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Comparison of the Antioxidant Activities of Extra Virgin Olive Oils

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The phenol content and antioxidant activity of extra virgin olive oils (EVOOs) differing in their origins and degradation degrees were studied. The *o*-diphenolic compounds typical of olive oil, namely, the oleuropein derivatives hydroxytyrosol (3′,4′-dihydroxyphenylethanol, 3′,4′-DHPEA), the dialdehydic form of elenolic acid linked to 3′,4′-DHPEA (3′,4′-DHPEA-EDA), and an isomer of oleuropein aglycon (3′,4′-DHPEA-EA), were analyzed by HPLC. The antioxidant activity was studied by (a) the xanthine oxidase (XOD)/xanthine system, which generates superoxide radical and hydrogen peroxide; (b) the diaphorase (DIA)/NADH/juglone system, which generates superoxide radical and semiquinonic radical; and (c) the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) test. Results showed that EVOOs with a low degradation level (as evaluated by acidity, peroxide number, and spectroscopic indices K_{232}, K_{270}, and ΔK according to the EU Regulation) had a higher content of 3′,4′-DHPEA-EDA and a lower content of 3′,4′-DHPEA than oils having intermediate and advanced degradation levels. EVOOs with a low degradation degree were 3−5 times more efficient as DPPH scavengers and 2 times more efficient as inhibitors of the XOD-catalyzed reaction than oils with intermediate and advanced degradation levels. The DIA-catalyzed reaction was inhibited by EVOOs having low or intermediate degradation levels but not by the most degraded oils.

KEYWORDS: Extra virgin olive oil; oleuropein derivatives; 2,2-diphenyl-1-picrylhydrazyl radical; xanthine oxidase; diaphorase

INTRODUCTION

In 1960 a phenolic compound present throughout the olive tree (*Olea europea*) was isolated from olive leaves and called oleuropein (*1*). Oleuropein is an ester of 3′,4′ -dihydroxyphenylethanol (3′,4′-DHPEA) and the oleosidic skeleton common to the secoiridoid glucosides of Oleaceae, which is characterized by an exocyclic 8,9-olefinic functionality, a combination of elenolic acid and a glucosidic residue.

Oleuropein and its derivative 3′,4′-DHPEA have been intensively studied with respect to their potential effect on human health. By using cell-free systems it was demonstrated that oleuropein and 3′,4′-DHPEA are powerful scavengers of peroxyl radicals, HClO, superoxide radical, hydrogen peroxide, and synthetic radicals (*2*−*4*). The antioxidant activity of these compounds has also been demonstrated by employing cell culture model systems. Manna et al. (*5*), by using the Caco-2 human cell line challenged with xanthine oxidase (XOD) and its substrate xanthine to induce oxidative stress, demonstrated that both oleuropein and 3′,4′-DHPEA prevented the loss of cell viability. Visioli et al. (*2*) demonstrated that both oleuropein and 3′,4′-DHPEA had a potent ability to scavenge superoxide anion produced by human neutrophils (after activation with phorbol 12-myristate 13-acetate). Manna et al. (*6*) demonstrated that 3′,4′-DHPEA protected human red blood cell from hydrogen peroxide damage. Della Ragione et al. (*7*) found that 3′,4′-DHPEA inhibited human leukemia HL60 cell proliferation.

In vivo studies have been conducted using animal models. The addition of oleuropein to a standard diet increased the in vivo low-density lipoprotein (LDL) resistance to oxidation in rabbits (*8*). Dose-dependent absorption was demonstrated for 3′,4′-DHPEA, which was found to increase the antioxidant capacity of rat plasma (*9*).

On the basis of the nutritional importance of oleuropein and 3′,4′-DHPEA, the phenolic content of extra virgin olive oil (EVOO), which is the fat of choice in the Mediterranean diet, has been extensively investigated. Although oleuropein is the major phenolic compound present in the olive fruit, it is not present in EVOO as during processing it concentrates into vegetation water because of its high water solubility (*10*). Phenyl acids and phenyl alcohols including 3′,4′-DHPEA and *p*-hydroxyphenylethanol (*p*-HPEA) are present in low amounts in fresh EVOO (*11*). However, the prevalent phenolic compounds in EVOO are the dialdehydic form of elenolic acid linked either to 3′,4′-DHPEA or to *p*-HPEA (3′,4′-DHPEA-EDA and *p*-HPEA-EDA, respectively) and an isomer of oleuropein aglycon (3′,4′-DHPEA-EA) (*12*). The presence of lipophilic derivatives of oleuropein in EVOO is due to oleuropein hydrolysis catalyzed by endogenous glycosidases during the

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Table 1. Antioxidant Activity of EVOO Extracted with Different Solvents As Measured by the DPPH Scavenging Test

extractant	relative antioxidant activity
100% methanol	100 ± 2
acetone/water, 30:70	84 ± 5
acetone/water, 40:60	89 ± 1
acetone/water, 50:50	95 ± 10
acetone/water, 60:40	95 ± 3
acetone/water, 90:10	100 ± 6

crushing of olive fruit (*13*). The concentration of these compounds in EVOO is strongly affected by the particular olive cultivar, by agronomic and environmental factors, and by the extraction and storage conditions (*14*, *15*).

The aim of the present work was to evaluate the phenol content and the antioxidant activity of EVOOs differing in their origins and oxidation degrees. Two different sources of reactive oxygen species (ROS), which are relevant to cell damage in vivo, were used, namely, XOD and diaphorase (DIA). For comparison, the radical scavenging activity toward the synthetic 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was also evaluated.

MATERIALS AND METHODS

Oil Samples. One fresh EVOO (S1) was supplied by a small producer and analyzed immediately. Two EVOOs (S2 and S3) were produced in a laboratory-scale oil mill consisting of a hammer crusher, a mixer, and a basket centrifuge. Seven different brands of commercial EVOOs were purchased at a local supermarket (S4−S10) and analyzed within their commercial lifetimes. Aliquots (500 mL each) of two commercial EVOOs (S9 and S10) were bottled in 1 L clear glass bottles, which were closed with screw caps and stored at 30 °C for 30 days to accelerate the aging of the samples. One EVOO (S11) was supplied by a small producer and analyzed 5 years after production.

Analytical Indices. Acidity, peroxide value, and spectroscopic indices K_{232}, K_{270}, and ΔK in the UV region were determined according to the EU official method (*16*).

Polyphenols. Polyphenols were determined by HPLC according to the method of Cortesi et al. (*17*). Methanol was used as an extraction solvent. Operating conditions were as follows: RP-18 Spherisorb ODS-2 column (25 cm × 0.46 cm, 5 μm) equipped with an RP-18 Waters precolumn; injection volume, 20 μL; mobile phase, elution with a binary gradient of methanol/acetonitrile (50:50 v/v) and 0.5% H_3PO_4 in water, at 1 mL/min; UV−vis detector set at 280 nm.

Reference Compounds. p-HPEA was obtained from Merck (Darmstadt, Germany); 3′,4′-DHPEA was synthesized according to the method of Montedoro et al. (*12*); 3′,4′-DHPEA-EA was obtained according to the method of Limirioli et al. (*18*) from oleuropein glycoside (Extrasynthese, Genay, France), by enzymatic reaction using β-glicosidase from almonds (Sigma, St. Louis, MO); 3′,4′-DHPEA-EDA was isolated from olive leaves according to the procedure of Paiva-Martins and Gordon (*19*).

Quantification. p-HPEA and 3′,4′-DHPEA contents were calculated by using a standard curve of *p*-HPEA. 3′,4′-DHPEA-EDA and 3′,4′-DHPEA-EA contents were calculated by using a standard curve of oleuropein.

Antioxidant Activity. *Extraction of the Polar Fraction.* EVOO (2 g) was extracted with methanol (5 mL) or with acetone/water mixtures (5 mL) at different volume ratios (from 30:70 to 90:10). The mixtures were vigorously stirred for 1 h at room temperature and then centrifuged (4500*g* at 15 °C for 10 min) to separate the polar and lipid fractions. Methanol and acetone/water extracts were analyzed for the antioxidant activity by the DPPH test (**Table 1**). Acetone/water (90:10) extracts were found to have the same antioxidant activity as the methanol extract. Therefore, acetone/water (90:10) extracts were used to measure the antioxidant activity when using the XOD/xanthine and DIA/NADH/juglone systems, as methanol itself inhibits these enzymes.

XOD/Xanthine System. This system contained 0.1 M phosphate buffer, pH 7.4; 0.5 mM xanthine (in 10 mM NaOH); 0.08 U XOD (from cow's milk, Roche, Monza, Italy); 1.25 mM α-keto-γ-methiolbutyric acid (KMB); and various aliquots of an acetone/water (90:10) extract. The final acetone concentration in the assay mixture was kept constant at 2.5% (v/v). The reaction was carried out at 37 °C for 30 min, followed by gas chromatographic determination of ethene released from KMB (*20*).

DIA/NADH/Juglone System. This system contained 0.1 M phosphate buffer, pH 7.4; 0.1 mM NADH; 0.05 mM 5-hydroxy-1,4-naphthoquinone (juglone); 0.75 unit of DIA (from pig heart, Roche, Monza, Italy); 1.25 mM KMB; and various aliquots of an acetone/water (90:10) extract. The final acetone concentration in the assay mixture was kept constant at 2.5% (v/v). The reaction was carried out at 37 °C for 30 min, followed by gas chromatographic determination of ethene released from KMB (*21*).

Control reactions were prepared for each enzymatic systems by adding the extraction solvent (acetone/water, 90:10) in place of the antioxidant extract. The antioxidant activity was calculated as the percent inhibition of the control reaction rate, and expressed as I_{30} (for the XOD/xanthine system) or I_{50} (for the DIA/NADH/juglone system), as interpolated by the dose−response curves. I_{30} and I_{50} are the amount of original oil sample (in milligrams) that caused 30 or 50% inhibition of the model reaction under the conditions described above.

DPPH Scavenging Test. This assay was performed as described previously (*2*) with some modifications. Different dilutions of the methanolic or various acetone/water extracts of EVOO samples were added to a 25 mg/L methanolic solution of DPPH (Sigma). The decrease in absorbance was determined at 515 nm until 15 min (when a constant value was reached). I_{50} was defined as the amount of original oil sample (in milligrams) required to lower the initial DPPH concentration by 50% and was extrapolated from a dose−response curve.

HPLC Equipment. The HPLC equipment consisted of an L-7100 Merck Hitachi pump and an L-7400 Merck Hitachi UV−vis detector or of an EG&G Instruments (Princeton Applied Research) model 400 electrochemical detector and a D-7500 Merck Hitachi integrator.

GC Equipment. The GC equipment consisted of a Varian Aerograph 3300 with a Varian integrator and a deactivated aluminum oxide column ($^1/_8$ in. × 100 cm). The column temperature was 60 °C, the injection temperature, 80 °C, and the FID temperature, 225 °C.

UV—Vis Spectrophotometer. UV−vis measurements were performed with a Jasco UVDEC-610 spectrophotometer.

RESULTS AND DISCUSSION

The EVOOs differed greatly in their degradation levels, as determined according to EU Regulation 2568/91 (**Table 2**). Samples used here may be clustered in three groups: Fresh EVOOs (S1−S3) had low acidity, low peroxide values, and low spectroscopic indices K_{232} and K_{270}. Commercial EVOOs (S4−S8) showed higher values for all degradation parameters but did not exceed the legal limits, except for S4 and S5, which exceeded the limit for K_{270} but not that of ΔK. All of these samples represented an intermediate level of degradation. Aged EVOOs (S9−S11) exceeded the legal limits for all parameters except for S9, which conformed to the legal limit for acidity. They represented an advanced level of degradation (and could not be labeled as "extra virgin" according to EU rules).

A clear distinction can also be made on the basis of the phenol content of the oils (**Table 3**). In particular, EVOOs having a low degradation level had a high content of the secoiridoid derivative 3′,4′-DHPEA-EDA and low contents of the simple phenolic compounds 3′,4′-DHPEA and *p*-HPEA, whereas oils having an intermediate or an advanced degradation level showed a lower content of 3′,4′-DHPEA-EDA and higher contents of 3′,4′-DHPEA and *p*-HPEA. These data are in agreement with the results of Pagliarini et al. (*15*), who found that an increase in EVOO storage time results in 3′,4′-DHPEA and *p*-HPEA formation. On the contrary, the 3′,4′-DHPEA-EA content did

Table 2. Physicochemical Parameters of the Oils As Determined According to UE Regulation 2568/91

oil	acidity (oleic acid %)	peroxide value (mequiv of O_2/kg)	K_{232}	K_{270}	ΔK
S1	0.16 ± 0.01	4.64 ± 0.03	1.60 ± 0.04	0.11 ± 0.02	0
S2	0.141 ± 0.001	1.80 ± 0.01	1.51 ± 0.02	0.083 ± 0.001	0
S3	0.18 ± 0.01	2.353 ± 0.001	1.42 ± 0.01	0.090 ± 0.001	0
S4	0.479 ± 0.001	9.6 ± 0.6	2.28 ± 0.08	0.23 ± 0.02	0
S5	0.254 ± 0.001	9.8 ± 0.3	2.32 ± 0.04	0.27 ± 0.03	0
S6	0.35 ± 0.2	13.64 ± 0.04	1.98 ± 0.07	0.16 ± 0.04	0
S7	0.414 ± 0.008	13.5 ± 0.2	2.25 ± 0.04	0.16 ± 0.03	0
S8	0.27 ± 0.02	12.8 ± 0.6	2.43 ± 0.02	0.157 ± 0.006	0
S9	0.63 ± 0.02	28.1 ± 0.4	2.6 ± 0.1	0.36 ± 0.04	0.010 ± 0.001
S10	1.04 ± 0.01	29.3 ± 0.1	2.53 ± 0.05	0.25 ± 0.02	0.008 ± 0.001
S11	1.76 ± 0.07	30.0 ± 1.0	5.9 ± 0.4	0.5 ± 0.1	0.006 ± 0.001
legal limit	1.0	20	2.40	0.20	0.01

Table 3. Polyphenol Content of the Oils

oil	3′,4′-DHPEA (mg/kg of oil)	*p*-HPEA (mg/kg of oil)	3′,4′-DHPEA-EDA (mg/kg of oil)	3′,4′-DHPEA-EA (mg/kg of oil)
S1	nd[a]	2.1 ± 0.2	379 ± 19	115 ± 8
S2	0.6 ± 0.2	2.67 ± 0.06	515 ± 5	51.4 ± 0.7
S3	1.39 ± 0.01	1.61 ± 0.01	499.7 ± 0.01	48.7 ± 0.1
S4	14.4 ± 1.1	17.1 ± 0.7	78.4 ± 0.8	123 ± 6
S5	35.35 ± 0.08	57 ± 1	40.9 ± 0.7	21.3 ± 0.4
S6	7.864 ± 0.005	11.0 ± 0.2	63.6 ± 9.8	24.5 ± 0.5
S7	8.3 ± 0.7	45.1 ± 0.9	17 ± 1	33.6 ± 1.5
S8	5.0 ± 0.7	6.0 ± 0.3	68.9 ± 3.5	29.8 ± 3.5
S9	7.8 ± 0.2	19.4 ± 0.2	9.0 ± 0.2	73.2 ± 0.4
S10	9.5 ± 0.1	20.64 ± 0.02	17.2 ± 1.8	61.0 ± 1.2
S11	5.42 ± 0.06	6.79 ± 0.08	12 ± 2	31.2 ± 0.7

[a] nd, not detectable.

Table 4. Antioxidant Actvity of the Oils

oil	DPPH (I_{50}, mg of oil)	XOD/xanthine (I_{30}, mg of oil)	DIA/NADH/juglone (I_{50}, mg of oil)
S1	32.0 ± 0.5	10.5 ± 1.8	9.93 ± 0.29
S2	30.60 ± 0.06	8.25 ± 0.48	13 ± 1
S3	27.9 ± 0.01	9.81 ± 0.29	20 ± 1
S4	51.3 ± 0.2	22.45 ± 1.70	14.53 ± 0.79
S5	51 ± 1	17.44 ± 0.91	15.3 ± 1.8
S6	74.5 ± 2.5	23.6 ± 2.9	14.15 ± 0.55
S7	91 ± 7	28 ± 4.2	34.35 ± 3.47
S8	87 ± 5	23.31 ± 3.62	32.3 ± 2.9
S9	94 ± 6	23.79 ± 4.00	nd[a]
S10	129 ± 1	17 ± 1.9	nd
S11	156 ± 1	nd	nd

[a] nd, not detectable.

not show any apparent relationship with the degradation degree of the sample.

In general, EVOOs having a low degradation level had a higher total phenol content than degraded EVOOs, and the most degraded oil had a very low phenol content. Therefore, it seems that the increase in 3′,4′-DHPEA concentration during storage of EVOOs did not balance the loss in 3′,4′-DHPEA-EDA content.

As a preliminary approach, the antioxidant activity of EVOOs was studied as their ability to reduce the DPPH radical (**Table 4**). In a previous study, Visioli et al. (*2*) found that both 3′,4′-DHPEA and its derivative oleuropein exhibit a strong radical-scavenging activity on DPPH. We found that all oils containing the lipophilic derivatives of 3′,4′-DHPEA were able to scavenge the DPPH radical. In particular, the antioxidant activity appeared to be related to the degradation level of oils. In fact, fresh oils were 3−5 times more efficient than aged oils, whereas the commercial oils had intermediate efficacy.

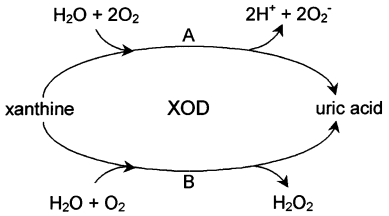

Figure 1. Scheme illustrating the possible routes of XOD-mediated xanthine oxidation by O_2.

The antioxidant activity of EVOOs was then studied using two model systems, which include XOD and DIA as ROS generators. Both of these enzymes are known to catalyze oxidative processes in vivo, producing ROS that may lead to tissue damage. In particular, XOD is involved in the oxidative damage occurring after reperfusion of ischemic tissues, in brain edema and injury, and in vascular permeability changes, and DIA is involved in damage due to quinoid xenobiotics (*23*−*25*).

XOD is present in several types of cells as a dehydrogenase enzyme that oxidizes xanthine or hypoxanthine to uric acid. Under certain conditions, the dehydrogenase is converted to an oxidase enzyme. Upon this conversion the enzyme reacts with the same electron donors, but in the reoxidizing steps it reduces oxygen instead of NAD^+, thus producing superoxide or hydrogen peroxide. **Figure 1** depicts the two possible routes for the reaction of XOD with O_2. The level of enzyme reduction determines whether hydrogen peroxide or the superoxide anion is produced (*26*). The oxidase reaction catalyzed by XOD can be followed by using KMB, which reacts with hydrogen peroxide and the superoxide anion producing ethene. Antioxidant compounds inhibit ethene formation from KMB by scavenging hydrogen peroxide and the superoxide anion. Some phenolic compounds were reported also to inhibit directly the enzymatic activity of XOD (*24*). The XOD-catalyzed reaction has been used to study the properties of plant extracts used in phytotherapy and the properties of tomato derivatives (*20*, *24*). Furthermore, it has been found that cell damage due to the XOD-catalyzed reaction can be prevented by both 3′,4′-DHPEA and oleuropein (*2*, *5*).

In the present work we found that the phenolic fraction of EVOOs was also able to inhibit ROS formation by XOD in vitro. As reported in **Table 4**, the inhibitory effect of EVOOs having a low degradation level was twice that of oils having intermediate and advanced degradation levels. The most degraded oil did not show any antioxidant activity in this system.

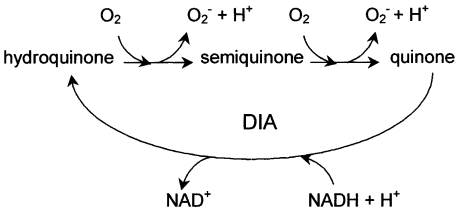

Figure 2. Scheme illustrating the DIA-mediated two-electron reduction of a quinonoid compound, followed by nonenzymatic one-electron hydroquinone reoxidation by O_2.

DIA [NAD(P)H:quinone oxidoreductase] is another flavoenzyme present in most animal tissues and involved in a number of electron-transfer processes. It also catalyzes the two-electron reduction of quinones to hydroquinones, allowing their disposal from the cells after conjugation of hydroquinones with glucuronide or sulfate. In analogy to other enzymatic systems involved in xenobiotic detoxification, DIA can also indirectly contribute to ROS production. In fact, as shown in **Figure 2**, the hydroquinones formed by DIA can autoxidize with transfer of an electron to molecular oxygen with formation of a superoxide radical and of semiquinone radical. The latter can further oxidize with the transfer of an electron to molecular oxygen with the formation of superoxide radical and quinone, which in turn can be reduced again by DIA, thereby closing a cycle of enzyme reduction/oxidation yielding ROS. Such a mechanism is believed to explain the toxicity of quinoid compounds (*25*). The reaction catalyzed by DIA can be followed using KMB as an oxidation target, which produces ethene. As discussed above for the XOD/xanthine system, inhibition of ethene formation occurring in the presence of antioxidants can be attributed either to the scavenging of superoxide anions or to the direct inhibition of DIA (*24*). The DIA-catalyzed reaction has been used to study the properties of plants extracts used in phytotherapy (*24*), but it has never been applied for studying olive oil phenolics.

By using the DIA-catalyzed reaction in vitro, we found that oils with a high degradation level were clearly distinguishable from the other samples, as they did not show an antioxidant activity (**Table 4**). However, EVOOs with low and intermediate degradation levels could not be distinguished.

According to Ninfali et al. (*27*) the antioxidant activity of methanol extracts of EVOO, measured as the ability to scavenge peroxyl radicals generated with 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), was correlated to the total phenolic content. Investigation of the antioxidant activity of pure compounds showed that EVOO phenolic compounds which share the *o*-diphenolic structures, such as 3′,4′-DHPEA and its derivatives, are mainly responsible for EVOO antioxidant activity, rather than p-HPEA and its derivatives. In fact, Baldioli et al. (*11*) found that the antioxidant activity of olive oil, as measured by the Rancimat test, correlated mainly with the oleosidic forms of 3′,4′-DHPEA. Along the same lines, Visioli et al. (*2*) found that oleuropein and 3′,4′-DHPEA (but not *p*-HPEA) had high antioxidant activity toward ROS. Synthetic lipid-soluble derivatives of 3′,4′-DHPEA have also been considered for their antioxidant activity (*22*, *28*).

In the present work we studied the relationship between the sum of 3′,4′-DHPEA derivatives present in the methanolic or in the acetone/water extracts of EVOOs and their antioxidant activity. By extending our approach to 22 EVOOs it was found that the total amount of 3′,4′-DHPEA derivatives correlated with the antioxidant activity as measured by the XOD/xanthine (squared correlation coefficient = 0.799) and DPPH methods (squared correlation coefficient = 0.866) (**Figure 3**).

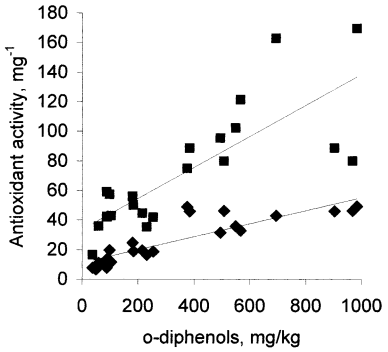

Figure 3. Relationship between the total content of 3′,4′-DHPEA derivatives and the antioxidant activity of EVOOs as measured by the XOD/xanthine ($1/I_{30}$, ■) and the DPPH ($1/i_{50}$, ◆) methods ($n = 22$).

On the basis of these correlations, the *o*-diphenol family can be identified as the main source of the overall antioxidant activity of EVOOs. However, because of the different antioxidant activities of the individual *o*-diphenol compounds present in oil extracts and of possible synergistic effects among individual molecules, a better correlation cannot be established. A poor correlation was found between the total amount of 3′,4′-DHPEA derivatives and the antioxidant activity measured by the DIA/NADH/juglone system (not shown). The lack of correlation in this latter system could be due to the presence in the extract of a DIA inhibitor of unknown nature.

In the hypothesis that the measurements carried out with the purified systems used in our study might be related to the antioxidant activity of EVOO in vivo, our results suggest that EVOOs have a different potential benefit for human health.

Whatever the measuring system, high levels of oil degradation lead to a loss of antioxidant activity. On the basis of the results presented here, three indices could be proposed to differentiate EVOOs, namely, (1) the 3′,4′-DHPEA-EDA content, (2) the antioxidant activity measured by DPPH reduction, and (3) their ability to inhibit ROS formation in the XOD/xanthine system.

According to the indices proposed above, EVOOs could be further differentiated with respect to the oxidation and hydrolysis indices already indicated by the EU Regulation. In particular, the proposed indices evidence a level of quality, which could correspond to “excellent”. In fact, we found that oils which can be labeled as “extra virgin” according to the present EU Regulation may show 20-fold differences in their 3,4-DHPEA-EDA contents and 2−3-fold differences in their antioxidant activities.

ABBREVIATIONS USED

3′,4′-DHPEA, 3′,4′-dihydroxyphenylethanol; 3′,4′-DHPEA-EDA, dialdehydic form of elenolic acid linked to 3′,4′-DHPEA; 3′,4′-DHPEA-EA, isomer of oleuropein aglycon; DIA, diaphorase; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; EVOO, extra virgin olive oil; *p*-HPEA, *p*-hydroxyphenylethanol; ROS, reactive oxygen species; XOD, xanthine oxidase.

LITERATURE CITED

(1) Panizzi, L.; Scarpati, M. L.; Oriente, E. G. Structure of the bitter glucoside oleuropein. Note II. *Gazz. Chim. Ital*. **1960**, *90*, 1449−1485.

(2) Visioli, F.; Bellomo, G.; Galli, C. Free radical scavenging properties of olive oil polyphenols. *Biochem. Biophys. Res. Commun*. **1998**, *247*, 60−64.

(3) Aruoma, O. I.; Deiana, M.; Jenner, A.; Halliwell, B.; Kaur, H.; Banni, S.; Corongiu, F. P.; Assunta-Dessi, M.; Aeschbach, R. Effect of hydroxytyrosol found in extravirgin olive oil on oxidative DNA damage and low-density lipoprotein oxidation. *J. Agric. Food Chem*. **1998**, *46*, 5181−5187.

(4) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem*. **2001**, *49*, 2532−2538.

(5) Manna, C.; Galletti, P.; Cucciolla, V.; Moltedo, O.; Leone, A.; Zappia, V. The protective effect of olive oil polyphenol (3,4-dihydroxyphenyl)ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *J. Nutr*. **1997**, *127*, 286−292.

(6) Manna, C.; Galletti, P.; Cucciolla, V.; Montedoro, G.; Zappia, V. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. *J. Nutr. Biochem*. **1999**, *10*, 159−165.

(7) Della Ragione, F.; Cucciolla, V.; Borriello, A.; Della Pietra, V.; Pontoni, G.; Racioppi, L.; Manna, C.; Galletti, P.; Zappia, V. Hydroxytyrosol, a natural molecule occurring in olive oil, induces cytochrome *c*-dependent apoptosis. *Biochem. Biophys. Res. Commun*. **2000**, *278*, 733−739.

(8) Coni, E.; Di Benedetto, R.; Di Pasquale, M.; Masella, R.; Modesti, D.; Mattei, R.; Carlini, E. A. Protective effect of oleuropein, an olive oil biophenol, on low-density lipoprotein oxidizability in rabbits. *Lipids* **2000**, *35*, 45−54.

(9) Visioli, F.; Caruso, D.; Plasmati, E.; Patelli, R.; Mulinacci, N.; Romani, A.; Galli, G.; Galli, C. Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Res*. **2001**, *34*, 301−305.

(10) Limirioli, R.; Consonni, R.; Ranalli, A.; Bianchi, G.; Zetta, L. ^{1}H NMR study of phenolics in the vegetation water of three cultivars of *Olea europaea*: similarities and differences. *J. Agric. Food Chem*. **1996**, *44*, 2040−2048.

(11) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(12) Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable compounds in virgin olive oil. 3. Spectroscopic characterization of the secoiridoid derivatives. *J. Agric. Food Chem*. **1993**, *41*, 2228−2234.

(13) Vierhuis, E.; Servili, M.; Baldioli, M.; Schols, H. A.; Voragen, A. G. J.; Montedoro, G. F. Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. *J. Agric. Food Chem*. **2001**, *49*, 1218−1223.

(14) Tsimidou, M. Polyphenols and quality of virgin olive oil in retrospect. *Ital. J. Food Sci*. **1998**, *10*, 99−116.

(15) Pagliarini, E.; Zanoni, B.; Giovanelli, G. Predictive study on Tuscan EVOO stability under several commercial conditions. *J. Agric. Food Chem*. **2000**, *48*, 1345−1351.

(16) Regulation 2568/91. *Off. J. Eur. Communities* **1991**, July 11.

(17) Cortesi, N.; Azzolini, M.; Rovellini, P. Dosaggio dei componenti minori polari (CMP) in oli vergini di oliva. *Riv. Ital. Sostanze Grasse* **1995**, *72*, 325−329.

(18) Limirioli, R.; Consonni, R.; Ottolina, G.; Marsilio, V.; Bianchi, G.; Zetta, L. ^{1}H and ^{13}C NMR characterization of new oleuropein aglycones. *J. Chem. Soc., Perkin Trans.* **1995**, *1*, 1519−1523.

(19) Paiva-Martins, F.; Gordon, M. H. Isolation and characterization of the antioxidant component 3,4-dihydroxyphenylethyl 4-formyl-methyl-4-hexenoate from *olive* (*Olea europaea*) leaves. *J. Agric. Food Chem.* **2001**, *49*, 4214−4219.

(20) Lavelli, V.; Peri, C.; Rizzolo, A. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase and copper-induced lipid peroxidation. *J. Agric. Food Chem.* **2000**, *48*, 1442−1448.

(21) Albrecht-Goepfert, E.; Schempp, H.; Elstner, E. Modulation of the production of reactive oxygen species by pre-activated neutrophils by aminoadamantane derivatives. *Biochem. Pharmacol*. **1998**, *56*, 141−152.

(22) Gordon, M. H.; Paiva-Martins, F.; Almeida, M. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *J. Agric. Food Chem.* **2001**, *49*, 2480−2485.

(23) Halliwell, B.; Gutteridge, J. M. C. Role of free radicals and catalytic metal ions in human disease. *Methods Enzymol*. **1990**, *186*, 1−85.

(24) Meyer, B.; Schneider, W.; Elstner, E. F. Antioxidative properties of alcoholic extracts from *Fraxinus excelsior*, *Populus tremula* and *Solidago virgaurea*. *Arzneim.-Forsch./Drug Res*. **1995**, *45*, 174−176.

(25) Buffinton, G. D.; Ollinger, K.; Brunmark, A.; Cadenas, E. DT-diaphorase-catalysed reduction of 1,4-naphthoquinone derivatives and glutathionyl-quinone conjugates. *Biochem. J.* **1989**, *257*, 561−571.

(26) Xia, M.; Dempski, R.; Hille, R. The reductive half-reaction of xanthine oxidase. *J. Biol. Chem.* **1999**, *274*, 3323−3330.

(27) Ninfali, P.; Aluigi, G.; Bacchiocca, M.; Magnani, M. Antioxidant capacity of extra-virgin olive oils. *J. Am. Oil Chem. Soc.* **2001**, *78*, 243−247.

(28) Briante, R.; La Cara, F.; Tonziello, M. P.; Febbraio, F.; Nucci, R. Antioxidant activity of the main bioactive derivatives from oleuropein hydrolysis by hyperthermophilic β-glycosidase. *J. Agric. Food Chem.* **2001**, *49*, 3198−3203.

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