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Comparison of the Radical Scavenging Potential of Polar and Lipidic Fractions of Olive Oil and Other Vegetable Oils under Normal Conditions and after Thermal Treatment

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The antioxidant activity (IC_{50}) of extra virgin olive oil (EVOO), commercial olive oil, and other vegetable oils (soybean, sunflower, and corn oil) was determined by UV−vis and by electron paramagnetic resonance (EPR) spectroscopy of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Also, we studied the antioxidant activity of the methanol soluble phase (methanolic, MF) and the nonsoluble phase (lipidic, LF) of oils by the same methods. Similarly, we studied the effect of heating on the antioxidant activity at 160 and 190 °C. Also, the MF, containing the polyphenolic substances, was used for measurements of the radical scavenging capacity toward the most important oxygen free radicals, superoxide anion ($O_2^{\bullet -}$) and hydroxyl ($HO^{\bullet}$) radicals. Results showed that soybean oil and EVOO had the highest antioxidant potential and thermal stability. In the case of soybean oil, the antioxidant capacity is the result of its high content of γ- and δ-tocopherols (with the highest antioxidant capacity and thermostabilities), whereas in EVOO, the antioxidant potential is the result of the combination of specific antioxidant polyphenols, which are acting additionally as effective stabilizers of α-tocopherol. The high content of EVOO in tyrosol, hydrotyrosol, and oleuropein and other polyphenolics with radical scavenging abilities toward superoxide anion and hydroxyl radical suggests that olive oil possesses biological properties that could partially account for the observed beneficial health effects of the Mediterranean diet.

KEYWORDS: Antioxidants; antioxidant activity; DPPH; electron paramagnetic resonance; polyphenols; hydroxyl radical; olive oil; radical scavenger; spin trapping; superoxide anion

INTRODUCTION

There has been much scientific interest regarding the "bioactive compounds", which are extranutritional constituents of plant products and lipid-rich foods, for their beneficial health effects, especially in the prevention of cardiovascular diseases and malignant neoplasms (*1*). Recent findings suggest that antioxidant vitamins and polyphenolic compounds in olive oil and other vegetable oils are endowed with several biologic capacities that may contribute to the lower incidence of cardiovascular diseases in the population of Mediterranean countries (*2*).

The role of olive oil and other vegetable oils in cancer prevention has been investigated by numerous epidemiological studies (*3*, *4*). Olive oil and other vegetable oils have been shown to reduce low-density lipoprotein (LDL) oxidizability in the postprandial state, which in part reflects their fatty acid profile (high in monounsaturated fatty acids) and their content in antioxidant tocopherols and polyphenols (*5*, *6*). Recent studies demonstrate that olive oil polyphenols are powerful antioxidants, both in vivo and in vitro, and possess other potent biological activities that could account for their beneficial health effects (*7*−*10*).

Olive oil, especially the known type extra virgin, has a high content of at least 30 phenolic compounds with high antioxidant potential (*11*). Polyphenols in vegetable oils are a complex mixture of compounds, that include oleuropein, 4-hydroxyphenylethanol (tyrosol), 3,4-dihydroxy-phenylethanol (hydroxytyrosol), 4-hydroxyphenylacetic acid, protocatechuic acid, syringic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and sinapic acid (*12*, *13*). The presence of antioxidants in vegetable oils is also an important factor in the stabilization of free fatty acids (*14*).

Phenolic compounds in vegetable oils are strong free radical scavengers. Studies showed that vegetable oil fractions scavenge the stable radical DPPH (*15*) and other oxygen free radicals (*16*, *17*). Similarly, polyphenols are effective stabilizers of

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α-tocopherol during olive oil heating, thus contributing to the nutritional value of cooked foods (*18*).

When olive oil and other vegetable oils were subjected to simulated common domestic processing, such as frying, microwave heating, and boiling with water in a pressure cooker, they showed a decrease in hydrotyrosol and tyrosol-like substance concentrations and rapid degradation of α-tocopherol and the glyceridic fraction of oils. So, the choice of cooking method with vegetable oils may be relevant for their antioxidant potential (*19*−*21*).

The aim of this work was to compare the free RSC of olive oil and other vegetables oils and their extracted phases under normal conditions and after thermal treatment. Oils were separated by extraction into two fractions: methanol soluble nonlipidic (polar), nonsoluble LF, and the TF oil without fractionation. The antioxidant activity of compounds in the polar fraction, such as simple phenols tyrosol, hydrotyrosol, and the secoiridoid oleuropein (aglycone), were tested toward DPPH$^•$. Also, we carried comparative studies for the antioxidant potential of the MF toward superoxide anion and hydroxyl radicals. Finally, we compared changes in the antioxidant capacity of olive and vegetable oils, due to degradation of antioxidant compounds, after thermal treatment, simulating frying temperatures at 160 and 190 °C.

MATERIALS AND METHODS

Chemicals and Reagents. Stable free radical DPPH$^•$, tyrosol, syringic acid, squalene, and DMPO were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). DMSO, hydrogen peroxide, ferrous sulfate, methanol, and other chemicals were purchased from E. Merck Co. (Darmstadt, Germany). 18-Crown-6 ether and KO_2 were purchased from Aldrich. Oleuropein was acquired from Extrasynthese (Genay, France). Hydrotyrosol was prepared by the procedure of Baraldi et al. (*22*) from the reduction of 3,4-dihydroxy-1-benzeneacetic acid with $LiAlH_4$ in tetrahydrofuran. All other chemicals were from Merck and Fluka.

Oil Samples and Preparation of Extracts. EVOO, commercial olive oil, edible corn oil, sunflower oil, and soybean oil were purchased in local supermarkets of Athens, from known commercial brands, and data were obtained for their fatty acid composition and acidity. Two samples of each vegetable oil were used. Quality characteristics of vegetable oils were as follows: EVOO (Greek origin), acidity 0.3−0.5, 12−13% saturated fatty acids, 76−78% monounsaturated, 7−8% polyunsaturated; olive oil (Greek origin), acidity 0.8−1.0, 14−15% saturated, 74−75% monounsaturated, 8−9% polyunsaturated; soybean oil, acidity >1, 13−14% saturated, 23−25% monounsaturated, 57−58% polyunsaturated; corn oil, acidity >1, 11−13% saturated, 30−22% monounsaturated, 55−59% polyunsaturated; and sunflower oil, acidity >1, 10−13% saturated, 27−30% monounsaturated, 58−60% polyunsaturated.

Oils were treated with methanol to assay the polar fraction. One hundred grams of the different oils was mixed with 100 mL of methanol, and the mixture was vigorously stirred for 1 h and further centrifuged at 2500*g* for 5 min to separate both MF and LF phases. Part of the MF samples was evaporated under reduced pressure, and the residues were weighted to quantify the amounts extracted (mg/g) by methanol. The antioxidant potential (IC_{50}) was separated into MF, LF, and TF. Various solvents were used, but the best results for the LF and TF measurements were achieved with ethyl acetate solutions.

Quantification of the Antioxidant Activity with DPPH. IC_{50} measurements were performed spectrophotometrically at 515 nm. This assay was performed as described by Lavelli (*23*) with some modifications. Different amounts (mg/mL) of methanolic extracts of oils were added to a 3 mL solution of DPPH (1×10^{-4} M in methanol) in small volumes, starting from 0.05 and up to a maximum of 0.4 mL. The decrease in absorbance was determined at 515 nm until 15 min (when a constant value was reached). IC_{50} was defined as the amount of original oil sample, in milligrams per mL solution of the total volume reached after the addition of extracts (taking into account the increased volume of the 3 mL initial solution), required to lower the initial DPPH$^•$ concentration by 50% and was extrapolated from the dose−response curve.

The IC_{50} for the LF and TF of vegetable oils was performed similarly with DPPH but in ethyl acetate solutions. DPPH solution was 1×10^{-4} M. Experiments were carried out in triplicate, and results were expressed as mean values ± standard deviation (SD). All experimental data of absorption decrease showed a linear correlation as to the amount of the antioxidant fraction (straight line resulting from the fit by linear regression, $r^2 = 0.99-0.97$). Experiments were always performed on freshly made up solutions.

The antioxidant capacity (IC_{50}) of oil fractions was measured also by EPR, using a Varian E-4 ESR spectrometer, following the decrease of the intensity of the EPR signal of DPPH at room temperature (results and EPR spectra not shown). Results of the IC_{50} by EPR were similar to the spectrophotometric ones, but the method was more cumbersome and with a lower degree of reproducibility as compared to the UV−vis measurements. Also, the antioxidant capacity of tyrosol, hydrotyrosol, and oleuropein was studied in methanol with DPPH.

Quantification of the Antioxidant Activity of the MF toward Superoxide Anion ($O_2^{•-}$) in DMSO. Quantitative EPR analysis was based on the spin trapping of $O_2^{•-}$ generated by potassium superoxide (KO_2) in DMSO with the addition of 18-crown-6 ether to complex K^+. Under these conditions, a typical DMPO-OOH adduct was observed. The spin trap DMPO was purified on active charcoal, aliquoted, stored frozen, and protected from light. The mixture was 18-crown-6 ether/KO_2 (1:1, 10 μM) was dissolved in DMSO, as reported (*24*, *25*). A 3 mL DMSO solution was prepared by adding the reagents in the following order: DMPO (80 mM), test compound (*x* mM) or MF (mg/mL), and the mixture 18-crown-6 ether/KO_2. The reaction mixture was stirred vigorously for 1 min and transferred to a flat quartz EPR cell and into the cavity of the EPR instrument. The EPR spectra were recorded for 4 min at room temperature. A control mixture, without the test compound, gave the reference intensity of the EPR signal. Fresh solutions were used except for DMPO. EPR recording conditions (Varian E-4) were as follows: field set, 3500 G; scan range, 100 G; modulation amplitude, 1.0 G; microwave power, 20 mW; time constant, 1.0 s; scan time, 4 min; receiver gain, $6 \times 10^3-8 \times 10^3$. The EPR parameters of the DMPO-OOH signal were as follows: $a_N = 1.42$ mT (or 14.2 G); $a_H^{\alpha} = 1.12$ mT; $a_H^{\beta} = 0.13$ mT. The intensity of the EPR signal was calculated by adding the height of the quadruplet peaks. The IC_{50} values represent the concentration necessary for 50% diminution of the signal. This IC_{50} was deduced graphically by plotting the percentage of inhibition. The antioxidant capacity of each compound and MFs was investigated by three independent experiments. At least five concentrations were used giving inhibition between 10 and 90% were assayed. Values are means ± SD. Representative EPR spectra are shown in **Figure 1**.

Quantification of the Antioxidant Activity of MF toward Hydroxyl Radicals (HO$^•$) Produced by the Fenton System in Aqueous Solution at pH 7.4. Hydroxyl radicals (HO$^•$) were produced by the mixture of H_2O_2/Fe^{2+}/EDTA-Na_2 and spin-trapped by DMPO. The mixture consisted of the reagents in the following order: 1 mL of 0.01 M of ferrous sulfate (phosphate buffer, pH 7.4) and 1 mL of aqueous solution of 0.02 M EDTA. The mixture was degassed with dry nitrogen for 1 min. Then, 1 mL of aqueous solution of DMPO (80 mM) and finally 1 mL of the test compound (various concentrations, mM) in phosphate buffer (pH 7.4) or MF (various concentrations, mg/mL) and 1 mL of 0.01 M of H_2O_2 were added to start the reaction. The mixture was stirred vigorously for 1 min and then degassed with dry nitrogen, filtered, and transferred to a flat EPR quartz cell. The EPR spectra were recorded for 4−8 min (Varian E-4, X-band). EPR instrument recording conditions were similar as above. The DMPO-OH adduct (g = 2.054) had spectrometric parameters: $a_N = a_H = 1.49$ mT (14.9 G). The diminution of the four line EPR signal was followed at concentrations of the test compounds in the range of 10−90%. The IC_{50} values represent the concentration necessary for the decrease by 50% of the EPR signal without antioxidant and was deduced graphically by plotting inhibition against the test material concentrations. The antioxidant

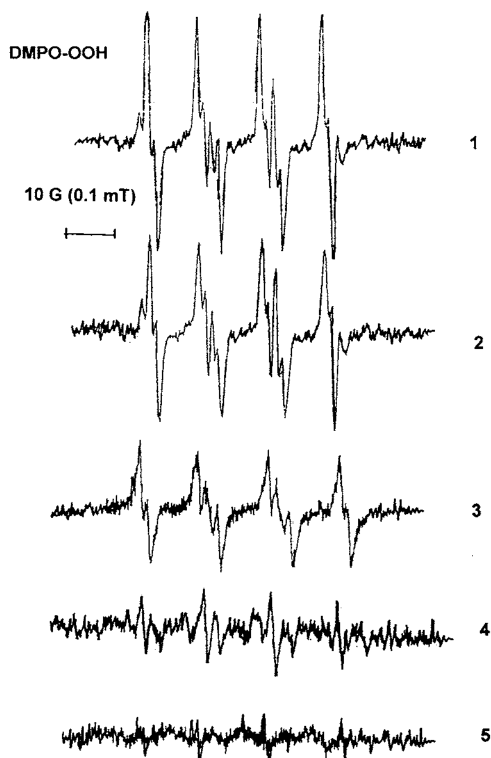

Figure 1. EPR spectra of superoxide anion spin-trapped by DMPO (spin adduct DMPO-OOH) and EPR signal intensity decrease by the addition of increasing amounts (mg/mL) of MF of olive and vegetable oils. Olive oil: 1, without olive oil; 2, with addition of 15 mg/mL; 3, 25 and 30 mg/mL; and 4, 35 mg/mL.

activity was investigated by three independent experiments. Values are means ± SD. Representative results of EPR spectra are shown in **Figure 2**.

Thermal Treatment. Vegetable oils were incubated at 160 and 190 °C for 2 h duration. The 180−190 °C is a common temperature in frying processes. During heating, the tubes were left open. After thermal treatment, oils were treated with methanol to assay the polar and lipidic (nonsoluble in methanol) fractions. Antioxidant measurements were carried as before with DPPH in methanol for the MF and in ethyl acetate for the LF and TF. Values are means ± SD.

High-Performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds. The HPLC method was used for the analysis of the major phenolics in vegetable oils. Tyrosol, hydrotyrosol, oleuropein (aglycone), and squalene were identified and quantified by HPLC. The HPLC was Hewlett-Packard Agilent 1100 Series, equipped with UV−vis. A column (Merck) LichroCART 250−4 mm, Lichrospher 100 RP-18 (5 μm particle size), with a precolumn of the same material, was used as the stationary phase with a flow rate of 1 mL min^{-1}. Separation was achieved with an elution gradient by using an initial composition of 90% water (pH adjusted to 3.1 with 0.2% acetic acid) and 10% methanol. The concentration of methanol was increased to 30% in 10 min and maintained for 20 min. Subsequently, the MeOH percentage was raised to 40% for 10 min and finally to 50, 60, 70, and 100% for 5 min periods. Phenolic compounds were analyzed at 280 mm (squalene at 208 nm) and quantified by using reference compounds from commercial suppliers.

A representative HPLC chromatogram of phenolic compounds in olive oil is shown in **Figure 3**.

Thin-Layer Chromatography (TLC) Experiments. MFs of the various vegetable oils were applied on silica gel 60 F254 TLC aluminum shets (20 cm × 20 cm) (Merck) at one of the extremes to run the chromatography. A mixture of toluene/ethyl acetate/formic acid (5:4:1) (v/v/v) was used as the mobile phase. When the front arrived at the end, the sheet was dried out and viewed under UV or stained with 0.4 mM DPPH$^•$ in methanol. TLC of the MFs of EVOO, olive oil, and other vegetable oils is shown in **Figure 4**.

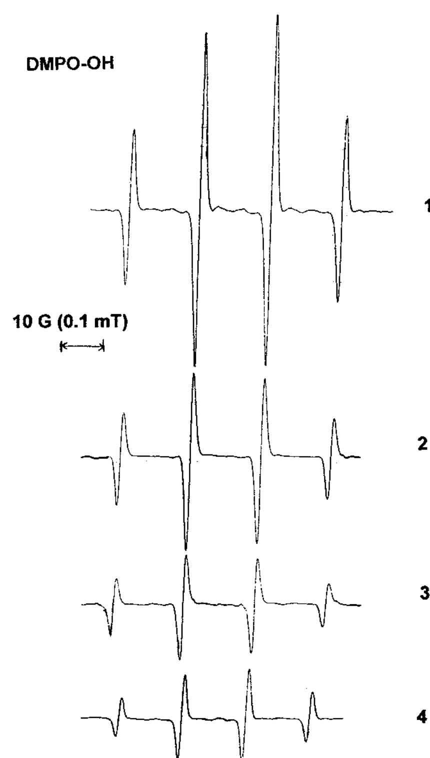

Figure 2. EPR spectra of DMPO-OH spin adduct and inhibition of signal intensity by different amounts (mg/mL) of MF factions added to the solution. EVOO: 1, without olive oil; 2, with addition of 15 mg/mL; 3, addition of 20 mg/mL; and 4, addition of 25 mg/mL.

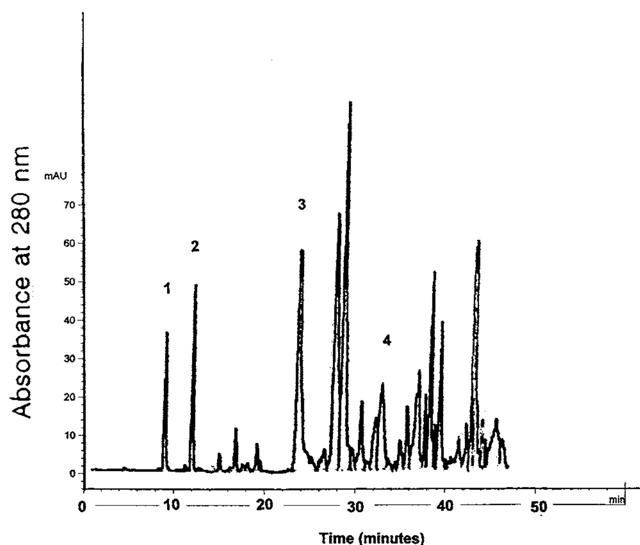

Figure 3. Representative HPLC of certain polyphenols in EVOO (see Materials and Methods for experimental details). Key: 1, hydrotyrosol; 2, tyrosol; 3, dialdehyde aglycone of oleuropein glucoside; and 4, oleuropein aglycone.

Total Polyphenol Determination. Polyphenols were extracted form the oils as described by Favati et al. (*26*), and the phenolic content was determined by the Folin−Ciocalteu method (*27*). Gallic acid was used as a standard for the calibration curve. Results were expressed as mg of gallic acid equivalents (GAE) per kg of oil.

RESULTS AND DISCUSSION

Radical Scavenging Effect of Vegetable Oil Fractions on DPPH$^•$. The model of scavenging stable radical DPPH is a widely used method to evaluate antioxidant capacities of natural

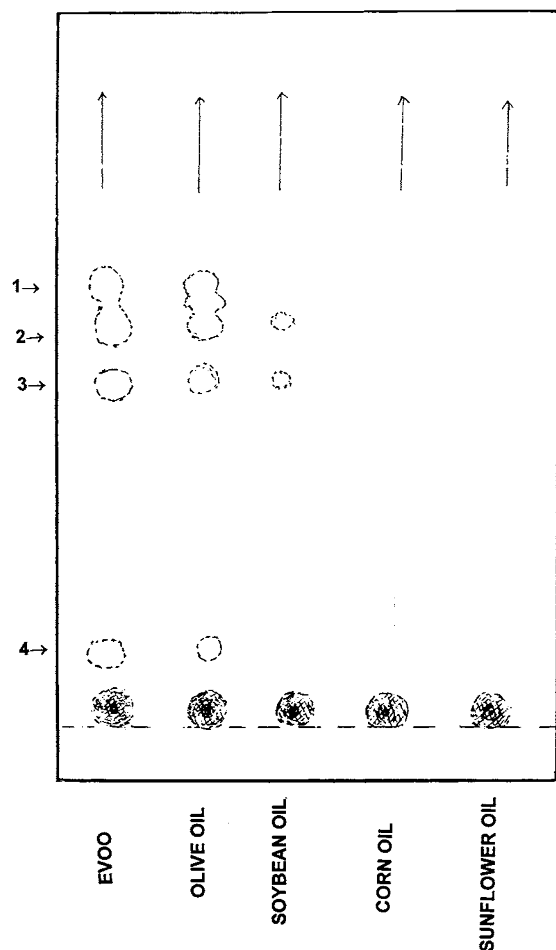

Figure 4. Combined TLC of MF factions of EVOO, olive oil, soybean oil, corn oil, and sunflower oil (see Materials and Methods for experimental details). Standards were syringic acid (1, R_f = 0.65), tyrosol (2, R_f = 0.52–054), hydrotyrosol (3, R_f = 0.45–0.47), and oleuropein (4, R_f = 0.16–0.18).

products, and it has been used for olive oil and other vegetable oils as well as for individual antioxidant polyphenols (*15*, *28*).

The actual reaction taking place between the DPPH stable radical and the antioxidant (AH) is

$$\mathrm{DPPH^{\bullet}} + (\mathrm{AH})_n \rightarrow \mathrm{DPPH{-}H} + (\mathrm{A^{\bullet}})_n \tag{1}$$

The radical formed ($A^{\bullet}$) in general is less reactive, depending on the structure of the molecule, or can follow radical−radical interaction to render a stable molecule.

In the present work, we calculated the IC_{50} as the amount of oil sample (in milligrams) required to reduce the initial DPPH concentration by 50% (it was extrapolated from a dose−response curve) divided by the total volume of DPPH solution with added solution of oil (e.g., 3 mL of DPPH + 0.1 mL of oil = 3.1 mL). Scientists used various ways of expressing IC_{50} or RSC in the scientific literature (*15*, *23*).

Soybean oil samples showed the highest value of IC_{50} among oils (**Table 1**). EVOO showed similarly high values of antioxidant capacity. Sunflower and corn oils showed relatively high antioxidant activity, but commercial refined olive oil showed a lower antioxidant activity, despite its high content in polyphenols and α-tocopherol. Soybean oil antioxidant activity is mainly the result of type and content of tocopherols in the LF, which are mainly δ- and γ-tocopherols with the highest antioxidant efficiencies, as compared to α-tocopherol (*29*, *30*). Soybean oil

Table 1. Inhibitory Capacity (IC_{50}) on $DPPH^{\bullet}$ of TF and Oil Fractions (MF and LF) in mg/mL and IC_{50} of Tyrosol, Hydrotyrosol, and Oleuropein (in μM)[a]

oil source	IC_{50} (TF) (mg/mL)	IC_{50} (MF) (mg/mL)	IC_{50} (LF) (mg/mL)
EVOO	11 ± 0.6	15 ± 1.4	22 ± 1.6
olive oil	17.5 ± 1.2	22 ± 2.2	28 ± 2.2
corn oil	15 ± 0.9	52 ± 4.5	20 ± 1.5
sunflower oil	14 ± 0.7	48 ± 3.8	18 ± 1.1
soybean oil	10 ± 0.5	45 ± 3.2	15 ± 0.7

phenolic antioxidants	IC_{50} (μM) (in methanol)
tyrosol	5.8 ± 0.5
hydrotyrosol	0.25 ± 0.02
oleuropein	0.35 ± 0.03
α-tocopherol	4.8 ± 0.3

[a] DPPH solution was 1×10^{-4} M. Note that the higher the inhibition potential, the smaller the amount (mg/mL) needed to scavenge the DPPH stable free radical.

was found to contain only a small amount of polyphenols (3−4 mg/kg of oil); thus, their contribution to its antioxidant capacity is only minor. The HPLC analysis showed that soybean oil contains only small amounts of tyrosol, hydrotyrosol, and oleuropein aglycone, and in the TLC analysis, soybean oil showed only trace bands for these polyphenols. Our results can be compared with that of Espin et al. study (*15*), measuring the RSC of vegetable oils. Espin et al. found that the TF of soybean oil had the highest value among 14 types of vegetable oils. The high RSC for soybean oil is very important considering that it is the predominant oilseed oil in the world. Soybean oil makes up about 75% of vegetable oil use for food in the U.S.A., and global consumption of soybean oil is almost 22% (1998−2002) out of a total of 17 oils and fats (*31*).

EVOO of Greek origin was second in antioxidant activity, but its antioxidant potential is mostly due to the high concentration of polyphenols in the polar fraction and partly to the high concentration of tocopherols (of which more than 90% is α-tocopherol). HPLC analysis showed that EVOO samples contain 0.5−0.7 μg/kg oil tyrosol, 0.7−0.9 μg/kg hydrotyrosol, and 40−60 μg/kg oleuropein. These results are comparable to recent ones with virgin olive oil samples (*32*, *33*). The presence of tyrosol, hydrotyrosol, and oleuropein was also observed by the TLC bands with R_f = 0.52−0.55, R_f = 0.45−0.45, and R_f = 0.17−0.19, respectively.

Total phenolic analysis showed that EVOO samples contain 180−210 mg (GAE)/kg oil. Also, the HPLC analysis showed that EVOO contained 4.200−4.700 mg/kg oil of squalene. Squalene plays an important role in the stability of olive oil toward autoxidation (*34*). The presence of polyphenols in EVOO is an advantageous aspect, because their antiradical activity can play a protective role in the degradation of tocopherols during storage and cooking processes (*29*).

Antioxidant Capacity of MF and LF. The MF of vegetable oils contains most of the phenolic antioxidants (hydrotyrosol, tyrosol, syringic acid, sinapic acid, protocatechuic acid, caffeic acid, etc.), lignans, and secoiridoids (oleuropein aglycone). It is also called the polar fraction. EVOO showed the highest value of IC_{50} as expected. Second was the commercial olive oil, followed by soybean, sunflower, and corn oils. The results showed that the radical scavenging potential of oils was proportional to their polyphenolic content. Total phenolics measurements showed that EVOO contains 170−210 mg (GAE)/kg, commercial (Greek) olive oil, 60−80 mg/kg soybean

oil, 3−4 mg/kg sunflower oil, and less than 1 mg/kg corn oil. Also, HPLC measurements and TLC showed that the most important antioxidant polyphenols, such as hydrotyrosol, tyrosol, and oleuropein, were absent (or in trace amounts in soybean) in sunflower and corn oils.

MF results for RSC are comparable with the MF results of vegetable oils by Espin et al. (although their RSC values for TF, MF, and LF were calculated for set conditions: 93 μM DPPH• and 5 μL of the corresponding oil or oil fraction). Sesame, safflower, and olive oils with high polyphenolic content showed the highest RSC values (*15*). Antioxidant polyphenols in virgin olive oil have been previously reported (*12*, *35*). Other studies showed that oleuropein ester (3′,4′-DHPEA dihydroxyphenylethanol), oleuropein, and hydrotyrosol exhibit a strong radical scavenging activity, which has been proven to be more effective than BHT (2,6-di-*tert*-butyl-4-methylphenol) or vitamin E (*16*, *36*).

The LF of oils contains mostly tocopherols, triglycerides, and phospholipids. The antioxidant capacity of LF is mainly due to the type and concentration of tocopherols, whereas phospholipids have a much lower RSC but affect the antioxidant capacity of vitamin E (*37*). It is well-known from other studies that tocopherols have different efficiencies as antioxidants. It has been shown that δ-tocopherol has the highest efficiency, then comes γ-tocopherol = β-tocopherol, and α-tocopherol is the least efficient antioxidant (*30*).

The highest IC_{50} in our experiments was observed for soybean oil. This result can be explained by the fact that soybean, according to other studies, is rich in δ-tocopherol (*38*). Second is corn oil, and third is sunflower (**Table 1**). The LF of EVOO, despite its high concentration of tocopherols [according to the scientific literature, 180−210 mg/kg (*34*)], showed a lower IC_{50}, as compared with other vegetable oils. An explanation is that more than 95% is in the form of α-tocopherol, the least efficient antioxidant. Commercial olive oil showed the lowest IC_{50}, since its tocopherol content (<80 mg/kg) was shown to be much lower than in EVOO (*34*). Similar results were observed by Espin et al. LF of soybean showed the highest RSC, among 14 vegetable oils, due to its high content in δ-tocopherol, and second and third were corn and sunflower oils, whereas EVOO showed much lower RSC (*15*).

Thermal Effects on Olive Oil and Other Vegetable Oils. Recent studies showed that fats and oils undergo various changes including hydrolytic, oxidative, isomerization, and polymerization reactions during heat treatment at elevated temperatures. Olive oil shows a high resistance to these changes as compared to other vegetable oils (*39*). Also, tocopherols in oils present different thermostabilities. Various studies showed that α-tocopherol is the least resistant to temperature and then β-tocopherol, and the highest thermostability is shown, equally, by γ- and δ-tocopherols (*40*).

Our experiments were focused on the antioxidant capacity TF at temperatures of 160 and 190 °C for a 2 h heating period. Soybean oil TF presented a high resistance to temperature, and its IC_{50} reduced substantially only after 2 h at a temperature of 190 °C (**Table 2**). Second in thermal stability was sunflower oil, and third in stability was corn oil. EVOO with high concentrations in polyphenols and squalene revealed high resistance to heating, but its high content in polyphenols and α-tocopherol did not contribute to its thermostability. Commercial olive oil showed the lowest thermostability. Similar results were presented by Espin et al. (*15*). Studies on the degradation of polyphenols under thermal treatment showed that concentrations of hydrotyrosol and oleuropein rapidly decreased with heating time (*41*). Also, other studies showed that α-tocopherol in VOO decreases rapidly and substantially with heating time (*18*). The resistance of olive oil to deterioration at elevated temperature is due to the presence of natural antioxidants: tocopherols, sterols, and polyphenolic compounds (*42*).

Table 2. Antioxidant Capacity (IC_{50}) of Olive Oil and Vegetable Oils (TF, MF, and LF) by the DPPH Method, after Thermal Treatment at 160 and 190 °C

oil source	IC_{50} (mg/mL) (without heating)	160 °C (2 h)	190 °C (2 h)
	TF		
EVOO	11 ± 0.6	22 ± 1.4	35 ± 2.2
soybean oil	10 ± 0.5	13 ± 0.9	25 ± 1.4
corn oil	15 ± 0.9	18 ± 1.4	33 ± 1.6
sunflower oil	14 ± 0.7	16 ± 1.2	30 ± 1.4
olive oil	17.5 ± 1.2	25 ± 2.2	35 ± 2.5
	MF		
EVOO	15 ± 1.4	19 ± 1.5	35 ± 2.2
olive oil	22 ± 2.2	27 ± 2.8	42 ± 3.1
soybean oil	52 ± 4.5	58 ± 4.8	63 ± 5.5
corn oil	48 ± 3.8	55 ± 4.3	59 ± 4.8
sunflower oil	45 ± 3.2	53 ± 3.8	57 ± 4.2
	LF		
EVOO	22 ± 1.6	27 ± 3.1	45 ± 3.8
olive oil	28 ± 2.2	32 ± 3.3	48 ± 4.2
soybean oil	20 ± 1.5	25 ± 2.3	40 ± 2.9
corn oil	18 ± 1.1	24 ± 1.8	45 ± 2.5
sunflower oil	15 ± 0.7	22 ± 2.3	44 ± 2.5

Changes in the IC_{50} of the oil fractions (MF and LF) at 160 and 190 °C under thermal treatment for the various vegetable oils showed a correlation with polyphenolic content in the MF fraction and tocopherol content in the LF fraction. The MF fractions of EVOO and olive oil with high content in polyphenols showed the highest radical scavenging activity (IC_{50}) after thermal treatment, whereas soybean, corn, and sunflower oils, which have very low or trace amounts of polyphenols, showed the lowest IC_{50} values after thermal treatment. In the case of LF fractions, soybean, corn, and sunflower oils, with high contents in δ- and γ-tocopherols (thermostable tocopherols) in their lipid fractions, showed as expected the highest IC_{50} values after thermal treatment, whereas the EVOO and olive oil showed the lowest antioxidant capacity. It is noted that the differences in the antioxidant activity for the LF of oils, after heating at 190 °C, is very small among the various vegetable oils.

Recent studies showed that hydrotyrosol and α-tocopherol significantly contribute to the stability of olive oil during potato frying (*43*). Pellegrini et al. found (with experimental oils) that polyphenols in EVOO are effective stabilizers of α-tocopherol during olive oil heating, thus contributing to the nutritional value of cooked foods, and at same time, their study elucidated the contribution of polyphenols for the prevention of antioxidant activity decay in olive oil under realistic heating conditions (*18*).

Quantification of Superoxide Anion Scavenging Capacity of MF. The antioxidant potential of olive and vegetable oils is the result of direct scavenging effect of their polyphenols on superoxide anion ($O_2^{\bullet -}$), which can influence oxidative stress in humans (*44*, *45*). Superoxide anion is unstable in aqueous solutions, so in our experiments, we used the system KO_2/18-crown-ether in DMSO, which produced a stable concentration of superoxide anion for more than 20 min. Representative EPR spectra are shown in **Figure 1**. The results of DMPO-OOH inhibition by MF fractions of vegetable oils are presented in **Table 3**.

The scavenging capacity toward superoxide anion, as expected, is proportional to the polyphenolic content of vegetable

Table 3. Combined Table of Antioxidant Activity (IC_{50}) of MF Fractions of Vegetable Oils toward the Superoxide Anion ($O_2^{\bullet-}$) Expressed as Inhibition of the DMPO-OOH EPR Signal and Antioxidant Capacity (IC_{50}) of MF Fractions of Vegetable Oils toward Hydroxyl Radicals ($HO^{\bullet}$) Produced by the Fenton Expressed as Inhibition of the DMPO-OH EPR Signal System[a]

oil source, MF fraction	IC_{50} (mg/mL) of MF toward $HO^{\bullet}$ radical	IC_{50} (mg/mL) of MF toward $O_2^{\bullet-}$ radical
EVOO	16 ± 2.2	15 ± 1.5
soybean oil	30 ± 3	35 ± 2.5
sunflower oil	37 ± 4	38 ± 2.7
corn oil	42 ± 6	45 ± 3.5
olive oil	22 ± 2.5	25 ± 2.2
olive oil polyphenols	**IC_{50} (μM)**	**IC_{50} (μM)**
hydrotyrosol	1.4 ± 0.2	3.5 ± 0.8
oleuropein	1.7 ± 0.3	4.3 ± 1.2
tyrosol	5.4 ± 0.5	15 ± 2.5

[a] EPR signal intensity was calculated by adding the height of the quadruplet peaks. IC_{50} is the amount in mg/mL necessary for 50% diminution of the EPR signal, and it was deduced graphically by plotting the % of inhibition against the amount of MF in mg/mL.

oils. EVOO and commercial olive oil showed the highest antiradical activity. Also, our results showed that hydrotyrosol, tyrosol, and oleuropein have antiradical activity toward $O_2^{\bullet-}$. A similar methodology in the scientific literature was carried out for the natural polyphenol *trans*-ε-viniferin (dimer of resveratrol, polyphenol with five hydroxyl groups, from grapevine) and various stilbene derivatives. The results showed that the IC_{50} was 0.14 mM (*trans*-ε-viniferin) and 0.82−1.68 mM for derivatives (*46*). The *trans*-ε-viniferin was about 25-fold more active antioxidant than α-tocopherol under the same conditions against $O_2^{\bullet-}$.

Hydrotyrosol and oleuropein have been tested toward their $O_2^{\bullet-}$ scavenging capacity in vitro by employing both cell-free (xanthine/xanthine oxidase system, X/XO) and neutrophil-based generation methodologies (PMN = human polymorphonuclear neutrophils; PMA = phorbol-12-myristate-13 acetate) by Visioli et al. (*16*). Both compounds showed a potent scavenging potential. The EC_{50} (50% inhibition capacity) values were found to be as follows: hydroxytyrosol (EC_{50}), 9.1 (X/XO) and 3.2 μM (PMN-PMA); oleuropein (EC_{50}), 14.3 (X/XO) and 29.3 μM (PMN-PMA). Hydrotyrosol is more active than oleuropein. According to the authors, vitamin E and BHT in experiments with the above methods had no effect on $O_2^{\bullet-}$ production (*16*).

The quantification of the antioxidant capacity of MF fractions of olive oil and other vegetable oils toward hydroxyl radicals ($HO^{\bullet}$) was performed with the Fenton system (Fe^{2+}/H_2O_2). The addition of EDTA-Na_2 increases the EPR signal intensity. Increasing amounts (mg/mL) of MF fractions decrease the EPR signal intensity of the four line spin-trapped DMPO-OH adduct (**Figure 2**).

The antioxidant capacity toward $HO^{\bullet}$ is proportional to the polyphenolic content of vegetable oils. The EVOO with more than 200 mg/kg of polyphenols was the most active, whereas sunflower and corn oils with the lowest concentration of polyphenols showed the least RSC toward hydroxyl radicals (**Table 3**). Also, hydrotyrosol is the most active toward hydroxyl radical, second is oleuropein, but equally active, whereas tyrosol had a much lower antiradical activity (**Table 3**).

There are no similar results in the scientific literature for the antioxidant capacity of olive oil polyphenols toward hydroxyl radicals for comparison, except one study in which, under different methodology, the antioxidant properties of oil polyphenols toward superoxide anion, hydroxyl radical, and hypochlorous acid were tested (*49*).

The results obtained from the comparative study of antioxidant capacity indicate that soybean oil has the highest antioxidant potential, as well as the higher thermal stability among the vegetable oils tested. Similar results were reported by Pellegrini et al. (*50*). It was shown that the total antioxidant capacity of soybean oil, using three different assays, had the highest antioxidant capacity, followed by EVOO. Despite of the low polyphenolic content of soybean oil, its high concentration of γ- and δ-tocopherols in the LF, with the highest antioxidant potentials and thermostabilities, makes this oil effective for cooking and beneficial for human health. World production of soybean oil has increased substantially from 15 (1968−1972) to 22% (1998−2002) of the total world production, making it the most consumed oil all over the world (*47*). Also, a survey of prices in Greek supermarkets revealed that soybean oil had the lowest price, 1.6−1.7 Euro per liter, as compared to 1.8−2.10 Euro for corn and sunflower oils, 3.60−4.0 Euro for olive oil, and 4.30−4.50 Euro for EVOO.

However, EVOO and olive oil have a major advantage with their high concentrations of polyphenols and α-tocopherol (more than 65−90% of tocopherols). Also, part of the antioxidant activity for olive oil, which cannot be neglected, can be assigned to the unsaturated fatty acids, such as linoleic acid, present in olive oil up to 11.5%, related to the presence of double bonds (*48*). Greek EVOO and commercial olive oils contain high concentrations of α-tocopherols (60% of Greek EVOO had α-tocopherol above 200 mg/kg, with a maximum at 370 mg/kg, and 60% of commercial olive oils above 180 mg/kg, with a maximum at ∼250 mg/kg) (*51*). Free forms of hydrotyrosol, tyrosol and their secoroid derivatives, and oleuropein and its ligstroide aglycones represent almost 50% of the total phenolic content of VOO (*52*). Experimental data showed that tyrosol and hydrotyrosol are absorbed at the intestinal level from realistic doses of VOO, and these phenolics can be used as biomarkers of VOO intake (*8*, *53*). Olive oil phenolics are dose depedently absorbed in humans after ingestion and are excreted in the urine as glucuronide conjugates (*54*).

It has been found that polyphenols in olive oil confer their health-promoting properties by the route of antioxidant activity and can play an important role in the protection against malignant neoplasms, such as colorectal, prostate, and breast cancer. Rates of incidence of these cancers in Mediterranean countries, where olive oil is one of the major constituents of their diet, is much lower than in countries of northern Europe (*3*, *4*, *55*). Also, phenolic antioxidants of olive oil have been recently implicated for the lower rates of cardiac disease mortality among people consuming a Mediterranean diet by inhibition of LDL oxidation (*2*, *56*).

ABBREVIATIONS USED

DMPO, 5,5-dimethyl-1-pyroline-N-oxide; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EPR, electron paramagnetic resonance; ROS, reactive oxygen species; EVOO, extra virgin olive oil; IC_{50}, inhibition capacity 50%; RSC, radical scavenging capacity; MF, methanolic fraction; LF, lipidic fraction; TF, total fraction.

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