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INFLUENCE OF OLIVE RIPENESS DEGREE AND HARVEST YEAR ON CHEMICAL AND SENSORY PROPERTIES OF KİLİS YAĞLIK AND MEMECİK OLIVE OIL

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ABSTRACT
The objective of this study was to investigate the effect of olive cultivar, olive ripeness degree and harvest year on the oil yield, oil quality, oxidative stability and sensory profiles of olive oils extracted obtained from Kilis Yağlık and Memecik cultivars. The effect of olive cultivar on the oil content was statistically significant and the oil content of Kilis Yağlık olive cultivar was determined to be higher than Memecik cultivar. Free fatty acidity, peroxide value, K_{270} value, total phenol content, palmitoleic, stearic, oleic, linoleic, linolenic acids and MUFA/PUFA ratio and sensory properties of olive oil samples were statistically significantly affected by cultivar of olive, ripening index and harvest year. While the spice aroma was recorded in the sensory analysis of Kilis Yağlık olive oil, flower and bitter almond aromas were perceived for Memecik olive oil. The ideal harvest time for the production of extra virgin olive oil was determined as at "medium maturity level" in december for both cultivars. Ripening index was measured as 3.96-3.96 and 4.09-3.87 in december for Kilis Yağlık and Memecik cultivars for two harvest years, respectively.
Keywords: olive ripeness degree, olive oil, sensory analysis, Kilis Yağlık, Memecik

KİLİS YAĞLIK VE MEMECİK ZEYTİNYAĞLARININ KİMYASAL VE DUYUSAL ÖZELLİKLERİNE ZEYTİNİN OLGUNLUK DERECESİ VE HASAT YILININ ETKİSİ

ÖZ
Bu çalışmanın amacı, Kilis Yağlık ve Memecik çeşitlerinden elde edilen zeytinyağlarının yağ verimi, yağ kalitesi, oksidatif stabilitesi ve duyusal profilleri üzerine zeytin çeşidinin, zeytin olgunluk derecesinin ve hasat yılının etkisini araştırmaktır. Yağ içeriği üzerinde çeşidin etkisi istatistiksel olarak önemli bulunmuş ve Kilis Yağlık zeytin çeşidinin yağ içeriğinin Memecik çeşidinden daha yüksek olduğu saptanmıştır. Zeytin çeşidi, olgunlaşma indeksi ve hasat yılından; zeytinyağı örneklerinin serbest yağ asitliği, peroksit değeri, K_{270} değeri, toplam fenol içeriği, palmitoleik, stearik, oleik, linoleik, linolenik asit ile tekli doymamış yağ asitleri/çoklu doymamış yağ asitleri oranı ve duyusal özellikleri istatistiksel olarak önemli derecede etkilenmiştir. Kilis Yağlık zeytinyağının duyusal analizinde baharat aroması kaydedilirken, Memecik zeytinyağı için çiçek ve acı badem aromaları algılandı. Natürel sızma zeytinyağı üretiminde ideal hasat zamanı, her iki çeşit için de aralık ayında "orta olgunluk seviyesi" olarak belirlendi. Kilis Yağlık ve Memecik çeşitlerinde olgunlaşma indeksi aralık ayında iki hasat yılı için sırasıyla 3.96-3.96 ve 4.09-3.87 olarak ölçülmüştür.
Anahtar kelimeler: zeytin olgunluk derecesi, zeytinyağı, duyusal analiz, Kilis Yağlık, Memecik

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INTRODUCTION

Virgin olive oil is extracted from the drupes of olive trees (*Olea europaea* L.) by mechanical procedures and requires no refining before consumption. It contains volatile substances and other minor compounds responsible for the delicate and fragrant taste that is highly appealing to consumers. In the past few years, there has been a more widespread consumption of virgin olive oil, even in countries where it is not produced, such as Canada and Japan. The importance of virgin olive oil is linked to its unique flavor, nutritional features, positive impact on human health and also its high content of oleic acid a balanced contribution quantity of polyunsaturated fatty acids (Capino et al., 2001; Bendini et al., 2007).

The effects of harvest time and olive ripeness on the oil yields, oil quality, oxidative stability and sensory characteristics are of particular interest to the grower. During the ripening process, the weight, pulp-to-stone ratio, color, oil content, chemical composition of the oil and enzyme activities change dramatically in the fruits (Dag et al., 2011).

Fatty acid composition, levels of polyphenols, tocopherols, sterols and pigments are strongly affected by several agronomical factors such as variety, pedoclimatic production conditions, agronomic techniques, olive ripening stage, harvest year, and growing area (Beltrán et al., 2004a; Morello et al., 2004; Mousa et al., 2004, Youssef et al. 2009).

In general, as the olive fruit matures, the oil becomes less stable due to an increase in polyunsaturated fatty acids and a decrease in total polyphenol content. Polyphenols are key to olive oil quality since they contribute significantly to stability against oxidation (Mateos et al., 2003; Nieto et al., 2010). In addition, total phenols of olive oil are the main contributor to olive oil bitterness, astringency, and pungency (Gutierrez et al.,1999; Andrewes et al., 2003; Nieto et al., 2010; Sevim et al., 2013).

Olive growers use traditional harvesting dates, changes in fruit color and natural fruit drop as guidelines to begin the harvest period; however these parameters are not really valid because the development and ripening process of olive fruit changes with the cultivar and environmental conditions and thus is different for each growing area and yield (Beltrán et al., 2004b). An appropriate index of fruit ripening must be established specifically for each individual olive cultivar (Rotondi et al., 2004).

The objectives of this study were to evaluate the effects of olive cultivar, olive ripeness and harvest year on the chemical and sensory properties of olive oil and to determine the optimal harvesting time and ripening index for Kilis Yağlık and Memecik cultivars. Kilis Yağlık cultivar is mainly located in the Southeastern Anatolia Region of Turkey that has high in oil content. Memecik is the most economically important cultivar in the Aegean Region of Turkey and constitutes more than 50% of olive production in that region.

MATERIALS AND METHODS

Plant material

Olive fruits were collected from olive trees of "Kilis Yağlık" and "Memecik" cultivars situated on the gen bank of Olive Research Institute of Ministry of Food, Agriculture and Livestock in Izmir, Turkey. Olive samples were harvested by hand at three different maturity levels (beginning in November, medium in December, and end in January) during the 2012/13 and 2013/14 successive harvest years. Each sampling was carried out in duplicate of two different olive trees in the same orchard (n=2).

Oil extraction

Olive oil extraction was performed using an Abencor system (MC2 Ingenieria y Sistemas, Sevilla, Spain) that simulates the industrial process of virgin olive oil at a laboratory scale. This system consists of three basic parts; a hammer mill, a thermo beater and a paste centrifuge. Deleafed and washed olive fruits were milled using a stainless steel hammer mill. The resulting olive paste was kneaded in a thermo beater at 28°C for 30 min at 50 rpm. Kneaded olive paste was centrifuged at 3500 rpm for 1 min to separate liquid phases (oil and vegetable water) from solid phase. The oil samples were filtered and stored at 4°C in darkness using amber glass bottles without headspace until analysis.

Ripening index

Ripening index (RI) of the olives was determined according to the method developed by Uceda and Frías (1975), based on the evaluation of olive skin and pulp colors. The procedure consists of distributing eight groups according to the color of 100 random fruits: intense green (group N=0), yellowish green (group N=1), green with reddish spots (group N=2), reddish-brown (group N=3), black with white flesh (group N=4), black with 50% purple flesh (group N=5), black with 50% purple flesh (group N=6), and black with 100% purple flesh (group N=7). The index is expressed as $\sum (N_i n_i)/100$ where N is the group number, and n is the fruit number in that group. RI values range from 0 to 7 (Ramón-Morelló et al., 2004).

Oil content

The oil content of olives was determined by Soxhlet extraction and expressed as % on a dry weight basis according to International Union of Pure and Applied Chemistry (IUPAC, 1979).

Free fatty acidity, peroxide value, UV spectrophotometric indices

Free fatty acidity **(**FFA, oleic acid %), peroxide value (PV, meqO_2/kg) and UV spectrophotometric indices (K_{232} and K_{270}) were determined according to the analytical methods described in the official journal of the European Communities, EEC regulation no. 2568/1991 and later modifications (EEC/2568/1991).

Fatty acid composition

The fatty acid composition of oil samples was determined by gas chromatography (HP 6890, Agilent Technologies, DE, USA) equipped with flame ionization detector (FID) using the fatty acid methyl ester (FAME) method described by International Olive Council (COI/T.20.Doc.no:17/1996) and International Union of Pure and Applied Chemistry (IUPAC, 1987). The capillary column (DB-23, 30m*0.25 mm*0.25 μm, Agilent J&W GC columns, USA) was used for analyses. Injector and detector temperatures were set to 250°C. The oven temperature was programmed from 170°C to 210°C with an increment of 2°C/min. The analyses were ended by maintaining the temperature to 210°C for 10 min. The injection volume was 1 μl.

Total phenols

Total phenols (TP) were determined spectrophotometrically by using Folin-Ciocalteu reagent (Gutfinger, 1981). 2.5 g of olive oil was dissolved in 5 ml n-hexane and phenol compounds extracted with 5 ml of a methanol:water mixture (60:40. v/v). Afterwards the solution was shaken vigorously by a vortex for 2 min and centrifuged at 3500 rpm for 10 min. 0.2 ml methanolic phase was put into flask and completed with distilled water to 5 ml then 0.5 ml Folin-Ciocalteu was added to mixture. After 3 min, 1 ml of sodium carbonate solution (35 %, w/v) was added, mixed and diluted with distilled water to 10 ml. The mixture allowed standing for 2 hours. The absorbance of the solution was measured after 2 hours against a blank sample by spectrophotometer (UV-1700, Shimadzu, Japan) at 725 nm. The results were expressed in mg caffeic acid/kg oil.

Oxidative stability

Oxidative stability was determined according to the Rancimat Method (Barmak et al., 2011) which evaluates the time (h) of resistance to oxidation of 3 g of oil samples exposed to a dry air stream (20 L h^{-1} air) at 120°C from measurements taken using a Rancimat 743 apparatus (Metrohm, Herisau, Switzerland). The inflection point of the curve was assigned as the Induction Period (IP).

Sensory analysis

Sensory analysis of the olive oil samples was carried out according to the method of described by International Olive Council (COI/T.20/Doc. No 15/2007). Analysis was performed by 10-12 selected and trained panelists from the panel of Olive Research Institute of Turkey that accredited by Turkish Accreditation Institution (TURKAK) and recognized by the IOC.

Statistical analysis

Statistical analysis was performed using JMP 7.0 software package program. The assays were carried out in duplicate. The discussion of the results is based on the analysis of variance applied

to each parameter using the test of Student's t to compare means and thereby the effect of the cultivar, ripening index and harvest year.

RESULTS AND DISCUSSION

Oil content

The change in oil content of Kilis Yağlık and Memecik olive cultivars during ripening period for two harvest years was given in Table 1. It was found that the change in oil content of olive fruits with ripening index was statistically insignificant *(P* >0.05*)*, and that of the cultivars and harvest year were significant *(P* <0.05*)*. The oil content of Kilis Yağlık cultivar was higher than Memecik cultivar. The oil content of native olive cultivars of the Southeastern Anatolian Region was generally higher than the other olive cultivars (Yavuz, 2008). The oil content of olives harvested in the second year was significantly higher than the first harvest year. That may be attributed to the more rainy days in the second harvest year. The oil content of olive fruits may vary depending on some parameters such as cultivar, harvest year, climate conditions, and harvest time (Lavee and Wonder 1991).

Table 1. Cultivar, ripening stage, harvest year, sample code, ripening index and oil content of olive fruits

Cultivar	Ripening Stage	Harvest Year	Sample Code	RI	Oil Content (%)
Kilis Yağlık (K)	Beginning(B)	2012/13(1)	KB1	2.04	59.37
	Medium(M)		KM1	3.96	64.89
	End(E)		KE1	4.80	73.55
	Beginning(B)	2013/14(2)	KB2	2.08	68.99
	Medium(M)		KM2	3.96	70.02
	End(E)		KE2	4.88	70.64
Memecik (M)	Beginning(B)	2012/13(1)	MB1	1.96	38.98
	Medium(M)		MM1	4.09	52.87
	End(E)		ME1	4.75	50.28
	Beginning(B)	2013/14(2)	MB2	1.92	56.40
	Medium(M)		MM2	3.87	51.05
	End(E)		ME2	4.79	59.46

Quality characteristics

Free fatty acidity, peroxide value, and UV spectrophotometric indices (K_{232}, K_{270}) of olive oil samples were presented in Table 2. The changes in free fatty acidity, peroxide value *(P* <0.01*)* and K_{270} *(P* <0.05*)* with cultivars, ripening index and harvest year were statistically significant, while these parameters did not display a clear trend during ripening. All oil samples remained within the limits of free fatty acidity (<0.8%), peroxide value (<20meq O_2 kg^{-1}) and UV spectrophotometric indices (K_{270}<0.22, K_{232}<2.5) determined by International Olive Council for extra virgin olive oil (COI/T.15/NC No: 3/2015). It is obvious that olive fruits were hand-picked and then processed immediately, olives were not exposed to hydrolytic and oxidative damage.

Fatty acid composition

The changes in mean values of palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3) acids and MUFA/PUFA ratio of olive oil samples were shown in Table 3.

The changes in palmitoleic, stearic, oleic, linoleic *(P* <0.01*)*, linolenic *(P* <0.05*)* acids and MUFA/PUFA ratio *(P* <0.01*)* of olive oil samples were significantly affected by cultivar, ripening index and harvest year (Table 3). Oleic acid was the most abundant fatty acid never less than 66.80% and it was generally increased with the exception of the second harvest year for Kilis

Yağlık olive oil. Kilis Yağlık olive oil also consists of higher oleic acid and lower linoleic acid and linolenic acid when compared to the Memecik oil. Oleic acid of Kilis Yağlık olive oil was found to be similar with the results of the study of Dıraman and Yüksel (2010).

All oil samples remained within the limits of fatty acid levels determined by International Olive Council for extra virgin olive oil (COI/T.15/NC No:3/2015).

Table 2. Quality characteristics of olive oil samples

Sample Code	FFA (oleic a. %)	PV (meq O_2/kg)	K_{232}	K_{270}	TP (mg caffeic a./kg)	IP (120°C, h)
KB1	0.60^{a}	13.55^{a}	1.60	0.15^{abc}	256de	10.40
KM1	0.26^{f}	6.38^{bc}	1.45	0.10^{e}	321^{cd}	13.34
KE1	0.39^{d}	5.85^{cde}	1.56	0.12^{de}	239^{ef}	10.99
KB2	0.33^{e}	5.12^{ef}	1.82	0.16^{a}	593^{a}	13.98
KM2	0.52^{bc}	6.62^{bc}	1.78	0.15^{ab}	525^{b}	11.73
KE2	0.51^{c}	6.23^{bcd}	1.63	0.13^{cd}	332^{c}	8.60
MB1	0.22^{g}	6.81^{b}	1.56	0.13^{cd}	131^{gh}	6.95
MM1	0.40^{d}	3.28^{g}	1.49	0.13^{bcd}	300^{cde}	8.59
ME1	0.28^{f}	6.09^{bcd}	1.52	0.12^{d}	184^{fg}	8.23
MB2	0.54^{b}	4.63^{f}	1.39	0.13^{cd}	116^{h}	5.71
MM2	0.35^{e}	6.92^{b}	1.54	0.12^{de}	109^{h}	4.39
ME2	0.39^{d}	5.38^{def}	1.43	0.12^{de}	159^{gh}	5.80
	**	**	NS	*	**	NS

Values are mean of two measurements. Different letters in the same column indicate significant differences. NS, not significant, (P> 0.05*); *P<* 0.05*; **P<* 0.01.

Table 3. Fatty acid composition and MUFA/PUFA ratio of olive oil samples

Sample Code	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	MUFA/ PUFA
KB1	13.19	0.89^{f}	4.33^{c}	71.68^{b}	6.17^{h}	0.60^{f}	11.03^{a}
KM1	12.70	0.84^{fg}	4.52^{b}	71.73^{ab}	6.60^{g}	0.54^{g}	10.45^{b}
KE1	12.13	0.72^{h}	5.03^{a}	72.16^{ab}	6.49^{gh}	0.59^{f}	10.54^{b}
KB2	14.95	0.87^{f}	3.85^{e}	70.34^{d}	7.86^{f}	0.61^{f}	8.47^{c}
KM2	14.85	0.89^{f}	3.86^{e}	69.04^{e}	9.17^{cd}	0.54^{g}	7.28^{d}
KE2	14.08	0.80^{g}	4.13^{d}	67.88^{f}	11.12^{b}	0.54^{g}	5.94^{f}
MB1	14.57	1.33^{a}	1.77^{g}	68.54^{ef}	8.89^{d}	0.98^{b}	7.43^{d}
MM1	13.50	1.06^{e}	1.89^{f}	66.80^{g}	12.68^{a}	0.85^{e}	5.21^{g}
ME1	12.94	1.12^{cd}	1.86^{f}	71.48^{bc}	8.32^{e}	0.95^{bc}	8.14^{c}
MB2	14.65	1.19^{b}	1.77^{g}	70.74^{cd}	9.48^{c}	1.03^{a}	6.90^{e}
MM2	14.26	1.16^{bc}	1.85^{f}	71.78^{ab}	8.94^{d}	0.92^{cd}	7.45^{d}
ME2	13.48	1.08^{de}	1.87^{f}	72.52^{a}	9.06^{d}	0.88^{de}	7.45^{d}
	NS	**	**	**	**	*	**

*Values are mean of two measurements. Different letters in the same column indicate significant differences. NS, not significant, (P> 0.05); *P< 0.05; **P< 0.01.*

The ratio of MUFA/PUFA has a great importance because of the effects on nutritional properties and oxidative stability of olive oils. MUFA/PUFA ratio of Kilis Yağlık oil was found to be higher than that of Memecik oil, while this ratio was slightly decreased during the ripening period.

Total phenols

The amount of phenolic compounds in virgin olive oil is an important factor when evaluating its quality given that the natural phenols improve its resistance to oxidation, and to certain extent are responsible for its sharp bitter taste (Bendini et al., 2007). It is found that the total phenols in olive oil samples were significantly affected by cultivar, ripening index and harvest years ($P < 0.01$) (Table 2). The total phenols in Kilis Yağlık oil were higher than that of Memecik oil. In general, the amount of total phenols was found to be high in december (medium maturity level). The amount of total phenols gradually increased to the half-pigmentation stage and reached to the maximum level then rapidly decreased as the ripening proceeded (Salvador et al., 2000; Rotondi et al., 2004; Beltrán et al., 2005; Benito et al., 2012; Köseoğlu et al., 2016). The increase in the total phenols at the last harvest period observed in some cases which is responsible for the corresponding increase in oxidative stability could be due to the reduction in water content observed with ripening; this can affect the extraction of partially soluble compounds (Salvador et al., 2000). The total phenols of the olive oils belonging the second harvest year were significantly higher than of the olive oils in the first harvest year ($P < 0.01$). Béltran et al., (2005) and Benito et al., (2012) have also reported that there was a difference between years in terms of total phenols.

Oxidative stability

Oxidative stability was an important property of olive oil which was improved by synergist effects between phenolic and non-phenolic antioxidants and lipid composition. Induction time of oil samples was given in Table 2. It is found that the effect of cultivar and harvest year on the induction period was statistically significant ($P < 0.01$), and that of ripening index was insignificant ($P > 0.05$). Induction period of Kilis Yağlık olive oil was found to be higher than that of the Memecik olive oil. Moreover, the relationship of induction period and total phenols was statistically significant ($P < 0.01$, $R^2=0.82$) (Table 4).

Table 4. Pearson correlation coefficients

	Ripening Index	Induction Period	Total Phenols	MUFA/ PUFA	Bitterness	Pungency
Ripening Index	1.00**	-0.07	-0.04	-0.15	-0.14	-0.16
Induction Period	-0.07	1.00**	0.82**	0.49	0.69**	0.79**
Total Phenols	-0.04	0.82**	1.00**	0.05	0.94**	0.91**
MUFA/PUFA	-0.15	0.49	0.05	1.00**	-0.04	0.10
Bitterness	-0.14	0.69**	0.94**	-0.04	1.00**	0.94**
Pungency	-0.16	0.79**	0.91**	0.10	0.94**	1.00**

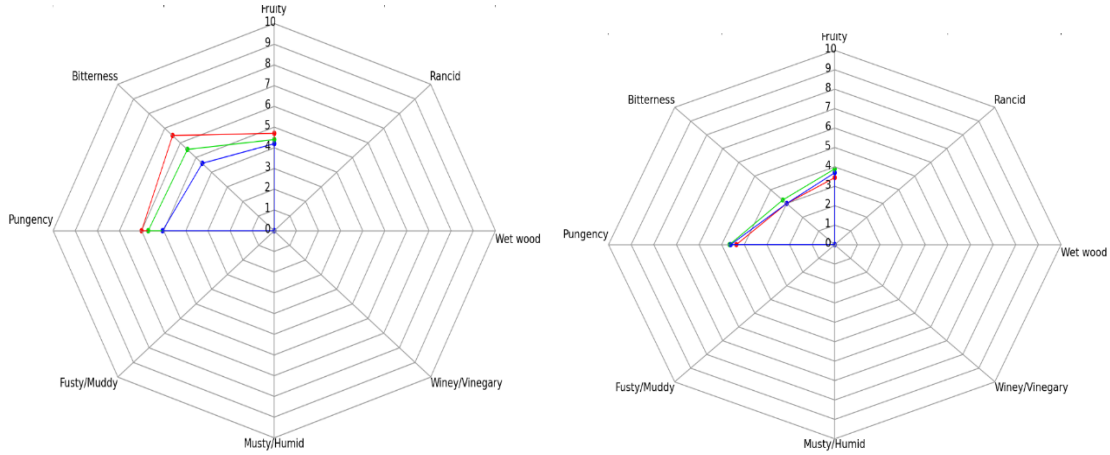
*** Correlation is significant at the* 0.01 *level (P<* 0.01*).*

Sensory profile

The sensory profile of Kilis Yağlık and Memecik olive oils was shown in Figure 1 and Figure 2, respectively.

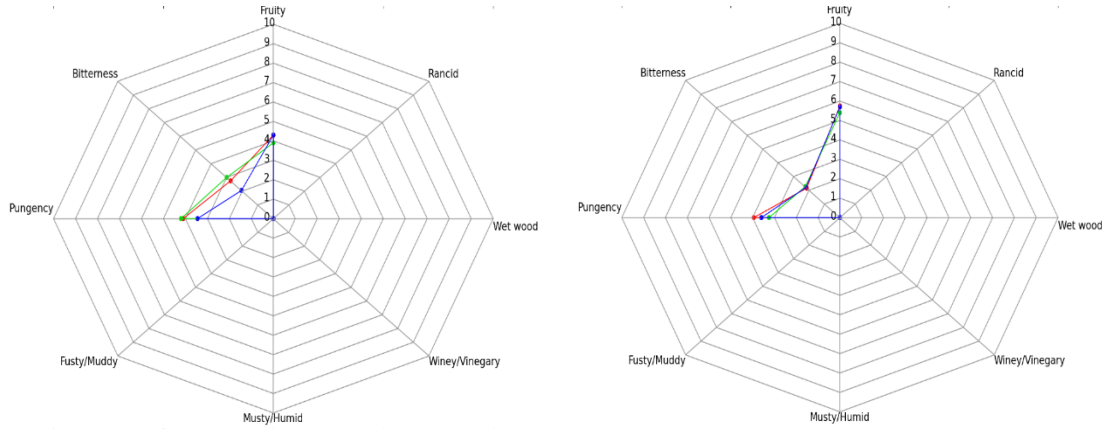
The olive oil samples were in the category of extra virgin olive oil according to European Union (EU/61/2011). No sensory defects were perceived in any of the samples studied and the fruitiness intensity of olive oil samples were perceived as greater than "0" by the panelists. Fruitiness medians of samples ranged from 3.73 to 5.75. It is found that the cultivar and ripening index on the fruitiness intensity of olive oils were statistically insignificant ($P > 0.05$), that of harvest year was statistically significant ($P < 0.05$).

Fruitiness medians of the olive oils in the second harvest year were higher than the olive oil samples in the first harvest year. The fruitiness medians of the samples generally showed a slight decrease as maturation progressed. Gutiérrez et al., (1999) have also reported that fruitiness intensity remained constant during maturation but a slight decrease has been observed on fruitiness of olive cultivar in the last phase of maturation.



2012/13 2013/2014

Figure 1. The changes in sensory profiles of Kilis Yağlık olive oil with ripening index and harvest year (–beginning in November, –medium in December, –end in January)



2012/13 2013/2014

Figure 2. The changes in sensory profiles of Memecik olive oil with ripening index and harvest year (–beginning in November, –medium in December, –end in January)

The olive oil bitterness medians varied between 2.03 and 6.48. It is found that the effect of cultivar, ripening index, and harvest year on the bitterness intensity of olive oils was statistically significant *(P* <0.05*)*. The bitterness median of the oil samples was the highest in at "the medium maturity level" in december. The bitterness median of the Kilis Yağlık olive oil was found to be higher than that of the Memecik olive oil.

The pungency medians of samples varied between 3.25 and 6.0. It is found that the effect of ripening index and harvest year on the pungency of olive oils was statistically insignificant *(P* >0.05*),* that of cultivar was statistically significant *(P* <0.05*)*. Pungency median of the Kilis Yağlık oil was found to be higher than that of the Memecik oil. The pungency medians of the samples generally showed a slight decrease in both years as maturation progressed. Previous study on two important Spanish olive cultivars showed that pungency intensity of olive oil samples remained constant as the maturation progressed (Gutierrez et al., l999).

Moreover, other positive attributes were perceived in olive oils by panelists such as floral, bitter almond and spicy. The floral and bitter almond attributes were more intense in the Memecik oil while the spicy attribute had higher intensity in the Kilis Yağlık oil.

In the present study, it was also observed a positive correlation between total phenols and the bitter (*P* <0.01*,* R^2= 0.94) and pungent (*P* <0.01*,* R^2= 0.91) sensory attributes (Table 4). Having knowledge about the chemical properties of olive oil allows us to have an idea about its sensory properties and vice versa (Mailer and Beckingham, 2006). The contribution of total phenol contents to the bitterness and pungency of positive sensory properties of olive oil has been shown in many studies (Rotondi et al., 2004; Nieto et al., 2010; Benito et al., 2012; Rivas et al., 2013). Benito et al., (2012) have reported that fruitiness, bitterness and pungency scores increased up to a maximum coinciding with the maximum total phenol content and then decreased. It is known that phenolic compounds are responsible for the bitterness and pungency of oils (Andrewes et al., 2003; Benito et al., 2012).

CONCLUSIONS

These results provide information on how different olive cultivars behave at different harvest time and harvest year during ripening. The results showed that, all of the olive oil samples were of extra virgin olive oil quality in terms of their chemical end sensory properties. The changes in palmitoleic, stearic, oleic, linoleic, linolenic acids, MUFA/PUFA ratio and total phenols content of olive oil samples were significantly affected by cultivar, ripening index and harvest year. It was found that the effect of cultivar on sensory properties of olive oils such as bitterness and pungency was statistically significant. Bitterness medians of the oil samples were the highest at the medium harvest period. It was observed a positive correlation between total phenols and the bitter and pungent sensory attributes. The optimum harvest time for the production of extra virgin olive oil was determined as at "medium maturity level" in december for Kilis Yağlık and Memecik cultivars.

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