SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 23(2) (2025) 91-100, DOI: 10.24323/akademik-gida.1746595

Research Paper / Araştırma Makalesi

Effect of Different Irrigation Treatments on Quality and Antioxidant Compounds of Virgin Olive Oils from cv. "Memecik"

Oya Köseoğlu[1], Ferişte Öztürk Güngör[1], Ünal Kaya[1], Gökhan Çamoğlu[2], Erhan Akkuzu[3], Didar Sevim[1], Şerafettin Aşik[3]

[1]Ministry of Agriculture and Forestry of İzmir Olive Research Institute, Üniversite Cad. No:43 35100 Bornova, İzmir, Türkiye
[2]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, Çanakkale, Türkiye
[3]Ege University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, 35100 İzmir, Türkiye

Received (Geliş Tarihi): 18.07.2024, Accepted (Kabul Tarihi): 24.06.2025
Corresponding author (Yazışmalardan Sorumlu Yazar): atamete35@hotmail.com (O. Köseoğlu)
+90 232 462 70 73-137 +90 232 435 70 42

ABSTRACT

The effect of different irrigation treatments on the quality and antioxidant compounds of the virgin olive oil obtained from Memecik variety was determined in this study, which was carried out in three consecutive years. For this purpose, six different irrigation treatments (25%, 50%, 75%, 100% and 125%) were applied to an olive orchard using the drip method. According to quality criteria, free acidity and peroxide value were not influenced by the amount of irrigation. Differences in fatty acids among oil samples were found statistically significant. Among the hydroxytyrosol, gallic acid, oleuropein, chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin, hydroxytyrosol and vanillic acid were influenced by the amount of irrigation water applied to olive trees, and there was a significant relationship among the irrigation treatments. Generally, an increase in the amount of irrigation water given was accompanied by a reduction in bitterness indices and total chlorophyll contents. In terms of oil yield and chemical quality, 25% irrigation treatment could be recommended under experimental conditions.

Keywords: Memecik cv, Phenolic compounds, Bitterness index, α-tocopherol, Irrigation

Farklı Sulama Uygulamalarının "Memecik" Çeşidinden Elde Edilen Sızma Zeytinyağlarının Kalitesi ve Antioksidan Bileşikleri Üzerine Etkisi

ÖZ

Bu araştırma üç yıl üst üste gerçekleştirilmiş olup farklı sulama düzeylerinin Memecik zeytin çeşidinden elde edilen sızma zeytinyağının kalitesi ve antioksidan bileşikleri üzerine etkisi incelenmiştir. Bu amaçla zeytin bahçesine damla sulama yöntemi kullanılarak altı farklı sulama oranları (%25, %50, %75, %100 ve %125) uygulanmıştır. Kalite kriterlerinden serbest asitlik ve peroksit değerlerinin sulama miktarından etkilenmediği tespit edilmiştir. Yağ asitleri açısından uygulamalar arasındaki farklar istatistiksel olarak anlamlı bulunmuştur. Hidroksitirozol, gallik asit, oleuropein, klorojenik asit, kafeik asit, vanilik asit, *p*-kumerik asit, ferrulik asit, luteolin ve apigenin arasında, hidroksitirozol ve vanilik asit değerleri uygulanan sulama suyu miktarından etkilenmiş ve sulama uygulamaları arasında önemli bir ilişki tespit edilmiştir. Genel olarak verilen sulama suyu miktarındaki artışa, acılık indeksi ve toplam klorofil içeriğinde azalma eşlik etmiştir. Yağ verimi ve kimyasal kalitesi açısından deneme koşullarında %25 oranında sulama uygulaması önerilmektedir.

Anahtar Kelimeler: Memecik cv, Fenolik bileşenler, Acılık indeksi, α-tokoferol, Sulama

INTRODUCTION

Türkiye is considered as the homeland of olives and is one of the important olive oil producers of the world [1]. One of the olive varieties which is widely grown in Türkiye and preferred by olive growers is Memecik, and this variety's trees represent about 45% of the total olive trees in Türkiye. Approximately 75-80% of virgin olive oil is obtained from Ayvalık and Memecik olive varieties. Memecik olive oil is distinguished by high levels of phenolic compounds, which also contribute to its high oxidative stability and distinctive sensory qualities (intense fruity and bitterness).

Olive trees can usually grow and produce adequate yields even with a low annual water supply, but irrigation is essential for high yields in times of drought [2]. To increase olive yield, substantial water resources for irrigation are necessary. Unfortunately, these quantities of water are not always available [3]. Irrigation is a very important factor in increasing olive oil production, productivity and quality, because quality olive oil cannot be obtained from an olive fruit lacking in water [4].

Virgin olive oil is a rich source of natural antioxidants due to minor components such as chlorophyll, carotenoids, tocopherols and phenolic compounds. These elements form a crucial first line of defence against free radical attacks made by various mechanisms. Because of its high polyphenol, α-tocopherol, and low polyunsaturated fatty acid content, extra virgin olive oil is very resistant to oxidation [5]. Irrigation had the greatest impact on antioxidant components, particularly polyphenol contents. Additionally, it has been observed that under conditions of water stress, the concentration of phenolic compounds in oil increases [6]. It is crucial for health to produce olive oil with high antioxidant activity and antioxidant compounds.

Early research on the effects of irrigation on olive oil focused on chemical components. It has been shown that the components most affected by irrigation are phenolic compounds that affect both oxidative stability and sensory properties, especially bitterness [7]. Since high sensory bitterness and pungency are preferred in extra virgin olive oil, achieving these characteristics is very important. Therefore, the right level of irrigation is very important for olive oil to improve its sensory properties [6].

The olive tree grows in semi-arid and arid regions in Türkiye. Irrigation is especially needed on summer days when the temperature is high and the rainfall is low. Today, and in the future water is very important for agricultural production and food security. Many researchers point out that, as a result of climate change, water scarcity is currently a major problem and a major challenge [8]. Considering the climate forecasts for the future, significant warming draws attention. Especially the Mediterranean Basin is considered as a "hot spot" in terms of climate change [9]. The quality of oils can be significantly impacted by irrigation, which can also have an impact on olive fruit development, production, and vegetation [10].

There is no study on the effect of different deficit irrigation program on extra virgin olive oils of Memecik olive variety grown in Türkiye. This variety is very important for oil production in Türkiye. On this basis, the aim of this study was to evaluate the effects of different water levels applied by the drip irrigation method on the antioxidant compounds (total phenol content (TPC), phenolic compositions, bitterness index (BI), total chlorophyll (TC) and α-tocopherol (α-Toc) and quality parameters (free fatty acid (FFA), peroxide value (PV), UV absorptions and fatty acid compositions (FACs) of the extra virgin olive oil obtained from Memecik olive variety grown in İzmir (western Türkiye). In the light of the findings, it is aimed to create the most suitable irrigation program for this variety and to ensure that the producers gain the right irrigation habit in this way.

MATERIALS and METHODS

Irrigation Practices and Olive Plantations

The Olive Research Institute experimental plantation served as the site for the research in Türkiye during the three successive crop seasons on Memecik olive (*Olea europaea* L. cv.) cultivar (Latitude:38°43'N; Longitude 27°25'E, 20 m above sea level). Flat land with medium clay-loam soil makes up the plot. For a crop root depth of 90 cm, there is 94 mm of available water capacity. With hot, dry summers, the climate is semi-arid Mediterranean. Mean annual temperature and rain varied from 26.5 to 27.3°C and 55.4 mm to 88.6 respectively.

Twenty-year-old olive trees make up the Memecik olive orchard. The trees were arranged in a 5 x 7 m pattern that ran from east to west. Fertilizer was applied to the soil and leaves in accordance with laboratory analyses of soil and leaf nutrients. Every season, these analyses were conducted again to ensure that they had access to the necessary nutrients. Six applications and three replications were used in the randomized blocks design of the study. Each research plot had four olive trees in it. The 6 applications included a fully irrigated control, 5 that applied irrigation at rates that represented 25% ($S_{0.25}$), 50% ($S_{0.50}$), 75% ($S_{0.75}$), 100% ($S_{1.00}$) and 125% ($S_{1.25}$) of the total evaporation over 5 days from a Class A evaporation pan, and one that was set up to reach field capacity for reducing moisture at a soil depth of 0-90 cm (the control, or Sc).

Extraction of Samples

Samples of olive oil were obtained using ABENCOR, a laboratory-scale extraction system (MC2 Ingenierias y Sistemas in Seville, Spain). After crushing the olive fruit, weighing approximately 5 kg, with a hammer crusher, the resulting paste was mixed at 30°C for 30 min. Following solid-liquid phase separation, the paste was centrifuged at 1500 rpm. Filtration was used to remove impurities from the liquid phase.

Quality Indices

According to Turkish Food Codex, the FFA (% in oleic acid), the PV (meqO_2/kg oil) and K_{232} and K_{270} measurements (were calculated from absorption at the precise wavelengths in nm) were determined [11].

Fatty Acid Composition by Gas Chromatography

The flame ionization detector supplied by system (HP 6890) described by Turkish Food Codex was used to determine the fatty acid composition (FAC) of the samples [11]. For analyses, a capillary column DB-23 (30m x 0.25mm x 0.250m) was employed. A standard FAME mixture was used as a standard (Sigma-Aldrich Chemicals 189-19). All fatty acid peak areas were calculated by HP 3365 Chemstation® program and recorded.

Total Chlorophyll Content

Spectrophotometry (Shimadzu UV 1700, Kyoto, Japan) was used to determine the TC content in accordance with the American Oil Chemists' Society's official method [12].

Total Phenolic Content

For TPCs analysis, the method recommended by Kiritsakis was used [13]. A Shimadzu UV 1700 (Kyoto, Japan) spectrophotometer was used to measure the samples' absorbance at 725 nm. Results are presented in milligrams of total polyphenols per kilogram of fat (as caffeic acid).

Phenolic Composition

SPE with Diol cartridges (Supelco Co., Bellefonte, USA) were used to extract the phenolic fraction. The oil (30 g) was dissolved in 30 mL of n-hexane and passed through the column after the cartridge had been preconditioned with 6 mL each of methanol and hexane. N-hexane was used to clean the cartridge. Finally, the phenols were extracted with methanol and dried in a rotary evaporator at 35°C under reduced pressure. The residue was filtered through a 0.25 m filter, dissolved in 2 mLof methanol, and then analyzed using an Agilent 6410 Triple Quad LC/MS-ESI system [14]. With a particle size of 1.8 m, C18 columns and Zorbax SB-50mm were used for the oil extract analysis (Agilent, USA). With a flow rate of 0.7 L/min, the mobile phase contained 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), with the gradient being as follows: 40% A / 60% B for the first four minutes, followed by 60% A / 40% B for the final minute.

Bitterness Index

Obsolete extraction columns for octadecyl (C18) were used for BI analysis (6 mL, J.T. Baker Company). Weighing 1 g, dissolving it in hexane, and running it through a C18 column were the steps in the experiment. Following elution, hexane was used to remove the oil, and the retained compounds were then washed to a volume of 25 mL with a solution of methanol and water (1:1 v/v) in a tared beaker. Using a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan) set to 225 nm, measurements were taken [15].

α-Tocopherol Content

According to the IUPAC standard method, α-Toc was calculated [16]. The HPLC system (Agilent-1100 series) received 1 g of oil solution in hexane, which was then filtered through a 0.45μm cellulose filter and washed with hexane/2-propanol (99:1) using an auto-sampler at a flow rate of 1 mL/min. The UV detector HP 1100 has a wavelength of 292 nm. The column was a Waters u Porasil (300mm x 3.9mm x 10m, Ireland; R^2=0.999). Analyses were performed at 25°C.

Statistical Analysis

In order to statistically analyze the data, SPSS version 13.0 was used. It was determined whether differences between data groups were significant using variance analysis (ANOVA). When significant differences were found, the Duncan's multiple range test was applied to determine which groups differed. A significance level of $p < 0.05$ was considered statistically significant. For each sample, all parameters were assessed in triplicate.

RESULTS and DISCUSSION

Quality Indices

FFA, PV and UV spectrophotometric indices (K_{232} and K_{270}) are the basic quality indices in National and International Standards of olive oil. Especially FFA parameter used in the classification of olive oils with respect to quality. These quality indices are highly affected by variety, olive harvest, harvest year and time, geography, agricultural practices, oil production, and storage practices [17].

Quality indices of extra virgin olive oils from Memecik variety grown under different irrigation treatments are seen at the Table 1. According to the Table 1, there were no significant effects determined between irrigation treatments of the Memecik olive oils of average FFA. In other words, free fatty acidity was not affected by the amount of irrigation. Olive oil samples FFAs were ranging from 0.26 ($S_{1.00}$) to 0.43 ($S_{0.25}$) (% in oleic acid). Dettori and Rusco [18] and Gomez-Rico et al. [6] have found similar results in their studies. On the other hand Dag et al. [19] reported that there was a significant difference in FFA between the waterless and irrigated subjects, but this value did not increase much with the amount of irrigation. The olive oils with FFA less than 0.8 (%in oleic acid) are defined as extra virgin olive oil (EVOO) in International Olive Council (IOC). All our samples were within the limit of the values established by IOC [20] All olive oil samples were categorized as "EVOO" according to the FFAs.

As seen at Table 1, average PV varied between 3.02 ($S_{1.00}$) and 3.81 meqO^2 kg^{-1} oil (S_C), and there were no statistically significant differences determined between the irrigation treatments. This was also obtained by Baccouri et al. [21] for Chétoui cultivar. The olive oils with PV less than 20 meqO_2 kg^{-1} oil are defined as "EVOO" in IOC standard. The results were within the limit of IOC [20].

According to the UV spectrophotometric indices, average K_{232} and K_{270} values ranged from 1.29 ($S_{1.00}$) to 1.54 (S_C) and ranged from 0.11 ($S_{1.00}$) to 0.14 ($S_{0.25}$), respectively. According to the statistical analysis, there were important differences detected only in the $S_{1.00}$ treatment ($p<0.05$) in the K_{232} values. For the K_{270} values, it is seen that the statistically differences between the treatments were determined. The highest value is obtained for the least irrigated $S_{0.25}$ treatment. When both parameters (K_{232} and K_{270}) evaluated together, it is seen that the lowest values were realized for $S_{1.00}$. The K_{232} and K_{270} values were given as ≤2.5 and ≤0.20, respectively by IOC. All the samples exhibited the values within the range of the limits for "EVOO" [20]. The PV and UV spectrophotometric characteristics were the important parameters describing the oxidative status of the oil samples. Although certain numerical discrepancies were noted among treatments, these variations remain within the bounds of natural variability and lack the consistency required to establish a definitive impact of irrigation on oil quality. This indicates that, within the evaluated spectrum of irrigation treatments, the olive trees preserve consistent oil quality irrespective of the water quantity provided. Therefore, water management practices aimed at optimizing irrigation may focus more on yield and water use efficiency without compromising the chemical quality and stability of the produced olive oil. In practical terms, olive growers can adjust irrigation density according to water availability and agronomic needs without fearing detrimental effects on the essential quality characteristics of the olive oil.

Table 1. Quality indices of extra virgin olive oils from Memecik variety grown under different irrigation treatments

Parameter	Year	Irrigation Treatment					
		$S_{0.25}$	$S_{0.50}$	$S_{0.75}$	$S_{1.00}$	$S_{1.25}$	S_C
FFA (% in oleic acid)	2008	0.66±0.03	0.42±0.01	0.35±0.03	0.26±0.01	0.22±0.03	0.41±0.03
	2009	0.37±0.02	0.20±0.03	0.27±0.02	0.27±0.01	0.37±0.02	0.40±0.03
	2010	0.26±0.02	0.23±0.02	0.33±0.03	0.25±0.02	0.30±0.02	0.28±0.01
	Average	0.43 NS*	0.28 NS	0.32 NS	0.26 NS	0.30 NS	0.36 NS
PV (meq O_2 kg^{-1} oil)	2008	2.96±0.05	2.27±0.03	2.33±0.04	2.39±0.03	2.11±0.02	2.56±0.02
	2009	3.90±0.06	4.40±0.04	3.87±0.03	3.07±0.04	3.03±0.03	5.00±0.05
	2010	3.72±0.03	3.96±0.03	5.19±0.05	3.62±0.04	4.18±0.04	3.87±0.03
	Average	3.53 NS	3.54 NS	3.80 NS	3.02 NS	3.11 NS	3.81 NS
K_{232}	2008	1.56±0.03	1.55±0.02	1.49±0.02	0.99±0.04	1.46±0.01	1.48±0.02
	2009	1.57±0.03	1.47±0.03	1.51±0.02	1.39±0.03	1.44±0.01	1.67±0.03
	2010	1.46±0.02	1.42±0.02	1.57±0.01	1.50±0.03	1.54±0.01	1.45±0.02
	Average*	1.53 A**	1.48 A	1.53 A	1.29 B	1.48 A	1.54 A
K_{270}	2008	0.13±0.01	0.11±0.03	0.10±0.01	0.07±0.03	0.10±0.01	0.08±0.02
	2009	0.16±0.01	0.13±0.02	0.13±0.01	0.11±0.02	0.12±0.01	0.14±0.03
	2010	0.13±0.02	0.15±0.02	0.16±0.02	0.14±0.01	0.16±0.02	0.13±0.03
	Average*	0.14 A	0.13 AB	0.13 AB	0.11 C	0.12 BC	0.12 BC

*NS: Not Significant, **Values with different letters in the same row present significant differences between irrigation treatments for each parameter ($p<0.05$).

Fatty Acid Composition by Gas Chromatography

Major fatty acids are palmitic acid (C16:0) (PA), palmitoleic acid (C16:1) (POA), stearic acid (C18:0) (SA), oleic acid (C18:1) (OA), linoleic acid (C18:2) (LO), linolenic acid (C18:3) (LN) acid and arachidic acid (C20:0) (AA) and Saturated fatty acids (SFAs), Monounsaturated fatty acids (MUFAs) and Polyunsaturated fatty acids (PUFAs) are given in Table 2 according to the years and the average of the years.

According to irrigation treatments, PA, POA, SA, OA, LO, LN, AA acid, SFAs, MUFAs and PUFAs content were ranging from 13.14 to 16.17%, ranging from 0.92 to 1.44%, ranging from 1.80 to 2.42%, ranging from 62.34 to 71.59%, ranging from 9.90 to 15.87%, ranging from 0.69 to1.06%, ranging from 0.40 to 0.66%, ranging from 16.13 to 18.83%, ranging from 64.19 to 72.96% and ranging from 10.74 to 16.93%, respectively.

When the results of the average fatty acids obtained according to the irrigation treatments were examined, it was seen that the differences between the subjects are statistically significant. Considering the subject of $S_{0.25}$, where irrigation was applied the least, it was seen that PA, POA and LO acid and SFAs and PUFAs content were the lowest, while SA, OA acid and MUFAs content were the highest.

Likewise, when the treatments of $S_{1.25}$, where irrigation was applied the most, was examined, it was seen that PA, POA and LO acid, SFAs and PUFAs content were the highest, while SA, OA acid and MUFAs content were the lowest. The values for all fatty acids were within the limits defined in the standard of IOC [20].

MUFAs content, which is an important factor in the oxidative stability of olive oils, decreased due to increasing irrigation, while PUFAs content increased. These results compatible with Toplu et al. [21]. The lowest PUFAs content was obtained from the $S_{0.25}$

subject, where the least irrigation was applied. This result was not found to be compatible with Stefanoudaki et al. [2]. This may be due to the olive cultivars and the different maturity indexes of the olives.

It is known that increasing the content of PUFAs (LO and LN acid) leads to increased oxidation of olive oil, shortened shelf life [2, 22]. Therefore, PUFA content should be a parameter to be considered in irrigation applications. The lowest PUFA value was obtained in subject $S_{0.25}$, where the lowest irrigation was applied. The high ratio of OA acid, which is the most important source of MUFAs, which is important for nutrition and oxidative stability of olive oil, makes olive oil more resistant to oxidation [23]. In this research, OA acid content decreased with increasing irrigation amount. During the ripening period of the fruit, there is an increase in OA acid content and a decrease in the relative percentage of PA acid content due to the biosynthesis of triacylglycerols. On the other hand, the increase in LO acid content is due to the conversion of OA acid to LIN acid due to oleate desaturase activity during triacylglycerol biosynthesis [24]. In this research, the highest OA acid content was determined in $S_{0.25}$, where irrigation was applied the least and the maturity index was the highest. The highest PA and LO acid content were determined in $S_{1.25}$, where irrigation was applied the most. These research findings have been in accordance with other studies [6].

Table 2. FACs of extra virgin olive oils from Memecik variety grown under different irrigation treatments (%)

Fatty Acid	Year	Irrigation Treatment					
		$S_{0.25}$	$S_{0.50}$	$S_{0.75}$	$S_{1.00}$	$S_{1.25}$	S_C
Palmitic C16:0	2008	13.47±0.11	14.23±0.16	14.40±0.14	14.43±0.11	15.03±0.12	14.67±0.14
	2009	13.21±0.15	13.30±0.11	14.10±0.12	13.14±0.12	14.16±0.15	14.21±0.12
	2010	14.30±0.14	15.22±0.13	15.73±0.11	15.31±0.11	16.17±0.13	15.80±0.16
	Average	13.66 C*	14.25 B	14.75 AB	14.29 B	15.12 A	14.89 A
Palmitoleic C16:1	2008	1.03	1.20	1.33	1.27	1.43	1.33
	2009	0.94	1.04	1.20	0.92	1.20	1.22
	2010	1.12	1.16	1.33	1.29	1.44	1.30
	Average	1.03 C	1.13 C	1.29 AB	1.16 BC	1.36 A	1.28 AB
Stearic C18:0	2008	2.40±0.02	1.93±0.03	1.83±0.02	1.93±0.07	1.80±0.02	1.80±0.04
	2009	2.25±0.04	2.08±0.02	1.96±0.04	2.27±0.05	1.96±0.04	1.87±0.05
	2010	2.42±0.02	2.10±0.05	1.98±0.07	2.01±0.04	1.92±0.05	1.94±0.02
	Average	2.36 A	2.04 BC	1.93 CD	2.07 B	1.89 D	1.87 D
Oleic C18:1	2008	66.87±0.47	66.23±0.64	66.20±0.53	67.70±0.57	66.03±0.39	65.40±0.52
	2009	71.59±0.65	70.18±0.55	68.83±0.41	71.08±0.35	66.10±0.57	67.12±0.61
	2010	69.36±0.55	68.39±0.74	65.88±0.65	65.11±0.74	62.34±0.47	64.09±0.49
	Average	69.27 A	68.27 AB	66.97 B	67.96 AB	64.82 C	65.54 C
Linoleic C18:2	2008	14.20±0.21	14.87±0.23	14.80±0.31	12.53±0.27	13.67±0.23	14.63±0.17
	2009	9.90±0.18	11.25±0.13	11.76±0.27	10.18±0.15	14.41±0.21	13.38±0.20
	2010	10.71±0.15	10.99±0.24	12.90±0.19	14.11±0.17	15.87±0.19	14.73±0.21
	Average	11.60 C	12.37 BC	13.15 B	12.28 BC	14.65 A	14.25 A
Linolenic C18:3	2008	0.90	0.97	0.90	0.93	1.00	0.97
	2009	0.84	0.91	0.93	0.69	0.89	1.00
	2010	0.86	0.93	0.97	0.96	1.06	0.95
	Average	0.87 B	0.94 AB	0.93 AB	0.86 B	0.98 A	0.97 A
Arachidic C20:0	2008	0.43	0.40	0.40	0.43	0.40	0.40
	2009	0.46	0.46	0.40	0.66	0.49	0.45
	2010	0.50	0.47	0.47	0.46	0.45	0.45
	Average	0.46 B	0.44 BC	0.42 C	0.52 A	0.45 BC	0.43 BC
SFAs	2008	16.63	16.80	17.00	17.10	17.47	17.20
	2009	16.30	16.13	16.74	16.55	16.93	16.82
	2010	17.52	18.10	18.49	18.07	18.83	18.49
	Average	16.82 D	17.01 CD	17.41 ABC	17.24 BCD	17.74 A	17.50 AB
MUFAs	2008	68.27	67.87	67.23	69.43	67.87	67.13
	2009	72.96	71.66	70.45	72.56	67.75	68.77
	2010	70.90	69.99	67.63	66.82	64.19	65.81
	Average	70.71 A	69.84 A	68.44 BC	69.61 AB	66.60 D	67.24 CD
PUFAs	2008	15.07	15.80	15.67	13.47	14.63	15.63
	2009	10.74	12.16	12.70	10.87	15.30	14.38
	2010	11.57	11.92	13.87	15.08	16.93	15.68
	Average	12.46 C	13.29 BC	14.08 B	13.14 BC	15.62 A	15.23 A

*Values with different letters in the same row present significant differences between irrigation treatments for each parameter ($p<0.05$).

Bitterness Index

The values of the BI, TPC, α-Toc and TC of the oils from the different irrigation treatments studied were shown in Table 3. The bitter taste is related to the content of polyphenols and in this sense is a positive characteristic. Although there is no clear or established limit, experience has shown that K_{225}, an analytical determination to evaluate the bitter taste, values of the order of 0.360 or higher correspond to quite bitter oils (high or extremely high intensities) which are rejected by many consumers and K_{225} values of the order of 0.14 correspond to not bitter oil [15]. It influences consumer acceptance. The BI value of the virgin olive oils obtained from different irrigation treatments are given in Table 3 together with their three-year average. The BI value of different irrigation treatment varied between 0.69 and 1.14 in 2008, 0.9 and 1.46 in 2009, and 0.64 and 1.21 in 2010. Significant differences in its concentration were observed between the irrigation treatments studied. There was a decrease in the BI of olive oil as the irrigation water applied to olive trees increased. The BI values, which is attributed to the polyphenols, were found to be higher in treatments $S_{0.25}$ than the control treatment (S_C). According to these data, we can also conclude that our virgin olive oil obtained from different irrigation treatments are quite bitter oils. This data can be explained as a property of Memecik olive variety. Because Köseoğlu et al. [25] also found that the BI of Memecik olive oil was between 0.33 to 1.05 according to the different olive oil extraction systems. The three irrigation treatments ($S_{1.00}$, $S_{1.25}$, S_C) were statistically located in the same groups (Table 3). These findings were very similar to the observations of Tovar et al. [3], Romero et al. [26], Gomez-Rico et al. [6] and also Garcia et al. [27] reported in their research that the lowest the BI were determined in the control oils that were fully irrigated.

Total Phenolic Content

The concentration of phenolic compounds affects both sensory characteristics of olive oil and oil stability to autoxidation. According to the reports which were made by a many researchers, as the irrigation level of the olive trees decreased, the total polyphenols in the olive oil significantly increased, especially at the lower levels of irrigation [6, 28]. Table 3 shows the values of the TPC of the virgin olive oils year by year and the averages of the years. In our research, total phenol content varied from 194.1 ($S_{1.25}$) to 263.2 ($S_{0.25}$) mg CAE/kg under the different irrigation treatment depending average values. The lowest and the highest values were obtained in $S_{0.75}$ and $S_{0.25}$ treatments in 2009. Irrigation threat had a significant effect on the total phenolic content of the oils, such that with increasing irrigation dose, the TPC content in the resulting virgin olive oil decreased significantly. The total phenolic concentration of the extra virgin olive oil from the group that was less irrigated was significantly higher at 263.2 mg CAE/kg. The extra virgin olive oil from the more irrigated group had a lower content (194.1 mg CAE/kg). It is well known that phenol biosynthesis in plants is very sensitive to environmental conditions. If we look at the values of the TPC in 2009, the TPC of $S_{0.25}$ was found very high according to the others as a result of less rainfall. According to average value, the four highest irrigation treatments ($S_{0.75}$, $S_{1.00}$, $S_{1.25}$, S_C) oils were not statistically different from each other (Table 3) and the total phenol content decreased with increasing the amount of irrigation water (Table 3).The highest TPCs were obtained from the treatments ($S_{0.25}$) in which the lowest amounts of irrigation water had been applied Caruso et al. [29] stated that trees with high water status yielded oils with lower concentrations of total phenols. According to the results of the study, it can be concluded that the irrigation applied to olive trees leads to significant decrease in the olive oil phenolic content. Similar studies have found that olive oils obtained from trees with deficit water contain more total polyphenolic content than those from trees with adequate water supply [2, 6, 27, 30]. Contrary to the study findings Inglese et al. [31] determined that there was an increase on the phenolic content of Carolea olive oil as irrigation water applied to olive trees increased. Also, Garcia et al. [27] reported that irrigation induces a delay of fruit olive ripening that results in greater phenol contents in olive oil.

α-Tocopherol Content

α-Toc are considered natural antioxidant compounds abundant in extra virgin olive oil. The tocopherol content of extra virgin olive oil is very important for oxidative stability, its known to act as antioxidant and protect the oils from oxidation [32]. Therefore, it’s very important to have more tocopherol content in the extra virgin olive oil. In our research, the average α-Toc contents of the extra virgin olive oils from the different irrigation treatment were varied from 285.6 ($S_{0.25}$) to 325.6 ($S_{0.50}$) mg/kg (Table 3). The α-Toc values were found high compared to those cited in the literature, such as 139 mg/kg [33], 165 mg/kg [34]. Even if it looks like the amount of tocopherol increased as the amount of irrigation water given increased, it was not statistically different. Similar to the findings obtained by reported by Gomez-Rico et al. [6], Palese et al. [35]. However, in some studies, found a negative linear relationship between the water supplied to olive trees and α-Toc contents of the extra virgin olive oils. α-Toc content was higher in the olive oil produced from the non-irrigated trees [2, 5]. Garcia et al. [27] were determined a positive effect of irrigation on the content of α-Toc.

Total Chlorophyll Content

Depending on the amount of pigment present, extra virgin olive oil can range in colour from very green to gold [36]. The green hues in the oil are thought to be caused by chlorophyll pigments. Table 3 shows the TC content of the tree oils produced under various irrigation methods. When an evaluation was made according to the average values, the lowest and the highest chlorophyll values were found in the ranges of 1.76 mg/kg (S_C) and 3.94 mg/kg ($S_{0.50}$), respectively. There was significant irrigation effect on chlorophyll content. The less-irrigated conditions favoured chlorophylls' production (3.78 mg/kg) compared to the more irrigated group (1.83 mg/kg). In the study, it was observed that as the amount of water given to olive trees increased, the chlorophyll levels in the oils decreased. Chlorophyll values also were higher in 2009 due to less rainfall. There was no statistical difference between. $S_{0.25}$ and $S_{0.50}$ treatments in terms of chlorophyll content. The results from this research are similar to those found by Garcia et al. [27] and who found significant negative effects of irrigation on chlorophylls. Contrary to these studies Gomez-Rico et al. [6] determined that the irrigation regime does not affect the chlorophyll content of the Cornicabra olive oils.

Table 3. Content of natural antioxidants and chlorophylls in Memecik variety extra virgin olive oils grown under different irrigation methods

Parameter*	Year	İrrigation Treatment					
		$S_{0.25}$	$S_{0.50}$	$S_{0.75}$	$S_{1.00}$	$S_{1.25}$	S_C
BI (K_{225})	2008	1.00±0.02	1.12±0.03	1.14±0.02	0.71±0.02	0.69±0.04	0.84±0.02
	2009	1.46±0.01	1.04±0.02	0.90±0.00	0.79±0.03	0.90±0.05	0.85±0.03
	2010	1.21±0.02	0.87±0.01	1.00±0.01	0.92±0.03	0.83±0.00	0.64±0.01
	Average	1.22 A**	1.01 B	1.01 B	0.81 C	0.81 C	0.78 C
TPC (mg kg^{-1})	2008	278.3±3.54	258.7±5.68	205.3±7.25	191.7±3.51	192.7±6.59	228.3±2.58
	2009	285.6±4.25	238.3±4.28	176.3±4.98	186.2±5.46	179.7±5.53	182.4±5.87
	2010	255.7±5.27	231.0±3.58	210.7±6.31	216.3±4,57	191.0±4.78	215.7±4.53
	Average	263.2 A	240.2 AB	210.8 BC	206.2 C	194.1 C	211.7 BC
α-Toc (mg kg^{-1})	2008	276.7±5.47	329.7±3.51	322.7±7.22	330.0±6.47	282.3±5.45	337.7±7.14
	2009	297.0±3.47	350.8±5.45	311.3±5.41	306.7±3.24	341.9±3.21	328.7±5.32
	2010	283.0±6.58	296.4±2.52	294.0±4.25	298.8±4.15	261.0±6.13	260.2±3.33
	Average	285.6 NS***	325.6 NS	309.3 NS	311.8 NS	295.1 NS	308.9 NS
TC (mg kg^{-1})	2008	2.86±0.22	5.04±0.55	2.27±0.35	3.28±0.26	2.20±0.65	2.29±0.25
	2009	5.19±0.45	3.38±0.73	2.80±0.16	2.24±0.23	1.40±0.35	1.45±0.39
	2010	3.30±0.28	3.39±0.25	2.46±0.35	2.32±0.58	1.88±0.33	1.52±0.21
	Average	3.78 A	3.94 A	2.51 B	2.62 B	1.83 B	1.76 B

*BI: Bitterness Index, TPC: Total Phenolic Content, α-Toc: α-Tocopherol, TC: Total Chlorophyll, **Values with different letters in the same row present significant differences between irrigation treatments for each parameter ($p<0.05$), ***NS: Not significant

Phenolic Composition

The most significant class of hydrophilic antioxidative compounds found in nature are phenolic compounds. Given that the natural phenols improve extra virgin olive oil's resistance to oxidation and, to some extent, are responsible for its intensely bitter flavor, the amount of phenolic compounds in the oil is a crucial consideration when assessing its quality [37]. The agronomic and technological conditions have a significant impact on the qualitative and quantitative composition of the hydrophilic phenols in extra virgin olive oil. The most researched factors are cultivar, fruit ripening, production pedoclimatic conditions, and some agronomic practices like irrigation. By increasing the amount of healthy bioactive compounds and the dry matter percentage, deficit irrigation can enhance fruit quality. Many different secondary metabolites, primarily phenolic compounds, must be specifically mentioned among these substances [38]. The concentrations of individual phenolics that were affected by irrigation regime are reported in Table 4. In this research conditions the most typical phenolic compounds that were identified in three years were hydroxytyrosol, gallic acid, oleuropein, chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ferulic acid, luteolin and apigenin. Hydroxytyrosol was the highest concentration was registered in extra virgin olive oil produced from the $S_{0,25}$ treatment (1650 µg/kg), while the lowest concentration (416 µg/kg) was found in extra virgin olive oil from treatment $S_{1.00}$. It can be seen that only hydroxytyrosol, and vanillic acid were affected by the amount of irrigation water applied. But phenolic compounds vary differently in response to the irrigation strategy applied. Artajo et al. [39] also showed that the content of hydroxytyrosol was significantly higher in samples from non-irrigated trees. Jiménez-Herrera et al. [40] determined important changes in the concentration of hydroxytyrosol in response to water stress by drought. Therefore, they put forward the need for suitable amount of water during the fruit development in order to obtain optimum growth and quality. In general, it can be concluded that the amount of irrigation water increase, the values of the hydroxytyrosol decrease, but the vanillic acid values increase. Decrease on the hydroxytyrosol is very important. Because it is known to possess much greater antioxidant. Romero et al. [26] also studied that vanillic acid values increase with increasing the amount of irrigation water; Stefanoudaki et al. [2] has found that at least water treatment has the maximum amount of hydroxytyrosol. These results are in concordance with those described in this study. The flavonoid content like luteolin was found higher but apigenin was found lower amount in the less irrigation treatments.

CONCLUSION

In conclusion, the data obtained in this present work that carried out during three harvest years showed that the impact of irrigation is obvious and remarkable, on the BI, TPC, TC and some of the phenolic compositions of the extra virgin olive oil. Overwatering had no positive effect on the α-Toc content of the Memecik olive oil. It can be seen that $S_{0.25}$ irrigation treatment under our experimental conditions was the necessary to achieve good oil extractability from the olives and obtain ideal oil chemical parameters. In this research deficit irrigation regimes of olive trees showed very important effects on the antioxidant properties of olive oil. Water, especially in arid and semiarid regions, is an increasingly problem so the pressure for a more sustainable use of water in agriculture will grow. Under our experimental condition it seems that $S_{0.50}$ treatment would be an ideal irrigation level to maintain oil yield and quality, while reducing water requirements If producers wanted to produce extra virgin olive oil with more bitter, greener and phenolic-rich olive oil they might choose to limit water treatments. The findings on antioxidant compounds in relation to irrigation of Memecik cultivar show that application of 25% of Class A pan (treatment $S_{0.25}$) evaporation can be recommended for limited water conditions.

Table 4. Phenolic compositions of extra virgin olive oils from Memecik variety grown under different irrigation treatments (µg/kg)

Parameter	Year	Irrigation Treatment					
		$S_{0.25}$	$S_{0.50}$	$S_{0.75}$	$S_{1.00}$	$S_{1.25}$	S_C
Gallic Acid	2008	2.12±0.22	2.30±0.17	2.27±0.24	2.28±0.25	2.43±0.18	2.25±0.27
	2009	2.36±0.18	2.31±0.32	2.41±0.21	2.23±0.11	2.44±0.31	2.83±0.25
	2010	2.12±0.35	2.27±0.19	2.18±0.13	2.10±0.15	2.09±0.21	2.10±0.19
	Average	2.20 NS*	2.29 NS	2.29 NS	2.20 NS	2.32 NS	2.39 NS
Hydroxytyrosol	2008	2956±25.2	1888±30.1	1622±23.5	632±10.7	804±17.2	1551±27.5
	2009	1332±15.3	506±9.20	310±8.10	294±6.50	918±20.1	983±11.3
	2010	662±10.3	551±11.3	554±11.2	321±5.70	674±18.5	629±9.20
	Average	1650 A**	981 B	829 BC	416 C	799 BC	1054 AB
Oleuropein	2008	1.60±0.11	1.37±0.13	0.78±0.08	0.62±0.07	0.78±0.07	0.90±0.10
	2009	0.64±0.09	0.65±0.10	0.47±0.02	0.68±0.09	0.82±0.12	0.53±0.05
	2010	3.79±0.10	2.41±0.11	3.07±0.11	2.11±0.10	2.11±0.11	2.16±0.17
	Average	2.01 NS	1.48 NS	1.44 NS	1.14 NS	1.24 NS	1.20 NS
Chlorogenic Acid	2008	2.24±0.18	2.24±0.21	-	2.17±0.13	2.21±0.12	2.23±0.10
	2009	2.24±0.18	2.24±0.19	2.23±0.11	2.25±0.17	2.25±0.11	-
	2010	2.27±0.20	2.26±0.17	2.32±0.17	2.26±0.15	2.26±0.15	2.26±0.11
	Average	2.26 NS	2.25 NS	2.328 NS	2.26 NS	2.25 NS	2.25 NS
Caffeic Acid	2008	5.15±0.48	10.03±0.61	15.52±0.55	10.32±0.5	8.55±0.87	12.92±0.94
	2009	15.46±0.87	9.16±0.48	4.72±038	6.01±0.54	8.11±0.77	10.46±0.65
	2010	1.76±0.21	5.39±0.38	1.94±0.21	1.10±0.48	1.56±0.33	1.90±0.23
	Average	7.46 NS	8.19 NS	7.39 NS	5.81 NS	6.07 NS	8.43 NS
Vanillic Acid	2008	34.43±1.25	42.22±2.27	80.22±6.25	145.5±8.5	164.8±11.1	65.18±4.15
	2009	92.14±4.11	128.91±11.2	137.7±13.4	169.0±9.3	155.1±13.5	152.9±12.2
	2010	47.00±3.21	56.37±5.33	39.25±3.23	35.41±2.8	40.47±2.23	56.60±3.33
	Average	57.86 B	75.83 B	85.74 AB	116.64 A	120.15 A	91.57 AB
p-Coumaric Acid	2008	302±13.2	543±11.1	838±21.2	831±17.1	786±11.5	545±10.9
	2009	579±17.7	1049±31.2	566±13.7	788±13.2	640±12.8	646±11.2
	2010	352±17.2	627±12.7	258±11.2	305±7.21	286±8.12	321±7.54
	Average	411 NS	740 NS	554 NS	641 NS	571 NS	504 NS
Ferulic Acid	2008	8.65±0.88	16.60±0.77	21.30±1.83	41.73±1.1	40.56±2.21	50.70±1.17
	2009	20.93±1.17	31.91±1.07	47.04±2.13	57.14±1.8	47.06±2.01	45.43±2.04
	2010	23.45±2.87	26.92±1.25	19.54±0.97	32.62±0.9	26.13±0.81	33.97±1.13
	Average	17.68 NS	25.14 NS	29.30 NS	43.83 NS	37.92 NS	43.37 NS
Luteolin	2008	344±13.7	341±10.7	532±18.1	536±11.5	658±13.2	311±9.21
	2009	810±11.2	562±23.1	694±14.2	590±9.22	536±11.1	375±8.91
	2010	302±15.2	281±17.1	168±11.1	211±7.54	252±8.71	156±8.11
	Average	485 NS	395 NS	465 NS	446 NS	482 NS	281 NS
Apigenin	2008	104±4.13	129±4.11	187±2.13	254±4.81	299±3.15	133±2.03
	2009	329±5.14	368±7.87	365±6.17	349±6.13	215±3.33	223±3.98
	2010	171±2.54	157±3.18	86±1.23	86±1.11	103±2.01	71±1.01
	Average	201 NS	218 NS	213 NS	230 NS	206 NS	142 NS

*NS: Not Significant, **Values with different letters in the same row present significant differences between irrigation treatments for each parameter ($p<0.05$).

DECLARATION OF COMPETING INTERESTS

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to TUBITAK for providing financial support for the study. Under project number TOVAG 108O135, TUBITAK (The Scientific and Technological Research Council of Turkey) provided funding for this study.

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