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Physical, Chemical and Antioxidant Properties of Olive Oil Extracted from Memecik Cultivar

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ABSTRACT

Physical, chemical and antioxidant properties of olive oil extracted from Memecik cultivar, one of the economically important cultivars of Turkey, were studied. Olive samples were handpicked at the last stage of ripening index (RI: 6.91) based on the degree of pulp and skin pigmentation. Olive samples were mechanically processed at laboratory conditions by using two-phase batch extraction equipment. Afterwards olive oil was analytically tested for fatty acid composition and tocopherols. Also main quality parameters such as free acidity, peroxide value, K_{232}, K_{270}, total chlorophyll, total caretonoids and refractive index were determined. Antioxidant activity of olive oil was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging method. Quality parameters of oil extracted from Memecik olives were in good agreement with the literature. Results indicated that late harvest olive oils can have low free fatty acid (FFA) and peroxide values (PV). Total phenolic content and antioxidant activity of oils were 169.25 mg gallic acid equivalent/kg and 89.55 (% inhibition of DPPH· radical), respectively.

Key Words: Memecik cultivar, Olive oil, Antioxidant activity, Ripeness index

Memecik Çeşidi Zeytinlerden Elde Edilen Yağların Fiziksel, Kimyasal ve Antioksidan Özellikleri

ÖZET

Bu çalışma Türkiye'nin ekonomik açıdan önemli zeytin çeşitlerinden biri olan Memecik çeşidinden elde edilen yağların fiziksel, kimyasal ve antioksidan özelliklerini içermektedir. Zeytin örnekleri et ve kabuk rengi dikkate alınarak olgunluk indeksinin son safhasında (Oİ: 6.91) elle hasat edilmiştir. İlk olarak meyve eni, boyu, ağırlığı, çekirdek ağırlığı, et ağırlığı, et/çekirdek oranı, nem ve yağ içeriği belirlenmiştir. Memecik zeytin örnekleri laboratuar koşullarında iki fazlı kesikli bir sistem kullanılarak mekanik olarak yağa işlenmiştir. Sonrasında elde edilen yağın yağ asidi kompozisyonu ile tokoferolleri analitik olarak belirlenmiştir. Zeytinyağları için kalite parametreleri olan serbest asitlik, peroksit değeri, K_{232}, K_{270}, toplam klorofil, toplam karetonoit ile kırılma indisi tespit edilmiştir. Zeytinyağlarının antioksidan aktivitesi 2,2-difenil-1-pikrilhidrazil (DPPH•) radikal giderme yöntemi kullanılarak belirlenmiştir. Memecik zeytinlerinden ekstrakte edilen yağın kalite parametreleri literatür değerleriyle uyumlu bulunmuştur. Sonuçlara göre, geç hasat edilen zeytinyağlarının serbest asitlik (FFA) ve peroksit değerleri (PV) düşük olabilmektedir. Yağların toplam fenolik madde miktarı ve antioksidan aktivitesi sırasıyla 169.25 mg gallik asit eşdeğeri/kg ve %85.55 (DPPH radikalinin yüzde inhibisyonu) olarak bulunmuştur.

Anahtar Kelimeler: Memecik çeşidi, Zeytinyağı, Antioksidan aktivite, Olgunluk indeksi

INTRODUCTION

The olive tree (*Olea europaea L.*) is known the oldest cultivated tree in the world [1] which originated in upper Mesopotamia and South Front Asia including a part of the south-eastern Anatolia region of Turkey and Syria [2], and it has been widely cultivated in southern Europe and played a significant role in the early civilizations of Egypt and Greece [3]. Olive trees are distributed all continents, 98% of the world production of olive is concentrated in the Mediterranean basin countries [4]. Turkey is the world's fifth largest olive oil producer, which exports a great majority of its production accounting for 10% of total world exports [5]. Turkey's economically important olive cultivars are Ayvalik, Memecik and Gemlik, respectively [6]. Memecik olive cultivar has more than 50% orchard area in the Aegean region of Turkey and 45,5% of Turkey's orchard area [7]. Its synonyms are Tas arasi, Asiyeli, Tekir, Gulumbe, Sehir and Yaglik. However Memecik cultivar was originated in Mugla province of the Aegean region, it has been cultivated widely in İzmir, Aydin, Manisa, Denizli, Antalya, Sinop, Kahramanmaras, Kastamonu and other provinces of Turkey [6]. Memecik is dual-use cultivar, used both as table olive and for extraction of oil. Fruits of this cultivar are big in size and spherical that is high in both oil content and quality. Its oil has dark, greenly-yellow color and pungently fruit smell. According to chemical and sensorial character, it is the second cultivar of Turkey after Ayvalik cultivar [8].

Virgin olive oil is obtained only by mechanic or physical procedure and it should not be exposed to any heat or refining procedure, not treated except for washing decantation, centrifugation and filtration. Virgin olive oil has a color changing from green to yellow and a distinctive taste and besides it can be consumed as a food in its natural oil form [9]. Its antioxidant capacity is stable due to its high monounsaturated fatty acid content with low polyunsaturated fatty acid content and the presence of natural antioxidants such as phenols, tocopherols and carotenoids. The fatty acid composition, especially the MUFA (monounsaturated fatty acid) content, and the natural antioxidants provide advantages for health [10-12]. These quality and uniqueness parameters of specific extra virgin olive oils are determined by different factors such as cultivar, environment and cultural practices [13]. Although most of them are controlled or being modified currently, the effect of geographical origin that includes the effects of cultivar, soil and climatic conditions altogether is becoming an important factor to identify and protect the affiliated quality [14]. Because of high monounsaturated and low unsaturated fatty acid composition and minor components such as phenolic compounds, tocopherols and sterols, virgin olive oil has stronger oxidation stability. But this stability is affected by a number of factors such as cultivar, location, harvesting time, processing, and storage. Oils of different cultivars can show different characteristics because of their chemical composition. Environmental conditions also influence oil properties such as phenolic compounds, tocopherols, and sterols in the same cultivar [2].

In this study, it was aimed to characterize the physical, chemical and antioxidant properties of fruit and oil, extracted from Memecik cultivar of Aydin province of Turkey. Traditionally most of the olives in Turkey continue to be harvested late, and it is more economical to harvest after they are fully ripened because all of the olives are collected at once [15]. Therefore, in this study, quality parameters, including free fatty acid content, peroxide value, UV spectrophotometric characteristics and other physico-chemical data such as antioxidant activity, chlorophyll content, carotenoid content, fatty acid composition, tocopherols and total phenolics of virgin oils from ripe or late harvest Memecik olives were also determined.

MATERIALS and METHODS

Olives: Commercial olive cultivar of Memecik located in Southern Aegean, province of Aydin, Turkey was chosen. Twelve old olive trees were identified and carefully marked in a conventional orchard. Olive samples were handpicked at the last stages of ripeness index (RI:6.91) based on the degree of skin and pulp pigmentation [16]. Only healthy fruits without any kind of infection or physical damage were processed. The ripeness index was determined on 100 randomly selected olives in triplicate sample to obtain a numerical value for the olive sample appearance.

Physical Properties of Olives: Physical properties of olive fruits determined on each olive sample included width and length of olive (cm), weights of olives, stones and pulp (g), pulp/stone ratio (g), moisture and oil contents (by Soxhlet method on dry weight basis) of olives (%). All parameters were determined in triplicate for each sample.

Extraction of Oils: Olive samples (Memecik) were mechanically processed at laboratory conditions by using two-phase batch equipment (Hakki Usta Machinery, Aydin Turkey). The steps of extraction process were as follows: (1) removing of leaves from olive lots, (2) milling of drupes by a disc miller, (3) kneading of the resultant paste for 45 minutes at 27˚C (Hakki Usta Machinery, Aydin Turkey). Both time and temperature were standardized during oil extraction, (4) centrifugation of paste by a two-phase vertical decanter (Hakki Usta Machinery, Aydin Turkey) and (5) separation of the oily must into oil and water by means of an automated discharge centrifuge. The paste during centrifugation was fluidized. Oil samples were stored in a freezer at – 20˚C until analyses.

Main Quality Parameters of Oils: Acid value, given as percent oleic acid, was determined in accordance with the AOCS (1989) [17], peroxide value (meq O_2/kg oil) was determined by the AOCS (1994) [18], and UV extinction coefficient K_{232} and K_{270} were determined by the Codex Alimentarius (2001) [19]. All parameters were determined in triplicate for each sample. Refractive index of virgin olive oil samples was measured in daylight with 60/70 Abbe Refractometer, calibrated against pure water at 25˚C.

Total Chlorophyll and Carotenoid Content: Extraction procedure of chlorophyll and carotenoid pigments from olive oil was carried out according to Minquez-Mosquera et al. (1991) method [20], chlorophyll and carotenoid fractions in the absorption spectrum were determined at 670 and 470nm, respectively, using a spectrophotometer (T70 + UV/VIS spectrophotometer, PG Instruments, England). Results are given as mg/kg of oil. Thus, chlorophyll and carotenoid content were calculated by using equation 1 and 2, respectively.

For chlorophyll (in mg/kg), $(A_{670} \times 10^6)/(613 \times 100 \times L)$ (1)
For carotenoid (in mg/kg), $(A_{470} \times 10^6)/(2000 \times 100 \times L)$ (2)

Chlorophyll and carotenoid pigments were expressed as milligrams of pheophytin 'α' per kilogram of oil with the equation 3 [21].

$$\text{'}\alpha\text{'} \text{ (mg /kg oil as Pheo } \alpha) = 345.3\,[A_{670}-(A_{630}+A_{710})/2]/L \quad (3)$$

where A_λ is the absorbance, and L is the spectrophotometer cell thickness (10 mm)

Total Phenols: Total phenolic (TP) content of the extracts was determined by the Folin-Ciocalteu method at 765 nm [22], using gallic acid calibration curve (r^2 = 0.999). The results were expressed as milligrams of gallic acid equivalent per kilogram of oil. The spectrophotometric analysis was repeated three times for each type of extract.

Antioxidant Activity: Antioxidant activity of oils was determined by DPPH• radical scavenging method according to Dorman et al. (2003), and results were expressed as percent inhibition [23]. A 50 µL aliquot of olive oil phenolic extract in Tris-HCl buffer (50 mM, pH 7.4) was mixed with 450 µL of Tris-HCl buffer (50 mM) and 1.0 mL of DPPH• (0.1 mM in methanol). After 30 minutes of incubation in darkness and at ambient temperature, absorbance was monitored at 517nm. Percent inhibition was calculated using the following equation;

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (4)$$

where A is the absorbance.

Estimated DPPH• inhibition values (%) are averages of quadruplicate analyses.

Tocopherol and Fatty Acid Composition: The HPLC method of AOCS (1997) was used to determine tocopherol composition of olive oils [24]. Fatty acid composition of olive oils was determined by gas chromatography (GC) using AOCS (1997) method [25].

RESULTS AND DISCUSSION

Results for physical fruit properties of conventional Memecik olive cultivar are presented in Table 1.

Physical properties of olives are important parameters for table olives and olive oil. These characteristics are influenced by cultivar, fruit ripeness, irrigation regimes, and environmental factors (geographical area, soil quality, type of cultivation, rainfall, etc.) [7]. Several investigations about the physical properties of Memecik olives at different fruit ripeness, irrigation regimes or environmental factors are reported in the literature. In a study, Dolgun et al. [26] studied the effects of cultivation type on Memecik cultivar and reported that weight of stone 0.83 (g), weight of pulp 3.28 (g), pulp/stone ratio 4.38 (g), weight of olive 3.95 (g), width of olive 1.79 (cm), length of olive 2.56 (cm), moisture of olives 58.16 (%), yield of oil in dry matter 31.47 (%) of the conventional Memecik olive cultivar (RI: 6.21). Similarly Efe et al. [27] investigated Milas region, representing the station Memecik olive varieties fruit weight (4.78 g), stone weight (0.56 g), fruit length (2.56 cm) and fat content (24.50 %). In a study on the effects of fertilization on physical properties of Memecik olives, Irget et al. [28] reported that the ranges of olives' width of stone (cm), length of stone (cm), fruit shape index and oil content (%) were 1.23-1.40, 1.89-2.15, 1.51-1.58, 25.94-32.42 %, respectively. Moisture content (%) and flesh to stone ratio of Memecik cultivar were found 48.9-54.5 and 3.6-4.8, respectively, at various stages of ripening [7]. In general, our findings were similar to those reported in the literature but small differences might be due to cultivar, ripeness degree, harvest time, climate and/or locality.

Table 1. Physical Fruit Properties of Memecik Olive cultivar (values are mean ± standard deviation)

Physical Fruit Properties	Value
Ripeness index	6.91 ± 0.04
Width of olive (cm)	1.88 ± 0.09
Length of olive (cm)	2.55 ± 0.13
Weight of stones (g)	0.95 ± 0.17
Weight of olives (g)	5.98 ± 0.00
Weight of pulp (g)	5.03 ± 0.17
Pulp/stone ratio(g)	5.48 ± 1.30
Moisture of olives (%)	50.52 ± 0.2
Yield of oil/ dry matter (%)	44.74 ± 0.2
Refractive index	1.47 ± 0.00
Fruit shape index	1.36 ± 0.08

According to [19], refractive index of virgin olive oils ranges from 1.4677 to 1.4705. Therefore, our results for refractive index were in good agreement with the fact that Memecik olive oil used in this present study should be considered as virgin olive oil. Refractive indices for some Turkish olive cultivars such as Ayvalik, Domat and Gemlik were found 1.4688, 1.4678 and 1.4689, respectively [29]. Results showed that the differences might be due to cultivar but Memecik olive oil was found in natural virgin olive oil category.

Table 2 shows that the olive oil quality parameters' mean value and standard deviations. Free fatty acid content (0.95 % oleic acid) exceeded the limit of 0.8 %, so the oil is classified as virgin olive oil. Peroxide value (3.20 meq/kg) of the oil was below 20 meq/kg limit. K_{232}, K_{270}, and ΔK values did not exceed the limit value established by the Turkish Food Codex's standard for olive oil and olive-pomace oil [30]. Some researchers reported that peroxide value (meq O_2/kg oil) of Memecik cultivar were 8.68 for 2005 harvest year and 13.45 for

2006 harvest year [31]. Dolgun et al. were reported that peroxide value (meq O_2/kg oil), free acidity as oleic acid (%) and K_{232} value of conventional Memecik olive cultivar (RI: 6.21) were 12.07, 1.71 and 1.86, respectively [26]. In general, results were in good agreement with those reported in literature but differences could arise from several factors including extraction methods, climatic and locality conditions and postharvest treatment [26]. K_{232} and K_{270} coefficients may be affected by cultivar, fruit quality, climatic and ecological conditions, harvest time, altitude, crop season, growing location and storage conditions [32].

Table 2. Physico-chemical Properties of Olive Oil Extracted from Memecik Olive cultivar (values are mean ± standard deviation)

Physico-chemical Properties of Olive oil	Value
Chlorophyll pigments (mg/kg oil)	0.493 ± 0.031
Chlorophyll pigments as pheo α (mg/kg oil)	0.285 ± 0.016
Carotenoids (mg/kg oil)	0.510 ± 0.008
K_{232}	1.4935 ± 0.0080
K_{270}	0.0985 ± 0.0005
Peroxide Value (meq O_2 / kg oil)	3.20 ± 0.06
Free Acidity as Oleic Acid (%)	0.95 ± 0.02

Color is an important determinant of the organoleptic quality of virgin olive oil affected by pigment contents of olives. Pigment profile has been proposed to serve as an indicator of typicality and authenticity of monovarietal olive oils [33]. Dolgun et al. were reported that chlorophyll pigments (mg/kg oil), chlorophyll pigments as pheo α (mg/kg oil), and carotenoids (mg/kg oil) of conventional Memecik olive cultivar (RI: 6.21) were 12.74, 0.28 and 7.95, respectively [26]. Pigment contents of Memecik olive oil at RI: 6.91 were found lower than those reported in the literature, and the reason could be that the pigment content of virgin olive oil may depend on several factors such as olive variety, geographical origin, environmental conditions, degree of olive ripeness, and extraction and storage conditions [33].

Fatty acid composition of Memecik olive cultivar is given in Table 3. Fatty acid composition of samples was found within the standard limits by Turkish Food Codex [30]. Oleic acid was at the highest concentration (76.915 %) followed by palmitic (11.605 %), linoleic (9.825 %), stearic (0.995 %) and palmitoleic acids (0.575 %). According to [10] and [12] virgin olive oils are classified into two types based on their fatty acid compositions. The first type is characterized by low linoleic and palmitic and high oleic acid contents, and Turkish virgin olive oils (like Spanish, Italian and Greek) are considered to belong to this type. Therefore, Memecik olive oil could be classified on this first type. In a study [26], fatty acid composition of the conventional Memecik olive cultivar (RI: 6.21) was at the highest concentration of oleic acid (61.96 %) followed by linoleic (17.14 %), palmitic (14.71 %), stearic (3.19 %) and linolenic acids (0.70 %). Nergiz and Engez [7] reported that palmitic (13.9-15.0 %), palmitoleic (0.94-1.60 %), stearic (2.23-2.73 %) and oleic (63.7-71.5 %) linoleic (7.7 -15.6 %) acid contents of Memecik cultivar vary at different ripeness index. Our results were in good agreement with the literature in terms of fatty acid composition, but differences could be explained by the fact that the fatty acid composition in olive oil is effected by several factors such as variety, growing conditions, altitude, zone of production, climate, ripeness index and harvest time.

Table 3. Fatty Acid Composition of Olive Oil Extracted from Memecik Olive cultivar (values are mean ± standard deviation)

Fatty Acids	Percent
Palmitic (C16:0)	11.38 ± 0.03
Palmitoleic (C16:1)	0.50 ± 0.02
Margaric (C17:0)	0.05 ± 0.00
Margaroleic (C17:1)	0.03 ± 0.00
Stearic (C18:0)	1.25 ± 0.05
Oleic (C18:1)	76.33 ± 0.42
Linoleic (C18:2)	10.34 ± 0.44
Linolenic (C18:3)	0.08 ± 0.02
Behenic (C22:0)	0.07 ± 0.01

Tocopherols are essential, and their content is highly variety-dependent, with concentrations ranging from 5 to 300 ppm [34]. The content of tocopherols in Memecik olive (RI: 6.91) is shown in Table 4. In this present study, α-tocopherol was the most abundant tocopherol compared to γ, β, δ- tocopherols. Similarly Ilyasoglu et al. [35] reported that the α-tocopherol content of Memecik olive oils ranged from 198.7 to 326.8 mg/kg. However, another study [26] reported that α- tocopherol, β- tocopherol , γ- tocopherol and δ- tocopherol contents of the conventional Memecik olive cultivar (RI: 6.21) were 443.50, 2.77, 7.56 and 0.6 ppm, respectively. Several factors such as variety, locality, climate, ripeness index and postharvest treatment can fluctuate tocopherol concentrations in olive oils.

Table 4. Tocopherol Composition of Olive Oil Extracted from Memecik Olive cultivar

Tocopherols	Mean ± Standard Deviation
α- tocopherol	205.45 ± 30.35
β- tocopherol	1.645 ± 0.205
γ- tocopherol	6.065 ± 0.885
δ- tocopherol	0.325 ± 0.045

Concentration of phenolic compounds is an important parameter in the evaluation of virgin olive oil quality because phenols contributing largely to oil flavour and protecting the free fatty acid fraction from oxidation [33]. Total phenolic contents of Memecik cultivar (RI: 6.91) are shown in Table 5. Ocakoglu et al. [31] reported that total phenolic contents of Memecik olive cultivar in 2005 and 2006 harvest years were 330.92 and 137.15 mg GAE/kg of oil, respectively. Ilyasoglu et al. [35] reported that total phenolic content of Memecik cultivar ranged from 121.59 to 254.30 mg GA/kg of oil. The differences from the previously reported values may be due to several factors such as differences in harvest years, climatic conditions, maturity stage of fruits and locality.

In extra virgin olive oils, different classes of compounds characterized by antioxidant activity are present, namely, tocopherols, carotenoids, chlorophylls, and in particular phenolic compounds. These natural antioxidants exert their antioxidant activity through

various mechanisms preventing free radical initiation by scavenging initially formed radicals, decreasing the localized oxygen concentration, and decomposing peroxides [32].

Kiralan et al. [2] investigated the oxidation stability of virgin olive oils from some important cultivars in the East Mediterranean Area of Turkey and found different correlations between oxidation stability and total phenolic content depending on cultivar. According to their results DPPH· radical scavenging activity of methanol:water extracts and total oil varied with respect to olive cultivar [2].

Inhibition (%) values are considered as good estimates for antioxidant activity, the ability of oil on scavenging free radicals. The inhibition values (%) of Memecik cultivar (RI: 6.91) are shown in Table 5. Karakus [36] studied Memecik olives from different locations in Turkey and analyzed the total phenolic content and DPPH· scavenging activity of extracted olive oils. The total phenolic contents of Memecik olive oil from four different locations were 151.71, 121.13, 130.00 and 65.49 mg caffeic acid equivalent per kg of oil. The DPPH· radical scavenging activities of Memecik olive oil from four different locations were 88.40, 67.75, 55.00 and 38.55%, respectively. And the researcher concluded that there was a positive correlation between total phenolic content and radical scavenging activity of oils. Memecik olive oil from Ortaklar location (in Aydin) exhibited the best antioxidant activity [36]. Our findings were similar to those reported in the literature but there were differences due to harvest year, climatic conditions, maturity stage of fruits and locality.

Table 5. Antioxidant Properties of Olive Oil Extracted from Memecik Olive cultivar

Antioxidant Properties of Olive Oil	Mean ± Standard Deviation
Total Phenols (mg/kg)	169.25 ± 4.83
Percent Inhibition of DPPH· Radical	9.55 ± 1.97

The antioxidant capacities of oleuropein, its aglycone, and minor phenols have been previously studied using methods like DPPH and ABTS [37]. Four different free radical scavenging methods (ABTS·, DPPH·, ORAC, β-carotene-linoleat model system) were used on 39 different extra virgin olive oils, and all test methods were found suitable for the determination of antioxidant capacity for olive oil. DPPH· radical scavenging activity was 587.01 µM for Picual cultivar on 2003-2004 crop seasons. Similarly Gorinstein et al. [38] studied four different antioxidant methods (TRAP, DPPH, β-carotene and ABTS tests) for five Spanish olive oils and reported that there was a positive correlation between total phenolic content and antioxidant methods. The researchers reported that the highest antioxidant potential was observed in extra virgin oil.

CONCLUSION

Physical properties of Memecik olive and oils were comparable with the Codex Alimentarius and values reported in the literature. However, differences were most probably due to factors such as locality, climate and soil conditions of Aydin. Present study showed that quality parameters of virgin olive oil extracted from conventional Memecik olives were also similar to the values reported previously in the literature. Today, early or fall harvest is practiced in different parts of Turkey because early harvest olives have low free fatty acid (FFA) and peroxide values (PV) which are important in high quality olive oil. But this study verified that the late harvest olive oils (RI:6.91) can have low free fatty acid (FFA) and peroxide values (PV), too. It makes olive oils more stable to oxidation. On the other hand, antioxidant activity of the studied oil samples decreased because pigment concentration of olive oils was lower than the values reported in the literature. This study suggested that DPPH· radical scavenging method can be used as an indicator for the antioxidant activity of olive oil together with its total phenolic content.

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