

Determination of deficit irrigation treatments on olive fruit quality and olive oil (Memecik cv.) chemical composition and antioxidant properties

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In this research, the influences of irrigation treatments (K1, K2, K3, K4, K5) on chemical properties, antioxidant compounds and activities of table olive and olive oils (Memecik) were investigated during three crop seasons (2012/13, 2013/14 and 2014/15). The three least irrigated (K3, K4 and K5) and non-irrigated (K1) regimes indicated that higher content of polyphenols of olive fruits were those with the most irrigated regime (K2). According to L*, a* and b*, results of statistical differences were determined between the treatments of K1 and K5 (P<0.05). There was no significant effect determined between irrigation treatments of the Memecik olive oil main fatty acids such as palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acid, except palmitic acid (first year) and linoleic acid (second year). When we look at the triacylglycerol (TAG) composition in the 2012/13 and 2013/14 crop seasons important statistical differences were determined between the irrigation treatments on some TAG contents (P<0.05) however these differences between irrigation treatments, although statistically significant, are very slight. During the last crop season, no significant effects were determined in the TAG composition between irrigation treatments. During the first and the second year, important statistical differences were observed between the irrigation treatments both for the total phenol content and bitterness index (K225) value of oils (P<0.05). The research showed that the K225 value of Memecik olive oils is above 0.360 value. Important differences were determined between the irrigation treatments on the alpha tocopherol content (P<0.05). DPPH* (2,2-diphenyl-1-picrylhydrazyl) content (first and second year) and ABTS*+ (2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid) content (all crop seasons) were also significantly influenced by irrigation treatments due to changes in antioxidant compounds (P<0.05). Whereas all these differences, although statistically important, are very slight. In the light of the findings of this research, it was concluded that the use of the restricted irrigation regimes enabled water to be saved with very slight change in fruit and oil quality.

Keywords: Deficit irrigation, Water Stress, Chemical composition, Antioxidant activity, Antioxidant properties.

INTRODUCTION

The olive tree is widely cultivated throughout the Mediterranean region and is an economically important product in terms of production regions [1]. According to National and International Standard, Virgin Olive Oils (VOO) are identified as oils which are obtained from the olive tree fruit (*Olea europaea* L.) solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, and that have not undergone any treatment other than washing, decantation, centrifugation and filtration. The International Olive Council (IOC) reported that 287.000 tonnes of VOO and 455.000 tonnes of table olive are produced in Turkey during the 2017/18 harvest year, and approximately

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31.35% and 18.68% of it is exported to other countries, respectively [2]. In Turkey, the (75-80%) of VOO is obtained from the Region of Aegean, where Ayvalık and Memecik olive cultivars are the main [3]. Memecik that is grown in the South Aegean area is an economically important olive cultivar in Turkey [4].

Chemical content of OO consists of TAG (~99%) and free fatty acids, mono and diacylglycerols, and lipids [4]. Extra virgin olive oil (EVOO) is a rich natural antioxidant source because it contains high amounts of chlorophyll and carotenoids, tocopherols and phenolic compounds. These compounds constitute an important defensive system against free radical attacks with different mechanisms [5]. Chlorophyll is a very important antioxidant and shows antioxidant properties in darkness. But they act as pro-oxidants in lighted environments, for that reason we need to keep them away from daylight [6]. OO is highly durable to oxidation due to the high α -tocopherol, polyphenol content and low polyunsaturated fatty acid content [3]. The content of minor components varies depending on many factors such as variety, climate and ecological conditions and agricultural application [7].

The olive tree is known to be resistant to aridness and appropriate for growing in the zones with a Mediterranean climate. Recently, olive agronomic practices have been changed. Even though olive trees are highly tolerant to aridity, most researches show that they react to irrigation [8]. There is little or no rain at the critical phenological time for olives in the Mediterranean Basin, for that reason it is difficult to grow olives without resorting to irrigation. That makes it difficult to grow olives economically. Looking at the olive growing areas, it is seen that there are arid or semi-arid regions and the precipitation is not enough to meet the water requirement of these olive trees [9]. An interest in irrigated agriculture is increasing day by day as irrigation increases the olive cultivation yield [10]. Some studies have shown that there are differences in the chemical composition of the OO obtained from irrigated and non-irrigated olive orchards [10]. Antioxidant components, especially polyphenol contents were mostly affected by irrigation [11, 12, 13]. It was also pointed out that the concentration of phenolic compounds in the oil increased with water stress [10, 14]. The production of OO with high antioxidant activity and antioxidant compounds is very important for the health. In these studies, it was determined that different irrigation regimes usually did not affect the fatty acid content of OO [10, 14]. Gucci [16], also reported that irrigation has little effect on the relationship between saturated and unsaturated fatty acids, or on single fractions. Only in very dry climates, irrigation determined significant variations in the acid composition of oil, increasing the oleic acid content. Yet, little is known about the effect of deficit irrigation to chemical properties, antioxidant content and table olives and OOs of Memecik variety. Therefore,

the objective of this study was to determine the effect of the different irrigation treatments on the chemical composition, antioxidant content and the activity of table olives and OOs extracted from Memecik olives in combination with the main component analysis (PCA) as a multivariate statistical method.

EXPERIMENTAL PART

IRRIGATION SYSTEM AND EXPERIMENTAL DESIGN

The study was conducted using Memecik olive cultivar during the 3 crop seasons (2012/13, 2013/14 and 2014/15) at the Olive Research Institute (ORI) in Izmir/Turkey. Water used for irrigation in the experiment was pH 7.5 and the electrical conductivity was 0.5 dS/m. The experiment consisted of five irrigation treatments, with three replicated in a random block design. Four trees were assessed on each plot. Irrigation was applied by drip irrigation.

Irrigation treatments;

K1: non-irrigated (rainfed),

K2: soil water deficit in a 90 cm soil depth was completed to field capacity for every 5 days,

K3: application of 33% of water given at K2,

K4: 3 times application of 50% of the soil water deficit in a 90 cm soil depth concerning 3 growing stages; seed hardening, fruit growth and oil accumulation,

K5: 3 times application of 25% of the soil water deficit in a 90 cm soil depth concerning 3 growing stages; seed hardening, fruit growth and oil accumulation. Irrigation started when the capacity of available soil water was down to half and ended with autumn rains. Harvesting was carried out by hand in November. Crop water use efficiency (WUE) was calculated as yield divided by seasonal evapotranspiration. Irrigation water-use efficiency (IWUE) was determined as:

$$IWUE = (Y_i - Y_o) / IRR$$

Where:

Y_i is yield of irrigated olive,

Y_o yield of rainfed olive

IRR the amount of applied irrigation (mm)

MATURITY INDEX (MI)

MI of olive was determined described by International Olive Council [17] where the calculation is based on the evaluation of olive skin and pulp colours.

FLESH WEIGHT/STONE WEIGHT RATIO (FW/SW)

Randomly selected olives (100 g) were weighed and picked manually to evaluate the FW/SW ratio. Then the olive stones were cleared, dried and measured. And then the SW was subtracted from the total weight

and the FW was determined, the SW was compared with the FW [18].

OLIVE ANALYSIS

The moisture (%) and oil (%) content were determined, described by Turkish Standard [19]. After weighing 15 grams of olive fruit, it was dried in the oven at 105°C until a constant weight and percentage of moisture was calculated. The oil content was measured with Soxhlet extraction. After that percentage of oil was calculated. Reducing Sugar (RS) was determined according to the Luff-Scroll method [20].

COLOUR MEASUREMENT (CM)

The colour of the olive was evaluated using a Minolta Chroma Meter with a CR-400 measuring head (Minolta, Osaka, Japan) according to Argyri et al. [21]. The results are described based on L^* , a^* , b^* parameters. L^* is a measure of the lightness component, which ranges from 0 to 100 (black to white). Parameters a^* and b^* are termed opponent colour axes; a^* represents red (positive) versus green (negative) colours, b^* is positive for yellow and negative for blue.

OLIVE OIL EXTRACTION

Memecik (M) olives were harvested (15 kg) in the ORI. OOs were obtained with Abencor laboratory oil mill (Mc2 Ingenieria y Sistemas, Sevilla, Spain). The olive fruits were crushed, malaxated and centrifugated. The maximum temperature was 30°C and the maximum duration was 30 minutes. After filtered the samples, they were storage in the dark glass bottles and at +4°C until they were analysed (Bottles contains 100 mL of OO).

FATTY ACID COMPOSITION (FAC)

FAC of OO was determined using the gas chromatography system (HP 6890, U.S.A), equipped with a flame ionisation detector (FID) described by IOC [22]. The methyl esters were obtained by cold alkaline transesterification with methanolic potassium hydroxide solution and extracted with n-hexane. The capillary column (DB-23, 30 m x 0.25 mm x 0.250 µm film thickness, Agilent J&W GC Columns, U.S.A.) was used for analyses. The temperature of the detector and injector was set to 250°C. The oven temperature was programmed from 170°C to 210°C with an increment of 2°C /min. The analysis was ended by maintaining the temperature to 210°C for 10 min. The injection volume was 1 µl. The results were expressed in percentage.

TRIACYLGLYCEROL ANALYSIS (TAG)

The analysis of TAGs was detected according to

method described in Regulation EEC/2568/91 of the European Union Commission [23] with the High-Performance Liquid Chromatography (HPLC) (1200 Agilent) system consisted of a degasser, quaternary pump, autosampler, differential refractometer detector. The column was a Superspher® 100 RP-18 HPLC column (Merck, Germany) (250 x 4 mm i.d. x 4 µm). The injection volume of the sample was 0.5 µL. Acetone (63.6%)/ acetonitrile (36.4%) were mobile phases with a flow rate linear gradient (800 mL/min) under nebuliser gas pressure 2.00 bar for 45 min. The results were expressed in percentage of the total TAG.

ALPHA TOCOPHEROL ANALYSIS (AT)

AT analysis was determined according to IUPAC [24]. OO (1 g) was weighed into a 10 mL volumetric flask and made up to volume with hexane. The AT content was determined by high-performance liquid chromatography (HPLC- Agilent 1100), with hexane:2-propanol (99:1) as the mobile phase, with a flow rate of 1 ml/min. A µ-porasil column (300 mm x 3.9 mm x 10 µm) (Waters, Ireland) was used. The temperature of the column was set to 25°C. The injection volume was 20 µl. The results were expressed in mg/kg.

TOTAL PHENOL CONTENT (TPC) OF OLIVE FRUIT AND OLIVE OIL

TPC of the olive fruit was evaluated by modifying according to Susamcı et al. [25] and Gutfinger [26]. 1 g of homogenised olive was weighed and mixed with 5 mL of methanol/water (60:40 v/v) at 2 min. And then it was centrifuged 10 min at 3500 rpm. After filtering through the coarse filter paper to the tape measure of 10 mL, the residue was centrifuged again with a 5 mL of methanol/water (60:40 v/v) and filtered again through the filter paper. And then it was filled to 10 mL with distilled water. 0.1 mL of this methanolic phase was added to 50 ml volumetric flask and completed with purified water to 5 mL a reagent blank using distilled water was prepared. 0.5 mL of Folin-Ciocalteu was added to the mixture. 3 min later 1 mL of sodium carbonate (35%, w/v) solution was added to the mixture and diluted to 50 mL with water.

TPC of the OO was determined according to Gutfinger [26]. 2.5 g of olive oil were diluted with 5 mL of hexane and then 5 mL of methanol/water (60:40 v/v) was added, followed by 10 min centrifugation at 3500 rpm. The analysis was done at methanolic phase. 0.2 mL of methanolic phase was added to 10 ml of a volumetric flask and completed with purified water to 5 mL, a reagent blank using distilled water was prepared. 0.5 mL of Folin-Ciocalteu was added to the mixture. 3 min later 1 mL of sodium carbonate (35%, w/v) solution was added to the mixture and diluted to 10 mL with water. After 2 hours the absorbance of the

solution was read at 725 nm with a spectrophotometer (UV-1700, Shimadzu, JAPAN). The results of olive TPC was expressed as mg of Caffeic Acid Equivalent per kg of olive (mg CAE/100 g olive) and the TPC of olive oil was expressed as mg CAE/kg oil.

TOTAL CHLOROPHYLL (TCH) AND CAROTENOID (TCA) ANALYSES

TCH and TCA compounds of the (7.5 g) OOs were measured calorimetrically at 670 and 470 nm, in cyclohexane, with a spectrophotometer (UV-1700, Shimadzu, JAPAN) and the results were expressed as mg/kg oil [27].

$$\text{TCH} = (A_{670} \times 10^6) / (613 \times 100 \times d)$$

$$\text{TCA} = (A_{470} \times 10^6) / (2000 \times 100 \times d)$$

ANTIOXIDANT ACTIVITY ANALYSES

ABTS• + RSA (Radical Scavenging Activity) analysis of OO samples was detected by Re et al. [28]. 0.5 g oil was dissolved in 5 mL hexane. ABTS was dissolved in a diluted water at a 7 mM concentration. ABTS• + was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and mixture was kept in darkness for 12-16 h at room temperature and diluted in ethanol until an absorbance of 0.70 (± 0.020) at 734 nm. The OO extract (150 μ L) was mixed with ABTS• + RSA (2.000 μ L) and the mixture allowed to stand for 15 min and the absorbance were determined with a spectrophotometer (UV-1700, Shimadzu, JAPAN) at 734 nm ($R_2 = 0.99$, μ mol Trolox Equivalent (TE) of 100 g oil).

DPPH• RSA was performed according to Lavelli [29], Carrasco-Pancorbo et al. [30] and Jiang et al. [31]. 1 g of OO was dissolved in 5 mL methanol and shaken approximately 1 h at room temperature with a homogenizer and then centrifuged (at 3500 rpm for 10 min) to separate polar and non-polar compounds. DPPH• RSA determination was carried out at the methanol phase. 1900 μ L of a 100 mM DPPH solution was added to 100 μ L of a sample extracts and allowed to react for 15 min at 25°C in the dark. After that the absorbance was determined at 517 nm with a spectrophotometer ($R_2 = 0.99$, μ mol TE of 100 g oil).

BITTERNESS INDEX

The bitterness index value was determined spectrophotometrically at 225 nm as absorbance (K_{225} values) with a Shimadzu Spectrophotometer (UV-1700 Pharma Spec, Japan) [32]. A Solid-Phase Extraction (SPE) was carried out for extraction of the bitter compounds. A sample of 1.0 ± 0.01 g of oil was dissolved in 4 mL hexane and placed on a C18 column (Bakerdond spe Columns, J.T. Baker, Phillipsburg, NJ, Hol-

land) previously activated with methanol (6 mL) and washed with hexane (6 mL). After elution, 10 mL hexane was placed to eliminate the fat, and the retained compounds were eluted with methanol/water (1:1) to 25 mL in a flask. The absorbance of the extract was measured at 225 nm against methanol/water (1:1) in a 1.0 cm cuvette. Results were expressed as the absorbance of 1.0 g in 100 g (K_{225} values).

STATISTICAL ANALYSIS

The analytical measurements were given as mean \pm standard deviation (SD). Analysis of variance (ANOVA) was applied to indicate the differences among the treatments using the Fisher's least significant difference test at $p < 0.05$ significance level. Data analyses were conducted with Minitab 17 (Minitab Inc., State College, USA) software. Multivariate data analysis was performed to discriminate OO samples with Principle Component Analysis (PCA) according to FAC, TAG and pigments of samples (cv. Memecik) for different irrigation regimes and crop seasons.

RESULTS AND DISCUSSION

WUE AND IWUE

Yield ranged from 5700 to 10 593 kg/ha (Tab. I). The highest water-use efficiency was in treatment K1, whereas the highest irrigation water-use efficiency was obtained from treatment K4. When average yield was evaluated according to WUE and IWUE, treatment K4 came out ahead. Thus, it may be rec-

Table I - Water use efficiency (WUE) and irrigation water use efficiency (IWUE) for olive

Treatment	Average Yield (kg/ha)	Average WUE (kg/m ³)	Average IWUE (kg/m ³)
K1	5 700	4.01	-
K2	10 593	1.40	0.65
K3	7 256	2.21	0.62
K4	8 860	3.46	2.0
K5	7 144	3.63	1.83

ommended K4 application when the water source is inadequate.

THE CHARACTERISTICS OF OLIVE FRUIT

In this work, the average maturity index values of the olive fruits showed variation between irrigation treatment regimens and the tears under study that were statistically important in 2012/13, 2013/14 and 2014/15 crop seasons. Kaya et al. [9] stated in their study that an increase in the amount of irrigation wa-

ter given was accompanied by a drop of maturity index values. No such relationship was found between irrigation issues and maturity index in our study. In the 2013/14 crop season, the differences between K2 and K1 and K5 was found as statistically significant. Like the work of Martinelli et al. [33], we can say that the maturity of olives was affected by the irrigation.

The irrigation regimes significantly ($p < 0.05$) affected L^* , a^* and b^* in 2012/13, 2013/2014 crop seasons; however, no significant effects were determined in the L^* , a^* , and b^* values in 2014/15. In the 2012/13 crop season, no clear distinction was found in terms of L^* and b^* values relative to irrigation issues. In addition, the red colour value (a^*) was not affected by irrigation. In the 2013/15 crop season, a significant difference in lightness (L^*), redness (a^*) and blueness (b^*) was noted (L^*), between K1 and K2 as a result of the irrigation.

The colour of K2 olives was lighter and had a higher yellow intensity than olives from the other treatments. Generally, L^* and b^* decreased as the deficit irrigation conditions become more severe; however, a statistical difference was determined between the treatments, K1 and K5. Pastor et al. [34] reported that when the stressed olives were used, the yellow colour of the OOs had less intensity. The result is in accordance with the author.

The irrigation regime seems to affect flesh/stone ratio in studied crop seasons. Flesh/ratio was found to be significantly higher for treatments K2 and, statistically, no differences were determined between the regimes, K1 and K5 (except for the 2013/2014 crop season). Proietti and Antognozzi [35] reported that

flesh/stone ratio increased with irrigation. Our results are in accordance with the authors.

Moisture and oil contents of the olive fruit were also affected by the irrigation treatments. There is no doubt that the moisture content of table olives depended on the water availability for trees, with K2 fruits having the highest content of moisture and K2 was found to be statistically different from the other regimes in the 2012/13, 2013/2014 crop seasons (Tab. II).

Except for the 2014/15 crop season, irrigation conditions significantly affected the oil ratio of olives. Cano-Lamadrid et al. [36] stated that a low level of water application significantly activated plant metabolism resulting in the highest oil content. Motilva et al. [12], reported that as irrigation water was reduced, the oil content increased. Contrary to researchers, the lowest amount of oil was determined K1 in our work. Generally, the oil content was higher in K3, K4 and K5 than K1 and K2. Parallel to our findings Goldhamer et al. [36] found that the oil content was significantly higher for all regulated deficit irrigation (RDI) strategies applied in comparison to the control. However, other studies indicated no differences in oil ration when RDI was imposed [10, 38]. The RS amount of the fruit is very important because they are the raw material for the fermentation in the process. The highest reducing sugar concentrations, 4.69, 4.37 and 3.39 g/100g, were found from the K1 treatment in the 2012/13, 2013/2014 and 2014/15 crop seasons respectively. The lowest reducing sugar content was found from K2. The results obtained from this study by Proietti and Antognozzi [35], report that irrigation slightly reduced the amount of sugar, which could be detrimental

Table II - Effect of the irrigation treatment on properties of olive fruit samples between 2012/13-2014/15 crops seasons

Irrigation treatment	Maturity Index	L^*	a^*	b^*	Flesh/Stone Ratio	Moisture (%)	Oil content (DM%)	Reducing sugar contents (g/100g)	Total phenol (mg CAE/100g olive)
2012/13									
K1	1.98±0.026 ^b	35.14±0.87 ^{ab}	4.49±0.28	6.12±1.013 ^b	1.35±0.05 ^c	36.30±20.82 ^b	19,27±1,22 ^a	4.69±0.01 ^a	491.7±46.7 ^a
K2	1.84±1.167 ^b	41.65±13.29 ^a	8.49±12.01	20.37±17.0 ^a	2.74±0.96 ^a	47.29±5.85 ^a	32,04±0,50 ^c	3.05±1.74 ^b	286.1±14.6 ^b
K3	2.93±0.025 ^a	30.80±1.67 ^b	3.74±1.03	7.46±1.116 ^b	1.64±0.10 ^{bc}	39.10±1.67 ^b	30,61±1,11 ^{bc}	4.37±0.23 ^{ab}	418.5±70.6 ^a
K4	2.93±0.007 ^a	29.94±0.71 ^b	4.80±0.53	6.53±0.375 ^{ab}	2.45±1.00 ^{ab}	41.92±0.75 ^{ab}	26,11±1,29 ^b	3.89±0.16 ^{ab}	387.4±17.0 ^{ab}
K5	2.79±0.029 ^a	32.84±1.22 ^{ab}	5.56±0.65	4.80±0.460 ^b	1.74±0.32 ^{abc}	38.95±2.76 ^b	20,91±1,86 ^a	4.60±0.07 ^a	429.8±52.9 ^a
2013/14									
K1	3.90±0.092 ^a	22.15±1.13 ^b	7.30±3.34 ^c	3.95±3.36 ^b	4.32±0.54 ^c	49.64±2.30 ^{bc}	28,24±3,05 ^a	4.37±0.431 ^a	467.4±41.4 ^a
K2	1.48±0.104 ^c	58.78±0.60 ^a	17.38±3.27 ^a	35.06±3.21 ^a	6.55±0.23 ^a	60.96±3.20 ^a	42,49±3,98 ^{ab}	2.41±0.448 ^b	256.9±20.2 ^b
K3	1.94±0.460 ^{bc}	49.71±11.10 ^a	12.83±2.35 ^{ab}	24.50±12.08 ^a	5.11±0.30 ^{bc}	54.77±3.32 ^b	49,41±5,33 ^{ab}	2.20±0.390 ^b	386.4±52.5 ^a
K4	2.57±0.445 ^b	27.05±4.04 ^b	11.20±1.82 ^{bc}	6.91±6.31 ^b	4.46±0.80 ^c	48.73±0.94 ^{bc}	42,88±11,55 ^{ab}	3.65±0.806 ^a	386.55±13.4 ^a
K5	3.72±0.479 ^a	24.51±2.94 ^b	12.05±0.70 ^{bc}	3.97±2.84 ^b	5.87±0.72 ^{ab}	49.21±3.07 ^c	47,92±4,90 ^b	3.44±0.197 ^a	443.5±47.5 ^a
2014/15									
K1	3.57±0.00 ^{ab}	24.11±0.71	4.62±2.25	0.93±0.48	4.94±1.38 ^b	56.58±5.26	36,59±1,07	3.39±0.983 ^a	469.6±102.2 ^a
K2	2.35±1.101 ^b	41.74±19.8	9.81±6.31	20.14±19.7	7.62±1.22 ^a	57.30±3.05	40,39±1,72	1.99±0.517 ^b	299.6±60.0 ^b
K3	3.20±0.940 ^{ab}	27.97±5.91	9.03±5.16	4.66±5.72	6.89±0.94 ^{ab}	51.63±2.95	45,37±3,48	2.31±0.269 ^{ab}	367.9±66.3 ^{ab}
K4	3.09±0.615 ^{ab}	29.31±8.19	4.66±2.20	4.88±7.25	6.14±0.94 ^{ab}	51.57±1.45	44,07±4,15	2.74±0.784 ^{ab}	379.7±52.7 ^{ab}
K5	3.74±0.015 ^a	24.09±0.16	4.57±1.32	1.53±1.24	5.65±0.81 ^b	50.99±4.54	39,09±6,89	2.50±0.436 ^{ab}	427.4±54.7 ^a

^{a-c} Different letters in each column per season indicates significance at which means differ at $P < 0.05$.

tal to the fermentation process, and results obtained from this study are like this.

TPC of Memecik olive fruits changed according to the irrigation applications because olives were collected on the same date and grow under the same conditions. In fact, the five regimes can be divided into two groups, in neither of which there are any important differences: the three least irrigated (K3, K4, K5) and non-irrigated (K1) regimes showed a higher level of phenols compared with the most irrigated regime (K2). Motilva et al. [12], Tovar et al. [39] and Romero et al. [40] remarked that a clear reduction of the TPC connected with the increase in irrigation. Our study is in accordance with the authors.

OLIVE OIL ANALYSES

Fatty acid composition (FAC)

As shown in Table III, there were no significant effects determined between irrigation treatments of the Memecik OOs main fatty acids likes palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acid, except for palmitic acid (first year) and linoleic acid (second year). The content of C18:1 ranged from 66.55 to 69.20%, 67.10-70.33%, 65.55-67.47% in the 2012/13, 2013/14 and 2014/15 crop seasons, respectively. The C18:1 content of the oils was within the limit of 55-83% by IOC. C18:1 is a significant source of MUFA and its high content in oil composition makes it more resistant to oxidative rancidity [8]. Previous research on different irrigation practices indicated varying results. Ahumada-Orellana et al. [41] observed there were no significant effects of irrigation cut-off on most of the Arbequina olive oil fatty acids such as palmitic, oleic, and linoleic acids, on the other hand it was reported that stearic and palmitoleic acids were affected by the irrigation cut-off strategies. Motilva et al. [12] observed that the water deficit did not affect FAC of Arbequina olive oil. Patumi et al. [15] found that the FAC of different varieties was not affected by irrigation practices. And, Berenguer et al. [42] did not determined differences in FAC in Arbequina olive oil except for a very slight increase in C18:1 due to a severe water deficit in one of the two seasons. Moreover, Campo and Garcia [43] observed that minor fatty acids were not significantly affected by the irrigation treatment. The results of our research coincide with the researches. Toplu et al. [8] reported that additional irrigation significantly decreased the C18:1 content although the C16:1 content of Gemlik OO increased. Our research showed that the C18:1 percentage was not affected by the irrigation treatments in all studied crop seasons. During the first harvest year, important differences were determined between the treatments on C16:0, C16:1, C17:0, C18:0 and C18:3 ($P < 0.05$). Gomez-Rico et al. [10] and Salas et al. [44] reported that irrigation treatments had an increase in C16:0 and C18:2 and a decrease in C18:1 and C18:3 in

virgin olive oil and statistical differences were found between rainfed and irrigation treatments. In the second harvest year, there were statistical important differences only in the C18:2 amounts, depending on the treatments ($P < 0.05$). In the last season, the percentage of C16:1, C17:0, C17:1, C18:0, C18:3 and C20:0 showed important differences between the irrigation treatments ($P < 0.05$). In any case, these differences, although statistically significant, are very slight, and these changes do not have any nutritional relevance. In general, we can say that C16:0, C18:1, C18:2, C20:0, C20:1, C22:0 and C24:0 percentage of OOs were not affected by the irrigation practices. It is known that FAC of olive oil is strongly affected from cultivar factors and environmental conditions especially temperature has an essential role in FAC.

PCA was applied to analyse the discrimination pattern of OOs according to their FAC and MUFA, PUFA, MUFA/PUFA values. PCA model was constructed with 4 principal components explaining 88.1% of the total variance. PC1 explained 44.5% of the total variance while PC2 explained 24.2% of the total variance (Fig. 1a). According to PCA biplot analysis, PUFA and C18:2 characterised the 2014/15 season K1 OOs. C17:1 characterised 2012/13 K4 OOs. C18:0 was effective on the characterisation of 2013/14 K1. C18:1 and MUFA was effective on the discrimination of 2012/13 and 2013/14 K2 samples (Fig. 1b).

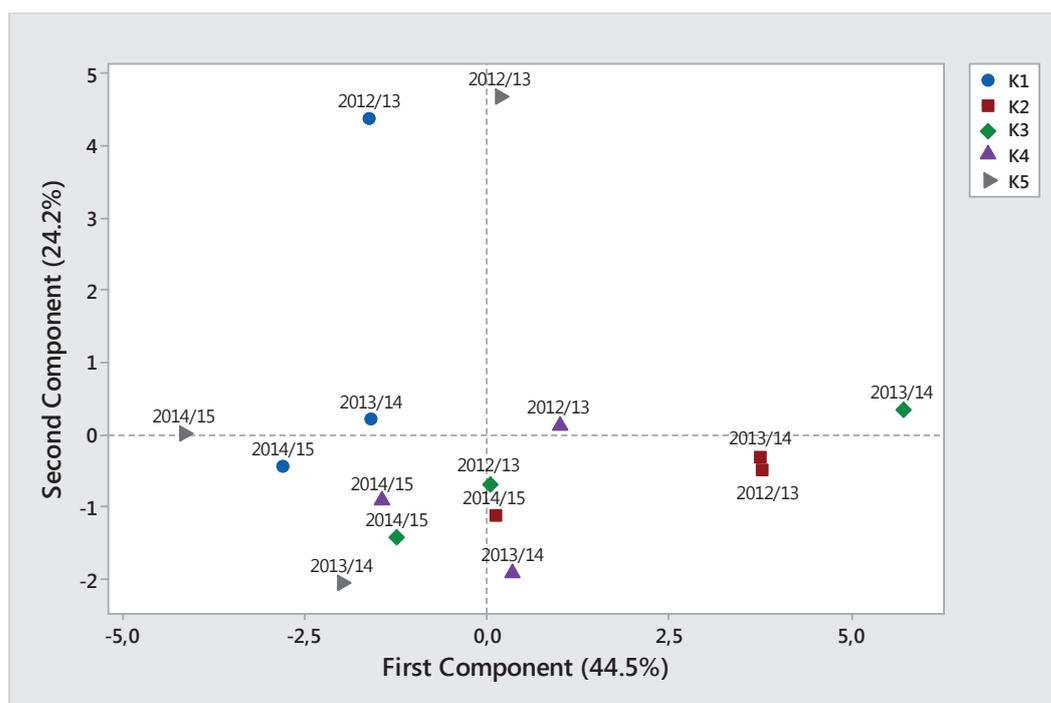
Triacylglycerol composition (TAG)

The composition of FA and TAG of the VOO can be change depending mainly on latitude, climate, cultivar and the olive maturity stage [45]. About 95-98% of olive oil consists of TAGs. TAG composition can also be used as a measurement of the quality and purity of vegetable oils. Table IV showed variations of TAG components of different irrigation treatments. In relation to the main TAGs (triolein (OOO), palmityldiolein (POO), linoleyldiolein (LOO) and palmityllinoleylein (PLO)), the percentage of OOO was determined to be the highest for Memecik olive oil. The percentage of OOO was followed by POO and LOO, respectively. The presence of OOO at high content showed the authenticity of OOs [46]. Oils obtained from all irrigation treatments were characterised by four major TAG fractions, OOO, POO, LOO and PLO. Yorulmaz et al. [47] determined OOO values of the Turkish monovarietal OOs between 24.72 and 48.64%, Köseoğlu et al. [48] determined OOO values of Memecik OOs between 28.88 and 32.91%. Our results are compatible with their reports. In our study, statistical important differences were determined in the 2012/13 crop season between the irrigation treatments on LLL, LOO, OOO, POO, Equivalent Carbon Number (ECN)44, ECN 46, ECN48 and ECN50 values ($P < 0.05$). In the 2013/14 crop season, important differences were demonstrated between the treatments according to LLL, LOO,

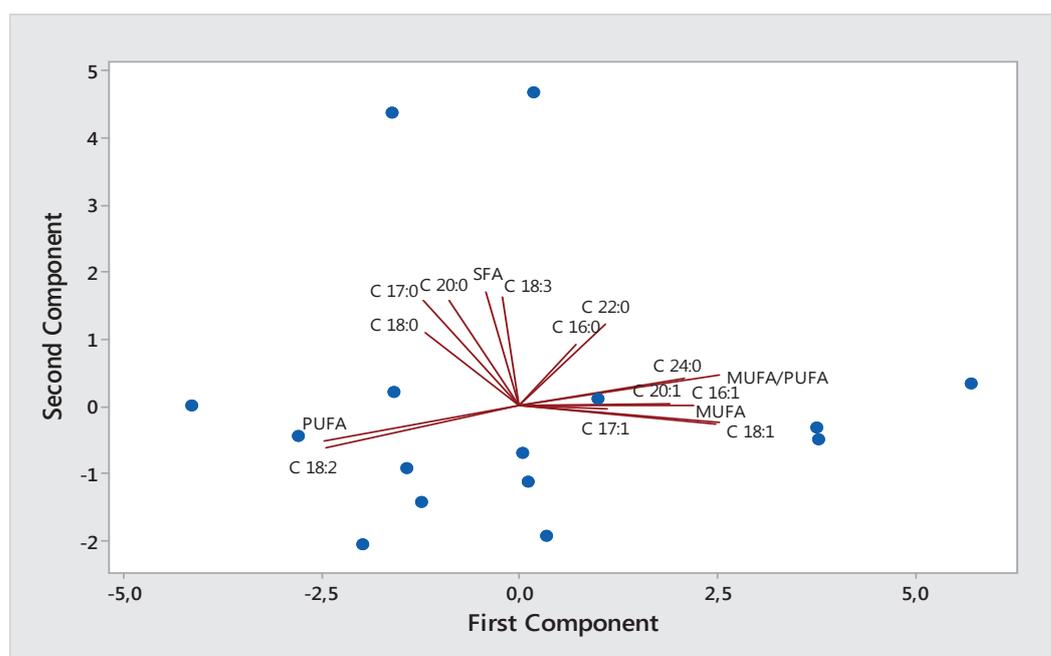
Table III - FAC (%) of olive oils (cv. Memecik) obtained from different irrigation treatments

Irrigation treatments	Palmitic acid (C 16:0)	Palmitoleic acid (C 16:1)	Margaric acid (C 17:0)	Margoleic acid (C 17:1)	Stearic acid (C 18:0)	Oleic acid (C 18:1)	Linoleic acid (C 18:2)	Linolenic acid (C 18:3)	Arachidic acid (C 20:0)	Gadoleic acid (C 20:1)	Behenic acid (C 22:0)	Lignoceric acid (C 24:0)
2012/13												
K1	14.87±0.35 ^a	1.04±0.01 ^b	0.06±0.01 ^a	0.06±0.01 ^{ab}	2.69±0.44 ^{bc}	66.55±2.21	12.17±2.97	1.23±0.06 ^a	0.66±0.11	0.35±0.00	0.20±0.04	0.09±0.02
K2	14.09±0.25 ^{ab}	1.21±0.12 ^a	0.04±0.00 ^c	0.07±0.01 ^a	2.08±0.35 ^c	69.96±1.28	10.43±1.41	0.95±0.06 ^b	0.47±0.06	0.37±0.01	0.21±0.05	0.11±0.01
K3	13.94±0.68 ^b	1.14±0.06 ^{ab}	0.05±0.01 ^{bc}	0.04±0.00 ^d	2.04±0.50 ^c	68.65±1.51	11.91±0.41	0.92±0.02 ^b	0.58±0.02	0.33±0.02	0.18±0.02	0.08±0.00
K4	13.32±0.37 ^b	1.14±0.03 ^{ab}	0.04±0.00 ^{bc}	0.05±0.00 ^{cd}	3.22±0.01 ^{ab}	69.20±0.10	10.88±0.48	0.98±0.03 ^b	0.56±0.00	0.36±0.00	0.17±0.00	0.08±0.00
K5	14.29±0.42 ^{ab}	1.05±0.03 ^b	0.06±0.01 ^{ab}	0.06±0.01 ^{bc}	3.70±0.46 ^a	68.13±0.85	10.01±0.76	1.16±0.06 ^a	0.64±0.02	0.35±0.03	0.20±0.01	0.09±0.01
2013/14												
K1	13.58±1.26	0.98±0.09	0.05±0.00	0.07±0.01	2.36±0.02	67.42±4.70	13.16±3.44 ^a	1.21±0.03	0.56±0.05	0.38±0.04	0.16±0.01	0.08±0.01
K2	14.58±0.53	1.16±0.03	0.04±0.00	0.08±0.00	1.87±0.03	69.66±0.90	10.32±0.98 ^{ab}	1.00±0.03	0.43±0.04	0.37±0.04	0.19±0.06	0.11±0.02
K3	14.58±1.48	1.23±0.26	0.04±0.01	0.08±0.03	2.20±0.22	70.33±2.41	8.92±0.57 ^b	0.99±0.18	0.45±0.01	0.38±0.07	0.14±0.01	0.19±0.17
K4	13.57±0.15	1.01±0.01	0.05±0.01	0.06±0.00	2.22±0.19	69.37±0.75	11.70±0.94 ^{ab}	0.98±0.08	0.48±0.05	0.35±0.01	0.14±0.02	0.07±0.01
K5	13.96±0.52	0.98±0.01	0.04±0.01	0.05±0.00	2.57±0.51	67.10±1.58	13.27±1.56 ^a	0.89±0.13	0.48±0.10	0.35±0.01	0.13±0.02	0.07±0.02
2014/15												
K1	14.11±0.25	0.98±0.01 ^b	0.06±0.00 ^a	0.07±0.00 ^a	3.03±0.37 ^a	65.56±0.94	13.98±0.25	1.08±0.04 ^a	0.54±0.03 ^a	0.37±0.04	0.14±0.00	0.08±0.00
K2	14.42±0.46	1.21±0.11 ^a	0.05±0.00 ^b	0.07±0.00 ^a	2.19±0.14 ^b	67.47±2.82	12.59±2.38	0.93±0.04 ^b	0.46±0.03 ^c	0.35±0.01	0.14±0.00	0.08±0.02
K3	13.97±0.24	0.97±0.06 ^b	0.05±0.00 ^b	0.06±0.00 ^b	2.54±0.24 ^b	67.41±1.16	13.09±1.25	0.90±0.01 ^b	0.47±0.02 ^{bc}	0.35±0.01	0.14±0.01	0.07±0.00
K4	14.19±0.32	1.02±0.09 ^b	0.05±0.00 ^b	0.06±0.00 ^b	2.58±0.17 ^{ab}	67.12±2.82	13.14±2.72	0.93±0.03 ^b	0.48±0.02 ^{bc}	0.36±0.01	0.14±0.01	0.08±0.01
K5	13.97±0.42	0.99±0.06 ^b	0.05±0.01 ^a	0.07±0.00 ^{ab}	2.99±0.26 ^a	65.73±1.14	14.14±0.39	0.99±0.11 ^{ab}	0.51±0.04 ^{ab}	0.30±0.09	0.14±0.01	0.08±0.01

^{a-c} Different letters in each column per season indicates significance at which means differ at $P < 0.05$.



(a)



(b)

Figure 1 - (a) Scores; (b) loading plots with PCA according to FAC of olive oils (cv. Memecik) at different irrigation treatments

POO, ECN42, ECN44, ECN46, ECN48 and ECN50 values ($P < 0.05$). These differences between irrigation treatments, although statistically significant, are very little. This trend coincides with those concerning the maturity index. In fact, as previously observed, significant differences determined on the maturity index. As we know, TAGs are composed of a mixture of three fatty acids. These differences in TAGs stem from the FAC of oils. ECN42, ECN44, ECN46, ECN48 and ECN50 values of TAGs are the results of the

calculation of triacylglycerols as shown on Table IV. These differences are due to their differentness. During the last crop season, no significant effects were determined in the TAG composition between irrigation treatments. Baccouri et al. [49] observed small changes in OOO, POO, OOL levels of Chemlali and Chetoui extra virgin olive oil with the irrigation regime; however, it was reported that these changes can be due to the maturity index of olive oils. Patumi et al. [50] says that some authors reported a bene-

ficial effect of irrigation on the TAG accumulation in olives. Moreover Baccouri et al. [51] reported that changes were determined in POO, OOL, OOO and POL values under the irrigation regime during olive maturation.

İlyasoğlu and Özçelik [52] determined that level of POO and LOO of Memecik OO ranged from 18.25 to 25.82% and from 6.01 to 9.18%, respectively. Aranda et al. [53] reported that the percentages of OOO, ECN 48 and ECN 50 of Cornicabra VOOs were 51.7, 74.7 and 8.68%, respectively. OOO, ECN 48 and ECN 50 values of Memecik OO were observed to be lower than Cornicabra VOO. Ben Temime et al. [54] determined that the TAG content of Chetoui oils ranged from 29.59 to 37.38, from 15.11 to 18.02 and from 19.03 to 24.74%, OOO, POO and LOO, respectively. Memecik olive oil LOO and OOO content were determined to be lower, POO content was determined as higher than Chetoui cv. oil.

PCA was applied to analyse the discrimination pattern of OOs according to their triacylglycerol composition. The PCA model was constructed with 4 principal components explaining 91.9% of the total variance. PC1 explained 54.3% of the total variance while PC2 explained 20.6% of the total variance (Fig. 2a). According to PCA biplot, 2012/13 K2 was characterised by PoOP, ECN48, LOO/PLO, OOO. 2012/13 K4 was characterised by POP; 2013/14 K4 was characterised by OOO/POO. 2014/15 K4 was characterised by LOO/PLnP; 2013/14 K5 was characterised by ECN46; 2014/15 K1 was characterised by OLL, 2012/13 K3 was characterised by PLL/OLL (Fig. 2b).

Antioxidant compounds and activity of olive oils

TPC is an important parameter for OOs affecting the antioxidative effect and sensory properties. Phenolic composition of OOs is influenced by irrigation, climatic conditions, cultivar, fruit maturing index and some extraction processes [4]. Polyphenols are potent antioxidants demonstrated to scavenge free radicals [55]. TPC of oils obtained from treatments were shown in Table V. Several researchers remarked that a negative relation has been detected between irrigation amounts and TPC [10, 11, 13]. Greven et al. [56] pointed out that severe water stress was determined to have decreased the TPC in OOs. During the first and second year, statistically important differences were determined between the irrigation treatments at the TPC analysis ($P < 0.05$). In the 2012/13 crop season, while the significant differences between K1 and K4 were observed, statistically, the similarities were determined between the other treatments. In the 2013/14 crop season, whereas K2 and K4 were determined, statistically, in different groups, the similarities were observed between the K1, K3 and K5 treatments. During the last year, statistical differences were not determined between the treatments. With Grijalva-Contreras et al. [57], no statistical differences

were determined on TPC of Manzallina olive oil between different regulated deficit irrigations (100% ETc, 75% ETc and 50% ETc) and also Baccouri et al. [58] reported that irrigation caused a delay of olive maturation that resulted in greater TPC in OO. The reaction to regulated deficit irrigation varies according to the olive variety [50].

Depending on the type of phenols, the bitterness intensity of OOs can vary (high or low) [59]. Due to the positive contribution of phenolic compounds to human health, consumers prefer consumption of oils with a high bitterness attribute [60]. Bitterness index (K_{225}) of the oils obtained from the different irrigation treatments was shown in Table V. During the two crop seasons statistical important differences between the irrigation treatments were detected on the bitterness index ($P < 0.05$). During the first year, the lowest K_{225} was determined statistically at K1. While K4 and K5 were in different groups, the similarities were observed between K2 and K3 irrigation treatments. During the second year, although statistically significant differences were determined between the treatments, these are very slight. During the 2014/15 crop season, statistical differences were not found between the treatments. Salas et al. [44] and Romero et al. [40] determined that irrigation reduced bitterness in olives, although Ramos and Santos [61] observed negligible differences in bitterness among oils obtained from different irrigation treatments. Garcia et al. [62] reported that, in the first year, they determined a higher bitterness index at low frequency irrigation treatments, but in the second year, there were no important differences between the treatments. There is no limit set for the bitterness index value at National or International standards. Consumers only refuse or consume the oil products according to their preference. Gutierrez et al. [32] have reported that K_{225} value ≥ 0.360 correspond to quite bitter olive oils that some consumers do not choose to consume. For this, due to the positive attribution of phenolic compounds to human health, some consumers are increasing their consumption of oils with high bitterness index values [60]. It was noticed in the research that the K_{225} value of Memecik OOs are above 0.360 value.

In OO, vitamin E is represented by AT. AT content accounts for about 90% of the total tocopherols in OO [63]. AT content of the oils obtained from different irrigation treatments was shown in Table V. In our case, important differences were found between the irrigation treatments. The highest AT content was observed in the 2012/13 crop season at the K1 and K5 irrigation treatments and in 2013/14 and 2014/15 crop seasons at the K1 irrigation treatment and the values were found as 625.3, 604.4, 361.3 and 295.3 mg/kg, respectively. Tovar et al. [11] determined greater tocopherol contents at the non-irrigated treatments. Our results were similar with researcher Tovar et al. [39], Gomez-Rico et al. [10] and Palese et al. [64] did not find differences in AT content of the oils

obtained from different irrigation treatments. TCH and TCA have an antioxidant effect in VOOs [65]. TCH and TCA contents of the OOs obtained from different irrigation treatments was shown in Table V. During the first and second year, significant differences were determined between the irrigation treatments for the TCH and TCA contents, especially between the rainfed and irrigation treatments ($P < 0.05$). In the 2012/13 crop season the highest chlorophyll and carotenoid contents were determined at the K1 and K5 irrigation treatments, 7.20 and 5.90 mg/kg and 7.70 and 4.10 mg/kg, respectively. In the 2013/14 crop seasons the highest chlorophyll and carotenoid contents were found at the K2 and K3 treatments, 3.97 and 4.43 mg/kg and 2.07 and 2.47 mg/kg, respectively. During the 2014/15 crops season differences were not observed. Motilva et al. [12] and Gomez-Rico et al. [10] detected that the chlorophyll and carotenoid contents were not influenced by irrigation treatments. But Tovar et al. [29] found that the TCH and TCA contents of OO were negatively associated with the amount of water supplied by irrigation. Inarejos-Garcia et al. [64] determined the highest carotenoid and chlorophyll contents at the sustained deficit irrigation treatment.

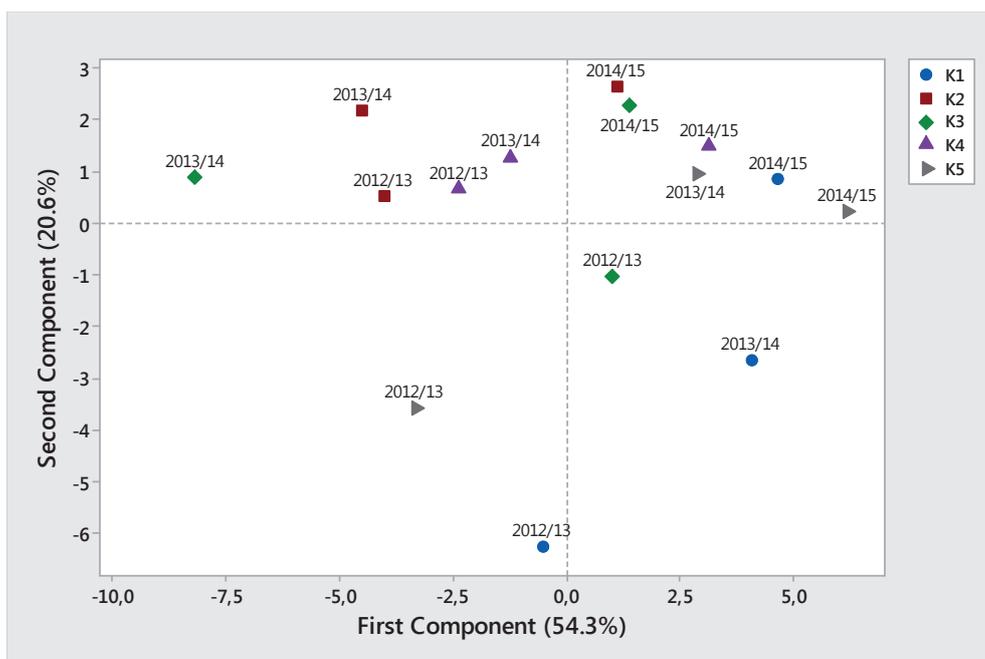
The antioxidant properties of OOs depend on several factors such as the cultivar, olive maturation index, agroclimatic conditions and olive growing methods [65]. The ability of antioxidant molecules or extracts to scavenge DPPH[•] and ABTS^{•+} RSA were measured in this study. They are usually used for assessment olive oil antioxidant activity as they give a good prediction of their sensitivity to oxidation degradation. According to the results, during the first and second year, significant differences were determined between the irrigation treatments on the DPPH[•] RSA ($P < 0.05$). During the first and second year, 2 statistically different groups were observed according to DPPH[•] RSA (Tab. V). During the 2012/13 crop season, K2 grouped the same with K3, and K4 grouped the same with K5 treatment. A statistical similarity was observed at the K1 treatment between all treatments. In the 2013/14 crop season, whereas statistically K1 and K2 were determined in different groups, there was a similarity observed between the other treatments. During the last year, no difference was observed between the treatments. As known, the DPPH antioxidant activity is affected by antioxidants of olive oils. This trend coincides with results of antioxidants such as TPC, AT, TCH, TCA and bitterness index. Different researchers reported that DPPH[•] RSA of OOs was influenced by TPC, AT content [31, 66] and TCH contents of oils [67]. All the years significant differences were observed between the irrigation treatments at the ABTS^{•+} RSA ($P < 0.05$). It was determined that ABTS^{•+} RSA of the oils significantly increased as the irrigation level decreased. As seen on Table V, in the first year, K1 grouped the same with K5 and K2 grouped the same with K3 treatment. And we determined statistical sim-

ilarities with the K4 treatment between all treatments. During the second year, the highest ABTS^{•+} RSA was observed at the K1 with 124.65 $\mu\text{molTE/kg}$ oil. These results coincide with Baccouri et al. [51] who observed that irrigation affected the oil ABTS^{•+} RSA in cv. Chemlali. Gorinstein et al. [66] reported that ABTS^{•+} RSA is positively affected by the TPC and AT content of oils. And also, Baccouri et al. [51] observed a positive correlation between TPC and ABTS^{•+} RSA ($r^2 = 0.9630$). Our results revealed that, during the first year ABTS^{•+} RSA was affected by TCH and TCA and AT contents of the oils, during the second year by AT content, and during the last year by TPC of OOs.

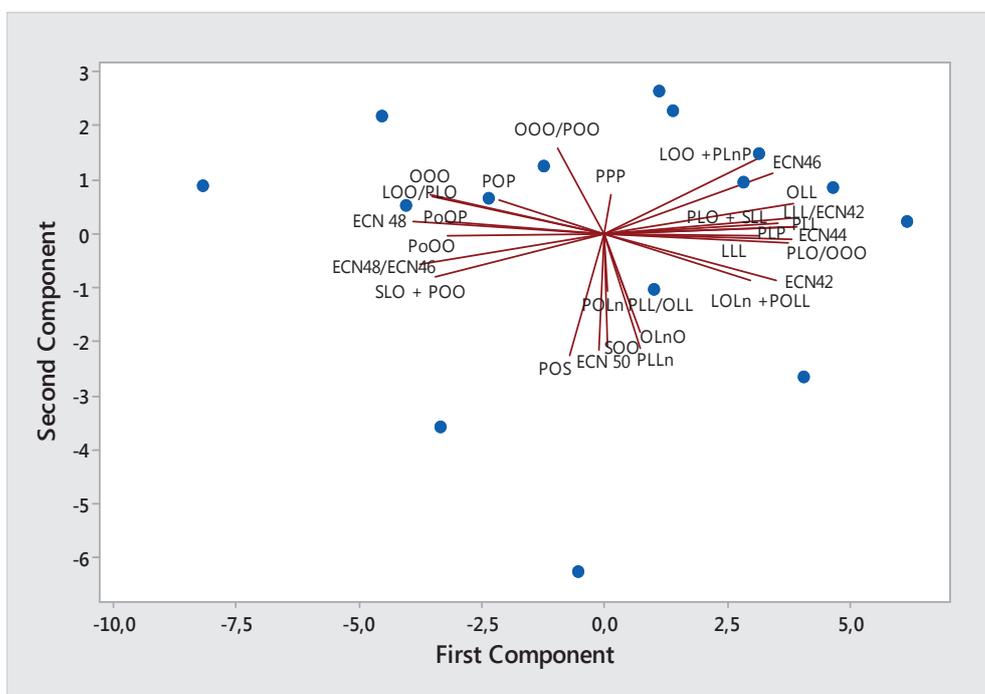
CONCLUSION

In Turkey, as in the world, 85% of olive fields are not irrigated. However, there has been a recent increase in irrigation practices in olive cultivation. It is very important for olive cultivation to gain habits towards the producers due to the irrigation novelty. A good determination of agricultural irrigation strategies is essential to obtain regular crops, high yields and high-quality olives and olive oil every year in olive growing.

In our research, depending on the spring rains, the irrigation started between the first and third week of June according to years. It was terminated right at the end of September. In brief, the results indicated that the deficit irrigation treatments significantly affected cv. Memecik fruit quality such as L^{*}, a^{*}, b^{*}, flesh/stone ratio, moisture content (%), oil content (%), reducing sugar content and total phenol content ($P < 0.05$). The three least irrigated (K3, K4 and K5) and non-irrigated (K1) regimes indicated that higher content of polyphenols of olive fruits compared with the most irrigated regime (K2). When we look at the effect of irrigation treatments on olive oil quality, our results showed that there were no significant effects determined on main fatty acids except palmitic acid (first year) and linoleic acid (second year). According to the TAG composition, during the first two years, statistical important differences were found between the irrigation treatments ($P < 0.05$); however, these differences between irrigation treatments, although statistically significant, are very slight. During the last crop season, no significant effects were determined in the TAG composition between irrigation treatments. Statistically important differences were observed between the irrigation treatments for the TPC, bitterness index (K_{225}) value, AT, of DPPH[•] RSA and ABTS^{•+} RSA of cv Memecik olive oils ($P < 0.05$). While these differences although statistically significant, are very minor. Water stress does not have a great difference in the quality of fruit and oil in Memecik olive variety. In the light of the findings of this research, it was concluded that the use of the restricted irrigation regimes enabled water to be saved with a very slight change



(a)



(b)

Figure 2 - (a) Scores; (b) loading plots with PCA according to triacylglycerol composition of olive oils (cv. Memecik) for different irrigation treatments

in fruit and oil quality. Considering the findings of yield, olive, and olive oil, K4 can be suggested where water resource is limited, and irrigation water cost is high. In this application, irrigation is performed 3 times considering the 50% of the undermined soil at 0-90 cm soil depth, including seed hardening, fruit growth and oil accumulation stages. 79% less water is applied according to the K2 context, provided that the mentioned proposal is applied. The choice of an optimal irrigation regime of the traditional growing regions of

olive orchards in the Aegean Region of Turkey, where water resources are easily accessible, requires an appropriate compromise between olive production, the quality of Memecik olive oil and water consumption.

Acknowledgements

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Table V - Antioxidant contents and antioxidant activities of olive oils (cv. Memecik) obtained from different irrigation treatments

Irrigation treatments	Total Phenol (mgCAE/kg oil)	Bitterness index (K ₂₂₅ .nm)	α-Tocopherol (mg/kg)	Total Chlorophyll (mg/kg)	Total Carotenoid (mg/kg)	DPPH-RSA (μmol TE/100g oil)	ABTS+ RSA (μmol TE/100g oil)
2012/13							
K1	32.07±12.68 ^c	0.30±0.08 ^c	625.33±37.8 ^a	7.20±0.824 ^a	4.40±0.419 ^a	42.63±1.80 ^{ab}	157.97±3.75 ^a
K2	78.60±0.29 ^{ab}	0.63±0.02 ^{ab}	318.38±9.02 ^c	1.85±0.894 ^b	1.55±0.631 ^b	33.90±4.21 ^b	111.25±2.88 ^b
K3	82.73±27.1 ^{ab}	0.67±0.05 ^{ab}	418.55±46.9 ^{bc}	2.23±0.199 ^b	2.06±0.197 ^b	37.83±4.69 ^b	116.97±23.10 ^b
K4	115.00±9.77 ^a	0.79±0.11 ^a	450.13±41.0 ^b	2.45±0.467 ^b	2.25±0.315 ^b	51.95±10.29 ^a	138.15±3.03 ^{ab}
K5	55.50±27.0 ^{bc}	0.51±0.13 ^b	600.42±89.0 ^a	5.90±1.74 ^a	4.10±1.018 ^a	48.70±5.21 ^a	155.70±12.59 ^a
2013/14							
K1	278.35±49.5 ^{ab}	1.30±0.237 ^{ab}	361.28±45.9 ^a	1.25±0.784 ^b	1.00±0.728 ^b	109.20±25.5 ^a	124.65±1.52 ^a
K2	185.17±16.62 ^b	0.77±0.061 ^b	192.04±26.4 ^b	3.97±0.398 ^a	2.07±0.151 ^a	67.20±6.27 ^b	97.43±2.90 ^c
K3	223.0±95.7 ^{ab}	1.10±0.469 ^{ab}	225.17±39.5 ^b	4.43±1.552 ^a	2.47±0.495 ^a	80.63±31.5 ^{ab}	107.87±5.47 ^b
K4	357.95±4.44 ^a	1.38±0.074 ^a	253.23±56.9 ^{ab}	1.50±0.721 ^b	1.05±0.232 ^b	90.450±6.09 ^{ab}	108.35±7.28 ^b
K5	315.13±91.9 ^{ab}	1.40±0.250 ^a	229.35±71.2 ^b	1.50±0.791 ^b	0.90±0.448 ^b	81.10±12.65 ^{ab}	112.97±3.10 ^b
2014/15							
K1	292.95±69.8	1.001±0.017	295.33±49.0 ^a	2.20±0.098	1.60±0.239	98.50±34.5	111.40±2.43 ^{ab}
K2	264.93±47.5	1.01±0.226	111.30±24.6 ^{bc}	2.43±1.091	1.57±0.524	72.33±15.18	98.10±4.94 ^b
K3	320.03±108.8	1.02±0.309	92.50±36.2 ^c	2.93±2.16	1.63±0.788	81.76±10.71	106.60±13.81 ^{ab}
K4	224.33±84.7	0.80±0.199	215.28±13.78 ^{ab}	3.10±1.709	1.73±0.894	63.53±35.3	94.80±13.17 ^b
K5	401.76±37.9	1.13±0.140	195.08±128.3 ^{abc}	1.50±1.189	1.20±1.111	110.60±23.5	119.30±8.84 ^a

^{a-c} Different letters in the same column concerning all samples significantly different values ($P < 0.05$)

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