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EVALUATION OF OLIVE OILS PRODUCED FROM OLIVES OF GAZIANTEP REGION IN TERMS OF DELTA-7 STIGMASTENOL CONTENT

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Mustafa Baki KAPUDERE

ABSTRACT

EVALUATION OF OLIVE OILS PRODUCED FROM OLIVES OF GAZIANTEP REGION IN TERMS OF DELTA-7 STIGMASTENOL CONTENT

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M.Sc. in Food Engineering

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Olive oil obtained from fatty olive fruits is directly consumable after suitable processing. Olive oil has a high nutritional and commercial value. Plant sterols, or phytosterols, belong to the group of the desmethylsterols make up the greatest proportion of the unsaponifiable fraction of vegetable oils. Sterols are the most important parameter in the olive oil composition for determining purity.

Maturity index, free acidity, fatty acid composition and sterol composition analysis were done for 39 samples. The average percentage of saturated fatty acids has been identified as 19.816 % in Kilis Yağlık variety, 20.249 % in Nizip Yağlık variety and 20.089 % in Yuvarlak Halhalı variety. In Gemlik and Ayvalık varieties, saturated fatty acids have been determined 16.899 %, and 16.974 %, respectively.

The percentage of monounsaturated fatty acids has been detected as the highest ratio (76.166 %) in Gemlik variety. In addition, the percentage of polyunsaturated fatty acids was the biggest with the ratio of 17.027 % in Yuvarlak Halhalı variety. The percentage of Δ7-Stigmastenol was 0.47 % and 0.46 % in Nizip Yağlık and Kilis Yağlık varieties, respectively, those were higher than Gemlik and Ayvalık varieties. Ayvalık (1793 mg/kg) and Kilis Yağlık (1541 mg/kg) varieties were observed to be rich from the point of view of total sterol content.

Keywords: Olive oil, Δ7-Stigmastenol, olive, Gaziantep

ÖZET

GAZİANTEP BÖLGESİNDEKİ ZEYTİN ÇEŞİTLERİNDEN ÜRETİLEN ZEYTİNYAĞLARININ DELTA-7 STİGMASTENOL İÇERİĞİ AÇISINDAN DEĞERLENDİRİLMESİ

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Zeytin meyvesinden elde edilen zeytinyağı uygun işleme teknikleriyle doğrudan tüketime uygundur. Zeytinyağı yüksek besleyici özelliğe ve ekonomik değere sahiptir. Bitkisel steroller veya fitosteroller bitkisel yağların sabunlaşmayan kısmının en büyük bölümünü oluşturan desmetilsteroller grubunda yer alır. Steroller zeytinyağının saflığının belirlenmesinde en önemli parametredir.

Toplanan 39 örnekte olgunluk indeksi, serbest asitlik, yağ asitleri kompozisyonu ve sterol kompozisyonu analizleri yapılmıştır. Doymuş yağ asitleri yüzdesi Kilis Yağlık çeşidinde ortalama %19,816, Nizip Yağlık çeşidinde %20,249, Yuvarlak Halhalı çeşidinde %20,089 olarak tespit edilmiştir. Gemlik ve Ayvalık çeşitlerinde ise sırasıyla %16,899 ve %16,974 olarak belirlenmiştir.

Tekli doymamış yağ asitleri yüzdesi %76,166 oranı ile en yüksek Gemlik çeşidinde tespit edilmiştir. Ayrıca çoklu doymamış yağ asitleri yüzdesi Yuvarlak Halhalı çeşidinde ortalama %17,03 oranı ile en yüksek bulunmuştur. Δ7-Stigmastenol yüzdesinin Nizip Yağlık çeşidinde ortalama %0,47, Kilis Yağlık çeşidinde ortalama %0,46 değerleri ile Gemlik ve Ayvalık çeşitlerine kıyasla yüksek olduğu tespit edilmiştir. Toplam sterol içeriği açısından Ayvalık (1793 mg/kg) ve Kilis Yağlık (1541 mg/kg) çeşitlerinin daha zengin olduğu gözlenmiştir.

Anahtar Kelimeler: Zeytinyağı, Δ7-Stigmastenol, zeytin, Gaziantep

To my son

Muhammet Talat KAPUDERE

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ABBREVIATIONS

ANOVA	Analysis of Variance
BOO	Blended Olive Oil
COPO	Crude Olive-Pomace Oil
EC	European Comission
EVOO	Extra Virgin Olive Oil
FA	Free Acidity
FAME	Fatty Acid Methyl Ester
FID	Flame Ionization Detector
G	Gaziantep
GC	Gas Chromatograpy
IOOC	International Olive Oil Council
K	Kilis
LVOO	Lampante Virgin Olive Oil
MI	Maturity Index
MUSFA	Monounsaturated Fatty Acid
N	Nizip
OO	Olive Oil
OPO	Olive-Pomace Oil
PUSFA	Polyunsaturated Fatty Acid
ROO	Refined Olive Oil
ROPO	Refined Olive-Pomace Oil
SFA	Saturated Fatty Acid
TFC	The Turkish Food Codex
TLC	Thin Layer chromatography
VOO	Virgin Olive Oil

1. INTRODUCTION

1.1. Olive Oil

Olive oil (OO) is the product of olive fruits. OO can be consumed directly after proper extraction process. The olive tree have been raised in Mediterranean countries for very long time. These countries have supplied 90 % of the world olive oil requirement (Arvanitoyannis and Vlachos, 2007).

The most olive oil producing country is Spain, with 40 % of world production. Other important olive oil producing countries are Italy, with 24 % of total production, Greece with 12 % of total production, followed by Tunisia and Turkey 7% and 4% respectively. Turkey has 112,000 tons of olive oil production yearly and about 70% of total production is exported. In the Anatolia regions of Turkey, olive oil production is done in Aegean, Marmara, Mediterranean and Southeastern parts (Arslan D. and Schreiner M. 2012).

Olive oil produced from mature fruits and directly consumable unlike other vegetable oils without refination process. Olive oil is also used in cosmetic and pharmaceutical industries.

1.1.1. Olive varieties in Gaziantep region

Eğriburun, Halhalı Çelebi, Hamza Çelebi, Kalembezi, Kan Çelebi, Tesbih Çelebi, Yağ Çelebi, Yağlık Çelebi, Yuvarlak Halhalı, Yün Çelebi, Nizip Yağlık, Kilis Yağlık olive varieties were grown around the Gaziantep region, while Ayvalık and Gemlik varieties have started to cultivate with the subvention given by the Ministry of Food, Agriculture and Stockbreeding.

Yuvarlak Halhalı variety is usually considered to table olive (Figure 1.1.). Their fruits size is large but oil content is low.

Figure 1.1. Yuvarlak Halhalı olive variety (Source: Özilbey, 2011)

Kilis Yağlık variety (Figure 1.2.) is cultivated a wide area including Kahramanmaraş, Şanlıurfa, Mardin, Gaziantep and Kilis provinces. Fruit size of Kilis Yağlık is small but oil content is very high.

Figure 1.2. Kilis Yağlık olive variety (Source: Özilbey, 2011)

Nizip Yağlık variety (Figure 1.3.) is cultivated a wide area including Kahramanmaraş, Mardin, Gaziantep and Kilis provinces. Nizip Yağlık olive fruits are processed for table olive as well as olive oil.

Figure 1.3. Nizip Yağlık olive variety (Source: Özilbey, 2011)

Gemlik variety (Figure 1.4.) is cultivated widely in Marmara region. Gemlik variety has cultivated a wide area in Turkey because of its high rooting property. Its

fruits are consumed especially table olive, but these fruits may be processed as olive oil, if the fruits are not suitable for table olive production.

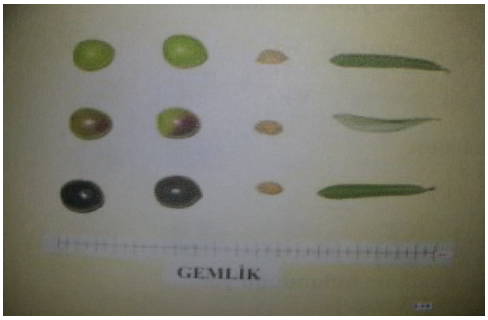

Figure 1.4. Gemlik olive variety (Source: Özilbey, 2011)

Ayvalık variety (Figure 1.5.) are cultivated widely in North Ege region. Ayvalık variety were cultivated a wide area in Turkey including Akdeniz and Güneydoğu Anadolu regions. Its fruits are especially processed as olive oil, but table olive is produced also. Ayvalık variety has large fruit size and contains high oil content.

Figure 1.5. Ayvalık olive variety (Source: Özilbey, 2011)

1.2. Properties of Olive Oil

Chemical composition of olive oil is composed of major and minor components. Triacylglycerol constitutes more than 98% of the major components. Aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds, antioxidants and more are included in the group of minor compounds and constitute about 2 % of total oil weight (Servili, et al., 2002).

1.2.1. Fatty acid profile

Fatty acids in olive oil that forms triglycerides are oleic, palmitic, linoleic, stearic and palmitoleic acids, however, linolenic, arachidic, behenic, lignoceric, eicosenoic, heptadecanoic and heptadecenoic acids are found in small amounts (International Olive Oil Council (IOOC), 1997). The variation on fatty acid composition is important factor for industrial use or human consumption. If the oleic acid content is more than other fatty acids, oils are more stable than others and assists to decrease the risk of cardiovascular diseases in humans (Msaada et al., 2009).

The amount of unsaturated fatty acids in olive fruit that planted in cool regions are more than the dry and warm regions. In addition, polyunsaturated fatty acids are very important for human nutrition. The fatty acid composition of olive oils varies with the region of cultivation and climate (Ben Temime et al., 2006).

The essential fatty acids are included in polyunsaturated fatty acids. Fatty acid composition is a useful indicator of purity or the presence of oils other than olive oil. The adulteration in olive oil with other cheaper oils under the limit of 5% can not be detected by using fatty acid composition. The predominant fatty acid in olive oil is the (mono unsaturated fatty acid) oleic acid (18:1, n-9) with percentages ranging from 56% to 84%, while the polyunsaturated fatty acid (poly unsaturated fatty acid) linoleic acid (18:2, n-6) is usually found at percentages between 3% and 21% (Cicero et al., 2008).

Fatty acid composition is influenced with the stability of oils, and the rancidification is contributed by polyunsaturated fatty acids of many oils. Fatty acid composition has been found to be responsible for the odors and flavors as a quality parameter (Leon et al., 2004).

1.2.2. Sterols

Sterols are important constituents of cell membrane and not only animals but also plants produce them. All sterols have steroid skeleton but they differ on the side chain. β-sitosterol is the most abundant sterol in plants and campesterol and stigmasterol is also found in large amounts (Clifton P., 2002).

Sterols are the most important constituents of unsaponifable part of the oil. Sterol composition is the most important criteria for determining purity of the olive oil (Bayrak et al. 2010).

The nutritional and chemical value of extra virgin olive oils (EVOO) is related with the presence of lots of components including antioxidants and sterols. Antioxidants and sterols and together with other minor components, contribute to the oxidative stability of the oil. However, the reaction mechanisms of some of these substances are not completely known (Cercaci et al., 2007). Many researchers mentioned about health benefits of plant sterols.

The sterols that found in plant origin oils are 4-monomethylsterols, 4-desmethylsterols, and 4,4'-dimethylsterols (triterpene alcohols) (Kochhar, 1983).

Plant sterols belong to the desmethylsterols steroid alcohols group present in all living organisms except bacteria. The contents of these sterols in different olive oils are limited by regulations established by the European Union, the IOOC, and the Codex Alimentarius of the FAO/ WHO, to control against fraud (Sanchez, et al., 2004).

Phytosterols are biologically active constituents of all vegetal foods. Plant sterols are alcohols that contain 28 or 29 carbon and they look like cholesterol from the point of view of structure and function (Figure 1.6). Phytosterols are in group of desmethylsterols steroid alcohols that exist in living organisms except bacteria. The sterol composition of various olive oils is determined and the legal limits regulated by the European Union, the IOOC, and the Codex Alimentarius of the FAO/ WHO, to prevent against adulteration (Lagarda et al., 2006).

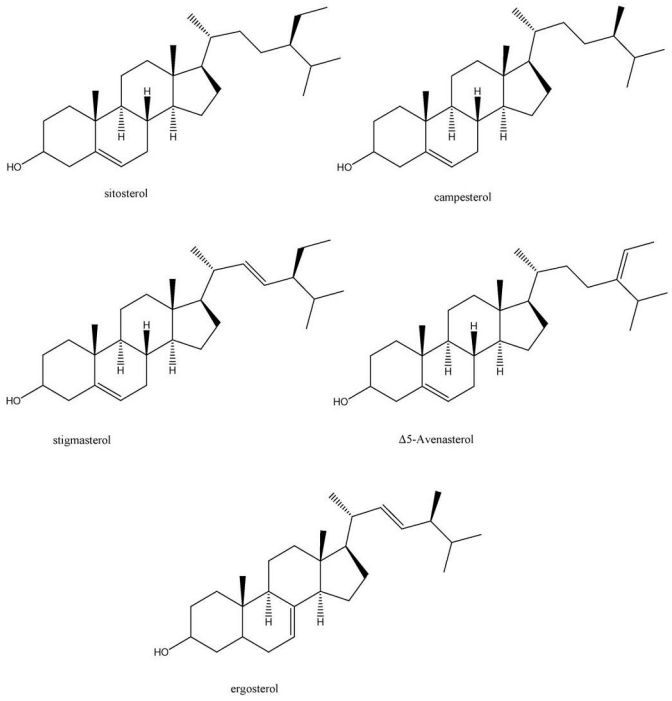

Figure 1.6. Structures of most common phytosterols (Source: Lagarda et al., 2006)

1.2.2.1. Types in olive oil

High amounts of olive oil is consumed by Mediterranean people and this means that high amounts of β-sitosterol, Δ5-avenasterol and campesterol consumption. In addition, several other sterols exist in olive oil such as cholesterol, brassicasterol, stigmasterol, clerosterol, sitostanol, Δ7-stigmastenol and Δ7-avenasterol (Alves et al. 2005).

1.2.2.2. Importance in terms of nutrition

Many clinical studies show that when a diet is involved in plantsterols or if phytosterols are taken as a supplement, blood cholesterol level is reduced by inhibiting absorption of cholesterol from the small intestine. Plant sterols also have antiinflammatory, antibacterial, antifungal, antiulcerative, antitumural activities and antioxidant activities (Alves et al., 2005).

The important constituents of cell membrane are sterols. The structure of phytosterols are similar with cholesterol steroid skeleton. Phytosterols are present in the form of both free and esterified. A diet that contain plant sterols caused to decrease in level of plasma cholesterol in living organisms. The esterified form of plant sterols are also used as cholesterol inhibitor (Clifton, 2002).

1.2.2.3. Importance in terms of quality

The major part of the unsaponifiable constituents of the olive oils are sterols, that have a characteristic composition and used to determine genuineness or adulteration (Alves et al., 2005).

Phytosterols have important health effects (Mailer et al., 2010). The International Olive Oil Council set limits according to natural levels for different olive oil types. If the sterol composition of an olive oil does not fit the regulations it can be said that the olive oil is not pure. The required sterol profile (as % of total sterols) is as follows: The content of cholesterol and Δ7-stigmastenol should not exceed 0.5% according to regulations. The level of brassicasterol is ≤0.1%, campesterol ≤4.0%, stigmasterol ≤campesterol, ∑β-Sitosterol (the sum of beta-sitosterol, Δ5-avenasterol, Δ5-23-stigmastadienol, clerosterol, sitostanol, Δ5-24-stigmastadienol)≥93.0% (IOOC, 2009). The Turkish Food Codex (TFC) has also set the same limits for sterol composition (TFC, Regulation No: 2010/35).

The oil quality and composition factors are cultivar, extraction method, origin, climatic conditions, maturity degree and rainfall and these also effect biosynthesis. The sterol composition as a part of unsaponifiable component is significant for determination of the oil authenticity (Firestone D., 2005). The campesterol/stigmasterol ratio is an index of quality of an oil (Guillaume et.al., 2010).

1.2.2.4. Use as marker for adulteration

Olive oil is adulterated with cheaper oils because of its high price (Passaloglou-Emmanouilidou, 1981). Different vegetable oils show a large variability of sterol compositions. As olive oil has a fairly stable sterol composition, this enables a relatively reliable detection of any adulterations with other types of vegetable oils. This fraction is determined by gas chromatography and it is not a quality measurement, it is an authenticity test (Guillaume et al., 2010).

Sterol composition of EVOO is very distinctive parameter and useful for detecting adulteration with cheaper oils. The free and esterified sterol parts should be distinguished well from each other, because each form has different content and gives data about source of olive oil. (Arvanitoyannis and Vlachos, 2007).

1.2.3. Effects of olive variety

The olive variety has a significant effect on sterol composition. There is a strong influence of the variety on sterol composition, particularly in the case of certain sterols such as campesterol, stigmasterol, apparent β-sitosterol and total sterols. Based on this variety specificity, it is possible to include in the current legislation specific references to those varieties that do not normally comply with the authorised levels for the different sterols (Guillaume et.al., 2010).

The fatty acid compositions of the oil samples depended mainly on olive variety. Year, location, latitude, longitude, elevation, and oil facility did not show significant correlations with individual fatty acids when data from all varieties were considered (Rondanini et al., 2011). The quality of olive oil is related to olive variety and the geographical region where the olive fruit grown up (İlyasoğlu and Özçelik, 2011).

1.2.4. Effects of factors before harvest

The chemical composition and quality of virgin olive oil (VOO) are influenced by a variety of factors, among them geographical production area (altitude, soil composition, latitude), climatic conditions prevailing in the production year, cultivar, and extraction process (Dag et al., 2011).

Climatic and pedologic factors, cultivation and agronomic techniques, harvesting, transport and storage systems of olives, ripening degree of fruits, genetic factors (cultivar), and processing techniques, effect the analytical characteristics of oil (Ranalli et al., 1999). Fatty acid composition of olive oil is strongly affected by several agronomical factors such as cultivar, fruit ripeness, crop yield, and growing medium (Beltran et al., 2004). During fruit maturation, fatty acid profiles varied significantly among the growing regions and stages of maturity (Msaada et al., 2009).

1.2.5. Free Acidity

Glycerides account for at least 97% of a virgin oil if the acidity is neglected. The free fatty acid content is used to distinguish the various classes of virgin oil, from extra virgin to crude (Firestone, 2005). Free acidity has been increased with increasing activity of lipolytic enzymes in olive fruit. The fruit quality, climate conditions, processing and agronomic conditions effect the activity of this enzyme (Bayrak et al., 2010).

1.3. Processing of Olive Oils

1.3.1. Olive processing

Ripe olives contain a variety of components, including water, oil, sugars, proteins, organic acids, and cellulose. Olive cultivars with medium-size fruits generally provide the best oil yields. The pulp-to-kernel ratio of olives for oil production ranges from 4:1 to 8:1 (Firestone, 2005).

The content of aliphatic and triterpenic alcohols, sterols tend to higher in the pressed oils (Ranalli et al., 2001). Virgin (or native) olive oils are oils obtained from the fruit of the olive tree by mechanical or other physical means. Virgin oils have not undergone any treatment other than washing, crushing, pressing, centrifugation, and filtration.

The flesh of olive fruit (Figure 1.7) is low in sugar (2 to 6% w/w), and high in oil content (10 to 30% w/w) and contains glycoside oleuropein that has a specific bitter taste. The ratio of components varies with variety, growing environment and cultural diversity. Growth and chemical composition of olive fruit depend on variety and climatic factors. The Flesh/Stone ratio is effective on oil quality and specific constituents. The fruit size depends on the amount of fruit on the tree.

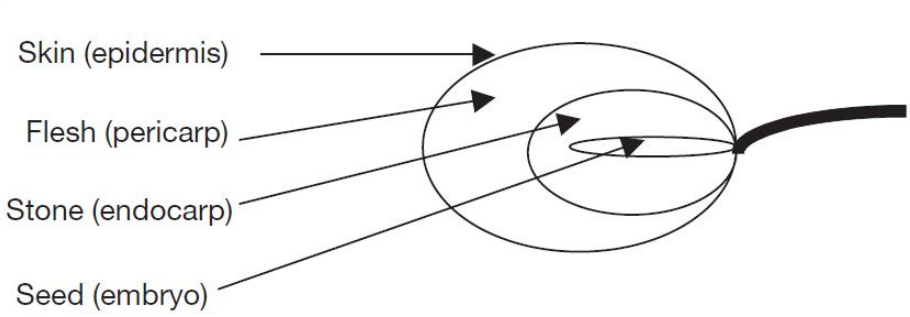

Figure 1.7. The olive fruit Structure (Source: Stan Kailis and David Harris, 2007)

Stages of olive fruit growth takes place into five steps: Fertilisation and fruit set, embryo and stone development, endocarp hardening, mesocarp development and oil accumulation, olive fruit ripening. Mesocarp development and oil accumulation is the main period for oil synthesis, oil starts to accumulate in the cells of the mesocarp (Stan Kailis and David Harris, 2007) .

Olive fruit were harvested by mechanically or by hand according to its degree of ripening. After harvesting the olives were transported to factory as soon as possible and foreign matters (branches, leaves, dirt, etc.) were separated and the olives were washed. Then the fruits were crushed and milled by metal crushers or millstone (Bayrak et al., 2010).

The malaxation, a basic step of the mechanical olive oil extraction process. An effective olive paste malaxing is crucial in producing VOO of exceptional quality. Then malaxation prepares the paste for separation of the oil. Traditionally, the malaxing step consisting of a low (20-30 rpm) and continuous kneading of olive paste at a carefully monitored temperature. This phase is especially useful for achieving high and satisfactory yields of extraction (Clodoveo, 2012).

After malaxation, separation of liquid and solid phases from each other takes place (mechanical extraction). This step includes pressing, percolation and

centrifugation. In pressing system, the olive paste were pressed under appropriate conditions to separate liquid phase. Generally, hydraulic presses are used in this system. Percolation system known as selective filtration, the olive paste is based on the fact that the liquid phase with a different surface tension. The steel plates were immersed to the olive cake and the plates were coated with olive oil. The principle of centrifugation system generates centrifugal force for the separation of the pomace, the black water and oil phases by using the density difference (Bayrak et al., 2010).

The flow diagrams of olive oil production by pressing system, by two phase system and by three phase system were given in Figure 1.8, Figure 1.9 and Figure 1.10, respectively.

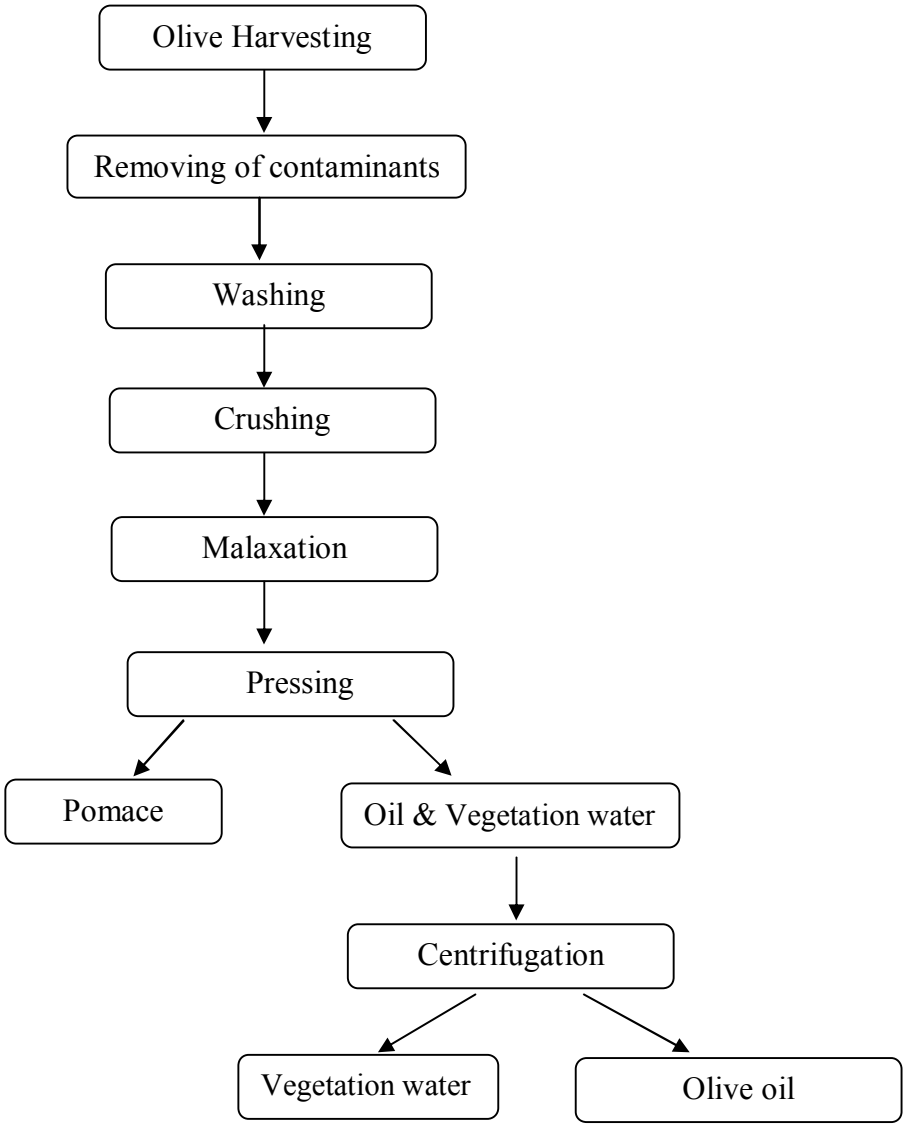

Figure 1.8. The flow diagram of olive oil production by pressing system (Source: Bayrak et al., 2010)

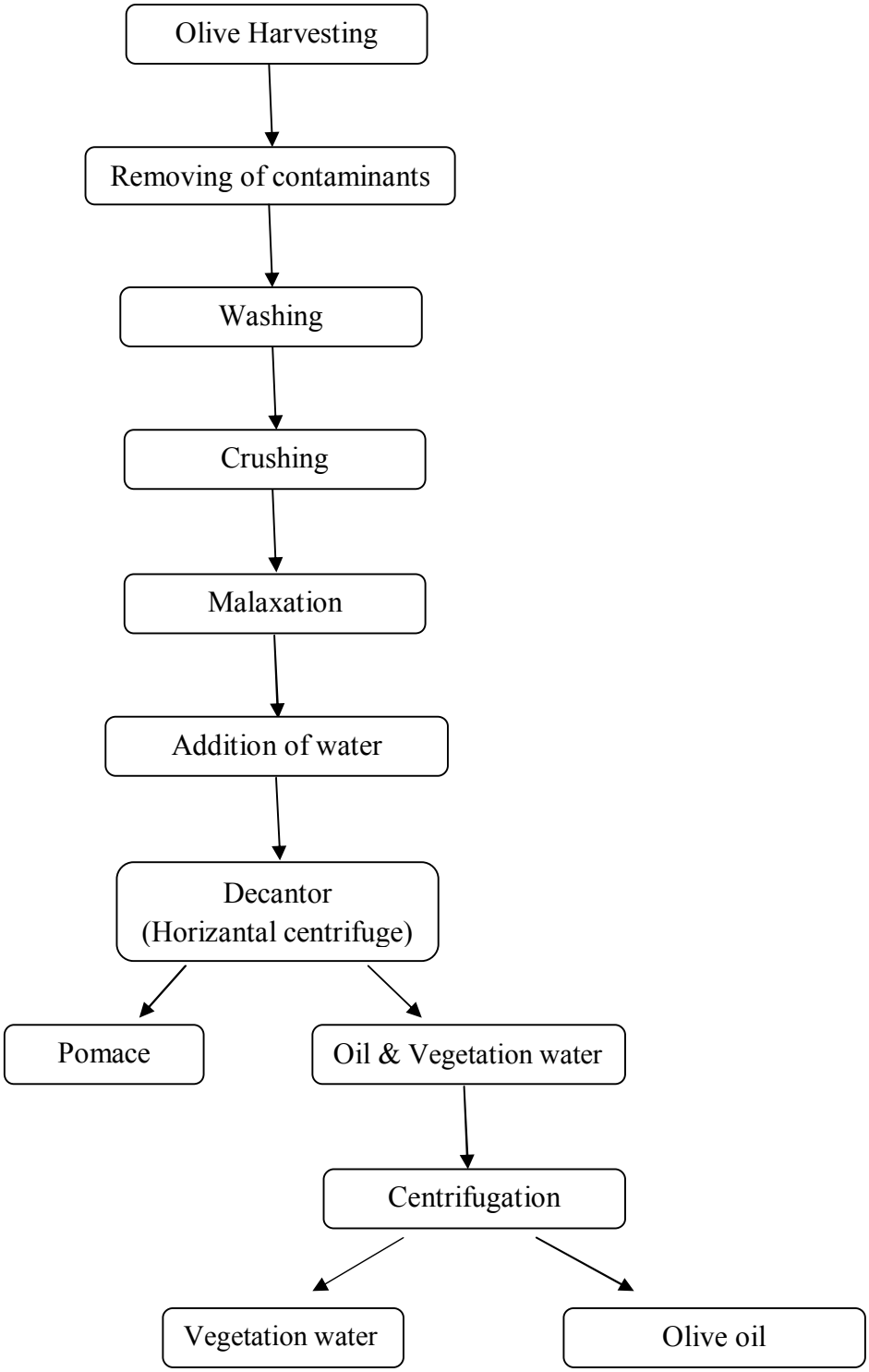

Figure 1.9. The flow diagram of olive oil production by two phase centrifugation system (Source: Bayrak et al., 2010)

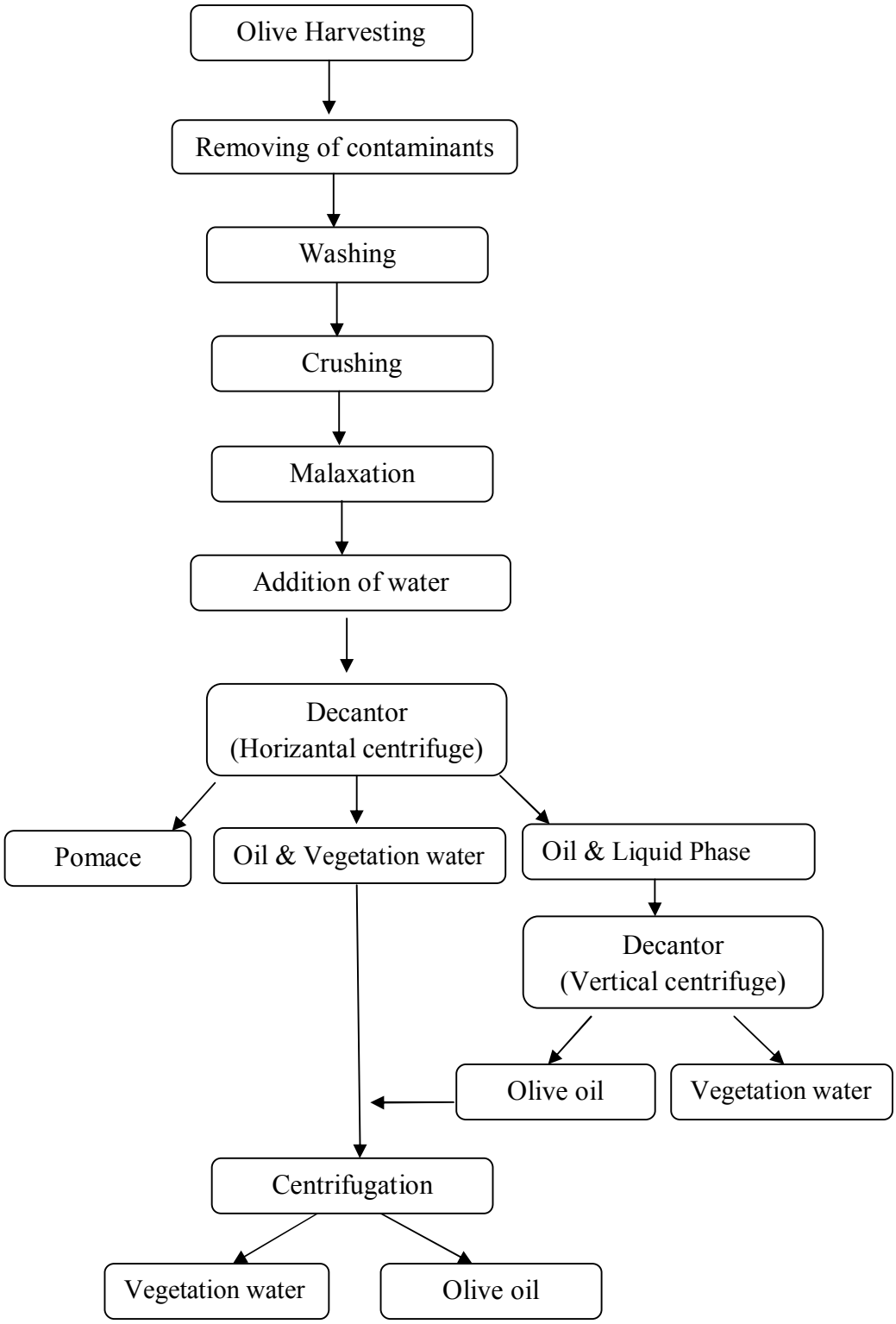

Figure 1.10. The flow diagram of olive oil production by three phase centrifugation system (Source: Bayrak et al., 2010)

1.3.2. Types of olive oils

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

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

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

Lampante Virgin olive oil (LVOO) not fit for consumption as it is, designated LVOO, is VOO which has a free acidity, expressed as oleic acid, of more than 2.0 grams per 100 grams. It is intended for refining or for technical use (TFC, Regulation No:2010/35).

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

Blended olive oil (BOO) is the oil consisting of a blend of refined olive oil and virgin olive oils fit for consumption as they are. It has a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams.

Olive-pomace oil (OPO) is the oil obtained by treating olive pomace with solvents or other physical treatments, to the exclusion of oils obtained by re esterification processes and of any mixture with oils of other kinds.

Crude olive-pomace oil (COPO) is olive pomace oil whose characteristics correspond to those fixed for this category in this standard. It is intended for refining for use for human consumption, or it is intended for technical use.

Refined olive pomace oil (ROPO) is the oil obtained from crude olive pomace oil by refining methods which do not lead to alterations in the initial glyceridic structure. It has a free acidity, expressed as oleic acid, of not more than 0.3 grams per 100 grams.

OPO is the oil comprising the blend of refined olive pomace oil and virgin olive oils fit for consumption as they are. It has a free acidity of not more than 1 gram per 100 grams (IOOC, 2013).

1.3.3. Olive Oil Regulations

Olive oil is very attractive product for adulteration with other cheaper edible oils. (Al-Ismail et al. 2010). Many methods and regulations published to control quality and genuineness of olive oil (Ruiz-Samblás et al. 2012).

In Table 1.1. the free acidity, FAME, sterol composition and total sterol limits as OO quality parameters set by the TFC (Regulation No: 2010/35) is given. The same limits have been set in European Commission (EC) regulations (EC Regulation 2568/1991)

Table 1.1. The free acidity, FAME, sterol composition and total sterol limits as OO quality parameters set by the TFC (Regulation No: 2010/35)

	LVOO	**EVOO**	**VOO**	**ROO**	**BOO**	**COPO**	**ROPO**	**OPO**
Acidity	> 2,0	≤ 0,8	≤ 2,0	≤ 0,3	≤ 1,0	-	≤ 0,3	≤ 1,0
Fatty acid composition, % m/m								
Myristic (C14:0)	≤ 0,05	≤ 0,05	≤ 0,05	≤ 0,05	≤ 0,05	≤ 0,05	≤ 0,05	≤ 0,05
Linolenic (C16:0)	7,5-20	7,5-20	7,5-20	7,5-20	7,5-20	7,5-20	7,5-20	7,5-20
Palmitoleic (C16:1)	0,3-3,5	0,3-3,5	0,3-3,5	0,3-3,5	0,3-3,5	0,3-3,5	0,3-3,5	0,3-3,5
Margaric (C17:0)	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3
Margoleic (C17:1)	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3	≤ 0,3
Stearic (C18:0)	0,5-5,0	0,5-5,0	0,5-5,0	0,5-5,0	0,5-5,0	0,5-5,0	0,5-5,0	0,5-5,0
Oleic (C18:1)	55,0-83,0	55,0-83,0	55,0-83,0	55,0-83,0	55,0-83,0	55,0-83,0	55,0-83,0	55,0-83,0
Linoleic (C18:2)	3,5-21,0	3,5-21,0	3,5-21,0	3,5-21,0	3,5-21,0	3,5-21,0	3,5-21,0	3,5-21,0
Linolenic (C18:3)	≤ 1,0	≤ 1,0	≤ 1,0	≤ 1,0	≤ 1,0	≤ 1,0	≤ 1,0	≤ 1,0
Arachidic (C20:0)	≤ 0,6	≤ 0,6	≤ 0,6	≤ 0,6	≤ 0,6	≤ 0,6	≤ 0,6	≤ 0,6
Eicosenoic (C20:1)	≤ 0,4	≤ 0,4	≤ 0,4	≤ 0,4	≤ 0,4	≤ 0,4	≤ 0,4	≤ 0,4
Behenic (C22:0)	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,3	≤ 0,3	≤ 0,3
Lignoceric (C24:0)	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2	≤ 0,2
Sterol Composition, % m/m								
Cholesterol	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5
Brassicasterol	≤ 0,1	≤ 0,1	≤ 0,1	≤ 0,1	≤ 0,1	≤ 0,2	≤ 0,2	≤ 0,2
Campesterol	≤ 4,0	≤ 4,0	≤ 4,0	≤ 4,0	≤ 4,0	≤ 4,0	≤ 4,0	≤ 4,0
Stigmasterol	< Campesterol							
Delta-7-stigmastenol	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5	≤ 0,5
Σ Beta-sitosterol (*)	≥ 93	≥ 93	≥ 93	≥ 93	≥ 93	≥ 93	≥ 93	≥ 93
Total Sterol, (mg/kg)	1000	1000	1000	1000	1000	2500	1800	1600

(*) Σ Beta-sitosterol is the sum of Beta-sitosterol, delta-5-avenasterol, delta-5,23-stigmastadienol, clerosterol, sitostanol, delta-5,24-stigmastadienol.

1.4. Factors Affecting Sterol Composition

1.4.1. Maturity and fruit size

The weight of fruit, pulp to stone ratio, color, oil content, chemical structure of the oil and enzyme activities are altered remarkable by fruit maturation. These steps are important factors for fruit stability, oil extraction and flavor components. During ripening the content of fatty acids, polyphenols, tocopherols, sterols and pigments change (Dag et al., 2011).

β-sitosterol, sitostanol, Δ5-avenasterol and Δ7-avenasterol are significantly ($P < 0.001$) affected by maturity index (Guillaume et.al., 2010). Chemical composition of olive fruit is changed by different activity of enzymes when the fruit became mature (Beltran et al., 2004).

1.4.2. Irrigation

24-methilene cholesterol, stigmasterol, Δ7-stigmasterol, apparent β-sitosterol and Δ7-avenasterol are amongst the significantly affected by irrigation. It is important point that while stigmasterol and Δ7-stigmasterol decrease with higher levels of irrigation, apparent β-sitosterol significantly increases (Guillaume et.al., 2010). The composition of fatty acid, alcohol and sterol are dramatically influenced by time of harvest. There is great interaction between irrigation and harvest date. Many of fatty acids, alcohols and sterols are affected by this interaction. (Inglese et al., 1996).

1.4.3. Malaxing time and temperature

The malaxation of olive cake is very important step because this step increases oil yield. The producers increase the time of malaxation to increase the oil yield. The average malaxing time changes from 45 to 60 min depending on olive characteristics (Clodoveo, 2012). The total level of sterols was significantly affected by this processing parameter showing increasing values at higher malaxing temperatures (Guillaume et.al., 2010).

VOO quality depends both on malaxing time and malaxing temperature. The malaxing temperature has a great influence on the process yield since the oil droplets are grouped due to a reduction in the oil viscosity. However for excessive heating undesirable effects can be observed: loss of phenolic compounds, loss of volatile

compounds responsible for oil flavour and fragrance and accelerates its oxidative process (Clodoveo, 2012). Erythrodiol + uvaol were significantly affected by malaxing temperature and stigmasterol was one of the few sterols affected (Guillaume et.al., 2012).

1.4.4. The time between harvest and process

The time between harvest and process has a remarkable effect on percentage of erythrodiol + uvaol and stigmasterol. The content of these parameters increase when the time between harvest and process is long. Campestanol is dramatically decreased with late processing time after harvest (Guillaume et.al., 2012). Any delay in pressing and fruit damage and can affect the quality of the oil as well as a stack, any delay in transport after harvesting should be avoided (IOOC, 1997).

1.4.5. Year

A research by Guillaume et.al. (2010) shows that cholesterol, campestanol, stigmasterol, Δ7-stigmasterol, apparent β-sitosterol, Δ5,23-stigmastadienol and Δ5,24-stigmastadienol. erythrodiol + uvaol levels are significantly affected by the year.

1.4.6. Variety

The sterol fraction is an important determinant of the genuineness of an olive oil, and the ratio of campesterol/stigmasterol has been reported as a quality index of oil (Koutsaftakis et al., 1999). There is significant interaction between variety of olive fruit and sterol composition, especially campesterol, stigmasterol, β-sitosterol and total sterol levels. Because of variety of olive fruit, it is possible to include in the current legislation specific references to those varieties that do not normally comply with the authorised levels for the different sterols (Guillaume et.al., 2012).

1.5. The aim of the thesis

Sterol determination, free and/or esterified in olive oil is an important quality and adulteration parameter. The variety, climate, geographical area, maturity, duration and the processes after harvest effect the quality of olive oil in terms of sterol content as well as the nature and quantity of constituent triglycerides and other organic compounds.

The plantation areas of oil olive trees in Gaziantep region are about 650 m high from the sea level, and as a general practise no irrigation was done, and the climate is worm and less rainy in winter, and hot in summer. The time of harvest is November and/or December depending on the climate conditions for that year. Local varieties are Nizip Yağlık, Kilis Yağlık and Yuvarlak Halhalı, and occasionally Δ7-Stigmastenol levels are reported to be higher than the limit decided by Turkish Food Codex.

In recent years new olive varieties from west of Turkey, Ayvalık, and Gemlik were planted to this region under the support of government. This gave us the opportunity to compare Δ7-Stigmastenol levels between local and newly planted varieties.

The aim of this study was the determination of sterol levels including Δ7-Stigmastenol and of fatty acid compositions in Nizip Yağlık, Kilis Yağlık, Yuvarlak Halhalı, Ayvalık, and Gemlik varieties. Altitude was considered for probable variance in the results. Maturity index and free acidity were also thought to be measured for the evaluation of our data as a whole.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample Collection

Plant materials of this study have constituted from Ayvalık, Gemlik, Nizip Yağlık, Kilis Yağlık and Yuvarlak Halhalı olive varieties (Table 2.1). Olive fruits were harvested by hand at the end of the November 2011. Samples were transported to laboratory into plastic bags and encoded according to olive variety and region.

Table 2.1. Olive samples used in this study (variety, geographical place, altitude)

Gemlik K1	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N- 37º07'00.29"E	633 m
Gemlik K3	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Nizip Yağlık K4	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Nizip Yağlık K5	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Yuvarlak Halhalı K6	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Yuvarlak Halhalı K7	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Ayvalık K8	İl Tarım Müdürlüğü Bahçe 36º42'29.70"N - 37º07'00.29"E	633 m
Kilis Yağlık K9	Demirışık Yolu 36º42'28.34"N - 37º05'46.40"E	680 m
Kilis Yağlık K10	Demirışık Yolu 36º42'28.34"N - 37º05'46.40"E	680 m
Kilis Yağlık K11	Demirışık Yolu 36º42'09.45"N - 37º05'25.19"E	652 m
Kilis Yağlık K12	Demirışık Yolu 36º42'09.45"N - 37º05'25.19"E	652 m
Gemlik K13	Demirışık Yolu 36º42'09.45"N - 37º05'25.19"E	652 m
Kilis Yağlık K14	Ziyaret Tepesi 36º42'36.63"N - 37º05'32.28"E	709 m
Kilis Yağlık K15	Ziyaret Tepesi 36º42'36.63"N - 37º05'32.28"E	709 m
Yuvarlak Halhalı N1	Belkız Yolu 37º02'11.49"N - 37º49'16.00"E	533 m
Yuvarlak Halhalı N2	Belkız Yolu 37º02'11.49"N - 37º49'16.00"E	533 m
Nizip Yağlık N3	Belkız Yolu 37º02'24.37"N - 37º48'55.66"E	560 m
Gemlik N4	Belkız Yolu 37º02'24.37"N- 37º48'55.66"E	560 m
Nizip Yağlık N5	Belkız Yolu 37º02'24.37"N- 37º48'55.66"E	560 m
Nizip Yağlık N6	Belkız Yolu 37º02'24.37"N- 37º48'55.66"E	560 m
Gemlik N8	Belkız Yolu 37º02'18.30"N- 37º49'00.20"E	551 m
Nizip Yağlık N9	Belkız Yolu 37º01'49.98"N- 37º49'01.00"E	532 m
Nizip Yağlık N10	Belkız Yolu 37º01'49.98"N- 37º49'01.00"E	532 m
Nizip Yağlık G1	İbrahimşehir köyü 37º05'09.71"N - 37º40'00.20"E	827 m
Nizip Yağlık G2	İbrahimşehir köyü 37º05'09.71"N - 37º40'00.20"E	827 m
Nizip Yağlık G3	İbrahimşehir köyü 37º05'09.71"N - 37º40'00.20"E	827 m
Nizip Yağlık G4	İbrahimşehir köyü 37º05'09.71"N - 37º40'00.20"E	827 m
Gemlik G5	İbrahimşehir köyü 37º03'47.28"N - 37º38'13.58"E	808 m
Gemlik G6	İbrahimşehir köyü 37º03'47.28"N - 37º38'13.58"E	808 m

Table 2.1. Olive samples used in this study (variety, geographical place, altitude) (continued)

Ayvalık G10	İbrahimşehir köyü 37º03'39.02"N - 37º38'19.69"E	800 m
Ayvalık G11	İbrahimşehir köyü 37º03'39.02"N - 37º38'19.69"E	800 m
Ayvalık G12	İbrahimşehir köyü 37º03'39.02"N - 37º38'19.69"E	800 m
Ayvalık G14	Sinan Köyü Yol ayrımı 37º02'36.40"N - 37º35'13.23"E	756 m
Gemlik G15	Sinan Köyü Yol ayrımı 37º02'36.40"N - 37º35'13.23"E	756 m
Gemlik G16	Sinan Köyü 37º02'12.40"N - 37º34'58.96"E	775 m
Gemlik G18	Sinan Köyü 37º02'12.40"N - 37º34'58.96"E	775 m
Ayvalık G19	Sinan Köyü 37º02'12.40"N - 37º34'58.96"E	775 m
Gemlik G20	Nizip Yolu 37º03'22.83"N - 37º30'35.18"E	794 m
Gemlik G21	Nizip Yolu 37º03'22.83"N - 37º30'35.18"E	794 m

K: Kilis; N: Nizip; G:Gaziantep

2.1.2. Reagents and solvents

Reagents used in this study were kindly provided by a local research institution namely Gaziantep Food Control Laboratory (Table 2.2). The common reagents used were all reagent quality and solutions were prepared using distilled water unless otherwise stated.

Table 2.2. Reagents and purchasing companies

REAGENT	**COMPANY**
Supelco® 37 Component FAME Mix, Cat.No: 47885-u	Sigma-Aldrich
Ethyl ether, GC grade, 99 %	Merck
Ethanol, GC grade, 99,9 %	Merck
Methanol, GC grade, 99,9 %	Merck
Heptane, GC grade, 99 %	Sigma-Aldrich
α-Cholestanol(5α-cholestan-3β-ol), ~95 %	Sigma-Aldrich
Chloroform, GC grade, 99-99,4 %	Merck
Hexane, GC grade, ≥95 %	Merck
Pyridine, Anhydrous, 99.8%	Sigma-Aldrich
2,7-dichlorofluorescein, bioreagent ≥ 90 %	Sigma-Aldrich
Hexamethyl disilazane, reagent grade, ≥ 99 %	Sigma-Aldrich
Trimethylchlorosilane, GC grade, ≥ 98 %	Sigma-Aldrich
Phenolphthalein, pH indicator	Merck

2.2. Methods

2.2.1. Preparation of Olive Oil Samples

The healthy and undamaged fruits were washed, separated from leaves and crushed by a hammer crusher after maturity index determination. The olive pastes were malaxed for at 25 ^{o}C for 30 minutes and centrifuged without addition of warm water. The supernatant (olive oil) was decanted into dark glass bottles (Baccouri et al., 2007).

2.2.2. Storage prior Measurements

Olive samples were stored at -18 ^{o}C prior to oil extraction. The olive oil samples were stored in dark glass bottles at 4 ^{o}C until analysis time.

2.2.3. Measurements

Ayvalık, Gemlik, Kilis Yağlık, Nizip Yağlık and Yuvarlak Halhalı olive varieties were analyzed in terms of fatty acid composition, sterol composition and content, maturity index and free acidity. Sterol composition and content and free acidity analyses of Yuvarlak Halhalı variety couldn't performed due to insufficient sample.

2.2.3.1. Maturity Index

The maturity index (MI) was determined according to Vinha et al. (2005). 100 olive fruits were selected randomly for each sample and classified into eight groups according to the skin color, flesh color and fruit pulp color. The groups were as follows:

0 – olives dark green skin color;

1 – olives yellow or yellowish green skin color;

2 – olives yellowish skin color but with reddish spots over less than half of the fruit;

3 – olives reddish or light violet skin color;

4 – olives black skin color and white pulp;

5 – olives black skin color and less than 50% purple pulp;

6 – olives black skin color and more than 50% purple pulp;

7 – olives black skin color and totally dark pulp.

Then number of fruits in each category were determined and MI was calculated by the following formula:

MI= (ax0+bx1+cx2+dx3+ex4+fx5+gx6+hx7)/100

Where a = the number fruits in group 0
b = the number fruits in group 1
c = the number fruits in group 2
d = the number fruits in group 3
e = the number fruits in group 4
f = the number fruits in group 5
g = the number fruits in group 6
h = the number fruits in group 7

2.2.3.2. Free Acidity

The free acidity (FA) analysis were performed by the method of Turkish Food Codex, Notification of Methods of Sampling and Analysis of Olive Oil and Olive Pomace Oil (TFC Regulation No: 2010/36). Firstly ethanol diethyl ether mixture of 1:1 (V/V) was prepared. The mixture was neutralized with 0.1 N ethanolic potassium hydroxide by using 3 mL of phenolphthalein as indicator. 20 g of olive oil was weighed with 0.005 g precision and the sample was dissolved in 50–150 mL of neutral ethanol diethyl ether mixture. Solution was titrated with 0.1 N ethanolic potassium hydroxide until light pink color appeared. Analysis was done in two parallel and the result is arithmetic mean of two results. FA was calculated by the following formula:

$$V \times c \times \frac{M}{1000} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m}$$

Where V = amount of 0.1 N ethanolic potassium hydroxide (mL)
c = concentration of ethanolic potassium hydroxide (N),
M = molecular weight of oleic acid (= 282);
m = weight of sample taken (g)

2.2.3.3. Fatty Acid Methyl Esters

The fatty acid methyl ester analysis (FAME) were prepared by the method of COI/T.20/ Doc.no.24 2001 (IOOC). 0.1 g of olive oil was taken into screwtube and 2 mL of heptane was added to it. The solution was shaked vigorously and 0.2 mL of 2 N methanolic potassium hydroxide was added for esterification and vortexed for 30 seconds. It was waited until upper phase become clear. The upper phase was taken into 2 mL of vials and injected to Gas Chromatograpy (GC).

FAME analyses were carried out on a Shimadzu GC-2010 series gas chromatography equipped with flame ionization detector and Supelco SP 2380 capillary column 100 m of length, 0.25 mm i.d., 0.2 μm film thickness. A flow rate of 0.94 mL min^{-1} of helium as a carrier gas was used. The Flame Ionization Detector (FID) detector was at 260 ^{o}C. The initial oven temperature was kept at 140 ^{o}C for 5 minutes and raised to 240 ^{o}C at a rate of 4 ^{o}C min^{-1}. Peaks were identified by comparison to their retention times with those of analytical standard (Supelco® 37).

2.2.3.4. Sterol composition and total sterol

The sterol composition analyses were carried out by the method of COI/ T.20/ Doc. no.10/ Rev 1 2001 (IOOC). 500 μL of the 0.2 % α-cholestanol solution was added into the 250 mL flask as internal standard and evaporated to dryness in a current of nitrogen. 5 g of olive oil sample was weighed into the same flask and 50 mL of 2 N ethanolic potassium hydroxide solution was added to it. It was fitted the reflux condenser and heated to gentle boiling on a water bath until saponification takes place. Then add 50 mL of distilled water from the top of the condenser. The sample was transferred the contents of the flask into a 500 mL separating funnel (Figure 2.1) and washed three times with ethyl ether. The lower aqueous phase was separated in each time. The ether extracts were washed with distilled water until the wash water gives a neutral reaction and lower phase was separated again. The upper phase was distilled with rotary evaporator and drying completed in a oven at l00ºC.

Figure 2.1 Phase separation in separating funnel

The thin layer chromatography silica gel plates (TLC) (Figure 2.2) were immersed completely in the 0.2 N ethanolic potassium hydroxide solution for 10 seconds, then allowed for two hours and finally placed in a oven at 100 ºC for one hour. 5% solution of the unsaponifiables was prepared in chloroform and it was streaked on TLC approximately 2 cm from one end.

Hexane/ethyl ether mixture 65:35 (V/V) was added to the plate-developing chamber and it was left at least half an hour so that liquid-vapour equilibrium is established. The TLC plates were put into developing chamber and allow to elute until the solvent front reaches approximately 1 cm from the upper edge of the plate. 2,7-dichlorofluorescein solution was sprayed to the plate uniformly to identify the sterol band under ultraviolet light.

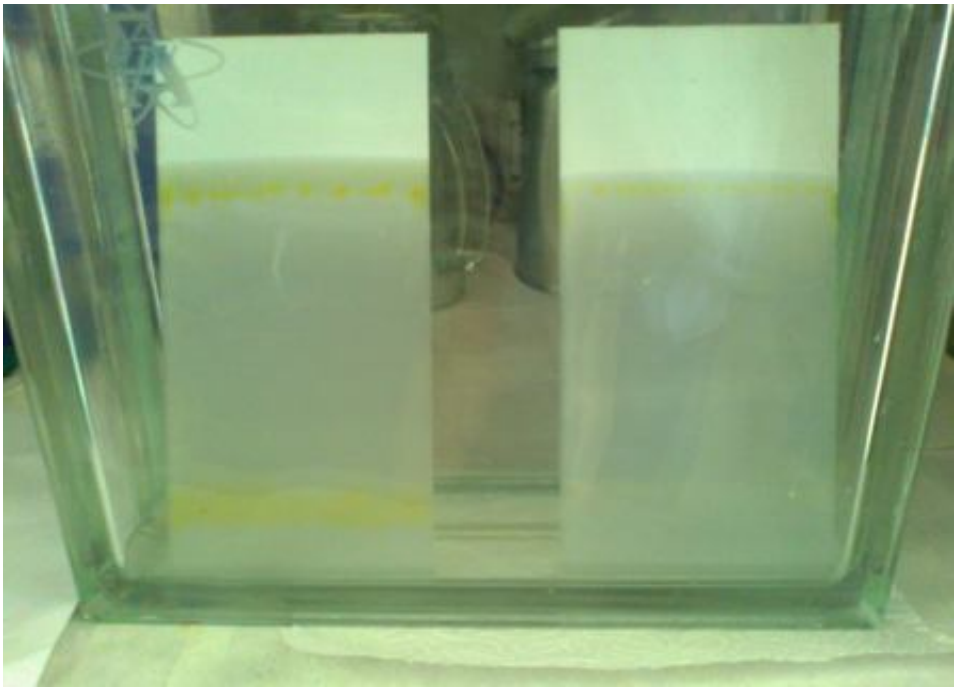

Figure 2.2 TLC plates in developing chamber

The limits of the sterol band was marked and the marked area of the TLC was scraped off using a metal spatula. 10 mL of hot chloroform was added to it. After that it was filtered. The silylation reagent 9:3:1 (V/V/V) mixture of pyridine/hexamethyl disilazane/trimethyl chlorosilane was added for preparation of the trimethylsilyl ethers and shaked carefully without overturning. It was waited for 15 minutes and centrifuged for a few minutes. The clear solution was injected to GC.

Sterol analyses were carried out on a Shimadzu GC-2010 series gas chromatography equipped with flame ionization detector and Agilent HP-Ultra 2 fused silica capillary column 25 m, 0.32 mm i.d., 0. 52 μm film thickness. A flow rate of 2.50 mL min^{-1} of helium as a carrier gas was used. The FID detector was at 300 ^{o}C. The initial oven temperature was kept at 260 ^{o}C.

Peaks were identified by comparison to their retention times with those of β-sitosterol and internal standard that purchased from Sigma-Aldrich.

Total sterol (mg/kg) was calculated by the summation of amount of each sterol;

$$\text{sterol x} = \frac{A_x \times m_s \times 1000}{A_s \times m}$$

Where,

A_x = peak area for sterol x, in square millimetres;

A_s = area of the α-cholestanol peak, in square millimetres;

m_s = mass of α-cholestanol added, in milligrams;

m = mass of the sample used for determination, in grams

2.2.4. Statistical Analysis

Statistical analysis was performed using the SPSS 17.0. The data were compared by using the analysis of variance (ANOVA) followed by Duncan's multiple range tests to determine significant differences. The differences between individual means were deemed to be significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Maturity Index

Maturity index of olive fruits was determined in 2011 harvest year. Maturation index of all olives examined in this study varied between 2.98 and 5.50. Nizip Yağlık and Yuvarlak Halhalı olives had low maturation indices whereas other olive varieties had high maturation indices (Table 3.1). It was not observed significant change of free acidity by maturity index.

Table 3.1. Mean values and The Duncan's new multiple range test of maturity index

		Subset for alpha = 0.05		
OLIVE VARIETIES	N	1	2	3
Yuvarlak Halhalı	4	3.4750 **a**		
Nizip Yağlık	11		4.3809 **b**	
Kilis Yağlık	6		4.4500 **b**	
Gemlik	12		4.8975 **bc**	4.8975 **bc**
Ayvalık	6			5.2283 **c**
Significance		1.000	0.069	0.213

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Ripening degree of Ayvalık and Gemlik varieties are close to each other. Yuvarlak Halhalı variety has the lowest maturity index level and in group of a.

3.2. Free Acidity

Free acidity was found to be not affected by the olive growing region. The amount of free acidity (Table 3.2) was much lower than the limits set for EVOO by TFC almost all samples (Ayvalık G14: 1.02 and Ayvalık K8: 1.08). Free acidity could be related to many factors for example processing conditions, variety, the time between harvest and processing.

Table 3.2. Mean values and The Duncan's new multiple range test of free acidity

OLIVE VARIETIES	N	Subset for alpha = 0.05			
		1	2	3	4
Nizip Yağlık	11	0.2445 **a**			
Gemlik	12		0.2842 **b**		
Kilis Yağlık	6			0.3450 **c**	
Ayvalık	6				0.9883 **d**
Significance		1.000	1.000	1.000	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

The difference in free acidity level for all varieties are statistically significant. Ayvalık variety has high free acidity among all other varieties.

3.3. Fatty Acid Methyl Esters

The average Saturated Fatty Acid (SFA) content of Nizip Yağlık, Kilis Yağlık and Yuvarlak Halhalı varieties were observed 20.25%, 19.82% and 20.09% respectively. The SFA content of these varieties were higher than Gemlik (16.90%) and Ayvalik (16.97%) varieties. Gemlik variety was the richest variety in terms of Monounsaturated Fatty Acid (MUSFA), especially the average amount of oleic acid ratio were 76.16% . MUSFA content of Yuvarlak Halhalı variety was determined that the lowest. In contrast the percentage Polyunsaturated Fatty Acid (PUSFA) content of Yuvarlak Halhalı was the highest value, 17.03% (Figure 1 to Figure 3). The average values of SFA, MUSFA and PUSFA of all samples in percentages were given in Appendix.

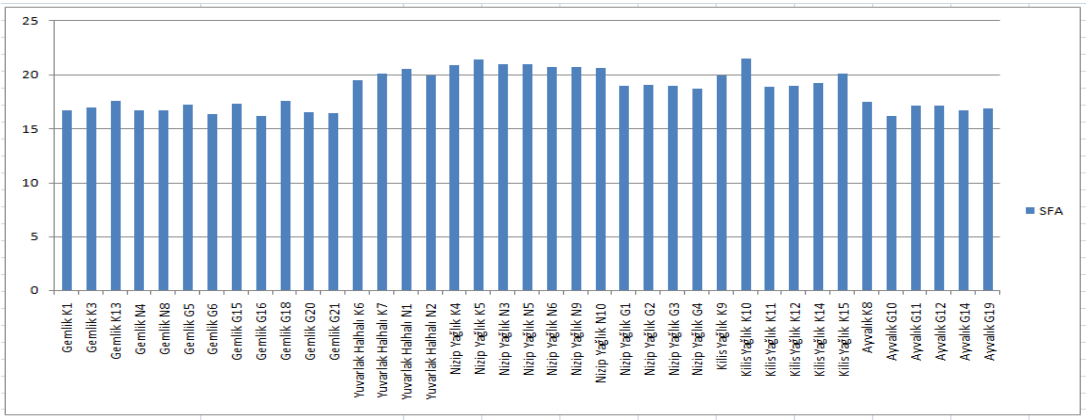

Figure 3.1. SFA contents of Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties

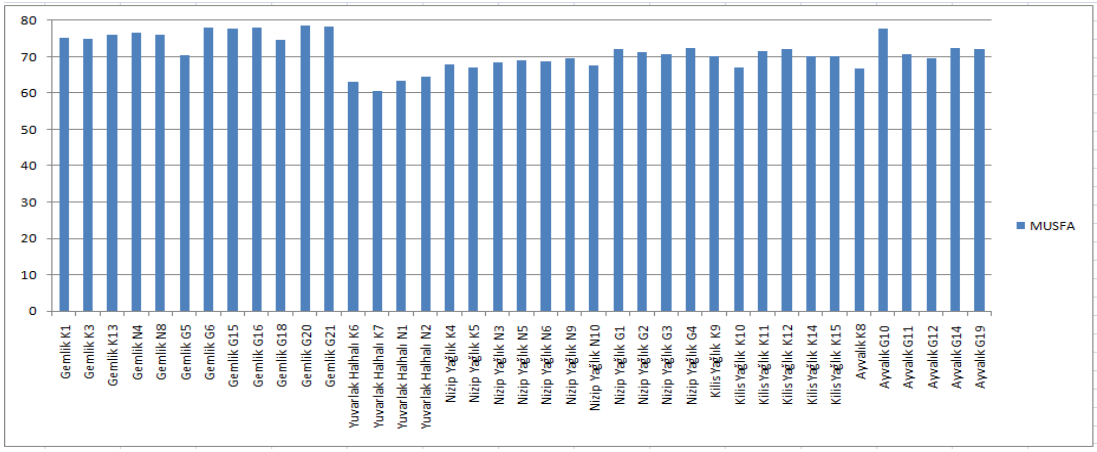

Figure 3.2. MUSFA contents of Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties

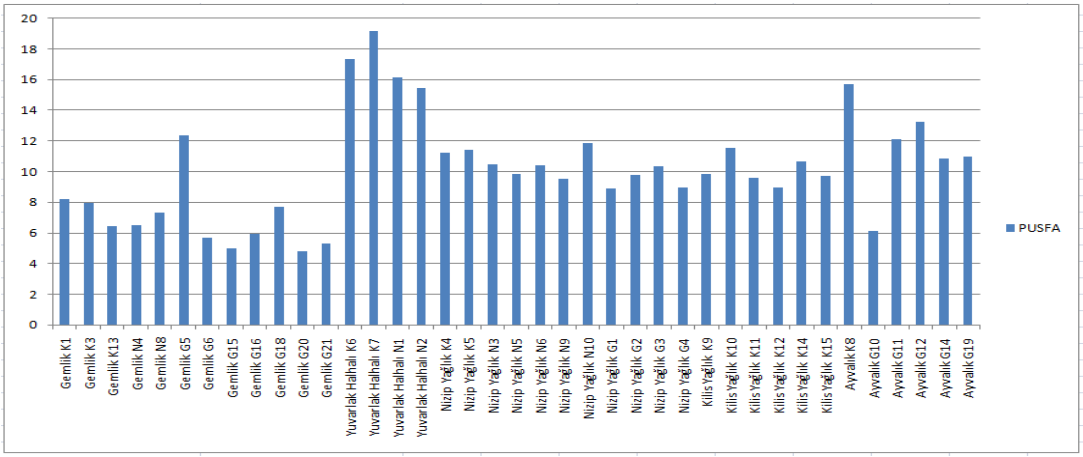

Figure 3.3. PUSFA contents of Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties

According to Table 3.3, the differences among varieties are significant in terms of SFA, MUSFA and PUSFA ($p<0.01$).

Table 3.3. Variance analysis of SFA, MUSFA and PUSFA

		Sum of Squares	df	Mean Square	F	Significance
SFA	Between Groups	98.166	4	24.541	43.080	0.000
	Within Groups	19.369	34	0.570		
	Total	117.535	38			
MUSFA	Between Groups	610.863	4	152.716	29.140	0.000
	Within Groups	178.189	34	5.241		
	Total	789.052	38			
PUSFA	Between Groups	324.487	4	81.122	23.190	0.000
	Within Groups	118.936	34	3.498		
	Total	443.423	38			

As shown in Table 3.4, the ratio of SFA in Ayvalık and Gemlik olive varieties has the lowest value and they involved in first group. Nizip Yağlık, Yuvarlak Halhalı and Kilis Yağlık olive varieties are in second group those have the high SFA level.

It is known that consumption of SFA must be reduced in a healthy diet (Zarrouk et al. 2009).

Table 3.4. Mean values and The Duncan's new multiple range test of SFA

		Subset for alpha = 0.05	
OLIVE VARIETIES	N	1	2
Gemlik	12	16.8999 **a**	
Ayvalık	6	16.9743 **a**	
Kilis Yağlık	6		19.8165 **b**
Yuvarlak Halhalı	4		20.0893 **b**
Nizip Yağlık	11		20.2491 **b**
Significance		0.859	0.334

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

The highest ratio of MUSFA was determined in Gemlik variety and the lowest in Yuvarlak Halhalı variety. Kilis Yağlık, Nizip Yağlık and Ayvalık varieties

get involved in same group (Table 3.5). MUSFA as a nutrient concerned with the decreasing cardiovascular disease, obesity, type 2 diabetes, metabolic syndrome and hypertension (Lopez-Miranda et al. 2010) .

Table 3.5. Mean values and The Duncan's new multiple range test of MUSFA

		Subset for alpha = 0.05		
OLIVE VARIETIES	N	1	2	3
Yuvarlak Halhalı	4	62.8840 **a**		
Nizip Yağlık	11		69.4957 **b**	
Kilis Yağlık	6		70.1288 **b**	
Ayvalık	6		71.5127 **b**	
Gemlik	12			76.1585 **c**
Significance		1.000	0.139	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

In contrast MUSFA, the highest ratio of PUSFA was determined in Yuvarlak Halhalı variety and the lowest in Gemlik variety. Kilis Yağlık, Nizip Yağlık and Ayvalık varieties have approximately same ratio (Table 3.6.). PUSFA have been associated with the rancidification. The odor and flavor of oils and also oil quality concerned with the fatty acid composition. (Leon et al., 2004). Essential fatty acids are belong to PUSFA.

MUSFA are more durable to oxidation than PUSFA and more stable (Kratz et al. 2002).

Table 3.6. Mean values and The Duncan's new multiple range test of PUSFA

		Subset for alpha = 0.05		
OLIVE VARIETIES	N	1	2	3
Gemlik	12	6.9342 **a**		
Kilis Yağlık	6		10.0542 **b**	
Nizip Yağlık	11		10.2550 **b**	
Ayvalık	6		11.5140 **b**	
Yuvarlak Halhalı	4			17.0273 **c**
Significance		1.000	0.190	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

Fatty acid composition of olive oils from all varieties under current study was given in Table 3.7.

Table 3.7. Results of fatty acid composition analysis of olive oils from Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region

OLİVE VARIETIES	Myristic Acid, (C14:0)	Palmitic Acid (C16:0)	Palmitoleic Acid (C16:1)	Heptadecanoic Acid (C17:0)	cis-10 Heptadecanoic Acid (C17:1)	Stearic Acid, (C18:0)	Oleic Acid (C18:1)
Gemlik K1	0,006	13,553	1,485	0,096	0,185	2,651	73,190
Gemlik K3	0,008	13,728	1,308	0,096	0,191	2,734	73,269
Nizip Yağlık K4	0,019	16,494	0,949	0,059	0,059	3,638	66,585
Nizip Yağlık K5	0,017	16,658	0,946	0,056	0,053	4,019	65,836
Yuvarlak Halhalı K6	0,007	14,736	0,736	0,050	0,047	4,235	62,103
Yuvarlak Halhalı K7	0,007	15,213	0,695	0,052	0,044	4,316	59,714
Ayvalık K8	0,019	14,686	0,831	0,126	0,221	2,149	65,474
Kilis Yağlık K9	0,011	14,950	0,536	0,185	0,184	3,960	69,120
Kilis Yağlık K10	0,014	16,636	0,539	0,203	0,202	3,772	65,880
Kilis Yağlık K11	0,010	14,752	1,083	0,107	0,156	3,363	69,968
Kilis Yağlık K12	0,012	14,468	0,904	0,127	0,165	3,693	70,775
Gemlik K13	0,009	12,771	0,777	0,196	0,273	3,993	74,662
Kilis Yağlık K14	0,012	14,424	0,737	0,158	0,189	3,857	68,880
Kilis Yağlık K15	0,013	14,973	0,575	0,200	0,206	4,077	69,110
Yuvarlak Halhalı N1	0,008	15,465	0,809	0,059	0,050	4,435	62,234
Yuvarlak Halhalı N2	0,007	14,812	0,767	0,059	0,054	4,545	63,538
Nizip Yağlık N3	0,014	15,885	0,783	0,185	0,212	4,037	67,224
Gemlik N4	0,010	13,027	1,119	0,162	0,263	3,033	75,075
Nizip Yağlık N5	0,014	15,679	0,844	0,194	0,224	4,251	67,801
Nizip Yağlık N6	0,013	15,884	0,914	0,148	0,186	3,852	67,458
Gemlik N8	0,009	13,261	1,327	0,111	0,214	2,850	74,190
Nizip Yağlık N9	0,013	16,328	1,205	0,122	0,168	3,610	68,061
Nizip Yağlık N10	0,013	16,525	1,372	0,115	0,168	3,343	65,716
Nizip Yağlık G1	0,010	14,673	0,870	0,127	0,156	3,559	70,807
Nizip Yağlık G2	0,010	14,786	0,970	0,111	0,150	3,450	69,770
Nizip Yağlık G3	0,011	14,700	0,999	0,111	0,152	3,466	69,248
Nizip Yağlık G4	0,009	14,256	0,880	0,117	0,155	3,578	71,012
Gemlik G5	0,015	14,444	1,090	0,110	0,217	2,133	68,822
Gemlik G6	0,009	12,818	1,171	0,129	0,249	2,884	76,302
Ayvalık G10	0,009	12,763	1,196	0,120	0,231	2,863	75,956
Ayvalık G11	0,013	14,388	1,185	0,114	0,219	2,161	69,004
Ayvalık G12	0,015	14,442	1,156	0,112	0,219	2,142	67,881
Ayvalık G14	0,000	13,719	1,100	0,116	0,228	2,372	70,789
Gemlik G15	0,000	13,354	1,260	0,182	0,314	3,183	75,864
Gemlik G16	0,008	13,092	1,378	0,121	0,244	2,523	75,995
Gemlik G18	0,010	13,818	1,263	0,153	0,277	3,093	72,955
Ayvalık G19	0,015	14,255	1,122	0,110	0,237	2,000	70,469
Gemlik G20	0,000	13,047	1,207	0,155	0,292	2,858	76,861
Gemlik G21	0,006	12,974	1,146	0,142	0,273	2,823	76,628

Table 3.7. Fatty acid composition of olive oils from Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region (continued)

OLİVE VARIETIES	Linoleic Acid (C18:2)	Arachidic Acid (C:20)	cis-11-Eicosenoic Acid (C20:1)	Linolenic Acid (C18:3)	Behenic Acid (C22:0)	Lignoceric Acid (C24:0)
Gemlik K1	7,684	0,321	0,206	0,511	0,073	0,041
Gemlik K3	7,479	0,336	0,223	0,503	0,079	0,046
Nizip Yağlık K4	9,853	0,518	0,222	1,392	0,122	0,090
Nizip Yağlık K5	10,166	0,543	0,219	1,269	0,130	0,087
Yuvarlak Halhalı K6	16,899	0,431	0,198	0,436	0,082	0,041
Yuvarlak Halhalı K7	18,714	0,452	0,196	0,476	0,082	0,039
Ayvalık K8	15,040	0,370	0,280	0,638	0,108	0,058
Kilis Yağlık K9	9,132	0,640	0,285	0,727	0,156	0,110
Kilis Yağlık K10	10,765	0,643	0,286	0,790	0,163	0,109
Kilis Yağlık K11	9,196	0,500	0,237	0,427	0,125	0,076
Kilis Yağlık K12	8,462	0,512	0,229	0,475	0,118	0,062
Gemlik K13	5,931	0,484	0,221	0,520	0,108	0,058
Kilis Yağlık K14	10,030	0,595	0,261	0,623	0,145	0,089
Kilis Yağlık K15	8,931	0,634	0,269	0,767	0,151	0,094
Yuvarlak Halhalı N1	15,732	0,474	0,173	0,426	0,090	0,044
Yuvarlak Halhalı N2	15,017	0,483	0,178	0,409	0,091	0,042
Nizip Yağlık N3	9,801	0,646	0,252	0,694	0,157	0,112
Gemlik N4	5,920	0,405	0,219	0,612	0,102	0,055
Nizip Yağlık N5	9,191	0,643	0,238	0,669	0,151	0,102
Nizip Yağlık N6	9,827	0,624	0,249	0,588	0,153	0,105
Gemlik N8	6,666	0,367	0,223	0,631	0,093	0,058
Nizip Yağlık N9	9,042	0,533	0,215	0,498	0,122	0,079
Nizip Yağlık N10	11,326	0,502	0,211	0,509	0,119	0,082
Nizip Yağlık G1	8,401	0,513	0,218	0,482	0,119	0,066
Nizip Yağlık G2	9,322	0,527	0,228	0,465	0,128	0,080
Nizip Yağlık G3	9,866	0,531	0,229	0,471	0,133	0,084
Nizip Yağlık G4	8,495	0,554	0,239	0,478	0,140	0,086
Gemlik G5	11,821	0,374	0,260	0,550	0,107	0,058
Gemlik G6	5,143	0,379	0,218	0,556	0,091	0,052
Ayvalık G10	5,586	0,368	0,217	0,553	0,090	0,050
Ayvalık G11	11,637	0,371	0,257	0,495	0,104	0,054
Ayvalık G12	12,737	0,364	0,254	0,524	0,101	0,054
Ayvalık G14	10,384	0,403	0,276	0,493	0,121	0,000
Gemlik G15	4,369	0,457	0,235	0,620	0,109	0,054
Gemlik G16	5,409	0,353	0,220	0,520	0,089	0,048
Gemlik G18	7,168	0,399	0,196	0,514	0,102	0,051
Ayvalık G19	10,494	0,363	0,274	0,503	0,102	0,056
Gemlik G20	4,273	0,404	0,227	0,534	0,093	0,048
Gemlik G21	4,791	0,379	0,218	0,485	0,090	0,048

Table 3.8 shows the variation of myristic, palmitic, palmitoleic, heptadecanoic, cis-10 heptadecanoic, stearic, oleic, linoleic, arachidic, eicosenoic,

behenic and lignoceric acid contents are statistically significant among varieties. However, linolenic acid content is statistically insignificant.

Table 3.8. Variance analysis of fatty acid methyl esters

		Sum of Squares	df	Mean Square	F	Significance
Myristic	Between Groups	0.000	4	0.000	3.993	0.009
	Within Groups	0.001	34	0.000		
	Total	0.001	38			
Palmitic	Between Groups	34.688	4	8.672	17.969	0.000
	Within Groups	16.409	34	0.483		
	Total	51.097	38			
Palmitoleic	Between Groups	1.284	4	0.321	11.013	0.000
	Within Groups	0.991	34	0.029		
	Total	2.275	38			
Heptadecanoic	Between Groups	0.031	4	0.008	6.827	0.000
	Within Groups	0.038	34	0.001		
	Total	0.069	38			
Cis-10 Heptadecanoic	Between Groups	0.145	4	0.036	25.048	0.000
	Within Groups	0.049	34	0.001		
	Total	0.194	38			
Stearicacid	Between Groups	16.121	4	4.030	35.753	0.000
	Within Groups	3.833	34	0.113		
	Total	19.954	38			
Oleicacid	Between Groups	546.866	4	136.717	26.875	0.000
	Within Groups	172.965	34	5.087		
	Total	719.831	38			
Linoleicacid	Between Groups	330.691	4	82.673	24.496	0.000
	Within Groups	114.746	34	3.375		
	Total	445.437	38			
Arachidicacid	Between Groups	0.304	4	0.076	33.707	0.000
	Within Groups	0.077	34	0.002		
	Total	0.381	38			

Table 3.8. Variance analysis of fatty acid methyl esters (continued)

		Sum of Squares	df	Mean Square	F	Significance
Eicosenoicacid	Between Groups	0.019	4	0.005	15.485	0.000
	Within Groups	0.011	34	0.000		
	Total	0.030	38			
Linolenicacid	Between Groups	0.242	4	0.061	1.636	0.188
	Within Groups	1.258	34	0.037		
	Total	1.500	38			
Behenicacid	Between Groups	0.018	4	0.004	26.629	0.000
	Within Groups	0.006	34	0.000		
	Total	0.023	38			
Lignocericacid	Between Groups	0.017	4	0.004	22.130	0.000
	Within Groups	0.006	34	0.000		
	Total	0.023	38			

Yuvarlak Halhalı variety is in group a which has the lowest myristic acid content. The highest value of myristic acid in Nizip Yağlık variety that in group c. Other varieties have values near each other (Table 3.9).

Table 3.9. Mean values and The Duncan's new multiple range test of myristic acid

		Subset for alpha = 0.05		
OLIVE VARIETIES	N	1	2	3
Yuvarlak Halhalı	4	0.0072 **a**		
Gemlik	12	0.0075 **ab**	0.0075 **ab**	
Ayvalık	6	0.0118 **abc**	0.0118 **abc**	0.0118 **abc**
Kilis Yağlık	6		0.0120 **bc**	0.0120 **bc**
Nizip Yağlık	11			0.0130 **c**
Significance		0.050	0.054	0.613

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

Gemlik and Ayvalık varieties have the low palmitic acid content than other varieties (Table 3.10).

Table 3.10. Mean values and The Duncan's new multiple range test of palmitic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Gemlik	12	13.3239 **a**	
Ayvalık	6	14.0422 **a**	
Kilis Yağlık	6		15.0338 **b**
Yuvarlak Halhalı	4		15.0565 **b**
Nizip Yağlık	11		15.6244 **b**
Significance		0.069	0.154

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

The lowest palmitoleic acid ratio was determined in Yuvarlak Halhalı and Kilis Yağlık varieties (group a). Ayvalık variety is in group b and Gemlik variety is in group c (Table 3.11).

Table 3.11. Mean values and The Duncan's new multiple range test of palmitoleic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Kilis Yağlık	6	0.7290 **a**		
Yuvarlak Halhalı	4	0.7518 **a**		
Nizip Yağlık	11		0.9756 **b**	
Ayvalık	6		1.0983 **bc**	1.0983 **bc**
Gemlik	12			1.2109 **c**
Significance		0.810	0.200	0.239

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

The lowest value of heptadecanoic acid ratio in Yuvarlak Halhalı variety that in group a. Other varieties have values close to each other (Table 3.12).

Table 3.12. Mean values and The Duncan's new multiple range test of heptadecanoic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Yuvarlak Halhalı	4	0.0550 **a**		
Ayvalık	6		0.1163 **b**	
Nizip Yağlık	11		0.1223 **b**	
Gemlik	12		0.1378 **bc**	0.1378 **bc**
Kilis Yağlık	6			0.1633 **c**
Significance		1.000	0.284	0.176

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

Ayvalık and Gemlik varieties have the high cis-10 heptadecanoic acid content than other varieties (group c and d) as was shown in Table 3.13.

Table 3.13. Mean values and The Duncan's new multiple range test of cis-10 heptadecanoic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05			
		1	2	3	4
Yuvarlak Halhalı	4	0.0488 **a**			
Nizip Yağlık	11		0.1530 **b**		
Kilis Yağlık	6		0.1837 **bc**	0.1837 **bc**	
Ayvalık	6			0.2258 **cd**	0.2258 **cd**
Gemlik	12				0.2493 **d**
Significance		1.000	0.152	0.052	0.269

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

The highest stearic acid level is in Yuvarlak Halhalı variety (group a) and the lowest in Ayvalık variety (group d) (Table 3.14).

Table 3.14. Mean values and The Duncan's new multiple range test of stearic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05			
		1	2	3	4
Ayvalık	6	2.2812 **a**			
Gemlik	12		2.8965 **b**		
Nizip Yağlık	11			3.7094 **c**	
Kilis Yağlık	6			3.7870 **c**	
Yuvarlak Halhalı	4				4.3828 **d**
Significance		1.000	1.000	0.677	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$).

The oleic acid level is higher than other samples in Gemlik variety (group c) and lower in Yuvarlak Halhalı variety (group a). Oleic acid is major fatty acid in olive oil. The high level of oleic acid in olive oil has the hypotensive effect and arranges lipid structure of membrane (Teres et al. 2008).

It can be said that Gemlik variety has a rich oleic acid content than Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties (Table 3.15).

Table 3.15. Mean values and The Duncan's new multiple range test of oleic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Yuvarlak Halhalı	4	61.8973 **a**		
Nizip Yağlık	11		68.1628 **b**	
Kilis Yağlık	6		68.9555 **b**	
Ayvalık	6		69.9288 **b**	
Gemlik	12			74.4844 **c**
Significance		1.000	0.188	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Yuvarlak Halhalı variety has high level of linoleic acid content and is in c group. Since the linoleic acid is an essential fatty acid, high linoleic acid content may be an advantage with respect to others (Table 3.16).

Table 3.16. Mean values and The Duncan's new multiple range test of linoleic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Gemlik	12	6.3878 **a**		
Kilis Yağlık	6		9.4193 **b**	
Nizip Yağlık	11		9.6380 **b**	
Ayvalık	6		10.9797 **b**	
Yuvarlak Halhalı	4			16.5905 **c**
Significance		1.000	0.154	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

The arachidic acid content of Ayvalık and Gemlik varieties are low than other varieties and they are in group a. Regional varieties have little higher arachidic acid levels (group b and c) (Table 3.17).

Table 3.17. Mean values and The Duncan's new multiple range test of arachidic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Ayvalık	6	0.3732 **a**		
Gemlik	12	0.3882 **a**		
Yuvarlak Halhalı	4		0.4600 **b**	
Nizip Yağlık	11			0.5576 **c**
Kilis Yağlık	6			0.5873 **c**
Significance		0.570	1.000	0.264

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Yuvarlak Halhalı variety is in group of a, which has low level eicosenoic acid, Gemlik and Nizip Yağlık varieties is in group b, which has moderate level eicosenoic acid. Kilis Yağlık and Ayvalık varieties have high eicosenoic acid content and they are in group c (Table 3.18).

Table 3.18. Mean values and The Duncan's new multiple range test of eicosenoic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Yuvarlak Halhalı	4	0.1862 **a**		
Gemlik	12		0.2222 **b**	
Nizip Yağlık	11		0.2291 **b**	
Ayvalık	6			0.2597 **c**
Kilis Yağlık	6			0.2612 **c**
Significance		1.000	0.481	0.878

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Yuvarlak Halhalı variety has the lowest level of linolenic acid (group a) in varieties under study. Nizip Yağlık variety is in group of b with the high level. Other varieties have taken part in the same group (Table 3.19).

Table 3.19. Mean values and The Duncan's new multiple range test of linolenic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Yuvarlak Halhalı	4	0.4367 **a**	
Ayvalık	6	0.5343 **ab**	0.5343 **ab**
Gemlik	12	0.5463 **ab**	0.5463 **ab**
Kilis Yağlık	6	0.6348 **ab**	0.6348 **ab**
Nizip Yağlık	11		0.6832 **b**
Significance		0.095	0.209

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Yuvarlak Halhalı, Gemlik and Ayvalık varieties are close to each other in terms of behenic acid content. The level of behenic acid in Kilis Yağlık and Nizip Yağlık varieties are higher than others (Table 3.20).

Table 3.20. Mean values and The Duncan's new multiple range test of behenic acid

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Yuvarlak Halhalı	4	0.0863 **a**		
Gemlik	12	0.0947 **ab**	0.0947 **ab**	
Ayvalık	6		0.1043 **b**	
Nizip Yağlık	11			0.1340 **c**
Kilis Yağlık	6			0.1430 **c**
Significance		0.242	0.181	0.212

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Yuvarlak Halhalı, Ayvalık and Gemlik varieties are in group of a. Kilis Yağlık and Nizip Yağlık varieties are in group of b with respect to lignoceric acid (Table 3.21).

Table 3.21. Mean values and The Duncan's new multiple range test of lignoceric acid

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Yuvarlak Halhalı	4	0.0415 **a**	
Ayvalık	6	0.0453 **a**	
Gemlik	12	0.0514 **a**	
Nizip Yağlık	11		0.0885 **b**
Kilis Yağlık	6		0.0900 **b**
Significance		0.222	0.838

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

3.4. Sterol Composition and total sterol

The olive oil samples from Gemlik, Ayvalık, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region were analyzed with respect to sterol composition (Table

3.22). The profiles of β-Sitosterol (Figure 3.4), Δ-7-Stigmastenol (Figure 3.5) and total sterol contents (Figure 3.6) were given below:

Table 3.22. Results of sterol composition analysis of olive oils from Gemlik, Ayvalık, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region

OLİVE VARIETIES	Cholesterol	Campesterol	Stigmasterol	Apparent β-Sitosterol *	Delta-7 Stigmastenol	Delta-7 Avenasterol	Total Sterol
	%	%	%	%	%	%	(mg/kg)
Gemlik K1	0,16	2,04	0,52	96,55	0,22	0,47	1201,44
Gemlik K3	0,28	1,43	0,52	97,41	0,28	0,09	1092,53
Nizip Yağlık K4	0,04	3,51	0,61	95,23	0,56	0,05	1901,52
Nizip Yağlık K5	0,19	2,97	0,87	95,54	0,43		1790,84
Ayvalık K8		3,00	0,48	96,17	0,29	0,06	2178,15
Kilis Yağlık K9		2,75	0,52	96,29	0,43		1653,51
Kilis Yağlık K10	0,09	2,57	0,32	96,48	0,53	0,02	1700,94
Kilis Yağlık K11	0,13	3,10	0,66	95,41	0,51	0,18	1261,10
Kilis Yağlık K12	0,15	2,40	0,50	96,16	0,43	0,36	1227,92
Gemlik K13	0,16	2,28	0,63	96,62	0,21	0,10	1171,41
Kilis Yağlık K14	0,04	3,11	0,32	96,03	0,45	0,05	1635,02
Kilis Yağlık K15	0,12	3,04	0,49	95,87	0,42	0,07	1764,93
Nizip Yağlık N3	0,22	2,90	0,68	95,61	0,51	0,09	1328,77
Gemlik N4	0,06	1,70	0,72	97,29	0,17	0,05	1616,55
Nizip Yağlık N5	0,08	2,86	0,60	96,14	0,41	0,05	1562,93
Nizip Yağlık N6	0,26	2,52	0,60	96,17	0,41	0,05	1608,57
Gemlik N8	0,11	1,97	0,72	96,95	0,18	0,07	1485,98
Nizip Yağlık N9	0,18	2,19	0,66	96,41	0,50	0,05	1261,61
Nizip Yağlık N10	0,12	2,23	0,68	96,30	0,54	0,13	1186,43
Nizip Yağlık G1	0,56	2,02	0,61	96,06	0,38		1285,78
Nizip Yağlık G2	0,31	2,05	0,45	96,54	0,44	0,39	1167,59
Nizip Yağlık G3	0,14	2,04	0,41	96,79	0,55	0,07	1173,94
Nizip Yağlık G4	0,09	2,46	0,58	96,39	0,43	0,05	1100,03
Gemlik G5	0,11	2,28	1,07	96,29	0,17	0,08	1645,62
Gemlik G6	0,05	1,95	0,84	96,88	0,25	0,03	1044,40
Ayvalık G10	0,07	1,90	0,50	96,78	0,30	0,45	1156,89
Ayvalık G11	0,17	2,75	0,65	96,00	0,27	0,16	1922,04
Ayvalık G12	0,02	2,49	0,54	96,69	0,26		2030,09
Ayvalık G14	0,07	2,98	0,56	96,00	0,27	0,11	1546,58
Gemlik G15	0,31	1,97	0,73	96,89		0,09	1296,86
Gemlik G16	0,27	1,95	0,88	96,75	0,15		1517,52
Gemlik G18	0,38	1,56	0,66	97,18	0,19	0,02	1522,32
Ayvalık G19	0,31	2,36	0,42	96,73	0,18		1921,91
Gemlik G20	0,23	1,50	0,65	96,81	0,29	0,52	1261,58
Gemlik G21	0,24	1,90	0,84	96,55	0,28	0,19	1386,88

* Apparent β-Sitosterol is the sum of percentage of Delta-5,23 Stigmastadienol, Clerosterol, ß-Sitosterol, Delta-5-Avenasterol and Delta-5,24 Stigmastadienol.

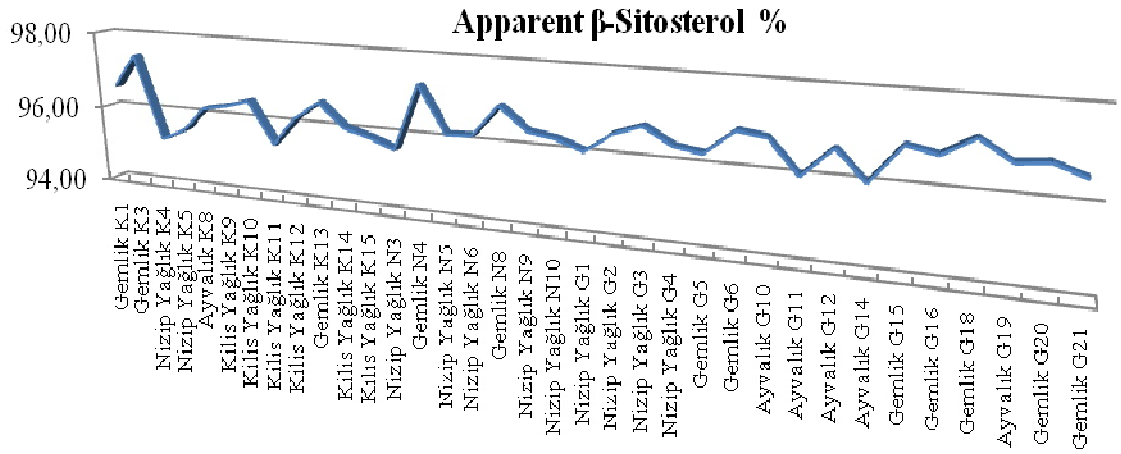

Figure 3.4. Apparent β-Sitosterol profiles of Gemlik, Ayvalık, Kilis Yağlık and Nizip Yağlık varieties

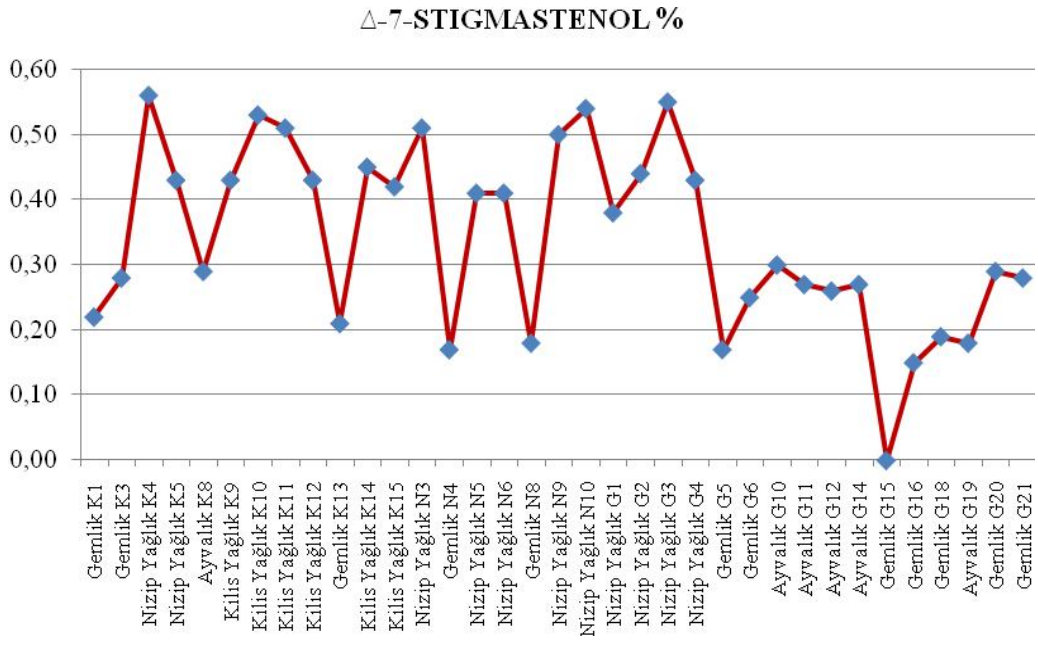

Figure 3.5. Percent Δ-7-Stigmastenol profiles of Gemlik, Ayvalık, Kilis Yağlık and Nizip Yağlık varieties

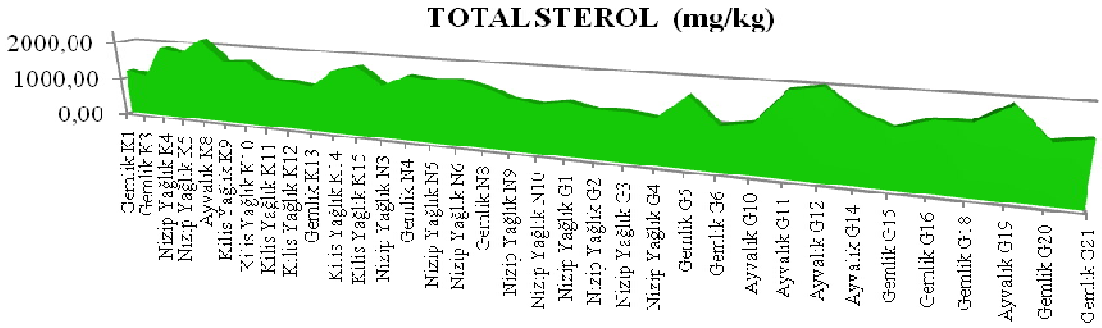

Figure 3.6. Total sterol contents of Gemlik, Ayvalık, Kilis Yağlık and Nizip Yağlık varieties

According to Table 3.23, the variation of campesterol, stigmasterol, apparent β-sitosterol, Δ-7stigmastenol, maturity index, total sterol and free acidity levels are statistically significant among varieties. However, the level of cholesterol, Δ-7avenasterol and altitude is statistically insignificant. It has been reported that most of sterols and triterpenic dialcohols are effected dramatically by maturation stage of the olive fruit (Koutsaftakis et al., 1999).

Table 3.23. Variance analysis of sterol composition, total sterol, maturity index, free acidity and altitude

		Sum of Squares	df	Mean Square	F	Significance
Cholesterol	Between Groups	0.080	3	0.027	2.019	0.132
	Within Groups	0.412	31	0.013		
	Total	0.492	34			
Campesterol	Between Groups	4.691	3	1.564	10.694	0.000
	Within Groups	4.533	31	0.146		
	Total	9.223	34			
Stigmasterol	Between Groups	0.341	3	0.114	6.598	0.001
	Within Groups	0.534	31	0.017		
	Total	0.875	34			
Δ-7stigmastenol	Between Groups	0.547	3	0.182	43.492	0.000
	Within Groups	0.130	31	0.004		
	Total	0.677	34			

Table 3.23. Variance analysis of sterol composition, total sterol, maturity index, free acidity and altitude (continued)

		Sum of Squares	df	Mean Square	F	Significance
Δ-7avenasterol	Between Groups	0.020	3	0.007	0.313	0.816
	Within Groups	0.676	31	0.022		
	Total	0.697	34			
Apparent Betasitosterol	Between Groups	4.123	3	1.374	8.874	0.000
	Within Groups	4.801	31	0.155		
	Total	8.925	34			
Totalsterol	Between Groups	874695.709	3	291565.236	4.129	0.014
	Within Groups	2189162.800	31	70618.155		
	Total	3063858.509	34			
Maturity index	Between Groups	9.151	4	2.288	10.190	0.000
	Within Groups	7.633	34	0.225		
	Total	16.784	38			
Free Acidity	Between Groups	2.522	3	0.841	727.86	0.000
	Within Groups	0.036	31	0.001		
	Total	2.557	34			
Altitude	Between Groups	88491.004	4	22122.751	2.375	0.071
	Within Groups	316751.765	34	9316.228		
	Total	405242.769	38			

All varieties are in group of a and the differences are statistically insignificant for cholesterol content (Table 3.24).

Table 3.24. Mean values and The Duncan's new multiple range test of cholesterol

OLIVE VARIETIES	N	Subset for alpha = 0.05
		1
Kilis Yağlık	6	0.0883 **a**
Ayvalık	6	0.1067 **a**
Gemlik	12	0.1967 **a**
Nizip Yağlık	11	0.1991 **a**
Significance		0.090

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

The campesterol content of Gemlik variety is lower than other varieties (group a) (Table 3.25).

Table 3.25. Mean values and The Duncan's new multiple range test of campesterol

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Gemlik	12	1.8775 **a**	
Nizip Yağlık	11		2.5227 **b**
Ayvalık	6		2.5800 **b**
Kilis Yağlık	6		2.8283 **b**
Significance		1.000	0.144

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Kilis Yağlık (group a) variety has low and Gemlik variety (group c) has high stigmasterol content than others (Table 3.26).

Table 3.26. Mean values and The Duncan's new multiple range test of stigmasterol

OLIVE VARIETIES	N	Subset for alpha = 0.05		
		1	2	3
Kilis Yağlık	6	0.4683 **a**		
Ayvalık	6	0.5250 **ab**	0.5250 **ab**	
Nizip Yağlık	11		0.6136 **bc**	0.6136 **bc**
Gemlik	12			0.7317 **c**
Significance		0.398	0.190	0.084

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Gemlik variety has higher β-sitosterol content than other varieties (Table 3.27). β-sitosterol content of all samples is higher than the minimum limit set by legislation (93 %).

The primary plant sterol is β-sitosterol (Bouic Patrick J.D. 2002). Phytosterols lower the amount of cholesterol in blood and also have many helpful effects such as anti-inflammatory (Brufau et al. 2008).

Table 3.27. Mean values and The Duncan's new multiple range test of Apparent β-sitosterol

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Kilis Yağlık	6	96.0400 **a**	
Nizip Yağlık	11	96.1073 **a**	
Ayvalık	6	96.3950 **a**	
Gemlik	12		96.8475 **b**
Significance		0.100	1.000

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Gemlik and Ayvalık varieties are in group of a those contain low Δ-7stigmastenol level than Kilis Yağlık and Nizip Yağlık varieties (Table 3.28). The mean Δ-7stigmastenol contents of Kilis Yağlık and Nizip Yağlık varieties are remarkably higher than that of Gemlik and Ayvalık varieties.

Six samples of total seventeen samples from Kilis Yağlık and Nizip Yağlık varieties are higher than the legal maximum 0.5 % in terms of Δ-7-stigmastenol content.

Variety has evident effect on Δ-7stigmastenol content. Type of soil, geographical area, degree of maturation and olive fly infestation have effected Δ-7stigmastenol contents. In addition Δ-7stigmastenol content is effected moderately by temperature of pressing, olive variety, olive oil storage conditions and olive storage time and conditions prior to processing. (Abu-Alruz et al. 2011).

The amount of stigmasterol, campesterol, Δ5,24-stigmastadienol, uvaol, and apparent β-sitosterol is high in fresh oils, but, the concentration of Δ7-stigmastenol and campestanol is high in stored oils (Lukic et al., 2013).

Table 3.28. Mean values and The Duncan's new multiple range test of Δ-7stigmastenol

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Gemlik	12	0.1992 **a**	
Ayvalık	6	0.2617 **a**	
Kilis Yağlık	6		0.4617 **b**
Nizip Yağlık	11		0.4691 **b**
Significance		0.065	0.821

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

As shown in Table 3.29. there is no significant difference between varieties in terms of delta7-avenasterol.

Table 3.29. Mean values and The Duncan's new multiple range test of Δ-7avenasterol

OLIVE VARIETIES	N	Subset for alpha = 0.05
		1
Nizip Yağlık	11	0.0845 **a**
Kilis Yağlık	6	0.1133 **a**
Ayvalık	6	0.1300 **a**
Gemlik	12	0.1425 **a**
Significance		0.485

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

Gemlik and Nizip Yağlık varieties get involved in same group (Table 3.30.) and have low total sterol level than Ayvalık variety. Kilis Yağlık variety is in both a and b groups.

The seeds, legumes and cereal grains contain high amount of squalene, tocopherol and plant sterols. In addition their fatty acid composition is useful for cardiovascular health (Ryan et al. 2007).

Table 3.30. Mean values and The Duncan's new multiple range test of total sterol

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Gemlik	12	1353.5908 **a**	
Nizip Yağlık	11	1397.0918 **a**	
Kilis Yağlık	6	1540.5700 **ab**	1540.5700 **ab**
Ayvalık	6		1792.6100 **b**
Significance		0.197	0.069

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

The obtained data are insufficient for determining whether the altitude affects the sterol composition, total sterol, free acidity and fatty acid composition or not (Table 3.31).

Table 3.31. Mean values and The Duncan's new multiple range test of altitude

OLIVE VARIETIES	N	Subset for alpha = 0.05	
		1	2
Yuvarlak Halhalı	4	583.0000 **a**	
Nizip Yağlık	11	665.2727 **ab**	665.2727 **ab**
Kilis Yağlık	6	680.3333 **ab**	680.3333 **ab**
Gemlik	12		711.5833 **b**
Ayvalık	6		760.6667 **b**
Significance		0.091	0.109

Means in a column with same letter group are insignificant according to Duncan's new multiple range test ($p < 0,05$)

4. CONCLUSIONS

1. Maturation indices were found to be low for Nizip Yağlık and Yuvarlak Halhalı olive oils whereas high for other olive varieties. Maturation stage had no significant effect on free acidity.
2. The differences in free acidity levels for all varieties were statistically significant. Ayvalık variety has the highest free acidity among all other varieties.
3. Local varieties, namely Nizip Yağlık, Kilis Yağlık and Yuvarlak Halhalı had rich SFA content whereas Gemlik variety was the richest variety in terms of MUSFA, and Yuvarlak Halhalı was the richest variety in terms of PUSFA.
4. The percentage of Δ7-Stigmastenol was 0.47 % and 0.46 % in Nizip Yağlık and Kilis Yağlık varieties, respectively. Gemlik and Ayvalık varieties exhibit lower Δ7-Stigmastenol level.
5. Ayvalık (1793 mg/kg) and Kilis Yağlık (1541 mg/kg) varieties were observed to be rich in terms of total sterol content.
6. Δ7-Stigmastenol levels of two local varieties, namely Nizip Yağlık and Kilis Yağlık were found higher than the newly planted varieties, Gemlik and Ayvalık.
7. It was observed that, there is significant relation between olive variety and composition of sterols.
8. Fatty acid composition was also remarkably affected by olive variety. The percentage oleic acid content was 61.90 % and 74.48 % in Yuvarlak Halhalı and Gemlik varieties, respectively. It was observed that Ayvalık, Nizip Yağlık and Kilis Yağlık varieties have close oleic acid levels.
9. In this study, it was tried to determine the differences between varieties in terms of fatty acid composition and sterol composition. However, a more detailed research should be needed to take into consideration of harvest year, climatic conditions, composition of soil, process conditions and producing technique of olive fruits.

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APPENDICES

Table.A.1. The results of SFA, MUSFA and PUSFA contents of olive oils from Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region

	SFA's (%)	**MUSFA's (%)**	**PUSFA's (%)**
Gemlik K1	16.741	75.066	8.195
Gemlik K3	17.027	74.991	7.982
Nizip Yağlık K4	20.940	67.815	11.245
Nizip Yağlık K5	21.510	67.054	11.435
Yuvarlak Halhalı K6	19.582	63.084	17.335
Yuvarlak Halhalı K7	20.161	60.649	19.190
Ayvalık K8	17.516	66.806	15.678
Kilis Yağlık K9	20.012	70.125	9.859
Kilis Yağlık K10	21.540	66.907	11.555
Kilis Yağlık K11	18.933	71.444	9.623
Kilis Yağlık K12	18.992	72.073	8.937
Gemlik K13	17.619	75.933	6.451
Kilis Yağlık K14	19.280	70.067	10.653
Kilis Yağlık K15	20.142	70.160	9.698
Yuvarlak Halhalı N1	20.575	63.266	16.158
Yuvarlak Halhalı N2	20.039	64.537	15.426
Nizip Yağlık N3	21.036	68.471	10.495
Gemlik N4	16.794	76.676	6.532
Nizip Yağlık N5	21.034	69.107	9.860
Nizip Yağlık N6	20.779	68.807	10.415
Gemlik N8	16.749	75.954	7.297
Nizip Yağlık N9	20.807	69.649	9.540
Nizip Yağlık N10	20.699	67.467	11.835
Nizip Yağlık G1	19.067	72.051	8.883
Nizip Yağlık G2	19.092	71.118	9.787
Nizip Yağlık G3	19.036	70.628	10.337
Nizip Yağlık G4	18.740	72.286	8.973
Gemlik G5	17.241	70.389	12.371
Gemlik G6	16.362	77.940	5.699
Ayvalık G10	16.263	77.600	6.139
Ayvalık G11	17.205	70.665	12.132
Ayvalık G12	17.230	69.510	13.261
Ayvalık G14	16.731	72.393	10.877
Gemlik G15	17.339	77.673	4.989
Gemlik G16	16.234	77.837	5.929
Gemlik G18	17.626	74.691	7.682
Ayvalık G19	16.901	72.102	10.997
Gemlik G20	16.605	78.587	4.807
Gemlik G21	16.462	78.265	5.276

Table.A.2. The results of maturity index, free acidity and altitude of olive oils from Gemlik, Ayvalık, Yuvarlak Halhalı, Kilis Yağlık and Nizip Yağlık varieties in Gaziantep region

	Maturity index	**Free Acidity**	**Altitude**
Gemlik K1	4.52	0.26	633 m
Gemlik K3	4.47	0.27	633 m
Nizip Yağlık K4	4.82	0.24	633 m
Nizip Yağlık K5	4.17	0.23	633 m
Yuvarlak Halhalı K6	3.51	-	633 m
Yuvarlak Halhalı K7	3.58	-	633 m
Ayvalık K8	4.67	1.08	633 m
Kilis Yağlık K9	4.47	0.35	680 m
Kilis Yağlık K10	4.80	0.36	680 m
Kilis Yağlık K11	4.40	0.36	652 m
Kilis Yağlık K12	2.98	0.31	652 m
Gemlik K13	4.49	0.27	652 m
Kilis Yağlık K14	5.21	0.37	709 m
Kilis Yağlık K15	4.84	0.32	709 m
Yuvarlak Halhalı N1	3.39	-	533 m
Yuvarlak Halhalı N2	3.42	-	533 m
Nizip Yağlık N3	4.91	0.26	560 m
Gemlik N4	4.67	0.24	560 m
Nizip Yağlık N5	4.73	0.24	560 m
Nizip Yağlık N6	4.50	0.25	560 m
Gemlik N8	4.90	0.25	551 m
Nizip Yağlık N9	2.99	0.22	532 m
Nizip Yağlık N10	4.19	0.26	532 m
Nizip Yağlık G1	4.41	0.24	827 m
Nizip Yağlık G2	4.53	0.25	827 m
Nizip Yağlık G3	4.65	0.26	827 m
Nizip Yağlık G4	4.29	0.24	827 m
Gemlik G5	5.25	0.30	808 m
Gemlik G6	4.98	0.29	808 m
Ayvalık G10	5.30	0.99	800 m
Ayvalık G11	5.48	0.97	800 m
Ayvalık G12	5.50	1.00	800 m
Ayvalık G14	5.14	1.02	756 m
Gemlik G15	4.47	0.29	756 m
Gemlik G16	5.23	0.31	775 m
Gemlik G18	5.12	0.32	775 m
Ayvalık G19	5.28	0.87	775 m
Gemlik G20	5.37	0.30	794 m
Gemlik G21	5.30	0.31	794 m