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The Effects of Bioactive Compounds and Fatty Acid Compositions on the Oxidative Stability of Extra Virgin Olive Oil Varieties

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Abstract The aim of this study was to determine the various bioactive components of five olive oil varieties, as well as to assess their contribution to the oxidative stability of the oils. Fatty acids, α-tocopherol, β-carotene, total flavonoids, total phenols, and certain phenolic compounds of extra virgin olive oils (EVOO; blended, arbequina, hojiblanca, and picual) and pure olive oil (POO) were examined. Oxidation stability was evaluated by the peroxide value (POV). The total content of all the studied antioxidant compounds was significantly higher in the EVOOs than the POO ($p<0.05$). Among the EVOOs, picual had the highest levels of α-tocopherol (10.18±0.40 mg/100 g), β-carotene (557±8 μg/100 g), and total phenols (110.7±1.3 mg/g), which correlated strongly with antioxidative capacity. Furthermore, the lowest POV occurred in picual EVOO and correlated with the highest monounsaturated fatty acid (MUFA, C16:1 and C18:1) and lowest polyunsaturated fatty acid (PUFA, C18:2 and C18:3) compositions, suggesting the ratio of MUFA to PUFA is a critical parameter for the oxidative stability of olive oil. Our results indicate that the oxidative stability and antioxidant potential of EVOO depends not only on the antioxidant vitamins, but also on the amount of phenolic compounds and fatty acid profile of the oil.

Keywords: bioactive component, oxidation stability, polyphenolic compound, olive oil grade, olive oil cultivar

Introduction

Epidemiological studies have shown that the traditional Mediterranean diet is associated with low incidences of cardiovascular disease and certain cancers (1, 2). These beneficial effects on human health have been attributed to the presence of antioxidants in the Mediterranean diet, such as phenolic compounds, carotenoids, and tocopherols that play an important role in disease prevention (3). Olive oil is the primary source of polyphenols in the Mediterranean diet; also, polyphenols are important markers for evaluating the quality of virgin oil (4).

Olives are usually used for direct consumption at meals, or are further processed for olive oil extraction. Olive oil can also be consumed in the natural unrefined state known as extra virgin olive oil (EVOO), or as a refined oil made from virgin olive oil. Pure olive oil (POO) is a commercial grade oil and is the mixed product of EVOO and refined olive oil. The kinds of olive oils generally consumed are POO and EVOO by olive oil grade, and then arbequina, hojiblanca, and picual EVOOs by olive cultivar. EVOO is commonly consumed in Korea and made from various olive cultivars as a blended form and is called blended EVOO (4-6).

EVOO and POO differ slightly in their physico-chemical characteristics such as fatty acid composition (7). Moreover, the nutritional and antioxidative quality of olive oil depends on the type and concentration of substances such as tocopherols, carotenoids, and polyphenolic compounds (8, 9). In particular, the contents of mono-unsaturated fatty acids (MUFA) and polyphenolic compounds in most olive oils are higher than in other edible oils such as soybean and corn oils. In addition to these components, olive oil contains tocopherols and carotenoids, which are of great importance to human health due to their antioxidant and free-radical scavenging activities (7-10).

Growing evidence points out that the MUFA content alone cannot fully explain olive oil's beneficial impacts on health. Rather the beneficial effects of olive oil have been attributed to other components in the oil, the phenolic compounds, which are effective defenders against reactive oxygen species. For example, during preservation, olive phenolic compounds were shown to have free radical scavenging activities that correlated with the antioxidation of the lipids present in the oil (11-14).

Olive oils are known to contain different classes of phenolic compounds such as hydroxytyrosol, tyrosol, caffeic acid, *p*-coumaric acid, vanillic acid, vanillin, oleuropein, luteolin, diosmetin, rutin, verbascoside, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside (11-14). In essence, the physico-chemical and antioxidant properties of olive oils can vary depending on several factors such as the processing method, type of cultivar, growing conditions, and the time of ripening (15, 16).

Although there is evidence for the importance of antioxidant phenolics in olive oil, very little is known about the other antioxidant compounds such as α-tocopherol and the carotenoids, and their influence relative to the phenolic compound and fatty acid profiles on the overall oxidative stability of the oils.

The aim of this study was to evaluate the contents and contributions of antioxidant compounds present in POO and four EVOOs of the blended, arbequina, picual, and hojiblanca varieties. Thus, we determined their fatty acid compositions and bioactive component contents, including

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α-tocopherol, β-carotene, and polyphenolics, and further correlated the contributions of these components to the oxidative stabilities of the five different oils during autoxidation, using the peroxide value test.

Materials and Methods

Materials The olive oils used in this study were four EVOOs (blended, arbequina, hojiblanca, and picual varieties) and POO, and were supplied from Borges Korea Co. (Gyeonggi, Korea). The names of the EVOOs correspond to different Spanish olive oil varieties. Picual and hojiblanca are the main cultivars from the south of Spain and arbequina is mainly grown in Catalonia.

Naringin, tannic acid, caffeic acid, ferulic acid, vanillin, *p*-coumaric acid, and DMSO (dimethylsulfoxide) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and oleuropein was obtained from Extrasynthese (Genay, France). All other reagents were analytical grade.

Fatty acid analysis The fatty acid compositions were determined according to the method of Lepage and Roy (17) by gas chromatography (HP-6860; Hewlett Packard, Palo Alto, CA, USA) with an FID detector on a DB-wax column (60×0.25 mm i.d.; J&W, Folsom, CA, USA). Helium was used as a carrier gas, and the split ratio was 30:1. The chromatographic conditions were as follows: an injection port temperature of 250°C; a detector temperature of 250°C; and an initial oven temperature of 90°C for 5 min, rising to 180°C at 10°C/min with a hold time of 3 min, and then rising to 230°C at 3°C/min with a hold time of 3 min, and again rising to 245°C at 2°C/min, and finally, rising to 250°C at 0.7°C/min with a final hold time of 10 min.

Determination of α-tocopherol and β-carotene contents The α-tocopherol contents of the olive oil samples were determined according to the method of Lee and Lee (18). A solution of oil in hexane was analyzed by HPLC (M 720; Younglin, Korea) on a silica gel Lichrosorb Si-60 column (250×4 mm, 5 mm; Hibar Fertigsaube RT, Darmstadt, Germany), which was eluted with hexane-2-propanol (99.2:0.8, v/v) at a flow rate of 1 mL/min. A fluorescence detector (Waters 470; Waters, Millipore, MA, USA), with excitation and emission wavelengths set a 290 and 330 nm, respectively, was used. The standard was α-tocopherol (Sigma). The β-carotene content was determined according to the method of Gimeno *et al.* (3). Briefly, the method involved a rapid saponification and subsequent extraction with a mixture of hexane-ethyl acetate. The chromatographic system consisted of an ODS-2 column (300×3.9 mm; Waters) with a photodiode array (PDA) detector.

Extraction of polyphenolic compounds The polyphenolic compounds were extracted from each olive oil sample according to the method described by Gutfinger (19). Each olive oil (100 g) was dissolved in 100 mL hexane and the solution was successively extracted with 200 mL of 80% aqueous ethanol. The extracted samples were concentrated with a vacuum evaporator and completely dried in a freeze drier.

Determination of total flavonoid and phenol contents The contents of total flavonoids were determined according to the method of Kang *et al.* (20). A sample solution (1 mL) was taken from each olive oil extract dissolved in DMSO (dimethylsulfoxide), and then placed in a test tube containing diethylene glycol (10 mL) and 1 N NaOH solution (1 mL), which were allowed to stand for 30 min at room temperature (RT). The absorbance was then measured at 420 nm. The total flavonoid contents (%) were reported as naringin equivalents.

The total phenol contents were determined according to the Folin-Denis Method (21). The sample solution (1 mL) was placed in a test tube with distilled water (7 mL), Folin-Denis reagent (0.5 mL), and saturated sodium carbonate solution (1 mL) and allowed to stand for 30 min at RT. The absorbance at 715 nm was measured. The total phenol contents (%) were reported as tannic acid equivalents.

HPLC analysis of polyphenolic compounds The HPLC analyses of the polyphenolic compounds in the olive oil extracts were performed according to the method of Benavente-Garcia *et al.* (22). The 80% ethanol extracts of these olive oils (10 mg) were dissolved in DMSO (10 mL) and filtered through a Millipore membrane filter (0.45 mm) prior to HPLC analysis. The HPLC equipment was a Younglin M 720 with a UV detector at 280 nm. HPLC analysis was carried out using a μ-Bondapak C_{18} (300×3.9 mm, Waters). The mobile phase was distilled water:acetonitrile = 8:2 (v/v), at a flow rate of 1.0 mL/min. The standards were caffeic acid, ferulic acid, vanillin, *p*-coumaric acid, and oleuropein.

Oxidation stability of the five olive oil varieties The olive oils (100 mL) were placed into 250 mL beakers and stored at 65±1°C for 35 days. Samples were taken on days 7, 14, 21, 28, and 35 of storage for further analysis. The oxidative stabilities of the olive oils were evaluated by measuring the peroxide values (POV) according to the AOCS official method (23). The POVs were expressed in milliequivalents of active oxygen per kg of oil (meq/kg) and determined as follows: a mixture of oil and chloroform-acetic acid was left to react with a solution of potassium iodide in the dark. The free iodine was then titrated with a sodium thiosulphate solution.

Statistical analysis All measurements were repeated three times. The results are shown as mean values and standard deviations. The data were statistically analyzed using ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $p<0.05$ (24).

Results and Discussion

Fatty acid compositions of all analyzed olive oil samples Table 1 shows the fatty acid compositions of the five olive oil varieties by oil grade and cultivar. Most of the olive oils analyzed in this study showed typical fatty acid compositions, and the contents of major fatty acids for POO and the EVOOs (blended, arbequina, picual, and Hojiblanca) were in the order of oleic acid (C18:1),

Table 1. Fatty acid compositions (%) in the five studied olive oil varieties[1)]

Olive oil (%) / Fatty acid	POO	EVOO			
		Blended	Arbequina	Picual	Hojiblanca
C16:0	10.6	12.0	14.7	11.0	10.1
C16:1	0.8	1.1	1.5	0.9	0.7
C18:0	3.1	3.1	2.2	3.1	3.2
C18:1	77.6	73.2	68.6	77.8	78.7
C18:2	6.5	9.1	11.5	5.8	5.8
C18:3	0.6	0.7	0.7	0.6	0.7
C20:0	0.4	0.4	0.4	0.4	0.4
C20:1	0.3	0.3	0.3	0.3	0.3
C22:0	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0
C18:1/C18:2 ratio	11.9	8.0	6.0	13.4	13.6

[1)]POO, pure olive oil; EVOO, extra virgin olive oil. Values were calculated as the % of the total fatty acids.

palmitic acid (C16:1), and linoleic acid (C18:2). The most abundant monounsaturated fatty acid (MUFA), oleic acid, was significantly higher in the hojiblanca and picual EVOOs compared with the blended and arbequina EVOOs. In contrast, the most abundant polyunsaturated fatty acid (PUFA), linoleic acid, was markedly higher in the arbequina and blended EVOOs than in hojiblanca and picual. The combined oleic and linoleic acid contents in all the studied oils exceeded 80% of the total fatty acids, and these results are in accordance with others (16). Also, the results obtained in this study confirm the variability in fatty acid compositions of different olive oil grades and cultivars. This variability of fatty acid content and composition in olive oils is likely due to many factors such as the nature of the cultivar, soil and climate conditions, maturity of the olive fruit, and food preparation and processing.

Determination of α-tocopherol and β-carotene contents To evaluate the contribution of antioxidant vitamins present in the 5 olive oils, we determined the contents of 2 principle vitamins, α-tocopherol and β-carotene, which are commonly found in both vegetable and olive oils.

Figure 1 and 2 show that the α-tocopherol and β-carotene contents of the EVOOs (blended, arbequina, picual, and Hojiblanca) were significantly higher than in the POO ($p<0.05$). The α-tocopherol contents in the POO and blended, arbequina, picual, and hojiblanca EVOOs were 5.31±0.04, 7.99±0.32, 9.02±0.48, 10.18±0.40, and 9.72±0.22 mg/100 g of olive oil, respectively (Fig. 1). Similarly, the contents of β-carotene in the POO and blended, arbequina, picual, and hojiblanca EVOOs were 59±1, 444±27, 531±18, 557±8, and 353±7 μg/100 g of olive oil, respectively (Fig. 2). Interestingly, the contents of α-tocopherol and β-carotene were significantly higher in the picual EVOO than the other studied EVOOs ($p<0.05$). These results are in agreement with a report by Gimeno *et al.* (3) where the α-tocopherol and β-carotene contents of

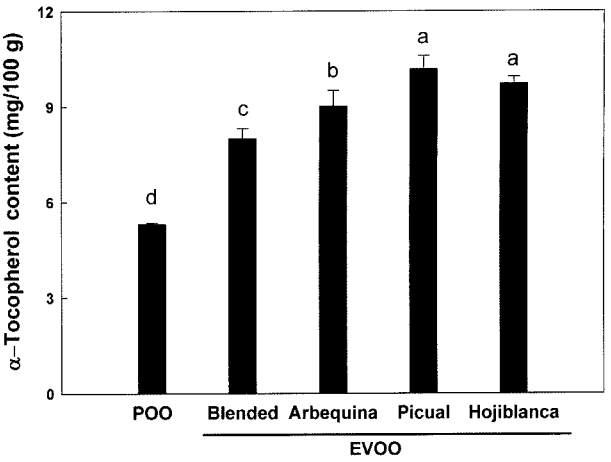

Fig. 1. α-Tocopherol content in the five studied olive oil varieties. POO, pure olive oil; EVOO, extra virgin olive oil. [a-d]Values are the means±SD of three samples. Bars with different letters indicate statistically significant differences among groups at $p< 0.05$.

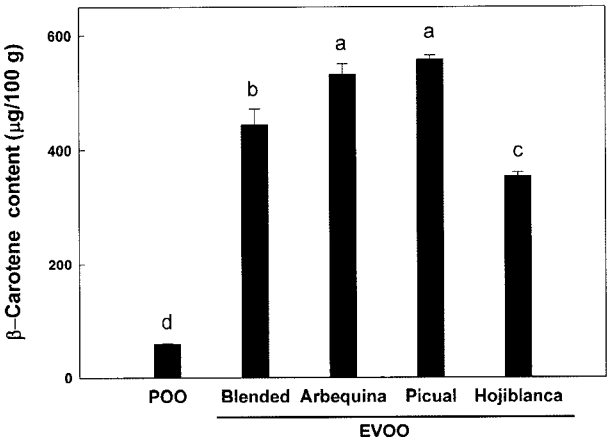

Fig. 2. β-Carotene content in the five studied olive oil varieties. POO, pure olive oil; EVOO, extra virgin olive oil. [a-d]Values are the means±SD of three samples. Bars with different letters indicate statistically significant differences among groups at $p< 0.05$.

EVOOs were in the range of 185.6-212.5 and 1.6-2.8 mg/kg, respectively.

Moreover, our data presented in Fig. 1 and 2 indicate that the contents of α-tocopherol and β-carotene in olive oil varieties are very different. The POO had the lowest α-tocopherol and β-carotene contents, which is likely due to the removal of these vitamins during the various refining stages. However, the substantial amounts of these vitamins found in the studied EVOOs may play an important role in not only the stability of the oils, but also in their antioxidant properties, along with the polyphenolic contents of the oils.

Total flavonoids and phenol contents The total flavonoid and phenol contents of olive oil have been reported numerous times in the literature; however, there are variations with the concentrations obtained. The reason for this discrepancy could be due to many factors,

including the olive oil grade, olive cultivar, the degree of ripening, and methods used (1, 7). Thus, we determined the contents of total flavonoids and phenols in the five studied olive oil varieties.

The total flavonoid and phenol contents of the 80% ethanol extracts of these olive oils are shown in Fig. 3. The total flavonoid and phenol contents for the EVOOs (blended, arbequina, picual, and hojiblanca) were significantly higher than for the POO ($p<0.05$). The total flavonoid contents from the 80% ethanol extracts of POO and the blended, arbequina, picual, and hojiblanca EVOOs were 0.6±0.4, 13.9±0.3, 22.3±3.1, 10.4±0.7, and 10.3±0.9 mg/g of olive oil extract, respectively. Also, the total phenol contents of the 80% ethanol extracts from POO and the blended, arbequina, picual, and hojiblanca EVOOs were 14.8±5.2, 68.7±3.3, 59.6±5.1, 110.7±1.3, and 77.5±13.4 mg/g of olive oil extract, respectively. On the contrary, the total flavonoid content by oil cultivar was markedly higher in the 80% ethanol extract of arbequina EVOO than in the other EVOOs ($p<0.05$). Total phenol content, however, was significantly higher in the 80% ethanol extract of the picual EVOO than the other EVOOs ($p<0.05$).

Polyphenolic compound compositions In addition to the total phenol contents, we also analyzed the phenolic constituents of the five olive oil varieties to assess their contribution to the overall quality and antioxidative properties of the oils. Table 2 shows the amounts of polyphenolic compounds in the 80% ethanol extracts of the five olive oil varieties by oil grade and cultivar. Oleuropein, caffeic acid, ferulic acid, *p*-coumaric acid, and vanillin were found to be the major polyphenolic compounds in the 80% ethanol extracts of POO as well as the blended, arbequina, picual, and hojiblanca EVOOs, which is consistent with other reports (12, 22). The two phenolic compounds in highest concentration in the five studied olive oils were oleuropein and *p*-coumaric acid.

The total amount of polyphenolic compounds was significantly higher in the EVOO extracts (blended, arbequina, picual, and hojiblanca) than in the POO extract ($p<0.05$). On the other hand, the total amount of polyphenolic compounds by oil cultivar was significantly higher in the picual, hojiblanca, and blended EVOO extracts than in the extract from the arbequina EVOO ($p<0.05$).

Our data indicate that total amounts of these analyzed phenolic compounds may be more important for the antioxidative activities of the EVOOs than the amounts of the individual components. However, it is also possible that other unidentified phenolic compounds may play important roles in the antioxidative properties, in addition to the representative phenolic compounds we measured in this study.

Assessment of oxidation stability To determine the antioxidative effects of the olive oils, changes in peroxide values (POV) for the five oils were evaluated during autoxidation for 35 days in the dark at 65±1°C (Fig. 4). The POVs of POO and the blended, arbequina, picual, and hojiblanca EVOOs increased to 156.8, 116.9, 167.7, 36.1, and 40.7 meq/kg oil, respectively. The picual EVOO and hojiblanca EVOO showed the lowest POVs during autoxidation at 65±1°C for 35 days. These results indicate that the oxidation stability of these olive oils depended on the amount of bioactive components such as α-tocopherol, β-carotene, polyphenolics, as well as the fatty acid compositions of the oils.

It is known that the oxidative stability of olive oil is greatly affected by the presence of phenolic compounds. These phenolic compounds possess a strong antioxidant activity by scavenging peroxides and free radicals during auto-oxidation, thus acting as inhibitors of biologically harmful oxidation reactions in the body. Baldioli *et al.* (4) showed that the oxidative stability of olive oil was mainly correlated with the phenolic compound concentration. In the present study, picual EVOO had the highest content of total phenols and showed the best oxidation stability compared to the other EVOOs. These results indicate that among the EVOOs tested, picual EVOO possessed not only a strong antioxidant activity, but also an enhanced oxidative stability, thus providing health benefits against oxidant-related diseases. Furthermore, picual EVOO, which exhibited the best oxidation stability among the olive oils, contained not only the highest concentration of total phenols (110.7±1.3 mg/g) (Fig. 3), but also the lowest concentration of PUFAs in its fatty acid composition (Table 1).

The highest stability values obtained from the picual and hojiblanca EVOOs correlated with the highest MUFA (C16:1 and C18:1) and the lowest PUFA (C18:2 and C18:3) contents (Table 1). Thus the ratio of MUFAs to PUFAs may be a critical parameter for determining the

Table 2. Polyphenolic compound contents in the five studied olive oil varieties[1)]

PPC \ Olive oil	POO (mg/ 100 g)	EVOO (mg/ 100 g)			
		Blended	Arbequina	Picual	Hojiblanca
Caffeic acid	80.4±4.1[a2)]	99.8±15.1[a]	88.5±3.4[a]	95.4±0.2[a]	101.0±24.8[a]
Ferulic acid	12.4±4.7[b]	45.0±26.2[b]	36.3±16.4[b]	65.0±49.6[ab]	116.5±32.7[a]
p-Coumaric acid	63.3±43.0[b]	211.9±100.7[ab]	129.1±23.8[ab]	233.6±130.6[a]	208.6±67.1[ab]
Vanillin	6.1±0.0[b]	48.3±33.0[a]	26.70±9.5[ab]	37.0±23.5[ab]	61.7±4.8[a]
Oleuropein	213.3±7.9[b]	298.6±92.2[a]	201.6±1.2[b]	208.7±3.2[b]	203.0±3.0[b]
Total	375.4±17.6[c]	703.7±39.9[a]	482.1±9.3[b]	639.6±53.6[a]	690.8±26.0[a]

[1)]PPC, polyphenolic compound; POO, pure olive oil; EVOO, extra virgin olive oil.
[2)][a-c]Values are mean±SD (n=3); Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Duncan's multiple test.

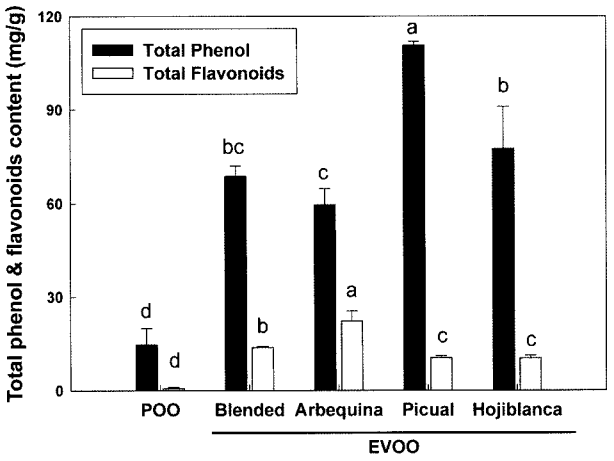

Fig. 3. Total flavonoid and phenol contents of 80% ethanol extracts obtained from pure olive oil (POO) and extra virgin olive oil (EVOO) varieties. [a-d]Values are the means±SD of three samples. Bars with different letters indicate statistically significant differences among groups at $p<0.05$.

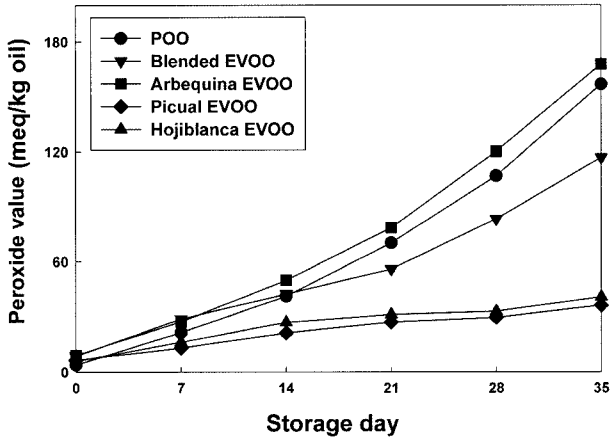

Fig. 4. Effect of the five olive oil varieties on the oxidation of olive oil stored up to 35 days at 65°C, assessed by the peroxide value (POV). POO, pure olive oil; EVOO, extra virgin olive oil. POV was expressed as meq/kg of oil.

quality of oil as well as its oxidative stability. It is also entirely plausible that there is a certain synergy between the ratio of fatty acids and the phenolic compounds. Some synergistic behavior between the polyphenolic compounds and flavonoids present in several olive oils has been reported previously (22, 25).

In conclusion, we found that the EVOOs had higher oxidative stabilities, which correlated well with higher contents of bioactive components, compared to the POO. The high oxidative stabilities observed in picual and hojiblanca EVOOs may be due to the oleic (C18:1) to linoleic (C18:2) acid ratio, as these varieties have lower contents of PUFAs in addition to bioactive compounds. Thus, further study is needed to evaluate the antioxidant capacities of these olive oils using different methods such as electron donating abilities, SOD-like activities, and hydrogen peroxide scavenging activities. Additionally, although all the EVOOs tested contained substantial amounts of α-tocopherol, which is the most powerful lipid soluble antioxidant, the present study did not examine the contents of the minor vitamin E isomers such as β-, γ-, and δ-tocopherols and tocotrienols. Consequently, we can not exclude these individual and/or mixtures of isomers that may contribute to the potentially beneficial effects of the EVOOs; for example, the non-antioxidant functions of α-tocopherol in the preventions of platelet aggregation (26) and atherosclerosis (27).

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