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

UTICAJ MASLINOVOG LIŠĆA NA HEMIJSKE I FENOLNE KOMPONENTE RAFINISANOG MASLINOVOG ULJA

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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} 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 .*

REZIME

Maslinova ulja, koja su dobijena od delimično oštećenih maslina, imaju biohemijske reakcije kao negativnu pojavu. Zbog toga se moraju rafinisati za svrhe potrošnje. Međutim, proces rafinisanja podrazumeva gubitke nekih bioaktivnih jedinjenja koja se prirodno nalae u maslinovom ulju. Osnovna svrha ovo istraživanja je da proceni hemijske osobine rafinisanih maslinovih ulja koja sadrže različite udele listova maslina (opranih, suvih, mlevenih i prosejanih) u poređenju sa devičanskim maslinovim uljem. U odnosu na devičansko maslinovo ulje , ukupni fenoli , gorki indeks (K225) i vrednosti antioksidativne aktivnosti maslinovog ulja obogaćenog listovima maslina u koncentracijama od 10% i 15% su veće, ali je utvrđeno da su vrijednosti koeficijenta ekstinkcije (K232 i K270) niže. Nivoi fenolnih jedinjenja, tj. Oleuropeina, luteolina, tirozola i hidroksi-trosola, za rafinisana maslinova ulja koji sadrže 10% i 15% lišća maslina, značajno su porasli sa količinom dodatog lišća . Dobijeni rezultati naglašavaju veliki potencijal sušenih maslina za poboljšanje hemijskih i fenolnih svojstava rafinisanih maslinovih ulja kao izvora fenolnih jedinjenja.

Ključne reči: *rafinisano maslinovo ulje, listovi maslina, fenolna jedinjenja, antioksidativna aktivnost.*

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 (*Bengana et al., 2013*). The total phenols of virgin olive oils undergo some modifications due to hydrolytic and oxidative reactions during storage at 18-25 ºC (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 vanilic acid), simple phenols (namely tyrosol, hydroxytyrosol, luteolin, quercetin, apigenin, rutin and secoiridoid) 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 lutelin, 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 impermanent 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 ºC), crushed, milled and sieved. The olive leave particles obtained (< 0.5 mm) were added to the experimental refined olive oils at different ratios (5-15 %), stirred every day and stored in a dark place using a glass container for 30 days at 20 ºC. The reagents, chemicals, and phenolic standards were supplied by the Merck (Germany), Sigma (USA), and Fluka (Switzerland) companies.

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 CH_3OH/H_2O was poured onto the samples (60:40 v v^{-1}). 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 CH_3OH/H_2O, 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 50 mL volumetric flask. A total of 5 mL of distilled water and Folin-Ciocalteu solution (0.5 mL) was poured into the flasks and the mixture was kept for 3 minutes. The volume was completed by adding distilled water (50 mL) and 1 mL of Na_2CO_3 (35% w v^{-1}). The mixture was kept in a dark place for 2 hours, and the absorbance value of the mixture was evaluated at 725 nm (Shimadzu UV 1800, Japan) relative to the replicate solution. The results obtained were expressed in mg of GAE per kg. Ultimately, the same analytical procedures were performed for olive leaves as well (*Hrncirik and Fritsche 2004*).

Quality Indices

The peroxide, free acidity and spectrophotometric absorbance (K_{232} and K_{270}) values obtained were in accordance with the EU standards. (Commission Regulation no. 2568/91).

Bitter Index (K_{225})

A total of 1 g of the olive oil sample was dissolved in hexane mixture (5 mL), and subsequently extracted with 5 mL of the CH_3OH/H_2O (60/40) mixture. The extracted solution was mixed using the Vortex equipment and centrifuged at 3,500 rpm for 10 minutes. After removing the hexane layer formed, the polar phase of mixture was transferred to a flask by adding 10 mL of the CH_3OH/H_2O 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:

$$K_{225} = A_{225}/C \quad (1)$$

where C is g of olive oil/100 mL and A_{225} 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*):

$$DPPH = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \times 100 \quad (2)$$

Chromatographic Analysis of Phenolic Constituents

Stock solutions of olive oil phenols were prepared in CH_3OH at 2 mg mL^{-1}, whereas standard stocks of chemicals were prepared by serial dilution of solutions containing the CH_3OH/H_2O mixture. Phenolic compounds were seperated using the HPLC (Thermo Dionex; CA, USA), which consisted of a solvent efficient pump attached to an array detector (diode) and a UV dedector. 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 % CH_3OH for 9 minutes and 100 % CH_3OH for 2 minutes at a rate of 1 mL per minute. The mobile portion ratio recorded was 19 to 1 (v v^{-1}), 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

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 % (*Tanilgan 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 O_2 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 O_2 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 vr.) containing olive leaves (1-10 %) during oil processing increas 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 olive 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}, 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 K_{232} and K_{270} at 232 and 270 nm, the values at which the absorbance of oxidation products indicates extensive oxidation reactions in oil/fat-containing products. The olive oil samples showed that the K_{232} and K_{270} 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 K_{232} and K_{270} levels. By contrast, the olive oil containing 15 % olive leaves had significantly higher K_{232} and K_{270} 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 (K_{232} ranging from 1.69 to 1.78; K_{270} ranging from 0.05 to 0.18 (*Bouarroudj et al., 2016*); the Tunisia olive star cultivar: K_{232} from 1.61 to 2.36 and K_{270} from 0.12 to 0.17; the 'Cobrançosa' olive oil that contains different amount of olive leaves: 0-10 %, K_{232} from 1.63 to 2.07 and K_{270} from 0.09 to 0.16 (*Bouaziz et al., 2005*); and higher than the 'Memecik' and 'Edremit' cultivars: K_{232} from 1.42 to 1.73 and K_{270} from 0.04 to 0.10 for Memecik, K_{232} from 1.49 to 1.59 and K_{270} from 0.03 to 0.13 for the 'Edremit' cultivar (*Yorulmaz et al., 2013*). The determination of K_{232} and K_{270} 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 K_{225} and the refined olive oil containing 15 % of olive leaves the highest K_{225} 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', 'Halhali', and 'Sarı Haşebi' cultivars grown in the Hatay province changed varied 0.31 and 0.72 (*Konuskan, 2008*), whereas in the 'Coratina', 'Ogliarola', 'Maiatica', '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., 2015, 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^{c} ±0.27	26^{c} ±0.31	93.5^{a} ± 0.85	92.5^{a} ±0.79	44.5^{b} ±0.44
Total Phenols (mg GAE kg^{-1})	109.8^{d} ±0.81	138.6^{c} ±0.88	217.9^{b} ±1.23	233.2^{a} ±1.44	143.41^{c} ±0.97
Free acidity (%)	1.29^{a} ±0.06	1.15^{b} ± 0.05	1.24^{a} ±0.07	1.11^{b} ±0.05	0.95^{c} ±0.03
Peroxide value (meq O_2 kg^{-1})	8.32^{d} ±0.11	8.57^{d} ±0.11	10.93^{c} ±0.10	12.19^{b} ±0.13	15.81^{a} ±0.14
K_{225}	1.5^{d} ±0.03	2.10^{c} ±0.05	4.20^{b} ±0.08	4.50^{a} ±0.09	4.18^{b} ±0.08
K_{232}	2.73^{b} ±0.02	2.53^{c} ±0.02	2.76^{b} ±0.02	3.53^{a} ±0.03	2.03^{d} ±0.02
K_{270}	0.74^{b} ±0.01	0.67^{b} ±0.01	0.75^{b} ±0.02	0.96^{a} ±0.02	0.210^{c} ±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 (r^2) > 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

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, flavones, ferulic acid and hydroxycinnamic acids. The results obtained in this study are consistent with the results of *Harp (2011)* and *Talhaoui et al., (2015).*

Luteolin, ferulic acid, vanillic 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 vanillic 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 GAE 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.

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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^{c} ±1.1	$0.801^{c,D}$ ±0.01	$1.423^{e,C}$ ±0.02	$2.885^{d,B}$ ±0.05	$3.144^{d,A}$ ±0.17	2.754^{c} ±0.03
Tyrosol	21.326^{d} ±0.8	$1.024^{b,D}$ ±0.01	$2.806^{c;C}$ ±0.04	$3.778^{c,B}$ ±0.06	$5.876^{c,A}$ ±0.03	3.698^{b} ±0.05
Oleuropein	734.13^{a} ±8	$1.429^{a,D}$ ±0.02	$3.722^{b,C}$ ±0.05	$13.028^{b,B}$ ±0.32	$26.354^{a,A}$ ±0.44	12.120^{a} ±0.27
o-coumaric acid	70.918^{b} ±1.4	$0.184^{d,D}$ ±0.009	$2.144^{d,C}$ ±0.03	$2.574^{d,B}$ ±0.09	$3.025^{d,A}$ ±0.04	0.800^{f} ±0.009
t-cinnamic acid	17.151^{e} ±0.6	$0.049^{e,C}$ ±0.007	$0.101^{g,B}$ ±0.004	$0.462^{h,A}$ ±0.008	$0.524^{h,A}$ ±0.009	0.077^{h} ±0.0008
Luteolin	39.177^{c} ±1.2	-	$13.127^{a,B}$ ±0.14	$15.028^{a,A}$ ±0.38	$14.438^{b,A}$ ±0.29	-
Ferulic acid	33.261^{c} ±1.0	-	-	-	-	-
Apigenin	87.14^{b} ±1.5	$0.045^{e,C}$ ±0.0009	$0.905^{f,B}$ ±0.008	$1.215^{g,A}$ ±0.02	$1.314^{g,A}$ ±0.03	1.141^{e} ±0.04
Vanillin	4.060^{g} ±0.07	$0.024^{f,D}$ ±0.0009	$0.418^{e,C}$ ±0.003	$0.591^{h,B}$ ±0.004	$0.740^{h,A}$ ±0.008	0.560^{g} ±0.007
Vanillic acid	3.651^{g} ±0.05	-	$1.490^{e,C}$ ±0.007	$1.794^{f,B}$ ±0.07	$1.988^{f,A}$ ±0.05	1.611^{d} ±0.07
p-coumaric acid	13.821^{f} ±0.3	-	$1.854^{d,C}$ ±0.05	$2.148^{e,B}$ ±0.09	$2.497^{e,A}$ ±0.07	0.834^{f} ±0.02

Small letters in a column, capital letters in a row indicate significantly different values ($p < 0.05$)

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