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COMPARISON OF SOME PHENOLIC COMPOUNDS OF ORGANIC AND CONVENTIONAL EXTRA-VIRGIN OLIVE OIL

Yasemin ŞAHAN [*]
Güler ÇELİK [**]
Esra DOĞANGÜN [**]

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Abstract: Olive oil is an important ingredient of the Mediterranean diet, because of its nutritional qualities and organoleptic characteristics. In addition olive oil has positive effects on human health, in particular to prevent of some types of cancer and cardiovascular diseases and as regards diabetes, inflammatory and autoimmune diseases. These properties are related not only to the fatty acid composition of its lipid matrix, but especially to the presence of the phenolic compounds. The changes in the phenolic compounds of EVOO can be an important quality control parameter. The aim of this study was to determine the changes in the some phenolic compounds of organic and conventional extra-virgin olive oil (EVOO) from Turkey. Five phenolic compounds oleuropein, hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid were quantified LC-DAD and justified by LC-MS. Ten extra virgin olive oils (organic and conventional extra-virgin olive oil), produced by different brands were analyzed. Oleuropein were found to be higher than other phenolic compounds. The amount decreased with the order of tyrosol, hydroxytyrosol, caffeic acid, p-coumaric acid, respectively. Oleuropein concentration varied between 3.8-39 mg/kg in organic production of extra virgin olive oils. As a conclusion, phenolic content are higher in organic products compared to conventional products of EVOO.

Keywords: Olive oil, oleuropein, hydroxytyrosol, organic production, LC-MS

Organik ve Konvansiyonel Olarak Üretilen Sızma Zeytinyağlarının Bazı Fenolik İçeriklerinin Karşılaştırılması

Öz: Zeytinyağı, besleyici kalitesi ve organoleptik özellikleri ile Akdeniz diyetinin önemli bir bileşenidir. Buna ek olarak, zeytinyağı bazı kanser türlerinin, kardiyovasküler hastalıkların, diyabet, inflamatuar ve otoimmün hastalıklarının önlenmesinde insan sağlığı üzerinde olumlu etkilere sahiptir. Bu özellikler yalnızca yağ asidi kompozisyonuna bağlı olmayıp, özellikle fenolik bileşiklerin varlığıyla ilgilidir. Sızma zeytinyağlarının (EVOO) fenolik bileşiklerindeki değişiklikler önemli bir kalite kontrol parametresi olabilmektedir. Bu çalışmanın amacı, Türkiye'deki organik ve konvansiyonel olarak elde edilmiş EVOO'nın bazı fenolik bileşiklerindeki değişiklikleri belirlenmesidir. Beş fenolik bileşik oleuropein, hidroksityrosol, tyrosol, kafeik asit ve p-kumarik asit LC-DAD ile belirlenmiş ve ayrıca LC-MS ile doğrulanmıştır. Farklı markalar tarafından üretilen on adet sızma zeytinyağı (organik ve konvansiyonel) analiz edilmiştir. Oleuropein'nin diğer fenolik bileşiklere göre daha yüksek düzeyde olduğu bulunmuştur. Fenolik bileşenler bulunma düzeyleri yüksekten aza doğru sıralandığında, tyrosol, hidroksityrosol, kafeik asit ve p-kumarik asit olarak belirlenmiştir. Organik olarak üretilen sızma zeytinyağlarının oleuropein konsantrasyonu 3,8-39 mg/kg arasında değişmiştir. Sonuç olarak, organik ve konvansiyonel olarak üretilmiş sızma zeytinyağları fenolik içerik açısından karşılaştırıldığında organik olarak üretilenlerin daha yüksek olduğu saptanmıştır.

Anahtar Kelimeler: Zeytinyağı, oleuropein, hidroksityrosol, organik üretim, LC-MS

[*] Uludag University, Faculty of Agriculture, Food Engineering Department, Bursa, TURKEY
Yasemin SAHAN (yasemins@uludag.edu.tr)
[**] The Scientific and Technological Research Council of Turkey, Bursa Test and Analysis Laboratory, (TUBITAK BUTAL), Turkey
Correspondence Author: Yasemin ŞAHAN (yasemins@uludag.edu.tr)

1. INTRODUCTION

The increased importance of organic farming in recent decades has come from a heightened consumer awareness of its associated food and environmental quality benefits. Worldwide land under organic farming in 2011 encompassed more than 37.2 million hectares (Sacco et al., 2015). Organic farming improves the environmental quality of the agricultural system (Gomiero et al., 2011; Gaudino et al., 2014) and the organoleptic quality of its products (Crecente- Campo et al., 2012). Recently, there are highly demand on olive oil among the different countries of world due to the healthy nutrition trends and natural foods consumption. In addition, increasing income levels and high life standards in the world gave rise to the new markets for the olive oil consumption (Öztürk et al., 2009).

Olive oils worldwide production has been estimated to be 2,988,500 tons for 2015/2016. The olive oil production of Turkey is as average 165.000 tones at 2010 -2016 years and our country is the world's fifth largest producer (IOOC, 2016). In Turkey, amongst more than about 50 cultivated varieties, Ayvalık, Memecik, Gemlik, and Kilis yağlık are the most widely used cultivars for VOO production (Öztürk et al., 2009).

Olive oil is a key component of the traditional Mediterranean diet, which is believed to be associated with a relatively long life in good health. Among vegetable oils, extra-virgin olive oil (EVOO) have nutritional and sensory characteristics that make them unique because of the high level of particular phenolic compounds, to which, together with the high content of unsaturated and monosaturated fatty acids, the health benefits of virgin olive oil are attributed (Dagdelen et al., 2013). A large number of study are present in literature, high concentrations of phenolic compounds in olive oil may contribute to the healthy action of the Mediterranean diet because they exhibit protective effects against neuro-degenerative and cardiovascular diseases and even show antiproliferative effects (Huang and Sumpio, 2008; Owen et al., 2000; Franco et al., 2014).

Olive oil phenolics also contribute to the characteristic taste and the high stability of virgin olive oil against oxidation. The phenolic fraction of virgin olive oil consists of a heterogeneous mixture of compounds, each of which vary in chemical properties and impact on the quality of virgin olive oil (Dagdelen et al., 2013). Hydroxytyrosol (3.4-dihydroxyphenethylalcohol), tyrosol (4-hydroxyphenethylalcohol) and their derivatives with elenolic acid, which derive from the glycosides ligstroside and oleuropein, are the most abundant phenolic compounds in EVOO (Servili et al., 2004; Segura-Carretero et al., 2010; Kotsiou and Tasioula-Margari, 2015).

Phenolic compounds are attracting considerable attention over the last decades. The qualitative and quantitative composition of EVOO hydrophilic phenols is strongly affected by intrinsic (cultivated variety, ripening stage), and extrinsic factors (climatic condition, soil and geography of the olive growing area, agricultural practice, harvesting methods and time, transformation methods and time, differences regarding the processing and storage conditions (Romero and Motilva, 2010; Lozano-Sánchez et al., 2011; Bajoub et al., 2016). The aim of the study was determine the changes in the some phenolic compounds of organic and conventional extra-virgin olive oil (EVOO) based on the selected EVOOs present on the Turkish market.

2. MATERIALS AND METHODS

2.1. Chemicals

Oleuropein (OP), tyrosol (TY), hydroxytyrosol (HTY), para-coumaric acid (p-CA), caffeic acid (CAA) were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany). All reagent used were analytical grade purity. High quality water, obtained using a Milli-Q system (Millipore, Bedford, MA, USA), was used exclusively.

2.2. Extra virgin olive oils sampling

Samples of extra virgin olive oil were obtained from local markets in Bursa, Turkey. Ten extra virgin olive oils [organic (n=5) and conventional extra-virgin olive oil (n=5)], produced by different brands, were chosen for the analyses. The selections were made randomly. All oil samples were kept at room temperature in dark bottles until analyses.

2.3. Extraction and sample preparation

The phenolic extracts from the extra virgin olive oils were prepared according to Murkovic et al., (2004) with slightly modifications. Two milliliters of methanol was added to a sample of EVOOS 2 g and mixed with a vortex for 2 min. After this process the upper methanolic phase is used for LC-DAD-MS analysis.

2.4. LC-DAD- MS Analyses

Analyses of the phenolic compounds of olive oil were performed on an Agilent 1100 series LC/MSD Trap consisting of a vacuum degasser, autosampler, and a pump equipped with C18 column (4.6 ×50 mm, 1.8 μm). The mobile phases were water with acetic acid (0.2%) (phase A) and methanol (phase B) and were degassed by ultrasonication before use. The flow rate was kept at 0.4 ml min−1. The solvent gradient changed according to the following conditions: 0 min, 5% B; 0.5 min, 5% B; 8 min, 90% B; 10 min, 90 % B. The injection volume was 10μl, and peaks were monitored at 280 nm.

All of the analyses used the ion-spray source in the negative mode with the following settings: nebulizer gas (N2) gas 45.0 psi, drying gas flow to 11 l/ min, and dry gas temperature to 325 ^{o}C. To transfer the ions into capillary, a voltage of 3500 V was used. Full scan data were acquired by scanning from m/z 50 to 2200. For mass selective detection, the negatively charged ions were analyzed. For hydroxytyrosol, the ion with molecular mass of 153, Tyrosol 137, Caffeic acid 179, p-coumaric 163, and Oleuropein 593 were selected (Godoy et al., 2012; Tóth et al., 2015).

Oleuropein (OP), tyrosol (TY), hydroxytyrosol (HTY), para-coumaric acid (p-CA), caffeic acid (CAA) quantified against their corresponding reference compounds were expressed as mg of each compound per kg of extra virgin and conventional olive oil. In order to validate the LC-MS-DAD method used, some parameters were evaluated. The linearity was established by calculating the calibration curves for each standard compound. The reference molecules were dissolved in 100% methanol and analyzed by using six increasing concentrations within a range of specific values. The sensivity of the assay was defined by determining limit of the detection (LOD) and limit of the quantitation (LOQ) were calculated as then corresponding the multiple times the standard deviation of the lower concentration standard signal, respectively.

2.5. Statistical Analyses

All the determinations above described were conducted in triplicate and results are reported as mean ± SD values. The one-way analysis of variance was applied for the statistical evaluation of the results. It was designed by using the pocket program of the Minitab version 14.0. (Minitab Inc., State College, PA, USA). LSD ($p < 0.05$) was used in the tests.

3. RESULTS AND DISCUSSION

The analysis of the phenolic substances by using mass selective detection after liquid chromatographic separation showed that oleuropein, hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid were found in EVOO samples. Figure 1-3 shows the chromatograms of an EVOO samples.

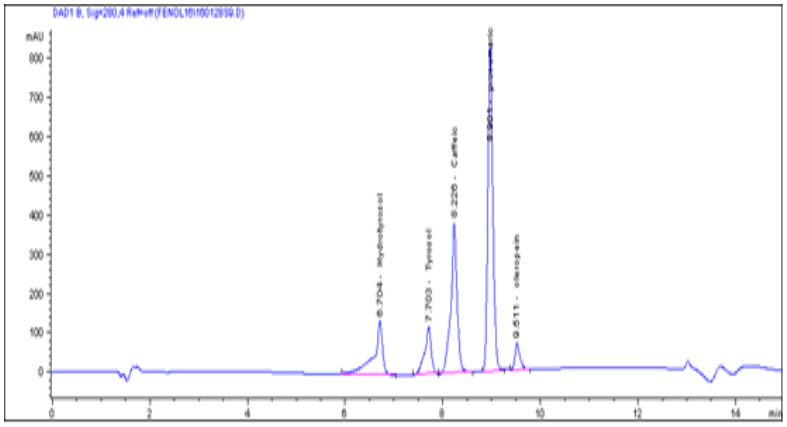

Figure 1:
Chromatogram of the standard of phenolic compounds in the optimal separation conditions.

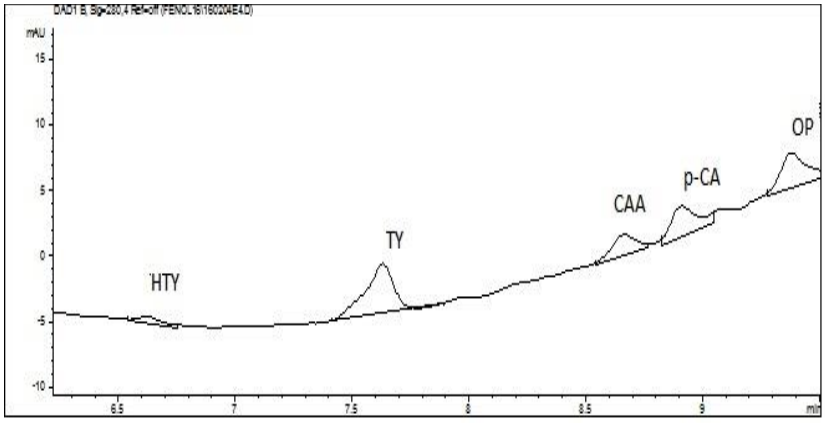

Figure 2:
Chromatogram of the CEVOO sample.

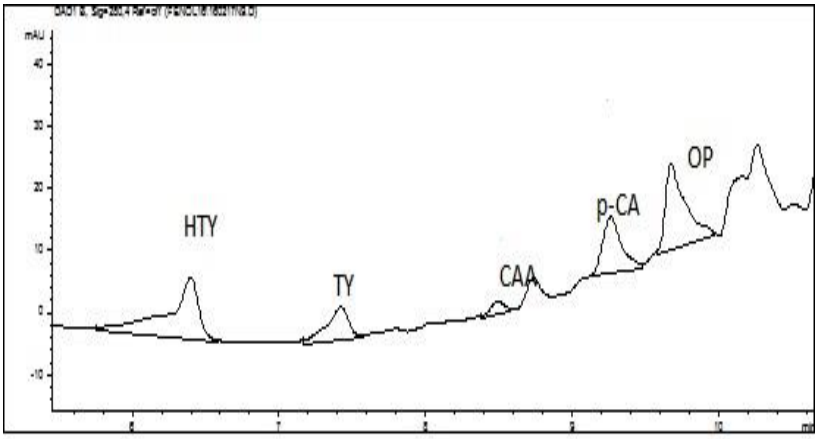

Figure 3:
Chromatogram of the OEVOO sample

The proposed method was validated by evaluating some parameters (Table 1). LOD and LOQ values for each molecule were lower than the working range, attesting the sensitivity of the method. As concerns the repeatability, the results were satisfactory, because RSD values

were lower than 5%. The linearity was established by construing calibration curves of each standard compound, obtained by plotting standard concentration as a function of the corresponding peak area. These curves were linear over the working range of study and the correlation coefficients (R^2) were higher than 0.9991 for all analytes.

Table 1. The Performance Characteristics of the Method

Phenolic compounds	Retention time (minute)	Detection limit (mg/ kg)	Quantification limit (mg/ kg)
Hydroxytyrosol	6.755	0.1	0.3
Tyrosol	7.750	0.3	1.0
Caffeic acid	8.273	0.2	0.7
Para-Coumaric acid	9.006	0.3	1.0
Oleuropein	9.548	0.3	1.0

Increasing evidences have supported the hypothesis that olive oil phenolic components, responsible for the bitter and pungent aroma and for oxidative stability of the olive oil, may play a major role in preventing oxidative damages. In this study, ten extra virgin olive oils obtained from organic and conventional products of EVOO were characterized some phenolic compounds.

The phenolic compounds that were quantified and identified in the samples were: tyrosol, hydroxytyrosol, p-coumaric acid, caffeic acid and oleuropein. The studied phenolic compounds are listed in Table 2.

Table 2. Selected Phenolic Compounds in EVOOS

Olive Oils	Phenolic Compounds (mg/kg)				
	Hydroxytyrosol	Tyrosol	Caffeic acid	Para-coumaric acid	Oleuropein
OEVOO1	1.1 ± 0.1*	1.5 ± 0.6	1.4 ± 0.2	1.1 ± 0.1	39.0 ± 2.1
OEVOO2	1.0± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	28.0 ± 2.0
OEVOO3	1.0 ± 0.1	6.4 ± 1.6	1.4 ± 0.2	1.1 ± 0.1	5.7 ± 1.3
OEVOO4	11.0 ± 1.0	16.0 ± 3.0	1.4 ± 0.2	ND	25.0 ± 2.1
OEVOO5	6.9 ± 1.1	14.0 ± 1.0	ND	1.0 ± 0.1	3.8 ± 0.6
CEVOO1	1.3 ± 0.1	1.9 ± 0.1	1.4 ± 0.1	ND	20.0 ± 2.1
CEVOO2	1.0 ± 0.1	1.1 ± 0.3	1.2 ± 0.1	ND	ND
CEVOO3	2.6 ± 0.1	3.2 ± 0.1	1.5 ± 0.1	ND	18.0 ± 1.3
CEVOO4	ND	1.3 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	14.0 ± 1.1
CEVOO5	8.4 ± 0.9	5.5 ± 1.4	1.3 ± 0.1	1.5 ± 0.3	28.0 ± 4.1

O-EVOO (organic extra-virgin olive oil), C-EVOO (conventional extra-virgin olive oil),
ND: The result is smaller than Method LOQ, *Mean value (n = 6) of standard division ($p < 0.05$).

Although with a large variability, OP was one of the most abundant phenolic compounds detected in the analyzed samples, ranging from 3.8 mg/kg of OEVOO5 to 39.0 mg/kg of OEVOO1. Oleuropein, which is found in olive oil, couldn't be identified in CEVOO2. With the exception of CEVOO2, OP was identified in all samples.

Hydroxytyrosol and tyrosol, ranging between 1.0–11.0 mg/kg and 1.1–16.0 mg/kg respectively, belong to the secoiridoid group and are the most characteristic compounds in olives and EVOO. Tyrosol and hydroxytyrosol concentrations decreased with increasing olive ripeness, because of they can be produced by the partial hydrolysis of their derivatives (Montedoro et al., 1992; Martinez et al., 2010; Franco et al., 2014). Olmo-Garcia et al. (2012) showed that the tyrosol concentration in Picholine Marocaine, Dohbia, Haouzia and Menara EVOO samples were in the range of 11.90-14.46 mg/kg, 2.03-2.17 mg/kg, 15.93-18.68 mg/kg and 10.01-14.05 mg/kg, respectively, values within those found in our study.

The contents of CAA and p- CA were similar in all olive oil samples. Also no significant differences of these phenolic compounds were found between olive oil types ($p > 0.05$). Our results were close to those obtained by Monaco et al., (2015), who characterized phenolic profile of extra virgin olive oils produced with typical Italian varieties. Franco et al.,(2014) analyzed virgin olive oil from Arbequina, Carrasqueña, Corniche, Manzanilla Cacereña,Morisca, Picual, and Verdial de Badajoz varieties and they found similar concentration (0.29-1.37 mg/kg) of para coumaric acid in our study. A higher amount of caffeic acid in Chemlali VOO was also reported by Hbaieb et al. (2017) compared to our results.

In this study, the phenolic compounds of extra virgin olive oils from the organic production were found higher than conventional production. Results showed a significant change in the phenolic profile according to olive growing condition (organic or conventional). It is possible to say that some evident differences were detected that OEVOO were the richest in terms of oleuropein and tyrosol.

4. CONCLUSION

The amount of phenolic compounds is an important factor when evaluating EVOO quality. Overall the tested oils showed qualitative and quantitative differences in phenol composition. The oleuropein levels were found to be higher than other phenolic compounds. The amount decreased with the order of tyrosol, hydroxytyrosol, caffeic acid and p-coumaric acid, respectively. In the comparison of the concentration of phenolic compounds among organic and conventional production of EVOO, differences were observed. As a conclusion, phenolic content are higher in organic products compared to conventional products of EVOO.

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