Changes in Chemical Composition and Olive Oil Quality of Turkish Variety 'Kilis Yağlık' with Regard to Origin of Plantation

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Abstract: 'Kilis yağlık' is the most common variety cultivated in the South-east region of Turkey and represents around 52% of the growing area. During the last few years new cultivars have been introduced in South regions being mostly the cultivar "Gemlik" without evaluating their behaviours in this environment. Unfortunately, it is now understood that this cultivar with good plantation characteristics did not adapt well to the environmental conditions of South Turkey. This has led to an increase in the need of research on traditional autochthonous cultivars. 'Kilis yağlık' although shows alternate; has a high yield of fruits. The aim of this work is was to study changes in virgin olive oil composition of 'Kilis yağlık' variety according to origin of plantation. Olives from this variety were collected in four characteristic and representative olive growing locations in South-east of Turkey. The analytical parameters studied were fatty acid composition, total phenolics, chlorophylls and carotenoids, free acidity, peroxide value, colour indexes and some individual phenolic compounds. The contents of some of the individual phenolics (tyrosol, hydroxyl tyrosol, oleuropein, 4-hydroxyphenyl acetic acid, 3-4 hydroxy benzoic acid, taxifolin, verbascoside, vanillic acid, luteolin, apigenin and rutin) were determined with a qualitative and quantitative analysis performed by HPLC-DAD.

Keywords: Olive oil, Kilis yağlık variety, phenolics, growing area.

1. INTRODUCTION

Turkey is one of the most important olive oil producing countries, coming after Spain, Italy, Greece and Tunusia [1]. Annually 112,000 tons of olive oil are produced in Turkey, and approximately 70% of it exported to other countries. Most of the production occurs in the Aegean, Marmara, Mediterranean and Southeastern Anatolia regions of the country. The Aegean region yields 80.5% of olive production, followed by 11.8% in Mediterranean, 6.1% in Marmara and 1.6% in Southeastern Anatolia regions of Turkey [2].

In Southeastern Anatolia region of Turkey; Gaziantep, Kilis, Adıyaman, Şanlıurfa, Mardin are the locations where olive plant is cultivated. High air temperatures and inadequate rainfall had a negative impact on the grow up of olive production in this region, even the region has a great potential on olive plant production. The region covers 4.9% of olive fruit production of Turkey. Kilis yağlık is the most common variety comprising 52% of total olive trees cultivated in this region.

The geographical origin of this variety is Kilis. It is characterized by medium vigor, is resistant to frost, produces medium-sized fruit and with high productivity; the plant shows high alternation. Fruits are generally used in olive oil extraction.

Little is known about the nature and/or concentrations of minor components and the chemical composition of the oils from Kilis yağlık variety grown in Southeastern region of Turkey. Thus, this study was undertaken to evaluate the chemical composition of the oil from this variety by analyzing several quality-related parameters (e.g., tocopherols, phenolic compounds and fatty acids).

2. MATERIALS AND METHODS

2.1. Olive Origin

The olive samples were collected in the 2006 and 2007 crop seasons from different olive groves located in the olive growing countryside areas of southeastern region of Turkey. The olive samples were collected from the olive trees in triplicate all located in close orchards and which benefited from the same cultural practices. The ripening indexes of olives ranged between 4.5-6. The characteristics of production area of olive varieties studied are shown in Figure **1**. Climatic data (temperature, rainfall and humidity) for the experimental years 2006 and 2007 were obtained from the TURKISH STATE METEOROLOGICAL SERVICE [3]. Olive fruits were handpicked. 5 kg of olives per variety were collected. After harvesting, the olive fruit samples were immediately transported to the laboratory, where the oil was extracted within 24h.

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2.2. Oil Extraction

The olives were washed and deleafed, crushed with a hammer crusher and the paste was mixed at 25°C for 20 min. The paste was pressed with a stainless steel manual press and the oil was extracted by means of a laboratory basket centrifuge (6,000Xg for over 5 min) without addition of warm water and then transferred into dark glass bottle. All samples were stored at 4°C in darkness using amber glass bottles without headspace until analysis.

2.3. Oil Sample Analysis

Free fatty acids and peroxide value of olive oils were determined following the analytical methods described in REGULATION EUROPEAN ECONOMIC COMMISSION [4].

2.4. Determination of Tocopherols

Tocopherols were evaluated according to IUPAC 2432 method [5]: 1.5 g oil was dissolved in 10 mL hexane and injected into the HPLC system with a LiChroCART, Si 60 column (25cm×4mm×5μm) (Merck, Darmstadt, Germany). The chromatographic separation was performed using a Shimadzu liquid chromatograph equipped with an isocratic pump LC-20AT prominence, a CTO-10AS VP heater (column temperature 22°C), a SIL-20A prominence autosampler and a SPD-M20A Prominence diode-array detector (Shimadzu, Kyoto, Japan). The mobile phase was 0.5% isopropanol in n-hexane. The total run time was 40 min and the injection volume was 20 µL. The detector was a DAD operated at a fixed wavelength of 295 nm. Tocopherols were quantified by an external standard method; α-, β-, γ- and δ-tocopherol standards were obtained from Sigma-Aldrich (St. Louis/MO).

2.5. Fatty Acid Analysis

For the determination of fatty acid composition of the oils, fatty acid methyl esters were prepared from olive oil, using a cold transmethylation [6]. The fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2-g oil and 3 mL of hexane with 0.4 mL of 2-N methanolic potassium hydroxide. A Shimadzu (Kyoto, Japan) gas chromatograph, equipped with a flame ionization detector and a split/splitless injector, was employed. Separations were made on a Teknokroma TR-CN100 (Barcelona, Spain) fused-silica capillary column (60 m·0.25 mm i.d.· 0.20 μm film thickness). The carrier gas was nitrogen, with a flow rate of 1 mL/min. The temperatures of the injector and the detector were held at 220 and 250°C, respectively. The initial oven temperature of 90°C was maintained for 7 min., raised to 240°C at a rate of 5°C/min, where it was maintained for 15 min. The injection volume was 1 µL. Peaks were identified by comparison of their retention times with those of authentic reference compounds (Sigma–Aldrich, St. Louis, MO, USA).

2.6. Carotenoids and Chlorophylls

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values, according to the method of MINGUEZ-MOSQUERA *et al*. [7].

2.7. Extraction of Phenolic Compounds

The extraction was performed according to the procedure described by PIRISI *et al*. [8]. Briefly, 2 g of oil were weighed into a centrifuge tube, added with 1 ml of *n*-hexane and 2.0 ml of methanol–water (60:40, v/v). Gallic acid (0.5 mL, 100 mg/L) was added to the oil as an internal standard. The mixture was stirred for 2 min in a vortex apparatus, and the tube was centrifuged at 3000 rpm /min for 5 min. The methanol layer was separated and the extraction repeated twice. The methanolic extracts were combined and evaporated to dryness under reduced pressure at a temperature not exceeding 35°C. Samples were dissolved in 1 ml of methanol–water (1:1, v/v) and filtered through a 0.45 μm nylon filter to be used for HPLC analysis as well as for determination of total phenols and antioxidant activity assays.

2.8. HPLC Analysis of Phenolic Compounds

The extracted phenolic fractions were analyzed by HPLC. The HPLC system included a LC 10A vp, an LC-20AT prominence pump, a CTO-10AS VP heater (column temperature 22°C), a SIL-20A prominence autosampler and a SPD-M20A Prominence diode-array detector (Shimadzu, Kyoto, Japan). The column was an Inertsil ODS-3 (5μm, 25cm×4.6mm i.d.) (GL Sciences, Tokyo, Japan). PC running Class VP chromatography manager software (Shimadzu, Japan) was used and chromatograms were obtained at 240, 280 and 320 nm. The eluents were a 2% aqueous formic acid solution and methanol, the flow rate was 0.85 mL/min, and the injection volume 40μL. The total run time was 76 min. Quantification was carried out by a four-point regression curve on the basis of standards obtained from commercial suppliers.

Table 1: Some Chemical and Physical Properties of Kilis yağlık Variety Oils from Different Locations

	Kilis		Maraş		Urfa		Antep	
	2006	2007	2006	2007	2006	2007	2006	2007
Alpha tocopherol	438.21±14.51*c*[‡],g	314.15±8.63b,h	365.89±29.98*b*	427.13±59.12c	275.35±21.01*a*,g	421.43±40.96c,h	232.11±46.41*a*	156.81±41.59a
Beta tocopherol	1.68±0.05*c*	1.39±0.30c	1.90±0.36*c*,g	0.01±0.01a,h	0.45±0.13*a*,g	1.32±0.38c,h	0.12±0.03*b*,g	0.59±0.11b,h
Gama tocopherol	6.58±0.38*c*,g	1.13±0.08b,h	2.89±0.63*b*,g	0.00±0.00a,h	0.00±0.00*a*	0.00±0.00a	0.00±0.00*b*	1.34±0.39a
Delta tocopherol	0.02±0.01*a*	0.03±0.00 b	0.63±0.08*c*,g	0.04±0.01b,h	0.00±0.00*b*	0.00±0.00a	0.00±0.00*b*	0.00±0.00 a
chlorophyll	7.36±0.94*ab*	6.79±1.01a	13.45±1.20*c*,g	9.70±0.66b,h	8.53±0.56*b*	8.90±0.50b	6.33±0.70*a*,g	10.01±0.38b,h
carotenoid	8.37±0.46*b*	7.59±1.15a	10.96±0.56*c*,g	6.58±1.26a,h	4.77±0.53*a*	5.89±1.40a	8.20±2.31*b*	7.33±1.77a
Peroxide value	5.62±0.70*b*	5.11±1.06a	2.33±0.24*a*,g	5.24±1.16a,h	6.57±0.66*b*	4.88±1.82a	5.67±0.81*b*	6.85±0.96a
Free acidity	0.57±0.03 *a*,g	0.50±0.03b,h	0.46±0.05*b*	0.43±0.11ab	0.36±0.03*c*	0.33±0.05a	0.86±0.06*d*,g	0.67±0.07c,h
L*	76.89±1.14*a*	77.96±1.85b	79.29±1.20*ab*,g	74.09±2.19ab,h	77.82±0.77*ab*,g	70.85±0.91ab,h	79.91±1.65*b*,g	69.53±7.10a,h
a*	-12.08±0.06*a*,g	-4.14±1.83c,h	-7.79±0.15*b*,g	-6.43±0.27b,h	-10.88±1.46*a*	-11.18±0.17a	-7.05±1.17*b*	-5.67±0.74bc
b*	47.59±1.49*a*,g	15.00±5.46 a,h	25.72±1.33*b*	26.20±2.95b	30.83±1.99*c*,g	53.00±0.63c,h	21.98±1.02*d*	33.82±15.61b

mean value±standard deviation.

a, b, c, d, e: Mean values of the the same crop year with a different superscript differ significantly (P≤0.05) [comparison between locations].

g, h : Mean values of the same location with a different superscript differ significantly (P≤0.05) [comparison between crop years].

[‡]Italic letters indicate significant differences between locations in the year 2006, normal letters indicate significant differences between locations in the year 2007.

2.9. Statistical Analysis

All parameters analyzed were determined in triplicate and reported as mean values of the three replicates and standard deviations. One-way analysis of variance was used to evaluate variety and harvest time depended differences regarding the parameters analyzed. In case of significance, differences between mean values of specific varieties and harvest times were evaluated using the Duncan's new multiple range test [9].

3. RESULTS AND DISCUSSION

3.1. Free Acidity and Peroxide Value, Chlorophyll and Carotenoids

The free acidity values of the oils varied between 0.33-0.86%, which were below the limit for extra VOO (Table **1**). The free acidity values were apparently affected by growing area of olives. The oils from Antep and Kilis had higher acidity values when compared to other location oils, while the oils from Urfa showed lowest free acidity values.

Peroxide values of the samples were in the range of 2.33-6.85 meqO_2/kg oil. There were not significant differences between the peroxide values of oil samples from three different locations, only with the exception of Maraş oils which had lower values in previous harvest year.

Apart from cultivation area or cultivar, factors causing damage to the fruits, such as olive fly attacks, improper systems of harvesting, carriage and storage of the olives, and by technological treatments, which may favor the hydrolysis of triglycerides, resulting in an increase of the free fatty acid concentration were reported to have the most significant influence on these quality parameters [10].

The oils from Kilis location showed lower chlorophyll content (6.37-13.45 mg/kg). The carotenoid level of Maraş oils was twice higher than the levels of Urfa oils in 2006, while in 2007 the variation was minimal among the locations where there were no statistically significant differences.

Generally, the differences in carotenoid content of oils from different locations between the two consecutive crop years were not statistically significant, only Maraş samples showed lower content in the following crop year.

The oils from Kilis location showed higher free acidity, peroxide values and carotenoid level as well, while the oils from this location had lower levels of chlorophylls than the other locations.

Urfa oils showed higher peroxide values. Maraş samples had higher carotenoid levels with lower free acidity when compared to the values of the remaining oil samples.

3.1. Color

The color measurement by tristimulus coordinates CIELAB (L*, a* and b*) of the oils showed significant differences related to location (Table **1**). Urfa oils had lower L* values (darker) and b* values (which means darker colour in terms of yellowness) than the other location oils. The values of a* were found in the green zone, the oils from Urfa location together with Kilis oils showed the highest a* values pointing to a darker colour in terms of greenness. The color changes were mainly attributed to the drop in the ratio of chlorophylls/carotenoids and that both, the carotenoid and, above all, the chlorophyll content diminish along the ripening process which is in line with the observation of MOYANO *et al*. [11]. The a* and b* (except Kilis oils) values decreased in the oils from next harvest year, whereas L* values were higher for the oils from 2007 harvest when compared to the values from the previous year's harvest.

3.2. Fatty Acid Composition

As shown in Table **2**, palmitic, oleic and linoleic acids were measured as major fatty acids. Linoleic, oleic and stearic acid ratios was higher in Urfa oils than the ratios of other location oils. Urfa had the lowest precipitation among the locations studied in this assay. The change in fatty acid ratios regarding the harvest year was lower for the oils from this location.

As compared to the oils of Urfa, Kilis and Antep locations, Maraş location produced oils with lower levels of oleic, linoleic and linolenic acids and higher palmitic and palmitoleic acids. Maraş had higher levels of rainfall than those of other locations.

Olive fruits from cooler areas were also reported to contain oil with more unsaturated fatty acids than the fruits from dry and warm areas [12,13].

But in this study oleic and linoleic acids were found at higher concentrations in oils from Urfa, which has higher average air temperatures; but is located at a considerable higher altitude than the other locations.

3.3. Tocopherols

The contents of tocopherols are shown in Table **1**. α-Tocopherol amounts of oils from Kilis and Maraş locations were close, as the mean levels were 300 and 400 mg/kg. Oils of Urfa location contained the lowest levels of total tocopherols.

Previous studies reported the α-tocopherol contents of olive oils from many cultivars of Mediterranean countries as 132-261 μg/g [14], 141.94-364.23 mg/kg [15], 193.7-349.7 mg/kg [16], 240-480 mg/kg [17]. BACCOURI *et al*. [18] reported the α-tocopherol contents of some Tunisian varieties at three different ripening stages as 121, 274 and 250 for Chetoi and as 321, 329 and 214 for Chemlali. Thus, the α-tocopherol contents of the oils can be considered high.

Table 2: Fatty Acid Composition of Kilis yağlık Variety Oils from Different Locations

	Kilis		Maraş		Urfa		Antep	
	2006	**2007**	**2006**	**2007**	**2006**	**2007**	**2006**	**2007**
Palmitic	14.44±0.34*_b_[‡]	14.41±0.13a	16.00±1.16 _c_	15.77±1.42a	11.95±0.29_a_,g	14.66±0.22a,h	13.29±0.84_ab_	13.96±0.74a
Palmitoleic	0.85±0.08	0.89±0.04b	1.00±0.47	0.97±0.34b	0.82±0.29	0.68±0.21a	0.99±0.13	1.05±0.11c
Stearic	2.14±0.56_b_	2.76±0.82b	3.32±0.83_a_	3.57±0.93ab	3.66±1.39_a_	3.98±0.11a	1.75±0.57_c_,g	4.00±0.76a,h
Oleic	69.63±3.62_b_	67.54±1.29a	66.93±3.53_c_	66.83±1.34a	74.25±3.03_a_,g	67.20±1.66a,h	71.73±1.49_b_	68.31±2.02a
Linoleic	8.87±1.89_a_	10.09±0.72a	8.10±1.01_a_	8.44±1.95a	6.82±0.37_a_,g	10.90±1.75a,h	8.05±1.00_a_	9.14±1.99a
Linolenic	1.50±0.08_b_,g	1.13±0.16a,h	1.06±0.13_a_	0.92±0.07a	0.94±0.07_a_,g	1.45±0.08 b,h	1.63±0.19_b_,g	0.96±0.68ab,h

*mean value±standard deviation

a, b, c, d, e: Mean values of the the same crop year with a different superscript differ significantly (P≤0.05) [comparison between locations]

g, h : Mean values of the same location with a different superscript differ significantly (P≤0.05) [comparison between crop years]

[‡] Italic letters indicate significant differences between locations in the year 2006, normal letters indicate significant differences between locations in the year 2007.

Table 3: Phenolic Compounds of Kilis yağlık Variety Oils from Different Locations (mg/kg)

	Kilis		Maraş		Urfa		Antep	
	2006	2007	2006	2007	2006	2007	2006	2007
Rutin	0.40±0.05a,g‡	0.10±0.05b,h	2.30±0.15b,g	1.30±0.25c,h	0.00±0.00c	0.00±0.00a	4.90±0.40d,g	1.00±0.20b,h
Oleuropein	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.15±0.05b,g	0.00±0.00a,h	0.00±0.00a,g	4.10±0.60b,h
Ferulic acid	0.00±0.00a	0.00±0.00a	0.05±0.00b,g	0.00±0.00a,h	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
H.phenyl carboxylic	0.05±0.00a,g	0.20±0.05a,h	0.25±0.05b,g	0.70±0.10b,h	0.40±0.05c,g	0.15±0.05a,h	0.05±0.00a,g	0.15±0.05a,h
Luteolin	1.75±0.50a,g	0.35±0.05a,h	1.15±0.35a,g	0.15±0.05b,h	3.25±0.65b,g	2.20±0.45c,h	1.35±0.30a	1.10±0.25d
Apigenin	0.10±0.05b,g	0.00±0.00a,h	0.05±0.05ab	0.05±0.05b	0.10±0.05b,g	0.05±0.05b,h	0.05±0.00a	0.05±0.00b
Cinnamic acid	0.25±0.05a	0.20±0.00b	0.15±0.05b,g	0.65±0.10c,h	0.15±0.05b,g	0.40±0.05a	0.20±0.05a,g	0.75±0.15c,h
4 H.phenyl acetic acid	3.20±0.30a,g	4.70±0.40c,h	5.50±0.65b,g	1.40±0.20b,h	4.20±0.70b,g	0.30±0.05a,h	20.45±2.15c,g	7.40±2.15d,h
3-4 H.benzoic acid	2.20±0.20c,g	0.15±0.05a,h	0.65±0.05b,g	0.25±0.00b,h	0.05±0.00a	0.05±0.00c	0.65±0.05b,g	0.05±0.00c,h
Caffeic acid	0.05±0.00a	0.05±0.00a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.05±0.00a	0.05±0.00a
Verbascoside	0.80±0.37a,g	0.00±0.00a,h	0.15±0.00a,g	0.05±0.00b,h	0.15±0.05a	0.20±0.05c	0.15±0.00a	0.10±0.05bc
Chlorogenic acid	0.20±0.05a,g	0.05±0.00a,h	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
H-tyrosol	5.35±0.60d,g	0.76±0.09a,h	0.40±0.04a,g	3.53±0.25b,h	1.07±0.13c,g	0.83±0.13a,h	0.90±0.08b,c	1.02±0.07a
p-coumaric acid	0.00±0.00a,g	0.10±0.00a,h	0.05±0.00b,g	0.00±0.00b,h	0.35±0.05d,g	0.50±0.05c,h	0.70±0.10c,g	0.95±0.25d,h
Syringic acid	0.00±0.00a,g	0.05±0.00b,h	0.00±0.00a	0.00±0.00a	0.05±0.00b,g	0.15±0.05c,h	0.10±0.00c	0.10±0.00c
Taxifolin	0.80±0.20c,g	0.55±0.05b,h	0.10±0.00a,g	0.25±0.05a,h	0.25±0.05b	0.30±0.05a	0.35±0.05b,g	0.50±0.10b,h
Tyrosol	0.29±0.09a,g	0.16±0.00a,h	0.26±0.09a	0.17±0.02a	14.57±2.32c,g	6.25±1.48b,h	1.61±0.16b	1.43±0.26c
Vanillic acid	2.85±0.25b,g	0.45±0.05a,h	1.30±0.20a,g	0.50±0.00a,h	1.70±0.25a,g	0.65±0.05b,h	2.40±0.25c	2.20±0.25c

*mean value±standard deviation

a, b, c, d, e: Mean values of the the same crop year with a different superscript differ significantly (P≤0.05) [comparison between locations]

g, h : Mean values of the same location with a different superscript differ significantly (P≤0.05) [comparison between crop years]

‡ Italic letters indicate significant differences between locations in the year 2006, normal letters indicate significant differences between locations in the year 2007.

3.4. Phenolic Compounds

Oils of Urfa and Antep locations showed higher levels of tyrosol and oleuropein respectively, even the oils of these locations had lower levels of individual phenolics when considered in total (Table **3**). Generally the levels of phenolics diminished in the following crop year.

MOUSA *et al*. [19] attributed the higher phenol content in oil from Tarragona to the lower altitude of the growing region [20] but in the present study this was not the case as Antep location is at the highest elevation among the locations studied.

In conclusion, the oils from locations with higher rainfall and altitude had lower concentrations of oleic acid and phenolic compounds and higher concentrations of α-tocopherol.

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