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THE EFFECT OF IRRIGATION ON OLIVE AND OLIVE OIL CHARACTERISTICS

Aslı Yorulmaz[1], Hakan Erinç[2*], Abidin Tatlı[3], Aziz Tekin[4]

[1]Adnan Menderes University, Faculty of Engineering, Food Engineering Department, Aydın, Turkey
[2]Nigde Omer Halisdemir University, Faculty of Engineering, Food Engineering Department, Nigde, Turkey
[3]Zer Group, Akhisar, Manisa, Turkey
[4]Ankara University, Faculty of Engineering, Food Engineering Department, Ankara, Turkey

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ABSTRACT

The aim of the study was to identify the changes in olive and corresponding olive oil characteristics in response to irrigation. Five olive cultivars (Halhalı, Sarı ulak, Nizip Yağlık, Kilis Yağlık and Karamani) from Turkish origin were harvested from their own traditional growing region (Mardin, Mersin, Gaziantep, Kilis and Hatay respectively) from both irrigated and non-irrigated trees (rain-fed) for two consecutive crop years. Olives were processed to oil with a laboratory scale system. Olive fruits were analyzed for their physico-chemical properties and phenolic composition and olive oils were analysed for fatty acid, triacylglycerol and sterol composition. Results have shown that average weight, flesh/pit ratio of olives were lower and dry matter and oil content were higher in rain-fed trees than irrigated ones. Phenolic content and composition of fruits were cultivar dependent and responded differently to water supplement. Fatty acid composition of olive oils mainly remained unchanged, however the concentration of individual triacylglycerols behaved in distinct ways. Total sterol and β-sitosterol contents increased and decreased for various cultivars whereas Δ-5-avenasterol increased in all cases. Sarı ulak variety was well separated from other varieties by principal component and hierarchical cluster analysis both in rain-fed and irrigated treatments.

Key words: Irrigation, fatty acid, olive oil, phenolic, sterol, triacylglycerol

SULAMA İŞLEMİNİN ZEYTİN VE ZEYTİNYAĞININ ÖZELLİKLERİNE ETKİSİ

ÖZ

Çalışmanın amacı zeytin ve bu zeytinlerden elde edilen zeytinyağının özellikleri üzerine sulama işleminin etkisinin belirlenmesidir. Çalışmada Türkiye orijinli 5 farklı zeytin çeşidi (Halhalı, Sarı ulak, Nizip Yağlık, Kilis Yağlık and Karamani), ard arda gelen 2 hasat yılı boyunca, sulanmış ve sulanmamış (yağmur suyuyla beslenmiş) ağaçlardan, yetiştirildikleri bölgelerden (sırasıyla Mardin, Mersin, Gaziantep, Kilis ve Hatay) hasat edilmişlerdir. Zeytinler laboratuvar ölçekli bir sistem ile yağa işlenmişlerdir. Zeytin meyveleri fiziko-kimyasal özellikleri ve fenolik kompozisyonları yönünden, zeytinyağları ise yağ asidi, trigliserit ve sterol bileşimleri yönünden analiz edilmişlerdir. Sonuçlar sulama yapılmamış örneklerde sulanan örneklere göre, zeytin ortalama ağırlıkları ile et/çekirdek oranının daha düşük, kuru madde ve yağ içeriğinin ise daha yüksek olduğunu göstermiştir. Fenolik madde içeriği ve kompozisyonunun çeşide bağlı olduğu görülmüş ve sulama işlemi ile farklı sonuçlar elde edilmiştir. Zeytinyağlarının yağ asidi bileşimleri değişmemiştir, ancak her bir trigliserit konsantrasyonunda farklı değişiklikler gözlenmiştir. Toplam sterol ve β-sitosterol miktarlarında çeşitler arasında artış azalışlar gözlenmiş, ancak Δ-5-avenasterol miktarı tüm durumlarda artmıştır. Sarı ulak çeşidi sulama yapılmamış ve sulanmış diğer çeşitlerden temel bileşen ve hiyerarşik kümeleme analizi ile net bir şekilde ayrılmıştır.

Anahtar kelimeler: Sulama, yağ asidi, zeytinyağı, fenolik, sterol, triaçilgliserol

[*] *Corresponding author* /Yazışmalardan sorumlu yazar;
✉ herinc@ohu.edu.tr, ✆ (+90) 388 225 4005 🖷 (+90) 388 225 0112

INTRODUCTION

Olive (*Olea europaea* L.) is a characteristic crop of Mediterranean basin, covering 96 % of the trees in the area. It is grown in subtropical climate with dry summers and wild winters. Olive trees are drought resistant and well adapted to arid and semi-arid regions with low water availability. Olive orchards have traditionally been dry farmed, however there has been increasing interest in water use during olive cultivation due to positive yield response of plantations to complementary irrigation. In olive trees, three main fruit development stages namely flowering, pit hardening, and post pit hardening periods show different tolerances to water stress; bloom and post pit hardening are the water sensitive stages whereas pit hardening is known to be a drought resistant phase (Goldhamer, 1999). Hence, supplemental irrigation during water sensitive phases of phenological plant cycle provides better vegetative growth and increases olive production. The increase in total oil production in response to irrigation is generally achieved by increment in size and quantity of drupes per tree rather than significant oil accumulation in fruit. Some contradictory and variety dependent results have been reported about the influence of water application on oil content in drupe mesocarps; positive (Stefanoudaki et al, 2001), negative (Dabbou et al, 2010) and invariable (Patumi et al, 1999; Zeleke et al, 2012) outcomes complicate to reach consistent conclusions.

Changes in quality, composition and sensory attributes of olive oil in response to irrigation have also been studied by several authors. Free acidity and peroxide value have been generally found to remain constant (Berenguer et al, 2006; Servili et al, 2007; Dabbou et al, 2010;), whereas ultraviolet spectrophotometric indices (K_{232}, K_{270}) have been determined to decrease with irrigation (Gómez-Rico et al., 2007). Phenolic compounds are important constituents for oxidative stability and organoleptic characteristics such as bitterness and pungency attributes of oils. Total phenol content and individual phenolics have been reported to be influenced and generally inversely correlated by irrigation both for olives and resulting olive oils (Motilva et al, 2000; Tovar et al, 2001), as hydric stress encourages the synthesis of phenolic compounds (Parr and Bolwell, 2000). Fatty acid composition was slightly altered or determined to be unchanged in the majority of the early papers (Inglese et al, 1996; Tovar et al, 2002; Berenguer et al, 2006), nevertheless there are some results indicating the adverse effect of water application on oleic acid and unsaturated fatty acid concentration (Gómez-Rico et al, 2007). The triglyceride composition is an important measure to determine the origin, purity and quality of olive oil and normally follow consistent trends with fatty acid composition (Yorulmaz et al, 2014). The major triglyceride of olive oil, triolein, was found either to fall (Stefanoudaki et al, 2001) or raise (Stefanoudaki et al, 2009) with irrigation. Sterols are the major components of the unsaponifiable part of lipids and they are important agents for evaluating the authenticity of virgin olive oil. Conflicting results have been reported about the tendency of individual sterols in response to irrigation, nonetheless, considerable number of studies have pointed the higher amount of total sterols of oils from water stressed trees when compared to irrigated ones (Inglese et al, 1996; Stefanoudaki et al, 2001; Stefanoudaki et al, 2009).

Turkey is one of the largest olive producer country in the world and olive tree population has increased recently with government support. While the trees are conventionally rain-fed, new orchards have mostly been planted with modern cultivation methods adopted to irrigation systems. However, little information is available on the influence of water supply on the quality and chemical properties of olives and corresponding olive oils. Hence, the current study investigates the fruit characteristics and chemical composition of virgin olive oils from irrigated and non-irrigated trees of five different varieties grown in Southern Anatolia, collected for two consecutive crop years.

MATERIALS AND METHODS

Reagents and standards

Methanol, hexane, pyridine, β-sitosterol, chloroform and formic acid were obtained from Merck (Darmstadt, Germany); stigmasterol, campesterol, 5α-cholestan-3β-ol (cholestanol),

luteolin, *p*-coumaric acid, and rutin were purchased from Sigma (St-Louis, USA); 4-(2-hydroxyethyl)phenol (tyrosol), cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside and apigenin were supplied from Fluka (Buchs, Switzerland); verbascoside (acteoside), luteolin-7-*O*-glucoside and oleuropein were purchased from Applichem (Darmstadt,Germany); *trans*-cinnamic acid was obtained from Aldrich (St- Louis, USA); while 37 fatty acid methyl ester (FAME) mix and N,O- bis (trimethylsilyl) trifluoro acetamide (BSTFA)+trimethyl chlorosilane (TMSC) were from Supelco (Bellefonte, USA). Hydroxytyrosol was synthesized from oleuropein as reported by Owen et al. (2000).

Olive sampling and oil extraction

Olea europaea L. fruits of five different cultivars were obtained from commercial orchards in five separate districts: Halhalı, Sarı ulak, Nizip Yağlık and Kilis Yağlık varieties were harvested from Mardin, Mersin, Gaziantep and Kilis provinces respectively in both crop years. Karamani cultivar was obtained from Hatay province in the first year of the experiment. The climate of the region is Mediterranean and annual rainfall was 317 mm and 809 mm for 2008 and 2009 respectively. Olive fruits of each variety were collected from both irrigated and rain-fed (non-irrigated) trees grown in the same environmental conditions and agricultural applications. Olive trees were irrigated by drip irrigation system with compensating emitters placed around trees. Four, eleven, eight, six and ten irrigation treatments were imposed for Halhalı, Karamani, Sarı ulak, Nizip Yağlık and Kilis Yağlık varieties respectively. Each of the treatments received 250, 50, 80, 30 and 50 L of water in the same order. All irrigation treatments ended in September for all cultivars. The fruits were picked in October-November, at appropriate harvest time for table olive production.

A representative batch of drupes were transported to laboratory and processed to olive oil. 2 kg of fruits were first washed, then crushed with a laboratory scale crusher. The resulting paste was kneaded for 30 minutes at 25°C. The oily paste was pressed to obtain the liquid phase and oil was separated from the liquid by centrifugation at 6000 rpm. Virgin olive oil samples were kept in nitrogen atmosphere at 4°C until analyses.

Analyses

Average weight, flesh/pit ratio, dry matter and oil content of fruits

Average weight and flesh/pit ratio of fruits were determined by weighing 10 individual olives and their stones. The dry matter content was measured by drying 10 g of olive paste in an oven at 105 °C to constant weight. Oil content was determined according to AOCS Official Method Am 2-93 (AOCS, 2003) by soxhelet apparatus using *n*-hexane as solvent and expressed as percentage of dry weight basis.

Phenolic profile of olive samples

Phenolic compound analysis of olives were performed according to the method described in Yorulmaz et al. (2012). High performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) apparatus equipped with Inertsil (250 mm x 4.6 mm, ODS-3, 5-µm particle size, GL Sciences, Tokyo, Japan) column was used for chromatographic analysis.

Fatty acid composition

Fatty acid methyl esters were prepared according to International Union of Pure and Applied Chemistry (IUPAC) (1987) and analyzed with Shimadzu GC-2010 (Shimadzu, Kyoto, Japan) gas chromatograph. DB-23 fused silica capillary column (60m x 0.25 mm i.d. x 0.25 µm film thickness) (J&W) was employed for chromatographic separation. Injector, column and detector temperatures were 230, 195 and 240°C respectively. The carrier gas was helium with a flow rate of 1 ml/min and the split ratio was 80:1.

Triglyceride composition

Chromatographic analysis of triacylglycerols was achieved by HPLC (Shimadzu, Kyoto, Japan) according to the method proposed by Herslöf (1981) and monitored with differential refractometer detector. Olive oil samples were dissolved (3%) in acetonitrile:2-propanol:hexane (2:2:1) prior to analysis. Nucleosil 100 C18 (25x0.46, 5µm particle size, Teknochroma,

Barcelona, Spain) column was employed for separation and mobile phase was acetonitril:2-propanol:hexan (500:118:100) with a flow rate of 1 ml/min. Oven temperature was 25°C. Triacylglycerols were separated according to the equivalent carbon number (ECN) and expressed as the percentage of each triacylglycerol. The peaks were identified by comparing the data given in literature (Ollivier et al, 2006).

Sterol composition

Sterol composition was determined according to AOCS Official Method Ch 6-91 (2003). Silyl ethers of the sterols were analyzed by GC 2010 gas chromatograph (Shimadzu, Kyoto, Japan). HP-5 fused silica capillary column (30m x 0.25 mm i.d. x 0.25 μm film thickness) (Chrom Tech., Apple Valley, MN, USA) was used for chromatographic elution. Injector, column and detector temperatures were 280, 260 and 290 °C respectively. Helium was the carrier gas with a flow rate of 0.8 ml/min and split ratio was 50:1.

Statistical Analysis

Statistical analysis was carried out using SPSS 9 statistical software (SPSS Inc., Chicago, USA). Data were evaluated by Students's t-test test to determine any significant differences between irrigation treatments. A *p*-value of less than 0.05 was considered significant. Data were also evaluated by principal component analysis (PCA) and hierarchical cluster analysis (HCA) using XLSTAT 2014 version (Addinsoft, New York, NY).

RESULTS

Olive Characteristics

Physical and chemical characteristics together with phenolic composition of olives from irrigated and rain-fed trees is listed in Table 1. Average fruit weight, flesh/pit ratio were lower and dry matter content were higher in rain-fed trees for all cultivars confirming former works (Gómez-Rico et al, 2007). The increase in water content of fruits due to irrigation resulted in higher average drupe weights. Oil contents of olives on dry weight basis, decreased with drip irrigation by 5.73% for Nizip Yağlık, 4.86 % for Kilis Yağlık, 4.44 % for Halhalı, 4.51 % for Karamani and 7.33% for Sarı ulak variety. Oil content results are in accordance with some previous works (Gómez-Rico et al, 2007; Dabbou et al., 2010), nonetheless there are a moderate number of studies indicating the positive effect of irrigation on oil content of fruit mesocarp (Inglese et al., 1996; Stefanoudaki et al, 2001). The higher oil content may be possibly because of the lower fruit load of rain-fed trees. Total phenol content of olive fruits were determined by adding up each individual phenolic compounds and ranged between 1526.39-11778.21 mg/kg. Irrigation increased phenolic contents of Nizip Yağlık, Kilis Yağlık and Halhalı but decreased that of Karamani and Sarı ulak varieties. The major phenolic, secoiridoid oleuropein, had similar respond to irrigation with total phenol content and determined between 201.15-8082.66 mg/kg. It increased in Nizip Yağlık, Kilis Yağlık, Halhalı but decreased in Karamani and Sarı ulak by extra water application. *Trans* cinnamic acid, a marker for Turkish olive cultivars grown in Anatolia (Yorulmaz et al., 2012), is the second predominant phenolic of olives and varied between 98.06-2372.18 mg/kg. Its concentration was higher than oleuropein in drip-irrigated Karamani variety. Similar to *trans* cinnamic acid, luteolin-7-glycoside and verbascoside also increased in Nizip Yağlık, Kilis Yağlık and Halhalı but decreased in Karamani and Sarı ulak fruits in response to irrigation. Hydroxytyrosol and tyrosol were two phenolic alcohols of olives, and both showed different attitudes by water treatment for various cultivars where statistically significant descents were observed for Karamani. Tyrosol had lower concentrations than hydroxytyrosol for all analyzed samples. Concentrations of two cyanidin glycosides, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside, responsible for black color of drupes increased with irrigation, except for Karamani cultivar. *p*-coumaric acid increased in all cultivars and the remaining flavonoids (rutin, apigenin and luteolin) did not have a common tendency by water supplement. There are very limited number of papers revealing the influence of irrigation to phenolic distribution of olives. Patumi et al (2002) reported higher values for tyrosol, vanillic acid, 3,4-dihydroxyphenylglycol, oleuropein aglycones, oleoside-11-methyl ester

and oleuropein but lower values for hydroxytyrosol in irrigated trees.

Table 1. Physical and chemical characteristics of olives from rain fed and irrigated trees

	Nizip yaglik		Kilis yaglik		Halhalı		Karamani		Sarı Ulak	
	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation
Average weight (g)	1.64	2.70	2.43	2.86	1.65^{X}	2.63^{Y}	3.37^{X}	4.56^{Y}	1.95	2.20
Flesh/Pit ratio	3.74	4.27	3.53^{X}	4.25^{Y}	4.47	4.59	5.14^{X}	6.37^{Y}	2.45	2.72
Dry matter (%)	71.83^{X}	54.41^{Y}	59.59	56.17	55.53	55.20	48.88	45.78	58.32	56.71
Oil (%, d.b.)	41.30	35.57	26.62	21.76	33.10	28.66	26.75^{X}	22.24^{Y}	31.93	24.60
Hydroxytyrosol (mg/kg)	234.52	637.35	159.09	164.69	143.46	131.52	341.20^{X}	197.57^{Y}	532.63	478.73
Tyrosol (mg/kg)	60.95	113.27	97.39	95.57	60.13	127.46	75.80^{X}	11.25^{Y}	200.08	212.84
Cyanidin 3-*O*-glucoside (mg/kg)	6.20	48.29	118.38	118.60	1.69	4.47	15.48^{X}	0.37^{Y}	2.13	0.80
Cyanidin 3-*O*-rutinoside (mg/kg)	53.62	214.19	224.10	296.34	14.06	42.36	69.18^{X}	1.62^{Y}	32.98	2.35
p-coumaric acid (mg/kg)	1.86	2.42	0.34	1.35	0.53	1.63	1.41^{X}	3.17^{Y}	1.64	1.90
Verbascoside (mg/kg)	69.93	90.22	41.39	81.10	14.54	33.63	41.15^{X}	4.17^{Y}	87.71	42.82
Luteolin-7-glucoside (mg/kg)	265.35	1383.42	110.15	265.70	117.38	267.30	438.26^{X}	110.07^{Y}	1684.95	1193.91
Rutin (mg/kg)	49.37	149.63	32.28	63.81	31.04	80.35	226.06^{X}	15.50^{Y}	405.66	278.22
Oleuropein (mg/kg)	890.58	3666.82	628.20	1053.24	1969.48	6046.92	3968.60^{X}	201.15^{Y}	8082.66	6741.13
trans-cinnamic acid (mg/kg)	890.53	2372.18	98.06	244.18	864.63	1465.16	1122.70^{X}	658.53^{X}	661.79	364.09
Luteolin (mg/kg)	22.74	57.33	16.67	39.21	115.29^{X}	57.27^{Y}	17.66^{X}	434.40^{Y}	84.74	61.00
Apigenin (mg/kg)	0.66	0.17	0.34	0.25	2.81	3.86	3.78	2.38	1.24	0.51
Total phenolic compounds (mg/kg)	2551.35^{X}	8735.36^{Y}	1526.44	2423.10	3335.41	8254.98	6331.31	1640.22	11778.26	9378.33

†Different superscript letters (X-Y) indicate significant differences (P <0.05) between water availibilities of each single cultivar.

Olive Oil Characteristics

The fatty acid composition of oils from dry-farmed and irrigated trees is presented in Table 2. Myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), gadoleic (C20:1), behenic (C22:0) and lignoceric (C24:0) acids were monitored in oil samples. Fatty acid ratios of all cultivars were within the limits established by Turkish Food Codex (2018), EU (2013) and IOC (2015) regulations except for C 17:1 ratios of Sarı ulak (both rain-fed and irrigated) and C20:0 percentage of rain-fed Halhalı samples. The main fatty acid was oleic acid ranging between 62.15 (Karamani)- 72.05 % (Halhalı). The prevailing polyunsaturated fatty acid was linoleic acid which had the values between 6.73 (Halhalı)- 15.38 % (Karamani). Palmitic acid, the major saturated fatty acid, varied between 12.12 (Sarı ulak) – 16.55 % (Karamani); whereas stearic acid existed between 3.14 (Karamani)- 3.78 % (Halhalı). The sum of the remaining fatty acids were lower than 4 %.

Only few fatty acids, namely, palmitic acid of Kilis Yağlık and gadoleic acid of Kilis Yağlık and Halhalı, were affected significantly by irrigation. The other fatty acids did not exhibit a constant relation with irrigation, approving some previous works (Inglese et al., 1996; Motilva et al., 2000; Ayton et al, 2007). Several studies stating the significant influence of water application on fatty

acid composition have also been reported (Stefanoudaki et al., 2001; Salas et al. 1997).

Table 2. Fatty acid composition of oils from rain fed and irrigated trees (%)

	Nizip yaglik		Kilis yaglik		Halhalı		Karamani		Sarı Ulak	
	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation
C14:0	0.01	0.01	0.01	0.01	0.01^{X}	0.02^{Y}	0.01	0.01	0.01	0.01
C16:0	14.25	15.60	14.04^{X}	15.00^{Y}	14.86	14.77	15.12	16.55	12.55	12.12
C16:1	1.08	1.01	0.91	1.04	0.98	1.15	1.58	0.96	0.92	0.88
C17:0	0.12	0.10	0.14	0.11	0.11	0.11	0.15	0.13	0.20	0.26
C17:1	0.18	0.17	0.20	0.19	0.19	0.14	0.20	0.18	0.32	0.35
C18:0	3.48	3.49	3.57	3.61	3.78	3.47	3.46	3.14	3.33	3.36
C18:1	67.86	69.48	71.50	68.49	71.39	72.05	66.21	62.15	69.50	70.64
C18:2	11.54^{X}	8.48^{Y}	8.07	10.13	7.04	6.73	11.66	15.38	11.40	10.59
C18:3	0.60	0.53	0.64	0.60	0.57	0.58	0.69	0.68	0.89	0.91
C20:0	0.50	0.43	0.49	0.48	0.62	0.59	0.52	0.44	0.49	0.46
C20:1	0.22	0.16	0.25^{X}	0.21^{Y}	0.28^{X}	0.23^{Y}	0.25	0.24	0.27	0.29
C22:0	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
C24:0	0.11	0.08	0.08	0.10	0.12	0.14	0.13	0.11	0.07	0.08
MUFA/PUFA	5.70^{X}	7.87^{Y}	8.69	6.84	9.67	10.06	5.52	3.96	5.94	6.66

†Different superscript letters (X-Y) indicate significant differences (P <0.05) between water availibilities of each single cultivar.

Triacylglycerol composition of olive oils obtained from rain fed and irrigated trees is shown in Table 3. Main triacylglycerols were triolein (OOO), palmitodiolein (OOP), dioleolinolein (OOL), palmitooleolinolein (PLO), dipalmitoolein (POP) and stearodiolein (SOO). Additionally palmitoleodiolein (OOPo), palitoleopalmitoolein (PoOP), trilinolein (LLL), oleodilinolein (OLL), oleolinoleolinolenin (OLLn), palmitodilinolein (PLL), palmitolinoleolinolenin (PLLn), dioleolinolenin (OOLn), palmitooleolinolenin (POLn), dipalmitolinolein (PPL) and palmitostearoolein (POS) were found in lower ratios. The main triglyceride, triolein, ranged between 27.29 (Karamani) – 38.69 % (Kilis Yağlık). Irrigation increased OOO ratio of Nizip and Kilis Yağlık, but decreased that of Halhalı, Karamani and Sarı ulak oils. OOP, existed as the second important triglyceride and varied between 23.36 (Sarı ulak) – 28.91 % (Nizip Yağlık). OOP values decreased in all cultivars' oils by water treatment except for Nizip Yağlık oils. Stefanoudaki et al (2001) reported higher values for OOO and lower percentages for POO(+SOL) in oils of water stressed trees than irrigated ones. OOL (+PPLn) was between 10.18 (Kilis Yağlık) – 17.15 % (Karamani) and decreased with additional water supply for Nizip Yağlık and Sarı ulak varieties, although statistically significant differences were not observed.

Sterol content and erythrodiol-uvaol ratio of olive oils from dry farmed and irrigated trees is tabulated in Table 4. Total sterol content of oil samples were all above the minimum established limit of 1000 mg/kg determined by national and international regulations for virgin olive oil. Total sterol content of the oils decreased in Nizip Yağlık and Kilis Yağlık by 25.74 %, 1.94 % but increased in Halhalı, Karamani, Sarı ulak varieties by 10.70 %, 16.19 % and 10.61 % respectively. Inglese et al (1996), Stefanoudaki et al (2001) and Stefanoudaki et al (2009) reported higher total sterol contents for the oils from water stressed trees, whereas Berenguer et al (2006) figured increments and invariable results respectively in a two year assay. β-sitosterol, Δ-5-avenasterol and campesterol were the main sterols, while cholesterol, brassicasterol, 24-methylene-cholesterol, campestanol, stigmasterol, Δ-7-campesterol, clerosterol, sitostanol, Δ-5,24-stigmastadienol, Δ-7-stigmastenol, Δ-7-avenasterol were also determined in small amounts. β-sitosterol existed between 934.44 (Nizip Yağlık)- 1316.00 mg/kg (Karamani) and had similar respond to irrigation with total sterol content since it covers the major part of sterol

fraction. Δ-5-avenasterol was between 50.96 (Karamani) – 166.66 mg/kg (Kilis Yağlık) and increased with irrigation for all cultivars in contrast with the results obtained by Inglese et al. (1996) and Stefanoudaki et al. (2009). β-sitosterol and Δ-5-avenasterol percentages were strong and inversely correlated (r = -0.93) probably because of desaturase enzyme activity altering β-sitosterol to Δ-5-avenasterol. Campesterol varied between 21.83 (Kilis Yağlık) – 52.92 mg/kg (Sarı ulak), below the established upper limit of 4.0 %. Stigmasterol ratios were lower than campesterols' for all samples compatible with regulation requirements. Increases in campestanol were recorded in all cultivars by water supplement. Individual sterols excluding campestanol and Δ-5-avenasterol did not reveal a common tendency in response to irrigation. The sum of two triterpene dialcohols (erythrodiol and uvaol) were below the regulatory limit of 4.5 % for all cultivars and increased only in Nizip Yağlık oils and decreased for the rest of the varieties by water treatment.

Table 3. Triacylglycerol composition of oils from rain fed and irrigated trees (%)

	Nizip yaglik		Kilis yaglik		Halhalı		Karamani		Sarı ulak	
	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation
LLL	0.17	0.06	0.05	0.11	0.06	0.22	0.17^{X}	0.52^{Y}	0.04	0.01
OLLn+PoLL	0.08	0.07	0.10	0.07	0.07	0.18	0.17	0.34	0.13	0.07
PLLn	0.02	0.04	0.01	0.03	0.05	0.04	0.08	0.08	0.02	0.04
OLL+OLPo	2.78^{X}	1.38^{Y}	1.20	1.24	0.89	2.42	2.72^{X}	4.30^{Y}	1.68	1.68
OOLn	0.90	0.64	0.72	0.71	0.42^{X}	0.96^{Y}	0.94	0.84	0.77	1.90
PLL	0.56^{X}	0.29^{Y}	0.18	0.27	0.13^{X}	0.60^{Y}	0.60^{X}	1.18^{Y}	0.36	0.55
POLn	0.08	0.02	0.04	0.04	0.03	0.05	0.04	0.11	0.03	0.05
OOL+PPLn	12.96	11.35	10.18	10.98	10.42	12.27	14.76	17.15	13.82	13.27
OOPo	0.74	1.55	1.25	0.83	2.91	1.35	0.71	0.92	1.53	1.46
PLO+SLL	9.87	7.17	7.13	7.13	5.64	8.26	8.91^{X}	11.87^{Y}	9.08	7.41
PoOP	0.48	0.62	0.32	0.54	0.37	0.72	0.56	1.82	1.59	1.48
PPL	0.16	0.30	0.16	0.38	0.10	0.14	0.16	0.85	0.13	0.18
OOO	33.02	35.13	36.08	38.69	36.77	32.88	33.52^{X}	27.29^{Y}	36.29	35.38
OOP	27.69	28.91	28.20	27.23	28.42	28.02	26.70	24.03	25.97^{X}	23.36^{Y}
POP	3.51	4.38	5.41	5.76	4.57	4.81	4.27	3.32	3.17	2.12
SOO	5.37	5.77	5.43	5.79	6.95	5.28	4.06	4.04	4.82	8.51
POS	1.51	2.23	0.85	2.72	2.10	1.70	1.56	1.26	0.50	2.55

†Different superscript letters (X-Y) indicate significant differences (P <0.05) between water availibilities of each single cultivar.

Table 4. Sterol content (mg/kg) and eritrodiol-uvaol ratio (%) of oils from rain fed and irrigated trees

	Nizip yaglik		Kilis yaglik		Halhalı		Karamani		Sarı ulak	
	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation	Rain-fed	Drip Irrigation
Cholesterol	9.42	5.15	5.50	4.15	6.35^{X}	5.30^{Y}	6.66	6.07	7.44	7.79
Brassicasterol	1.33^{X}	0.61^{Y}	0.71	0.32	0.56	0.16	0.71	0.61	0.53	0.54
24-methylene-cholesterol	0.66	1.11	0.85	1.58	1.70	0.98	0.88	0.42	1.36	1.61
Campesterol	48.69^{X}	33.97^{Y}	25.11	21.83	36.37	38.18	31.63	35.16	47.32	52.92
Campestanol	9.33	2.83	3.50	1.39	2.23	2.19	2.35	1.57	3.27	2.86
Stigmasterol	21.49	19.69	17.34^{X}	13.51^{Y}	11.74	14.57	13.43^{X}	17.40^{Y}	11.84	12.07
Δ-7-campesterol	2.06^{X}	0.96^{Y}	1.32^{X}	0.54^{Y}	0.40	0.43	0.24	0.61	1.24	1.03
Clerosterol	13.63^{X}	9.90^{Y}	11.93	11.10	10.27	11.03	12.41	14.34	11.67	13.59
β-sitosterol	1282.93^{X}	934.44^{Y}	1013.13	961.00	1052.49	1170.62	1134.62	1316.00	1014.82	1147.14
Sitostanol	19.91	8.79	9.57^{X}	6.38^{Y}	9.62	14.83	6.50	11.70	8.23	8.81
Δ-5-avenasterol	107.86	111.67	127.03	166.66	72.17	86.07	50.96	93.99	55.88	56.27
Δ-5,24-stigmastadienol	7.52^{X}	5.43^{Y}	6.94	7.30	4.88	6.59	5.08	5.59	5.53	5.63
Δ-7-stigmastenol	9.68^{X}	5.72^{Y}	3.30	3.41	5.02	7.49	4.37	9.06	6.42	5.08
Δ-7-avenasterol	14.54^{X}	9.89^{Y}	5.91	9.02	7.13	8.87	4.63	8.28	5.76	6.23
Total sterols	1549.07^{X}	1150.19^{Y}	1232.18	1208.16	1220.96	1367.35	1274.48	1520.82	1181.36	1321.62
Erythrodiol+uvaol	1.52	2.25	2.14	1.57	3.72^{X}	2.01^{Y}	2.96^{X}	0.52^{Y}	2.27	1.96

†Different superscript letters (X-Y) indicate significant differences (P <0.05) between water availibilities of each single cultivar.

Multivariate analysis

Principal component analysis was performed to identify the differences between cultivars based on their attitudes in response to irrigation. Analytical data with adequate selected variables (average weight, flesh/pit ratio, dry matter, oil, hydroxytyrosol, tyrosol, *p*-coumaric acid, luteolin-7-glucoside, rutin, oleuropein, *trans* cinnamic acid, luteolin content of olives; C14:0, C 16:0, C 16:1, C17:0, C17:1, C18:1, C18:2, C18:3, C20:0, C20:1, C22:0, C24:0, LLL, PLLn, OLL+OLPo, OOLn, PLL, OOL+PPLn, PLO+SLL, PoOP, PPL, OOO, OOP, POP, SOO, cholesterol, brassicasterol, campesterol, campestanol, Δ-7-campesterol, Δ-7-stigmastenol, Δ-7-avenasterol and erythrodiol+uvaol ratio of corresponding oils) were arranged in a matrix. The first (F1), second (F2) and third (F3) principal components had eigen values of 14.42, 11.11, 6.59 and accounted for 32.04, 24.77 and 14.65 of the variance respectively. Loading and factor score plots are given in Figure 1 and 2. F1 showed high and positive correlations with average weight, LLL, OLL+OLPo, PLL, PPL; high and negative correlations with C18:1 and OOO. F2 was strong and positively correlated with C17:0, C18:3 and high and negatively correlated with POP. Score plot revealing the relations among variables showed that KI, KR, HI had positive scores on F1; while NI, KLI, KLR, HR had positive scores on both F1 and F2.

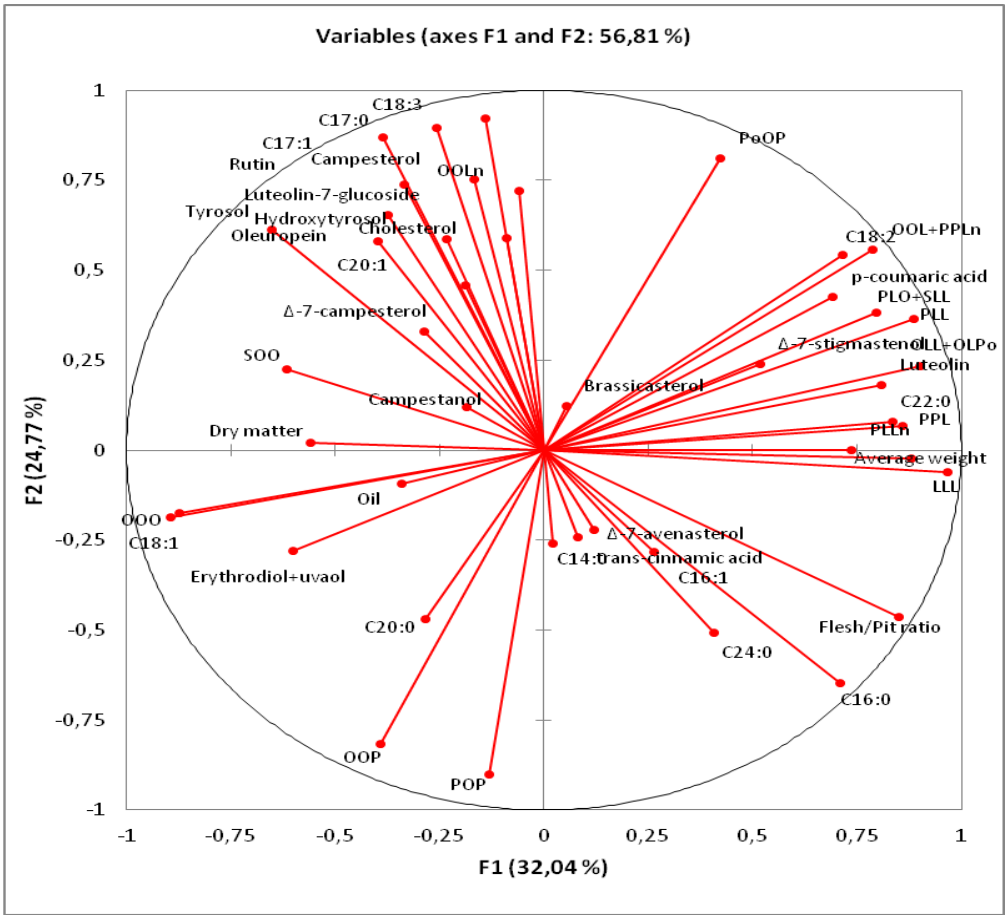

Figure 1. Loading plot of cultivars from rain-fed and irrigated trees obtained from PCA of data using selected variables on the plane identified by two principal components (Kilis Yağlık rain-fed: KLR, Kilis Yağlık irrigated: KLI, Nizip Yağlık rain-fed: NR, Nizip Yağlık irrigated: NI, Karamani rain-fed: KR, Karamani irrigated: KI, Halhalı rain-fed: HR, Halhalı irrigated: HI, Sarı ulak rain-fed: SR, Sarı ulak irrigated: SI)

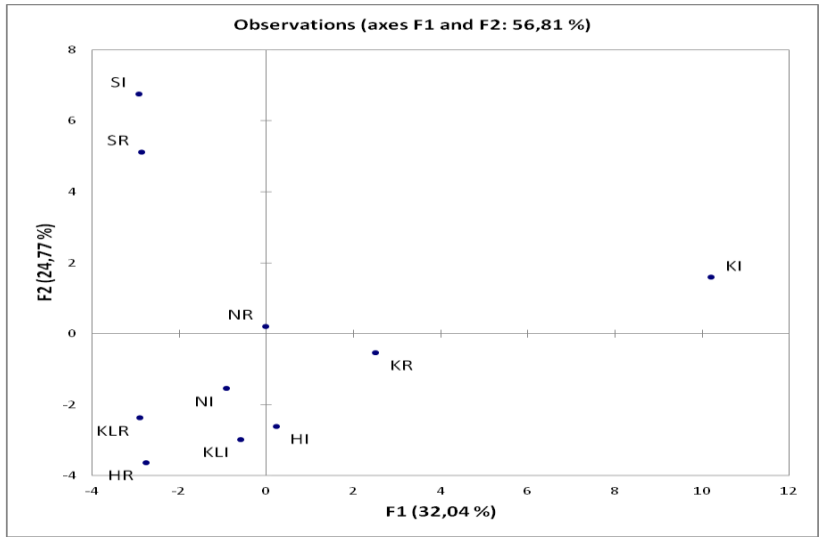

Figure 2. Factor score plot of cultivars from rain-fed and irrigated trees obtained from PCA of data using selected variables on the plane identified by two principal components (Kilis Yağlık rain-fed: KLR, Kilis Yağlık irrigated: KLI, Nizip Yağlık rain-fed: NR, Nizip Yağlık irrigated: NI, Karamani rain-fed: KR, Karamani irrigated: KI, Halhalı rain-fed: HR, Halhalı irrigated: HI, Sarı ulak rain-fed: SR, Sarı ulak irrigated: SI)

Olive and the oil samples were also subjected to further PCAs using i) all physical and chemical parameters, ii) olive phenolics, iii) fatty acids, iv) triacylglycerols, v) sterols as variables. All PCAs revealed that Sarı ulak variety (both rain-fed and irrigated samples) were well separated from other cultivars. Fatty acids were more evident than sterols and triacylglycerols in discriminating the samples.

Hierarchical cluster analysis was carried out to differentiate olive oils according to their response to water treatment utilizing the selected variables, as in PCA. Euclidean distance between samples and Ward algorithm were used to perform the analysis. A dendrogram obtained from HCA is given in Figure 3, where 3 main groups were identified. The first group contained NR, KLR, KLI, HR, KI where a high similarity between KLR and KLI was observed. The second group consisted of NI and KR. The third group was built by HI, SR, SI where SR and SI formed a couple being surrounded by HI.

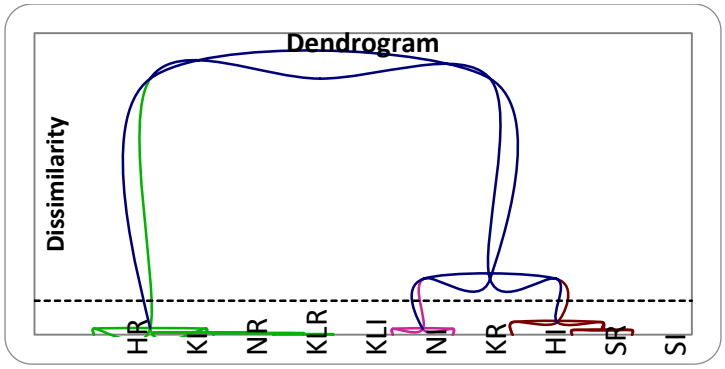

Figure 3. Hierarchical cluster analysis dendrogram of cultivars from rain-fed and irrigated trees obtained using selected variables (Kilis Yağlık rain-fed: KLR, Kilis Yağlık irrigated: KLI, Nizip Yağlık rain-fed: NR, Nizip Yağlık irrigated: NI, Karamani rain-fed: KR, Karamani irrigated: KI, Halhalı rain-fed: HR, Halhalı irrigated: HI, Sarı ulak rain-fed: SR, Sarı ulak irrigated: SI)

CONCLUSION

Thus, in conclusion, the results indicate that drip irrigation affects the characteristics of both olives and olive oils, however various cultivars respond differently to water application. So, further studies should be carried out for the other varieties cultivated in different regions of Turkey, including more parameters such as shelf life and sensorial quality. It is also important to determine the right amount and time of water supplement to increase the yield and quality of the products.

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