

## Chemical Composition of Turkish Olive Oil—Ayvalik

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Received: 14 January 2008 / Revised: 16 November 2008 / Accepted: 20 November 2008 / Published online: 17 December 2008  
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**Abstract** Although large amounts of olive oil are produced in Turkey, not much information on its chemical composition is available in the literature to date. The aim of this study was to evaluate the chemical composition of commercial olive oils produced from the Ayvalik olive cultivar in Canakkale, Turkey. Five different samples corresponding to the olive oil categories of extra virgin (conventional, extra virgin olive oil (EVOO), and organic extra virgin olive oil (OGOO) production), virgin olive oil (OO-1), ordinary virgin olive oil (OO-2) and refined olive oil (RFOO) were evaluated. Olive oils were collected from two consecutive production years. According to the free fatty acids, the absorbance values ( $K_{232}$  and  $K_{270}$ ), and peroxide values of all the samples conformed to the European standards for olive oil. The level of oleic acid was in the range of 68–73%; while the linoleic acid content was significantly lower in the refined olive oils. The tocopherol and polyphenol content was in the lower range of some European olive oils. However, pinoresinol was a major phenolic compound (5–77 mg/kg depending on the oil category). Its content was markedly higher than in many other oils, which would be a useful finding for olive oil authentication purposes.

**Keywords** Turkish olive oil · Chemical composition · Harvest year · Tocopherol · Polyphenols · Pinoresinol

### Introduction

Olive trees have been grown along the Aegean coast of Turkey for over 8,000 years. This has had an impact on a wide range of olive growing regions and olive fruit cultivars in Turkey today. There are five distinct olive growing regions: the Aegean region, the Marmara Region, the Mediterranean Region, the Southeast Anatolia Region, and the Black Sea Region. Around 75–80% of the total olive oil production in Turkey is located within the Aegean region [1].

Ayvalik (Edremit Yaglik) is one of the major olive oil producing cultivars in the Turkish Aegean region. Although Turkey is the world's sixth largest producer of olive oil (FAO, 2006) [2] and uses different olive varieties for oil production (e.g. Ayvalik, Memeli, Memecik and Gemlik) data on the chemical composition of these oils is scarce.

The chemical characterization of olive oils from both Mediterranean and non-Mediterranean regions is essential for conducting a geographical characterization of the oil. Previous studies on olive oil were mainly done on Spanish, Italian, and French olive cultivars [3–5], and with only a few papers published on Tunisian olive oil [6]. However, despite these many studies on olive oil there is a lack of data elucidating the quality and chemical composition of olive oil produced and marketed in Turkey. Therefore, this study addresses the need to evaluate specific chemical properties of olive oil produced in Turkey. For this purpose, polyphenols contents were studied together with these of tocopherols. Moreover, their antioxidant activity

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was determined by evaluating the ferric reducing ability (ferric reducing ability of plasma, FRAP) of the samples. The quality of the olive oil samples and the content of the fatty acids, phenolics and tocopherols were compared between two consecutive harvest years.

## Experimental Procedures

### Olive Oil samples

Ten commercial Ayvalik (Edremit yaglik) olive oil samples were produced from olives grown in the olive growing region of Canakkale, Turkey during two harvesting seasons: 2005/06 and 2006/07. The extra virgin olive oil (EVOO), organic extra virgin olive oil (OGOO), virgin olive oil (OO-1), and ordinary virgin olive oil (OO-2) samples were obtained from a local producer, while the refined olive oil (RFOO) samples were supplied by another partner-company responsible for further refining. EVOO was produced to reach the demands of extra virgin olive oil, OGOO was produced from organically grown olives whereas OO-1 and OO-2 complied with the quality parameters of 2 g/100 g free acidity and 3.3 g/100 g free acidity both expressed as oleic acid, respectively. All olives were harvested during the period October–December by hand and immediately processed to olive oil in the plant. After processing with a 3-phase centrifugal decanter, unrefined oil samples were collected. For RFOO, all refining processes were carried out in the partner plant. Prior and during analyses, all samples were kept at +4 °C and were protected from light.

### Chemical Analyses

The acidity (% oleic acid) was measured by the AOCS official method Ca 5a-40 and spectrophotometric parameters ( $K_{232}$  and  $K_{270}$ ) by the European Union official methods (EC 796/2002). The peroxide value (PV) was determined as described in the AOCS method Cd 8-53, and expressed as milliequivalents (mequiv) of active oxygen per kilogram of oil.

### Fatty Acid Composition

Fatty acids methyl esters (FAME) were analyzed by gas chromatography (GC) to identify and quantify the individual fatty acid in the oils. The procedure was adapted from AOCS Official method Ce 1f-96.

Briefly, approximately 0.05 mg (3 drops) oil was weighed into a 10 mL tube, subjected to alkaline saponification (2 mL, 0.5 N NaOH) and flushed with nitrogen for 1 min. After heating the test tube for 7 min, it was cooled for 5 min.

Then BF<sub>3</sub>/methanol reagent was added to the tube and then closed. Prior to heating for 5 minutes, the tube was vortexed for 30 s. Internal standard (6 mL) and NaCl solution (5 mL) were added to the tube, flushed with nitrogen for 1 min, and again shaken for 30 s. Afterwards, adding small amount of dry MgSO<sub>4</sub> and shaking, the sample was subsequently diluted with isooctane into a vial until the end concentration of 1.5–1.8 mg/mL was reached and it was then injected into the GC. The content of each fatty acid was expressed as a percentage of fatty acid methyl esters.

The identification of FAME was carried out by Agilent Technologies 6890N gas chromatography using a 50-mm long capillary column CP-Sil 88 (Varian-Chrompack) with a flame ionization detector (FID). The column temperature was isothermal at 190 °C and the injector and detector temperatures were 220 °C. The carrier gas hydrogen was at 0.8 bar pressure and the quantity of the injection was 1 μL. All measurements were carried out in triplicate.

### Tocopherols

Tocopherols ( $\alpha$ -tocopherol) were analyzed according to the AOCS Official Method Ce 8-89.

### Polyphenols

The phenolic fractions of olive oil were isolated according to the method described by Mateos et al. [7]. The method is based on a solid-phase extraction (SPE) with SPE C18 diol-bond cartridges (Supelco, Bellefonte, PA). The extraction of each sample was performed in triplicate for each test using the extract of polyphenols for further analysis.

Phenolics were separated by reversed-phase high performance liquid chromatography (HPLC) and detected by both diode array UV detection system and mass spectroscopy (MS) using a validated chromatographic method by Andjelkovic et al. [8]. By this method detection was performed at 280 nm. The elution solvents used were A (0.2% acetic acid in water), B (methanol), C (acetonitrile). B and C solvents were mixed in a 50:50 (v/v). The flow rate was 1 mL/min and run time 72 min. The run was performed at 35 °C. The sample injection volume was 20 μL. Identification of compounds was achieved by comparing their retention time absorbance spectra, and  $m/z$  ratios to those of the standards. Individual phenolic compounds were quantified by using two internal standards. One was *ortho*-coumaric used for quantification of following compounds: hydroxytyrosol, tyrosol, *para*-coumaric acid, vanillin, vanillic acid, ferulic acid, luteolin and apigenin. For these compounds calibration curves were obtained by plotting the response ratio of the area of the reference compound over the area of the internal standard versus the concentration of the internal standard. The other internal standard

was *para*-hydroxyphenylacetic acid and was used for calculation of dialdehydic form of decarboxymethyl oleuropein aglycon, pinoresinol, 1-acetoxypinoresinol, aldehydic form of oleuropein aglycon and aldehydic form of ligstroside aglycon using the response factors as suggested by Mateos et al. (2001).

#### Total Phenols

The Folin–Ciocalteu method [9] was used for assessing the amount of total phenols in olive oil. The results were expressed as mg gallic acid equivalents per kilogram of olive oil (mg GAE/kg) after generating the calibration curve in the range 0–400 mg per liter of methanol.

#### Ferric Reducing Capacity

The ferric reducing ability (FRAP) assay was employed according to the procedure described by Benzie and Strain [10]. The ferric reducing capacity of phenolic extracts was expressed as mmol Fe<sup>2+</sup>/kg olive oil based on a calibration curve with aqueous solution of FeSO<sub>4</sub> in the range 0–1 mM ( $y = 0.6379x + 0.2464$ ;  $R^2 = 0.99$ ).

#### Statistical Analysis

All experiments were carried out in triplicate and results are expressed as means  $\pm$  standard deviation (SD). Statistical differences between the oil categories were estimated by applying one-way ANOVA and using the Tukey test at the significance level of 5% ( $P < 0.05$ ) for the pair-wise

comparisons between means of triplicate measurements. The software used for the statistical analysis was the SPSS for Windows statistical package (v.15.0.1; SPSS).

## Results and Discussion

#### Quality Indices

According to the quality indices (free fatty acids and UV absorption parameters,  $K_{232}$  and  $K_{270}$ ), all samples were in line with the limit values for EVOO, OO and RFOO set by the European Regulation on Olive Oils and Olive Pomace Oils (EC 796/2002). K-values showed some differences between the samples from 2 years, in particular in EVOO ( $K_{232; 2005} = 2.33$  and  $K_{232; 2006} = 1.90$ ;  $K_{270; 2005} = 0.56$  and  $K_{270; 2006} = 0.15$ ) and OGOO ( $K_{232; 2005} = 2.25$  and  $K_{232; 2006} = 1.80$ ;  $K_{270; 2005} = 0.31$  and  $K_{270; 2006} = 0.20$ ) samples. This could be attributed to the different storage time whereby samples from 2005 were stored 3 months longer prior to the analysis compared to the samples taken in 2006.

#### Fatty Acids

The distribution of fatty acids in the samples was in agreement with the European Standard for Olive Oils and Olive Pomace Oils (EC 796/2002) and the Codex Alimentarius (Codex Alimentarius, 2001). The major fatty acids of the Ayvalik cultivar olive oil are presented in Table 1. Although the linolenic acid content was clearly different among the oils from two different crop seasons, its content was always

**Table 1** Fatty acid composition (expressed in %) of Ayvalik olive oil determined in two consecutive years

	Year	EVOO	OGOO	OO-1	OO-2	RFOO
Palmitic acid, C16:0	2005	12.4 $\pm$ 0.1	12.8 $\pm$ 0.1	12.7 $\pm$ 0.2	13.1 $\pm$ 0.2	12.3 $\pm$ 0.1
	2006	13.2 $\pm$ 0.1	13.6 $\pm$ 0.3	13.2 $\pm$ 0.3	13.4 $\pm$ 0.3	12.3 $\pm$ 0.1
Palmitoleic acid, C16:1	2005	0.6 $\pm$ 0.0	0.5 $\pm$ 0.0	0.5 $\pm$ 0.0	0.6 $\pm$ 0.0	0.6 $\pm$ 0.0
	2006	0.7 $\pm$ 0.0	0.7 $\pm$ 0.0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.0	0.7 $\pm$ 0.0
Heptadecanoic acid, C17:0	2005	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0
	2006	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0
Heptadecenoic acid, C17:1	2005	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0
	2006	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0
Stearic acid, C18:0	2005	2.6 $\pm$ 0.0	2.5 $\pm$ 0.1	2.5 $\pm$ 0.1	2.7 $\pm$ 0.0	2.9 $\pm$ 0.0
	2006	2.5 $\pm$ 0.0	2.3 $\pm$ 0.1	2.6 $\pm$ 0.0	2.6 $\pm$ 0.1	2.8 $\pm$ 0.0
Oleic acid, C18:1	2005	72.3 $\pm$ 0.6	72.0 $\pm$ 1.2	71.6 $\pm$ 1.7	70.0 $\pm$ 0.5	73.3 $\pm$ 0.4
	2006	69.5 $\pm$ 0.3	69.1 $\pm$ 1.9	68.9 $\pm$ 1.5	68.3 $\pm$ 1.5	72.0 $\pm$ 0.3
Linoleic acid, C18:2	2005	10.3 $\pm$ 0.0	10.4 $\pm$ 0.2	10.9 $\pm$ 0.3	11.8 $\pm$ 0.3	9.6 $\pm$ 0.1
	2006	10.8 $\pm$ 0.0	10.9 $\pm$ 0.1	11.2 $\pm$ 0.4	11.9 $\pm$ 0.4	9.8 $\pm$ 0.2
Linolenic acid, C18:3	2005	0.8 $\pm$ 0.0	0.7 $\pm$ 0.0	0.8 $\pm$ 0.1	0.9 $\pm$ 0.0	0.7 $\pm$ 0.0
	2006	0.7 $\pm$ 0.0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.0	0.6 $\pm$ 0.0	0.7 $\pm$ 0.0

Values represent the average of three replicate analyses  $\pm$  standard deviation

**Table 2** Mean values of the quantified phenolic compounds (mg/kg),  $\alpha$ -tocopherol (mg/kg) and antioxidant activity (expressed as FRAP values in mmol FeSO<sub>4</sub>/kg oil) in olive oil samples produced in 2005 and 2006 crop season with the standard deviations

Olive oil	Year	EVOO	OGOO	OO1	OO-2	RFOO
Hydroxytyrosol	2005	4.9 ± 0.3	3.3 ± 0.4	1.2 ± 0.2	1.6 ± 0.1	0.8 ± 0.1
	2006	2.7 ± 0.4	0.5 ± 0.1	2.3 ± 0.2	1.3 ± 0.1	0.8 ± 0.2
Tyrosol	2005	4.0 ± 0.2	1.9 ± 0.3	3.6 ± 0.1	1.9 ± 0.0	1.5 ± 0.2
	2006	4.2 ± 0.5	1.0 ± 0.0	2.0 ± 0.2	1.7 ± 0.1	4.2 ± 0.9
Vanillin	2005	1.9 ± 0.3	3.6 ± 0.8	2.9 ± 0.2	1.8 ± 0.2	ND
	2006	6.0 ± 1.0	1.5 ± 0.3	2.4 ± 0.8	2.1 ± 0.2	ND
<i>para</i> -Coumaric acid	2005	2.4 ± 0.1	2.0 ± 0.3	2.5 ± 0.0	3.9 ± 0.1	ND
	2006	4.3 ± 0.4	2.8 ± 1.3	2.6 ± 0.3	3.6 ± 0.2	ND
Luteolin	2005	5.0 ± 0.3	7.6 ± 0.7	32.1 ± 2.7	4.7 ± 0.4	ND
	2006	18.4 ± 4.6	9.4 ± 0.6	6.7 ± 0.6	5.8 ± 0.5	ND
Apigenin	2005	2.1 ± 0.1	3.1 ± 0.2	4.0 ± 1.1	1.8 ± 0.1	ND
	2006	8.0 ± 1.7	4.5 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	ND
diAOle agl	2005	0.8 ± 0.2	0.4 ± 0.0	ND	0.5 ± 0.1	ND
	2006	0.8 ± 0.1	0.6 ± 0.1	ND	0.6 ± 0.1	ND
Pinoresinol	2005	50.8 ± 9.4	49.0 ± 1.0	49.3 ± 1.0	32.0 ± 4.9	7.8 ± 7.8
	2006	59.7 ± 9.8	77.1 ± 2.8	48.6 ± 4.4	43.5 ± 3.2	4.9 ± 4.9
1-Acetoxy-pinoresinol	2005	ND	ND	ND	ND	2.0 ± 0.1
	2006	ND	ND	ND	ND	2.4 ± 0.1
AOle agl	2005	3.1 ± 0.6	2.0 ± 0.1	2.6 ± 0.0	1.2 ± 0.1	0.3 ± 0.0
	2006	2.6 ± 0.4	1.7 ± 0.0	2.2 ± 0.3	1.8 ± 0.2	0.2 ± 0.1
ALig agl	2005	2.7 ± 0.5	1.5 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	ND
	2006	2.1 ± 0.3	0.7 ± 0.0	1.5 ± 0.6	1.2 ± 0.1	ND
Total phenolic compounds by LC/MS	2005	77.8 ± 11.7	64.0 ± 14.1	99.2 ± 3.4	50.2 ± 5.3	10.4 ± 0.2
	2006	108.7 ± 18.8	99.7 ± 3.4	71.1 ± 6.7	64.2 ± 4.6	10.2 ± 1.2
Total phenolic compounds by Folin	2005	105.9 ± 15.3	89.2 ± 3.2	89.6 ± 16.0	128.7 ± 10.2	104.6 ± 8.3
	2006	106.9 ± 16.7	89.5 ± 2.7	90.3 ± 18.6	132.0 ± 11.1	110.3 ± 8.1
$\alpha$ -Tocopherol	2005	141.5 ± 2.0	144.1 ± 0.5	88.9 ± 0.3	87.8 ± 1.8	115.4 ± 0.8
	2006	162.9 ± 1.6	131.5 ± 2.4	151.3 ± 1.5	124.8 ± 2.8	110.6 ± 3.5
FRAP	2005	45.0 ± 3.5	38.8 ± 3.8	39.6 ± 2.4	20.4 ± 3.6	11.5 ± 2.7
	2006	45.8 ± 0.7	36.2 ± 2.5	55.3 ± 6.3	40.0 ± 3.2	16.5 ± 0.9

Values represent the average of three replicate analyses ± standard deviation

*Abbreviations:* ND not detected, *diAOle agl* dialdehydic form of oleuropein aglycone, *AOle agl* aldehydic form of oleuropein aglycone, *ALig agl* aldehydic form of ligstroside aglycone, Folin colorimetric method (Folin-Ciocalteu, 1999)

very low and not of great importance. On the other hand, the major fatty acid, oleic acid was dominant and present in similar amounts in all oil samples. The content of oleic acid content in Ayvalik olive oil samples (EVOO and OGOO) was in the lower range than in some Spanish olive oils (Picual and Cornicabra) [11], but similar to other Spanish olive oils (Arbequina) [12] and some Italian olive oils (Leccino and Coratina) [13].

#### Tocopherol and Phenolic Compounds Composition

$\alpha$ -Tocopherol was detected in the samples of Ayvalik olive oil (Table 2). This compound was significantly different in

the oils from two crop seasons ( $P = 0.018$ ). The amount of  $\alpha$ -tocopherol in Ayvalik was lower than in some Greek EVOO [14], or Italian [15] and Spanish olive oil [11, 16]. However, Ayvalik had a higher  $\alpha$ -tocopherol content than Italian Caroleo olive oil [17] and some Portuguese virgin olive oils [18].

The total phenolic content (expressed as mg GAE/kg) and the sum of phenolic compounds quantified by LC-MS was different in oils harvested in 2005 and 2006 (Table 2). The main phenolic compound detected in Ayvalik monovarietal oil was pinoresinol followed by luteolin. Results showed higher hydroxytyrosol and tyrosol contents in samples of the 2005 crop season in comparison to those of 2006 season.

Furthermore, it was found that Ayvalik monocultivar virgin olive oil (EVOO) had a lower total polyphenol content than many other cultivars, such as Picual, Cornicabra, Manzanilla, and Arbequina [4, 12]. Additionally, a higher content of pinosresinol was found in Ayvalik oils, which may be used as a marker in the authentication process of these Turkish olive oils. These discrepancies in oil composition described above may be related to the specific variety and/or the olive oil production characteristics as frequently suggested [19].

It is important to note that Turkish olive oils from different crop seasons were slightly different. The explanation for the varied amount of  $\alpha$ -tocopherol in the oils from different seasons could be the temperature difference in the harvest periods [20]. In contrast to positive air temperatures during the 2006 harvest period (November–December), the weather during the 2005 harvest period was characterized by severe frosts inducing a substantial degradation of the olives (climatic data collected from Canakkale region, Turkey). In addition to climate effects, storage of oils prior to analysis was different and might have influenced the tocopherol and polyphenol content as suggested previously [14, 16, 21]. Accordingly, the samples produced in 2005 were kept longer in storage and had a lower amount of  $\alpha$ -tocopherol. The difference in polyphenols may be explained by more profound acid hydrolysis of the secoiridoid aglycons which probably occurred during longer storage of 2005 samples prior to analyses [22].

With respect to the categories of olive oil, refined olive oil had a notably lower content of linoleic acid whereas other fatty acids were similar contrasting the findings of Satue et al. [23]. In contrast to  $\alpha$ -tocopherol, which is probably added to RFOO during production, the amount of phenolic compounds was lower in the refined olive oil [24], with the exception of pinosresinol and 1-acetoxypinosresinol. This is in accordance with the findings of Garcia et al. [25] who reported removal of phenolic compounds during refining in a clear order.

#### Antioxidant Capacity

The negative influence of processing was observed by a decreased antioxidant power from EVOO through RFOO ( $P < 0.05$ ) with only one exception being the sample OO-1, which resulted in very high FRAP values similar to those of EVOO (Table 2). This result had an influence on the relationship between total phenolic content and FRAP values of the samples. Whereas the correlation between the FRAP and the total amount of phenolic compounds by both methods (Folin and LC-MS) was found to be moderate in the season 2005 ( $r = -0.53$  and  $r = 0.88$ , respectively), no correlation or lower correlation was in the season 2006 ( $r = -0.27$  and  $r = 0.69$ , respectively). In contrast, the

correlation between FRAP and  $\alpha$ -tocopherol content was low in 2005 ( $r = -0.42$ ) but high in 2006 ( $r = 0.89$ ).

Saura-Calixto et al. [26] reported the antioxidant capacity of Spanish olive oil to be 152  $\mu\text{mol}$  Trolox equivalent per gram of dry matter. Due to different equivalent ( $\text{FeSO}_4$ ) in the present study and that of Saura-Calixto et al. [26] it is not possible to compare these results directly. However, the FRAP values of the evaluated Turkish olive oil appear to be similar to those of mint, dry apricot, and chocolate (milk) consumed in Italy [27].

In this study no significant difference in organically and conventionally produced olive oil was noticeable for the fatty acid composition and the  $\alpha$ -tocopherol content. The reported findings are in agreement with the earlier studies suggesting a similarity in the nutritional quality of food products made from organic and conventional commodities [28].

**Acknowledgments** This research was financially supported by the Special Research Fund of Ghent University (BW/04749/01) providing a PhD fellowship for Mirjana Andjelkovic. The authors are grateful to the olive oil producer for providing the samples. We would like to express our thanks to Dr. Artemis Karaali from the Istanbul Technical University for her technical support during the study.

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