CHEMICAL CHARACTERIZATION OF 'HURMA' OLIVE GROWN IN KARABURUN PENINSULA

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
Ayşe Burcu AKTAŞ

July 2013
İZMİR

We approve this thesis of **Ayşe Burcu AKTAŞ**

Examining committee members:

Assoc. Prof. Dr. Banu ÖZEN
Department of Food Engineering, Izmir Institute of Technology

Assist. Prof. Dr. Handan BAYSAL
Department of Food Engineering, Izmir Institute of Technology

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

10 July 2013

Assoc. Prof. Dr. Banu ÖZEN
Supervisor,
Department of Food Engineering
Izmir Institute of Technology

Assoc. Prof. Dr. Figen TOKATLI
Co-advisor,
Department of Food Engineering
Izmir Institute of Technology

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

Prof. Dr. R. Tuğrul ŞENGER
Dean of the Graduate Schools of
Engineering and Sciences

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Banu ÖZEN for her supervision, guidance, support, encouragement and endless patience during my studies.

I would like to appreciate deeply to my co-supervisor Assoc. Prof. Dr. Figen TOKATLI,for her all kind of supports and valuable comments.

I express my special thanks to Eğlenhoca village and their kind habitants (Karaburun-Izmir, Turkey) for providing olive samples. This study is a part of TUBITAK project (TOVAG-110O780) and supported by The Scientific and Technological Research Council of Turkey.

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

I would like to thank to Specialist İlknur ŞEN for her help during my analysis.

Lastly, I offer sincere thanks to my parents for their endless support, encouragement and love during all my education life.

ABSTRACT

CHEMICAL CHARACTERIZATION OF ‘HURMA’ OLIVE GROWN IN KARABURUN PENINSULA

Olive type, mostly Erkence, grown in nearby area around Karaburun peninsula of Izmir-Turkey, goes through a natural debittering phase on the tree during its ripening. This olive is known by the name of Hurma and loses its bitter taste while still on the tree and can be consumed directly at the end of this natural process.

The aim of this study is to investigate the changes in the chemical composition of Hurma, Erkence and Gemlik olives throughout their maturation period and to determine some chemical compositional differences between Hurma and other types of olives to obtain more insight about the natural debittering phenomena. For this purpose, the chemical parameters measured are pH, water activity, total fat amount, fatty acids, sugar and organic acid amounts, total phenol content and phenol profile. All analyses were performed for two harvest years. Data were analyzed by ANOVA and principal component analysis (PCA) to investigate the differences regarding the olive types, ripening period and harvest year.

Total phenol content and generally concentration of individual phenolic compounds of Hurma olive were lower than Erkence and Gemlik olives. Both fatty acid and phenol profiles allowed a differentiation with respect to type and also harvest year according to PCA while organic acid and sugars provided a separation only in terms of harvest year.

ÖZET

KARABURUN YARIMADASINDA YETİŞEN ‘HURMA’ ZEYTİNİNİN KİMYASAL KARAKTERİZASYONU

Karaburun yarımadasında yetişen zeytin türü, çoğunlukla Erkence, olgunlaşma periyodu sırasında acılık kaybetme aşamasından geçer. Bu zeytin ‘Hurma’ adı ile bilinir ve bu doğal işlem sonucunda henüz ağaç üzerindeyken acılığını kaybederek doğrudan tüketilebilir hale gelir.

Bu çalışmanın amacı olgunlaşma süreci boyunca, Hurma, Erkence ve Gemlik zeytinlerinin kimyasal özelliklerinin belirlenmesi ve doğal olarak gerçekleşen acılık kaybetme işlemini açıklayabilmek için Hurma zeytinin diğer zeytinlerden bazı kimyasal içerik farklarının belirlenmesidir. Bu amaçla yapılan analizler, pH, su aktivitesi, toplam yağ miktarı, yağ asidi profili, şeker ve organik asit miktarları, toplam fenol içeriği ve fenol profilidir. Bütün analizler iki hasat sezonu için gerçekleştirilmiştir. Zeytin tipinin, hasat yılının ve hasat zamanın etkisini belirlemek üzere veriler ANOVA ve Asal Bileşenler Analizi ile çözümlenmiştir.

Hurma zeytininde toplam fenolik madde miktarı ve genel olarak bireysel fenolik bileşen konsantrasyonları Erkence ve Gemlik zeytinlerine göre nispeten daha düşüktür. Asal bileşenler analizine göre fenol ve yağ asidi profilleri hem hasat yılına hem de zeytin tipine bağlı ayrıma imkân vermekte iken organik asit ve şekerler sadece hasat yılına bağlı olarak bir ayrım sağlamaktadır.

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CHAPTER 1

INTRODUCTION

Olive is an important agricultural product for Turkey and especially for Aegean Region. Olives, which are rich in minor components such as phenolics, outshine as one of healthy food products which are trendy in recent years. Turkey is very rich in terms of olive varieties. Olive type, mostly Erkence, grown in nearby area around Karaburun peninsula of Izmir-Turkey, goes through a natural debittering phase on the tree during its ripening. This naturally debittered olive is known by the name of Hurma. Hurma olive, which has the characteristic sensorial properties, is a noteworthy product for both its growers and its consumers. Hurma olive has the characteristic of losing its bitterness throughout its maturation period; therefore, this type of olive does not require further processing steps for debittering. It was stated that a fungus called *Phoma olea* is the reason for this phenomena with the help of climactic conditions. According to a few studies in the literature, it was reported that similar types of olives were also grown in countries like Greece and Tunisia.

Although there are many studies related to olive oil composition and factors affecting the compositional parameters in the literature research related with olive fruit itself is relatively less. However, there are reports of beneficial health effects of consuming table olives and a study claims that consuming 5-10 table olives might cover the daily intake of polyphenols, which are associated with the prevention of cardiovascular disease, degenerative disease protection, anti-inflammatory and anti-carcinogenic activities (Boskou et al., 2006). Especially, current knowledge on natural debittering of olive while still on the tree is very limited.

The studies about sweet Thasos olive which is grown in Thasos island of Greece shows that oleuropein responsible for bitter taste is hydrolyzed to hydroxytyrosol and its derivatives by an enzyme, β-glucosidase, which is produced by fungi and bacteria during ripening (Zoidou et al., 2009). Same trend was also observed in a study about Dhokar olives which are cultivated in the southern region of Tunisia (Jemai et al., 2009). Therefore, while oleuropein concentration decreases during maturation,

hydroxytyrosol concentration increases. In addition to this, total phenol content and reducing sugar concentration increase relatively (Jemai et al., 2009).

Sugars and organic acids are significant components of olive fruit. Sugars not only provide energy for metabolic changes that take place in the fruit but also are related to textural properties of the olive. In addition, sugars are the precursor for fatty acid biosynthesis and they act as carbon source of microorganism during table olive processing (Marsilio et al., 2001). In Thasos olives, glucose and mannitol were detected as the main sugar and sugar alcohol, respectively and their concentration levels were very close to each other (Marsilio et al., 2001). In sweet Dhokar olives, glucose and mannitol reached their highest level at the last stage of ripening and their concentration were really higher compared to regular Chemlali olives (Jemai et al., 2009).According to a study about Turkish olives, succinic, malic and citric acids were found as major organic acids (Ergönül and Nergis, 2010). It was reported that malic and citric acids are the major organic acids in olive and they affect the color of the fruit. In addition, they have an important role in olive processing by affecting the buffering activity of olive tissue. It has also known that organic acids influence the stability, quality and aroma of the olive fruit (Joslyn, 1970).

One of the major components of olive is fatty acids. The fatty acid composition of olive oil varies widely depending on the cultivar, maturity of the fruit, altitude, climate, and several other factors (Rondanini et al., 2011).Olive oil contains more oleic acid and less linoleic and linolenic acids than other vegetable oils, that is, more monounsaturated than polyunsaturated fatty acids. This makes olive oil more resistant to oxidation.

The aim of this study is to investigate the changes in the chemical composition of Hurma (naturally debittering Erkence), same variety olive which does not sweeten up on tree, Erkence, and a regular olive variety commonly used as a table olive, Gemlik, throughout their maturation period for two harvest years and to show some chemical compositional differences between Hurma and other types of olives using multivariate statistical analysis. For this purpose, pH, water activity, total fat amount, fatty acids, sugar, organic acids, total phenol content and phenol profile were determined for two harvest years. The data were analyzed by ANOVA and principal component analysis (PCA) to investigate the differences regarding the olive types, ripening period and harvest year.

CHAPTER 2

LITERATURE VIEW

2.1. Description of Olive and Brief History

2.1.1. Description of Olive

The olive tree, *Olea europaea*, is an evergreen tree or a shrub and natively grows in Mediterranean, Asian and African countries. Its scientific classification is provided in Table 2.1. It is short and squat, and rarely exceeds 8–15 meters (26–49 ft) in height. The fruit is a small drupe 1–2.5 centimeters (0.39–0.98 in) long, thinner-fleshed and smaller in wild plants than in orchard cultivar (Encyclopedia Wikipedia, 2013).

Table 2.1. Scientific Classification of Olives
(Source:Encyclopedia Wikipedia, 2013)

Scientific Classification	
Kingdom	Plantae
(unracked)	Angiosperms
(unracked)	Eudicots
(unracked)	Asterids
Order	Lamiales
Family	*Oleaceae*
Genus	*Olea*
Species	*Oleaeuropaea*
Binomial Name	
OleaEuropaea L.	

The olive tree has been cultivated for olive oil, fine wood (2.5 times the energy generated by burning the same amount of wood), olive leaf, and the olive fruit. Its

harvesting starts in the green to purple stage from early of October until the end of December.

2.1.2. Brief History

Olive has 6,000 years long history which is documented by legends, traditions, religious texts and archaeological discoveries. First wild olives were collected by Neolithic people early in the 8th millennium BC. The olive tree is believed to have originated in the Middle East and the last studies about homeland of olive indicate Mardin, Andırın, Anamur triangle of Turkey (East Mediterranean Olive Association, Turkey, 2011)(Figure 2.1).First, the olive spread to the Fertile Crescent area then to the rest of the World by two different ways (Figure 2.1). First way is from Mardin- Andırın-Anamur triangle to the west of Anatolia, Greece, the territories of Greece, Aegean islands, the coastal area of the Balkans, Italy, Spain and Portugal (East Mediterranean Olive Association, Turkey, 2011).The second way is from Syria, Israel and Lower Egypt to North Africa. Due to the positive recognition of its fruit and by the favorable environmental conditions of the Mediterranean climate, its cultivation spread out to Morocco, Algeria, Tunisia and the oases of Libya. After the discovery of America, olive cultivation spread southwards to Peru, Argentina, Chile and Uruguay and northwards to the coastal regions of Mexico and the United States where it found an ideal environment in the southern part of California (East Mediterranean Olive Association, Turkey, 2011).

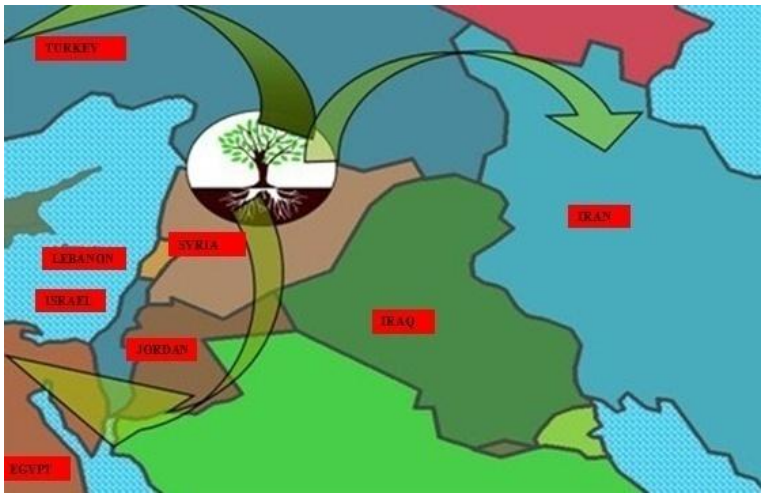

Figure 2.1.Homeland of olive and its distribution
(Source: East Mediterranean Olive Association, 2013)

In recent times, olive trees have also been introduced in other countries without an earlier tradition of olive oil production or consumption. As a result, nowadays this fruit found more and more widely in countries like South Africa, Australia, New Zealand and China. Major olive growing areas of the World is shown in Figure 2.2.

Figure 2.2. Geographical distribution of olive growing areas
(Source: International Olive Oil Council, 2010)

Major progress in olive processing started with the invention of screw press by Greeks. Then, Romans set and disseminated the equipment. After the fall of Roman Empire, there was a reduction in olive cultivation until middle Ages. During the 1900s, mechanical extraction systems started to be used as a result of improvements inpercolation and centrifugation systems. The first industrial decanter based on the continuous centrifugation of the olive paste was used toward the end of 1960s. Despite

the improvement in pressing systems, old pressing system is still in use in some countries (Aparicio and Aparicio-Ruíz, 2000).

2.2. World Olive Growth and Production

2.2.1. Cultivation Conditions

Olive trees, *Olea europaea*, prefer calcareous soils, and coastal climate conditions for the best growth. They can grow in any light soil, but in rich soils they are prone to disease and produce poorer oil. Olives like hot weather, and temperatures below −10 °C may injure even a mature tree. Their tolerance to drought is well because of their sturdy and extensive root system. Olive trees can live for several centuries, and can remain productive if pruned correctly and regularly (Encyclopedia Wikipedia, 2013).

2.2.2. World Olive Production

Olive tree finds the best growth conditions in the Mediterranean region which is called as 'the Civilization of Olive'. According to the statistics, 98 percent of olive production which is around 18.5 million tons produced in Spain, Italy, Greece, Turkey, Tunisia, Syria, Portugal, France and Algeria. Major table olive producing countries and their production numbers between 2005 and 2011 are given in Table 2.2. According to 2010 statistics, the total World olive oil production is 2,950,000 tons. Spain is in the first rank followed by Italy and Greece in the second and third ranks, respectively. Turkey is the fifth country in terms of tree numbers and the fourth one in terms of olive oil production (International Olive Oil Council, 2010).

Turkey has 159,473,907 olive trees; 43,904,206 of these trees are fruitless while 115,569,647 olive trees have fruit according to 2010 statistics. Turkey produced 1,076,601 tons of olives in 2010; 305,045 tons of these are used as table olive and 771,556 tons are for olive oil production .In 2011-2012 season, Turkey had 123,375,388 olive trees which had fruit. In 2011, 1,446,171 tons olive produced and 534,376 tons of

these were used as table olive and 903,535 tons were for olive oil (International Olive Oil Council, 2012).

It is expected for 2012-2013 season that with 131,263,255 olive trees which has fruit 1,438,481 tons of olive will be produced.455, 030 tons of these olives will be used for table olive and 983,450 tons will be utilized in olive oil production.

Table 2.2.Production of Table Olives (1.000 tones)

(Source: IOOC, 2011)

YEARS / **COUNTRIES**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
Algeria	85.0	75.0	100.0	95.0	220.0	250.0	200.0
Egypt	200.0	436.0	432.0	440.0	409.0	200.0	500.0
Syria	120.0	200.0	100.0	120.0	135.0	142.0	165.0
Tunisia	26.5	15.0	18.0	18.0	22.0	20.0	22.0
Turkey	280.0	240.0	200.0	300.0	390.0	330.0	450.0
Morocco	100.0	90.0	100.0	100.0	90.0	110.0	100.0
Argentina	85.0	75.0	100.0	95.0	220.0	250.0	200.0
EU	623.5	714.5	720.5	677.0	675.0	809.0	667.5

2.3. Production of Table Olives and Its Types

‘Table olives are the sound fruit of varieties of the cultivated olive trees (*Olea europaea* L.) that are chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh taste, firmness and ease of detachment from the stone make them particularly suitable for processing; treated to remove its bitterness and preserved by natural fermentation; or by heat treatment, with or without the addition of preservatives; packed with or without covering liquid’ (IOOC, 2004).

A complete definition of all trade preparations can be found in the ‘Trade Standard Applying to Table Olives’ by International Olive Council (IOOC, 2004). The main commercial preparations as described by IOOC are explained below:

Treated olives.“Green olives, olives turning color or black olives that have undergone alkaline treatment, and then taken in brine where they undergo fermentation, and

preserved or not by the addition of acidifying agents". The most common preparation is 'treated green olives in brine' also known as 'Spanish style' or 'Seville style'.

Natural olives. 'Green olives, olives turning color or black olives are placed directly in brine in which they undergo fermentation, preserved or not by the addition of acidifying agents'. The most prevalent preparation is 'natural black olives' also known as 'Greek style'.

Olives darkened by oxidation. 'Green olives or olives turning color are preserved in brine, fermented or not, darkened by oxidation in an alkaline medium and preserved in hermetically sealed containers subjected to heat sterilization; they shall be a uniform black color'. These are also known as 'ripe olives' or 'black olives'.

2.3.1. Spanish Style Green Olives

For this process, green olives are obtained from fully developed green fruits during the maturation period, prior to darkening. These olives must be firm, sound, resistant to a slight pressure between the fingers, and without marks other than natural pigmentation. The color of the fruits may be green to yellow.

The cultivar is one of the most important criteria for green table olives and this type of olives must have the following characteristics: good size and proper shape, high relationship of flesh/stone, ease in releasing the pit as well as good color and texture in the final product. The most popular cultivars used are: Sigoise (Algeria), Arauco (Argentina), Kalamata (Greece), Gordal, Manzanilla,Hojiblanca (Spain),Ascolana (Italy),Picholine, Marocaine (Morocco),Meski (Tunisia), and Domat (Turkey) (COI, 2000).

Figure 2.3.Process of Spanish Table Olives

(Source: Fernández et al., 1997)

Typical steps of a Spanish table olive processing are listed in Figure 2.3. The lye treatment with a diluted solution of NaOH is the essential step for green olive processing. The main purpose in this step is to eliminate the bitter taste of the fresh fruits, which can be achieved by the chemical hydrolysis of the oleuropein.

The penetration of the lye into the flesh is considered sufficient if the chemical reaches about 2/3 of the distance from the exterior to the pit. A homogeneous penetration could be obtained by using the olives having similar size and maturation degree. Lye treatment is normally achieved in 10,000 kg tanks. The diffusion coefficient of NaOH through the skin ranges from 43.3 to $9.32x10^{-12}$while through the flesh is 7.18 x 10^{-11} to 1.18 x 10^{-9} m^2/s (García et al.,2006). Currently, it is preferred to apply the lye treatment at a controlled temperature (18°C) to avoid peeling (García et al., 2006) and allow a more homogeneous lye penetration.

When the lye treatment ends, the alkaline solution is removed by covering olives with tap water (‘lavado’). The main purpose of this washing is to remove the excess of

the alkali which is penetrated into the flesh. An excessive washing is not desirable because it may cause losses of various soluble compounds which will be later required for fermentation (Sánchez et al., 2000).

The use of warm water does not improve removing alkali either (Sánchez, García et al. 1995) HCl in the concentrations of up to 0.07 eq/l is used in the washing step. This much of acid is under detection level of any sensory analysis; however, the number of washing waters given to the alkali treated olives is increased for precaution.

With the removal of washing waters olives are immersed in a 10-11% (w/v) brine in which olives are maintained during the fermentation and storage periods. Normally, underground fermenters are used. Brine stabilization is fairly rapid and in a few days NaCl concentration stabilizes at a level of 5-6%.In Tunisia, for Meski cultivar, the best debittering conditions were established as: 2% (w/v) NaOH lye concentration and a brine concentration of 9% (Chammem et al., 2005).

At the early stages of the brining process, the pH value of the brine is higher than 10 units due to the alkali that is released by the fruits. The development of microorganisms especially lactic acid bacteria causes production of different acids and lowers the pH to around 4 units.

The fermentation is still spontaneous in most cases since the use of starter cultures is not common. However, the use of *Lactobacillus pentosus* 5138 starter culture which is resistant to alkaline pH (around 9 units) causes initiation and acceleration of the fermentative process (Sánchez et al., 2001).

During the fermentation, there is a slow hydrolysis of the elenolic acid glycoside with the production of elenolic acid and glucose. These hydrolysis products are used by the microorganisms in the brine and maintain the microbial activity for a longer period of time (Brenes and de Castro, 1998).

At the end of fermentation, the olives must have reached the proper physicochemical characteristics for consumption. Therefore, series of complementary operations should be applied to green fermented olives to adapt them to the different commercial presentations.

2.3.2 Natural Black Olives

For this type of olives, the fruit should be harvested when it is ripe but not over-ripe. Degrees of ripeness affect the texture and over-ripe olives don't get firm enough. In this type of process, olives are placed into brine with a salt concentration between 8 and 10 % (w/v) and lower concentrations (about 6 %) can be used for colder areas. The fermentation process takes a long time because of diffusion of fermentable compounds through the skin. Debitterness process can be only achieved by solubilization of the oleuropein into the brine and equilibrium is reached in 8-12 months (Fernández et al., 1997)

During anaerobic fermentation of the olives a variable proportion of fruits with "gas-pocket" spoilage are produced. This spoilage is characterized by the development of blisters in the flesh of olives which may extend to the pits of the fruits. This is due to the CO_2 accumulation that is produced by the effect of olive respiration and the activity of the responsible microorganisms during the fermentative process (Borcakli et al., 1993).

To avoid the appearance of "gas pocket" spoilage, fermentation under aerobic conditions is carried out. The species of gram-negative bacteria are used in the traditional process. Yeasts are stable during the whole fermentation process with a higher population than under anaerobic conditions. Lactic acid bacteria can grow only if the salt concentration is below 8%.Microbial flora almost exclusively include *Leuconostoc* and *Pediococcus* at the early stages of fermentation, but after 20 days *Lactobacillus* predominate (Borcakli et al., 1993).

Fermentation under both aerobic and anaerobic conditions is influenced by the initial pH and NaCl concentration. In order to prevent excessive growth of gram-negative bacteria, acetic acid must be added to the brining solution to reduce pH below 4.5. If the pH is high, the population of gram-negative bacteria is excessive and produces a great volume of CO_2, which causes gas-pocket spoilage in the olives (Fernández et al., 1997).

In the past, olives processed with this technique were not packed. They were only sold in bulk. Glass jars or cans are rarely used for this product. In general, there are two presentation forms: naturally black olives in brine (Greek style) and Kalamata style. For Greek style, the most common values for commercial products are: pH about 4.0-

4.2 and salt concentration between 6-8%. For Kalamata style, the pH values are lower because wine vinegar must be added; furthermore, olive oil is also included into the formulation (Borcakli et al., 1993).

2.3.3. Black (ripe) Olives

The stages of this process are shown in Figure 2.4.Olives that will be used in this process should be collected when the fruit has a green color as in Spanish style. However, fruits can be directly exposed to the oxidation process without any preservation in order to produce the ripe olives. All the fruits can't be processed immediately because factories do not have the required capacity and it is not desirable to store large amounts of canned product. In addition to this, it is possible to use green Spanish style olives as raw material although the working conditions for obtaining a good final product are different (Fernández et al., 1997).

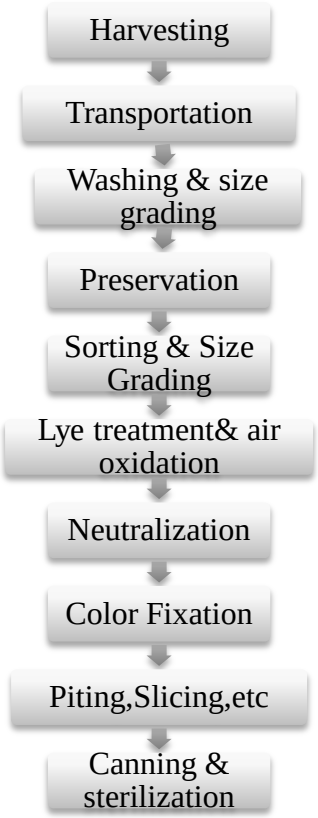

Figure 2.4. Flow scheme of ripe black olive elaboration process

(Source: Fernández et al., 1997)

In Spain, the procedure for storage of naturally ripe (black) olives is used commonly. Briefly, the olives are put into fermentation vessels in 4-6 % NaCl (w/v) brine. This concentration is increased progressively to 8-9 % salt which is maintained during the storage period (Fernández et al., 1997). However, this system causes serious damage to fruits such as shriveling and gas-pocket (‘alambrado’) formation which is produced by the accumulation of respiratory gases (CO_2) of the olives themselves (García et al.,1995).In addition to this, the activities of gram negative bacteria and yeast are responsible for this fermentation (Fernández et al., 1997).

To prevent both types of spoilage this method is modified. Calibrating the initial pH of brines to 3.8-4.0 by acetic acid can inhibit the growth of gram-negative rod and CO_2 accumulation are prevented by aeration in a similar trend to naturally black olives (García et al., 1995).

In the USA, combination of salt-free and acidulated water storage (lactic and acetic acid) in anaerobic conditions is used. This method was formed to diminish the problem of brine disposal. Sodium benzoate is also necessary for this process and calcium chloride is usually added to the liquid to improve the olive texture (Vaughn et al., 1969).

The industrial process of the production of ripe olives consists of successive treatments with dilute NaOH solution (lye). Between lye treatments the fruits are placed in water with bubbling of air. Throughout this operation, the olives get darker progressively due to the oxidation of ortho-diphenols, hydroxytyrosol (3,4 dihydroxyphenyl ethanol) and caffeic acid (Brenes et al., 1992).The number of lye treatments applied generally changes between 2 and 5. Penetration into the fruits must be controlled so that NaOH of the first treatment barely passes through the skin. Other treatments are applied so that they can penetrate to deeper part of the flesh and the final lye treatment must reach to the stone (Fernández et al., 1997). It is possible to make only one lye treatment but the concentration of NaOH in the lye solution should be between 1-4 %(w/v) and the concentration depends on the ripeness of the fruit, olive variety, preservation system, environmental temperature and the desired penetration speed. After each NaOH treatment, water is added to complete a 24 hour cycle. To reduce waste-water it is possible to reuse the storage liquid diluted with tap water (Brenes and de Castro, 1998).

After the last lye treatment, olives are washed several times with water to remove the most of NaOH and lower the pH in the flesh to around 8 (Fernández et al., 1997).

The black surface color obtained from lye treatment is not stable and fades progressively after oxidation and during the shelf life of the packed product. To prevent this deterioration, only the use of ferrous gluconate and ferrous lactate is legally permitted for ripe olive processing (García et al., 2006).Normally, ferrous salts were added at a concentration of 100 ppm (parts per million) of iron in the liquid. Iron diffusion into the flesh is complete in 10 hours, but normally, this phase is allowed to continue about 24 hours (García et al., 2006).

The black (ripe) olives (whole, pitted, slices, quarters or paste) are packed in cans or glass containers with a liquid that contains 2-4% of NaCl and 10-40 ppm of iron to prevent deterioration of their black color. Ripe olives could be packed in plastic pouches by addition of lactic o-gluconic acid, and applying pasteurization for preservation (García et al., 1999).

2.4. Composition of Olive and Olive Oil

The major components of olive chemical structure are fatty acids (e.g. oleic, linoleic, linolenic, stearic and palmitic acids) and phenolics (e.g. oleuropein, verbascoside, apigenin, hydroxytyrosol and phenolic acids).Sugars (e.g. glucose, fructose, mannitol, sucrose, mannose), organic acids (e.g. citric, succinic, acetic, lactic, malic acids), amino acids, minerals and vitamins constitute the minor components.

2.4.1. Phenolic Compounds and Their Importance

In recent years, there has been growing interest in phenolics of olives due to their antioxidant and antimicrobial activities, health and sensory properties. Phenolic compounds regulate the nutritional properties, sensory characteristics and the shelf life of olive oil. These compounds have an important effect on human health because of their anti-inflammatory, anti-allergic, antimicrobial, anti-carcinogenic, and antiviral activities (Yorulmaz et al., 2012).

Phenolic compounds are a large and a diverse group of molecules, which include many different families of aromatic secondary metabolites in plants. These phenolics are most abundant secondary metabolites in plants and can be classified into non-soluble compounds such as condensed tannins, lignin, cell wall bound hydroxycinnamic acids, and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids and quinines. Different phenolic classes that exist in plants and their chemical structures are provided in Table 2.3. All these groups are involved in various processes in plants and animals. One family, the flavonoids, is of special interest because of its multiple roles in plants and its impact on human health (Harborne and Williams, 2000).

Table 2.3.Phenolic classes in plants
(Source: Naczk and Shahidi, 2004)

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

Olive fruits, olive oil and derived products (table olive, olive paste) are good sources of some phenolics with important antioxidant activities. There are large numbers of phenolics in olive belonging to different classes such as phenolic acids, phenolic alcohols, flavonoids, lignans and hydroxy-isocromans. In addition to these, secoiridoids which are derivatives of oleuropein, demethyloleuropein and ligstroside are the major phenolics that can be detected (Table 2.4) (Yorulmaz et al., 2012).

Table.2.4. Amount of phenolics in table olives and derived products

(Source: East Mediterranean Olive Association, 2013)

Table olive and derivatives	Amount of phenolic compounds
Natural black olives	16.40 g/kg
Spanish style green olives	4.48 g/kg
Brine of black olives	0.93 g/L
Brine of green olives	1.36 g/L
Olive oil	0.1-0.8 mg/kg
Olive-mill waste water	2-10 g/kg

The previous studies about olives show that main phenolics are tyrosol, oleuropein, p-coumaric acid, verbascoside, luteolin 7-*O*-glucoside, rutin, trans-cinnamic acid, luteolin, apigenin,cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside (Ryan et al., 1999).Some phenolics always exist in all olive varieties like oleuropein, but some of them can be detected in some varieties like demethyloleuropein and verbascoside. Oleuropein and trans-cinnamic acid are existed in higher amounts in all olive types (Yorulmaz et al., 2012).

Oleuropein is a heterosidic ester of β-glycosylated elenolic acid and hydroxytyrosol. It is the major secoiridoids of olive fruit and responsible for the bitter taste of olives. Different forms of oleuropein exist in olives such as demethyloleuropein, ligstroside and oleuropein aglycone (Yorulmaz, et al., 2010).

Verbascoside, a hydroxycinnamic acid derivative, and its isomeric forms are also the important phenolics of olive fruit. The (3, 4-dihydroxyphenyl) ethanol (3, 4-DHPEA) and (*p*-hydroxyphenyl) ethanol (*p*-HPEA) are predominant phenyl alcohols and both of them exist in olive oil and olive fruit (Yorulmaz et al., 2012).

Phenolic acids with the basic chemical structure C_6-C_1 (benzoic acid) and C_6-C_3 (cinnamic acid) was detected in polar phenol fraction of olives. Caffeic, vanillic, syringic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic, and *p*-hydroxybenzoic are the groups of phenolics which are found in very small amounts (Yorulmaz et al., 2010).

Flavonol glycosides such as luteolin-7-*O*-glucoside, rutin, apigenin-7-glucoside and anthocyanins such as cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside are the most abundant flavonoids in olives (Yorulmaz et al., 2012).

Table 2.5.Major classes of phenolics in olive

Major classes of phenolic compounds in olive
Phenolic acids and derivatives
Vanilic acid
o-coumaric acid
p-coumaric acid
Caffeic acid
Ferulic acid
p-hydroxybenzoic acid
Protocatechuic acid
Cinnamic acid
Benzoic acid
Verbascoside
Phenyl ethyl alcohols
Hydroxytyrosol
Tyrosol
(3,4-Dihydroxyphenyl)ethanol-glycoside
Secoiridoids
3,4-DHPEA (3,4-DHPEA-EDA)
p-HPEA-EDA
(3,4-DHPEA-EA)
Ligstroside aglycone
Oleuropein
p-HPEA-derivative
Oleuropein aglycone
Ligstroside aglycone
Flavones
Apigenin
Luteolin
Rutin
Flavonol
Quercetin

2.4.2. Fatty Acid Content of Olive Oil

One of the major components of olive is fatty acids. The fatty acid composition of olive oil varies widely depending on the cultivar, maturity of the fruit, altitude, climate, and several other factors. The major fatty acids in olive oil are:

- Oleic acid (C18:1), a monounsaturated omega-9 fatty acid. It makes up 55 to 83% of olive oil.
- Linoleic acid (C18:2), a polyunsaturated omega-6 fatty acid that makes up about 3.5 to 21% of olive oil.
- Palmitic acid (C16:0), a saturated fatty acid that makes up 7.5 to 20% of olive oil.
- Stearic acid (C18:0), a saturated fatty acid that makes up 0.5 to 5% of olive oil.
- Linolenic acid (C18:3), a polyunsaturated omega-3 fatty acid that makes up 0 to 1.5% of olive oil.

Olive oil contains more oleic acid and less linoleic and linolenic acids than other vegetable oils, that is, more monounsaturated than polyunsaturated fatty acids. This makes olive oil more resistant to oxidation. Greater the number of double bonds in the fatty acids they are more unstable and easily broken down by heat, light, and other factors. It is generally accepted that cooler areas will yield oil with higher oleic acid than warmer climates. That means a cool region' olive oil may have more monounsaturated fatty acid content than warmer region oil (IOOC, 2006).

Fatty acid composition is important for the commercial properties of oils. It has an influence on the stability of oils due to the contribution of polyunsaturated fatty acids to oil rancidity. In addition to this, several studies have shown that a diet rich in monounsaturated fatty acids may result in a wide range of health benefits such as an improvement in cholesterol levels, and, in turn, prevention of cardiovascular disorders (Gillingham et al., 2011).

2.4.3. Sugar and Organic Acid Content of Olive

Sugars are one of the minor components of olives. Although they exist in very low concentration, they play an important role in both maturation and processing of table olives. Particularly, sugars provide energy for metabolic changes during

maturation and contribute to olive fruit texture. In addition to this, they are really good carbon source which is necessary for the fermentation of table olives (Menz and Vriesekoop, 2010).

Acetyl Co-A is a molecule that is needed for fatty acid synthesis in the seeds and carbohydrates serve as a source for this compound. (Wodner et al., 1988) Glucose, fructose and mannitol were found to be the predominant sugars in the olive fruit (Patumi et al., 1989).In a study about the oil and sugar content during the development and maturation of the fruit in different cultivars, it was observed that if the cultivar has the least amount of oil, there is a parallel rise in oil and sugar levels at the beginning of the fruit ripening period. However, if the sugar level decreases in other cultivars, they have higher oil content (Wodner et al., 1988).

Major soluble sugars in olive fruit are glucose, fructose, sucrose, xylose rhamnose and mannitol (López et al., 2007) . In Thassos olives, glucose and mannitol were detected as the main sugar and sugar alcohol, respectively and their concentration levels were very close to each other (Marsilio et al., 2001).In sweet Dhokar olives, glucose and mannitol reached their highest level at the last stage of ripening and this concentration are really higher compared to regular Chemlali olives (Jemai et al., 2009).

Organic acids are the compounds that are naturally found in vegetables and fruits. They can be formed during processes like fermentation or can be added into food during the production process. Organic acids are another minor component of olive fruit and their amount is approximately 1.5% of the flesh part. Organic acids which are produced during the formation and degradation of the other components like carbohydrates in olive play an important role in metabolic activity (Cunha et al., 2001).

According to a study about Turkish olives, succinic, malic and citric acids are found as major organic acids in Memecik and Domat varieties (Ergönül and Nergis, 2010). Organic acids not only affect the color of the fruit but also have an important role in olive processing due to their buffering capacity. It has also known that organic acids influence the stability, quality and aroma of the olive fruit (Joslyn, 1970).

2.5. Changes in Olive during Maturation Period

During maturation period, a number of physical and chemical changes occur in olive fruit. Many of these changes have important roles in the production of both table olives and olive oil. These changes can be influenced by various factors such as the cultivar, fruit ripeness, irrigation regimes, and environmental factors (geographical area, soil quality, type of cultivation, rainfall, etc.). Changes in the composition, in turn, affect the quality, sensorial, and nutritional properties of the fruit and the oil (Yorulmaz et al., 2010).

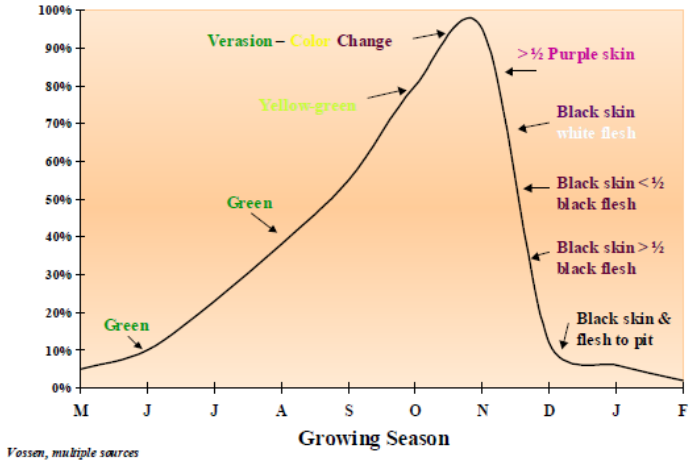

Figure 2.5.Fruit polyphenol level and color during maturation period (Source: Vossen, 1998)

The phenolic profile is considerably influenced by the type of cultivar, maturity stage, climatic conditions, (Amiot et al., 1986) and irrigation management (Tovar et al., 2001). Cultivar is the most important factor affecting the phenolic profile of the olive fruit (Amiot et al., 1986). Maturation degree affects phenolic contents of the olive fruit and olive oils as well. Figure 2.5 shows the relation between phenol content of olives and maturation. A negative correlation between oleuropein concentration, hydrophilic phenol amounts of oils and maturity stage of olive is reported (Garcia et al., 1996). Climatic conditions (especially temperature and rainfall) influence the physiology of the olive. Particularly, cumulative rainfall and temperature changes during maturation might have a correlation with phenolic distribution of olive and olive oil (Romero et al., 2003).

According to another study performed with Turkish olives, oleuropein concentration decreases whereas hydroxytyrosol and demethyloleuropein increase throughout maturation. In addition to this, while trans-cinnamic acid content of olive fruits decreases, cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside concentrations increase (Yorulmaz et al., 2010).

The concentration of phenolic compounds in olive and olive oil is related to the activity of several endogenous enzymes present in olives such as β-glucosidase, polyphenoloxidases(PPO), peroxidases (POD) and lipoxygenase (LPO)(Yorulmaz et al., 2010).

Maturation process is divided into three phases. First one is the growth phase in which accumulation of oleuropein occurs. Second phase is a green maturation phase that coincides with a reduction in the levels of chlorophyll and oleuropein (Charoenprasert and Mitchell, 2012). Extensive lipid synthesis is observed in this phase. Rapid reduction of oleuropein and accumulation of verbascoside is related to the second phase (Amiot et al., 1986). The last phase is a black maturation phase that is characterized by the appearance of anthocyanins and flavonoids and during which the oleuropein levels continue to fall (Charoenprasert and Mitchell, 2012). Oleuropein is the most abundant phenolic in the early stages of fruit development even in young fruits it can reach to 14% of dw. Earlier studies showed that hydroxytyrosol increased as the fruit matured; however, recent studies do not provide this finding (Mitchell, 2012). Levels of oleuropein decrease in olive pulp during maturation and the glucoside forms of flavonoids, luteolin-7-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside and quercetin-3-rutinoside are more abundant in the pulp of the mature olive fruit (Charoenprasert and Mitchell, 2012).

In a study about Gordal Sevillana olives during the ripening period, the olive size, flesh/pit ratio, and oil content all increased, while the moisture and total sugar contents decreased (Menz and Vriesekoop, 2010). In addition, total phenolics initially decreased and then gradually increased during maturation. Furthermore, at the optimal maturity level maximum sugar content was detected. The olives in the onset of the turning color phase resulted in a decrease in sugars in the fruit, which minimized the available substrate for the subsequent fermentation of the green table olives (Menz and Vriesekoop, 2010).

Organic acids show metabolic activity and are intermediate products resulting from formation and degradation of other compounds (Cunha et al., 2001). The

maturation stage and geographical origin have an effect on the amounts and types of organic acids in different kinds of fruits (Cámara et al., 1994). The concentrations of organic acids in plants decrease with respect to ripening due to their usage as respiratory substrates or their conversion to sugars (Islam et al., 1996).In addition to this, the amount and structure of the chemical components of olive fruit show considerable changes during growth and ripening (Boskou, 1996).

Oil synthesis and accumulation in the olive fruit begins about 10thweek and continue to 34th week of season (Vossen, 1998).The oil content varies with cultivar and ripening degree, ranging from 3 to 38% on a fresh weight basis (Charoenprasert and Mitchell, 2012). Oil synthesis increases rapidly up until fruit reaches maximum maturity (color change and softening), then the rate of accumulation decreases, but still continues. It seems like that there is a much larger increase in the beginning of maturation than the late phases of ripening due to the loss of moisture in the fruit (Figure 2.6). When the fruit gets very over-ripe, oil synthesis stops completely (Vossen, 1998).

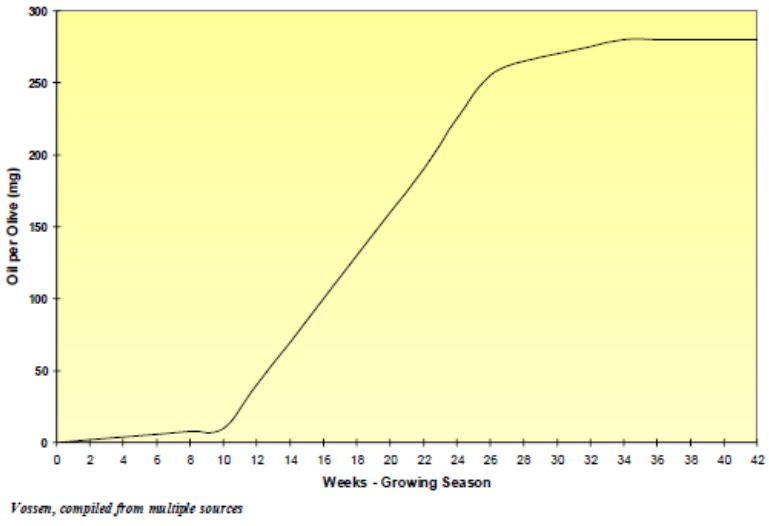

Figure 2.6. General oil accumulation during maturation (Mid May to end of February) (Source: Vossen, 1988)

2.6. Hurma Olive

Olive type, mostly Erkence, grown in nearby area around Karaburun peninsula of Izmir-Turkey, goes through a natural debittering phase on the tree during its ripening. This naturally debittered olive is known by the name of Hurma. At the end of this process, olive loses its bitter taste while still on the tree and has a dark brownish color in the inside and a wrinkled outer layer which is its differentiating appearance characteristics from olives not going through this process. Therefore, this type of olive does not require further processing steps for debittering.

It was stated that a fungus called *Phoma olea* is the reason for this phenomena with the help of climactic conditions (Buzcu, 1969).

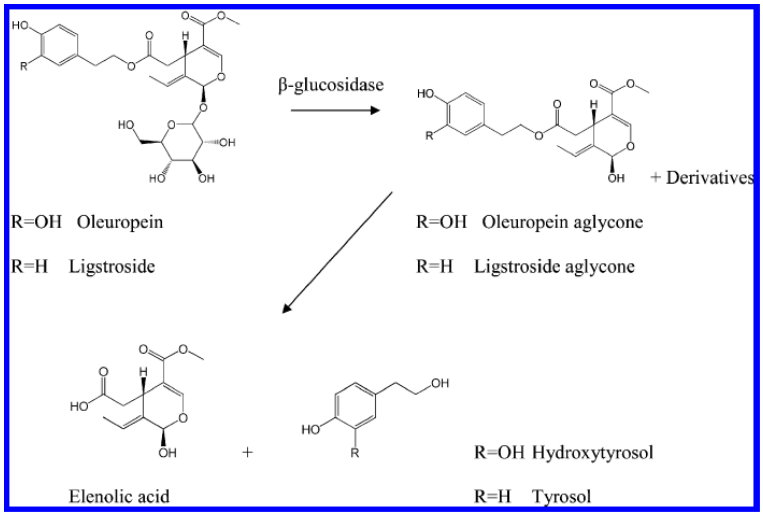

Figure 2.7.Hydrolysis of oleuropein and ligstroside
(Source: Charoenprasert and Mitchell, 2012)

According to several studies in literature, similar types of olives were also reported in countries like Greece and Tunisia. The studies about sweet Thasos olive which is grown in Thasos island of Greece show that oleuropein is responsible for bitter taste. It is hydrolyzed to hydroxytyrosol and its derivatives by an enzyme, *β*-glucosidase (Figure 2.7), which is produced by fungi and bacteria during ripening (Zoidou et al., 2009).Same trend was also observed in a study about Dhokar olives, which are cultivated in southern region of Tunisia (Jemai et al., 2009).Therefore, oleuropein

concentration decreases while hydroxytyrosol concentration increases during maturation. In addition , total phenol content and reducing sugar concentration increase relatively (Jemai et al., 2009).

In Thassos olives, glucose and mannitol were detected as the main sugar and sugar alcohol, respectively and their concentration levels were very close to each other (Marsilio et al., 2001).In sweet Dhokar olives, glucose and mannitol reached their highest level at the last stage of ripening and these concentrations were really higher compared to regular Chemlali olives (Jemai et al., 2009).

CHAPTER 3

EXPERIMENTAL STUDY

3.1. Materials

3.1.1. Olive Samples

Three different types of olives were used in the analysis. These types are Gemlik, Erkence and Hurma olives (Table 3.1).Actually, Hurma olive is not a variety, it is naturally debittered form of Erkence type. Hurma and Erkence olives were hand-picked from an olive orchard (latitude: 38°54'07"N, longitude: 26°57'24"E) which is located in Karaburun peninsula (Figure 3.1) of Izmir while Gemlik type was obtained from another orchard located in Izmir Institute of Technology campus area (latitude: 38°19'30.84"N, longitude 26°37'48.87"E) which is 30 km south of the first orchard (Figure 3.2).

Figure 3.1. Karaburun peninsula map
(Source: Encyclopedia Wikipedia, 2013)

Figure 3.2.IZTECH campus area map
(Source: Encyclopedia Wikipedia, 2013)

For the two harvest years 2011 (1st) and 2012 (2nd), all olives were picked up during eight weeks of maturation period from the end of October to the beginning of December. Every week approximately half a kilogram of olives were picked up from the all sides of three trees for each type.

After harvesting stones of olives were separated from the fruit immediately. For the storage, olives were first immersed into liquid nitrogen, then dried with a freeze-dryer (Labconco, The United States). All analyses were completed within a couple of months after harvesting.

Table 3.1.Codes of olive varieties

Sample Name	Sample Code
Hurma (debittered Erkence)	H
Erkence	E
Gemlik	G

*first number after olive type represents the harvest time (1st week, second week etc.) and second number shows the harvest years

3.1.2. Chemical Agents

Reagents used in chemical analysis were obtained from Riedel-de Haën (Germany) and Sigma-Aldrich (Germany) and they are either HPLC or analytical grade. In chromatographic analysis, 37 component fatty acid methyl esters (FAME) mixture containing C4-C24 (2-4% relative concentration) was used as a reference standard (Supelco # 47885-U).

Following abbreviations are used in the text: ††MI: maturity index, †††TPC: total phenol content (averages of 3 measurements), OLE: oleuropein, HYT: Hydroxytyrosol, TY: tyrosol, API: apigenin, VER: verbascoside, RTN:rutin, L-7-Glu:luteolin-7-glucoside, LTLN:luteolin, QUE:quercetin-3-glucoside, o-cou:o-coumaric acid, p-cou:p-coumaric acid, FA: ferulic acid, VA: vanillic acid, CA: caffeic acid, VN: vanillin. Concentrations are the averages of 2 measurements.

3.2. Methods

3.2.1. Routine Analysis

Before lyophilization process, maturity index of olives was determined and pH and a_w measurements were performed with fresh olive pulp. Procedure described by Morello et al. (2005) was used for maturity index determination. In order to calculate maturity index, 100 olives were selected randomly, classified into seven groups according to their color (green, black, reddish brown etc.) and olives in each group were counted. Black olives were cut up to examine the percentage of olive flesh turning to black or purple. Counted olive samples were multiplied with different coefficient numbers for each class and following formula was used to determine the maturity index.

MI=Maturity index N=total number of olives

$$MI = \sum_{i}^{7} \frac{coefficient number of group \times number of olives}{N}$$

For pH measurement, approximately 5 gram fresh olive flesh was kneaded in a blender with the addition of approximately 5 mL pure water. pH of the samples is measured by a pH meter (WTW 720 Series, Germany).

For water activity, same mixture was prepared without adding pure water and a_w was measured by a water activity measurement device (Hygrolab-3, Rotronic Instruments, The United States).All measurements were repeated three times.

3.2.2. Total Phenol Content

The concentrations of total phenolic compounds of olives were determined with the Folin–Ciocalteu assay (Bouaziz et al., 2004).0.3 gram of lyophilized olive pulp was weighed and extracted with 5 mL methanol five times. Methanol in the extract was evaporated at 45 ^{0}C with a rotary evaporator (Laborato 4000 Heidolph, Germany) in 20 minutes. Remaining extract was dissolved in 5 mL methanol again.100 μL of this extract was taken into a glass tube and 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water) was added. Then, 2 mL of Na_2CO_3 (75 g/L) was added within time interval from 0.5 to 8 min. The sample was incubated at 40 ^{0}C for 15 min and then cooled to room temperature. The absorbance of the sample was measured at 765 nm in a UV spectrophotometer (PG Instrument, England) and distilled water was used as the blank. Same procedure was applied to gallic acid standard solutions at different concentrations (100-1000 ppm) and a standard curve was plotted (Appendix). Total phenol content of the extracts was calculated using the standard curve and the results were expressed in milligram of gallic acid per 100 g of dry matter (mg GA/100 g dw). Each sample was analyzed three times.

3.2.3. Phenolic Compound Profile

3.2.3.1. Sample Preparation

Extraction of phenolics and HPLC analysis were performed according to a method in literature (Bouaziz et al., 2004). First 1 gram lyophilized olive pulp was extracted with 15 mL EtOH: water (80:20) twice. 1 mL internal standard (150 ppm 4-

hydroxyl acetic acid) was added and the mixture was mixed with a homogenizer (Heidolph, Germany) at 15,000 rpm for 5 minutes. Then, the mixture was filtered through a vacuum filtration unit with 125 mm pore size filter paper. Filtered extract was taken to a rotary evaporator (Laborato 4000 Heidolph; Germany), and ethanol was evaporated at 40 ^{0}C for 20 minutes under vacuum, then traces of ethanol were removed by using a nitrogen flow. Extract was washed with 30 mL hexane twice in order to remove the oil. Hexane, oil and extract separated from each other by the help of separation funnel. Traces of hexane were removed by using nitrogen flow. The extract was completed to 25 mL with water: MeOH (70:30) and filtered by 0.45μm syringe filter into vials. Finally, it was injected to the high pressure liquid chromatography (HPLC) equipment (Agilent 1200). Each sample was analyzed twice with HPLC.

3.2.3.2. Analytical Conditions

Chromatographic analyses were performed with an Agilent 1200 HPLC (The United States), equipped with refractive index (RI) detector and photodiode array detectors (DAD), a column oven, and an auto sampler. The instrumental configuration and analytical conditions are presented in Table 3.2 and mobile phase program is provided in Table 3.3. Some sample calibration curves for phenolic standards are provided in Appendix.

Table 3.2. Chromatographic conditions for the analysis of phenolic compounds of olive samples

Instrumentation	
Chromatographic system	Agilent 1200
Detector	DAD
Automatic sampler	ALS G1329A
Column	SGE 8211 C18 (250*4mm,5μm)
Experimental conditions of HPLC-DAD	
Injection Temperature	30 ^{0}C
Injection volume	20 μL
Flow Rate	1 mL/min
Mobile Phase A	water: acetic acid (99.9:0.1)
Mobile Phase B	ACN: MeOH: acetic acid (50:50:0.1)
Wavelength	280 nm

Table 3.3. Chromatographic conditions for the analysis of phenolic compounds of olive samples.

Time	% Mobile Phase A	% Mobile Phase B
0	95	5
45	45	55
55	0	100
60	0	100
65	95	5
70	95	5

Phenolic compounds used as standards in the analysis were oleuropein, oleuropein aglycone, tyrosol, rutin, hydroxytyrosol, quercetin-3-glucoside, quercetin, luteolin, luteolin-7-glucoside, verbascoside, vanillin, vanilic acid, ferulic acid, o-coumaric acid, p-coumaric acid, syringic acid, caffeic acid, apigenin and apigenin-7-glucoside.

3.2.4. Sugar-Organic Acid Analysis

3.2.4.1. Sample Preparation

Sugar and organic acid analyses of the olive samples were performed according to a procedure in the literature (López et al., 2007). Same extracts were used for sugar and organic acid analyses.5 gram lyophilized olive was weighed.5 mL of 1000 ppm sorbitol solution was added to the sample as internal standard and the solution was completed to 50 mL by adding ultra-pure water at 60 ^{0}C. The mixture was mixed for 30 minutes. Then, it was centrifuged at 9000 rpm (Sigma-2-16KC Centrifuge, The United Kingdom) for 15 minutes. Supernatant was collected and filtered by 0.45 µ syringe filter into vials and injected into HPLC. Each sample was analyzed twice. Sample calibration curves for sugar standards are given in Appendix.

3.2.4.2. Analytical Conditions

Chromatographic analyses were performed with an HPLC (Agilent 1200), equipped with a detector, a column oven and an auto-sampler. The instrumental configuration and analytical conditions were presented in Table 3.4:

Table 3.4.Chromatographic conditions for the analysis of sugar content of olive samples

Instrumentation	
Chromatographic System	Agilent 1200
Detector	RID
Automatic Sampler	ALS G1329A
Column	Biorad HPX-87C (300*7.8 mm ID, 9mm)
Experimental conditions of HPLC-RID	
Injection Temperature	65 ^{0}C
Injection volume	50 µL
Flow Rate	0.7 mL/min
Mobile Phase	0.05M H_2SO_4

Sugar standards used in the analysis were glucose, fructose, lactose, mannose, mannitol and sucrose.

For organic acid analysis same extracts were used. However, external standard settings were done in order not to use sorbitol as internal standard. Sample calibration standards of organic acids are shown in Appendix.

Table 3.5. Chromatographic method for the analysis organic acid content

Instrumentation	
Chromatographic System	Agilent 1200
Detector	RID
Automatic Sampler	ALS G1329A
Column	Biorad HPX-87H (300*7.8 mm ID, 9mm)
Experimental conditions of HPLC-RID	
Injection Temperature	65 ^{0}C
Injection volume	50 µL
Flow Rate	0.7 mL/min
Mobile Phase	0.05M H_2SO_4

Organic acid standards used in this analysis were lactic, acetic, malic, citric and succinic acids.

3.2.5. Fatty Acid Profile

3.2.5.1. Sample Preparation

For this analysis, oil extraction was done firstly. For this purpose, 5 gram lyophilized olive was extracted with n-hexane at 180 ^{0}C by an automatic Soxhelet extraction unit (Gerhard Multistat, Germany).

In order to determine the fatty acid profile, fatty acid methyl esters were formed. European Official Methods of Analysis (EEC, 1991) was performed to prepare methyl esters. 100 mg oil was weighed into 25 mL centrifuge tubes. The samples were dissolved in 10 mL n-hexane and saponified to their methyl esters with the addition of methanolic potassium hydroxide solution (11.2 g in 100 mL methanol). The sample solution was vortexed for 30 s and centrifuged at 5000 rpm for 15 min. Supernatant was collected and filtered by 0.45 μ syringe filter into vials and injected to a gas chromatography (GC) equipment. Each sample was analyzed at least two times with GC.

3.2.5.2. Analytical Conditions

Chromatographic analyses were performed with an Agilent 6890 GC equipped with Agilent 7683 auto-sampler and FID detector. The instrumental configuration and analytical conditions were presented in Table 3.6:

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

Instrumentation	
Chromatographic system	Agilent 6890 GC
Inlet	Split/splitless
Detector	FID
Automatic Sampler	Agilent 7683
Column	100 m x 0.25 mm ID, 0.2 μm HP-88 (J&W112-88A7)
Liner	Split liner (p/n 5183-4647)
Experimental Conditions of GC-FID	
Inlet temperature	250 ^{0}C
Injection volume	1μ
Split ratio	1/50
Carrier Gases	Helium
Head pressure	2 mL/min constant flow
Oven temperature	175 ^{0}C, 10 min, 3^{0}C/min, 220^{0}C, 5 min
Detector temperature	280 ^{0}C
Detector Gases	Hydrogen:40 mL/min;Air:450 mL/min; Helium make-up gas: 30 mL/min

3.2.6. Statistical Analyses

3.2.6.1 .Principal Component Analysis

Use of principal component analysis (PCA) is preferred when measurements obtained on a number of observed variables and if it is desired to develop a smaller number of artificial variables (called principal components) that will account for the most of the variance in the observed variables. The principal components (PCs) may then be used as predictor or criteria on variables in subsequent analyses (Cattell,1966).

PCA can be defined as a linear combination of optimally-weighted observed variables. To be able to show the meaning of this definition, firstly it is necessary to describe how subject scores on a PC are computed. PCA is a powerful tool for reducing a number of observed variables into a smaller number of artificial variables that account for most of the variance in the data set. This statistical analysis technique is particularly useful when a data reduction is needed (Stevens, 2012).

It is often desirable to reduce the dimensionality of large multivariate data sets. PCA is a good technique to achieve this goal. It replaces the original variables by smaller number of derived variables, the PCs, which are linear combinations of the original variables. Often, it is possible to retain most of the variability in the original variables with a smaller number of PCs. Due to its apparent simplicity, PCA has a number of distinctions, and it has many uses and extensions (Jolliffe, 2002).

In this study, the multivariate data matrix consists of observations represented by samples from three different olive types for two harvest years and variables represented by the results of chemical measurements. The same analysis was also performed for each harvest year by separating the data into two to observe the differences between olive types more clearly. Data were auto scaled before multivariate analysis. The multivariate analyses were performed by SIMCA-P v.11.5 (Umetrics, Umea, Sweden). Results of PCA were visualized by scores and loading plots. Scores plots were constructed to observe principal groupings among observations. Loadings indicate the importances of each variable for the model so loading plots were used to interpret the relations among variables and clusters observed in the score plots.

3.2.6.2. ANOVA

Analysis of variance (ANOVA) is a statistical method to analyze the differences between group means and their associated procedures.

In this study, ANOVA was used in order to determine the effect of harvest year, harvest time and variety on pH, a_w and total phenol content with triple replicates. General factorial design was applied by Minitab 16 (USA).

Table 3.7.Factors and their levels for ANOVA

Factors	**Levels of Factors**
Olive Type	Hurma Erkence Gemlik
Harvest year	2011/12 season 2012/13 season
Harvest time(weeks)	1 2 3 4 5 6 7 8

CHAPTER 4

RESULTS & DISCUSSIONS

4.1. Maturity Index, pH and aw of Olive Varieties

Results of maturity index, pH and aw values for Hurma, Erkence and Gemlik types of olives during eight weeks of sampling for 2011/12 and 2012/13 harvest years are provided in Table 4.1. Both in 2011/12 and 2012/13 harvest years olive samples were obtained from the same trees in the same locations from the end of October till the beginning of December. This time interval corresponds to the season when the appearance of Hurma olives allowed us to differentiate them from regular Erkence olives which did not go through debittering. Maturity index of Hurma ranged from 3.14-5.34 in the first season while it was between 4.56 and 6.37 in the next year. Erkence had a range of maturity index of 0.5-3.67 in 2011/12 while the range was 2.16-5.94 in 2012/13. Maturity index of Gemlik varied between 1.1-5.88 in the first harvest year and 2.19-4.16 in the second year.

Maturity index shows the ripening degree of the fruit and overall, maturity index increased during the sampling weeks. Olive samples were collected from the all sides of the same designated trees throughout the sampling period; however, not all olives ripen at the same time. Depending on the position of the trees some sides ripen earlier than the other. Therefore, maturity index had its ups and downs in some weeks.

Figure 4.1 shows pH values for olive samples throughout sampling period for two harvest years. pH values of Hurma olives changed between 5.06-5.5 in 2011/12 and 5.6-5.67 in 2012/13 harvest years, respectively. Erkence variety has pH values of 5.12-5.39 in the first harvest year and 5.08-5.46 in the second harvest year. pH range of Gemlik cultivar varied between 5.05-5.29 in 2011/12 harvest year and 5.19-5.44 in 2012/13 harvest year.

In general, pH values are higher in 2011/12 harvest year than 2012/13 harvest year for all olive types. This increase can be associated with total organic acid amount which was in higher concentrations in the second harvest year.

Figure 4.1 shows a_w values for olive samples throughout sampling period for two harvest years. a_w values of Hurma olives changed between 0.94-0.98 in 2011/12 and 0.92-0.94 in 2012/13 harvest years, respectively. Erkence variety has 0.92-0.97 a_w values in the first harvest year and 0.94-0.96 in the second harvest year. a_w range of Gemlik cultivar varied between 0.92-0.97 in 2011/12 harvest year and 0.94-0.96 in 2012/13 harvest year.

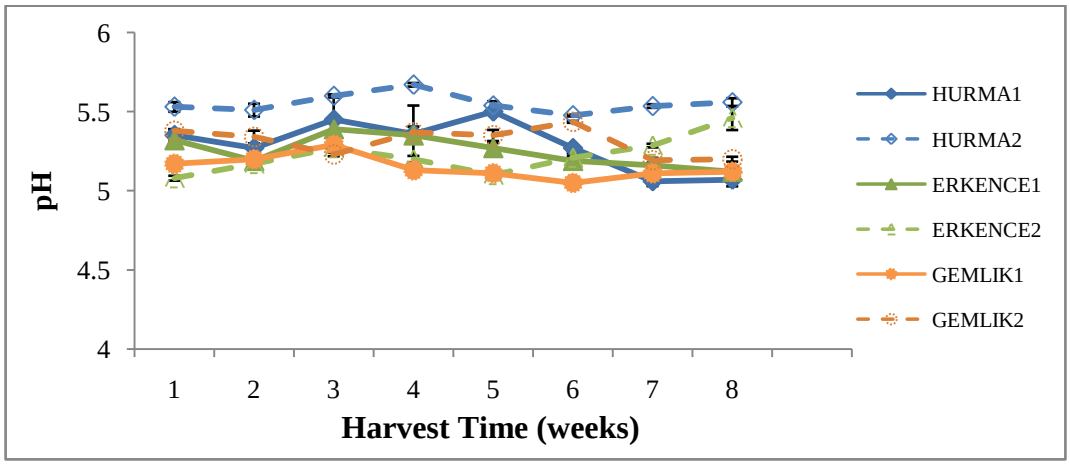

Figure 4.1.pH values of Hurma, Erkence and Gemlik types of olives during eight weeks of maturation for two harvest years (Numbers after olive types refer to first and second harvest year)

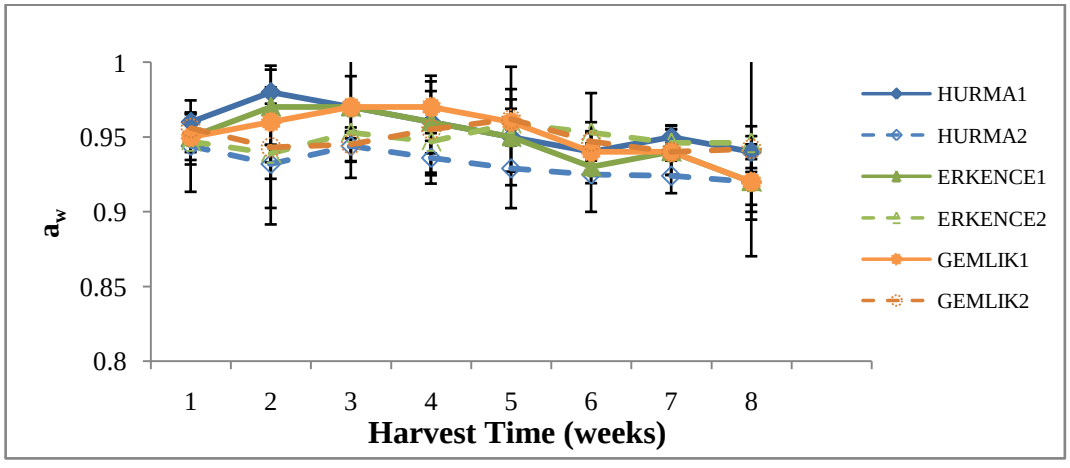

Figure 4.2.$a_w$values of Hurma, Erkence and Gemlik types of olives during eight weeks of maturation for two harvest years (Numbers after olive types refer to first and second harvest year)

Table 4.1.pH, a_w and maturity index (MI) values for Hurma, Erkence and Gemlik olives for two harvest year

Olive type	pH	a_w	MI		pH	a_w	MI
2011/12				**2012/13**			
H11	5.35±0.03	0.96±0.006	3.9	**H21**	5.53±0.03	0.94±0.014	5.43
H12	5.27±0.01	0.98±0.001	4.1	**H22**	5.51±0.04	0.93±0.003	4.56
H13	5.45±0.15	0.97±0.004	5.34	**H23**	5.6±0.01	0.94±0.003	5.92
H14	5.36±0.17	0.96±0.002	4.95	**H24**	5.67±0.01	0.94±0.002	5.6
H15	5.50±0.01	0.95±0.005	3.74	**H25**	5.54±0.02	0.93±0.01	6.18
H16	5.27±0.02	0.94±0.014	4.37	**H26**	5.48±0.005	0.93±0.006	5.42
H17	5.06±0.03	0.95±0.007	3.99	**H27**	5.54±0.011	0.92±0.012	6.37
H18	5.07±0.04	0.94±0.01	3.94	**H28**	5.56±0.02	0.92±0.004	6
E11	5.32±0.01	0.95±0.004	0.5	**E21**	5.08±0.01	0.95±0.002	2.53
E12	5.19±0.02	0.97±0.004	1.27	**E22**	5.17±0.005	0.94±0.006	2.16
E13	5.39±0.04	0.97±0.003	2.25	**E23**	5.27±0	0.95±0.017	2.34
E14	5.35±0.02	0.96±0.008	1.65	**E24**	5.2±0.02	0.95±0.004	2.53
E15	5.27±0.03	0.95±0.009	1.69	**E25**	5.1±0.01	0.96±0.011	3.17
E16	5.19±0.03	0.93±0.007	3.65	**E26**	5.21±0.02	0.95±0.016	3.18
E17	5.16±0.005	0.94±0.01	3.56	**E27**	5.29±0.01	0.95±0.012	3.49
E18	5.12±0.002	0.92±0.07	3.67	**E28**	5.46±0.07	0.95±0.011	5.94
G12	5.2±0.03	0.96±0.002	1.1	**G21**	5.38±0.01	0.96±0.002	2.42
G13	5.29±0.02	0.97±0.003	1.38	**G22**	5.34±0.04	0.94±0.003	2.19
G14	5.13±0.01	0.97±0.011	2.41	**G23**	5.23±0.01	0.95±0.008	3.32
G15	5.11±0.01	0.96±0.003	2.1	**G24**	5.37±0.03	0.96±0.016	3.09
G16	5.05±0.005	0.94±0.007	3.65	**G25**	5.35±0.03	0.96±0.005	3.92
G17	5.11±0.01	0.94±0.016	4.82	**G26**	5.44±0.005	0.95±0.004	4.6
G18	5.12±0.01	0.92±0.006	5.88	**G27**	5.19±0.015	0.94±0.01	4.26
				G28	5.2±0.015	0.94±0.006	3.84

*first number after olive type represents the harvest time (1st week, second week etc.) and second number shows the harvest years

In order to investigate the effect of olive variety, harvest time and year on pH and a_w statistically General Factorial Design by ANOVA was used. Factors are harvest year (2011/12 and 2012/13), variety (Hurma, Erkence, Gemlik) and time (8 weeks of maturation period) and responses are pH and a_w with triple replicates.

Table 4.2. ANOVA table for pH

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Variety	2	1.105276	1.105276	0.552683	304.55	0.00
Harvest Year	1	0.592900	0.592900	0.592900	326.74	0.00
Time	7	0.286100	0.286100	0.040871	22.52	0.00
Variety*harvest year	2	0.545113	0.545113	0.272556	150.20	0.00
Variety*time	14	0.275512	0.275512	0.019679	10.85	0.00
Harvest year*time	7	0.356889	0.019679	0.050984	28.10	0.00
Variety*harvest year*time	14	0.479165	0.050984	0.034226	18.86	0.00
Error	96	0.174200	0.034226	0.001815		
Total	143	3.815156	0.001815			

According to ANOVA (Table 4.2), all factors and their interactions are significant for the pH model because p values are less than 10^{-3} and model R^2 and adjusted R^2 values are 95.43% and 93.20%, respectively. It means pH values of olives fruit change significantly with respect to year, variety and time. Normal probability and residual plots of the model are provided in Figure 4.3 and Figure 4.4, respectively. These plots show that there is no problem regarding the normality and there are no outliers among the data. Since the model and all parameters are significant it can be concluded that olive variety, harvest time and season as well as their interactions influence the pH.

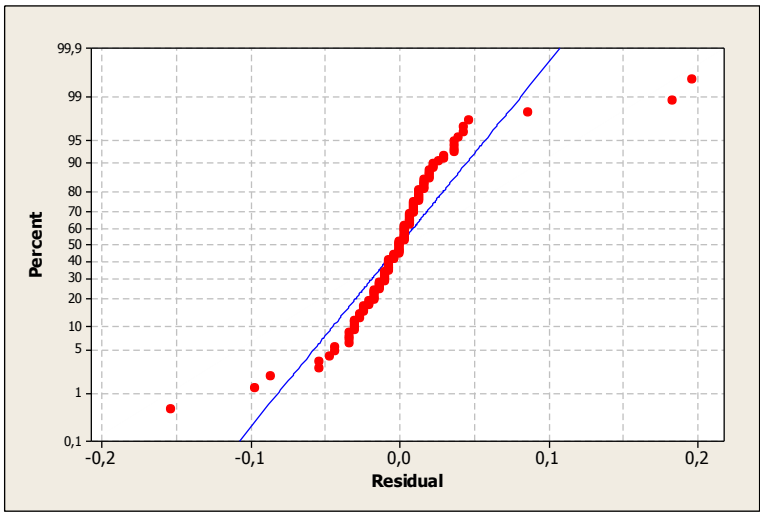

Figure 4.3. Normal probability plot of pH

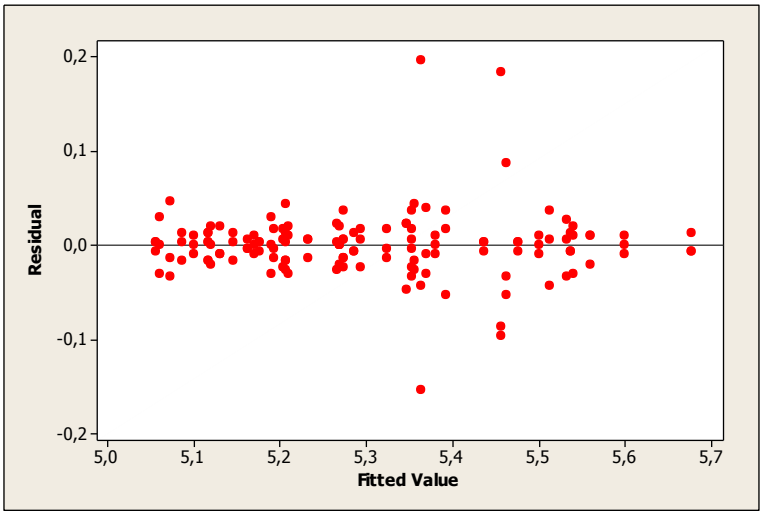

Figure 4.4.Residual plot of pH

Table 4.3 ANOVA table of aw

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Variety	2	0.0016822	0.0016822	0.0008411	11.57	0.00
Harvest Year	1	0.0053900	0.0053900	0.0053900	74.14	0.00
Time	7	0.0101355	0,0101355	0.0014479	19.92	0.00
Variety*harvest year	2	0.0037776	0,0037776	0.0018888	25.98	0.00
Variety*time	14	0.0020619	0.0020619	0.0001473	2.03	0.00
Harvest year*time	7	0.0059718	0.0059718	0.0008531	11.73	0.00
Variety*harvest year*time	14	0.0015507	0.0015507	0.0001108	1.52	0.117
Error	96	0.0069793	0.0069793	0.0000727		
total	143	0.037492				

ANOVA results for test the effect of olive variety, harvest time and season on a_w values are listed in Table 4.3 and normal probability and Residual plots are shown in Figure 4.5-4.6. As it can be seen from the ANOVA table (Table 4.3) all factors and their interactions are significant except variety*harvest year*time interaction since p value is greater than 0.05, R^2 value is 91.70% and R^2 (adj) equals to 87.63%. Therefore, a_w is also affected by the olive variety, harvest time and year.

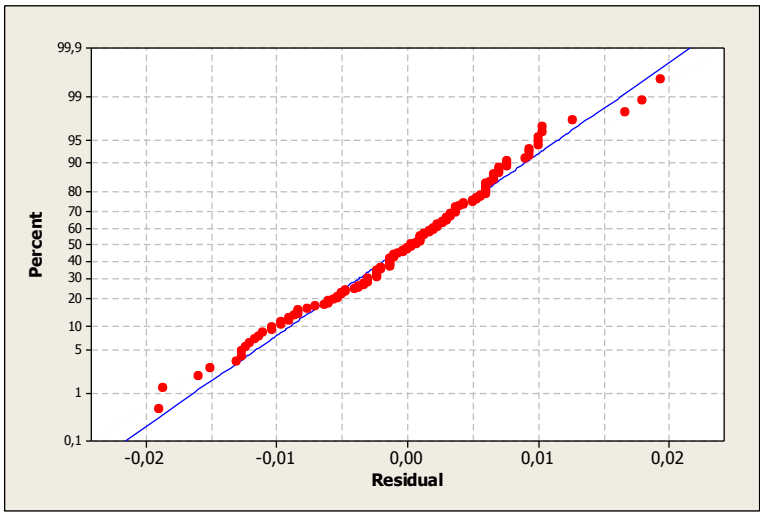

Figure 4.5. Normal probability plot of a_w

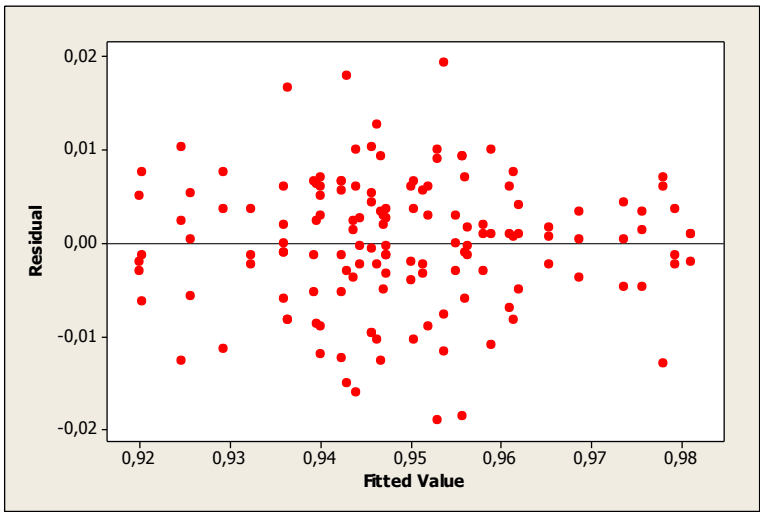

Figure 4.6.Residual plot of a_w

4.2. Sugar Composition of Olive Varieties

Glucose, fructose, sucrose, mannose and mannitol are the sugars that are detected in Hurma, Erkence and Gemlik varieties. Concentrations of these sugars for different varieties with respect to harvest time and year are listed in Table 4.4.

For the first harvest year, glucose and mannitol were detected as the dominant sugars as in sweet Dhokar olives (Jemai, Bouaziz et al. 2009). Sucrose disappeared after the second week of sampling in Hurma olive. At the same time, fructose and glucose concentrations increased implying a conversion of sucrose to these sugars. For all olive types, there is an increase in the amounts of all sugars for the last three weeks of ripening except mannitol. Mannitol was not detected after 5th week of maturation for all olive types.

The highest concentration of glucose was mostly detected in Hurma type and it varied between 21,256-296,787.05 mg/kg dw in the first harvest year. While in Erkence glucose concentration changed between the ranges of 30,700-163,449.44 mg/kg dw, in Gemlik type it was between 39160-88883.15 mg/kg dw. For Dhokar variety, glucose was determined as the main sugar with concentrations of 40,830 mg/kg fw followed by fructose (45,170 mg/kg fw). These sugars were in higher concentrations compared to a regular olive variety grown in the same region (Rigane et al., 2011).

The lowest concentrations of mannitol in the first harvest year were detected in Gemlik type and its range is between 7,360-30,500 mg/kg dw. Erkence type had the highest concentrations of mannitol and it changed between 4,386.3-18,971.63 mg/kg dw. While in Dhokar olives mannitol reaches to 79,800 mg/kg at the end of ripening in Hurma its level is 11,681.49 mg/kg and does not show any linear increasing trend as opposed to Dhokar (Jemai et al., 2009).

In contrast to the first harvest year, sucrose was detected throughout the ripening period and mannitol didn't disappear after 5th week of maturation in the second year. Olives have higher concentrations of mannose than the first harvest year.

The highest concentration of glucose in the second year was in Erkence type, and it varied between 13,218.97-53,439.29 mg/kg dw. Similar to glucose, the highest concentrations of fructose were found between the ranges of 11,075.2-50,872.33 mg/kg dw in Erkence type. Gemlik type had the lowest amounts of mannitol (3,587.83-11,853.94 mg/kg dw) as in the first harvest year.

The total sugar content of olive varieties investigated in this study is shown graphically in Figure 4.7. The total sugar content in the first season increased significantly in the last three weeks of harvesting. Other than that there is no significant trend regarding the total sugar content. There are increases and decreases throughout the sampling period. These changes are associated with the continuous synthesis of sugar during the ripening period and its use in the fatty acid biosynthesis (Menz and Vriesekoop 2010).Although some studies reported a decrease in total sugar content during ripening (Menz and Vriesekoop, 2010 ; Nergiz and Engez, 2000; Ergönül and Nergiz, 2010) an increase in reducing sugar content was observed for Chemlali and Dhokar varieties in another study (Jemai et al., 2009).

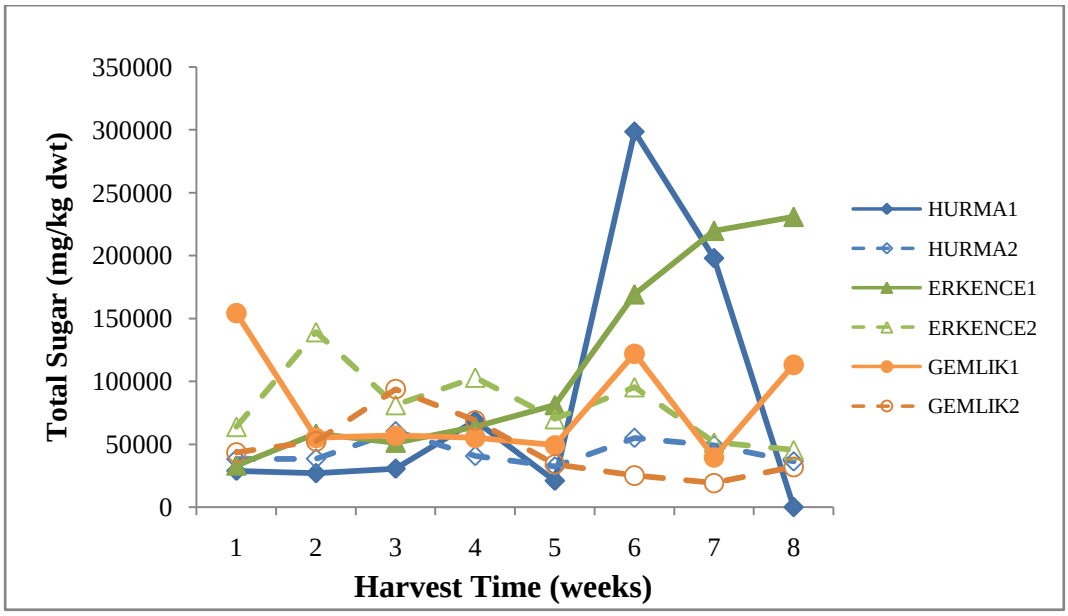

Figure 4.7. Total sugar content of Hurma, Erkence and Gemlik olives for both harvest years (Numbers after olive types refer to first and second harvest year)

In order to investigate the relation between sugar profile and the parameters of olive variety, harvest time and year statistically, principal component analysis (PCA) was applied. PCA was run for the sugar data of each year separately and also two harvest years in the same data matrix.

Table 4.4. Sugar Concentrations (mg/kg dw) of olive varieties for the two harvest years

2011/12 OLIVE TYPE	GLUCOSE	FRUCTOSE	SUCROSE	MANNOSE	MANNITOL	TOTAL	2012/13 OLIVE TYPE	GLUCOSE	FRUCTOSE	SUCROSE	MANNOSE	MANNITOL	TOTAL
H11	21256	nd	3371.2	nd	4227.2	28854.4	**H21**	8009.5	11036.1	5631.4	4997.8	8556.3	38231.2
H12	20546.1	nd	2156.9	1291.7	2942.6	26937.4	**H22**	18828.3	7047.2	5709.6	3361.8	3534.6	38481.7
H13	21859.1	1785.3	nd	1124.6	5770.7	30539.7	**H23**	23122.9	13137.2	4955.6	4411.9	14748.4	60376.5
H14	46305.5	5780.1	nd	5000.2	11681.4	68767.3	**H24**	12879	9475.9	5426.9	3598.3	9259.4	40639.8
H15	14399.7	2003.7	nd	nd	4564.4	20967.8	**H25**	13378.5	7569.4	3223.6	nd	8083.4	32255
H16	296787	1723.7	nd	nd	nd	298510.8	**H26**	20619	14885.5	6177.3	2587.4	10851.1	55120.4
H17	197919.6	nd	nd	nd	nd	197919.6	**H27**	14663.8	15827.6	5409.9	2333.7	10934.9	49170.1
H18	118058.6	4180.3	nd	nd	nd	122238.9	**H28**	11677.4	9690	3861.6	3682.4	7348.5	36260.1
E11	19364.4	nd	4284.4	5026.7	4386.3	33061.8	**E21**	13457.7	24904.3	9172.2	6530.9	9817.1	63882.5
E12	26777.4	5385.4	4573.1	2838.8	18971.6	58546.3	**E22**	53439.2	33284.2	8037.5	21459	22882.4	139102.5
E13	30700	5780.1	5070	1729.6	7970	51249.7	**E23**	33454	15464.9	16040	1278	14962.4	81199.5
E14	42440	3220	4580	2700	10990	63930	**E24**	13218.9	50872.3	16287.2	nd	22627.9	103006.4
E15	41400	5960	14110	5590	14110	81170	**E25**	23352.1	15889.8	3541.4	23714	3541.4	70038.9
E16	135115.6	7148.5	26978.6	nd	nd	169242.7	**E26**	38161.4	31263.9	5542.1	2847.7	17594.4	95409.7
E17	163449.4	7743.52	32613.5	15953.9	nd	219760.4	**E27**	21440.4	11363.6	7001.1	2099.7	6545.7	51450.6
E18	153751.4	12382.1	50059.9	14654.3	nd	230847.8	**E28**	19500.6	11075.2	4390.9	2797.7	7680.7	45445.2
G12	35620	5740	4950	nd	8740	55050	**G21**	13163.6	13858.9	8365.7	nd	6058.6	43446.9
G13	39160	4351	4580	1321.8	7360	56772.8	**G22**	18368.6	16477.9	6061.7	6711.7	4778.4	52368.5
G14	33333.8	4530	2720	4770	9700	55053.8	**G23**	29216.1	16478.1	23581.6	14742	9737.2	93755.2
G15	40160	6890	nd	nd	9110	49270	**G24**	30741.3	19786	6616.7	nd	11853.9	68998.1
G16	88883.1	15346.7	9313.7	8483	nd	122026.6	**G25**	8933	14465.7	4693	1970.8	4103.9	34166.5
G17	33807.8	nd	5768	nd	nd	39575.9	**G26**	8628.5	5946.4	3575.7	2714.3	4274.3	25139.4
G18	73598.3	5624	21733.5	12260.7	nd	113216.6	**G27**	4701.8	6109.9	3379.2	1467.7	3587.8	19246.5
							G28	11118.7	12205.6	2383	1643	4609.4	31959.9

*first number after olive type represents the harvest time (1st week, second week etc.) and second number shows the harvest year,nd: not detected

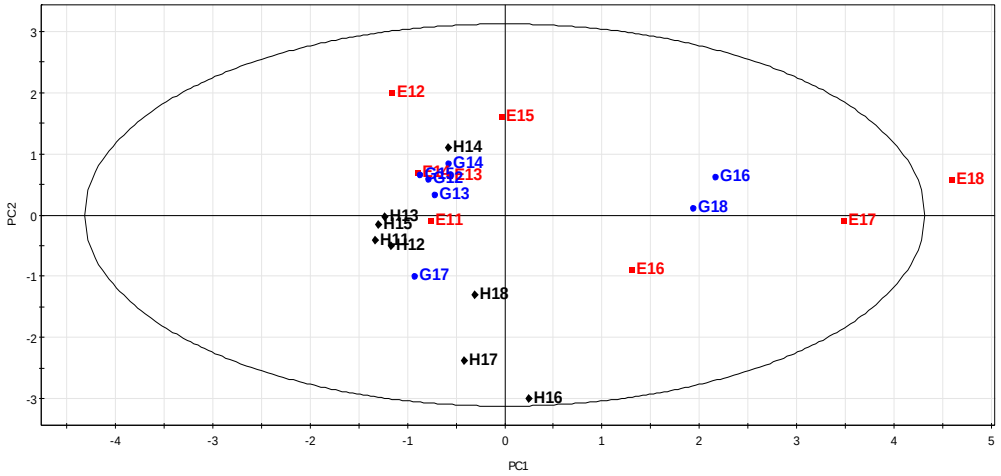

Figure 4.8.Score plot obtained with PCA for sugar concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year(H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

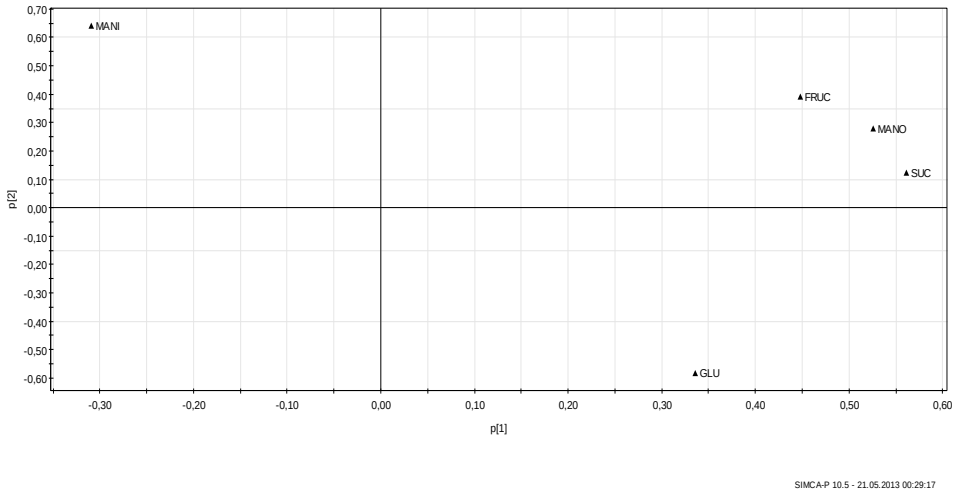

Figure.4.9.Loading plot for sugar concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year

PCA model for the data containing the first harvest year is made from 2 components and R^2 value is 0.782 for this model. According to the score plot of the first harvest year (Figure 4.8), first five weeks' samples are clustered together and there is no differentiation with respect to olive type. However, Hurma olives of the last three weeks are placed in the left down quartile of the plot, Gemlik olives of the same period are in

the left upper part of the plot. Erkence samples of the last weeks are situated more closely to Gemlik and separated well from Hurma olives. Loading plot (Figure 4.9) indicates that mannitol is the sugar that separates the first five weeks' samples from the rest since it was not detected after the first five weeks. Glucose is the differentiating sugar for Hurma having the highest concentration of this sugar at 6th week according to loading plot. On the other hand, fructose, mannose and sucrose concentrations are identified as the parameters separating Erkence and Gemlik from Hurma in the last three weeks.

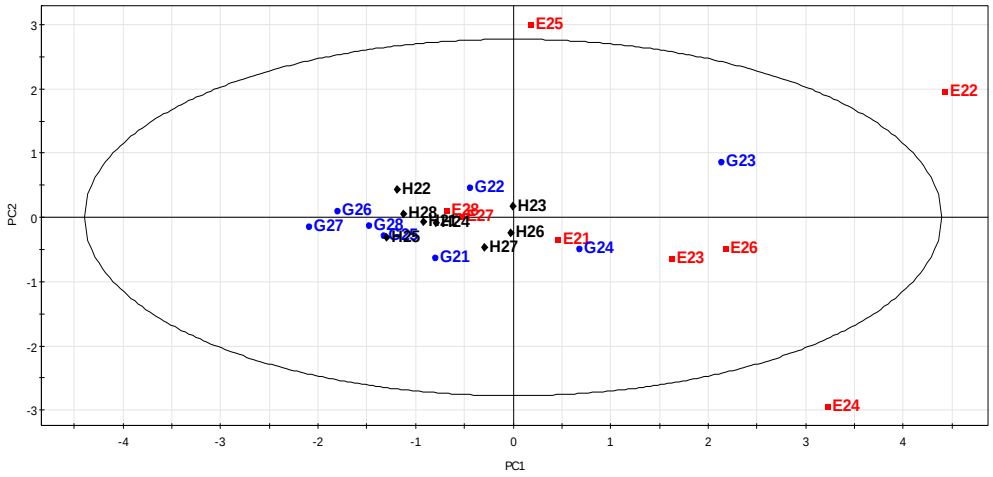

Figure 4.10. Score plot obtained with PCA for sugar concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year (H:Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

R^2 value of the PCA model for the second harvest year is 0.977 and 4 components are used for constructing the model. As the score plot shows (Figure 4.10) there is not a significant discrimination in terms of sugar concentration among varieties in the second harvest year.

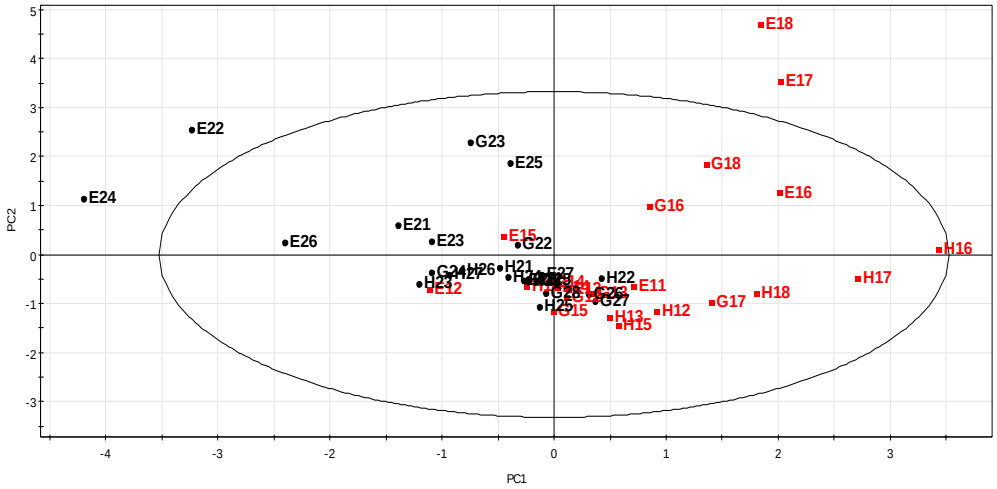

Figure 4.11.Score plot obtained with PCA for sugar concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years(H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

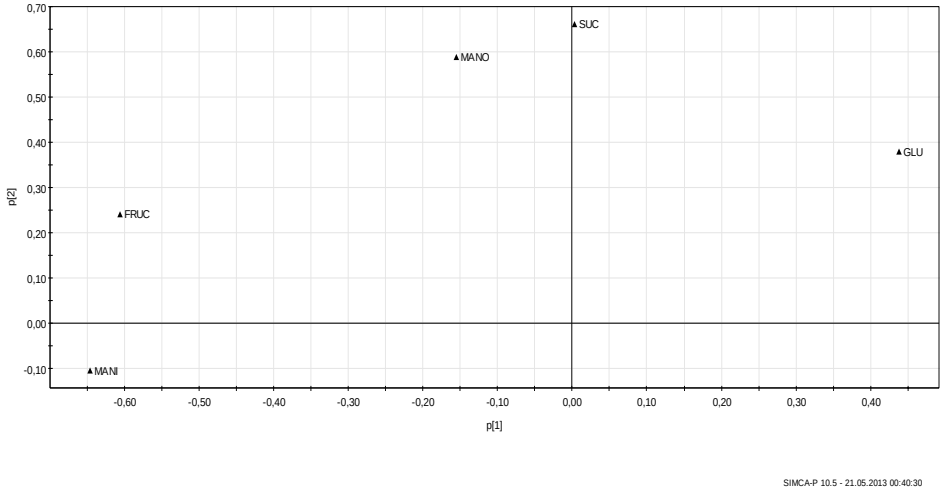

Figure.4.12.Loading plot for sugar concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

PCA model for the data containing two harvest years is made from 2 PCs and R^2 value of this model is 0.716.Score plot for this model shows that in terms of sugar composition of olive samples there is a difference between harvest years although some

samples are not placed in their category (Figure 4.11). While the first year samples are mostly placed in the right part of the plot, second year samples are in the left part of it. According to loading plot (Figure 4.12) sucrose and glucose are the sugars that cause differentiation of the first year from the second year. Sucrose has lower concentration in the first year samples and mannitol, fructose and mannose which are the differentiating parameters in the second year have more regular distribution throughout sampling period compared to the first year.

As a result, it can be concluded that sugar profile do not provide much differentiation among olive varieties while the effect of harvest year is identified as an important factor in the determination of sugar concentrations of olive varieties investigated in this study.

4.3. Organic Acid Composition of Olive Varieties

Organic acid composition of Hurma, Erkence and Gemlik olive types during eight weeks of maturation in 2011/12 and 2012/13 harvest years is provided in Table 4.5. For both harvest years, dominant organic acid was citric acid for all olive types. Citric acid was detected for all olive samples during whole maturation period. In addition, malic and succinic acids were also found in olive samples. Lactic acid was only detected in the last weeks of the second harvest year for Gemlik olives.

Higher concentrations of citric acid were measured in Gemlik type between the ranges of 6,907-16,412 mg/kg dw in the first harvest year. According to a study about Turkish olives, malic acid was determined as the dominant organic acid with high concentrations (Ergönül and Nergiz, 2010) while another study reported citric acid as the main organic acid followed by succinic acid in olives grown in several locations of Turkey (Arslan and Özcan, 2011) Although malic acid couldn't be detected until 6th week of maturation in Hurma, after that it increased and reached the highest concentration (6,390.7 mg/kg dwt) at the last week of the first year. Succinic acid was not detected after 5th week in Hurma olive. The highest amounts of succinic acid were detected in Erkence as 47,636 mg/kg dw. In Domat and Memecik olives succinic acid was detected at lower concentrations of 539-614 mg/100 g (Ergönül and Nergiz, 2010). Acetic acid was detected only in Hurma samples; however, it disappeared after the 5th week of ripening.

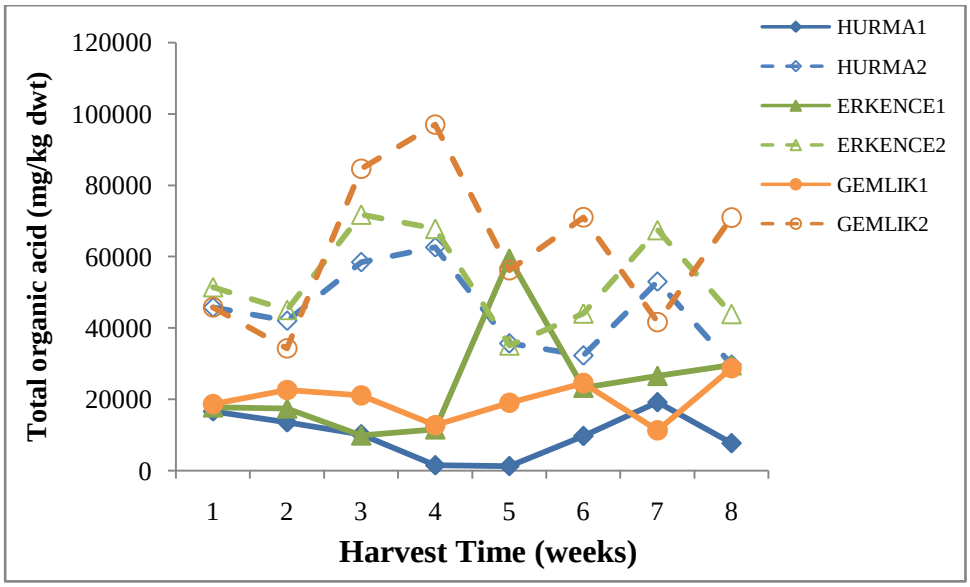

Figure 4.13.Total organic acid content of Hurma, Erkence, Gemlik olives for both harvest years (Numbers after olive types refer to first and second harvest year)

In the second harvest year, almost all organic acid concentrations were higher than the first year. Similar to the first year, Gemlik had the highest amounts of citric acid between the ranges of 26,055.9-86,098.7 mg/kg dwt. Citric acid was detected for all varieties in higher concentrations than other Turkish olives (Ergönül and Nergiz 2010). Citric acid was between the ranges of 1,024 and 702-mg/100g dw in Memecik and Domat, respectively (Ergönül and Nergiz, 2010). Contrary to the first year, malic acid existed for all olive types during maturation and it was found in higher amounts in Erkence type (4,583.79-12,935.2 mg/kg dw).Succinic acid was not found in Erkence for the second year. In addition to this, it disappeared from Hurma after 5th week as in the first harvest year.

In order to investigate the organic acid results with respect to olive variety, harvest time and year statistically, PCA analysis was used.

Table 4.5. Organic concentrations (mg/kg dwt) of olive varieties for two harvest years

2011/12 Olive type	CITRIC	MALIC	SUCCINIC	LACTIC	ACETIC	TOTAL	2012/13 Olive type	CITRIC	MALIC	SUCCINIC	LACTIC	ACETIC	TOTAL
H11	11546.2	nd	3644.1	690.6	743.7	16624.6	**H21**	34306.5	9651.3	1873	nd	nd	45830.9
H12	13402	nd	103.4	nd	39.3	13544.8	**H22**	32140	7702.1	2223.1	nd	nd	42065.2
H13	9859.4	nd	288.3	nd	59.4	10207.2	**H23**	46080.7	9644.5	2780	nd	nd	585053.2
H14	1170.4	nd	403.6	nd	nd	1574	**H24**	52511.7	5168.4	837.6	1989.4	2171.4	62678.6
H15	1134.8	nd	148.8	nd	462.6	1329.9	**H25**	27357.3	3549.5	1030.8	1850.7	1952.7	35741.1
H16	7087.4	2655.4	nd	nd	nd	9742.8	**H26**	27252.1	5088.2	nd	nd	nd	32340.4
H17	13145.2	6055.1	nd	nd	nd	19200.3	**H27**	41919.8	11129.5	nd	nd	nd	53049.3
H18	1343.9	6390.7	nd	nd	nd	7734.6	**H28**	23880.6	5519.2	373.7	nd	nd	29773.6
E11	8113.6	9701.5	nd	nd	nd	17815.1	**E21**	38514.9	12935.2	nd	nd	nd	51450.1
E12	9715.7	7778.3	nd	nd	nd	17494.1	**E22**	34925.3	10146.7	nd	nd	nd	45072
E13	8644.4	nd	1300.4	nd	nd	9944.9	**E23**	62574.9	9191.8	nd	nd	nd	71766.8
E14	10036	nd	1612.1	nd	nd	11648.2	**E24**	58357.9	9424.4	nd	nd	nd	67782.3
E15	11872.4	nd	47636.7	nd	nd	59509.2	**E25**	30521.5	4583.7	nd	nd	nd	35105.3
E16	12240.1	11077.6	nd	nd	nd	23317.7	**E26**	36880.2	7183.5	nd	nd	nd	44063.7
E17	10932.4	15701.1	nd	nd	nd	26633.6	**E27**	33711.4	33711.4	nd	nd	nd	67422.8
E18	13185.9	16432.8	nd	nd	nd	29618.8	**E28**	39251.7	4666.8	nd	nd	nd	43918.6
G12	7071.6	15594.1	nd	nd	nd	22665.6	**G21**	39145.1	6669.5	nd	nd	nd	45814.6
G13	12143.4	8996.2	nd	nd	nd	21139.6	**G22**	26055.8	8243.2	nd	nd	nd	34299.1
G14	10091.8	nd	2663.6	nd	nd	12755.4	**G23**	73957.7	10667.3	nd	1931.4	nd	84625.1
G15	15489	nd	3541.3	nd	nd	19030.3	**G24**	86098.6	9515.3	nd	1392.2	nd	97006.2
G16	9294.5	15279.3	nd	nd	nd	24573.9	**G25**	47637	7500.6	nd	1020.2	nd	56157.9
G17	1387.6	9938.1	nd	nd	nd	11325.7	**G26**	58393.2	6454.433	1424.2	2234.4	2526.6	71033
G18	16412.8	12338.8	nd	nd	nd	28751.7	**G27**	33768.1	5164803	1548.9	1182.5	nd	41664.4
							G28	64755	4366.9	1837	nd	nd	70958.9

*first number after olive type represents the harvest time (1st week, second week etc.) and second number shows the harvest year,nd: not detected

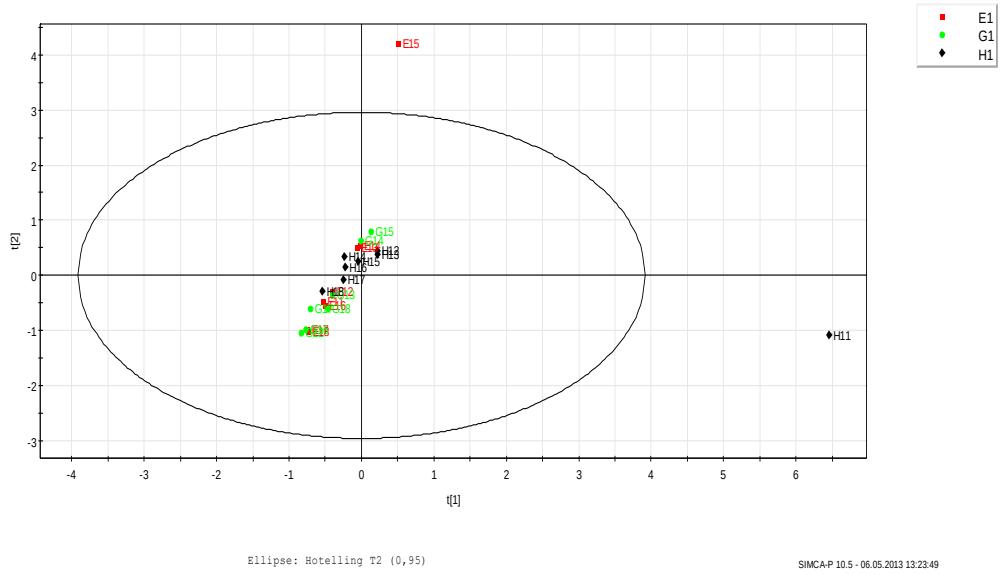

Figure 4.14.Score plot obtained with PCA for organic acid concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

PCA model constructed with organic acid data of the first harvest year consists of 4 components with R^2 of 0.999. As it is shown in the score plot (Figure 4.14), for the first harvest year there was no discrimination between olive varieties in terms of organic acids. This means that organic acid profile is not a good parameter to characterize olive varieties for the first harvest year.

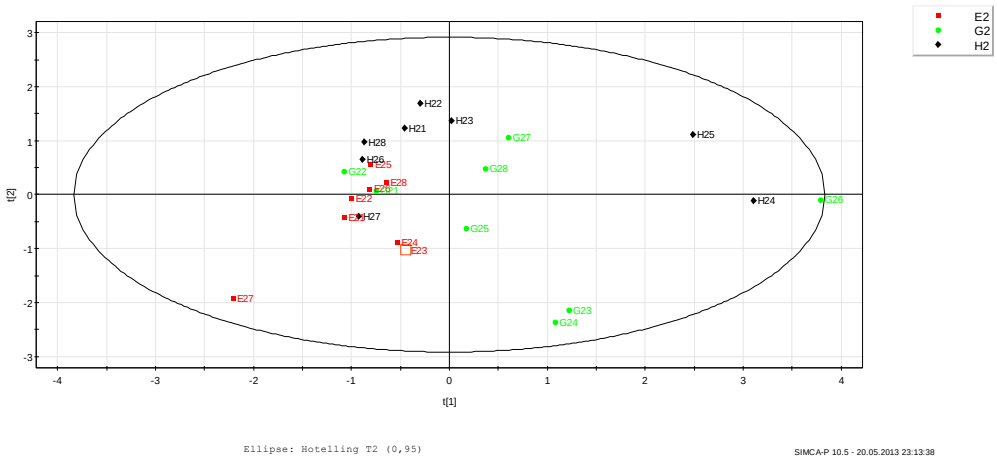

Figure 4.15.Score plot obtained with PCA for organic acid concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year(H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

PCA model created with the second year organic acid profiles of three olive varieties for eight weeks of maturation has 4 components and R^2 of 0.996. According to the score plot (Figure 4.15), there is not much differentiation regarding the olive varieties as in the first year.

Finally, data for both harvest years were combined and PCA was performed to see the effect of harvest year. PCA model has $R^2$of 0.966 and 4 components. As it is shown in the score plot (Figure 4.16) there is a good separation between harvest years in terms of organic acids. Most of the first harvest year samples are located on the left side of the plot. First year samples are more closely clustered while there is more spread out in the second year samples. Another study also reported the significant effect of harvest year on organic acid concentrations of Turkish olives (Arslan and Özcan, 2011).According to loading plot malic and succinic acid concentrations are the differentiating parameters for the first year. Malic acid exists throughout maturation period in the second year while in the first year it exists on and off form depending on the variety. As a result, although organic acid profile of investigated olive varieties do not provide separation with regard to variety organic acid is an important parameter that cause differentiation between harvest years.

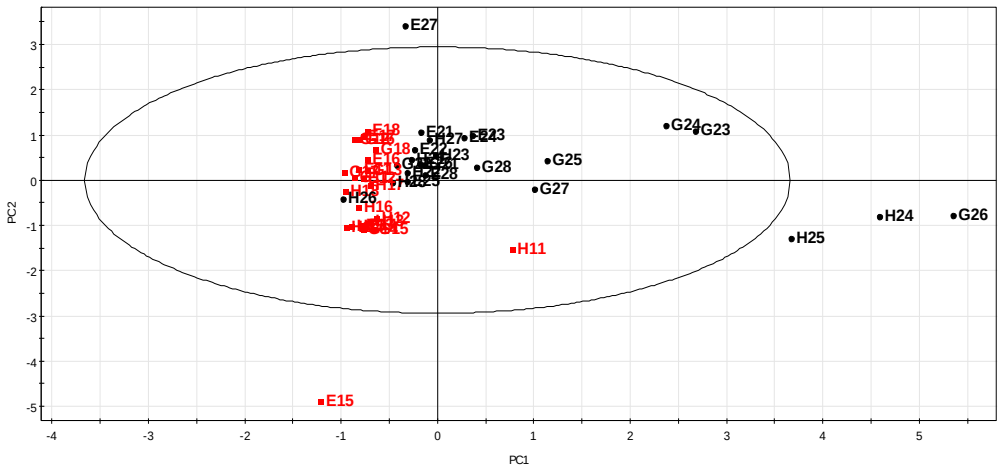

Figure 4.16.Score plot obtained with PCA for organic acid concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

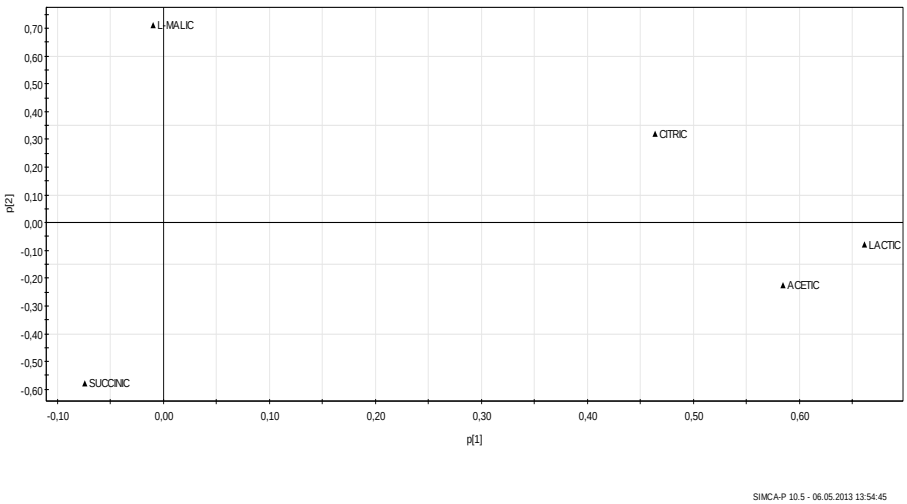

Figure 4.17.Loading plot obtained with PCA for organic acid concentrations of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

4.4. Total Phenol Content of Olive Varieties

The total phenol contents of all three types of olives are listed in Table 4.6. TPC of Erkence variety changed between 504.7-1,230.4 mg GAE/100g in 2011/12 and 335.9-664.8 mg GAE/100g in 2012/13 harvest years, respectively. Hurma had 337.7-649.6 mg GAE/100g in the first and 29.2-468.2 mg GAE/100g in the second harvest years while TPC of Gemlik varied between 416.8-701.9 mg GAE/100g in the first and 103.2-452.4 mg GAE/100g in the second year. In general, Erkence variety had the highest TPC in both harvest years while Hurma type, although from the same variety, had lower phenol content. This difference between these two types of olives might be resulted from debittering stage which Hurma goes through. Dhokar, Tunisian sweet olive variety, (Jemai et al., 2009) also had lower TPC (508-768 mg GAE/100g dw) when compared to another olive variety, Chemlali (698-1300 mg GAE $100g^{-1}$ dw) and researchers also attributed the lower TPC of this variety to its sweet character. (Bouaziz et al., 2004)

During the ripening period TPC of all olive types have ups and downs. Mostly, there is a decreasing trend after 2^{nd} week until 5^{th} week and an increase in TPC follows this decreasing phase. This trend of increase and decrease was also observed in other studies (Morello et al., 2004).

In the second harvest year (2012/13), all three olive types had lower TPC compared to the first harvest year (2011/2012). Change in the phenol content depending on the harvest year is expected and well-documented for olive oil in the literature. However, TPC for different types has similar trend in both years; Erkence having the highest and Hurma having the lowest average TPC content. ANOVA was used to show the significant differences among TPC values. The p-values were found to be less than 10^{-3}.

Table 4.6.ANOVA table for total phenol content

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Variety	2	1412364	1412364	706182	102.43	0.00
Harvest Year	1	2802655	2802655	2802655	406.50	0.00
Time	7	684942	684942	97849	14.19	0.00
Variety*harvest year	2	116157	116157	58079	8.42	0.00
Variety*time	14	690419	690419	49316	7.15	0.00
Harvest year*time	7	918877	918877	131268	19.04	0.00
Variety*harvest year*time	14	685990	685990	48999	7.11	0,00
Error	96	661878	661878	6895		
Total	143	7973283				

ANOVA results obtained to test the effect of olive variety, harvest time and season on TPC values are listed in Table 4.6 and normal probability and residual plots are shown in Figure 4.17-4.18. As it can be seen from the ANOVA table (Table 4.6) all factors and their interactions are significant and model R^2 value is 91.70% and R^2 (adj) equals to 87.63%. Therefore, total phenol content is affected by the olive variety, harvest time and year.

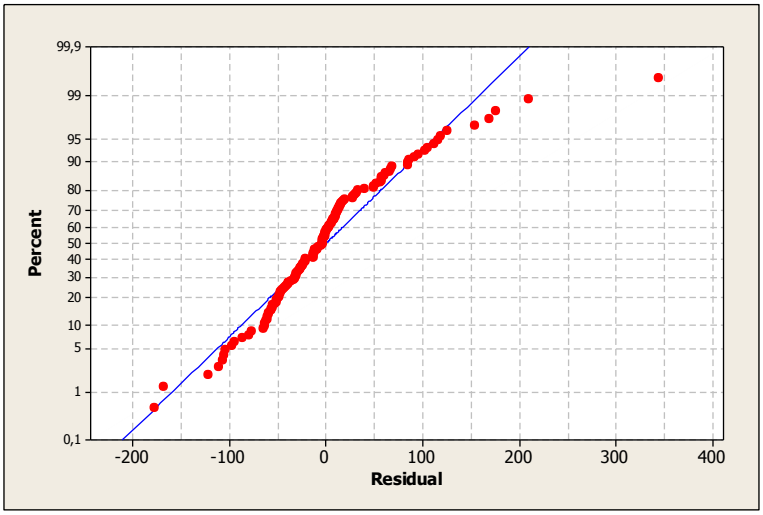

Figure 4.18. Normal probability plot of total phenol content

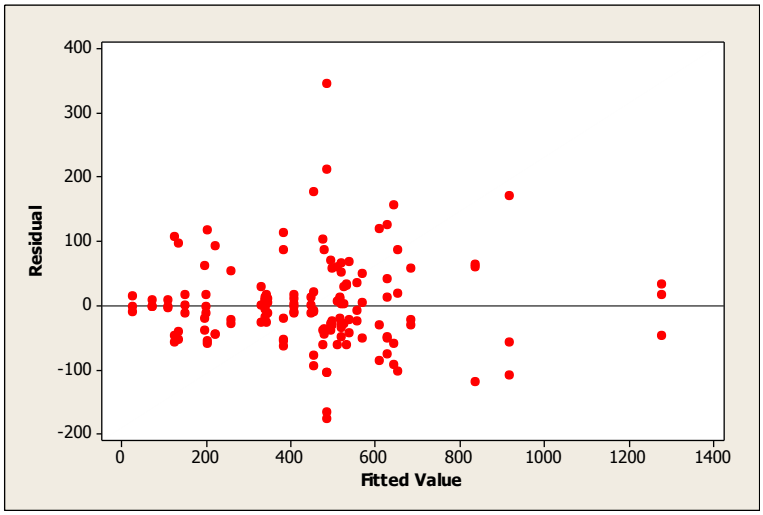

Figure 4.19.Residual plot of total phenol content

4.5. Phenol Profile Results of Olive Varieties

Chromatograms of phenolic profiles of Hurma, Erkence and Gemlik olive types are provided in Figure 20.1,2 and 3 and Table 4.7 and 4.8 show the concentration of individual phenolics.

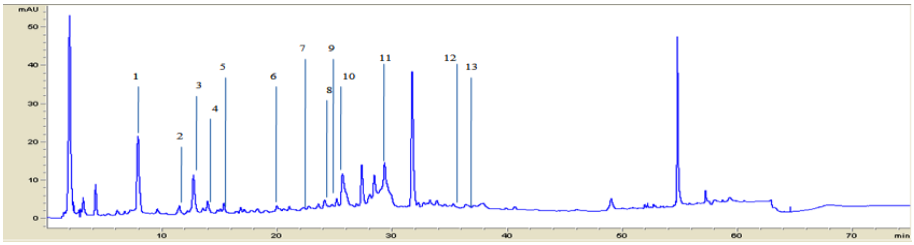

Figure 4.20.1. HPLC chromatogram of phenolics of Hurma belonging to the first week of the first harvest year (1-hydroxytyrosol, 2-tyrosol, 3-ISTD (p-hydroxy acetic acid), 4-apigenin, 5-vanilic acid, 6-p-coumaric acid, 7-verbascoside, 8-L-7-glucoside,9-rutin,10-o-coumaric acid, 11-oleuropein, 12-quercetin, 13-luteolin)

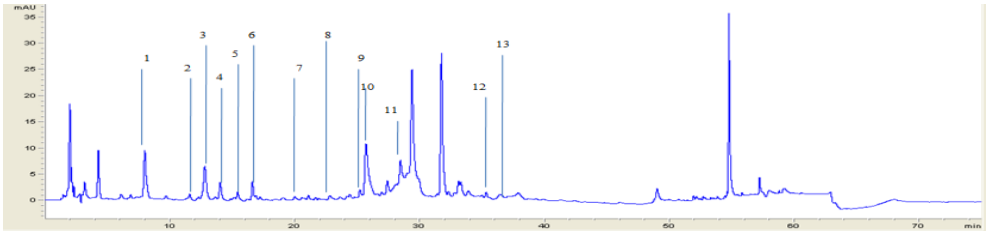

Figure 4.20.2. HPLC chromatogram of phenolics of Erkence belonging to the first week of the first harvest year (1-hydroxytyrosol, 2-tyrosol, 3-ISTD (p-hydroxy acetic acid), 4-apigenin, 5-vanilic acid, 6-caffeic acid, 7- p-coumaric acid, 8-verbascoside, 9-rutin,10-o-coumaric acid, 11-oleuropein, 12-quercetin, 13-luteolin)

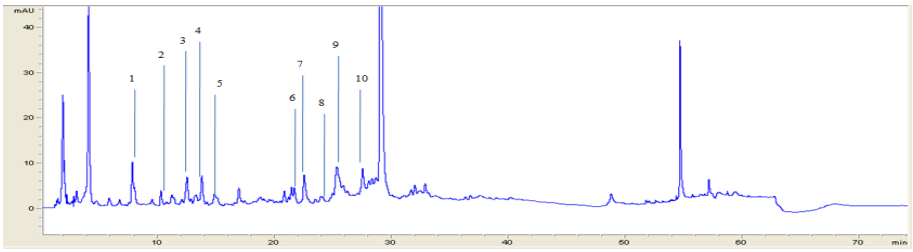

Figure 4.20.3. HPLC chromatogram of phenolics of Gemlik belonging to the first week of the first harvest year (1-hydroxytyrosol, 2-tyrosol, 3-ISTD (p-hydroxy acetic acid), 4-apigenin, 5-vanilic acid, 6-ferulic acid, 7-verbascoside, 8-rutin, 9-o- coumaric acid,10-oleuropein)

As it can be seen from the Table 4.7 quercetin-3-glucoside is the only phenol which was detected in the first harvest year in all olive types but not in the second year. In addition, while luteolin was observed in the first year in Hurma and Gemlik samples, this phenolic was not measured in Hurma and Gemlik had it only on the first three weeks of the second harvest year. To see the differences between varieties, harvest time and harvest year a multivariate classification technique, PCA, was applied to the data and both TPC and individual phenols were used in the data matrix. Although R^2 values of the models obtained are not very high PCA plots are still helpful in visualizing the differences regarding the olive type, harvest season and year. Without this multivariate analysis it would be hard to draw conclusions considering all phenolic compounds and TPC at the same time.

For the whole data, a model with 2 principal components and R^2 of 0.48 was obtained and score plot for this model showing the classification of olive types is provided in Figure 4.21. According to this plot, a differentiation could be observed between the first and the second harvest year olives (except first season Hurma at 5th weeks of maturation). First five week samples from all types of 2011/12 harvest year are more closely positioned to 2012/13 harvest year samples. The later weeks (6th, 7th and 8th weeks) of the first harvest year are totally separated from the rest of the samples and are on the right side of the plot. Overall, all phenolic compounds except vanillic acid exist in higher amounts for the samples harvested in the 2011/12 season. Therefore, they are located on the right side of the loading plot (Figure 4.22) like the first year olives since they are the differentiating parameters for the first year olives.

Table 4.7.MI, TPC (mg 100 g^{-1}) and concentration of individual phenolic compounds (mg kg^{-1}) of Hurma, Erkence and Gemlik olive types during eight weeks of maturation for the first harvest season

Olive type†	MI††	TPC†††	OLE	HYT	TY	API	VER	RTN	L-7-Glu	LTLN	QUE	o-cou	p-cou	FA	VA	CA	VN
H11	3.90	560.40	527.63	2013.44	17.49	10264.62	517.20	9.571	709.97	36.36	18.6	61.41	0.59	nd	2.16	2.4	0.79
H12	4.10	649.64	786.15	2722.70	36.90	1022.18	66.37	12.99	84.071	34.20	23.2	118.9	0.70	0.56	3.01	2.37	1.61
H13	5.34	523.26	1166.89	1424.46	18.68	297.04	54.05	10.03	128.85	nd	35.12	122.78	1.05	nd	2.06	3.87	nd
H14	4.95	412.68	87.701	3357.91	11.56	1727.42	81.92	nd	123.80	4.98	nd	126.61	nd	0.81	3.03	nd	1.88
H15	3.74	337.68	nd	827.59	nd	377.30	57.20	nd	7.49	nd	nd	nd	5.09	nd	3.49	4.44	nd
H16	4.37	533.97	103.71	1602.36	52.51	15502.65	519.73	9.56	189.07	72.98	35.96	16.09	1.99	231.72	5.74	1.65	4.57
H17	3.99	579.68	753.07	4104.61	73.10	8003.25	1995.76	21.21	334.76	nd	50.40	23.34	3.86	282.28	10.72	2.14	4.27
H18	3.94	644.63	241.91	3239.12	32.53	5561.70	391.08	24.88	532.48	nd	47.51	47.82	3.08	nd	9.41	nd	2.29
E11	0.50	518.43	1388.21	1001.16	14.94	3993.86	258.75	12.23	167.92	69.50	26.18	103.98	nd	nd	nd	2.98	nd
E12	1.27	518.02	1265.98	1627.43	24.19	1270.94	31.024	13.75	141.59	98.41	27.66	170.52	1.45	nd	4.93	3.03	nd
E13	2.25	526.22	625.70	2222.26	30.42	1391.48	39.24	59.50	63.43	nd	33.73	12.51	nd	nd	11.52	2.85	nd
E14	1.65	520.95	137.99	497.86	0.94	990.58	14.52	nd	22.13	109.30	17.56	nd	nd	nd	3.43	1.48	nd
E15	1.69	504.69	470.90	471.66	11.03	1673.20	40.32	13.13	56.83	95.02	12.54	30.48	2.91	nd	3.63	2.11	nd
E16	3.65	900.69	329.34	2011.45	29.03	15454.53	312.87	14.99	1387.22	168.42	37.88	74.07	1.9	nd	nd	1.41	2.07
E17	3.56	691.32	608.61	1281.41	19.14	9804.94	566.54	29.23	1833.21	351.19	95.63	143.82	2.3	nd	2.61	2.7	2.17
E18	3.67	1230.4	705.14	876.30	12.36	4443.14	424.94	29.53	2207.42	252.29	63.11	203.62	2.85	nd	1.5	2.12	1.74
G12	1.10	544.61	4786.76	3070.73	nd	4788.42	594.30	nd	78.70	nd	nd	135.07	nd	nd	nd	nd	nd
G13	1.38	452.33	2057.32	5399.42	nd	2030.05	700.34	56.84	239.38	154.24	nd	111.69	nd	4.33	nd	nd	nd
G14	2.41	637.10	294.53	6596.22	nd	1835.13	66.63	24.77	133.99	171.68	nd	107.23	nd	5.63	7.95	nd	11.5
G15	2.10	416.78	nd	2277.51	nd	2704.81	35.45	0.16	85.43	167.16	nd	31.67	1.19	0.87	5.14	nd	nd
G16	3.65	806.88	683.95	3803.16	17.36	24689.41	473.78	22.11	619.82	298.15	152.08	132.75	4.39	nd	3.66	nd	2.13
G17	4.82	524.48	237.300	3704.21	6.99	22139.33	689.24	19.59	957.05	128.93	30.9	112.54	3.24	nd	2.37	1.53	1.07
G18	5.88	701.84	307.42	8183.35	17.09	31838.78	1942.04	74.96	1081.14	nd	nd	116.46	7.24	nd	nd	9.53	nd

*for abbreviations look at Chapter 3.Experimental study,3.1.2. Chemical Agents

Table 4.8. MI, TPC (mg 100 g^{-1}) and concentration of individual phenolic compounds (mg kg^{-1}) of Hurma, Erkence and Gemlik olive types during eight week of maturation

Olive type†	MI††	TPC†††	OLE	HYT	TY	API	VER	RTN	L-7-Glu	LTLN	QUE	o-cou	p-cou	FA	VA	CA	VN
H21	5.43	208.36	780.76	61.83	nd	1251.18	76.7	33.64	22.28	nd	nd	8.26	nd	12.02	11.03	nd	nd
H22	4.56	344.34	190.94	22.55	nd	539.02	10.81	28.9	7.87	nd	nd	3.13	nd	nd	5.03	nd	0.43
H23	5.92	73.89	60.28	33.39	nd	378.71	nd	nd	9.56	nd	nd	0.84	0.32	5.1	nd	nd	nd
H24	5.60	29.21	291.09	105.61	7.55	906.06	39.89	7.78	39.28	nd	nd	1.09	1.02	2.96	nd	4.41	nd
H25	6.18	245.56	nd	29.5	nd	552.43	nd	3.53	9.06	nd	nd	nd	nd	nd	nd	0.02	nd
H26	5.42	152.11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
H27	6.37	160.95	145.34	75.34	5.54	585.26	17.17	8.57	65.29	nd	nd	0.76	0.21	6.14	6.49	1.15	nd
H28	6.00	159.09	17.9	7.17	nd	714.23	nd	nd	nd	nd	nd	nd	nd	nd	3.52	nd	nd
E21	2.53	335.88	1490.37	101.51	10.95	929.82	29.91	61.84	21.73	1.03	nd	9.45	0.49	4.63	9.03	nd	1.14
E22	2.16	359.17	505.48	126.92	13.19	1229.2	34.77	21.19	46.06	0.95	nd	6.56	0.27	4.18	3.5	nd	nd
E23	2.34	535.66	431.9	116.45	13.17	1641.2	16.75	15.14	54.24	2.76	nd	0.42	2.34	5.03	2.93	5.02	nd
E24	2.53	518.32	307.2	123.31	10.83	1412.5	43.73	19.95	34.26	1.19	nd	nd	7.85	6.66	3.51	2.64	0.43
E25	3.17	519.82	126.38	24.11	1.78	946.92	19.16	11.85	12.45	0.64	nd	0.49	2.91	1.7	2	1.84	nd
E26	3.18	347.00	334.58	78.58	24.7	1222.1	75.16	17.15	25.99	13.32	nd	1.73	1.60	11.01	78.58	2.39	1.38
E27	3.49	664.81	139.11	79.39	5.12	920.2	11.07	8.11	39.07	nd	nd	5.97	3.77	1.99	2.05	1	0.15
E28	5.94	514.07	58.66	97.33	28.03	1211.1	80.42	30.28	21.33	56.23	nd	8.02	0.36	8.37	2.43	1.89	nd
G21	2.42	343.29	242.32	118.05	7.53	634.36	88.57	34.76	13.55	39.22	nd	6.54	0.45	14.73	3.96	nd	0.41
G22	2.19	411.37	751.48	484.79	10.07	1615.7	160.85	22.32	90.50	nd	nd	2.66	0.93	33.11	1.95	nd	nd
G23	3.32	244.92	166.65	427.43	nd	981.71	47.1	12.75	37.64	1.33	nd	0.91	0.38	8.06	2.12	2.12	nd
G24	3.09	229.63	280.47	616.73	5.66	1420.9	194.56	24.62	28.68	nd	nd	1.84	0.69	10.05	2.38	nd	0.07
G25	3.92	242.80	456.82	346.14	8.42	888.65	110.62	15.09	41.79	nd	nd	nd	0.66	15.53	4.31	nd	0.61
G26	4.60	103.19	163.24	374.06	2.65	694.54	37.16	6.51	22.31	nd	nd	0.72	0.19	15.93	8.18	nd	nd
G27	4.26	452.36	385.87	424.92	5.65	838.93	105	10.78	49.94	nd	nd	3.91	0.33	8.13	3.04	nd	0.53
G28	3.84	228.39	290.94	288.62	nd	701.73	37.6	4.14	8.14	nd	nd	nd	0.5	4.14	nd	nd	nd

*for abbreviations look at Chapter 3.Experimental study,3.1.2. Chemical Agents

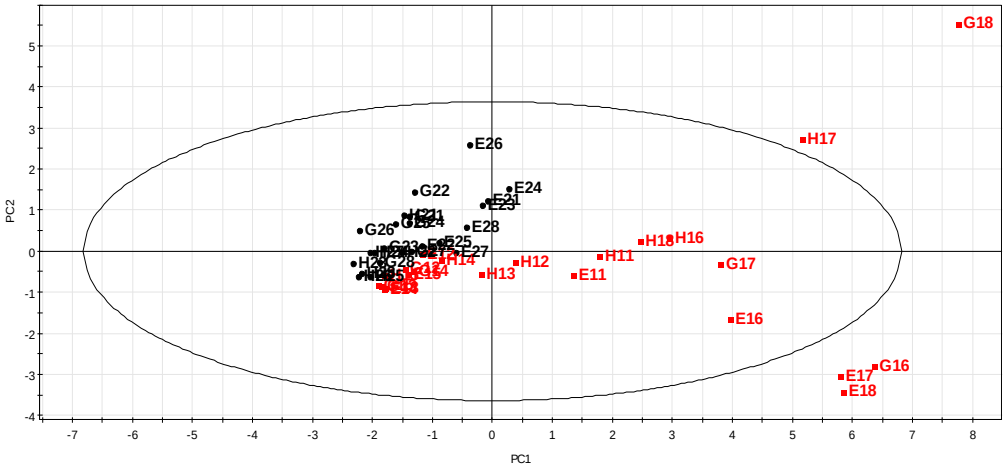

Figure 4.21.Score plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the both harvest year (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

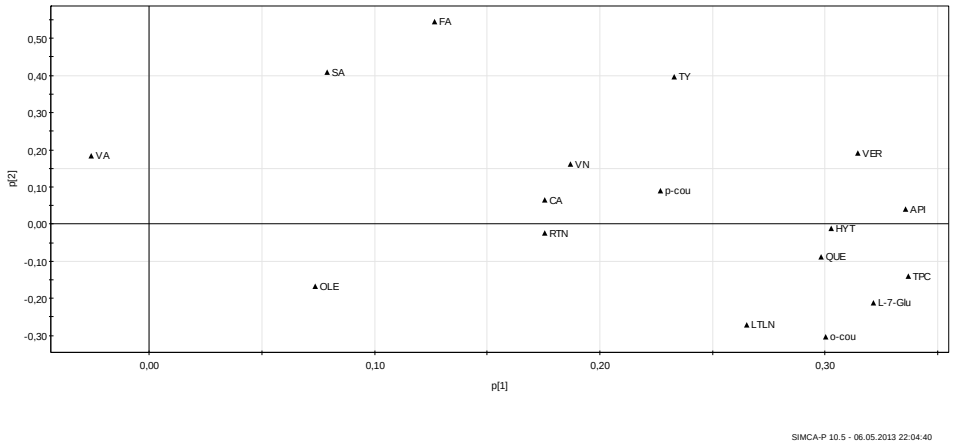

Figure 4.22.Loading plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

To better understand the differences between each type of olives, PCA was run separately for each harvest year and score and loading plots are shown in Figure 4.23 and 4.24. PCA constructed for the first harvest year consists of 2 principal components

with R^2 of 0.46. Score plot (Figure 4.23) shows also that there is not much differentiation with regard to olive type in the first 5 weeks of sampling. There is a clear separation between the first 5 weeks' olives and the olives harvested in the last 3 weeks. Actually, those last three weeks, in general, correspond to time where most of the harvesting is done locally. According to loading plot (Figure 4.24) oleuropein and vanillic acid are the phenols that differentiate early harvest period from the rest. Actually, oleuropein and vanillic acid contents of early samples are higher compared to later period (Table 4.7). When the late period is considered; however, different olive types, especially Erkence and Hurma, could be clearly separated from each other since Erkence is located on the lower right quartile and Hurma on the upper right quartile of the score plot (Figure 4.23).

Gemlik variety in the last 3 weeks of harvest has high hydroxytyrosol (3,704.2-8,183.3 mg/kg) and apigenin (22,139.3-31,838.7 mg/kg) content compared to others. Generally, oleuropein content of Erkence (137,9-1,388.2 mg/kg) is higher compared to Hurma (0-1,166.9 mg/kg) throughout the sampling period. According to loading plot (Figure 4.24), luteolin, o-coumaric acid, luteolin-7-glucoside and TPC are the differentiating parameters for Erkence. In fact, Erkence has the highest TPC (504.7-1,230.4 mg/100g) especially in the late period of harvesting while luteolin (168.4-351.2 mg/kg) exists in high amounts in the last 3 weeks. Vanillin, ferulic acid and tyrosol content of Hurma olives in the last 3 weeks of harvesting are the parameters that separate out this olive from the rest (Figure 4.24).

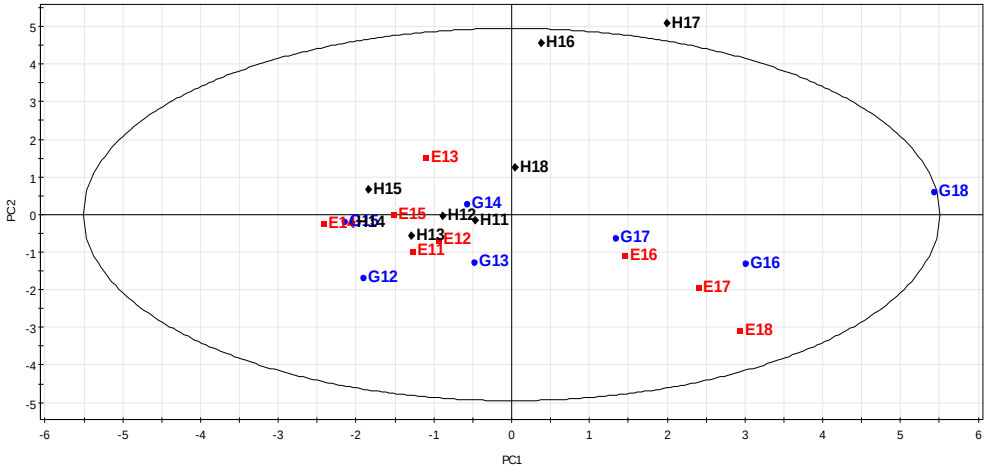

Figure 4.23.Score plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

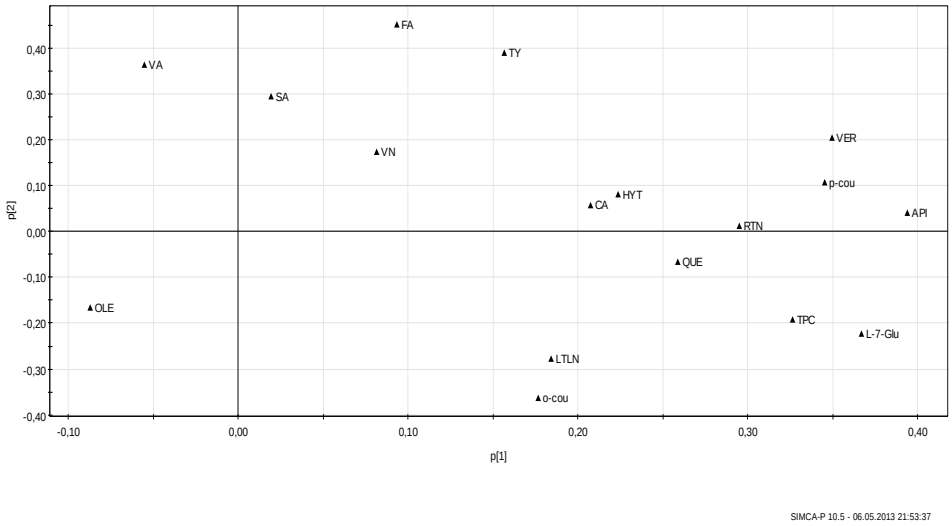

Figure 4.24.Loading plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year

PCA model for the second harvest year has R^2 of 0.63 and 3 principal components. PCA in this case provided better classification for different olive types although some samples are not located in their class (Figure 4.25 and 4.26). First week Hurma (H21) and Gemlik (G21) and second week Erkence (E22) samples are close to each other in the score plot; therefore, separation in the early harvest period is not that clear as it was observed in the first harvest year. Other than fifth week Erkence (E25), Erkence and Hurma are separated from each other quite well indicating that phenolic compounds are very much affected from debittering phase during maturation. According to loading plot (Figure 4.25), hydroxytyrosol, ferulic acid, verbascoside and luteolin-7-glucoside are the phenolics which provide separation of Gemlik type compared to others. Oleuropein, apigenin, rutin and o-coumaric acid are also important phenolics in Gemlik differentiation but they also play a role in Erkence classification since they are located close to horizontal axis. Caffeic acid, p-coumaric acid, vanillin, tyrosol and TPC are the differentiating parameters for Erkence according to the loading plot (Figure 4.26) and this type olive contains these phenols in higher amounts and its TPC is the highest compared to others as in the first year. Caffeic acid is mostly present in Erkence for the second harvest year. Tyrosol was in significant amounts throughout ripening for Erkence while Hurma did not contain much of this phenolic as opposed to first year. Hurma type has always lower content of every phenolic compounds and especially its oleuropein concentration is very low after 4 weeks in the second harvest year.

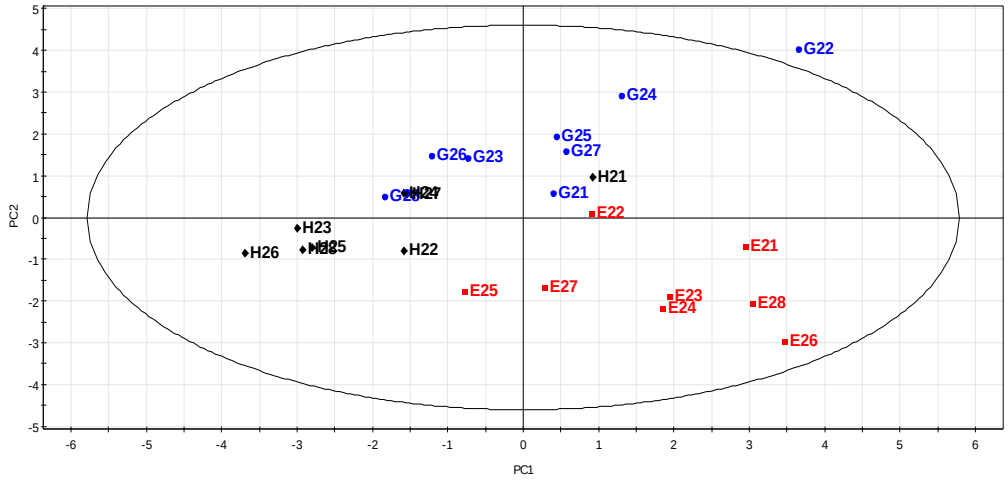

Figure 4.25.Score plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

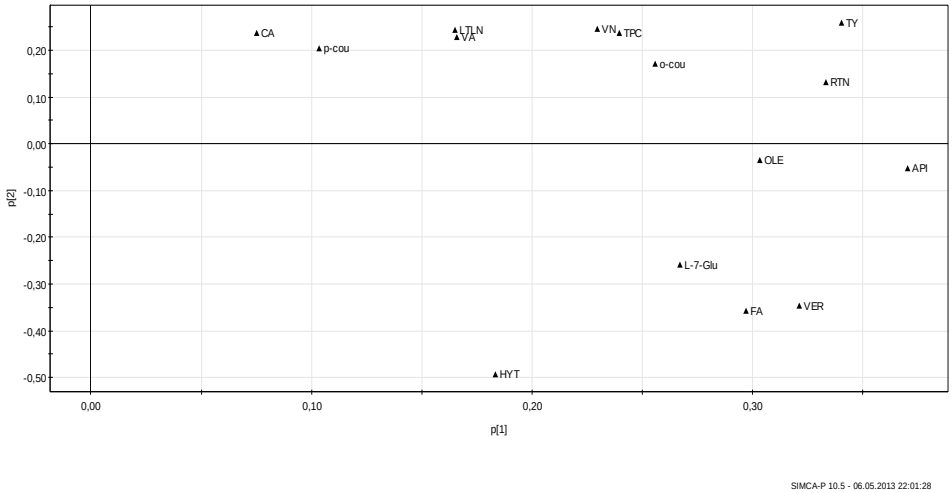

Figure 4.26.Loading plot obtained with PCA for phenolics of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year

As it was reported in the literature, an overall decreasing trend for oleuropein was observed although there are ups and downs depending on the harvest time. This type of trend during maturation was also observed by other researchers (Dağdelen et al., 2013).Increasing and decreasing trend observed for almost all phenols could be explained by the conclusion provided by a study by Menz and Vriesekoop (2010) that is the continuous synthesis of phenolic compounds during maturation. Since phenolic compounds are secondary metabolites of plants and part of their defense mechanisms they could be synthesized when they are needed depending on the environmental conditions or the other factors.

Although a decreasing trend is not that clear for Hurma in the first year it could still be said oleuropein is becoming less with increasing maturity for this olive type also. According to a study performed with sweet Dhokar and regular Chemlali olives there is a clear decrease in oleuropein content and increase in hydroxytyrosol contents of both olives with ripening (Jemai et al., 2009; Bouaziz et al., 2004). While oleuropein content reduced to almost zero level for Dhokar with time it stayed at a certain level for Chemlali. Although same observation applies for oleuropein in our case, a decreasing trend for hydroxytyrosol is not seen not only for Hurma but also for other olive types. Studies on naturally debittered olive varieties were concentrated on the oleuropein content of these olives since oleuropein is the phenolic compound that gives the bitter taste of the olives. However, as it is observed in this study not only oleuropein but almost all phenolics are affected from this debittering process. This observation is also confirmed by the lower TPC of Hurma variety.

Erkence, Hurma and Gemlik can be differentiated from each other using their phenolics profiles. Since Hurma is Erkence type olive which debitters on the tree, the separation between Erkence and Hurma shows that natural debittering is related to changes in phenolic composition and this phenomenon results in a reduction in phenolic composition of Hurma. As it was hypothesized by other researchers these changes in phenolic composition could be related to the activities of β-glucosidase and esterase enzymes (Jemai et al., 2009). In addition, phenolic profiles of olive types investigated in this study depend on harvest year. Therefore, more data on phenolics content obtained at multiple harvest years will be helpful to enlighten the natural debittering phenomena of olives.

4.6. Oil Content and Fatty Acid Profile of Olive Varieties

4.6.1. Oil accumulation of olive varieties

The oil accumulation behavior observed in olive varieties are provided in Table 4.8.The amount of oil on a dry weight basis varied with cultivar and harvest year as well as harvest time. Higher oil contents are detected in Hurma olive, between the ranges of 14.57- 61.71 % dw in 2011/12 harvest year. In the first harvest year, oil content of Erkence type changed between the ranges of 17.07-65.92% dw and in Gemlik type it was between 12.11-66.82% dw.

Generally, in the second harvest year oil accumulation was really low compared to the first year. For the first two weeks of the maturation, oil content is higher than the first harvest year for all olive types as in Chemlali Gafsa and Chemlali Zarsis olives (Issaoui et al., 2008). While oil content begins to decrease after the second week in Hurma, it decreases after third week in Gemlik and Erkence. Higher amounts of oil are observed in Gemlik type between the ranges of 29.24-58.75% dw. In 2012/13 harvest year, oil content of Hurma changes between the ranges of 15.25-31.04 %dwt and in Erkence it varies between 19.63-46.65%.

It is reported that sugar concentration is proposed as a ripening index for oil accumulation in olives. Since minimum sugar amounts correspond to maximum oil content (Cherubini et al., 2009). In a study about Kadesh and Manzanillo olives, it is found out that mannitol levels and oil accumulations of olives are related (Wodner et al., 1988). However, it was not observed any relation between sugar, polyol and oil content in this study.

Table 4.9. Percent oil content of olive varieties for two harvest years

HURMA	% oil content	ERKENCE	% oil content	GEMLIK	% oil content
2011/12					
H11	34.91	**E11**	28.77	**G11**	12.11
H12	14.57	**E12**	17.07	**G12**	48.59
H13	41.37	**E13**	13.68	**G13**	39.17
H14	43.99	**E14**	44.21	**G14**	59.49
H15	49.91	**E15**	37.98	**G15**	40.59
H16	61.52	**E16**	51.16	**G16**	66.82
H17	61.71	**E17**	62.61	**G17**	64.94
H18	47.46	**E18**	65.92	**G18**	63.94
2012/13					
H21	31.04	**E21**	30.1	**G21**	39.22
H22	28.46	**E22**	26.53	**G22**	58.75
H23	27.43	**E23**	46.65	**G23**	51.91
H24	37.52	**E24**	21.16	**G24**	58.53
H25	21.64	**E25**	19.64	**G25**	40.17
H26	16.21	**E26**	25.79	**G26**	29.24
H27	15.25	**E27**	39.56	**G27**	50.98
H28	19.17	**E28**	28.03	**G28**	44.83

4.6.2. Fatty Acid Profile of Olive Varieties

Fatty acid compositions of olive varieties during two harvest years are listed in Table 4.10.and Table 4.11.A typical GC profile of is shown in Figure 4.27.

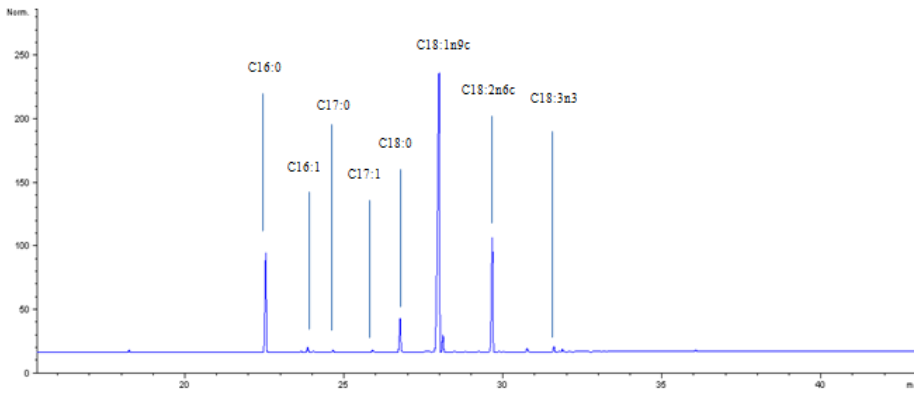

Figure 4.27.GC chromatogram of Hurma belonging to the first week of the first harvest year

For both harvest years, oleic acid is the main fatty acid for all olive types with higher percentages as in Chemlali and Zarsis olives (Issaoui et al., 2008). In the previous studies, as the ripening process goes on, the oleic acid content rises throughout the maturation period (Issaoui et al., 2008). However, it was not observed a linear increasing trend between ripening and oleic acid content of Hurma, Erkence and Gemlik olives in this study. In addition, oleic acid content is lower than the first season for all olive varieties in the second season. As it is shown in Table 4.9, the highest percentage of oleic acid (66.85%) is observed in the 3th week of the maturation for Hurma type for the 2011/12 season and it reaches the highest level at the 6th week of maturation (64.95%) in 2012/13. In the first harvest year, Erkence type has the highest oleic acid among other types and it varies between the ranges of 68.75-71.83%. However, in the second year its range decreases to 60.5-66.82%.In Gemlik type, similar amounts of oleic acid are detected for both harvest years and it is between the ranges of 61.86-65.87% in the first season and 63.05-66.79% in the second season.

Palmitic acid is the other fatty acid that is detected in higher concentrations. Palmitic acid (C16:0) is found between the ranges of 8.55-18.94% dw in Gemlik in 2011/12 season. In Erkence type, its concentration decreases from 13.81 to 11 % throughout the ripening period. This decrease has been observed before by other researchers during maturation (Beltran et al.,2004; Ayton et al.,2007; Manai et al., 2007).While in Hurma, palmitic acid is observed between the ranges of 14.17-15.94% in the first harvest year, it changes between 12.55-14.28% in the second year. There is a decrease in palmitic acid content of Hurma olive until 7th week of maturation after that it increases slightly.

Higher concentrations of linoleic acids (C18:2) are observed in Hurma type in both harvest years. In the first year, it follows an up and down trend during sampling period. Linoleic acid content of Hurma reaches to a maximum of 17.19% in the first year and 22.47% in the second year at the end of the maturation. In sweet Dhokar variety also linoleic acid content (22.29%) was higher compared to other varieties (Rigane et al., 2013). In Gemlik olives, linoleic acid increases at the last three weeks of maturation and it reaches the highest concentration (%15.97) at the last week of ripening in the first season. However, it increases during maturation period in the second season (11.78-16.11%). In Erkence olives, linoleic acid content is almost the same during the first four weeks of maturation. After that, it decreases until the end of maturation (% 14.3-12.80) in the first harvest year. In the second harvest year, higher

content of linoleic acid is observed at beginning of ripening, 18.25%, and then it decreases and reaches to the lowest level (13.56%) at the 6th week of maturation.

Stearic acid has been observed in lower values between 1.66–4.39% for all varieties in both harvest years. According to a study about Tunisian olives, stearic acid is observed at values between 2.05–4.43% and its content does not depend on maturation (Issaoui et al., 2008).

As it can be seen in Table 4.9, palmitoleic (16:1), linolenic (18:3n3), linoleadic (C18:2n6t), behenic (22:0), arachidic (20:0), arachidonic (20:4n6), lignoceric (C24:0), heptadecanoic (C17:0), nervoic (C24:1), cis-11-eicosanoic (C20:1) acids are also detected for both harvest years at lower concentrations. Cis-10-heptadecanoic acid (C17:1) is detected only in the second harvest year.

It was observed that oleic to linoleic acid ratio of Hurma olives are generally lower compared to Erkence and Gemlik for both harvest year and Hurma has higher linoleic acid content compared to other types for two years. (Figure 4.29). This ratio was also lower for sweet Dhokar olive compared to another regular olive variety (Rigane et al.,2013). Therefore, this might be an indication for increased desaturase activity for the conversion of oleic acid to linoleic acid during debittering. Lower MUFA/PUFA of Hurma compared to Erkence and Gemlik olives for both harvest years also strengthen this hypothesis (Figure 4.30). Fatty acid desaturases are the enzyme which catalyzes the formation of double bonds (Figure 4.28).

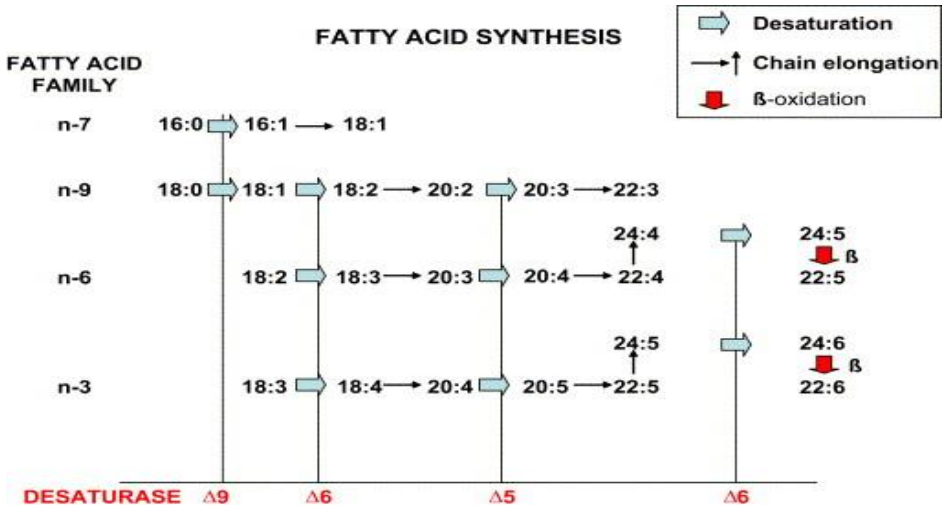

Figure 4.28. General synthesis of unsaturated fatty acids
(Source: sciencedirect.com)

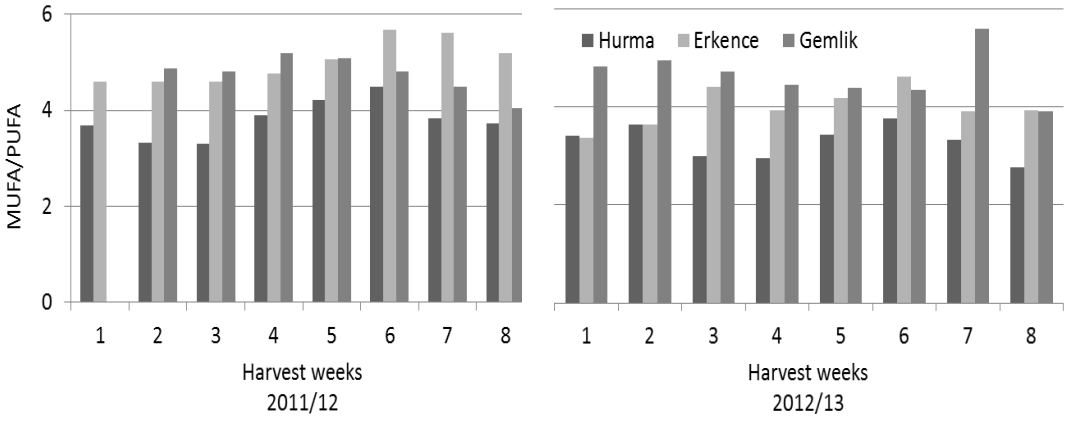

Figure 4.29. MUFA/PUFA ratio of the investigated olives

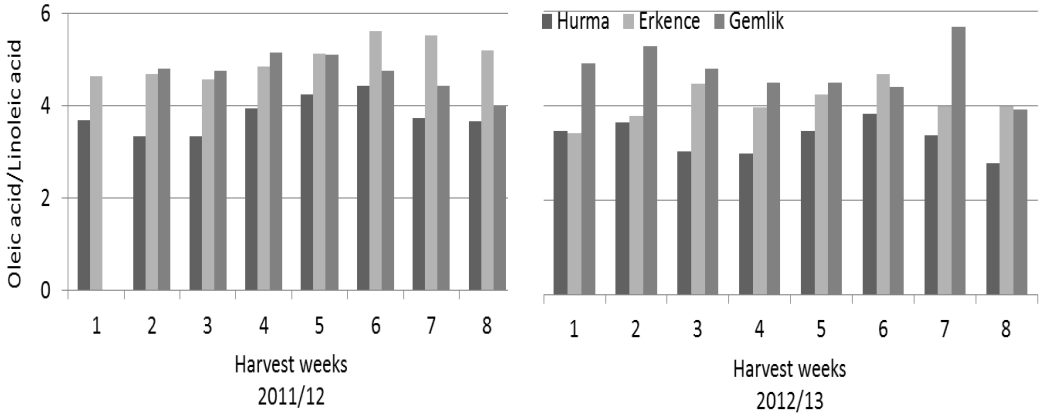

Figure 4.30. Oleic acid/linoleic acid ratio of the investigated olives

To see the differences between varieties, harvest time and harvest year a multivariate classification technique, PCA, was applied to the data. Although R^2 values of the models obtained are not very high PCA plots are still helpful visualizing the differences regarding the olive type, harvest season and year.

For the whole data, a model with 4 principal components and R^2 of 0.611 was obtained and score plot for this model showing the classification of olive types is provided in Figure 4.31. According to this plot, a differentiation could be observed between the first and the second harvest year olives with respect to their fatty acid profiles. First five week samples from Hurma type of 2011/12 harvest year are placed in the left lower quartile of the plot. The later weeks (6^{th}, 7^{th} and 8^{th} weeks) of the first harvest year are totally separated from the rest of the samples and are on the right upper quartile of the plot. There is a clear separation between the first and the second year samples. Higher concentrations of oleic acid are detected in 2011/12 season. Therefore,

both oleic acid and the first harvest year samples are located on the left side of the loading plot (Figure 4.32).Gemlik type for 2012/13 season had higher concentrations of palmitic acid. Therefore, both palmitic acid and second year Gemlik samples are located in the right side of loading plot (Figure 4.31).

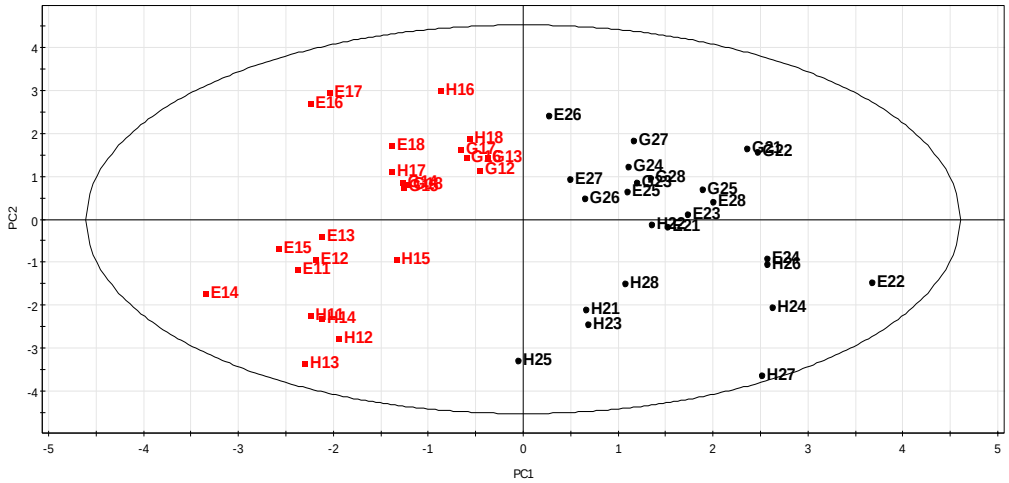

Figure 4.31.Score plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

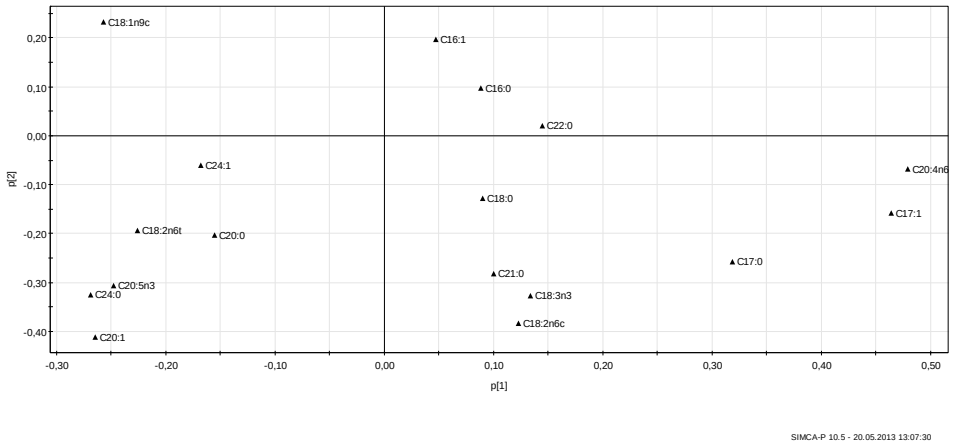

Figure.4.32.Loading plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

To better understand the differences between each type of olives, PCA was run separately for each harvest year and score and loading plots are shown in Fig.4.33 and 34, respectively. PCA constructed for the first harvest year consists of 3 principal components with R^2 of 0.679. Gemlik samples are mostly located around the ellipsoid center. There is a clear separation between Hurma and Erkence and between Gemlik and Erkence with respect to their fatty acid profiles (Figure 4.33). According to loading plot (Figure 4.34), palmitic, palmitoleic and stearic acids are the fatty acids that caused gathering of Gemlik samples at the center of the score plot. Actually, Gemlik type generally have the higher concentrations of these fatty acids compared to others throughout sampling period. Main fatty acid causing separation of Erkence from Hurma and Gemlik is its oleic acid content (Figure 4.34). Oleic acid content of Erkence (66.38-72.19%) is the highest among others during ripening and it increased with harvest time and reached to the highest level at the 7^{th} week. Linolenic and gondoic (20:1n9c) acid contents of Erkence are comparable and higher than Gemlik; therefore, these fatty acids are located between Hurma and Erkence in the loading plot. First 5 week samples of Hurma are located separately from the last 3 weeks since fatty acids such as eicosopentaenoic (20:5n3) and heneicosanoic (21:0) acids exist in small amounts only in Hurma in early period and disappear later. Another fatty acid that causes separation of Hurma from the rest is linoleic acid which is observed in this olive in higher amounts (14.79-18.45%).

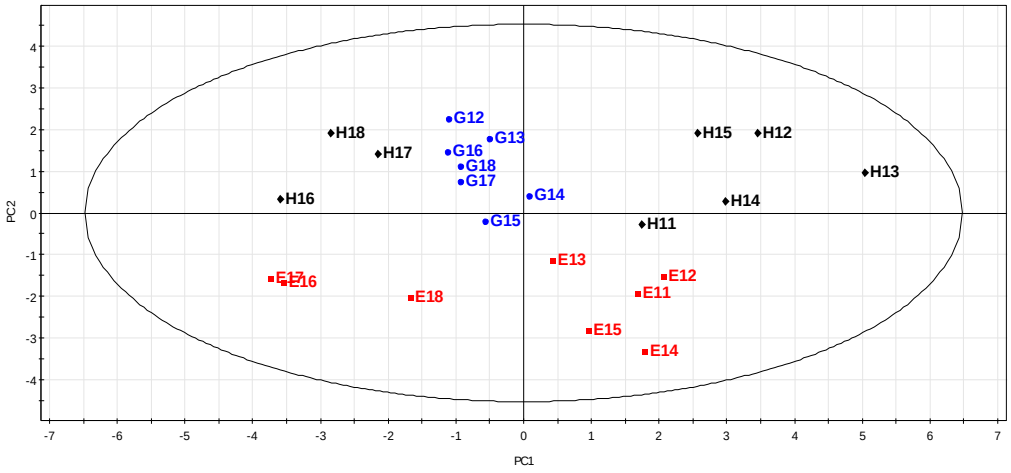

Figure 4.33.Score plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

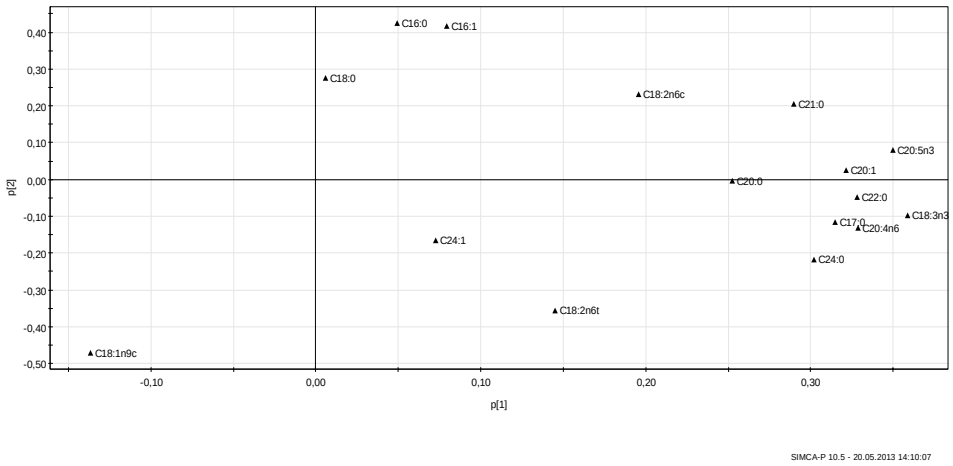

Figure.4.34.Loading plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the first harvest year

PCA model for the second harvest year has R^2 of 0.475 and 2 principal components. PCA in this case provided better classification for Hurma and Gemlik olive types (Figure 4.35). Other than the first week sample of Erkence, Hurma and Erkence samples separated from each other with respect to their fatty acid profiles. Some samples of Erkence and Gemlik types are located away from their groups but it could be still concluded that there is a differentiation between these types of olives. According to loading plot (Figure 4.36), palmitic and palmitoleic acids are the main parameters causing separation of Gemlik as in the first year. Hurma can be differentiated from other olives mainly owing to its higher content of stearic and linoleic acid content. Hurma also had higher linoleic acid in the first harvest year. Erkence and Gemlik have comparable levels of oleic acid in the second year; therefore this fatty acid is located in between Erkence and Gemlik in the loading plot.

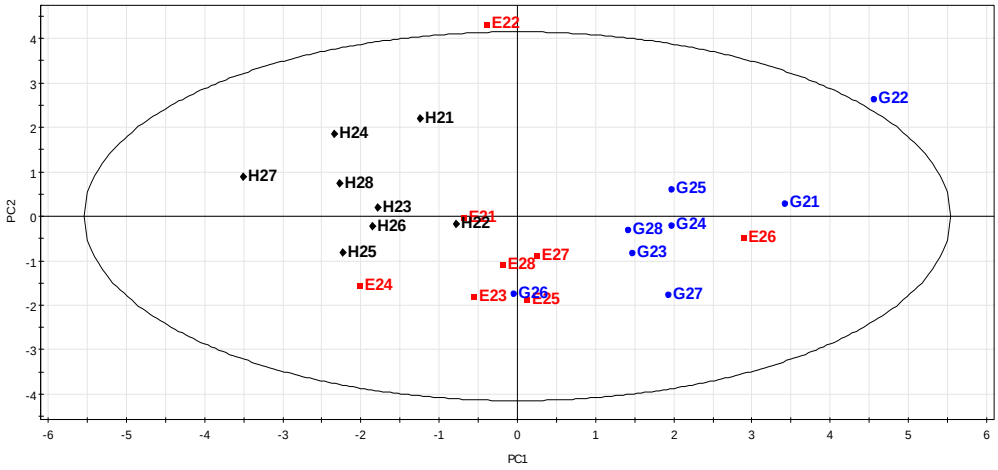

Figure 4.35.Score plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

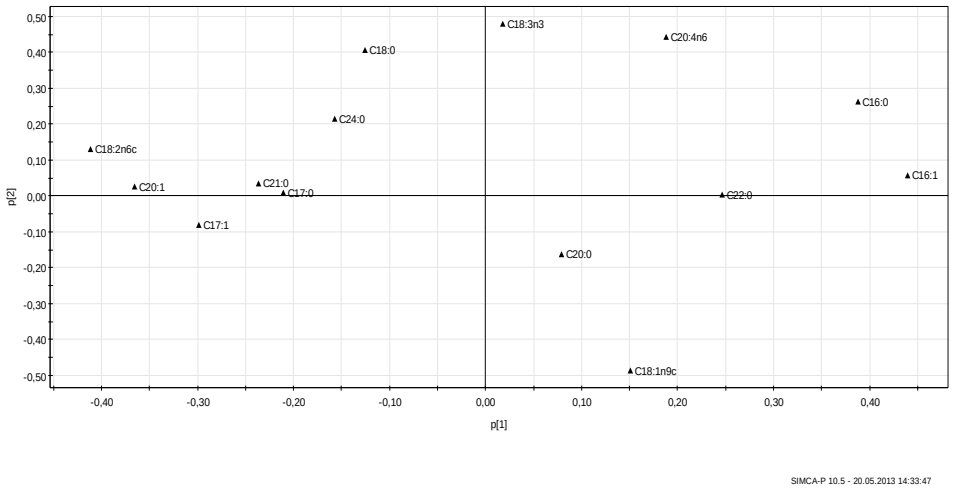

Figure.4.36.Loading plot obtained with PCA for fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for the second harvest year

Table 4.10.Percentage of individual fatty acids of olive varieties (Hurma, Erkence, and Gemlik) for the first harvest year

Olive type	C16:0	C16:1	C17:0	C18:0	C18:1n9c	C18:2n6c	C18:3n3	C18:2n6t	C20:0	C20:1	C20:4n6	C20:5n3	C22:0	C24:0	C24:1
H11	14.64	0.77	0.12	2.72	62.80	17.01	0.30	0.038	0.41	0.85	0.037	0.11	nd	0.07	nd
H12	15.32	0.96	0.11	2.67	60.38	18.04	0.28	nd	0.42	0.86	0.034	0.37	0.11	0.06	0.04
H13	14.17	0.75	0.14	2.96	66.85	19.97	0.33	nd	0.47	0.87	0.042	0.43	0.25	0.06	nd
H14	13.59	0.66	0.12	3.01	64.19	16.25	0.31	0.014	0.47	0.80	0.036	0.25	0.13	0.06	nd
H15	15.9	1.19	0.13	3.01	62.80	14.78	0.27	nd	0.46	0.67	0.034	0.27	0.12	0.05	nd
H16	14.68	0.55	nd	2.62	66.53	15.038	nd	nd	nd	0.55	nd	nd	nd	nd	nd
H17	14.9	0.64	nd	2.85	63.63	16.97	nd	nd	0.37	0.69	nd	nd	nd	nd	nd
H18	15.43	0.66	nd	3.09	62.85	17.19	nd	nd	nd	0.66	nd	nd	nd	nd	nd
E11	13.81	0.58	0.14	2.65	66.50	14.29	0.30	0.042	0.43	0.74	0.046	0.08	0.15	0.05	0.02
E12	13.53	0.58	0.13	2.74	66.38	14.17	0.32	nd	0.45	0.65	0.069	0.19	0.11	0.08	0.20
E13	13.89	0.55	0.16	3.07	65.78	14.37	0.29	nd	0.37	0.62	nd	nd	0.10	0.07	0.45
E14	12.13	0.47	0.14	2.62	68.59	14.15	0.30	0,051	0.41	0.69	0.023	0.11	0.11	0.14	0.037
E15	12.34	0.50	0.15	2.63	69.01	13.43	0.32	0,04	0.41	0.67	0.024	0.064	0.11	0.06	0.044
E16	11.60	0.27	nd	2.66	71.82	12.80	nd	nd	0.30	0.58	nd	nd	nd	nd	nd
E17	11	0.34	nd	2.86	72	13	nd	nd	0.30	0.51	nd	nd	nd	nd	nd
E18	11.81	0.38	0.12	2.33	70.59	13.58	0.20	nd	0.30	0.58	nd	nd	0.09	nd	nd
G11	18.94	1.17	nd	2.86	62.90	12.17	nd	nd	0.63	1.62	nd	nd	nd	nd	nd
G12	16.79	1.17	0.12	3.33	61.86	12.87	0.20	nd	0.48	0.59	nd	nd	nd	nd	nd
G13	16.79	1.40	0.12	2.91	63.90	13.43	0.26	nd	0.41	0.59	nd	nd	0.11	nd	nd
G14	15.70	1.28	0.12	2.82	65.86	12.78	0.26	nd	0.43	0.59	nd	nd	0.11	0.09	nd
G15	8.55	1.17	0.11	3.16	65.33	12.79	0.25	nd	0.46	0.54	nd	0.14	0.11	nd	nd
G16	15.56	0.99	0.10	3.60	64.80	13.62	0.18	nd	0.41	0.51	nd	nd	0.08	0.03	nd
G17	15.88	1.24	0.09	2.27	64.59	14.57	0.23	nd	0.32	0.549	nd	nd	0.08	0.03	nd
G18	15.48	0.95	0.01	2.67	63.89	15.97	0.2	nd	0.56	0.55	nd	nd	0.07	0.03	nd

Table 4.11.Percentage of individual fatty acids of olive varieties (Hurma, Erkence, and Gemlik) for the second harvest year

Olive type	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9c	C18:2n6c	C18:3n3	C20:0	C20:4n6	C20:1	C21:0	C22:0	C24:0
H21	14.28	0.53	0.20	0.16	4.39	61.012	17.64	0.24	0.36	0.39	0.65	nd	0.14	0.058
H22	13.84	0.55	0.21	0.22	3.7	62.82	17.24	0.23	0.31	0.16	0.56	nd	0.14	nd
H23	13.65	0.53	0.17	0.35	2.76	60.66	19.99	0.21	0.41	0.23	0.64	nd	0.38	0.063
H24	13.76	0.46	0.27	0.42	2.75	60.30	20.24	0.31	0.31	0.38	0.61	0.013	0.13	0.046
H25	12.7	0.44	0.18	0.27	2.30	63.32	18.36	0.24	0.35	0.29	0.69	0.14	nd	nd
H26	12.28	0.46	0.19	0.54	3.15	64.95	16.99	0.22	0.33	0.50	0.61	0.06	nd	nd
H27	12.55	0.45	0.30	0.37	2.93	62.99	18.65	0.42	0.32	0.30	0.71	0.25	nd	nd
H28	13.08	0.51	0.15	0.17	2.70	62.45	22.48	0.28	0.28	0.33	0.72	nd	nd	nd
E21	13.68	0.49	0.21	0.20	2.46	62.50	18.28	0.21	0.32	0.38	0.57	nd	nd	nd
E22	15.18	0.58	0.3	0.34	5.22	60.49	16.03	0.47	0.31	0.54	0.53	nd	nd	nd
E23	12.59	0.57	0.31	0.38	2.69	66.81	15.02	0.23	0.33	0.29	0.6	nd	0.28	nd
E24	13.029	0.45	0.59	0.43	2.86	65.10	16.48	0.19	0.33	0.23	0.58	nd	nd	nd
E25	12.96	0.46	0.21	0.23	2.57	66.36	15.7	0.21	0.34	0.245	0.54	nd	0.32	nd
E26	15.29	1.24	nd	nd	2.42	63.24	13.56	0.16	0.34	0.36	0.47	nd	nd	nd
E27	12.60	0.59	nd	0.19	2.48	64.49	16.24	0.26	0.32	0.29	0.51	nd	nd	nd
E28	12.57	0.489	0.19	0.36	2.48	64.17	16.10	0.21	0.32	0.33	0.51	nd	0.36	nd
G21	16.46	1.42	0.23	0.17	3.003	63.48	12.99	0.20	0.34	0.38	0.51	nd	0.79	nd
G22	16.66	1.46	0.10	0.08	2.65	64.37	12.26	0.48	0.33	0.65	0.46	nd	0.47	nd
G23	15.57	1.15	0.14	0.31	2.55	64.92	13.60	0.26	0.39	0.32	0.61	nd	0.15	nd
G24	16.24	1.35	0.12	0.22	1.67	64.30	14.34	0.28	0.32	0.36	0.62	nd	0.15	nd
G25	15.69	1.25	0.26	0.17	2.48	64.01	14.28	0.30	0.35	0.47	0.56	nd	0.14	nd
G26	12.75	0.48	0.14	0.24	2.83	66.79	15.17	0.24	0.37	0.28	0.58	nd	0.10	nd
G27	14.63	1.18	0.20	0.29	2.34	66.63	11.78	0.22	0.35	0.28	0.51	nd	0.13	nd
G28	15.42	1.10	0.12	0.21	2.39	63.05	16.10	0.21	0.35	0.32	0.54	nd	0.17	nd

As a result, it can be concluded that the fatty acid profile provides differentiation with respect to olive varieties and harvest year. The effect of harvest year is identified as an important factor determining fatty acid profile of olive varieties investigated in this study.

4.7. PCA of Combination of Various Parameters

In order to better understand and explain the differentiation of olives in terms of variety, harvest year and harvest time PCA analysis with different combinations were conducted.

Firstly, PCA constructed with sugar and organic acid data for the both harvest year consists of 3 principal components with R^2 of 0.639. As it is seen in Figure 4.37 there is a good separation between harvest years with combined sugar and organic acid data. Sucrose, glucose and succinic acid are the components that differentiate the second harvest year and they are located in the right side of loading plot (Figure 4.38).In the first harvest year, all samples of Hurma separated from other types and located in the right lower quartile of the plot. There is no separation between Erkence and Gemlik olives in the first harvest year using both organic acid and sugar data. In the second harvest year, there is no clear separation between olive types (Figure 4.37).

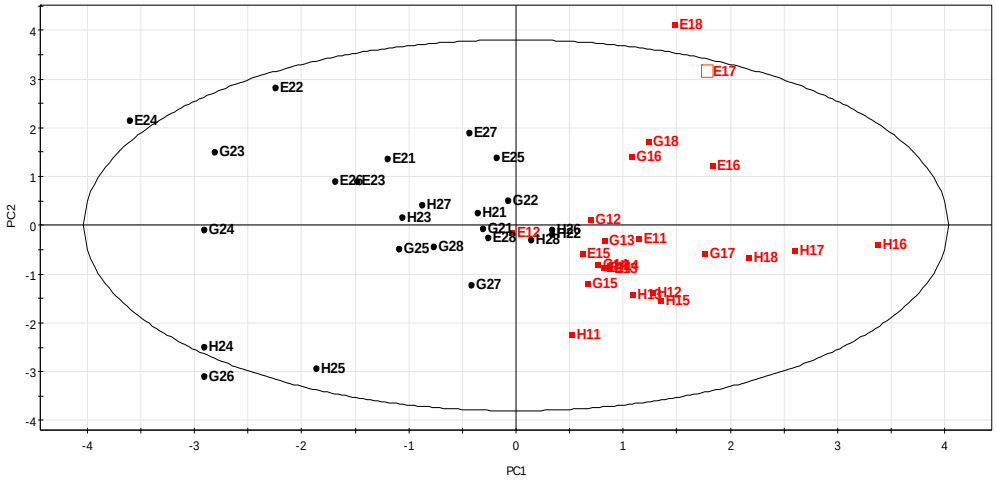

Figure 4.37.Score plot obtained with PCA for sugar and organic acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

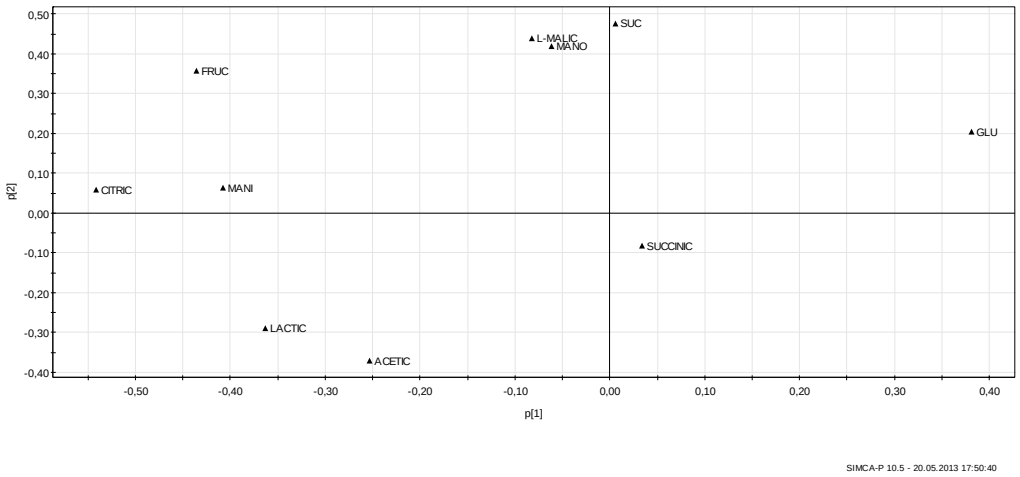

Figure.4.38.Loading plot obtained with PCA for sugar and organic acid data of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

PCA constructed with sugar and fatty acid data for the both harvest year consists of 4 principal components with R^2 of 0.605. As it is seen in Figure 4.39 there is a good separation between harvest years by combining data of sugar and fatty acid. Sucrose, glucose and oleic acid are the main components that differentiate the second harvest year and they are located in the right side of loading plot (Figure 4.40). First five week samples of both Hurma and Erkence are located in the right upper part of the plot. All samples of Gemlik belong to the first year is located on the horizontal axis of the plot. It was not observed a separation in terms of olive varieties using combined sugar and fatty acid data in the first season. In the second season, on the other hand, Hurma olives are mostly located on the left upper quartile of the score plot while Erkence mixed with Gemlik are on the lower left part of the plot. Mainly, linoleic and linolenic acids are responsible from this differentiation according to the loading plot.

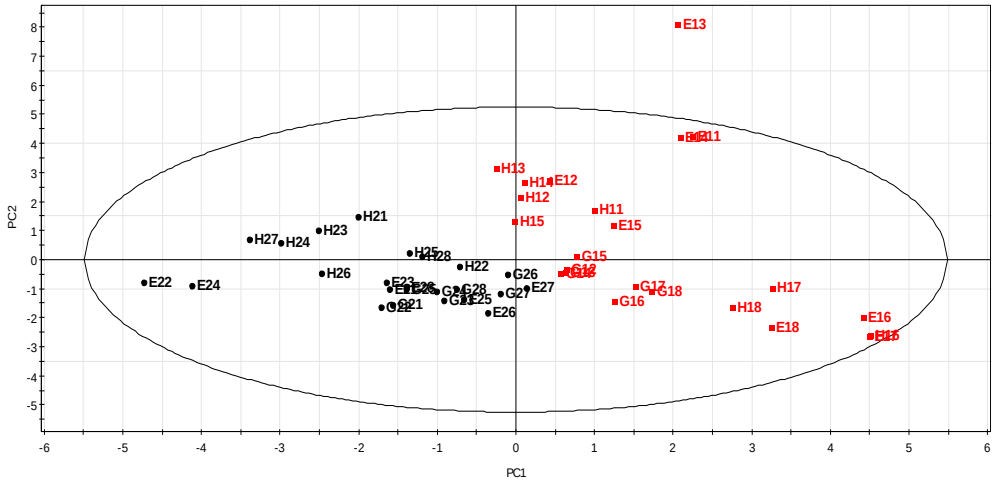

Figure 4.39.Score plot obtained with PCA for sugar and fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkence, G:Gemlik, first number after the letter is the harvest year and second number is the harvest week)

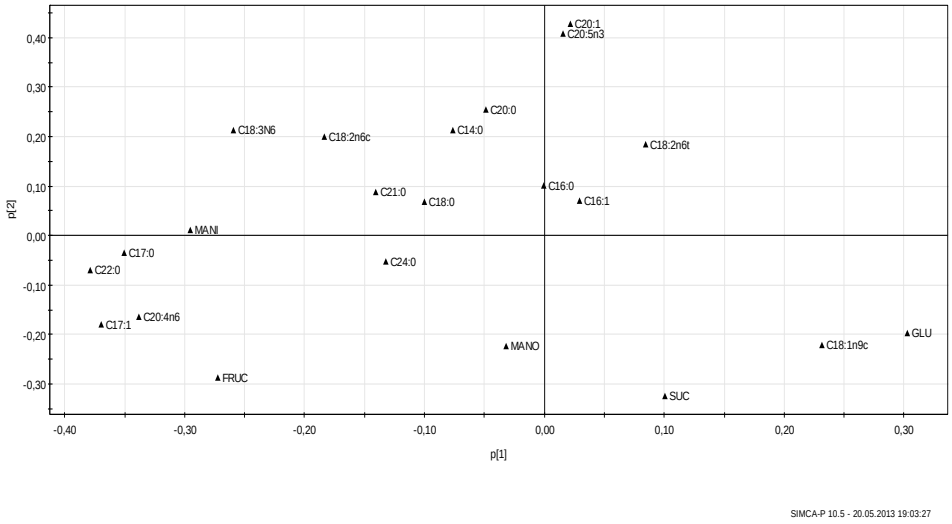

Figure.4.40.Loading plot obtained with PCA for sugar and fatty acids of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

Lastly, PCA was conducted with whole data including sugars, organic acids, phenolics, total phenol content, and fatty acids for both harvest years. The model consists of 3 PCs and R^2 is 0.418.All samples of Hurma type belong to the first year are located around the horizontal axis of left side (Figure 4.41). Linolenic and linoleic acids, mannitol and acetic acid are in the same place with Hurma in the loading plot and these compounds differentiate this olive from the other types (Figure 4.42). Both Gemlik and Erkence samples are in the left quartile of the plot. In the second harvest year, last three weeks' samples of all types are located in the left upper part of the plot. Remaining samples belong to all types are in the left lower quartile of the plot.

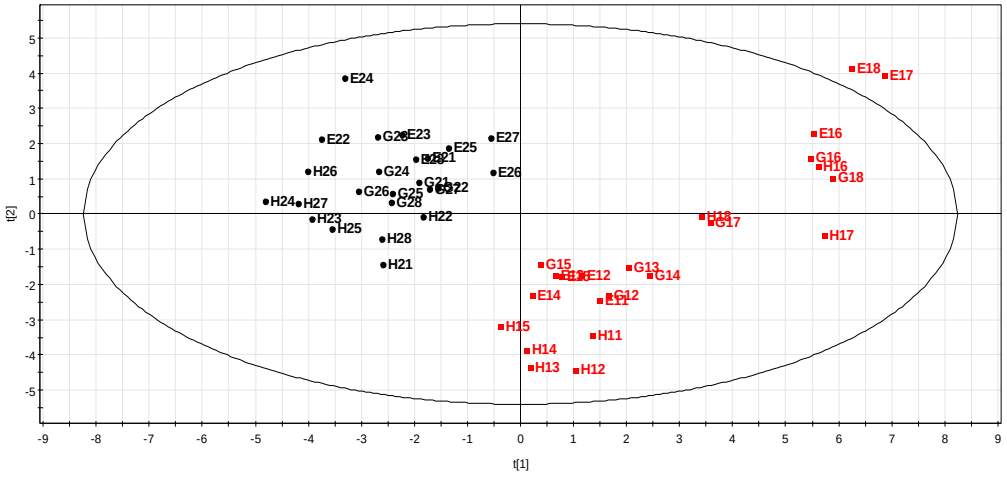

Figure 4.41.Score plot obtained with PCA for whole data of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years (H: Hurma, E: Erkence, G: Gemlik, first number after the letter is the harvest year and second number is the harvest week)

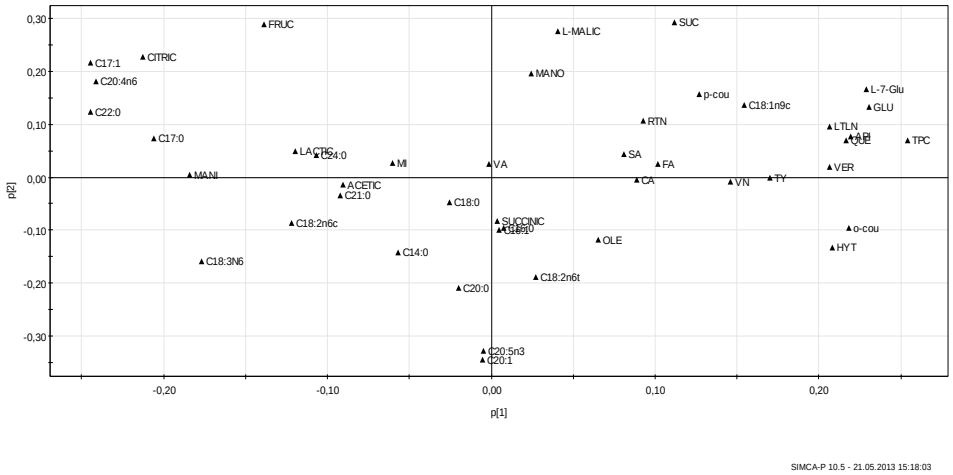

Figure.4.42.Loading plot obtained with PCA for whole data of Hurma, Erkence and Gemlik types of olives during 8 weeks of maturation for both harvest years

In conclusion, PCA analysis of several combinations of chemical parameters resulted in a differentiation based on the harvest year but not on variety.

CHAPTER 5

CONCLUSIONS

In this study changes in the chemical compositions of Hurma (naturally debittering Erkence), Erkence and Gemlik olives throughout their maturation period were investigated for two harvest years and some chemical compositional differences between Hurma and other types were determined.

It is found out that olive variety, harvest time and year have significant effects on pH and a_w statistically. Total phenol content and concentration of individual phenolic compounds of Hurma olive are lower than Erkence and Gemlik olives. PCA could separate Hurma from Erkence and Gemlik olives with respect to phenolic content and phenolic profile. In addition, harvest year is also an important parameter that for differentiation of olives with respect to their phenolic profile.

Glucose and mannitol are detected as the main sugar and polyol for all olive types. No differentiation was obtained in terms of olive type depending on the sugar content according to PCA. However, there is some separation with respect to harvest year when sugar profiles of olive types is analyzed statistically.

Main organic acids for the olives investigated are the citric and malic acids. Gemlik variety has the highest citric acid content compared to other types for both harvest years. Total organic acid composition of olives in the second harvest year is significantly higher compared to the first year. There was no differentiation of olive types depending on their organic acid content as in the sugars; however, organic acid profile provided a clear separation between harvest years.

Oleic acid is identified as the main fatty acids for Hurma, Erkence and Gemlik as expected. Hurma has higher content of linolenic acid in both harvest years compared to other types. Fatty acid profile allowed a differentiation with respect to variety and also harvest year according to PCA.

It was hypothesized that the changes during natural debittering of olives could be related to the activities of β-glucosidase and esterase enzymes and cause a decrease in phenolic compounds (Jemai et al., 2009). However, as this study shows not only phenolic compounds but also fatty acids are affected from this process since there is a

separation between Erkence and Hurma olives depending on their fatty acid profiles. This difference could be associated with the esterase activity. In addition, decreased oleic acid to linoleic acid ratio might be an indication of increased desaturase activity.

In a study about microbiological characterization of Hurma olive, it was found out that microbial population on Hurma olive is higher compared to Erkence and Gemlik olives (Karslı, 2013).This result can be also associated with increased activity of enzymes that might originate from microorganisms for Hurma type. During ripening period, microbial growth on Hurma may lead to increases in the enzyme activities which degrade phenolic compounds and cause debittering stages of Hurma olive

In conclusion, Hurma, Erkence and Gemlik varieties could be differentiated in terms of their fatty acid and phenolic profiles; however, sugar and organic acid content of these olives do not provide a varietal separation. Harvest season has a significant effect on all chemical parameters; therefore, it needs to be taken into consideration on studies about chemical composition of olive varieties.

As the future study, activity of enzymes involved in natural debittering of Hurma olive could be investigated during maturation period to further enlighten the sweetening on the tree phenomena. In addition, investigations about packaging options and changes during storage of Hurma olive will help to increase the quality and shelf-life of this special olive.

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APPENDIX A

CALIBRATION GRAPHICS

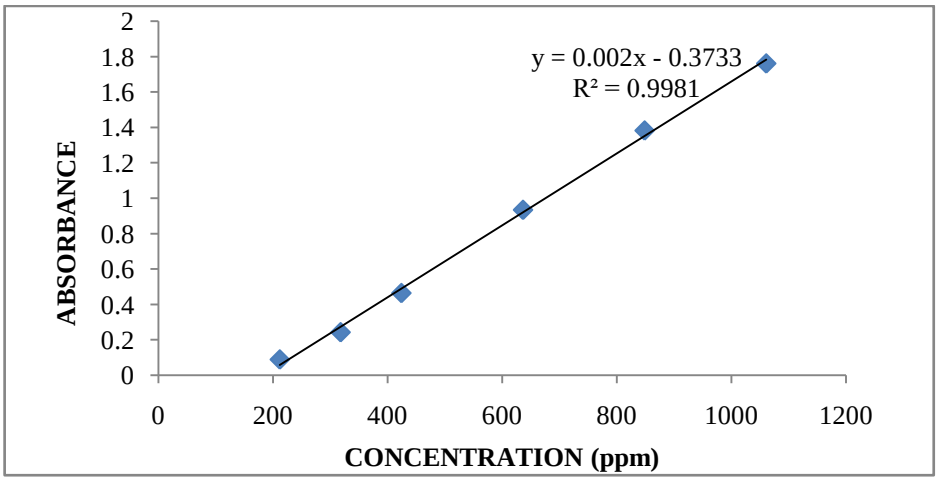

Figure A.1. Calibration curve of gallic acid

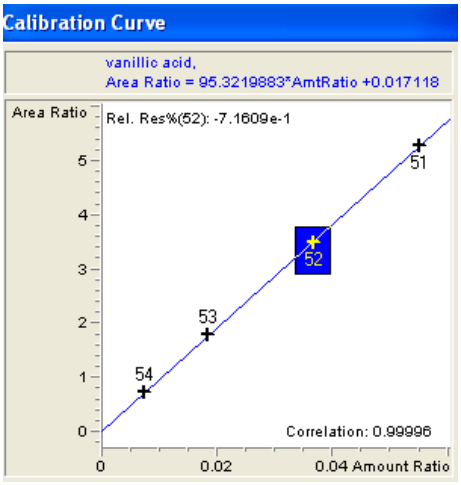

Figure A.2. Calibration curve of vanilic acid

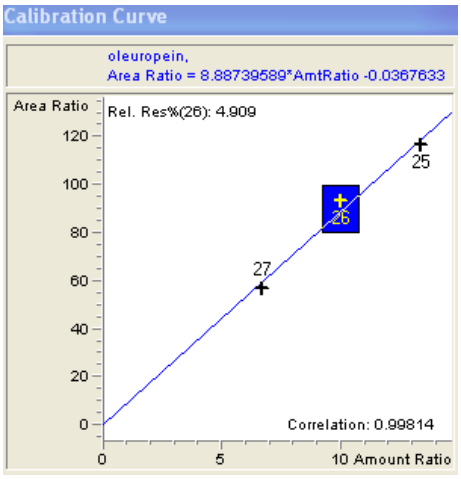

Figure A.3. Calibration curve of oleuropein

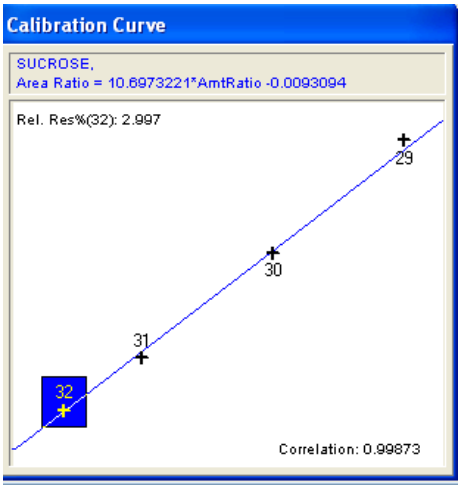

Figure A.4 .Calibration curve of sucrose

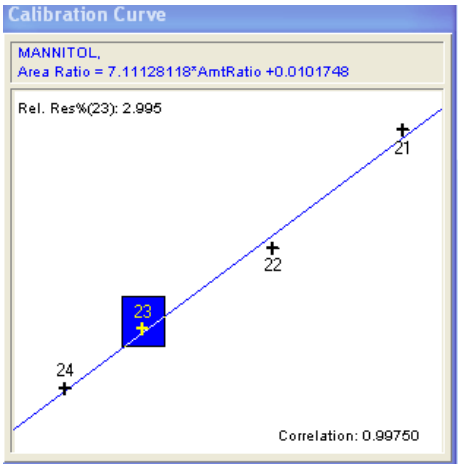

Figure A.5. Calibration curve of mannitol

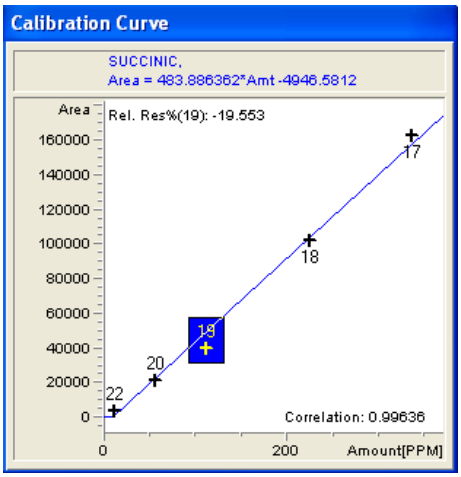

Figure A.6. Calibration curve of succinic acid

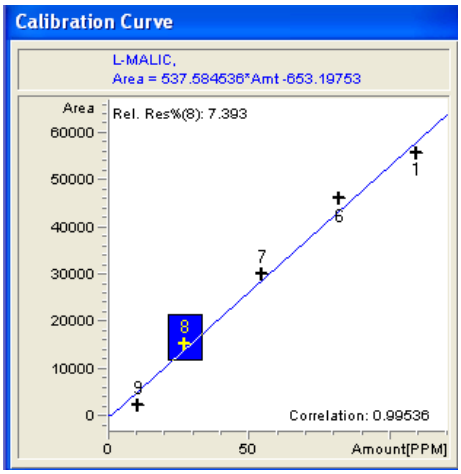

Figure A.7. Calibration curve of L-malic acid