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ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · SEPTEMBER 2009

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J. Agric. Food Chem. 2009, 57, 7785–7789 7785
DOI:10.1021/jf901528v

Evaluation of Potential Genotoxicity of Virgin Olive Oil (VOO) Using the *Drosophila* Wing-Spot Test

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Edible and nonedible grades of virgin olive oil (VOO), differing in quality characteristics, were evaluated for potential genotoxicity in the *Drosophila* somatic mutation and recombination test (SMART) before and after heating at high temperatures. *Drosophila* larvae were fed on medium containing 6 and 12% v/v of each of the examined oils. Edible VOOs did not exhibit any mutagenic or recombination activity even after thermal treatment. Lower grade VOO gave negative results at the concentration of 6% and inconclusive ones at 12%. However, after its thermal treatment, a statistically significant increase of large single spots was observed, giving a positive result for this spot category at both concentrations. Evaluation of the possible contribution of olive phenolic compounds to the nongenotoxic effects observed was carried out using a polar olive leaf extract and pure oleuropein. No significant increase in the frequency of any category of mutant spots was recorded for leaf extract (0.8−12 mg of total polar phenols/dose) or pure oleuropein (0.8−8 mg/dose). These results are expected to contribute to the ongoing interest in the inherent properties of VOO as part of the everyday diet.

KEYWORDS: SMART assay; genotoxicity; virgin olive oil; heating; oleuropein; olive leaf extract

INTRODUCTION

Virgin olive oil (VOO) is the most popular dietary lipid used for food cooking or salad dressing in the Mediterranean diet. Its daily intake is related to reduction of risks resulting from cardiovascular disorders and certain types of cancer due to its abundance in monounsaturated fatty acids (~58.0−83.0% oleic acid) and the presence of endogenous polar antioxidants, for example, derivatives of oleuropein and ligstroside, α-tocopherol, and other minor compounds (*1*). Findings presenting the positive biological importance of VOO and/or of its minor components are many and well documented (see, e.g., ref *2*). Nevertheless, because lipid consumption is an adherent part of the human diet, scientists investigate all possible effects these nutrients could induce. In this view, recently, two olive oil samples, one virgin and one refined, were comparatively studied with a series of vegetable oils (sesame, sunflower, wheat germ, flax, and soy oil) employing the *Drosophila melanogaster* somatic mutation and recombination test (SMART) (*3*). The authors reported that olive oil was clearly nongenotoxic and suggested the possible contribution of monounsaturates and/or the polar phenolic compounds, although they did not carry out either fatty acid or phenol analysis.

In an effort to highlight this point, in the present study, samples of edible and nonedible grades of VOO were chemically characterized and evaluated for genotoxicity. To further explore olive oil genotoxicity aspects associated with olive oil intake, the samples were assessed in the same assay after heating at high temperature. It is well-known that exposure of oils at such temperatures affects their chemical characteristics and gives rise to nondesirable compounds (*4*). Thus, values for quality indices (peroxide value, absorbance indices (K_{232}, K_{270}), % free acidity, total polar phenol and α-tocopherol contents, oleic acid/linoleic acid ratio) as well as for the DPPH$^{\bullet}$ scavenging ability of the samples were used in an effort to relate the findings on genotoxicity with chemical composition. In addition, the contribution of olive biophenols to the observed effects was assessed on a polar olive leaf extract (chemically characterized) and pure oleuropein.

The evaluation of the genotoxic potential of the samples before and after thermal treatment, the olive leaf extract and pure oleuropein, were assessed employing the somatic mutation and recombination test in *Drosophila*. This test is a well-known eukaryotic in vivo assay, which not only detects the different kinds of mutational events but also allows the detection of mitotic recombination (*5*). The use of two genetic markers, multiple wing hair (*mwh*) and flare (*flr*) in the third chromosome, makes it possible to discern the formation of mutant clones on the wing blade. Single spots are produced by somatic point mutation, deletion, etc., and mitotic recombination occurring between the two markers. Twin spots are produced exclusively by mitotic recombination occurring between the proximal marker *flr* and the centromere of chromosome 3. Because both somatic mutation and mitotic recombination events are found to be associated with

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Published on Web 08/13/2009

carcinogenesis (*6*), our results are expected to contribute to the ongoing interest in the inherent properties of virgin olive oil as part of the everyday diet.

MATERIALS AND METHODS

Solvents, Specific Reagents, and Standards. All chemicals used were of the appropriate purity and were purchased from various suppliers. Methanol, acetonitrile, and *n*-hexane (HPLC grade) were obtained from Merck (Darmstadt, Germany), and 2-propanol (HPLC grade) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Iso-octane (spectral grade) was from Riedel-de Haën (Seelze, Germany). Folin−Ciocalteu reagent, potassium iodide, sodium thiosulfate, sodium hydroxide, and anhydrous sodium sulfate were purchased from Panreac Quimica S.A. (Barcelona, Spain). Potassium hydroxide was from Merck and anhydrous sodium carbonate from Riedel-de Haën. DPPH (90% purity) and caffeic acid (98% purity) were obtained from Sigma-Aldrich Chemie. α-Tocopherol (>98% purity) was a product of Fluka Chemie GmbH (Buchs, Switzerland) and oleuropein (98% purity) of Extrasynthese (Genay, France). Ethyl methanesulfonate (EMS) (99% purity) was obtained from Sigma Chemical Co. Before use, EMS was dissolved in distilled water.

Oil Samples. Two commercial Greek virgin olive oil (VOO1 and VOO2) samples and one lampante (LOO) were used in the present study. The three samples were stored at 4 °C for 1 year before use.

Heating Experiment. Oil samples (500 mL) were placed in a deep fryer and were heated in a domestic electric heater at 100, 180, and 220 ± 5 °C for 15, 30, and 30 min, respectively. Then, the temperature was elevated at 250 ± 5 °C for 5 min. Oil temperature was monitored by a digital thermometer. Heating gradient for 80 min in total was chosen to ensure complete destruction of polar and nonpolar antioxidants. All assays were run in duplicate. After thermal treatment, the samples were cooled to room temperature and kept at 4 °C until analysis.

Determination of Quality Parameters and Composition of Oil Samples. Peroxide value (PV), acidity as percent oleic acid, and absorbance measurements at 232 and 270 nm of the unheated oils were determined in duplicate according to official methods (*7*). Total polar phenol content (expressed as mg of caffeic acid/kg of oil using the Folin−Ciocalteu colorimetric method) and HPLC analysis of α-tocopherol in both unheated and heated samples were carried out as described in Psomiadou and Tsimidou (*8*). The measurement of radical scavenging activity in the total fraction of both unheated and heated oils using the DPPH$^{\bullet}$ assay and the determination of fatty acid composition were carried out according to the methods of Kalantzakis et al. (*9*). All absorbance measurements were recorded in a U-2000 Hitachi spectrophotometer (Tokyo, Japan). HPLC analysis was carried out on a system consisting of two Marathon IV series HPLC pumps (Rigas Laboratories, Thessaloniki, Greece) and a Rheodyne injection valve (model 7125) with a 20 μL fixed loop (Rheodyne, Cotati, CA). The chromatographic system was equipped with a UV−vis spectrophotometric detector SPD-10AV (dual wavelength) from Shimadzu (Kyoto, Japan). Fatty acid methyl esters (FAMEs) were analyzed on a WCOT fused-silica capillary column (25 m length × 0.25 mm i.d.) coated with HP-FFAP (0.33 mm film thickness; Hewlett-Packard) using an Agilent 6890 GC equipped with a flame ionization detector.

Olive Leaf Sample and Extract Preparation. New and mature leaves from 1-year-old shoots were collected (October 2007) from 10 olive trees (Chondrolia Chalkidikis cultivar) grown in the university orchard (Aristotle University of Thessaloniki, Greece). Leaves were selected from branches within arm reach, with a north orientation. After sampling, leaves were immediately cleaned of dust and subsequently freeze-dried. Dried samples were then stored in sealed opaque glass jars purged with nitrogen and kept in a dry, dark, and cool place until analysis. Analysis was accomplished as close as possible to sampling date. Prior to the analysis, freeze-dried leaves were powdered with the aid of a mechanical electric mill (particle size < 2 mm). Phenols extraction from the freeze-dried plant material was carried out with methanol (1/40 w/v) in an ultrasonic bath at room temperature for 5 min. The extract was evaporated to dryness under vacuum at ∼40 °C and redissolved in distilled water for larvae treatment. Concentrations applied as indicated under Bioassay for Genotoxicity were based on Folin−Ciocalteu determination using oleuropein as reference compound.

HPLC Analysis of Phenolic Compounds in Olive Leaf Extract. The phenol analysis was performed according to the procedure described by Papoti and Tsimidou (*10*). The characterization of the olive leaf extract was carried out using the following elution protocol: aqueous acetic acid solution (3%, v/v) (A) and ACN (B); 0−1 min, 4% B; 1−26 min, 4−30% B; 26−36 min, 30−60% B; 36−46 min, 60−98% B; 46−50 min, 98% B; 50−60 min, 98−4% B. The flow rate was 0.9 mL/min, and analysis was carried out on a Chromolith RP-18e (100 × 4.6 mm) column (Merck KGaA) at room temperature. The injection volume was 10 μL. The HPLC system used consisted of a pump, model P4000 (Thermo Separation Products, San Jose, CA), a Midas autosampler (Spark, Emmen, The Netherlands), and a UV 6000 LP diode array detector (DAD; Thermo Separation Products) in series with an SSI 502 fluorescence detector (FLD; Scientific Systems Inc., State College, PA). Phenolic compounds in the tested extracts were monitored at 245, 280, and 335 nm using DAD and at 280 nm excitation and 320 nm emission using FLD.

Fly Stocks. Two *D. melanogaster* strains, the multiple wing hair strain (*mwh*), with genetic constitution *fs(1)K10 w/Y; mwh se e /mwh se e*, and the flare (*flr*^{3}) strain, with genetic constitution *y w*^{co}*/y w*^{co} *; flr*^{3} *se/TM2 Ubx*^{130} *se e*, were used in the present study (*11*, *12*). Larvae from the cross between *flr*^{3} virgin females with *mwh* males were used for testing (*5*). The stocks and the crosses were maintained at 24 ± 1 °C in photoperiod of 16 h of light−8 h of darkness on a yeast−glucose medium.

Bioassay for Genotoxicity. For screening the genotoxic activity of the tested olive oils, the somatic mutation and recombination test (SMART) (*5*, *13*) was used. The experiments were carried out following the principles and the basic procedures presented by Graf et al. (*5*). Thus, eggs obtained by parental crosses between *flare-3* virgin females and *mwh* males were collected during a 6 h period. Larvae emerging 72 ± 3 h later were washed with Ringer solution, and a series of 30 larvae were transferred to treatment vials, containing 0.85 g of *Drosophila* Instant Medium (Carolina Biological Supply, Burlington, NC) rehydrated with 4 mL of a mixture of distilled water and one of the tested samples (oils, olive leaf phenolic extract, or standard oleuropein). Oils were incorporated into the medium at concentrations of 6 and 12% v/v, which correspond to the usual range of oil intake by humans (*3*). In the case of olive leaf extract and pure oleuropein aqueous solutions of different concentrations were prepared so that the medium tested to contain finally 0.8, 2, 4, 8, or 12 mg of total polar phenols expressed as oleuropein or 0.8, 2, 4, or 8 mg of pure oleuropein. The larvae were fed in the medium until pupation. A parallel experiment using only distilled water was carried out as the negative control. A well-known mutagen, EMS at a concentration of 0.1 mM, served as the positive control. The trans-heterozygous (*mwh*/*flr*^{3}) female flies emerging after chronic treatment were stored in 70% v/v ethanol/glycerol (1:1, v/v) solution, and their wings were mounted in Euparal solution and scored at 400× magnification for the presence of mosaic spots (Figure S1 of the Supporting Information). Following the methods and criteria of Graf et al. (*5*), the spots were grouped into four categories based on the size, number, and type of cells showing malformed wing hairs: (a) small single spots (with one or two affected cells, either *mwh* or *flr*^{3}); (b) large single spots (with three or more affected cells, either *mwh* or *flr*^{3}); (c) twin spots (consisting of both *mwh* and *flr*^{3} subclones); and (d) total spots. All of the experiments were carried out at 24 ± 1 °C.

Data Analysis. For the evaluation of induced effects, the frequency of spots per wing in the treated series was compared with that of the negative control. Statistical analysis of the data was done using the multiple-decision procedure (*14*, *15*). The procedure is based on the conditional binomial test (*16*, *17*) and the x^2 test (K. Pearson's criterion). Each statistical test was carried out at the 5% significance level.

RESULTS AND DISCUSSION

In recent years, a growing body of evidence concerning the beneficial properties of virgin olive oil has dramatically increased. The association of olive oil intake with nongenotoxicity and/or cancer protection as well as anticlastogenic effects of olive oil has been most frequently addressed (see, e.g., refs *3* and *18*−*21*). Nevertheless, olive oil, except for raw, is also used to cook various foods. Even though such practices (heating, frying) have been reported to affect the antioxidant and polyphenol content as well as the nutritional properties of oils (*9*, *22*, *23*), the available

Table 1. Quality Parameters and Genotoxicity Results Obtained in the Mutation and Recombination Test after Treatment of mwh/flr^3 Larvae of *D. melanogaster* with Unheated Virgin Olive Oil Samples

	quality parameters							genotoxicity parameters					
										spots per wing (no. of spots) diagnosis[j]			
sample	PV[a]	% free acidity[b]	K_{232} (K_{270})	TPP[c]	α-toc[d]	OAME/ LOAME[e]	% RSA[f]	level of dosing	wings analyzed	small single spots (1−2 cells) $m = 2.0$	large single spots (>2 cells) $m = 5.0$	twin spots $m = 5.0$	total spots $m = 2.0$
control								0	110	0.22 (25)	0.03 (3)	0.03 (3)	0.28 (31)
VOO1[g]	24.0	0.4	2.64 (0.21)	157 ± 3.0	122 ± 0.3	11.9 ± 0.1	54.2 ± 2.7	6%	56	0.22 (12) −	0.05 (3) i	0.05 (3) i	0.32 (18) −
								12%	53	0.20 (11) −	0.04 (2) i	0.04 (2) i	0.28 (15) −
VOO2[g]	16.9	1.7	2.08 (0.18)	198 ± 7.0	96.6 ± 4.2	7.7 ± 0.1	52.7 ± 1.7	6%	61	0.20 (12) −	0.06 (4) i	0.03 (2) i	0.29 (18) −
								12%	52	0.19 (10) −	0.05 (3) i	0.04 (2) i	0.29 (15) −
LOO[h]	50.0	5.6	6.70 (0.67)	0	0	8.9 ± 0.0	<5	6%	47	0.19 (9) −	0.06 (3) i	0.04 (2) i	0.29 (14) −
								12%	43	0.23 (10) −	0.07 (3) i	0.07 (3) i	0.37 (16) i
EMS[i]								0.1 mM	140	0.36 (50) +	0.1 (15) +	0.08 (11) i	0.54 (76) +

[a] PV, peroxide value (mequiv of O_2/kg of oil), $n = 2$. [b] As % oleic acid, $n = 2$. [c] TPP, total polar phenol content (mg of caffeic acid/kg of oil), $n = 3$. [d] α-tocopherol as mg/kg of oil, $n = 3$. [e] Area ratio of oleic acid methyl ester/linoleic acid methyl ester, $n = 3$. [f] % RSA, % DPPH radical scavenging activity, $n = 3$. [g] VOO, virgin olive oil. [h] LOO, lampante olive oil. [i] EMS, ethyl methanesulfonate. [j] Statistical diagnosis according to Frei and Würgler (*15*): +, positive; −, negative; i, inconclusive; *m*, multiplication factor. Probability levels: $\alpha = \beta = 0.05$.

information for the possible mutagenic activity of heated olive oils is very limited (*24*−*26*).

In an effort to further explore genotoxicity aspects with regard to oil oxidative status, in the present study, the three oil samples (VOO1, VOO2, and LOO) were evaluated for potential genotoxicity before and after thermal treatment (100−250 °C). A heating gradient for 80 min in total was chosen to ensure complete destruction of polar and nonpolar antioxidants. Values for the quality parameters (PV, K_{232}/K_{270}, % free acidity, total polar phenol and α-tocopherol contents, oleic acid/linoleic acid ratio) and the DPPH$^{\bullet}$ scavenging ability as well as the results obtained in the wing spot assay for the three samples are presented in **Table 1**. VOO1 and VOO2 were significantly less oxidized forms of virgin olive oil in comparison to LOO in terms of PV and K_{232}/K_{270} values and also contained significant levels of total polar phenols and α-tocopherol. LOO was devoid of these two classes of phenolic antioxidants. The concentration of minor components was within the range reported for other VOO samples of Greek origin (*27*, *28*). The levels of such minor components contributed to the overall antioxidant potential of the samples as reflected in the respective % RSA values.

Results obtained on the wing spot test showed that none of the three samples exhibited mutagenic or recombinagenic activity at applicable concentrations with the exception of LOO, which gave inconclusive results at the concentration of 12% (**Table 1**). On the other hand, the positive control carried out with EMS showed a clear positive response; this fact supports the validity of the results obtained for the olive oil samples. Present data on virgin olive oil performance were consistent with those of Rojas-Molina et al. (*3*). In both studies VOO samples were found to be clearly nongenotoxic, whereas the lampante oil (used in the present study) and the refined one (used in the previous study) (*3*) gave inconclusive results. The refined olive oil is devoid of minor constituents, such as polar phenols and tocopherols, but it is also free of oxidized forms of fatty acids. On the other hand, the lampante oil sample (see **Table 1**) was also devoid of phenols and tocopherols but presented high PV and free acidity values. The inconclusive result found for both refined and lanpante oils could be judged as having minimal biological significance, because no increase in the frequency of any of the three categories of mutant spots (e.g., small single, large single, and twin spots) was recorded in relation to the negative control (*3*) (**Table 1**).

Taking into account that various treatments of oil, such as cooking, may lead to the formation of nondesirable compounds that could induce genotoxicity, the three samples were then thermally treated as described under Matierals and Methods. Thermal treatment had a dramatic effect on the quality of the samples. For example, a complete loss of polar phenols was evidenced for VOO1 and VOO2; α-tocopherol loss was complete in the case of VOO2, whereas a 2.5-fold lower amount in VOO1 (50 ± 1.2 mg/kg) remained. Loss of the antioxidant potential of the samples was verified by the very low % RSA values measured after heating (% $RSA_{LOO} < 5$; % $RSA_{VOO1} = 15.1 \pm 0.1$; % $RSA_{VOO2} = 8.4 \pm 0.7$) in line with literature data (*9*). In addition to antioxidant loss, the fatty acid composition of the samples was also affected. In particular, an increase in oleic acid content was observed due to degradation of linolenic acid. The changes were significantly higher in the case of the LOO sample as indicated by the value of oleic acid/linoleic acid ratio (16.1 ± 0.1) when compared with the respective ones for VOO1 (13.8 ± 0.1) and VOO2 (10.4 ± 0.2). This is obviously due to the absence of antioxidant constituents that contribute to the oxidative stability of lipids. A genotoxicity test was carried out for the same doses as in the case of the nonthermally treated oils (6 and 12% v/v).

The derived genotoxicity data for the heated oil samples are presented in **Table 2**. As evidenced, both VOO1 and VOO2 retained their clear nongenotoxic activity, because no significant increase in the frequency of any of the three categories of mutant spots was recorded (e.g., small single, large single, and twin spots) in relation to the negative control. As shown in **Table 2**, when LOO was fed to the larvae at both concentrations, a statistically significant increase of the large single spots was observed, giving a positive result for this spot category. The large single spots can be due to either mutation (point mutation, deletion, etc.) or mitotic recombination (*5*). However, only at the concentration of 12% of the heated LOO the total spots gave inconclusive results (**Table 2**). Even though this inconclusive statistical result could be judged as having minimal biological significance (nongenotoxic), the

Table 2. Genotoxicity Results Obtained in the Mutation and Recombination Test after Treatment of mwh/flr^3 Larvae of *D. melanogaster* with Heated Virgin Olive Oil Samples

			genotoxicity parameters			
			spots per wing (no. of spots) diagnosis[d]			
sample	level of dosing	wings analyzed	small single spots (1−2 cells) m = 2.0	large single spots (>2 cells) m = 5.0	twin spots m = 5.0	total spots m = 2.0
control	0	110	0.22 (25)	0.03 (3)	0.03 (3)	0.28 (31)
VOO1[a]	6%	53	0.21 (11) −	0.07 (4) i	0.04 (2) i	0.32 (17) −
	12%	51	0.17 (9) −	0.04(2) i	0.04 (2) i	0.25 (13) −
VOO2[a]	6%	79	0.16 (13) −	0.09 (7) i	0.04 (3) i	0.29 (23) −
	12%	75	0.18 (14) −	0.07 (5) i	0.07 (5) i	0.32 (24) −
LOO[b]	6%	70	0.14 (10) −	0.12 (8) +	0.04 (3) i	0.3 (21) −
	12%	62	0.19 (12) −	0.13 (8) +	0.06 (4) i	0.38 (24) i
EMS[c]	0.1 mM	140	0.36 (50) +	0.1 (15) +	0.08 (11) i	0.54 (76) +

[a] VOO, virgin olive oil. [b] LOO, lampante olive oil. [c] EMS, ethyl methanesulfonate. [d] Statistical diagnosis according to Frei and Würgler (*15*); +, positive; −, negative; i, inconclusive; m, multiplication factor. Probability levels: $\alpha = \beta = 0.05$.

Table 3. Genotoxicity Results Obtained in the Mutation and Recombination Test after Treatment of mwh/flr^3 Larvae of *D. melanogaster* with Olive Leaf Phenolic Extract and Pure Oleuropein

			genotoxicity parameters			
			frequency of spots per wing (no. of spots) and diagnosis[c]			
sample	level of dosing [a]	wings analyzed	small single spots (1−2 cells) m = 2.0	large single spots (>2 cells) m = 5.0	twin spots m = 5.0	total spots m = 2.0
control	0	98	0.20 (20)	0.02 (2)	0.04 (4)	0.26 (26)
olive leaf phenolic extract	0.8	49	0.20 (10) −	0.02 (1) i	0.04 (2) i	0.28 (13) −
	2	54	0.18 (10) −	0.04 (2) i	0.05 (3) i	0.27 (15) −
	4	47	0.19 (9) −	0.04 (2) i	0.04 (2) i	0.27 (13) −
	8	50	0.18 (9) −	0.04 (2) i	0.04 (2) i	0.26 (13) −
	12	52	0.17 (9) −	0.07 (4) i	0.04 (2) i	0.28 (15) −
oleuropein	0.8	61	0.22 (13) −	0.05 (3) i	0.03 (2) −	0.29 (18) −
	2	55	0.2 (11) −	0.05 (3) i	0.02 (1) −	0.27 (15) −
	4	75	0.2 (15)	0.04 (3) i	0.04 (3) −	0.28 (21) −
	8	82	0.22 (18) −	0.02 (2) i	0.03 (3) −	0.26 (22) −
EMS[b]	0.1 mM	140	0.36 (50) +	0.1 (15) +	0.08 (11) i	0.54 (76) +

[a] Milligrams of TPP expressed as oleuropein/4 mL aqueous medium. [b] EMS, ethyl methanesulfonate. [c] Statistical diagnosis according to Frei and Würgler (*15*); +, positive; −, negative; i, inconclusive; m, multiplication factor. Probability levels: $\alpha = \beta = 0.05$.

positive result for the large single spots observed after the thermal treatment of this sample needs further investigation.

As suggested by Rojas-Molina et al. (*3*), for the same level of saturates, the ratio monounsaturates/polyunsaturates of the oils may be a major contributory factor for observing genotoxic effects. However, in our study, this ratio varied slightly among VOO1, VOO2, and LOO. It is interesting that the increase in the value of the ratio OAME/LOAME caused by the heating treatment did not affect positively genotoxicity results, possibly due to the oxidized forms of the lipid matrix. Therefore, contribution of minor constituents such as polar phenols to the nongenotoxicity could not be excluded. To examine this aspect and because of difficulties in isolating an adequate quantity of polar phenols from the available oil samples, we decided to isolate phenols from olive leaves. The latter is a well-documented rich source of various oleuropein type secoiridoids and derivatives structurally similar—though not always identical—to those found in olive oil and other olive products (*10*, *29*−*31*). The phenolic profile of the extract is given in Figure S2 of the Supporting Information. According to Figure S2 and our experience in the field (see, e.g., ref *10*) various wavelengths and/or detection means reveal different constituents as the dominant ones. However, it can be suggested that hydroxytyrosol, luteolin, and its 7-*O* glucoside, along with oleuropein and related secoiridoids, are the major extract constituents. In the present study, different doses of the olive leaf phenolic extract, as well as of pure oleuropein were evaluated for genotoxicity, and the results are given in **Table 3**. The concentrations used were based on recommended levels of dosing for various commercially available dietary supplement forms. The results obtained (**Table 3**) indicate that no significant increase in the frequency of any category of mutant spots was recorded independently of the concentrations of oleuropein and olive leaf extracts applied. Our results are consistent with the biological properties of these substances reported in previous studies (*1*, *32*−*34*).

In summary, the results obtained for the genotoxicity of edible and nonedible grades of VOO, differing in quality characteristics, in the *Drosophila* wing spot test increase the genotoxicity database of olive oil. The clear nongenotoxic activity of the edible olive oil samples, even after thermal treatment, contributes to the ongoing interest on safety aspects of this widely consumed product. Moreover, our results add to the knowledge on the safe use of phenol-rich olive oil by humans and the repeatedly

suggested anticancer and other biological properties of oleuropein. However, further examination using additional tests is required. Due to the complex nature of the virgin olive oil matrix, the presence of other compounds, which can act independently or in a synergistic way under the aforementioned experimental conditions, cannot be precluded.

ABBREVIATIONS USED

ACN, acetonitrile; DAD, diode array detector; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EMS, ethyl methanesulfonate; FAMEs, fatty acid methyl esters; FLD, fluorescence detector; flr, flare; LOAME, linoleic acid methylester; LOO, lampante olive oil; mwh, multiple wing hair; OAME, oleic acid methyl ester; PV, peroxide value; RSA, radical scavenging activity; SMART, somatic mutation and recombination test; TPP, total polar phenol; VOO, virgin olive oil.

Supporting Information Available: Figure S1, trichomes of the *Drosophila* wing blade. Two mwh trichomes (marked by the circle) surrounded by normal ones. Figure S2, RP-HPLC phenolic profile of methanol leaf extract. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received May 7, 2009. Revised manuscript received July 14, 2009. Accepted July 27, 2009. V.T.P. thanks the EU−European Social Fund (75%) and the Greek Ministry of Development-GSRT (25%) for financial support of this project (PENED 03EΔ596).