The effects of processing methods on polyphenol content of some Turkish black table olives

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In this study, Gemlik, Memecik and Uslu olive varieties, which are important for the Turkish black table olive sector, were analysed to determine the effects of processing methods on the types and amounts of the polyphenols which have potentially high antioxidant activities. The effects of the processing techniques on the phenolic compounds were found statistically significant in the level of $p<0.01$. It was determined that the number of phenolic compounds decreased due to the diffusion into brine especially in the processing of ripe olives. In particular, the application of caustic to remove the bitterness of the olive and the darkening of the olive through oxidation applied in the California type processing caused the hydrolysis of polyphenols and increased diffusion. However, the production techniques applied for natural black olive in brine and traditional Turkish-style natural turning olive consist of fewer processes, and less washing process is applied. For this reason, it is thought that these kinds of olives contain higher amounts of phenolic compounds.

Keywords: HPLC, Natural, Polyphenols, Processing methods, Table olive

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1. INTRODUCTION

Table olive from *Olea europaea* L. is a traditional product and an important component of the Mediterranean diet. Olive is considered as a different kind of fruit with its low sugar content, high levels of oil content and a specific bitter taste [1].

The pulp fraction of olive contains of flavonoids, secoiridoids and phenolic compounds with simple phenol structure such as C2-C6 in the amounts of 1-3% [2]. 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 determining the phenolic properties of olive [3]. Table olive and olive oil are considered as one of the most valuable sources of "functional foods" with their phenolic antioxidant compounds [4, 1].

In several studies, it is stated that along with the variety and the maturity of olive, processing techniques and systems are the major factors affecting the type and the amount of the phenolic compounds in olive [5].

Each country has its own traditional methods for the consumption of olive in addition to the industrial production methods for the market. In Turkey, traditional methods used for the production of table olive are green split and cracked olive, natural turning colour olive, dry-salted olive, turning olive and black olive in brine. Industrial processing techniques: treated black olive, olive darkened by oxidation, Spanish style green olive, and stuffed olive are applied properly for the world trade.

In Turkey, different olive varieties are used in the production of olive oil and table olive. While Gemlik, Domat and Uslu varieties are generally preferred to produce table olives, Ayvalık and Memecik varieties are mostly preferred in olive oil production. The variety of Ayvalik is generally processed as turning colour split olive while Gemlik is processed as natural black table olive. Domat is used to produce green split olive, Spanish style green olive and stuffed olive. Memecik is mostly suitable to produce olive darkened by oxidation, treated black olive and Spanish style green olive whereas Uslu is also used for the purpose of producing natural black table olive in addition to these production techniques.
Researchers point out that studies 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, unprocessed, and additive-free food products have been increasing. It is observed that organic and additive-free food products, which are assumed to be safer, tastier, and more natural than the foods produced on an industrial scale are more preferred. For this reason, hedonistic and functional subjects become more prominent for the qualification of nutritional value [6].
Several papers have reported on the effects of table olive processing methods on phenolic compounds. This study has an importance due to lack of the detailed studies related to this subject in Turkey, although olive is a significant source of phenolic compounds and the phenolic compounds are effective on human health with their antioxidant activity. In addition to determining the phenolic profiles of some important olive varieties used for table olive consumption or oil production, it is aimed to ensure that the varieties rich in biophenol are widely cultivated.
Thus, by determining the effect of different production methods on phenolic compounds, it is aimed to provide consumers with access to better quality and healthier products.

2. MATERIAL AND METHODS

2.1. MATERIALS

In this study, Gemlik, Memecik and Uslu olive varieties harvested from the collection plant of Bornova Olive Research Institute were used. For each processing, a total of about 240 kg olives were collected and put into two containers. Then, three sample were analysed in three replicates. The harvest times for the olive varieties were determined according to the specific process techniques stated in Turkish Food Codex [7]. Memecik olives were harvested in the first week of October while Gemlik and Uslu variety olives were harvested in the second week of November.

2.2. PROCESSING OF OLIVES

2.2.1. Traditional Turkish-style natural turning olive processing

Gemlik variety olives were harvested (5,3 MI) and washed. Then the olives were transferred into the plastic vessels and 6-10% salt was added on the olives. The covers of the vessels were closed tightly. The olives were kept in their own water until the end of fermentation. Olive vessels were turned every two days to provide fermentation [8].

2.2.2. Processing natural black olives in brine

Uslu variety olives were harvested in the period of maturity index (5,3 MI) and sizing. 200 L capacity fiberglass industrial containers were used. Olives were placed in the container and covered with brine including 2-4% salt. The olives in brine were exposed to the air. The incorporation of air was performed for 8 h per day, air at a rate of 0.25 L/h for L of brine was bubbled from a circular ring at the bottom of the container. Consequently, fermentation took place under normal conditions and lasted approximately three months [8]. The analyses were carried out at the end of fermentation.

2.2.3. Processing olives darkened by oxidation (Californian-style)

The Californian method was applied, and this method included lye treatment, washing, iron-salt treatment and air-oxidation, washing, sizing, canning and sterilisation [2]. Memecik variety olives were harvested in the green period and taken into the plastic container containing 2% NaOH solution to provide the permeability as a first process step. After the lye solution penetrated the first layer of the fruit, the lye solution was removed and washing process was carried out. During the washing process, air was given to the olives in water by air compressor. After 24 hours, in the second step of the alkali process, the olives were treated with 1,5% NaOH solution until it penetrated 2/3 part of the flesh. The washing process was repeated with the application of air by compressor. After 4 hours, in the last step of the alkali process, the olives were treated with 1% NaOH until the solution penetrated the fruit's seed. The washing process was repeated by the application of air by compressor and for the fixation of the colour, ferrogluconate was put into the brine. The washing process was carried

out for the last time and then the pH balancing process was applied, the product was ready for consumption [9].

2.3. CHEMICALS

The chemicals used in the project were obtained from "Merck" as LC grade. Standards, Hydroxytyrosol was obtained from "Extrasynthese" (France), Gallic acid, Tyrosol, Chlorogenic acid, Vanillic acid, Caffeic acid, Syringic acid, p-Coumaric acid, Ferulic acid, Cinnamic acid, Quercetin, Luteolin, and Apigenin were kindly obtained from "Sigma" (USA).

2.4. 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 50 ml methanol: water (80:20). The applications were repeated for 3 times. The collected methanol phase was evaporated at 35°C in rotary evaporator. After dissolving and mixing with 2.5 ml methanol, the sample was filtered through 0.45 µm (Millex-FH Filter, 0.45 µm) and injected (20 µl) onto the column for HPLC analysis [10].

A high-performance liquid chromatography (HPLC) system was used to determine the phenolic compounds. It was an Agilent HP 1100 series, equipped with a vacuum degasser, a gradient pump, diode array UV detector (200-400 nm) and Phenomenex C18 RP (250 mm × 4.6 mm, 5 µm) 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 was 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 was facilitated using a photodiode receiver detector. Detection was done at 280 nm.

2.5. STATISTICAL ANALYSIS

In this study, three extractions of each sample were performed, and the extracts were analysed three times by HPLC. The data were statistically analysed by ANOVA. Statistical significance was accepted at a level of $p<0.01$.

3. RESULTS AND DISCUSSION

3.1. PHENOLIC COMPOUNDS IN OLIVE VARIETIES

HPLC analyses were carried out on raw and the processed olive samples belonging to the varieties of Gemlik, Memecik and Uslu to determine the phenolic profile and the amount of phenolics. The analysed phenolic compounds were as follows; 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 analysed phenolic compounds were depicted in Figure 1.

3.2. PHENOLIC COMPOUNDS IN RAW OLIVES

The amounts of the phenolic compounds in the raw olive samples were demonstrated in Table I according to the varieties. Uslu was found to be the richest sample in hydroxytyrosol with 58.70 mg/100 g, whilst Memecik was determined as the variety having the lowest value with 27.53 mg/100 g. The values for Gemlik were 55.61 mg/100 g (Tab. I).

The number of phenolic compounds vary according to the variety of olives. The amount of hydroxytyrosol of Crete olive varieties (cv. Tsakistes and Thrubes Crete) was measured 114 and 2 mg/100 g, respectively [11]. Othman *et al.,* (2009) detected hydroxytyrosol concentration 135 mg/100 g dw in Tunisian black olives [12]. The level of hydroxytyrosol was determined between 7.5 with 10.3 mg/100g in olive varieties of Portugal [13].

According to the results, Uslu demonstrated a significant difference and had the highest amount of tyrosol with 21.23 mg/100 g, whereas Memecik were determined as the sample having the lowest amount of tyrosol with 9.75 mg/100g. The tyrosol levels of Crete olives were between 1 and 21 mg/100 g [11]. Tyrosol content was determined between 0.548 with 1.349 mg/100 g in olive varieties of Portugal [13].

In terms of luteolin, which is regarded as one of the most characteristic phenolic compounds of the olives, Memecik became prominent. The amount of luteolin in Memecik was determined 55.73 mg/100g whereas it was found 6.87 mg/100 g, and 4.72 mg/100 g in Uslu and Gemlik varieties, respectively. Level of luteolin was determined in Cobrancosa variety about 7.5 mg/kg [13]. Sousa *et al.* (2015) stated that luteolin characterised for Verdeal Transmontana olives from the third and fourth (10th Nov.) sampling dates, due to the higher content on this flavone [14].

In terms of apigenin, the highest value was determined in Memecik olives with 24.43 mg/100 g; whereas lower amounts of apigenin were found in Uslu, and Gemlik olives, 8.41 and 5.58 mg/100 g, respectively. The results obtained for luteolin and apigenin became prominent for Memecik olives as the evaluation criteria of the phenolic profile.

Gallic acid, which is one of the phenolic compounds, was only determined in olive samples belonging to Uslu and Memecik varieties. Thus, gallic acid could be evaluated as a characteristic property of these olive varieties. The amount of gallic acid were determined as 6.58 mg/100 g for Uslu and 4.48 mg/100 g for Memecik.

Considering the absence of phenolic compounds such as quercetin, vanillic acid and caffeic acid only in Memecik olives, it is possible to conclude that this result might be evaluated as a phenolic profile for the variety Memecik. Quercetin was determined in Portuguese olive varieties between 0.59-0.85 mg/100 g [13].

The olive samples belonging to Uslu differed from the other varieties with the presence of syringic acid only in the raw samples of this variety. While the highest amount of cinnamic acid was found in Uslu raw olives with a value of 10.97 mg/100 g, it was determined 3.05 and 1.26 mg/100 g in Gemlik and Memecik varieties, respectively.

The differences noticed were statistically significant at $p<0.01$ level in terms of the type and composition of the phenolic compounds in raw samples of the olive varieties. 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 [15]. The phenolic composition of olives is overly 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.

Levels of hydroxytyrosol are ranged from 0.2 to ~71 g/kg (dry weight). 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. Phenolic acids such as p-coumaric acid, chlorogenic acid, vanillic acid, syringic, ferulic, and homovanillic acid, and

Table I - The amounts of the phenolic compounds in the raw and processed olive samples according to the varieties, mg/100 g.

	Gemlik		Memecik		Uslu	
	Raw	**Turning**	**Raw**	**Ripe olive**	**Raw**	**Nat. brined**
Gallic acid	ND	2,04 ± 0,02	4,48 ± 0,024	1,13 ± 0,032	6,58 ± 0,034	4,21 ± 0,018
Hidroxytyrosol	47,57 ± 0,124	56,55 ± 0,175	27,53 ± 0,167	89,46 ± 0,238	58,70 ± 0,123	72,74 ± 0,248
Tyrosol	18,25 ± 0,216	19,83 ± 0,248	9,75 ± 0,053	4,73 ± 0,042	21,23 ± 0,264	32,42 ± 0,197
Chlorogenic acid	3,21 ± 0,018	1,17 ± 0,024	ND	ND	6,99 ± 0,043	1,62 ± 0,046
Vanillic acid	4,32 ± 0,012	6,16 ± 0,032	ND	ND	5,65 ± 0,038	6,72 ± 0,032
Caffeic acid	5,08 ± 0,023	5,12 ± 0,029	ND	ND	6,72 ± 0,042	5,83 ± 0,018
Syringic acid	ND	2,38 ± 0,015	ND	ND	5,21 ± 0,051	3,33 ± 0,035
p-Coumaric acid	4,36 ± 0,034	6,75 ± 0,019	1,70 ± 0,028	0,64 ± 0,032	4,91 ± 0,041	6,84 ± 0,029
Ferulic acid	5,25 ± 0,015	3,1 ± 0,034	1,13 ± 0,034	0,96 ± 0,027	5,84 ± 0,038	3,99 ± 0,043
Cinnamic acid	3,05 ± 0,022	8,68 ± 0,047	1,26 ± 0,046	ND	10,97 ± 0,142	15,43 ± 0,187
Quercetin	6,17 ± 0,011	3,37 ± 0,013	ND	ND	4,12 ± 0,053	1,82 ± 0,045
Luteolin	4,72 ± 0,027	11,91 ± 0,254	55,73 ± 0,351	3,50 ± 0,056	6,87 ± 0,038	14,78 ± 0,163
Apigenin	5,58 ± 0,018	9,38 ± 0,025	24,43 ± 0,292	6,33 ± 0,037	8,41 ± 0,029	18,68 ± 0,231

*ANOVA was applied for the obtained data.

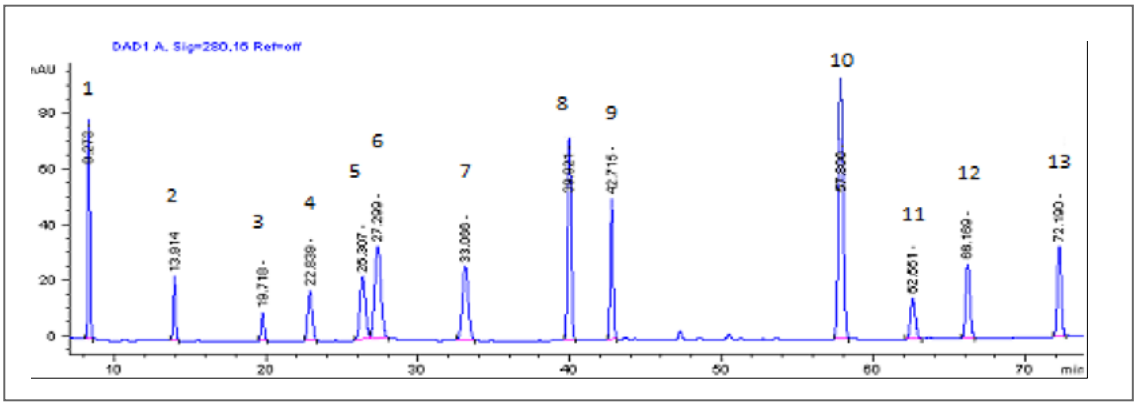

Figure 1 - Standard material chromatogram belonging to the phenolic compounds
Phenolic compounds: 1) Gallic acid, 2) Hydroxytyrosol, 3) Tyrosol, 4) Chlorogenic acid, 5) Vanillic acid, 6) Caffeic acid, 7) Syringic acid, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 11) Quercetin, 12) Luteolin, 13) Apigenin

caffeic acid are also present in the pulp. The level of phenolic compounds is generally in the milligram per kilogram range [16].
Syringic and vanillic acid were not found in a study that 5 Portuguese olive varieties were used [14]. As reported, hydroxytyrosol and tyrosol are the most abundant biophenols in the pulp of both fresh and processed olives [17].
When comparing our results with those of the mentioned studies, Turkish olive varieties are quite rich in phenolic fraction.

3.3. THE AMOUNT OF THE PHENOLIC COMPOUNDS DETERMINED ACCORDING TO THE OLIVE VARIETIES

3.3.1. The phenolic profile of Gemlik variety naturally black turning olives

After fermentation, changes in the profile and the quantity of simple phenolic compounds are mainly due to the diffusion of substances from olive fruit to brine and vice versa [18]. Total simple phenolic content in flesh increased after the fermentation of pink and black olives, especially in the controlled fermentation [12]. This result could be explained by the freeing of simple phenolic compounds after acid and enzymatic hydrolysis of polymerised phenolic compounds, which are in high levels in pink and black olives [12].
In the samples of Gemlik variety, as it was in other varieties, the amount of hydroxytyrosol determined as 47.57 mg/100 g in raw samples increased during fermentation and reached to 56.55 mg/100 g in samples processed with the turning olive technique (Tab. I). Chromatograms of phenolic compounds of raw and processed Gemlik variety table olive samples are shown in Figure 2 and 3. In our study, hydroxytyrosol is lower than the results of Aktaş (2013) [19]. Boskou et al. (2006) also determined the amount of hydroxytyrosol in black Greek olives (Crete and Kalamon) as 21 and 39 mg/100g, respectively [11]. Our results are higher than those of this study. Among the other phenolics, tyrosol increased from 18.25 mg/100 g to 19.83 mg/100 g; vanillic acid

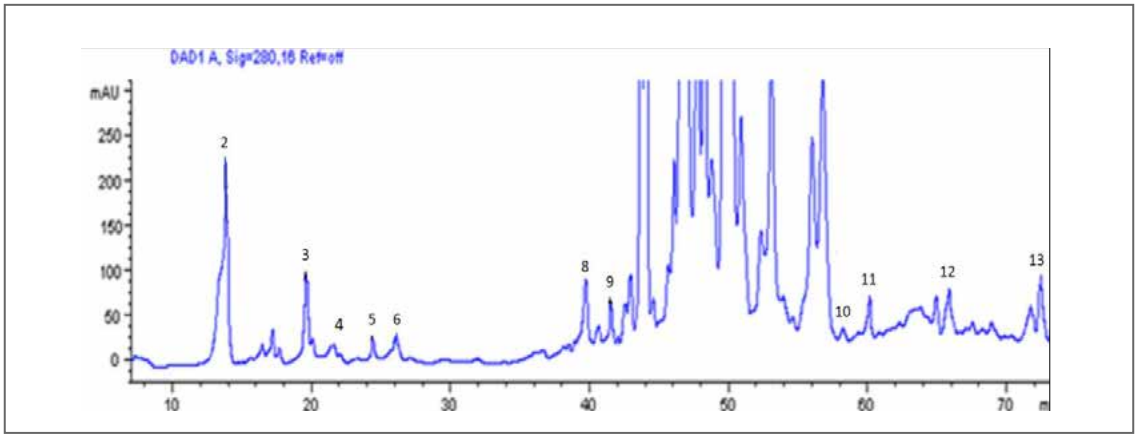

Figure 2 - Phenolic profiles in the raw olive samples of Gemlik variety
Phenolic compounds: 2) Hydroxytyrosol, 3) Tyrosol, 4)Chlorogenic acid, 5) Vanillic acid, 6) Caffeic acid, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 11) Quercetin, 12) Luteolin, 13) Apigenin

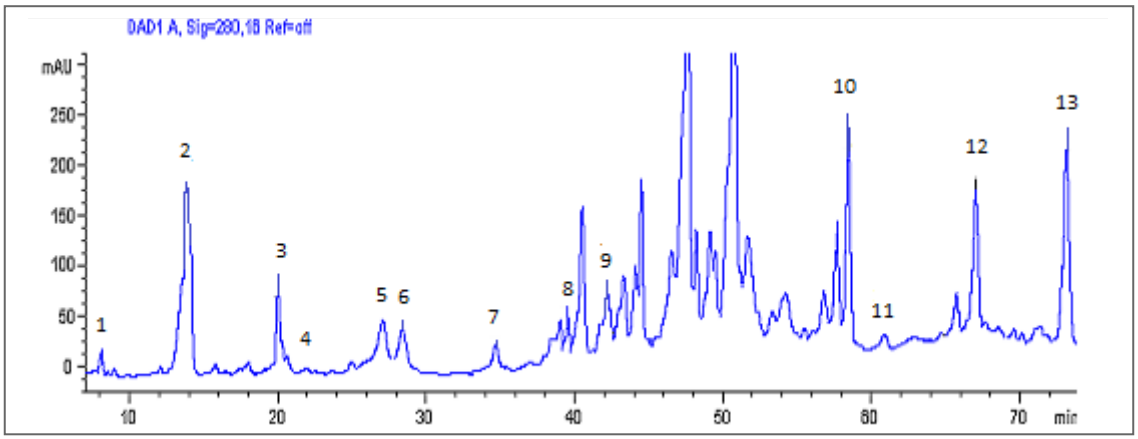

Figure 3 - Phenolic profiles in the turning olive samples of Gemlik variety
Phenolic compounds: 1) Gallik acid, 2) Hydroxytyrosol, 3) Tyrosol, 4) Chlorogenic acid, 5) Vanillic acid, 6) Caffeic acid, 7) Syringic acid, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 11) Quercetin, 12) Luteolin, 13) Apigenin

increased from 4.32 mg/100 g to 6.16 mg/100 g; p-coumaric acid increased from 4.36 mg/100 g to 6.75 mg/100 g; cinnamic acid increased from 3.05 mg/100 g to 8.68 mg/100 g; luteolin increased from 4.72 mg/100 g to 11.91 mg/100 g; and apigenin increased from 5.58 mg/100 g to 9.38 mg/100 g. The amount of the increase were determined higher in some phenolics such as luteolin, cinnamic acid and apigenin; whereas the levels of the increase were lower in the phenolics such as hydroxytyrosol, tyrosol, vanillic acid, p-coumaric acid and caffeic acid (Fig. 2 and 3).

Concentrations of hydroxytyrosol and caffeic acid increased after fermentation. A great interest is particularly shown to hydroxytyrosol, because of its important antioxidant activity. This compound is abundant in fresh olives and its concentration increased after fermentation, due to acid and enzymatic hydrolysis of oleuropein [12].

In the study carried out in Algeria, it was determined that the amount of hydroxytyrosol increased from 24 mg / 100 g to 95 mg / 100 g in the Sigoise (Relizane) variety processed with the dry salt method [22].

As generally observed, the increase in the amount of the phenolic compounds of the raw Gemlik samples proved that the turning process technique was a suitable method for Gemlik type olives in terms of phenolic compounds. It was observed that fermentation of this type of olives in their own water contributed to the phenolic compound content and the quality. The phenolics such as gallic acid, chlorogenic acid and syringic acid, which could not be determined in the raw Gemlik samples, were found in the olive samples processed via turning technique as 2.04/1.17/2.38 mg/100 g, respectively. Moreover, these mentioned phenolics that could not be determined in the raw samples, could be considered as a significant distinctive feature in determining the phenolic profile.

It was determined that turning technique had no significant effect on the amount of caffeic acid considering the similarity of the amounts between the raw samples (5.08 mg/100 g) and the processed olives (5.12 mg/100 g). It was found out that the turning process technique had a decreasing effect only on the phenolics such as ferulic acid and quercetin in Gemlik olive samples (Fig. 2 and 3). The obtained values are relatively like those of dry salted olives reported by Blekas *et al.* (2002) and Melliou *et al.* (2015) [20, 21]. Othman *et al.* 2009 reported that this compound forms with verbascoside degradation [12]. There was no caffeic acid in fresh olives and it appeared after fermentation in all types of olives.

Melliou *et al.* (2015) reported that the dry salted Mission and Throuba Thassos olives presented relatively higher amounts of almost all studied compounds [21]. This data indicated that California-style black ripe olive processing methods led to significant reductions in the levels of the secoridoids and phenolic compounds evaluated in the study. These findings support the notion that dry salt debittering methods are advantageous for the retention of polyphenolic and secoiridoid components in table olives.

The comparison between fresh and salted olives confirms previous literature data that reported a decrease in oleuropein content during processing, paralleled to an increase in hydroxytyrosol which derived from hydrolysis of oleuropein [22, 9].

The main change for all types of olives was the decrease in concentrations of ferulic acid. This compound is a bitter glucoside, and the elimination of this compound is among the aims of olive processing. After the fermentation of all olive types, the concentration of this compound decreased [12].

3.3.2. The phenolic profiles of olives darkened by oxidation in Memecik Variety

The production process of ripe olives, the so-called "Californian style" table olives, begins with an alkaline (NaOH) treatment, followed by several treatments with air until the fruit acquire the typical black appearance induced by the oxidation and polymerisation of phenol compounds [27].

It was determined that the phenolic compound content of the raw samples belonging to Memecik variety demonstrated a completely different profile when compared to the other olive varieties. As can be seen in Table I, the absence of the phenolics such as chlorogenic acid, vanillic acid, caffeic acid and syringic acid in the raw samples and in the samples darkened by oxidation, made these phenolics as indicators in determining the phenolic profiles of Memecik olives. The only phenolic compound found in raw samples was hydroxytyrosol, and its amount in raw samples was determined as 27.53 mg/100 g; whereas this determined as 89.46 mg/100 g in olive samples processed by California-style ripe olive technique by demonstrating an increase during the fermentation period (Tab. I). Chromatograms of phenolic compounds of raw and processed Memecik variety table olive samples are shown in Figure 4 and 5. Among the other phenolics; the amount of gallic acid decreased from 4.48 mg/100 g to 1.13 mg/100 g, tyrosol decreased from 9.75 mg/100 g to 4.73 mg/100 g, p-coumaric acid decreased from 1.70 mg/100 g to 0.64 mg/100 g, ferulic acid decreased from 1.13 mg/100 g to 0.96 mg/100 g, luteolin decreased from 55.73 mg/100 g to 3.50 mg/100 g, and the amount of apigenin decreased from 24.43 mg/100 g to 6.63 mg/100 g (Tab. I). The highest decrease was observed in the amount of luteolin and apigenin, while the decrease in the amounts of gallic acid, tyrosol, p-coumaric acid and ferulic acid was less. In general, considering other varieties and

processing techniques, a significant decrease in phenolic compounds was determined in Memecik olive samples.

On the other hand, cinnamic acid, which was determined in the raw samples of Memecik type, could not be found in the olive samples darkened via oxidation process. Quercetin was regarded as one of the most distinctive properties in determining the phenolic profile of Memecik type olives due to its absence in both raw samples and the samples processed with darkening by oxidation. Besides, luteolin and apigenin, which were determined in high amounts in the raw samples, were evaluated as the main phenolic compounds of the olives belonging to Memecik variety. It is considered that the amount of luteolin and apigenin determined in the raw samples would be distinctive in determining differences between varieties.

In olive flesh, hydroxytyrosol is at the highest level, followed by oleanolic acid and tyrosol. Table olives have a different phenol composition than olive oil and non-processed olives. This is due to the debittering, which causes a diffusion of phenols from the fruit to the water or brine and vice versa. When lye is used, sodium hydroxide and constituents with carboxylic and hydroxyl groups react and the hydrophilic derivatives are washed away. Oleuropein and verbascoside are highly hydrolysed during lye treatment [11].

Changes in phenolic composition of olive fruits (Olea europaea Intosso cv.) during California-style ripe olive processing were investigated by Marsilio et al. (2001). Hydroxytyrosol and tyrosol were the main phenols identified by GC and GC-MS techniques. During the darkening process, only hydroxytyrosol decreased markedly and its decrease in flesh was directly related to the olive fruit browning development. Iron salts, used for colour fixation, seem to play a catalytic role in the oxidation of hydroxytyrosol and mechanisms involved in the browning are proposed. The effect of NaOH and air oxidation on phenolic compounds of Intosso variety was determined as hydroxytyrosol and tyrosol components increased from 57 and 40 mg/100 g dw to 1030 and 152 mg/100 g dw [2]. Charoenprasert and Mitchell (2012) determined the

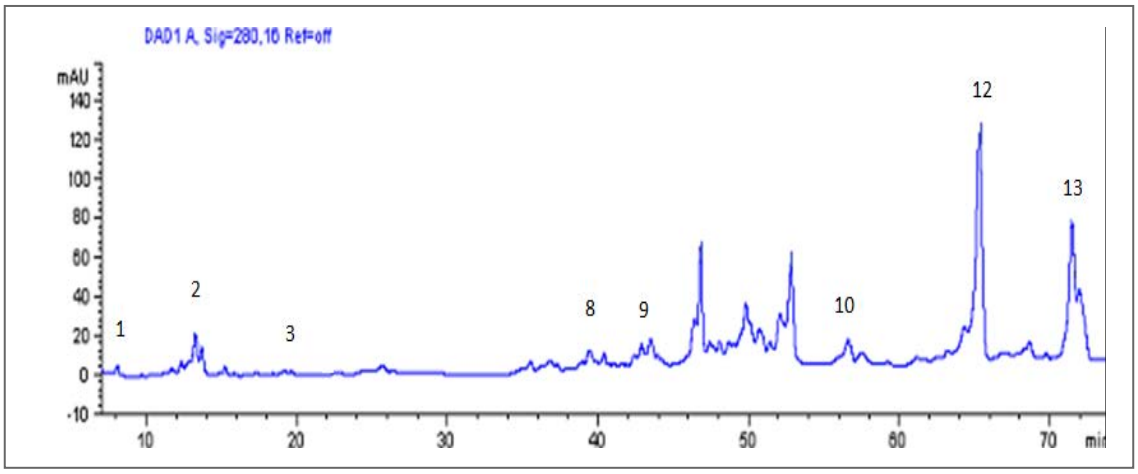

Figure 4 - Phenolic profiles in the raw olive samples of Memecik variety
Phenolic compounds: 1) Gallic acid, 2) Hydroxytyrosol, 3)Tyrosol, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 12) Luteolin, 13) Apigenin

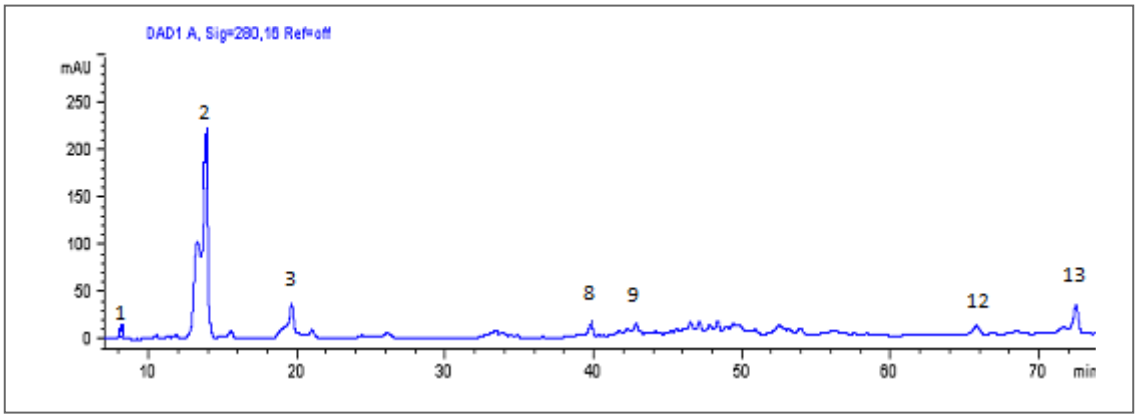

Figure 5 - Phenolic profiles in the olives darkened by oxidation samples of Memecik variety.
Phenolic compounds: 1) Gallic acid, 2) Hydroxytyrosol, 3) Tyrosol, 8) p-Coumaric acid, 9) Ferulic acid, 12) Luteolin, 13) Apigenin

number of phenolic compounds in Californian-style table olives (Cobrançosa variety) as 75.27 mg/100 g, 11.20 mg/100 g, 1.36 mg/100g, 6.39 mg/100g and 7.49 mg/100g for hydroxytyrosol, tyrosol, chlorogenic acid, quercetin and luteolin, respectively. [16].

Hydroxytyrosol and caffeic acid are eliminated during the preparation of California-type black olives. The reduction of ortho-diphenols in the flesh of this type of oils is related to the browning. Iron salts used for colour fixation catalyze the oxidation of hydroxytyrosol, which disappears or is decreased significantly [11]. The Californian method of treatment contains lye treatment, washing, iron-salt treatment and air-oxidation, washing, sizing, canning and sterilisation. All these procedures result to a decrease of the total amount of phenols [11].

In the Spanish-style green olive processing, Brenes *et al.* (1995) studied the changes in phenolic compounds and noticed that the NaOH treatment hydrolysed oleuropein into hydroxytyrosol and elenolic acid glucoside, and that caffeic acid, oleuropein, and p-coumaric acid contents reduce during fermentation period, while tyrosol concentration remained constant [23]. Marsilio *et al.* (2001) showed that Californian-style ripe olive processing also influences the phenolic composition [2]. Especially, tyrosol and hydroxytyrosol content increased while vanillic acid and oleuropein decreased [13].

During the washing with water to remove excess NaOH prior to the iron salt treatments, all the phenolic compounds reduced markedly, because of the effects of diffusion and dilution in the water. This effect was more evident for hydroxytyrosol, perhaps due to its higher water solubility. In the presence of ferrous ions with air bubbling, hydroxytyrosol content in flesh rapidly fell to an exceptionally low level and tyrosol remained practically unchanged, while the end-product showed a content similar to that of fresh fruits. A phenolic oxidation process has been suggested as a major factor responsible for this behaviour.

Throughout the alkali aerobic treatments, a progressive darkening of fruits occurred. While the oxidative browning of phenols in food generally results in a loss of nutritional value, in some processed foods, such as ripe olives, these reactions are a part of desirable changes, essential to the product, and could contribute to the colour complexity. The browning of fruits during processing could also be due to the polymerisation of phenols to dark pigments [2].

Melliou *et al.* (2015) reported that the level of

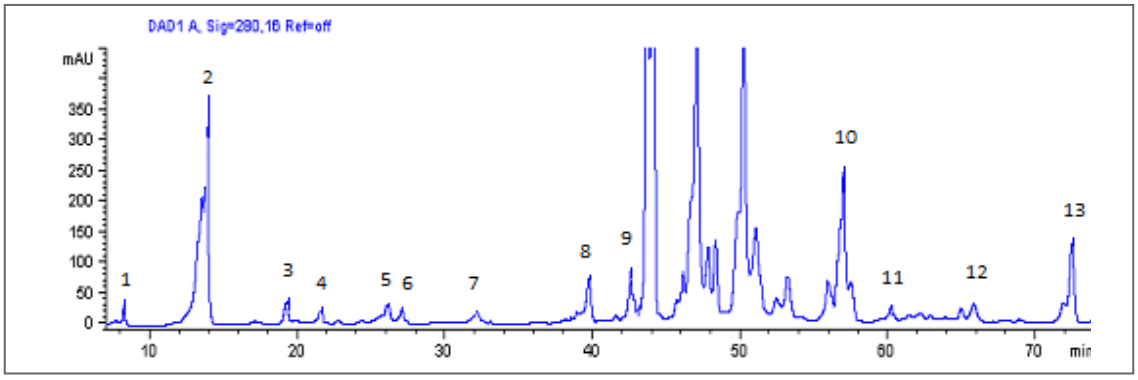

Figure 6 - Phenolic profiles in the raw olive samples of Uslu variety
Phenolic compounds: 1) Gallic acid, 2) Hydroxytyrosol, 3) Tyrosol, 4) Chlorogenic acid, 5) Vanillic acid, 6) Caffeic acid, 7) Syringic acid, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 11) Quercetin, 12) Luteolin, 13) Apigenin

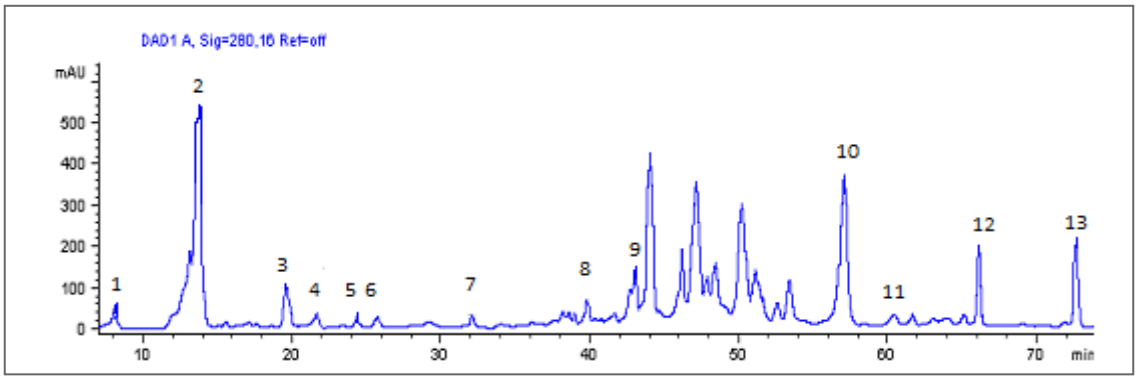

Figure 7 - Phenolic profiles in the naturally black olive in brine samples of Uslu variety
Phenolic compounds: 1) Gallic acid, 2) Hydroxytyrosol, 3) Tyrosol, 4) Chlorogenic acid, 5) Vanillic acid, 6) Caffeic acid, 7) Syringic acid, 8) p-Coumaric acid, 9) Ferulic acid, 10) Cinnamic acid, 11) Quercetin, 12) Luteolin, 13) Apigenin

hydroxytyrosol measured in fresh olives and after Californian-style black ripe processing was (894.5 µg/g) and 210 µg/g (wet weight), respectively [21]. Melliou *et al.* (2015) stated that all the other compounds were in concentrations lower than 10 µg/g (wet weight), and chlorogenic acid and o-coumaric acid could not be detected. These data indicate that California-style black ripe olive processing methods lead to significant drops in the levels of the secoridoid and phenolic compounds evaluated in their study [21].

3.3.3. The phenolic profiles of natural black olive in brine in Uslu variety

It was detected that, among the phenolics of the olives belonging to Uslu variety, hydroxytyrosol increased from 58.70 mg/100 g to 72.74 mg/100 g; tyrosol increased from 21.23 mg/100 g to 32.42 mg/100 g; vanillic acid increased from 5.65 mg/100 g to 6.72 mg/100 g; p-coumaric acid increased from 4.91 mg/100 g to 6.84 mg/100 g; cinnamic acid increased from 10.97 mg/100 g to 15.43 mg/100 g; luteolin increased from 6.87 mg/100 g to 14.78 mg/100 g and apigenin increased from 8.41 mg/100 g to 18.68 mg/100 g (Tab. I). Chromatograms of phenolic compounds of raw and processed Uslu variety table olive samples are shown in Figure 6 and 7.

Acid hydrolysis of hydroxytyrosol, tyrosol and luteolin glycosides takes place during the fermentation in brine when naturally black olives are prepared. Thus, the prevailing phenols in table olives are hydroxytyrosol, tyrosol, luteolin and phenolic acids [20, 11]. High levels of hydroxytyrosol, verbascoside and luteolin were determined in naturally black olives [11].

Particularly, tyrosol, which is one of the main compounds of the olive phenols, were found in significant amounts in the olive samples belonging to Uslu. And, it was stated that this amount demonstrated an increase with the application of process technique. Moreover, the increase determined in the amounts of luteolin, apigenin and cinnamic acid were found higher than the increase observed in the amount of vanillic acid.

As for the other phenolic compounds, such as gallic acid, chlorogenic acid, caffeic acid, syringic acid, ferulic acid and quercetin, a decrease was observed in their amounts when compared to the amounts determined in the raw samples.

As clearly seen in Table I, the presence of all the phenolic compounds investigated in this study in of Uslu variety showed a completely different phenolic profile both for the raw and the processed samples. Phenolic compounds were detected in both raw and processed olives in phenolic compound analysis made on Uslu olive variety. For this reason, Uslu variety was regarded as one of the richest olive varieties in terms of phenolic compounds. The amount of total phenolic substances in Uslu variety olives supports this information.

The diffusion of phenolic compounds from olive flesh to the brine depends on several parameters such as cultivar characteristics, fruit skin permeability, type of phenolic compounds in olive flesh and their ability to diffuse outside the fruit. After the 40th day of brining, the phenolic content starts to decrease. This decline may be due to the degradation of phenolic acids by *Lactobacillus plantarum*. It has been demonstrated that *L. plantarum* contains phenolic acid decarboxylases, which decarboxylate p-coumaric, m-coumaric, ferulic and caffeic acids to their corresponding vinyl derivatives [24].

Piscopo *et al.* (2014) stated that it is well-known that oleuropein is hydrolysed during fermentation, whereas hydroxytyrosol rises due to acid and enzymatic hydrolysis of this compound. In their study, the concentration of caffeic acid and quercetin, a phenolic derived by verbascoside hydrolysis, also tended to increase after brining [25]. A similar trend was demonstrated for a greater part of the phenolics except for caffeic acid and quercetin. In contrast, caffeic acid and quercetin showed a decrease in our study.

Hydroxytyrosol is the main degradation product of oleuropein as also indicated by results concerning black olives (cv. Kalamata) where the mean hydroxytyrosol content increased from 44% to 62 and 64% in olives fermented in Brine A and B, respectively [17].

As black olives are harvested ripe, they have a minor initial concentration of phenolic compounds compared to fresh green olives. The flesh phenolic content in black olives is lower than green olives because total phenolic content diminishes during maturation [26]. A major distinction between the phenol composition of fresh and fermented olives was observed because of the hydrolysis of the initial glycosides during fermentation; hydroxytyrosol was the most important phenol determined in fermented olives [17].

4. CONCLUSION

In this study, the phenolic properties of the Turkish olive varieties such as Gemlik, Memecik and Uslu that have a huge field of production and an industrial value in Turkey were determined. In addition, the effects of the processing techniques applied to make these olives available as table olives on the phenolic compounds were also compared.

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 noticed to be statistically significant at the level of $p<0.01$. All the olive varieties were generally found to be rich in the phenolic compounds such as hyrdoxytyrosol, tyrosol, luteolin and apigenin.

The use of lye solution in the process techniques such

as darkening by oxidation and the processing of olives with NaCl to remove the bitter taste of the olives caused a diffusion and hydrolysis of polyphenols. Thus, in particular, these mentioned olive processing techniques were found to be effective on decreasing the amounts of the phenolic compounds in olive samples.

Natural black olive in brine and natural turning olive processing techniques contain fewer process steps and thus fewer washing steps when compared to the other processing techniques. For this reason, it was determined that these table olive processing techniques provided more phenols.

Table olives obtained with the methods of Turkish-style naturally black olive (cv. Uslu) and turning olive (cv. Gemlik) were richer in phenolic compounds compared to the olives obtained by Californian-style processing method.

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