EFFECT OF STORAGE TIME ON OLIVE OIL QUALITY

A Thesis Submitted to
The Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Food Engineering

by
Gaye YILDIRIM

July 2009

İZMİR

We approve the thesis of **Gaye YILDIRIM**

Assoc. Prof. Dr. Figen TOKATLI
Supervisor

Assoc. Prof. Dr. Banu ÖZEN
Co-Supervisor

Assoc. Prof. Dr. Figen KOREL
Co-Supervisor

Assist. Prof. Dr. Sevcan ÜNLÜTÜRK
Committee Member

Assoc. Prof. Dr. Durmuş ÖZDEMİR
Committee Member

09 July 2009

Prof. Dr. Şebnem HARSA
Head of the Department of
Food Engineering

Prof. Dr. Hasan BÖKE
Dean of the Graduate School of
Engineering and Science

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor Assoc Prof. Dr. Figen TOKATLI for her guidance, supervision, patience, and support during this study. I also wish to express my thanks to my co-advisors Assoc. Prof. Dr. Banu ÖZEN and Assoc. Prof. Dr.Figen KOREL for their all kind of support and valuable comments.

I would like to thank to the Union of Taris Olive and Olive Oil Agricultural Sales Co-operatives in İzmir for the commercial olive oil samples.

Also, technical support of IZTECH R&D Center for Environmental Studies in this research is gratefully acknowledged.

I would also like to thank my friends Derya OCAKOĞLU, Pınar KADIROĞLU, Gözde GÜRDENİZ, Bahar YEŞİLBULUTLU and Ürün ŞENYURDUSEV for their help.

Lastly, I offer sincere thanks to my family members for their endless support, encouragement and love.

ABSTRACT

EFFECT OF STORAGE TIME ON OLIVE OIL QUALITY

This work reports changes in the major quality parameters of Turkish extra virgin olive oils stored at room and refrigerator temperatures in dark and monitored for 14 months. Peroxide values, specific absorbance values, total phenol content, free fatty acidity, fatty acid and phenolic profiles of extracted (Erkence and Ayvalık-Edremit) and commercial extra virgin olive oil samples (Altınoluk, Ezine, Bayındır and Ortaklar) from South and North of the Aegean were determined. The acidity and K232 values of samples were within the acceptable limits, the peroxide and K270 values exceeded the limits after 7 and 9 months storage. Hydroxytyrosol, tyrosol, caffeic acid, vanillic acid, vanilin, p-coumaric acid, ferulic acid, m-coumaric acid, cinnamic acid, luteolin, and apigenin were determined as the major phenolics in Turkish extra virgin olive oils. The concentration of hydroxytyrosol and tyrosol increased, while a decrease was observed in the amounts of other phenolics during storage.

The highly unsaturated flaxseed oil was mixed with olive oil at 5-15 % levels and stored at room and refrigerator temperature to examine the effect of olive oil on the oxidative stability of flaxseed oil. 15% olive oil addition to flaxseed oil increased its oxidative stability.

Fourier Transform (FT-IR) spectral data were used to predict the oxidative quality parameters, total phenol content and the fatty acid compositions by partial least square analysis (PLS). FT-IR spectra of samples subjected to accelerated oxidation were examined to determine the bandwidths, which can be considered as the finger-prints of the oxidation phenomenon (2924, 2852, 1746-1743, 1163 and 967-976 cm^{-1}).

ÖZET

DEPOLAMA SÜRESİNİN ZEYTİNYAĞI KALİTESİ ÜZERİNE ETKİSİ

Bu çalışmada, oda sıcaklığı ve buzdolabında 14 ay boyunca depolanan naturel zeytinyağlarının başlıca kalite parametreleri, yağ asitleri ve fenolik madde kompozisyonlarındaki değişimleri gözlemlenmiştir. Ekstre edilmiş (Erkence ve Ayvalık) zeytinyağları ile Kuzey ve Güney Ege bölgelerine ait ticari zeytinyağlarının (Altınoluk, Ezine, Bayındır ve Ortaklar) peroksit değerleri, spesifik absorbans değerleri, toplam fenol içerikleri, serbest yağ asitliği, yağ asidi ve fenol profilleri belirlenmiştir. Zeytinyağı örneklerinin serbest asitliği ve K232 absorbans değerleri kabul edilir sınırlar içersinde kalırken, peroksit değerleri ve K270 absorbans değerleri 7 ve 9 aydan sonra sınır değerlerini geçmişlerdir. Hidroksitirosol, tirosol, kafeik asit, vanilik asit, m-kumarik asit, sinamik asit, luteolin ve apigenin Türk naturel sızma zeytinyağlarının başlıca fenolikleri olarak belirlenmiştir. Depolama sırasında hidroksityrosol ve tirosol miktarları artarken, diğer fenolik maddelerin miktarlarında azalma görülmüştür.

Yüksek miktarda doymamış yağ asidi içeren keten tohumu yağı % 5-15 oranlarında zeytinyağı ile karıştırılmış, keten tohumu yağının oksidasyon özelliklerindeki değişiklikler gözlenmiştir. %15 oranında keten tohumu yağına eklenen zeytinyağı, keten tohumu yağının oksidatif stabilitesini arttırmıştır.

Oksidasyon kalite parametrelerini, toplam fenol içeriğini ve yağ asidi kompozisyonlarını orta bölge kızıl ötesi Fourier Transform (FT-IR) spektra verileriyle tahmin etmek için, kısmi en küçük kareler analizi (PLS) uygulanmıştır. Hızlı oksidasyona tabi tutulan yağ örneklerinin FT-IR spektrasında oksidasyonun parmak izleri olan bant genişlikleri tanımlanmıştır (2924, 2852, 1746-1743, 1163, 967-976 ve 721 cm^{-1}).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

A	Altınoluk Olive Oil
AE	Ayvalık-Edremit Olive Oil
ALA	Alpha-Linolenic Acid
AV	Anisidine Value
B	Bayındır Olive Oil
CIE	Commission Internationale d'Eclairage
DAG	Diacylglycerols
DSC	Differential Scanning Calorimetry
E	Ezine Olive Oil
Erk	Erkence Olive Oil
EVOO	Extra Virgin Olive Oil
FFA	Free Fatty Acids
GA	Gallic Acid
HATR	Horizontal Attenuated Total Reflectance
IOOC	International Olive Oil Council
IV	Iodine Value
LOX	Lipoxygenase Pathway
MAG	Monoacylglycerols
MUFA	Monounsaturated Fatty Acid
O	Ortaklar Olive Oil
Q^2	The Goodness of Prediction Parameter
PCA	Principle Component Analysis

PCR	Principle Component Regression
PDO	Protected Denomination of Origin
PLS	Partial Least Square
PLS-DA	Partial Least Square Discriminant Analysis
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
R^2	The Goodness of Fit Parameter
SIMCA	Soft Independent Modelling Class Analogy
TAG	Triacylglycerols
TPC	Total Phenol Content
UV	Ultraviolet
VOO	Virgin Olive Oil
WOSC	Wavelet Orthogonal Signal Correction

CHAPTER 1

INTRODUCTION

Olive oil is a very important agricultural product of the Mediterranean region, especially, Spain, Italy, Greece, Tunisia, Syria, Turkey and Morocco. Turkey produces 4.3% of world's olive oil and is located in the sixth place in the world both in production and consumption (International Olive Oil Council 2007).

Olive oil's characteristic aroma, taste, color and nutritive properties, stability distinguish it from other edible vegetable oils. Therefore there is a matter of great concern for the olive industry to preserve its product without loss of these positive attributes. The positive influence of olive oil on health include an improvement in blood lipid profile by lowering the bad LDL-cholesterol (Low Density Lipoprotein) level while significantly raising the level of good HDL-cholesterol (High density Lipoprotein) in the blood stream. Olive oil consumption reduces coronary hearth diseases, diabetes, certain cancer risks such as breast, prostate and colon cancers, certain malignant tumors (endometrium, digestive tract, skin tumors) and some other chronic diseases (Perez-Jimenez, et al. 2007).

One of the primary causes of loss of olive oil quality is oxidation. Among the technological factors that influence the composition and oxidative stability of olive oils, the extraction method and storage conditions play a critical role in its quality. Oxidation takes place either in the presence of light (photooxidation) or in the dark (autoxidation) and also by the effect of enzymes (enzymatic oxidation). Olive oil is considered to be resistant to oxidation in comparison with other vegetable oils because of its low content of polyunsaturated fatty acids and the presence of natural antioxidants. Abundance of oleic acid, ranging from 56 to 84% of total fatty acids, is the feature that sets olive oil apart from other vegetable oils. Olive oil provides a rich source of natural antioxidants. These include carotenoids, tocopherols and phenolic compounds which may act, by different mechanisms, to confer an effective defence system against free radical attack. Although the interest in phenolic compounds is related primarily to their antioxidant

activity, they also show important biological activity in vivo and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defence capacity of the human body (Aparicio, et al. 1999).

There is a need in developing reliable analytical methods to ensure compliance with labeling, i.e. the control of geographical origin giving also support to the denominated protected origin policy, and the determination of the genuineness of the product by the detection of eventual adulterations, quality maintenance during processing and storage periods. One of the agricultural products designated with the Protected Denomination of Origin (PDO) is olive oil. An important European (EU) regulation allows the PDO labeling of some EU EVOOs and this designation guarantees that the geographical origin of the product is closely in conjunction with the quality of the product (Cosio, et al. 2006). Furthermore, the International Olive Oil Council (IOOC) and European Communities Legislation (EC) define the identity characteristics of olive oil and olive-pomace oil, specify analytical methods and standard limit values of the quality parameters such as peroxide value (PV), acidity, UV absorbance values (K232 and K270) and organoleptic characteristics (odor, taste and color) for olive oils in order to improve product quality, to expand international trade, to raise its consumption. In this study, the compliance of Turkish extra virgin olive oil samples to these legal limits were reported during storage in order to determine quality.

Effect of storage conditions, time and their consequences were studied for olive oils produced in Europe (Manzi, et al. 1998, Mastrobattista 1990, De Leonardis and Macciola 1998, Cinquanta, et al. 1997, Okogeri and Tasioula-Margari 2002, Gomez-Alanso, et al. 2007, Capino, et al. 2005, Kalua, et al. 2006, Torres, et al. 2006). To our knowledge, there are not published studies corresponding to the effect of storage conditions and time on quality of olive oils grown in Turkey.

In this study, oxidative quality of Turkish extra virgin olive oils from different geographical region were monitored during 14 months to reveal the changes in chemical compositions at different storage conditions. In another phase of this study, flaxseed oil, which is known as alpha-linolenic acid (ALA) source and easily oxidized plant oil because of its highly poly-unsaturated fatty acid composition, was mixed with olive oil and the effect of olive oil on the oxidation of flaxseed oil was determined. Several studies about chemical characterization of Turkish olive oils were reported in the literature. However, there is a lack of knowledge about the changes in quality parameters of Turkish extra virgin olive oils over time. The findings of this study can

reveal the oxidative properties of Turkish olive oils of different varieties in long term storage and also the changes in their phenolic fractions and fatty acid compositions.

CHAPTER 2

OLIVE OIL AND QUALITY

2.1. Olive Oil

The olive tree (*Olea europea* L.) grows in a subtropical climate as a traditional main crop in Mediterranean countries. It probably originates from Mesopotamia and has been cultivated from many centuries in southern European countries bordering the Mediterranean and in North Africa (Murkovic, et. al. 2004). Olive oil is one of the oldest known vegetable oils mainly produced in the countries surrounding the Mediterranean Sea. It is a natural fruit juice, obtained from the fruit of the tree *Olea europea*, with a unique composition and quality. Beside, olive oil is one of the very few oils that can be consumed in its natural form, thus preserving all its natural constituents.

Olive oil is a key component of the traditional Mediterranean diet, which is believed to be associated with a relatively long life in good health. Consumption of olive oil has also increased in non-Mediterranean areas because of the growing interest in the Mediterranean diet and the tendency of consumers to select least-processed foods. Consumers are increasingly demanding that high food quality be maintained during the period between purchase and consumption. These expectations are a consequence not only of the primary requirement that the food should remain safe but also of the need to minimize unwanted changes in sensory quality (Morello, et al. 2003).

Virgin olive oil quality depends on many factors related to olive tree cultivation and to the harvesting, storage and olive processing steps and time. Of particular importance for olive oil quality are the olive cultivar, the pedoclimatic conditions of cultivation, as well as the pruning, fertilization and irrigation of olive trees. Harvest timing can have a significant effect on oil quality as well as on yield, oil stability and sensory characteristics. In order to obtain a characteristically fragrant and delicately

flavored olive oil, it is imperative that it is properly extracted from undamaged fruits at its best degree of ripeness. This illustrates the need to determine the quality of olive oil from a range of harvest times and cultivars to establish an optimum harvest time (Baccouri, et al. 2006). The maturity of the olives *(Olea europaea* L.) and the method of processing have been shown to contribute to a wide range of quality aspects in oil produced within individual olive groves.

In harvest periods olives are often piled into large heaps and stored at ambient temperatures for up to several weeks prior to processing for oil extraction, and during this period the greatest deterioration takes place. Pressure within the olive pile during storage can cause fluid secretion from the fruit that can provide an optimum medium for growth of fungi and bacteria. Under these conditions, anaerobiosis can occur in the inner part of the pile while aerobic losses occur in the outer part. Furthermore, heat production from respiratory activity may accelerate the deterioration of the fruit and eventually cause the breakdown of cell structure. Oil extracted from these damaged olives can be high in acidity and low in stability and can develop a high content of volatile acids (acetic or butyric) that causes a characteristic musty smell (Agar, et al. 1998).

Olive processing consists of the following stages: milling, mixing, pressure or centrifugation for classic and centrifugal systems respectively, and separation of the oil phase. The centrifugal force produced by high-speed rotating machines increases the difference between the specific weight of the immiscible liquid and the solid matter and this is used to extract the oil from the olives.

Proper storage techniques for olive oil are very important, not only to preserve the delicate taste of the oil, but also to ensure that it does not spoil and become rancid, which will have a negative effect on its nutritional profile. Olive oil can be kept longer than any other edible oil, and if stored properly it will take years before it becomes rancid. Even though olive oil's monounsaturated fats are more stable and heat-resistant than the polyunsaturated fats that predominate in other oils (especially the easily damaged omega-3 fatty acids found in flaxseed oil, which should always be refrigerated and never heated), olive oil should be stored properly and used within a few months to ensure its healthy phytonutrients remain intact and available. Virgin olive oil is more stable than other edible oils because of its high content of phenolic compounds, tocopherol, carotenoids and monounsaturated fatty acids. On the other hand, high quality olive oils are rich in polyphelons, which apart from the health benefits they

offer; they can also give a shelf life that is considerably longer. The most significant factors affecting the olive oil quality after processing and during storage are environmental, temperature, exposure to light and contact with oxygen. Light is an initiator of reactions that ultimately result in deterioration of the oil. Sensitizers such as chlorophyll may play a role in promoting photooxidation. The type of packaging has a dramatic effect on the shelf life of the oils. Oil that has been carefully processed to maximize palatability may be damaged by improper selection of the storage container. It is desirable to maintain the product quality at an optimum level for the longest time period. Papers concerning the behavior of various packaging materials have been published by Mendez and Falque (2006), Koutsaftakis, et al. (1999). Kanavouras, et al. (2004), Gutierrez and Fernandez (2002), Cecchi, et al. (2006) studied selected characteristic flavor compounds evolved in extra virgin olive oil when packaged in various packaging materials and stored under different conditions. Pagliarini, et al. (2000) has studied the stability of extra virgin olive oil by different types of bottles and under different commercial conditions and proved that it was not significantly influenced by different controlled bottling, transport and storage conditions in supermarkets. Otherwise, Vekiari, et al. (2007) eludicated that glass acted as a barrier to oxygen, which can not pass through it, avoiding the loss of certain components that deteriorate under its presence but it allows the direct action of light on the olive oil and this could promote oxidative rancidity as a consequence of its sensibility to photo-oxidation. Therefore, the storage of extra virgin olive oil in PVC bottles, can not be suggested as the most appropriate mean for maintaining the quality of the extra virgin olive oil. The best containers for storage are glass (especially tinted glass), ceramic, porcelain, or non-reactive metals such as stainless steel. Olive oil should not be stored in containers made of reactive metals such as copper or iron for the reason of the chemical reaction between the olive oil and the metal will damage the oil and may produce toxins.

Olive oil should be stored in a cool**,** dark place in consideration of accelerated oxidation effect of light factor. Caponio, et al. (2005) touched on the influence of light on EVOO quality during storage. They concluded that the shelf life of oils exposed to light was shorter that the ones kept in dark and after only 2 months of exposure to light oils could not be considered as extra virgin. Olive oil can be refrigerated without significantly affecting its quality or flavor. Beyond, it should be stored at normal room temperature (21-25°C) if olive oil is kept in a dark area where the temperature remains

fairly constant. The stability of olive oil usually ranges 9 to more than 18 months, assuming that it is properly stored (Gomez-Alonso, et al. 2007). As olive oil ages, it continually degrades and the acidity level rises. Rancidity, a wine smell or taste and a metallic flavor are the three key signs which indicate that olive oil is no longer desirable for consumption.

2.2. Designations and Definitions of Olive Oil

Olive oil is produced by mechanical means without using solvents or reesterification processes. It is marketed according to the following designations and definitions: (International Olive Council 2007)

Virgin olive oil: The oil obtained from the fruit of the olive tree only by mechanical or other physical conditions, peculiarly thermal conditions, that do not cause alterations in the oil, and which has not undergone any treatment other than washing, decantation, centrifugation, and filtration. Virgin olive oils are classified according to free fatty acid content (g oleic acid/100 g olive oil):

Extra virgin olive oil: Free fatty acidity (expressed as oleic acid) of a virgin olive oil should not exceed 0.8 grams per 100 grams.

Virgin olive oil: Virgin olive oil which has a free fatty acidity (expressed as oleic acid), of not more than 2 grams per 100 grams.

Ordinary virgin olive oil: Virgin olive oil which has a free acidity (expressed as oleic acid), should not exceed 3.3 grams per 100 grams.

Virgin olive oil not fit for consumption as it is, designated lampante virgin olive oil: Virgin olive oil having a free acidity (expressed as oleic acid), more than 3.3 grams per 100 grams. It is intended for refining or for technological use.

Refined olive oil: The olive oil obtained from virgin olive oils by refining methods which do not alter in the initial glyceridic structure. It has a free fatty acidity (expressed as oleic acid), not more than 0.3 grams per 100 grams.

Olive oil: The oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption. It has a free fatty acidity (expressed as oleic acid), not more than 1 gram per 100 grams.

Olive-pomace oil: The oil obtained by treating olive pomace with solvents or other physical treatments not including the 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: Olive pomace oil is intended for refining for use for human consumption, or for technical use.

Refined olive pomace oil: The oil obtained from crude olive pomace oil by refining methods which do not alter in the initial glyceridic structure. It has a free fatty acidity (expressed as oleic acid), not more than 0.3 grams per 100 grams.

Olive pomace oil: The oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption. Free fatty acidity of this oil should not exceed 1 gram per 100 grams.

Riviera olive oil: The oil that is obtained by mixing refined olive oil with natural olive oil that can directly be consumed as a food. The free fatty acidity should not be more than 1.5 gram per 100 gram (Turkish Food Codex 2000). According to EU (1991) and IOOC (2007), the mixture of refined and virgin olive oil is named as olive oil with free fatty acidity not more than 1 gram per 100 gram.

2.3. Olive Oil Chemical Composition

Olive oil chemical composition can be classified into two groups as major and minor compounds. Nearly 98% fraction of the olive oil structure includes triacylglycerols (TAG) and the group of glyceridic compounds made up of free fatty acids (FFA) and mono-(MAG) and diacylglycerols (DAG). Minor components that constitute about 2% of its composition, which are phospholipids, waxes and esters of sterols, aliphatic and triterpenic alcohols, carotenoids, chlorophylls, hydrocarbons, antioxidants, volatile compounds etc. Minor compounds play an important role in the

quality and purity analyses, in the studies of authentication and genuineness, and more recently in olive oil trace ability and health.

2.3.1. Major Components

Major components of olive oil are also called saponifiable substances which include triacylglycerols (TAGs). In a molecule of olive oil, the fatty acids are bound in groups of three together with a unit of glycerol. These units are called triacylglycerol molecules or TAGs. Only when the fatty acids are bound in these small units are they considered to be good quality oil. A triacylglycerol unit may lose one fatty acid to become a diacylglycerol and if it loses two fatty acids it is a monoacylglycerol. The fatty acid which is lost from the triacylglycerol is then called free fatty acid. The glycerol unit can have any three of several fatty acids attached to form TAGs. The carbon chains may be different lengths and they may be saturated, monounsaturated or polyunsaturated. It is the relative proportion of these that make one oil different from another.

The most important components in olive oil are the fatty acids (Figure 2.1). Fatty acids are simple structures made up of long chains of various numbers of carbon atoms. There are only a few types of fatty acids in olive oil, but the proportions of each strongly influence the characteristics and nutritive value of the oil. The main fatty acids in olive oil are comprised between the myristic (14 carbon atoms) and lignoceric (24 carbon atoms). The most prominent are the monounsaturated oleic and palmitoleic, the polyunsaturated linoleic and linolenic. Fatty acid compositions of olive oil, pomace oil and extra virgin olive oil are presented in Table 2.1.

Oleic acid, as the characteristic monounsaturated fatty acid of olive oil, constitutes 55-83% of total fatty acids. In point of health aspects, high intake of oleic acid in the Mediterranean region was reported to be the reason for decreases in the rates of coronary artery disease. Also, olive oil improves the lipid profile of cardiovascular risk by decreasing the ratio of LDL/HDL (Martinez-Gonzalez and Sanchez-Villegas 2004).

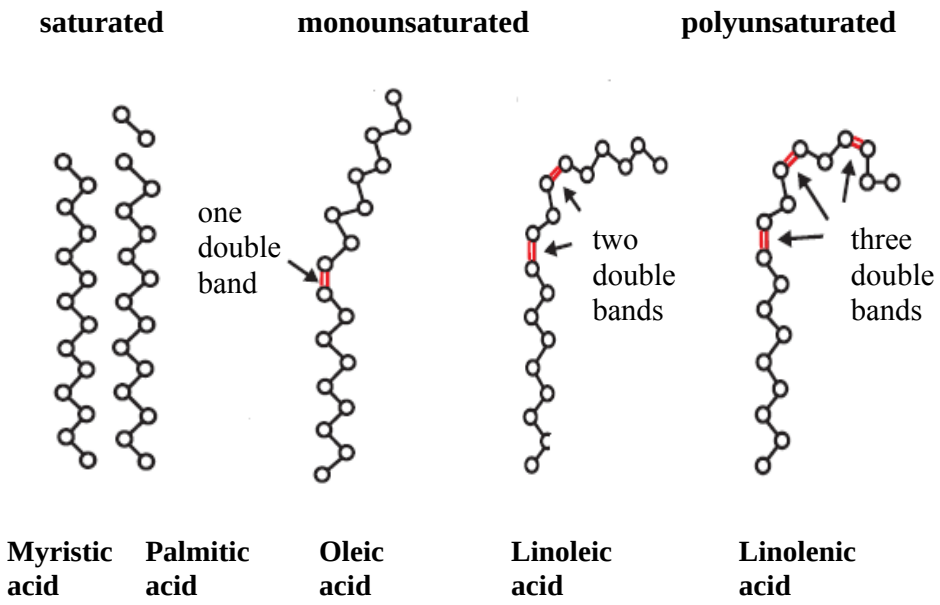

Figure 2.1. Forms of some fatty acids in olive oil
(Source: NSW Department of Primary Industries 2006)

Table 2.1. Fatty acid compositions of olive oil, pomace oil and extra virgin olive oil

	Olive and olive-pomace oil (Turkish Food Codex 2000, IOOC 2007)	Extra virgin olive oil (EU 1991)
Myristic acid (C14:0)	≤ 0.05	≤ 0.1
Palmitic acid (C16:0)	7.5-20	-
Palmitoleic acid (C16:1)	0.3-3.5	-
Heptadecanoic/margaric acid (C17:0)	≤ 0.3	-
Heptadecenoic/margoleic acid (C17:1)	≤ 0.3	-
Stearic acid (C18:0)	0.5-5.0	-
Oleic acid (C18:1)	55.0-83.0	-
Linoleic acid (C18:2)	3.5-21.0	-
Linolenic acid (C18:2)	≤ 0.9[1]	≤ 0.9
Arachidic acid (C20:2)	≤ 0.6	≤ 0.7
Gadoleic/eicosenoic acid (C20:1)	≤ 0.4	-
Behenic acid (C22:0)	≤ 0.2[2]	≤ 0.3
Lignoceric acid (C24:0)	≤ 0.2	≤ 0.5

[1] IOOC states this value as ≤ 1

[2] This value of olive-pomace oil should be ≤ 0.3

2.3.2. Minor Components

Sterols: Sterols are nutritionally important lipids that need to be routinely determined in foods. 4-desmethylsterols, β-Sitosterol are the predominant sterols in olive oils. Minor sterols include Δ^5-avenasterol, stigmasterol, sitostanol, and cholesterol. The triterpene dialcohols erythrodiol and uvaol are also present in olive oil, at concentrations ranging from 10 to 200 mg/kg oil. The predominant sterol was β-sitosterol and total sterol content depended on the type of oil, and ranged from 687 to 2.479 mg/kg. Stigmasterol and the amount of erythrodiol plus uvaol can be used in order to distinguish between olive oil and seed oil (Martinez-Vidal, et al. 2007)**.** Compositional analysis of the sterol fraction of olive oil can be used to assess the degree of purity of the oil and the absence of other plant oils. This determination also permits characterization of the type of olive oil.

Squalene: Squalene is the major olive oil hydrocarbon and makes up more than 90% of the hydrocarbon fraction ranging from 200 to 7500 mg/kg oil or even higher (800−12000 mg/kg oil). Squalene is regarded as partially responsible for the beneficial effects of olive oil against certain cancers. In a recent study on the content of minor constituents of Italian olive oils, derived from olives of six cultivars and different degrees of ripeness, it was found that squalene loss during storage of oil samples in the dark was greater than that of α-tocopherol (Manzi, et al. 1998). This was attributed to a possible regeneration of α-tocopherol from squalene implying thus an antioxidant activity of this highly unsaturated hydrocarbon.

Pigments: The color of a virgin olive oil is due to the solubilization of the lipophilic chlorophyll and carotenoid pigments present in the source fruit. Virgin olive oil contains 1.0 to 2.7 ppm *β*-carotene as well as 0.9 to 2.3 ppm lutein (Psomiadou and Tsimidou 2002). Carotenoids and especially β-carotene can slow down oil oxidation by light filtering, singlet oxygen quenching, sensitizer inactivation, and free radical scavenging. In the absence of light, carotenoids and their oxidation products may act as prooxidants in vegetable oils (Velasco and Dobarganes 2002).

These components can transfer energy from light into chemical molecules. Thus, they act as prooxidants during storage in light. Although chlorophylls are strong prooxidants under light acting as a sensitizer to produce $^1O_2$, they act as antioxidants in

the dark possibly by donating hydrogen to free radical (Endo, et al. 1985, Francisca and Isabel 1992). Both chlorophylls and carotenoids are considered to have an important role in keeping the quality of edible oils, mainly due to their action as photo-sensitizers or singlet oxygen quenchers respectively (Cert, et al. 2000).

Tocopherols: There are four natural tocopherols α, β, γ, δ- forms are available in olive oil. The α-tocopherol or vitamin E is the major antioxidant present in olive oil and the amount is in the range of 150 and 300 ppm. These compounds display antioxidant properties and they are active as vitamins (vitamin E), which makes them particularly important for human health. The antioxidant activities are mainly depended on their concentration and presence of other antioxidants in olive oil. Tocopherols act as singlet oxygen quenchers and increase the oxidative stability of vegetable oils during storage in light and when chlorophyll is present (Cert, et al. 2000).

Phospholipids: The amount of phospholipids in olive oils changes between 40-135 mg/kg. Phosphatidylcholine, phospatidylethanolamine, phosphatidylinositol, and phosphatidylserine, phosphatidylglycerol, phosphatidic acid are the main phospholipids detected in olive oils. Their presence in the olive oils oils may affect their oxidative stability or the physicochemical state of cloudy (veiled) olive oil. The antioxidant functions of phospholipids based on an amino group that has the capacity to chelate metals and keep them in an active form. They can act as synergists with phenolic compounds and tocopherols contributing to enhance their antioxidant activity (Velasco and Dobarganes 2002). Phospholipids have hydrophilic and hydrophobic groups in the same molecule. The hydrophilic groups of the phospholipids are on the surface of oil and hydrophobic group are in the edible oil. The phospholipids decrease the surface tension of edible oil and may increase the diffusion rate of oxygen from the headspace to the oil to accelerate oil oxidation (Choe and Min 2006).

Phenolic Compounds: VOO contains at least 30 phenolic compounds. The major phenolic compounds are oleuropein derivatives, based on hydroxytyrosol which are strong antioxidants and radical scavengers. Phenolic compounds are complex class of chemicals including a hydroxyl group on a benzene ring. The pulp of olives contains these compounds, which are hydrophilic, but they are also found in the oil. The class of phenols includes numerous classes, such as simple phenolic acids and derivates like vanillic, coumaric and caffeic acids, tyrosol and hydroxytyrosol and more complex compounds like the secoiridoids of oleuropein and ligstroside, the lignans (1-

acetoxypinoresinol and pinoresinol), flavones (apigenin, luteolin). Table 2.2 refers to the major phenolic compounds in virgin olive oil.

Phenolic acids contains two distinguishing constitutive carbon frameworks, namely the hydroxycinnamic and hydroxybenzoic structures. They present in olives with the chemical structure of C6–C1 (benzoic acids) and C6–C3 (cinnamic acid) (Garrido Fernandez, et al. 1997). Phenolic acids have been associated with color and sensory qualities, as well as with the health-related and antioxidant properties of foods (Nergis and Unal 1991). Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruit and vegetables, against diseases that may be related to oxidative damage (coronary heart disease, stroke, and cancers) (Masaki, et al. 1997). In particular, several phenolic acids such as gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*- and *o*-coumaric, ferulic and cinnamic acid have been identified and quantified in VOO (in quantities lower than 1 mg of analyte kg^{-1} of olive oil). Phenolic acids may be conjugated with organic acids, sugars, amino compounds, lipids, terpenoids, or other phenolics. Bianco, et al. (2002) investigated the presence of hydroxy-isochromans in VOO. In fact, during the malaxation step of VOO extraction, hydrolytic processes through the activity of glycosidases and esterases augment the quantity of hydroxytyrosol and carbonylic compounds, thus favouring the presence of all compounds necessary for the formation of isochroman derivatives. Two hydroxy-isochromans, formed by the reaction between hydroxytyrosol and benzaldehyde or vanillin, have been identified by HPLC-MS/MS technique and quantified in commercial VOOs.

The secoiridoids oleuropein, demethyloleuropein, and ligstroside are the main complex phenols in virgin olive oil. Secoiridoids are characterised by the presence of elenolic acid in its glucosidic or aglyconic form (Bianco and Uccella 2000). The secoiridoids, which are glycosidated compounds, are produced from the secondary metabolism of terpenes as precursors of several indole alkaloids (Soler-Rivas, et al. 2000) and are characterised by the presence of elenolic acid in its glucosidic or aglyconic form. Especially, they are formed from a phenyl ethyl alcohol (hydroxytyrosol and tyrosol), elenolic acid and, eventually, a glucosidic residue. Oleuropein is the ester between 2-(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) and the oleosidic skeleton common to the glycosidic secoiridoids of the Oleaceae. Oleuropein and demethyloleuropein are hydrolyzed by endogenous β-glycosidases to

the dialdehydic form of elenolic acid linked with 3,4-dihydroxyphenylethanol (3,4-DHPEA-EDA) and 3,4-dihydroxyphenylethanol-elenolic acid (3,4-DHPEA-EA) during crushing and malaxation (Bendini, et al. 2007).

Hydroxytyrosol, which is the major phenolic alcohol, can be present as a simple or esterified phenol with elenoic acid, forming oleuropein and its aglycone, or as part of the molecule of verbascoside (Servili, et al. 1999); it can also be present in several glycosidic forms, depending on the hydroxyl group to which the glucoside is bound (Bianco, et al. 1998, Ryan, et al. 2001).

Another group of substances present in the phenolic fraction is lignans, (+)-1-acetoxypinoresinol and (+)-pinoresinol (Owen, et al. 2000). The substance (+)-pinoresinol is a common compound of the lignan fraction of several plants such as *Forsythia* species *and Sesamum indicum* seeds, while (+)-1 acetoxypinoresinol, (+)-1-hydroxypinoresinol and their glycosides have been found in the bark of the Olea europeae L. (olive) (Kato, et al. 1998).

The phenolic compounds in olive oil acted as antioxidants mainly at the initial stage of autoxidation (Deiana, et al. 2002) by scavenging free radicals and chelating metals. Changes in the phenolic compounds of virgin olive oils during storage are also reported. Cinquanta, et al. (1997) studied the evolution of simple phenols during 18 months of storage in the dark. They found a great increase in the tyrosol and hydroxytyrosol contents due to hydrolysis of their complex derivatives in a first stage and a rapid loss of hydroxytyrosol as compared with that of tyrosol at the end of the storage period. Hydroxytyrosol was the most effective antioxidant in olive oil oxidation. Among phenolic compounds, o-diphenols such as caffeic acid are oxidized to quinones by ferric ions and become ineffective in inhibiting iron-dependent free radical chain reactions in oil (Keçeli and Gordon 2002). However, hydroxytyrosol, tyrosol, vanilic acid, p-coumaric acid were not oxidized by the ferric ions.

Flavonoids also have a great concern because of their beneficial health effects related to cancer and coronary heart diseases. Flavonoid aglycones are subdivided into flavones, flavonols, flavanones, and flavanols depending upon the presence of a carbonyl carbon at C-4, an OH group at C-3, a saturated single bond between C-2 and C-3, and a combination of no carbonyl at C-4 with an OH group at C-3, respectively. Rovellini, et al. (1997) and Morello, et al. (2005) revealed the luteolin and apigenin in flavonoid group of phenolic compounds in VOO. Luteolin may originate from rutin or luteolin-7- glucoside, and apigenin from apigenin glucosides.

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

The concentrations of the phenolic compounds have a great importance through these compounds are responsible for the sensory and antioxidant properties of high-quality olive oils. The quality of the olives and the oil is affected by the amount of oleuropein and its hydrolytic products (Limiroli, et al. 1995). Separately, the absolute content of the phenolic compounds of the olive oil depends on the place of cultivation, the climate, the variety, and the olives' level of maturation during of harvesting time (Cinquanta, et al. 1997, Visioli and Galli 1998, Brenes, et al. 1999).

Volatile Compounds: Volatile compounds are low molecular weight compounds which vapourise readily at room temperatures. Characteristic aroma and in particular green and fruity features of olive oil originates from many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway which takes place during the oil extraction process (Angerosa, et al. 2000, Angerosa, et al. 2004).

Table 2.3. Defined major volatile compounds in VOO

aldehydes	alcohols	esters	hydrocarbons	ketones	furans
3-Methylbutanal	Methanol	Methyl acetate	Octane	2-Butanone	Ethylfuran
(E)-2-Pentenal	Ethanol	Ethyl acetate	2-Methylbutane	3-Pentanone	
(Z)-2-Pentenal	1-Hexanol	Butyl acetate	Nonane	1-Penten-3-one	
Hexanal	1-Penten-3-ol	Hexyl acetate	Hexane	2-Octanone	
(E)-2-Hexenal	(E)-3-Hexen-1-ol	(Z)-3-Hexenyl-acetate			
(Z)-3-Hexenal	(Z)-3-Hexen-1-ol	Ethyl propanoate			
Heptanal	(E)-2-Hexen-1-ol				
2-4-Heptadienal	1-Octen-3-ol				
Octanal	Terpineol				
Nonanal	3-Methylbutan-1-ol				
2,4-Nonadienal					
2,4-Decadienal					
(E)-2-Undecenal					

Some of the volatiles found in virgin olive oil are present in the intact tissue of the fruit, and others are formed during disruption of cell structure during the virgin olive oil production due to enzymatic reactions in the presence of oxygen. The main

precursors of volatile compounds are fatty acids (particularly linoleic and alpha-linolenic) and amino acids (leucine, isoleucine and valine) (Morales and Tsimidou 2000). In fact, it has been reported that the concentrations of volatile compounds depend on the enzymatic activity (Salas, et al. 2005) though external parameters (e.g. climate, soil, harvesting and extraction conditions) may alter the inherent olive oil sensory profile (Morales and Aparicio 1999).

The aroma of olive oil is attributed to aldehydes (hexanal, trans-2-hexenal, acetaldehyde), alcohols (methanol, hexan-1-ol, 3-methylbutan-1-ol), esters (methyl acetate, ethyl acetate, hexyl acetate), hydrocarbons (2-methylbutane, hexane, nonane), ketones (2-butanone, 3-methyl-2-butanone, 3-pentanone), furans and other undefined volatile compounds. The major volatiles in virgin olive oils are C6 and C5 volatile compounds (Angerosa, et al. 2004, Cimato, et al. 2006). Table 2.3 also demonstrates the major volatile compounds identified in VOO.

Volatile compounds, whether major or minor, contribute olive oil quality crucially and provide useful quality indicators. Beside volatile compounds, non-volatile compounds such as phenolic compounds also stimulate the tasting perception of bitterness, the latter pungency, astringency and metallic attributes.

2.4. Quality of Olive Oil

Quality is defined as the combination of attributes or characteristics of a product that have significance in determining the degree of acceptability of that product by the user. Olive oil quality may be defined from commercial, nutritional or organoleptic perspectives. The nutritional value of EVOO originates from high levels of oleic acid content and minor components, such as phenolic compounds, whereas the aroma is strongly influenced by volatile compounds. Nutritional value and unique pleasant flavor promotes consumption demands and price of olive oil in comparison with other edible oils. In order to fulfill the expectations of consumers, good quality control of olive oil should be assured in the course of production and storage line. The quality of olive oils is maintained in terms of measurement of analytical parameters for which certain limit values are set.

The International Olive Oil Council, which was created by olive oil producing countries to raise olive productivity and improve olive oil quality, has proposed a conventional index which expresses numerically the quality of the oil and provides indirectly a warranty for its authenticity. The International Olive Oil Council and the EEC have designated the quality of olive oil, based on parameters that include free fatty acid (FFA) content, peroxide value (PV), UV specific extinction coefficients (K232 and K270) and sensory score. Especially, the quantity of FFA is an important factor for classifying olive oil into commercial grades. Apart from the quality parameters that IOOC and EEC defined, the changes in major and minor compounds and their concentrations in olive oils give an idea for providing and improving quality of olive oil, especially for storage, marketing and packaging issues. In order to protect producers of high quality olive oils and ensure consumer awareness of product quality, European Community legislation has introduced the Protected Designation of Origin (PDO) mark that allows the labeling of virgin olive oils with the names of the areas where they are produced. This certification increases the oil value. The question of geographical identification becomes more interesting when it regards small production areas, where high quality products are often obtained.

Finally, during storage of the oil the hydrolysis, esterification and oxidation also originate changes in the minor constituents. Accordingly, the determination of the minor constituents is essential for the analytical assessment of the quality, origin, extraction method, refining procedure and adulteration of the olive oils. In the following sections, the quality parameters of olive oil that are used to define oxidative quality of olive oil and its color.

2.4.1. Acidity Determination (Free Fatty Acid Content) (FFA)

The acidity expresses the percentage content (in weight) of the free fatty acids in the oil under examination. Free fatty acids are normally present also in oils when the triglycerides are formed, there is a progressive increase in acidity due to the action of enzymes (lipase) naturally present in the olive fruit, which help the fatty acids to detach from the molecule of triglyceride (lipolysis). The lipolytic action of lipase produces free

fatty acids which are responsible for the acidity of the oil. The same lipolytic action can be caused by enzymes produced by micro-organisms which grow on the fruit. Thus, in order to obtain a product which is organoleptically better and has lower acidity, it is necessary to preserve the olives well. Consequently, FFA reflects the stability of oil and its susceptibility to rancidity.

Acidity determination is mainly accomplished by titration using potassium hydroxide. The method determines the amount of free fatty acids (FFA) present in the oil, which is expressed as percentage of oleic acid. The free fatty acidity is a measure of the quality of the oil, and reflects the care taken in producing and storage processes of the oil**.** As well, acidity values are used as a basic criterion for classifying the different categories of olive oil. In contrast, according to Kiritsakis, et al. (1998), acidity is not considered as the best criterion for evaluating olive oil quality, since one oil with relatively high acidity may have a good aroma while another one with low acidity may not have so good a taste and aroma. For extra virgin olive oil the maximum acidity is 0.8%, according to EU (European Union Commission 1991).

2.4.2. Peroxide Value (PV)

Peroxide value (PV) is a measure of total peroxides in olive oil expressed as miliequivalent of O_2 kg^{-1} oil (meq O_2 /kg oil) and so this value is known as a major guide of quality. The official EU method is based on the titration of iodine liberated from potassium iodide by peroxides present in the oil. In other words, the peroxide value is a measure of the active oxygen bound by the oil which reflects the hydroxyperoxide value, and is one of the simplest measures of the degree of lipid peroxidation. The higher the number means the greater degradation due to oxidation. Peroxide value usually increases gradually over time after pressing. The upper standard value of the peroxide is 20 meq O_2 /kg oil. The upper standard peroxide indexes, which are established by European Regulation for other types of olive oil, are displayed in Table 2.4. In general, peroxide levels higher than 10 may mean less stable oil with a shorter shelf life (Nouros, et al. 1999).

2.4.3. Specific Absorption Coefficients (UV Absorbance Values) (K232 and K270)

Determination of the specific absorption coefficients (specific extinction) in the ultraviolet region is needed for estimating the oxidation stage of olive oil. The absorption at specified wavelengths at 232 and 270 nm in the ultra violet region is related to the formation of conjugated diene and triene in the olive oil system, due to oxidation or refining processes. Compounds of oxidation of the conjugated dienes contribute to K232 while compounds of secondary oxidation (aldehydes, ketones etc.) contribute to K270 (Kiritsakis, et al. 2002). The upper standard UV absorbance values are shown in Table 2.4.

Table 2.4. European Regulation Standard limit values for olive oil quality parameters

Quality Indexes	Acidity (oleic acid %)	Peroxide Index (meq/kg)	K232	K270
EVOO	≤ 1.0	≤ 20	≤ 2.5	≤ 0.20
VOO	≤ 2.0	≤ 20	≤ 2.6	≤ 0.25
Ordinary VOO	≤ 3.3	≤ 20	≤ 2.6	≤ 0.25
Lampante olive oil	> 3.3	> 20	≤ 3.7	> 0.25
Refined olive oil	≤ 0.5	≤ 5	≤ 3.40	≤ 1.20
Olive oil	≤ 1.5	≤ 15	≤ 3.30	≤ 1.00
Crude olive-pomace oil	> 0.5	-	-	-
Refined olive-pomace oil	≤ 0.5	≤ 5	≤ 5.5	≤ 2.5
Olive-pomace oil	≤ 1.5	≤ 15	≤ 5.3	≤ 2.0

2.4.4. Anisidine Value (AV)

Anisidine value (AV) determination is an empirical test for assessing advanced oxidative rancidity of oils and fats. It estimates the secondary oxidation products of unsaturated fatty acids, principally conjugated dienals and 2-alkenals. Aldehydes are largely considered responsible for the off-flavors in fats and oils due to their low sensory threshold values. The AV test is particularly useful for oils of low peroxide value (PV) and for assessing the quality of highly unsaturated oils. The test involves a condensation reaction between the conjugated dienals or 2-alkenals and p-anisidine to form colored products. The AV is empirically defined as 100 times the absorbance of a solution resulting from 1 g of fat or oil mixed with 100 mL of isooctane/acetic acid/*p*-ansidine reagent, measured at 350 nm in a 10 mm cell under the conditions of the test. As the absorbance maximum shifts towards longer wavelengths with increasing unsaturation and as the color intensity is greater with conjugated dienals than 2-alkenals, the absorption maximum varies from oil to oil. The AV obtained is only comparable within each type of oil. In spite of the low specificity of the AV test and has no a standard limit in olive oil codex, it is reported as a very useful indicator of oil quality and complements the PV test (Labrinea, et al. 2001).

2.4.5. Iodine Value (IV)

Iodine value is a measure of the unsaturated linkages in fat and is expressed in terms of percentage if iodine absorbed. The decline in iodine value can be used to monitor lipid oxidation. The unsaturated fatty acid residues of the glycerides react with iodine, and thus the iodine value indicates the degree of unsaturation of the fatty acid residues of the glycerides. The iodine value is often most useful in identifying the source of an oil. Generally, the higher iodine values indicate oils and the lower values fats. Iodine values are normally determined using Wijs or Hanus methods.

2.4.6. Color

Color is a sensory property with a strong influence on food acceptance as it contributes decisively to the initial perception that one can acquire of the condition, ripeness, degree of processing, and other characteristics of foods (Alos, et al. 2006). As virgin olive oil is a natural product whose color depends exclusively on biological compounds such as the chlorophyll and carotenoid pigments, their identification and individual evaluation make it possible to relate oil color with the content and type of these compounds present.

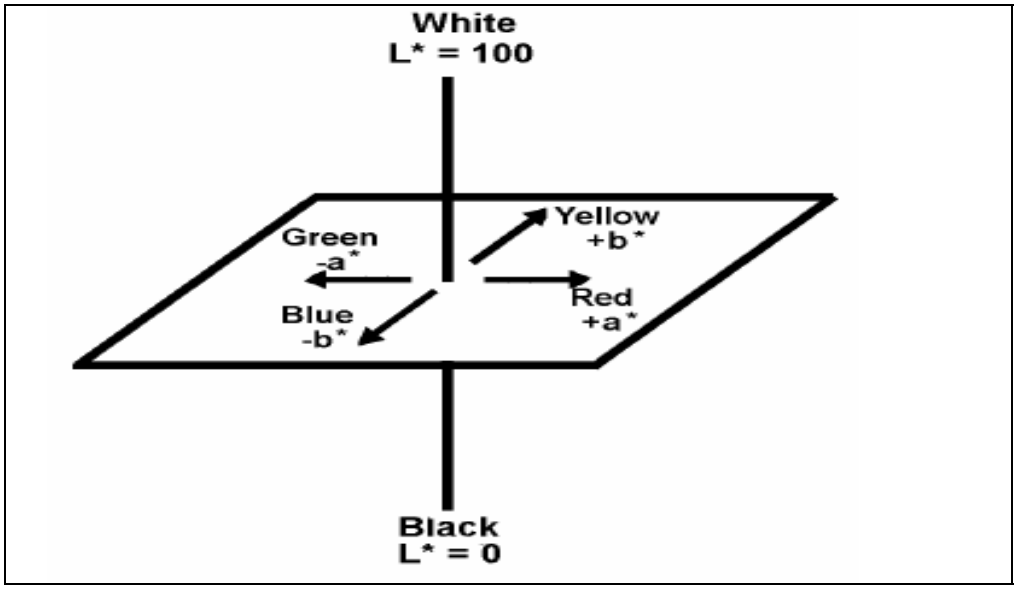

Figure 2.2. Coordinates of chromatic components of L*, a*, b*
(Source: Hunterlab 2008)

Oil appearance might be an indicator of a quality problem having occurred during blending, storage, crushing, and extraction or the refining process. The American Oil Chemists' Society (AOCS) has proposed four official methods for the color determination of fats and oils. Related methods are Lovibond color, Wesson color, spectrophotometer color and chlorophyll color. Presently, CIE L*a*b*, XYZ, Hunter Lab, and RGB (Red, Green, Blue) are the alternative color models that might be used in objective oil color evaluation. L*a*b* is an international standard for color

measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L* is the lightness component ranging from 0 to 100. a* refers to the color ranges from green to red and b* displays the colors from blue to yellow. These two chromatic components range from -120 to 120 (Figure 2.2).

2.5. Instrumental Techniques in Olive Oil Analysis

2.5.1. Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared (IR) spectra contain significant information about the individual components of complex mixtures; Fourier transform infrared (FT-IR) spectroscopy is able to enhance greatly the quantitative analysis capabilities of IR spectroscopy. It has an important role in the analysis of edible oils by yielding simpler and more rapid techniques for determining common oil quality parameters and identification of molecular structure originates from the high information content of IR spectra and to assign specific absorption bands refers to functional groups. The FT-IR spectroscopy technique brings in several advantages in comparison with the dispersive IR technique: improvement in the signal-to-noise ratio, higher resolution and accuracy in wavelength measurement, as well as advantages in storing and manipulating data, among others. For these reasons, the application of FT-IR spectroscopy is acquiring a great concern in the study of edible fats and oils. Recently, methods have been improved for determination of iodine value and saponification number (Van der Voort, et al. 1992), free fatty acids (Ismail, et al. 1993), peroxide value (Van der Voort, et al. 1994), fatty acid composition (Maggio, et al. 2008) and cis-trans content (Van der Voort, et al. 1995) of edible oils from FT-IR.

This technique is also able to characterize edible oils with multivariate statistical techniques such as partial least square (PLS) and principle component analysis (PCA), which allows accenting the differences between spectra, and the classification of the samples. Bendini, et al. (2007) discriminated the VOO samples from Italy by FT-IR and PCA techiques. Moreover, this technique has shown to be a useful tool in detecting

mixtures or adulterations. The degree of unsaturation of oils and fats can be determined by infrared spectroscopy, from the ratio of absorbances of olefinic and aliphatic –C–H stretching vibration bands or from the absorbance of a single band such as the –C=C– stretching band at 1658 cm^{-1} or the olefinic band at 3007 cm^{-1}, although, in these cases, only cis- unsaturation is taken into account. The degree of unsaturation is closely related to the iodine value and can be determined by means of calibrations between both parameters including cis- and trans- unsaturations.

Other important parameter for oils such as saponification number can be determined from infrared spectra by means of calibrations applying partial least square analysis (PLS) to several spectral regions. Determination of FFA content has also been carried out by infrared spectroscopy through quantification of the intensity of the band at 1710 cm^{-1} by different methods or of the band at 1570 cm^{-1} after derivatisation of the sample to obtain carboxylate anions. Parameters related to the concentration of primary or secondary oxidation products of oils and fats, such as peroxide value and anisidine value, can also be obtained from infrared spectroscopic data in combination with PLS calibrations in good agreement with chemical methods. This technique has shown to be useful to monitoring the oil oxidation process.

At higher frequencies between 3400 and 3700 cm^{-1}, the bands observed in the spectrum of edible oil can be related to the –OH stretching vibration of water (H–OH), hydroperoxides (ROOH) and their breakdown products, namely alcohols (ROH). The 2850–3025 cm^{-1} region is known as the absorption zone of C–H stretching vibration of methylene and terminal methyl groups of fatty acid chains. In the center of the spectrum, a band due to the C=O stretching absorption of the triglyceride ester linkages is present. Vlachos and co-workers (2006) evidenced that both the spectral region between 3050 and 2740 cm^{-1} and the band at 1746 cm^{-1} undergo several changes (because of the production of saturated aldehydes functional groups or other secondary oxidation products) during the oxidation process of corn oil samples heated at various temperatures or/and exposed to UV radiation. At lower frequencies (1650–1500 cm^{-1}), the carbonyl absorption bands of aldehydes (R–CHO) and ketones (R–CO–R) can be observed. The next region (1500–900 cm^{-1}) is the so-called “fingerprint region” because the pattern of the bands is particularly characteristic of molecular composition and can be used to identify minor substances (Table 2.5).

Table 2.5. Frequencies of bands (b) or shoulders (s) of some edible oils and fats in infrared spectra (Source: Guillen and Cabo 1997)

Frequency (cm^{-1})	*Functional group*	*Mode of vibration*	*Intensity*[a]
3468 (b)	−C=O (ester)	Overtone	w
3025 (s)	=C−H (*trans*-)	Stretching	vw
3006 (b)	=C−H (*cis*-)	Stretching	m
2953 (s)	−C−H (CH_3)	Stretching (asym)	m
2924 (b)	−C−H (CH_2)	Stretching (asym)	vst
2853 (b)	−C−H (CH_2)	Stretching (sym)	vst
2730 (b)	−C=O (ester)	Fermi resonance	vw
2677 (b)	−C=O (ester)	Fermi resonance	vw
1746 (b)	−C=O (ester)	Stretching	vst
1711 (s)	−C=O (acid)	Stretching	vw
1654 (b)	−C=C− (*cis*-)	Stretching	vw
1648 (b)	−C=C− (*cis*-)	Stretching	vw
1465 (b)	−C−H (CH_2, CH_3)	Bending (scissoring)	m
1418 (b)	=C−H (*cis*-)	Bending (rocking)	w
1400 (b)		Bending	w
1377 (b)	−C−H (CH_3)	Bending (sym)	m
1319 (b,s)		Bending	vw
1238 (b)	−C−O, $-CH_2-$	Stretching, bending	m
1163 (b)	−C−O, $-CH_2-$	Stretching, bending	st
1118 (b)	−C−O	Stretching	m
1097 (b)	−C−O	Stretching	m
1033 (s)	−C−O	Stretching	vw
968 (b)	−HC=CH− (*trans*-)	Bending out of plane	w
914 (b)	−HC=CH− (*cis*-)	Bending out of plane	vw
723 (b)	$-(CH_2)_n-$, −HC=CH−(*cis*-)	Bending (rocking)	m

[a] w, weak; vw, very weak; m, medium; vst, very strong; st, strong.

2.5.2. Gas Chromatography (GC)

Gas chromatography (GC) is a powerful and widely used tool for the separation, identification and quantitation of components in a mixture. In this technique, a sample is converted to the vapor state and a flowing stream of carrier gas (often helium or nitrogen) sweeps the sample into a thermally-controlled column. In the case of gas-liquid chromatography, the column is usually packed with solid particles that are coated with a non-volatile liquid, referred to as the stationary phase. As the sample mixture moves through the column, sample components that interact strongly with the stationary

phase spend more time in the stationary phase vs. the moving gas phase and thus require more time to move through the column.

Retention time is defined as the time from injection of the sample to the time a specific sample component is detected. Components with higher volatility (lower boiling points) tend to spend more time in the moving gas phase and therefore tend to have shorter retention times. After exiting the column the separated components are detected and a detector response is recorded (Figure 2.3).

The most application field of GC in olive oil analysis is the determination of methyl esters of fatty acids. The aim of this determination is to establish the percentage composition of fatty acids in olive oil, more commonly known as fatty acid composition, which is influenced by the olive variety, production zone, climate and stage of maturity of the drupes when they are collected. Determination of fatty acid composition of olive oil is not only a quality indicator but also is used for classification and characterization of the oils. Some studies have been carried out with the aim of characterizing particular productions of olive oil, which have given great importance to the fatty acid profile. Kotti, et al. (2008) characterized the two varieties Chetoui and Chemlali from Tunisian, Poiana and Mincione (2004) evaluated the fatty acid and compositions of olive oils extracted from different olive cultivars grown in Calabrian area. Ollivier, et al. (2006) differentiated the French virgin olive oil by fatty acid compositions and triacylglycerol and sensory characteristics. Additionally, Spugnoli et al. (1998) characterized the fatty acid profile of some monovariety olive oils from Tuscany. Stefanoudaki, et al. (1999) made classification virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. Gurdeniz, et al. (2008) had an investigation related to classification of Turkish olive oils with respect to with respect to cultivars, geographic origin and harvest year, and using fatty acid profile and mid-IR spectroscopy. The fatty acid profile evolution of oils produced by typical Spanish olive cultivars like Picual and Hoijblanca (Gutierrez, et al. 1999) and Moroccan Picholine (Ajana, et al. 1998) has also been studied.

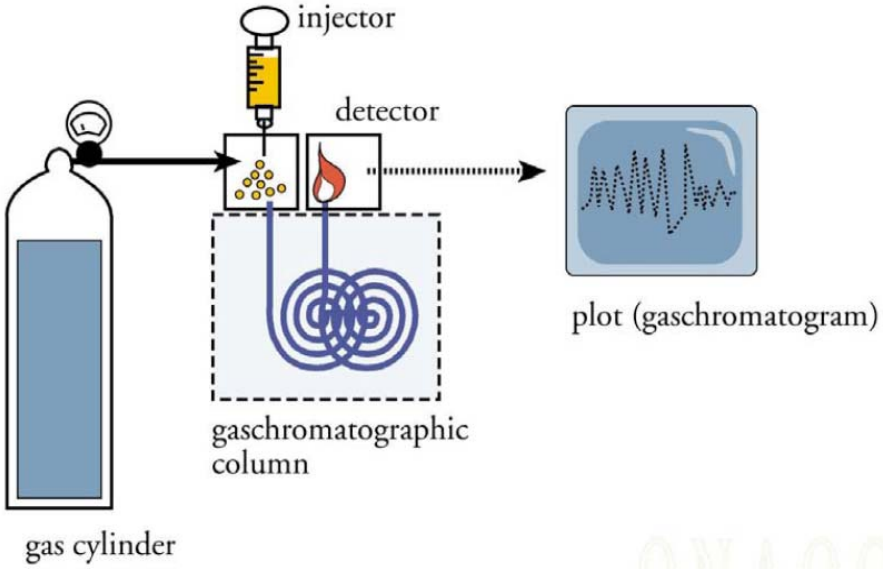

Figure 2.3. Schematic representation of a system for gas chromatography (Source: Oliveoil 2003)

Apart from fatty acid compositon of olive oil, sterols, triglycerides and the volatile compounds are also analyzed by using various GC methods and combined chromatographic methods. For instance, GC-MS technique was used for detection of phenols, secoroids and sterols (Angerosa, et al. 1996, Cinquanta, et al. 1997). Capillary GC has also been used to separate triglycerides, not only according to their carbon number but also by their degree of unsaturation. Finally, solid-phase micro extraction (SPME) method coupled to GC–MS and GC–FID combined technique is effective for isolation and the characterization of volatile components in olive oil. Characterization of VOO from Tunisia according to their volatile componds using SPME- GC method was achieved by Zarrouk, et al. (2007) and Manai, et al. (2007).

2.5.3. High Performance Liquid Chromatography (HPLC)

HPLC is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds which is used to find the amount of a chemical compound within a mixture of other chemicals. High performance liquid chromatography (HPLC) has the

ability to separate, identify and quantitate the compounds that are present in any sample that can be dissolved in a liquid.

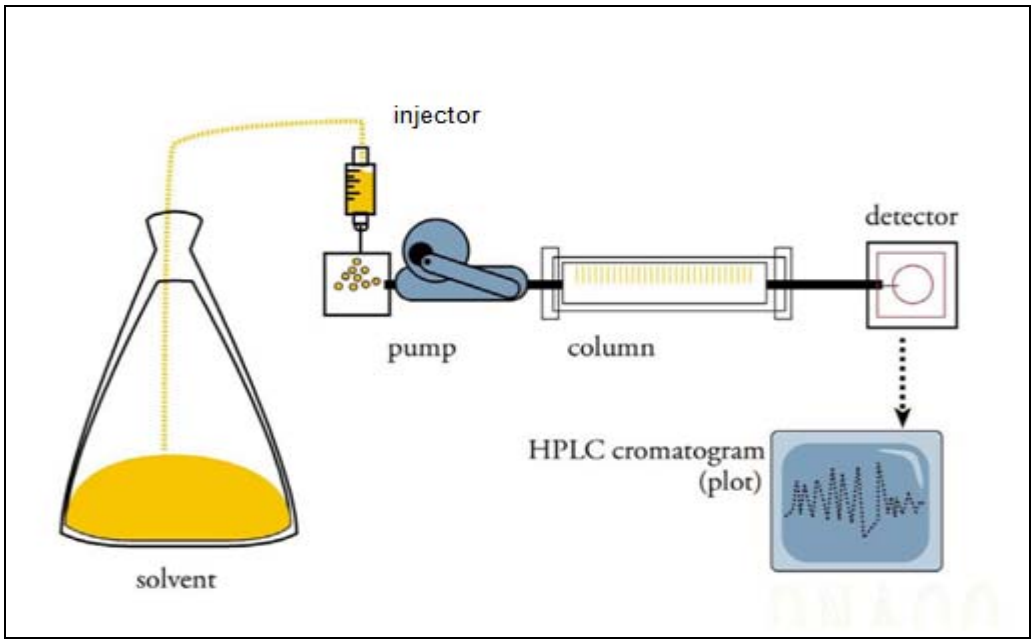

Figure 2.4. Schematic representation of a system for high performance liquid chromatography (Source: Oliveoil 2003)

HPLC and combined chromatographic methods has a great emphasis in olive oil analysis techniques. Several minor components of olive oil such as phenolic compounds, pigments, sterols, tocopherols and triacylglycerols can be identified and quantitated with this technique. Reversed-phase high performance liquid chromatography (RP-HPLC) currently is the most popular and reliable technique for the determination of phenolic compounds. Numerous mobile phases have been employed with different modifiers, which include methanol, acetonitrile or tetrahydrofuran, acids (acetic or formic) and/or salt (ammonium phosphate) (Ryan, et al. 1999). Detection is typically based on the measurement of ultraviolet (UV) absorption, usually at 280 nm, which represents a suitable compromise as most phenols absorb considerably at this wavelength (Ryan and Robards 1998). Several compounds such as: tyrosol, hydroxytyrosol, phenolic acids, (ferulic, syringic, caffeic and *p*-coumaric), oleuropein aglycone, deacetoxyoleuropein aglycone, elenolic acid and derivatives, other

secoiridoid compounds and flavone aglycones (luteolin and apigenin), have been identified by HPLC in olive oils obtained from different olive cultivars in many studies. Phenolic compounds in Spanish VOO were characterized by HPLC. Simple phenols such as tyrosol, hydroxytyrosol, vanilic acid, p-coumaric acid, ferulic acid and vanilin were detected. The flavonoids apigenin and luteolin; the dialdehydic form of elenolic acid linked to tyrosol and hydroxytyrosol were found in most Spanish olive oils (Brenes, et al. 1999). Gomez-Rico, et al. (2008) also evaluated the effect of cultivar and ripening stage of olive fruit on the phenolic profile of six different Spanish varietes by HPLC analysis. The phenolic compounds present in 29 samples of Portuguese olive fruits were analyzed by RP-HPLC method by Vinha, et al. (2005). Ocakoglu, et al. (2008) depicted phenolic characterization of Turkish olive oils.

RP-HPLC method was also used to determine carotenoids in olive oil, lutein and β-carotene as major pigmets in concentrations varying with the type of oil and process of manufacture. Cichelli and Pertensana (2004) distinguished the VOO according to their pigments such as chlorophylls, pheophytins and carotenoids by using HPLC with fluorescence detection, alternatively, triglycerides can be analyzed by RP-HPLC coupled with refractive index (RI). Percentage determination of the various triglycerides present in virgin olive oil or high performance liquid chromatography offers a way of detecting possible adulterations with oils which, while having a similar fatty acid composition to olive oil, have a different triglyceride composition. Canabate-Diaz, et al. (2006) applied the LC-MS with atmospheric pressure chemical ionisation (APCI) and evaluated for the first time identification and characterization sterol fraction in the olive oil sample. In the result of this study, a sufficient separation of cholesterol, stigmasterol, b-sitosterol, sitostanol, fucosterol, erythrodiol and uvaol was achieved.

HPLC is the most common methodology used for the analysis of tocopherols and tocotrienols. Cunha, et al. (2006) compared the three different HPLC detection systems, which are fluorescence and diode array connected in series, ultraviolet, and evaporative light scattering for determination of tocopherols and tocotrienols in 18 samples of Portuguese olive oils. The best results were obtained with the fluorescence detector in the quantification of tocopherols and tocotrienols.

2.5.4. Electronic nose (e-nose)

Electronic nose is a system that mimics the human olfaction by combining the response of a set of chemical sensors with partial specificity for the measurement of volatiles and develops techniques to recognize patterns for data interpretation (Gardner and Bartlett 1993). Electronic nose combined with pattern recognition techniques; offer a fast, simple and efficient tool for classification purposes. The electronic nose consists of an array of gas sensors, a signal collecting unit and pattern-recognition software. The principle is the transfer of the total headspace containing different chemical volatile compounds to a sensor array, where each sensor has partial specificity to a wide range of aroma molecules.

In food industry it has been applied for assessment of food properties (Brezmes, et al. 2001, Garcia-Gonzalez and Aparicio 2003, Guadarrama, et al. 2000), detection of adulteration (Oliveros, et al. 2002), sensory properties prediction (Buratti, et al. 2007). Application of this method has advantages of cheapness, no time-consuming, simplicity, little or no sample pre-preparation. There has been several researchs for usage of e-nose in characterization and differentiation of olive oils in regard to their geographical origin (Guadarrama, et al. 2001, Cosio, et al. 2006, Oliveros, et al. 2002) and determination the quality control of olive oil aroma profiles (Guadarrama, et al. 2001).

2.5.5. High-Resolution Nuclear Magnetic Resonance (H-NMR) Spectroscopy

H-NMR is useful for a variety of quantitative analytical purposes in the chemistry of fats and oils. This technique is mainly focused on the quantitative information that can be obtained from the oils by H-NMR spectra, which regards minor and major components. One or two dimensional high-resolution NMR spectroscopy has been recognized as a viable technique to analyze oils for their fatty acid composition, authenticity, adulteration and nature of unsaturation. Mannina, et al. (2001) cited the

wide application of H-NMR in monitoring olive oil quality and adulteration by determination of the free acidity, fatty acid profiles, unsaturated fatty acids, phenolic compounds, aldehydes and volatile compounds, sterols, squalene and chlorophyll and monitoring oxidation of olive oil, analysis of mixtures of olive oil with other fatty compounds.

The unsaturated fatty acids (oleic, linoleic, linolenic) in oil can be quantified using H-NMR. The results agree well with GC conducted as a control, although reportedly ^{13}C-NMR results correlate even better with GC (Miyake, et al. 1998). Rezzi, et al. (2005) studied the profile of olive oil samples in relation to its geographical origin and processing with the combination of H NMR fingerprinting with multivariate analysis. Petrakis, et al. (2008) also analyzed the olive oils by means of ^{1}H and ^{31}P NMR spectroscopy and characterized according to their content in fatty acids, phenolics, diacylglycerols, total free sterols, free acidity, and iodine number.

2.5.6. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is a solventless, simple and relatively inexpensive method used to examine the physical state and properties of food components. Modified DSC can adequately describe changes related to the level of oxidation due to structural conformations of the triacylglycerols and their oxidation derivatives inside the olive oil mass. Since this technique is sensitive to composition changes resulting from oxidation, it could be used as a rapid and effective method to characterize the quality of olive oil at different degrees of oxidation (Kiritsakis, et al. 2002). Kanavouras, et al. (2004) applied modulated DSC to extra virgin olive oil and to oil subjected to various accelerated oxidation treatments. They found a very good correlation between thermograph parameters selected for the main crystallization peak and the various off-flavors derived mainly from the oleic acid degradation. Thermal properties (measured both in cooling and heating regimes) of monovarietal EVOO samples were found to correlate well with the chemical composition (Chiavaro, et al. 2008). DSC has application in assessment of olive oil adulteration with other vegetable or seed oils of lower quality and/or economic value. Jimenez-Marquez (2003) evaluated

DSC heating thermograms of admixtures of virgin olive oil with low-quality olive oils: melting transition enthalpy and peak temperatures discriminated virgin olive oil from other olive oils, as well as from their admixtures.

CHAPTER 3

OLIVE OIL OXIDATION

Extra virgin olive oil, is one of the few oils being consumed without any chemical treatment, has high resistance to oxidative deterioration mainly due to two reasons;

1) Its fatty acid composition which is characterized by a high monounsaturated to polyunsaturated ratio,

2) The presence of certain minor components is equally significant and initial content of natural antioxidants, especially phenolic compounds (total polyphenols, *o*-diphenols or olesidic forms of hydroxytyrosol) carotenoids, tocopherols, and polyphenols are natural antioxidants that delay the oxidation of lipids and the production of the undesirable volatile compounds (Gutierrez, et al. 2002, Velasko and Dobarganes 2002).

Olive oil quality and stability are principally affected by lipid oxidation, a general term for a complex process that results in generation of off-flavour and reduction in nutritional value and causes health risks. This deterioration is characterized by physicochemical changes, a marked decrease in the nutritional value, an unpleasant flavor called "rancid," and even some toxicity. The process is complex because of the influence of multiple factors, such as light, temperature, enzymes, and metals. Furthermore, it always takes place by the same mechanism: chain reactions involving free radicals, which are called autoxidation. The oxidation taking place in light is known as photooxidation (Gutierrez, et al. 2002).

The oxidation level of oil is an important quality criteria for food industry. As stated before, oxidation of oils not only produces rancid flavours but also decrease the nutritional quality and safety by the formation of oxidation products, which may play a role in the development of diseases (Muik 2005). Lipid peroxidation has been proposed as a possible mechanism in production of toxic compounds which causes lung damage. In addition to this effect, reactions between peroxidized lipids and proteins have been

shown to cause loss of enzyme activities, polymerization, accelerated formation of brown pigments and the destruction of essential amino acids such as histidine, lysine, tryptophan and methionine. Aldehydes, ketones, hydrocarbons and furans, which are known as the cleavage products of hydroperoxides, cause reduction in protein solubility, and reduction in nutritional value of proteins, too. As well, lipid oxidation provokes a decrease in nutritional values of some vitamins such as A, D, E and K. From the health point of view, lipid radicals and oxidation products contribute formation of some diseases such as aging, DNA damage, parkinsonism, carcinogenesis, tumor formation and coronary heart diseases.

3.1. Mechanisms of Lipid Oxidation

As lipids oxidize, they form hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products, such as aldehydes, ketones, acids, and alcohols. In many cases, these compounds adversely affect flavor, aroma, taste, nutritional value, and overall quality. Many catalytic systems, including light, temperature, enzymes, metals, metalloproteins, pigments, and microorganisms, can accelerate lipids oxidation. Most of these reactions involve some type of free radical and oxygen species. The oxidation can be produced either in the dark (autoxidation) or in the presence of light (photooxidation), or the process of oxidative deterioration can occur by the effect of enzymes (Harwood and Aparicio 2000).

3.1.1. Lipid Autoxidation

The many catalytic systems, which are light, temperature, enzymes, metals, metalloproteins, pigments, and microorganisms, stimulate oxidation mechanism of lipids. In these reactions, many free radicals and oxygen species, such as singlet oxygen are involved. The main substrates for these reactions are unsaturated fatty acids and oxygen.

The free radical mechanism of lipid oxidation is usually described as three stages of initation, propagation, and the termination steps. Free radicals are produced during peroxide formation - hydroperoxides are the highly reactive substance defined as a molecular entity having single unpaired electron.

1. Initiation - formation of free radicals: In initation stage of autoxidation, free radicals are formed directly from lipid components where initiator, such as temperature, light, and other radicals or metals, are involved. In other words, initiation takes place by the abstraction a hydrogen radical from the allylic methylene group in double bonds of unsaturated fatty acids and results in formation of lipid free radical.

$$RH \longrightarrow R\bullet + H\bullet \qquad (3.1)$$

Initiation starts with the abstraction of a hydrogen atom adjacent to a double band in a fatty acid (RH) molecule, and this may be catalyzed by light, heat, or metal ions to form a free radical. Direct reaction of fatty acid molecule with oxygen does not take place frequently, because of the high activation energy.

The resultant free radical (R•) reacts with atmospheric oxygen to form an unstable peroxy free radical (ROO •), which may in turn abstract a hydrogen atom from another unsaturated fatty acid to form a hydroperoxide (ROOH) and a new alkyl free radical initiates further oxidation and contributes to the chain reaction. This chain reaction is called propagation stage of autoxidation. The chain reaction may be terminated by formation of nonradical products resulting from combination of two radical species.

$$R\bullet + {}^3O_2 \longrightarrow ROO\bullet \qquad (3.2)$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \qquad (3.3)$$

2. Propagation - free-radical chain reaction: The propagation stage in autoxidation process includes an induction period when hydroperoxides formation is minimal. The rate of oxidation of fatty acids increases in relation to their degree of unsaturation. The relative rate of autoxidation of oleate, linoleate, and linolenate is in order of 1:40-50:100 on the basis of oxygen uptake and 1:12:25 on the basis of peroxide formation. Therefore, oils that contain high proportions of polyunsaturated fatty acids

may experience stability problems. The breakdown products of hydroperoxides, such as alcohols, aldehydes, ketones, furans, esters, lactones and hydrocarbons, generally cause off-flavors. These compounds may also interact with other food components and change their functional and nutritional properties (Akoh and Min 2002).

3. Termination - formation of nonradical products: In termination stage of the autoxidation, free radicals interact or react with each other and turn to normal state cause formation of non-radicals (R – R, ROOR).

$$R\bullet + R\bullet \longrightarrow R - R \quad (3.4)$$

$$R\bullet + ROO\bullet \longrightarrow ROOR \quad (3.5)$$

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2 \quad (3.6)$$

3.1.2. Photooxidation

Another important way of oxidation involves exposure to light and a sensitizer such as chlorophyll. Exposure to light can cause formation of hydroperoxides when both oxygen and the photosensitizer are present. The process of formation of hydroperoxides can take place by photooxidation and photolytic autoxidation. This process is initiated and propagated by the free radical reactions, and similar isomers of hydroperoxide are formed as during autoxidation without light.

Photooxidation of olive oil in the presence of naturally occurring chlorophyll pigments produces singlet oxygen, which acts with unsaturated fatty acids and produces fatty acid hydroperoxides. This photooxidation results in a change in color and because of the formation of hydroperoxide decomposition products, develops undesirable odor and flavor constituents (Rahmani and Csallany 1998).

During photosensitized oxidation, energy is transferred from light to the sensitizer and into oxygen as follows:

$$\text{sensitizer}_{\text{ground}} + h\nu \longrightarrow \text{sensitizer}_{\text{excited}} \quad (3.7)$$

Energy (hv) is transferred from light onto sensitizer ($sensitizer_{excited}$), as seen in equation (3.7) which, in turn, can react directly with lipid (RH)-forming radicals (R•), which, in turn, initiate autoxidation.

$$\text{sensitizer}_{\text{excited}} + RH \longrightarrow \text{sensitizer H} + R\bullet \qquad (3.8)$$

More damaging is the reaction of excited sensitizer with ground state oxygen to form singlet oxygen.

$$\text{sensitizer}_{\text{excited}} + {}^3O_2 \longrightarrow \text{sensitizer}_{\text{ground}} + {}^1O_2 \qquad (3.9)$$

The transformation of energy onto an acceptor, unsaturated fatty acid, causes formation of a hyroperoxide.

$${}^1O_2 + RH \longrightarrow ROOH + {}^3O_2 \qquad (3.10)$$

Singlet oxygen has been found to react with linoleic acid about 1500 times faster than does normal oxygen (Harwood and Aparicio 2000). In addition, singlet oxygen is very harmful and capable of damaging endogenous DNA, lipids and enzymes. Therefore, this reactive component is defined as the most important initiator in the free radical autoxidation of vegetable oils. Singlet oxygen can interact with other molecules such as carotenoids in two different ways: it can provoke a chemical reaction or an energy transfer reaction, either of them leading to the deactivation of singlet oxygen (Viljanen, et al. 2002).

Carotenoids are effective inhibitors of photo-oxidation by quenching singlet oxygen and triplet excited states of photosensitizers. The physical quenching mechanism of carotenoids is based on their low singlet energy state, which facilitates the acceptance of energy from singlet oxygen. Chlorophyll is also functioned as a photosensitizer resulting in rapid oxidation of the oil and the added components and loss of color (Psomiodou and Tsimidou 2002).

3.1.3. Enzymatic Oxidation of Unsaturated Fatty Acids

Decomposition of unsaturated fatty acids begins with hydrolysis of various glycerides by lipases, lipolytic acyl hydrolases, and phospholipases, during which the polyunsaturated fatty acids are, freed (Figure 3.1). Lipoxygenases then convert unsaturated fatty acids into hydroperoxides, mainly 9 and 13 isomers, which are unstable. In last step, lyases, isomerases, and dehydrogenases transfer hydroperoxides into a variety of volatile and nonvolatile products. The flavor components formed, such as aldehydes and alcohols, can be directly responsible for off-flavor.

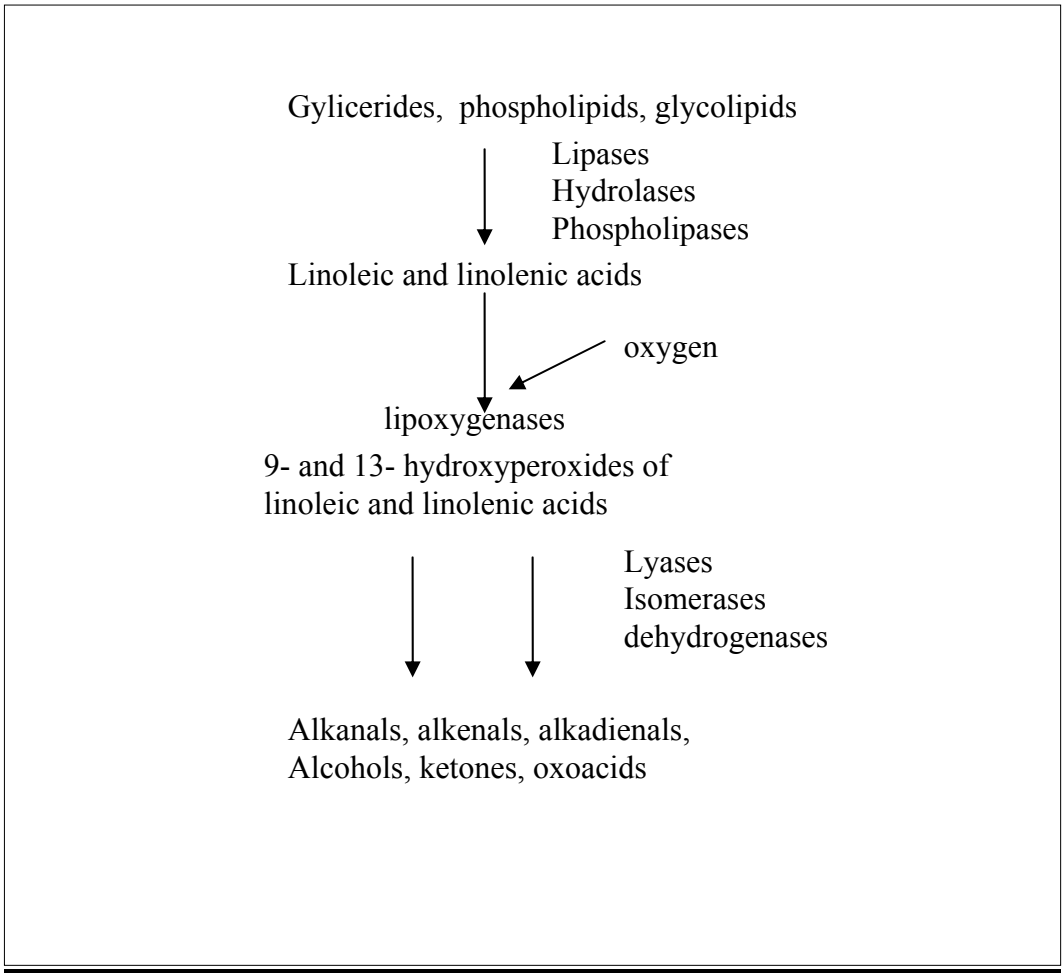

Figure 3.1. Enzymatic oxidation of unsaturated fatty acids
(Source: Harwood and Aparicio 2000)

Free unsaturated fatty acids, particularly linoleic and linolenic acids in plants, are the preferred substrate for oxidation by lipoxygenases. Certain lipoxygenase isoenzymes can also catalyze the oxidation of unsaturated fatty acids when they are esterified in lipids. Preferred conditions for oxidation enzymes are when oxygen is present, and then oxidation is performed at the highest rate. Lack of oxygen does not halt oxidation because some forms of lipoxygenases can oxidize fatty acids without the presence of oxygen, thus forming free radicals. The presence of trace amounts of hydroperoxides accelerates the oxidation of unsaturated fatty acids by lipoxygenase, particularly under anaerobic conditions. Free radical formed from the decomposition of hydroperoxides can elevate further oxidation, which causes earlier than expected off-flavor formation and results in lower oil stability during storage (Harwood and Aparicio 2000).

3.2. Factors Affecting Lipid Oxidation

The oxidation of oil is influenced by the fatty acid composition of the oil, oil processing, energy of heat or light, the concentration and type of oxygen, and free fatty acids, mono- and diacylglycerols, transition metals, peroxides, thermally oxidized compounds, pigments, and antioxidants. These factors interactively affect the oxidation of oil and differentiation of the individual effect of the factors is not easy (Choe and Min 2006).

3.2.1. Fatty Acid Composition of Oils

Oils that are more unsaturated are oxidized more quickly than less unsaturated oils (Parker, et al. 2003). As the degree of unsaturation increases, both the rate of formation and the amount of primary oxidation compounds accumulated at the end of the induction period increase. The relative autoxidation rate of oleic, linoleic, and

linolenic acids was reported as 1:40 to 50:100 on the basis of oxygen uptake (Choe and Min 2006).

3.2.2. Oil Processing

During olive oil milling process, hammer crushing should be applied instead of stone mill. Because hammer crushing increases polyphehol concentration as compared with stone mills. Application of different temperatures during malaxation resulted in an increase in polyphenols and olive oil stability increase with temperature but decrease with time and water addition (Velasco and Dobarganes 2002). In addition, according to the studies, which were about observation of the olive oil stability during extraction methods, it was found that pressure extraction had higher polyphenol and ortho-diphenol contents and consequently higher stabilities towards oxidation than obtained by centrifugation (Di Giovacchino, et al. 1994, Cert, et al. 1996, Piacquadio, et al. 1998, De Stefano, et al. 1999, Sciancalepore, et al. 2000).

Filtering should be avoided to increase shelf life of the oil because of the suspended materials play role of stabilizer by acting as antioxidants. After olive oil extraction, in order to prevent oxidation and extend the shelf life of the oil, storage should be done in dark, with certain materials promote metal contamination and minimum headspace and also should be kept away from light sources (Pagliarini, et al. 2000, Ahmed-Khan and Shahidi 1999).

3.2.3. Temperature and Light

Source of energy that can lead to the formation of radical initiators. UV light is particularly harmful. It is well known that minor compounds can be excited electronically due to the absorption of light. Therefore, the prevention of photoxidation during storage is great of importance to improve the oxidative stability of olive oil. Light is much more important than temperature in oxidation. Reportedly, the effect of

light on oil oxidation becomes less as temperature increases. Autoxidation of oils and the decomposition of hydroperoxides increase as the temperature increases.

At low and moderate temperatures, the formation of oxidation products during the induction period is slow, ROOH are the major compounds formed and their concentration increases until the advanced stages of oxidation. In these stage of oxidation, polymerisation compounds and the minor volatile compound, in particular carbonyl compounds, which have enormous sensory significant, are formed (Marquez-Ruiz, et al. 1996).

Autoxidation of oils and the decomposition of hydroperoxides increase as the temperature increases (St. Angelo 1996). The concentration of the hydroperoxides increases until the advanced stages of oxidation (Velasco and Dobarganes 2002).

3.2.4. Oxygen

The oxidation of oil can often take place when oil, oxygen, and catalysts are in contact. Both concentration and type of oxygen affect the oxidation of oils. The oxygen concentration in the oil is dependent on the oxygen partial pressure in the headspace of the oil. A higher amount of oxygen is dissolved in the oil when the oxygen partial pressure in the headspace is high. Oxidation of the oil increased with the amount of dissolved oxygen. The effect of oxygen concentration on the oxidation of oil increase at high temperature and in the presence of light and metals such as iron or copper. Higher oxygen dependence of oil oxidation at high temperature is due to low solubility of oxygen in the oil at high temperature. Consequently, vacuum packaging or flushing the oil with nitrogen, having minimum headspace, using the packaging materials which are impermable to oxygen should be considered while packaging or storage of the olive oil (Velasco and Dobarganes 2002).

3.2.5. Minor Components Present in Oil

Edible oil consists of mostly triacylglycerols, but it also contains minor components such as free fatty acids, mono- and diacylglycerols, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols. Some of them accelerate the oil oxidation and others act as antioxidants. Phospholipids, color compounds, tocopherols, phenolic compounds were discussed in section 2.3. Free fatty acids act as prooxidants in edible oil (Miyashita and Takagi 1986, Mistry and Min 1987). They have hydrophilic and hydrophobic groups in the same molecule and prefer to be concentrated on the surface of edible oils. The hydrophilic carboxy groups of the free fatty acids will not easily dissolve in the hydrophobic edible oil and are present on the surface of edible oil. Mistry and Min (1987) reported that free fatty acids decrease the surface tension of edible oil and increase the diffusion rate of oxygen from the headspace into the oil to accelerate oil oxidation.

3.3. Measurement of Lipid Oxidation

The measurement of oxidative stability and oxidation products is essential to determine shelf life, acceptability, and nutritional quality of edible oils. Lipid oxidation measurement involves a variety of techniques because the oxidation process involves several stages. Many methods and parameters have been developed to access the extent of oxidative deterioration, which are related to measurement of the concentration of primary or secondary products or of both (Harwood and Aparicio 2000). These parameters related to olive oil deterioration are included in official regulations with regard to olive oil and olive-pomace oil (EC 1991). The peroxide value (PV) is most commonly used to assay oxidation in oils and is applicable to the early stages of oxidation. A second early-stage oxidation measure is the conjugated diene (absorption coefficients) value. This technique measures the conjugation that is formed as the unsaturated lipid oxidizes. To monitor accumulated secondary oxidation products, usually carbonyl-type compounds, and the anisidine value (AV) test is a widely

accepted method. The test estimates the level of aldehydes, principally 2-alkenals, present in the oil (Yıldız, et al. 2001).

CHAPTER 4

FLAXSEED OIL (LINSEED OIL)

4.1. Flaxseed

Flax (*Linum usitatissimum* L.) is a multi-purpose crop. Its production goes back to ancient history. Its remnants were found in Stone Age dwellings in Switzerland and ancient Egyptians made fine from flax fiber. Two types of flax are grown, seed flax for the oil in its seed and fiber flax for the fiber in its stem. It is mainly grown in Canada, Argentina, America, China and India (Wang, et al. 2007). The plants range in height from 30 to 100 cm and have narrow leaves flowers that are in different shades of blue. Its seeds containing about 36 to 40% of oil have long been used in human and animal diets and in industry as a source of oil and as the basic component or additive of various paints or polymers (El-Beltagi 2007).

The main components of flaxseed, expressed on a moisture-free basis, are protein (21%), dietary fiber (28%), and fat (41%). Flaxseed has a unique fatty acid profile. It is high in polyunsaturated fatty acids (73% of total fatty acids), moderate in monounsaturated fatty acids (18%), and low in saturated fatty acids (9%). Linoleic acid, an omega-6 fatty acid, constitutes about 16% of total fatty acids, whereas alpha-linolenic acid (ALA) constitutes about 57% (Morris 2001), the highest of any seed oil (Thompson, et al. 1991). The growing concern that the linoleic acid content of the typical Western diet is too high has led some experts to recommend replacing dietary omega-6 fatty acids with those from the omega-3 family. Flaxseed is the richest source of food lignans, and can contain 75–800 times more than other food sources (2.0 mg/g based on seed dried weight). The flaxseed lignans secoisolariciresinol (SECO) and its diglucoside secoisolariciresinol diglucoside (SDG) are reported to have a number of health benefits associated with their consumption that have in part been attributed to

their antioxidant properties. Flaxseed also contains phospholipids from which 2.9% of sterol glycosides can be extracted. The sterols can be fractionated into cholesterol (2% sterol fraction), campesterol (26%), stigmasterol (7%), sitosterol (41%), Δ^5– avenasterol (13%), cycloartenol (9%), and 24- methylenecycloartanol (2%).

Flaxseed has recently gained attention as a "functional food" because of its unique nutrient profile and potential to affect the risk and course of cardiovascular disease and some cancers (Thompson, et al. 1991), particularly hormone-dependent cancers such as prostate and breast (Ingram, et al. 1997). Functional foods are those that resemble traditional foods, but render benefits beyond their nutrition and energy value in promoting health and preventing certain chronic diseases, especially cardiovascular disease, cancer, diabetes, autoimmune disorders, arthritis, and arrhythmia (Shahidi 2002). Thus, use of flaxseed in foods has increased during the past decade due to the presence of functional compounds alpha-linolenic acid (ALA), lignans, and fiber (Schorno, et al. 2003).

4.2. Flaxseed Oil

Flaxseed oil is derived from the seeds of the flax plant. Unrefined flaxseed oil from good seed has an attractive golden color, a pleasant, nut-like flavor, and mild odor. Flaxseed oil is qualitatively different from the more common vegetable oils with high PUFA proportions, such as soya oil, sunflower oil, rape oil, olive oil, etc. Flax oil is a rich source of the following unsaturated fatty acids: oleic (C18:1, 16–24%), linoleic (C18:2, 18–24%), and linolenic acid (C18:3, 36–50%) (Figure 4.1). Beside that, it contains about 6% palmitic acid, 2.5% stearic acid and 0.5% arachidic acid (Flachowsky, et al. 1997). Unsaponifiable lipid constituents of seed oils naturally contain hydrocarbons, terpene alcohols, sterols, tocopherols and other phenolic compounds which may act as oxidation inhibitors under a range of conditions. In flax grains, lipids are protected against oxidation by various mechanisms, for example, the presence of antioxidants such as lignans, phenols, tocopherols (vitamin E) and flavonoids. In addition to preventing rancidity, these antioxidants could increase commercial value of food products and have beneficial effects on human health. When

consumed together with essential unsaturated fatty acids, they can reduce the risk of various diseases. The antioxidant ability of phenols, tocopherols (vitamin E) and flavonoids is related to the presence of OH groups which may directly bind to free radicals and chelate metals (Romieu and Trenga 2001).

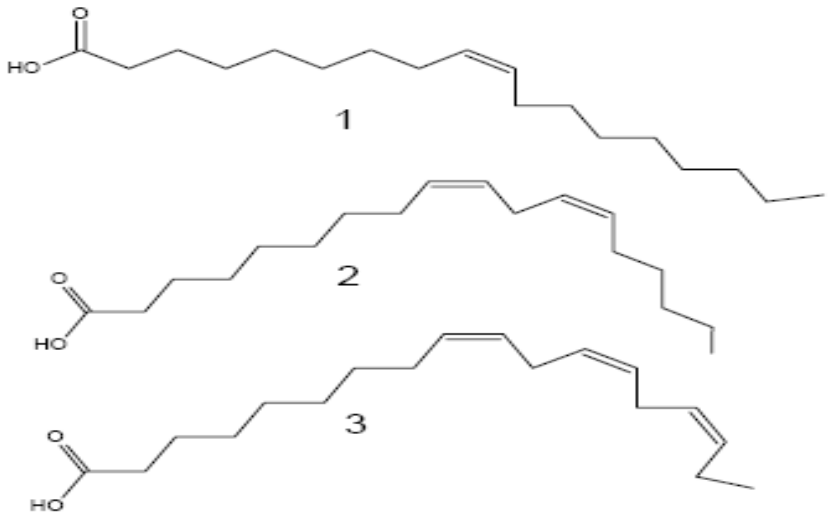

Figure 4.1. Structures of common fatty acids in flaxseed oil: 1- oleic acid, 2- linoleic acid, 3- linolenic acid (Source: Flachowsky, et al. 1997).

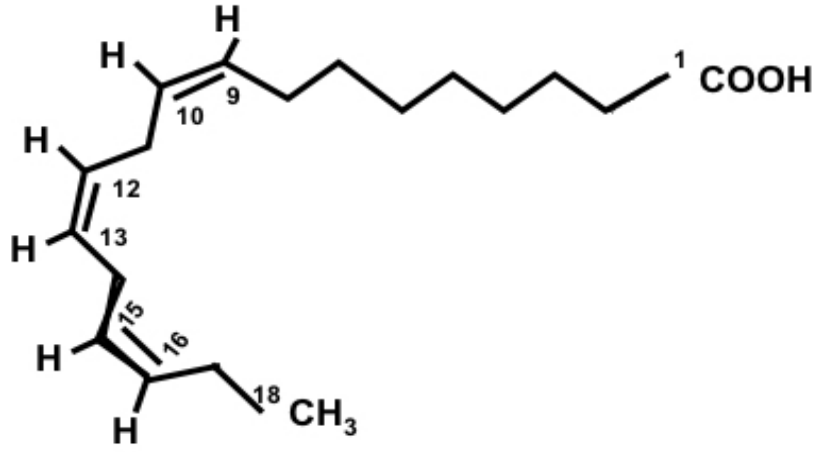

Figure 4.2. Structure of alpha-linolenic acid (ALA)
(Source: Schorno, et al. 2003)

There have been few studies related to the composition of flaxseed oil. It has gained attention because of its high content of alpha-linolenic acid content (Figure 4.2).Choo and coworkers (2006) had a study which remarked the physicochemical and quality characteristics of seven New Zealand-grown flaxseed oils. The major tocopherol in the seven cold-pressed flaxseed oils was in the γ-form. The amount of γ-tocopherol was between 10.56 and 15.00 mg/100 g oil. The β- and δ-tocopherol were not detected in the seven cold-pressed flaxseed oils. As far as the authors were aware, there were no reports available on the occurrence of β-tocopherol in flaxseed or flaxseed oil. Oomah, et al. (1997) reported the presence of δ -tocopherol in flaxseed but at very low levels (0.17–0.30 mg/100 g of seed) and Velasco and Goffman (2000) reported the occurrence of δ -tocopherol at 0.0–0.3 mg/100 g of seed in 288 accessions of the genus Linum. The acid values of the seven cold-pressed flaxseed oils were within the limit of 4.0 mg KOH/g of oil. These acid value measurements correlated well with the free fatty acid measurements, which were determined as oleic acid (%) and ranged between the values of 0.25 and 0.98%. Moreover, unsaponifiable matter content (%), chlorophyll content (mg of pheophytin/ kg of oil), total flavonoid content (lutein equivalents mg/ 100 g) and total phenolic acids as ferulic acid equivalents (mg/100 g) were stated in ranges of 0.39-0.71, 0.80-3.72, 12.7-25.6, 76.8-307.3, respectively. The peroxide values of all the cold-pressed flaxseed oils were found within the limit of up to 10 milliequivalents peroxide/ kg of oil under the New Zealand Food Regulations (1984) for edible fats and oils and 15 milliequivalents peroxide/kg of oil under the Codex Alimentarius Commission (1999) standard for virgin oils and cold-pressed fats and oils.

CHAPTER 5

CHEMOMETRICS

Chemometrics has been defined as the application of mathematical and statistical methods to problems of chemistry and has been used with success in different areas. The progressive use of chemometrics in diverse fields is explained by its capability of treating large quantities of information. In the food and beverage industries, chemometrics is applied in many instances, such as monitoring and controlling processes, determinations of geographical origin and sources of food and detection of fraudulent practices (Bortoleto, et al. 2005).

Chemometric methods implicate procedures for multivariate data analysis. Multivariate analysis comprises a set of techniques dedicated to the analysis of data sets with more than one variable. These methods are intended at projecting the original data set from a high dimensional space onto a line, a plane, or a 3D coordinate system (Diaz, et al. 2004). The most common projection methods of multivariate data analysis used in olive oil studies, especially for classification and authentication, are principle component analysis (PCA), partial least square analysis (PLS), discriminant analysis techniques (linear discriminant analysis (LDA) and partial least-squares discriminant analysis (PLS-DA).

5.1. Principle Component Analysis (PCA)

PCA is an unsupervised pattern recognition technique. This means that there is no prior knowledge of the classes that the samples will fall into. It is a way of identifying patterns in data, and expressing the data in such a way as to reveal their similarities and differences. PCA is also known as a variable reduction procedure. It is

useful when having data on a number of variables (possibly a large number of variables), and is believed that there is some redundancy in those variables. In this case, redundancy means that some of the variables are correlated with one another, possibly because they are measuring the same construct. Because of this redundancy, it is considered that it should be possible to reduce the observed variables into a smaller number of principal components (PCs) that will account for most of the variance in the observed variables.

The general purposes of PCA are: dimensionality reduction, determination of linear combinations of variables, feature selection: choosing the most useful variables, visualization of multidimensional data, identification of underlying variables, and identification of groups of objects or of outliers.

The basic concept of the method can be described geometrically as a reduction of multidimensional data sets by projecting the data **X** of p dimensions onto a subspace of a few dimensions, k ($k < p$). Score matrix **T** (n x k) and loading matrix **P** (k x k) can construct the **X** matrix (n x p) which is the prediction matrix of actual observations, **X**. The mathematical background is decomposing the original variable matrix **X** into the product of the score matrix **T** and the transposed loading matrix **P** plus a residual matrix **E** (Eriksson, et al. 2001).

$$\mathbf{X} = \mathbf{TP} + \mathbf{E} \tag{5.1}$$

Principal components (PC) account for the majority of the variability in the data. This enables to describe the information with considerably few variables than originally present. PC is defined as a linear combination of optimally weighted observed variables. "Optimally weighted" refers to the fact that the observed variables are weighted in such a way that the resulting components account for a maximal amount of variance in the data set. The loadings are the coefficients of the original variables that define each principal component (Brereton 2003). In reality, the number of components extracted in a principal component analysis is equal to the number of observed variables being analyzed. The first component extracted in a principal component analysis accounts for a maximal amount of total variance in the observed variables. Under typical conditions, this means that the first component will be correlated with at least some of the observed variables. The second component extracted will have two important characteristics. First, this component will account for a maximal amount of variance in the data set that was not accounted for by the first component. Again under typical conditions, this

means that the second component will be correlated with some of the observed variables that did not display strong correlations with component 1. The second characteristic of the second component is that it will be uncorrelated with the first component.

When the analysis is complete, the resulting components will display varying degrees of correlation with the observed variables, but are completely uncorrelated with one another. Thus, PCA develops an alternative set of coordinate axes; PCs are orthogonal to each other. After determination of significant components determination, the grouping of data is formed by plotting the latent variables, which are called score plots. Scores are the projections of the objects on the new axes. In score plots, the horizontal axis indicates the scores of first PC and the vertical one refers to the second PC. By plotting the principal components, one can view interrelationships between different variables, and detect and interpret sample patterns, groupings, similarities or differences (Gan, et al. 2005).

Application of PCA chemometric method combined with many spectroscopic and chromatographic techniques has been carried out in the classification, characterization and authentication studies of olive oil. There are several examples of olive oil classification and characterization studies, for instance, Diaz, et al. (2005) characterized the VOOs obtained from olives of Manzanilla Cacerena of the North of Caceres (Extremadura-Spain) according to content of the various triglycerides, sterols, or both by application of PCA method with soft independent modeling class analogy (SIMCA). D'Imperio, et al. (2007) and Rezzi, et al. (2005) had studies related to classification of olive oils from Italy and from various Mediterranean areas, respectively, by the combination of NMR with multivariate analysis techniques of PCA and linear discriminant analysis (LDA). Aranda, et al. (2004) have measured triglycerides, total and 2- position fatty acid composition by HPLC and achieved 90% correct classification using PCA and LDA in differentiating Spanish olive oil cultivars. Else, the rancid defects of Portuguese virgin olive oil with different percentages (0−100%) of rancid standard oil, was detected by using electronic-nose. In this research, PCA was applicated to analyze the structure of data sets and detect abnormal information (outliers), successfully (Aparicio, et al. 2000). Oliveras, et al. (2004) discriminated the 105 EVOO from five different Mediterranean areas depended on their volatile fractions by using headspace mass spectrometry with PCA and LDA methods. Bendini, et al. (2007) collected FT-IR spectra of 84 monovarietal virgin olive oil

samples from eight Italian regions and manipulated fingerprint region by PCA in order to control the geographic origin and quality of virgin olive oils.

The application of PCA with different methods, in particular, infrared spectroscopy (mid-infrared and near IR) has growing interest in detection of the adulterated olive oils with different vegetable oils in recent years. Christy, et al. (2004) have detected and quantified adulterated olive oil by NMR and using chemometric techniques: PCA and partial least square (PLS) and applied methods for data pretreatments such as multiplicative signal correction. Gurdeniz and co-workers (2008) reported the efficiency of PCA and mid-infrared techniques combination for detection of the adulteration in Turkish olive oils, which were mixed with different vegetable oils such as rapeseed, cottonseed and corn–sunflower binary mixtures. Webster, et al. (1999) analyzed the samples of VOO and refined olive oil for *n*-alkanes by gas chromatography with flame ionisation detection to determine if the hydrocarbon patterns could be used as determinants for assessing adulteration of olive oil. *n*-alkane data of olive oil was added to an existing database that included rapeseed, safflower, sunflower, corn, palm, palm kernel, coconut, groundnut and soybean oils and analysed by PCA. Analysis of the *n*-alkane pattern by PCA made it possible to identify adulterants at levels as low as 0.5% w/w. Lastly, Park and Lee (2003) investigated adulterated of olive oils mixed with soybean oil using triacylglycerol profiles by high temperature gas chromatography and PCA methods together.

5.2. Partial Least-Squares Analysis (PLS)

PLS is known as the regression extension of PCA, working with two matrices, X and Y. However, PLS methods differ from PCA methods as the X variables and the corresponding Y variables (response data of each object) are projected simultaneously on a subspace with respect to a maximum covariance between X and Y data for the final goal to predict Y from X. In another words, the principle of PLS is based on determination of the components in the input matrix (X) that describe as much as possible of the relevant variations in the input variables, and at the same time have maximal correlation with the target value in *Y*, but without including the variations that

are irrelevant or noisy (Rezzi, et al. 2005). The mathematical demonstration of this method is given in equations 5.2 and 5.3. In these equations, the first terms $1*\overline{x'}$ and $1*\overline{y'}$ refer the variable averages and originates from the pre processing step. The information related to the observations is given by the scores matrices T and U; the information related to the variables is stored in the X- loading matrix P' and Y-weight matrix C'. The variation in the data that is left out of the modeling forms the E and F residual matrices.

$$X = 1*\overline{x'} + T*P' + E \tag{5.2}$$

$$Y = 1*\overline{y'} + U*C' + F \tag{5.3}$$

First PLS component is usually not enough to describe the variation in the Y-data. So, second PLS component describes the remaining variation as much as possible. Second PLS component is also a line orthogonal to first one and it improves the description of X data and provides good correlation with Y remained after first component (Eriksson, et al. 2001).

As well PCA has been used in order to classify and to detect the adulteration studies of olive oil, the PLS method has also applied in many studies. Tapp, et al. (2003) distinguished 60 EVOO from different four European countries by using FT-IR in combination with PLS and LDA methods with a cross-validation success rate of 96%. Gurdeniz, et al. (2007) were able to differentiate the mixtures of monovarietal olive oils from Turkey (Erkence-Nizip and Ayvalik-Nizip) by mid-infrared spectroscopy with 94 – 96 % success ratio. Tay, et al. (2002) illustrated the authenticity of EVOO by using FT-IR and multivariate quantification based on PLS successfully and quantified the composition of sunflower oil, the degree of adulteration in the olive oil. Pena, et al. (2005) also developed a new methodology to detect and quantify adulteration of virgin olive oil and olive oil with hazelnut oil through direct analysis of oil samples by headspace-mass spectrometry and various multivariate analysis techniques such as PLS and PCR. Davis and McEwan (2007) evaluated the oxidative status of New Zealand EVOO samples according to their volatile organic compounds by ion flow tube mass spectrometry. In this research, PLS model including 13 volatile organic compounds was constructed in order to predict the peroxide values (PV) for oxidized olive oils.

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

PLS-DA is a PLS application for the optimum separation of classes using dummy variables 0 and 1 as variables of the Y matrix. Each class is assigned by 0 or 1 and a regression of Y onto X is calculated. For a two-dimensional subspace, the PLS-DA operation can be described as the positioning of a plane in the X space ensuring an optimum separation of assigned classes. So, the principle of PLS-DA is to find a model that separates classes of observations on the basis of their X-variables. Beyond, this method is applicable for two intents: for consideration of classification adequacy, given the group memberships of the objects under study; or for assignment of objects to one of a number of (known) groups of objects. Consequently, discriminant analysis may thus have a descriptive or a predictive objective.

PLS-DA method is usually applied to determine the classification of the olive oils. Galtier, et al. (2007) achieved the quantification of fatty acids and triacylglyceols in French VOO samples by NIR spectra and used PLS-DA technique for classification. In another study, VOO samples were grouped into four classes according to their storage conditions in order to evaluate their freshness and classified by PLS-DA method (Sinelli, et al. 2007).

5.4. SIMCA (Soft Independent Modelling of Class Analogy)

Simca is supervised classification technique, which is solely based on PCA to find out local groupings, a general PCA can be run first if one does not know the classes before. Each class in data set is modelled separately by PCA. These are called local models. When new observations come into the data, they are tested to which one of the classes, previously defined they belong. Leave-one-out cross-validation is used to set up dimension of each class.

Cooman's plots (continuation of Simca method) are used to define the bound area of each class and prevent them in a two-dimensional plot scale refers to the distance of observations to class 1, while the scale refers to that of observations class 2.

When a new observation joins in, it will be placed within that class to which it was the lowest distance or it may plot outside of two regions.

CHAPTER 6

MATERIALS AND METHODS

6.1. Materials

6.1.1. Extracted Extra Virgin Olive Oil Samples

Extracted EVOO samples were produced with olives obtained from cultivated garden of Olive Research Institute (İzmir, Turkey) and İzmir Institute of Technology (IYTE) campus (İzmir, Turkey) in the 2007 harvest year. The extracted olive oil samples used in this study, Ayvalık-Edremit (AE) oilves (Olive Research Institute) and Erkence (Erk) olives (IYTE-Gulbahce Campus) were milled with a maximum 5 kg capacity laboratory scale olive oil mill (TEM Spremoliva, Italy) and then stored in dark at refrigerator temperature (8°C) during analysis period which consists of 3 months time intervals in a year (2007-2008).

6.1.2. Commercial Extra Virgin Olive Oil Samples

Commercial EVOO samples were supplied by Tariş Olive Oil Company (İzmir, Turkey). The commercial olive oil samples used in the study were Altınoluk (A), Ezine (Erk), Bayındır (B) and Ortaklar (O). Each olive oil sample was separated into two groups according to their storage conditions as the samples stored in dark at refrigerator temperature (8°C) and stored in dark at room temperature during analysis period which consists of 3 months time intervals for 14 months (2008-2009) (Table 6.1). Commercial EVOO samples which were subjected to extended oxidation conditions were also

obtained from North and South of Aegean region (Tariş Olive Oil Company İzmir, Turkey). The samples used in this study were 'South Aegean Extra Virgin Olive Oil' (Güney Ege Naturel Sızma Zeytinyağı) which was referred as 'S' and 'North Aegean Extra Virgin Olive Oil' (Kuzey Ege Naturel Sızma Zeytinyağı) which was referred as 'N' in this study. These olive oil samples were kept in an incubator at 60°C.

Table 6.1. Abbreviations used for commercial EVOO samples

sample name	olive oil samples stored at room temperature	olive oil samples stored at refrigerator temperature
Altınoluk	aro	are
Ezine	ero	ere
Bayındır	bro	bre
Ortaklar	oro	ore

Table 6.2. Abbreviations used for olive oil and flaxseed oil samples

oil sample name	oil samples stored at room temperature	oil samples stored at refrigerator temperature
pure olive oil	oro	ore
pure flaxseed oil	fro	fre
5% olive oil+95% flaxseed oil	5ro	5re
10% olive oil+90% flaxseed oil	10ro	10re
15% olive oil+85% flaxseed oil	15ro	15re

6.1.3. Flaxseed Oil Samples

Flaxseed oil samples were purchased from Bükaş (İzmir, Turkey). These flaxseed oil samples were blended with olive oil sample (Burhaniye), which was supplied by Tariş Olive Oil Company (İzmir, Turkey), at the percentages of 5, 10 and

15% (v/v). These mixed oil samples were put in test tubes and kept at both refrigerator temperature (8°C) and room temperature (20-22°C) during analysis period which consists of 21 days time intervals (Table 6.2).

6.1.4. Chemicals Reagents

Chemical reagents used for determination of fatty acid compositions, phenolic compounds and chemicals used for determination of PV, specific extinction coefficients (K232 and K270), AV, IV, FFA and TPC analysis of oil samples are given in Table 6.3.

Table 6.3. Chemicals used in the analysis

NO	CHEMICAL	CODE
Peroxide value (PV)		
1	Acetic acid	Riedel-deHaen 27225
2	Chloroform	Riedel-deHaen 24216
3	Potassium iodate KIO_3	Fluka 60390
4	Potassium iodure (KI)	Riedel-deHaen 03214
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
Specific extinction coefficient (K232 & K270)		
8	Cyclohexane	Labscan
Anisidine value (AV)		
9	Acetic acid	Riedel-deHaen 27225
10	Isooctane	Merck
11	p-Anisidine	Fluka
Iodine value (IV)		
12	Acetic acid	Riedel-deHaen 27225
13	Cyclohexane	Labscan
14	Potassium iodate KIO_3	Fluka 60390
15	Potassium iodure (KI)	Riedel-deHaen 03214
16	Sodium thiosulphate ($Na_2O_3S_2$)	Fluka 72049
17	Starch	Carlo Erba 417587
18	Wijs reagent	Merck-1.00713.2500
Free fatty acids (FFA)		
19	Diethyl ether	Riedel-deHaen
20	Ethanol	Riedel-deHaen

(cont. on next page)

Table 6.3. (cont.) Chemicals used in the analysis

21	Phenolphthalein	Riedel-deHaen
22	Potassium hydrogen phthalate ($(KHC_8H_4O_4)$)	Fluka
23	Potassium hydroxide (KOH)	Riedel-deHaen
Gas chromatography analysis (GC)		
24	F.A.M.E mix C8-C24	Supelco # 18918
25	n-Hexane	Sigma-Aldrich 34859
26	Potassium hydroxide (KOH)	Riedel-deHaen
Total phenol content (TPC)		
27	Folin-Ciocalteau reagent	Fluka 47641
28	Gallic acid	Fluka 48630
29	Methanol	Sigma-Aldrich 34885
30	Sodium carbonate ($NaCO_3$)	Riedel-deHaen 13418
31	Tween 20	Sigma-Aldrich P1379
HPLC analysis of phenolic compounds		
32	Acetonitril	Sigma-Aldrich
33	Gallic acid	Fluka 48630
34	Methanol	Sigma-Aldrich 34885
35	n-Hexane	Sigma-Aldrich 34859
Standard phenolic compounds		
36	Apigenin	Fluka 10798
37	Caffeic acid	Fluka 60020
38	Chlorogenic acid	Fluka 25700
39	Cinnamic acid	Fluka 96340
40	Ferulic acid	Fluka 37528
41	Gallic acid	Fluka 48630
42	4-hydroxybenzoic acid	Fluka 54630
43	4-Hyroxyphenylacetic acid	Fluka 56140
44	Hydroxytyrosol	Extrasynthese 4986
45	Luteolin	Fluka 62696
46	M-coumaric acid	Fluka 28180
47	O-coumaric acid	Fluka 28170
48	Oleuropein	Extrasynthese 0204
49	P-coumaric acid	Fluka 28200
50	Syringic acid	Fluka 86230
51	Tyrosol	Fluka 56105
52	Vanilic acid	Fluka 94770
53	Vanilin	Fluka 94750

6.2. Methods

6.2.1. Determination of Peroxide Value (PV)

Determination of PV of olive oil samples were made according to the analytical method described in European Official Method of Analysis (Commision Regulation EEC N-2568/91- Determination of peroxide value). The PV was expressed in milliequivalents of oxygen per kg of oil (mequiv of O_2/kg). For standardization of 0.01 M sodium thiosulphate; 2 g of potassium iodate (KIO_3) was dried in an incubator (Memmert) at 90-100 $°C$ for 1-2 hours. After 1-2 hours, 0.001 mol/L KIO_3 solutions ($\approx$0.1070 gr KIO_3/500 ml dH_2O) was prepared with potassium iodate taken from the incubator. Exact weight of KIO_3 was recorded. In order to prepare 0.5 M H_2SO_4 solutions, 2.8 mL of H_2SO_4 (96% purity) was diluted to 100 mL with deionized water. For preparation of starch solution; 1 g of starch was weighed and dissolved in 10 mL of deionized water. 90 mL of boiling deionized water was added to starch solution and boiling continued for 2-3 minutes.

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

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

$$M_{KIO_3} = \frac{m_{KIO_3}(g) / MW_{KIO_3}(g/mol)}{V_{KIO_3}(mL) solution} \tag{6.1}$$

$$M_{sodiumthiosulphate} = \frac{6 * M_{KIO_3}(mol/L) * V_{KIO_3}(mL)}{V_{sodiumthiosulphate}(mL)} \tag{6.2}$$

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)

$V_{sodiumsulphate}$ = amount of sodium thiosulphate used in titration (mL)

After standardization part of the experiment; 10 mL of chloroform, 15 mL of acetic acid and 1 mL of potassium iodide solution were added into 3 g of an olive oil sample and mixed for 1 minute. Then, the sample was kept in dark and at the room temperature for 5 minutes. Lastly, 75 mL of deionized water and 0.5 mL of starch solution were added to oil sample. Titration of free iodine was carried out with 0.002 M sodium thiosulphate solution until the dark blue colour of solution turns to colourless and the amount of total sodium thiosulphate solution spent during the titration was recorded.

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

$$PV = \frac{V\,(mL) * M\,(mol/L) * 1000}{m\,(g)} \tag{6.3}$$

V: volume of sodium thiosulphate solution spent during titration (mL)

M: molarity of sodium thiosulphate solution

m: the weight of the sample (g)

6.2.2. Specific Extinction Coefficient at 232 and 270 nm (K232 and K270)

European Official Method of Analysis (Commision Regulation EEC N-2568/91) was used for the determination of specific extinction coefficients of the olive oil samples. 250 mg of olive oil was weighed. The weighed sample was placed into a 25 mL graduated flask and diluted to 25 mL with cyclohexane (spectrophotometric grade). The sample was homogenized using vortex (Velp Scientifika, Europe) for 30 seconds and then resulting solution was placed into a quartz cuvette. Absorbance at 232 and 270

nm was determined in a spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Japan), using the pure cyclohexane as the blank.

6.2.3. Determination of Anisidine Value (AV)

Anisidine value was determined by the standard 2504 IUPAC method (IUPAC, 1987) using a Shimadzu UV-2450 UV-Visible Spectrophotometer. In accordance with the method, 100 mg of olive oil sample was weighed into a 25 mL volumetric flask. The sample was dissolved and diluted to 25 mL of volume with isooctane. Then the absorbance of the solution was measured in a cuvette at 350 nm with the spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Japan), using the reference cuvette filled with isooctane solvent as a blank. After the measurement of absorbance, 2.5 mL of this solution was taken and added to 0.5 mL of p-anisidine analytical reagent (0.5 % w/v p-anisidine in acetic acid). After exactly 10 minute the absorbance of the solvent was measured at 350 nm, using the p-anisidine analytical reagent solution as a blank in the reference cuvette.

The method used for calculation of anisidine values;

$$AV = 25 * (1.2 A_2 - A_1) / W \qquad (6.4)$$

A_2: the absorbance of solution prepared with p-anisidine analytical reagent

A_1: the absorbance of solution prepared with isooctane

W: weight of the sample (g)

6.2.4. Determination of Iodine Value (IV)

The Wijs method was used for the determination of the iodine value (IV). With respect to this method, 2.1 g KIO_3 (potassium iodate) was dried in an incubator at 90-100 C for 1-2 hours. Then, 0.01 mol/L KIO_3 solution was prepared with potassium

iodate taken from the incubator. 0.5 M H_2SO_4 solution was prepared. 2 g KI (potassium iodine) was weighed and 10 mL of 0.5 M H_2SO_4, 50 mL of 0.01 M KIO_3 solution were added. Solution was titrated with thiosulphate until the solution has lost its initial reddish brown color and has become pale yellow. 2 mL of starch indicator was added and titration was completed. Sodium thiosulphate spent during titration was recorded. This part of the method was used for determination of molarity of standardization of 0.1 M sodium thiosulphate. After standardization, 0.1 mg of oil sample was weighed in to 500 mL flask and 20 mL of the solvent (prepared by mixing equal volumes of cyclohexane and acetic acid) was added. Similarily a blank with the solvent and the reagent was prepared. Following the addition of 25 mL of the Wijs reagent into this sample, it was left in the dark for 1 hour. At the end of 1 h, 20 mL of the potassium iodide solution (100 g/l) and 150 mL of deionized water were added into sample flask. Solution was titrated with the standard volumetric sodium thiosulphate solution until the yellow color due to iodine has almost disappeared. A few drops of the starch solution were added and titration was completed.

The iodine value is given by the expression;

$$IV = \left[12.96 * c * \left(V_1 - V_2\right)\right] / m \tag{6.5}$$

c: the exact concentration (mol/L)

V_1: volume of the standard sodium thiosulphate solution used for the blank test (mL)

V_2: volume of the standard sodium thiosulphate solution used for oil sample (mL)

m: weight of the sample (g)

6.2.5. Total Phenol Content (TPC)

The total phenol content (TPC) 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.

An oil sample of 2 g was weighed. 10 mL of methanol/water solution (80:20 v/v) and 1-2 drops of Tween-20 was added to oil sample. For extraction the sample was

homogenized with crusher (Heidolph – SilentCrusher M, Germany) at 15000 g for 1 minute and then centrifuged at 5000 rpm for 10 minutes (Nüve NF 615, Ankara, Turkey). After centfifugation the supernatant part of the sample was collected in a tube. Extraction with remaining oil residue is repeated two more times without Tween 20 addition and each time the supernatant part of the sample was collected in the same tube containing supernatant extracted before. The collected volume was recorded as total volume of the olive oil sample at the end of the extraction part. Following the extraction, 1 mL of the collected supernatant was taken and then 1mL of the aqueous-methanolic solution (80:20 v/v) was added and diluted with 5 mL of deoinized water. The 0.5 mL Folin-Ciocalteu reagent, 2 mL of sodium carbonate ($NaCO_3$) solution (15 % w/v) were added respectively. The mixture is diluted with deoinized water by adding 1.5 mL. The homogenization was applied by a vortex (Velp Scientifika, Europe) for 30 seconds. The mixture was kept in dark for 2 hours. For blank the same procedure was repeated with 1 mL of methanol-water (80:20 v/v) instead of phenolic extract. Finally, the absorbance was measured at 765 nm in a spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Japan). The total phenol content of extract was determined by using a gallic acid calibration curve.

Gallic acid (GA) calibration curve was constructed by means of the standard gallic acid solution which was prepared with different concentrations changing from 0.005 mg/mL to 0.09 mg/mL. Three parallel analyses were prepared for standard Gallic acid solution (0.005 mg/mL–0.09 mg/mL) obtained from mother solution of gallic acid (25 mg gallic acid/250 mL deionized water) and blank sample. Gallic acid calibration curve was obtained with 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 gallic acid calibration curve and total phenol content was determined in terms of gallic acid as mg gallic acid / kg oil.

$$TPC = \frac{GA\,(mg/\mathrm{mL}) * V_{sample}\,(mL) * 1000}{W_{\mathrm{sample}}\,(\mathrm{g})} \qquad (6.6)$$

6.2.6. FT-IR Analysis

All infrared spectra (4000-650 cm^{-1}) were acquired with a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer Inc., Wellesley, MA). This instrument was equipped with a horizontal attenuated total reflectance (HATR) sampling accessory (ZnSe crystal) and a deuterated tri-glycine sulphate (DTGS) detector. HATR accessory was used to collect the spectral data of oil. The resolution was set at 2 cm^{-1} and the number of scans collected for each spectrum was 128. ZnSe crystal was cleaned with hexane in between sample runs. Measurements were conducted duplicate or triplicate for each olive oil sample. In Figure 6.1, the typical FT-IR spectrum of an olive oil sample is given. The x-axis refers to the wavelengths among 4000-650 cm^{-1} and the y-axis refers to the absorbance values. The regions of the wavelength of 3050-2800, 1740 and 1500-650 cm^{-1} indicate the C-H stretching vibrations, C=O double bond stretching vibrations and deformations and bending of C-H and stretching vibrations of C-O, respectively. The significant differences between the examined oil samples can be observed by overlapping the spectra of samples.

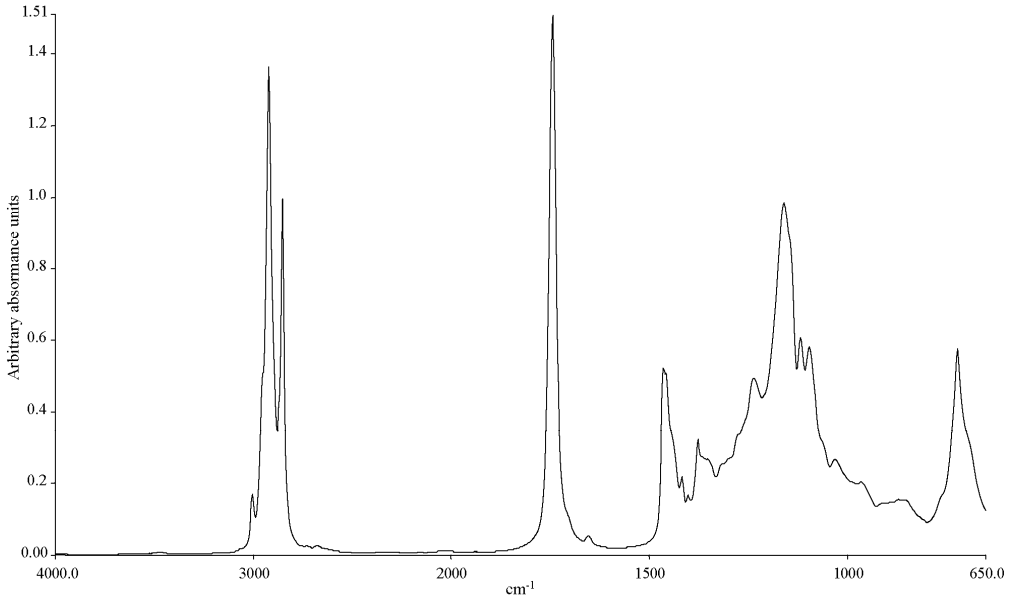

Figure 6.1. Typical FT-IR spectrum of olive oil

6.2.7. Determination of the Free Fatty Acids (FFA)

European Official Methods of Analysis (EEC 1991) was used for the determination of free fatty acid value in terms of % oleic acid. Approximately 1 g of potassium hydrogen phthalate ($KHC_8H_4O_4$) was weighed and dried in an oven at 110º C for 2 hours. Accurately 0.4 g of potassium hydrogen phthalate was weighed into an erlenmayer flask. 75 mL of deionized water and 3 drops of phenolphthalein indicator (0.5 g phenolphthalein in 50 mL 95% ethanol (v/v)) were added into the flask before titration with potassium hydroxide (KOH). 1 mol/L potassium hydroxide (KOH) was prepared with deionized water and it was standardized with potassium hydrogen phthalate.

50 mL of 95% ethanol-water solution (95:5 v/v) and 50 mL of diethyl ether mixture (1:1 v/v) were prepared. 3 drops of phenolphthalein indicator was added into the mixture. The ether-ethanol mixture was titrated with KOH solution until a sudden color change has occured. 20 g of olive oil sample was weighed. The titrated ether-ethanol mixture was added to the 20 g of sample and 3 drops of phenolphthalein indicator was added into the mixture before titration. Then, the mixture was titrated with 0.1 mol/L solution of KOH and the volume of solution spent was recorded.

Acidity was expressed as percentage of oleic acid with the equation given below:

$$V * c * \frac{M}{1000} * \frac{100}{m} = \frac{V * c * M}{10 * m} \qquad (6.7)$$

V : the volume of titrated KOH (mL)

c: exact concentration of the titrated solution of KOH (mol/L)

M: the molar weight of the oleic acid (282g/mole)

m: weight of the sample (g)

6.2.8. Gas Chromatography Analysis (GC)

Sample Preparation Method: European Official Methods of Analysis (EEC, 1991) was used for the preparation of methyl esters. 100 mg oil sample was weighed in 20 mL test tube. The sample was dissolved in 10 mL n-hexane and 100 µL 2 N potassium hydroxide in methanol was added (2.8 g in 25 mL). The sample solution was vortexed for 30 seconds and centrifuged for 15 minutes. After centrifugation, supernatant phase was transferred into 2 mL autosampler vial for chromatographic analysis.

Analytical Conditions: Chromatographic analyses were performed on an Agilent 6890 GC (Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector (FID). The instrumental configuration and analytical conditions were summarized in Table 6.4.

Table 6.4. Chromatographic method for the analysis of fatty acid methyl esters

Chromatographic system	Agilent 6890 GC
Inlet	Split/spitless
Detector	FID
Automatic sampler	Agilent 7683
Liner	Split liner (p/n 5183-4647)
Column	100 m x 0.25 mm ID, 0.2 µm HP-88 (J&W 112-88A7)
Inlet temperature	250 ^{o}C
Injection volume	1µL
Split ratio	1/50
Carrier gas	Helium
Head pressure	2 mL/min constant flow
Oven temperature	175^{o}C, 10 min, 3^{o}C/min, 220^{o}C, 5 min
Detector temperature	280 ^{o}C
Detector gas	Hydrogen:40mL/min; Air:450mL/min; Helium make-up gas:30mL/min

Fatty acids used in the analysis were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0) and behenic acid (C22:0) (Figure 6.2). Each sample was analyzed at least two times. 9 main fatty acids in olive oil samples were determined by retention time of each one according to the reference of standard fatty acids. The area of the each peak which belonged to these fatty acids was integrated by using Chem-station software. The integrated area of each fatty acid was converted to the % concentration by dividing the calculated area of each acid to total area content of all related fatty acids existed in olive oil.

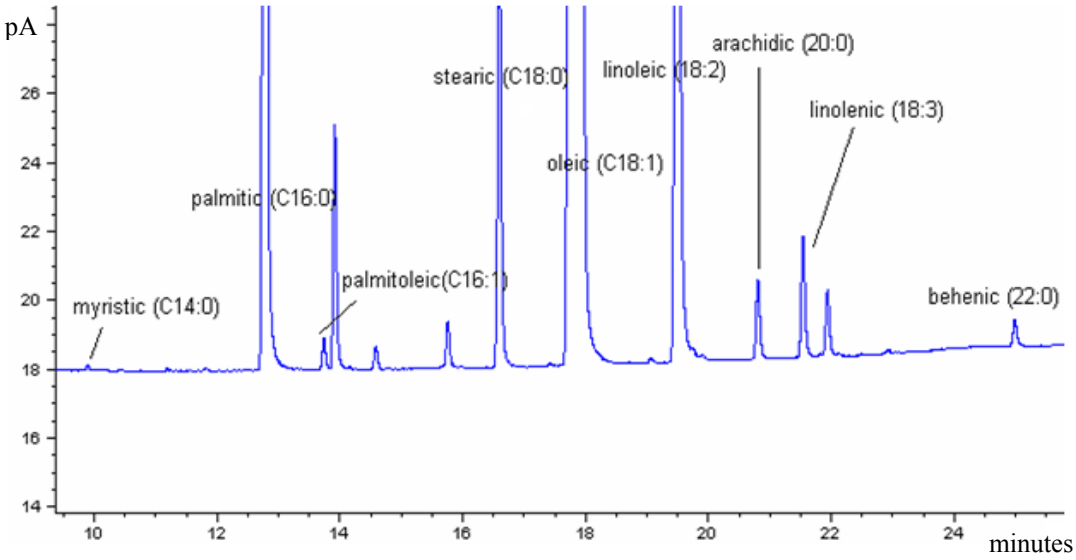

Figure 6.2. Typical GC chromatogram of olive oil

6.2.9. HPLC Analysis of Phenolic Compounds

Phenolic Extraction: The phenolic extracts of olive oil samples were carried out according to the procedure of Brenes et al. (1999). Firstly, 14 g olive oil sample was extracted by using 14 mL of methanol/water (80:20 v/v) for 4 times, 0.01 mol of gallic acid solution (0.05g GA / 25 mL methanol-water) as the internal standard was added to oil sample and homogenized, then centrifuged to separate the supernatant phase. After centrifugation, supernatant phase was collected in a clean tube and in a rotary

evaporator (Heidolph Laborota-4000, Germany) for 22 minutes. Methanol was removed, and then 15 mL of acetonitrile was added to the residue and washed with ×20 mL of n-hexane for 3 times. Acetonitrile solution was evaporated under vacuum for 37 minutes and the residue was exposed to nitrogen for 10 minutes and dissolved in 1 mL of methanol/water. Final extract was filtered with a 0.45 μm pore-size membrane filter (Minisart, Sartorious, and Goettingen, Germany) and transferred into a tube. The extract was injected to HPLC as 20 µL.

HPLC Analysis : HPLC system with Agilent 1100 series with quat pump, diode array detector (DAD), and a 5 µm, 25 cm × 4.6 mm, C18 column (Ace, Aberdeen, Scotland) was used to identify 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, the 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.

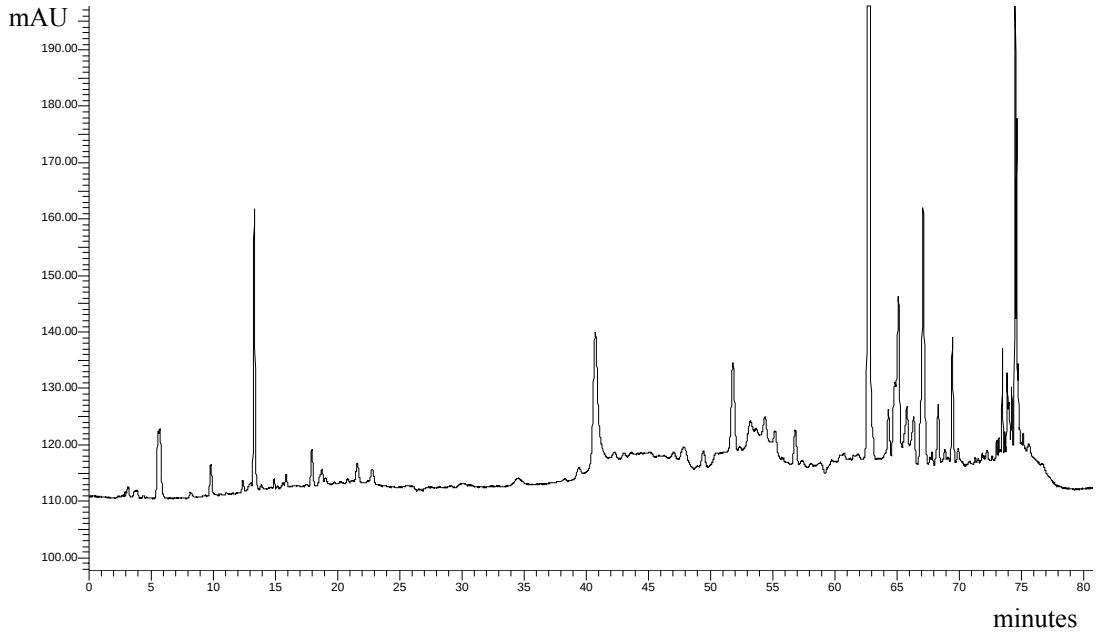

Figure 6.3. Typical HPLC chromatogram of olive oil at 280 nm

Phenolic compounds were stated with commercial standards at 280 and 320 nm, according to their retention time. The phenolic area of each compound and the area of gallic acid area as an internal standard were integrated (Figure 6.3). The peak ratio of the phenolic compounds was calculated by dividing phenolic area to gallic acid area. This peak ratio was also quantified by using their related 4-point calibration standard curves. Hydroxytyrosol, dialdeyhdic forms of elenolic acid and ligstroside aglycon were quantified by tyrosol standard and oleuropein aglycon was also quantified by oleuropein standard. Calibration curves of phenolic standards were given in Appendix B.

6.2.10. Color Analysis

In order to determine the oil color, a colorimeter (chromometer type CR-400, Minolta Sensing, Osaka, Japan) was used. Before measurement the oil color, calibration of the colorimeter was done according to the color coordinates by acquiring the values as Y=93.5, x=0.3140, y=0.3318. After calibration of the instrument, 40 mL of oil sample was put into the glass cell and measurement of the oil samples color was carried out at three different positions, in terms of L*, a* and b*. The oil color was reported as the average of three readings for L*, a*, b*. L*, a*, b* values refer to lightness value; red and green color; yellow and blue color respectively.

6.2.11. Data Analysis

6.2.11.1. Univariate Statistical Analysis

Analysis of variance (ANOVA) was applied to the data of the measurements comprising TPC, PV, K232, K270 and color coordinates of the extracted and commercial EVOO samples. Tukey's test at 5% significance level was used to establish

differences oil samples and storage time (Minitab 14, Minitab Inc., State College, USA). Significance was accepted at $p < 0.05$.

6.2.11.2. Multivariate Statistical Analysis

The multivariate analysis was carried out by using soft independent modelling of class analogy (SIMCA) software (Umetrics, Sweden). The quality parameters were predicted by FT-IR spectra using PLS. In this technique, FT-IR between 3620-2520 cm^{-1} and 1875-675 cm^{-1} were taken as X-matrix and PV, K232, K270, FFA, TPC, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid values were taken as Y-variables. Wavelet orthogonal signal correction (WOSC), which provides an increase in predictability and efficiency of PLS, was performed for each quality parameter prediction by FT-IR spectra. By the application of WOSC, number of X-variables was reduced in order to remove X-spectral data, which were not related with Y-variables.

Simca-P software determines 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. R^2, the fraction of the variation of Y explained by the model after each components, and Q^2, the fraction of the variation of Y that can be predicted by the model according to the cross validation. Q^2 value is always less than R^2 value and it is desired to be close to R^2 for a good model.

CHAPTER 7

RESULTS AND DISCUSSION

7.1. Monitoring EVOO samples for 14 months

In this section, the results of 14-month storage of north (Altınoluk and Ezine) and south (Bayındır and Ortaklar) Aegean olive oil samples, which belonged to 2007 harvest year, are presented. The changes in PV, K232, K270, FFA, TPC, color coordinates, fatty acid and phenolic profiles of EVOO samples were tabulated during 14 months in Appendix A.

7.1.1. Peroxide Value (PV)

The initial PVs of all samples were within the legal limit of 20 meq/kg. Bayındır olive oil had the highest and Ezine olive oil had the lowest initial PV among the olive oil samples monitored (Figure 7.1). All samples showed a progressive increase in PVs, which indicated the greater primary oxidation, after 7-month storage. Except Ezine and Ortaklar olive oils stored at refrigerator, all other olive oils exceeded the upper limit in 7^{th} month of storage. Ezine and Ortaklar olive oils exceeded the maximum value with a slight amount at 10^{th} month of the storage in comparison to other olive oil samples. Bayındır olive oil stored at room temperature had the highest PV at the end of the storage time. It was clear that the increase in PVs of the olive oils stored at room temperature was more than the oils stored at refrigerator temperature during 14-month storage.

According to the study of Gomez-Alanso, et al. (2007), the PVs of the Cornicabra VOO samples, PVs of which were found lower than 6.6 meq/kg at the beginning of the assay, did not exceed the upper limit established by European regulation for EVOO during the storage at 21 months and room temperature. The differences can be based on the lower initial values of PVs of the Cornicabra oils than our olive oil samples.

In a study of evaluation of the extent of degradation in the quality of some Greek virgin olive oils during storage, olive oil samples stored in darkness an increase in PV was observed during the first 7 months. After this time, a reduction occurred probably due to the break up of peroxides into secondary products. So the PVs decreased until the 9th month of the storage time. The peroxide value of oil samples extracted using the classic system and stored in dark did not exceed the limit of 20 meq/kg of oil until the 5th month of storage and values remained close to this during the remaining storage period in the same study (Vekiari, et al., 2002). Kiritsakis (1998) also searched the changes in PVs of six olive oil samples from olive fruits of the cultivar Koroneiki, which were stored in glass bottles and in dark for 2 years. According to results of this study, the PVs did not exceed the legal limit during storage period.

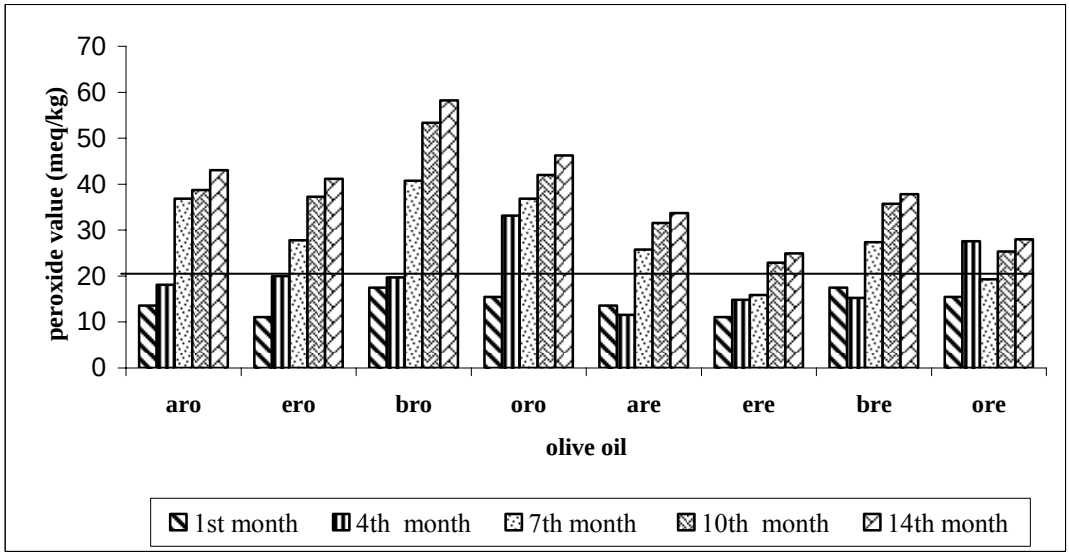

Figure 7.1. Changes in PVs of EVOO samples during 14-month storage period

According to the result of this statistical analysis significance differences were obtained among the olive oils ($p<0.05$). To reveal the effect of storage time and olive oil type on PVs of the oils, two-way ANOVA was performed and significant differences were found (p-value < 0.05).

7.1.2. K232 and K270 Values

None of the oil samples exceeded the upper limit of 2.5 during 14-month storage. Altınoluk olive oil had the lowest and Ortaklar olive oil had the highest initial K232 value. All K232 values decreased on 4th month of the storage time and then they began to increase to the end of the storage time (Figure 7.2). The olive oil samples, which were stored at room temperature, had higher final K232 values compared to those.

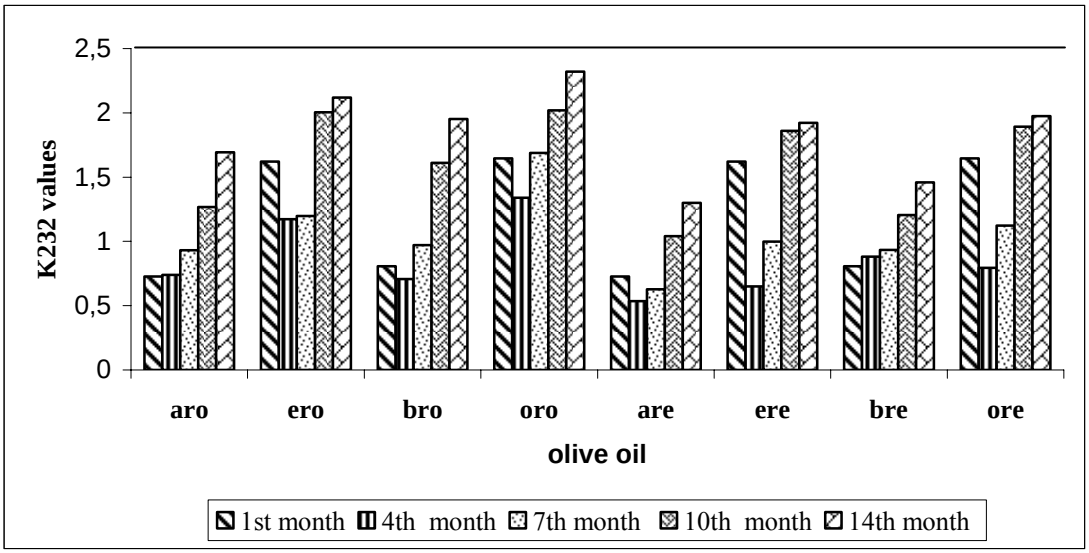

Figure 7.2. Changes in K232 values of EVOO samples during 14-month storage period

Vekiari and friends (2002) reported that the values of K232 in samples of Greek olive oils stored in dark showed an increase during the first 7 month and the K232 values of these samples did not exceed the upper limit value for K232. In addition,

Gutierrez and Fernandez (2002) pointed out the olive oils stored at 2°C and in dark for 6 months, K232 values remained practically constant or very slightly increased. The limit value of 0.25 for extra virgin olive oils exceeded at 85 and 63 days in Picual and Hojiblanca olive oils, respectively in the same study.

To determine the effect of time and oil type on K232 values, two-way ANOVA was performed to all oil samples. Significant differences were obtained among oils and storage months.

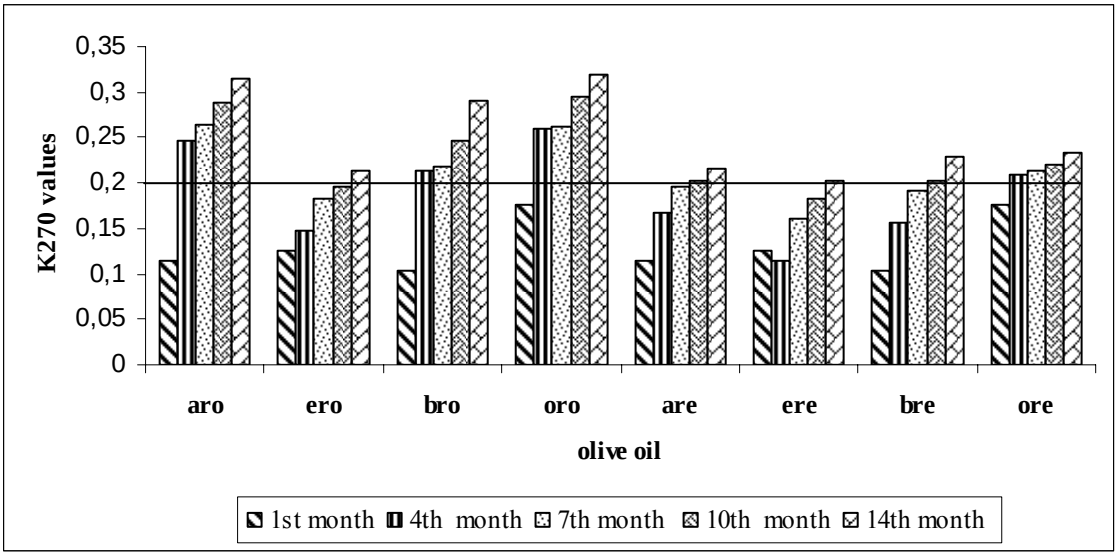

Figure 7.3. Changes in K270 values of EVOO samples during 14-month storage period

The initial K270 values of olive oil samples were less than the limit of 0.2. Ortaklar olive oil had the highest and Bayındır olive oil had the lowest initial K270 values. Altınoluk, Bayındır and Ortaklar olive oils, which were stored at room temperature, and Ortaklar olive oil, which were stored at refrigerator, exceeded the limit on 4th month of storage time. Sharp increase was observed in Altınoluk and Bayındır olive oil samples after 1st storage month. The increase occured in oils, which were stored at room temperature, was more than the oils stored at refrigerator, as the changes in K232 values during storage period. Figure 7.3 refers to the changes in K270 values of EVOO samples for 14 months.

Caro and co-workers (2006) reported the K270 values of the Bosana oil samples stored in dark and at room temperature for 16 months, and observed a significance

increase in K270 values, which were remained below the legal limit of 0.2. For the determination of quality of Picual and Hojiblanca olive oils during storage at 2°C, the K270 values of the samples were observed as unchanged (Gutierrez and Fernandez 2002).

Two-way ANOVA was performed to the samples to check the effect of time and oil types. Significant differences were observed in K270 values with respect to olive oil and time.

7.1.3. Free Fatty Acid (FFA)

The initial FFA values of the oil samples ranged from 0.3 to 0.41. The highest initial FFA value belonged to Ortaklar olive oil, while Altınoluk olive oil had the lowest FFA. The initial FFA value of Bayındır olive oil (0.38) was close to Ezine olive oil (0.39). During storage period of the oil samples, the FFA values increased slightly (Figure 7.4). None of the samples reached to limit of 0.8 %.

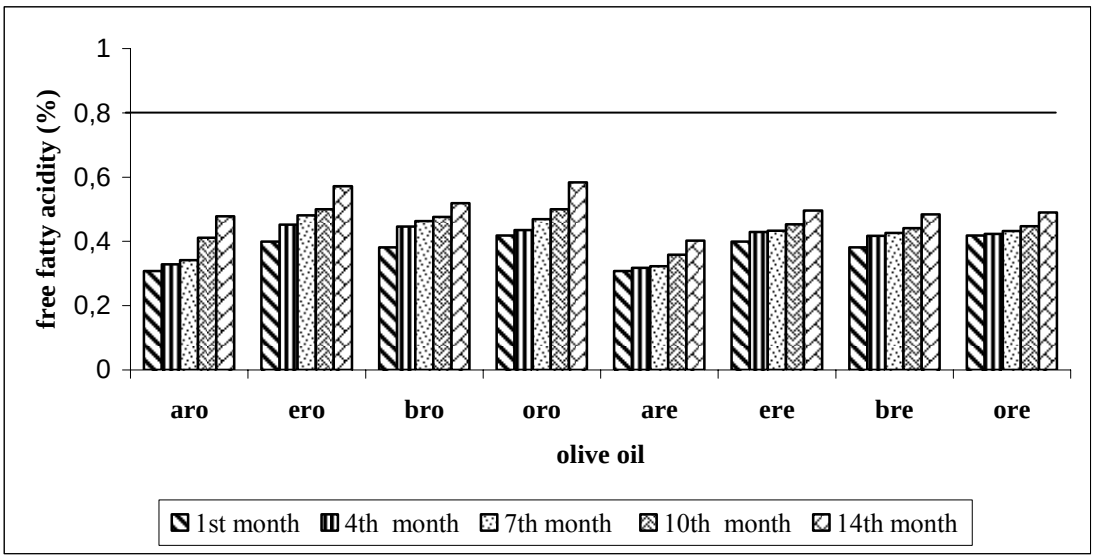

Figure 7.4. Changes in FFA values of EVOO samples during 14-month storage period

Gutierrez and Fernandez (2002) studied on quality indices of Picual and Hojiblanca EVOO samples, which were extracted from from several olive cultivars grown in the same Italian region and stored at 2°C at dark for 6 months. The FFA values of the oil samples maintained their initial acidity values for 6 months. In the same study, the oils stored at 30°C showed an increase in acidity with the storage time that became significant ($p < 0.05$) at the end of the storage period.

7.1.4. Total Phenol Content (TPC)

Ortaklar olive oil had the highest initial TPC value among the oil samples monitored. An increase in TPCs of all oil samples was observed in 4th month, and then these values decreased. The sharp decrease was observed in 14th month of storage (Figure 7.5).

Cinquanta, et al. (1997) studied the changes in phenolic compounds of olive oil samples from Molise region during 18-month storage in darkness and at room temperature. The reduction in TPC of these oils appeared after 6, 12, and 18 months of storage is a result of oxidation and hydrolytic activities, which increase during storage.

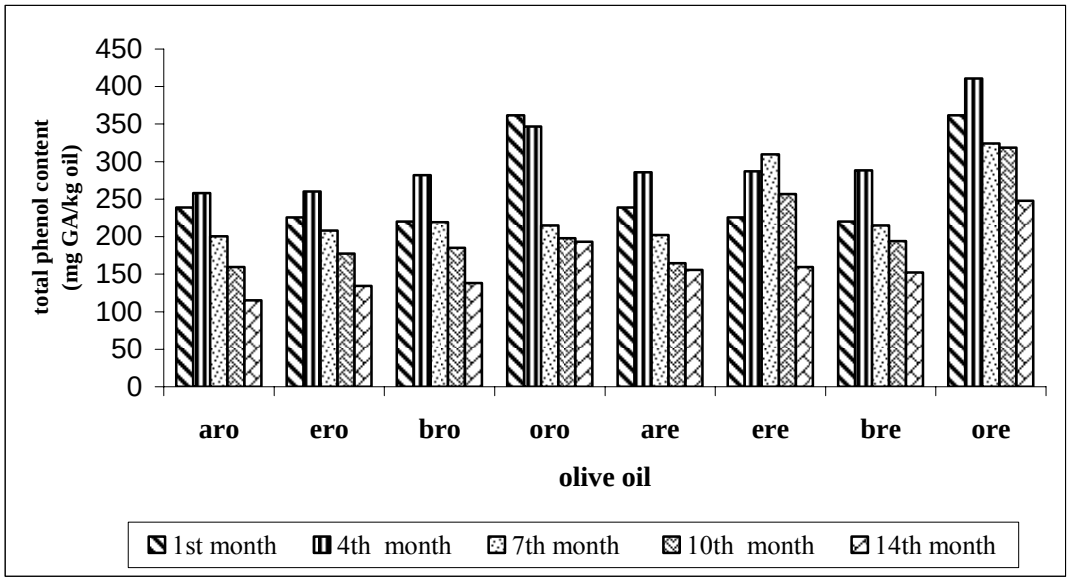

Figure 7.5. Changes in TPC values of EVOO samples during 14-month storage period

Two way ANOVA was applied to olive oil samples both stored at room and refrigerator temperatures individually including all months and significant differences were observed between the samples stored at room temperature. On the other hand, no significant differences ($p < 0.05$) were observed among the oils stored at refrigerator.

7.1.5. Color

Ezine oil had the higher L* value than others. During storage period, the luminosity values of the oil samples, which were kept at room temperature, increased regularly (Figure 7.6). The regular change in the L* values of the oil samples, which were stored at refrigerator, were not observed. The L* values of Altınoluk and Bayındır oils increased until the 7th month of storage time, but a decrease was seen in 10th month of the storage. The increase for Ortaklar oil was in a regular way like the oils, which were stored at room temperature during 10-month storage period. Two-way ANOVA was applied to the L* values for determination of the differences between both oil samples and storage months. Therefore, significance difference was found with the result of p-value < 0.05.

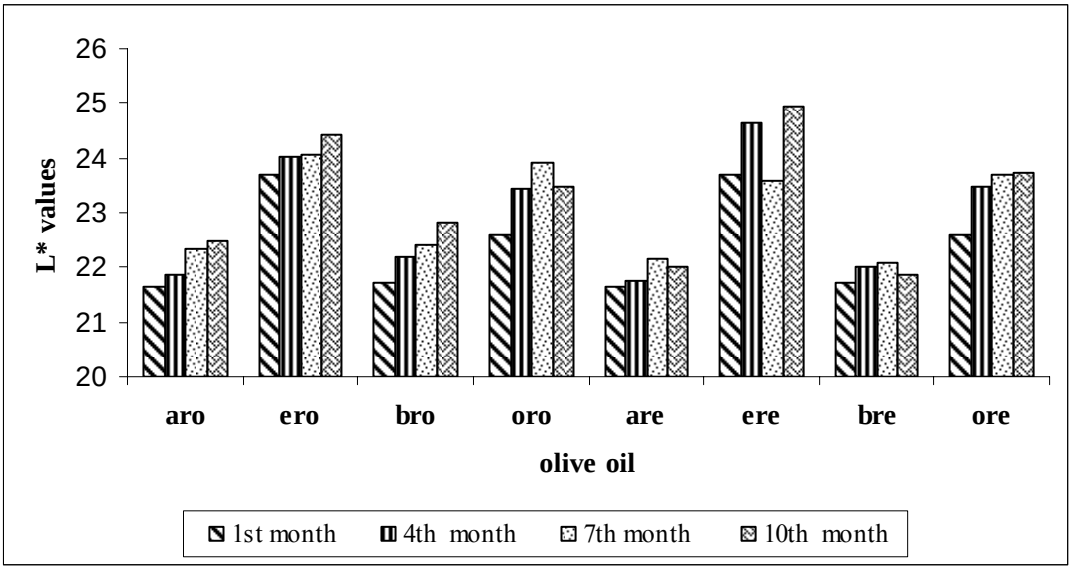

Figure 7.6. Changes in L* values of EVOO samples during 10-month storage period

The highest and the lowest a* values were 1.08 and 0.39, for Altınoluk and Ortaklar olive oils (Figure 7.7). The initial b* values of the samples were almost similiar and varied from 8.00 to 11.61 (Figure 7.8). Although no sharp increase was observed in b* values of the samples during storage, significant differences were found among the b* values of oil samples statistically.

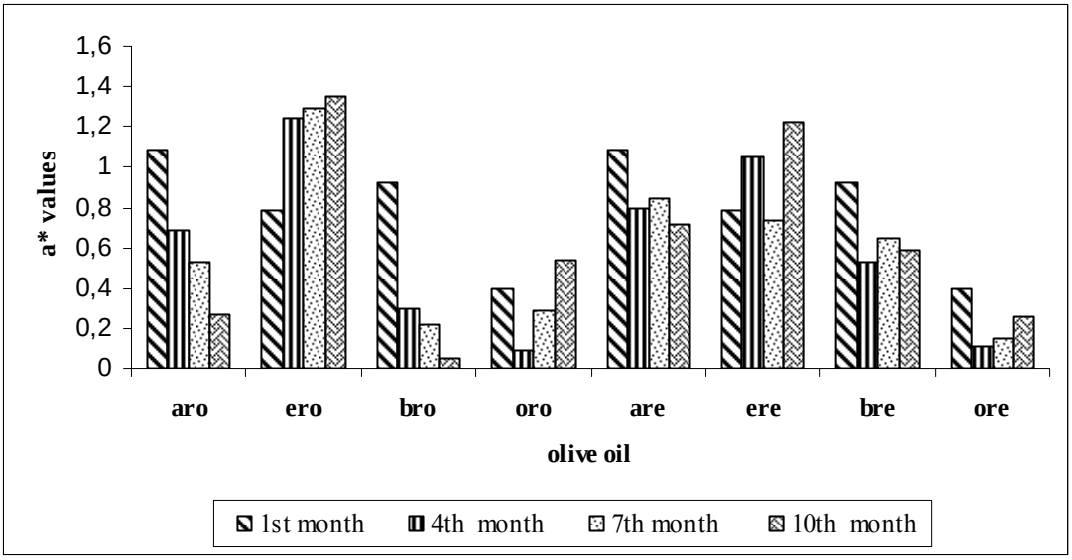

Figure 7.7. Changes in a* values of EVOO samples during10-month storage period

Morello and co-workers (2003) reported that storage did not have a significance effect on chromatic coordinate b* values of commercial olive oils of the Arbequina cultivar after 12 months of storage.

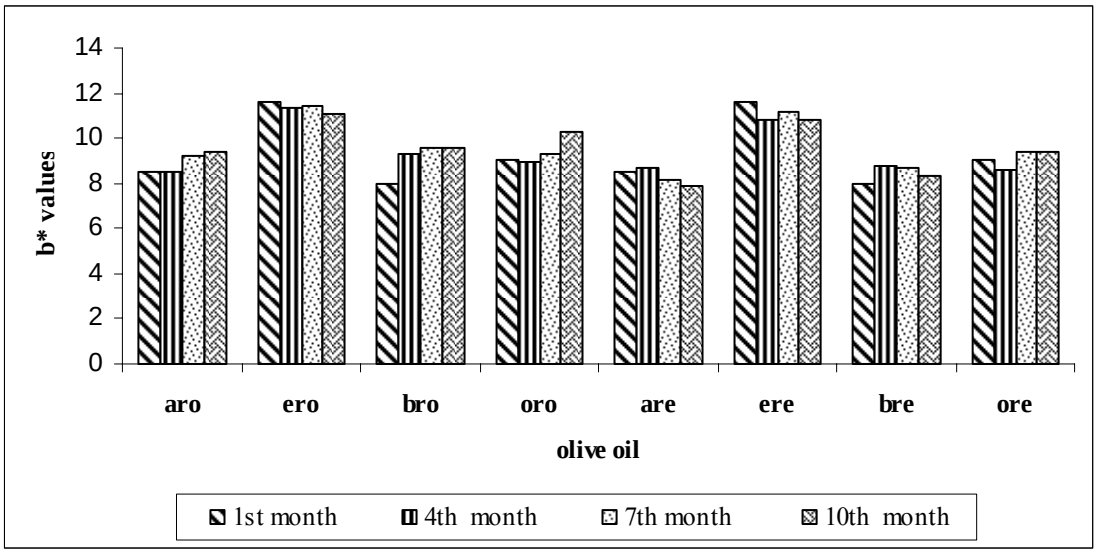

Figure 7.8. Changes in b* values of EVOO samples during 10-month storage period

7.1.6. Fatty Acid Profiles

Fatty acid composition suffered slight changes during storage. An increase in the percentage of oleic acid as a consequence of the decrease in of linoleic and linolenic acid contents were observed.

The initial oleic acid content of olive oil samples varied from 71.52 to 76.10. An increase was observed in oleic acid contents of the samples from 4th month to the end of the storage period (Figure 7.9).

The initial linoleic acid contents of Altınoluk and Ezine olive oils were higher than Bayındır and Ortaklar olive oils. A steady decrease was observed in linoleic acid contents of olive oils after 4th month of storage (Figure 7.10). The decrease in linolenic acid content was also observed olive oils after 4th month of storage (Figure 7.11).

Gomez-Alonso, et al. (2007) evaluated the changes occured in fatty acids of Spanish olive oils stored at room temperature for 21 months. In their study, there were no detectable changes in oleic acid after 21 months of storage time. The reductions observed in linoleic and linolenic acids of the olive oil samples ranging between 2.1% and 3.8% for linoleic acid and between 5.8% and 10.0%.

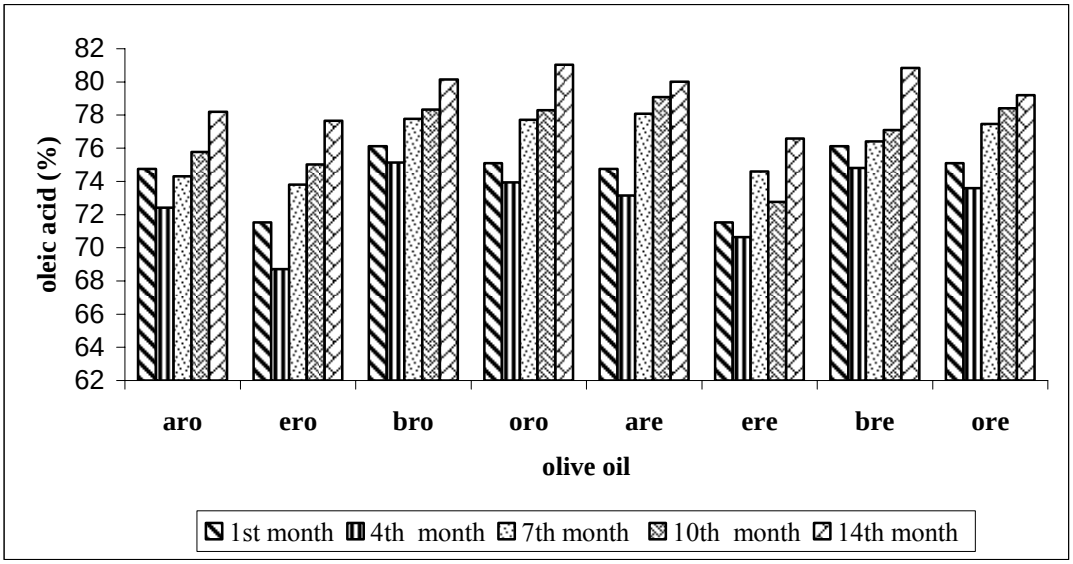

Figure 7.9. Changes in oleic acid contents of EVOO samples during 14-month storage period

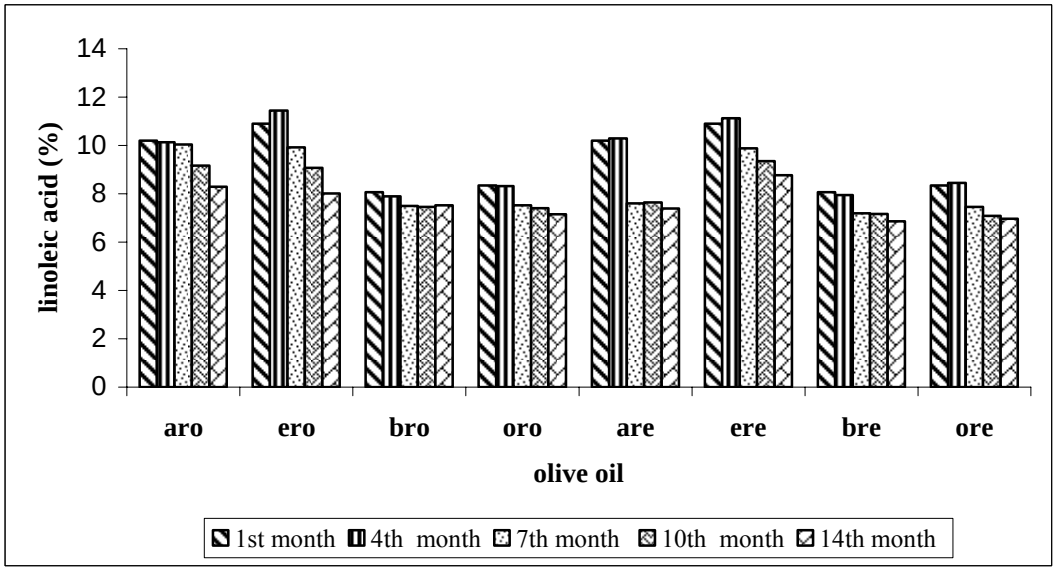

Figure 7.10. Changes in linoleic acid contents of EVOO samples during 14-month storage period

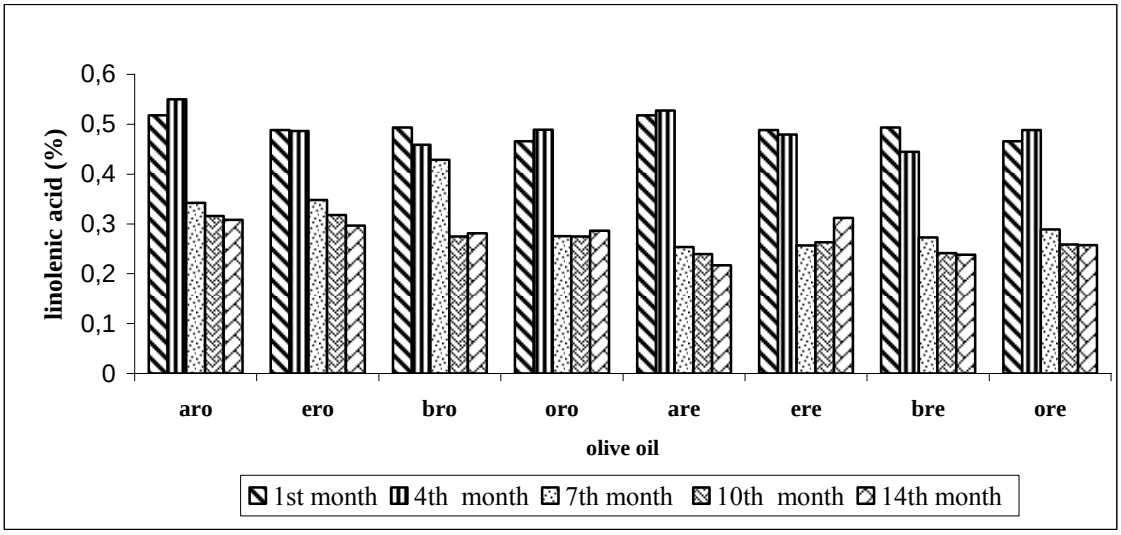

Figure 7.11. Changes in linolenic acid contents of EVOO samples during 14-month storage period

In the study of Morello, et al. (2003) an increase in oleic acid contents in commercial olive oil of Arbequina cultivar stored for 12 months ranging from 70.1 to 76.8%, which is similar to our results.

7.1.7. Anisidine Value (AV)

The highest initial AV belongs to Altınoluk olive oil. The other EVOO samples had approximately the same initial AVs. As a result of decomposition of hydroperoxides, a non-volatile portion of the fatty acid that remains a part of the glyceride molecule occurs. This non-volatile reaction product causes an increase in AV. From the 1st month of storage period AVs increased continuly.

The increase in AVs of oils kept at room temperature was greater than the oils stored at refrigerator (Figure 7.12).

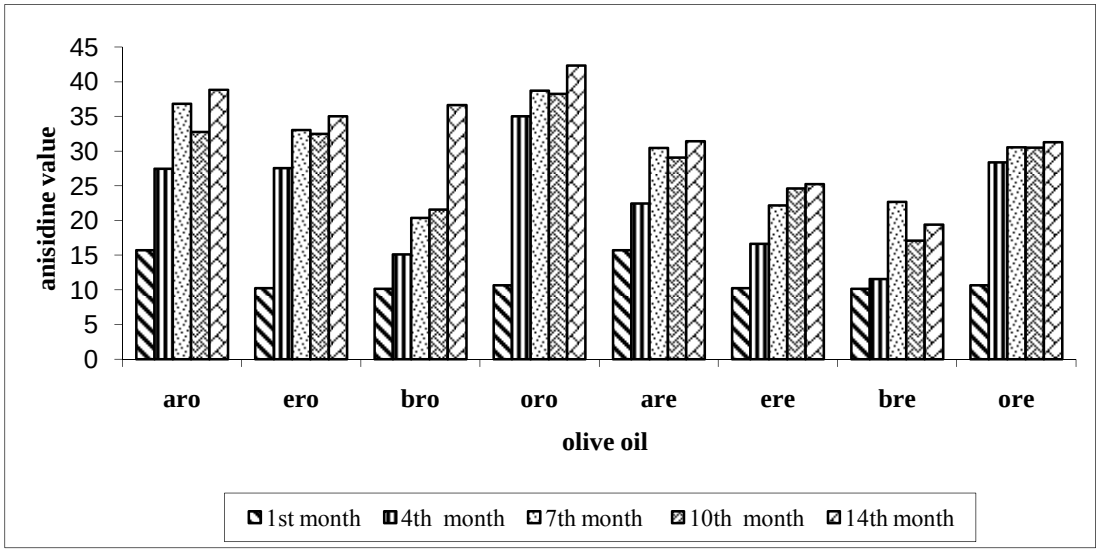

Figure 7.12. Changes in AVs of EVOO samples during 14-month storage period

7.1.8. Iodine Value (IV)

The initial IVs of the EVOO samples were close to each other and varied from 84.48 to 87.18. Among the oils, Altınoluk olive oil had the highest initial IV. As expected, unsaturated fatty acids declined during storage period. Decreasing of unsaturated fatty acids was observed for all storage period (Figure 7. 13).

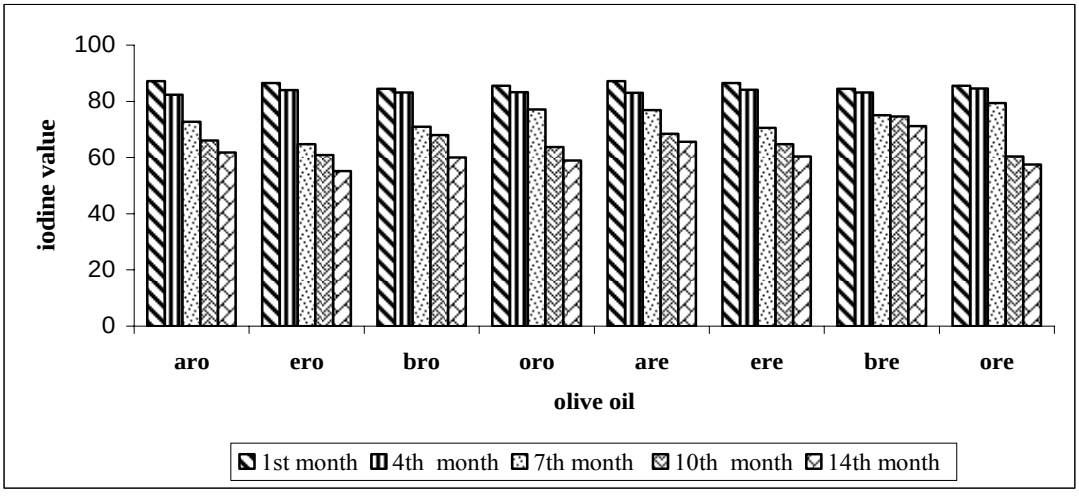

Figure 7.13. Changes in IVs of EVOO samples during 14-month storage period

7.1.9. Determination of Differences in FT-IR Spectra during 14-Month Storage Period

The changes in the FT-IR spectra of EVOO samples during storage, some of the most significant wavenumbers (cm^{-1}), which are useful indicators of the oxidation status, are defined in this part of the study. In all FT-IR spectra, X-axis indicates the wavenumbers (cm^{-1}) and Y-axis indicates the absorbance values (A). In order to emphasize the changes in FT-IR spectra of Altınoluk olive oil at the beginning (1st month) and at the end of the storage period (14th month) are compared in figures 7.14, 7.15, 7.16, 7.17, 7.18 and 7.19.

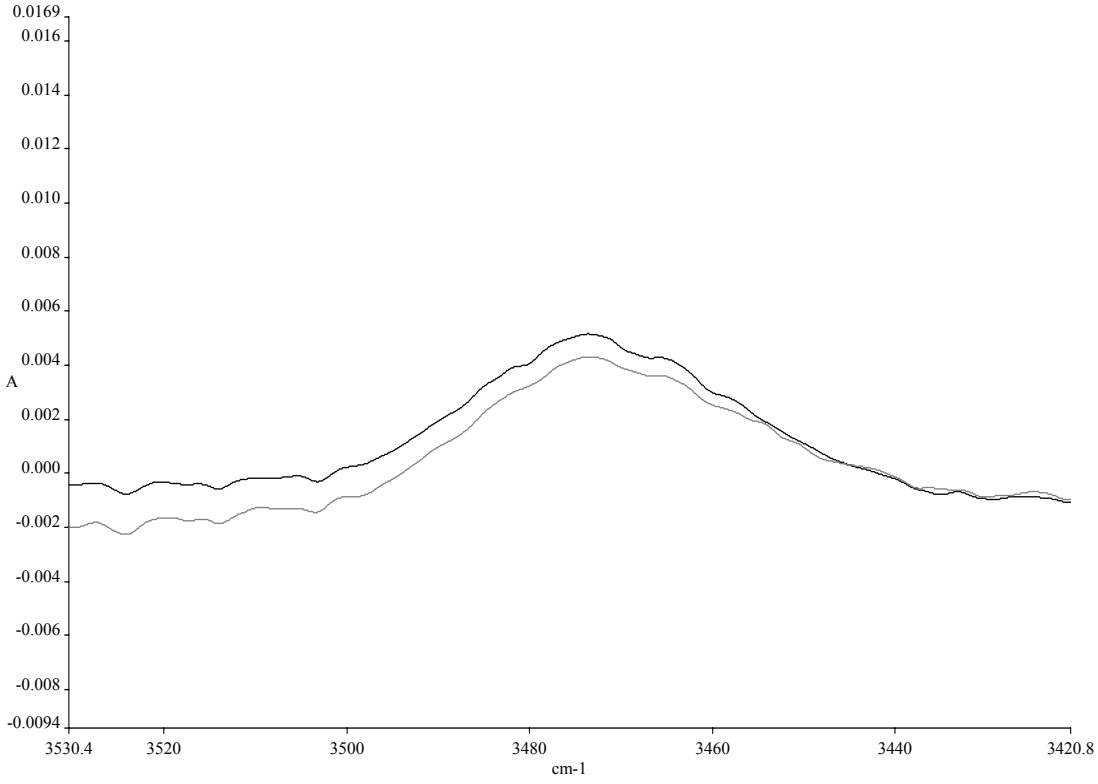

Figure 7.14. Changes in FT-IR spectra between 3530 and 3420 cm^{-1} for Altınoluk olive oil (—1st month, — 14th month)

In bands near 3445 and 3468 cm^{-1}, broading of bands indicates the presence of hydroperoxides and appreciable proportion of alcohols in high amounts. So this region of the spectra refers to the oxidized oils. The figure 7.14 shows the changes in bands between 3530-3420 cm^{-1}. In this spectrum, broading is observed around the region of 3500 and 3460 cm^{-1}.

Near the bands 2925-2852 cm^{-1} a reduction is seen in absorbance which is attributed to the symmetric stretching vibration of the aliphatic CH_2 group (Figure 7.15 and 7.16).

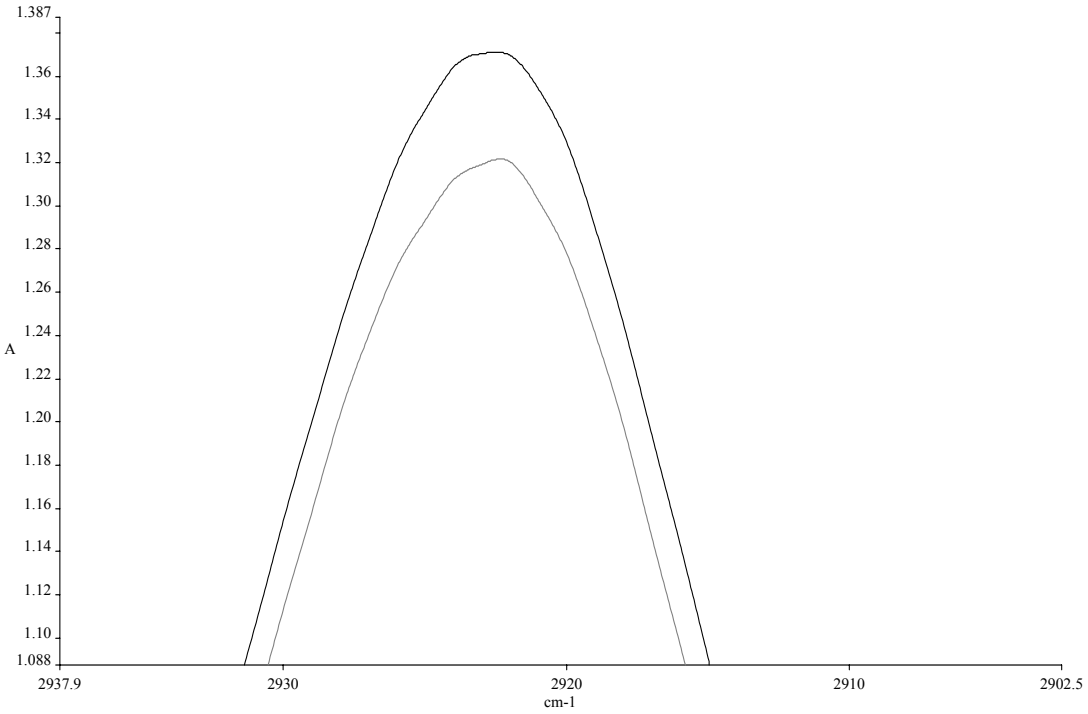

Figure 7.15. Changes in FT-IR spectra between 2930 and 2910 cm^{-1} for Altınoluk olive oil (—1^{st} month, — 14^{th} month)

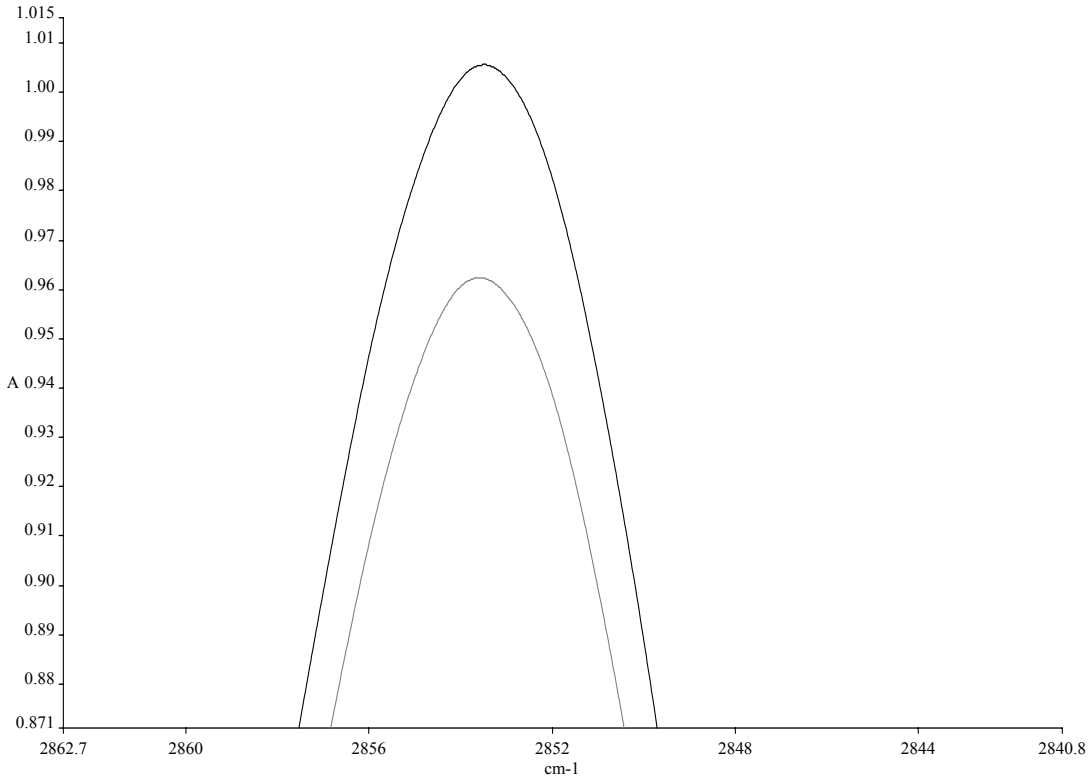

Figure 7.16. Changes in FT-IR spectra between 2856 and 2852 cm^{-1} for Altınoluk olive oil (—1^{st} month, — 14^{th} month)

The region between the wavenumbers of 1746 and 1743 cm^{-1}, is the indicator of production of saturated aldehyde functional groups or other secondary oxidation products. The major peak at 1743 cm^{-1} arises from C=O streching vibrations. The absorbance of the band at approximately 1746 $cm^{-1,}$ due to the ester carbonyl functional group of triglycerides, also change during oxidation. In this region, a slight decrease in absorbance value of band is determined (Figure 7.17).

The bands near 1238 and 1163 cm^{-1} associate with the stretching vibration of the C-O ester groups and with bending vibration of changes during oxidation period. Sharp decrease in absorbance value of band near 1163 cm^{-1} refers to the oxidation process. The lower absorbance value is the marker of the more advanced oxidation (Figure 7.18).

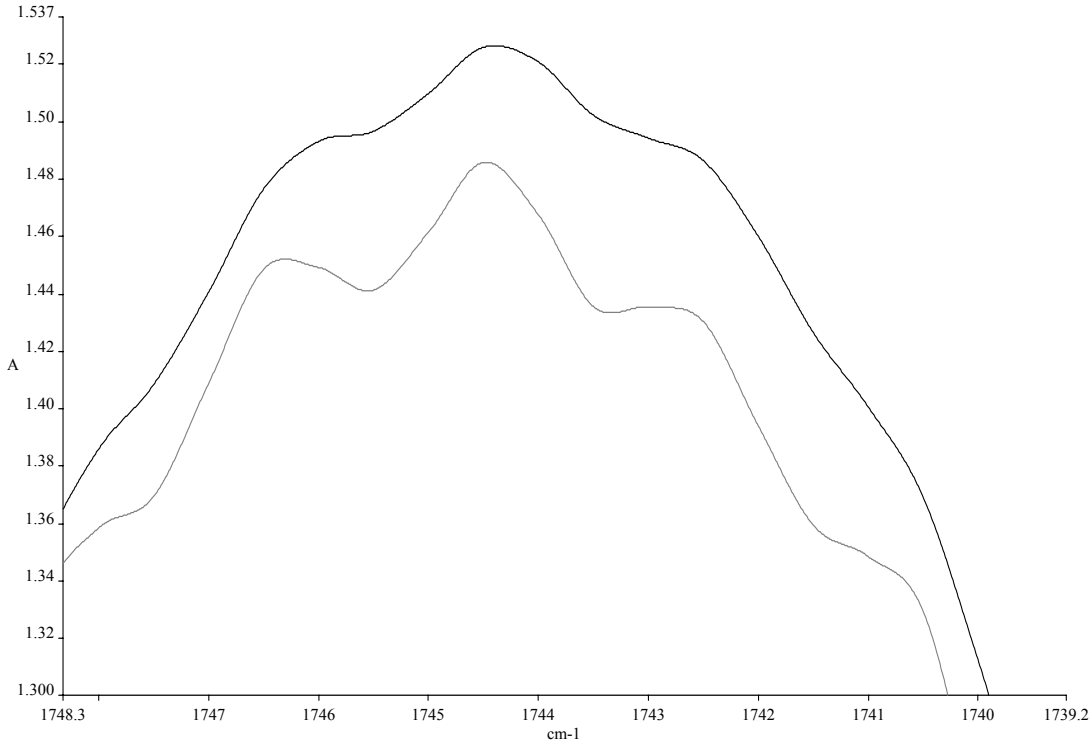

Figure 7.17. Changes in FT-IR spectra between 1748 and 1740 cm^{-1} for Altınoluk olive oil (—1^{st} month, — 14^{th} month)

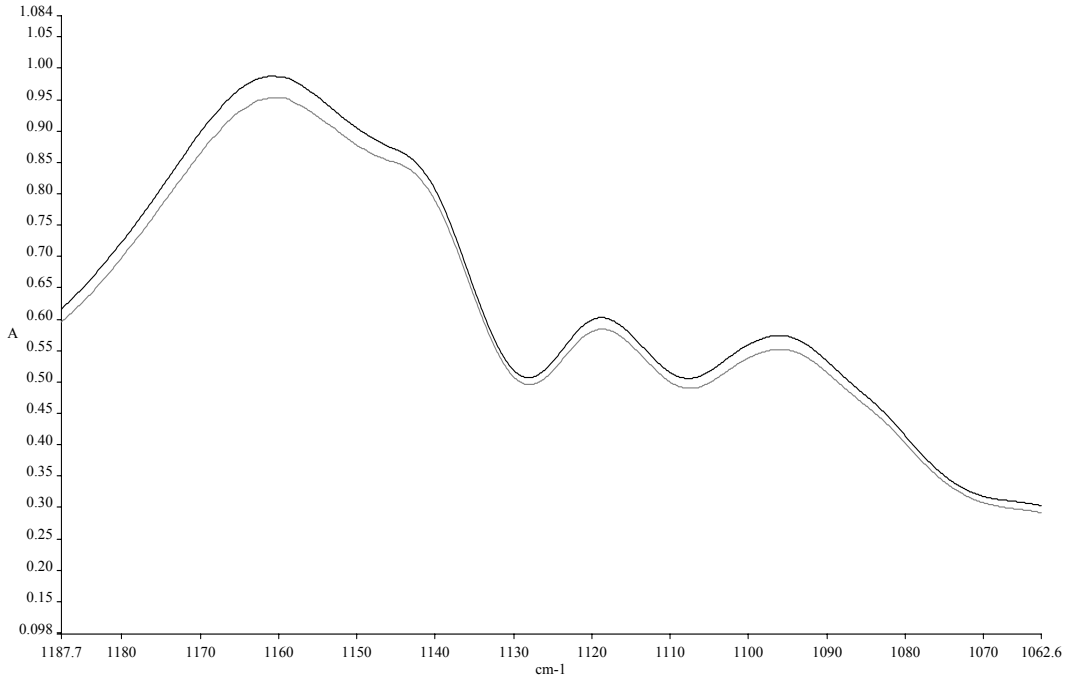

Figure 7.18. Changes in FT-IR spectra between 1187 and 1062 cm-1 for Altınoluk olive oil (—1st month, — 14th month)

The absorbance of the band at 967 cm^{-1}, associated with bending vibrations of CH functional groups of isolated trans-olefins, increases as oxidation advances. Bands at wavelengths of 973 and 976 cm^{-1} assigns to secondary products such as aldehydes and ketones. Therefore the region of 976 – 950 cm^{-1} refers to the possible aldehydic and ketonic groups with isolated trans double bonds, which an increase in the absorbance values of spectra occurs. The higher absorbance values are the indicator of the advanced oxidation process (Figure 7.19) (Vlachos, et al. 2006, Guillen and Cabo 1997).

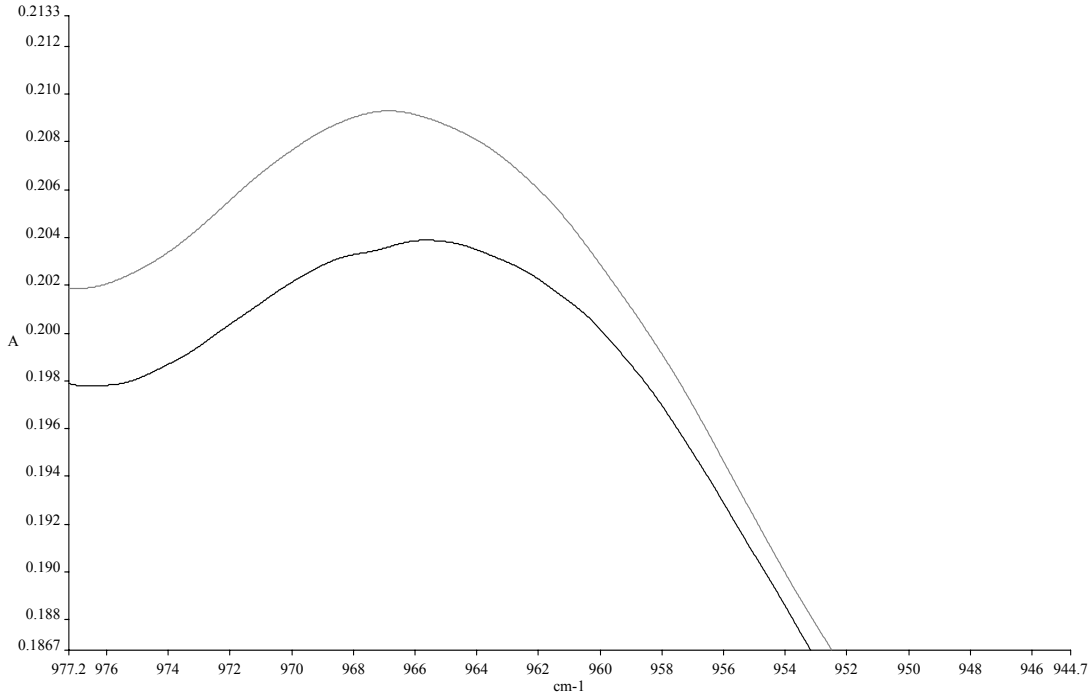

Figure 7.19. Changes in FT-IR spectra between 977 and 952 cm^{-1} for Altınoluk olive oil (—1st month, — 14th month)

7.1.10. Phenol Composition of EVOO Samples

The amount of phenolic compounds in olive oil is an important factor when evaluating the quality of olive oil because natural phenols contribute to its resistance to oxidation especially during storage period and improve its taste. These compounds correlate with the shelf life of oil and, in particular, its resistance to oxidation.

The common phenolic compounds, which were identified in this study, were hydroxytyrosol, tyrosol, chlorogenic acid, caffeic acid, vanillic acid, vanilin, p-coumaric acid, ferulic acid, m-coumaric acid, cinnamic acid, luteolin, and apigenin. The common phenolic compounds identified in this study; such as hydroxytyrosol, tyrosol, vanillic acid, p-coumaric acid, cinnamic acid, luteolin, and apigenin were also determined previously in Turkish extra virgin olive oils (Ocakoglu, et al., 2008). Several phenolic compounds, such as 4-hydroxybenzoic acid, 4-hydroxyphenylacetic and syringic acid were not present in oil samples. Cinnamic acid was found in high amount in all oils in forth month of storage time. This phenolic acid was identified and quantified in high levels in olive oils previously by Montedoro, et al. (1992). Altınoluk and Ezine olive oil samples separate from Bayındır and Ortaklar olive oils due to their chlorogenic acid content. The amount of chlorogenic acid in Altınoluk and Ezine olive oils ranges between 0.02 to 0.007 mgGA/kg oil. Phenolic compounds concentrations from 4^{th} month to 14^{th} month storage are presented in Appendix-A.

For identification of some of the secoiridoids and lignans qualitatively, which appeared between 37^{th} and 62^{th} minutes of HPLC analysis, study of Brenes and friends (2000) was used. The peaks between 37–39 minutes (undefined 1) can be qualitified as dialdehydic forms of elenolic acid and lignans (1-acetoxypinoresinol and pinoresinol. The unidentified peak 2, which existed between 46 and 49 and before cinnamic acid might be considered as oleuropein and oleuropein aglycon. Lastly, the unidentified peak 3 between luteolin and apigenin (between 59-62 minutes) might be described as ligstroside aglycon. As a sample, chromatograms of Ortaklar olive oil stored at room and refrigerator temperatures were given in Appendix A. The changes (%) in phenolic compounds between 4^{th} month and 14^{th} month storage are given in Table 7.1.

Calculation of change (%) in concentrations of phenolic compounds:

$$\Delta C(\%) = \left[\left(C_{14} - C_4 \right) / C_4 \right] * 100 \qquad (7.1)$$

ΔC: change in concentration (%)

C_{14}: concentration of phenolic compound in 14^{th} month (mg GA/kg oil)

C_4: concentration of phenolic compound in 4^{th} month (mg GA/kg oil)

Table 7.1. Changes (%) in phenolic compounds during 14-month storage

	olive oil samples							
phenolic compounds	aro	ero	bro	oro	are	ere	bre	ore
Hydroxytyrosol	-75.42	-44.34	-20.86	93.63	-52.57	-24.69	-15.74	23.03
Chlorogenic acid	-58.88	-79.76	0.00	0.00	71.53	28.33	0.00	0.00
Tyrosol	66.16	-19.47	0.74	12.61	52.00	-48.37	22.99	12.21
4- Hydroxybenzoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Hydroxyphenylacetic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caffeic acid	-86.79	-71.54	-35.53	-49.31	-25.72	-52.95	-48.35	-81.19
Vanilic acid	-66.62	-4.09	-33.74	-28.97	-23.59	-49.92	-41.67	-90.89
Syringic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vanilin	-66.37	-58.46	-69.08	-43.64	-76.16	-75.19	-17.69	-15.16
P-coumaric acid	-71.99	-56.49	-35.89	-8.00	-43.21	-70.95	-7.79	-48.86
Ferulic acid	-59.51	-91.77	-77.17	-93.74	-2.33	-55.75	-65.87	28.29
M-coumaric acid	-71.72	-64.84	-47.94	-25.92	-49.24	-37.57	29.41	-47.68
Undefined 1	-83.47	79.18	63.60	-63.16	-0.92	58.26	49.16	-24.46
Undefined 2	-69.05	-29.46	-51.90	-92.29	-29.95	-41.23	-215.0	-33.67
Cinamic acid	-14.25	-44.85	-59.14	-18.41	-65.74	-80.50	-28.62	-6.96
Luteolin	-87.15	-90.65	-69.30	36.31	-15.65	-57.42	-60.04	-48.96
Undefined 3	-64.16	-21.11	-70.62	-39.30	-55.67	-48.81	-29.31	-57.03
Apigenin	-93.19	-30.75	-77.84	-52.87	-8.18	-40.75	-10.05	-59.39

For Altınoluk olive oil sample stored at room temperature hydroxytyrosol, tyrosol, p-coumaric acid and m-coumaric acid had the highest concentrations among the others in 4th month. During storage the amount of p-coumaric and m-coumaric acids decreased slightly, whereas the concentration of hydroxytyrosol and tyrosol increased during storage time. The amount of cinnamic acid increased after 4 months and then decrease, while the apigenin concentration decreased sharply after 7 months of storage time.

Altınoluk olive oil stored at refrigerator temperature was rich in amount of the phenolics of vanilin, p- and m-coumaric acids, cinnamic acid and luteolin, initially. The amount of hydroxytyrosol and tyrosol increased as observed in Altınoluk olive oil stored at room temperature. Moreover, a decrease was observed in the concentrations of p- and m-coumaric acids, ferulic acid and vanilin. The concentrations of cinnamic acid, luteolin and apigenin decreased after 4 months storage, but they began to increase in 7th month.

The high amount of phenolics in Ezine olive oil stored at room temperature were hydroxytyrosol, p-coumaric and m-coumaric acids and cinnamic acid with the related concentrations 2.83, 3.10, 2.52 and 2.33, respectively. The amount of tyrosol in this sample had no a steady increase during all storage period as occured in other samples. After 7 months a decrease was observed in the concentration of tyrosol. All phenolic compounds had their lowest values at the end of the storage period.

Ezine olive oil stored at refrigerator temperature also had hydroxytyrosol, p-coumaric and m-coumaric acids, cinnamic acid and vanilin in high amounts, initially as Ezine olive oil stored at room temperature had. Unlike the Ezine oil stored at room temperature, high amount of vanilin was observed in refrigerated Ezine oil during storage. The concentrations of vanilic acid, ferulic acid, m-coumaric acid, cinnamic acid and luteolin remained constant first 7 months, but after 7^{th} month of storage period a decrease was occured in their concentrations.

The major phenolics were detected in Bayındır olive oil stored at room temperature were, tyrosol, p-coumaric and m-coumaric acids and cinnamic acid. The amounts of the hydroxytyrosol and tyrosol increased while the amounts of p-coumaric, cinnamic acid and luteolin decreased during all storage period.

For Bayındır olive oil stored at refrigerator temperature tyrosol, p-coumaric and m-coumaric acids and cinnamic acid had the highest initial concentrations. The amount of tyrosol, had an increase during storage time. The other phenolic compounds detected in this sample decreased after 4 months of storage but began to increase after 7 months of storage period, except hydroxytyrosol, luteolin and apigenin.

In Ortaklar olive oil stored at room temperature, hydroxytyrosol, tyrosol, vanilin, p-coumaric and m-coumaric acids, cinnamic acid were the major phenolics. During storage period, hydroxytyrosol and tyrosol values increase, and varied 1.51-3.04 and 2.04-4.44, respectively. The other phenolics in this sample, declined after 4 month of storage.

Ortaklar olive oil, stored at refrigerator temperature had the common major phenolic compounds. Hydroxytyrosol and tyrosol phenolics of Ortaklar olive oil stored at refrigerator temperature increased until 7^{th} month then began to decrease till the end of the storage period. The identical behavior was also observed for all other phenolic compounds in this sample.

Morello, et al. (2003) determined noticeable secoiridoid derivatives of Spanish olive oil samples, while lignans remained stable after 12 months storage. Beside, the

amount of vanilic acid and vanilin did not change during storage period due to their low initial concentrations in the same study. Cinquanta, et al. (1997) reported an increase in the content of hydroxytyrosol and tyrosol during 18-month storage, and they attributed this phenomenon to the hydrolysis of complex phenols which resulted in the formation of hydroxytyrosol and tyrosol. Additionally, Gomez-Alonso, et al. (2007) evaluated the changes in hydroxytyrosol and tyrosol concentrations of Cornicabra virgin olive oil samples during 21-month storage time. The hyroxytyrosol and tyrosol concentrations increased linearly in particularly all oil samples confirming that their secoiridoid derivates undergo partial non-oxidative hydrolysis.

7.2. Monitoring of Erkence (Erk) and Ayvalık-Edremit (AE) EVOO for 12 months

In this section, 12-month storage results for oils of Erkence (Erk) and Ayvalık-Edremit (AE) olives, harvested in 2006 are presented. The changes in PV, K232, K270, FFA, TPC, color coordinates and fatty acid profiles of EVOO samples were tabulated during 12 months in Appendix A.

Peroxide value (PV): PV is a measure of primary oxidation. The initial PV of AE was in the legal limit (PV < 20 meq/kg) and it also remained in this limit during storage period. Therefore, AE olive oil showed a low initial oxidation status according to PV. The initial PV of Erk was 23.57 meq/kg, exceeded the limit of 20 meq/kg. The PVs of AE oil showed more stable profile aganist to oxidation than Erk olive oil during storage (Figure 7.20). The PVs of Erk and AE remained almost constant in the first 10 months of storage. An increase in PVs appeared for both of the samples in the 12^{th} month of storage time. In the study of Ocakoglu et al. (2008), the initial PV of AE olive oil was reported as 10.64 meq/kg, which was similar to our result. The PVs of twenty olive oil samples, obtained from Lianolia variety olives in Greece stored both in light and dark conditions, reached to the maximum limit on the 7^{th} month and also they exceeded the limit value after storage of 12 months (PVs ranged 25.8-36.00 meq/kg (Okogeri and Tasioula-Margari, 2002).

In order to determine whether significant difference occurred between the oil samples among the storage months, two-way ANOVA was applied to the data. According to this statistical analysis, a significant difference was reported (p-value < 0.05).

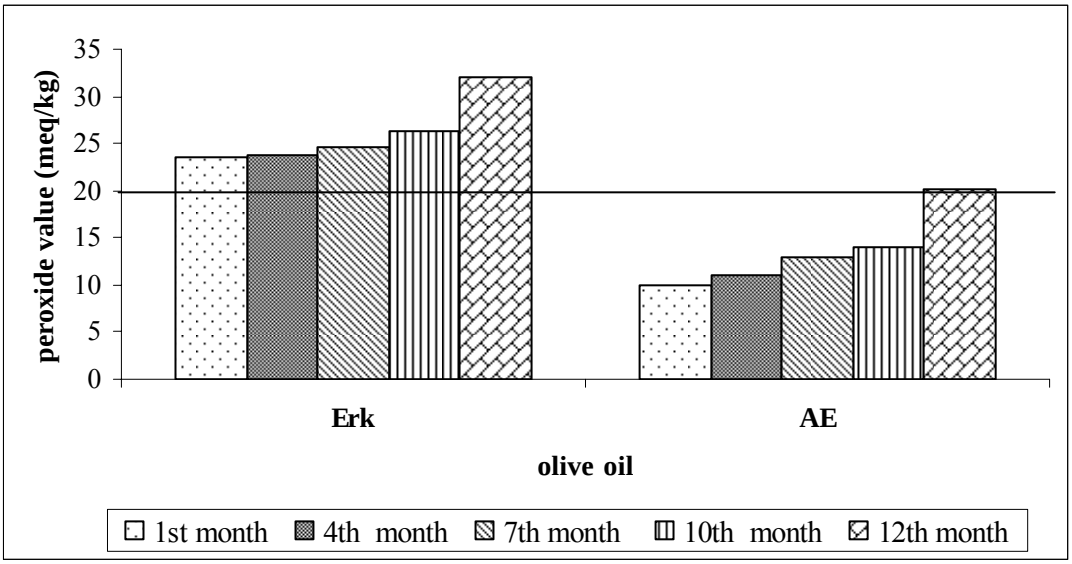

Figure 7.20. Changes in PVs of Erk and AE oils during 12-month storage period at 8°C

K232 and K270 Values: The maximum permitted value of K232 for EVOO is 2.5. The initial absorbance values at 232 nm of the oil samples were below limit of 2.5. The K232 values of both oils maintained under the limit of 2.5 until the 12th month of the storage. Erk and AE olive oils exceeded the upper legal limit in the 12th month. Figure 7.21 refers the changes in K232 values of the oils during 12-month storage. Capino, et al. (2005) analyzed the EVOOs from Corantina cultivars quality parameters during 12-month storage. With respect to the results of this search the K232 values showed significantly higher values and reached to the maximum limit at the end of the storage time. In another survey, it was reported dark-stored samples were ranged 1.8 to 3.3 in 6th month and 2.3 to 4.7 in 12th month of storage time (Okogeri and Tasioula-Margari, 2002).

Significant differences were observed among the olive oil samples and storage months at 5% level.

The upper limit of K270 value, which is known as a secondary oxidation product marker, is less than 0.20. The initial K270 values of both Erk and AE oils were observed within the limit. An increase was observed in K270 values of both oil samples. Increase in K270 values of AE oil was more than Erk oil (Figure 7.22).

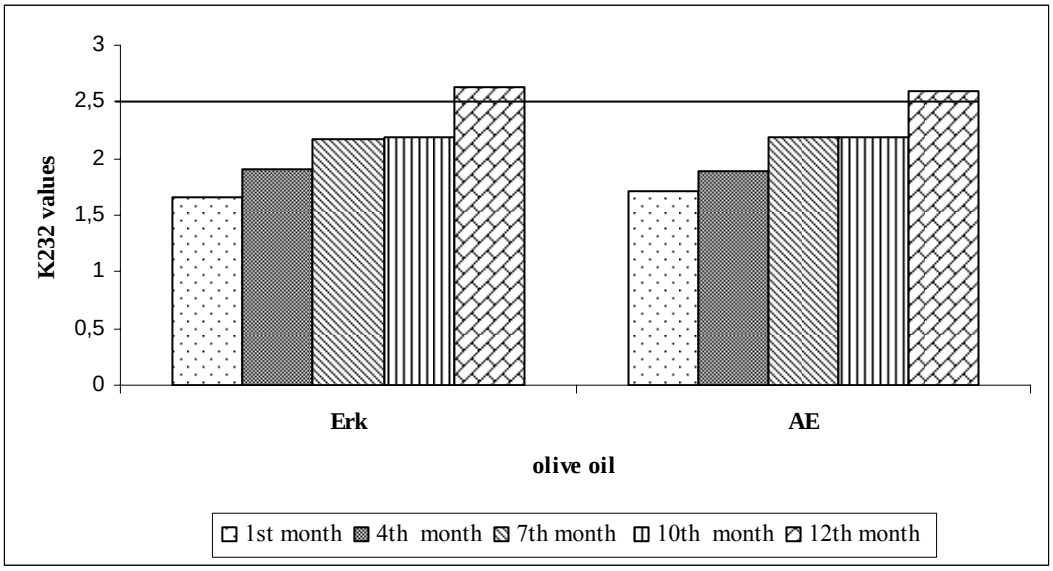

Figure 7.21. Changes in K232 values of Erk and AE oils during 12-month storage period at 8°C

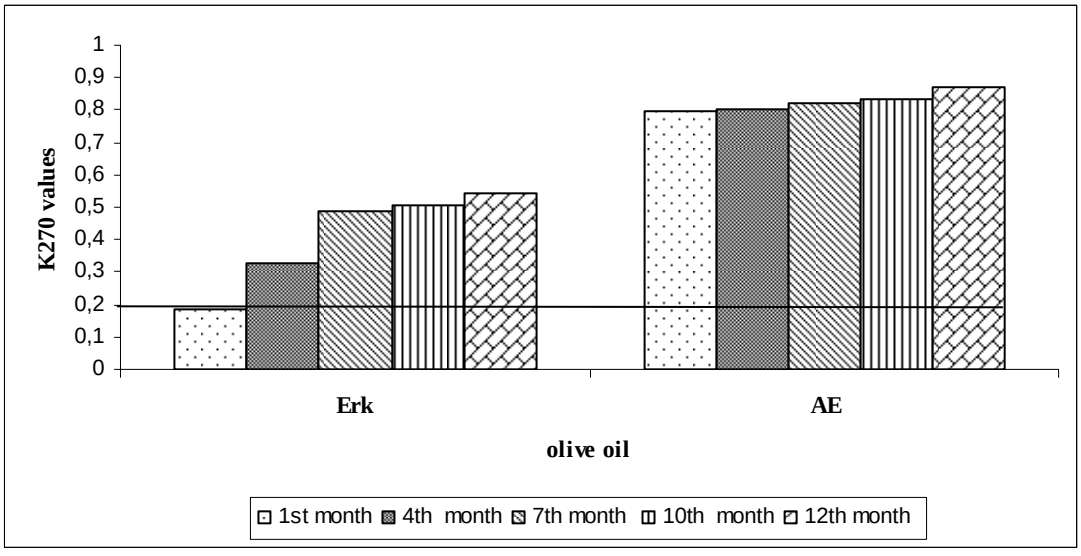

Figure 7.22. Changes in K270 values of Erk and AE oils during 12-month storage period at 8°C

Sinelli and friends (2007) exhibited the changes in K270 values of the olive oil samples from Garda region in dark one year and two years. The initial K270 values of the analyzed oil samples range from 0.05 to 0.24; but after storage of oils for 1 and 2 years, these ranges were found 0.1-0.54 and 0.16-0.6, respectively.

Significant differences were obtained between oils and storage months, statistically (p-value = 0.041 < 0.05).

Free Fatty Acid (FFA): FFA is also one of the most important factors in order to determine olive oil quality. The established acidity value of EVOO by EU regulation is less than 0.8%. The initial acidity of Erk and AE were 0.18 and 0.79, respectively. The initial acidity value of AE oil was higher than Erk olive oil (Figure 7.23).

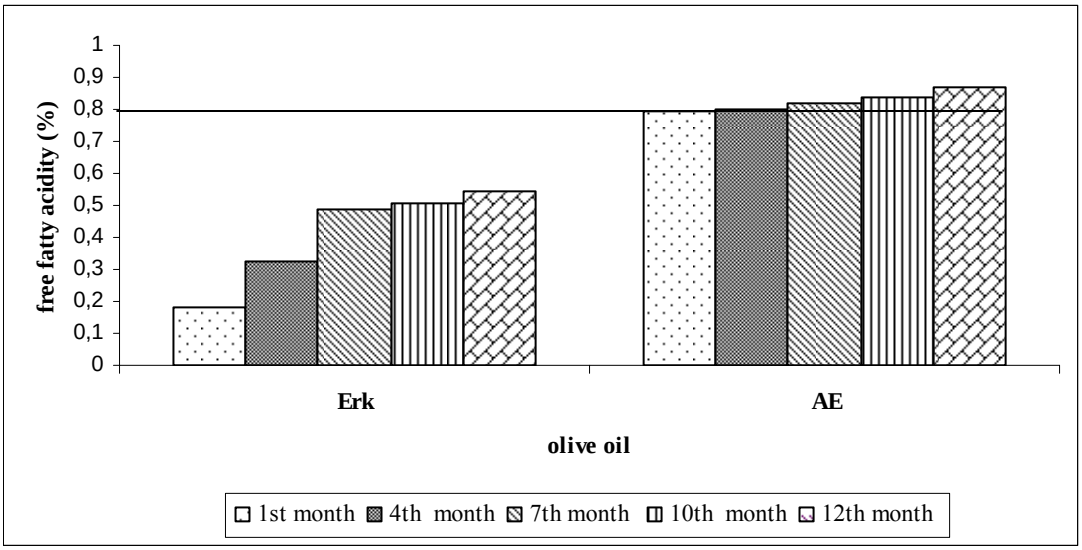

Figure 7.23. Changes in FFA values of Erk and AE oils during 12-month storage period at 8°C

During the storage, an increase in FFA was observed in two oil samples till the end of the storage. This increase in acidity is due to the free fatty acid hydrolysis. Oils of both cultivars maintained their extra quality (< 1%) at the end of storage time.

Total Phenol Content (TPC): TPC of Erk oil was higher than the TPC of AE oil. The initial TPC of Erk oil was 178.79 whereas the TPC value of AE oil was 61.06. Total phenol contents of olive oils decreased during storage (Figure 7.24). This reduction of the total phenol content of oils during storage is a result of the

decomposition processes that occur in the oxidation activities. Capino, et al. (2005) expressed the decrease in TPC values of Corantina cultivar olive oils during 12-month storage in darkness. The sharp decrease in TPC values were observed after storage of 8 months.

The changes among the olive oils during the storage period were not found significant, statistically ($p < 0.05$).

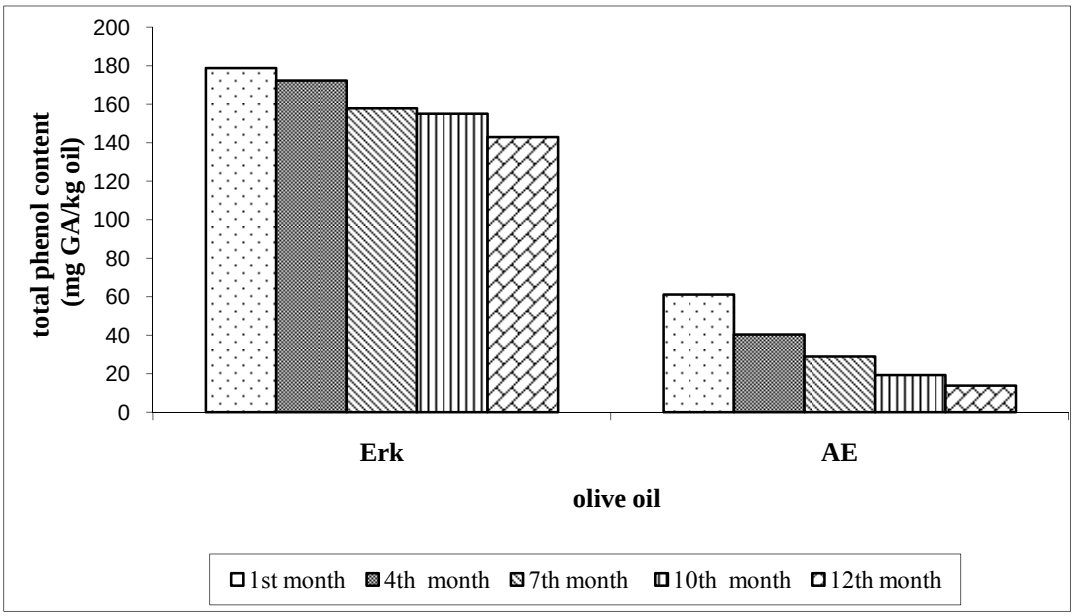

Figure 7.24. Changes in TPC values of Erk and AE oils during 12-month storage period at 8°C

Color: The initial luminosity values (L*) of Erk and AE oils were 23.58 and 24.88, respectively. L* value decreased in 4th and 7th month for Erk oil and a decrease was also observed in 4th month for AE oil. This value increased after the 10 month storage time probably as a consequence of the reduction on the pigment content.

AE oil showed higher negative initial a* value than Erk oil. Nevertheless, initial b* value, which corresponds to yellow zone, of Erk oil showed similarity to AE oil. Figures 7.25, 7.26 and 7.27 refer to the changes in color coordinates during 12-month storage period of EVOO samples. Significance differences between L*, a* and b* values of two types of EVOO samples were found at 5% level among the storage months.

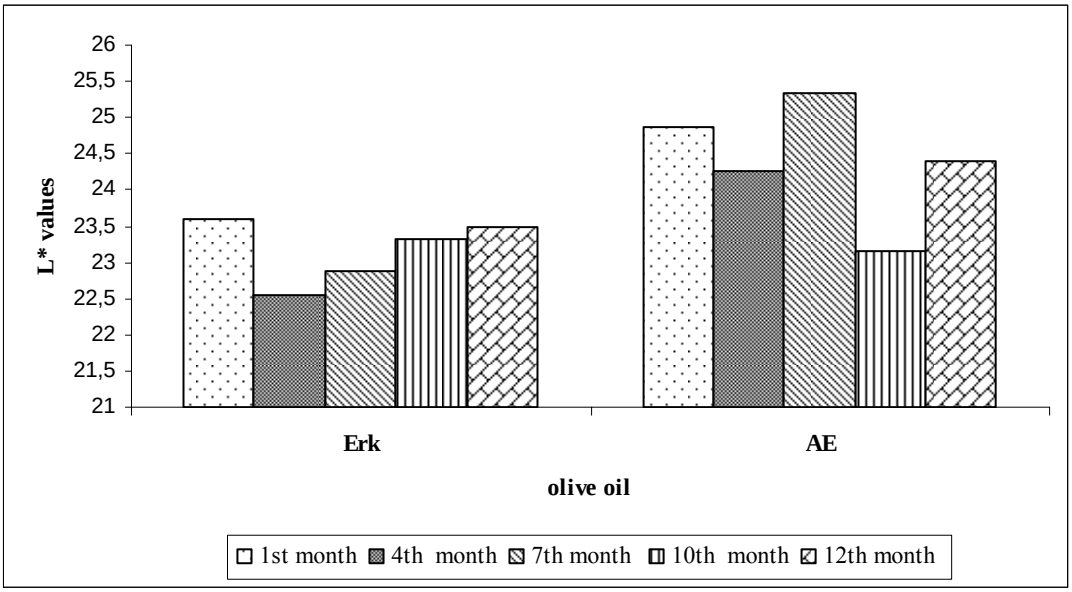

Figure 7.25. Changes in L* values of Erk and AE oils during 12-month storage period at 8°C

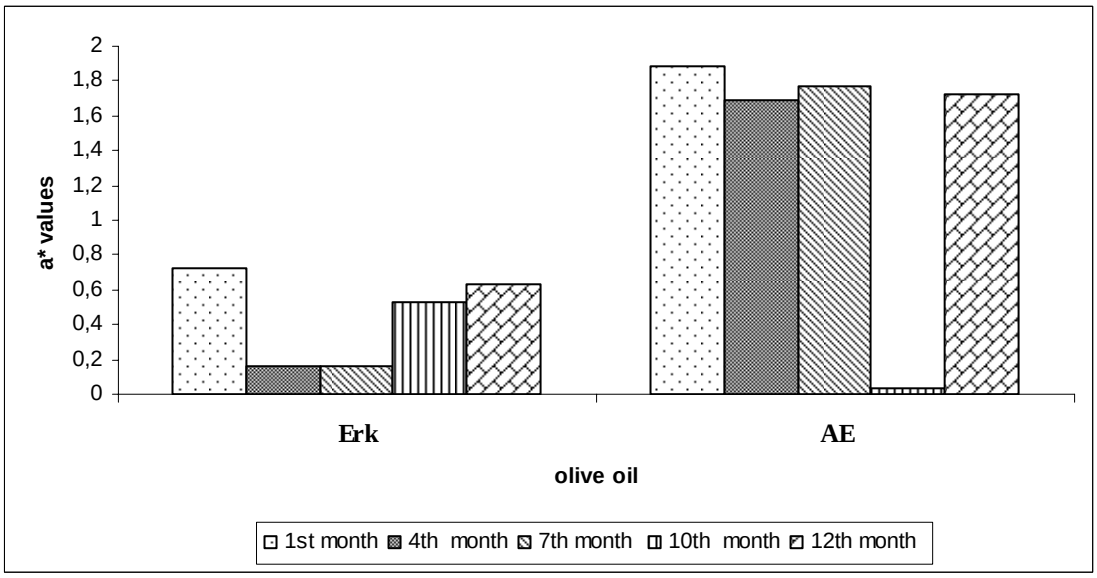

Figure 7.26. Changes in a* values of Erk and AE oils during 12-month storage period at 8°C

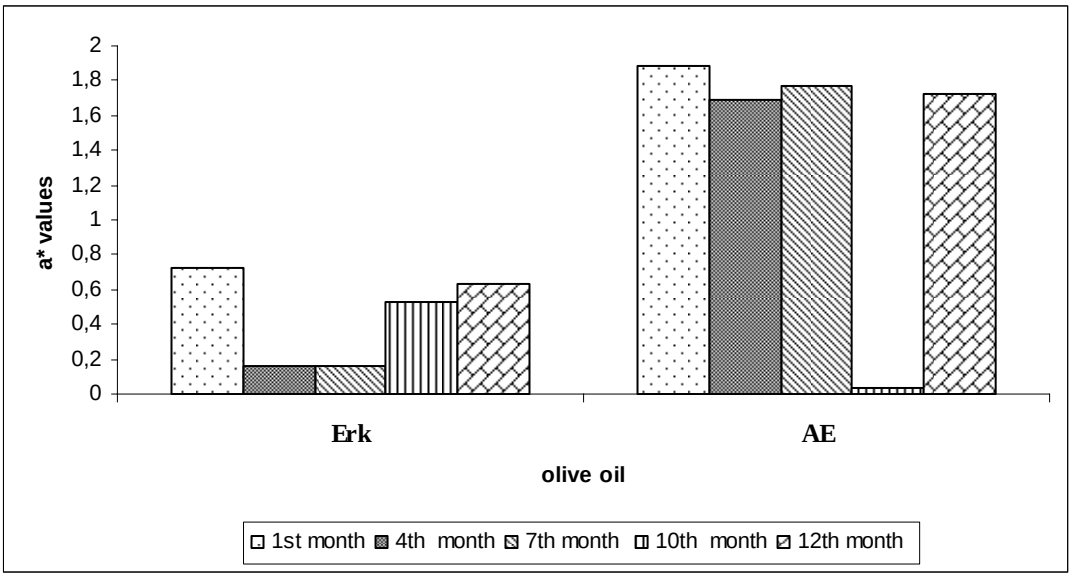

Figure 7.27. Changes in b* values of Erk and AE oils during 12-month storage period at 8°C

Fatty Acid Profiles: In this section, results for oleic, linoleic and linolenic acids were presented. The most abundant fatty acid in olive oil is oleic acid. The initial oleic acid content of Erk olive oil sample (68.10 %) was higher than that of AE olive oil (67.40%). During storage period, an increase was observed in the oleic acid contents of both oil samples (Figure 7.28).

The initial linoleic acid content of Erk oil was also higher than the AE oil. A decrease was observed in linoleic acid content of Erk oil while the decrease in AE oil was not regular like Erk oil (Figure 7.29). In addition, the initial linolenic acid amounts of Erk and AE oil samples was found 0.73 and 0.65, respectively. The linolenic acid contents of both oil samples decreased during 12-month storage time (Figure 7.30).

However, the other fatty acid contents of Erk and AE olive oil samples decreased during 12-month storage time.

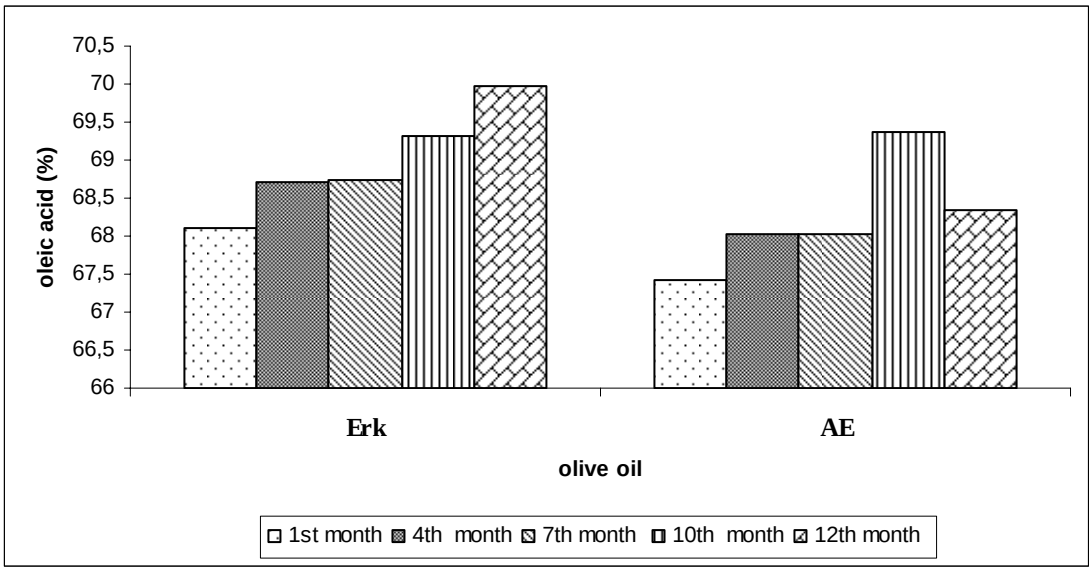

Figure 7.28. Changes in oleic acid contents of Erk and AE oils during 12-month storage period at 8°C

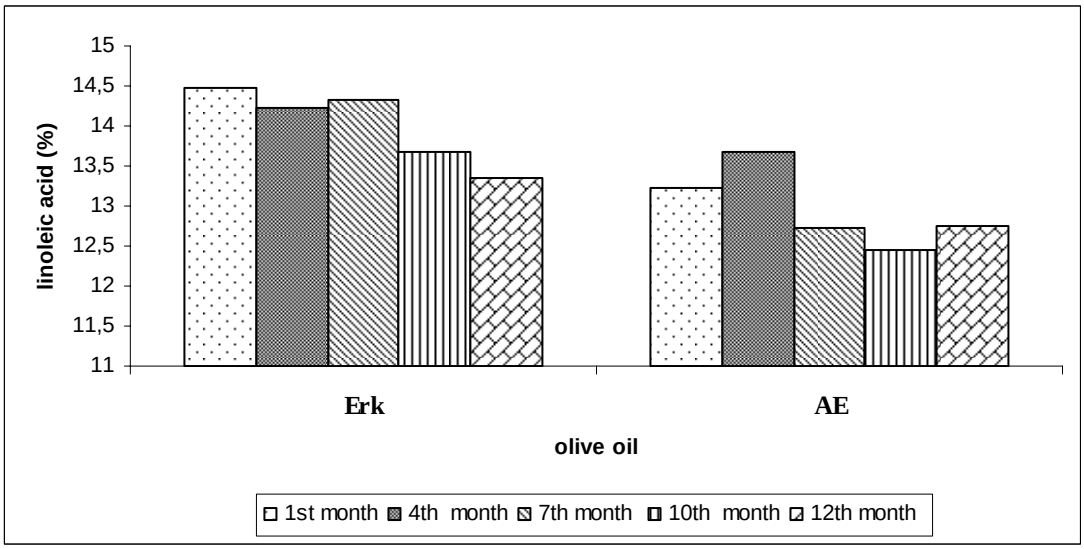

Figure 7.29. Changes in linoleic acid contents of Erk and AE oils during 12-month storage period at 8°C

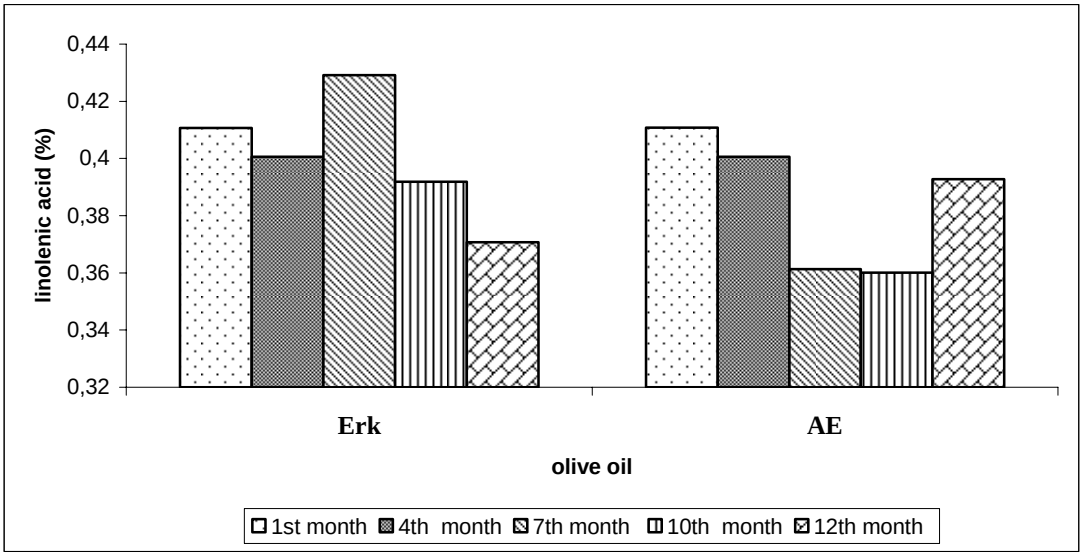

Figure 7.30. Changes in linolenic acid contents of Erk and AE oils during 12-month storage period at 8°C

7.3. Monitoring Accelerated Oxidation of North and South Aegean EVOO during 19 Days

In this section, the results of the olive oil samples exposed to accelerated oxidation for 19 days are given. The tables showed the changes in PV, K232 and K270 values of EVOO samples during 19 days were presented in Appendix A.

7.3.1. Peroxide Value (PV)

During autoxidation, the peroxide value of oil reaches a maximum followed by a decrease at more advanced stages varying according to the fatty acid composition of the oil and the conditions of oxidation. Thus, measurement of PV is an indicator of the initial oxidation.

The initial PVs of both South (S) and North (N) Aegean EVOO samples were below the upper limit value, which is 20 meq/kg. The initial peroxide concentration of northern olive oil samples was lower than those from south Aegean oil samples.

Otherwise, PVs of both oil samples exceed the related legal limit when the first day of exposed to oxidation period.

PVs increased considerably when compared the initial values, then began to decline and this decline in PV kept on until 9th day of oxidation. After 10th day of oxidation process the related PVs of both samples increased at the end of oxidation period. However, the increase took place in southern Aegean oil samples was more than northern Aegean oil samples, so this pointed out the stability of olive oil obtained from north region of Aegean is more stable against to oxidation depending on PV results (Figure 7.31).

A significance difference between PVs of two types of EVOO samples was found at 5% level.

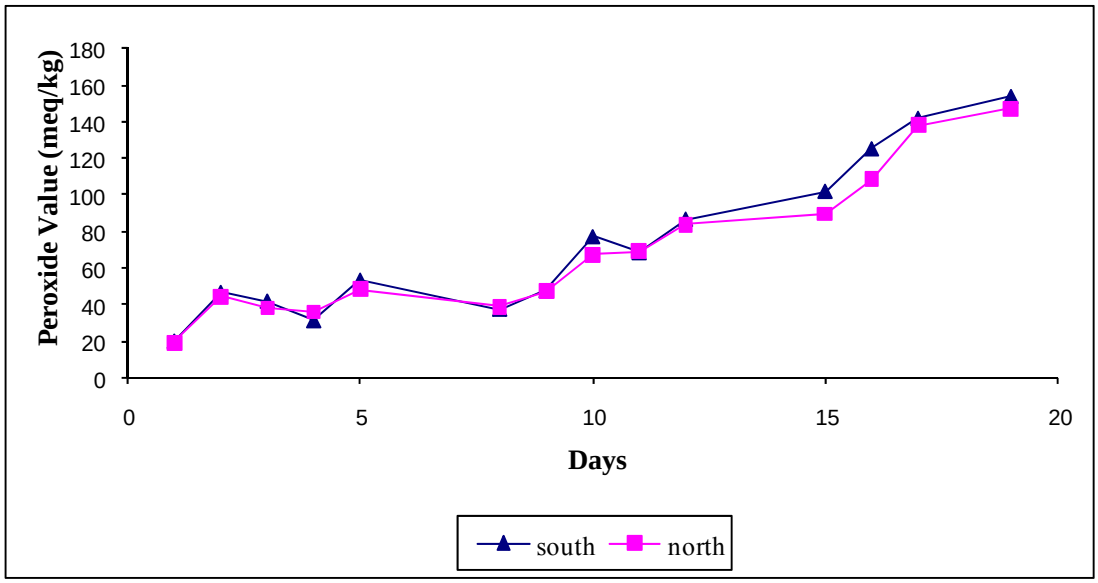

Figure 7.31. Changes in PVs of North and South Aegean EVOOs during 19 day accelerated oxidation

7.3.2. K232 and K270 Values

Specific extinction coefficients express the oxidation stage of olive oil. The absorption value at 232 nm in the ultra violet region refers to the conjugated diene and the absorption at 270 nm refers to the conjugated triene concentrations in olive oil due

to oxidation process. The higher concentration of conjugated dienes and trienes induce greater amounts of K232 and K270.

The initial K232 values of the oil samples were less than the limit of 2.5 and remained within this limit during one week of oxidation process. In the consequent days of oxidation period the K232 values exceeded this upper limit (Figure 7.32).

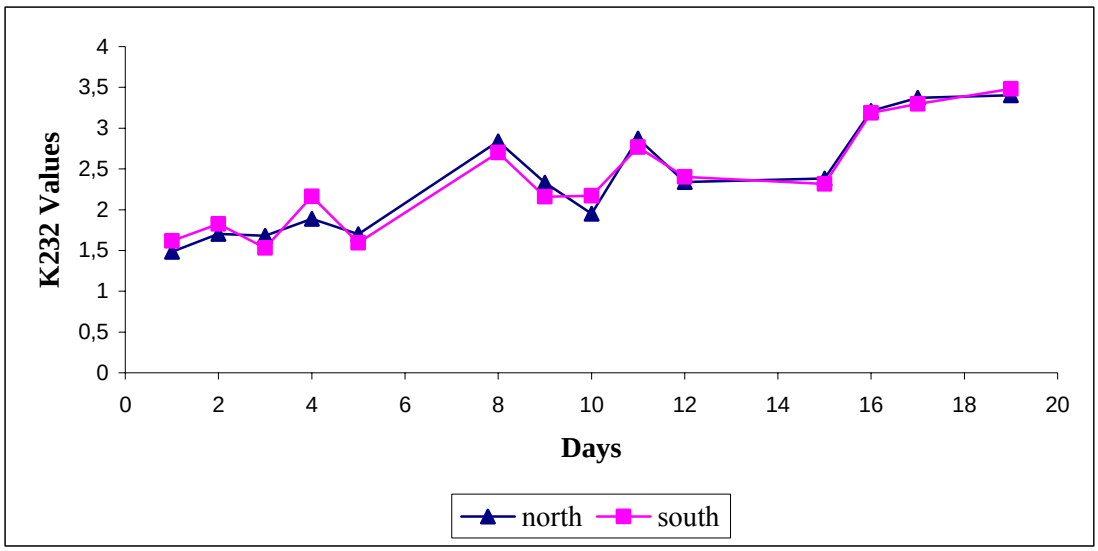

Figure 7.32. Changes in K232 values of North and South Aegean EVOOs during 19 day accelerated oxidation

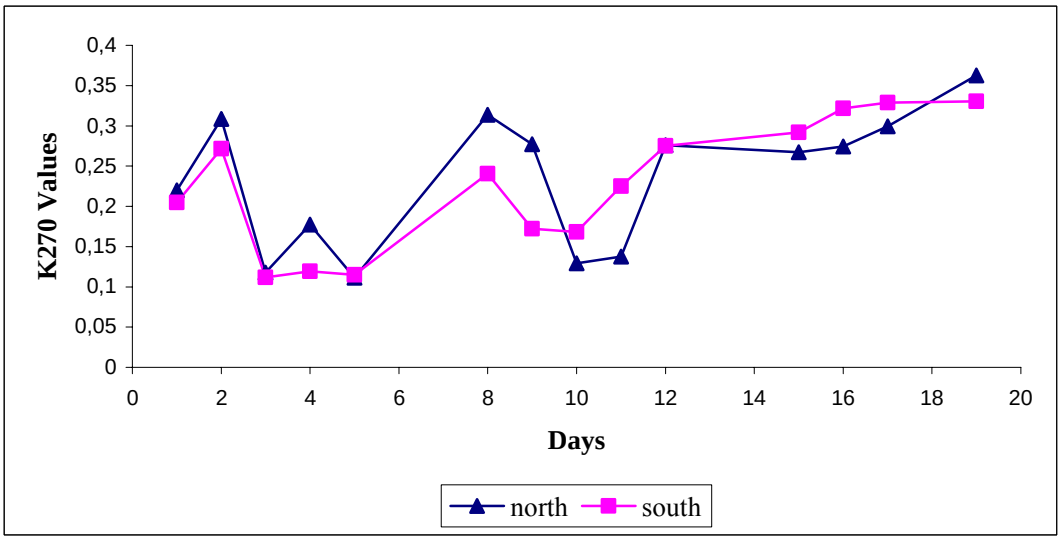

Figure 7.33. Changes in K270 values of North and South Aegean EVOOs during 19 day accelerated oxidation

K232 values of north and south Aegean olive oil samples were found significantly different for each day ($p < 0.05$).

The initial K270 values of oil samples were also within the limit of K270 ≤ 0.20, which was established by EU and Turkish Food Codex. However, this limit value was exceeded in the 2nd day of oxidation period for both north and south Aegean EVOO samples. Between the 2nd and 8th days of oxidation, the values varied 0.11 to 0.17 for the sample of north Aegean EVOO, additionally in these days stability was observed in K270 values of south Aegean EVOO. After the 8th day, an increase was observed in K270 values to end of the oxidation period (Figure 7.33).

The significance of differences at a 5% level among the K270 values during oxidation was detected.

7.3.3. Determination of Differences in FT-IR Spectra During Oxidation

The differences in the FT-IR spectra, due to oxidation are presented in figures 7.34, 7.35 and 7.36 for the 1st and 19th days.

In the region of wave numbers 2855-2851 cm^{-1}, stretching vibration of the aliphatic CH_2 functional groups form, and then absorbance declines and a narrowing is observed in bands. Guillen and Cabo (2000) reported on the significant changes in the spectra of FT-IR on different edible oils under oxidative conditions of 70°C temperature. Apart from these changes, they also emphasized the changes occurred in bands of 3600-3100 cm^{-1}, which depicts the presence of hydroperoxides during oxidation and a sharp decrease in frequency of band near 3006 cm^{-1}, which represents the disappearance of cis double bands, and the band at 988 cm^{-1}, was characterized as the indicator of presence of trans- and cis, trans conjugated olefinic double bonds. Any changes in these regions were not observed in this study.

Another important change in the spectra of oxidized olive oils formed among the 1743-1746 cm^{-1}. A slight decrease in absorbance value of the band near 1746 cm^{-1} was detected. This region corresponds to carbonylic compounds. Lower absorbance values indicate the more advanced oxidation process.

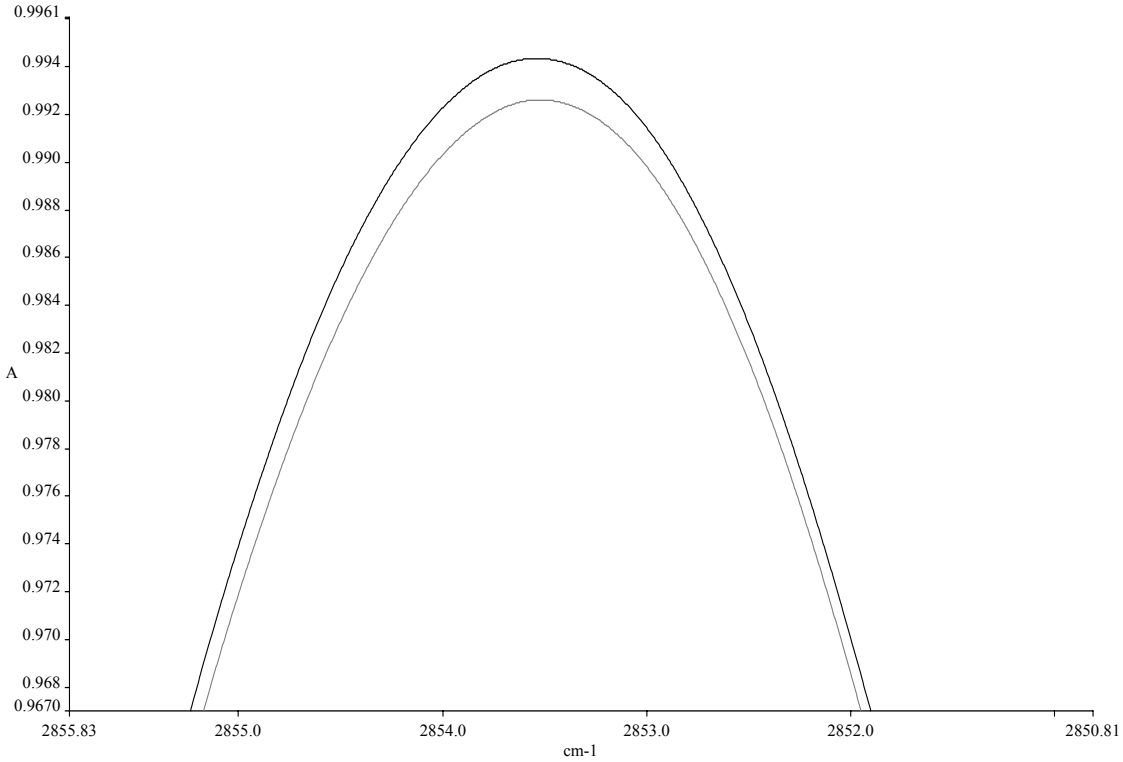

Figure 7.34. Changes in FT-IR spectra between 2855-2852 cm^{-1} (—1^{st} day, — 19^{th} day)

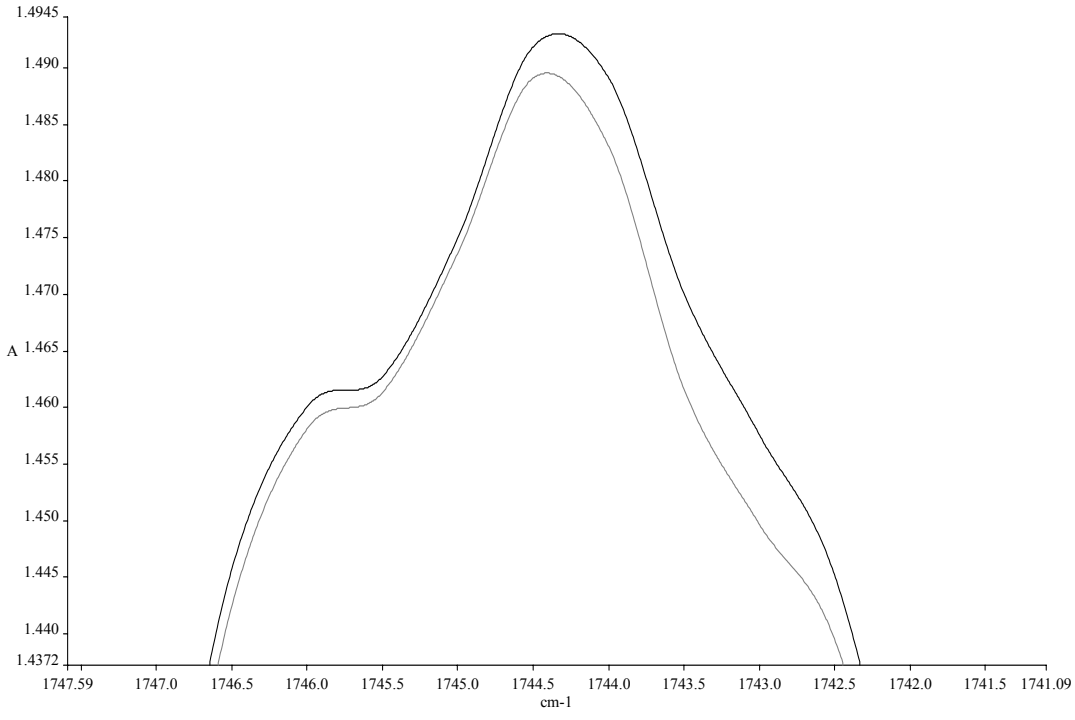

Figure 7.35. Changes in FT-IR spectra between 1746-1742 cm^{-1} (—1^{st} day, — 19^{th} day)

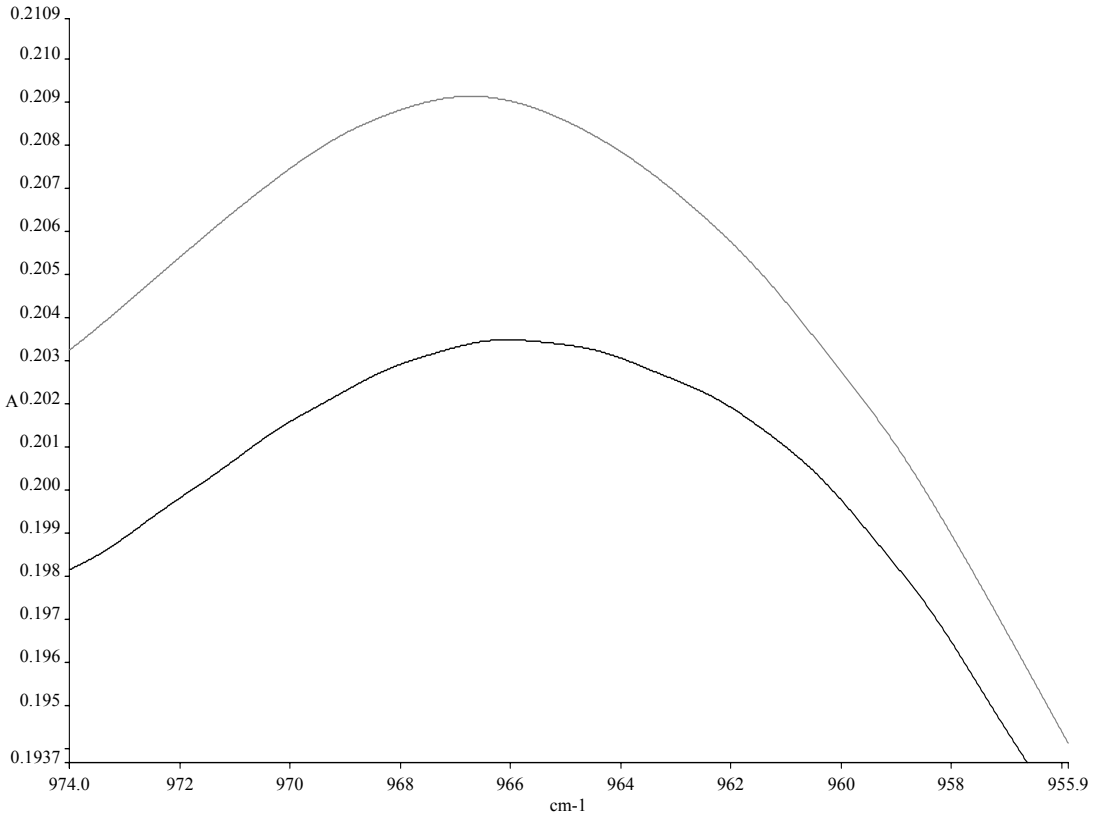

Figure 7.36. Changes in FT-IR spectra between 974-956 cm^{-1} (—1st day, — 19th day)

The region of 974-950 cm^{-1}, where considerable change occurred during oxidation process, is the indicator of the possible presence of aldehydic or ketonic groups with isolated trans double bonds. In this stage an increase was seen in the absorbance values of the spectra.

7.4. Results of Flaxseed-Olive Oil Mixtures

The tables of the measurements were provided in Appendix-A for pure olive oil, pure flaxseed oil and flaxseed oil-olive oil mixtures (5%, 10%, 15%).

7.4.1. Peroxide Value (PV)

Pure olive oil sample had the highest initial PV in comparison to other oil samples. The initial PV of flaxseed oil also higher than the 5, 10, 15% flaxseed-olive oil mixtures. The initial PVs of the oil mixtures of 5, 10 and 15% were 2.81, 2.54 and 2.34, respectively. During storage period, the PVs of the samples, which contained flaxseed oil, ranged 2.34 to 6.73. As expected, oil samples stored at room temperature had higher PVs than the samples kept at refrigerator at the end of the storage, because of the negative effect of temperature to oxidation of oil. In this study some of the oil samples stored at room temperature had slightly lower final PVs at the end of the 126-day storage. According to the initial PVs and the other PVs measured during storage, the PVs of the oil mixture samples decreased with the increase in amount of olive oil addition to flaxseed oil. However, as stated in the previous sections, peroxides are the primary oxidation products and peroxide concentration may fluctuate over time since peroxides turn to other oxidation products in time.

During storage of the samples, increases in PVs of all oil samples were observed (Figure 7.37). In the figures suffix ‘ro’ and‘re’ in the sample names stand for the room and refrigerator conditions. ‘o’ is for pure olive oil, whereas ‘f’ is for pure flaxseed oil. 5, 10, 15 represent the percentages of olive oil in the flaxseed oil. The increase in PVs of the oil samples, stored at room temperature, was higher than the oil samples, which were kept at refrigerator.

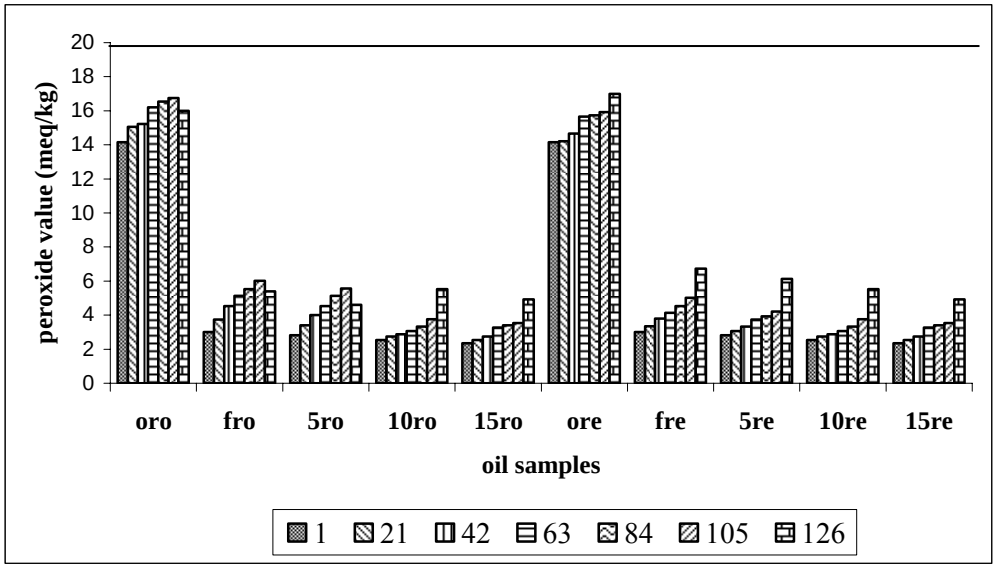

Figure 7.37. Changes in PVs of oil samples during 126-day storage period

7.4.2. K232 and K270 Values

Olive oil sample had the highest and 15% flaxseed-olive oil mixture sample had the lowest initial K232 values among all samples. As shown in Figure 7.38, the samples maintained their stability in terms of K232 values during storage time, especially the oil samples stored at refrigerator. It is clear that K232 values of pure flaxseed oil samples remained approximately constant, as in oil samples during 100 days. However, an increase was observed in pure oil samples as opposed to flaxseed and olive oil samples after 100 days. None of the samples exceeded the upper limit value for K232 ($K232 \leq 2.5$) during storage time.

The initial K270 values of oil samples were within the maximum limit and varied from 1.64 to 0.20. Flaxseed oil had the highest initial K270 value while 15% mixtured sample had the lowest one. Pure flaxseed oil exceeded the upper limit of K270 after 21-day storage (Figure 7.39). Moreover, olive oil sample, which was kept at room temperature, reached the limit value in 105th day of the storage.

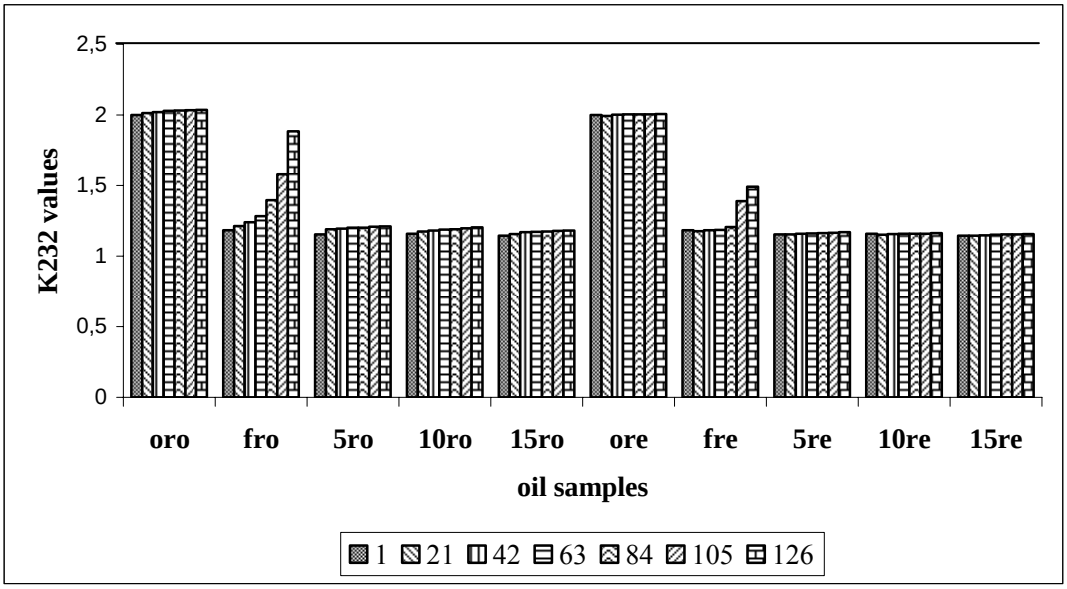

Figure 7.38. Changes in K232 values of oil samples during 126-day storage period

At room temperature, the K270 values of flaxseed oil showed an increase after 63-day storage time, whereas the changes in K270 values of this sample were not as high as flaxseed oil stored at room temperature.

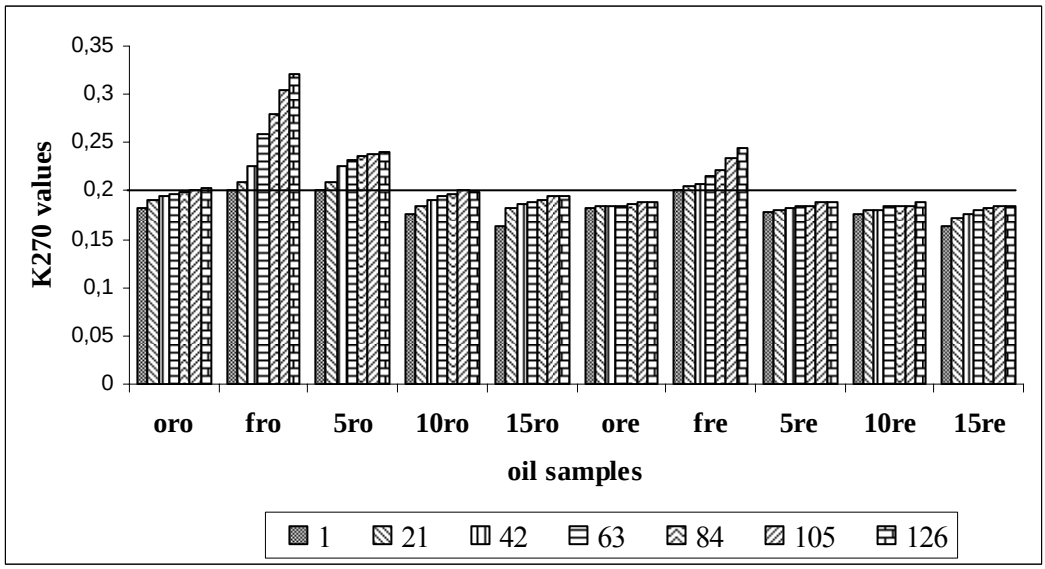

Figure 7.39. Changes in K270 values of oil samples during 126-day storage period

7.4.3. Total Phenol Content (TPC)

As given in Figure 7.40, the high content of total phenol belonged to olive oil sample. Flaxseed had the lowest initial TPC value among the oil samples. The olive oil addition contributes a small increase in TPCs of flaxseed-olive oil mixtures. TPC of the samples decreased during the storage regularly. The changes in TPCs of the oil samples, which were stored at room temperature, were higher than the oils stored at refrigerator during 126-day storage period.

Choo and co-workers (2007) studied the physicochemical and quality characteristics of seven cold-pressed flaxseed oils sold in New Zealand. They measured the total phenolic acids of these samples as ferulic acid equivalents at 725 nm. According to their results the total phenol acid content of the flaxseed oil samples ranged from 87.2 to 307.3 as ferulic acid equivalents (mg/100g).

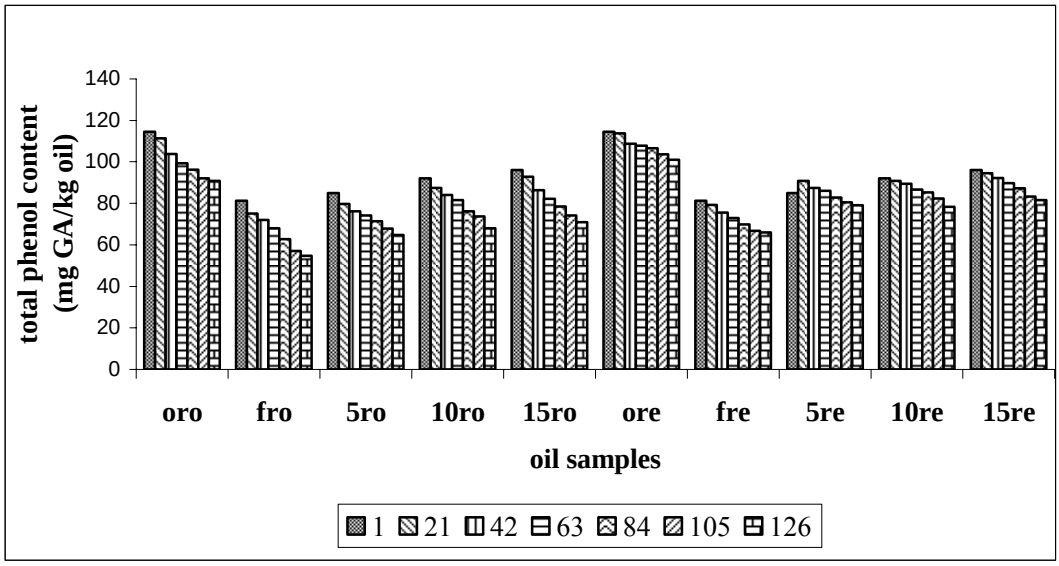

Figure 7.40. Changes in TPC values of oil samples during 126-day storage period

7.4.4. Fatty Acid Profiles

The initial oleic acid contents of pure olive oil and flaxseed oil samples were 70.42 and 17.94, respectively. Based on the high content of oleic acid stability of olive oil is about 6 times higher than that for linseed oil. By the addition of olive oil to flaxseed oil the initial oleic acid contents of the flaxseed-olive oil mixture samples became higher than the initial oleic acid content of pure flaxseed oil. Slight changes were observed in oleic acid contents of oil samples during 126-day storage (Figure 7.41). The increase was observed in oleic acids after 63 and 84 days of storage.

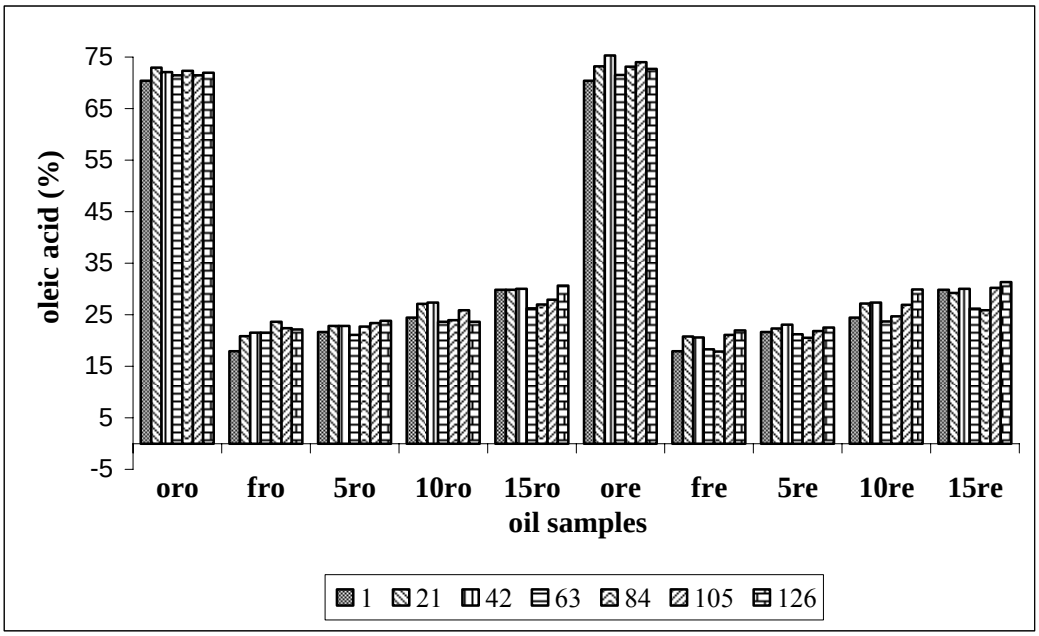

Figure 7.41. Changes in oleic acid contents of oil samples during 126-day storage period

Significant changes were observed in linoleic acid contents of oil samples until 105th day of the storage period, after 105 days linoleic acid contents of the all oil samples decreased (Figure 7.42).

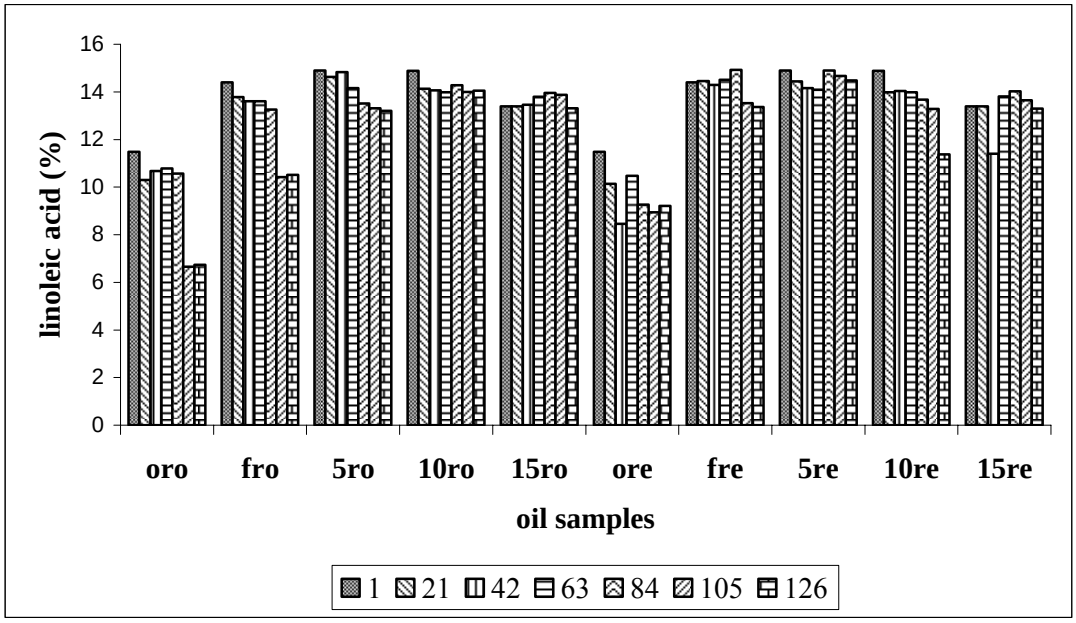

Figure 7.42. Changes in linoleic acid contents of oil samples during 126-day storage period

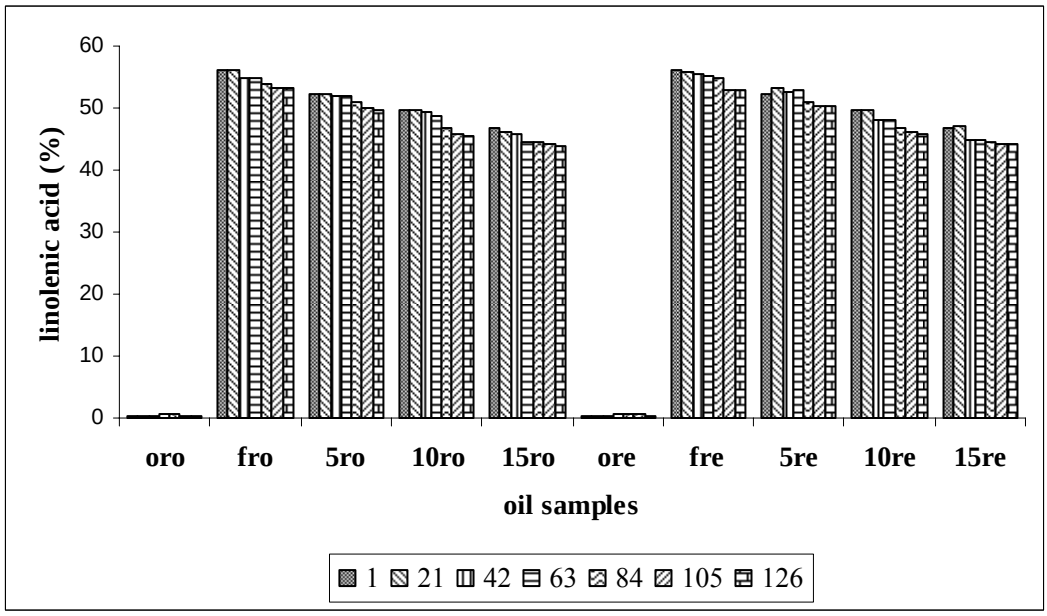

Figure 7.43. Changes in linolenic acid contents of oil samples during 126-day storage period

As shown in Figure 7.43, linolenic acid content of pure flaxseed oil and flaxseed-olive oil mixtures are higher than pure olive oil. The initial linolenic acid contents of pure flaxseed oil and pure olive oil were 54.88 and 0.36, respectively. The

reduction in linolenic acid content of pure olive oil was steady as expected, but also the linolenic acid contents of pure flaxseed oil and flaxseed-olive oil samples decreased continually during storage period.

7.4.5. Determination of Differences In FT-IR Spectra of Olive Oil and Flaxseed Oil

The overall appearance of the FT-IR spectra of olive oil and flaxseed oil are not similiar, they show differences both in exact absorbance values in presence and absence of some functional groups in their structure and different oxidative behavior (Figure 7.44).

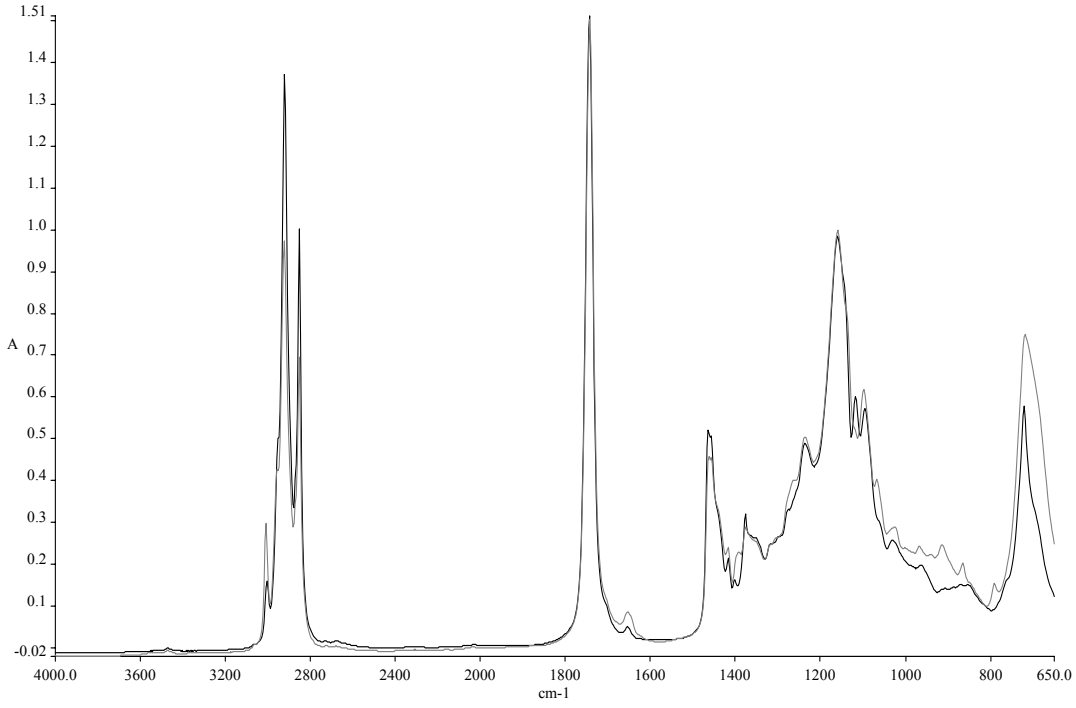

Figure 7.44. The spectra of olive oil and flaxseed oil (— flaxseed oil, — olive oil)

The observed differences between these oil types around the regions of 3500-3440, 2924-2840, 1746-1743, 1654, 1463, 1377, 1255-1210, 1140-1110, 1000-800 and 721 cm^{-1}. Absorbance at 2924 and 2852 cm^{-1} are due to bands arising from CH_2 strecthing vibrations, asymmetric and symmetric, respectively. The large peak around 1743 cm^{-1} results from C=O double bond stretching vibration of carbonyl groups. Peaks of 1463-1377 cm^{-1} arise from CH_2 and CH_3 scissoring vibration. Fingerprint region lay between 1250-700 cm^{-1} which is due to stretching vibration of C-O ester group and CH_2 rocking vibration (Guillen and Cabo 1999, Vlachos, et al. 2006).

7.5. Relationship Between FT-IR and PV, UV Absorbance Values (K232 and K270) and TPC Values

The predictability of oxidation and quality parameters (PV, K232, K270 values and TPC) and fatty acid profiles by FT-IR spectra was studied by PLS technique. In order to check the correlation between these variables and FT-IR spectra, 13 Y-variables and 4603 X-variables (FT-IR spectral data) with 80 observations were used. Wavelet orthogonal signal correction (WOSC), which provides an increase in predictability and efficiency of PLS, was performed for each quality parameter prediction by FT-IR spectra. By the application of WOSC, number of X-variables was reduced to 128, so X-spectral data, which were not related with Y-variables were removed. Results of quality parameters and fatty acid predictions by FT-IR readings are presented in Table 7.2. Figures 7.45, 7.46, 7.47, 7.48, 7.49 and 7.50 show the PV, K232, TPC, stearic, oleic and linolenic acid values, respectively.

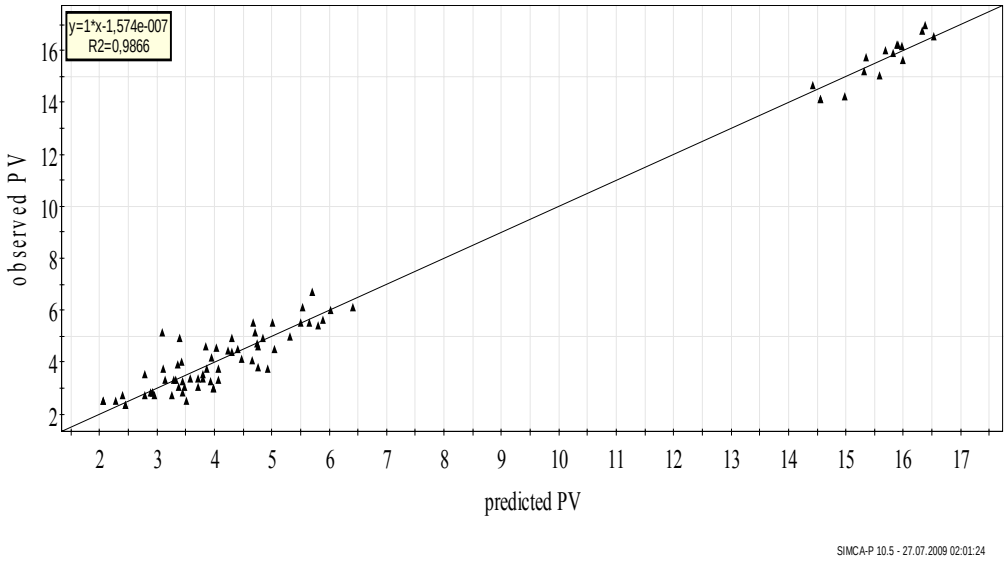

Figure 7.45. Results of PLS model: Predicted vs observed PV

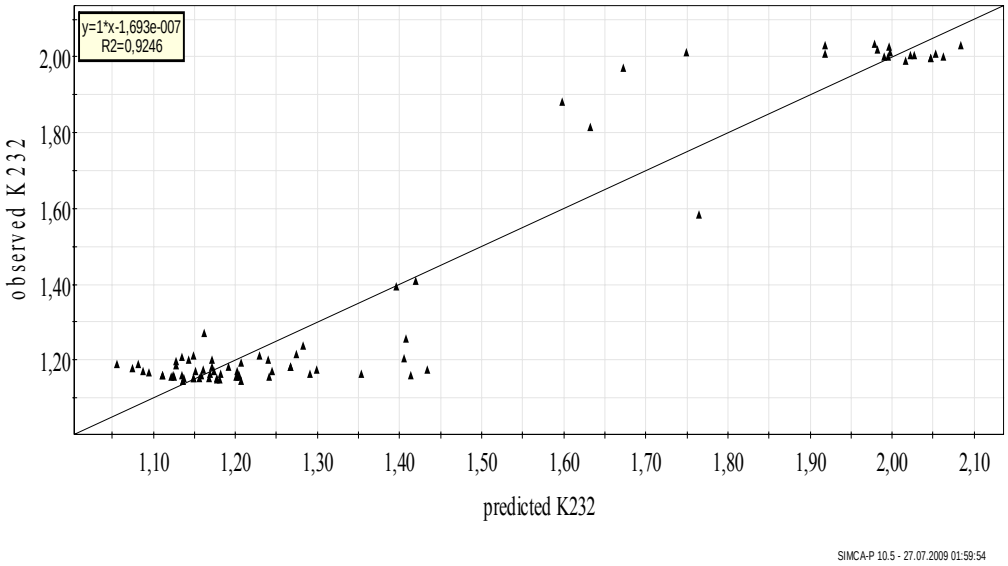

Figure 7.46. Results of PLS model: Predicted vs observed K232 values

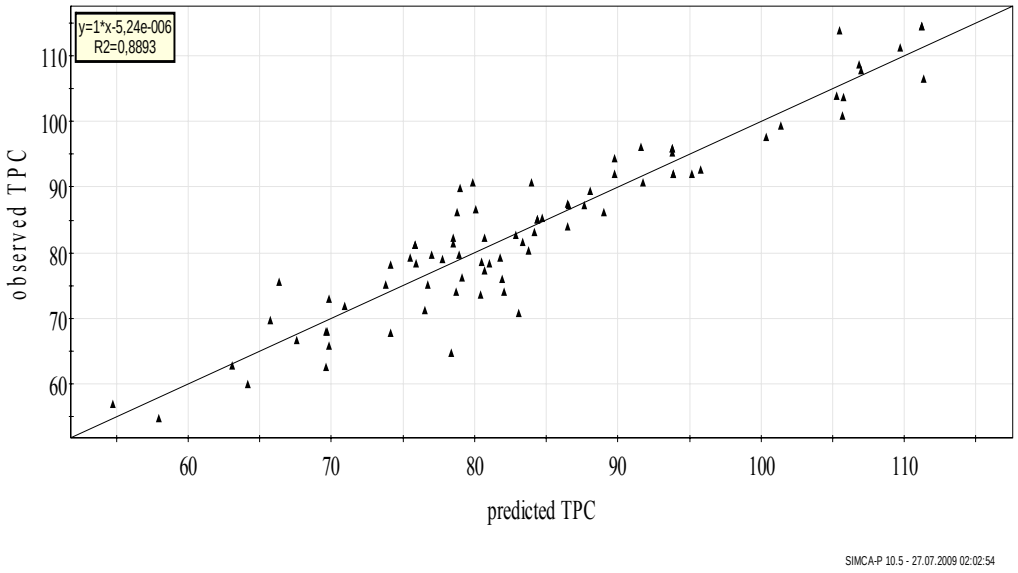

Figure 7.47. Results of PLS model: Predicted vs observed TPC values

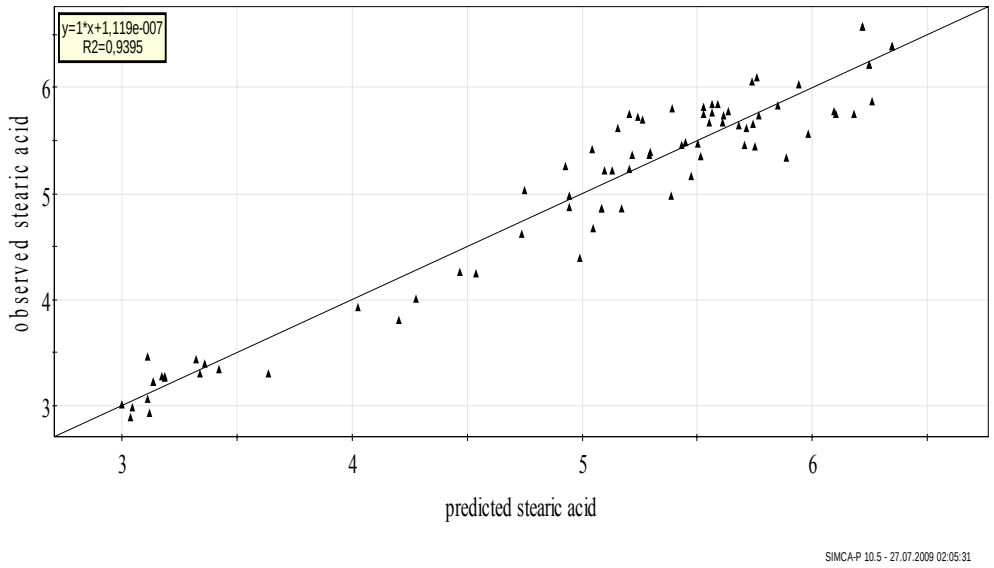

Figure 7.48. Results of PLS model: Predicted vs observed stearic acid values

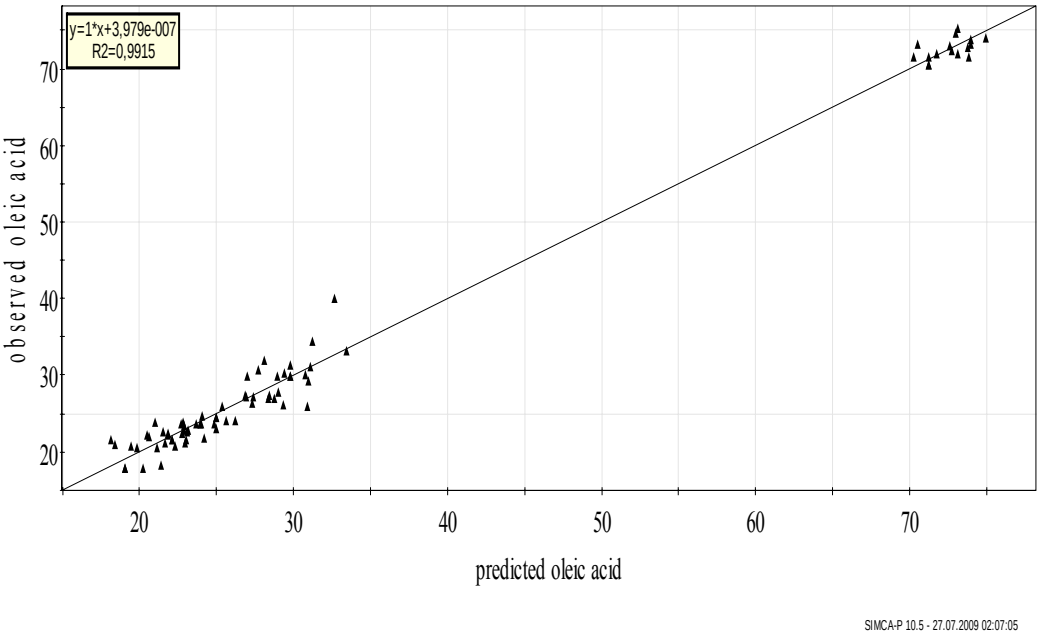

Figure 7.49. Results of PLS model: Predicted vs observed oleic acid values

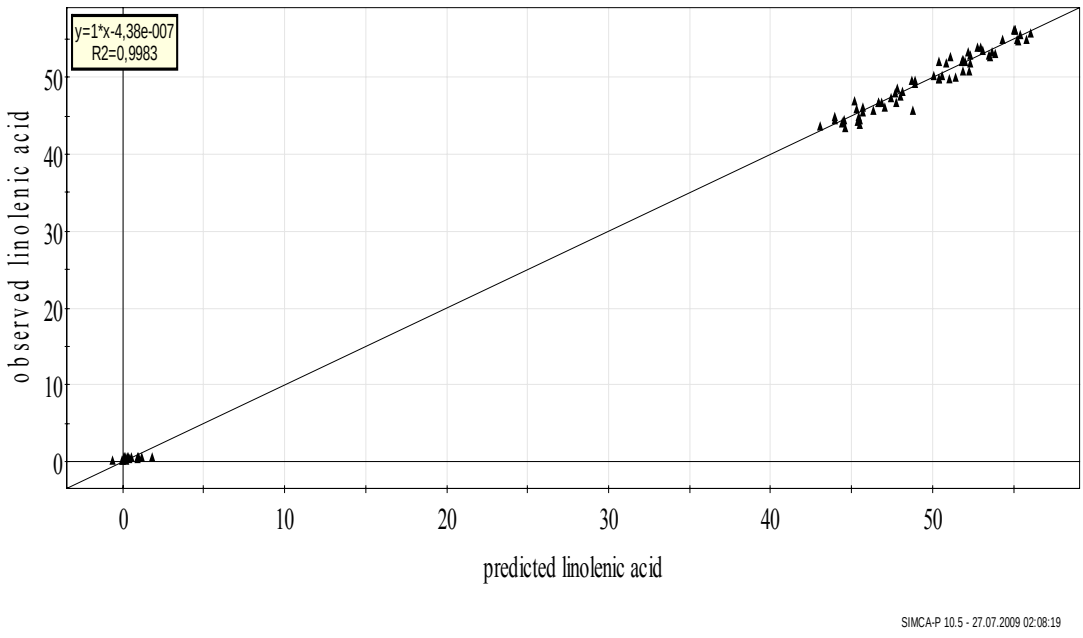

Figure 7.50. Results of PLS model: Predicted vs observed linolenic acid values

It was observed that all quality parameters (PV, K232, K270 and TPC) and fatty acids except arachidic and behenic acids can be described with a high accuracy by using FT-IR spectra.

Table 7.2. Parameters of PLS models of quality variables and fatty acid profiles

quality parameters	number of components	R^2	Q^2
PV	1	0.933	0.927
K232	1	0.925	0.923
K270	1	0.795	0.764
TPC	1	0.889	0.887
myristic acid	2	0.794	0.758
palmitic acid	1	0.979	0.978
palmitoleic acid	1	0.992	0.992
stearic acid	1	0.939	0.938
oleic acid	1	0.991	0.991
linoleic acid	1	0.870	0.867
linolenic acid	1	0.998	0.998
arachidic acid	1	0.718	0.652
behenic acid	2	0.732	0.695

CHAPTER 8

CONCLUSION

This study evaluated the changes in chemical compositions and oxidative quality of Turkish extra virgin olive oils from north and south Aegean regions during 14 month storage at two temperatures in the dark. In addition, contribution of olive oil on the oxidation rate of flaxseed oil and prediction of quality variables with FT-IR spectra by using PLS technique was studied.

During storage an increase took place in the values of quality parameters PV, UV absorbance values (K232 and K270), FFA and AV that are the measure of oxidative degradation of oils. The oxidative stability of olive oil samples stored at room temperature was less than the oils stored at refrigerator. Significant differences were found among the oil samples during storage period at 5% significance level. PVs exceeded the upper acceptable level of 20 meq/kg after 7 and 9 months of storage. Nevertheless, K232 values of oil samples remained within the limit until 10^{th} month while K270 values of oil samples reached the maximum value earlier. It was also concluded that when the initial quality of of oil samples were poor, they reached to the maximum allowable attained to the limit values of these quality parameters rapidly. Acidity of oil samples were also within the limit (< 0.8 %) and no significance increase were observed in this value during storage period.

The decrease in total phenol content of oil samples varied 20 to 70% during storage time. The individual phenolic concentrations of oil samples were qualified by HPLC. Hydroxytyrosol, tyrosol, caffeic acid, vanillic acid, vanilin, p-coumaric acid, ferulic acid, m-coumaric acid, cinnamic acid, luteolin, and apigenin were determined as the major phenolic compounds in Turkish extra virgin olive oils. In storage period of oil samples, the concentrations of hydroxytyrosol and tyrosol increased, while the other phenolic compounds decreased.

Addition of olive oil to flaxseed oil, which is known as highly-unsaturated plant oil, had positive effect on oxidation parameters during 126-day storage. While UV

absorbance values of flaxseed oil showed an increase during storage, the olive oil-flaxseed oil samples maintained their stability. Flaxseed oil and 5% olive oil-flaxseed oil mixture exceeded the legal limit of K270. In addition, the other quality parameters (PV and K232) of the oil samples remained within the limits during 126-day storage time. Considering all quality parameters, 15% olive oil addition to flaxseed oil increased the oxidative stability.

PLS processing with FT-IR data also allowed to set up mathematical models which were able to predict EVOO degradation in terms of peroxide value (PV), UV absorbance values (K232 and K270), total phenol content (TPC) and fatty acid profiles with prediction values ranged between 0.99 and 0.65. In addition, the peaks at 3468-3445, 2924, 2852, 1746-1743, 1163, 1119 and 967-976 cm^{-1} in FT-IR spectra were assigned as the useful markers of oxidative status of oil samples.

REFERENCES

Agar, T. I., Hess-Pierce, B., Sourour, M.M., Kader, A.A. 1998. Quality of fruit and oil of black-ripe olives is influenced by cultivar and storage period. *Journal of Agricultural and Food Chemistry* 46: 3415-3421.

Ahmed-Khan, M. and Shahidi, F. 1999. Rapid oxidation of commercial extra virgin olive oil stored under fluorescent light. *Journal of Food Lipids* 6: 331-339.

Ajana, H., El Antarl, A., Hafidi, A. 1998. Fatty acids and sterol evolution during the ripening of olives from the Moroccan Picholine cultivar. *Grasas y Aceites* 49: 405-410.

Akoh, C.C.and Min, D.B. 2002. Lipid Oxidation of Edible Oils. *Food Lipids, Chemistry, Nutrition and Biotechnology* 54: 283–296.

Alos, E., Cercos, M., Rodrigo, M.J., Zacarias, L., Talon, M. 2006. Regulation of color break in citrus fruits. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. *Journal of Agricultural and Food Chemistry* 54: 4888- 4895.

Amelio, Mauro. 2003. Chemical-Physical Characteristics of Olive Oil. http://oliveoil.org (accessed May 1, 2009).

Angerosa, F., d'Alessandro, N., Konstantinou, P.,Di Giacinto, L. 1996. GC-MS evaluation of phenolic compounds in virgin olive oil. *Journal of Agricultural and Food Chemistry* 43:1802-1807.

Angerosa, F., R. Mostallino, C. Basti, R. Vito. 2000. Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. *Food Chemistry* 68:283-287.

Angerosa, F., M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro. 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. *Journal of Chromatography A* 1054:17–31.

Aparicio, R., Roda, L., Albi, M. A., Gutierrez, F. 1999. Effect of various compounds on virgin olive oil stability measured by rancimat. *Journal of Agricultural and Food Chemistry* 47:4150–4155.

Aparicio, R., Rocha, S.M., Delgadillo, I., Morales, M.T. 2000. Detection of rancid defect in virgin olive oil by electronic nose. *Journal of Agricultural and Food Chemistry* 48(3):853-860.

Aranda, F., Gomez-Alanso, S., Riveradel, R.M., Salvador, M.D., Fregapane, G. 2004. Triglyceride, total and 2-position fatty acid composition of Cornicabra virgin olive oil: Comparison with other Spanish cultivars. *Food Chemistry* 86:485-492.

Baccouri, B., Temime, S.B., Campeol, E., Cioni, P.L., Daoud, D., Zarrouk, M. 2006. Application of solid-phase microextraction to the analysis of volatile compounds in virgin olive oils from five new cultivars. *Food Chemistry* 102:850–856.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A.M., Segura-Carretero, A., Fernandez-Gutierrez, A., Lercker, G. 2007. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* 12:1679-1719.

Bianco, A., Mazzei, R. A., Melchioni, C., Romero, G., Scarpati, M. L., Soriero, A., Uccella, N. 1998. Microcomponents of olive oil ─ III. Glucosides of 2 (3,4-dihydroxy-phenyl) ethanol. *Food Chemistry* 63: 461−464.

Bianco, A. and Uccella, N. 2000. Biophenolic components of olives. *Food Research International* 33: 475-485.

Bianco, A., Coccioli, F., Guiso, M., Marra, C. 2002. The occurrence in olive oil of a new class of phenolic compounds: Hydroxyl-isochromans. *Food Chemistry* 77: 405–411.

Bortoleto, G.G., Pataca, L.C.M., Bueno, M.I.M.S. 2005. A new application of X-ray scattering using principal component analysis – classification of vegetable oils. *Analytica Chimica Acta* 539:283-287.

Brenes, M., Garcia, A., Garcia, P., Rios, J.J., Garrido, A. 1999. Phenolic compounds in Spanish olive oils. *Journal of Agricultural and Food Chemistry* 47(9):3535-3540.

Brenes, M., Hidalgo, F.J., Garcia, A., Rios J.J., Garcia, P., Zamora, R., Garrido, A. 2000. Pinoresinol and 1-Acetoxypinoresinol, two new phenolic compounds identified in olive oil. *Journal of American Oil Chemists' Society* 77:715-720.

Brereton, Richard G. 2003. Chemometrics: *Data Analysis for the Laboratory and Chemical Plant.* UK: John Wiley & Sons Ltd.

Brezmes, J., Llobet, E., Vilanova, X., Orts, J., Saiz, G., Correig, X. 2001. Correlation between electronic nose signals and fruit quality indicators on shelf-life measurements with pinklady apples. *Sensors and Actuators B* 80: 41–50.

Buratti, S., Ballabio, D., Benedetti, S., Cosio, M. S. 2007. Prediction of Italian red wine sensorial descriptors from electronic nose, electronic tongue and spectrophotometric measurements by means of Genetic Algorithm regression models. *Food Chemistry* 100:211-216.

Canabate-Diaz, B., Segura-Carretero, A., Fernandez-Gutierrez, A., Belmonte Vega, A., Garrido Frenich, A., Martinez-Vidal, J. L. 2006. Separation and determination of sterols in olive oil by HPLC-MS. *Food Chemistry* 102: 593–598.

Capino, F., Bilanca, T., M., Pasqualone, A., Sikorska, E., Tommaso, G. 2005. Influence of the exposure to light on the extra virgin olive oil quality during storage. *European Food Research and Technology* 221:92-98.

Caro, A.D., Vacca, V., Poiana, M., Fenu, P., Piga, A. 2006. Influence of technology, storage and exposure on components of extra virgin olive oil (Bosana cv) from whole and de-stoned fruits. *Food Chemistry* 98:311–316.

Cecchi, T., De Marco, C., Passamonti, P. 2006. Analytical definition of the quality of extra virgin olive oil stored in polyethylene terephthalate bottles. *Journal of Food Lipids* 13:251–258.

Cert, A., Alba, J., Leon-Camacho, M., Moreda, W., Perez-Camino, M.C..1996. Effects of talc addition and operating mode on the quality and oxidative stability of virgin olive oils obtained by centrifugation. *Journal of Agricultural and Food Chemistry* 44:3930–3934.

Cert, A., Moreda W., Perez-Camino M.C. 2000. Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A* 881:131–148.

Chemistry and Quality of Olive Oil. NSW Department of Primary Industries. 2006. http://dpi.nsw.gov.au (accessed May 3, 2009).

Chiavaro, E., Vittadini, E., Rodriguez-Estrada, M.T., Cerretani, L., Bendini, A. 2008. Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. *Food Chemistry* 110:248–256.

Choe, E. and Min, D.B. 2006. Mechanisms and factors for edible oil oxidation. *Food and Biotechnology* 1:104-110.

Choo, W., Birch, J., Dufour, J. 2007. Physicochemical and quality characteristics of cold-pressed flaxseed oils. *Journal of Food Composition and Analysis* 20: 202-211.

Christy, A.A., Kasemsumran S., Du, Y., Ozaki, Y. 2004. The detection and quantification of adulteration in olive oil by near-infared spectroscopy and chemometrics. *Analytical Sciences* 20:935-940.

Cichelli, A. and Pertesana, G.P. 2004. High-performance liquid chromatographic analysis of chlorophylls, pheophytins and carotenoids in virgin olive oils: chemometric approach to variety classification. *Journal of Chromatography A* 1046 :141–146.

Cimato, A., Dello Monacoa, D., Distante, C., Epifani, M.,Siciliano, P., Taurino, A.M., Zuppa, M., Sani, A. 2006. Analysis of single-cultivar extra virgin olive oils by means of an electronic nose and HS-SPME/GC/MS methods. *Sensors and Actuators B* 114:674–680.

Cinquanta, L., Esti, M., La Notte, E. 1997. Evoulation of pheolic compounds in virgin olive oil during storage. *Journal of American Oil Chemists' Society* 74:1259-1264.

Codex Alimentarius Commission. 1999. Codex Stan 19. Edible Fats and Oils Not Covered by Individual Standards. http://codexalimentarius.net (accessed April 21, 2009).

Cosio, M.S., Ballabio, D., Benedetti, S., Gigliotti, C. 2006. Geographical origin and authentication of extra virgin olive oils by an electronic nose in combination with artificial neural networks. *Analytica Chimica Acta* 567:202–210.

Cunha, S.C., Amaral, J.S., Fernandes, J.O., Oliveira, M.B.P.P. 2006. Quantification of tocopherols and tocotrienols in Portuguese olive oils using HPLC with three different detection systems. *Journal of Agricultural and Food Chemistry* 54:3351–3356.

Davis, B.M. and McEwan, M.J. 2007. Determination of olive oil oxidative status by selected ion flow tube mass spectrometry. *Journal of Agricultural and Food Chemistry* 55 (9):3334–3338.

De Leonardis, A. and Macciola, V. 1998. Evaluation of the shelf-life of virgin olive oils. *Rivista Italiana Sostanze Grasse* 75: 391–396.

De Stefano, G., Piacquadio, P., Servili, M., Di Giovacchino, L., Sciancalepore, V. 1999. Effect of extraction system on the phenolic composition of virgin olive oils. *Fett / Lipid* 101:328–332.

Deiana, M., Rosa, A., Cao, C.F., Pirisi, F.M., Bandino, G., Dessi, M.A. 2002. Novel approach to study oxidative stability of extra virgin olive oils: importance of α-tocopherol concentration. *Journal of Agricultural and Food Chemistry* 50:4342–4346.

Di Giovacchino, L., Solinas, M., Miccoli, M. 1994. Effect of extraction systems on the quality of virgin olive oil. *Journal of the American Oil Chemists' Society* 71: 1189-1194.

Di Giovacchino, L., Sestili, S., Di Vincenzo, D. 2002. Influence of olive processing on virgin olive oil quality. *European Journal of Lipid Science and Technology* 104:587–601.

D'Imperio, M., Dugo, G., Alfa, M., Mannina, L., Segre, A.L. 2007. Statistical analysis on Sicilian olive oils. *Food Chemistry* 102:956–965.

Diaz, T.G., Meras, I.D., Cabanillas, A.G., Franco, M.F. 2004. Voltammetric behavior and determination of tocopherols with partial least squares calibration: analysis in vegetable oil samples. *Analytica Chimica Acta* 511:231–238.

Diaz, T.G., Meras, I.D., Casas, J.S., Franco, M.F.A. 2005. Characterization of virgin olive oils according to its triglycerides and sterols composition by chemometric methods. *Food Control* 16:339-347.

El-Beltagi, H. S., Salama, Z. A., El-Hariri, D. M. 2007. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (Linum Usitatissimum L.). *General and Applied Plant Physiology* 33:187-202.

Endo Y, Usuki R, Kaneda T. 1985. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. *Journal of the American Oil Chemists' Society* 62:1375–1378.

Eriksson, Lennart, Erik Johansson, Nouna Kettaneh-Wold and S. Wold, eds. 2001. *Multi-and Megavariate Data Analysis: Principles and Applications*. Sweden: Umetrics AB.

European Union Commission. 1991. Regulation EEC 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of European Communities L248*.

Flachowsky, G., Langbein, T., Böhme, H., Schneider, A., Aulrich, K. 1997. Effect of false flax expeller combined with short-term vitamin E supplementation in pigs feeding on the fatty acid pattern, vitamin E concentration and oxidative stability of various tissues. *Journal of Animal Physiology and Animal Nutrition* 78: 187–195.

Francisca, G. and Isabel M. 1992. Action of chlorophylls on the stability of virgin olive oil. *Journal of the American Oil Chemists' Society* 69:866–871.

Galtier, O., Dupuy, N., Le Dreau Y., Ollivier, D., Pinatel, C., Kister, J., Artaud, J. 2007. Geographic origins and compositions of virgin olive oils determinated by chemometric analysis of NIR spectra. *Analytica Chimica Acta* 595:136-144.

Gan, H.L., Che Man, Y.M., Tan, C.P., NorAini, I., Nazimah, S.A.H. 2005. Characterization of vegetable oils by surface acoustic wave sensing electronic nose. *Food Chemistry* 89:507–518.

Garcia-Gonzalez, D.L., Aparicio-Ruiz, R., Aparicio, R. 2003. Virgin olive oil-chemical implications on quality and health. *European Journal of Lipid Science and Technology* 110:1-6.

Gardner, J. W. and Bartlett, P. N. 1999. Brief history of electronic nose. *Sensor and Actuator B* 18: 211–217.

Garrido- Fernandez, Antonio, Fernandez- Diez, M. J., and Adamos, Martin E. 1997. *Physical and chemical characteristics of the olive fruit, in: Table olives.* Chapman & Hall, London, UK. http://books.google.com.tr (accessed June 1, 2009)

Gomez- Alonso, S., Mancebo- Campos, V., Salvador, M. D., Fregapane, G. 2007. Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. *Food Chemistry* 100: 36–42.

Gomez-Rico, A., Fregapane, G., Desamparados Salvador, M. 2008. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Research International* 41:433-440.

Guadarrama, A., Fernandez, J.A., Iniguez, M., Souto, J., Saja, J.A. 2000. Array of conducting polymer sensors for the characterisation of wines. *Analytica Chimica Acta* 411:193–200.

Guadarrama, A., Rodriguez-Mendez, M. L, Sanz, C., Rios, J. L. 2001. Electronic nose based on conducting polymers for the quality control of the olive oil aroma. Discrimination of quality, variety of olive and geographic origin. *Analytica Chimica Acta* 432: 283–292.

Guillen, M.D. and Cabo, N. 1997. Characterization of Edible Oils and Lard by Fourier Transform Infrared Spectroscopy. Relationships Between Composition and Frequency of Concrete Bands in the Fingerprint Region. *Journal of the American Oil Chemists' Society* 74: 1281-1286.

Guillen, M.D. and Cabo, N. 1999. Usefulness of the Frequency Data of the Fourier Transform Infrared Spectra to Evaluate the Degree of Oxidation of Edible Oils. *Journal of Agricultural and Food Chemistry* 47:709–719.

Guillen, M.D. and Cabo, N. 2000. Some of the most significant changes in the Fourier transform infrared spectra of edible oils under oxidative conditions. *Journal of the Science of Food and Agriculture* 80:2028-2036.

Gurdeniz, G., Tokatlı, F., Ozen, B. 2007. Differentiation of mixtures of monovarietal olive oils by mid-infrared spectroscopy and chemometrics. *European Journal of Lipid Science and Technology* 109: 1194–1202.

Gurdeniz, G., Ozen, B., Tokatli, F. 2008. Classification of Turkish olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy. *European Food Research and Technology* 227:1275-1281.

Gutierrez, F., Jimenez, B., Ruiz, A., Albi, M. A. 1999. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry* 47: 121–127.

Gutiearrez, F. and Fernandez, J.L. 2002. Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "Extra" quality. *Journal of Agricultural and Food Chemistry* 50: 571-577.

Harwood, John and Aparicio Ramon eds. 2000. *Handbook of olive oil. Analysis and Properties.* Gaithersburg. Aspen publications.

Hunterlab, L, a, b Color Space.2008. http://hunterlab.com (accessed April 13, 2009).

Ingram, D., Sanders, K., Kolybaba, M., Lopez, D. 1997. Case-control study of phytooestrogens and breast cancer. *Lancet* 350:990–994.

International Olive Oil Council. 2007. http:/.internationaloliveoil.org (accessed April 10, 2009).

Ismail, A.A., Van de Voort, F.R., Emo, G., Sedman, J. 1993. Rapid quantitative determination of free fatty acids in fats and oils by Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society.* 70:335-341.

IUPAC. Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th ed. International Union of Pure and Applied Chemistry Blackwell Scientific Publications. Oxford, UK, 1987; Methods 2.501, 2.505.

Jimenez-Marquez, A. 2003. Preliminary results of the characterization of mixtures of olive oil by differential scanning calorimetry. *Ciencia y Tecnologia Alimentaria* 4: 47–54.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D. 2005. Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. *Journal of Agricultural and Food Chemistry* 53:8054-8062.

Kanavouras, A. and Coutelieris, F.A. 2004. Shelf-life predictions for packaged olive oil based on simulations. *Food Chemistry* 96: 48–55.

Kato, M. J., Chu, A., Davin, L. B., Lewis, N. G. 1998. Biosynthesis of antioxidant lignans in Sesamum indicum seeds. *Phytochemistry* 47: 583–591.

Keceli, T. and Gordon, M.H. 2002. The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *Journal of the Science of Food and Agriculture* 81:1391–1396.

Kiritsakis, A.K. 1998. Flavour components of olive oil—A review. *Journal of the American Oil Chemists' Society* 75(6):673-681.

Kritsakis, A., Kanavouras, A., Kritsakis, K. 2002. Chemical analysis, quality control and packaging issues of olive oil. *European Journal of Lipid Science and Technology* 104:628–638.

Kotti, F., Chiavaro, E., Cerretani, L., Barnaba, C., Gargouri, M., Bendini, A. 2008. Chemical and thermal characterization of Tunisian extra virgin olive oil from Chetoui and Chemlali cultivars and different geographical origin. *European Food Research and Technology* 228:735-742.

Koutsaftakis, A., Kotsifaki, F., Stefanoudaki, E. 1999. Effect of extraction system, stage of ripeness and keading temperature on the sterol composition of virgin olive oils. *Journal of the American Oil Chemists' Society* 76:1477–1481.

Labrinea, E. P., Thomaidis, N. S., Giorgiou, C. A. 2001. Direct olive oil anisidine value determination by flow injection. *Analytica Chimica Acta* 448:201–206.

Limiroli, R., Consonni, R., Ottolina, G., Marsilio, V., Bianchi, G., Zetta, L. 1995. 1H and 13C NMR characterization of new oleuropein aglycones. *Journal of American Oil Chemistry Society* 1:1519–1523.

Maggio, R. M., Kaufman, T. S., Carlo, M., Cerretani, L., Bendini, A., Cichelli, A., Compagnone, D. 2008. Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infrared spectroscopy coupled with partial least squares. *Food Chemistry* 114:1549–1554.

Manai, H., Haddada, M. F., Daoud, D., Fernandez, X., Lizzani-Cuvelier, L., Zarrouk, M. 2007. Profiles of volatile compounds from some monovarietal Tunisian virgin olive oils. Comparison with French PDO. *Food Chemistr*y 103:467-476.

Mannina, L., Patumi, M., Proietti, N., Bassi, D., Segre, A. L. 2003. Geographical characterization of Italian extra virgin olive oils using high-field 1H NMR spectroscopy. *Journal of Agricultural and Food Chemistry* 49: 2687–2696.

Manzi, P., Panfili, G., Esti, M., Pizzoferrato, L.1998. Natural antioxidants in the unsaponifiable fraction of virgin olive oils from different cultivars. *Journal of the Science of Food and Agriculture* 77: 115−120.

Marquez-Ruiz, G., Martin-Polvillo, M., Dobarganes, M.C. 1996. Quantitation of oxidized triglyceride monomers and dimers as an useful measurement for early and advanced stages of oxidation. *Grasas Aceites* 47: 48-53.

Martinez-Gonzalez, M.G. and Sanchez-Villegas, A. 2004. Review. The emerging role of Mediterranean diets in cardiovascular epidemiology: Monounsaturated fats, olive oil, red wine or the whole pattern? *European Journal of Epidemiology* 19: 9–13.

Martinez-Vidal, J.L., Garrido-Frenich, A., Escobar-Garcia, M.A., Romero-Gonzales, R. 2007. LC–MS determination of sterols in olive oil. *Chromatographia* 65:695-699.

Masaki, H., Okamoto, N., Sakaki, S., Sakurai, H. 1997. Protective effects of hydroxybenzoic acids and their esters on cell damage induced by hydroxyl radicals and hydrogen peroxides. *Biological and Pharmaceutical Bulletin* 20:304-308.

Mastrobattista, G. 1990. Effect of light on extra virgin olive oils in different types of glass bottles. *Itaia Journal Food Science* 3:191-195.

Mendez, A.I. and Falque, E. 2006. Effect of storage time and container type on the quality of extra-virgin olive oil. *Food Control* 18:521-529.

Mistry, B. S. and Min, D. B. 1987. Effects of fatty acids on the oxidative stability of soybean oil. *Journal of Food Science* 52:831–832.

Miyake, Y., Yokomizo, K., Matsuzaki, N. 1998. Determination of unsaturated fatty acid composition by high-resolution nuclear magnetic resonance spectroscopy. *Journal of the American Oil Chemists' Society* 75: 1091–1094.

Miyashitau, K. and Takagi, T. 1986. Study on the oxidative rate and prooxidant activity of free fatty acids. *Journal of the American Oil Chemists' Society* 63:1380–1384.

Montedoro, G., Servili, M., Baldioli, M., Miniati, E. 1992. Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry* 40(9):1571-1576.

Morales, M.T. and Aparicio, R. 1999. Effect of extraction conditions on sensory quality of virgin olive oil. *Journal of American Oil Chemists' Society* 76:295-300.

Morales, Martin T. and Tsimidou Maria, eds. 2000. *The role of volatile compounds and polyphenols olive oil sensory quality. in: Handbook of Olive Oil. Analysis and Properties.* USA: Aspen Publication.

Morello, J., Motilva, M., Tovar, M., Paz-Romero, M. 2003. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. *Food Chemistry* 85:357-364.

Morello, J. R. Vuorela, S., Romero, M.P., Motilva, M. J., Heinonen, M. 2005. Antioxidant activity of olive pulp and olive oil phenolic compounds of the Arbequina cultivar. *Journal of Agricultural and Food Chemistry* 53: 2002-2008.

Morris, D. H. 2001. Essential nutrients and other functional compounds in flaxseed. *Nutrition Today* 36(3):159–162.

Muik, B., Lendl, B., Molina-Diaz, A., Ayora-Canada, M.J. 2005. Direct monitoring of lipid oxidation inedible oils by Fourier transform Raman spectroscopy. *Chemistry and Physics of Lipids* 134:173–182.

Murkovic, M., Lechner, S., Pietzka, A., Bratacos, M., Katzogiannos, E. 2004. Analysis of minor components in olive oil. *Journal of Biochemistry Methods* 61:155-160.

Nergiz, C. and Unal, K., 1991. Determination of phenolic acids in virgin olive oil. *Food Chemistry* 39:237-240.

New Zealand Food Regulation, 1984. General standard for edible fats and edible oils. http://foodstandarts.gov.au (accessed April 2, 2009).

Nouros, P.G., Georgiou, C.A., Polissiou, M.G. 1999. Direct parallel flow injection multichannel spectrophotometric determination of olive oil peroxide value. *Analytica Chimica Acta* 389:239-245.

Ocakoglu, D., Tokatlı, F., Ozen, B., Korel, F. 2008. Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chemistry* 113:401-410.

Okogeri, O. and Tasioula-Margari, M. 2002. Changes in phenolic and α-tocopherol of virgin olive oil during storage. *Journal of Agricultural and Food Chemistry* 50:1077-1080.

Oliveros, M.C.C., Pavon, J.L.P., Pinto, C.G.B., Laespada, M.E.F., Cordero, B.M., Forina, M. 2002. Electronic nose based on metal oxide semiconductor sensors as a fast alternative for the detection of adulteration of virgin olive oils. *Analytica Chimica Acta* 459:219–228.

Oliveros, C.C., Boggia, R., Casale, M., Armanino, C., Forina, M. 2004. Optimisation of a new headspace mass spectrometry instrument Discrimination of different geographical origin olive oils. *Journal of Chromatography A* 1076:7–15.

Ollivier, D., Artaud, J., Pinatel, C., Durbec, J., Guerere, M. 2006. Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. *Food Chemistry* 97:382–393.

Oomah, B.D., Kenaschuk, E.O., Mazza, G. 1997. Tocopherols in flaxseed. *Journal of Agricultural and Food Chemistry* 45:2076–2080.

Owen, R.W., Giacosa, A., Hull, W.E., Haubner, R., Würtele, G., Spiegelhalder, B., Bartsch, H. 2000. Olive-oil consumption and health: the possible role of antioxidants. *The Lancet Oncology* 1:107-111.

Pagliarini, E., Zanoni, B., Giovanelli, G. 2000. Predictive study on Tuscan extra virgin oil stability under several commercial conditions. *Journal of Agricultural and Food Chemistry* 48:1345–1351.

Park, J., R. and Lee, D. 2003. Detection of Adulteration in Olive Oils Using Triacylglycerols Compositions by High Temperature Gas Chromatography. *Bulletin Korean Chemistry Society* 24: 529-534.

Parker, T.D., Adams, D.A., Zhou, K., Harris, M., Yu, L. 2003. Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Journal of Food Science* 68:1240–1243.

Pena, F., Cardenas, S., Gallego, M., Valcárcel, M. 2005. Direct olive oil authentication: Detection of adulteration of olive oil with hazelnut oil by direct coupling of headspace and mass spectrometry, and multivariate regression techniques. *Journal of Chromatography A* 1074: 215-221.

Perez-Jimenez, F., Ruano, J., Perez-Martinez, P., Lopez-Segura, F., Lopez-Miranda, J. 2007. The influence of olive oil on human health: not a question of fat alone. *Molecular Nutrition Food Research* 51:1199-1208.

Petrakis, P., Agiomyrgianaki, A., Christophoridou, S., Spyros, A., Dais, P. 2008. Geographical characterization of Greek virgin olive oils (Cv. Koroneiki) using 1H and 31P NMR fingerprinting with canonical discriminant analysis and classification binary trees". *Journal of Agricultural and Food Chemistry* 56: 3200-3208.

Piacquadio, P., De Stefano, G., Sciancalepore, V. 1998. Quality of virgin olive oil extracted with the new centrifugation system using a two-phases decanter. *Lipids* 100: 472–474.

Poiana, M. and Mincione, A. 2004. Fatty acids evolution and composition of olive oils extracted from different olive cultivars grown in Calabrian area. *Grass y Aceites* 55(3):282-290.

Psomiadou, E. and Tsimidou, M. 2002. Stability of virgin olive oil. 1. Autoxidation studies. *Journal of Agricultural and Food Chemistry* 50:716-721.

Rahmani M, Saari Csallany A. 1998. Role of minor constituents in the photooxidation of virgin olive oil. *Journal of the American Oil Chemists' Society* 75:837–843.

Rezzi, S., Axelson, D.E., Heberger, K., Reniero, F., Mariani, C., Guillou, C. 2005. Classification of olive oils using high throughput flow 1H NMR fingerprinting with principal component analysis, linear discriminant analysis and probabilistic neural networks. *Analytica Chimica Acta* 552:13–24.

Romieu, I. and Trenga, C. 2001. Diet and obstructive lung diseases. *Epidemiologic Review* 23: 268–287.

Rovellini, P., Cortesi, N., Fedeli, E. 1997. Analysis of flavonoids from Olea europaea by HPLC-UV and HPLC-electrospray-MS. *Rivista Italiana Sostanze Grasse* 74:273-279.

Ryan, D. and Robards, K. 1998. Phenolics compounds in olives. *Analyst* 123:41-44.

Ryan, D., Robards, K., Lavee, S. 1999. Changes in phenolic content of olive during maturation. *International Journal of Food Science and Technology* 34:265–274.

Ryan, D., Lawrence, H., Prenzler, P. D., Antolovic, M. 2001. Recovery of phenolic compounds from Olea europeae. *Analytica Chimica Acta* 445: 67–77.

Salas, J. J., Sanchez, C., Garcia-Gonzalez, D. L., Aparicio, R. 2005. Impact of the suppression of lipoxygenase and hydroperoxide lyase on the quality of the green odor in green leaves. *Journal of Agricultural and Food Chemistry* 53:1648–1655.

Schorno, A. L., Tulbek, M. C., Hall, C., Manthey, F. 2003. Evaluation of physical and chemical properties of roasted flaxseed. *Institute of Food Technologists* 14: 6-26.

Sciancalepore, V., De Stefano, G., Piacquadio, P. 2000. Effects of the cold percolation system on the quality of virgin olive oil. *European Journal of Lipid Science and Technology* 102: 680-683.

Shahidi, F. 2002. Nutraceuticals and functional foods: research addresses bioactive components. *Food Technology* 56:23-28.

Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A., Montedoro, G. F. 1999. High-performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D-and 2D-nuclear magnetic resonance characterization. *Journal of the American Oil Chemists' Society* 76: 873−882.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G., Morozzi, G. 2004. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A* 1054:113–127.

Sinelli, N., Cosio, M., S., Gigliotti, C., Casiraghi, E. 2007. Preliminary study on application of mid infrared spectroscopy for the evaluation of the virgin olive oil "freshness". *Analytica Chimica Acta* 598: 128–134.

Soler-Rivas, C., Espın, J. C., Wichers, H. J. 2000. Oleuropein and related compounds. *Journal of the Science of Food and Agriculture* 80: 1013–1023.

Spugnoli, P., Parenti, A., Cardini, D., Modi, G., Caselli, S. 1998. Characterization of single variety extra virgin olive oils of three cultivars from Tuscany. Rivista *Italiana Sostanze Grasse* 75: 227-233.

St. Angelo AJ. 1996. Lipid oxidation in foods. *Critical Reviews in Food Science and Nutrition* 36:175–224.

Stefanoudaki, E., Kotsifaki, F., Koutsaftakis, A.1999: Classification of Virgin Olive Oils of the Two Major Cretan Cultivars Based on Their Fatty Acid Composition. *Journal of the American Oil Chemists' Society* 76:623–626.

Tapp, H. S., Defernez, M., Kemsley, E.K. 2003. FT-IR spectroscopy and multivariate analysis can distinguish the geographic origin of extra virgin olive oils. *Journal of Agricultural and Food Chemistry* 51:6110-6115.

Tay, A., Singh, R.K., Krishnan, S.S., Gore, J.P. 2002. Authentication of olive oil adulterated with vegetable oils using Fourier Transform Infrared Spectroscopy. *Lebensm.-Wiss. u.-Technology* 35:99–103.

Thompson, L. U., Robb, P., Serraino, M., Cheung, F. 1991. Mammalian lignan production of various foods. *Nutrition and Cancer* 16:43–52.

Torres, M.M. and D.M. Maestri. 2006. The effects of genotype and extraction methods on chemical composition of virgin olive oils from Traslasierra Valley (Cordoba, Argentina). *Food Chemistry* 96:507-511.

Turkish Food Codex. 2000. Communiqué on Cooking Olive Oil and Cooking Pomace Oil. *The Official Gazette*.

Van de Voort, F. R., Sedman, J., Emo, G., Ismail, A. A. 1992. Rapid and direct iodine value and saponification number determination of fats and oils by attenuated total reflectance/Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society* 69: 1118−1123.

Van de Voort, F. R., Ismail, A. A., Sedman, J., Dubois, J., Nicodemo, T. 1994. The determination of peroxide value by fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society* 71: 921−926.

Van de Voort, F. R., Ismail, A. A., Sedman, J. A. 1995. Rapid, automated method for the determination of cis and trans content of fats and oils by fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society* 72: 873−880.

Vekiari, S.A., Papadopouloua, P., Koutsaftakisb., A. 2002. Comparison of different olive oil extraction systems and the effect of storage conditions on the quality of the virgin olive oil. *Grasas y Aceite* 53:324-329.

Vekiari, S. A., Papadopoulou, P., Kiritsakis, A. 2007. Effects of processing methods and commercial storage conditions on the extra virgin olive quality indexes. *Grasas y Aceites* 58(3): 237–242.

Velasco, J. and Dobarganes, C. 2002. Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*. 104:661–676.

Velasco, L. and Goffman, F.D. 2000. Tocopherol, plastochromanol and fatty acid patterns in the genus Linum. *Plant Systematisc and Evaluation* 222:77–88.

Viljanen, K., Sundberg, S., Ohshima, T., Heinonen, M. 2002. Carotenoids as antioxidants to prevent photoxidation. *European Journal of Lipid Science and Technology* 104: 356–359.

Vinha, A. F., Ferreres, F., Silva, B. M., Valentao, P., Goncalves, A., Pereira, J. A., Oliveira, B., Seabra, R. M., Andrade, P. B. 2005. Phenolic profiles of Portuguese olive fruits (Olea europaea L.): Influences of cultivar and geographical origin. *Food Chemistry* 89:561–568.

Visioli, F. and Galli, C. 1998. Olive oil phenols and their potential effects on human health. *Journal of Agricultural and Food Chemistry* 46:4292-4296.

Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A., Tegou, E. 2006. Applications of Fourier transform-infrared spectroscopy to edible oils. *Analytica Chimica Acta* 573:459-465.

Wang, B., Li, D., Wang, L.J., Huang, Z.G., Zhang, L., Chen, X.D., Mao, Z.H., 2007. Effect of moisture content on the physical properties of fibered flaxseed. *International Journal of Food Engineering* 5:1–11.

Webster, L., Simpson, P., Shanks, A.M., Moffat, C.F. 1999. The authentication of olive oil on the basis of hydrocarbon concentration and composition. *Analyst* 125:97–104.

Yildiz, G.,Wehling, R.L., Cuppett, S.L. 2001. Method for determining oxidation of vegetable oils by near-infrared spectroscopy. *Journal of the American Oil Chemists' Society* 78 (5):495–502.

Zarrouki, W., Haddadai, F.M., Baccourin, B., Queslatin, I., Taamallin, W. 2008. Characterization of virgin olive oil from Southern Tunisia. *European Journal of Lipid Science and Technology* 110:81–88.

APPENDIX A

EXPERIMENTAL DATA

Table A.1. PVs of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	13.56±0.02	18.12±0.61	36.85±0.06	38.74±0.81	43.05±0.23
ero	11.05±0.04	19.96±1.87	27.78±0.14	37.28±0.65	41.14±0.51
bro	17.49±0.02	19.74±0.02	40.77±0.42	53.36±1.88	58.24±0.85
oro	15.48±0.06	33.14±0.23	36.87±0.16	41.98±0.03	46.22±0.10
are	13.56±0.02	11.56±0.13	25.76±0.06	31.52±0.89	33.7±0.71
ere	11.05±0.04	14.82±0.37	15.85±1.18	22.92±0.57	24.9±0.71
bre	17.49±0.02	15.25±0.06	27.38±0.62	35.75±0.18	37.81±0.52
ore	15.48±0.06	27.6±0.94	19.29±0.29	25.33±0.19	27.97±0.65

Table A.2. K232 values of EVOO samples during14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	0.72±0.05	0.73±0.03	0.93±0.03	1.26±0.02	1.69±0.00
ero	1.62±0.37	1.17±0.20	1.19±0.01	2.00±0.00	2.11±0.00
bro	0.8±0.11	0.7±0.11	0.97±0.11	1.61±0.00	1.95±0.00
oro	1.64±0.03	1.34±0.24	1.68±0.00	2.01±0.00	2.32±0.00
are	0.72±0.05	0.53±0.06	0.62±0.04	1.04±0.05	1.29±0.00
ere	1.62±0.37	0.65±0.11	0.99±0.00	1.85±0.00	1.92±0.00
bre	0.8±0.11	0.88±0.06	0.93±0.02	1.20±0.00	1.45±0.00
ore	1.64±0.03	0.79±0.07	1.12±0.00	1.89±0.00	1.97±0.00

Table A.3. K270 values of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	0.11±0.05	0.24±0.02	0.26±0.01	0.28±0.01	0.31±0.00
ero	0.12±0.02	0.14±0.02	0.18±0.00	0.19±0.00	0.21±0.00
bro	0.10±0.02	0.21±0.02	0.21±0.00	0.24±0.02	0.29±0.00
oro	0.17±0.03	0.25±0.00	0.26±0.00	0.29±0.00	0.32±0.00
are	0.11±0.05	0.16±0.04	0.19±0.00	0.2±0.00	0.21±0.00
ere	0.12±0.37	0.11±0.01	0.16±0.00	0.18±0.00	0.2±0.00
bre	0.10±0.11	0.15±0.02	0.19±0.00	0.2±0.01	0.22±0.00
ore	0.17±0.03	0.20±0.02	0.21±0.00	0.21±0.00	0.23±0.00

Table A.4. FFA values of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	0.3	0.32	0.34	0.41	0.47
ero	0.39	0.45	0.48	0.5	0.57
bro	0.38	0.44	0.46	0.47	0.51
oro	0.41	0.43	0.46	0.5	0.58
are	0.3	0.31	0.32	0.35	0.4
ere	0.39	0.42	0.43	0.45	0.49
bre	0.38	0.41	0.42	0.44	0.48
ore	0.41	0.42	0.43	0.44	0.49

Table A.5. TPC values of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	238.84±24.91	258.15±11.59	200.45±5.02	159.38±5.16	115.2±2.54
ero	225.75±23.11	260.11±10.97	208.26±4.63	177.50±2.75	134.31±9.38
bro	220.24±12.17	281.98±5.70	219.41±4.81	185.02±6.37	138.18±3.81
oro	361.5±17.76	346.8±52.52	215.05±6.21	197.77±3.65	193.29±2.66
are	238.84±24.91	285.82±10.62	202.01±4.27	164.49±5.01	155.71±4.20
ere	225.75±23.11	286.94±5.69	309.61±5.24	256.90±7.38	159.46±4.28
bre	220.24±12.17	288.39±6.37	214.78±7.43	193.97±5.89	152.3±4.87
ore	361.5±17.76	410.66±9.50	324.30±9.15	318.46±4.63	247.75±3.34

Table A.6. L* values of EVOO samples during 10-month storage period

olive oil	time (months)			
	1	**4**	**7**	**10**
aro	21.66±0.08	21.86±0.10	22.34±0.07	22.47±0.15
ero	23.68±0.24	24.02±0.08	24.05±0.07	24.42±0.06
bro	21.71±0.13	22.21±0.04	22.42±0.04	22.83±0.25
oro	22.59±0.26	23.44±0.12	23.92±0.19	23.48±0.30
are	21.66±0.08	21.74±0.04	22.17±0.43	22.02±0.24
ere	23.68±0.24	24.64±0.50	23.57±0.04	24.92±0.15
bre	21.71±0.13	22.01±0.09	22.10±0.13	21.88±0.06
ore	22.59±0.26	23.48±0.11	23.68±0.09	23.74±0.18

Table A.7. a* values of EVOO samples during 10-month storage period

olive oil	time (months)			
	1	**4**	**7**	**10**
aro	1.08 ±0.02	0.68±0.04	0.53±0.05	0.27±0.14
ero	0.79±0.05	1.24±0.03	1.30±0.03	1.35±0.11
bro	0.92±0.01	0.29±0.02	0.22±0.08	0.05±0.11
oro	0.40±0.08	0.09±0.01	0.29±0.00	0.54±0.25
are	1.08±0.02	0.80±0.05	0.84±0.05	0.72±0.14
ere	0.79±0.05	1.05±0.06	0.73±0.3	1.22±0.06
bre	0.92±0.01	0.53±0.05	0.65±0.05	0.58±0.07
ore	0.40±0.08	0.11±0.05	0.15±0.00	0.26±0.01

Table A.8. b* values of EVOO samples during 10-month storage period

olive oil	time (months)			
	1	**4**	**7**	**10**
aro	8.463±0.07	8.54±0.09	9.20±0.09	9.41±0.14
ero	11.61±0.22	11.35±0.22	11.44±0.22	11.08±0.11
bro	8.00±0.20	9.27±0.03	9.56±0.03	9.53±0.11
oro	9.00±0.13	8.99±0.14	9.30±0.14	10.26±0.25
are	8.46±0.07	8.67±0.31	8.17±0.31	7.92±0.14
ere	11.61±0.22	10.80±0.06	11.20±0.06	10.77±0.06
bre	8.00±0.20	8.75±0.14	8.70±0.14	8.33±0.07
ore	9.00±0.13	8.60±0.07	9.42±0.08	9.43±0.26

Table A.9. AVs of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	15.69	27.45	36.81	32.75	38.83
ero	10.22	27.56	33.00	32.49	34.98
bro	10.12	15.09	20.33	21.53	36.61
oro	10.64	35.01	38.68	38.23	42.31
are	15.69	22.41	30.44	29.07	31.40
ere	10.22	16.62	22.17	24.59	25.23
bre	10.12	11.53	22.66	17.07	19.40
ore	10.64	28.34	30.55	30.48	31.27

Table A.10. IVs of EVOO samples during 14-month storage period

olive oil	time (months)				
	1	**4**	**7**	**10**	**14**
aro	87.18	82.31	72.72	66.05	61.74
ero	86.54	84.01	64.70	60.88	55.16
bro	84.48	83.13	70.95	68.04	60.03
oro	85.51	83.23	77.14	63.70	58.94
are	87.18	83.05	76.92	68.47	65.64
ere	86.54	84.12	70.49	64.67	60.32
bre	84.48	83.17	75.06	74.60	71.15
ore	85.51	84.52	79.45	60.32	57.48

Table A.11. Fatty acid profiles of (% total fatty acids) of EVOO samples during 14-month storage

time (months)	olive oil samples	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
1	aro	0.015	10.285	0.549	2.837	74.739	10.204	0.518	0.691	0.161
	ero	0.017	12.845	0.652	2.844	71.521	10.895	0.489	0.584	0.153
	bro	0.018	11.295	0.637	2.519	76.110	8.065	0.432	0.782	0.143
	oro	0.018	11.822	0.708	2.669	75.097	8.344	0.466	0.723	0.152
	are	0.015	10.285	0.549	2.837	74.739	10.204	0.518	0.691	0.161
	ere	0.017	12.845	0.652	2.844	71.521	10.895	0.489	0.584	0.153
	bre	0.018	11.295	0.637	2.519	76.110	8.065	0.432	0.782	0.143
	ore	0.018	11.822	0.708	2.669	75.097	8.344	0.466	0.723	0.152
4	aro	0.042	11.986	0.602	3.194	72.421	10.133	0.550	0.884	0.193
	ero	0.020	13.467	0.645	2.938	68.708	11.439	0.486	1.001	0.162
	bro	0.023	12.323	0.675	2.540	75.139	7.890	0.459	0.800	0.152
	oro	0.021	12.810	0.730	2.799	73.934	8.320	0.489	0.736	0.162
	are	0.028	11.248	0.600	3.194	73.146	10.291	0.528	0.777	0.188
	ere	0.019	13.218	0.642	2.915	70.640	11.122	0.479	0.801	0.163
	bre	0.023	12.407	0.674	2.531	74.805	7.944	0.445	1.036	0.142
	ore	0.022	12.744	0.732	2.765	73.866	8.456	0.488	0.760	0.167
7	aro	0.015	10.879	0.542	3.142	74.298	10.039	0.342	0.663	0.079
	ero	0.015	11.469	0.607	2.998	73.797	9.925	0.348	0.772	0.070
	bro	0.013	9.648	0.647	2.698	77.193	8.138	0.728	0.832	0.102
	oro	0.014	10.498	0.664	2.501	77.723	7.529	0.275	0.712	0.082
	are	0.011	9.860	0.495	3.030	78.087	7.602	0.254	0.590	0.072
	ere	0.019	10.923	0.579	2.952	74.588	9.884	0.256	0.717	0.081

(cont. on next page)

Table A.11. (cont.) Fatty acid profiles of (% total fatty acids) of EVOO samples during 14-month storage

	bre	0.015	11.870	0.649	2.645	76.400	7.186	0.273	0.886	0.077
	ore	0.016	10.826	0.672	2.451	77.473	7.462	0.289	0.748	0.064
10	aro	0.011	10.707	0.570	3.186	74.869	9.719	0.354	0.509	0.076
	ero	0.013	11.683	0.535	2.769	75.024	9.072	0.318	0.524	0.062
	bro	0.012	10.331	0.564	2.303	78.333	7.457	0.274	0.655	0.069
	oro	0.012	10.418	0.633	2.208	78.295	7.404	0.275	0.681	0.075
	are	0.010	9.008	0.463	2.956	79.084	7.647	0.239	0.522	0.071
	ere	0.012	13.010	0.706	3.324	72.752	9.355	0.263	0.496	0.081
	bre	0.012	11.794	0.569	2.290	77.103	7.172	0.241	0.743	0.076
	ore	0.015	10.436	0.664	2.234	78.420	7.087	0.259	0.702	0.057
14	aro	0.009	9.738	0.517	2.427	78.196	8.293	0.308	0.467	0.045
	ero	0.009	8.859	0.551	1.969	80.132	7.714	0.285	0.431	0.050
	bro	0.008	8.266	0.704	2.514	80.154	7.518	0.281	0.500	0.053
	oro	0.011	7.886	0.547	2.410	81.090	7.166	0.288	0.548	0.055
	are	0.010	9.033	0.426	2.360	80.008	7.394	0.217	0.482	0.069
	ere	0.010	9.897	0.720	3.298	76.587	8.764	0.312	0.349	0.063
	bre	0.010	8.428	0.528	2.542	80.845	6.855	0.238	0.496	0.057
	ore	0.010	10.079	0.613	2.117	79.207	6.962	0.258	0.699	0.055

Table A.12. Individual phenolic compounds of EVOOs in 4th month

		olive oil samples							
Month 4	**phenolic compounds**	**aro**	**ero**	**bro**	**oro**	**are**	**ere**	**bre**	**ore**
	Hydroxytyrosol	1.649	2.839	0.850	1.513	0.331	1.866	0.538	1.551
	Chlorogenic acid	0.007	0.025	0.000	0.000	0.001	0.004	0.000	0.000
	Tyrosol	1.164	1.517	2.517	2.056	0.181	0.953	1.916	2.026
	4 Hydroxybenzoic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4 Hydroxyphenylacetic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Caffeic acid	0.189	0.323	0.149	0.251	0.039	0.113	0.126	0.229
	Vanilic acid	1.048	0.908	0.816	0.235	0.216	0.861	0.807	0.296
	Syringic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Vanilin	0.333	4.478	1.459	0.989	0.784	3.998	1.527	1.203
	P-coumaric acid	3.106	2.328	3.998	3.576	0.937	3.549	3.565	3.930
	Ferulic acid	0.104	0.328	0.149	0.101	0.035	0.116	0.246	0.151
	M-coumaric acid	2.521	2.995	4.964	2.087	0.702	2.395	2.983	2.662
	undefined 1	10.184	11.905	4.008	5.516	3.929	10.755	3.206	7.324
	undefined 2	1.764	1.975	1.649	1.079	0.560	1.477	1.125	1.114
	Cinamic acid	2.335	4.183	4.060	3.968	3.525	3.457	4.535	3.861
	Luteolin	1.681	2.149	0.420	0.210	0.641	1.740	0.265	0.247
	undefined 3	0.547	0.374	1.004	0.419	0.301	0.713	0.189	0.222
	Apigenin	0.896	0.286	1.164	0.315	0.075	0.194	0.273	0.374

Table A.13. Individual phenolic compounds of EVOOs in 7th month

		olive oil samples							
Month 7	**phenolic compounds**	**aro**	**ero**	**bro**	**oro**	**are**	**ere**	**bre**	**ore**
	Hydroxytyrosol	1.949	2.860	0.909	1.816	0.864	2.050	0.505	2.048
	Chlorogenic acid	0.008	0.015	0.000	0.000	0.005	0.041	0.000	0.000
	Tyrosol	1.444	1.988	2.492	2.283	0.039	1.512	1.633	2.588
	4 Hydroxybenzoic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4 Hydroxyphenylacetic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Caffeic acid	0.101	0.215	0.126	0.094	0.053	0.117	0.079	0.092
	Vanilic acid	0.691	1.562	0.096	0.828	0.104	0.855	0.584	0.814
	Syringic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Vanilin	1.071	4.351	0.585	1.176	0.299	3.346	1.069	0.659
	P-coumaric acid	1.465	3.412	3.219	3.723	1.938	1.724	3.119	4.503
	Ferulic acid	0.023	0.391	0.042	0.118	0.051	0.114	0.085	0.141
	M-coumaric acid	1.004	2.175	0.358	1.897	1.270	2.083	3.153	2.324
	undefined 1	2.694	5.366	1.643	3.497	6.062	0.741	2.179	7.309
	undefined 2	1.027	3.027	1.284	1.290	0.284	1.970	1.131	9.037
	Cinamic acid	2.970	3.499	2.499	4.613	1.431	3.099	1.504	3.292
	Luteolin	0.268	1.883	0.282	0.335	0.579	1.660	0.365	0.375
	undefined 3	0.656	0.610	0.401	0.402	1.427	0.628	0.433	0.431
	Apigenin	0.101	0.210	0.265	0.319	0.073	0.267	0.412	0.245

Table A.14. Individual phenolic compounds of EVOOs in 10th month

		olive oil samples							
Month 10	**phenolic compounds**	**aro**	**ero**	**bro**	**oro**	**are**	**ere**	**bre**	**ore**
	Hydroxytyrosol	2.037	1.981	1.050	3.043	0.699	1.248	0.865	1.759
	Chlorogenic acid	0.006	0.015	0.000	0.000	0.008	0.007	0.000	0.000
	Tyrosol	1.634	1.023	2.973	4.442	0.328	0.606	2.512	2.166
	4 Hydroxybenzoic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4 Hydroxyphenylacetic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Caffeic acid	0.048	0.144	0.125	0.154	0.047	0.068	0.084	0.058
	Vanilic acid	0.417	1.171	0.852	0.404	0.305	0.529	0.638	0.039
	Syringic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Vanilin	0.170	2.079	0.644	1.767	0.228	1.126	1.446	1.414
	P-coumaric acid	1.008	1.361	3.067	3.528	1.634	1.241	3.327	2.032
	Ferulic acid	0.072	0.058	0.065	0.247	0.043	0.066	0.108	0.217
	M-coumaric acid	1.014	1.085	2.906	1.807	1.064	1.545	4.149	1.478
	undefined 1	1.708	2.628	1.551	2.257	4.295	4.531	2.271	5.633
	undefined 2	0.574	1.588	0.964	5.453	0.827	0.883	4.930	1.755
	Cinamic acid	2.243	2.124	2.053	3.548	2.674	0.805	3.502	3.888
	Luteolin	0.358	0.235	0.196	0.338	0.725	0.860	0.136	0.389
	undefined 3	0.219	0.434	0.323	0.872	0.524	0.349	0.388	0.572
	Apigenin	0.093	0.174	0.313	0.545	0.086	0.128	0.289	0.170

Table A.15. Individual phenolic compounds of EVOOs in 14th month

		olive oil samples							
Month 14	**phenolic compounds**	**aro**	**ero**	**bro**	**oro**	**are**	**ere**	**bre**	**ore**
	Hydroxytyrosol	2.892	1.580	0.673	2.930	0.505	1.405	0.623	1.908
	Chlorogenic acid	0.003	0.005	0.000	0.000	0.004	0.005	0.000	0.000
	Tyrosol	1.934	1.222	2.536	4.650	0.275	0.492	2.356	2.274
	4 Hydroxybenzoic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4 Hydroxyphenylacetic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Caffeic acid	0.025	0.092	0.096	0.127	0.029	0.053	0.065	0.043
	Vanilic acid	0.350	0.945	0.541	0.303	0.267	0.431	0.471	0.027
	Syringic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Vanilin	0.112	1.860	0.451	1.421	0.187	0.992	1.257	1.385
	P-coumaric acid	0.870	1.013	2.563	3.290	1.342	1.031	3.287	2.01
	Ferulic acid	0.042	0.027	0.034	0.196	0.0354	0.0512	0.084	0.194
	M-coumaric acid	0.713	1.053	2.584	1.546	1.047	1.495	3.86	1.393
	undefined 1	1.683	2.479	1.459	2.032	3.893	4.489	1.63	5.532
	undefined 2	0.546	1.393	0.793	2.074	0.392	0.868	3.545	1.489
	Cinamic acid	2.002	2.307	1.659	3.238	2321	0.674	3.237	3.592
	Luteolin	0.216	0.201	0.129	0.286	0.541	0.741	0.106	0.368
	undefined 3	0.196	0.295	0.295	0.583	0.469	0.365	0.245	0.348
	Apigenin	0.061	0.198	0.258	0.482	0.0692	0.984	0.300	0.152

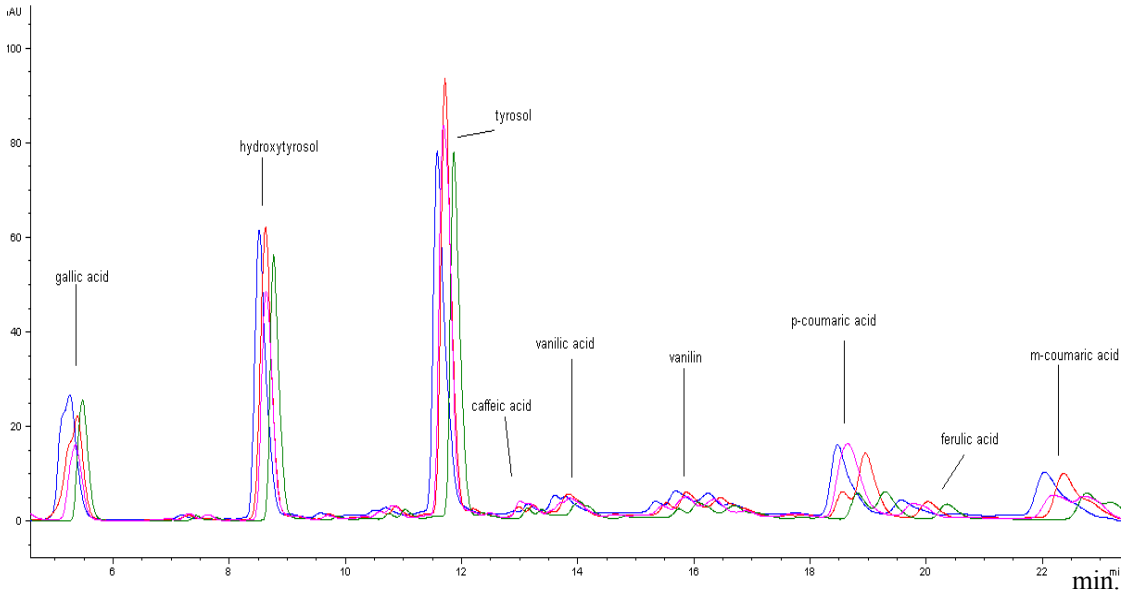

Figure A.1. HPLC chromatogram of Ortaklar olive oil sample stored at room temperature between 0 and 24 minutes, at 280 nm (— 4th month, — 7th month, —10th month, — 14th month)

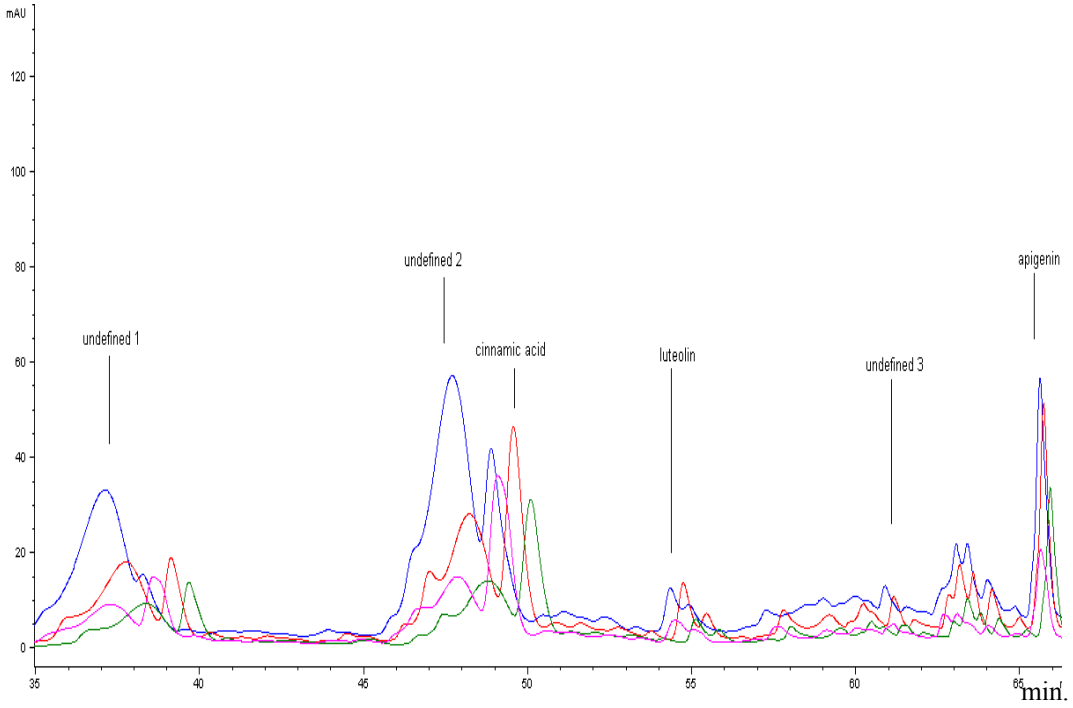

Figure A.2. HPLC chromatogram of Ortaklar olive oil sample stored at room temperature between 35 and 66 minutes, at 280 nm (— 4th month, — 7th month, —10th month, — 14th month)

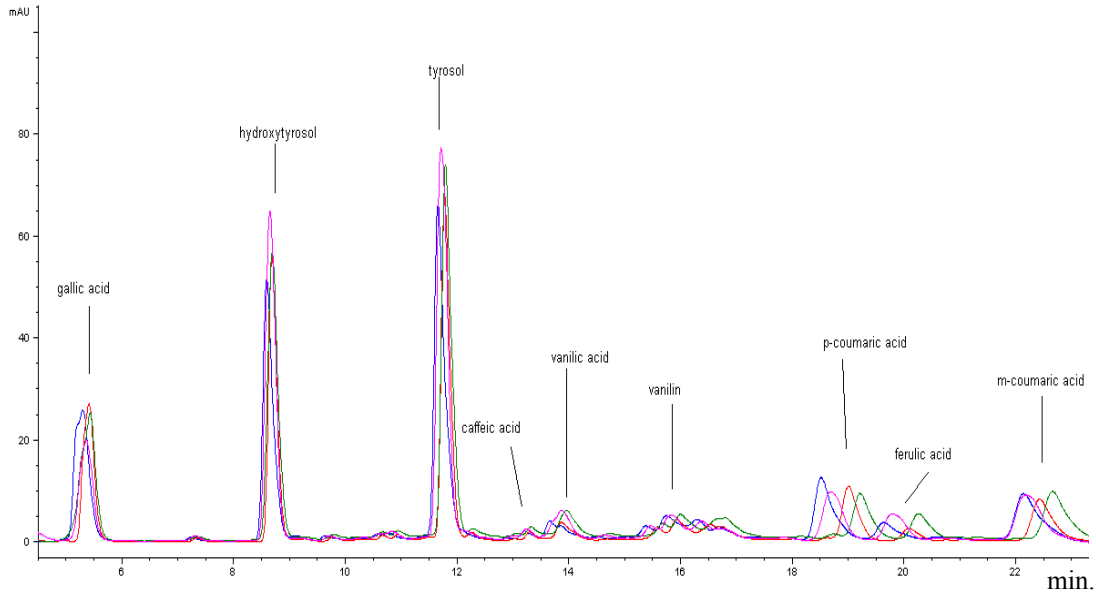

Figure A.3. HPLC chromatogram of Ortaklar olive oil sample stored at refrigerator temperature between 0 and 24 minutes, at 280 nm (— 4th month, — 7th month, —10th month, — 14th month)

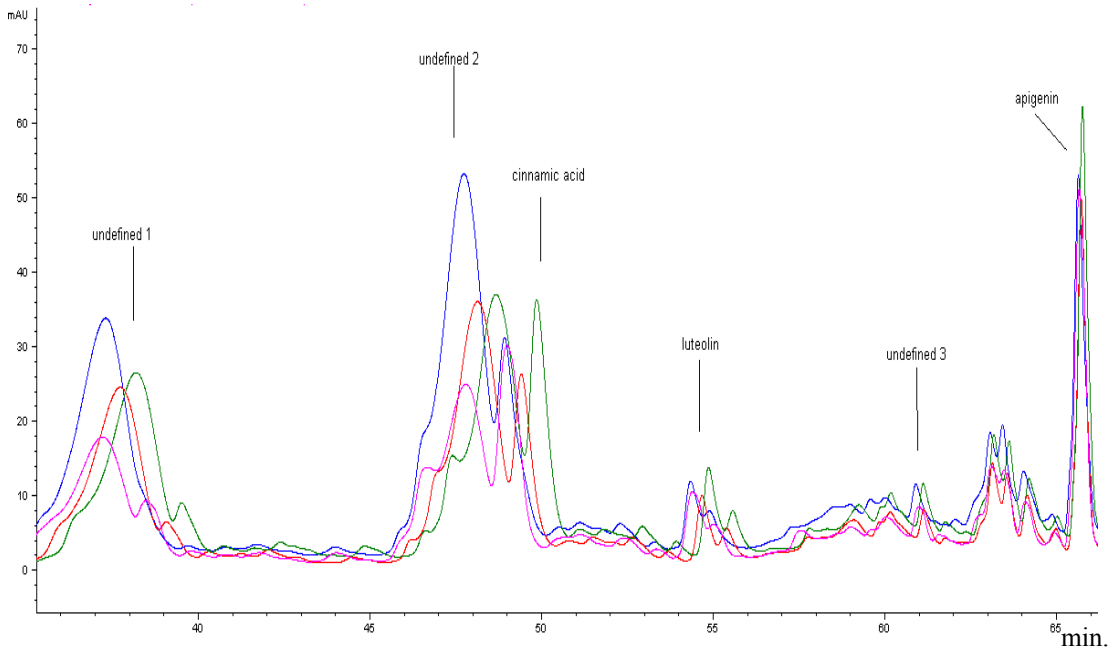

Figure A.4. HPLC chromatogram of Ortaklar olive oil sample stored at refrigerator temperature between 35 and 65 minutes, at 280 (— 4th month, — 7th month, —10th month, — 14th month)

Table A.16. PVs values in Erk and AE olive oils during 12- month storage

time (months)	PV	
	Erk	AE
1	23.57 ± 0.00	10.07 ± 0.00
4	23.71 ± 0.10	11.07 ± 0.32
7	24.7 ± 0.04	12.91 ± 0.23
10	26.26 ± 0.31	13.92 ± 1.00
12	32.06 ± 0.10	20.18 ± 0.04

Table A.17. K232 values in Erk and AE olive oils during 12-month storage

time (months)	K232	
	Erk	AE
1	1.66 ± 0.03	1.70 ± 0.03
4	1.90 ± 0.01	1.89 ± 0.00
7	2.17 ± 0.00	2.18 ± 0.00
10	2.18 ± 0.00	2.18 ± 0.00
12	2.62 ± 0.00	2.60 ± 0.43

Table A.18.. K270 values in Erk and AE olive oils during 12-month storage

time (months)	K270	
	Erk	AE
1	0.13 ± 0.00	0.17 ± 0.04
4	0.14 ± 0.00	0.18 ± 0.02
7	0.16 ± 0.00	0.20 ± 0.00
10	0.17 ± 0.00	0.20 ± 0.00
12	0.20 ± 0.00	0.22 ± 0.00

Table A.19. FFA values in Erk and AE olive oils during 12-month storage

time (months)	FFA	
	Erk	AE
1	0.18	0.79
4	0.32	0.8
7	0.48	0.81
10	0.5	0.83
12	0.54	0.87

Table A.20. TPC values in Erk and AE olive oils during 12-month storage

time (months)	TPC	
	Erk	AE
1	178.79 ± 4.84	61.06 ± 30.07
4	172.29 ± 2.08	40.30 ± 1.95
7	157.81 ± 3.25	28.92 ± 3.45
10	154.97 ± 6.16	19.22 ± 1.60
12	142.93 ± 9.52	13.78 ± 1.95

Table A.21. Color coordinates of Erk and AE olive oils during 12-month storage

time (months)	L *		a *		b *	
	Erk	AE	Erk	AE	Erk	AE
1	23.58 ± 0.04	24.88± 0.06	-0.72 ± 0.05	-1.89 ± 0.02	11.78 ± 0.07	11.92 ± 0.11
4	22.54 ± 0.38	24.26± 0.25	-0.15± 0.06	-1.69 ± 0.26	9.28 ± 0.34	11.48 ± 0.39
7	22.86± 0.26	25.32± 0.30	-0.16 ± 0.08	-1.76 ± 0.09	10.45 ± 0.42	10.68 ± 0.30
10	23.31± 0.05	23.16± 0.12	-0.52 ± 0.01	-0.03 ± 0.00	11.22 ± 0.04	9.65 ± 0.03
12	23.49± 0.09	24.39± 0.02	-0.62 ± 0.01	-1.72 ± 0.05	11.45 ± 0.22	10.91 ± 0.07

Table A.22. Fatty acid profiles of (% total fatty acids) of Erk and AE olive oil samples during 12-month storage

time (months)	olive oil samples	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
1	**Erk**	0.018±0.00	13.24±0.03	0.15±0.00	2.74±0.00	68.10±0.03	14.47±0.03	0.73±0.00	0.41±0.02	0.11±0.02
	AE	0.019±0.00	14.84±0.01	1.12±0.00	2.12±0.02	67.40±0.06	13.23±0.03	0.65±0.01	0.41±0.01	0.15±0.01
4	**Erk**	0.014±0.00	13.00±0.19	0.12±0.00	2.69±0.03	68.72±0.24	14.21±0.09	0.70±0.00	0.40±0.01	0.10±0.00
	AE	0.016±0.00	14.06±0.06	1.01±0.06	2.08±0.01	68.02±0.17	13.67±0.24	0.60±0.00	0.40±0.01	0.13±0.00
7	**Erk**	0.012±0.00	12.72±0.07	0.13±0.00	2.82±0.02	68.73±0.23	14.31±0.20	0.69±0.01	0.42±0.00	0.12±0.00
	AE	0.018±0.00	14.20±0.18	1.02±0.00	2.02±0.01	68.02±0.17	12.71±0.43	0.53±0.00	0.36±0.00	0.14±0.00
10	**Erk**	0.011±0.00	13.10±0.07	0.10±0.00	2.62±0.06	69.30±0.25	13.67±0.15	0.64±0.00	0.39±0.00	0.11±0.00
	AE	0.017±0.00	14.00±0.18	1.06±0.01	2.03±0.03	69.37±0.37	12.44±0.16	0.56±0.00	0.36±0.00	0.12±0.00
12	**Erk**	0.011±0.00	12.68±0.28	0.12±0.00	2.72±0.02	69.97±0.81	13.35±0.5	0.64±0.02	0.37±0.01	0.11±0.00
	AE	0.015±0.00	14.68±0.05	1.04±0.00	2.05±0.00	68.33±0.04	12.75±0.05	0.59±0.00	0.39±0.00	0.12±0.00

Table A.23. PV values of South (S) and North (N) Aegean EVOO samples during oxidation

	PVs	
Time (days)	**North (N)**	**South (S)**
1	19.18 ± 0.003	19.81 ± 0.26
2	44.19 ± 1.47	46.56 ± 1.96
3	38.23 ± 1.37	41.77 ± 1.00
4	35.78 ± 2.40	31.08 ± 0.62
5	48.17 ± 2.88	53.28 ± 1.33
8	39.00 ± 2.92	37.34 ± 0.18
9	47.05 ± 1.15	47.6 ± 3.17
10	67.42 ± 1.54	77.12 ± 3.42
11	69.19 ± 12.52	68.35 ± 0.25
12	83.68 ± 0.24	86.74 ± 7.25
15	89.72 ± 2.53	102.03 ± 1.75
16	108.66 ± 4.39	125.34 ± 1.41
17	137.99 ± 3.21	142.05 ± 5.05
19	147.06 ± 2.15	154.04 ± 0.31

Table A.24. K232 values of South (S) and North (N) Aegean EVOO samples during oxidation

	K232 Values	
Time (days)	**North (N)**	**South (S)**
1	1.48 ± 0.24	1.61 ± 0.23
2	1.70 ± 0.29	1.82 ± 0.08
3	1.68 ± 0.12	1.52 ± 0.40
4	1.88 ± 0.23	2.16 ± 0.34
5	1.70 ± 0.19	1.59 ± 0.04
8	2.83 ± 0.15	2.70 ± 0.16
9	2.33 ± 0.41	2.15 ± 0.08
10	1.95 ± 0.16	2.17 ± 0.18
11	2.87 ± 0.02	2.76 ± 0.15
12	2.33 ± 0.18	2.40 ± 0.58
15	2.38 ± 0.09	2.31 ± 0.08
16	3.21 ± 0.07	3.18 ± 0.10
17	3.37 ± 0.00	3.29 ± 0.10
19	3.40 ± 0.04	3.48 ± 0.06

Table A.25. K270 values of South (S) and North (N) Aegean EVOO samples during oxidation

	K270 Values	
Time (days)	**North (N)**	**South (S)**
1	0.21 ± 0.01	0.20 ± 0.01
2	0.30 ± 0.01	0.27 ± 0.009
3	0.11 ± 0.001	0.11 ± 0.02
4	0.17 ± 0.0007	0.11 ± 0.02
5	0.11 ± 0.006	0.11 ± 0.05
8	0.31 ± 0.04	0.24 ± 0.02
9	0.27 ± 0.12	0.17 ± 0.02
10	0.12 ± 0.02	0.16 ± 0.002
11	0.13 ± 0.004	0.22 ± 0.02
12	0.27 ± 0.009	0.27 ± 0.02
15	0.26 ± 0.01	0.29 ± 0.009
16	0.27 ± 0.003	0.32 ± 0.01
17	0.29 ± 0.004	0.32 ± 0.01
19	0.36 ± 0.009	0.33 ± 0.01

Table A.26. PVs of oil samples during 126-day storage time

oil samples	**time (days)**						
	1	**21**	**42**	**63**	**84**	**105**	**126**
oro	14.15	15.05	15.22	16.21	16.54	16.75	15.99
fro	3.01	3.74	4.52	5.13	5.52	6.02	5.39
5ro	2.81	3.40	4.00	4.53	5.12	5.55	4.59
10ro	2.54	2.73	2.86	3.07	3.32	3.70	5.52
15ro	2.34	2.53	2.73	3.27	3.40	3.53	4.92
ore	14.15	14.21	14.65	15.65	15.74	15.92	16.99
fre	3.01	3.34	3.80	4.14	4.52	5.01	6.73
5re	2.81	3.06	3.33	3.73	3.92	4.20	6.12
10re	2.54	2.73	2.86	3.07	3.32	3.75	5.52
15re	2.34	2.53	2.73	3.27	3.40	3.53	4.92

Table A.27. K232 values of oil samples during 126-day storage time

oil samples	time (days)						
	1	21	42	63	84	105	126
oro	1.99±0.00	2.01±0.01	2.02±0.00	2.02±0.00	2.03±0.00	2.03±0.00	2.03±0.00
fro	1.18±0.01	1.21±0.00	1.24±0.00	1.28±0.00	1.39±0.00	1.58±0.00	1.88±0.00
5ro	1.15±0.00	1.18±0.01	1.19±0.00	1.20±0.00	1.20±0.00	1.20±0.00	1.21±0.00
10ro	1.15±0.00	1.17±0.00	1.18±0.00	1.18±0.00	1.19±0.00	1.19±0.00	1.2±0.00
15ro	1.14±0.01	1.15±0.00	1.16±0.00	1.17±0.00	1.17±0.00	1.17±0.00	1.77±0.00
ore	1.99±0.00	1.99±0.01	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
fre	1.18±0.01	1.17±0.00	1.20±0.00	1.21±0.00	1.25±0.00	1.41±0.00	1.81±0.00
5re	1.15±0.00	1.15±0.00	1.15±0.00	1.16±0.00	1.16±0.00	1.16±0.00	1.17±0.00
10re	1.15±0.00	1.15±0.00	1.15±0.00	1.15±0.00	1.15±0.00	1.15±0.00	1.16±0.00
15re	1.14±0.01	1.14±0.00	1.14±0.00	1.15±0.00	1.15±0.00	1.15±0.00	1.15±0.00

Table A.28. K270 values of oil samples during 126-day storage time

oil samples	time (days)						
	1	21	42	63	84	105	126
oro	0.18±0.00	0.19±0.00	0.19±0.00	0.19±0.00	0.19±0.00	0.2±0.00	0.2±0.00
fro	0.2±0.02	0.21± 0.01	0.22±0.01	0.25±0.00	0.27±0.00	0.3±0.00	0.32±0.00
5ro	0.20±0.00	0.21±0.00	0.22±0.00	0.23±0.00	0.23±0.00	0.23±0.00	0.23±0.00
10ro	0.17±0.00	0.18±0.00	0.19±0.00	0.19±0.00	0.19±0.00	0.2±0.00	0.19±0.00
15ro	0.16±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.19±0.00	0.19±0.00	0.19±0.00
ore	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00
fre	0.2±0.02	0.2±0.00	0.2±0.00	0.21±0.00	0.22±0.00	0.23±0.00	0.24±0.00
5re	0.20±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00
10re	0.17±0.00	0.17±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00	0.18±0.00
15re	0.16±0.00	0.17±0.00	0.17±0.00	0.17±0.00	0.18±0.00	0.18±0.00	0.18±0.00

Table A.29. TPC values of oil samples during 126-day storage time

oil samples	time (days)						
	1	21	42	63	84	105	126
oro	114.57±1.41	111.31±2.16	103.83±5.46	99.3±2.45	96.15±5.54	92.08±4.25	90.74±3.44
fro	81.23±0.81	75.14±5.95	71.95±5.68	67.93±2.45	62.77±2.94	57.03±5.53	54.8±5.36
5ro	85.02±2.41	79.77±1.57	76.12±1.57	74.18±5.72	71.37±2.16	67.8±3.73	64.68±2.94
10ro	92.1±4.25	87.37±2.94	84.06±1.63	81.48±1.63	76.19±3.87	73.70±2.23	68.03±2.83
15ro	96.01±1.39	92.79±1.60	86.29±2.41	82.25±4.91	78.51±2.23	74.19±0.84	70.88±0.84
ore	114.57±1.41	113.78±2.08	108.77±1.57	107.78±4.07	106.47±4.25	103.67±3.95	100.97±2.16
fre	81.23±0.18	79.19±4.09	75.55±4.38	72.96±0.81	69.84±0.81	66.69±2.83	65.94±2.94
5re	85.02±2.14	90.78±3.75	87.47±4.09	86.09±1.69	82.79±1.63	80.45±1.63	79.14±1.41
10re	92.1±4.25	90.83±2.53	89.37±2.53	86.65±3.26	85.31±3.04	82.39±1.68	78.35±6.74
15re	96.01±1.39	94.5±4.55	92.14±4.91	89.78±1.69	87.23±0.84	83.25±3.39	81.62±9.94

Table A.30. Fatty acid profiles of (% total fatty acids) of oil samples during 126-day storage

time (days)	oil samples	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
1	oro	0.026	11.970	0.815	3.224	70.427	11.481	0.577	0.367	0.120
	fro	0.039	5.660	0.089	6.579	17.949	14.403	56.278	0.282	0.115
	5ro	0.047	5.732	0.120	6.386	21.623	14.904	52.302	0.215	0.107
	10ro	0.080	6.654	0.176	6.225	24.453	14.886	49.804	0.604	0.118
	15ro	0.062	6.783	0.206	4.857	29.850	13.389	46.873	0.235	0.089
	ore	0.026	11.970	0.815	3.224	70.427	11.481	0.577	0.367	0.120
	fre	0.039	5.660	0.089	6.579	17.949	14.403	56.278	0.282	0.115
	5re	0.047	5.732	0.120	6.386	21.623	14.904	52.302	0.215	0.107
	10re	0.080	6.654	0.176	6.225	24.453	14.886	49.804	0.604	0.118
	15re	0.062	6.783	0.206	4.857	29.850	13.389	46.873	0.235	0.089
21	oro	0.022	11.460	0.649	2.981	72.930	10.296	0.554	0.324	0.095
	fro	0.038	5.342	0.087	5.625	20.833	13.782	56.198	0.264	0.101
	5ro	0.041	6.221	0.103	5.747	22.808	14.626	52.183	0.246	0.080
	10ro	0.047	6.711	0.143	5.745	27.090	14.128	49.624	0.260	0.092
	15ro	0.062	6.783	0.206	4.857	29.850	13.389	46.139	0.235	0.089
	ore	0.022	11.544	0.631	2.940	73.187	10.135	0.564	0.314	0.084
	fre	0.040	5.595	0.083	5.849	20.781	14.447	55.724	0.265	0.089
	5re	0.073	6.581	0.113	5.828	22.309	14.444	53.332	0.301	0.084
	10re	0.057	6.722	0.172	5.781	27.172	13.984	49.566	0.268	0.094
	15re	0.076	6.501	0.204	5.261	29.198	13.395	47.031	0.320	0.092
42	oro	0.022	12.263	0.618	3.012	72.055	10.673	0.547	0.269	0.082
	fro	0.039	5.432	0.088	5.748	21.522	13.610	54.885	0.257	0.087
	5ro	0.041	6.294	0.098	5.825	22.795	14.830	51.904	0.238	0.075
	10ro	0.046	6.768	0.140	5.626	27.382	14.067	49.324	0.252	0.078
	15ro	0.057	7.038	0.211	4.879	30.044	13.460	45.967	0.232	0.083

(cont. on next page)

Table A.30. (cont.) Fatty acid profiles of (% total fatty acids) of oil samples during 126-day storage

	ore	0.021	11.285	0.607	2.891	75.318	8.460
	fre	0.049	5.916	0.093	5.807	20.563	14.294
	5re	0.070	6.402	0.094	5.671	23.076	14.156
	10re	0.039	6.510	0.158	5.356	27.375	14.032
	15re	0.054	4.830	0.169	4.399	40.007	11.401
63	oro	0.017	12.770	0.687	3.300	71.457	10.783
	fro	0.038	5.402	0.087	5.749	21.503	13.606
	5ro	0.050	5.947	0.090	6.030	21.086	14.152
	10ro	0.036	6.376	0.136	5.869	23.606	13.981
	15ro	0.039	6.761	0.151	5.746	26.261	13.789
	ore	0.017	12.910	0.693	3.395	71.506	10.480
	fre	0.040	5.092	0.056	6.054	18.308	14.506
	5re	0.043	5.706	0.094	5.721	21.200	14.090
	10re	0.036	6.365	0.135	5.849	23.671	13.983
	15re	0.035	6.754	0.164	5.669	26.178	13.803
84	oro	0.016	12.250	0.676	3.275	72.307	10.573
	fro	0.028	3.883	0.064	4.010	23.605	13.261
	5ro	0.042	5.598	0.075	5.562	22.715	13.506
	10ro	0.034	6.214	0.122	5.776	23.915	14.281
	15ro	0.035	6.648	0.135	5.762	26.958	13.953
	ore	0.013	12.689	0.664	3.305	73.125	9.270
	fre	0.039	5.017	0.057	6.094	17.837	14.928
	5re	0.041	5.249	0.095	5.465	20.548	14.895
	10re	0.033	6.188	0.140	5.649	24.675	13.674
	15re	0.032	6.467	0.163	5.700	25.892	14.017

(cont. on next page)

Table A.30. (cont.) Fatty acid profiles of (% total fatty acids) of oil samples during 126-day storage

105	oro	0.019	13.016	0.688	3.435	71.469	10.424
	fro	0.027	3.756	0.058	3.926	22.408	13.282
	5ro	0.039	5.528	0.069	5.482	23.366	13.307
	10ro	0.031	5.648	0.116	5.477	25.864	13.991
	15ro	0.033	6.056	0.124	5.664	27.912	13.882
	fre	0.030	4.586	0.045	5.465	21.057	13.525
	5re	0.035	4.782	0.080	5.365	21.808	14.669
	10re	0.027	5.678	0.133	5.415	26.912	13.286
	15re	0.026	5.877	0.142	5.393	30.230	13.647
126	oro	0.018	12.689	0.637	3.280	71.953	10.512
	fro	0.027	3.453	0.040	3.816	22.137	13.922
	5ro	0.036	5.413	0.064	5.340	23.792	13.199
	10ro	0.040	6.286	0.116	5.751	23.617	14.054
	15ro	0.024	5.849	0.115	5.447	30.660	13.310
	ore	0.015	13.095	0.682	3.466	72.708	9.204
	fre	0.026	4.259	0.044	5.223	21.985	13.366
	5re	0.030	4.617	0.072	5.236	22.511	14.487
	10re	0.027	5.631	0.121	4.676	29.907	11.370
	15re	0.022	5.423	0.121	5.363	31.322	13.304

APPENDIX B

STANDARD CALIBRATION CURVES FOR PHENOLIC COMPOUNDS

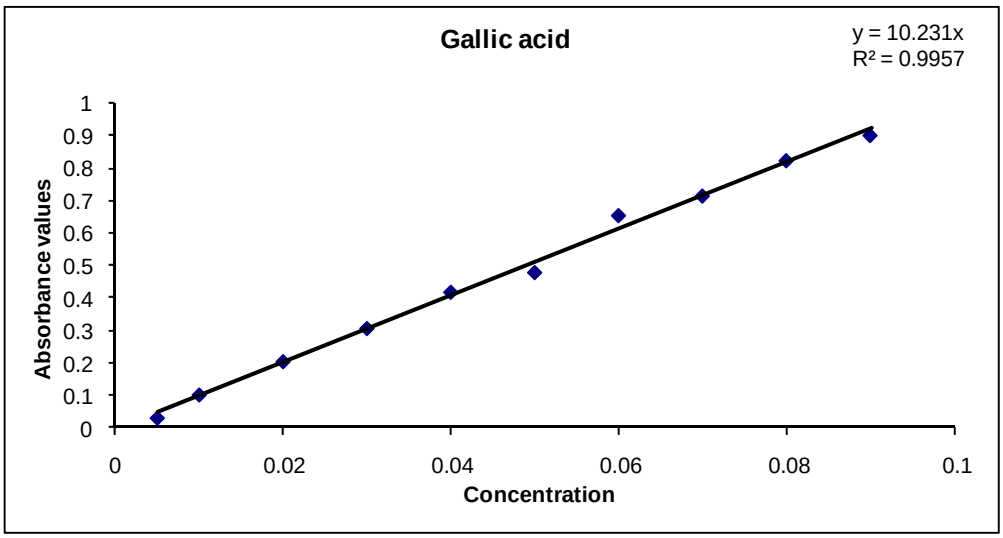

Figure B.1. Standard calibration curve for gallic acid

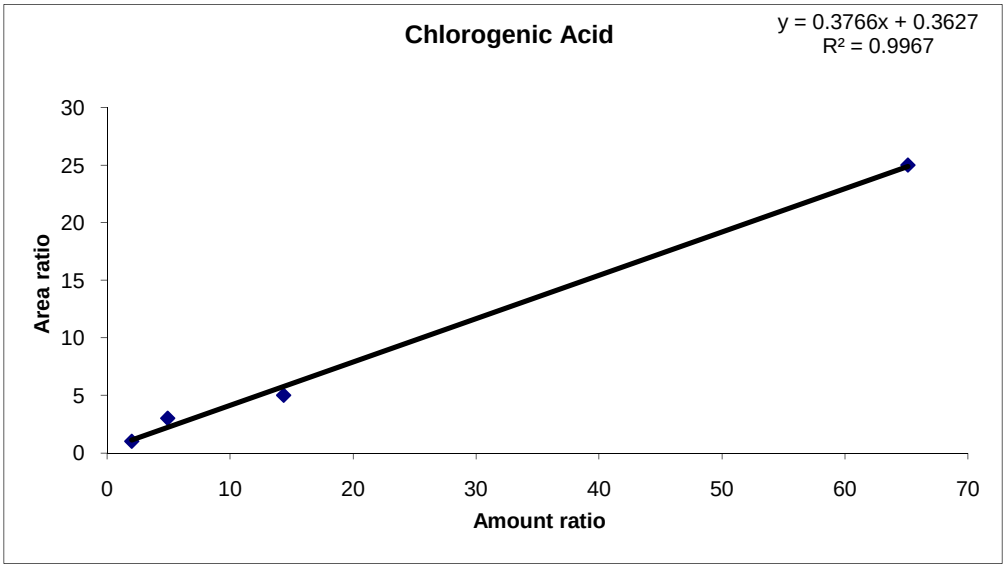

Figure B.2. Standard calibration curve for chlorogenic acid

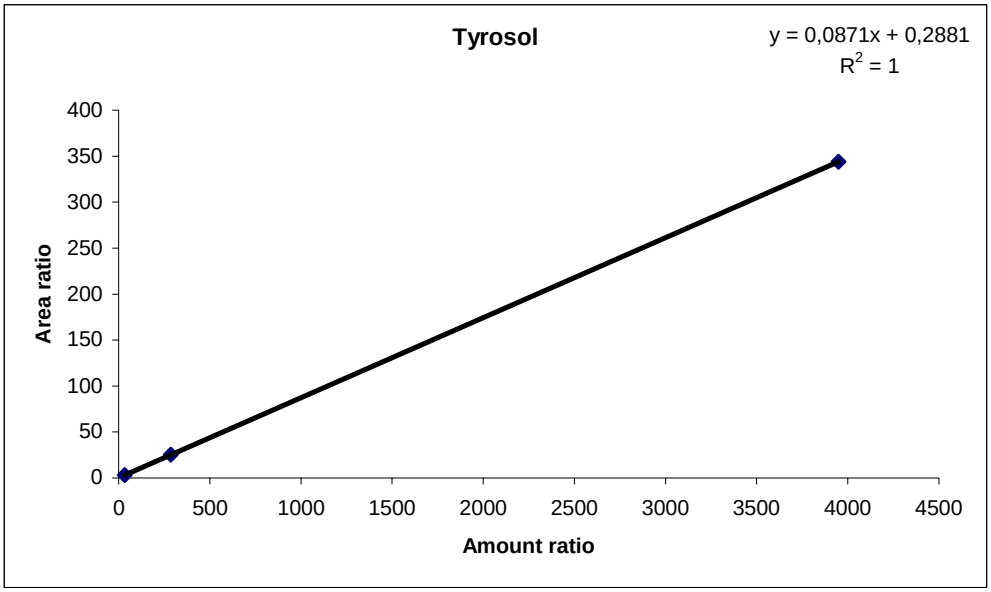

Figure B.3. Standard calibration curve for tyrosol

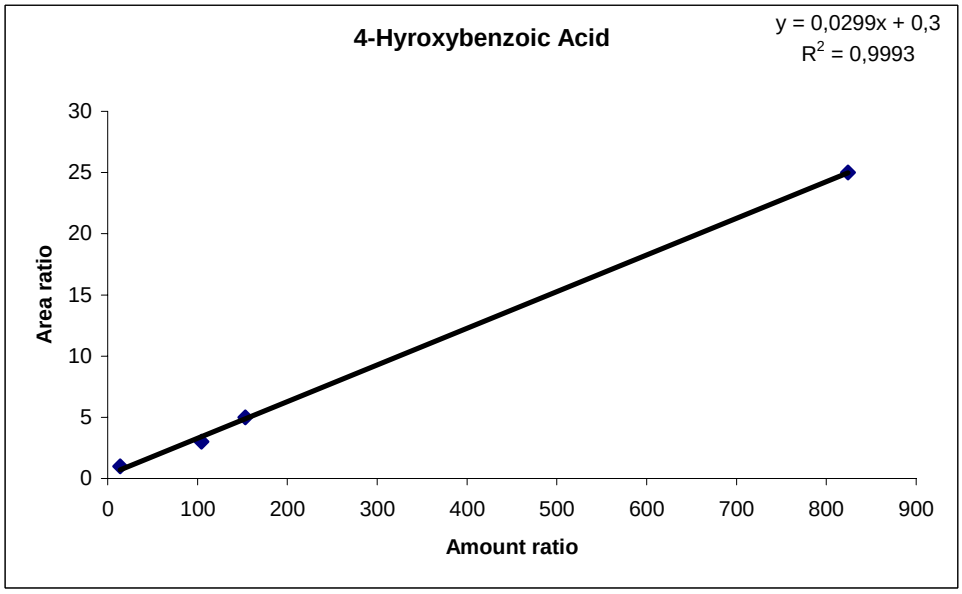

Figure B.4. Standard calibration curve for 4-hydroxybenzoic acid

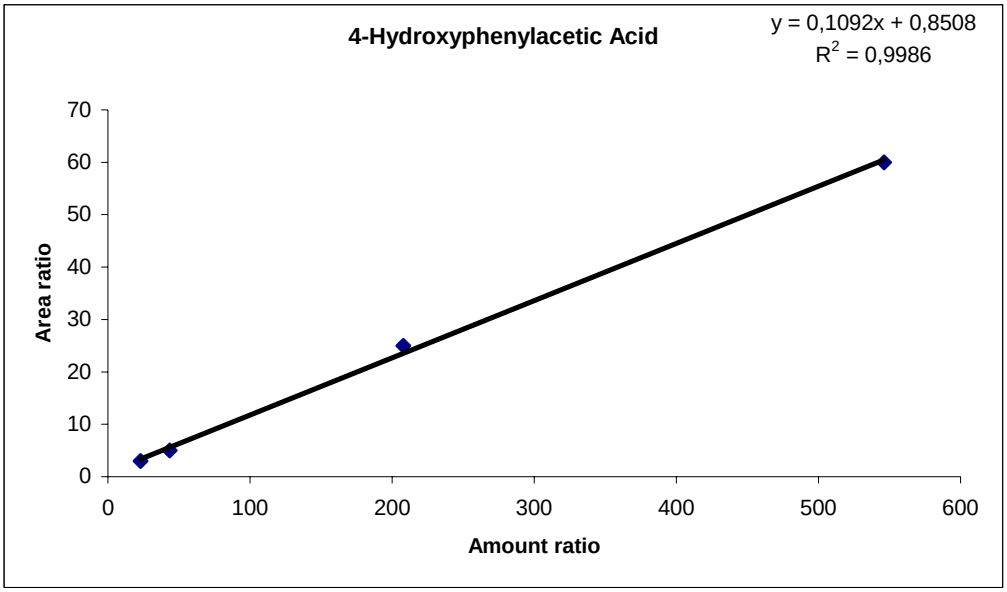

Figure B.5. Standard calibration curve for 4-hydroxyphenylacetic acid

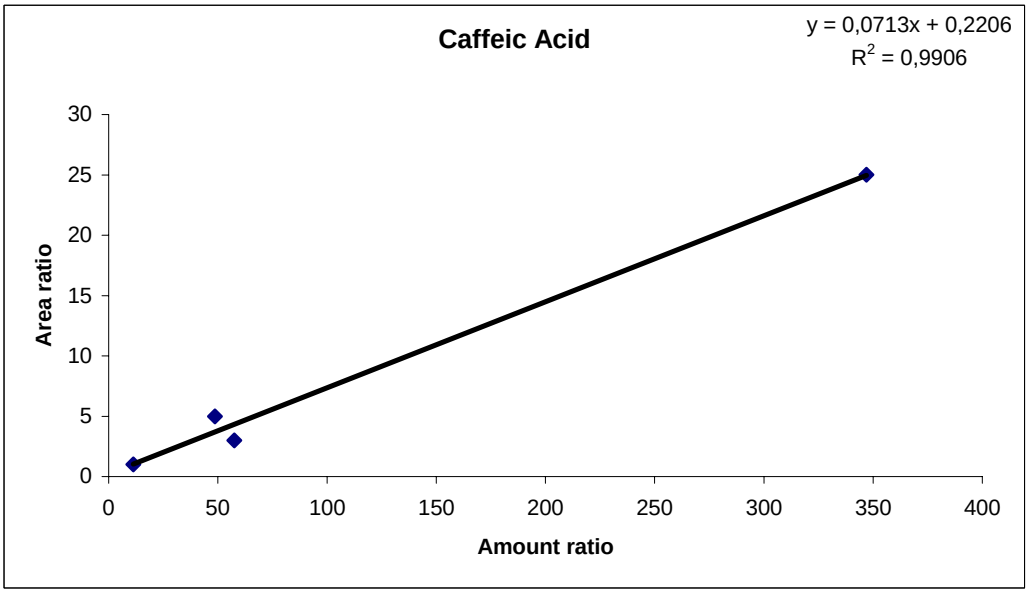

Figure B.6. Standard calibration curve for caffeic acid

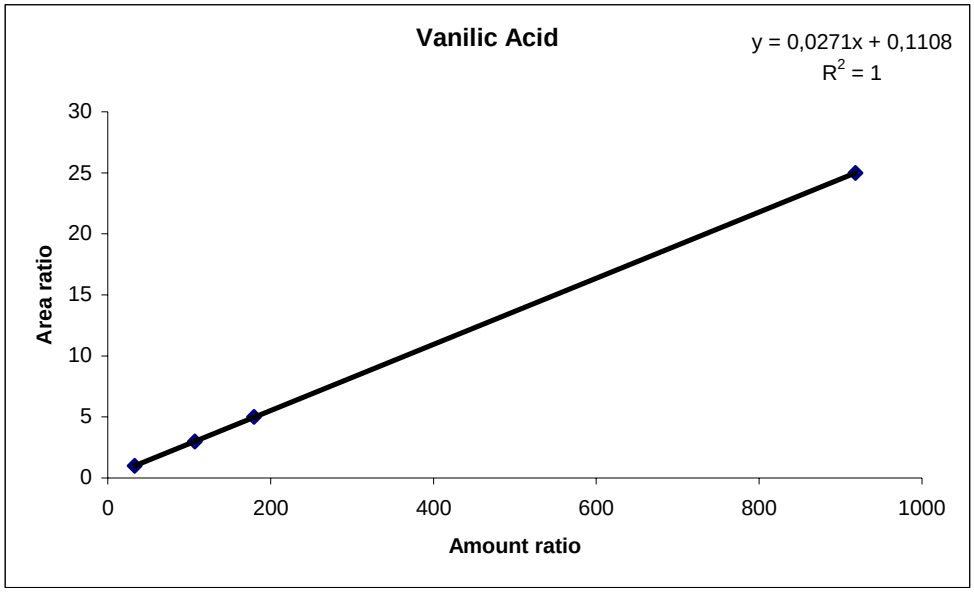

Figure B.7. Standard calibration curve for vanilic acid

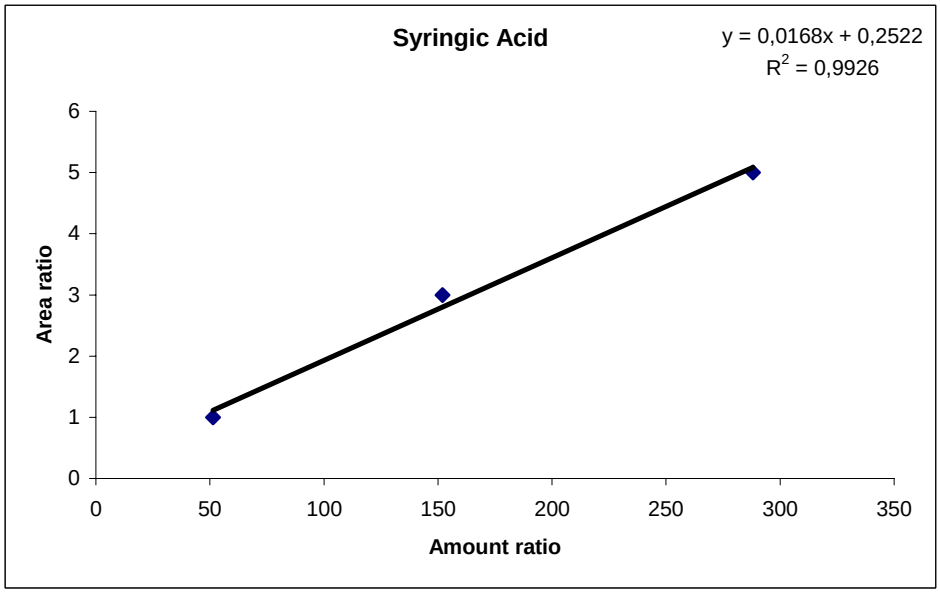

Figure B.8. Standard calibration curve for syringic acid

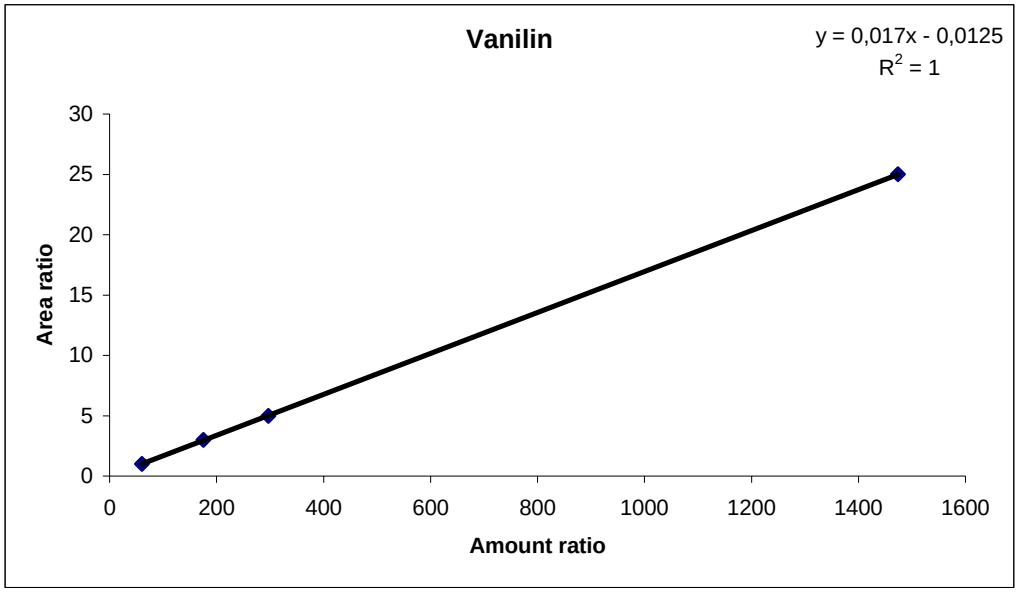

Figure B.9. Standard calibration curve for vanilin

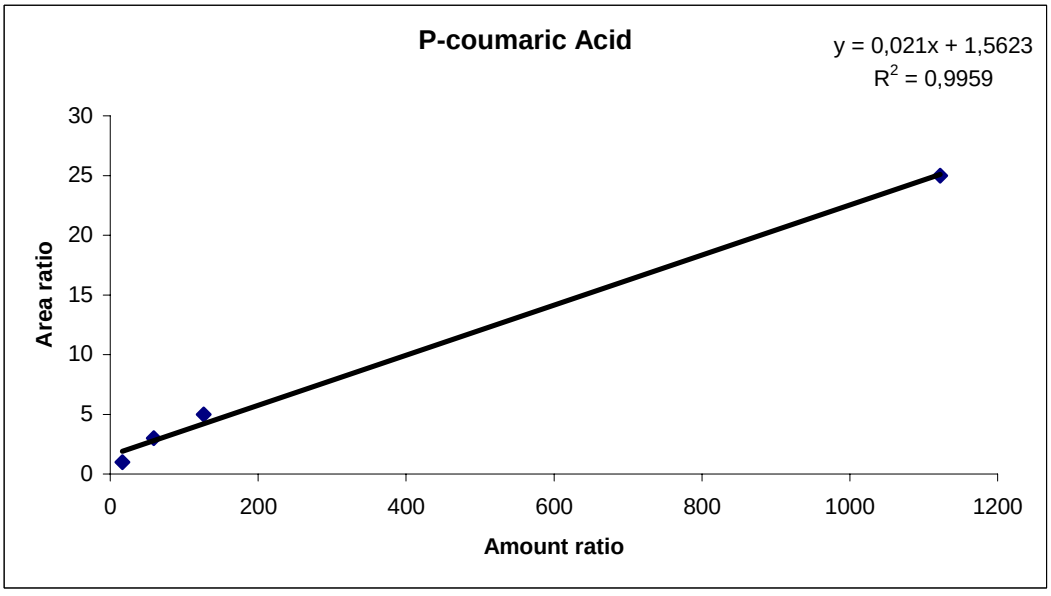

Figure B.10. Standard calibration curve for p-coumaric acid

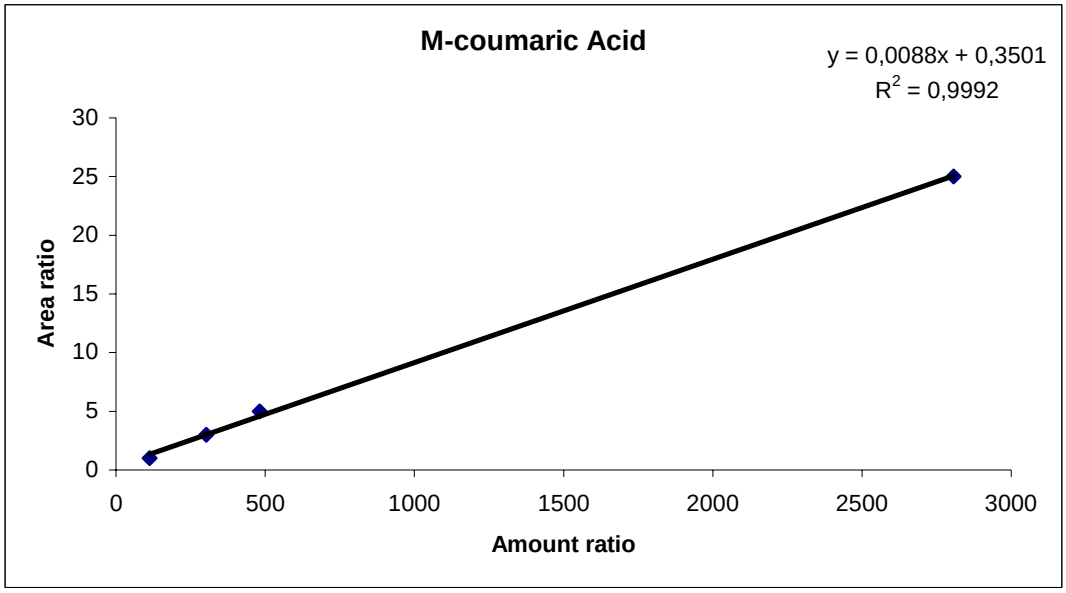

Figure B.11. Standard calibration curve for m-coumaric acid

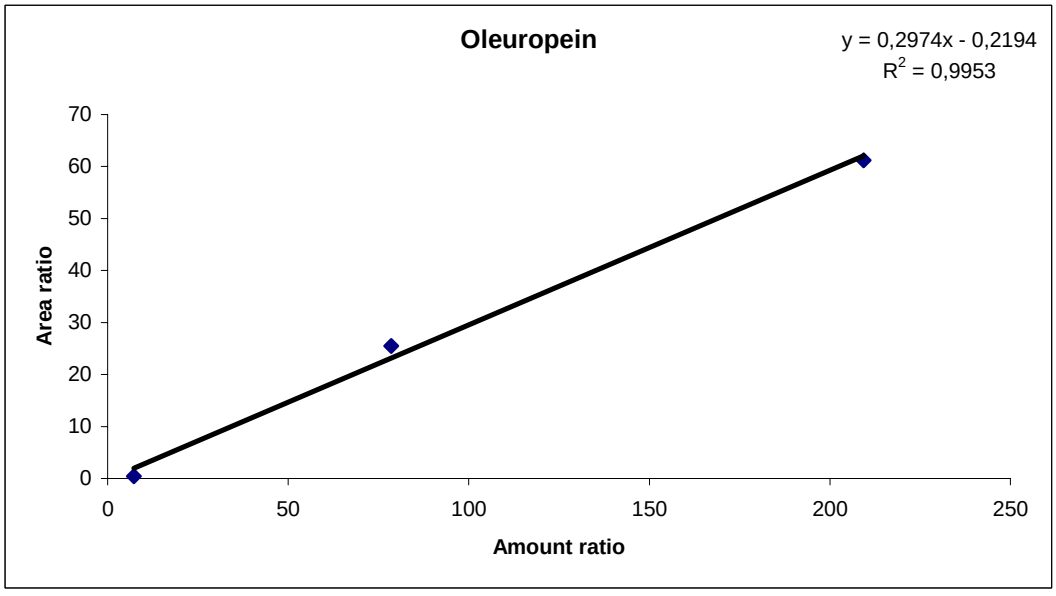

Figure B.12. Standard calibration curve for oleuropein

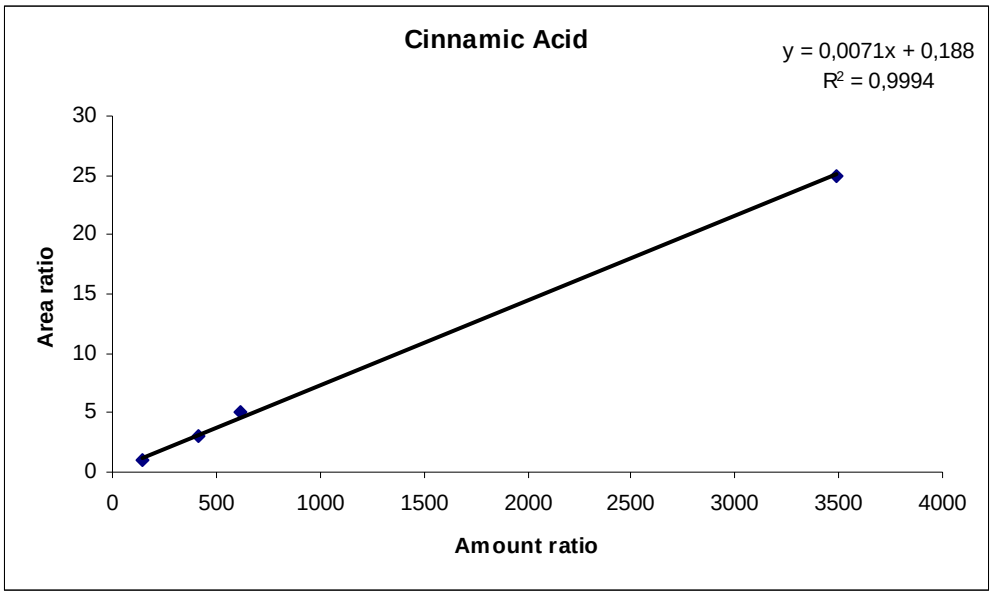

Figure B.13. Standard calibration curve for cinnamic acid

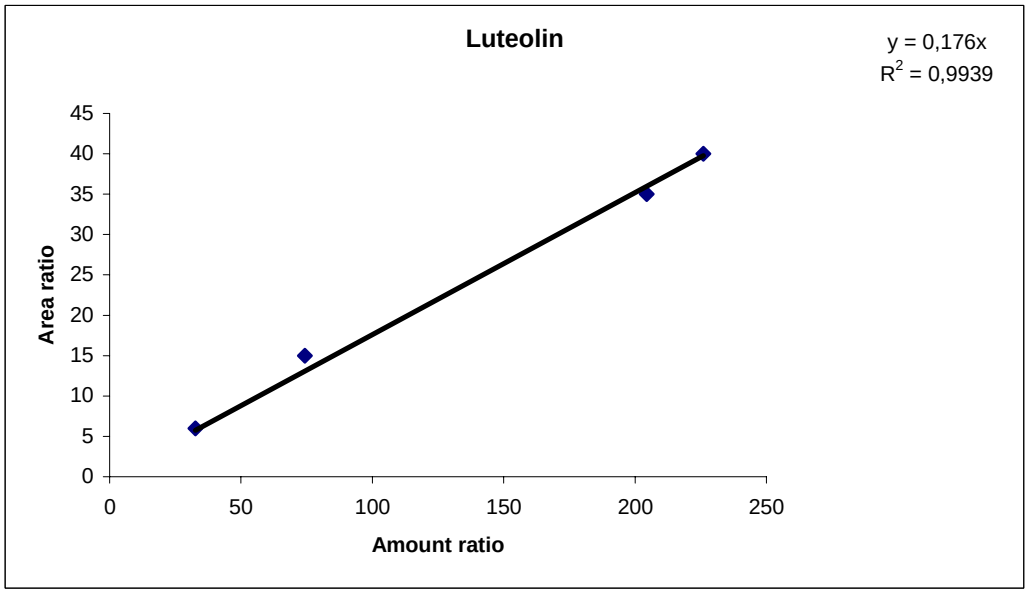

Figure B.14. Standard calibration curve for luteolin

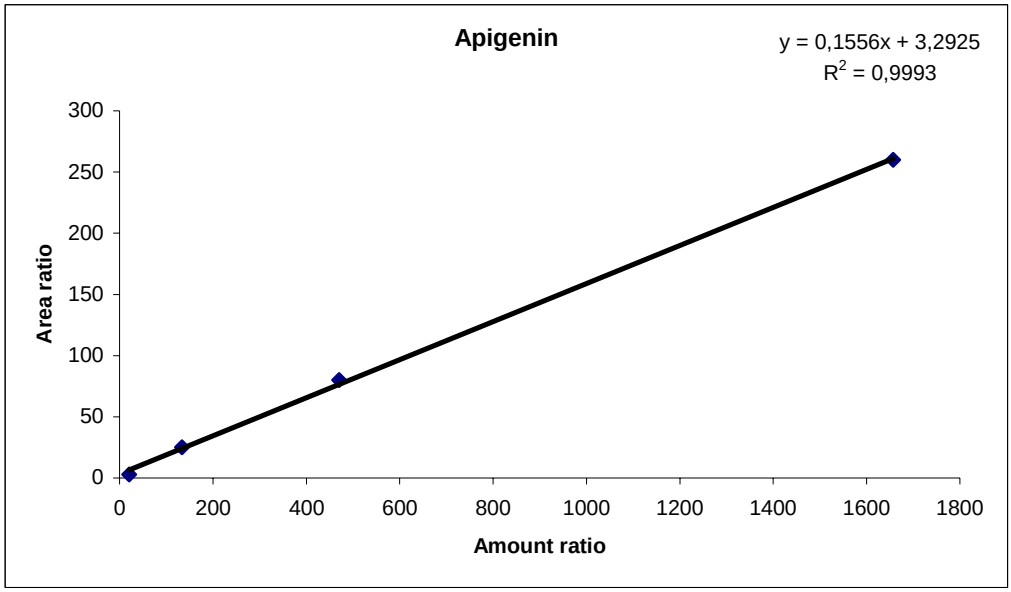

Figure B.15. Standard calibration curve for apigenin