. (2008). They found that the distribution of secoiridoid derivatives of hydroxytyrosol and tyrosol varied in the different cultivars, whereas simple phenol contents, hydroxytyrosol and tyrosol values; phenolic acids such as *p*-coumaric acid, vanillic acid, and ferulic acid values were not affected by the cultivar. Our results showed that oleuropein aglycon and apigenin in addition to phenolic acids were effective parameters to characterize olive oils from different cultivars. In another study, genetic and biologic characteristics were used to characterize some olive cultivars grown in Turkey and it was found that there were big differences among cultivars according to the genetic and biochemical results (Özkaya, et al. 2004).

Considering commercial EVOOs in both harvest years, the concentrations of phenolic compounds highly depend on geographical origin. Concentrations of tyrosol, *p*-coumaric acid, ferulic acid, cinnamic acid, and apigenin are higher in oils from south Aegean. This result is similar to the result obtained for extracted EVOOs. Memecik oil, which is found in the south Aegean, can be characterized by high content of *p*-coumaric acid, ferulic acid, and apigenin. High concentrations of vanillin were observed in olive oils coming from north Aegean similar to ayvalik and gemlik oils coming from the north Aegean. Our findings agree with the previous works where the geographical origin affected the concentrations of phenols of virgin olive oils (Salvador, et al.; 2003, Sacco, et al. 2000).

Phenol compositions presented significant differences with respect to harvest year for both extracted EVOOs and commercial EVOOs. The amounts of some phenols varied considerably from one year to the next. While syringic acid was not found in olive oils for the first year, it was observed in the second year. The concentrations of vanillin decreased whereas *p*-coumaric acid and cinnamic acid contents increased in the in the second year for all olive oils.

CHAPTER 6

CONCLUSION

This study can be considered as a preliminary characterization of Turkish olive oils in terms of phenolic compounds since the demand for authenticated food products and also olive oil has been increasing. Phenolic concentrations of extracted and commercial extra virgin olive oils from two successive harvest years were determined by high performance liquid chromatography.

Distribution of phenolic components in olive oils of six different olive cultivars studied was different. Major phenolic compounds in Turkish extra virgin olive oils are hydroxytyrosol, tyrosol, vanillic acid, *p*-coumaric acid, cinnamic acid, luteolin, and apigenin. The oxidative stability in terms of PV over an extended period at an elevated temperature was found weakly related to vanillin, syringic acid, and colorimetric ordinate a^*. Principal component and partial least square-discriminant analyses allowed the separation of erkence, domat and nizip oils from gemlik and ayvalik oils for two harvest years. In terms of phenolic composition, memecik oils were similar to gemlik and ayvalik oils. The discrimination among olive oil samples with respect to the cultivar were carried out with PCA class models.

High concentrations of tyrosol, p-coumaric acid, ferulic acid, cinnamic acid, and apigenin were the most effective parameter to characterize commercial extra virgin olive oils of south Aegean whereas the content of vanillin was higher in olive oils of north Aegean.

Phenolic content of olive oils was influenced not only by the cultivar and geographical area but also by harvest year. Partial least square-discriminant analyses showed that harvest year was an effective parameter for discrimination of oils. The concentrations of vanillin, syringic acid and p-coumaric acid in two years affected the separation of extracted and commercial extra virgin olive oils according to harvest year. Determination of characteristic phenols of Turkish olive oils may be used in the authentication of oils from different regions.

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Table A.1. Peroxide Values for the extracted EVOOs of 2005 harvest year

			Days											
Sample No	**Sample Code**	**Sample**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	M1	Memecik 1	10.243	15.61	18.994	16.68	23.751	24.089	-	31.853	-	41.392	-	53.435
2	M2	Memecik 2	7.075	9.677	14.626	17.447	21.157	37.349	-	43.343	-	60.041	-	68.349
3	M3	Memecik 3	8.732	13.971	16.099	14.431	21.829	22.669	-	34.904	-	42.06	-	60.126
4	E1	Erkence 1	17.348	20.862	21.182	24.459	42.777	35.589	-	48.016	-	55.327	-	67.702
5	E2	Erkence 2	12.282	14.147	22.014	26.092	26.124	47.185	-	54.441	-	78.717	-	81.608
6	E3	Erkence 3	18.601	20.289	22.161	18.756	30.368	27.654	-	36.257	-	52.396	-	59.17
7	G1	Gemlik 1	9.199	13.825	16.089	14.031	18.98	24.192	-	32.541	-	40.195	-	49.235
8	G2	Gemlik 2	10.867	16.006	18.818	13.929	19.488	23.701	-	30.609	-	43.382	-	49.077
9	G3	Gemlik 3	9.709	12.854	17.889	15.069	19.134	25.669	-	31.302	-	39.209	-	44.655
10	A3	Ayvalık 3	10.179	15.079	13.33	12.908	15.97	17.571	-	29.334	-	35.944	-	39.241
11	A4	Ayvalık 4	7.812	10.585	14.301	14.047	14.289	25.949	-	31.521	-	46.06	-	43.875
12	A5	Ayvalık 5	10.214	11.312	17.316	12.336	17.973	19.32	-	29.585	-	41.241	-	47.502
13	D1	Domat 1	12.883	12.194	18.509	20.152	23.217	44.797	-	48.757	-	48.212	-	52.498
14	D2	Domat 2	11.678	13.189	17.448	19.772	24.236	44.465	-	50.978	-	55.589	-	71.256
15	N1	Nizip 1	18.512	27.497	34.501	38.557	34.872	55.216	-	55.971	-	53.802	-	66.328
16	N2	Nizip 2	26.091	34.239	41.525	46.584	41.132	68.299	-	66.126	-	76.663	-	73.389
17	GE1	Gemlik Edremit 1	6.678	8.464	11.827	13.671	14.196	23.748	-	28.143	-	38.291	-	58.597
18	GE2	Gemlik Edremit 2	6.783	8.891	12.201	11.948	12.689	21.266	-	29.291	-	41.595	-	39.863
19	GE3	Gemlik Edremit 3	8.634	13.844	16.082	11.496	23.756	18.739	-	27.422	-	27.325	-	36.369
20	AE1	Ayvalık Edremit 1	12.767	12.827	16.646	11.922	20.062	19.493	-	29.354	-	30.15	-	40.219
21	AE2	Ayvalık Edremit 2	7.189	7.936	15.229	15.271	15.946	27.034	-	31.754	-	42.021	-	65.946

Table A.2. Peroxide Values for the extracted EVOOs of 2006 harvest year

			Days											
Sample No	**Sample Code**	**Sample**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	M1	Memecik 1	19.464	39.928	24.896	13.081	17.072	21.079	-	25.576	24.903	26.604	28.878	33.768
2	M2	Memecik 2	10.752	26.211	16.339	11.531	13.418	20.349	-	20.107	23.78	21.042	24.493	26.926
3	M3	Memecik 3	10.136	9.616	10.613	15.242	13.691	-	23.943	22.315	26.251	27.716	29.274	25.893
4	E1	Erkence 1	16.899	20.664	16.297	17.077	20.253	25.772	-	31.135	33.121	34.676	39.846	41.428
5	E3	Erkence 3	16.267	15.612	16.072	17.063	20.483	29.249	-	31.254	37.737	37.998	43.022	45.606
6	E5	Erkence 5	10.477	16.57	8.351	13.182	11.384	-	16.199	14.927	16.617	19.526	19.923	21.571
7	G1	Gemlik 1	11.563	11.576	11.734	11.572	11.747	15.346	-	16.913	22.342	21.206	23.152	24.691
8	G2	Gemlik 2	11.208	10.66	8.066	12.053	11.382	-	15.808	16.743	20.336	21.744	22.862	22.768
9	G3	Gemlik 3	5.931	6.543	7.366	8.897	9.829	-	11.129	15.884	16.209	18.299	21.984	19.614
10	A1	Ayvalık 1	9.812	17.511	11.657	10.008	12.284	14.333	-	18.23	23.583	23.837	25.339	28.338
11	A2	Ayvalık 2	6.608	8.0373	8.6	16.625	12.274	-	17.786	20.932	24.963	42.863	32.378	31.395
12	D2	Domat 2	8.768	11.665	13.066	24.02	19.696	-	24.315	24.622	35.022	35.161	35.783	42.359
13	D3	Domat 3	10.916	13.623	14.775	14.566	16.391	20.384	-	28.826	35.054	30.288	37.981	37.3
14	N1	Nizip 1	13.418	29.135	19.911	12.24	13.45	19.022	-	22.208	21.832	22.668	28.437	35.214
15	N2	Nizip 2	7.203	9.574	8.119	16.275	11.292	-	25.362	20.106	18.306	21.673	26.892	24.423
16	GE1	Gemlik Edremit 1	10.811	22.608	15.809	9.054	8.805	12.791	-	13.102	16.427	15.877	20.407	17.301
17	GE2	Gemlik Edremit 2	10.107	21.685	17.818	10.291	10.289	14.303	-	13.057	20.513	17.246	18.928	22.684
18	GE4	Gemlik Edremit 4	10.192	11.588	9.97	18.883	13.359	-	22.192	19.728	24.205	25.581	27.047	34.229
19	AE1	Ayvalık Edremit 1	11.504	26.205	13.26	11.962	13.324	18.324	-	20.159	23.281	22.429	24.493	27.568
20	AE3	Ayvalık Edremit 3	7.889	11.677	10.162	28.486	9.259	-	22.682	25.422	25.339	25.897	30.654	41.229
21	AE5	Ayvalık Edremit 5	12.539	10.143	10.061	20.174	18.408	-	24.059	27.816	23.479	28.802	31.757	32.627

Table A.3. Colour Coordinates for the extracted EVOOs of 2005 and 2006 harvest years

2005					
Sample No	**Sample Code**	**Sample**	**L***	**a***	**b***
1	M1	Memecik 1	25.72	-2.04	12.57
2	M2	Memecik 2	25.857	-1.97	12.76
3	M3	Memecik 3	25.197	-1.9	13.53
4	E1	Erkence 1	23.633	-0.36	11.17
5	E2	Erkence 2	22.913	0.103	11.01
6	E3	Erkence 3	22.22	0.037	9.77
7	G1	Gemlik 1	25.697	-1.75	13.27
8	G2	Gemlik 2	24.81	-0.99	13.82
9	G3	Gemlik 3	25.117	-1.42	14.19
10	A3	Ayvalık 3	24.693	-1.25	13.36
11	A4	Ayvalık 4	24.487	-1.06	13.15
12	A5	Ayvalık 5	24.647	-1.14	13.15
13	D1	Domat 1	23.947	-0.88	12.32
14	D2	Domat 2	24.823	-1.48	12.48
15	N1	Nizip 1	24.823	-0.52	13
16	N2	Nizip 2	24.207	-0.45	13.12
17	GE1	Gemlik Edremit 1	25.59	-1.86	13.17
18	GE2	Gemlik Edremit 2	25.457	-1.8	13.86
19	GE3	Gemlik Edremit 3	25.167	-1.75	13.97
20	AE1	Ayvalık Edremit 1	24.903	-1.59	13.31
21	AE2	Ayvalık Edremit 2	24.983	-1.65	13.14
2006					
Sample No	**Sample Code**	**Sample**	**L***	**a***	**b***
1	M1	Memecik 1	24.703	-1.58	13.1
2	M2	Memecik 2	26.003	-2.19	11.41
3	M3	Memecik 3	25.407	-2.02	11.84
4	E1	Erkence 1	25.163	-1.79	13.63
5	E2	Erkence 2	24.69	-1.51	13.4
6	E3	Erkence 3	25.01	-1.55	12.98
7	E4	Erkence 4	24.853	-1.41	12.89
8	E5	Erkence 5	24.08	-1.35	12.49
9	G1	Gemlik 1	25.2	-1.94	13.51
10	G2	Gemlik 2	25.213	-1.93	13.27
11	G3	Gemlik 3	25.56	-2.14	12.09
12	A1	Ayvalık 1	24.133	-1.7	10.45
13	A2	Ayvalık 2	25.183	-1.88	9.83
14	D2	Domat 2	25.097	-1.79	10.32
15	D3	Domat 3	24.24	-1.69	9.9
16	N1	Nizip 1	24.917	-1.6	13.42
17	N2	Nizip 2	24.703	-1.21	13.25
18	GE1	Gemlik Edremit 1	25.837	-2.09	12.74
19	GE2	Gemlik Edremit 2	25.673	-1.99	12.89
20	GE3	Gemlik Edremit 3	25.487	-2.07	13.19
21	GE4	Gemlik Edremit 4	25.09	-1.84	13.47
22	AE1	Ayvalık Edremit 1	25.773	-2.18	11.21
23	AE2	Ayvalık Edremit 2	25.893	-2.04	9.87
24	AE3	Ayvalık Edremit 3	26.04	-2.03	9.873
25	AE5	Ayvalık Edremit 5	25.647	-2.15	11.67

Table A.4. TPC for the extracted EVOOs of 2005 and 2006 harvest years

2005			
Sample No	**Sample Code**	**Sample**	**TPC**
1	M1	Memecik 1	324.91
2	M2	Memecik 2	369.23
3	M3	Memecik 3	298.62
4	E1	Erkence 1	310.38
5	E2	Erkence 2	423.36
6	E3	Erkence 3	336.21
7	G1	Gemlik 1	253.59
8	G2	Gemlik 2	296.66
9	G3	Gemlik 3	272.01
10	A3	Ayvalık 3	342.23
11	A4	Ayvalık 4	306.44
12	A5	Ayvalık 5	340.6
13	D1	Domat 1	360.96
14	D2	Domat 2	243.01
15	N1	Nizip 1	79.291
16	N2	Nizip 2	125.51
17	GE1	Gemlik Edremit 1	208.47
18	GE2	Gemlik Edremit 2	282.42
19	GE3	Gemlik Edremit 3	244.75
20	AE1	Ayvalık Edremit 1	190.37
21	AE2	Ayvalık Edremit 2	182.14
2006			
Sample No	**Sample Code**	**Sample**	**TPC**
1	M1	Memecik 1	117.14
2	M2	Memecik 2	156.97
3	M3	Memecik 3	137.36
4	E1	Erkence 1	297.4
5	E2	Erkence 2	320.86
6	E3	Erkence 3	399.81
7	E4	Erkence 4	295.42
8	E5	Erkence 5	353.36
9	G1	Gemlik 1	44.09
10	G2	Gemlik 2	87.92
11	G3	Gemlik 3	142.71
12	A1	Ayvalık 1	90.4
13	A2	Ayvalık 2	43.67
14	D1	Domat 1	332.14
15	D2	Domat 2	139.95
16	D3	Domat 3	147.65
17	N1	Nizip 1	100.1
18	N2	Nizip 2	125.29
19	GE1	Gemlik Edremit 1	37.87
20	GE2	Gemlik Edremit 2	75.12
22	GE3	Gemlik Edremit 3	79.12
23	GE4	Gemlik Edremit 4	84.01
24	AE1	Ayvalık Edremit 1	87.93
25	AE2	Ayvalık Edremit 2	77.59
26	AE3	Ayvalık Edremit 3	65.63
27	AE4	Ayvalık Edremit 4	43.7
28	AE5	Ayvalık Edremit 5	102.46

Table A.5. Chemical parameters for the commercial EVOOs of 2005 and 2006 harvest years

2005							
Sample No	**Sample Code**	**Sample**	**PV**	**L***	**a***	**b***	**TPC**
1	Ez	Ezine	20.24	23.64	-0.4	11.92	275.03
2	Ez-or	Ezine Gulpinar Organik	12.05	24.79	-1.3	12.99	137.48
3	Kk1	Kucukkuyu	25.86	23.32	-0	11.13	268.2
4	Kk2	Kkuyu	12.88	24.82	-1.3	13.32	137.68
5	Aol	Altinoluk	13.66	23.72	-0.3	11.98	202.62
6	Aol-su	Altinoluk-sulubaski	7.212	24.54	-1.2	12.34	94.57
7	Ed	Edremit	25.48	23.43	-0.2	11.74	274.08
8	Ha	Havran	9.674	23.32	-0.2	11.5	128.67
9	Bu	Burhaniye	22.82	21.89	0.93	9.17	269.12
10	Go	Gomec	23.51	22.13	0.89	9.63	220.7
11	Ay	Ayvalik	19.63	21.6	1.09	8.753	236.74
12	Aov	Altinova	10.42	22.88	0.2	10.69	152.49
13	Ze	Zeytindag	18.49	24.8	-1.1	12.86	253.66
14	Ak	Akhisar	12.41	24.78	-1.2	13.54	126
15	Me	Menemen	11.84	25.7	-2.1	12.85	179.51
16	Te	Tepekoy	11.72	23.02	0.48	11.14	355.31
17	Ba	Bayindir	18.38	23.24	0.2	11.47	231.07
18	Se	Selcuk	17.9	25.13	-1.5	13.84	160.93
19	Ayd	Aydin	34.1	23.4	-0.3	11.58	244.58
20	Or	Ortaklar	12.47	24.85	-1.5	13.58	111.57
21	Ko	Kocarli	23.51	24.18	-0.7	12.76	198.6
22	Mi	Milas	18.57	24.63	-0.8	12.01	185.85
2006							
Sample No	Sample Code	Sample	PV	L*	a*	b*	TPC
1	Ez	Ezine	8.45	23.88	-0.7	11.14	222
2	Kk	Kucukkuyu	16	24.47	-1.3	12.35	254.98
3	Aol	Altinoluk	10.77	22.17	0.48	9.29	195.42
4	Ed	Edremit	8.994	24.62	-1.3	12.57	200.1
5	Ha	Havran	10.72	24.56	-1.1	11.92	188.33
6	Bu	Burhaniye	11.75	23.78	-0.6	11.68	342.93
7	Go	Gomec	9.64	23.73	-0.6	11.62	265.29
8	Ay	Ayvalik	16.57	22.8	0.08	10.16	165.66
9	Aov	Altinova	12.18	23.64	-0.6	11.66	285.16
10	Ze	Zeytindag	9.336	24.48	-1.4	12.38	187.2
11	Te	Tepekoy	13.73	21.54	0.82	8.137	130.09
12	Ba	Bayindir	15.42	23.01	0.06	10.25	287.24
13	Od	Odemis	10.15	24.4	-1.1	12.4	291.07
14	Ti	Tire	10.13	24.07	-0.8	12.14	330.27
15	Se	Selcuk	13.43	22.51	0.34	9.423	295.5
16	Ku	Kusadasi	11.24	24.33	-1.1	12.41	358.05
17	Ge	Germencik	11.44	22.98	0.13	10.02	305.54
18	Ayd	Aydin	9.73	24.53	-1.2	12.5	348.92
19	Or	Ortaklar	11.8	22.16	0.63	8.87	343.85
20	Kos	Kosk	9.81	24.64	-0.9	11.95	306.62
21	Da	Dalama	8.49	24.37	-1	12.45	277.99
22	Koc	Kocarli	14.07	24.53	-1	12.03	301.83
23	Er	Erbeyli	11.71	24.76	-1.5	12.96	205.74
24	Ci	Cine	11.19	24.35	-1	12.41	260.75
25	Mi	Milas	15.91	23.64	-0.6	11.35	266.86

Table A.6. Individual phenolic compounds in extracted EVOOs of 2005 harvest year

Sample Code	Sample	Hyt	dba	Chla	Tyr	Hdba	Hpha	Va	3 hpha	Ca	Sya	Val	Pco	Fa	Mco	Oco	peak(RT:44)	peak(RT:47.5)	peak(RT:4
M1	Memecik 1	5.045	0	0	37.04	0	0	0	0	0	0	0	0.811	0.447	0	0	0	0	3.579
M2	Memecik 2	0.94	0	0	2.609	0	0	0.067	0.09	0	0	0.174	0.729	0.226	0	0	0.582	1.124	2.074
M3	Memecik 3	0.985	0	0	2.864	0	0	0.13	0	0	0	0.183	0.846	0.139	0	0	0.423	0	0
E1	Erkence 1	1.138	0	0	3.433	0	0	0.299	0	0	0.255	0.188	0.129	0	0	0	0	2.52	6.875
E2	Erkence 2	1.283	0	0	7.852	0	0.264	0.348	0	0	0	0.204	0.17	0	0	0	0.661	2.328	9.647
E3	Erkence 3	0.523	0	0	1.604	0	0	0.194	0	0	0.167	0.17	0.064	0	0	0	1.073	0	3.572
G1	Gemlik 1	0.792	0	0	1.522	0.47	0	0.145	0	0	0	0.124	0	0	0	0	0	0	0
G2	Gemlik 2	3.79	0.337	0.084	13.58	0.078	0.131	0.483	0	0	0	0.166	0.031	0	0	0	0	0.176	0
G3	Gemlik 3	4.463	0	0	13.15	0	0.125	0.598	0	0	0	0.106	0.048	0	0	0	0	0.228	0
A3	Ayvalık 3	0.902	0	0	0.57	0	0	0.118	0	0	0	0.228	0	0	0	0	0	0	4.416
A4	Ayvalık 4	1.566	0	0	0.931	0.059	0.089	0.161	0	0	0	0.466	0.068	0	0	0	0	0.15	2.908
A5	Ayvalık 5	0.842	0	0	0.5	0	0	0.111	0	0	0	0.27	0.065	0	0	0	0.488	0	2.678
D1	Domat 1	8.493	0	0	14.73	0	0.291	0.172	0	0.055	0	0.03	0.121	0.091	0	0	0	2.995	18.395
D2	Domat 2	0	0	0	6.28	0	0	0.766	0	0	0	0.217	0	0	0	0	0	2.687	20.649
N1	Nizip 1	0.131	0	0	0.284	0	0.225	0.033	0	0	0	0.097	0.038	0	0.02	0	0	0	0.86
N2	Nizip 2	0	0	0	0.209	0	0.258	0.025	0	0	0	0	0.05	0	0	0	0	0	0
GE1	Gemlik Edremit 1	0.376	0	0	2.029	0	0	0.697	0	0	0	0.355	0.023	0	0	0	0.252	0.193	0
GE2	Gemlik Edremit 2	2.527	0	0	8.904	0	0	0.697	0	0	0	0.296	0	0	0	0	0	0	0
GE3	Gemlik Edremit 3	0.185	0	0	1.13	0	0	0.4	0	0	0	0.217	0.018	0	0	0	0.179	0.151	0
AE1	Ayvalık Edremit 1	0.278	0	0	0.302	0.017	0.115	0.442	0	0	0	0.509	0.062	0.032	0	0	0.981	0.203	5.676
AE2	Ayvalık Edremit 2	0.235	0	0	0.596	0.039	0.215	0.423	0	0	0	0.661	0.06	0	0	0	1.028	0.321	7.045

Table A.7. Individual phenolic compounds in extracted EVOOs of 2006 harvest year

Sample No	Sample Code	Sample	Hyt	Hdba	Tyr	Chla	dba	Hpha	3 hpha	Ca	Va	Val	Sya	Pco	Fa	Mco	Oco	peak(RT:44)	peak(RT:47.5)	peak(RT:49.5)	Cina	Lut	peak(RT:61.5)	Apg
1	M1	Memecik 1	0	0	3.423	0	0	0	0	0.018	0.104	0	0.13	0.613	0.172	0	0	0.282	1.044	2.301	0.928	2.377	0.494	15.2
2	M1	Memecik 1	0	0	3.412	0	0	0	0	0.018	0.11	0	0.129	0.614	0.165	0	0	0.257	0.996	2.383	0.915	2.321	0.501	14.8
3	M2	Memecik 2	0.598	0.092	2.898	0	0	0	0	0	0.219	0	0.084	1.846	0.469	0	0	0	0.984	3.153	1.223	1.456	1.167	14
4	M2	Memecik 2	0.591	0.101	2.79	0	0	0	0	0	0.207	0	0.08	1.825	0.499	0	0	0	0.926	2.981	1.187	1.664	1.18	13.7
5	M3	Memecik 3	0.156	0	3.678	0	0	0	0	0	0.043	0	0.048	0.415	0.193	0	0	0	0.838	2.106	0.774	1.861	0	4.62
6	M3	Memecik 3	0.152	0	3.661	0	0	0	0	0	0.041	0	0.049	0.423	0.191	0	0	0	0.737	2.024	0.789	1.786	0	4.67
7	E1	Erkence 1	4.294	1.179	18.47	0	0	0	0	0.058	0.382	0	0.063	0.21	0.084	0	0	0	0.566	4.46	2.267	2.097	0.956	19
8	E1	Erkence 1	4.314	1.187	18.51	0	0	0	0	0.06	0.375	0	0.063	0.202	0.096	0	0	0	0.522	4.465	2.291	2.574	0.889	20.5
9	E2	Erkence 2	13.59	0.101	44.17	0	2.508	0	0	0.114	0.515	0	0.086	0.381	0.123	0.083	0	0	0.605	3.958	3.786	2.469	1.149	33.5
10	E2	Erkence 2	13.56	0.125	42.18	0	2.247	0	0	0.095	0.442	0	0.081	0.176	0.223	0.914	0	0	0.674	3.664	3.575	1.918	0.923	29.2
11	E3	Erkence 3	0.896	0.027	3.177	0	0	0.092	0	0.011	0.186	0	0.059	0.142	0.047	0	0	0.654	0.228	1.292	1.483	0.67	0.404	13.5
12	E3	Erkence 3	0.919	0.03	3.237	0	0	0.09	0	0.012	0.191	0	0.055	0.149	0.055	0	0	0.691	0.396	1.417	1.491	0.554	0.548	12.7
13	E4	Erkence 4	9.1	2.08	26.03	0	0	0.152	0	0.075	0.367	0	0.109	0.242	0.106	0	0	0.852	2.406	3.947	3.32	2.818	1.183	27.4
14	E4	Erkence 4	9.347	2.12	26.63	0	0	0.041	0	0.084	0.398	0	0.113	0.241	0.114	0	0	0.811	0.963	3.983	3.357	2.582	1.423	28.4
15	E5	Erkence 5	0.882	0	5.266	0	0	0	0	0	0.071	0	0.064	0.13	0.032	0	0	2.196	0	2.661	1.961	0	1.864	28.1
16	E5	Erkence 5	0.944	0	5.366	0	0	0	0	0	0.107	0	0.065	0.151	0.041	0	0	2.139	0	2.742	1.99	0	1.776	28.4
17	G1	Gemlik 1	0	0.044	0.58	0	0	0.094	0	0	0.399	0	0.094	0.035	0.023	0	0	0.322	0.747	0.847	0.565	0.704	0.287	3.82
18	G1	Gemlik 1	0	0.05	0.542	0	0	0.091	0	0	0.366	0	0.093	0.04	0.02	0	0	0.316	0.643	0.982	0.561	0.629	0.255	3.49
19	G2	Gemlik 2	0.065	0.144	0.703	0	0	0	0	0	0.592	0	0.151	0.073	0	0	0	0.438	0.778	0.996	0.726	0.173	0	3.4
20	G2	Gemlik 2	0.069	0.146	0.682	0	0	0	0	0	0.554	0	0.151	0.074	0	0	0	0.518	0.761	1.073	0.711	0	0	3.46
21	G3	Gemlik 3	0.14	0	0.691	0	0	0	0	0	0.195	0	0.05	0.118	0.041	0	0	0.208	0.357	1.652	0.582	0.692	0.334	7.58
22	G3	Gemlik 3	0.163	0	0.662	0	0	0	0	0	0.197	0	0.049	0.122	0.038	0	0	0.178	0.475	1.671	0.62	0.985	0.458	8.11
23	A1	Ayvalık 1	0.253	0	0.384	0	0.64	0	0	0.022	0.56	0	0.118	0.188	0.059	0	0	0.793	0.778	1.696	0.048	0	0.331	2.5
24	A1	Ayvalık 1	0.239	0	0.34	0	0.642	0	0	0.025	0.498	0	0.114	0.18	0.053	0	0	0.747	0.649	1.575	0.046	0	0.329	2.39
25	A2	Ayvalık 2	0.167	0	0.417	0	0	0	0	0.039	0.899	0	0.06	0.195	0.043	0	0	0.494	1.349	6.174	0.082	1.3	1.668	2.57
26	A2	Ayvalık 2	0.172	0	0.44	0	0	0	0	0.051	0.929	0	0.063	0.203	0.055	0	0	0.557	1.394	6.712	0.076	1.359	1.741	2.63

(cont. on next page)

Table A.7. (cont.) Individual phenolic compounds in extracted EVOOs of 2006 harvest year

Sample No	Sample Code	Sample	Hyt	Hdba	Tyr	Chla	dba	Hpha	3 hpha	Ca	Va	Val	Sya	Pco	Fa	Mco	Oco	peak(RT:44)	peak(RT:47.5)	peak(RT:49.5)	Cina	Lut	peak(RT:61.5)	Apg
27	D1	Domat 1	5.460	0	9.647	0	0	0	0	0.023	0.207	0	0.036	0.024	0	0	0	0.713	0.343	6.745	0.340	0.106	0.259	1.265
28	D1	Domat 1	5.728	0	9.848	0	0	0	0	0.025	0.213	0	0.036	0.020	0.001	0	0	0.727	0.364	7.007	0.348	0.110	0.238	1.445
29	D2	Domat 2	0.078	0.037	1.039	0	0	0.104	0	0.011	0.364	0	0.099	0.027	0	0	0	0.531	2.050	14.087	0.720	0.077	0.410	2.006
30	D2	Domat 2	0.081	0.033	1.050	0	0	0.116	0	0.011	0.356	0	0.097	0.019	0	0	0	0.470	1.906	13.974	0.705	0.109	0.387	2.016
31	D3	Domat 3	0.229	0.335	2.161	0	0	0	0	0	0.673	0	0.145	0	0	0	0	0.667	1.623	21.184	0.888	0	0.330	1.966
32	D3	Domat 3	0.246	0.338	2.208	0	0	0	0	0	0.669	0	0.140	0	0	0	0	0.636	1.598	21.885	0.922	0	0.298	1.986
33	N1	Nizip 1	0.643	0.062	2.850	0	0	0.118	0	0.072	0.321	0	0.075	0.081	0	0	0	0	0.525	2.062	0.644	0.747	1.015	1.502
34	N1	Nizip 1	0.676	0.064	2.900	0	0	0.156	0	0.032	0.156	0	0.075	0.092	0	0	0	0	0.677	1.848	0.703	0.000	1.021	1.482
35	N2	Nizip 2	0.176	0	1.243	0.331	0	0	0	0.014	0.033	0	0.046	0.007	0	0	0	0.140	0	0.490	0.080	0.282	0.189	2.179
36	N2	Nizip 2	0.187	0	1.256	0.297	0	0	0	0.011	0.038	0	0.046	0.010	0	0	0	0.172	0.161	0.490	0.080	0.221	0.185	2.224
37	N2	Nizip 2	0.229	0	1.553	0	1.991	0.065	0	0.017	0.041	0	0.060	0.013	0	0	0	0.427	0	0.482	0.100	0	0.156	2.427
38	N2	Nizip 2	0.206	0	1.683	0	1.990	0.088	0	0.015	0.041	0	0.057	0.017	0	0	0	0.291	0	0.561	0.101	0	0.177	2.038
39	GE1	Gemlik Edremit 1	0.420	0.040	1.341	0	1.050	0	0	0	1.243	0	0.209	0.112	0.047	0	0	0.469	0.928	1.480	0.701	2.048	0.380	3.653
40	GE1	Gemlik Edremit 1	0.364	0.037	1.423	0	0.366	0	0	0	1.288	0	0.219	0.119	0.047	0	0	0.570	0.920	1.530	0.746	2.116	0.403	4.023
41	GE2	Gemlik Edremit 2	0.480	0	1.639	0	0	0.251	0	0	1.368	0	0.427	0.115	0.047	0	0	1.057	1.378	3.028	1.126	2.283	0.539	5.981
42	GE2	Gemlik Edremit 2	0.475	0	1.563	0	0	0.243	0	0	1.169	0	0.419	0.106	0.045	0	0	0.988	1.428	3.107	1.107	2.183	0.539	5.916
43	GE3	Gemlik Edremit 3	0.105	0.245	0.411	0	0	0	0	0	0.564	0	0.102	0.075	0.034	0	0	0.251	0.338	0.305	0.316	0.303	0.166	1.011
44	GE3	Gemlik Edremit 3	0.096	0.246	0.415	0	0	0	0	0	0.561	0	0.107	0.086	0.037	0	0	0.215	0.403	0.426	0.338	0.286	0.208	1.086
45	GE4	Gemlik Edremit 4	0	0	0.358	0	0	0.093	0	0	0.340	0	0.135	0.027	0	0	0	0.382	0.628	1.501	0.509	1.198	0	3.358
46	GE4	Gemlik Edremit 4	0	0	0.376	0	0	0.092	0	0	0.348	0	0.138	0.028	0	0	0	0.418	0.706	1.612	0.528	1.045	0	3.464
47	AE1	Ayvalık Edremit 1	0.129	0	2.051	0	0.834	0	0	0.026	0.828	0	0.268	0.519	0.167	0	0	1.014	0.651	5.495	0.080	1.655	0	1.123
48	AE1	Ayvalık Edremit 1	0.117	0	2.071	0	0.801	0	0	0.026	0.860	0	0.275	0.522	0.181	0	0	1.050	0.696	5.797	0.080	1.851	0	1.118
49	AE2	Ayvalık Edremit 2	0.214	0	1.126	0	0	0	0	0.173	0.298	0	0.387	0.757	0.153	0	0	1.180	0.957	8.517	0.145	0	0.618	2.076
50	AE2	Ayvalık Edremit 2	0.242	0	1.090	0	0	0	0	0.152	0.379	0	0.381	0.767	0.177	0	0	1.241	0.961	8.068	0.171	0	0.474	1.716
51	AE3	Ayvalık Edremit 3	0.245	0	1.091	0	0.685	0	0	0	0.798	0	0.402	0.564	0.218	0	0	1.273	1.017	11.003	0.173	3.769	0	2.347
52	AE3	Ayvalık Edremit 3	0.240	0	1.034	0	0.516	0	0	0	0.786	0	0.395	0.568	0.203	0	0	1.269	1.225	10.945	0.180	4.023	0	2.410
53	AE4	Ayvalık Edremit 4	0.251	0	0.539	0	0	0	0	0.016	0.400	0	0.451	0.336	0.093	0	0	1.610	0.903	6.026	0.119	0	0.546	1.139
54	AE4	Ayvalık Edremit 4	0.259	0	0.580	0	0	0	0	0.015	0.211	0	0.453	0.319	0.075	0	0	1.630	0.775	5.955	0.121	0	0.593	1.150
55	AE5	Ayvalık Edremit 5	0.273	0	0.739	0	0.635	0	0	0	0.645	0	0.498	0.236	0.083	0	0	1.524	1.216	0.823	0.117	2.776	0	1.724
56	AE5	Ayvalık Edremit 5	0.294	0	0.789	0	0.662	0	0	0	0.674	0	0.513	0.246	0.089	0	0	1.507	1.156	8.553	0.126	2.652	0	1.802

Table A.8. Individual phenolic compounds in commercial EVOOs of 2006 harvest year

Sample No	Sample Code	Sample	Hyt	Hdba	Tyr	Chla	dba	Hpha	3 hpha	Ca	Va	Val	Sya	Pco	Fa	Mco	Oco	peak(RT:44)	peak(RT:47.5)	peak(RT:49.5)	Cina	Lut	peak(RT:61.5)	Apg
1	Ez	Ezine	17.568	0	15.582	0	0	0.529	0	0	0.595	0	0.437	0.397	0.149	0	0	0.457	0.476	11.958	0.097	4.041	0	1.829
2	Ez	Ezine	18.459	0	17.505	0	0	0.577	0	0	0.597	0	0.462	0.415	0.151	0	0	0.438	0.461	12.189	0.081	3.464	0	1.575
3	Kk	Kucukkuyu	1.715	0	1.957	0	0.498	0.124	0.279	0	0.084	0.024	0.296	0.228	0.105	0	0	0.167	0.829	8.914	0.045	1.477	0.215	3.678
4	Kk	Kucukkuyu	1.715	0	2.004	0	0.389	0.129	0	0	0.131	0.022	0.291	0.229	0.129	0	0	0.125	0.431	9.037	0.041	1.219	0.238	3.660
5	Aol	Altinoluk	1.683	0	0.813	0	0	0.059	0.112	0	0.127	0.021	0.294	0.201	0.045	0.016	0	0.347	0.345	3.606	0.016	0.199	0.062	1.143
6	Aol	Altinoluk	1.680	0	0.836	0	0	0.046	0.111	0	0.129	0.024	0.310	0.200	0.055	0.009	0	0.320	0.333	3.697	0.016	0.164	0.057	0.989
7	Ed	Edremit	2.334	0.026	1.898	0	0	0.091	0	0.034	0.163	0	0.020	0.242	0.090	0.020	0	0.246	0.297	4.940	0.025	0.688	0.577	1.233
8	Ed	Edremit	2.407	0.023	1.868	0	0	0.096	0	0.033	0.168	0	0.018	0.247	0.098	0.018	0	0.270	0.323	5.043	0.023	0.726	0.674	1.230
9	Ha	Havran	18.388	0.057	10.395	0	0.534	0.472	0	0.092	0.074	0.048	0.210	0.414	0.178	0.022	0	0.143	0.552	4.857	0.028	0.224	0.186	0.696
10	Ha	Havran	18.761	0.049	10.729	0	0.590	0.476	0	0.104	0.101	0.046	0.216	0.425	0.186	0.019	0	0.175	0.545	4.999	0.027	0.228	0.236	0.837
11	Bu	Burhaniye	10.272	0.061	4.285	0	1.310	0.391	0.726	0	0.182	0.046	0.673	0.848	0.497	0.052	0.038	0.562	1.020	9.360	0.046	1.763	1.045	1.706
12	Bu	Burhaniye	10.540	0.068	4.370	0	1.084	0.424	0.743	0	0.172	0.045	0.714	0.876	0.549	0.050	0.025	0.633	1.028	9.745	0.043	1.752	1.083	1.641
13	Go	Gomec	6.336	0.034	2.745	0	0.577	0.031	0.183	0.089	0.124	0.039	0.641	0.283	0.078	0.023	0	0.453	0.765	6.640	0.038	1.305	1.292	1.483
14	Go	Gomec	6.200	0.042	2.403	0	0.478	0.042	0.181	0.089	0.134	0.039	0.635	0.276	0.069	0.020	0	0.509	0.584	6.623	0.037	1.095	1.225	1.499
15	Ay	Ayvalik	2.372	0.033	2.083	0	0.447	0.163	0	0.093	0.111	0.021	0.457	0.225	0.189	0.021	0	0.107	0.784	5.598	0.041	0.543	0	1.943
16	Ay	Ayvalik	2.431	0.033	2.102	0	0.416	0.170	0	0.089	0.108	0.017	0.468	0.221	0.188	0.025	0	0.439	0.565	5.893	0.037	0.467	0	1.907
17	Aov	Altinova	10.442	0	6.945	0	0.244	0.237	0	0.062	0.074	0.031	0.238	0.075	0.035	0.019	0	0.346	0.521	3.932	0.167	0.556	0.163	1.120
18	Aov	Altinova	10.413		6.644	0	0.292	0.233	0	0.067	0.073	0.025	0.233	0.082	0.031	0.020	0	0.331	0.556	4.020	0.168	0.450	0.142	1.126
19	Ze	Zeytindag	1.744	0	1.740	0	0	0.051	0	0.024	0.168	0.020	0.312	0.274	0.083	0.024	0	0.231	0.745	5.883	0.081	1.200	0	0.911
20	Ze	Zeytindag	1.732	0	1.468	0	0	0.066	0	0.025	0.143	0.014	0.297	0.267	0.079	0.024	0	0.293	0.501	5.666	0.080	0.943	0	0.922
21	Te	Tepekoy	2.223	0	2.951	0	0	0	0.036	0.041	0.055	0	0.186	0.564	0.103	0	0	0.126	0.559	1.410	0.498	0.738	0.130	1.103
22	Te	Tepekoy	2.294	0	2.994	0	0	0	0.023	0.039	0.055	0	0.177	0.559	0.087	0	0	0.092	0.561	1.338	0.470	0.621	0.129	1.098
23	Ba	Bayindir	2.469	0.050	24.339	0	0.677	0.305	0	0.141	0.176	0	0.046	1.259	0.358	0.042	0	0.117	1.510	3.692	0.867	0.341	0.141	3.334
24	Ba	Bayindir	2.592	0.058	25.072	0	0.584	0.318	0	0.139	0.161	0	0.038	1.329	0.381	0.046	0	0.138	0.934	3.800	0.899	0.355	0.145	3.296
25	Od	Odemis	4.945	0.150	7.550	0.064	0.423	0.226	0	0	0.095	0.021	0.213	0.624	0.149	0.052	0	0.123	1.167	5.323	1.066	2.041	0.399	3.896
26	Od	Odemis	4.980	0.164	7.248	0.057	0	0	0	0	0.071	0.017	0.210	0.615	0.167	0.048	0	0.161	1.266	5.210	1.057	2.102	0.495	3.731

(cont. on next page)

Table A.8. (cont.) Individual phenolic compounds in commercial EVOOs of 2006 harvest year

Sample No	Sample Code	Sample	Hyt	Hdba	Tyr	Chla	dba	Hpha	3 hpha	Ca	Va	Val	Sya	Pco	Fa	Mco	Oco	peak(RT:44)	peak(RT:47.5)	peak(RT:49.5)	Cina	Lut	peak(RT:61.5)	Apg
27	Ti	Tire	1.482	0	4.903	0	0.476	0.093	0	0.134	0.058	0	0.132	0.959	0.425	0.044	0	0.182	1.259	3.603	0.844	0.823	0.315	1.325
28	Ti	Tire	1.565	0	5.149	0	0.510	0.092	0	0.134	0.065	0	0.113	0.993	0.445	0.048	0	0.136	1.360	3.659	0.872	0.695	0.343	1.034
29	Se	Selcuk	7.834	0.063	7.514	0	0.368	0.085	0	0.052	0.067	0.025	0.324	0.851	0.270	0.059	0	0.138	0.654	3.613	0.650	1.053	0.290	1.822
30	Se	Selcuk	8.299	0.050	8.017	0	0.264	0.086	0	0.056	0.073	0.026	0.301	0.855	0.282	0.061	0	0.121	0.885	3.581	0.641	0.800	0.254	1.886
31	Ku	Kusadasi	3.280	0.063	5.968	0	0	0.054	0	0.050	0.109	0	0.183	0.420	0.291	0.033	0	0.115	0.402	1.708	0.542	2.032	0.702	2.476
32	Ku	Kusadasi	3.349	0.058	6.138	0	0	0.062	0	0.052	0.104	0	0.167	0.448	0.325	0.023	0	0.116	0.291	1.615	0.531	1.703	0.703	2.500
33	Ge	Germencik	5.877	0.046	4.263	0	0	0.037	0.063	0.043	0.081	0	0.170	0.795	0.205	0.045	0	0	0.853	2.942	0.567	1.165	0.267	1.166
34	Ge	Germencik	6.155	0.047	4.293	0	0	0.041	0.066	0.035	0.068	0	0.173	0.825	0.223	0.053	0	0	1.015	3.047	0.585	0.875	0.285	1.256
35	Ayd	Aydin	2.185	0.036	7.463	0	0	0.056	0	0.011	0.026	0.012	0.075	0.167	0.053	0.016	0	0.052	0.334	1.753	0.239	0.200	0.204	1.665
36	Ayd	Aydin	2.275	0.038	7.416	0	0	0.055	0	0.011	0.024	0.012	0.064	0.168	0.066	0.017	0	0.077	0.307	1.730	0.245	0.238	0.212	1.650
37	Or	Ortaklar	7.529	0	16.147	0	0.523	0.196	0	0	0.435	0.027	0.263	1.954	0.498	0.044	0	0.806	1.702	4.435	0.882	2.761	0.381	1.597
38	Or	Ortaklar	7.671	0	16.077	0	0.543	0.195	0	0	0.449	0.026	0.262	1.942	0.522	0.052	0	0.820	1.508	4.487	0.896	2.699	0.390	1.552
39	Kos	Kosk	4.474	0.096	6.364	0	0.192	0.080	0	0.025	0.039	0	0.015	0.411	0.119	0.025	0	0.055	0.683	2.136	0.576	0	1.377	1.537
40	Kos	Kosk	4.805	0.101	6.892	0	0	0.097	0	0.022	0.044	0	0.016	0.433	0.126	0.027	0	0.081	0.577	2.329	0.621	0	1.612	1.676
41	Da	Dalama	2.132	0.085	4.805	0	0.323	0.065	0	0.024	0.069	0	0.243	0.367	0.242	0.039	0	0.129	1.440	4.663	0.765	2.299	0.227	4.345
42	Da	Dalama	2.013	0.097	4.730	0	0.240	0.057	0	0.038	0.093	0	0.235	0.345	0.243	0.036	0	0.102	1.016	4.775	0.733	2.289	0.223	4.116
43	Koc	Kocarli	3.884	0.121	9.874	0	0	0	0	0.034	0.149	0.019	0.160	0.390	0.184	0.058	0.078	0.511	0.313	1.821	0.430	2.216	1.235	5.993
44	Koc	Kocarli	4.000	0.134	10.078	0.151	0	0	0	0	0.149	0.013	0.030	0.398	0.155	0.027	0.079	0.403	0.278	1.864	0.418	1.389	0.382	5.236
45	Koc	Kocarli	3.109	0.123	7.221	0	0	0.050	0	0.043	0.089	0.025	0.159	0.383	0.167	0.033	0	0.133	0.760	3.599	0.521	2.124	0.385	4.500
46	Koc	Kocarli	3.153	0.119	7.183	0	0	0.064	0	0.036	0.090	0.028	0.164	0.369	0.177	0.036	0	0.113	0.750	3.424	0.490	1.824	0.366	4.511
47	Er	Erbeyli	0.432	0	26.221	0	0	0.301	0	0.026	0.043	0	0.053	0.422	0.202	0.024	0	0	0.643	2.871	0.610	0.977	0.141	3.052
48	Er	Erbeyli	0.433	0	27.217	0	0	0.321	0	0.056	0.096	0	0.055	0.428	0.200	0.026	0	0	0.607	2.922	0.618	0.646	0.148	3.090
49	Ci	Cine	1.010	0.048	15.843	0	0	0.116	0	0.039	0.046	0.033	0.063	0.255	0.298	0.022	0	0.064	0.915	2.969	0.462	0.674	0.379	3.780
50	Ci	Cine	1.040	0.039	16.183	0	0	0.125	0	0.018	0.023	0.037	0.072	0.263	0.319	0.023	0	0.077	0.639	2.867	0.470	0.592	0.370	3.819
51	Mi	Milas	8.166	0.090	15.153	0	0	0	0.112	0.069	0.116	0.068	0.343	0.823	0.433	0.077	0	0.180	2.021	4.227	0.803	2.537	0.272	3.547
52	Mi	Milas	8.198	0.077	16.050	0	0	0	0.130	0.065	0.131	0.062	0.334	0.859	0.461	0.080	0	0.140	1.398	4.233	0.827	2.671	0.352	3.865

Standard Calibration Curves for Phenolic Compounds

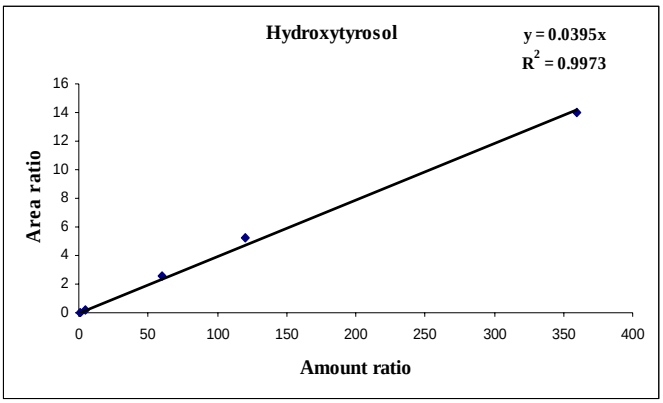

Figure A.1. Standard calibration curve for hydroxytyrosol

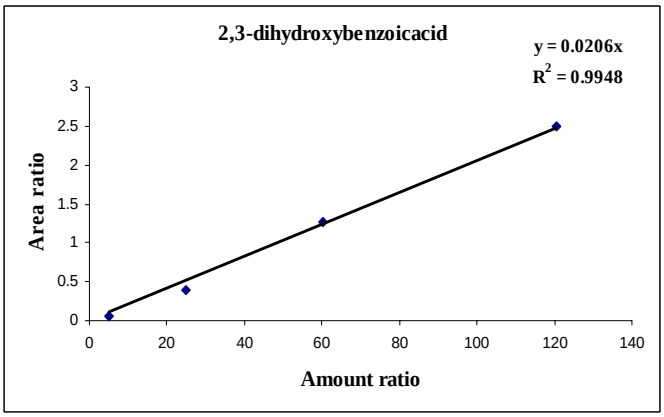

Figure A.2. Standard calibration curve for 2.,3 dihydroxybenzoic acid

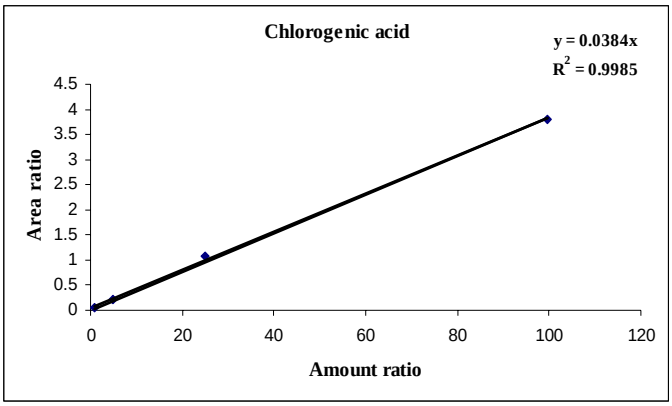

Figure A.3. Standard calibration curve for chlorogenic acid

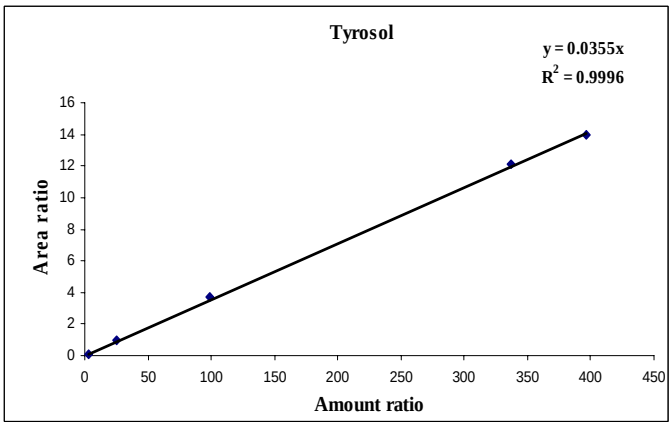

Figure A.4. Standard calibration curve for tyrosol

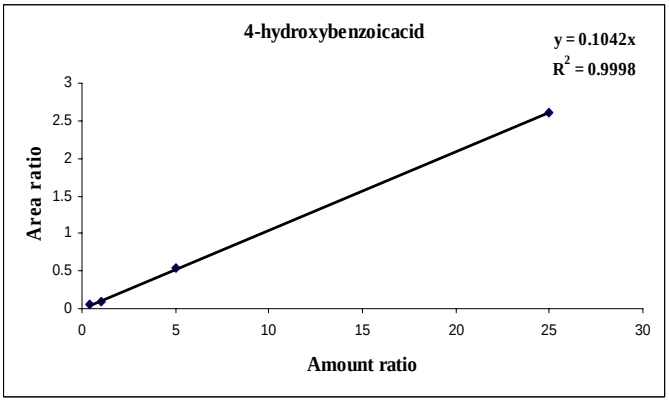

Figure A.5. Standard calibration curve for 4-hydroxybenzoic acid

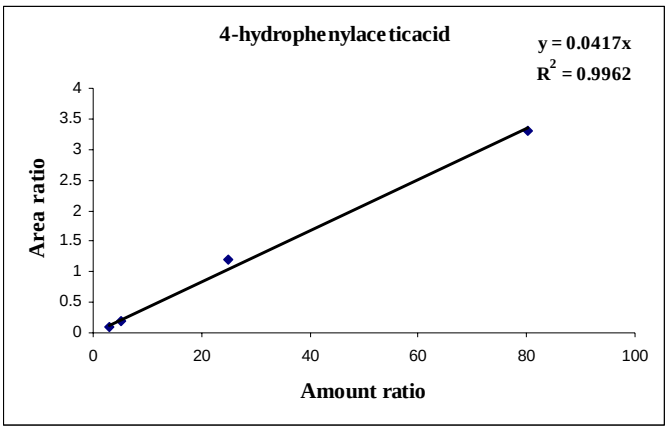

Figure A.6. Standard calibration curve for 4-hydroxyphenylacetic acid

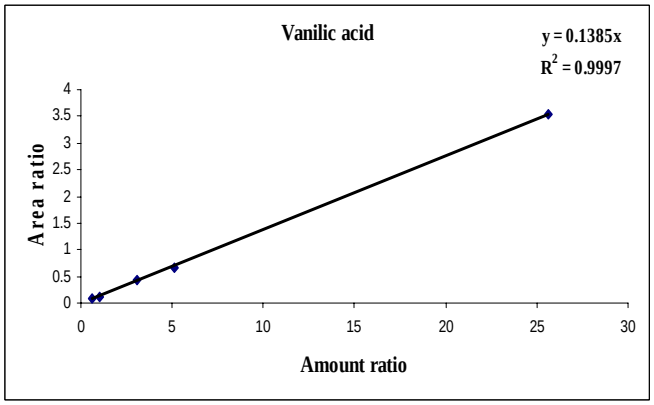

Figure A.7. Standard calibration curve for vanilic acid

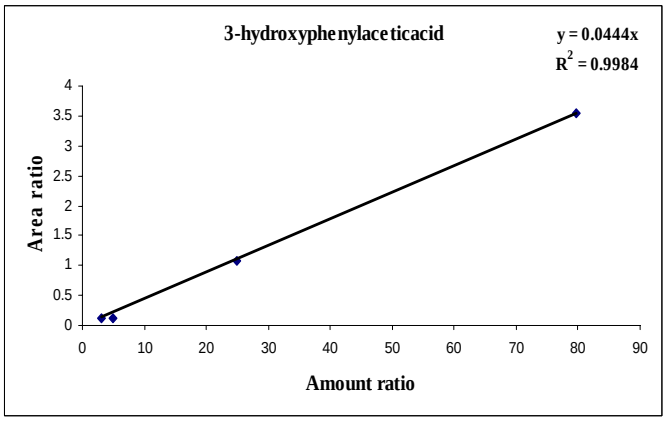

Figure A.8. Standard calibration curve for 3-hydroxyphenylacetic acid

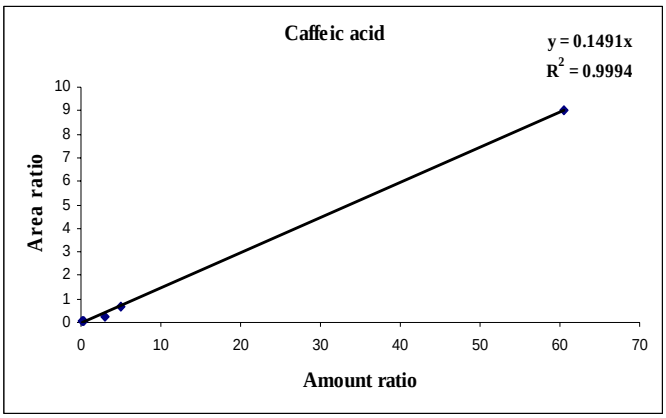

Figure A.9. Standard calibration curve for caffeic acid

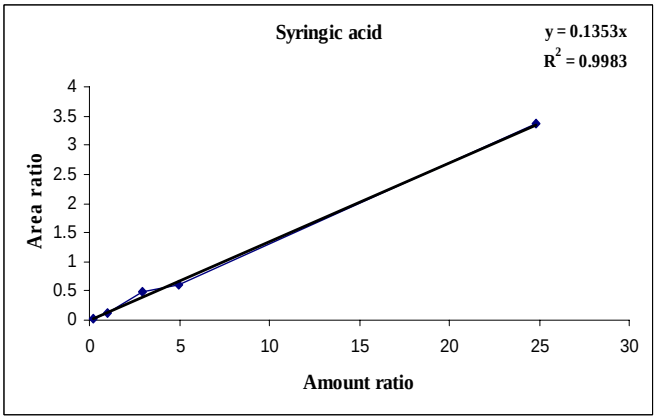

Figure A.10. Standard calibration curve for syringic acid

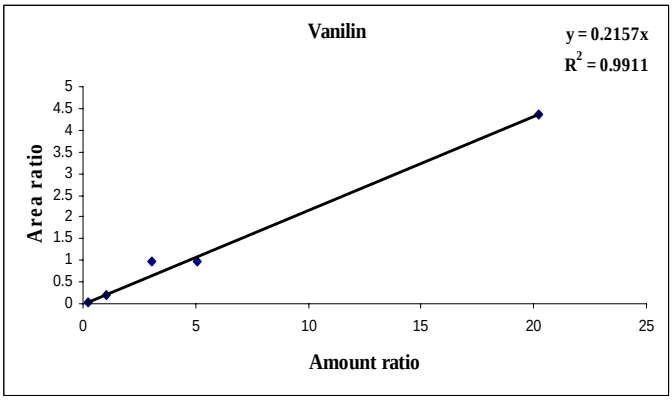

Figure A.11. Standard calibration curve for vanillin

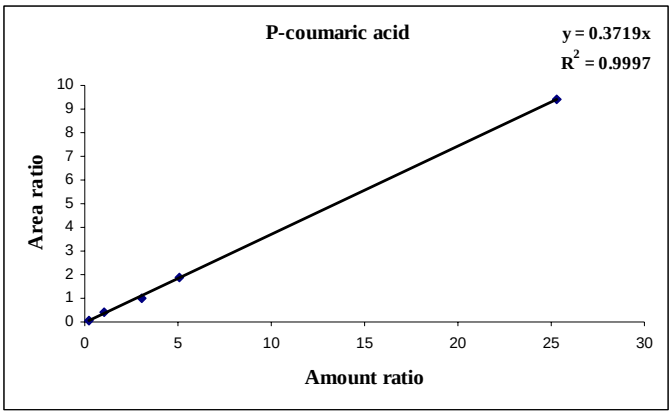

Figure A.12. Standard calibration curve for p-coumaric acid

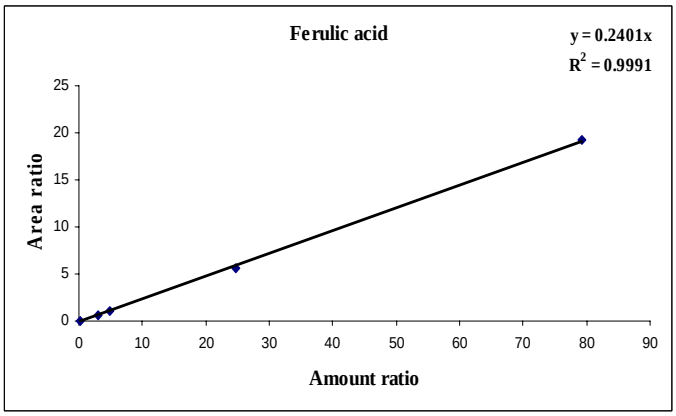

Figure A.13. Standard calibration curve for ferulic acid

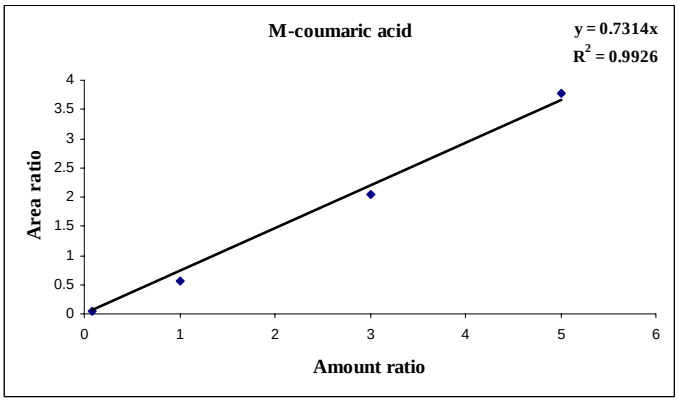

Figure A.14. Standard calibration curve for m-coumaric acid

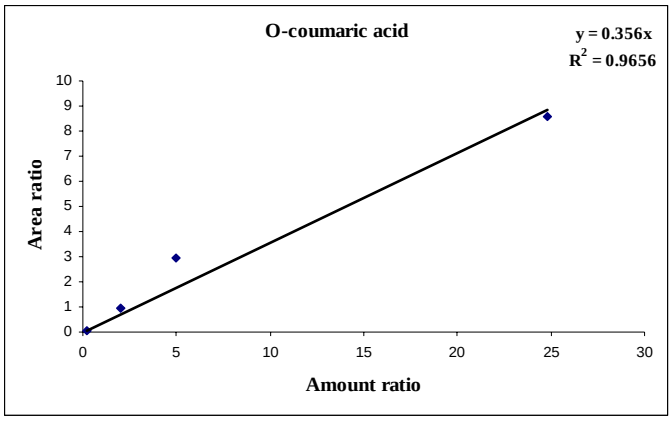

Figure A.15. Standard calibration curve for o-coumaric acid

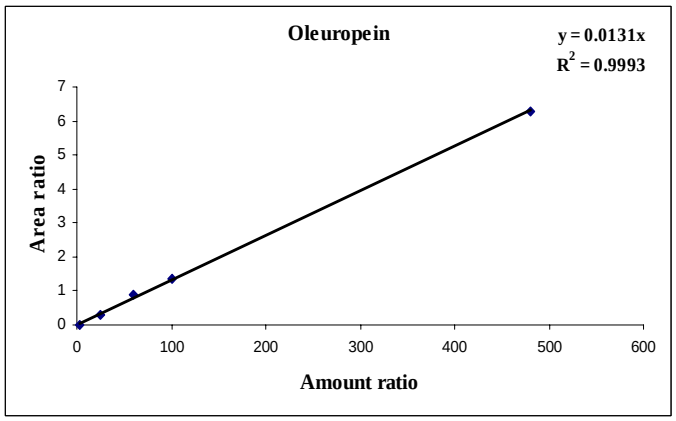

Figure A.16. Standard calibration curve for oleuropein

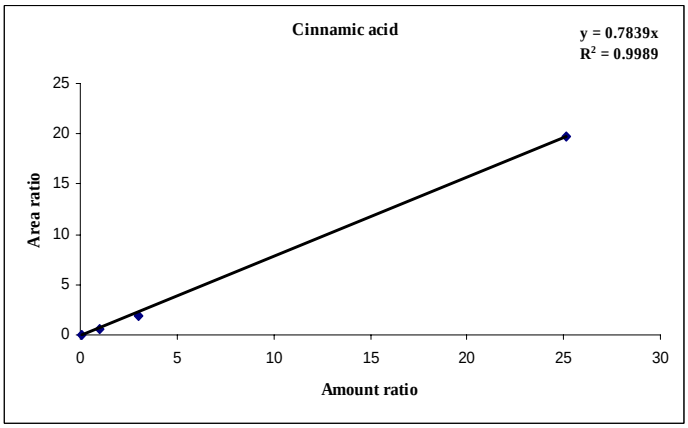

Figure A.17. Standard calibration curve for cinnamic acid

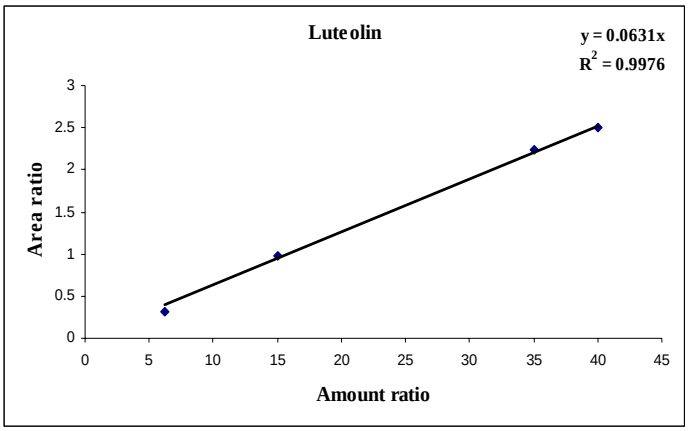

Figure A.18. Standard calibration curve for luteolin

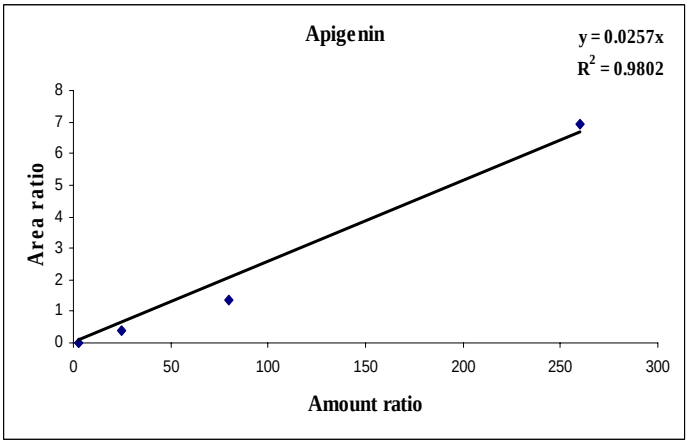

Figure A.19. Standard calibration curve for apigenin

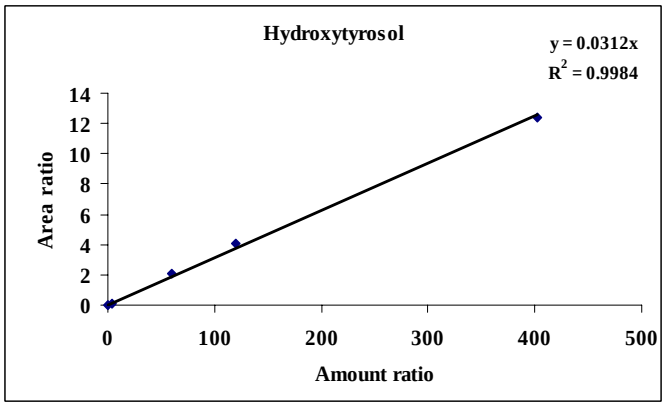

Figure A.20. Standard calibration curve for hydroxytyrosol

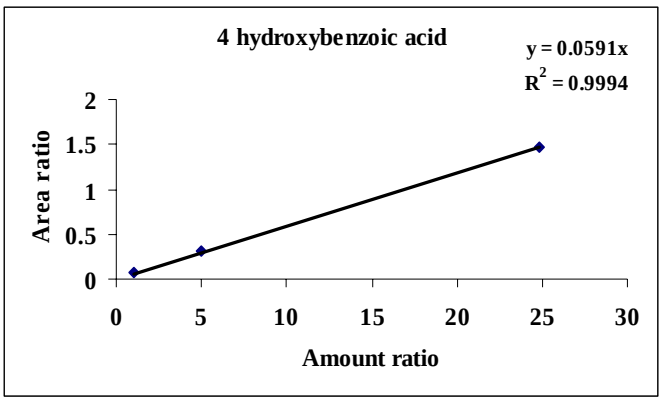

Figure A.21. Standard calibration curve for 4-hydroxybenzoic acid

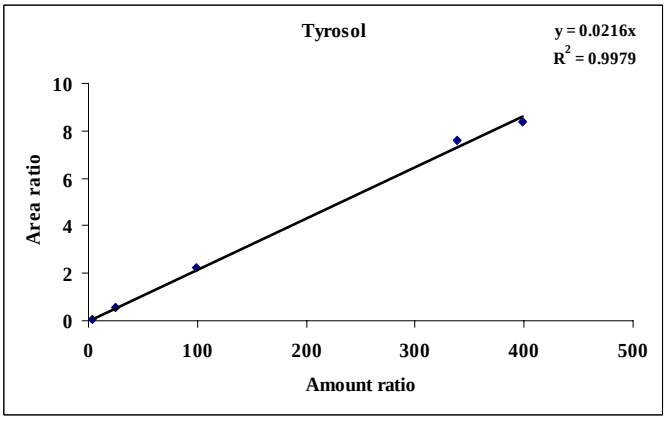

Figure A.22. Standard calibration curve for tyrosol

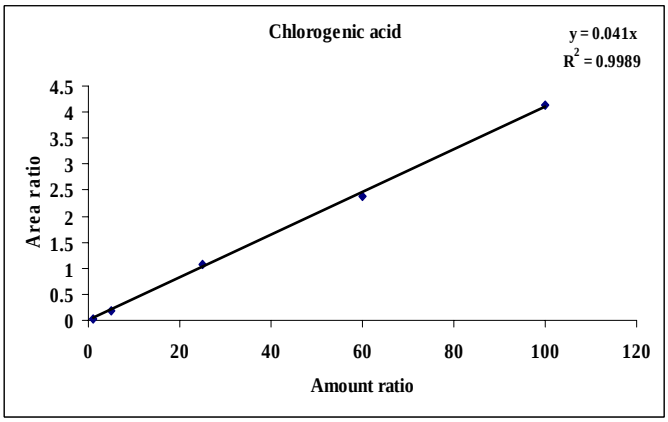

Figure A.23. Standard calibration curve for chlorogenic acid

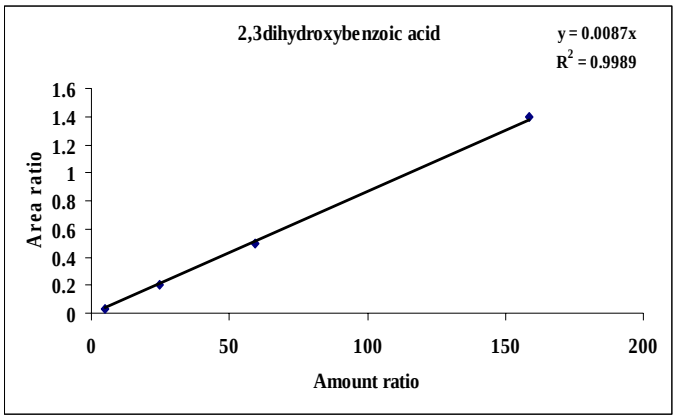

Figure A.24. Standard calibration curve for 2,3 dihydroxybenzoic acid

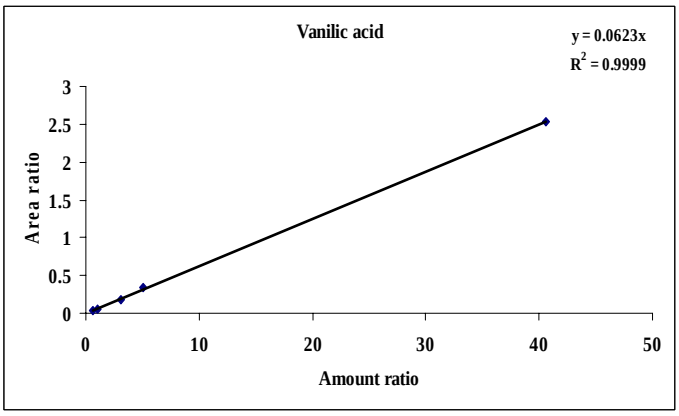

Figure A.25. Standard calibration curve for vanilic acid

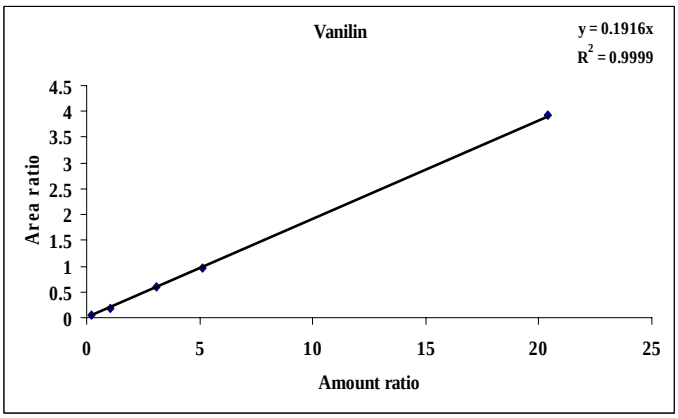

Figure A.26. Standard calibration curve for vanillin

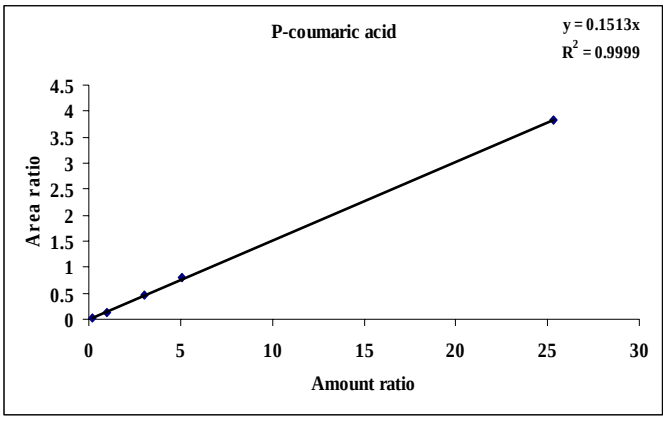

Figure A.27. Standard calibration curve for p-coumaric acid

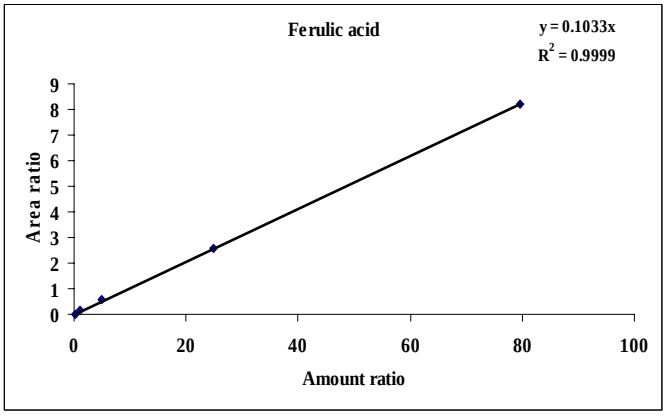

Figure A.28. Standard calibration curve for ferulic acid

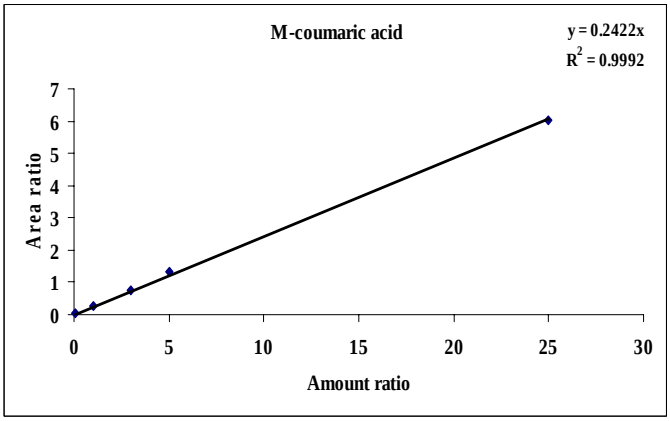

Figure A.29. Standard calibration curve for m-coumaric acid

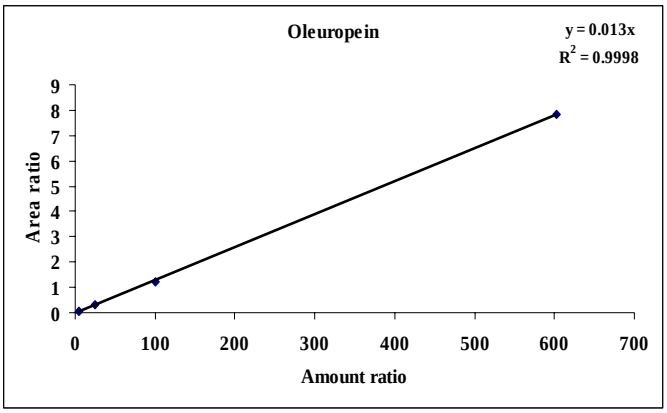

Figure A.30. Standard calibration curve for oleuropein

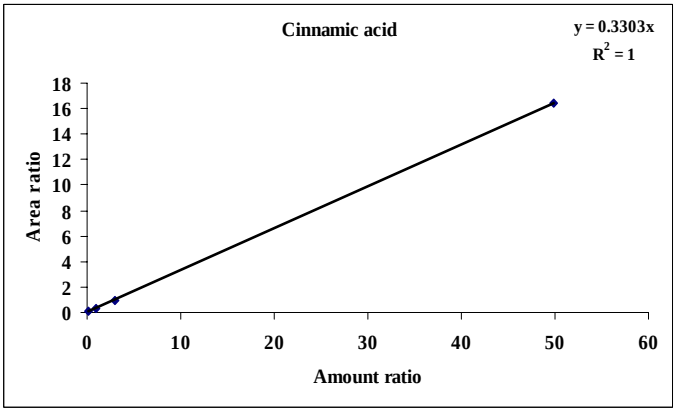

Figure A.31. Standard calibration curve for cinnamic acid

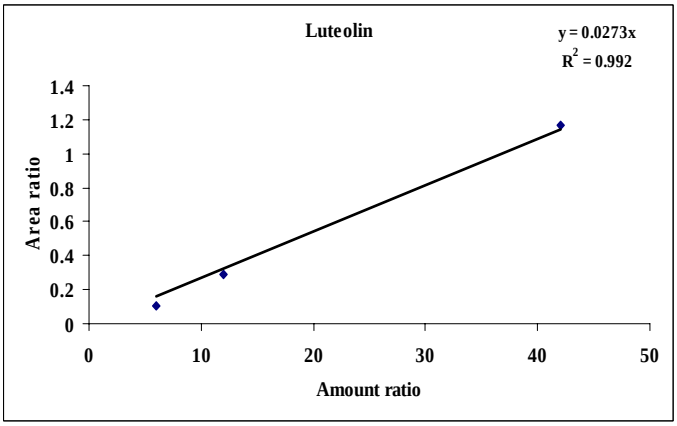

Figure A.32. Standard calibration curve for luteolin

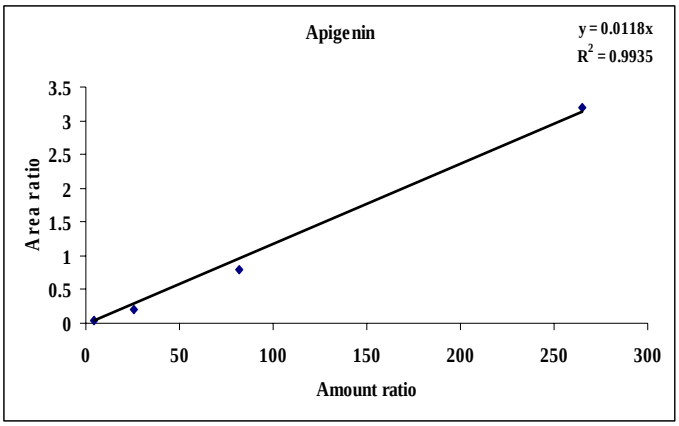

Figure A.33. Standard calibration curve for apigenin