

# The Effects of Green Table Olive Processing Methods on Polyphenol Content of Some Turkish Table Olive Varieties

Şahnur IRMAK<sup>1</sup> \* Hülya IRMAK<sup>2</sup>

## Abstract

In this manuscript, some olive varieties having an importance for the Turkish table olive sector such as Ayvalik and Domat variety olives are analyzed in order to determine the types and the amounts of the phenolic compounds of which potential antioxidant activities are extremely high. The effects of the processing techniques on the phenolic compounds belonging to Turkish table olive varieties are found statistically significant in the level of  $p > 0,01$ . It is determined that the amount of phenolic compounds decreases particularly in the processing of split olive due to the diffusion of phenolic compounds into the brine as well as in the processes of olives with due to the use of caustic, that increases the hydrolysis of polyphenols and diffusion, in order to remove the bitterness of olives.

**Keywords:** Table olive, Phenolic compounds, Total phenol, Processing, HPLC

## Introduction

Olive is considered as a different kind of fruit with its low sugar content, high levels of oil content and specific bitter taste (Mafra et al., 2006). One of the three main characteristics that makes the olive fruit different from the other fruits is that olive contains sugar in the amounts of 2-6% and oil in the amounts of 20-35%, whereas the other fruits contain higher levels of sugar such as 12% and lower levels of oil such as 1-2%. Another characteristic that separates olive from the other fruits is oleuropein, which is a glucosidic matter giving the specific bitter taste to olive.

The pulp fraction of olive consists of flavonoids, secoiridoids and phenolic compounds having simple phenol structure

such as C2-C6 in the amounts of 1-3% (Marsilio et al., 2001). Oleuropein is the first defined matter among these compounds (Brenes et al., 1992). Oleuropein, which is the most bioactive compound of olive, consists of three main structures such as a polyphenol, 4-(2-hydroxyethyl)benzene-1,2 diol that is also known as hydroxytyrosol; a secoiridoid known as elenolic acid and a glucose molecule. Oleuropein constitutes an importance also for human health due to its antiatherogenic, anticancerogenic, anti-inflammatory and antimicrobial effects (Gikas et al., 2007; Rivas et al., 2000).

Although many studies have been carried out related to the olive oil, the phenolic properties of the olive fruit have not been completely described in Turkey yet. The complexity of the structure, the existence of numerous varieties, the differences between maturation degrees of the varieties, and the factors related to geography, variety, process and agronomy result in difficulties in describing the phenolic properties of olive (Savarase et al., 2007). Table olives and olive oil are assumed as one of the most precious sources of the “functional foods” with the phenolic antioxidant compounds they contain (Garcia et al., 2000 ; Marsilio et al., 2001).

Free radicals, which are compounds with high activity and naturally existing in human body, increase in cases such as smoking and exposing to radiation. It is reported that these radicals initiate the coronary heart diseases and cancer via damaging lipids, proteins and DNA, whereas the phenolic compounds represent an effect on decreasing the risk of coronary heart diseases via strengthen the LDL (Low Density Lipoprotein) proteins against oxidation (Gaulejag et al., 1999; Romani et al.,1999; Visioli and Galli, 1994; Romero et al., 2004; Sousa et al., 2006; Boskou et al., 2006).

In addition to their antioxidant properties, phenolic compounds are the constituents that primarily affect the quality parameters due to their contribution to shelf-life, taste, flavour, colour; creating the sensory characterization depending on the formation of taste in table olives an olive oil; increasing the stability against otooxidation (Bianco and Uccella,2000; Garcia et al.,2000; Kalua et al.,2005; Savarase et al., 2007).

In several studies, it is stated that process techniques and the systems are assumed as the major factors affecting the types and the amounts of the phenolic compounds in olive as well as the variety and the maturity of olive (Ryan et al., 1999).

Each country has their own traditional methods for the consumption of olive in addition to the industrial production methods aimed at market. In Turkey, traditional methods used for the productions of green split and cracked olive, dry-salted olive, turning olive and olive in brine as well as the industrial processing techniques used for the productions of treated black olives, olives darkened by oxidation, Spanish style green olives, natural turning color olives and stuffed olives are applied properly for the world trade.

In Turkey, some of the olives used for the production technology of table olives are mostly produced for the purpose of table consumption (Gemlik, Domat and Uslu variety olives etc.), whereas some of the olives are evaluated in the sector for oil production (Ayvalik, Memecik variety olives etc.). Ayvalik variety olive is generally processed into green cracked and split, and turning color split olive; Domat variety olive is used for the productions of green cracked and split olive, Spanish style green olive and stuffed olives.

Researchers point out that the studies carried out in order to determine the quality characteristics of food products should not only focus on the characteristics of the final product, but also focus on the composition, texture, taste and the flavour of the raw materials. Recently, consumers are known to be more critical towards the modern production processes and thus the demand for the natural, un-processed foods and for the food products without additives increases. It is observed that the organic food products and the food products without additives, which are assumed as more reliable, tastier and more natural, are mostly preferred rather than the food products produced as a result of mass production in industrial scale. For this reason, hedonistic and functional subjects become more prominent for the qualification of nutritional value (Bianco and Uccella, 2000).

This study has an importance due to lack of the detailed studies related to the

subject in Turkey, although olive is a significant source of phenolic compounds and the phenolic compounds are effective matters on human health with their antioxidant activity. It is aimed to determine the phenolic profiles of some major olive varieties used for the purpose of green table olive consumption, in addition to provide the varieties rich in biophenols to be cultured widely. Furthermore, it is believed that the study contribute to the determination of the methods that have less effects on the decrease of phenolic capacity during the production of table olives. Thus, it is aimed at providing these methods to be applied widely and providing consumers to reach more qualified and healthy products after obtaining an increase in the quality characteristics of the products via the application of the mentioned methods.

## Material and Methods

### Materials

In this study, Ayvalik and Domat variety olives harvested from the collection plant of Bornova Olive Research Institute were used. For each processing collected about 240 kg olives and put into two containers. Then, three sample analyzed in three replicate.

The harvest times for the olive varieties were determined according to the specific process techniques stated in Turkish Food Codex. Domat olives were harvested in the first week of October, whilst Ayvalik were harvested in the third week of October.

### Processing olives

#### Processing green split olives

Domat and Ayvalik olives were harvested in the period of green-yellow and sized; then they were washed and taken into the polyester tanks after they were split. They were stored in brine consist of 2% NaCl and 0.2% citric acid during 4-6 weeks changing the solution per week. After the bitter taste was removed, olives were stored in brine consist of 8% NaCl and 1% citric or lactic acid.

### Processing Spanish-style green olives

Domat olives were harvested in green-yellow maturity and then separated according to their size. The process consisted of treating the olives with 1,8 g/100 mL NaOH solutions for Domat olives until the alkali reached 2/3 of the flesh. Then the fruits were washed with tap water for 24 h, brined in a 8 g/100 mL NaCl solution, and left to follow spontaneous fermentation. The acidity level of the olives was balanced at 0,3% the addition of lactic acid. The acidity level of the olives was 0,9-1,2% at the end of the fermentation.

### Chemicals

The chemicals used in the project were obtained from “Merck” as LC grade. Standards, Hydroxytyrosol (HTY) was obtained from “Extrasynthese” (France), Gallic acid (GA), Tyrosol (TY), Chlorogenic acid (CHL), Vanillic acid (VA), Caffeic acid (CA), Syringic acid (SYA), p-Coumaric acid (CO), Ferulic acid (FA), Cinnamic acid (CIN), Quercetin (QUE), Luteolin (LUT), and Apigenin (API) were kindly obtained from “Sigma” (USA).

### Extraction and determination of table olives phenolic compounds by HPLC

For the extraction of phenolic compounds, 5 grams of sample was centrifuged at 4000 rpm for 20 minutes with (80:20) % methanol: water (400ppm Sodium metabisulfite). The applications were repeated for 3 times. The collected methanol phases were evaporated at 35°C in rotary evaporator. Extraction with n-hexane and ethyl acetate was carried out for 3 times. The collected ethyl acetate phase was evaporated at 35°C in rotary evaporator. After it was solved with 2.5 ml methanol and filtrated through 0.45 µm, the sample was injected to 20 µl liquid chromatography device for the measurements (Morello et al., 2005).

In order to be able to determine the phenolic compounds analysis, we used a high performance liquid chromatography

(HPLC) system. It is an Agilent HP 1100 series, equipped with a vacuum degasser, a gradient pump, diode array UV detector (280 nm) and Phenomenex C18 RP (250mm x 4,6mm, 5µm) column. The temperature of the column was at ambient temperature. The injection volume was 20 µl, and elution was performed at a flow rate of 0,9 ml/min, using a mixture of formic acid 5% (solvent A) and methanol (solvent B) as mobile phases. The gradient elution program was changed as follows: to 98% (A) and (2%) for 3 min, 95% (A) and 5% (B) in 2 min, 90% (A) and 10% (B) in 5 min, 85% (A) and 15% (B) in 5 min, 80% (A) and 20% (B) in 15 min, 75% (A) and 25% (B) in 6 min, 65% (A) and 35% (B) in 3 min, 60% (A) and 40% (B) in 4 min, 55% (A) and 45% (B) in 6 min, 53% (A) and 47% (B) in 3 min, 50% (A) and 50% (B) in 17 min, 33% (A) and 67% (B) in 4 min and 100% solvent B in maintained for 10 min. Phenolic compounds were identified by comparing their retention times with those of commercial standards. The registration of spectra by an identification test is facilitated by the use of a photodiode receiver detector. Detection was done at 200 and 400 nm.

### *Statistical analysis*

In this project, three extractions of each sample were done and the extracts were analysed three times by HPLC. After applying variance analyses, the data were evaluated via Duncan's new multiple range test to different table olive methods (raw material, turning color split and Spanish style green).

## **RESULTS AND DISCUSSION**

### **Phenolic Compounds in Olive Varieties**

As a result of the HPLC analyses carried out on raw and the processed olive samples belonging to Ayvalik and Domat variety olives in order to determine the phenolic profile and the amounts. About thirteen phenolic compounds were established in olive varieties. These phenolic compounds are analyzed;

hydroxytyrosol, tyrosol, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, cinnamic acid, quercetin, luteolin, and apigenin. The standard chromatograms belonging to the analyzed phenolic compounds are depicted in Fig.1.

### **Phenolic Compounds Determined in Raw Olives**

All the phenolic compounds were identified in raw olive samples except for gallic acid and syringic acid. Chlorogenic acid was only determined in the raw olives. Gallic acid and syringic acid weren't found anyone olive samples. Also, while hydroxytyrosol, tyrosol, chlorogenic acid, caffeic acid and apigenin were determined as major phenolics in Ayvalik olive fruits, hydroxytyrosol, tyrosol, quercetin, caffeic acid, vanilic acid and ferulic acid were found as major phenolic compounds in Domat olive fruits. Concentrations, expressed as mg/100g of fresh weight, of the major biophenolic compounds found in the Ayvalik and Domat olive varieties studied at different table olive processing styles are reported in Table 1.

HTY quantity of Domat variety olive was found to be more rich according to Ayvalik variety olive. The values belonging to Domat and Ayvalik olives were found respectively as 55.61 and 42.46 mg/100g (Table 1).

Verdeal Transmontana (752 mg/kg) and Madural (830 mg/kg) variety olives in Portuguese presented higher HTY amounts (Sousa et al.2015). Levels of HTY are not consistent in the literature, ranging from 0.2 to ~71 g/kg (dw) (Charoenprasert and Mitchell, 2012). Concentration of HTY was determined as 57 mg/100g in the Intosso cultivar (Marsilio et al.2001). Also, Melliou et al.(2015) reported that HTY content (89,4 mg/100g) were measured in fresh olives (wet weight). HTY content was determined between from 18,9 to 89,18 mg/100g in Gemlik variety olives (Uylaser, 2015). HTY concentrations in our study were found similar or closely with other studies.

Domat had the highest amount of tyrosol demonstrating a significant difference with 11.21 mg/100g, whereas Ayvalik were determined as the samples having the lowest amount of tyrosol with 6.13 mg/100g.

Tyrosol content was found as 40 mg/100g in the Intosso cultivar by Marsilio et al.(2001). The results of the present study are in good agreement with the findings of Marsilio et al. (2001) who observed a decline in phenolic compound content with fresh olive. Also, Dagdelen et al.(2013) determined tyrosol concentration more in Domat olives according to Ayvalik olives. We have found lower values in our study than in other studies. It is seen that the phenolic constituents change according to the varieties.

The amount of luteolin in Domat olive was determined as 2.27 mg/100g whereas in Ayvalik olive was found as 3.66 mg/100g. Luteolin was determined in Cobrancosa variety about 7,5 mg/kg (Malheiro et al.,2011). Sousa et al. (2015) stated that luteolin characterized mainly Verdeal Transmontana olives from the third and fourth (10th Nov.) sampling dates, due to higher content on this flavone.

In terms of apigenin, the highest value was determined in Ayvalik olives with 7.54 mg/100g; whereas lower amounts of apigenin were found in Domat olive as 3.64 mg/100g. Apigenin was the most abundant phenolic compound in Ayvalik olives after HTY. Due to the amount of apigenin, it can be separated from other olives. Thus, apigenin may be evaluated as a characteristic property for these olive varieties. Vanillic acid (3 mg/100g) and flavanoid content (Luteolin-7-O-glucoside – 2 mg/100g) were low in fresh olives (Marsilio et al.2001).

Chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, cinnamic acid and quercetin contents were determined between 5,9 and 2,13 mg/100g. Phenolic acids such as p-coumaric acid, chlorogenic acid, vanillic acid, syringic, ferulic, and homovanillic acid, and caffeic

acid are also present in pulp, and their levels are generally in the milligram per kilogram range (Charoenprasert and Mitchell, 2012). Also, chlorogenic acid was identified varying between 6,1 and 1,2 mg/100g in Portuguese olive cultivars (Sousa et al.2015).

Gallic and syringic acids were not determined in the raw olive samples. Also, Sousa et al. (2006) couldn't detect syringic and vanillic acids in 5 olive varieties of Portuguese.

The differences in terms of the types and the compositions of the phenolic compounds that the raw samples of the olive varieties contain were found statistically significant in the level of  $p < 0.01$ . These significant differences could be explained by the cultivated variety and the specific processes applied on the fruits.

Pereira et al. (2006) informed that such changes on both quantitative and qualitative fractions of phenolic compounds in the studied table olives are related to olive cultivar. The phenolic composition of olives is very complex and depends upon many factors such as fruit maturation stage, part of the fruit (e.g., pulp or seed), cultivar, and season. There are considerable differences in the levels of these phenolics among cultivars (Charoenprasert and Mitchell, 2012).

Hydroxytyrosol, tyrosol, and their glycosidic forms are the predominant phenolic alcohols in olive pulp. Flavonoids and phenolic acids are present at low concentration (usually  $< 100$  mg/kg dry weight) and include luteolin-7-glucoside, rutin, apigenin-7-glucoside, luteolin-4-glucoside, luteolin-7-rutinoside, and quercetin-3-rhamnoside (Charoenprasert and Mitchell, 2012). According to the results of other study, the highest levels of hydroxytyrosol (253.67 mg/kg), vanillic acid (30.98 mg/kg), tyrosol (28.70 mg/kg), syringic acid (3.28 mg/kg), p-coumaric acid (2.94 mg/kg), ferulic acid (0.85 mg/kg) and cinnamic acid (0.21 mg/kg) were determined in the fresh Gemlik variety olives (Uylaser, 2015).

## The Amounts of the Phenolic Compounds Determined According to the Olive Varieties

### Phenolic Profiles of Ayvalik Variety Split Olives

In international olive council table olives preparing methods, “split olives” are known that whole olives are splitted lengthwise by cutting into the skin and part of the flesh. HTY, luteolin and apigenin contents were found higher in the olive samples that had been processed with split olive technique than the raw olive samples had (Table 1).

During the fermentation period, the HTY amount showed a constant increasing trend after 180 days. HTY compound is considered a marker to determine the oleuropein degradation and the diffusion of phenols from drupes to brine (Randazzo et al., 2011). The increase observed in the HTY content may be explained with the decomposition of oleuropein during the fermentation period and as a result of that creating HTY. Several researchers also support the approach of the increase in the amount of HTY as a result of oleuropein decomposition during the fermentation period. Thus, it is assumed as an expected result (Esti et al.1998 ; Gikas et al.2007; Marsilio et al.2001; Morello et al.2005a ; Rivas et al.2000). Similarly, the concentration of the simple phenol HTY increased during fermentation due to the increased activity of some hydrolytic enzymes.

HTY, luteolin and apigenin content which demonstrated increase with the application of process technique. It is considered that the increase observed in the amount of luteolin was due to the hydrolysis reactions occurred on phenolic compounds during the production processes. In some references, it is pointed out that the amount of luteolin demonstrated an increase during the maturation period. Furthermore, it is stated that the increase in the amount of luteolin should be regarded as a determination criterion for the maturation level.

HTY was the main simple phenolic compound identified in all brines, its proportion was up to 84% of total simple phenolic compounds. Actually, this finding is in a good agreement with the literature data where HTY was found to be the most abundant phenol in green table olives. This compound results from the hydrolysis of oleuropein, which is the major phenolic in fresh green olive fruit (Kiai and Hafidi, 2014). During fermentation, HTY was 25 mg/L after 15 days, and it became 155 mg/L after 270 days (Poiana and Romeo, 2006).

Chlorogenic acid content was determined as 5.9 mg/100g in the processed olive samples. This amount is assumed as lower than the limit levels that might be determined in processed samples. For this reason, this data is leading to the consideration of the loss of chlorogenic acid during the fermentation period.

The phenolic compounds that are absent in the raw and processed samples of Ayvalik olives were determined as syringic acid and gallic acid. The absence of these acids in Ayvalik olive variety may be regarded as an important criterion in evaluating the phenolic profile of this olive variety. In case this result is supported by the further studies, the absence of syringic acid and gallic acid in Ayvalik variety may be approved as one of the typical characteristics of this variety.

A decrease with the application of process techniques was observed in the amounts of the other phenolic compounds such as tyrosol, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, cinnamic acid and quercetin. Chlorogenic acid, vanillic acid and p-coumaric acid contents of Ayvalik fruits were established as 4.05, 4.74 and 3.92 mg/kg, respectively (Dagdelen et al. (2013). Our values has found more according to Dagdelen et al. (2013). This differences may be due to fruit maturation stage, part of the fruit and agronomic conditions (climate, fertilization etc.).

During storage in brine, in our study, the amount of tyrosol has decreased while it increased in the study of Marsilio et al.

(2001). Brenes-Balbuena et al. (1992) reported caffeic acid as the product of verbascoside degradation which appeared after fermentation in all types of olives. p-Coumaric acid can be diffused moderately into the brine due to its low solubility.

As Sousa et al. (2006) reported that the cracked olives underwent more losses during the washing and debittering stages. During removal of bitterness, characteristic of green olives, the loss of hydrosoluble compounds is unavoidable. The cultivar phenolic amount is, therefore, of major importance for the residual amounts of phenolics in processed “splitting”.

Pistarino et al. (2013) stated that more than 75% of phenolic compounds were reduced in the olive pulps after 100th days of the fermentation.

#### *Phenolic Compounds of Domat Variety Split Olives and Spanish Style Green Olives*

As it was observed in the results of Ayvalik olives, HTY content demonstrated an increase during the fermentation period in the split samples (61.16 mg/100g) and the samples with caustic (84.07 mg/100g) when compared to the raw olive samples (55.61 mg/100g) that belong to the Domat variety as well (Table 1).

The studies carried out also indicated that HTY content increased in the olive samples processed with caustic as a result of the hydrolysis of oleuropein (Kiai and Hafidi, 2014; Marsilio et al. 2001). It was determined that although HTY was a water-soluble matter, it could still exist in the composition of olive in high amounts. This compound results from the hydrolysis of oleuropein, which is the major phenolic in fresh green olive fruit. Moreover, an increase of HTY content in brine during the brining process is reported due to its diffusion from the olives into the brine and also because of the acid hydrolysis of oleuropein, and phenols that decreased during the brining process (Romero et al., 2004). Thus, at the end of processing, HTY

become the main phenol in brine (Kiai and Hafidi, 2014).

As well as the samples processed with caustic, HTY content increased due to the hydrolysis of oleuropein in split-type olive samples; whilst it is also estimated that some part of HTY diffusion into the brine due to its water-soluble characteristic (Morello et al., 2005).

Luteolin and apigenin contents were found higher in the samples that the splitting process was applied in comparison to the raw samples, whereas lower amounts were determined in the samples processed with caustic (Table 1). As well as in the Ayvalik samples processed via splitting technique, the increase in the amounts of luteolin and apigenin in split samples and the decrease in samples processed with caustic were found to be related to the processing techniques in Domat variety.

A decrease was observed in the values of tyrosol and caffeic acid existing in both of the processed olive samples in comparison to the raw olive samples. The amounts of the other phenolics such as vanillic acid, p-coumaric acid, ferulic acid, cinnamic acid and quercetin demonstrated decrease in the samples processed with splitting technique, whilst none of the mentioned phenolics were found in the sample group processed with caustic. Vanillic acid, p-coumaric acid, ferulic acid, cinnamic acid and quercetin weren't identified in the Spanish style table olives. Dağdelen et al. (2013) stated that HTY, oleuropein, tyrosol, vanilic acid, rutin, luteolin and p-coumaric acid were determined as major phenolics in Domat olive fruits.

It was determined that Domat type raw samples contained 2.38 mg/100g of chlorogenic acid. However, it was found out that chlorogenic acid content in both of the processed Domat olive samples were below the limit levels. Thus, it is considered that chlorogenic acid content decreased during the fermentation period.

Considering the absence of the phenolic compounds such as syringic acid

and gallic acid in raw and the processed samples belonging to Domat variety, it may be indicated that this data would be a significant parameter in determining the phenolic profile of Domat type olives.

In another study, antioxidant capacity of table olives was evaluated according to processing techniques. Processing methods were showed significant differences. The average antioxidant capacity of processed olives was in the following order; untreated black olives in brine > Californian style black olives > untreated black olives in dry salt > Spanish style green olives (Sahan et al.2013). It is revealed that the Spanish style process causes significant loss of phenolic compounds.

#### 4. Conclusion

In this study, the phenolic properties of Ayvalik and Domat olive varieties that have a huge field of production and an industrial value in Turkey were determined. In addition, the effects of the processing techniques applied in order to make these olives available as table olives on the phenolic compound were also determined. As a result, the effect of the processing techniques on the amounts and the characteristics of the phenolic compounds of the table olive samples were found statistically significant in the level of  $p > 0.01$ . These significant differences could be explained by the cultivated variety and the specific processes applied on the fruits, especially the use of brine or lye.

Hydroxytyrosol, tyrosol, apigenin and luteolin were identified for Ayvalik and Domat at the raw and processed olives. Both of the olive varieties were generally found to be rich in the phenolic compounds such as hydroxytyrosol, tyrosol, luteolin and apigenin.

Split-type olive processing caused a diffusion of the phenolic compounds to the brine due to the split existing on the olive sample. Moreover, the usage of lye solution in the process techniques and the processing of olives with NaCl in order to remove the bitter taste of the olives caused a diffusion

and hydrolysis of polyphenols. Thus, particularly these mentioned olive processing techniques were found to be affective on decreasing the amounts of the phenolic compounds in olive samples.

Malheiro et al.(2011) also reported that individual amounts of phenolic compounds are significantly affected ( $P < 0.001$ ), with the exception of quercetin, by the olive cultivar used for table olive processing, and among the phenolic compounds identified, the most abundant were hydroxytyrosol, tyrosol and verbascoside.

Melliou et al.(2015) indicated that the rightness of the consumers in tending to prefer mostly natural food products in recent days was underlined once again in their study. Our study also support this findings. The significance of the amount of the determined phenolic compounds existing in olive samples proved the necessity of olive to take more place in tables.

The information presented in this investigation shows variation in the composition of a range of key phenolic compounds in olives that is dependent upon both the variety and processing method used to create the olive product, and these effects must be considered when developing possible health claims for table olives and their products.



## References

- Bianco A. & Uccella N. (2000). Biophenolic components of olives. *Food Research International* 33, 475-485
- Boskou G., Fotini N.S., Chrysostomou S., Mylona A., Chiou A., Andrikopoulos N.K. (2006). Antioxidant capacity and phenolic profile of table olives from the Greek market. *Food Chemistry*, 94, 558–564
- Brenes, M., Garcia, P., Fernandez, A., (1992). Phenolic Compounds Related to the Black Color Formed during the Processing of Ripe Olives. *J. Agric. Food Chem.*, 40, 1192-1196.
- Brenes Balbuena, M., Garcia, P., Fernandez, A., (1992). Phenolic compounds related to the black color formed during the processing of ripe olives. *J. Agric. food Chem.*, 40, 1192-1196.
- Charoenprasert, S. and Mitchell, A., 2012, Factors Influencing Phenolic Comp. in Table Olives (*Olea europaea*), *J. Agric. Food Chem.*, 60, 7081-7095pp.
- Dağdelen, A., Tümen G., Özcan M.M., Dündar E., 2013, Phenolics profiles of olive fruits (*Olea europaea* L.) and oils from Ayvalık, Domat and Gemlik varieties at different ripening stages. *Food Chem.* 136, 41-45
- Esti M., Cinquanta L., La Notte E., (1998), Phenolic Compounds in Different Olive Varieties. *J. Agric. Food Chem.*, 46, 32-35
- Garcia, O.B., Castillo, J., Lorente, J., Ortuno, A., Del Rio, J.A., (2000). Antioxidant Activity of Phenolic Extracted from *Olea europaea* L. leaves. *Food Chemistry*, 68, 457-462.
- Gaulejac, N.S., Provost, C., Vivas, N., (1999). Comparative Study of Polyphenol Scavenging Activities Assessed by Different Methods, *J. Agric. Food Chem.*, 47, 425-431.
- Gikas E., Bazoti F.N., Tzarbopoulos A. (2007) Conformation of oleuropein, the major bioactive compound of *Olea europea*. *Journal of Molecular Structure: THEOCHEM* 821, 125–132
- Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., (2005). Discrimination of Olive Oils and Fruits into Cultivars and Maturity Stages Based on Phenolic and Volatile Compounds. *Journal of Agricultural And Food Chemistry*, 53, 8054-8062.
- Kiai, H. and Hafidi, A., 2014, Chemical composition changes in four green olive cultivars during spontaneous fermentation, *Food Science and Technology*, 57; 663-670pp.
- Mafra I., Barros A.S., Coimbra M.A. 2006 Effect of black oxidising table olive process on the cell wall polysaccharides of olive pulp. *Carbohydrate Polymers* 65, 1-8.
- Malheiro, R., Sousa, A., Casal, S., Bento, A., Pereira, J.A., 2011, Cultivar effect on the phenolic composition and antioxidant potential of stoned table olives, *Food and Chemical Toxicology* 49, 450–457.
- Marsilio V., Campestre C., Lanza B., (2001), Phenolic compounds change during Californian style ripe olive processing. *Food Chemistry*, 74, 55-60.
- McDonald, S., Prenzler, P.D., Antolovich, M., Robards, K., (2001). Phenolic Content and Antioxidant Activity of Olive Extracts. *Food Chemistry*, 73, 73-84.
- Melliou E., Zweigenbaum J.A. and Mitchell A.E., 2015, Quantitation of Polyphenols and Secoiridoids in California-Style Black Ripe Olives and Dry Salt-Cured Olives. *J. Agric. Food Chem.*, 63, 2400–2405
- Morello J., Vuorela S., Romero M., Motilva M.J., Heinonen M., (2005). Antioxidant Activity of Olive Pulp and Olive Oil Phenolic Compounds of the Arbequina Cultivar. *J. Agric. Food Chem.*, 53, 2008.
- Morello J., Romero M., Ramo T., Motilva J. 2005a, Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe from fruit setting period to harvesting time. *Plant Science* ,168, 65–72
- Pereira, J.A., Pereira, A.P.G., Ferreira, I.C.F.R., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., 2006, Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *J. Agric. Food Chem.* 54, 8425– 8431.
- Pistarino, E., Aliakbarian, B., Casazza, A. A., Painsi, M., Cosulich, M. E. and Perego, P. 2013. Combined effect of starter culture and temperature on phenolic compounds during fermentation of Taggiasca black olives. *Food Chemistry* 138: 2043-2049

- Secoiridoids in California-Style Black Ripe Olives and Dry Salt-Cured Olives. *J. Agric. Food Chem.*, 63, 2400–2405
- Morello J., Vuorela S., Romero M., Motilva M.J., Heinonen M., (2005). Antioxidant Activity of Olive Pulp and Olive Oil Phenolic Compounds of the Arbequina Cultivar. *J. Agric. Food Chem.*, 53, 2008.
- Morello J., Romero M., Ramo T., Motilva J. 2005a, Evaluation of L-phenylalanine ammonia-lyase activity and phenolic profile in olive drupe from fruit setting period to harvesting time. *Plant Science* ,168, 65–72
- Pereira, J.A., Pereira, A.P.G., Ferreira, I.C.F.R., Valentão, P., Andrade, P.B., Seabra, R., Estevinho, L., Bento, A., 2006, Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *J. Agric. Food Chem.* 54, 8425–8431.
- Pistarino, E., Aliakbarian, B., Casazza, A. A., Paini, M., Cosulich, M. E. and Perego, P. 2013. Combined effect of starter culture and temperature on phenolic compounds during fermentation of Taggiasca black olives. *Food Chemistry* 138: 2043-2049
- Poiana, M. and Romeo, F.V., 2006, Changes in Chemical and Microbiological Parameters of Some Varieties of Sicily Olives During Natural Fermentation, *Grasas y Aceites*, 57 (4); 402-408.
- Rivas, C.S., Espin, J.C., Wichers, H.J., (2000). Review Oleuropein and Related Compounds. *Journal of the Science of Food and Agriculture* 80, 1013-1023.
- Romero, C., Brenes, M., Yousfi, K., Garcia, P., Garcia, A., Garrido, A., (2004). Effect of Cultivar and Processing Method on the Contents of Polyphenols in Table Olives. *J. Agric. Food Chem.*, 52, 479-484.
- Romani, A., Mulinacci, N., Pinelli, P., Vincieri, F.F., Cimato, A., (1999). Polyphenolic Content in Five Tuscany Cultivars of *Olea europaea* L. *J. Agric. Food Chem.*, 47, 964-967.
- Ryan D., Robards K., Lavee S.(1999). Changes in phenolic content of olive during maturation. *International J. of Food Science. and Technology*, 34, 265-274.
- Sahan, Y., Cansev A. and Gulen H., 2013, Effect of Processing Techniques on Antioxidative Enzyme Activities, Antioxidant Capacity, Phenolic Compounds, and Fatty Acids of Table Olives, *Food Sci. Biotech.* 22(3): 613-620
- Savarese M., Marco E. De., Sacchi R. (2007) Characterization of phenolic extracts from olives (*Olea europaea* cv. Pisciotana) by electrospray ionization mass spectrometry. *Food Chemistry* 105, 761–770.
- Sousa, A., Ferreira I.C.F.R., Calhella, R., Andrade, P.B., Valentao, P., Seabra, R., Estevinho, L., Bento, A., Pereira J.A., (2006). Phenolics and Antimicrobial Activity of Traditional Stoned Table Olives "Alcaparra", *Bioorganic and Medicinal Chemistry*, 14, 8533-8538.
- Sousa A., Malheiro R., Casal S., Bento A. and Pereira J.A. 2015, Optimal harvesting period for cvs. Madural and Verdeal Transmontana, based on antioxidant potential and phenolic composition of olives. *LWT - Food Science and Technology* 62 1120-1126
- Visioli F., Galli C., (1994). Oleuropein protects low density protein from oxidation. *Life Science*,55, 1965 -1971.
- Uylaşer V. 2015, Changes in phenolic compounds during ripening in Gemlik variety olive fruits obtained from different locations. *CyTA - Journal of Food*, 13:2, 167-173

## TABLES

Table 1. Amounts of phenolic compounds of raw and processed olive samples

## FIGURE CAPTIONS

### Fig.1. Standard Material Chromatogram Belonging to the Phenolic Compounds

(Phenolic compounds: 1;Gallic acid, 2:Hydroxytyrosol, 3: Tyrosol, 4:Chlorogenic acid, 5:Vanillic acid, 6:Caffeic acid, 7: Syringic acid, 8:p-Coumaric acid, 9:Ferulic acid, 10:Cinnamic acid, 11:Quercetin, 12: Luteolin, 13:Apigenin)

### Fig.2 Phenolic Profiles in the Raw Olive Samples of Ayvalik Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3:Chlorogenic acid, 4:Vanillic acid, 5:Caffeic acid, 6:p-Coumaric acid, 7:Ferulic acid, 8:Cinnamic acid, 9:Quercetin, 10: Luteolin, 11:Apigenin)

### Fig.3 Phenolic Profiles in the Turning Colour Split Olive Samples of Ayvalik Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3: Vanillic acid, 4:Caffeic acid, 5:p-Coumaric acid, 6:Ferulic acid, 7:Cinnamic acid, 8:Quercetin, 9: Luteolin, 10:Apigenin)

#### Fig.4 Phenolic Profiles in the Raw Olive Samples of Domat Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3:Chlorogenic acid, 4: Vanillic acid, 5:Caffeic acid, 6:p-Coumaric acid, 7:Ferulic acid, 8:Cinnamic acid, 9:Quercetin, 10: Luteolin, 11:Apigenin)

#### Fig.5 Phenolic Profiles in the Split Olive Samples of Domat Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3: Vanillic acid, 4:Caffeic acid, 5:p-Coumaric acid, 6:Ferulic acid, 7:Cinnamic acid, 8: Luteolin, 9:Apigenin)

#### Fig.6 Phenolic Profiles in the Spanish Style Olive Samples of Domat Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3:Caffeic acid, 4: Luteolin, 5:Apigenin)

**Table1.** Amounts of phenolic compounds of raw and processed olive samples (mg/100g).

	<b>Ayvalık</b>		<b>Domat</b>		
	<b>Raw</b>	<b>Split</b>	<b>Raw</b>	<b>Split</b>	<b>Spanish style</b>
Gallic acid	ND	ND	ND	ND	ND
Hydroxytyrosol	42,46 ± 0,342	87,6 ± 0,247	55,61 ± 0,154	61,16 ± 0,028	84,07 ± 0,55
Tyrosol	6,13 ± 0,027	4,68 ± 0,036	11,21 ± 0,304	2,74 ± 0,031	1,15 ± 0,028
Chlorogenic acid	5,9 ± 0,031	ND	2,38 ± 0,012	ND	ND
Vanillic acid	3,13 ± 0,014	0,75 ± 0,021	6,55 ± 0,023	2,38 ± 0,025	ND
Caffeic acid	4,25 ± 0,025	0,12 ± 0,034	7,14 ± 0,026	3,71 ± 0,028	1,77 ± 0,044
Syringic acid	ND	ND	ND	ND	ND
p-Coumaric acid	3,19 ± 0,042	1,03 ± 0,048	3,47 ± 0,034	1,19 ± 0,047	ND
Ferulic acid	3,85 ± 0,038	0,25 ± 0,014	4,41 ± 0,028	1,63 ± 0,038	ND
Sinnamic acid	2,13 ± 0,036	0,41 ± 0,018	2,74 ± 0,016	0,43 ± 0,023	ND
Quercetin	3,74 ± 0,022	1,86 ± 0,027	7,97 ± 0,021	3,65 ± 0,023	ND
Luteolin	3,66 ± 0,063	16,7 ± 0,118	2,27 ± 0,017	6,88 ± 0,034	1,59 ± 0,029
Apigenin	7,54 ± 0,019	8,32 ± 0,043	3,64 ± 0,031	5,14 ± 0,049	2,13 ± 0,051

\*ANOVA was applied for the obtained data.

## FIGURES

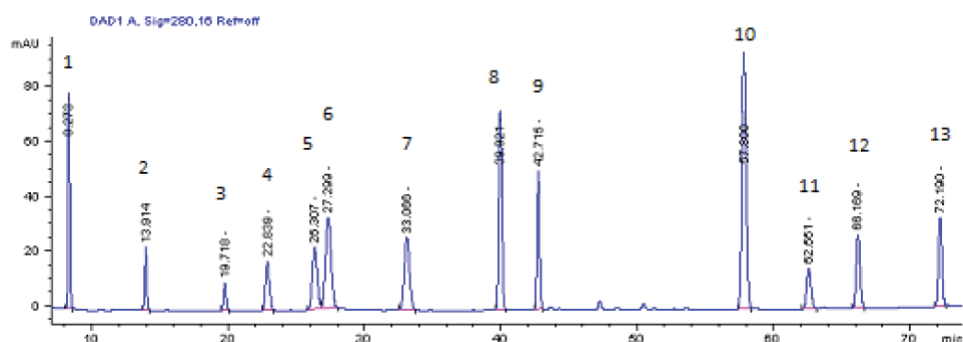


Fig.1. Standard Material Chromatogram Belonging to the Phenolic Compounds

(Phenolic compounds: 1:Gallic acid, 2:Hydroxytyrosol, 3: Tyrosol, 4:Chlorogenic acid, 5:Vanillic acid, 6:Caffeic acid, 7: Syringic acid, 8:p-Coumaric acid, 9:Ferulic acid, 10:Cinnamic acid, 11:Quercetin, 12: Luteolin, 13:Apigenin)

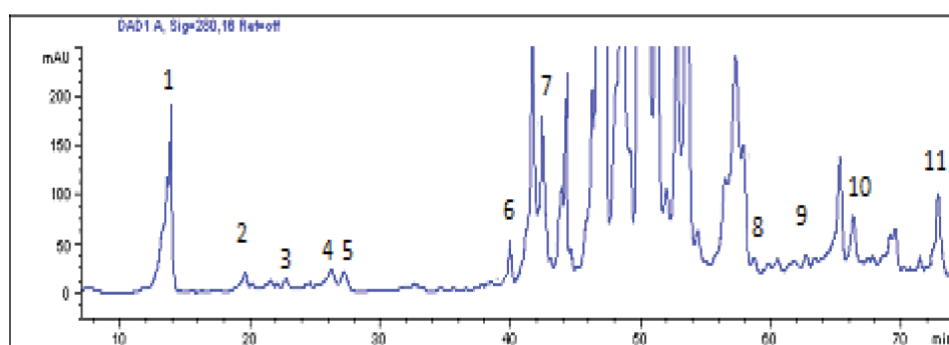


Fig.2 Phenolic Profiles in the Raw Olive Samples of Ayvalik Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3:Chlorogenic acid, 4:Vanillic acid, 5:Caffeic acid, 6:p-Coumaric acid, 7:Ferulic acid, 8:Cinnamic acid, 9:Quercetin, 10: Luteolin, 11:Apigenin)

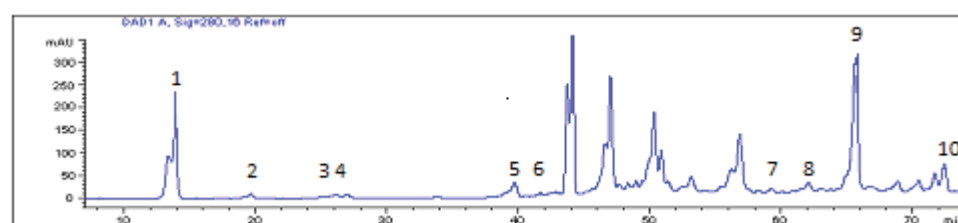


Fig.3 Phenolic Profiles in the Turning Colour Split Olive Samples of Ayvalik Variety

(Phenolic compounds: 1:Hydroxytyrosol, 2: Tyrosol, 3: Vanillic acid, 4:Caffeic acid, 5:p-Coumaric acid, 6:Ferulic acid, 7:Cinnamic acid, 8:Quercetin, 9: Luteolin, 10:Apigenin)

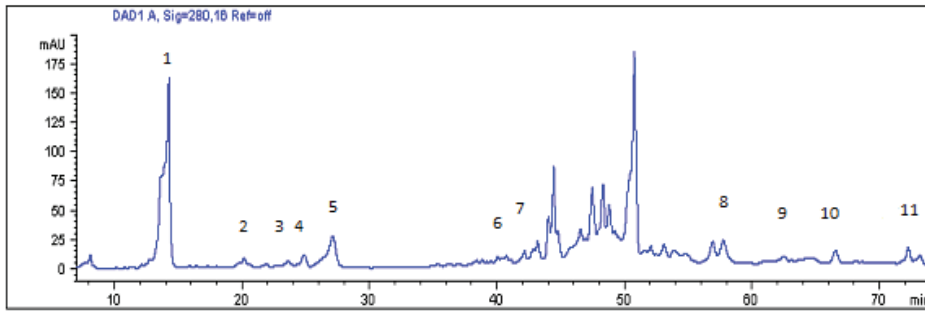


Fig.4 Phenolic Profiles in the Raw Olive Samples of Domat Variety

(Phenolic compounds: 1;Hydroxytyrosol, 2: Tyrosol, 3:Chlorogenic acid, 4: Vanillic acid, 5:Caffeic acid, 6:p-Coumaric acid, 7:Ferulic acid, 8:Cinnamic acid, 9:Quercetin, 10: Luteolin, 11:Apigenin)

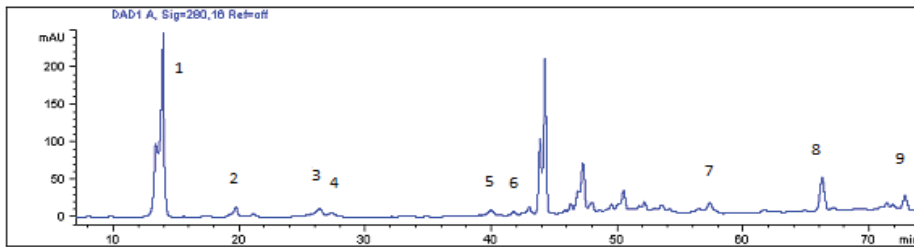


Fig.5 Phenolic Profiles in the Split Olive Samples of Domat Variety

(Phenolic compounds: 1;Hydroxytyrosol, 2: Tyrosol, 3: Vanillic acid, 4:Caffeic acid, 5:p-Coumaric acid, 6:Ferulic acid, 7:Cinnamic acid, 8: Luteolin, 9:Apigenin)

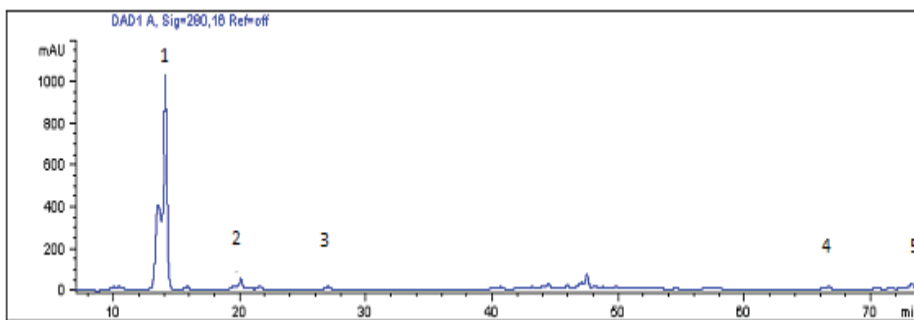


Fig.6 Phenolic Profiles in the Spanish Style Olive Samples of Domat Variety

(Phenolic compounds: 1;Hydroxytyrosol, 2: Tyrosol, 3:Caffeic acid, 4: Luteolin, 5:Apigenin)