# EFFECT OF DEEP-FRYING ON THE QUALITY PARAMETERS OF VEGETABLE OILS

**A Thesis Submitted to**
**the Graduate School of Engineering and Sciences of**
**Izmir Institute of Technology**
**in Partial Fulfillment of Requirement for Degree of**

**MASTER OF SCIENCE**

**in Food Engineering**

**by**
**Başak AVCI**

**December 2015**
**İZMİR**

We approve this thesis of **Başak AVCI**

**Examining Committee Members:**

**Prof. Dr. Figen TOKATLI**
Department of Food Engineering, Izmir Institute of Technology

**Prof. Dr. Durmuş ÖZDEMİR**
Department of Chemistry, Izmir Institute of Technology

**Assist. Prof. Dr. Fahri YEMİŞÇİOĞLU**
Department of Food Engineering, Ege University

**22 December 2015**

**Prof. Dr. Figen TOKATLI**
Supervisor, Department of Food Engineering, Izmir Institute of Technology

**Prof. Dr. Banu ÖZEN**
Co-Supervisor, Department of Food Engineering, Izmir Institute of Technology

**Prof. Dr. Ahmet YEMENİCİOĞLU**
Head of the Department of Food Engineering

**Prof. Dr. Bilge KARAÇALI**
Dean of the Graduate School of Engineering and Sciences

# ACKNOWLEDGEMENT

I wish to express my sincere gratitute to my supervisor, Prof. Dr. Figen TOKATLI for her guidance, support, encouragement and supervision during my study. I also wish to express my thanks to my co-supervisor Prof. Dr. Banu ÖZEN for her support and valuable comments.

I would like to thank my colleague Olusola Samuel JOLAYEMI and specialist İlknur ŞEN for their help and support. I would also like to thank to my friends Sinem ÜNEY, Esra KAÇAR and Gökçen KÖMEN for their kindness and support during my studies.

I would like to thank to Assist. Prof. Dr. Fahri YEMİŞÇİOĞLU and Orkide Küçükbay Inc. in İzmir for their help in the analysis of total polar compounds.

Lastly, I want to express my thankfulness to all my family members and my close friends Burcu KARAÖZ, Eylem DAĞAŞAR and Ayşe İŞLİ.

# ABSTRACT


## EFFECT OF DEEP-FRYING ON THE QUALITY PARAMETERS OF VEGETABLE OILS

It was aimed to determine the changes in several chemical parameters, fatty acid composition and phenolic profiles of different types of olive oils (extra virgin, virgin, blended, refined) and sunflower oil in the deep-frying process. In addition, UV and FTIR spectroscopic data were collected during frying and they (UV, FTIR and the combination of FTIR and UV) were analyzed with multivariate statistical techniques (PCA, OPLS-DA and OPLS) to classify the oil samples and predict the chemical parameters. A domestic deep-fat electrical fryer was used to fry french fries for 4 minutes at 180ºC ten times without oil replenishment and oil samples were removed after 1st, 2nd, 3rd, 4th, 5th, 7th and 10th frying.

Free fatty acid, peroxide value, $K_{232}$, $K_{270}$, total chloropyll and carotenoid contents, color, oxidative stability, total phenol content, total polar compounds, and fatty acid profiles of all oil samples and phenolics of extra virgin and virgin olive oils were determined. At the end of the tenth frying, no significant changes were detected in acidity, stability, and fatty acid compositions. Phenolic contents of extra virgin and virgin olive oils decreased after the fourth frying cycle.

According to PCA and OPLS-DA results, UV spectra was able to discriminate oil samples. Chemical parameters, except peroxide value, were predicted with high accuracy by spectroscopic data. The fatty acids were predicted successfully with FTIR data, while the total phenol content was predicted by UV data with a higher $R^2$ value. The combination of FTIR and UV spectra predicted the rest of the chemical measures in OPLS regression with a high accuracy.

# ÖZET

## DERİN YAĞDA KIZARTMA İŞLEMİNİN BİTKİSEL SIVI YAĞLARIN KALİTE PARAMETRELERİ ÜZERİNE ETKİSİ

Bu çalışmada derin yağda kızartma işlemi sırasında değişik zeytinyağlarında ve ayçiçek yağında meydana gelen kimyasal değişiklerin belirlenmesi amaçlanmıştır. Ultraviyole ve Fourier dönüşümlü kızıl ötesi spektrometrelere ait verilerin (UV, FTIR) ve bu iki spektrumun birleşimi ile oluşan verilerin çok değişkenli istatistiksel yöntemlerle analizi ile (PCA, OPLS-DA, OPLS), kızartma işleminde kullanılan yağ örneklerinin sınıflandırılması ve bazı kimyasal parametrelerin tahmini de çalışılmıştır. Kızartmalık donmuş patates örnekleri, belirtilen yağların kullanılmasıyla (sızma, natürel, reviera ve rafine zeytinyağları ve ayçiçek yağı), ev tipi elektrikli bir fritözde 4 dakika boyunca, 180ºC de kızartılmış, işlemler, taze yağ eklenmeksizin, 10 kez tekrarlanmıştır.

Serbest yağ asitliği, peroksit, $K_{232}$, ve $K_{270}$ değerleri, toplam klorofil ve karotenoid, oksidatif stabilite, toplam fenol, toplam polar madde miktarları ve yağ asitleri dağılımı bütün yağ örnekleri için, fenolik madde dağılımları ise sızma ve natürel zeytinyağları için belirlenmiştir. Onuncu kızartma işlemi sonunda, asitlik, stabilite ve yağ asidi dağılımlarında bir değişiklik bulunmamıştır. Sızma ve natürel zeytinyağlarında bulunan fenolik madde miktarlarında dördüncü kızartmalardan sonra bir azalma görülmüştür.

Çok değişkenli istatistiksel modeller, ultraviyole verilerinin yağ örneklerinin sınıflandırmasında başarılı olduğunu göstermiştir. Peroksit sayısı dışındaki kimyasal parametreler spektroskopik veriler yardımı ile yüksek $R^2$ değerleri ile tahmin edilebilmiştir. Yağ asitleri kompozisyonları FTIR verileri ile başarılı bir şekilde tahmin edilirken, Ultraviyole verilerinin toplam fenol miktarını tahmin etmede daha etkili olduğu görülmüştür. FTIR ve ultraviyole spektrumlarının birleşimi ile elde dilen veriler diğer kimyasal parametrelerin OPLS regresyon tekniği ile belirlenmesinde başarılı olmuştur.

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23Dhyb 2,3dihydroxybenzoic acid

34 Dhyb 3,4 dihdroxybenzoic acid

3Hyp 3Hydroxyphenylacetic acid

4Hyp 4Hydroxyphenylacetic acid

Apg Apigenin

B Blended Olive Oil

Caf Caffeic acid

CHL Chlorophyll

Cin Cinnamic acid

Cla Chlorogenic acid

CRT Carotenoid

DAG Diacylglycerols

E Extra Virgin Olive Oil

Fer Ferulic acid

FFA Free Fatty Acids

GA Gallic Acid

GC Gas Chromatography

HATR Horizontal Attenuated Total Reflectance

HPLC High Performance Liquid Chromatography

Hyt Hydroxytyrosol

IOC International Olive Council

IR Infrared

Lut Luteolin

MAG Monoacylglycerols

Mcou M-coumaric acid

MUFA Monounsaturated Fatty Acid

MVA Multivariate Statistical Methods

ND Not Detectable

Ocou O-coumaric acid

OO Olive Oil

OPLS Orthogonal Partial Least Square

OPLS-DA Orthogonal Partial Least Square Discriminant Analysis

OSI Oxidative Stability Index

PCA Principle Component Analysis

Pcou P-coumaric acid

Pin Pinoresinol

PLS Partial Least Square

PLS-DA Partial Least Square Discriminant Analysis

PUFA Polyunsaturated Fatty Acid

PV Peroxide Value

R Refined Olive Oil

$R^2$ Regression Coefficients for Calibration

$R^2_{CV}$ Regression Coefficients for Cross Validation

$R^2_{val}$ Regression Coefficients for Validation Set

RMSECV Root Mean Square Error of Calibration

RMSECV Root Mean Square Error of Cross Validation

S Sunflower Oil

SIMCA Soft Independent Modelling Class Analogy

Syr Syringic acid

TAG Triacylglycerols

TPC Total Phenol Content

Tyr Tyrosol

UV Ultraviolet

V Virgin Olive Oil

Vna Vanillic acid

# CHAPTER 1

# INTRODUCTION

Extra virgin or virgin olive oil is produced from the olive fruit (Olea europaea L.) by mechanical extraction, without being exposed to refining process. Therefore, olive oil can keep some components of olive fruit including phenolic compounds, sterols, hydrocarbons, phospholipids, waxes, and unique aroma compounds which could be lost during refining. Olive oil is different than most of the other types of vegetable oils with its rich content of monounsaturated fatty acids, which is one of the main causes for being less inclined to oxidation. The olive oils with high acidity goes under refining process like other vegetable oils in order to be consumable.

The consumption of fried fruits has always been high in human diet. For example, in 1970 Americans spent $6000 million on fast foods in which fried products have significant proportion and in 2000 this case grew to $110,000 million. Therefore, it is probable that a quarter of the adult people goes to a fast food restaurant almost every day and fried foods have an important role in this remarkable growth. (Saguy and Dana, 2003).

Frying is basically a dehydration operation, that is, the process causes removal of water from the food. In deep-fat frying method, food material is immersed in hot oil at high temperature and it is considered as the most common and popular procedure used in food preparation (Sánchez-Gimeno et al., 2008). Fried foods have desirable aroma compounds, color, flavor, and texture, which make fried foods highly appreciated by the consumers. In deep-fat frying process, the unsaturated fatty acid content decreases, whereas polymeric materials, polar contents, foaming, color, viscosity, density, specific heat, and free fatty acids of oil medium increase (Choe and Min, 2007).

During frying, the oil is subjected to air under high temperatures with the presence of water from the food. Series of chemical reactions occur in the oil, such as hydrolysis, oxidation, polymerization, and cyclization. After thermal oxidation, hydroperoxides, hydrocarbons, aldehydes, ketones, polymers, and oxidized monomers can be formed as oxidation products and they can cause adverse health effects (Li et al., 2015; Yemiscioglu et al., 2015).

Several studies have been conducted on the quality of continuously heated oils at high temperatures for many hours in different kinds of frying processes, but reports on the real domestic frying of foods are limited (Casal et al., 2010; Andrikopoulos et al., 2002).

The aim of this study is to examine the changes of certain parameters in different types of olive oil in several frying stages (1 to 10) in terms of phenolic, fatty acid compositions, oxidative stability index, and chlorophyll-carotenoid content. Different types of olive oils, extra-virgin, virgin, blended and refined, were compared because they can be used as frying medium in home cooking. Sunflower oil, which is a commonly used frying oil, was also used in the study for comparison.

Fatty acid and phenolic profiles were determined by gas chromotography (GC), and high performance liquid chromotography (HPLC), respectively. Chemical parameters like total phenol content (TPC), chlorophyll-carotenoid contents, and specific extinction coefficients ($K_{232}$ and $K_{270}$) were determined spectrophotometrically, the quantitive parameters like free fatty acid (FFA) and peroxide value (PV) were determined by titrimetric methods. Rancimat method was used in the determination of oxidative stability index (OSI). The color of oils were defined by CIELab parameters. Spectrometric scans by UV and FTIR spectrometers were taken to be used in classification of oils and prediction of chemical parameters. Data were analyzed by multivariate statistical methods. Orthogonal partial least square (OPLS) was used in the prediction of several chemical parameters (e.g. FFA, PV, $K_{232}$, $K_{270}$, and OSI) by spectrometric profiles, such as FTIR, UV and FTIR and UV combination. Also orthogonal partial least-squares discriminant analysis (OPLS-DA) was used in classification of oil samples.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Frying Process

Deep frying is commonly used to prepare foods both for industrial and domestic food cooking techniques. Deep-fried foods have palatable flavor, golden brown color and crunchy texture (Chen et al., 2013). Throughout the frying process, the oil is incessantly heated at high temperature of 160ºC to 200ºC in the presence of water and air. Eventually, many unwelcome changes may occur like loss of nutrients (Juárez et al., 2011).

During the frying process, two main changes may occur as physical and chemical changes in oils. Physical changes are increasing of viscosity, darkening in color and foaming and decreased the smoke point of the oil. On the other hand, frying causes some adverse effects like free fatty acids and carbonyl compounds will increase with accelerating frying time and temperature. In deep-frying process, oxidation, hydrolysis and polymerization reaction occur, and the structure changes to form volatile compounds and nonvolatile monomeric and polymeric components (Gupta et al., 2004).

Diacyl glycerides and free fatty acids are the products of hydrolysis of frying oil by water. Hydrolysis of the diacyl glycerides produces monoglycerides, glycerol and fatty acid. Some of these products like fatty acids are very reactive and the can change the quality of flavor. A summary of hydrolysis process is shown in Figure 2.1.

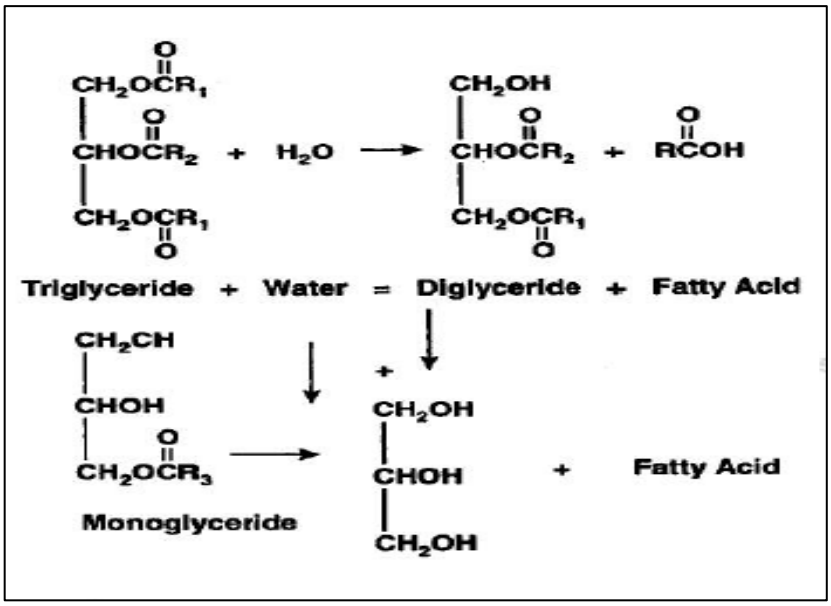


Figure 2.1. Hydrolysis process for frying oils
(Source: Warner, 2004)

Oxidation occurs in frying process in the presence of air. Heat, with the supplement with food substances, causes a complex series of reactions like the formation of hydroperoxides, free radicals and conjugated acids. In frying oil, the oxidation technique is like autooxidation at room temperature. In autooxidation, hydroxylperoxides formed and immediately decompose into primary and secondary products such as aldehydes and ketones. Autooxidation has three main steps such as initiation, propagation and termination, respectively. The first step (initiation) is catalyzed by many factors such as heat, light and metal ions and at the end of the initiation step free radicals ($L^{\cdot}$) occur. In the propagation step firstly, lipid radicals combined with oxygen and peroxy radicals ($LOO^{\cdot}$) consists. Then peroxy radical turns into lipid radicals. Thus, lipid radicals play the role of both product and reaction substrate in the propagation step. The last step includes the synthesis of free radicals like aldehydes, ketones and organic acids. The products of termination step prevent the progression of propagation step (Zhang et al., 2012). The details about the autooxidation is shown in Figure 2.2.

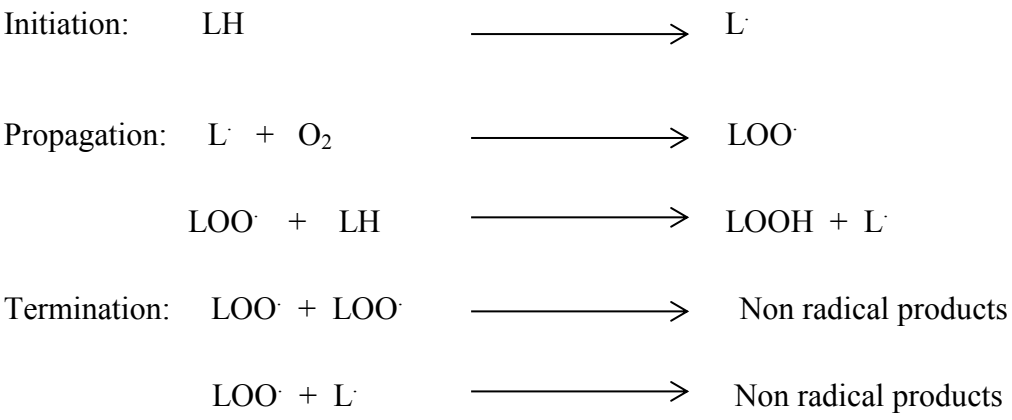


Figure 2.2. Autooxidation main steps
(Source: Hh and Gs, 2013)

Products of polymerization process in the formation of compounds with high molecular weight and polarity. Free radicals or triacylglycerols causes the formation of polymers. Cyclic fatty acids can form with one fatty acid while dimeric fatty acids can form between two fatty acids and polymers with high molecular weight can form as these molecules maintain to cross link. Viscosity of the oil is effected by polymerization process.

## 2.2. Olive Oil

The olive tree belongs to the family Oleacea and the genus Olea. The name is especially used for *Olea europaea*, the well-known olive which is grown for its edible fruits. Olive trees are inherent to Greece, Italy, Palestine, and Syria, but species change according to areas. It is believed that cultivation of olives started around the fourth millennium B.C. in the area which is today Syria and Palestine (Boskuo D., 2006).

Several studies indicate a relationship between the Mediterranean diet and a lower ratio of various important sickness like cardiovascular diseases and cancer. Olive oil in Mediterranean diet is the main source of fat. The advantages of olive oil can be associated with high amount of phenolic substances and monounsaturated fatty acid content, namely oleic acid (Martin-Palaez et al., 2013).

Marketing of olive oil is an important sector and the most considerable role in this sector are Mediterranean countries. According to International Olive Council (IOC) statistics in 2014/15, 97 % of the world's olive oil have been produced by the members of the IOC. Spain is the biggest olive oil producer followed by Italy and Greece (IOC,

2014). Turkey is sixth country in this production list. Between 2009 and 2013 seasons, production exceeded consumption, however increase in consumption was higher than production (Table 2.1).

Table 2.1. Consumption and production of olive oil in Turkey between 2009 and 2013 seasons in weight (x $10^3$ tonnes) (Source: International Olive Council 2015)

| Year | Production | Consumption |
|---|---|---|
| 2009/10 | 147,0 | 110,0 |
| 2010/11 | 160,0 | 131,0 |
| 2011/12 | 191,0 | 150,0 |
| 2012/13 | 195,0 | 150,0 |
| 2013/14 | 190,0 | 160,0 |

According to International Olive Council (IOC) definition, olive oil is produced by mechanical means without using solvents. The operation does not include any process other than washing, decantation, centrifugation and filtration. Olive oil is classified in the following categories.

**Extra virgin olive oil:** Olive oil having a free acidity, (as oleic acid), of not more than 0.8 grams per 100 grams.

**Virgin olive oil:** Free fatty acidity of a virgin olive oil should not exceed 2.0 grams per 100 grams.

**Ordinary virgin olive oil:** Virgin olive oil which has a free of not more than 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 fatty acidity higher than 2.0 grams per 100 grams. It is to be refined or for technological use.

**Refined olive oil:** The olive oil obtained from by refining process. Free fatty acidity of refined olive oil is smaller than 0.3 grams per 100 grams.

**Riviera (Blended) olive oil:** The oil that is obtained by mixing refined olive oil with natural olive oil that can directly be consumed as a food. Free fatty acidity should not be more than 1.0 gram per 100 gram.

**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 to be refined 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. Free fatty acidity (expressed as oleic acid) of a refined olive pomace oil should exceed 0.3 grams per 100 grams.

**Olive pomace oil:** The oil obtained by blending refined olive pomace oil and virgin olive oils fit for consumption. Free fatty acidity of this oil is not more than 1 gram per 100 grams.

## 2.3. Chemical Composition of Olive Oil

Olive oil chemical composition divided into major and minor fractions. The major components which were present more than 98% of total oil weight and minor fractions constituting approximately two-percent of weight, include compounds such as phenolic compounds, sterols, hydrocarbons, aliphatic and triterpenic alcohols, volatile compounds, carotenoids and chlorophylls (Servili et al., 2004). These minor components that are present almost exclusively in virgin olive oil, are removed as a result of some processes like refining.

### 2.3.1. Major Components

Major component fraction is also known as the saponifiable fraction and it is mainly contains triacylglycerols (TAGs) (Gunstone et al., 1994). If a triacylglycerol unit loses one fatty acid, it is named diacylglycerol (DAG) and if it loses two fatty acids, it

becomes a monoacylglycerol (MAG). A triacylglycerol molecule consists of one glycerol and three groups of saturated or unsaturated fatty acids . The fatty acid which is seperated from the TAG becomes free fatty acid.

The number of carbon atoms and bond structure effects chemical and physical properties of fatty acids. According to carbon chains fatty acids can be divided into two groups such as saturated and unsaturated. While saturated fatty acid means carbon atoms attached by single bonds, unsaturated fatty acid means double bond exists in the structure. If one double bond exists in structure, it means monounsaturated fatty acid and more than one double bond means polyunsaturated fatty acid. The main fatty acids of olive oil are oleic and palmitoleic acids, which are monounsaturated; palmitic and stearic acids, which are saturated; and linoleic and linolenic acids, which are polyunsaturated fatty acids.

Olive oil is characterized by a high degree of monounsaturated fatty acids (MUFA) particularly oleic acid. According to Comission Regulation (EEC) No 1348/2013, olive oil samples should include oleic acid between the values of 55 and 83 %. Important fatty acids chemical sturucture is shown in Figure 2.3.

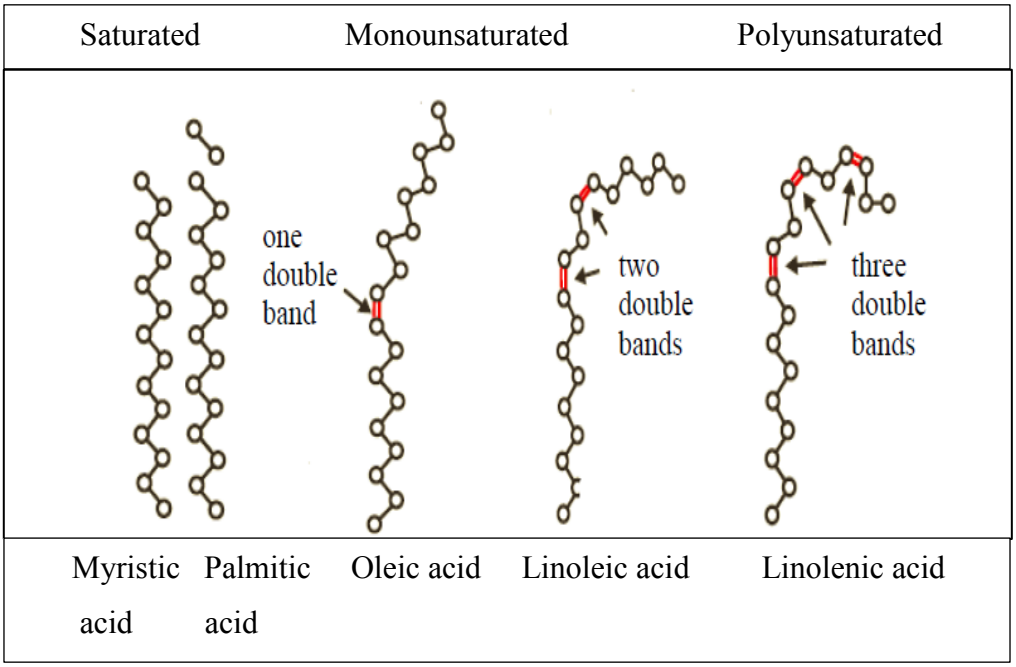


Figure 2.3. Sturucture of some fatty acids in olive oil
(Source: NSW Department of Primary Industries 2006)

## 2.3.2. Minor Components

The minor components of olive oil are classified into two groups: the unsaponifiable fraction, defined as the components extracted with solvents after the saponification of the oil, and the soluble fraction, which contains phenolic compounds. Fractions of the unsaponifiable fraction of olive oil are: hydrocarbons, tocopherols (particularly α-tocopherol), fatty alcohols, triterpenic alcohols, 4-methylsterols, sterols, other terpenic compounds and color pigments (Covas et al., 2006).

**Pigments:** Chlorophylls and carotenoids are the most common color pigments, where they have an important role in photosynthesis (Giuffrida et al., 2007). Chlorophylls a and b and their oxidation products, pheophytins a and b, are naturally present in olive oil and are liable for the green color of the oils. The quantity of chlorophylls in olive oil depends on a number of parameters like the variety, the degree of maturity of the olives, method of oil extraction, and some other biological and technical factors. In virgin olive oil from mature olives the levels of chlorophyll are about 1 to 10 mg/kg and those of pheophytins are about 0.2 to 24 mg/kg (Rahmani and Saari, 1991).

Lutein and β-carotene are the major yellow pigments of virgin olive oils. The amount of β-carotene and lutein in virgin olive oil is 1–2.7 ppm and 0.9–2.3 ppm, respectively (Psomiadou and Tsimidou, 2002).

The reason for decreasing green color fraction during processing and storage is relying on to the conversion of chlorophylls to pheophytins because ogf the acidity pH. Magnesium in the chlorophyll chain is altered by two hydrogen ions and green chlorophylls are converted to the olive brown pheophytins. The amount of chlorophylls and their derivatives based on the stage of olive maturity decreasing continuously from the beginning to the end of the olive picking period (Aparicio et al., 1999).

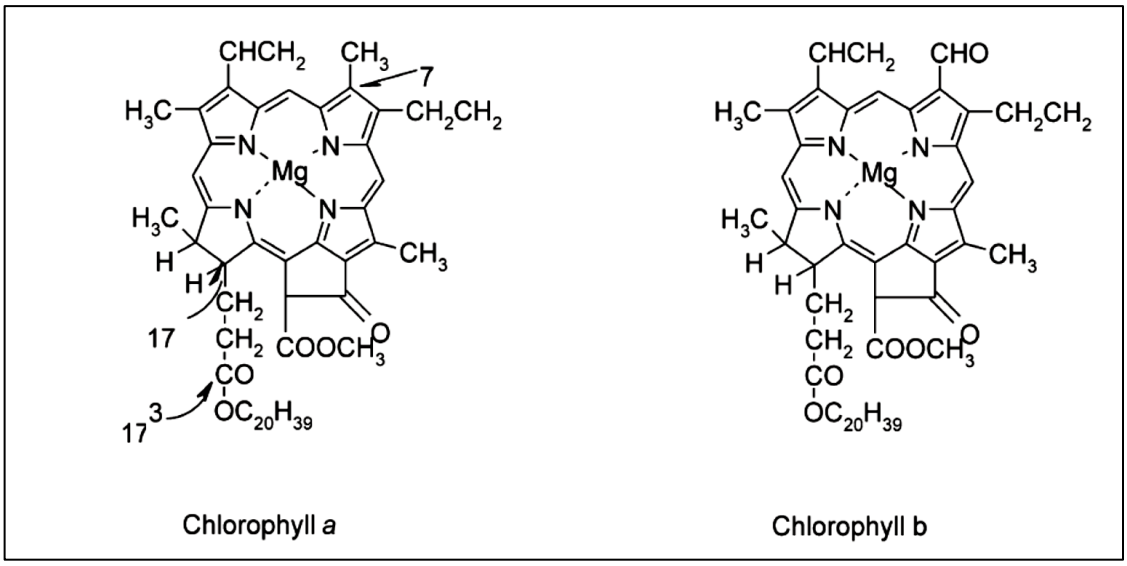


Figure 2.4. Structure of chlorophyll a and chlorophyll b
(Source: Schoefs, 2002)

**Phenolic Compounds:** Phenolic compounds can be classified as lipophilic and hydrophilic phenols. Lipophilic phenols are heteroacids of high molecular weight and tocopherols and tocotrienols are the most important lipophilic phenols in olive oil. α-tocopherol can be found between 12 and 400 mg/kg (Quiles et al., 2006).

Hydrophilic phenolics are responsible for the antioxidant characteristics of virgin olive oil (VOO) and the amount in olive oil is 40-1000 mg/kg (Boskuo, 2006). This class of phenols can be divided into several groups such as phenolic alcohols, phenolic acids, flavonoids, hydroxy-isochromans, lignans, and secoiridoids. Chemical structures of phenols are shown in Figure 2.5.

Phenolic acids that have the chemical structure of benzoic acids (C6–C1) and cinnamic acid (C6–C3) are present in olive fruit. Those phenols found in VOO are p-coumaric, caffeic, vanillic, ferulic, syringic, and p-hydroxybenzoic acids being present in small amounts.

The phenolic alcohols are hydroxytyrosol (3,4-dihydroxyphenyl-ethanol or 3,4-DHPEA) and tyrosol (p-hydroxyphenyl-ethanol or p-HPEA) in olives. As flavonoid compounds, luteolin and apigenin are responsible for aroma occurrence with volatile compounds (Bendini et al., 2007).

Secoiridoids are those compounds defined by the existence of either elenolic acid or elenolic acid derivatives in the structure. The secoiridoids in olive oil are the dialdehydic form of decarboxymethyl elenolic acid attached to hydroxytyrosol, the

dialdehydic form of decarboxymethyl elenolic acid attached to tyrosol, oleuropein, ligstroside and oleuropein aglycon (Quiles et al., 2006).

Phenolic compounds in olive oil affect nutritional properties, storage time, and also the sensory characteristics of olive oil. Those derived from the hydrolysis of oleuropein is the reason for the bitterness of virgin olive oil; hydroxytyrosol, tyrosol, caffeic acid, coumaric acids, and p-hydroxybenzoic acid have effect on the sensory properties (Kiritsakis, 1998). Phenolic compounds have various proven health effects such as antimicrobial, anti-inflammatory, and anticarcinogenic activities in the body. Phenolics of olive oil may help enhancing vascular function and minimise cardiovascular diseases (Scoditti et al., 2014).

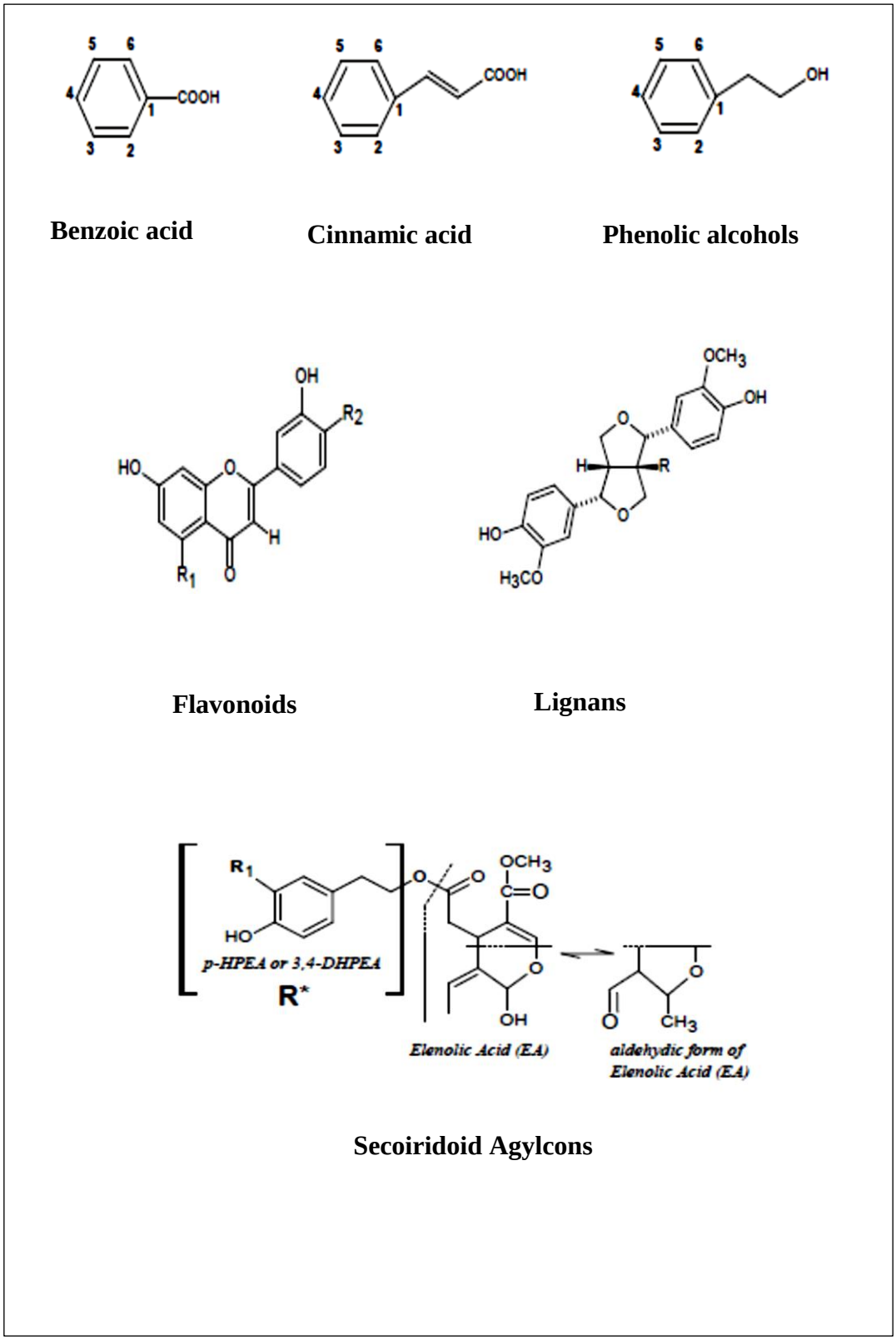


Figure 2.5. Chemical structures of phenolic compounds in olive oil
(Source: Bendini et al., 2007)

## 2.4. Quality of Olive Oil

Olive oil quality can be defined in terms of commercial, nutritional or organoleptic points of views. The nutritional value of virgin olive oil comes from high levels of oleic acid content and minor components such as phenolic compounds. Nutritional value and welcome flavor encourages consumption demands and price of olive oil in comparison with other edible oils. High level of quality control of olive oil should be predetermined in the production and storage period to carry out the expectations of consumers. The quality of olive oils is sustained in terms of measurement of analytical parameters for which specific limit values are set.

The European Comission Regulation (EEC) and the International Olive Council (IOC) defined the olive oil quality based on parameters such as peroxide value (PV), free fatty acid (FFA) value, UV specific extinction coefficients ($K_{232}$ and $K_{270}$) and finally sensory prpoerties. The changes in major and minor compounds and their concentrations in olive oils give an information in order to improve quality, especially for marketing, packaging, and storage.

**Free fatty acid** (FFA) is the oldest parameter used for detection of the olive oil quality because it symbolizes the content of hydrolytic activities. The acid determination is made by titration of free fatty acids of oils, diluted in a proper mixture of solvents, with an ethanolic potassium hydroxide solution. Limit values have been identified by regulations to establish the category. Results are expressed as percentage of oleic acid.

**Peroxide value** (PV) is an important parameter which increases, and related with the storage conditions like entry of oxygen, light, time, and preservation temperature. After reaching a maximum level, the formation of secondary products cause the decreasing of peroxide value (Angerosa, 2006). One way of the evaluation of degree of olive oil oxidation is depending upon determinations of both the primary and the secondary products of oxidation. The first step of oxidation is the formation of hydroperoxides from polyunsaturated fatty acids through a radicalic mechanism. The results of hydroperoxides (PV) is expressed as milliequivalents of active oxygen per kilogram of oil (meq $O_2$ /kg oil).

**Specific absorbances**, conventionally indicated as K, are measured in the UV region at 232 and 270 nm ($K_{232}$ and $K_{270}$). These wavelengths equaled to the maximum

absorption of the conjugated dienes at 232 nm and conjugated trienes at 270 nm. The conjugated dienes and trienes are formalised in the autoxidation process from the hydroperoxides of unsaturated fatty acids and their deterioration products.

Table 2.2. Commision regulation EEC, 2013, Official Journal of the European Union, No 1348/2013) limit values for olive oil quality parameters

| Category | Acidity (Oleic acid%) | Peroxide Value (meq $O_2$/kg) | $K_{232}$ | $K_{270}$ |
|---|---|---|---|---|
| Extra Virgin OO | ≤ 0.8 | ≤ 20 | ≤ 2.5 | ≤ 0.22 |
| Virgin OO | ≤ 2.0 | ≤ 20 | ≤ 2.60 | ≤ 0,25 |
| Lampante OO | > 2.0 | - | - | - |
| Refined OO | ≤ 0.3 | ≤ 5 | - | ≤ 1.10 |
| Blended OO | ≤ 1.0 | ≤ 15 | - | ≤ 0.90 |
| Olive-pomace oil | ≤ 1.0 | ≤ 15 | - | ≤ 1.70 |
| Refined olive-pomace oil | ≤ 0.3 | ≤ 5 | - | ≤ 2.0 |

## 2.5. Instrumental Techniques in Olive Oil Analysis

### 2.5.1. Gas Chromatography (GC)

Gas chromatography (GC) is the most largely used technique which is used in analytical chemistry because of advanced selectivity and sensitivity. In the analysis of GC, the sample is turned to the vapor state on to the chromatographic column. On the column, the solubility of every component is connected with its vapor pressure. The molecules of every component to diffuse between the mobile gas phase and stationary phase because of vapor pressure. At each rotation a molecule goes into the gas phase it is swept towards the detector by the carrier gas flow. After all, compounds will reach at the detector at different times due to the specific physical and chemical properties (Hübschmann, 2008). The basic components of GC is shown in Figure 2.6.

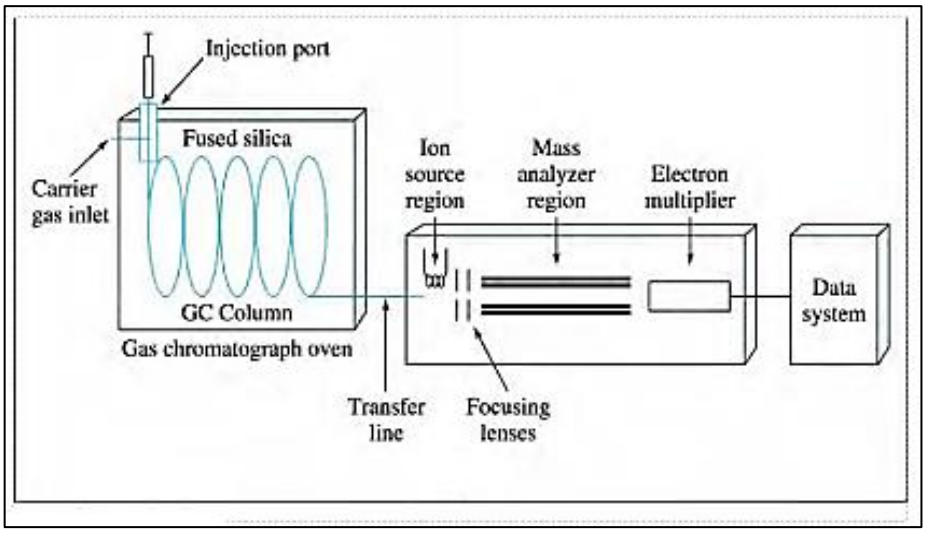


Figure 2.6. Schematic diagram of chromatography system
(Source: Joint FAO/WHO Expert Committee on Food Additives, 2005)

Retention time is the time taken from the moment the sample is given to the column up to the moment the chromatographic zone of the substance output arrives its maximum. If the volatility of the components increase, retention time will decrease.

In olive oil analysis, GC uses in the determination of methyl esters of fatty acids. The main purpose of this analysis is to detect the percentage composition of fatty acids in olive oil which is influenced by the olive variety and climate. Fatty acid composition analysis in GC can be also used for characterization and classification (Mcnair and Miller, 2009).

## 2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared (IR) technology is a vibrational spetroscopic method depends on the interaction between electromagnetic radiation that is rapid, sensitive and simple technology.

The most important advantage of FTIR spectroscopy is qualitative analysis of complex food materials having structural characteristics that can be extracted from the infrared spectra. The infrared region of the spectrum is seperated in three areas: the near-, mid-, and far-IR. The mid infrared region (4000-400 $cm^{-1}$) which includes 'fingerprint' region, is the most commonly used area for analysis. As an another important point, FTIR spectroscopy can easily combined with computer software and

multivariate data analysis (Man, 2010). FTIR technique is able to characterize edible oils and predict some of the chemical parameters with multivariate statistical techniques such as principle component analysis (PCA) and partial least square (PLS) , which underline the discrepancies between spectra, and the classification of the samples (Lerma-Garcia et al., 2010; Uncu and Ozen, 2015). Basic components of FTIR spectrometer was shown in Figure 2.7.

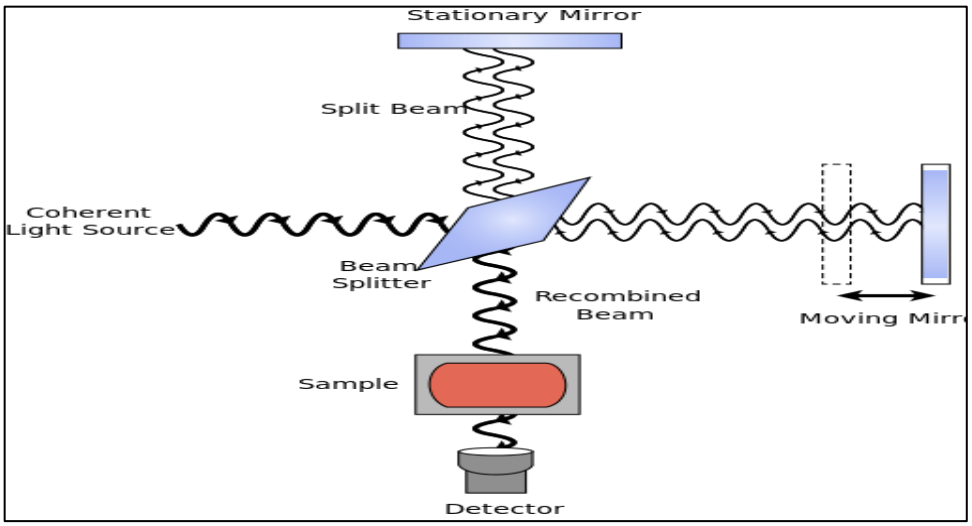


Figure 2.7. Basic components of an FTIR spectrometer
(Source: Davis and Mauer, 2010)

In FTIR spectroscopy each spectral region have different and specific meaning. The peaks in the range of 3025 and 2850 $cm^{-1}$ region are due to C-H stretching vibrations of methylene and terminal methyl groups of fatty acid chains. In the center of the spectrum, that is about 1740 $cm^{-1}$, a band due to the C=O stretching absorption of the triglyceride ester linkages is present. Another important region (1250–700 $cm^{-1}$) is the named "fingerprint region" which is characteristic of molecular composition and can be used to determine minor substances (Figure 2.8).

| No. | Frequency ($cm^{-1}$) [a] | Functional Group | Mode of vibration | Intensity [b] |
|---|---|---|---|---|
| 1 | 3468 (b) | –C=O (ester) | Overtone | w |
| 2 | 3025 (s) | =C–H (*trans*-) | Stretching | vw |
| 3 | 3006 (b) | =C–H (*cis*-) | Stretching | m |
| 4 | 2953 (s) | –C–H ($CH_3$) | Stretching (asym) | m |
| 5 | 2924 (b) | –C–H ($CH_2$) | Stretching (asym) | vst |
| 6 | 2853 (s) | –C–H ($CH_2$) | Stretching (sym) | vst |
| 7 | 2730 (b) | –C=O (ester) | Fermi resonance | vw |
| 8 | 2677 (b) | –C=O (ester) | Fermi resonance | vw |
| 9 | 1746 (b) | –C=O (ester) | Stretching | vst |
| 10 | 1711 (s) | –C=O (acid) | Stretching | vw |
| 11 | 1654 (b) | –C=C– (*cis*-) | Stretching | vw |
| 12 | 1648 (b) | –C=C– (*cis*-) | Stretching | vw |
| 13 | 1465 (b) | –C–H ($CH_2$,$CH_3$) | Bending (scissoring) | m |
| 14 | 1418 (b) | =C–H– (*cis*-) | Bending (rocking) | w |
| 15 | 1400 (b) | | Bending | w |
| 16 | 1377 (b) | –C–H ($CH_3$) | Bending (sym) | m |
| 17 | 1319 (b,s) | | Bending | vw |
| 18 | 1238 (b) | –C–O, –$CH_2$ – | Stretching, bending | m |
| 19 | 1163 (b) | –C–O, –$CH_2$ – | Stretching, bending | st |
| 20 | 1118 (b) | –C–O | Stretching | m |
| 21 | 1097 (b) | –C–O | Stretching | m |
| 22 | 1033 (s) | –C–O | Stretching | vw |
| 23 | 968 (b) | –HC=CH– (*trans*-) | Bending out of plane | w |
| 24 | 914 (b) | –HC=CH– (*cis*-) | Bending out of plane | vw |
| 25 | 723 (b) | –$(CH_2)_n$–, –HC=CH– (*cis*) | Bending (rocking) | m |

[a] b, band; s, shoulder.
[b] w, weak; vw, very weak; m, medium; vst, very strong; st, strong.

Figure 2.8. Important Frequency Bands And Shoulders Of Edible Oils In The Mıd-Infrared Region, Along With The Assigned Functional Group, Mode of Vibration And The Intensity (Source: Guillen and Cabo, 1997)

### 2.5.3. High Performance Liquid Chromatography (HPLC)

The utilization of HPLC is increasing day by day because compounds in any sample that can be dissolved in a liquid can be seperated and identified by using HPLC. There are variety of mobile phases, the extensive library of column packings and the variation in modes of operations. Also HPLC can be combined with several types of detectors (Nollet, 1992).

HPLC and other chromatographic methods take an important place in food material analysis. A lot of minor components of olive oil such as sterols, phenolic

compounds, tocopherols, pigments (chlorophyll and carotenoid), and triacylglycerols can be analyzed with HPLC method (Ryan et al. 1999).

HPLC starts with the injection of a small amount of liquid sample into the mobile phase that moves through a column packed with particles of a stationary phase. As in the gas chromatography, the principle of HPLC is based on specific retention times of each component in the column .

A schematic drawing of a typical high performance liquid chromatograph is shown in Figure 2.9.

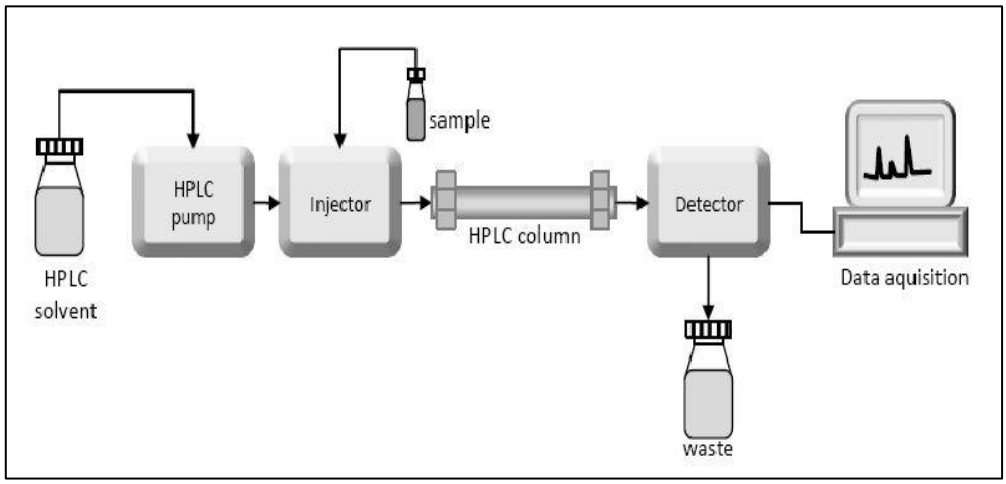


Figure 2.9. Schematic diagram of the HPLC system
(Source: Czaplicki, 2013)

## 2.6. Sunflower Oil

Sunflower oil is acquired from the seed of the plant Helianthus annuus and oil content percentage of it is 22-36. Color of crude sunflower oil is light amber but it changes according to steps of refining process. For example after bleaching process, the color of sunflower oil is a pale yellow (Sonntag, 1979). Sunflower oil is chracterized by a high amount of polyunsaturated fatty acids (PUFA) particularly linoleic acid. According to Codex Alimentarius (2001), sunflower oil samples should contain linoleic acid between the values of 48.3-74 % and oleic acid range should be 14-39.4 %. Important fatty acids percentage is shown in Table 2.3.

Table 2.3. Fatty Acid Composition Ranges for Sunflower Oil (expressed as % of total fatty acids) (Source: Codex Standard for Vegetable Oils, 2001)

| **Fatty acid** | **Sunflower oil** |
|---|---|
| Palmitic acid (C 16:0) | 2.0-7.6 |
| Stearic acid (C 18:0) | 1.0-6.5 |
| Oleic acid (C 18:1) | 14.0-39.4 |
| Linoleic acid (C 18:2) | 48.3-74.0 |
| Linolenic acid (C 18:3) | ≤ 0.3 |
| Behenic acid (C 22:0) | 0.3-1.5 |

Sunflower oil is a good source of tocopherols, especially α-tocopherol content. The level of α-tocopherol in crude sunflower oil ranged from 403-935 mg/kg oil. The levels of the phytosterols are very important because of their properties for inhibiting the deterioration of frying oils and lowering cholesterol and. In Table 2.4. showed the amounts of sterols that ranges for the sterols were within the ranges for sunflower oil.

Table 2.4. Levels of sterols in crude sunflower oils as a percentage of total sterols (Source: Codex Alimentarius, 2001)

| Sterols | Sunflower oil (% of total) |
|---|---|
| Cholesterol | ND-0.7 |
| Brassicasterol | ND-0.2 |
| Campesterol | 6.5-13.0 |
| Stigmasterol | 6.5-13.0 |
| Beta-sitosterol | 50-70 |
| Delta-5-avenasterol | ND-6.9 |
| Delta-7-stigmasterol | 6.5-24.0 |
| Delta-7-avenasterol | 3.0-7.5 |
| Others | ND-5.3 |
| Total sterols | 2400-5000 mg/kg |

ND = not detectable

## 2.6.1. Refining Process

Free fatty acids (FFA), partial glycerides, phosphatides, pigments, water, oxidation products and trace elements like sulfur, iron and halogens are the main impurities of oil (Subramanian, 1998). These foreign matters are removed at several steps in the chemical refining process (details are shown in Figure 2.10). The steps of refining are degumming, neutralization, washing, drying, bleaching, filtration, and deodorization (Subramanian, 1999).

In the step of degumming, the level of phospholipids (PL) decreases because of water and/or phosphoric acid. Free-fatty acids are removed in the neutralization step with alkali hydroxides at 85–90°C. Bleaching is done to remove undesirable colored components with addition of an adsorptive reagent at 90–120°C. Undesirable colored componenets are removed by bleaching with adding an adsorptive reagent. In the last step (deodorization) by steam stripping at 220–240°C under low pressure (0.2 kPa) and

undesirable volatile and odoriferous materials are removed in deodorization step (Pioch et al., 1998).

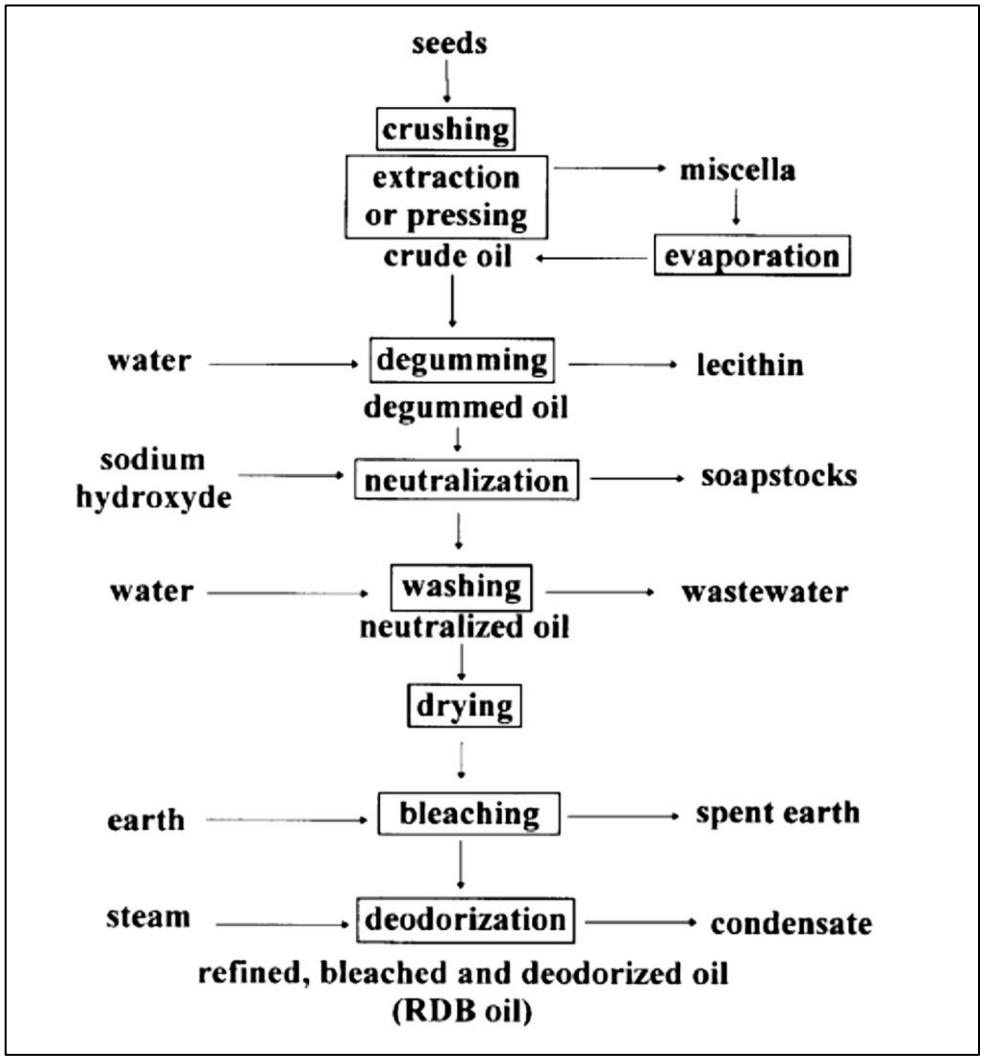


Figure 2.10. Refining process for crude vegetable oil
(Pioch et al., 1998)

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1. Samples

Extra virgin and virgin olive oil samples were obtained from Manisa region while blended and refined olive oil were supplied by Tariş Olive Oil Company (İzmir, Turkey). The sunflower oil was obtained from a local market in İzmir. During analysis period samples were kept at refrigerator (8ºC). Oil samples and their codes are listed in Table 3.1. The french fries were used in frying experiments (Nimet, İstanbul, Turkey).

Table 3.1. Oil Samples and sample codes

| Sample Name | Sample Code |
|---|---|
| Extra virgin olive oil | E |
| Virgin olive oil | V |
| Blended olive oil | B |
| Refined olive oil | R |
| Sunflower oil | S |

### 3.1.1. Chemical Reagents

Reagents used for determination of fatty acid compositions, phenolic compounds and chemicals used in FFA, PV, specific extinction coefficients, total chlorophyll and carotenoid, and TPC analysis of oil samples are given in Table 3.2.

Table 3.2. Chemicals used in the analysis

| | | |
|---|---|---|
| **Free Fatty Acid (FFA)** | | |
| 1 | Diethyl eter | Sigma Aldrich 24004 |
| 2 | Ethanol | Sigma Aldrich 32221 |
| 3 | Phenolphthalein | Riedel-deHaen 33518 |
| 4 | Potassium hydrogen phthalate | Sigma Aldrich |
| 5 | Potassium hydroxide (KOH) | Riedel-deHaen 06009 |
| **Peroxide Value (PV)** | | |
| 6 | Acetic acid | Riedel-deHaen 27225 |
| 7 | Chloroform | Riedel-deHaen 24216 |
| 8 | Potassium iodate ($KIO_3$) | Fluka 60390 |
| 9 | Potasium iodide (KI) | Carlo Erba 472735 |
| 10 | Sodium thiosulphate ($Na_2O_3S_2$) | Fluka 72049 |
| 11 | Starch | Carlo Erba 417587 |
| 12 | Sulfuric acid ($H_2SO_4$) | Fluka 84721 |
| **Specific Extinction Coefficients ($K_{232}$ and $K_{270}$)** | | |
| 13 | Cyclohexane | Sigma Aldrich |

**(Cont. on next page)**

Table 3.2. (Cont.)

| | | |
|---|---|---|
| **Gas Chromotography (GC)** | | |
| 14 | F.A.M.E mix C8-C24 | Supleco CRM47885 |
| 15 | n-hexane | Sigma Aldrich 34859 |
| 16 | Potassium hydroxide | Riedel-deHaen 06009 |
| **Total Chlorophyll and Carotenoid** | | |
| 17 | Cyclohexane | Sigma Aldrich |
| **Total Phenolic Content (TPC)** | | |
| 18 | Folin-Ciocalteau reagent | Fluka 47641 |
| 19 | Gallic acid | Sigma G7384 |
| 20 | Methanol | Sigma Aldrich 34885 |
| 21 | Sodium carbonate | Riedel-deHaen 13418 |
| 22 | Tween 20 | Sigma Aldrich P13379 |
| **High Pressure Liquid Choromotography (HPLC)** | | |
| 23 | Acetonitril | Sigma Aldrich 34851 |
| 24 | Gallic acid | Sigma G7384 |
| 25 | Methanol | Sigma Aldrich 34885 |
| 26 | n-haxane | Sigma Aldrich 34859 |
| **Standard Phenolic Compounds** | | |
| 27 | Apigenin | Fluka 10798 |
| 28 | Caffeic acid | Fluka 60020 |
| 29 | Chlorogenic acid | Fluka 25700 |
| 30 | Cinnamic acid | Fluka 96340 |

**(Cont. on next page)**

Table 3.2. (Cont.)

| | | |
|---|---|---|
| 31 | Ferulic acid | Fluka 46278 |
| 32 | 4-hydroxybenzoic acid | Fluka 54630 |
| 33 | 4-Hyroxyphenylacetic acid | Fluka 56140 |
| 35 | Luteolin | Fluka 62696 |
| 36 | m-coumaric acid | Fluka 28180 |
| 37 | o-coumaric acid | Fluka 28170 |
| 38 | p-coumaric acid | Fluka 28200 |
| 39 | Tyrosol | Fluka 56105 |
| 40 | Vanilic acid | Fluka 94770 |
| 41 | Vanilin | Fluka 94750 |

## 3.2. Deep Frying Experiments

A classical home fryer was used in frying process (Arçelik Fritty, İstanbul, Turkey). According to the instructions given on the fryer frying temperature and time was set up to 180°C and 4 minute, respectively. In every frying cycle, 150 g french fries were used without adding fresh oil and sampling was done after frying stages of 1, 2, 3, 4, 5, 7 and 10. The frying processes were carried out in duplicate for five different types of oil, which were extra virgin olive oil (E), virgin olive oil (V), blended olive oil (B), refined olive oil (R) and sunflower oil (S). Details about deep frying process is given in Table 3.3.

Table 3.3. Frying process parameters

| **Operation** | **Condition** |
|---|---|
| Type of fryer | Home fryer (Arçelik Fritty, İstanbul, Turkey) |
| Fryer dimensions | Diameter = 26 cm , Height =14 cm<br>Width = 21 cm |
| Frying method | Deep frying |
| Surface area (exposed to air) | 475 $cm^2$ |
| Oil samples | Extra virgin, virgin, blended and refined olive oils and sunflower oil |
| The amount of oil (at the begining) | 2000 mL |
| Frying temperature | 180$^{\circ}$C |
| Frying time | 4 minutes |
| The number of frying cycles | 10 |
| Food material | French fries |
| The amount of food material | 150 g |
| Stage of sampling | Beginning (0, control) and after 1, 2, 3, 4, 5, 7 and 10 frying |
| The amount of oil at the end | 1450-1500 mL |

## 3.3. Determination of Free Fatty Acid (FFA)

Acidity of oil samples was determined based on the analytical method given in European Official Method of Analysis (Commision Regulation EEC N-2568/91, 1991). The results were expressed as % oleic acid. First, 1.0 g potassium hydrogen phthalate ($KHC_8H_4O_4$) was dried in an incubator (Memmert, Wisconsin, USA) for 2 hours at 110°C for standardization. Then, 75 mL distilled water was added to 0.4 g of dried potassium hydrogen phthalate. As an indicator, a few drops of phenolphthalein (0.5 g phenolphthalein in 50 mL 95% ethanol (v/v)) was added and 0.1 mol/L (2.850g KOH in 500 mL distilled water) potassium hydroxide (KOH) was prepared with distilled water. It was standardized with priorly prepared potassium hydrogen phthalate and the volume of KOH was recorded. Molarity of KOH was calculated:

$$\mathrm{M_{KOH}(mol/L)} = \frac{\text{mass of } \mathrm{KHC_8H_4O_4}\ (\mathrm{g})/204.22}{\text{Volume of KOH}} * 1000 \qquad (3.1)$$

Ten-gram olive oil sample was dissolved in 75 mL diethyl ether-ethanol (1:1) mixture with the addition of phenolphthalein. The mixture was titrated with 0.1 mol/L solution of KOH until the indicator color changes as pink.

Acidity was calculated as oleic acid percentage:

$$\mathrm{FFA(oleic\ acid\ \%)} = \mathrm{V} * \mathrm{c} * \mathrm{M}\ \ /10 * \mathrm{m} \qquad (3.2)$$

Where:

V = volume of titrated KOH solution used (mL)

C = exact (Actual Molarity) concentration in mol/L of the titrated solution of KOH

M= molar weight in g/mol of the acid used (Oleic acid molecular weight: 282 g/mol)

m = sample weight (g)

## 3.4. Determination of Peroxide Value (PV)

The peroxide values of samples were determined based on the analytical method given in European Official Method of Analysis (Commision Regulation EEC N-2568/91, 1991). Results were expressed in milliequivalents of oxygen per kg of oil. For standardization of 0.01 M sodium thiosulphate; approximately 2 g of potassium iodate ($KIO_3$) was oven-dried at 100ºC for 1-2 hours. After potassium iodate ($KIO_3$) was taken from the incubator, 0.001 mol/L $KIO_3$ solution (0.1070 g $KIO_3$ in 500 mL deionize $H_2O$) was prepared and the weight of $KIO_3$ was recorded. Then, 2.8 mL of $H_2SO_4$ (96% purity) was diluted to 100 mL with deionized water to obtain 0.5 M $H_2SO_4$. In order to prepare starch solution, 1 g of starch was weighed and dissolved in 100 mL of deionized water and boiled for 3 minutes.

1 mL of 0.5 M $H_2SO_4$ and 50 mL of 0.001 M potassium iodate ($KIO_3$) solutions were added to 0.2 g of potassium iodide (KI) and titrated with sodium thiosulphate (0.01 mol/L) until the solution was turned to reddish brown color and has become pale yellow. As indicator 2 mL starch was added into the pale yellow solution and titration continued until it became colorless and the volume of sodium thiosulphate spent was recorded.

Molarity of standardized sodium thiosulphate was calculated using these following equations:

$$M_{\ KIO_3} = \frac{m_{KIo_3}\,(g)/MW_{KIO_3}(g/mol)}{V_{KIO_3}\,(mL)solution} \tag{3.3}$$

$$M_{sodiumthiosulfate} = \frac{6 * M_{KIO_3}(mol/L\ ) * V_{KIO_3}\,(mL)}{V_{sodiumthiosulfate}(mL)spent} \tag{3.4}$$

Where:

$m_{KIO3}$ = weight of $KIO_3$ (0.1070g)

$MW_{KIO3}$ = molecular weight of $KIO_3$ (212 g/mol)

$V_{KIO3}$ solution = Total volume of $KIO_3$ solution (500 mL)

$V_{KIO3}$ = Volume of $KIO_3$ solution (50ml)

$V_{sodiumthiosuphate}$ = Amount of sodium thiosulphate used in titration (mL)

After the standardization of sodium thiosulphate; 3 g olive oil sample was weighted and 1 mL of potassium iodide, 15 mL of acetic acid, and 10 mL of chloroform, were added. Then solution was mixed for 1 minute and the sample was kept in dark place and at room temperature for 5 minutes. In the last step, 75 mL of deionized water and 0.5 mL of starch solution were added to oil sample. Titration was completed when until the dark blue color of solution turns to colorless and the amount of total sodium thiosulphate solution used during the titration was recorded.

The method used for calculation of peroxide values as meq $O_2$ /kg oil;

$$PV = \frac{V(mL) * M(mol/L) * 100}{m(g)} \quad (3.5)$$

Where:
V: sodium thiosulphate solution required to titrate the sample (mL)
M: molarity of sodium thiosulphate solution
m: sample weight (g)

## 3.5. Specific Extinction Coefficient at 232 and 270 nm ($K_{232}$ and $K_{270}$)

Specific extinction coefficients were determined according to European Official Method of Analysis (Commision Regulation EEC N-2568/91, 1991). Firstly 0.25 g of olive oil sample was weighed and completed with 25 mL cyclohexane in a flask. The sample was homogenized with the help of vortex (Velp Scientifika, Europe) for 30 seconds and put into a quartz cuvette (10 mm). The absorption was measured at 232 and 270 nm in a spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Tokyo, Japan). Pure cyclohexane was used as a blank.

## 3.6. Total Chlorophyll and Carotenoid Analysis

Total carotenoids and chlorophylls (mg/kg oil) were determined according to a method in the literature (Mosquera et al., 1991). Three-gram oil sample was put in a

falcon tube and completed to 10 mL with cyclohexane. The absorption was measured by a UV spectrophotometer (Shimadzu UV-2450 Spectrophotometer, Tokyo, Japan) at 470 and 670 nm.

Total chlorophyll (CHL) and carotenoid (CRT) contents were calculated by the following equations:

$$\text{Chlorophyll (mg/kg)} = A_{670} * \frac{10^6}{613 * L} \quad (3.6)$$

$$\text{Carotenoid (mg/kg)} = A_{470} * \frac{10^6}{2000 * L} \quad (3.7)$$

Where;

L = path-length in decimeter

$A_{670}$ = Absorbance at 670nm

$A_{470}$ = Absorbance at 470nm

## 3.7. Oxidative Stability Index (OSI)

Oxidative stability index was determined by Rancimat apparatus (873 Biodiesel, Metrohm, Gallen, Switzerland) in terms of hour. Glass measuring vessel was filled with ultrapure water up to 60 mL as carrier medium while 3 g oil sample was inserted in the glass reaction vessel. Reaction temperature was set up to 120ºC with a constant flow rate of 20 L/h. In order to prevent any contamination, accessories were washed with acetone and detergent. Then rinsed with deoinized water and oven dried at 80ºC. A typical Rancimat profile is shown in Figure 3.1.

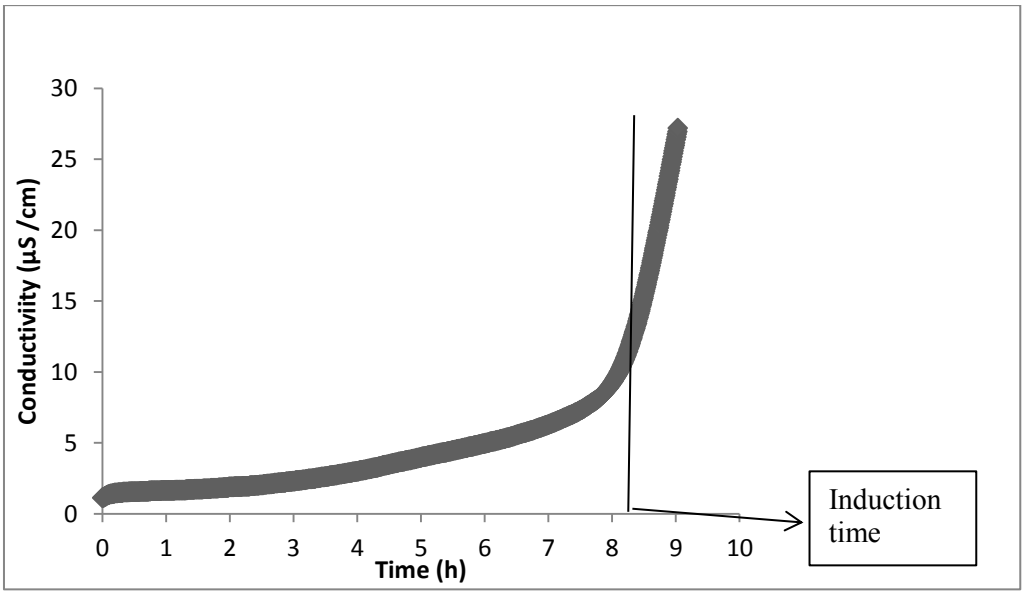


Figure 3. 1. Typical Rancimat curve

## 3.8. Color Parameters

The CIELAB color parameter was determined from the recorded transmission spectra of the oil samples. UV spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Tokyo, Japan) was used to measure color parameters in a quartz cuvette. Transmittance were taken over the range of 380–780 nm at 120 nm/min scan speed. Transmittance spectrum of each sample with the standard illuminant D65 and 10° observation angle was used to calculate color parameters. L*, a* and b* color parameters were determined in the CIELAB system. L* indicates lightness, a* indicates hue on the green (-) to red (+) axis, b* indicates hue on the blue (-) to yellow (+) axis. Chroma or saturation index (C*ab), describes the brightness of a color while hue (<HΘ(a*)) is the aspect of color that is described by words such as green, red, yellow or blue (Sánchez-Gimeno et al., 2008; Eissa et al., 2010).

## 3.9. Total Polar Compounds

The principle of total polar compounds estimation was based on the changes in dielectric constant. Measurements were done directly on the oil; oil samples were previously heated in a water bath to reach 50±5ºC, with a Test 270 cooking oil tester

(Testo, Lenzkirch, Germany). The temperature of the oil were shown on the display for 10 seconds. Total polar amounts of control sample (before frying) and oil sample in the last frying cycle were quantified. The analyses were carried out in duplicate.

## 3.10. Total Phenol Content (TPC)

Folin–Ciocalteau spectrophotometric method, at 765 nm, was used to determine total phenol content (TPC) of the oil samples and expressed in terms of gallic acid as mg GA / kg oil (Montedoro et al., 1992). Extracted samples and gallic acid standard curve measurements were repeated for three times.

**Extraction Procedure**: Oil sample (2 g) was weighed and 10 mL of methanol/water mixture (80:20 v/v) with 2 or 3 drops of Tween 20 was added. Then, this mixture was homogenized (Heidolph–Silent Crusher M, Schwabach, Germany) at 25.000 rpm for 1 min and centrifuged at 9000 rpm (Nüve NF 615, Ankara, Turkey) for 5 min. The supernatant (methanolic extract) was collected in a tube and extraction was repeated three times totally (without Tween 20) only with the addition of 10 mL methanol/water, and total volume of extract was recorded.

**Folin-Ciocalteu Method**: One mL of oil extract was diluted to 6 mL with deionized water. After that 0.5 mL of Folin-Ciocalteu reagent was added and waited for 1 minute. Then, 2 mL of sodium carbonate ($Na_2CO_3$) solution (15% g/mL) was added and diluted with 1.5 mL of deionized water and mixed with a vortex (Velp Scientifika, Usmate Velate, Italy) for 30 seconds. The same method was repeated for the blank sample with methanol/water mixture only instead of phenolic extract. The mixtures were left in a dark place for 2 hours. The absorbance of the mixtures was measured at 765 nm in a spectrophotometer (Shimadzu UV-2450 UV-Visible Spectrophotometer, Tokyo, Japan). Finally, gallic acid (GA) calibration curve was used to determine total phenol content of samples.

Gallic acid calibration curve was obtained using solution of GA prepared in several concentrations changing from 0.01 mg/mL to 1.0 mg/mL. Three parallel analyses were prepared for each concentration and obtained from stock solution (25 mg GA in 25 mL methanol). GA calibration curve was prepared by using the UV absorbances at 765 nm. Converted absorbance values to total phenol content were expressed as mg gallic acid/kg oil.

$$TPC = GA\,(mg/mL) * Vsample(mL) * 1000 / Wsample(g) \quad (3.8)$$

Where:

$W_{sample}$ = sample weight (g)

$V_{mL}$ = sample volume (mL)

## 3.11. High Performance Liquid Chromotography (HPLC) Analysis

**Extraction Procedure:** Phenolic extraction of oil samples were made according to procedure of Brenes et al. (1999). As the internal standard, gallic acid solution was prepared (0.05 g GA / 25 mL methanol/water (80:20 v/v)) and added to oil samples before extraction (1 mL). Firstly, 14 g of oil sample was weighed and 14 mL of methanol/water mixture was added. Then the solution was homogenized and centrifugated at 9000 rpm for 10 minutes. Supernatant of the each step was collected in a beaker. After the four extraction steps all of the supernatants were collected in the same beaker and carried into the bottom flask. A rotary evaporator (Heidolph Laborota-4000, Schwabach, Germany) was used to remove the methanol at 35ºC for 22 minutes. Then 15 mL of acetonitrile was added and washed with 20 mL of n-hexane for 3 times. In order to remove acetonitrile from the phenolic extract, further evaporation process was implemented under vacuum for 37 minutes and the residue was exposed to nitrogen for 10 minutes and dissolved in 1 mL of methanol/water mixture. Lastly, the extract was filtered with 0.45 μm pore-sized membrane (Minisart, Sartorious, and Goettingen, Germany) and transferred into vial. Injection volume was arranged as 20 μL in HPLC system.

**HPLC Analysis:** In order to measure individual phenolic compound amounts HPLC system with a Perkin Elmer series 200 pump (Norwalk CT 06859, USA) was used. Other system specifications are; PE series 200 diode array detector, PE-Nelson 900 series interface, Meta Therm HPLC column heater (series no:9540, Torrance, USA) and 5μm, 25 cm*4.6 mm, C18 column (Ace, Aberdeen, Scotland). All the necessary details about HPLC system are listed in Table 3.4 and Table 3.5.

Internal standard (ISTD) method was preferred to quantify any loss of phenolic compounds. Gallic acid is not expected to be found in olive oil sample so that it was choosen as an internal standard. Major phenolic compounds like cinnamic, caffeic,

vanilic, syringic, p-coumaric, o-coumaric, 4-hydroxyphenyl acetic, 3-hydroxyphenyl acetic and 2-3 dihydroxybenzoic acids, apigenin, luteolin, vanilin, tyrosol, and hydroxytyrosol were determined by using their standard form. For each standard points calibration curves were plotted to quantify phenolic compounds in terms of mg/kg olive oil. Standard curves are given in Appendix B.

Table 3.4. Analytical conditions for HPLC system

| **Analytical conditions** | |
|---|---|
| Mobile Phase, A | Water/acetic acid (99,8:0,2 v/v) |
| Mobile Phase, B | Methanol |
| Column Temperature | 35 $^{o}$C |
| Flow Rate | 1 mL/min |
| İnjection Volume | 20 µL |
| Wavelength | 280 and 320 nm |

Table 3.5. Gradient of mobile phase concentrations for the HPLC analysis

| Mobile Phase Concentrations | | |
|---|---|---|
| Time(min) | % Mobile Phase, A | % Mobile Phase, B |
| 0 | 90 | 10 |
| 10 | 70 | 30 |
| 30 | 70 | 30 |
| 40 | 60 | 40 |
| 45 | 60 | 40 |
| 50 | 50 | 50 |
| 55 | 50 | 50 |
| 60 | 40 | 60 |
| 65 | 30 | 70 |
| 70 | 0 | 100 |
| 85 | 90 | 10 |

## 3.12. Fatty Acid Profile

**Sample Preparation:** In the preparation of methyl esters European Official Methods of Analysis (EEC, 1991) was used. Oil sample (0.1 g) was weighed in 20 mL centrifuge tubes and samples were dissolved in 10 mL of pure n-hexane and saponified to their methyl esters with the addition of methanolic potassium hydroxide (KOH) solution (2.8 g KOH in 25 mL methanol). Then the sample solution was vortexed for 30 s and centrifuged at 5000 rpm for 15 min. Clear supernatant was transferred into the vial via syringe and 0.45 µm syringe filter. After filtration supernatant was injected into the gas chromatography (GC) instrument.

**Analytical Conditions:** Chromatographic analyses were carried out on an Agilent 6890 GC (Agilent Technologies, Santa Clara, USA) equipped with Agilent 7683 auto-sampler. The analytical conditions and equipment specifications are shown in Table 3.6.

Table 3.6. Chromatographic conditions for the analysis of fatty acid methyl esters

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

## 3.13. Fourier Transform Infrared (FTIR-ATR) Spectroscopy Analysis

Infrared spectra, within the wavenumber range of 4000-650 cm$^{-1}$, were acquired by Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., Wellesley, MA, USA). FTIR instrument was equipped with a horizontal attenuated total reflectance (HATR) accessory with ZnSe crystal plate and a DTGS (deuterated tri-glycine sulphate) detector. For each measurement, the resolution was set at 4 cm$^{-1}$ and the number of scans were 64. ZnSe crystal was cleaned with hexane and ethanol in between sample runs. Measurements were repeated two times as randomly. A typical spectrum of an olive oil sample is given in Figure 3.2.

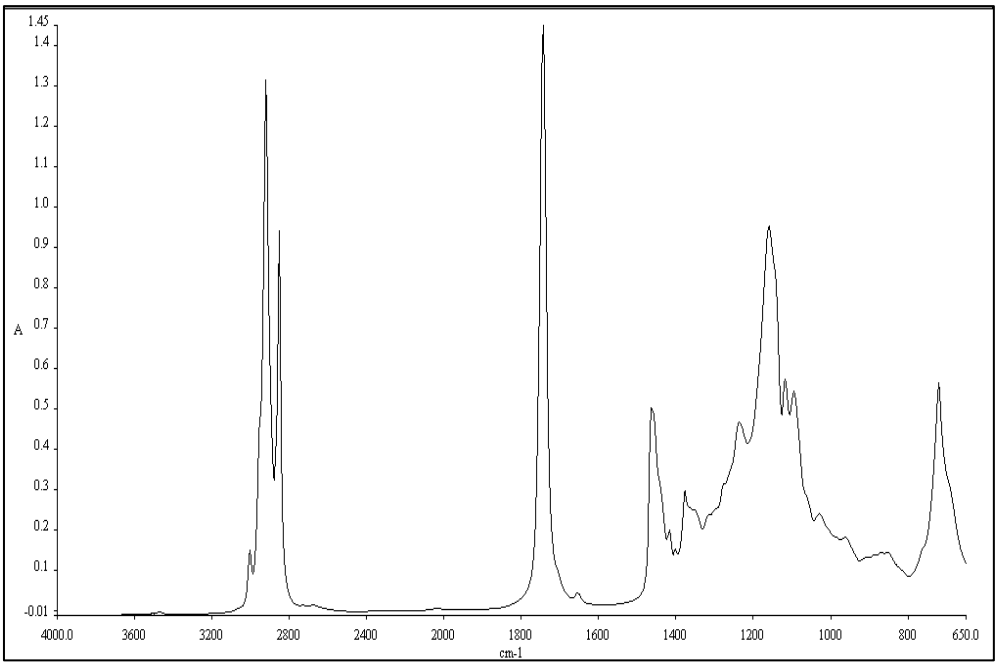


Figure 3.2. Typical mid-infrared spectrum of olive oil

In the thesis, the FTIR-ATR spectra are called as FTIR for simplicity and the profiles of oil samples from FTIR-ATR spectroscopy are given wavenumber ($cm^{-1}$) versus absorbance value.

## 3.14. Multivariate Statistical Analysis

In classification and regression analysis, multivariate statistical methods (MVA) were used with chemical (quality variables and fatty acid profiles) and spectrometric data (UV-Visible and FTIR spectra). Principal component analysis (PCA) and orthogonal partial least square – Discriminant Analysis (OPLS-DA) were used in the classification of oil samples. OPLS was used in the prediction of some chemical parameters by spectrometric profiles. The variables such as FFA, PV and OS (as predicted variable, i.e. Y variables) were modelled by FTIR, UV and combination of FTIR plus UV spectra (i.e. X variables). SIMCA software (ver. 13.0.3, Umetrics, Umea, Sweden) was used in MVA. In FTIR spectra of the samples, some regions were removed from the analysis since these portions do not contain any information related to the sample. Two portions of whole spectra (3080-2650 and 1850-650 $cm^{-1}$) where there were the most significant differences in the peak intensities, were selected to be employed in the models.

### 3.14.1. Principal Component Analysis (PCA)

The Principal Components Analysis (PCA) as an unsupervised MVA, a popular statistical tool, is used to decrease the number of a set of variables in a data set, while retaining the maximum variability in terms of the variance–covariance structure and as a result, revealing the similarities/differences in the observations (Jolliffe, 2002).

PCA on a data matrix **X** of size (n x k) with n observations (samples) and k variables reduces original variables, k, to a new, uncorrelated **T** variables named scores (**T**<k). The loadings (**P**) and scores are derived from the correlation matrix of **X** (Brereton 2003).

$$\mathbf{X} = \mathbf{TP} + \mathbf{E} \tag{3.9}$$

where **X** is the original data matrix; **T** is the score matrix of size of (n x p); **P** is the loading matrix of size (p x k), **E** is the error matrix of size (n x k).

The main objective of PCA are: the reduction of dimension from k to p principal components, determination of linear combinations of variables (new score variables), feature selection: choosing the most beneficial variables, visualization of multidimensional data (as score and loading plots), identification of emphasizing variables, and identification of groups of objects or of outliers.

### 3.14.2. Orthogonal Projections to Latent Structures (OPLS)

Partial least square analysis (PLS) is the regression extension of PCA and it is used in for modeling quality variables stored in **Y** matrix with the information in **X** matrix by maximizing correlation between them. A PLS model can be described as shown in Eqs.3.10 and Eqn. 3.11; **X** and **Y** are the datamatrices between which a relationship is going to be set up **T** and **U** are the scores matrices, **P** and **Q** are the loadingmatrices, and **E** and **F** are the residual matrices (Galindo-Prieto, 2015).

$$\mathbf{X} = \mathbf{TP} + \mathbf{E} \tag{3.10}$$

$$\mathbf{Y} = \mathbf{UQ} + \mathbf{F} \tag{3.11}$$

Orthogonal projections to latent structures (OPLS) is an improved and modified PLS method that is used in spectroscopic data analysis for calibration, classfication and

calibration transfer in the last decade (Trygg et al., 2002; Pinto et al., 2012). The main objective of the OPLS is to develop interpretation of PLS models and minimise model complexity. OPLS derives a way to eleminate systematic variation from an input data set **X** not correlated to the response set **Y**. An OPLS model can be described as shown in Eqn. 3.12.

$$\mathbf{X} = \mathbf{t_p p_p} + \mathbf{T_o P_o} + \mathbf{E} \qquad (3.12)$$

Where **T** is the score and **P** is the loading, and the subscript of p and o indicate predictive and orthogonal variation, respectively. The matrix **E** accounts for the residual or noise (Pinto et al., 2012).

In this study, OPLS was used to predict Y variables (FFA, PV, $K_{232}$, $K_{270}$, total chlorophyll and carotenoid contents, OSI, TPC, and color parameters) from X variables (FTIR, UV, and FTIR and UV combination). In order to enhance the predictive ability of the OPLS model first derivative of X variables was used to eliminate noises and shifts.

There are major parameters to determine the reliability of prediction model like root mean square error of calibration (RMSEC) and cross validation (RMSECV), regression coefficients for calibration ($R^2$) and cross validation ($R^2_{CV}$). And other parameters are root mean square error of validation set (RMSEP) and regresion coefficients for validation set ($R^2$val).

Generally, a good model should have low values of root mean square of error and high values of regression coefficients and differences between calibration and validation $R^2$ values should be small (Zhang et al., 2015). In OPLS models, the number of principal components is given as $p_p + p_o$, which is to indicate the number of predictive and orthogonal components.

### 3.14.3. Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA)

Orthogonal partial least-squares discriminant analysis (OPLS-DA), as a supervised MVA is used in the classification of samples by a given (user-defined) class information in a user-created Y matrix (for example extra virgin olive oil samples as class 1, virgin olive oil samples as class 2, blended olive oil samples as class 3, etc.). The information in this categorical response matrix Y is evaluated to decompose the X

matrix into three seperate parts as described in Equation 3.12.

OPLS-DA utilizes variations in the areas between groups: variation data can be partitioned into two groups, first one containing variations that correlate with the identifier of class and the other category containing variations that are orthogonal to the first block and consequently do not conduce to discrimination between the groups (Foti et al., 2007; Blasco et al., 2015).

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1. Deep Frying of Different Types of Oils

Palatable taste of fried food makes the deep-fat frying one of the most popular food cooking methods used in household kitchen. In this study, four different types of olive oil and sunflower oil were used in the frying experiments. Sunflower oil was chosen as an alternative for olive oil because of its preferability in home cooking. The oil samples and their codes are: extra virgin olive oil (E), virgin olive oil (V), blended olive oil (B), refined olive oil (R) and sunflower oil (S). Samples were taken after 1, 2, 3, 4, 5, 7, and 10 frying cycles. All calculations were shown in Appendix-A with the details.

### 4.1.1. Free Fatty Acid (FFA)

The initial FFA values of the oil samples ranged from 0.8 to 1.45. Virgin olive oil had the highest and sunflower oil had the lowest initial FFA value. The initial FFA value of blended olive oil and refined olive oil were close. After frying process, the FFA values increased slightly (Figure 4.1). Even the increase in FFA values were 13 %, 5 %, 15 %, 48 %, and 69 % for E, V, B, R and S, respectively, the values stayed within the defined levels (Chapter 2, Section 2.2).

Andrikopoulos et al. (2002) studied the performance of virgin olive oil during 10 successive deep fryings at 170ºC for a total period of 120 min. Also Akil et al. (2015) studied oxidative stability and changes in chemical composition of extra virgin olive oil and sunflower oil after deep frying of french fries at 180ºC for up to 75 min of successive frying. In both studies, it was reported that FFA values increased insignificantly.

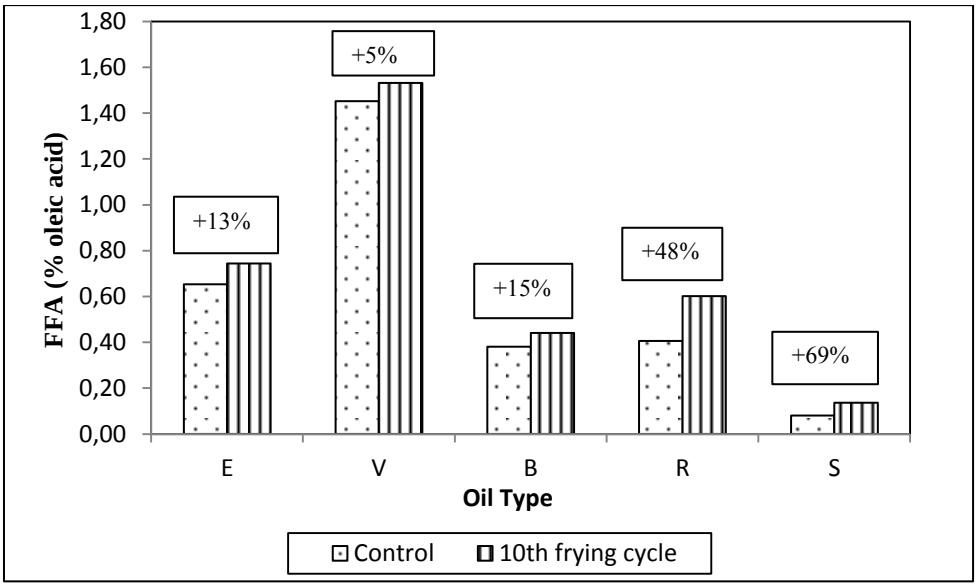


Figure 4.1. Changes in FFA values of oil samples after frying process

## 4.1.2. Peroxide Value (PV)

Oxidation of fatty acids leads to formation of hydroperoxides, which are often estimated by PV, another important oil quality parameter. The peroxide values of oil samples (without frying) within the legal limits: below 20 meq $O_2$/kg for extra virgin and virgin olive oils, 15 meq $O_2$/kg for the blended olive oil, 5 meq $O_2$/kg for the refined olive oil, and 10 meq $O_2$/kg for the sunflower oil. Only refined olive oil exceed the upper limit of PV.

Virgin olive oil had the highest and sunflower oil had the lowest initial PV among the oil samples (Figure 4.2). Extra virgin and virgin olive oil samples showed a sharp drop in PVs while other oil samples showed a progressive increase. Decrease in PV values were 27 % and 37 % for E and V and the percentages of increase in PV values of B, R and S were 13, 11, and 55, respectively.

Blended and refined olive oils presented comparatively smaller peroxide values, as expected due to the refining process, and PV during frying process was also quite constant.

The PV value of sunflower oil started from 6 meq $O_2$/kg and at the end of $10^{th}$ frying cycle it was measured as 9.3 meq $O_2$/kg. This increase can be explained by the high polyunsaturated fatty acid levels, mostly linoleic acid as the characteristic of sunflower oil, since it is highly inclined to oxidation when compared to

monounsaturated oils.

In a study of deterioration of refined olive oil due to deep frying, oils fried at 180ºC for varying periods of time i.e. 30, 60 and 90 min., PV values of olive oils increased in time (Naz et al., 2005). In an another study, olive oil samples were fried until the total polar compounds achieved the maximum legal value (25%) and in this period PV values of extra virgin and virgin olive oils did not change signficantly (Casal et al., 2010).

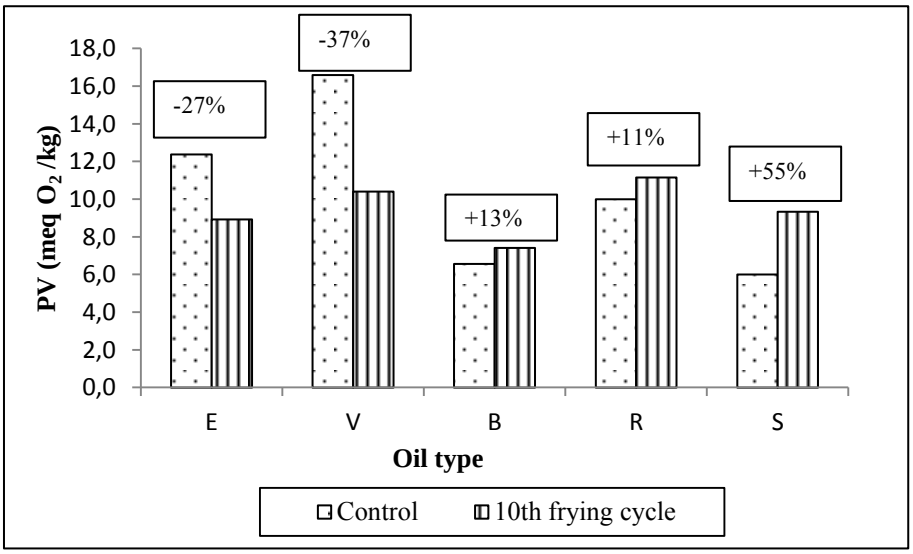


Figure 4.2. Changes in PV values of oil samples after frying process

### 4.1.3. $K_{232}$ and $K_{270}$ Values

The degree of oxidation of olive oil can also be determined by analyzing the specific extinction values of the oils. The $K_{232}$ is related with the formation of conjugated dienes of polyunsaturated fatty acids and $K_{270}$ is correlated with conjugated trienes. The upper limits for $K_{232}$ and $K_{270}$ are 2.50 and 0.22 for extra virgin olive oils, 2.60 and 0.25 for virgin olive oils, respectively. The limit $K_{270}$ values are 1.10 and 0.90 for refined and blended olive oils, respectively. For sunflower oil the $K_{270}$ limit is 0.90 .

In the control samples (non-fried), extra virgin olive oil had the lowest value for both K values and the sunflower oil had the highest value for both K values. K values increased with frying. The difference in $K_{270}$ was sharper than $K_{232}$ for all oil samples (Figure 4.3 and Figure 4.4).

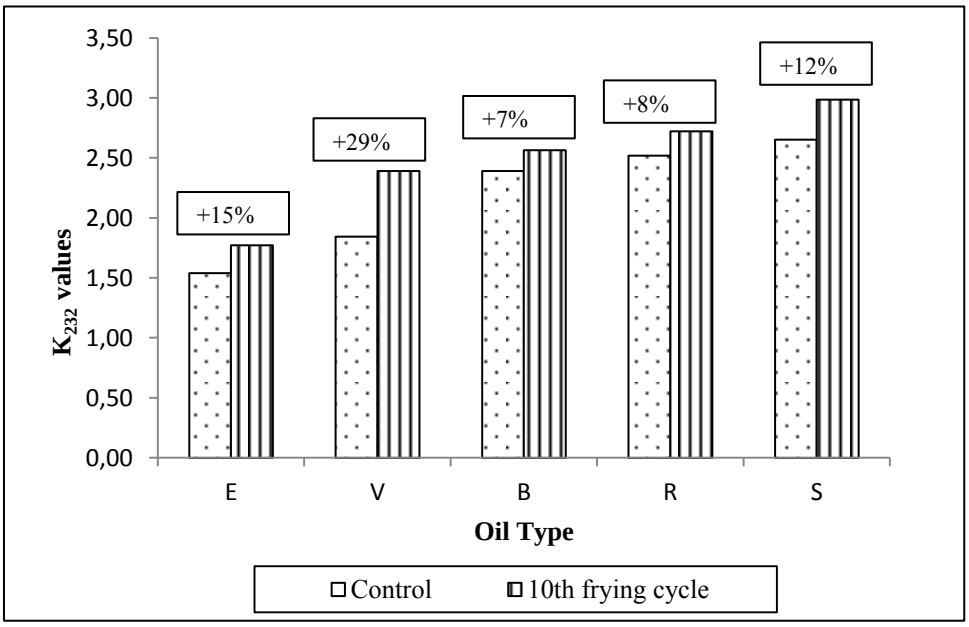


Figure 4.3. Changes in $K_{232}$ values of oil samples after frying process

Andrikopoulos et al. (2002) studied about performance of virgin olive oil during deep frying at 170ºC for 120 min. In this study, $K_{232}$ value increased during frying process. In an another study, Quiles et al. (2002) was studied about physicochemical chenges during short time deep frying. They were also observed that $K_{232}$ and $K_{270}$ values increased during frying.

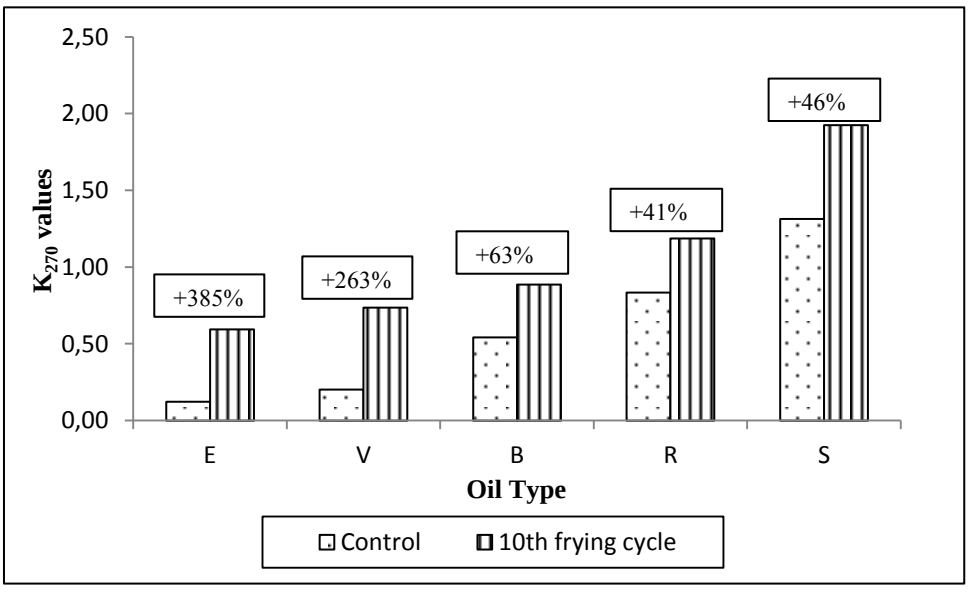


Figure 4.4. Changes in $K_{270}$ values of oil samples after frying process

## 4.1.4. Total Chlorophyll and Carotenoid Contents

Chlorophyll compounds are responsible for the green color, while the carotenoid content indicates the yellow fraction. Color pigments, total chlorophyll and carotenoid values were determined in the range of 0.19-1.42 mg/kg oil and 0.09-1.62 mg/kg oil for control samples, respectively.

Virgin olive oil had the highest total chlorophyll value while the extra virgin olive oil had the highest total carotenoid amount. Refined olive oil and sunflower oil had the lowest value for both category because of the refining process. After frying, chlorophyll and carotenoid values of extra virgin and virgin olive oil decreased sharply (Figure 4.5 and Figure 4.6). Similar to our results, it was also concluded in another study that chlorophylls showed more stable sturucture than carotenoids by heat (Aparicio-Ruiz and Gandul-Rojas, 2014).

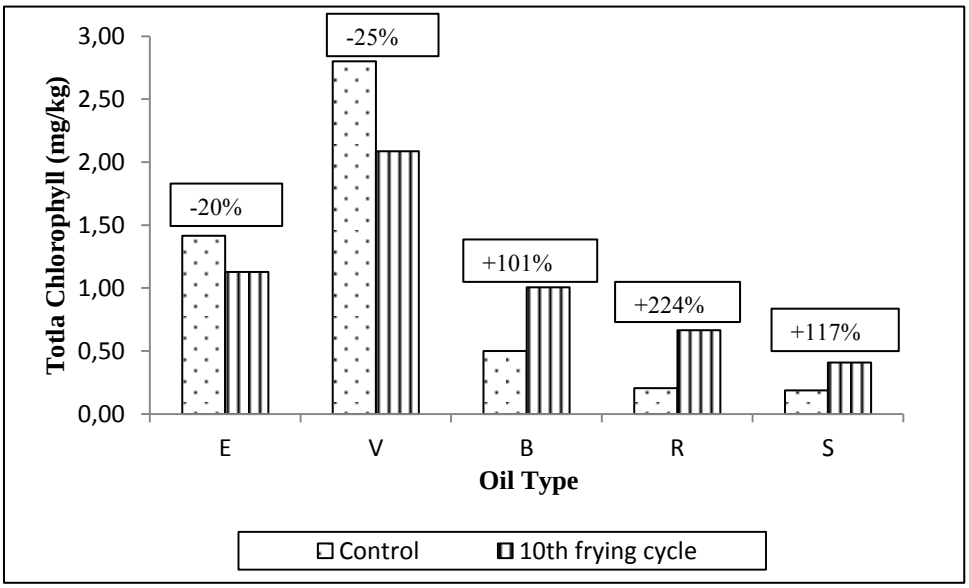


Figure 4.5. Changes in total chlorophyll values of oil samples after frying process

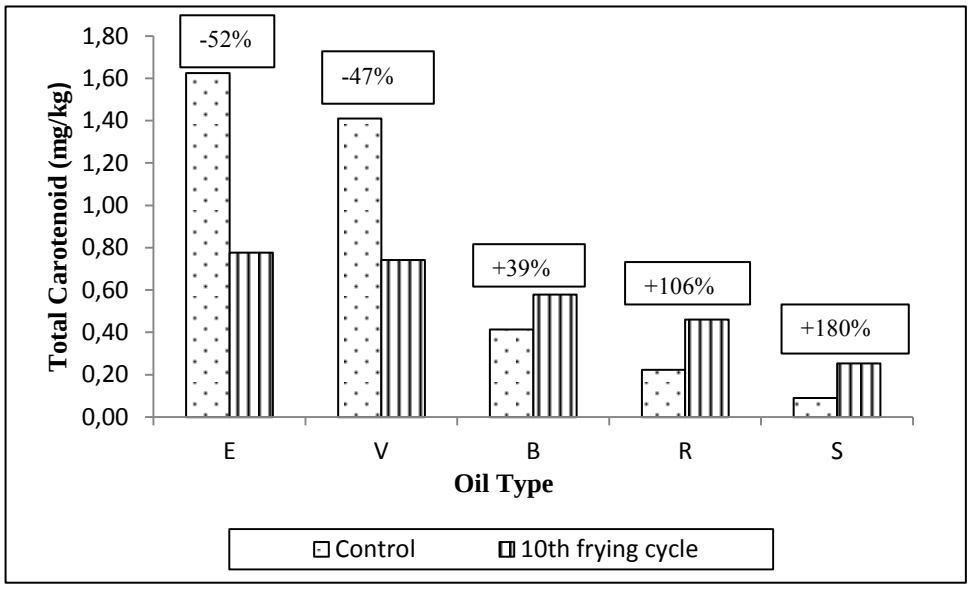


Figure 4.6. Changes in total carotenoid values of oil samples after frying process

## 4.1.5. Oxidative Stability Index (OSI)

The oxidative stability of oil samples can be determined by the Rancimat method, which estimates oxidative stability index (reported as induction time in hours) under heating and exposed air conditions. The highest initial OSI was observed by the extra virgin olive oil with 8.35 h and the lowest value was depicted by the sunflower oil with 2.65 h (Figure 4.7). No significant change was observed between control and $10^{th}$ frying cycle.

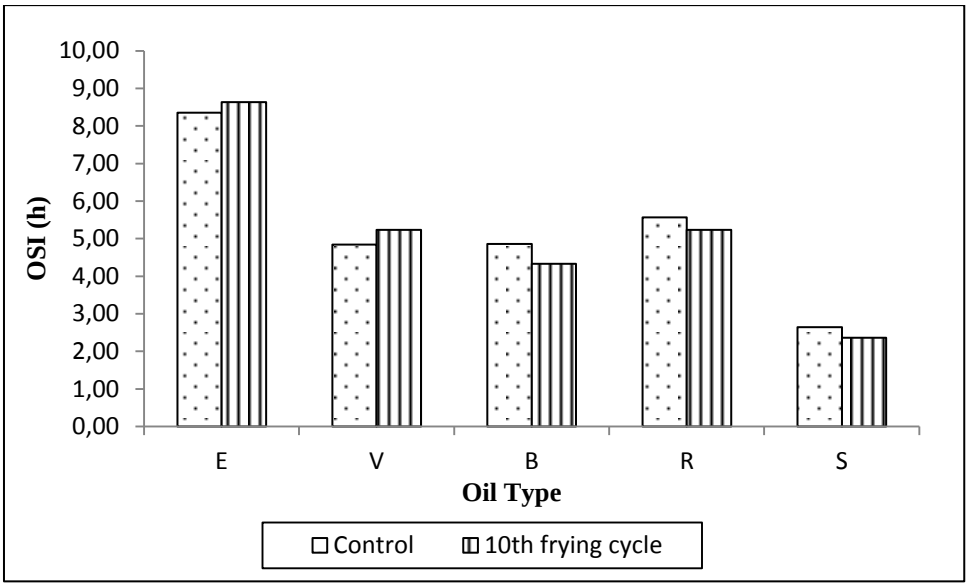


Figure 4.7. Changes in oxidative stability index values of oil samples after frying process

Casal et al. (2010) studied about olive oil stability under deep frying conditions at 170 °C. They concluded that extra virgin olive oil more resistant than virgin and blended olive oil and sunflower oil under the frying conditions.

## 4.1.6. Color Parameters

Sunflower oil had the highest initial L* value among the oil samples. The L* values of blended and refined olive oils were close to each other, while extra virgin olive oil had the lowest L* value. L* values of oil samples decreased due to frying except extra virgin olive oil (Figure 4.8).

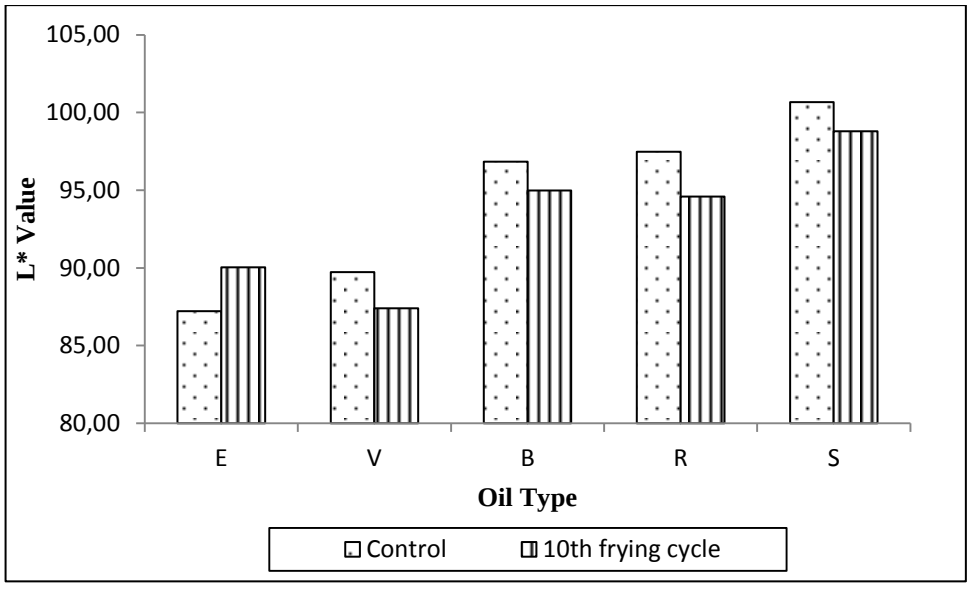


Figure 4.8. Changes in L* values of oil samples after frying process

The highest and the lowest initial a* values were -0.43 for extra virgin olive oil and -5.18 for refined olive oils (Figure 4.9). The a* values after frying were close to each other for all olive oil samples. Decrease was sharp for extra virgin and virgin olive oil, while the change was slight for blended and refined olive oil. Decrease was sharp for extra virgin and virgin olive oil, while the change was slight for blended and refined olive oil.

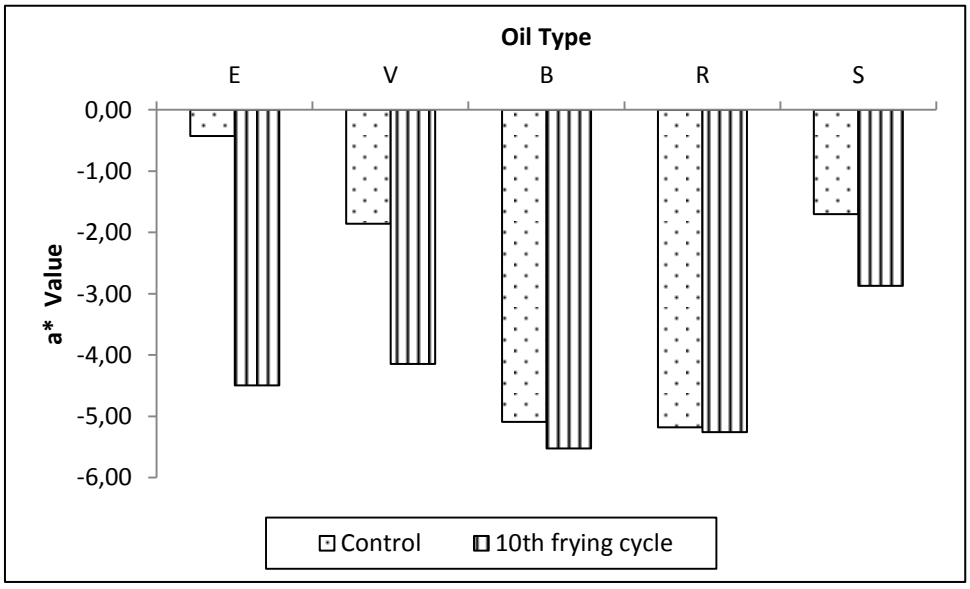


Figure 4.9. Changes in the a* values of oil samples after frying process

Virgin olive oil had the highest initial b* value, which was close to extra virgin olive oil, while the sunflower oil had the lowest initial b* value (Figure 4.10). There was a decrease in b* values of extra virgin and virgin olive oil before and after frying values.

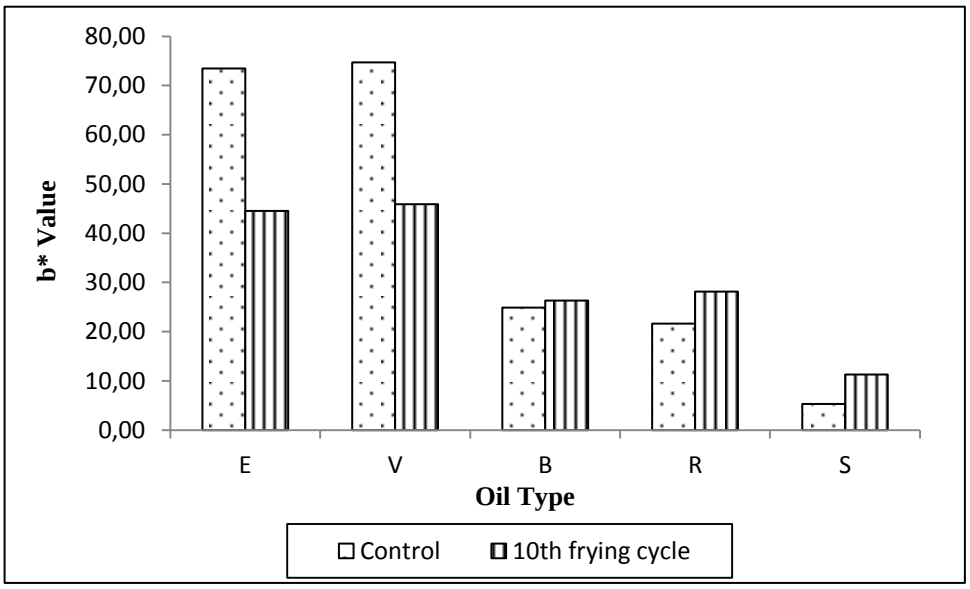


Figure 4.10. Changes in b* values of oil samples after frying process

Carotenoid compounds are responsible for yellow fraction in oil samples and in the CIELAB color scale, b* value indicates blue to yellow axis. So that if the carotenoid amount decreases, yellow fraction (b* value) should decrease. The same kind of change was observed in this study as can be seen in Figure 4.6 and Figure 4.10.

Sanchez-Gimeno et al. (2008) studied the physical changes in Bajo Aragon extra virgin olive oil during frying at 170ºC. They concluded that L* values increased for olive oil after frying while the opposite happened for high oleic sunflower oil also they reported that frying process caused and increase of b* values for extra virgin olive oil.

## 4.1.7. Total Polar Compounds

Total polar compound measurement (%) is widely accepted by the regulator agencies as one of the indicators of the state of the fried oil and maximum level is defined as 25 % (Tan and Man, 1999). The control oils presented total polar compounds

values (%) of 3.75 (extra virgin olive oil), 4.5 (virgin olive oil), 7.0 (blended olive oil), 7.25 (refined olive oil), and 8.25 (sunflower oil). The total polar compounds of refined oils were significantly higher than natural olive oils and frying process caused a raise in all oil samples (Figure 4.11). After frying, none of the oil samples exceeded the limit value of total polar compounds.

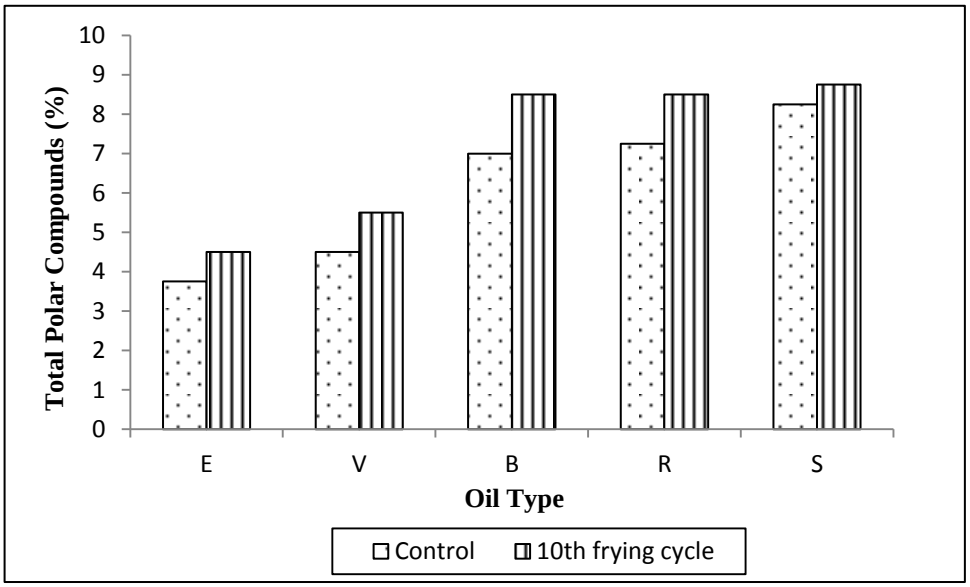


Figure 4.11. Changes in total polar compounds values of oil samples after frying process

Chen et al. (2013) studied total polar compounds of repeatedly used frying oils. As a result of deep frying of palm and soybean oils for 48 h and with three types of food (French fries, chicken fillet and pork chop), they concluded that oil type was a significant parameter for total polar compounds. They realized that soybean oil exceeded the limit value of total polar compounds after 23 h and palm olein exceeded after 25 h.

## 4.1.8. Total Phenol Content (TPC)

Before and after frying process, the changes in TPC values are given in Figure 4.12. After frying, the value of TPC decreased vastly for extra virgin and virgin olive oils, while it did not change for other oils.

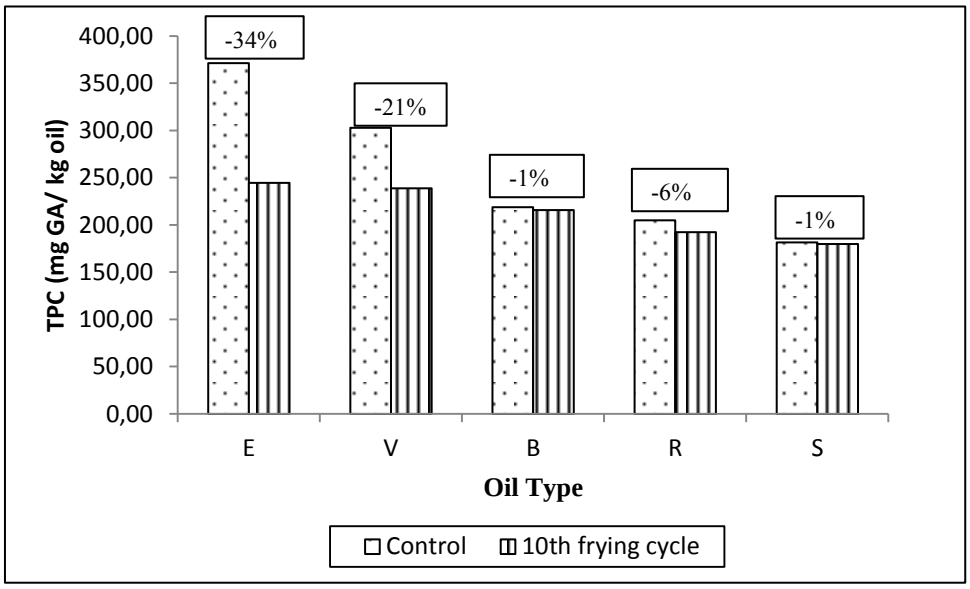


Figure 4.12. Changes in TPC values of oil samples after frying process

As seen in Figure 4.13, the sharp decrease in the value of E and V at the fourth frying was followed by a slow but continous decline up to frying cycle seven, then the TPC values of both olive oils were found close to each other.

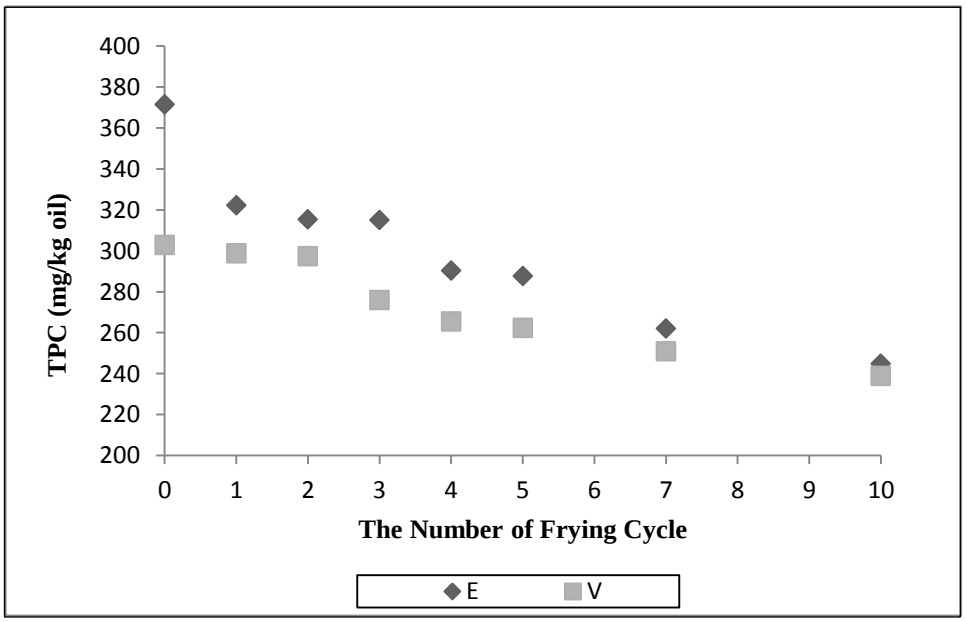


Figure 4.13. Changes in TPC values of E and V oil samples in frying process

## 4.1.9. Phenolic Composition

Phenolic compounds in olive oil is a significant parameter from many points of view (taste, color, nutritional) when determining the quality of olive oil, but at the same time natural phenols contribute to resistance of olive oil to oxidation.

Phenolic profiles were identified for extra virgin and virgin olive oil between control and tenth cycle, and for blended olive oil between control and third cycle. Phenolic compounds were studied in six groups: hydroxytyrosol, tyrosol, pinoresinol, apigenin, luteolin, and total phenolic acids (sum of 4-Hydroxyphenylacetic, 3-Hydroxyphenylacetic, caffeic, p-coumaric, vanilic, 2,3dihydroxybenzoic, m-coumaric, o-coumaric, chlorogenic, and ferulic acids). Some phenolic compounds, such as 3,4-dihydroxybenzoic, cinnamic and syringic acid and vanilin were not detected in oil samples.

In control sample of extra virgin olive oil, luteolin was the predominant phenolic compound, followed by pinoresinol and apigenin (Figure 4.14). After frying process, all phenolic compounds values decreased. Decreasing was sharp for pinoresinol, apigenin and lutelion.

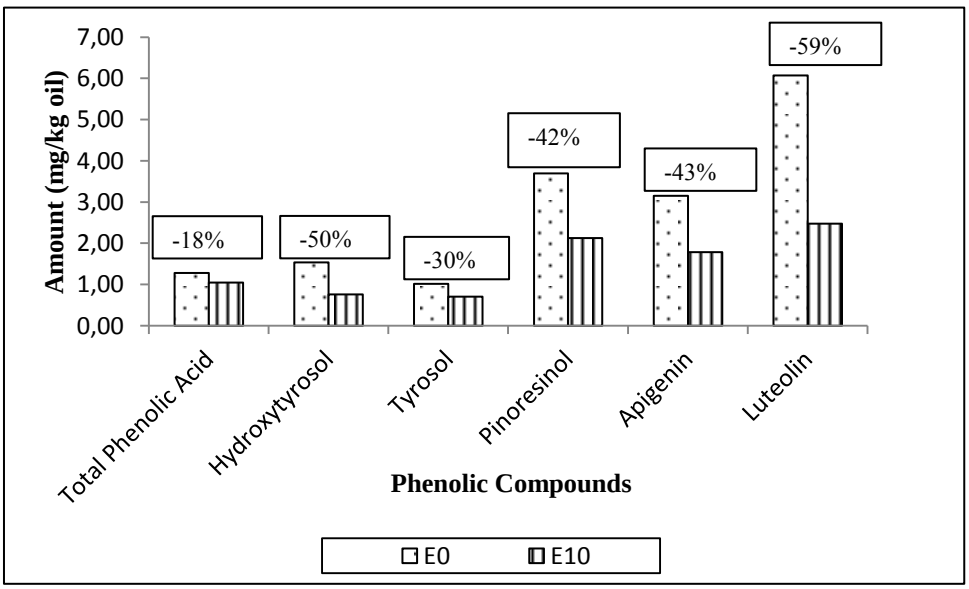


Figure 4.14. Changes in the amounts of phenolic compounds of extra virgin olive oil control sample (E0) and after frying (E10) process

.

In terms of phenolic compounds, the main difference between extra virgin and virgin olive oils was the concentrations of luteolin and pinoresinol contents. The amount of pinoresinol content of control and tenth frying cycle was 5.25 and 4.40 mg/kg oil, respectively (Figure 4.15). On the other hand, luteolin content of virgin olive oil was lower than that of extra virgin olive oil.

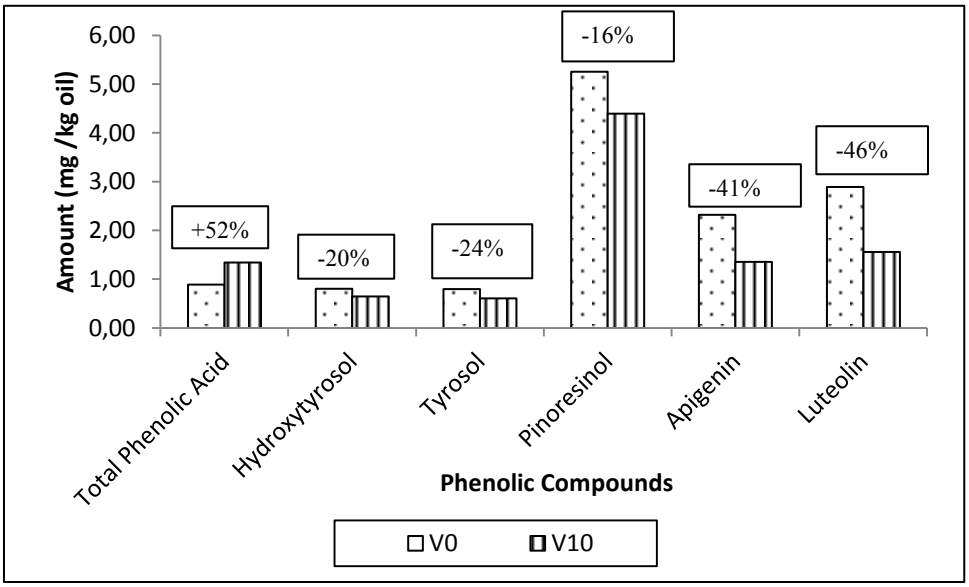


Figure 4.15. Changes in the amounts of phenolic compounds of virgin olive oil control sample (V0) and after frying (V10) process

Predominant phenolic compounds of blended olive oil, which consists of 80% refined olive oil, was pinoresinol like virgin olive oil. After the third frying cycle the values of all phenolic compounds decreased (Figure 4.16). Several phenolic compounds, such as vanilic, m-coumaric, o-coumaric, 2,3dihydroxybenzoic, chlorogenic, and ferulic acids, were not present at the end of 3$^{rd}$ frying cycle in blended olive oil sample.

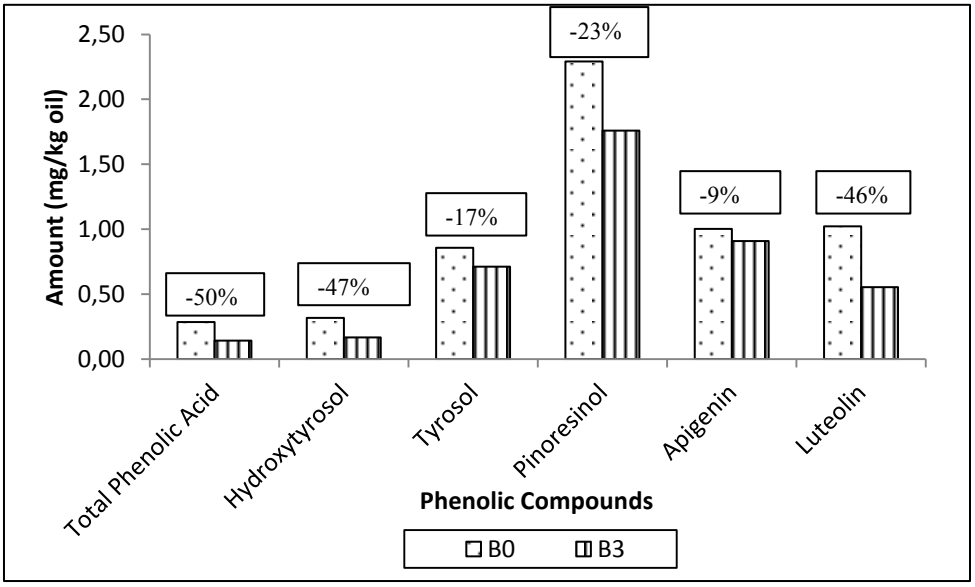


Figure 4.16. Changes in the amounts of phenolic compounds of blended olive oil control sample (B0) and in the 3rd frying cycle (B3)

The initial tyrosol concents were 1.01 and 0.80 for extra virgin and virgin olive oil, respectively and the tendency of decrease is not sharp for both olive oils. Olive oil with the highest tyrosol, also had the highest luteolin. This conclusion was similar with studies of Dagdelen et al. (2013).

## 4.1.10. Fatty Acid Profile

Olive oil was characterized by a high degree of oleic acid while the sunflower oil was characterized by high amount of linoleic acid and low amount of oleic acid.

According to EEC (No 1348/2013), olive oil fatty acid amount should be in the range of 55.0-83.0 (%) for oleic acid, 3.5-21.0 (%) for linoleic acid and ND (not detectable)-1.0 (%) for linolenic acid. In the study of Andrikopoulos et al. (2002) a decrease of oleic acid from 73.0 to 72.85% was observed.

According to Codex Alimentarius (2001), fatty acid composition of sunflower oil should be in the range of 14.0-39.4 (%) for oleic acid, 48.3-74.0 (%) for linoleic acid and ND-0.3 (%) for linolenic acid. In sunflower oil, oleic and linolenic acid amounts increased very slightly and the linoleic acid content decreased.

In this study, deep frying of potatoes did not cause any detectable increase in the trans fatty acids in the oils. In the studies of Andrikopoulos et al. (2002) and Akil et al. .

The fatty acid compositions of the olive oil samples were within the regulated levels. There was a small reduction in the fatty acid composition for olive oils after frying process (2015), the same conclusion was reported.

All the values about fatty acid compositions are shown in Figure 4.17, Figure 4.18 and Figure 4.19.

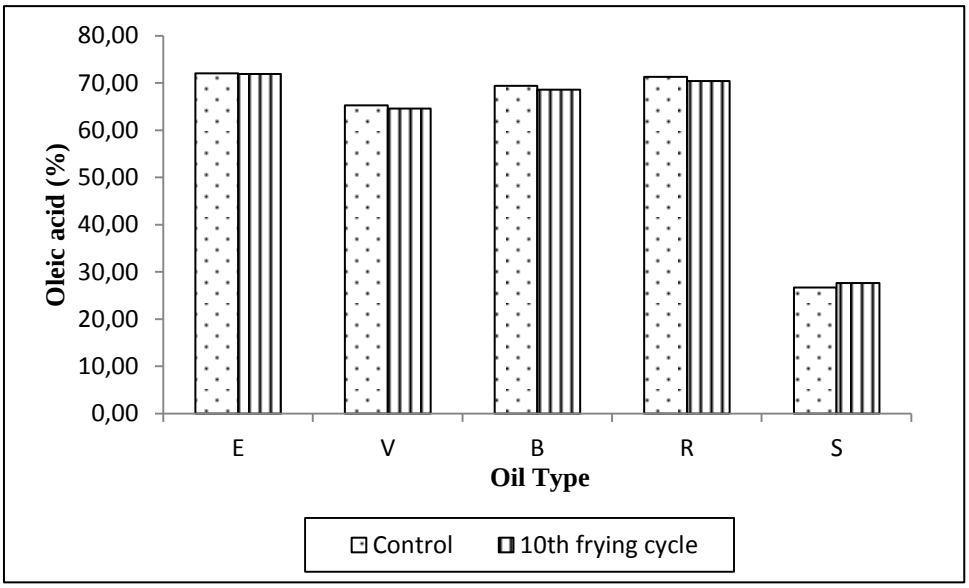


Figure 4.17. Changes in oleic acid values of oil samples after frying process

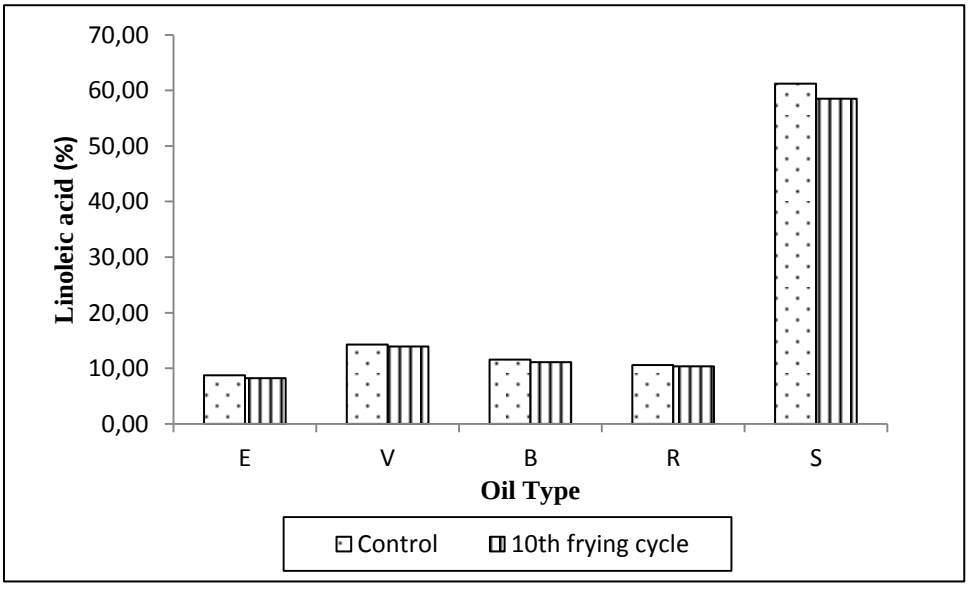


Figure 4.18. Changes in linoleic acid values of oil samples after frying process

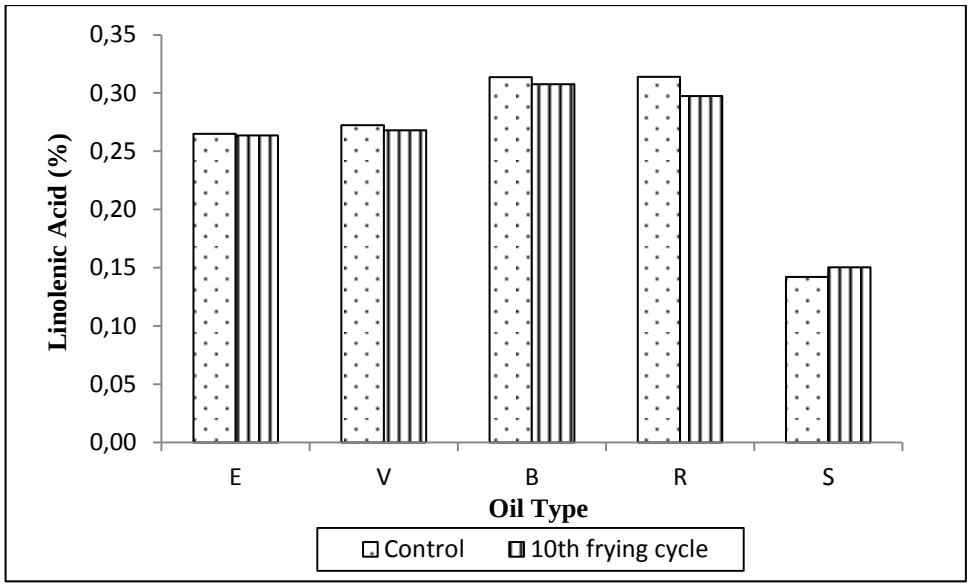


Figure 4.19. Changes in linolenic acid values of oil samples after frying process

As the quality variables some of the parameters which can be choice as a better indication of state of the frying oils. The increase in $K_{270}$ values (% increase) were between 41-385 %, the refined olive oil as the lowest and extra virgin olive oil as the highest. At the end of the $10^{th}$ frying cycle, the $K_{270}$ values were 0.60 and 0.74 for extra virgin and virgin olive oils respectively. A decrease in chlorophyll and carotenoid content was observed in extra virgin and virgin olive oils, whereas some amount of increase in blended and refined oils at the end of the frying. The increase can be explained by the transportation of those pigments from the food to the frying medium. However for different types of foods, the change in pigments concentration would be different. So the amount of chlorophyll and carotenoid content can not a good indication for the quality of frying oil. The total polar compounds is included in the food codex (Turkish and EU). However for short term-home frying cases, it might be lower than maximum legal value. No significant change were observed in OSI values. The decrease in the TPC was only seen in extra virgin and virgin olive oils not in the blended and refined oils. For the individual phenolic compounds the biggest decrease was seen in flavonoids (apigenin and luteolin).

Overall, in terms of these individual parameters, the only variable with a significant change in all types of oils was $K_{270}$, which could be a sign of frying effect.

Hypothesis test was applied to determine the significance levels for the differences in quality parameters of fresh oils and fried oils at the end of tenth cycle.

Two-sample t-test was used for each quality and chemical parameters of olive oils and sunflower oil. The results of the test is given in terms of p-values of t-test (Table 4.1).

Table 4.1. Comparision of fresh and fried oils (cycle 10): The p-values as a result of two-sample t test

| Parameters | E | V | B | R | S |
|---|---|---|---|---|---|
| **FFA** | * | * | ** | *** | ** |
| **PV** | * | NS | NS | NS | ** |
| **$K_{232}$** | *** | ** | NS | *** | NS |
| **$K_{270}$** | ** | *** | *** | ** | ** |
| **OSI** | NS | ** | NS | NS | NS |
| **TPC** | * | * | NS | NS | NS |
| **CHL** | NS | ** | * | NS | NS |
| **CRT** | * | *** | NS | NS | * |
| **L*** | NS | NS | ** | *** | *** |
| **a*** | ** | NS | * | NS | *** |
| **b*** | *** | *** | ** | *** | *** |
| **Palmitic acid** | NS | *** | *** | *** | ** |
| **Palmitoleic acid** | NS | NS | NS | ** | * |
| **Stearic acid** | NS | *** | ** | *** | ** |
| **Oleic acid** | NS | * | ** | ** | NS |
| **Linoleic acid** | NS | *** | NS | NS | * |
| **Linolenic acid** | NS | NS | * | NS | NS |

**(*, p-value less than 0.1; **, p-value less than 0.05; ***, p-value less than 0.01; NS, not significant )**

## 4.1.11. FTIR Profile

Spectrophotometric methods like FTIR can be alternative to traditional measurement techniques due to their advantages of short analysis time and easy sample preparation for the classification of olive oils (Gurdeniz et al., 2010). In a typical FTIR spectra of oils, one of the most considerable absorption band at 1743 $cm^{-1}$ is due to C=O stretching of aliphatic esters. Bands at around 2922 and 2852 $cm^{-1}$ are associated with symmetrical and assymmetrical C-H stretching of $CH_2$. Spectra were taken between 4000 and 650 $cm^{-1}$ but regions with no sample peaks (4000-3080 and 2650-1850 $cm^{-1}$) were excluded to eliminate unnecessary data (Du et al., 2012).

FTIR spectra of control samples in the whole range is shown in Figure 4.20. The most noticable point is difference of sunflower oil especially in the fingerprint region (1250-700 $cm^{-1}$).

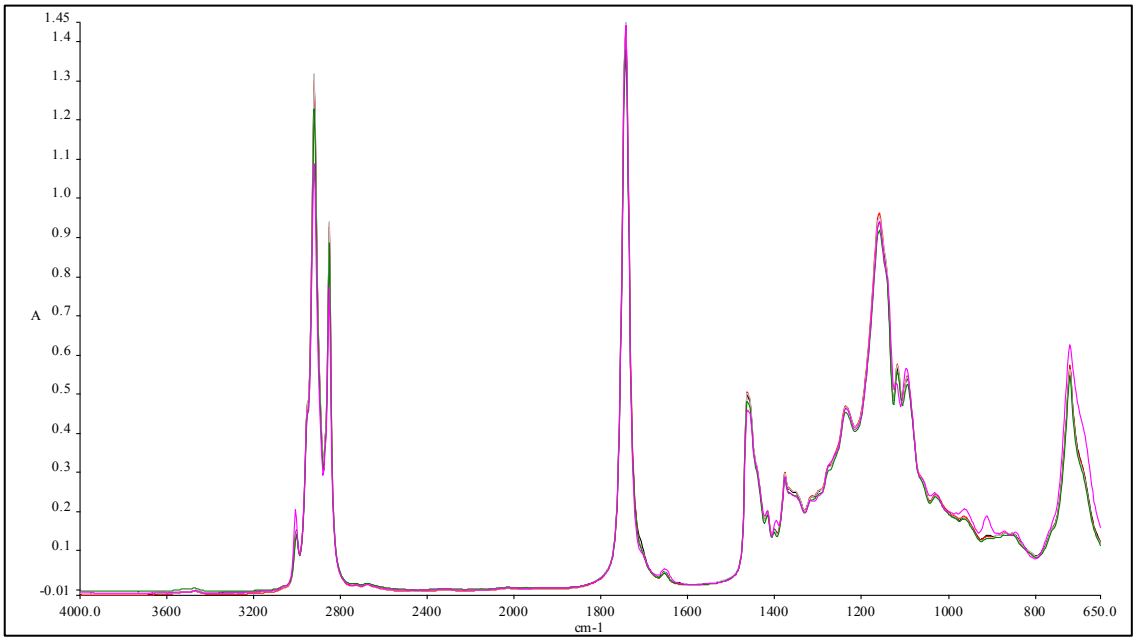


Figure 4.20. FTIR spectra of control samples (— E, — V, — B, — R, — S )

Near the bands 2950-2850 $cm^{-1}$ change in the intensity is seen in absorbance which is related with the symmetric stretching vibration of the aliphatic $CH_2$ group (a sections of Figures 4.21, 4.22, 4.23, 4.24 and 4.25). Band around the wavenumber of 1746 $cm^{-1}$, is the indicator of production of saturated aldehyde functional groups or

other secondary oxidation products. The absorbance of the band at 1746 $cm^{-1}$, represents C=O ester carbonyl of triglycerides. In this region, change in absorbance values of the band can be seen (Figures 4.21, 4.22, 4.23, 4.24, and 4.25). The band intensity changed along the fingerprint region while the most significant change in absorbance of band near 1163 $cm^{-1}$ refers to the oxidation process. In FTIR figures, X-axis is the wavenumbers ($cm^{-1}$) and Y-axis is the absorbance values.

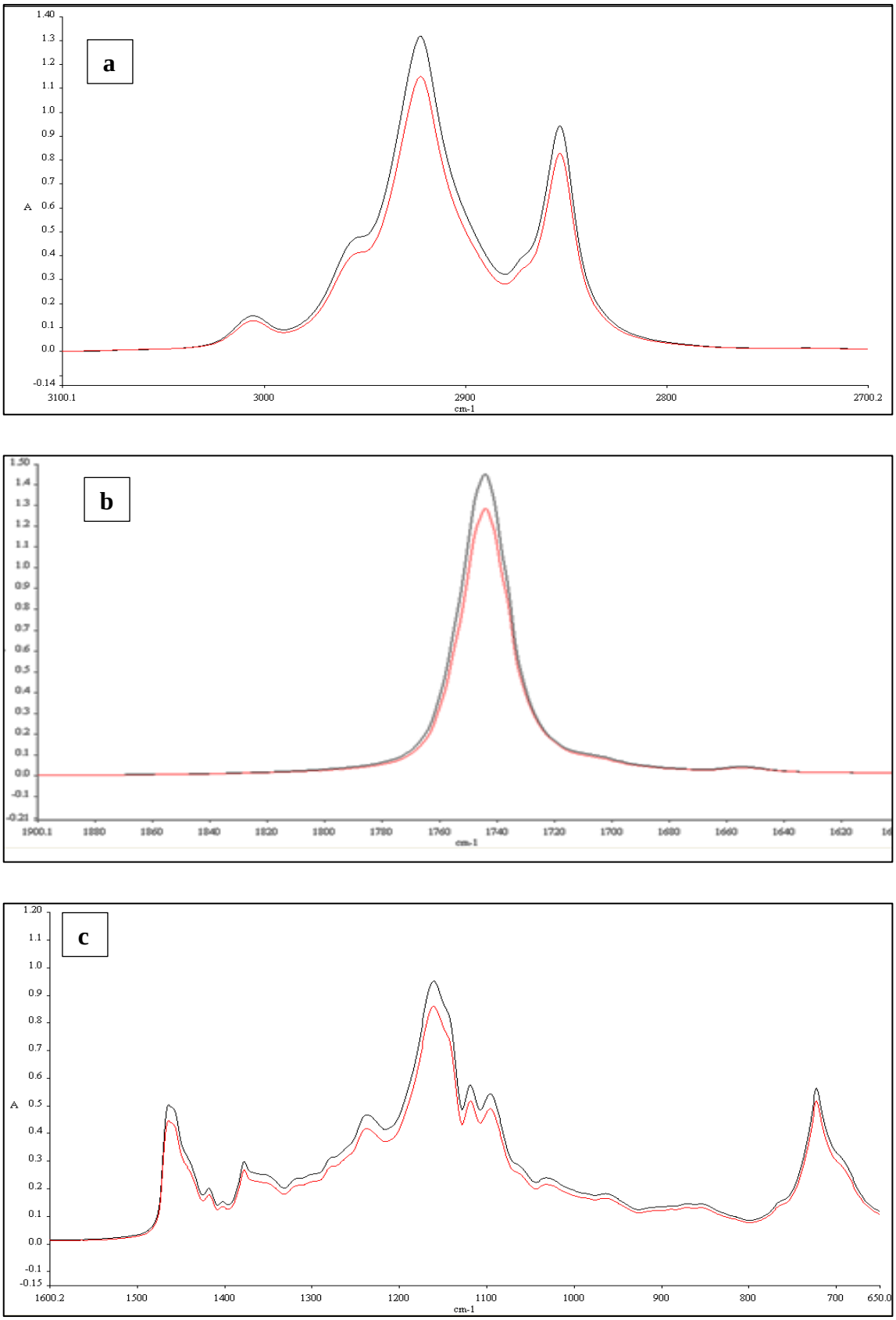


Figure 4.21. Changes in FTIR spectra for E a) 3100-2700 $cm^{-1}$ b) 1900-1600 $cm^{-1}$ c)1600-650 $cm^{-1}$ (—— control, —— after the $10^{th}$ frying cycle)

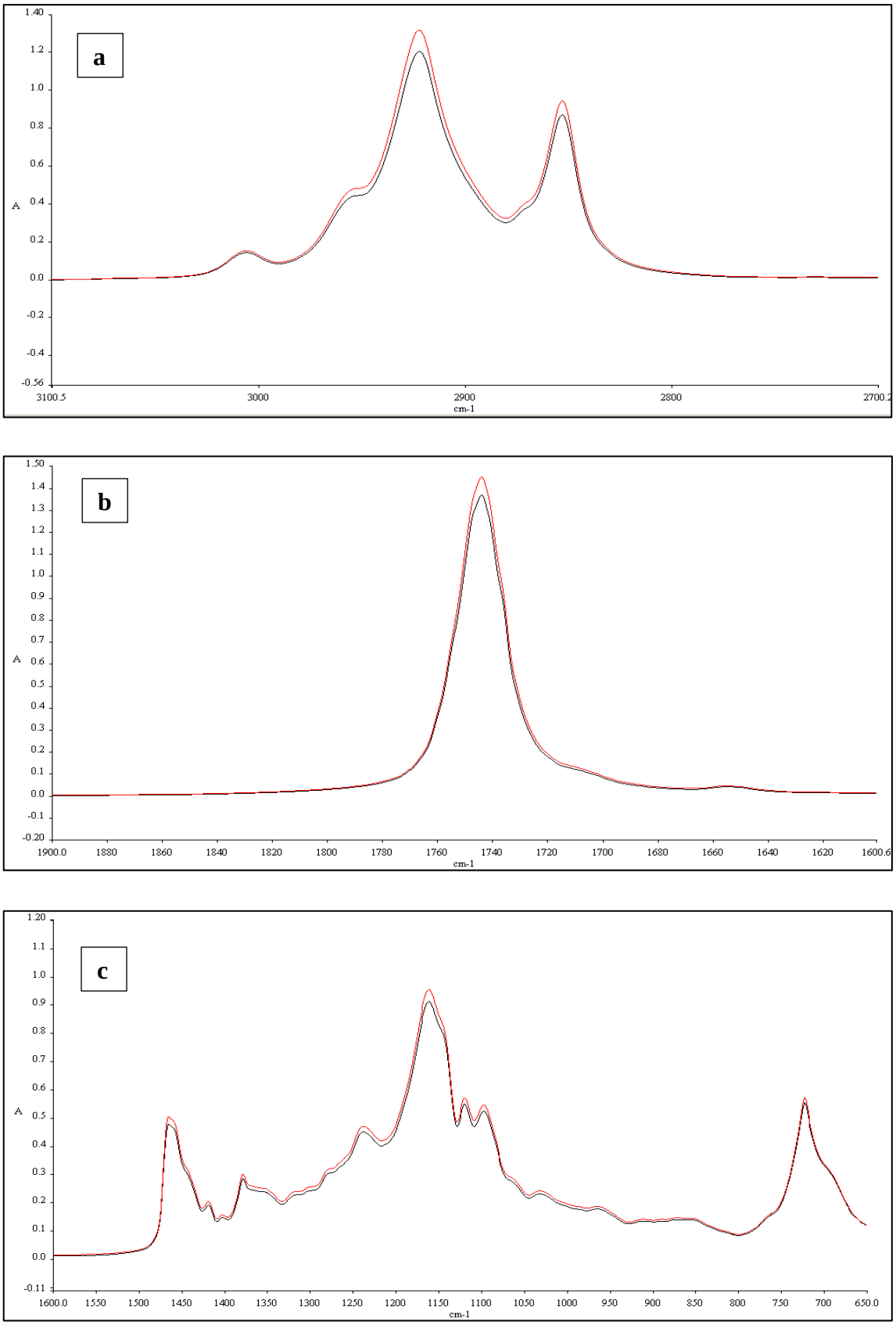


Figure 4.22. Changes in FTIR spectra for V a) 3100-2700 $cm^{-1}$ b) 1900-1600 $cm^{-1}$ c) 1600-650 $cm^{-1}$ ( —control, — after the $10^{th}$ frying cycle)

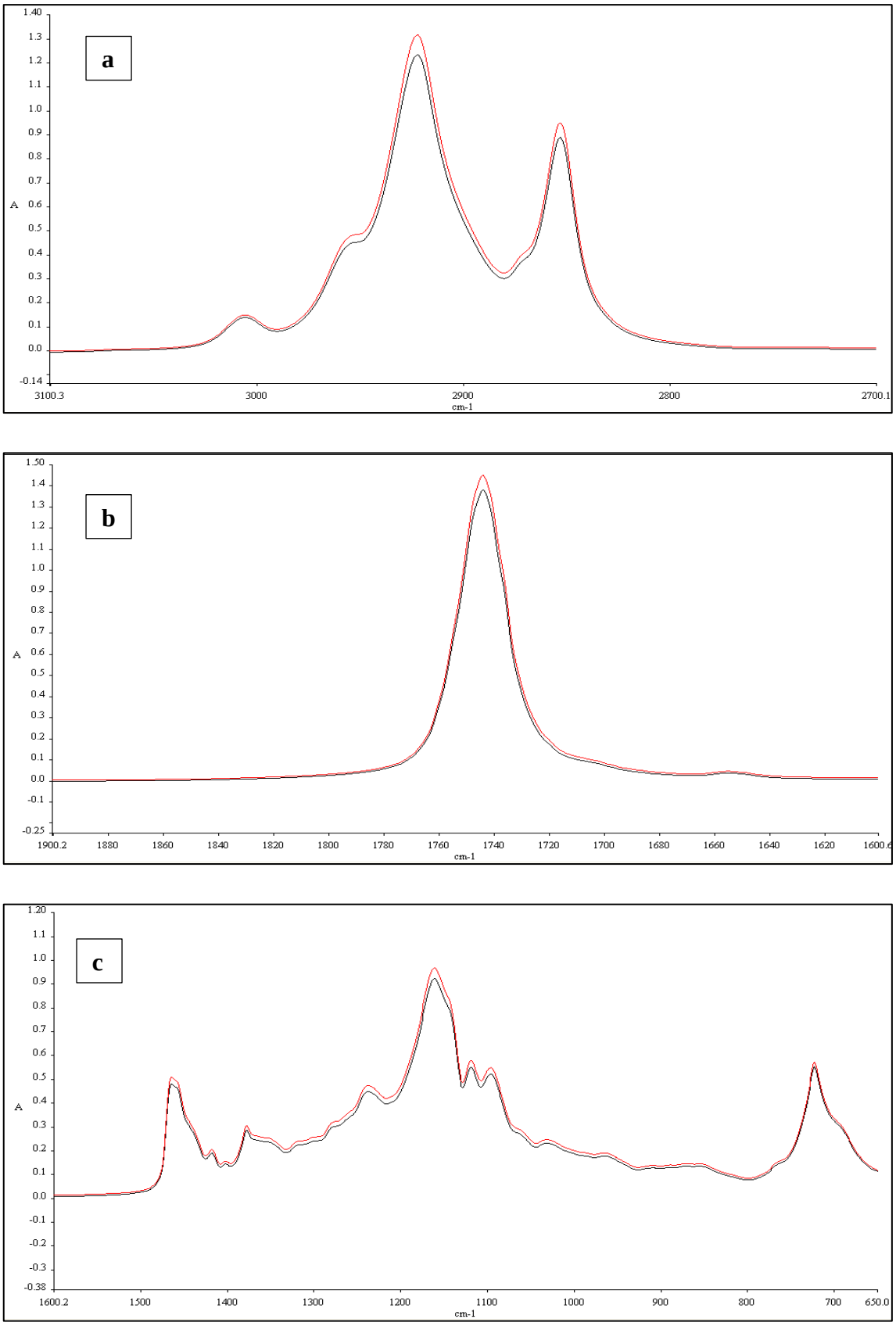


Figure 4.23. Changes in FTIR spectra for B a) 3100-2700 $cm^{-1}$ b) 1900-1600 $cm^{-1}$ c) 1600-650 $cm^{-1}$ ( —— control, —— after the 10$^{th}$ frying cycle)

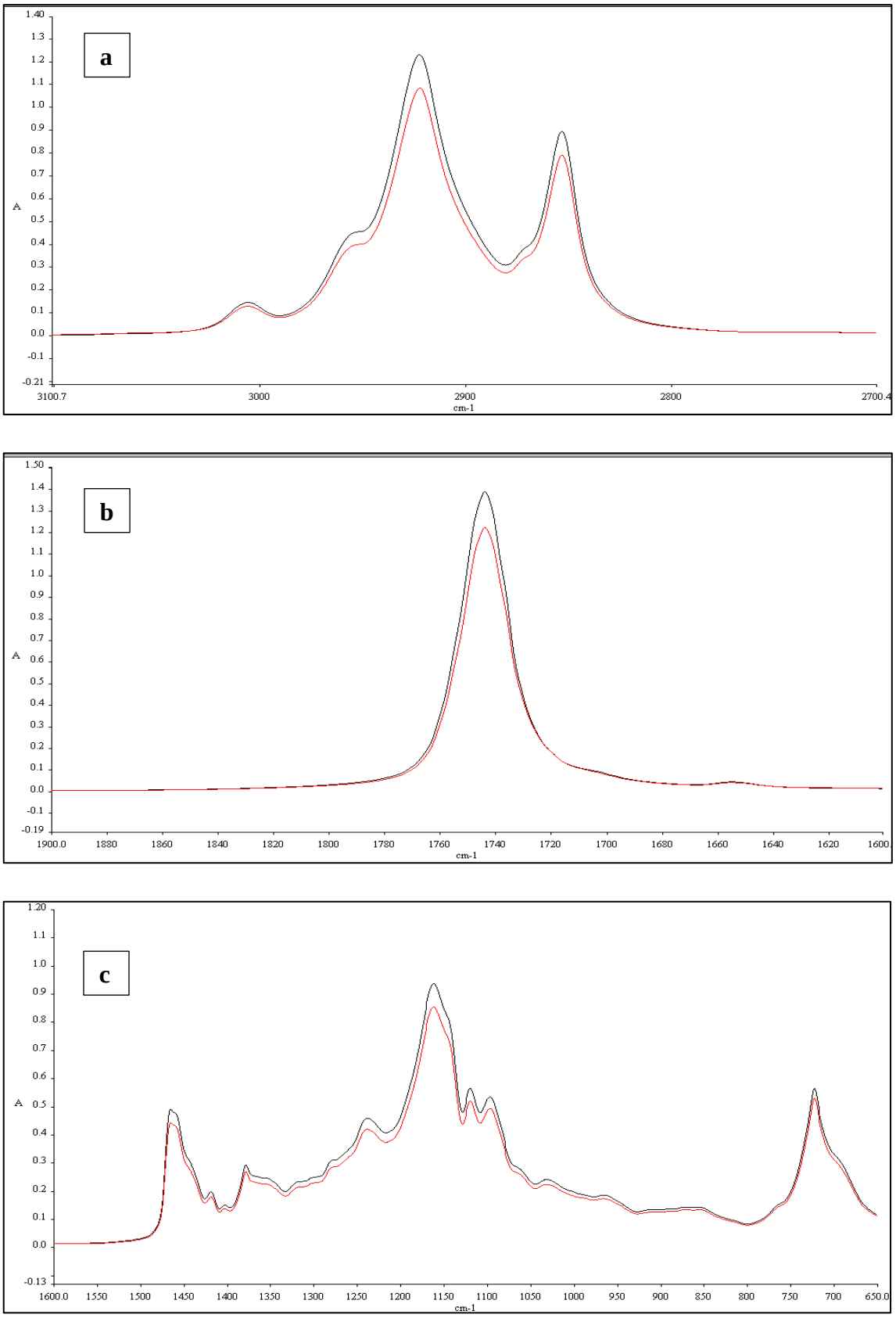


Figure 4.24. Changes in FTIR spectra for R a) 3100-2700 $cm^{-1}$ b) 1900-1600 $cm^{-1}$ c) 1600-650 $cm^{-1}$ (—— control, —— after the 10$^{th}$ frying cycle)

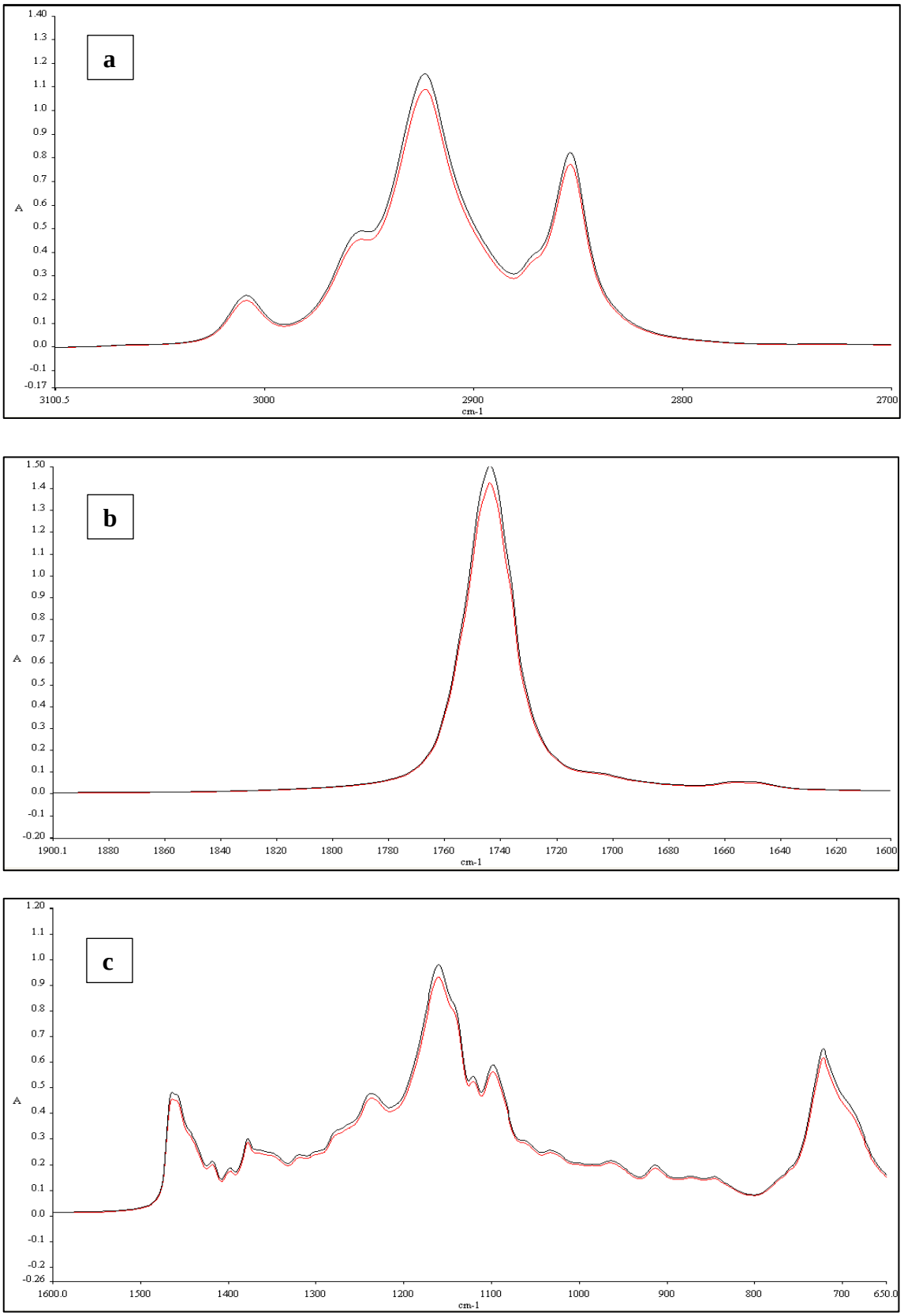


Figure 4.25. Changes in FTIR spectra for S a) 3100-2700 $cm^{-1}$ b) 1900-1600 $cm^{-1}$ c)1600-650 $cm^{-1}$ ( ——control, ——after the $10^{th}$ frying cycle)

The changes in the FTIR spectra can not be explained with the frying effects since in some of the spectra, an increase in the absorbance was observed while in the others decrease was observed. There was no general pattern that could be related with the frying process. Therefore, slight differences in absorbances (both decrease and increase) were observed in the FTIR spectra among fresh oils and fried oils.

## 4.2. Classification of Oil Samples

### 4.2.1. Classification Using UV-Vis Spectrometry

UV spectra between 200 and 800 nm data were used in PCA model. The 1st derivative filter was used in the pretreatment of the data. The score plot of 12-components model with $R^2 = 0.99$ and $R^2_{CV} = 0.98$ is shown in Figure 4.26. Blended and refined olive oils were not separated from each other while the sunflower oil differentiated clearly from the others.

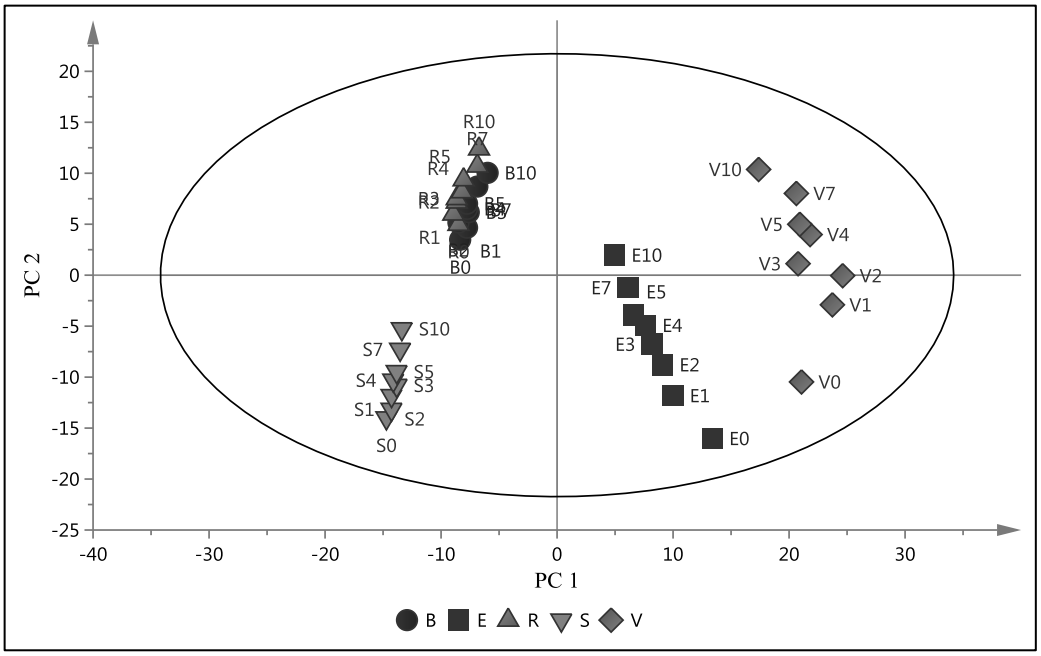


Figure 4.26. Score plot of PCA of oil samples with UV data

The PLS-DA and OPLS-DA models were created with the 1st and 2nd derivative data, respectively. The score plot of OPLS-DA model with 4+2 principal components, $R^2$= 0.94 and $R^2_{CV}$ = 0.89 shows a similar result to PCA model (Figure 4.27). No differentiation between B and R samples was achieved.

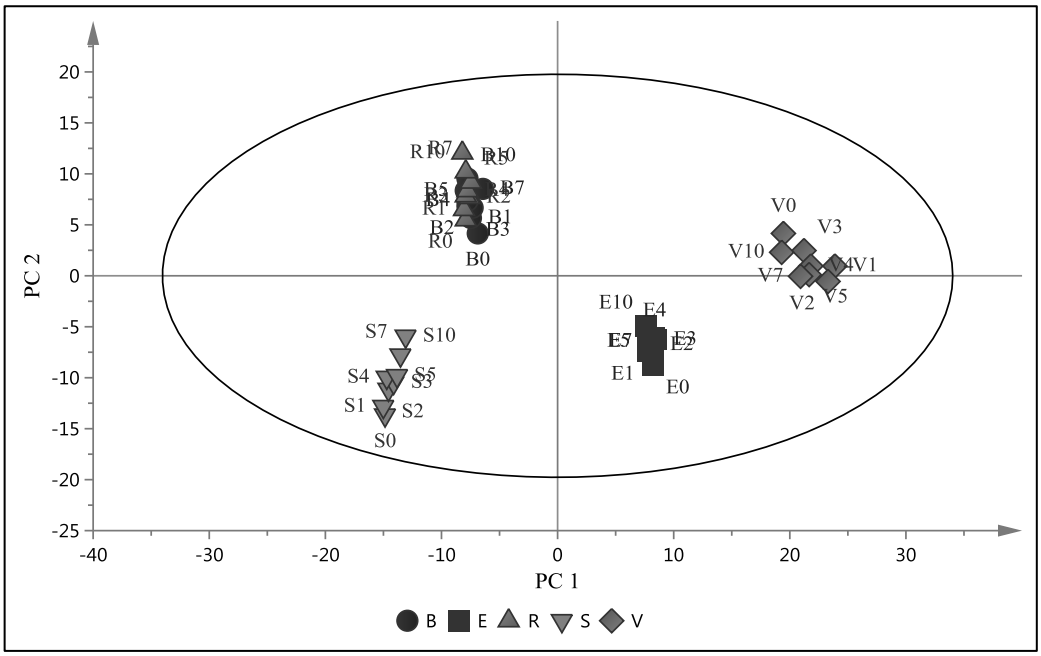


Figure 4.27. Score plot of OPLS- DA of oil samples with UV data

The clusters of oil samples in the classification with UV spectra showed the differences in fresh and fried oils as well. As in Figure 4.26, the fresh samples for all types of oils could be distinguished. The frying cycles were well separated within the clusters, too. In the OPLS-DA model of UV spectra (Figure 4.27), the same trend was observed in all fried oils except virgin olive oil samples.

## 4.2.2. Classification Using FTIR Data

The first derivative spectra of the FTIR data of a selected range (3080-2650 $cm^{-1}$ and 1850-650 $cm^{-1}$) were modeled with PCA and OPLS-DA. Score plot of PCA model with 3 components, $R^2$ = 0.94 and $R^2_{CV}$ = 0.93 shows the seperation of refined oils only (Figure 4.28).

OPLS-DA model (Figure 4.29) with 4+4 components, $R^2 = 0.98$ and $R^2_{CV} = 0.95$ shows a clear separation of refined oils, however, no distinction can be seen for olive oil samples.

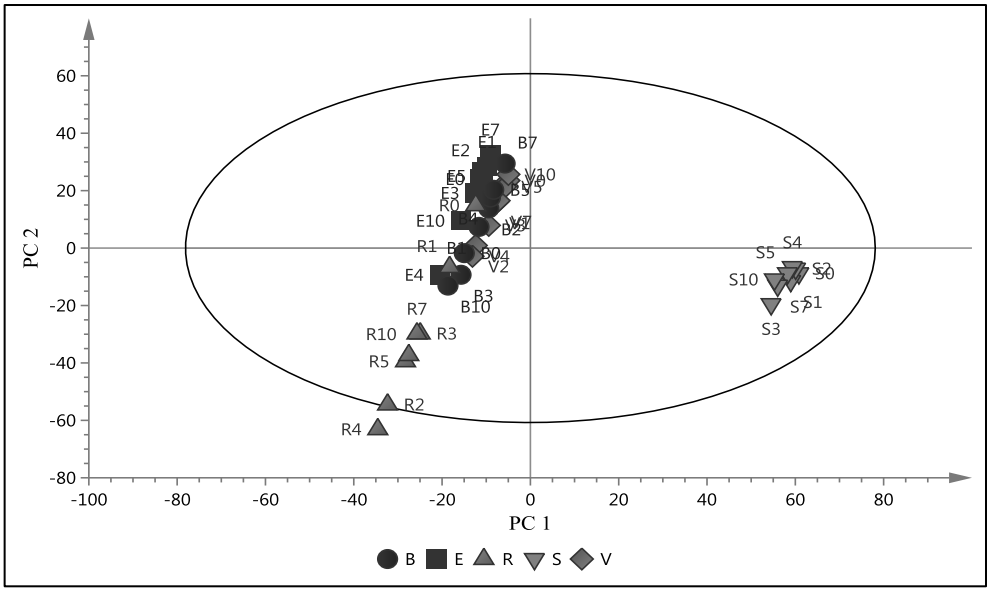


Figure 4.28. Score plot of PCA of oil samples with FTIR data

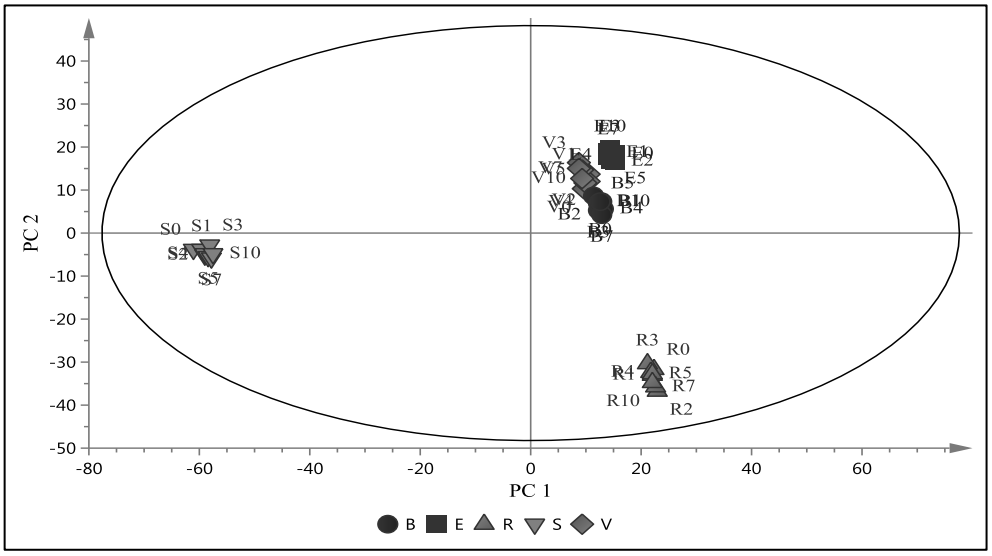


Figure 4.29. Score plot of OPLS- DA of oil samples with FTIR data

### 4.2.3. Classification Using the Combination of FTIR and UV Spectra

The combined data of FTIR and UV spectra (with 1st derivative filter) were also used for the classification. The PCA model with 11 components, $R^2 = 0.99$ and $R^2_{CV} = 0.96$ gave similar results to the FTIR (Figure 4.30). The sunflower oils were seperated clearly from the each other but the differentiation of olive oils was not good. Another PCA model, in which the sunflower oil samples were excluded, could not discriminate the samples clearly except virgin olive oil (Figure 4.31). Since size of the FTIR data set is larger compared to UV data FTIR data might have a dominating effect in PCA and the combination result is similar to model generated with FTIR data only.

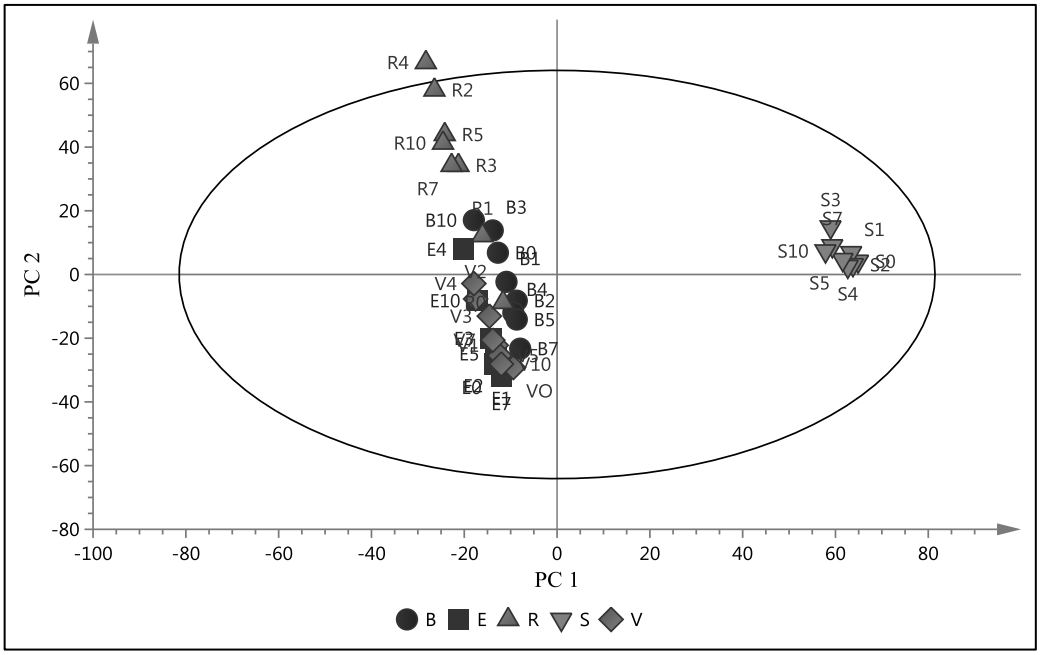


Figure 4.30. Score plot of PCA of oil samples with the combination of FTIR and UV data

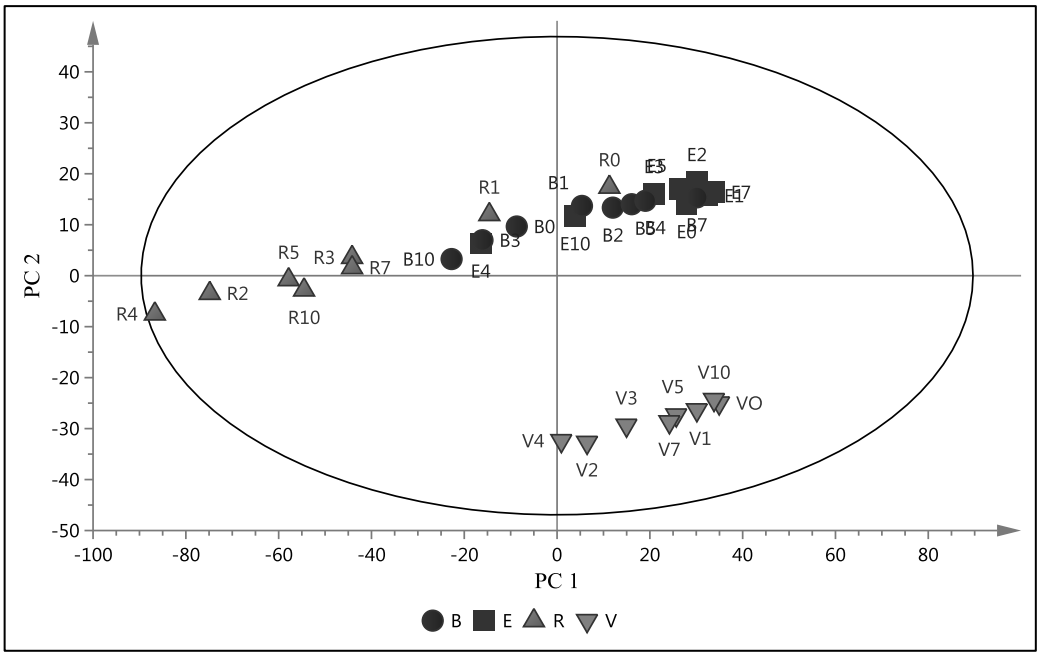


Figure 4.31. Score plot of PCA of olive oil samples with the combination of FTIR and UV data

The combination of FTIR and UV data set was modeled using OPLS-DA (4+4 components, $R^2$= 0.99 and $R^2_{CV}$= 0.96). Figure 4.32 shows the score plot of the model. The oil samples except virgin and extra virgin olive oil were clustered distinctly.

In the classification with FTIR spectra and the combination of UV and FTIR spectra, the clusters did not show the fresh oil samples ans those from different frying cycles explicitly.

In the classification of frying oils, the multivariate analysis of UV spectra with OPLS-DA models performed better than FTIR spectra or combination of FTIR and UV spectra.

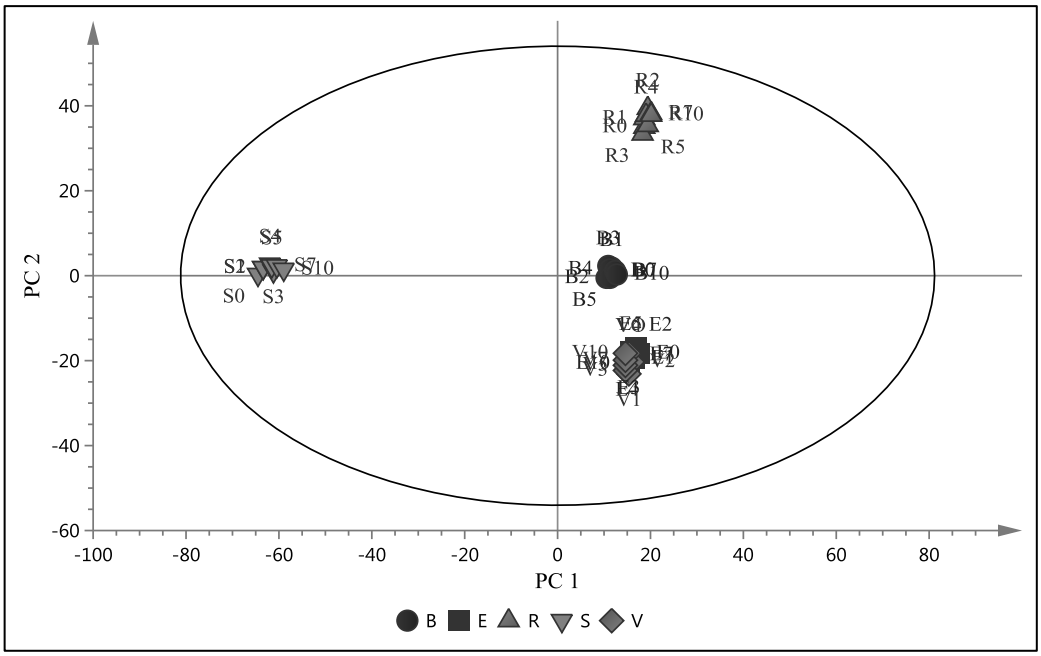


Figure 4.32. Score plot of OPLS-DA of oil samples with the combination of FTIR and UV data

## 4.2.4. Classification Using Chemical Parameters

Classification with chemical parameters data set include 80 oil samples with FFA, PV, $K_{232}$, $K_{270}$, CHL content, CRT content, OSI, TPC, L*, a*, b*, <HΘ(a*), C*ab, oleic acid, linoleic acid, and linolenic acid variables. The score and loading plots of OPLS-DA model with 4+2 PCs, $R^2 = 0.94$ and $R^2_{CV} = 0.90$ is given in Figure 4.33 and 4.34.

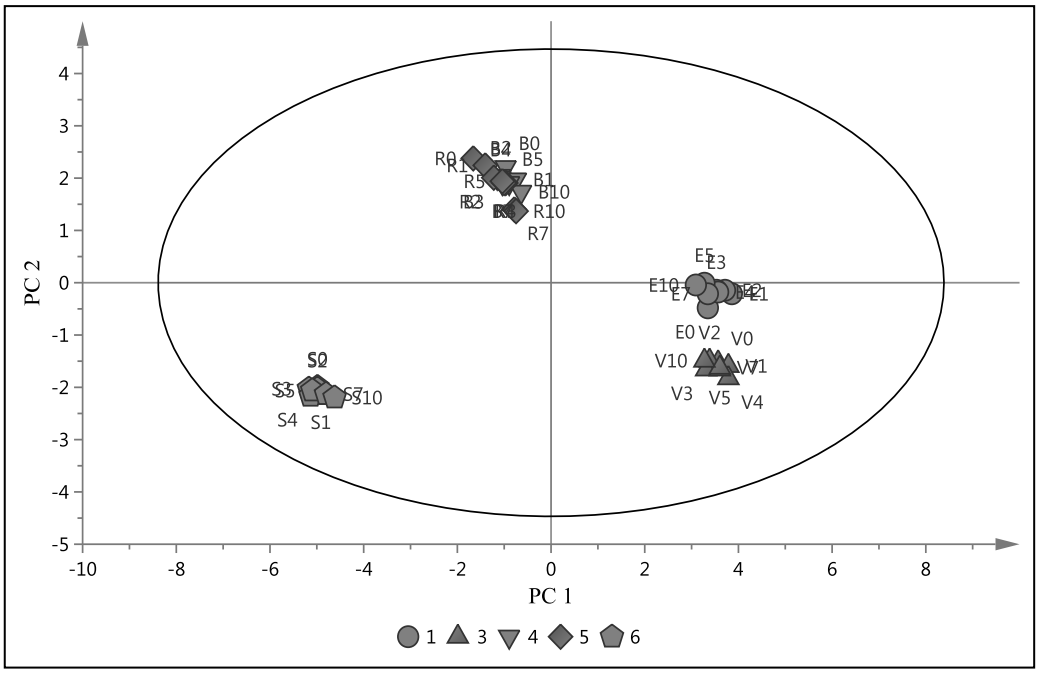


Figure 4. 33. Score plot of OPLS-DA of oil samples with the chemical parameters

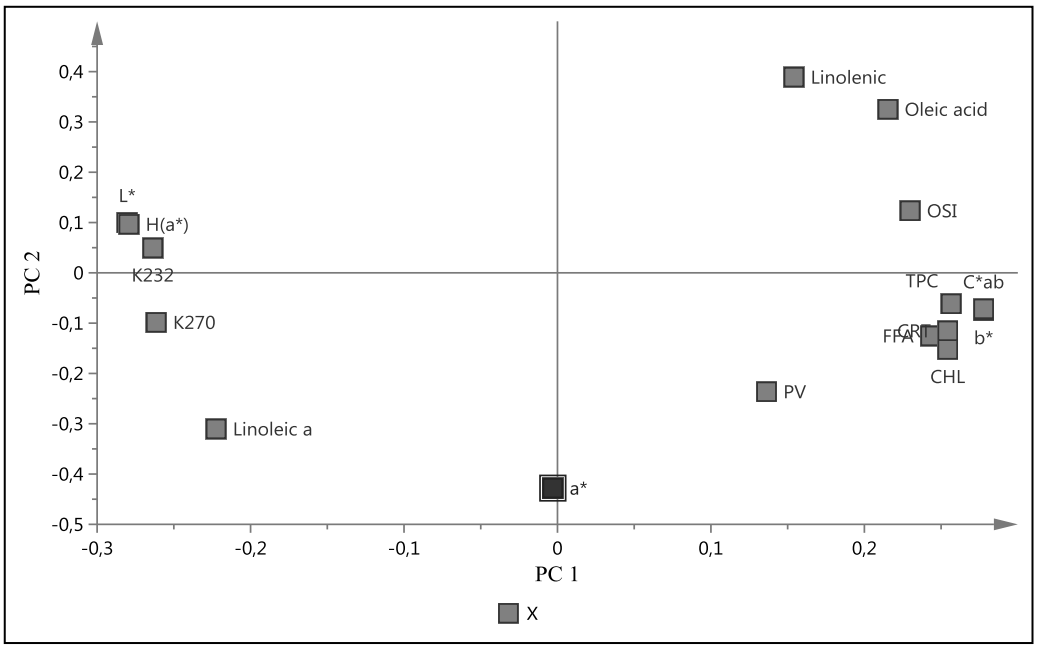


Figure 4. 34. Loading plot of OPLS-DA of oil samples with the chemical parameters

## 4.3. Prediction of Chemical and Quality Parameters with Spectrometric Data

In the prediction of quality variables with the spectrometric and chemometric methods, the 80-sample data set was split into a calibration set (n= 60) and prediction set (n= 20). The UV, FTIR and the combination of both were studied separately. The PLS and OPLS analysis were performed with filtered data. The highest $R^2$ and lowest error values were obtained in OPLS model. Best solutions were observed in the OPLS model of the first derivative spectra. In this section, the results of regression models as the plots of reference values versus predicted values of the chemical parameters were given for the calibration and validation set.

**Prediction of Chemical and Quality Parameters with UV Spectra:** The regression coefficient values for the variables were between 0.82 and 0.98 (Table 4.1). However, the prediction of PV parameter was not satisfactory ($R^2_{CV}$ = 0.44). It was seen that the UV spectra predicted FFA and fatty acid contents with cross-validation $R^2$ ($R^2_{CV}$) values greater than 0.91. For carotenoid contents, UV spectra was more promising than they were for chlorophyll.

Zhang et al. (2015) studied the effect of heating on edible oils (corn, sunflower, rapeseed, peanut, soybean, and sesame oil) using UV-Vis spectroscopy coupled PLS regression and they estimated free fatty acids with PLS. They concluded that UV-Vis spectrometry with chemometric analysis had a good potential for quantitive analysis of acid value of the oil samples.

**Prediction of Chemical and Quality Parameters with FTIR Spectra:** The $R^2_{CV}$ values of the variables were found between 0.73 and 0.99. The prediction of PV with FTIR spectra was not satisfactory ($R^2_{CV}$ = 0.30). Results of predictions with OPLS models by using FTIR data were presented in Table 4.2.

In a previous study, FTIR spectroscopic method and PLS models were used for the prediction of total polar compounds, carbonyl value, conjugated diene, and conjugated triene in frying of canola oil at 180ºC (Talpur et al., 2015).

In an another study from literature (Maggio et al., 2011) three different types of olive oils were subjected to heating treatment at intervals of 30 min at 180ºC. PLS technique was used with FTIR (mid infrared) and UV-NIR (near infrared) data to predict the oxidative stability, $K_{232}$, $K_{270}$ values and fatty acid compositions. They

concluded that spectroscopic data coupled with PLS was a useful approach for determining the oxidative status of cooked oil samples.

**Prediction of Chemical and Quality Parameters with the Combination of UV and FTIR Spectra:** The regression coefficients for variables except PV ($R^2_{CV}$ = 0.44) were found between 0.87 and 0.99 (Table 4.3). The prediction of PV parameter was not different compared to using only UV or FTIR spectra. The combination of UV and mid-infrared spectra for the classification of oil samples and prediction of chemical parameters were studided for the first time.

In terms of cross validation and validation $R^2$ values ($R^2_{CV}$, $R^2$val), it was concluded that UV spectra gave the best results for total phenol content (Figure 4.33). The OPLS model for TPC produced $R^2_{CV}$ of 0.90 and $R^2$val of 0.93. The FTIR spectra predicted the fatty acids better than UV data or combination of both did. The $R^2$ of validation sets were found as 0.99 for oleic, linoleic and linolenic acids (Figures 4.34, 4.35 and 4.36). For the rest of the quality and chemical parameters, the combination of UV and FTIR spectra estimated the reference values successfully. The regression plots af actual versus predicted values of variables FFA, PV, $K_{232}$, $K_{270}$, CHL, CRT and OSI are given in figures 4.37, 4.38, 4.39, 4.40, 4.41, 4.42, and 4.43.

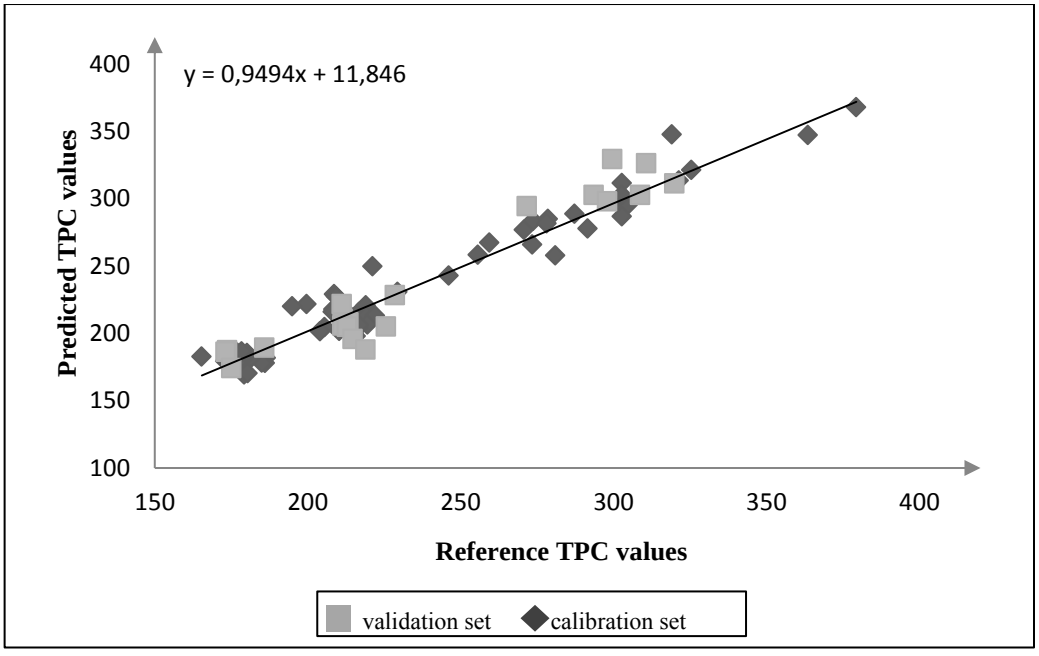


Figure 4.35. Actual vs predicted TPC (mg GA/kg oil) obtained from OPLS analysis using UV spectra

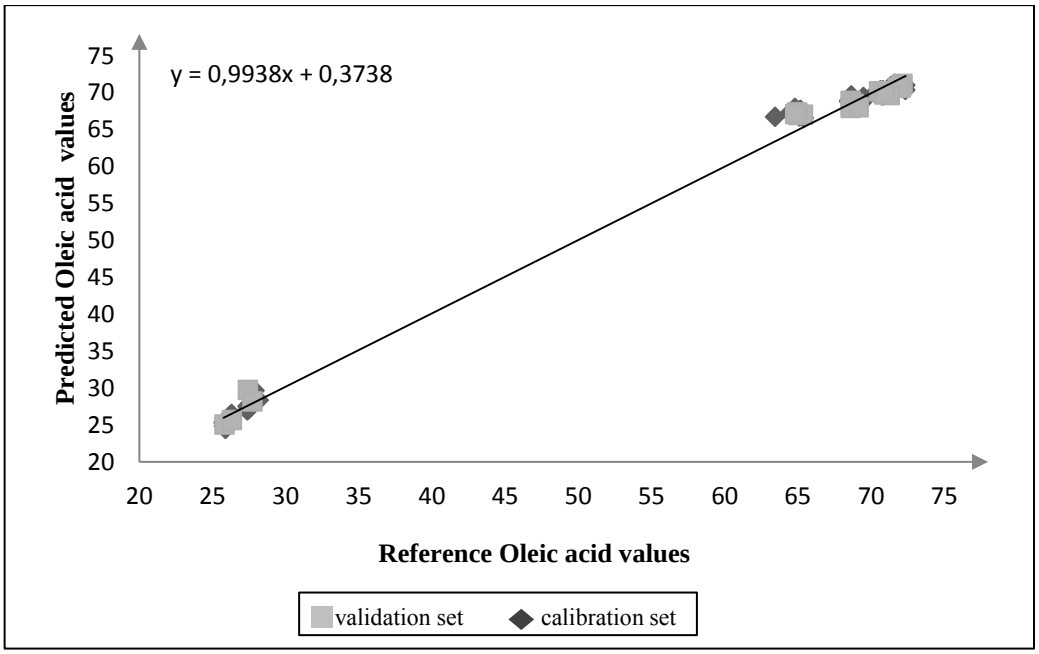


Figure 4.36. Actual vs predicted values of oleic acid percentages obtained from OPLS analysis using FTIR spectra

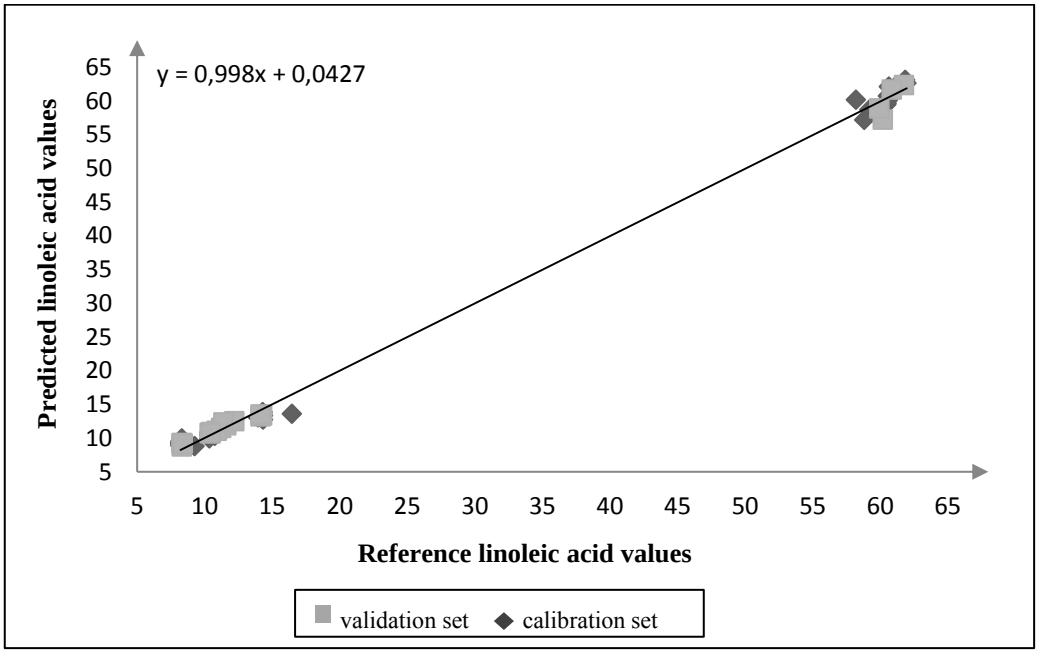


Figure 4.37. Actual vs predicted values of linoleic acid percentages obtained from OPLS analysis using FTIR spectra

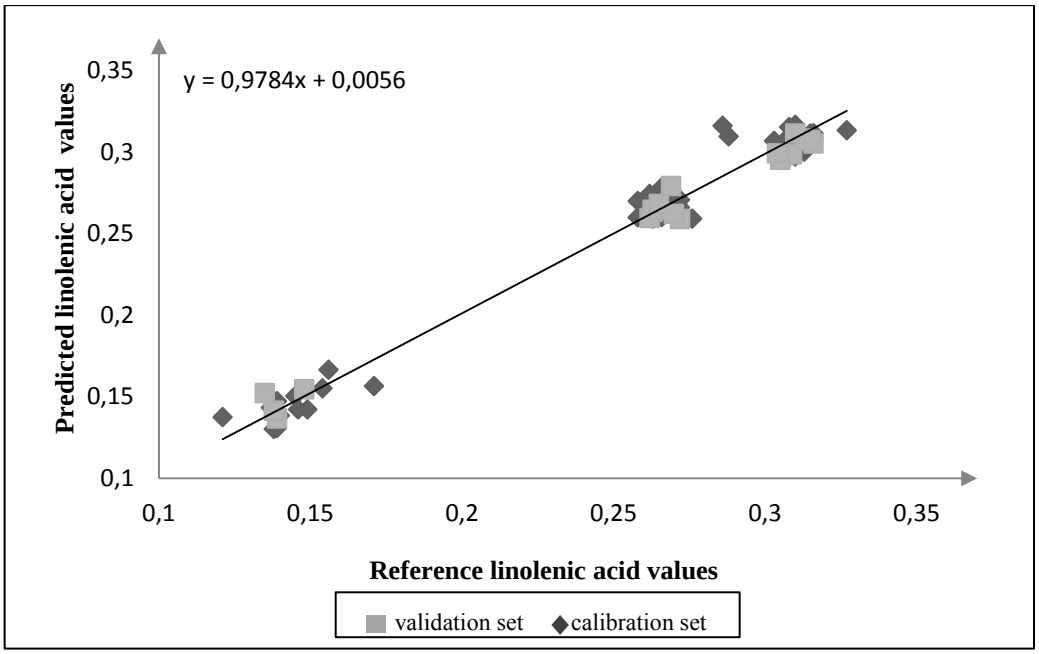


Figure 4.38. Actual vs predicted values of linolenic acid percentages obtained from OPLS analysis using FTIR spectra

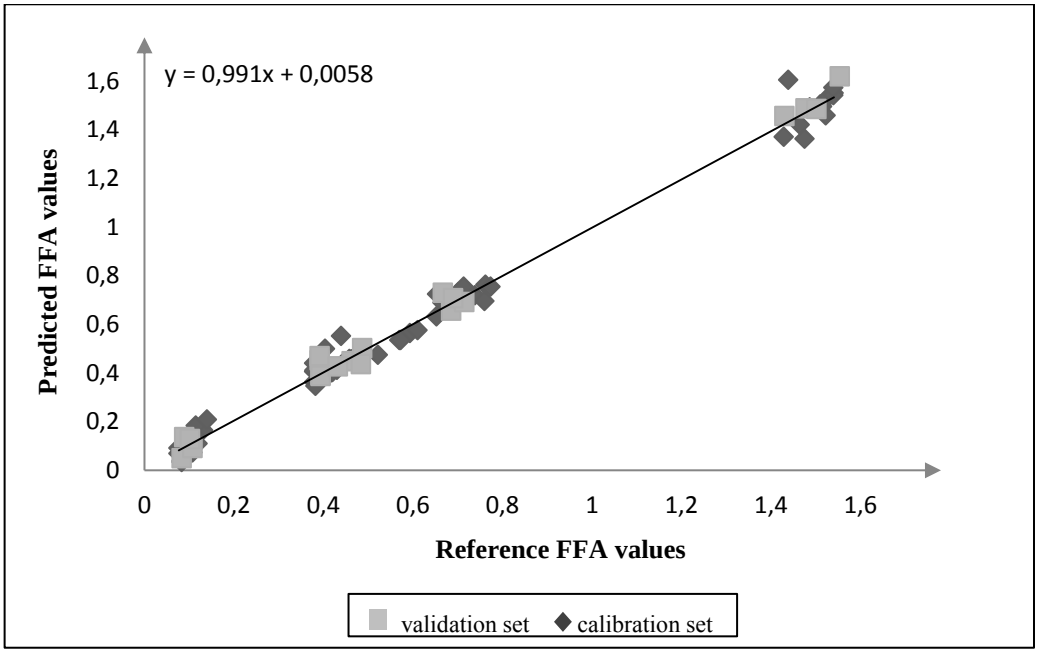


Figure 4.39. The actual vs predicted values of FFA (oleic acid%) obtained from OPLS analysis using the combination of FTIR and UV spectra

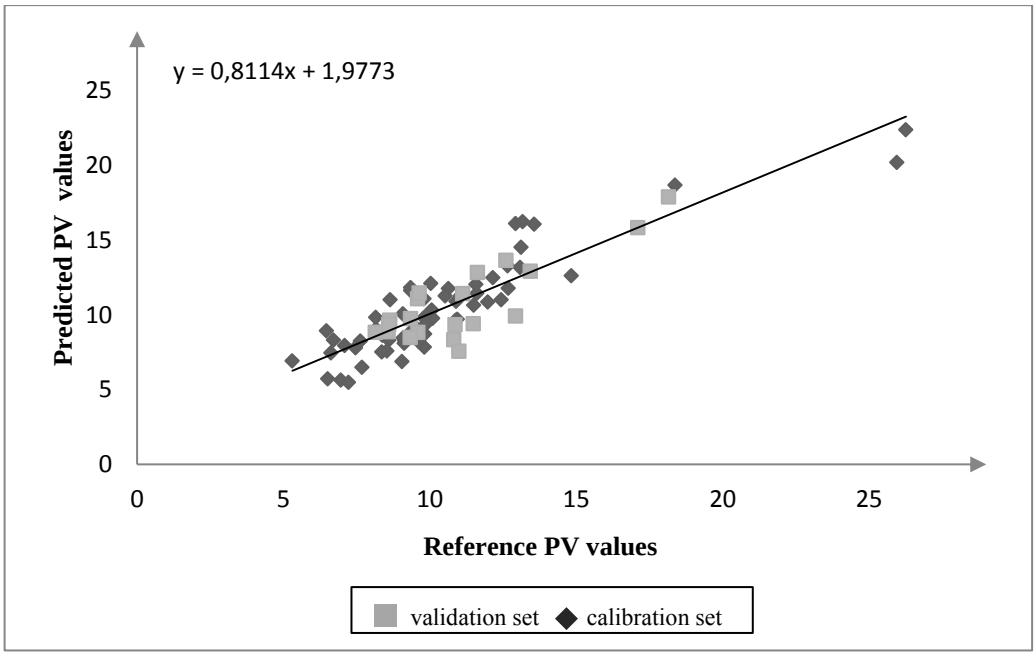


Figure 4.40. The actual vs predicted values of PV (meq $O_2$/kg oil) obtained from OPLS analysis using the combination of FTIR and UV spectra

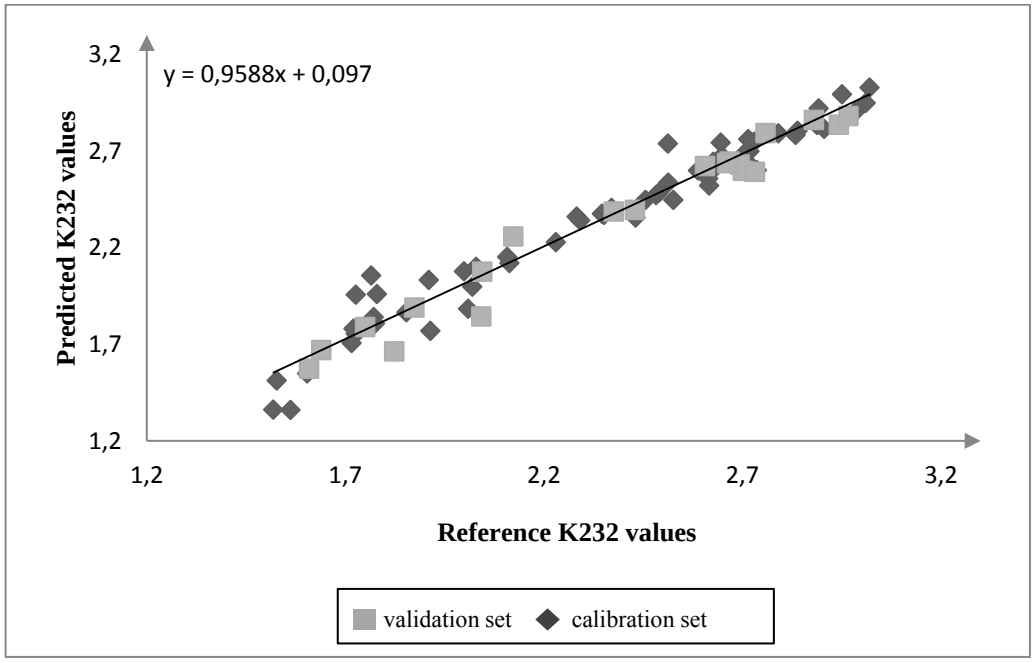


Figure 4.41. The actual vs predicted values of $K_{232}$ values obtained from OPLS analysis using the combination of FTIR and UV spectra

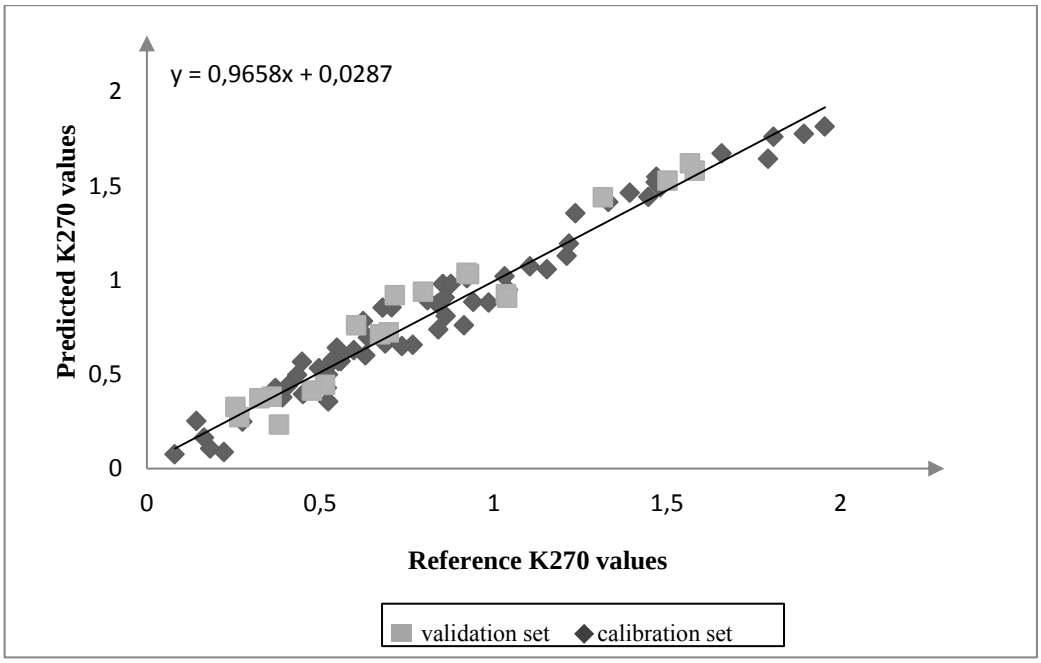


Figure 4.42. The actual vs predicted values of $K_{270}$ values obtained from OPLS analysis using the combination of FTIR and UV spectra

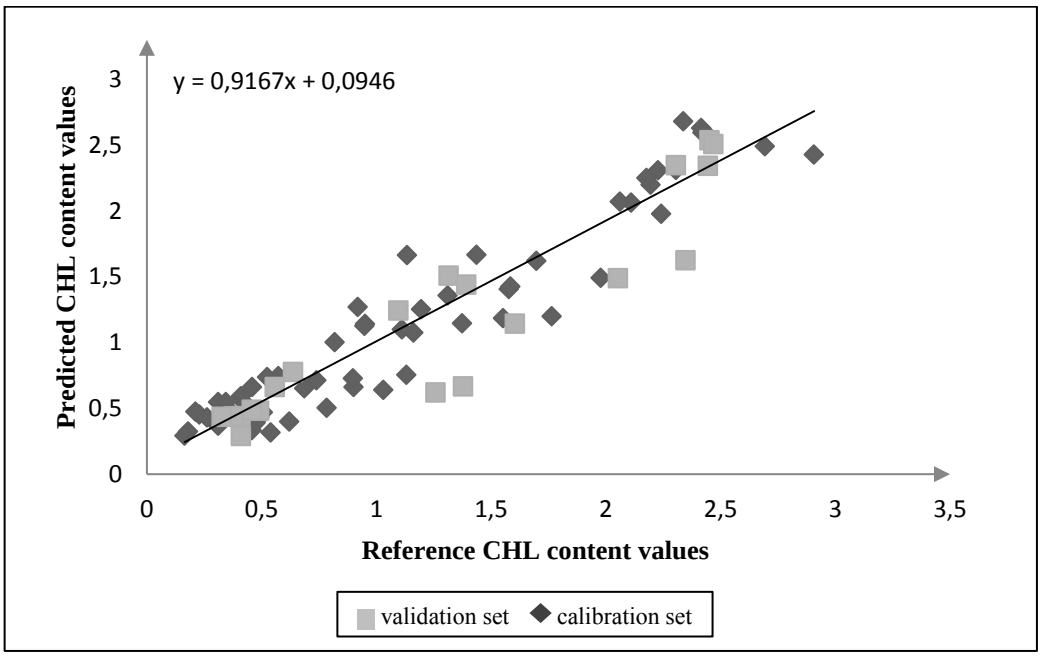


Figure 4.43. The actual vs predicted values of chlorophyll content (mg/kg oil) obtained from OPLS analysis using the combination of FTIR and UV spectra

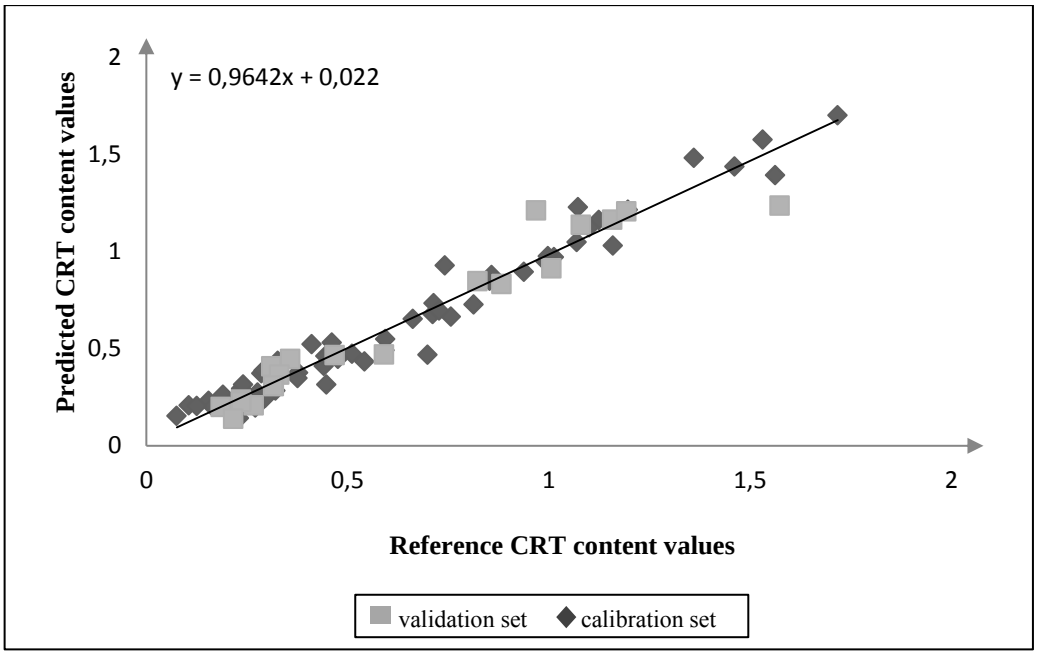


Figure 4.44. The actual vs predicted values of carotenoid content (mg/kg oil) obtained from OPLS analysis using the combination of FTIR and UV spectra

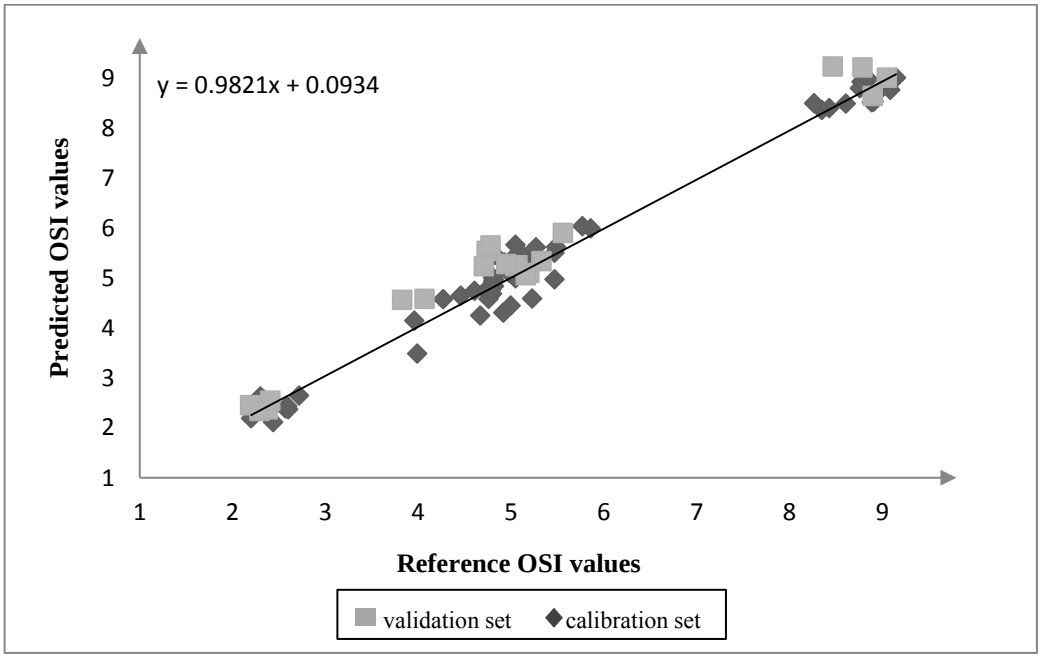


Figure 4.45. The actual vs predicted values of OSI (h) obtained from OPLS analysis using the combination of FTIR and UV spectra

Table 4.2. Range of quality variables used in prediction models in terms of [minimum-maximum]

| Variable (unit) | Range of data | Calibration data | Validation data |
|---|---|---|---|
| FFA (%oleic acid) | [0.08-1.55] | [0.08-1.54] | [0.08-1.55] |
| PV (meq O2/kg) | [5.30-26.26] | [5.30-26.26] | [8.14-18.15] |
| K232 value | [1.52-3.02] | [1.52-3.02] | [1.61-2.97] |
| K270 value | [0.08-1.96] | [0.08-1.96] | [0.26-1.58] |
| CHL (mg/kg) | [0.16-2.91] | [0.16-2.91] | [0.33-2.47] |
| CRT (mg/kg) | [0.08-1.72] | [0.08-1.72] | [0.19-1.57] |
| OSI (h) | [2.19-9.15] | [2.20-9.15] | [2.19-9.05] |
| L* value | [85.25-100.66] | [85.25-100.66] | [85.63-100.16] |
| a* value | [-5.67-0.21] | [-5.67-0.21] | [-5.50-2.09] |
| b* value | [5.23-74.82] | [5.23-74.82] | [7.09-68.94] |
| TPC (mg GA/kg) | [165.36-379.41] | [165.36-379.41] | [173.14-319.92] |
| Palmitic acid (%) | [6.53-15.21] | [6.53-15.21] | [6.93-14.47] |
| Palmitoleic acid (%) | [0.09-0.60] | [0.09-0.24] | [0.11-0.60] |
| Heptadecanoic acid (%) | [0-0.87] | [0-0.87] | [0-0.87] |
| Cis-10-Heptadecanoic acid (%) | [0-0.26] | [0-0.26] | [0-0.26] |
| Stearic acid (%) | [2.66-3.93] | [2.67-3.93] | [2.66-3.77] |
| Oleic acid (%) | [25.76-72.37] | [25.76-72.37] | [25.83-72.16] |
| Linoleic acid (%) | [8.21-61.97] | [8.21-61.97] | [8.30-61.76] |
| Arachidic acid (%) | [0.26-0.55] | [0.26-0.55] | [0.26-0.54] |
| Cis-11-Eicosenoic acid (%) | [0.05-0.79] | [0.05-0.79] | [0.09-0.78] |
| Linolenic acid (%) | [0.12-0.33] | [0.12-0.33] | [0.14-0.32] |
| Behenic acid (&) | [0.09-0.72] | [0.09-0.71] | [0.11-0.72] |
| Tricosanoic acid (%) | [0-1.25] | [0-1.25] | [0-1.17] |
| Lignoceric acid (%) | [0.04-0.26] | [0.04-0.26] | [0.05-0.26] |

Table 4.3. Results of OPLS models of UV spectra for prediction of quality parameters

| Variable | Number of PCs | $R^2$ | $R^2_{CV}$ | $R^2$val | RMSEC | RMSECV | RMSEP |
|---|---|---|---|---|---|---|---|
| **FFA** | 1+2 | 0.99 | 0.98 | 0.99 | 0.05 | 0.06 | 0.03 |
| **PV** | 1+3 | 0.63 | 0.44 | 0.66 | 2.35 | 2.79 | 1.59 |
| **$K_{232}$** | 1+4 | 0.93 | 0.89 | 0.92 | 0.12 | 0.15 | 0.15 |
| **$K_{270}$** | 1+6 | 0.93 | 0.86 | 0.94 | 0.13 | 0.17 | 0.12 |
| **CHL** | 1+0 | 0.83 | 0.82 | 0.79 | 0.32 | 0.33 | 0.39 |
| **CRT** | 1+2 | 0.92 | 0.91 | 0.92 | 0.12 | 0.13 | 0.12 |
| **OSI** | 1+6 | 0.95 | 0.90 | 0.94 | 0.50 | 0.64 | 0.61 |
| **L*** | 1+2 | 0.97 | 0.96 | 0.98 | 0.85 | 0.99 | 0.70 |
| **a*** | 1+5 | 0.98 | 0.94 | 0.94 | 0.24 | 0.35 | 0.32 |
| **b*** | 1+3 | 0.99 | 0.99 | 0.99 | 1.76 | 2.07 | 1.93 |
| **C*ab** | 1+3 | 0.99 | 0.99 | 0.99 | 1.76 | 2.06 | 1.93 |
| **<HΘ(a*)** | 1+1 | 0.97 | 0.97 | 0.99 | 0.87 | 0.92 | 0.63 |
| **TPC** | 1+5 | 0.95 | 0.90 | 0.93 | 12.30 | 16.10 | 14.46 |
| **C18:1n9c** | 1+5 | 0.94 | 0.94 | 0.97 | 3.63 | 4.33 | 3.19 |
| **C18:2n6c** | 1+5 | 0.94 | 0.94 | 0.97 | 3.95 | 4.73 | 3.69 |
| **C18:3n3** | 1+5 | 0.94 | 0.91 | 0.97 | 0.02 | 0.02 | 0.01 |

Table 4.4. Results of OPLS models of FTIR spectra for prediction of quality parameters

| Variable | Number of PCs | $R^2$ | $R^2_{CV}$ | $R^2$val | RMSEC | RMSECV | RMSEP |
|---|---|---|---|---|---|---|---|
| **FFA** | 1+3 | 0.98 | 0.97 | 0.98 | 0.06 | 0.07 | 0.06 |
| **PV** | 1+4 | 0.66 | 0.30 | 0.55 | 2.29 | 3.10 | 1.84 |
| **$K_{232}$** | 1+3 | 0.94 | 0.92 | 0.95 | 0.11 | 0.12 | 0.11 |
| **$K_{270}$** | 1+5 | 0.98 | 0.95 | 0.96 | 0.07 | 0.11 | 0.09 |
| **CHL** | 1+3 | 0.89 | 0.84 | 0.85 | 0.27 | 0.31 | 0.33 |
| **CRT** | 1+3 | 0.82 | 0.73 | 0.84 | 0.19 | 0.22 | 0.17 |
| **OSI** | 1+4 | 0.96 | 0.94 | 0.93 | 0.41 | 0.51 | 0.63 |
| **L*** | 1+5 | 0.97 | 0.92 | 0.97 | 0.88 | 1.31 | 0.79 |
| **a*** | 1+5 | 0.91 | 0.80 | 0.93 | 0.47 | 0.65 | 0.40 |
| **b*** | 1+3 | 0.93 | 0.91 | 0.97 | 5.59 | 6.32 | 3.84 |
| **C*ab** | 1+3 | 0.93 | 0.91 | 0.97 | 5.55 | 6.28 | 3.80 |
| **<HΘ(a*)** | 1+5 | 0.97 | 0.93 | 0.97 | 0.92 | 1.34 | 0.83 |
| **TPC** | 1+3 | 0.86 | 0.80 | 0.92 | 20.38 | 23.38 | 13.81 |
| **C18:1n9c** | 1+1 | 0.99 | 0.99 | 0.99 | 0.91 | 0.92 | 0.95 |
| **C18:2n6c** | 1+1 | 0.99 | 0.99 | 0.99 | 0.91 | 0.92 | 0.95 |
| **C18:3n3** | 1+3 | 0.98 | 0.97 | 0.99 | 0.01 | 0.01 | 0.01 |

Table 4.5. Results of OPLS models of the combination of FTIR and UV data for prediction of quality parameters

| Variable | Number of PCs | $R^2$ | $R^2_{CV}$ | $R^2$val | RMSEC | RMSECV | RMSEP |
|---|---|---|---|---|---|---|---|
| **FFA** | 1+3 | 0.99 | 0.99 | 0.99 | 0.05 | 0.05 | 0.04 |
| **PV** | 1+6 | 0.81 | 0.44 | 0.70 | 1.73 | 2.77 | 1.54 |
| **$K_{232}$** | 1+3 | 0.96 | 0.95 | 0.97 | 0.09 | 0.10 | 0.09 |
| **$K_{270}$** | 1+4 | 0.97 | 0.94 | 0.95 | 0.09 | 0.11 | 0.10 |
| **CHL** | 1+3 | 0.92 | 0.88 | 0.85 | 0.23 | 0.27 | 0.11 |
| **CRT** | 1+5 | 0.96 | 0.92 | 0.93 | 0.08 | 0.12 | 0.11 |
| **OSI** | 1+5 | 0.98 | 0.95 | 0.98 | 0.29 | 0.45 | 0.43 |
| **L*** | 1+3 | 0.98 | 0.96 | 0.98 | 0.75 | 0.97 | 0.68 |
| **a*** | 1+3 | 0.93 | 0.89 | 0.94 | 0.42 | 0.48 | 0.36 |
| **b*** | 1+3 | 0.99 | 0.98 | 0.99 | 2.69 | 2.96 | 1.49 |
| **C*ab** | 1+3 | 0.99 | 0.98 | 0.99 | 2.68 | 2.95 | 1.49 |
| **<HΘ(a*)** | 1+3 | 0.98 | 0.97 | 0.99 | 0.83 | 0.91 | 0.40 |
| **TPC** | 1+3 | 0.90 | 0.87 | 0.96 | 16.74 | 18.61 | 10.12 |
| **C18:1n9c** | 1+2 | 0.99 | 0.99 | 0.99 | 1.06 | 1.08 | 0.91 |
| **C18:2n6c** | 1+0 | 0.98 | 0.98 | 0.98 | 2.65 | 2.62 | 2.89 |
| **C18:3n3** | 1+3 | 0.98 | 0.97 | 0.99 | 0.01 | 0.01 | 0.01 |

# CHAPTER 5

# CONCLUSION

In this study, different types of olive oils were studied in deep-frying experiments of french fries to investigate the changes in chemical compositions and oxidative stability and to compare the results with those of sunflower oil. As chemical parameters, free fatty acid, peroxide, and K values, oxidative stability index, total phenol, chlorophyll and carotenoid contents, color parameters, fatty acid and phenolic profiles were monitored over 10 frying cycles. Furthermore, classification of oil types and prediction of chemical parameters with OPLS technique by using FTIR, UV and the combination of FTIR and UV spectra was studied with multivariate statistical methods.

During frying, changes were detected in total polar compounds, $K_{232}$ and $K_{270}$ values, being still under the regulatory levels. Total chlorophyll and carotenoid content and b* values decreased for extra virgin and virgin olive oil and increased for refined oil types. No significant changes were detected between the first and the last frying cycle for major fatty acids (oleic, linoleic and linolenic acid), FFA, OSI, and L* values. TPC values and all phenolic compounds decreased in fried extra virgin and virgin oil samples after tenth frying cycle. Decrease in TPC values were 34 % and 21 % for extra virgin and virgin oils. The highest decrease in the individual phenols was detected in luteolin concentration with 59% and 46% for extra virgin and virgin olive oils. The significant decreases in phenolic compounds were found after fourth frying cyle, however no significant changes were detected in FFA and oxidative stability of oil samples. The changes in PV did not follow any trend irregular during frying process.

The first derivative of the UV and FTIR spectra and the combination of both were modeled separately with PCA and OPLS-DA to classify oil samples. It was seen that the UV spectra was effective in the differentiation of different oil types. General problem in FTIR and combination FTIR and UV data was that the extra virgin, virgin and blended olive oil were inseparable in the score plots.

In the prediction of chemical and quality parameters with UV, FTIR, and the combination of FTIR and UV combination spectra was used with the first derivative and OPLS model. The $R^2_{CV}$ values of the models built with the combination of FTIR and

UV spectra were found in the range of 0.87-0.99, whereas those with the FTIR spectra were between 0.73-0.99 and those with the UV spectra were between 0.82-0.98. It was concluded that the FTIR spectra were preferable in the prediction of major fatty acids, while the UV spectra were preferable in the prediction of TPC parameter. For the rest of the parameters, the combination of FTIR and UV spectra was successful. The prediction of PV parameter with the any spectral data was unsatisfactory.

In conclusion, extra virgin, virgin and blended olive oils were found as healty alternatives in home frying as they can be used repeatedly. Spectrometric techniques coupled with multivariate techniques were found very effective and practical in the predictions of chemical parameters such as FFA, K values, TPC, total chlorophyll and carotenoid content. They have advantages of being low-labor, time saving and environmentally friendly analysis with no chemical waste.

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# APPENDIX A

# EXPERIMENTAL DATA

In the tables, the sample codes were arranged as: the first letter stands for the name of oil sample, the following numbers refer to the frying cycle.

Table A.1. FFA, PV, $K_{232}$, and $K_{270}$ values of fried oil samples

| Sample | FFA | PV | $K_{232}$ | $K_{270}$ |
|---|---|---|---|---|
| **E0** | 0.65 | 12.38 | 1.54 | 0.12 |
| **E1** | 0.67 | 12.09 | 1.57 | 0.21 |
| **E2** | 0.68 | 11.71 | 1.62 | 0.30 |
| **E3** | 0.70 | 11.97 | 1.77 | 0.41 |
| **E4** | 0.71 | 11.28 | 1.74 | 0.43 |
| **E5** | 0.73 | 9.87 | 1.75 | 0.48 |
| **E7** | 0.73 | 8.84 | 1.79 | 0.55 |
| **E10** | 0.74 | 8.94 | 1.77 | 0.60 |
| **V0** | 1.45 | 16.60 | 1.85 | 0.20 |
| **V1** | 1.47 | 15.79 | 1.96 | 0.32 |
| **V2** | 1.48 | 18.39 | 1.96 | 0.39 |
| **V3** | 1.50 | 17.99 | 2.01 | 0.49 |
| **V4** | 1.51 | 13.46 | 2.04 | 0.51 |
| **V5** | 1.51 | 11.70 | 2.11 | 0.54 |
| **V7** | 1.53 | 11.13 | 2.18 | 0.62 |
| **V10** | 1.53 | 10.41 | 2.39 | 0.74 |

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Table A.1. (Cont.)

| Sample | FFA | PV | $K_{232}$ | $K_{270}$ |
|---|---|---|---|---|
| **B0** | 0.38 | 6.56 | 2.39 | 0.54 |
| **B1** | 0.39 | 7.02 | 2.45 | 0.61 |
| **B2** | 0.39 | 8.13 | 2.57 | 0.69 |
| **B3** | 0.39 | 8.99 | 2.56 | 0.76 |
| **B4** | 0.40 | 9.70 | 2.52 | 0.70 |
| **B5** | 0.40 | 8.13 | 2.53 | 0.77 |
| **B7** | 0.42 | 8.30 | 2.59 | 0.85 |
| **B10** | 0.44 | 7.43 | 2.57 | 0.89 |
| **R0** | 0.41 | 10.01 | 2.52 | 0.84 |
| **R1** | 0.41 | 9.99 | 2.60 | 0.91 |
| **R2** | 0.45 | 10.15 | 2.61 | 0.95 |
| **R3** | 0.46 | 8.43 | 2.70 | 0.98 |
| **R4** | 0.49 | 9.86 | 2.66 | 0.98 |
| **R5** | 0.50 | 10.31 | 2.66 | 1.04 |
| **R7** | 0.57 | 9.39 | 2.71 | 1.16 |
| **R10** | 0.60 | 11.15 | 2.72 | 1.19 |
| **S0** | 0.08 | 6.01 | 2.65 | 1.31 |
| **S1** | 0.08 | 9.23 | 2.74 | 1.39 |
| **S2** | 0.09 | 9.59 | 2.83 | 1.39 |
| **S3** | 0.09 | 11.25 | 2.89 | 1.49 |
| **S4** | 0.10 | 11.96 | 2.93 | 1.52 |
| **S5** | 0.11 | 12.46 | 2.93 | 1.62 |
| **S7** | 0.12 | 9.94 | 2.95 | 1.80 |
| **S10** | 0.14 | 9.34 | 2.99 | 1.93 |

Table A.2. Total CHL and CRT, OSI and TPC values of fried oil samples

| | CHL | CRT | OSI | TPC |
|---|---|---|---|---|
| **E0** | 1.42 | 1.62 | 8.35 | 371.49 |
| **E1** | 1.78 | 1.38 | 8.96 | 322.30 |
| **E2** | 1.72 | 1.33 | 8.76 | 315.30 |
| **E3** | 1.77 | 0.95 | 8.82 | 315.07 |
| **E4** | 1.67 | 0.97 | 8.92 | 290.41 |
| **E5** | 1.26 | 0.94 | 8.84 | 287.72 |
| **E7** | 1.36 | 0.70 | 8.85 | 261.96 |
| **E10** | 1.13 | 0.78 | 8.63 | 244.79 |
| **V0** | 2.80 | 1.41 | 4.84 | 302.79 |
| **V1** | 2.46 | 1.18 | 5.01 | 298.78 |
| **V2** | 2.38 | 1.09 | 4.95 | 297.31 |
| **V3** | 2.38 | 1.03 | 4.93 | 275.95 |
| **V4** | 2.43 | 1.00 | 5.06 | 265.53 |
| **V5** | 2.20 | 0.86 | 4.90 | 262.35 |
| **V7** | 2.25 | 0.85 | 5.13 | 250.97 |
| **V10** | 2.09 | 0.74 | 5.23 | 238.82 |
| **B0** | 0.50 | 0.41 | 4.86 | 218.83 |
| **B1** | 0.83 | 0.49 | 4.93 | 202.76 |
| **B2** | 0.83 | 0.40 | 4.60 | 211.43 |
| **B3** | 0.86 | 0.36 | 4.49 | 209.20 |
| **B4** | 0.92 | 0.37 | 4.45 | 222.60 |

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Table A.2. (Cont.)

| | CHL | CRT | OSI | TPC |
|---|---|---|---|---|
| **B5** | 1.00 | 0.48 | 4.22 | 213.97 |
| **B7** | 0.74 | 0.42 | 4.35 | 209.48 |
| **B10** | 1.01 | 0.58 | 4.33 | 215.94 |
| **R0** | 0.20 | 0.22 | 5.57 | 205.00 |
| **R1** | 0.58 | 0.32 | 5.63 | 212.99 |
| **R2** | 0.82 | 0.27 | 5.13 | 211.57 |
| **R3** | 0.97 | 0.27 | 5.36 | 214.05 |
| **R4** | 0.90 | 0.34 | 5.25 | 215.06 |
| **R5** | 0.84 | 0.34 | 5.21 | 217.40 |
| **R7** | 0.95 | 0.40 | 4.97 | 193.51 |
| **R10** | 0.66 | 0.46 | 5.24 | 192.31 |
| **S0** | 0.19 | 0.09 | 2.65 | 181.63 |
| **S1** | 0.50 | 0.19 | 2.60 | 183.09 |
| **S2** | 0.47 | 0.21 | 2.36 | 179.48 |
| **S3** | 0.41 | 0.20 | 2.32 | 179.09 |
| **S4** | 0.36 | 0.22 | 2.29 | 178.29 |
| **S5** | 0.42 | 0.24 | 2.28 | 176.51 |
| **S7** | 0.48 | 0.26 | 2.25 | 177.69 |
| **S10** | 0.41 | 0.25 | 2.36 | 179.90 |

Table A.3. Color parameters L*, a*, b*, C*ab, and < HΘ(a*) of fried oil samples

| | **L*** | **a*** | **b*** | **C*ab** | **<HΘ(a*)** |
|---|---|---|---|---|---|
| **E0** | 87.21 | -0.43 | 73.49 | 73.49 | 90.33 |
| **E1** | 89.52 | -3.00 | 66.82 | 66.89 | 92.58 |
| **E2** | 89.37 | -3.40 | 62.47 | 62.56 | 93.13 |
| **E3** | 90.07 | -3.86 | 59.33 | 59.46 | 93.78 |
| **E4** | 89.75 | -4.00 | 55.86 | 56.00 | 94.07 |
| **E5** | 90.33 | -4.15 | 52.81 | 52.97 | 94.52 |
| **E7** | 89.99 | -4.28 | 48.54 | 48.73 | 95.05 |
| **E10** | 90.03 | -4.50 | 44.54 | 44.77 | 95.79 |
| **V0** | 89.73 | -1.86 | 74.73 | 74.75 | 91.43 |
| **V1** | 87.49 | -2.63 | 68.85 | 68.89 | 91.58 |
| **V2** | 87.64 | -2.71 | 63.81 | 63.84 | 91.86 |
| **V3** | 87.37 | -2.92 | 60.62 | 60.70 | 92.77 |
| **V4** | 86.38 | -2.90 | 56.89 | 56.97 | 92.93 |
| **V5** | 86.87 | -3.19 | 54.41 | 54.51 | 93.38 |
| **V7** | 87.21 | -3.70 | 50.21 | 50.35 | 94.23 |
| **V10** | 87.39 | -4.15 | 45.87 | 46.07 | 95.18 |
| **B0** | 96.83 | -5.09 | 24.89 | 25.41 | 101.57 |
| **B1** | 96.32 | -5.19 | 25.30 | 25.82 | 101.60 |
| **B2** | 96.57 | -5.30 | 25.19 | 25.74 | 101.89 |
| **B3** | 96.65 | -5.38 | 25.21 | 25.78 | 102.05 |
| **B4** | 96.51 | -5.45 | 25.39 | 25.96 | 102.11 |

**(Cont. on next page)**

Table A.3. (Cont.)

| | L* | a* | b* | C*ab | <HΘ(a*) |
|---|---|---|---|---|---|
| **B5** | 95.99 | -5.46 | 25.60 | 26.17 | 102.02 |
| **B7** | 95.62 | -5.52 | 25.79 | 26.37 | 102.09 |
| **B10** | 94.98 | -5.53 | 26.35 | 26.92 | 101.86 |
| **R0** | 97.47 | -5.18 | 21.65 | 22.27 | 103.46 |
| **R1** | 97.26 | -5.36 | 23.00 | 23.61 | 103.13 |
| **R2** | 96.42 | -5.22 | 23.69 | 24.26 | 102.43 |
| **R3** | 96.50 | -5.29 | 24.30 | 24.87 | 102.27 |
| **R4** | 96.33 | -5.40 | 24.90 | 25.48 | 102.23 |
| **R5** | 95.96 | -5.39 | 25.23 | 25.80 | 102.07 |
| **R7** | 95.47 | -5.30 | 26.40 | 26.92 | 101.37 |
| **R10** | 94.59 | -5.26 | 28.17 | 28.66 | 100.58 |
| **S0** | 100.66 | -1.70 | 5.34 | 5.60 | 107.67 |
| **S1** | 100.28 | -2.06 | 6.88 | 7.19 | 106.67 |
| **S2** | 100.07 | -2.18 | 7.57 | 7.88 | 106.12 |
| **S3** | 99.95 | -2.25 | 8.02 | 8.33 | 105.71 |
| **S4** | 99.85 | -2.36 | 8.54 | 8.86 | 105.44 |
| **S5** | 99.34 | -2.42 | 9.02 | 9.34 | 105.09 |
| **S7** | 99.16 | -2.61 | 10.02 | 10.36 | 104.63 |
| **S10** | 98.80 | -2.87 | 11.30 | 11.66 | 104.21 |

Table A.4. Phenolic compositions of extra virgin, virgin and blended olive oils at 280 nm

| | Hyt | Tyr | 4Hyp | 3Hyp | Caf | Pcou |
|---|---|---|---|---|---|---|
| **E0** | 1.54 | 1.01 | 0.32 | 0.18 | 0.17 | 0.18 |
| **E1** | 1.04 | 0.56 | 0.14 | 0.06 | 0.11 | 0.15 |
| **E2** | 0.78 | 0.70 | 0.20 | 0.11 | 0.18 | 0.23 |
| **E3** | 1.05 | 0.74 | 0.25 | 0.10 | 0.16 | 0.22 |
| **E4** | 0.86 | 0.69 | 0.19 | 0.08 | 0.08 | 0.23 |
| **E5** | 1.17 | 0.96 | 0.25 | 0.13 | 0.07 | 0.31 |
| **E7** | 0.79 | 0.65 | 0.22 | 0.11 | 0.07 | 0.22 |
| **E10** | 0.76 | 0.71 | 0.22 | 0.12 | 0.06 | 0.27 |
| **V0** | 0.80 | 0.80 | 0.08 | 0.08 | 0.15 | 0.28 |
| **V1** | 1.38 | 0.71 | 0.05 | 0.09 | 0.10 | 0.26 |
| **V2** | 1.23 | 0.64 | 0.06 | 0.09 | 0.13 | 0.26 |
| **V3** | 1.10 | 0.63 | 0.05 | 0.08 | 0.09 | 0.25 |
| **V4** | 0.95 | 0.58 | 0.06 | 0.09 | 0.06 | 0.25 |
| **V5** | 0.78 | 0.64 | 0.07 | 0.09 | 0.03 | 0.27 |
| **V7** | 0.74 | 0.73 | 0.06 | 0.10 | 0.05 | 0.29 |
| **V10** | 0.64 | 0.60 | 0.06 | 0.10 | 0.04 | 0.29 |
| **B0** | 0.32 | 0.86 | 0.04 | 0.08 | 0.05 | 0.07 |
| **B1** | 0.32 | 0.80 | 0.03 | 0.05 | 0.06 | 0.07 |
| **B2** | 0.23 | 0.65 | 0.01 | 0.04 | 0.07 | 0.06 |
| **B3** | 0.17 | 0.71 | 0.00 | 0.03 | 0.05 | 0.06 |

**(Hyt, hydroxytyrosol; Tyr, tyrosol; 4Hyp, 4Hydroxyphenylacetic acid; 3Hyp, 3Hydroxyphenylacetic acid; Caf, caffeic acid; Pcou, p-coumaric acid)**



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Table A.4. (Cont.)

| | Vna | 23Dhyb | Mcou | Ocou | Pin | Syr |
|---|---|---|---|---|---|---|
| **E0** | 0.08 | 0.18 | 0.01 | 0.03 | 3.70 | Nd |
| **E1** | 0.02 | 0.06 | 0.03 | 0.21 | 3.05 | Nd |
| **E2** | 0.04 | 0.04 | 0.09 | 0.20 | 3.76 | Nd |
| **E3** | 0.04 | 0.03 | 0.12 | 0.10 | 3.06 | Nd |
| **E4** | 0.03 | 0.05 | 0.12 | 0.08 | 3.19 | Nd |
| **E5** | 0.03 | 0.05 | 0.18 | 0.16 | 3.49 | Nd |
| **E7** | 0.02 | 0.04 | 0.12 | 0.09 | 2.89 | Nd |
| **E10** | 0.03 | 0.02 | 0.14 | 0.09 | 2.12 | Nd |
| **V0** | 0.01 | 0.24 | 0.00 | 0.01 | 5.25 | Nd |
| **V1** | 0.03 | 0.78 | 0.04 | 0.06 | 5.31 | Nd |
| **V2** | 0.02 | 0.65 | 0.06 | 0.06 | 4.50 | Nd |
| **V3** | 0.02 | 0.59 | 0.08 | 0.05 | 4.92 | Nd |
| **V4** | 0.02 | 0.70 | 0.08 | 0.06 | 4.27 | Nd |
| **V5** | 0.02 | 0.69 | 0.10 | 0.04 | 4.43 | Nd |
| **V7** | 0.02 | 0.73 | 0.12 | 0.05 | 4.87 | Nd |
| **V10** | 0.02 | 0.64 | 0.10 | 0.04 | 4.40 | Nd |
| **B0** | 0.03 | Nd | Nd | Nd | 2.29 | Nd |
| **B1** | Nd | Nd | Nd | Nd | 2.06 | Nd |
| **B2** | Nd | Nd | Nd | Nd | 1.66 | Nd |
| **B3** | Nd | Nd | Nd | Nd | 1.76 | Nd |

**(Vna, Vanilic acid; 23Dhyb, 2,3dihydroxybenzoic acid; Mcou, m-coumaric acid; Ocou, o-coumaric acid; Pin, pinoresinol; Syr, Syringic acid)**

Table A.5. Phenolic compositions of extra virgin, virgin and blended olive oils at 320 nm

| | Cla | Fer | 34Dhyb | Cin | Apg | Lut |
|---|---|---|---|---|---|---|
| **E0** | 0.09 | 0.06 | Nd | Nd | 3.15 | 6.07 |
| **E1** | 0.04 | 0.07 | Nd | Nd | 2.54 | 5.03 |
| **E2** | 0.11 | 0.07 | Nd | Nd | 2.60 | 4.24 |
| **E3** | 0.09 | 0.08 | Nd | Nd | 2.39 | 3.90 |
| **E4** | 0.09 | 0.08 | Nd | Nd | 2.24 | 3.57 |
| **E5** | 0.09 | 0.08 | Nd | Nd | 2.66 | 3.92 |
| **E7** | 0.02 | 0.06 | Nd | Nd | 1.81 | 2.73 |
| **E10** | 0.06 | 0.03 | Nd | Nd | 1.79 | 2.48 |
| **V0** | Nd | 0.02 | Nd | Nd | 2.32 | 2.89 |
| **V1** | Nd | 0.08 | Nd | Nd | 2.68 | 3.65 |
| **V2** | Nd | 0.08 | Nd | Nd | 2.43 | 3.38 |
| **V3** | Nd | 0.07 | Nd | Nd | 2.05 | 2.77 |
| **V4** | Nd | 0.06 | Nd | Nd | 1.98 | 2.48 |
| **V5** | Nd | 0.06 | Nd | Nd | 2.20 | 2.40 |
| **V7** | Nd | 0.05 | Nd | Nd | 1.87 | 2.11 |
| **V10** | Nd | 0.05 | Nd | Nd | 1.36 | 1.56 |
| **B0** | Nd | 0.01 | Nd | Nd | 1.00 | 1.02 |
| **B1** | Nd | Nd | Nd | Nd | 0.41 | 0.32 |
| **B2** | Nd | Nd | Nd | Nd | 0.77 | 0.38 |
| **B3** | Nd | Nd | Nd | Nd | 0.91 | 0.55 |

**(Cla, Chlorogenic acid; Fer, ferulic acid; 34Dhyb, 3,4 dihdroxybenzoic acid; Cin, cinnamic acid; Apg, apigenin; Lut, luteolin)**

Table A.6. Fatty acid compositions of oil samples

| | C16:0 | C16:1 | C17:0 | C17:1 | C18:0 | C18:1n9c |
|---|---|---|---|---|---|---|
| **E0** | 12.96 | 0.16 | 0.15 | 0.26 | 3.25 | 72.01 |
| **E1** | 13.31 | 0.16 | 0.15 | 0.26 | 3.57 | 72.09 |
| **E2** | 13.26 | 0.16 | 0.14 | 0.26 | 3.24 | 72.10 |
| **E3** | 13.31 | 0.16 | 0.15 | 0.26 | 3.25 | 72.04 |
| **E4** | 13.40 | 0.16 | 0.14 | 0.26 | 3.25 | 71.90 |
| **E5** | 13.72 | 0.16 | 0.15 | 0.26 | 3.26 | 71.59 |
| **E7** | 13.85 | 0.16 | 0.14 | 0.26 | 3.26 | 71.55 |
| **E10** | 13.55 | 0.16 | 0.16 | 0.26 | 3.26 | 71.89 |
| **V0** | 13.29 | 0.16 | 0.19 | 0.24 | 3.71 | 65.28 |
| **V1** | 13.39 | 0.37 | 0.19 | 0.24 | 3.71 | 65.17 |
| **V2** | 13.54 | 0.14 | 0.19 | 0.24 | 3.75 | 65.02 |
| **V3** | 13.50 | 0.11 | 0.19 | 0.24 | 3.73 | 64.25 |
| **V4** | 13.80 | 0.14 | 0.19 | 0.24 | 3.76 | 65.11 |
| **V5** | 13.89 | 0.14 | 0.19 | 0.24 | 3.75 | 65.00 |
| **V7** | 14.05 | 0.14 | 0.18 | 0.24 | 3.77 | 64.92 |
| **V10** | 14.51 | 0.13 | 0.19 | 0.23 | 3.77 | 64.60 |
| **B0** | 13.85 | 0.17 | 0.11 | 0.19 | 2.68 | 69.38 |
| **B1** | 14.04 | 0.15 | 0.11 | 0.19 | 2.68 | 69.32 |
| **B2** | 14.12 | 0.15 | 0.12 | 0.19 | 2.70 | 68.99 |
| **B3** | 14.20 | 0.15 | 0.48 | 0.19 | 2.69 | 68.93 |

**(C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, Heptadecanoic acid; C17:1, Cis-10-Heptadecanoic acid; C18:0, stearic acid; C18:1n9c, oleic acid)**



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Table A.6. (Cont.)

| | C18:2n6c | C20:0 | C20:1 | C18:3n3 | C22:0 | C23:0 | C24:0 |
|---|---|---|---|---|---|---|---|
| **E0** | 8.73 | 0.44 | 0.64 | 0.27 | 0.12 | 0.95 | 0.06 |
| **E1** | 8.29 | 0.44 | 0.65 | 0.26 | 0.11 | 0.97 | 0.07 |
| **E2** | 8.31 | 0.44 | 0.65 | 0.26 | 0.12 | 0.99 | 0.06 |
| **E3** | 8.34 | 0.44 | 0.64 | 0.26 | 0.12 | 0.98 | 0.06 |
| **E4** | 8.35 | 0.44 | 0.66 | 0.26 | 0.11 | 1.01 | 0.06 |
| **E5** | 8.29 | 0.44 | 0.63 | 0.26 | 0.11 | 1.07 | 0.06 |
| **E7** | 8.28 | 0.44 | 0.62 | 0.26 | 0.11 | 1.01 | 0.06 |
| **E10** | 8.22 | 0.44 | 0.63 | 0.26 | 0.12 | 1.00 | 0.07 |
| **V0** | 14.29 | 0.54 | 0.78 | 0.27 | 0.16 | 1.00 | 0.08 |
| **V1** | 14.19 | 0.54 | 0.77 | 0.27 | 0.16 | 0.92 | 0.09 |
| **V2** | 14.33 | 0.54 | 0.78 | 0.27 | 0.17 | 0.91 | 0.12 |
| **V3** | 15.36 | 0.53 | 0.75 | 0.27 | 0.15 | 0.85 | 0.07 |
| **V4** | 14.20 | 0.54 | 0.76 | 0.27 | 0.14 | 0.77 | 0.07 |
| **V5** | 14.17 | 0.54 | 0.76 | 0.27 | 0.14 | 0.79 | 0.11 |
| **V7** | 14.16 | 0.54 | 0.75 | 0.27 | 0.14 | 0.72 | 0.12 |
| **V10** | 13.95 | 0.54 | 0.74 | 0.27 | 0.16 | 0.81 | 0.11 |
| **B0** | 11.60 | 0.44 | 0.63 | 0.31 | 0.14 | 0.42 | 0.09 |
| **B1** | 11.50 | 0.44 | 0.63 | 0.31 | 0.13 | 0.42 | 0.08 |
| **B2** | 11.76 | 0.44 | 0.62 | 0.31 | 0.14 | 0.41 | 0.07 |
| **B3** | 11.39 | 0.44 | 0.62 | 0.31 | 0.13 | 0.43 | 0.06 |

**(C18:2n6c, linoleic acid; C20:0, arachidic acid; C20:1, Cis-11-eicosenoic acid; C18:3n3, linolenic acid; C22:0, behenic acid; C23:0, tricosanoic acid; C24:0, lignoceric acid)**



**(Cont. on next page)**

Table A.6. (Cont.)

| | C16:0 | C16:1 | C17:0 | C17:1 | C18:0 | C18:1n9c |
|---|---|---|---|---|---|---|
| **B4** | 14.35 | 0.15 | 0.49 | 0.19 | 2.69 | 68.91 |
| **B5** | 14.47 | 0.15 | 0.11 | 0.19 | 2.71 | 68.87 |
| **B7** | 14.69 | 0.14 | 0.11 | 0.19 | 2.73 | 68.75 |
| **B10** | 15.16 | 0.14 | 0.12 | 0.18 | 2.78 | 68.60 |
| **R0** | 12.85 | 0.18 | 0.08 | 0.14 | 2.78 | 71.32 |
| **R1** | 13.05 | 0.15 | 0.08 | 0.13 | 2.79 | 71.10 |
| **R2** | 13.12 | 0.18 | 0.10 | 0.11 | 2.79 | 71.23 |
| **R3** | 13.22 | 0.18 | 0.08 | 0.14 | 2.79 | 71.12 |
| **R4** | 13.34 | 0.17 | 0.06 | 0.17 | 2.81 | 70.75 |
| **R5** | 13.48 | 0.17 | 0.09 | 0.16 | 2.80 | 70.83 |
| **R7** | 13.51 | 0.17 | 0.09 | 0.13 | 2.82 | 70.76 |
| **R10** | 14.05 | 0.17 | 0.09 | 0.12 | 2.84 | 70.39 |
| **S0** | 6.62 | 0.22 | 0.00 | 0.00 | 3.65 | 26.69 |
| **S1** | 6.78 | 0.13 | 0.00 | 0.00 | 3.66 | 26.67 |
| **S2** | 6.86 | 0.14 | 0.00 | 0.00 | 3.66 | 26.62 |
| **S3** | 7.08 | 0.14 | 0.00 | 0.00 | 3.70 | 26.59 |
| **S4** | 7.14 | 0.11 | 0.00 | 0.00 | 3.67 | 27.03 |
| **S5** | 7.33 | 0.12 | 0.00 | 0.00 | 3.67 | 27.25 |
| **S7** | 7.50 | 0.11 | 0.00 | 0.00 | 3.71 | 27.10 |
| **S10** | 8.11 | 0.14 | 0.00 | 0.00 | 3.73 | 27.64 |

**(C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, Heptadecanoic acid; C17:1, Cis-10-Heptadecanoic acid; C18:0, stearic acid; C18:1n9c, oleic acid)**



**(Cont. on next page)**

Table A.6. (Cont.)

| | C18:2n6c | C20:0 | C20:1 | 3C18:3n3 | C22:0 | C23:0 | C24:0 |
|---|---|---|---|---|---|---|---|
| **B4** | 11.28 | 0.44 | 0.62 | 0.31 | 0.12 | 0.39 | 0.07 |
| **B5** | 11.52 | 0.44 | 0.62 | 0.31 | 0.13 | 0.41 | 0.09 |
| **B7** | 11.45 | 0.44 | 0.61 | 0.31 | 0.13 | 0.38 | 0.07 |
| **B10** | 11.10 | 0.45 | 0.60 | 0.31 | 0.13 | 0.37 | 0.06 |
| **R0** | 10.60 | 0.44 | 0.62 | 0.31 | 0.15 | 0.40 | 0.09 |
| **R1** | 10.72 | 0.45 | 0.62 | 0.31 | 0.12 | 0.41 | 0.08 |
| **R2** | 10.40 | 0.45 | 0.62 | 0.32 | 0.15 | 0.46 | 0.09 |
| **R3** | 10.45 | 0.45 | 0.62 | 0.31 | 0.13 | 0.42 | 0.09 |
| **R4** | 10.68 | 0.44 | 0.61 | 0.31 | 0.13 | 0.44 | 0.08 |
| **R5** | 10.39 | 0.44 | 0.61 | 0.32 | 0.16 | 0.45 | 0.09 |
| **R7** | 10.36 | 0.45 | 0.60 | 0.30 | 0.13 | 0.38 | 0.13 |
| **R10** | 10.36 | 0.45 | 0.60 | 0.30 | 0.16 | 0.39 | 0.09 |
| **S0** | 61.23 | 0.26 | 0.15 | 0.14 | 0.70 | 0.00 | 0.26 |
| **S1** | 61.21 | 0.26 | 0.09 | 0.14 | 0.70 | 0.00 | 0.25 |
| **S2** | 61.12 | 0.26 | 0.12 | 0.14 | 0.70 | 0.00 | 0.25 |
| **S3** | 60.43 | 0.27 | 0.08 | 0.14 | 0.70 | 0.00 | 0.25 |
| **S4** | 60.51 | 0.26 | 0.08 | 0.13 | 0.70 | 0.00 | 0.25 |
| **S5** | 60.04 | 0.26 | 0.12 | 0.15 | 0.69 | 0.00 | 0.25 |
| **S7** | 59.96 | 0.26 | 0.14 | 0.16 | 0.70 | 0.00 | 0.25 |
| **S10** | 58.53 | 0.27 | 0.12 | 0.15 | 0.68 | 0.00 | 0.25 |

**(C18:2n6c, linoleic acid; C20:0, arachidic acid; C20:1, Cis-11-eicosenoic acid; C18:3n3, linolenic acid; C22:0, behenic acid; C23:0, tricosanoic acid; C24:0, lignoceric acid)**

# APPENDIX B

# STANDARD CALIBRATION GRAPHICS FOR PHENOLIC COMPOUNDS

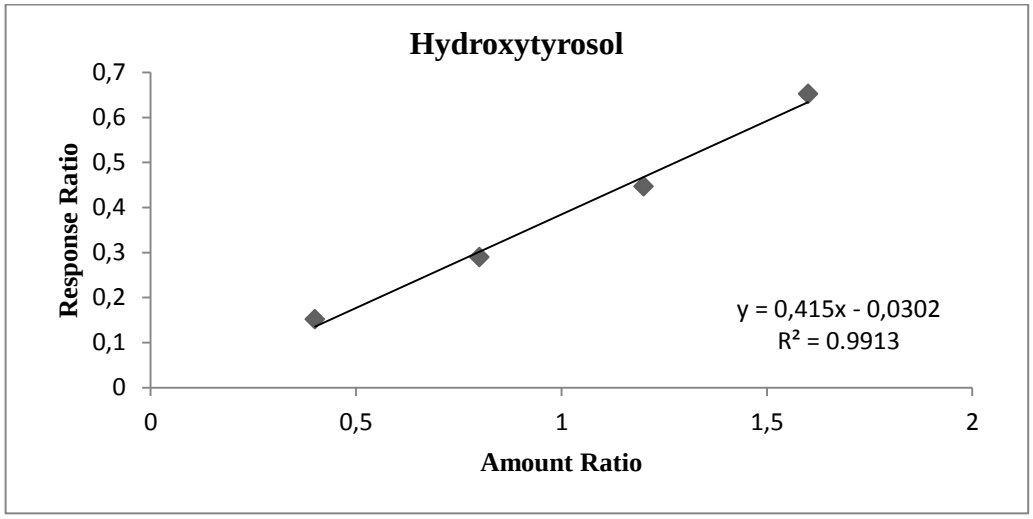


Figure B.1. Standard calibration curve for hydroxytyrosol

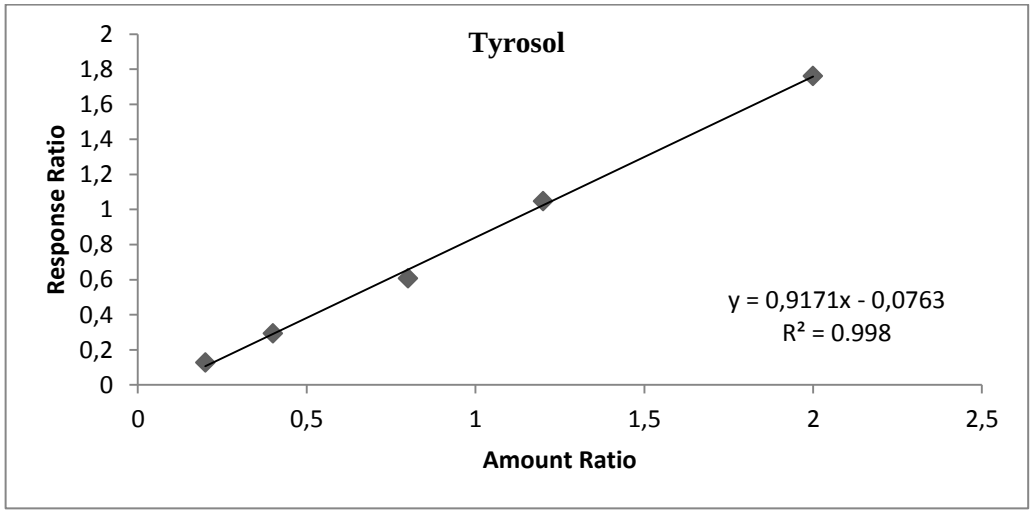


Figure B.2. Standard calibration curve for tyrosol

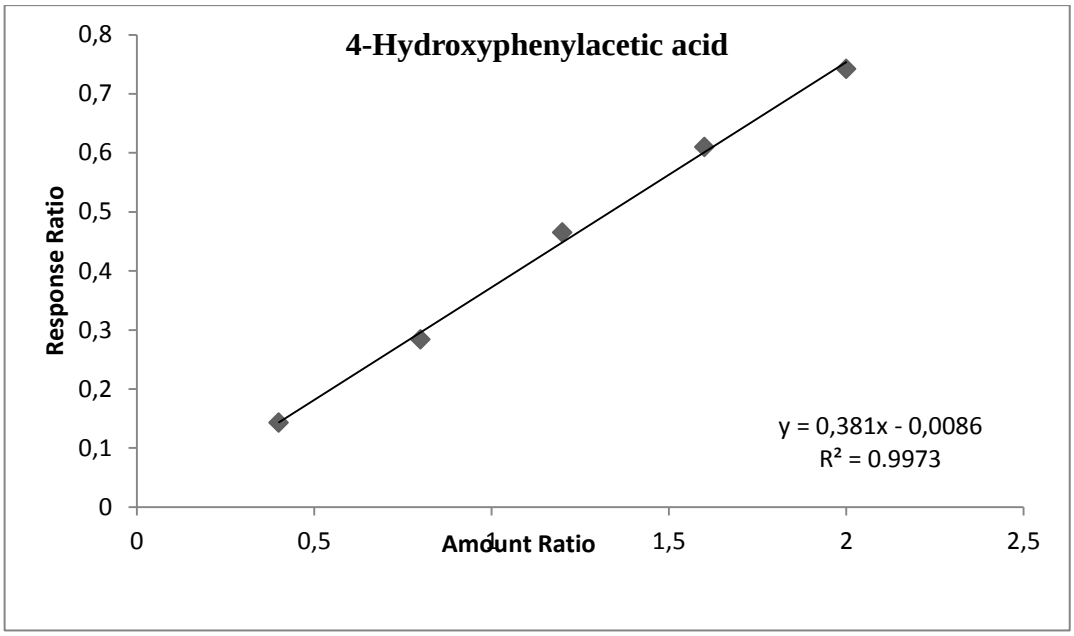


Figure B.3. Standard calibration curve for 4-Hydroxyphenylacetic acid

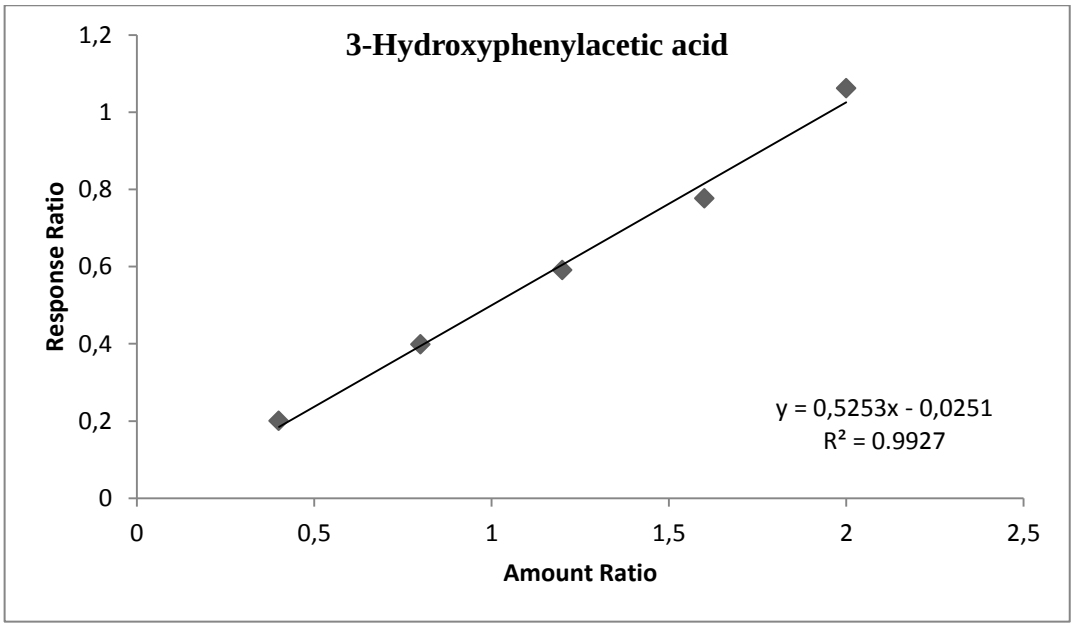


Figure B.4. Standard calibration curve for 3-Hydroxyphenylacetic acid

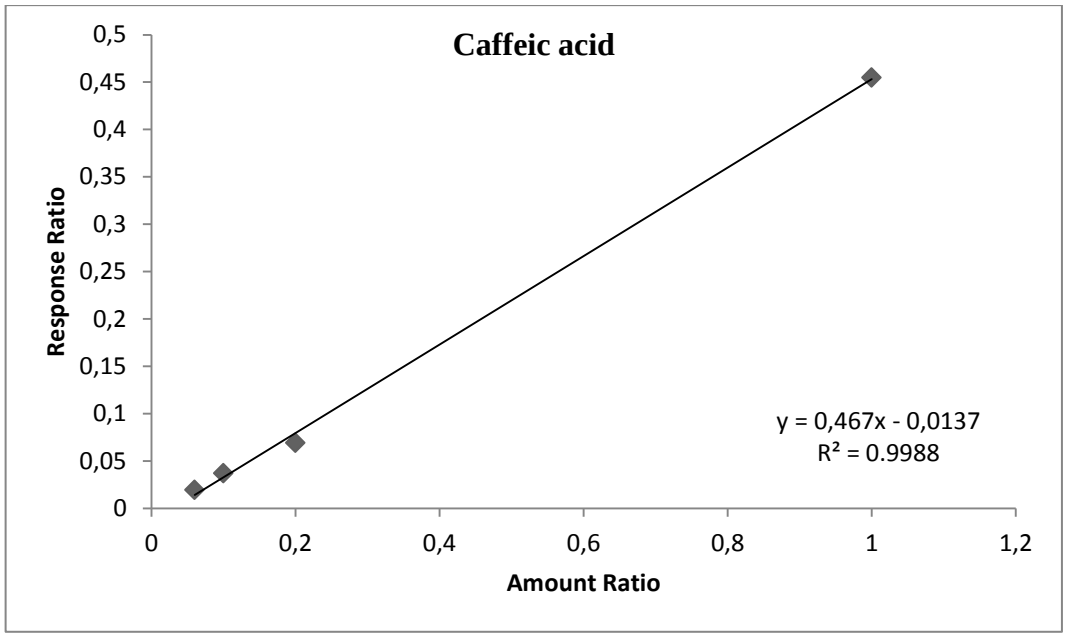


Figure B.5. Standard calibration curve for caffeic acid

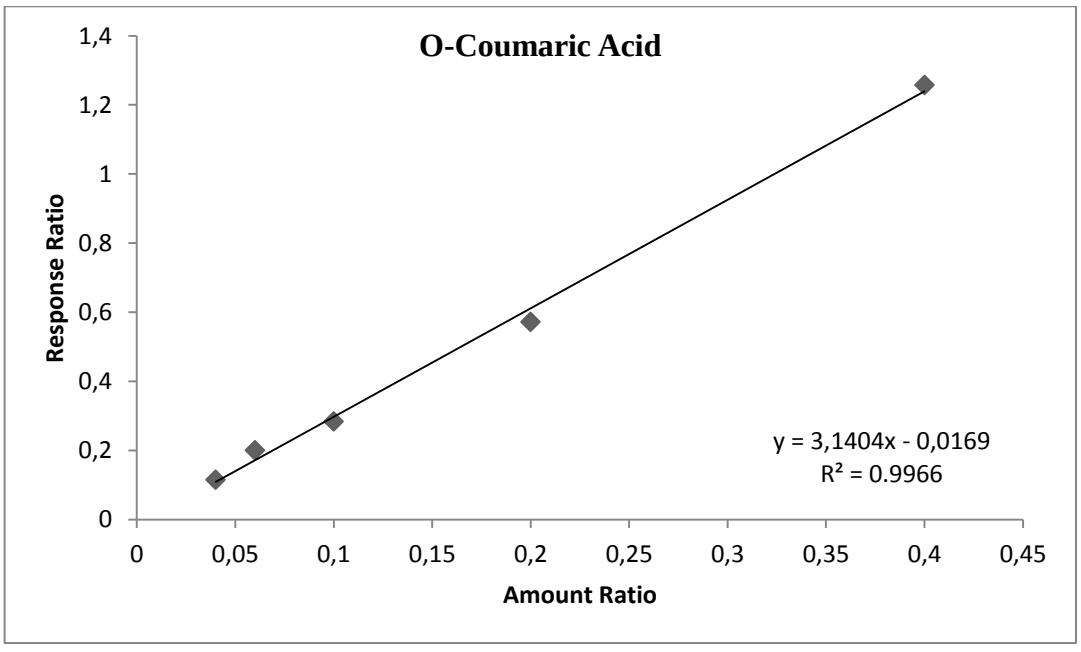


Figure B.6. Standard calibration curve for o-coumaric acid

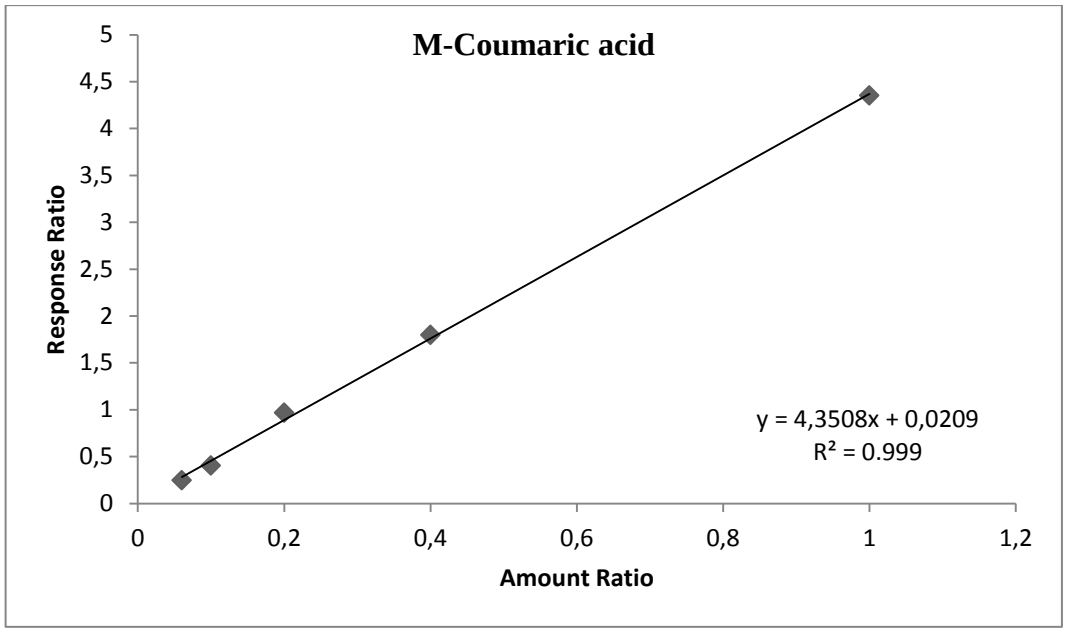


Figure B.7. Standard calibration curve for m-coumaric acid

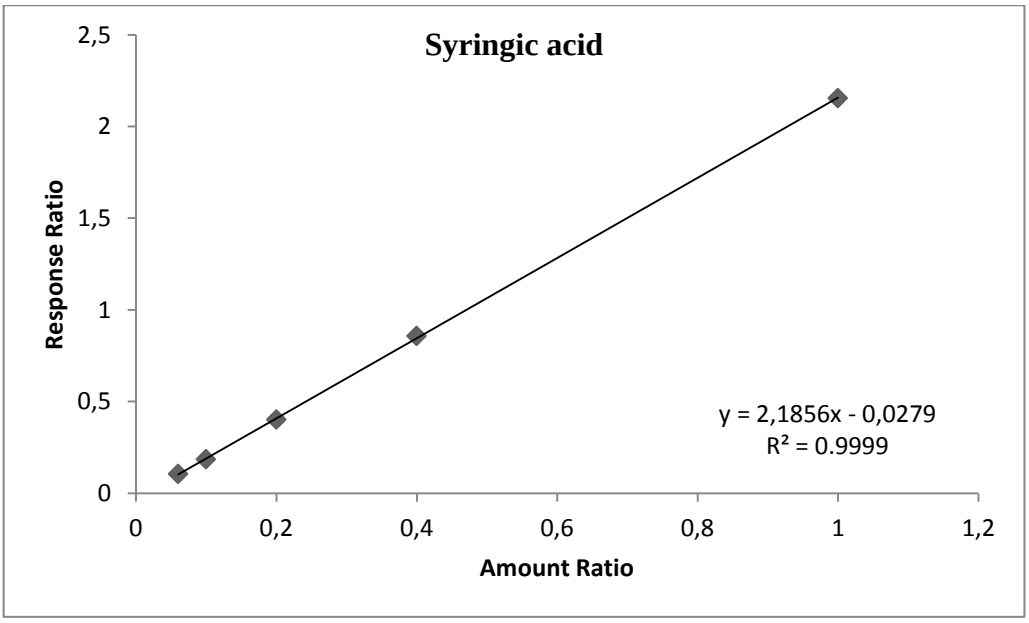


Figure B.8. Standard calibration curve for syringic acid

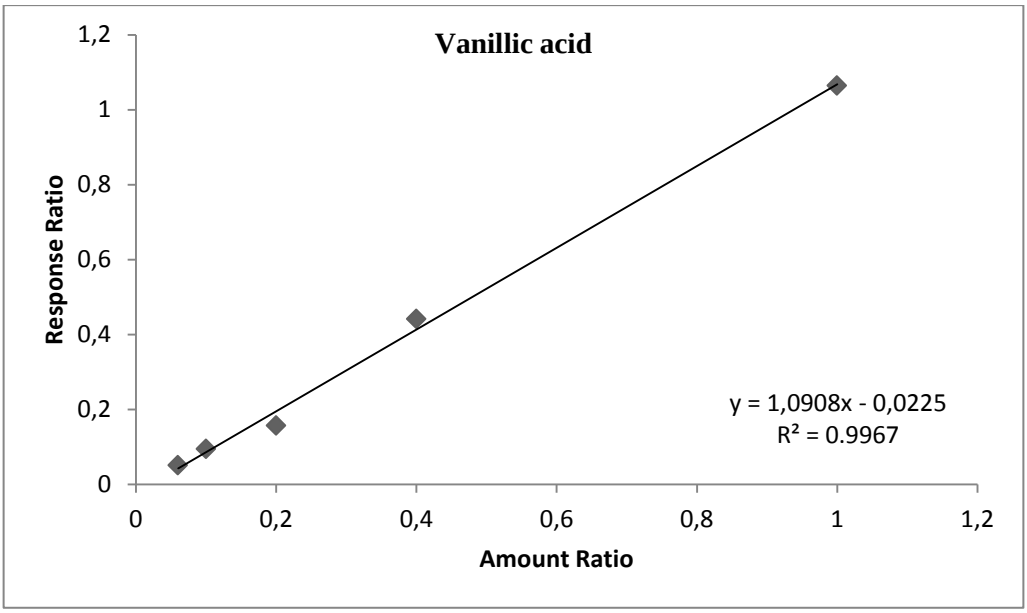


Figure B.9. Standard calibration curve for vanillic acid

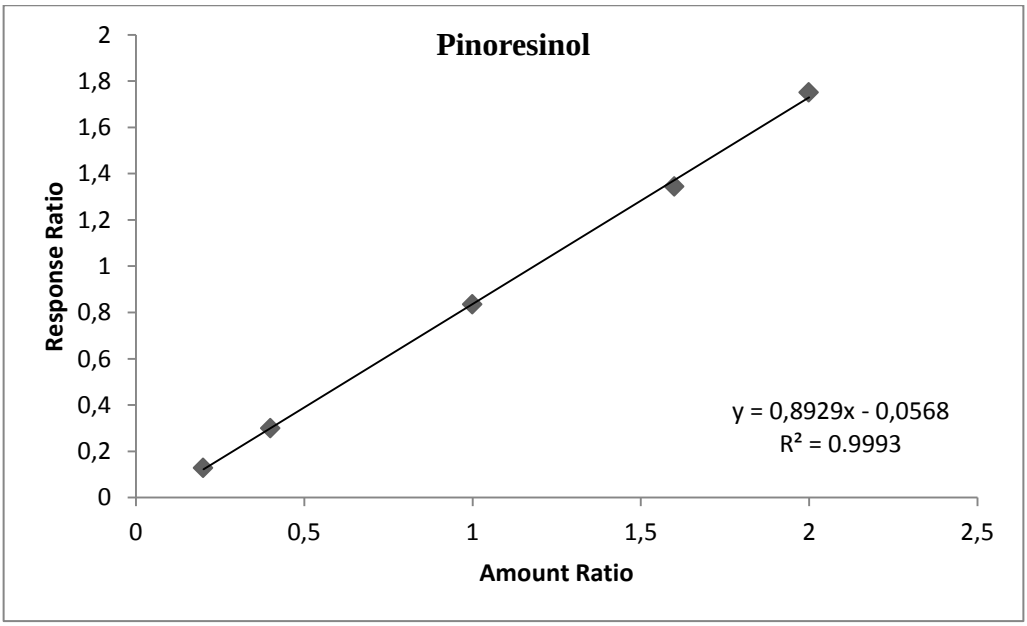


Figure B.10. Standard calibration curve for pinoresinol

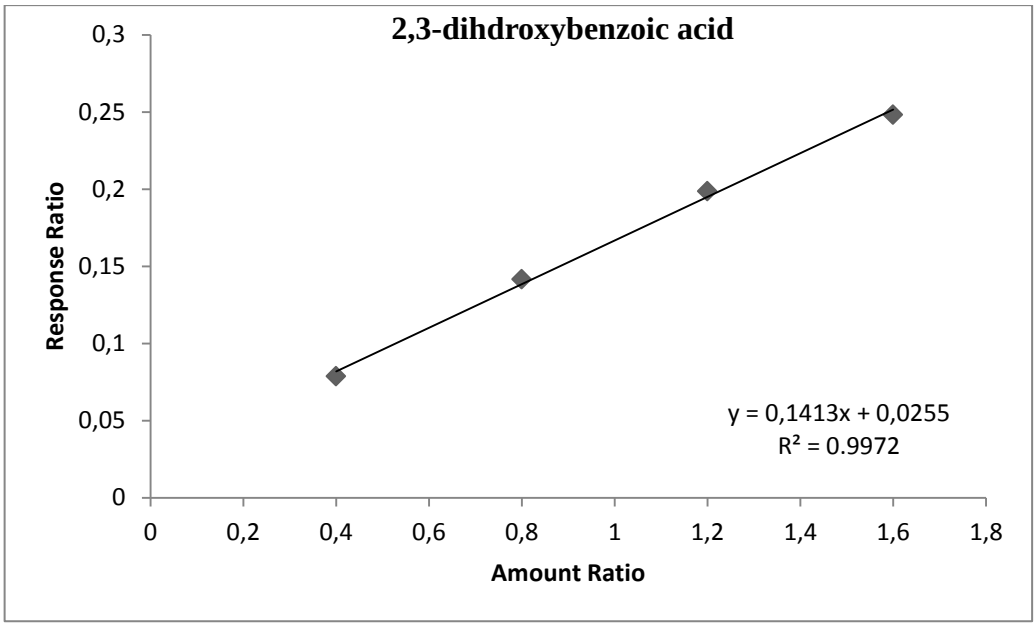


Figure B.11. Standard calibration curve for 2-3 dihdroxybenzoic acid

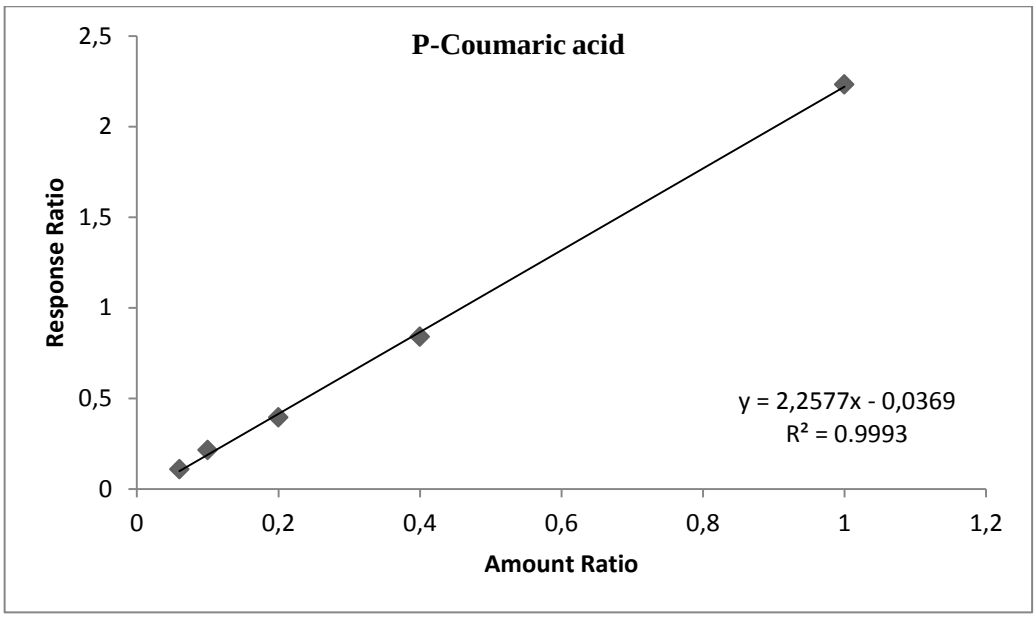


Figure B.12. Standard calibration curve for p-coumaric acid

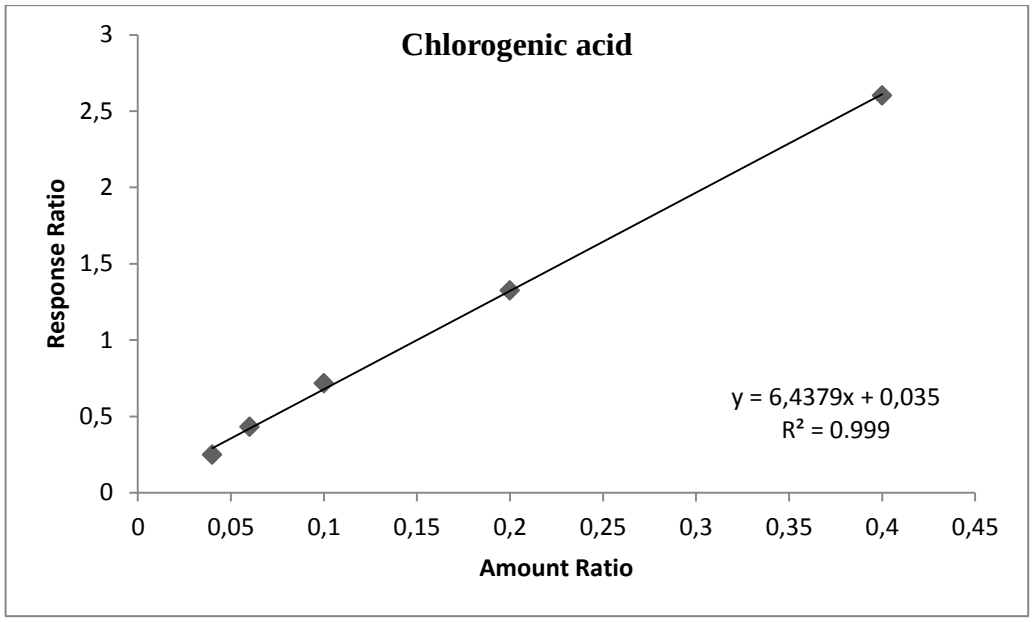


Figure B.13. Standard calibration curve for chlorogenic acid

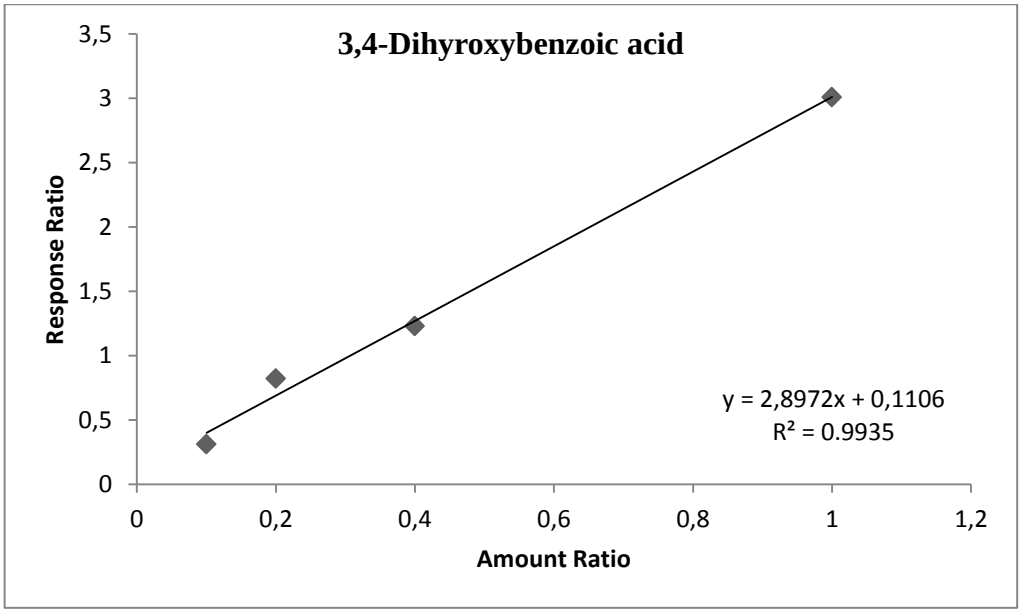


Figure B.14. Standard calibration curve for 3,4-Dihyroxybenzoic acid

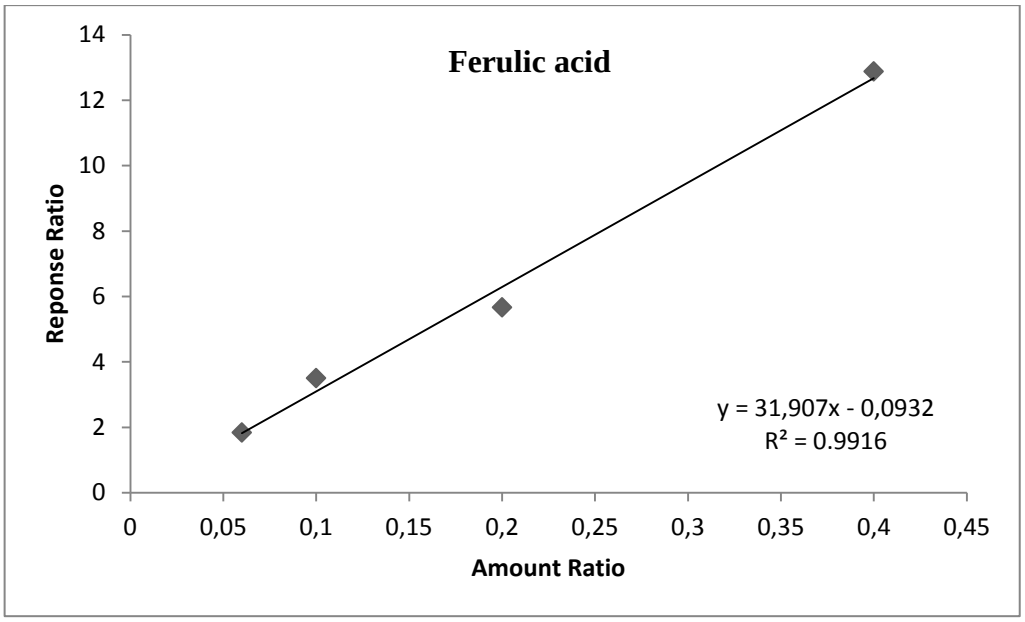


Figure B.15. Standard calibration curve for ferulic acid

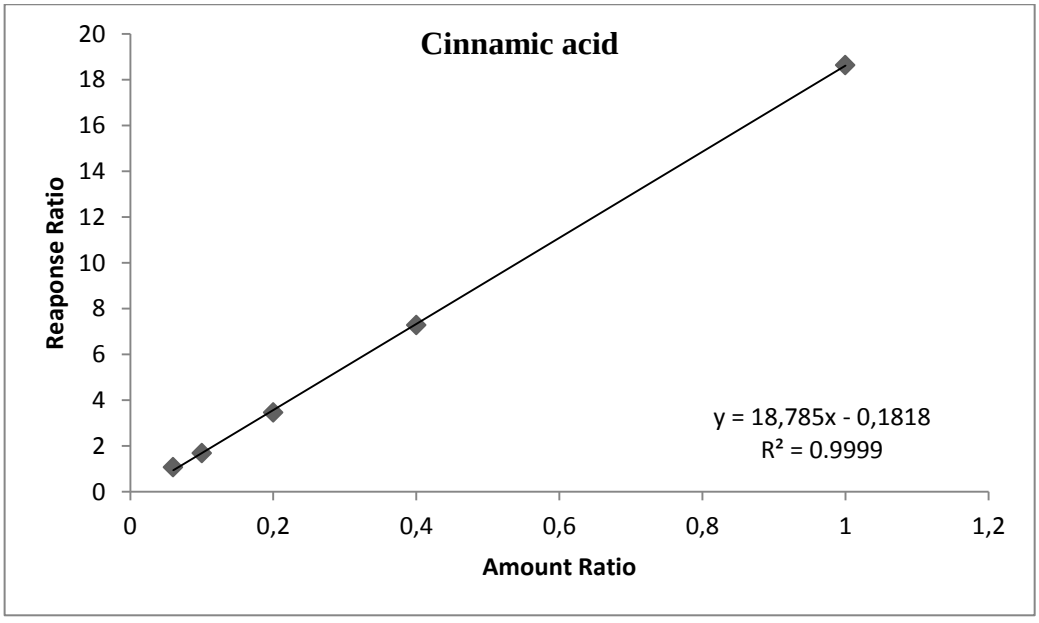


Figure B.16. Standard calibration curve for cinnamic acid

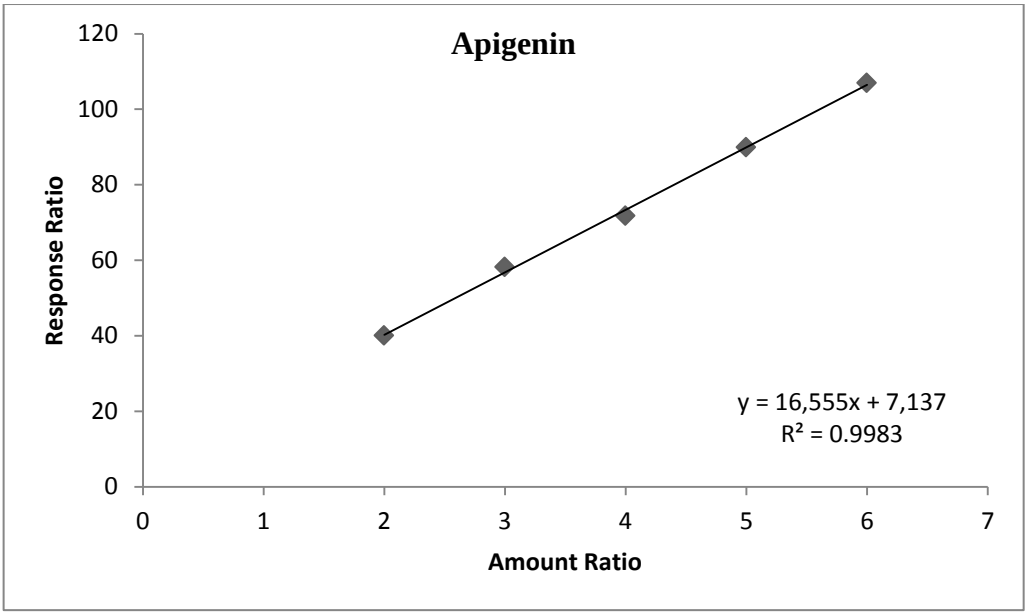


Figure B.17. Standard calibration curve for apigenin

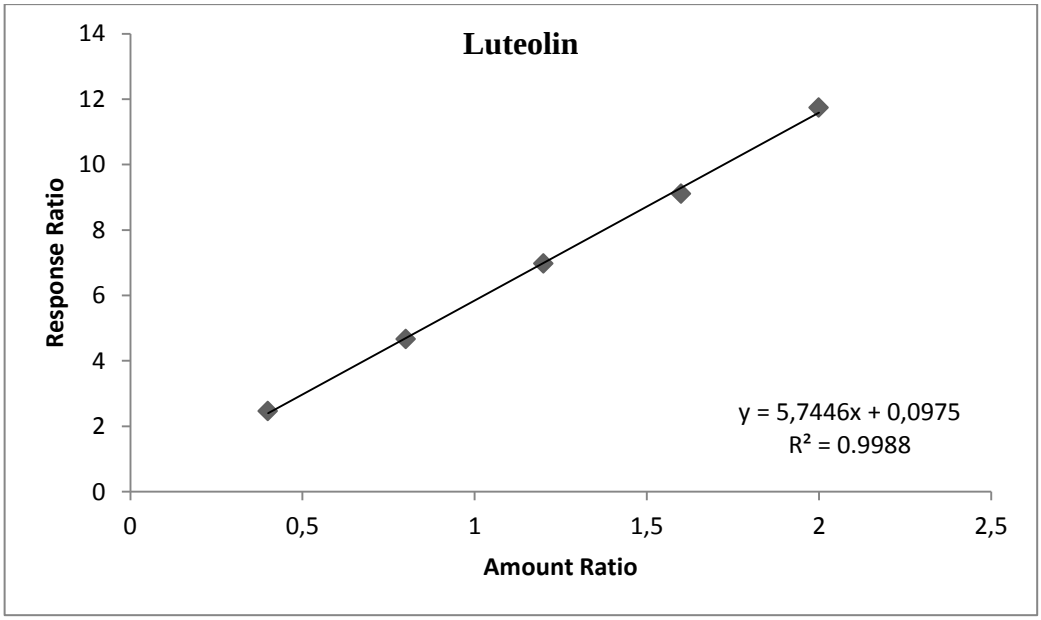


Figure B.18. Standard calibration curve for luteolin