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PHENOLIC COMPOUNDS AND STEROL CONTENTS OF OLIVE (*OLEA EUROPAEA* L.) OILS OBTAINED FROM DIFFERENT VARIETIES

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Abstract

Oil obtained from 5 different olive cultivars was analyzed for phenolic and sterol composition. Total phenolic contents of oils were determined between 94.99 mg GAE/kg oil (Al-Joif) to 405.71 mg GAE/ kg oil (Sarıulak) ($p<0.05$). Phenolic compounds of oils obtained from different olive verities (Ayvalık, Sarıulak, Savrani, Al-Joif and Gemlik) when fully ripened were evaluated using reversed phase high performance liquid chromatography (RP-HPLC). Hydroxytyrosol and tyrosol were identified to have higher concentrations than other compounds. Tyrosol contents were between 3.65 mg/kg to 21.47 mg/kg oil ($p<0.05$) in different verities. The contents of hydroxytyrosol of oils for Ayvalik and Gemlik were 1.23 and 14.42 mg/kg, respectively. Cinnamic acid was detected only in Al-Joif olive oil sample. Low amounts of syringic, vanillin, p-cumaric, quercetin and luteolin were observed in different varieties' oils.

Key words: Olive oil, Total phenol, Phenolic compounds, Sterols, GC, HPLC.

Introduction

The olive tree (*Oleo europaea* L.) is widely cultivated in different parts of the world for producing olive fruits and obtaining oil which has economic and nutritional significance. The virgin olive oil is distinctive due to its large amounts of unsaturated fatty acids having important health benefits which are also attributed to the high amounts of phenolic compounds present there (Visioli & Galli, 1998). Olive oil phenolics prevent oxidation of virgin olive oil and hence contribute to the stability of oil. These compounds also give a characteristic taste to the oil (Ryan & Robards, 1998). The fraction of phenolics in olive oil comprises of various compounds that have different chemical properties and has great influence on the oil quality (Ryan & Robards, 1998; Brenes *et al.,* 1999). The use of olive oil has risen in majority of non-Mediterranean countries due to its beneficial properties (Duga *et al.,* 2004). Traditionally, olive oil is also considered as a healing agent and an excellent food by Mediterranean people. Recently, research interest in the olive with regards to its nutritional and health properties has increased as it is considered to be a healthy food and consumed regularly in many countries in different food preparations (Visioli & Galli, 1998; Tanılgan *et al.,* 2007).

The olive cultivar is considered to exert strong influence on various compositional attributes of olive oil such as fatty acids, phenolics and other components all of which have high nutritional significance in addition to the fruit maturity stage and geographical origin. It has been reported that among these different parameters, varietal difference can be the most determining and significant factor for oil fatty acid composition from different Tunisian cultivars (Zarrouk *et al*., 2009). Hence, the objective of carrying out this study was to assess total phenols, phenolic compounds and sterol contents of oils obtained from Ayvalık, Gemlik, Savrani, Al-Joif and Sarıulak varieties cultivated in Turkey and Saudi Arabia in order to observe compositional differences with references to these components.

Material and Methods

Sarıulak, Savrani, Al-Joif, Gemlik and Ayvalık were collected from Turkey and Saudi Arabia and their oils were extracted at olive production stations in Edremit (Balıkesir) and Hatay, Mersin (Silifke), Turkey. Oil samples were filtered, poured in dark bottles and nitrogen was introduced before storage at -80°C until use.

Determination of total phenolics: Analysis of total phenolics was done according to the method described by Singleton & Rossi, (1965) using Folin-Ciocalteu colorimetric method. Experiments were carried out in triplicates and the total phenolics of the samples were expressed as gallic acid equivalent (mg GAE g^{-1} extract) which was calculated using the equation of the calibration curve obtained from the standard (gallic acid).

Phenolic compounds in olive oils: About 10 g of virgin olive oil sample was added to 50 ml hexane and the mixture was homogenized for 1 min. This was followed by the addition of 20 ml methanol (60%) to the mixture and homogenized further for 2 min. Then, the methanol phase was pipetted from the mixture into balon joje and removed from the balon joje by drying under vacuum at 40°C with the aid of rotary evaporator. About 5 ml of methanol was added to the phenolic residue and the phenolic compounds were determined by reverse phase high performance liquid chromatography (RP-HPLC). Gradient program was used to analyze profile of the phenolic compounds. Caponioet *et al*. (1999) described the method for quantifying the phenolic compounds. A controller (SCL-10 Avp System), Autosampler (SIL–10AD vp), pump (LC-10AD vp), degasser (DGU-14a), column heater (CTO-10 A vp), diode array detector set at 278 nm and Agilent Eclipse XDB C-18 column (250 x 4,6

mm) were used in the detection and quantification of the phenolic compounds by RP-HPLC. Sample injection volume (10 µL), flow rate (0.8 mL/min), and the column temperature (30ºC) were also controlled.

The gradient elution of two solvents was set as solvent A [acetic acid–water (2:98, v/v)], and solvent B (methanol). The integration and analysis of data was performed using Shimadzu Class-VP Chromatography Laboratory Automated Software System (Chiyoda-ku, Tokyo, Japan). Filtration of mobile phases, extract samples and standard solution was done with the aid of membrane filter obtained from Vivascience AG, Hannover, Germany (pore size 0.45 µm). Calibration curve of pure phenolic standard was used to calculate the quantity of phenolic compounds in the oil extracts. All determinations were done in triplicate and the mean of the data were recorded. Phenolic compounds were detected at 278 nm and syringic acid was used as internal standard for the determination of response factors (Matos *et al.*, 2007).

Sterol analyses: The composition of sterols was analyzed as described by ISO/FIDS 12228:1999 (E). Briefly, saponification of about 250 mg of oil sample was carried out by boiling the oil with ethanolic potassium hydroxide solution under reflux. Solid-phase extraction was used to isolate the unsaponifiable matter using Al_2O_3 column (Merck, Darmstadt, Germany). The fatty acids anions were retained whereas sterol passage were allowed to pass. A thin layer chromatography or TLC (Merck, Darmstadt, Germany) system was used to separate sterol fractions from unsaponifiable matter followed by re-extraction from the TLC material and thereafter HP 6890 Series GC System was used to determine sterol fraction composition while beulin was used as standard. The separation of compounds was carried out on a Supelco 24034 Sterol column (30 mm x 0.25 mm x 0.25 µm film thickness), a fused silica capillary column. Further parameters were as follows:

GC working conditions

Instrument: HP 6890 Series GC System
Column: Supelco 24034 Sterol column (30 mm x 0.25 mm x 0.25 µm film thickness) FUSED SILICA Capillary Column
Detector: FID (Flame Ionization Detector)
Temperatures: Column: max 320ºC
Oven: 260ºC
Inlet: 280ºC
Detector: 295ºC
Flow rates: 1.2 ml/min.
Carrier gas (H_2): 30.0 ml /min.
Auxiliary gas (Air): 300 ml/min.
Injection amount: 2.5 µl

Identification of peaks was done using standard compounds (β-sitosterol, campesterol, stigmasterol) from a sterols mixture prepared using brassicasterol (from rapeseed oil) and Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol (from sunflower oil). A GC-MS was used for identification of all other sterols for the first time followed by retention time comparison (Matthaus & Özcan, 2005).

Statistical analysis: All the experiments were carried out in triplicates and data were statistically analyzed for significance differences by using analysis of variance (ANOVA) (Püskülcü & İkiz, 1989).

Results and Discussion

Phenolic compounds and phenolic composition of olive oils: The oils obtained from different olive varieties were analyzed for total phenols, phenolic compounds and sterol content. The total phenolic contents were in the range of 94.99 mg GAE/kg in Al-Joif) to 405.71 mg GAE/ kg in Sarıulak oils ($p<0.05$) and the values for the phenolic content of oils from Ayvalık, Sarıulak, Savrani, Al-Joif and Gemlik varieties are shown in Table 1. The concentrations of the individual phenolic compounds of oils obtained from the five varieties as expressed as mg/kg virgin olive oil is shown in Table 2. The major phenolic compounds in oils of all the varieties were hydroxytyrosol and tyrosol secoiridoid derivatives in varying quantities. Furthermore, as shown in Table 2, a range of 0.07 to 0.15 mg/kg of *p*-cumaric contents was observed in different oil samples. The occurrence of good amounts of these phenolics in virgin olive oil renders it nutritional and health significance. The tyrosol amount in oils samples were in the range of 3.65 to 21.47 mg/kg oil ($p<0.05$). The oil sample containing the highest tyrosol contents (21.47 mg/kg) were observed in Gemlik olive oil which also had highest hydroxytyrosol contents (14.42 mg/kg). The luteolin contents were in the range of 0.23 to 0.55 mg/kg (Table 2), however, the syringic, *p*-cumaric, vanilin, quercetin and luteoil contents of the oils were quite low. The vanillic acid contents were in the range between 0.02 and 0.14 mg/kg. Tyrosol values of oils changed between 3.65 mg/kg and 21.47 mg/kg.

Table 1. Total phenol contents of olive oils.

Olive varieties	Total phenol (mg GAE/kg oil)
Sarıulak	405.71 ± 4.15[*a]
Savrani	249.76 ± 2.11b[**]
Al-Joif	94.99 ± 1.19[d]
Gemlik	105.21 ± 1.13[c]
Ayvalık	99.02 ± 1.52[c]

*Mean ± standard deviation

**Values within each column followed by different letters are significantly different ($p<0.05$)

The hydroxytyrosol contents were also reported in Arbequina (2.1 and 2.9 mg/100 g), Cornicabra (2.1 and 2.8 mg/100 g), Marisca (0.4 and 0.6 mg/100 g), Picolimon (0.6 and 0.8 mg/100 g) and Picual (1.8 and 2.2 mg/100 g) olive varieties by Gomez-Rice *et al.* (2008). Also, tyrosol content of arbequina (2.4 and 2.1 mg/100 g), in Cornicabra (1.5 and 1.2 mg/100 g), Morisca (5.5 and 6.4 mg/100 g), Picolimon (4.2 and 3.9 mg/100 g) and unripe

and ripe Picual (3.3 and 3.3 mg/100 g) olive varieties were found. It is an important finding as hydroxytyrosol and its complex derivative are reported to have higher antioxidant activity and sensory importance than that of tyrosol group (Baldioli *et al.*, 1996; Gennaro *et al.*, 1998). Generally, comparing the results obtained in this study as shown in Table 2 with that of literatures showed some differences and similarities. The variations in reports can be attributed to differences in studied material, varities and the possibilities of using mixed analytical samples Cimato *et al.* (1990) that may contain oils from fruits having variable maturation indices. The factors that generally influence the phenolic composition of olive oil are cultivar, climate and geographical distribution (Vinha *et al.*, 2005). Similar variations in different compositional attributes with reference to varietal and cultivar differences have been reported in fruits such as dates due to genetic variability and other climatic and geographical factors (Mırbahar *et al*., 2014).

Sterol composition of olive oil samples: The sterol compositions of the olive oils are summarized in Table 3. The major sterol fractions were campesterol, β-sitosterol, D5-avenasterol and erythrodiol+uvaol. The campesterol contents of olive oils changed between 2.33 mg/Kg (Sarıulak) to 3.86 mg/Kg (Ayvalık); D5-avenasterol contents were between 1.24 mg/kg (Al-Joif) and 5.11 mg/kg (Gemlik) ($p<0.05$) and β-stosterol and sum β-stosterol contents were detected at higher levels, i.e. 82.17 mg/g to 89.75 mg/kg and 89.34 mg/kg to 94.08 mg/kg, respectively. D7-avenasterol and Erythrodiol+uvaol contents of Sarıulak oil were found at the high levels compared with that of other samples. Other minor components were clerosterol (1.03 to 1.27%); stigmasterol (0.67 to 2.34%) and a small amount of cholesterol (0.15 to 0.28%) (Table 3) ($p<0.05$).Total sterol contents of samples were between 1297 mg/kg (Savrani) to 3283 mg/kg (Al-Joif).

Giocametti & Milin (2001) detected cholesterol (0.002 - 0.056%), Brassicasterol 0-(0.048%), campesterol (1.260 - 3.320%), stigmastenol (0.06 - 2.810%), β-sitostenol (93.88-98.47%) and δ^7-stigmastenol (0.003 - 0.440%) in virgin olive oils. Boggia *et al.* (2005) also reported campestenol (1.72 - 2.98 mg/Kg), β-sitestenol (91.9 - 94.81 mg/Kg) and δ-7-stigmastenol in Colabaia olive oils. In addition, El-Agaimy *et al.* (1994) reported campesterol (9.5%), stigmasterol (1.9%), beta-sitosterol (88.6%) in olive oil. Duga *et al*. (2004) found values of 75.5-86.3 mg/Kg β-sitestenol, 2.1-3.9 mg/Kg campestenol, δ-5-avenestenol 5.4-16.8 mg/Kg and 1.3-7.1 mg/Kg campestenol in Sicilion virgin olive oils.

Table 2. Phenolic compounds of olive oils (mg/kg oils).

Olive varieties	Hydroxytyrosol	Tyrosol	Syringic	Vanillin	Vanillic acid	p-coumaric acid	sinnamic	Quercetin	Luteolin
Sarıulak	14.36±0.02^{*a}	9.39±0.02^{b}	0.0±0.0	0.06±0.01^{d}	0.0±0.0	0.9±0.0^{b}	0.0±0.0	0.06±0.01^{c}	0.30±0.01^{c}
Savrani	2.56±0.01^{b**}	3.65±0.01^{d}	0.0±0.0	0.14±0.0^{a}	0.03±0.01^{a}	0.15±0.0^{a}	0.0±0.0	0.06±0.0^{c}	0.49±0.01^{b}
Al-Joif	2.29±0.01^{b}	3.94±0.0^{d}	0.08±0.01	0.09±0.0^{b}	0.02±0.0^{b}	0.07±0.0^{c}	0.02±0.0	0.10±0.0^{a}	0.55±0.01^{a}
Gemlik	14.42±0.02^{a}	21.47±0.03^{a}	0.0±0.0	0.02±0.0^{e}	0.03±0.01^{a}	0.12±0.01^{a}	0.0±0.0	0.07±0.0^{b}	0.37±0.0^{c}
Ayvalık	1.23±0.11c	7.15±0.01^{c}	0.0±0.0	0.07±0.0^{c}	0.0±0.0	0.09±0.0^{b}	0.0±0.0	0.06±0.01^{c}	0.23±0.01^{d}

*Mean ± standard deviation

**Values within each column followed by different letters are significantly different ($p<0.05$)

Table 3. Sterol contents (mg/kg) of olive oils from different olive varieties.

Phenolics	Sarıulak	Savrani	Al-Joif	Gemlik	Ayvalık
Cholesterol	0.15 ± 0.03^{*c}	0.28 ± 0.05^{a}	0.23 ± 0.03^{b}	0.27 ± 0.07^{a}	0.23 ± 0.06^{b}
24-metylen Cholesterol	0.01 ± 0.0^{c**}	0.04 ± 0.01^{b}	0.01 ± 0.0^{c}	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}
Campesterol	2.33 ± 0.17^{b}	2.56 ± 0.21^{b}	3.62 ± 0.28^{a}	3.09 ± 0.11^{a}	3.86 ± 0.25^{a}
Campestanol	0.08 ± 0.01^{c}	0.14 ± 0.03^{a}	0.06 ± 0.01^{d}	0.07 ± 0.01^{c}	0.10 ± 0.03^{b}
Stigmasterol	0.67 ± 0.11^{c}	0.81 ± 0.09^{b}	2.34 ± 0.28^{a}	0.99 ± 0.11^{b}	2.16 ± 0.22^{a}
Δ-7-campesterol	0.20 ± 0.03^{b}	0.12 ± 0.01^{c}	0.06 ± 0.01^{d}	0.17 ± 0.03^{b}	0.45 ± 0.05^{a}
Δ-5.23-stigmastadienol	-***	-	-	-	0.14 ± 0.01
clerosterol	1.03 ± 0.05^{c}	1.27 ± 0.08^{a}	1.27 ± 0.03^{a}	1.09 ± 0.11^{b}	1.11 ± 0.09^{b}
Beta-sitosterol	87.20 ± 1.28^{b}	87.37 ± 1.32^{b}	89.75 ± 1.38^{a}	86.59 ± 1.42^{b}	82.17 ± 1.23^{c}
Stostanol	0.78 ± 0.04^{a}	0.69 ± 0.03^{b}	0.35 ± 0.05^{c}	0.46 ± 0.02^{c}	0.50 ± 0.09^{c}
Δ-5 -avenasterol	2.88 ± 0.23^{c}	4.10 ± 0.21^{b}	1.24 ± 0.18^{d}	5.11 ± 0.34^{a}	4.59 ± 0.36^{b}
Δ-5.24-stigmastadienol	0.79 ± 0.09^{a}	0.66 ± 0.04^{c}	0.42 ± 0.07^{d}	0.71 ± 0.09^{b}	0.82 ± 0.11^{a}
Δ-7-stigmastenol	1.79 ± 0.13^{b}	0.81 ± 0.09^{c}	0.32 ± 0.03^{d}	0.48 ± 0.07^{d}	2.59 ± 0.11^{a}
Δ-7-avenasterol	2.07 ± 0.09^{a}	1.14 ± 0.21^{b}	0.34 ± 0.03^{d}	0.90 ± 0.13^{c}	1.17 ± 0.17^{b}
Erythrodiol+uvaol	2.08 ± 0.13^{a}	1.76 ± 0.09^{b}	0.84 ± 0.08^{e}	1.58 ± 0.11^{c}	1.29 ± 0.13^{d}
Total (mg/Kg)	**2777**	**1297**	**3283**	**2061**	**1549**

*Mean ± standard deviation

**Values within each column followed by different letters are significantly different ($p<0.05$)

***Unidentified

Conclusions

It can be observed that varieties do significantly affect phenolic and sterol composition of olive oils. Furthermore it was also observed that good amounts of these health promoting constituents of olive oils are present in some olive varieties obtained from Turkey and Saudi Arabia. The current study reported important analytical characterization of olive oils with reference to phenolic and sterol compounds. The studies with reference of these compounds in relation to different olive cultivars is scanty and further studies on oils obtained from these varieties may be carried out to report about essential and non-essential fatty acids, in vitro antioxidant properties and other physico-chemical characteristics.

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