Effects of Variety and Extraction Methods on Phenolic Compounds and Chemical Composition of Olive Oils

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This work was carried out to assess the influence of two extraction methods (three phase centrifugation system and solvent extraction) on oil quality from three olive varieties (Gemlik, Halhali and Sari Hasebi) cultivated at Hatay, Turkey. Analysis of variance showed that extraction systems were significant ($p \leq 0.05$) and affected on some quality parameters mainly in phenolic compounds, total tocopherol, chlorophyll, carotenoid contents and bitter index of olive oil. Varieties were also significant and many of the analytical parameters and fatty acids varied among olive varieties. Solvent extracted oil from Halhali variety had the highest values of oleic acid, whereas Sari Hasebi has the highest total phenolic and pigment contents.

Keywords: olive oil, extraction method, quality, phenolic compounds

Virgin olive oil is significant due to its use without refining that distinguishes it from other edible vegetable oils. It is characterized by a unique sensory, nutritive qualities and oxidation stability [1-3]. The composition of virgin olive oil is mainly constituted by triacylglycerols and minor compounds (0.5-2%) which is called unsaponifiable or non-glycerol fraction [4,5]. The main minor compounds such as polyphenols, tocopherols and pigments, are the most important antioxidants compounds since they delay the oxidation of fatty acids [6].

The chemical composition of virgin olive oils depends on many factors, such as the variety, climatic conditions, maturity, agronomic factors and oil extraction methods [7-9].

Turkey is one of the most important Mediterranean countries, such as Spain, Italy, Greece because of olive and olive oil production [10]. Hatay province which is located in the southern part of Turkey and borders of the Mediterranean Sea, has adequate climate and soil conditions for olive production. The most important olive varieties cultivated in this province are *Halhali*, *Sari Hasebi* and *Gemlik* [11]. Usually, olive oil is mechanically extracted from olives small plants which utilize pressure or centrifugation system. Extraction methods are the most important factor affecting extra virgin olive oil quality. The methods have some disadvantages, such as the reduction amounts of phenols, tocopherols, chlorophyll and carotenoid content of the oil due to the addition of warm water to dilute the olive past through [9, 12-14].

Phenolic compounds affect olive oil stability and contribute to its sensorial and nutritional characteristics. The compounds are main antioxidants compounds within virgin olive oil [3,15,16]. Tocopherols, including vitamin E, can act as antioxidants by singlet oxygen quenchers or a chain-breaking electron donor mechanism [3,17]. Chlorophyll and carotenoids are major pigments of olive oil, which gives colour characteristic and desirable green-yellow colour to olive oil. Chlorophyll pigments act as photosensitisers, promoting oxidation, while antioxidant activity was reported in the dark [18,19]. Carotenoids, as singlet oxygen quenchers, protect oils from photo-oxidation [18]. The extraction process causes losses of oil pigments [20,21].

The aim of this work was to determine the effect of the variety and extraction methods on the some chemical composition of three different virgin olive oils with special emphasis on the phenolic compounds and related oil quality parameters, such as fatty acids, tocopherol, chlorophyll, carotenoid contents and bitter index.

Experimental part

Materials and Methods

The study was conducted during the 2005/2006 crop years. Olive fruits of the varieties *Gemlik*, *Halhali* and *Sari Hasebi* were cultivated from Hatay province in Turkey. Three samples (80 kg each) of each variety were picked by hand at an optimal stage of ripeness (the first half of November). After homogenization and cleaning, each variety was divided into two portions. One of them was extracted using a three-phase centrifugation system, and the other one by a solvent method.

Centrifugation: Olives were crushed with a metal hummer crusher and the olive paste was kneaded for 40 min at 40±2°C and then diluted with water (60 L/100kg). The paste was then centrifuged with a three-phase centrifugation system. The oils phases were extracted with a horizontal centrifugal decanter and liquid obtained was separated with an automated discharge vertical centrifuge.

Solvent: The olive samples were blended in a mechanical blender and homogenized. The sample of about 200 g of paste was placed into a dish. It was dried at 100±2°C for 5 h by oven-drying. The dried sample was extracted by soxhalet apparatus, using petroleum ether (40-60°C) for 2 h. Subsequently, olive oil was extracted misella (oil + petroleum ether) by evaporated of petroleum ether.

All oil samples were filtered and stored at 4°C in dark glass bottles prior to analyses.

Milli Q water (Millipore, Bedford, MA) was used in all work. Petroleum ether, methanol, *n*-hexan, cyclohexane, acetonitrile and formic acid were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Germany). All

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chemicals and solvents used were either of analytical or HPLC grade. Fatty acid and phenolic standards were purchased from Sigma Aldrich (Germany) and Extrasynthes (Lyon-Genay, France), respectively.

Determination of free faty acidity and peroxide value were carried out, following the analytical methods described in IUPAC [22].

For the determination of fatty acid composition, the methyl-esters were prepared by vigorous shaking of a solution of oil in *n*-heptane (0.1 g in 2 mL) with 0.2 M of 2N methanolic potash. The methyl esters were performed by using a Shimadzu GC apparatus (Model 14 B) equipped with a hydrogen flame ionization detector (FID) and a cappilary column DB-23 of 60 m length x 0.25 mm i.d. and 0.25μm of film thickness (Agilent J&W, U.S.). Helium was used as carrier gas and the temperatures of injector, oven and detector were 270, 230 and 280°C, respectively. The results were expressed as peak area (relative) percent. The injection volume was 1 μL [23].

Polyphenol content analyses were determined according to Gutfinger et al. [24], using the Folin-Ciocalteau reagent and absorbance measurement at 725 nm, the results were expressed as mg/kg of caffeic acid. The determination of phenolic compounds was performed by analyzing the phenolic extracts with HPLC. Phenols were extracted from olive oil by following the procedure of Murkovic et al. [25]. 500 milligrams of the olive oil was extracted with 500 μL of methanol in 2-mL eppendorf vials. After vigorous shaking, the vials were centrifuged at 13.000 rpm for 5 min. The upper methanolic phase was used for HPLC analysis. An Agilent 1100 HPLC system operated by Windows NT based ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). System consisted of a binary pump, degasser and auto sampler. The column used was a Beckman Ultrasphere ODS (Roissy CDG, France): 4.6 mm x 250 mm, 5μm equipped with a precolumn 4.6 mm x 10 mm (same granulometry). The mobile phase consisted of two solvents: Solvent A, water/formic acid (95:5; v/v) and Solvent B, methanol/formic acid (95:5; v/v). Phenolic compounds were eluted according to the following gradient: 100% A at 1 min, 95% at 10 min, 90 at 20 min, 80 at 50 min, 70 at 75 min, 0% at 76 min, 100% at 87 and 88 min. The flow rate was 1 mL/min and run time, 88 min. The run was performed at 25°C. The ultra-violet-visible spectra (scanning from 200 nm to 600 nm) were recorded for all piks. The identification of phenolics was confirmed by using authentic standards and by comparing the retention times and ultra viole-visible-spectra with those found in the literature [3,16,26,27] while quantification was performed by external calibration with standards.

Total tocopherols were evaluated by the method of Wong et al. [28]. 200 mg of the oil sample were weighed accurately in to a 10 mL volumetric flask. 5 mL of toluene were added by pipette and the oil taken into solution. 3.5 mL of 2.2'-dipyridine and 0.5 mL of $FeCl_3$. $6H_2O$ were added in that order. This solution is made up to 10 mL with 95% aqueous ethanol. Then one minute the absorbtion at 520 nm determined using as a reference a blank solution. The tocopherol contents were expressed as mg/kg α-tocopherol.

Chlorophyll and carotenoid compounds were determined at 670 and 470 nm, respectively, in cyclohexane using the specific extinction values, by the method of Minguez-Mosquera et al. [29]. The chlorophyll and carotenoid contents are expressed as mg of pheophytin "a" and lutein per kg of oil, respectively.

Bitter index K_{225} was determined by solid phase extraction and absorbance measurement at 225 nm [3]. All tests were performed in triplicate.

The results are presented as mean values ±SD. Analysis of variance and significant difference tests were conducted to identify differences among means by one-way and three-way analysis of variance (ANOVA) using SPSS software (version 10.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results and discussions

The quality parameters of olive oils from the three different varieties were extracted by two different systems i.e. three phase centrifugation and solvent extractions, are listed in table 1. It was found that free fatty acid content of all analyzed samples was lower than 1. Varieties and extraction systems were not significant.

Peroxide values (PV) in centrifuges-extracted oils varied from 3.54 to 4.92 meq/kg; solvent- extracted oils were in the range of 5.66-6.20 meq/kg. Observed PV of oils obtained from the solvent-extracted oils were higher than centrifuges-extracted oils. It was possible that solvent-extracted methods can use higher temperature and longer time. When the oils from all varieties were compared to the each extraction system, there were no significant differences in among the solvent system. However, oils of Halhal variety extracted from centrifugation system had a PV significantly higher than oil of the other varieties.

The total pigment content and tocopherol content of olive oils are important quality indices, since they correlate with colour and oxidative stability, respectively. Chlorophyll, carotenoid and tocopherols contents of olive oils obtained from the solvent system were higher than those obtained from the centrifuge system. Chlorophyll and carotenoid contents of oil obtained from the solvents systems were

Table 1
CHEMICAL COMPOSITION OF OLIVE OILS FROM 'GEMLIK' 'HALHALI' AND 'SARI HASEBI'

Chemical Composition	Gemlik		Halhalı		Sarı Hasebi	
	Solvent	Centrifugation	Solvent	Centrifugation	Solvent	Centrifugation
Free fatty acid (% oleic)	0.76^{a1}	0.82^{a1}	0.81^{a1}	0.92^{a1}	0.70^{a1}	0.80^{a1}
Peroxide value (meq/kg)	5.66^{a1}	3.83^{b2}	5.97^{a1}	4.92^{b1}	6.20^{a1}	3.54^{b2}
Chlorophylls (mg/kg)	16.43^{a3}	9.73^{b2}	24.77^{a2}	19.63^{b1}	57.57^{a1}	11.40^{b2}
Carotenoids (mg/kg)	5.43^{a3}	2.11^{b3}	6.63^{a2}	3.43^{b2}	16.57^{a1}	4.93^{b1}
Tocopherol (mg/kg)	171.23^{a1}	152.21^{b1}	115.91^{a2}	109.52^{b2}	93.17^{a3}	76.30^{b3}
Total phenols (mg/kg)	65.07^{a1}	32.17^{b2}	45.21^{a2}	23.27^{b3}	65.17^{a1}	37.73^{b1}
Bitter index (K_{225})	0.71^{a1}	0.54^{b1}	0.31^{a3}	0.25^{b3}	0.42^{a2}	0.35^{b2}

Values in each row with different superscript letters present significant differences ($P<0.05$) between extraction systems for each variety. Values in each row with different superscript numbers present significant differences ($P<0.05$) between olive varieties for extraction system.

Phenolic Compounds (mg/kg)	Gemlik		Halhalı		Sarı Hasebi	
	Solvent	Centrifugation	Solvent	Centrifugation	Solvent	Centrifugation
hydroxytyrosol	0.95^{xa1}	0.31^{b1}	1.03^{a1}	0.18^{b1}	1.52^{a1}	0.23^{b1}
tyrosol	0.39^{a1}	0.07^{a1}	0.29^{a1}	0.05^{a1}	0.51^{a1}	0.22^{a1}
verbascoside	0.91^{a1}	0.29^{a1}	0.24^{a1}	0.14^{a1}	0.61^{a1}	0.27^{a1}
luteolin	0.10^{a1}	0.07^{a1}	0.34	0.16^{a1}	0.62^{a1}	0.19^{a1}
rutin	0.09^{a2}	0.02^{a1}	0.16^{a1}	0.12^{a1}	0.19^{a1}	0.04^{a1}
oleuropein	1.87^{a1}	0.58^{b1}	1.89^{a1}	0.62^{b1}	2.39^{a1}	0.71^{b1}

Values in each row with different superscript letters present significant differences ($P<0.05$) between extraction systems for each variety. Values in each row with different superscript numbers present significant differences ($P<0.05$) between olive varieties for extraction system.

Table 2
PHENOLIC COMPOUNDS (mg/kg) OF OLIVE OILS FROM 'GEMLIK' 'HALHALI AND 'SARI HASEBI

Fatty acids(%)	Gemlik		Halhalı		Sarı Hasebi	
	Solvent	Centrifugation	Solvent	Centrifugation	Solvent	Centrifugation
Palmitic	14.98^{a2}	15.06^{a2}	15.21^{a1}	15.45^{b1}	14.55^{a3}	14.45^{a3}
Palmitoleic	1.17^{a1}	1.20^{a1}	0.83^{a2}	0.83^{a2}	0.77^{a3}	0.67^{b3}
Stearic	3.46^{a3}	3.41^{a3}	3.74^{a2}	3.62^{b2}	3.85^{a1}	3.88^{a1}
Oleic	71.04^{b1}	71.64^{a1}	68.88^{a2}	69.13^{a2}	68.55^{b3}	69.25^{a3}
Linoleic	7.58^{a3}	6.75^{a3}	9.19^{a2}	8.87^{a2}	10.24^{a1}	9.69^{a1}
Linolenic	0.54^{a3}	0.52^{b2}	0.59^{b2}	0.57^{a1}	0.62^{a1}	0.56^{a1}

Values in each row with different superscript letters present significant differences ($P<0.05$) between extraction systems for each variety. Values in each row with different superscript numbers present significant differences ($P<0.05$) between olive varieties for extraction system.

Table 3
FATTY ACID COMPOSITION (%) OF OLIVE OILS FROM 'GEMLIK' 'HALHALI AND 'SARI HASEBI'

high because of the minor loss during extraction process [20, 29]. There was a significant difference between extraction systems in the concentrations of chlorophyll, carotenoid pigments and tocopherol content. Chlorophyll pigment content varied from 9.73 mg/kg (Gemlik) to 19.63 mg/kg (Halhali) in the centrifuged-extracted oils. Oils obtained from the solvent-extracted had values between 16.43 mg/kg (Gemlik) -57.57 mg/kg (Sari Hasebi). Carotenoid pigment content varied from 2.11 mg/kg (Gemlik) to 4.93 mg/kg (Sari Hasebi) in the centrifuged-extracted oils. The solvent-extracted oils had values between 5.43 mg/kg (Gemlik) -16.57 mg/kg (Sari Hasebi).

The carotenoids content, by solvent system extracted oils was higher than centrifugation extracted oils. Sari, Hasebi and Gemlik varieties had significantly higher carotenoid and tocopherol contents, than the other oils varieties, extracted by both extraction systems.

The total tocopherol contents and bitter index were statistically higher in solvent extracted than in centrifugation system extracted oils. Total tocopherol content varied from 76.3 mg/kg (Sari Hasebi) to 152.2 mg/kg (Gemlik) in the centrifuged-extracted oils. Oils by the solvent-extracted had values between 93.17 mg/kg (Sari Hasebi) -171.23 mg/kg (Gemlik). Bitter index varied from 0.25 K 225 (Halhali) to 0.54 K 225 (Gemlik) in the centrifuged-extracted oils. Oils of the solvent-extracted had values between 0.31 K 225 (Halhali) – 0.71 K 225 (Gemlik).

Phenolic compounds effect of olive oil stability, also contribute to oil flavour and aroma, especially to the typical bitter taste of olive oil [2]. A number of studies have shown that there were a positive correlation between bitter index and total phenol concentration [30-32].

Varieties and extraction systems were significant in terms of total olive oil phenolic compounds (table 1). The total polyphenol contents of oils extracted by centrifugation were significantly lower than oils extracted with the solvent system for three varieties. The phenol content varied from 23.27 mg/kg (Halhali) to 37.73 mg/kg (Sari Hasebi) in the centrifuged-extracted oils. Oils with the solvent-extracted had values between 45.21 mg/kg (Halhali) -65.17 mg/kg (Sari Hasebi). Decreasing phenol content of varieties may be explained by their water-solubility. Higher water/paste ratios were used in the centrifugation system, and therefore larger amounts of phenols were removed with water wastes [33,34]. Kiritsakis [20] confirmed that phenolic compunds of solvent-extracted system oils higher than of centrifugation systems that result were parallel to our research results.

Results of phenolic compounds of olive oils were listed in table 2.

Extraction systems of cultivars were significant ($p<0.05$) in terms of hydroxytrosol content. Hydroxytrosol content of solvent extraction system was higher than the other extraction systems. Extraction systems of cultivars were significant ($p<0.05$) in terms of Oleuropein content. Oleuropein content of solvent extraction system was higher than the other extraction systems. Cultivars were not significant ($p>0.05$) in terms of solvent extraction and mechanical extraction methods. Boskou [35] reported that polyphenol content of olive oil extracted by mechanical methods was lower than those of solvent extracted olive oil. This finding explained by polar feature of phenolic compounds of olive oil and removal of these compounds via water in mechanical extraction systems.

Results of fatty acid composition were listed in table 3. The composition of olive oils revealed that varieties were significant. The fatty acid composition differed slightly by the extraction method. The differences were mainly remarkable for oleic acid content. The major fatty acids were oleic (68.55-71.64%), palmitic (14.5-15.5%), linoleic (6.8-10.2%), stearic (3.4-3.9%), and palmitoleic (0.7-1.2%). These results were in agreement with those obtained by Torres and Maestri [9]. The oleic acid content was the lowest in Sari Hasebi variety (less than 69%); while was highest in Gemlik (>71%). Fatty acid composition may differ, depending on the variety of olive, location and degree of fruit ripeness [10,34,36].

Conclusions

The results confirmed that varieties and extraction methods were significant in terms of important phenolic compounds and chemical compounds of olive oil. Fatty acid composition of olive oil was changed when the varieties changed but did not changed when the extraction methods changed. Solvent extraction systems did not changed the phenolic content and pigments amount of olive oil. Because the centrifugation extraction systems which use water were the most used of these compounds of phenolic, tocopherol and pigments may be lost because of water washing.

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