EFFECTS OF OLIVE LEAVES ON CHEMICAL AND PHENOLIC CONSTITUENTS OF REFINED OLIVE OILS

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ABSTRACT

Olive oils obtained from partly damaged olive fruits are negatively affected by biochemical changes and thus need to be refined for consumption purposes. However, the refining process precipitates losses of some bioactive compounds naturally found in olive oil. The primary purpose of this study is to evaluate the chemical properties of refined olive oils containing different rates of olive leaves (washed, dried, milled and sieved) in comparison with virgin olive oil. Relative to virgin olive oil, the total phenols, bitter index \((K_{225})\) and antioxidant activity values of olive oils enriched with olive leaves at concentrations of 10 % and 15 % were found to be higher, whereas the extinction coefficient values \((K_{232} \text{ and } K_{270})\) were found to be lower. The phenolic compound levels, i.e. the oleuropein, luteolin, tyrosol and hydroxytyrosol levels of refined olive oils containing 10 % and 15 % olive leaves increased significantly with the amount of leaves added. The results obtained emphasize the great potential of dried olive leaves for enhancing the chemical and phenolic properties of refined olive oils as a phenolic compound source.

Keywords: refined olive oil, olive leaves, phenolic compounds, antioxidant activity.

INTRODUCTION

Olive oil is characterized by nutritional and sensory properties such as bitterness, pungency, fruitiness and greenness, which are considered positive attributes of olive oils. Phenolic compounds are the major constituents responsible for the pungency and bitterness of olive oils (virgin). They protect oil against autoxidation, acting as antioxidants. Phenolic compounds also attract great interest because of their health benefits (Aguilera et al., 2015), and their contents in olive oils vary widely, depending on olive fruits, ripening levels, environmental conditions, growing region, crushing and paste malaxation processes (Rigane et al., 2013). Additionally, pigments and tocopherols act as antioxidants that play important roles in the olive oil (virgin) stability, as well as in the inhibition of some illnesses (Bengaia et al., 2013). The total phenols of virgin olive oils undergo some modifications due to hydrolytic and oxidative reactions during storage at 18-25 °C (a decrease in polyphenolic content of approximately 25-31 % occurs after 18-24 months of storage (Kotsiou and Tasioula-Margari 2016)).

Olive oils contain phenolic compounds such as phenolic acids (namely caffeic, p-coumaric, cinnamic, ferulic, benzoic, gallic, elenolic and vanillic acid), simple phenols (namely tyrosol, hydroxytyrosol, luteolin, quercetin, apigenin, rutin and secoiridoids) and glycoside derivatives that contain oleuropein and its constituents (Caruso et al., 2000; Ryan et al., 2002). In addition to the olive oil composition, olive leaves contain simple phenols such as hydroxytyrosol, acids such as tyrosol, gallic, caffeic, ferulic, p-coumaric, cinnamic and verbascoside acid, flavonoids such as quercetin, rutin, catechin, apigenin, and luteolin, and secoiridoids such as oleuropein, ligstroside and oleuropein aglycone (Talhaoui et al., 2015; Tsimidou and Papoti 2010). A significant similarity was found between the composition of olive oil and olive leaves relative to phenolic compounds.

Although olive oils are produced from olive fruits as virgin and extra virgin olive oils, considerable amounts of olive oils are refined to be edible. Refined olive oils are susceptible to deterioration during storage due to lower contents of phenolic compounds and pigments, especially chlorophylls. The enrichment of refined oils with natural additives in order to minimize oil deterioration is of paramount importance. Natural antioxidants and pigments, especially chlorophyll, carotenoid, tocopherol and polyphenols, play an important role in preventing oxidation reactions (Jaber et al., 2012). Olive leaves are the major by-products of the oil processing industry, which contain numerous phenolic compounds featuring strong radical scavenging activities. Moreover, olive leaves are rich in pigments, antioxidants and phenolic compounds, which have been used to remedy neurotic disorders, cancer, hypotension, hypoglycemia and microbial activities (Bouaziz et al., 2005; Mkaouar et al., 2015; Lafka et al., 2013). The pharmacological effects of olive leaves or their extracts are attributed to the...
presence of polyphenols such as oleuropein, hydroxytyrosol, verbascoside, tyrosol, apigenin and luteolin-7-glucoside (Yorulmaz et al., 2013; Arslan, 2012). In addition, a study on the pharmacological effects of phenolic compounds involving rats has shown that the oral administration of olive leaf extracts decreases infarct volume, brain edema and the barrier permeability of brain, ameliorating the neurologic deficit scores after permanent cerebral artery clogging (Mohaghegi et al., 2011). The addition of olive leaves (3 %) before olive fruit crushing improves the phenolic content, oxidation stability, aroma profiles and sensory properties such as greenness, fruitiness, bitterness and pungency of olive oils (tested during a sensory evaluation of olive oil samples) (Sonda et al., 2014). The pigment extract of olive leaves, especially chlorophyll, improves the stability of refined oil by inhibiting the thermal deterioration and enhancing the hydrolytic stability of refined olive oils during storage (Jaber et al., 2012). Moreover, it significantly improves the oxidation resistance, nutritional qualities and appearance of olive oils (Malheiro et al., 2013).

The purpose of the present study is to evaluate the effects of olive leaves addition on the chemical and phenolic constituents of refined olive oils. Different refined olive oils with increasing quantities of olive leaves (5 %, 10 % and 15 %) were processed in the study. Refined olive oils, olive leaves-enriched refined olive oils and virgin olive oil used as a control were characterized for several quality parameters, oxidative stability (free fatty acidity, peroxide value and specific extinction coefficients), phenolic composition and antioxidant activity.

**MATERIAL AND METHOD**

**Material**

Refined and virgin olive oils (from the ‘Gemlik’ cultivars) were obtained from a local olive oil factory in Osmaniye, Turkey. In October, fresh olive leaves were collected from the ‘Gemlik’ olive cultivar, which is commonly cultivated in Osmaniye. The leaves collected were subsequently washed, dried under atmospheric conditions (25 °C), crushed, milled and sieved. The olive leave particles obtained (< 0.5 mm) were sieved. The olive leaf particles obtained (< 0.5 mm) were dried under atmospheric conditions (25 ºC), crushed, milled and sieved. The olive leave particles obtained (< 0.5 mm) were dried under atmospheric conditions (25 ºC), crushed, milled and sieved.

**Methods**

**Total Phenols**

Refined olive oils containing olive leaves were filtered by filter paper before the analytical process. The mass of olive oil samples obtained from the control, virgin and refined olive oil containing olive leaves totaled 1 g, and the mixture of 5 mL CH₃OH/H₂O was poured onto the samples (60/40 v/v). Thereafter, the samples were shaken for 2 minutes, filtered using a filter of 0.45 µm (PTFE) and centrifuged at 3,500 rpm for 10 minutes. The procedure was repeated with the remainder of the portion by adding 5 mL of CH₃OH/H₂O, and subsequently 10 mL of pure water was poured onto the extracted volume. A mixture of 0.1 mL was taken from the extract and poured into a mixture (5 mL), and subsequently extracted with 5 mL of the CH₃OH/H₂O mixture (60/40). A 5 mL of methanol/water solution was added, and the absorbance values of the solution were measured at 225 nm. The bitter index of the olive oil samples was calculated using the following equation:

\[ K_{225} = A_{225}/C \]  (1)

where C is g of olive oil/100 mL and A₂₂₅ is the absorbance at 225 nm (Bouarroudj et al., 2016).

**DDPH (2,2-diphenyl-1-picrylhydrazyl radical)**

The radical scavenging activity method was used to determine the inhibition percentage of total phenols obtained from the oil samples. A mixture of 0.1 mL of the extracted volume was poured to a 2.9 mL DPPH solution (0.24 mg/L, in methanol) and kept in a dark place for 30 minutes, after which the absorbance was measured at 515 nm using a spectrophotometer (Shimadzu UV 1800). The radical scavenging activities of the olive samples (DPPH, %) were measured as the inhibition percentage as follows (Ferreira et al., 2007):

\[ DDPH = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \]  (2)

**Chromatographic Analysis of Phenolic Constituents**

Stock solutions of olive oil phenols were prepared in CH₃OH at 2 mg mL⁻¹, whereas standard stocks of chemicals were prepared by serial dilution of solutions containing the CH₂OH/H₂O mixture. Phenolic compounds were separated using the HPLC (Thermo Dionex; CA, USA), which consisted of a solvent efficient pump attached to an array detector (diode) and a UV detector. The 1 µL samples obtained from phenolic extracts and olive leaves were split on the column (250 x 4.6 mm x 5 µ) (Interstil, Japan). The separation process on the C18 ODS-3 column was performed using a gradient elution volume ranging from 10 % to 70 % CH₂OH for 9 minutes and 100 % CH₂OH for 2 minutes at a rate of 1 mL per minute. The mobile portion recorded was 19 to 1 (v/v), water-formalin and methanol-formalin mixtures (Solvent A and B). The photodiode array detector and absorbance values were used to determine the phenolic compounds (280 and 320 nm). The quantities of phenolic compounds (namely hydroxytyrosol, tyrosol (2-4-hydroxyphenylethanol), oleuropein) and acids (namely vanillic, caffeic, p-coumaric, o-coumaric, vanillin, cinnamic, ferulic, apigenin, syringic, luteolin and gallic acid) were determined according to the peak areas relative to the phenolic standards (Yorulmaz et al., 2013; Cardoso et al. 2005; Bouarroudj et al., 2016).

**RESULTS AND DISCUSSIONS**

Free acidity of olive oils is referred to as a quality factor for classifying the samples. The free acidity of refined, virgin and enriched with olive leaves refined olive oil samples (with the

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free acidity values ranging 0.91-1.29 %) exceeded the upper limit of 0.8 % according to the council standard set for olive oil (IOOC, 2011). The refined olive oils containing olive leaves exhibited a slight decrease in the free acidity percentage, whereas higher acidity values were found in the refined olive oils, with and without olive leaves, compared to the virgin olive oil samples. The acidity of refined olive oils obtained showed a similarity between the Turkish olive cultivars examined (ranging from 0.5 % to 1.17 % (Tamligan et al., 2007)). However, the values obtained were higher than those recorded in the Tunisian (Sonda et al., 2014) and Algerian wild olive oil samples (Bouarroudj et al., 2016). At a ratio of 3 %, leaves added to olive varieties increase the free acidity (Sonda et al., 2014), whereas the addition of olive leaves to refined olive oils showed a similarity between both refined and virgin olive oils in our study.

The peroxide values of olive oils, which are the main analytic parameters showing lipid oxidation measurements, ranged from 8.32 to 15.81 meq O2 kg⁻¹, which proved lower than the values stipulated by the International Olive Oil Council (IOOC, 2011) (EEC 2568/91). The highest peroxide value was observed in the virgin olive oil samples (15.81 meq O2 kg⁻¹), whereas olive leaves added to the refined oil samples at a ratio of 10 % and 15 % led to an increase in the peroxide values (p < 0.05). It has been reported that olive oils (Cobrançosa var.) containing olive leaves (1-10 %) during oil processing increase the peroxide values by facilitating the respiration process of gases and the obtainable oxygen level (Malheiro et al., 2013). Moreover, the addition of olive leaves (3 %) before the oil extraction procedure significantly increases the peroxide values (monovarietal Tunisian olives) (Sonda et al., 2014). Our results are in agreement with the results of Sonda et al. (2014) for the ‘Chemlali’, ‘Zalmati’, ‘Chetoui’ olive cultivars in Tunisia and the ‘Cobrançosa’ cultivar in Portugal (Malheiro et al., 2013).

The amount of phenolic compounds in the refined and virgin olive oil samples ranged between 109.8 mg GAE kg⁻¹ and 143.4 mg GAE kg⁻¹, whereas their contents ranged between 138.6 mg GAE kg⁻¹ and 217.9 mg GAE kg⁻¹ and 233.2 mg GAE kg⁻¹ for the refined olive oil containing 5 %, 10 %, and 15 % olive leaves, respectively (Table 1). The DPPH activity of the olive oil samples extracted with methanol/water (80/20) and the total phenols are shown in Table 1 as the inhibition percentage of the DPPH radical. The refined olive oil samples containing 10 % and 15 % of olive leaves exhibited higher amounts of the phenolic contents inhibited, i.e. 93.5 % and 92.5 % of the DPPH radical, respectively.

The spectrophotometric characteristics of the oil samples were expressed by determining the coefficients K232 and K270 at 232 and 270 nm, the values at which the absorbance of oxidation products indicate extensive oxidation reactions in oil/fat-containing products. The olive oil samples showed that the K232 and K270 values varied from 2.03 to 3.53 and from 0.210 to 0.96, respectively (Table 1). The refined olive oil (the control sample) and the olive oils containing 5 % and 10 % olive leaves exhibited similar K232 and K270 levels. By contrast, the olive oil containing 15 % olive leaves had significantly higher K232 and K270 values than the virgin olive oil samples. The specific extinction coefficients of the olive oil samples containing 5 % and 10 % of olive leaves were higher than the Algerian wild olive oils (K232 ranging from 1.69 to 1.78; K270 ranging from 0.05 to 0.18 (Bouarroudj et al., 2016); the Tunisia olive star cultivar: K232 from 1.61 to 2.36 and K270 from 0.12 to 0.17; the ‘Cobrançosa’ olive oil that contains different amount of olive leaves: 0-10 %, K232 from 1.63 to 2.07 and K270 from 0.09 to 0.16 (Bouazizi et al., 2005); and higher than the ‘Memecik’ and ‘Edremit’ cultivars: K232 from 1.42 to 1.73 and K270 from 0.04 to 0.10 for Memecik, K232 from 1.49 to 1.59 and K270 from 0.03 to 0.13 for the ‘Edremit’ cultivar (Yorulmaz et al., 2013). The determination of K232 and K270 spectrophotometrically provides the information about oil quality and possible changes in olive oil samples. The refined olive oil containing olive leaves, especially the 15 % sample, indicated an increase in the oxidation and exhibited higher specific extinction coefficients than other olive oil samples. These results are in agreement with the results of Malheiro et al., (2013) and Sonda et al., (2014) respectively.

The bitter index values ranged from 1.50 to 4.50, whereas the refined olive oil exhibited the lowest K225 and the refined olive oil containing 15 % of olive leaves the highest K225 value. The bitterness value of the virgin olive oil and the refined olive oil were 4.18 and 1.50, respectively. Olive leaves added to the refined oils significantly increased the bitterness. The bitter index of ‘Gemlik’, ‘Haltali’, and ‘Sari Hashebi’ cultivars grown in the Hatay province changed varied 0.31 and 0.72 (Konuskan, 2008), whereas in the ‘Coratina’, ‘Ogliarola’, ‘Maïtica’, ‘Leccino’, and ‘Blend’ cultivars grown in Italy, it varied between 0.21 and 0.40 (Favati et al., 2013). Therefore, the results obtained in this study were higher for these olive cultivars. There is a close relationship between the bitter index of olive oil samples and the phenol contents (mg GAE kg⁻¹) as the increase in phenol contents of olive oils leads to the increase in bitterness (Aguilera et al., 2013, Condelli et al., 2015).

Table 1. Extinction coefficients, bitter index, DPPH, free acidity, peroxide value and total phenols of the refined, virgin and olive leaves added refined olive oil samples

| Quality index | Refined olive oil (control) | Refined olive oil (5%) | Refined olive oil (10%) | Refined olive oil (15%) | Virgin olive oil (control) |
|---------------|----------------------------|------------------------|-------------------------|-------------------------|---------------------------|
| DPPH (%)      | 22.0 ± 0.27                | 26.0 ± 0.31            | 93.5 ± 0.85             | 92.5 ± 0.79             | 44.5 ± 0.44               |
| Total Phenols (mg GAE kg⁻¹) | 109.8 ± 0.81              | 138.6 ± 0.88           | 217.9 ± 1.23            | 233.2 ± 1.44            | 143.4 ± 0.97              |
| Free acidity (meq O₂ kg⁻¹) | 1.29 ± 0.06               | 1.15 ± 0.05            | 1.24 ± 0.07             | 1.11 ± 0.05             | 0.95 ± 0.03               |
| Peroxide value | 8.32 ± 0.11                | 8.57 ± 0.11            | 10.93 ± 0.10            | 12.19 ± 0.13            | 15.81 ± 0.14              |
| K225          | 1.50 ± 0.03                | 2.10 ± 0.05            | 4.20 ± 0.08             | 4.50 ± 0.09             | 4.18 ± 0.08               |
| K232          | 2.73 ± 0.02                | 2.53 ± 0.02            | 2.76 ± 0.02             | 3.53 ± 0.03             | 2.03 ± 0.02               |
| K270          | 0.74 ± 0.01                | 0.67 ± 0.01            | 0.75 ± 0.02             | 0.96 ± 0.02             | 0.210 ± 0.01              |

Different letters in row indicate significantly different values (p<0.05)

The phenolic substances of the olive leaves and olive oils were defined and measured using the HPLC system, i.e. the corresponding standard curves that were linear over the studied range and the regression coefficient (r²) > 0.98 for the phenolic constituents examined. The analytical procedure of phenolic constituents allowed the separation and determination of 11 phenolic constituents among 14 phenolic standards, all of which were identified using the HPLC system (Table 2). It is noteworthy that caffeic, syringic and gallic acids were not determined in olive leaves. Among the phenolic constituents, luteolin, ferulic acid, vanillic acid, and p-coumaric acid were not found in the refined olive oil samples (the control samples). Statistically significant differences were found between the phenolic constituents of

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olive leaves and olive oil samples ($p < 0.05$). The major phenolic constituent of olive leaves was oleuropein and its concentration amounted to 739.13 mg GAE kg$^{-1}$. Moreover, it was determined that olive leaves were a good source of apigenin, $o$-coumaric acid, luteolin, ferulic acid, $t$-cinnamic acid, and tyrosol as shown in Table 2. The hydroxytyrosol concentration, i.e. phenolic alcohol with a high antioxidant activity, was 38.694 mg GAE kg$^{-1}$. Vanillin, $p$-coumaric acid, and tyrosol, with concentrations varying from 4.060 to 21.326 mg GAE kg$^{-1}$, were the first group to be identified by the HPLC method in olive leaves and olive oil samples. Our results showed that leaves are fine sources of luteolin, flavonoids, ferulic acid and hydroxycinnamic acids. The results obtained in this study are consistent with the results of Bayram et al., 2012.

Luteolin, ferulic acid, vanillin acid and $p$-coumaric acid were not found in the refined olive oil sample (the control sample) as indicated in Table 2, and the amount of phenolic constituents were low with values varying from 0.024 to 1.429 mg GAE kg$^{-1}$. However, the vanillin acid, luteolin and $p$-coumaric acid concentrations, which were not found in the refined olive oil (the control sample), increased significantly. When comparing the increased amount of phenolic constituents of refined olive oils to the virgin olive oil, all the phenolic constituents, except ferulic acid, increased significantly due to the addition of olive leaves ($p < 0.05$). The oleuropein concentrations responsible for bitterness changed from 3.722 to 26.354 mg GEA kg$^{-1}$, whereas the luteolin concentration changed between 13.127 and 14.438 mg GAE kg$^{-1}$, which was not found in the olive oil (virgin) sample.

The $t$-cinnamic acid content, an important marker of Turkish olive cultivars (Bayram et al., 2012), changed between 0.101 and 0.524 mg GAE kg$^{-1}$. In the studies regarding the amount of phenolic constituents, the concentration of these substances varies greatly in different olive cultivars. The tyrosol concentration changed from 5.1 to 105, the luteolin concentration from 0.97 to 13 mg GAE kg$^{-1}$, the hydroxytyrosol concentration from 0.68 to 10, the apigenin concentration from 0.07 to 1.1, and the oleuropein concentration from 24 to 90 mg GAE kg$^{-1}$ in the Algerian wild olive cultivars examined, respectively (Bouarroudj et al., 2016). With regard to the Turkish olive cultivars examined, the tyrosol concentration changed from 4.1 to 22, the hydroxytyrosol concentration from 1.1 to 26 mg GAE kg$^{-1}$, and the oleuropein concentration from 0.48 to 0.72 mg GAE kg$^{-1}$, respectively (Bayram et al. 2012).

Relative to the Spanish olive cultivar examined, the luteolin concentration changed from 0.72 to 2.96 and the apigenin concentration from 0.17 to 0.91 mg GAE kg$^{-1}$ (Franco et al., 2014). In the case of the Turkish ‘Edremit’ and ‘Memecik’ olive cultivars, the luteolin concentration changed from 29.26 to 63.42 mg GEA kg$^{-1}$, and the apigenin concentration from 21.60 to 76.35 mg GAE kg$^{-1}$, respectively (Yorulmaz et al., 2013). Our results showed that the addition of olive leaves to refined olive oils enhanced the quality of refined olive oils relative to phenolic constituents, particularly oleuropein, luteolin, vanillic acid, $p$-coumaric acid, tyrosol and hydroxytyrosol. Finally, the results obtained in the present study are consistent with the results of Franco et al., (2014) and Yorulmaz et al., (2013).

**CONCLUSION**

Olive leaves, as an important by-product of the olive processing and oil industry, contain a large variety of phenolic constituents, simple phenols, secoiridoids and pigments, which are similar to those found in the olive oil composition. The total phenols increased with the addition of olive leaves to refined olive oils. The total phenols, DPPH (%), extinction coefficients ($K_{232}$ and $K_{270}$) and bitter index ($K_{225}$) of the refined olive oils containing 10% and 15% of olive leaves exhibited superior properties compared to virgin olive oils. Moreover, the phenolic constituents of the refined olive oils containing 10% and 15% were also improved, especially oleuropein, luteolin, tyrosol and hydroxytyrosol. A total of 11 phenolic constituents out of 14 compounds were identified in olive leaves, whereas the oleuropein, luteolin, hydroxytyrosol, tyrosol and vanillic acid concentrations of refined olive oils increased significantly.

**REFERENCES**

Aguilera, M.P., Jimenez, A., Sanchez-Villascaras, S., Uceda, M., Beltran, G. (2015): Modulation of bitterness and pungency in virgin olive oil from unripe ‘Pical’ fruits. European Journal of Lipid Science and Technology, 117, 1463-1472.

Arslan D. (2012). Physico-chemical characteristics of olive fruits of Turkish varieties from the province of Hatay. Grasas Y Aceites, 63, 2, 158-166.

Bayram, B., Ozcelik, B., Grimm, S., Roeder, T., Schrader, C., Ernst, I. M., ... & Rimbach, G. (2012). A diet rich in olive oil phenolics reduces oxidative stress in the heart of N nr2-dependent gene expression. Rejuvenation Research, 15(1), 71-81.

Bengana, M., Bakhouce, A., Lozano-Sanchez, J., Amir, Y., Youyou, A., Segura-Carretero, A., Fernandez-Gutierrez, A. (2013). Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil. Food Research International, 54, 1868-1875.

Bouarroudj, K., Tamendjari, A., Larbat, R. (2016). Quality, composition and antioxidant activity of Algerian wild olive (Olea europaea L. subsp. Oleaster) oil. Industrial Crops and Products.

**Table 2. Phenolic constituents of olive leaves, virgin and refined olive oils containing olive leaves (mg GAE kg$^{-1}$)**

| Phenolic Constituents | Olive leave | Refined olive oil (control) | Refined olive oil (5%) | Refined olive oil (10%) | Refined olive oil (15%) | Virgin olive oil (control) |
|----------------------|-------------|----------------------------|------------------------|-------------------------|-------------------------|--------------------------|
| Hydroxytyrosol       | 38.694 ±1.1 | 0.801 ±0.01                | 1.423 ±0.02            | 2.885 ±0.05             | 3.144 ±0.17              | 2.754 ±0.03              |
| Tyrosol              | 21.326 ±0.8 | 1.024 ±0.01                | 2.806 ±0.04            | 3.778 ±0.06             | 13.028 ±0.32             | 26.354 ±0.44             |
| Oleuropein           | 734.13 ±8   | 1.429 ±0.02                | 3.722 ±0.05            | 5.876 ±0.03             | 5.798 ±0.05              | 12.120 ±0.27             |
| $o$-coumaric acid    | 70.918 ±1.4 | 0.184 ±0.009               | 2.144 ±0.03            | 2.574 ±0.09             | 3.025 ±0.04              | 8.004 ±0.009             |
| $t$-cinnamic acid    | 17.151 ±0.6 | 0.049 ±0.007               | 0.101 ±0.004           | 0.462 ±0.008            | 0.524 ±0.009             | 0.077 ±0.0008            |
| Luteolin             | 39.177 ±1.2 | -                          | 13.127 ±0.14           | 15.028 ±0.38            | 14.438 ±0.29             | -                        |
| Ferulic acid         | 33.261 ±1.0 | -                          | -                      | -                       | -                       | -                        |
| Apigenin             | 87.14 ±1.5  | 0.045 ±0.0009              | 0.905 ±0.008           | 1.215 ±0.02             | 1.314 ±0.03              | 1.141 ±0.04              |
| Vanillin             | 4.060 ±0.07 | 0.024 ±0.0009              | 0.418 ±0.003           | 0.591 ±0.004            | 0.740 ±0.008             | 0.560 ±0.007             |
| Vanilliac acid       | 3.651 ±0.05 | -                          | 1.490 ±0.007           | 1.794 ±0.07             | 1.988 ±0.05              | 1.617 ±0.07              |
| $p$-coumaric acid    | 13.821 ±0.3 | -                          | 1.854 ±0.03            | 2.148 ±0.09             | 2.497 ±0.07              | 0.834 ±0.02              |

Small letters in a column, capital letters in a row indicate significantly different values ($p < 0.05$).
Bouaziz M., Grayer R. J., Simmonds, M. S. J., Damak, M., and Sayadi, S. (2005). Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar Chemlali growing in Tunisia. Journal of Agriculture and Food Chemistry, 53, 236-241.

Condelli, N., Caruso, M.C., Galgano, F., Russo, D., Milella, L., Fava, F. (2015) Prediction of the antioxidant activity of extra virgin olive oils produced in the Mediterranean. Food Chemistry, 177, 233-239.

Cardoso, S.M., Guyot, S., Marnet, N., Lopes da Silva, J. A., Renard, C. M., & Coimbra, M. A. (2005). Characterisation of phenolic extracts from olive pulp and olive pomace by mass spectrometry. Journal of the Science of Food and Agriculture, 85(1), 21-32.

Caruso, D., Colombo, R., Patelli, R., Giavarini, F., Galli, G. (2000). Rapid evaluation of phenolic component profile and analysis of oleuropein aglycon in olive oil by atmospheric pressure chemical ionization– mass spectrometry (APCI–MS). Journal of Agricultural and Food Chemistry, 48(4), 1182-1185.

Favati, F., Condelli, N., Galgano, F., Caruso, M. C. (2013) Extra virgin olive oil bitterness evaluation by sensory and chemical analyses. Food Chemistry, 139, 949-954.

Ferreira, I.C.F.R., Barros, L., Soares, M.E., Bastos, M.L., Pereira, J.A. (2007). Antioxidant activity and phenolic contents of olea europaea L. leaves sprayed with different copper formulations. Food Chemistry, 103,188-19.

Franco, M. N., Galeano-Diaz, T., Lopez, O., Fernandez-Bolaños, J. G., Sanchez, J., De Miguel, C., Martin-Vertedor, D. (2014). Phenolic compounds and antioxidant capacity of virgin olive oil. Food Chemistry, 163, 289-298.

Harp, F. (2011). Determination of antioxidant effects of Gemlik, Domat, Adana Topagi and Adana local type olive leaves. MSc Thesis. Cukurova University, Natural and Applied Science. Adana

Hrncirik, K., S. Fritsche. (2004). Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. European Journal of Lipid Science and Technology, 106: 540.

IOOC. (2011). Guide for the determination of the characteristics of oils-olives. International Olive Oil Council.

Jaber, H., Ayadi, M., Makni, J., Rigane, G., Sayadi, S., Bouaziz, M. (2012). Stabilization of refined olive oil by enrichment with chlorophyll pigments extracted from Chemlali olive leaves. European Journal of Lipid Science and Technology, 114, 1274-1283.

Kotsiou, K., Tasioula-Margari, M. (2016). Monitoring the phenolic compounds of Greek extra-virgin olive oils during storage. Food Chemistry, 200, 255-262.

Lafka, T.L., Lazou, A.E., Sinanoglou, V.J., Lazoza, E.S. (2013). Phenolic extract from wild olive leaves and their potential as edible oils antioxidants. Foods, 2, 18-31.

Malheiro, R., Casal, S., Teixeira, H., Bento, A., Pereira, J.A. (2013). Effect of olive leaves addition during the extraction process of overmature fruits on olive oil quality. Food Bioprocess Technology, 6, 509-521.

Mohagheghi, F., Bigdeli, M.R., Rasoulian, B., Hashemi, P., Pour, M.R. (2011). The Neuroprotective effect of olive leaf extract is related to improved blood-brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia, Phytomedicine, 18, 170-175.

Mkaouar, S., Bahloul, N., Gelicus, A., Allaf, K., Kechaou, N. (2015). Instant controlled pressure drop texturing for intensifying ethanol solvent extraction of olive leaf polyphenols. Separation and Purification Technology, 145, 139-146.

Rigane, G., Bouaziz, M., Sayadi, S., Salem, R. B. (2013). Effect of storage on refined olive oil composition: stabilization by addition of chlorophyll pigments and squalene. Journal of Oleo Science, 62 (12), 981-987.

Ryan, D., Antolovich, M., Prenzler, P., Robards, K., Shimon L. (2002). Biotransformations of phenolic compounds in Olea europaea L. Scientia Horticulturae, 92: 147-176.

Sonda Akram, Z., Boutheina, G., Guido, F., Mohamed, B. (2014): Effect of addition of olive leaves before fruits extraction process to some monovarietal Tunisian extra-virgin olive oils using chemometric analysis. Journal of Agriculture and Food Chemistry, 62, 251-263.

Tanilgan, K., Özcanb, M. M., & Ünverb, A. (2007). Physical and chemical characteristics of five Turkish olive (Olea europea L.) varieties and their oils. Grasas y aceites, 58(2), 142-147.

Talhaoui, N., Taamalli, A., Gomez-Caravaca, A.M., Fernandez-Gutierrez, A., Segura-Carretero A. (2015). Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits. Food Research International, 77,92-108.

Tsimidou, M.Z., Papoti, P.V. (2010). Bioactive ingredients in olive leaves, olives and olive oil in health and disease prevention, Preedy V, R, Watson R, R Ed. Elsevier, London, Chapter 39, 349-356.

Yorulmaz, A., Ercin, H., Tekin, A. (2013) Changes in olive oil characteristics during maturation. Journal of American Oil Chemists Society, 90, 647-658.

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