Antioxidants and Total Peroxyl Radical-Trapping Ability of Olive and Seed Oils

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The presence of the oxidized and reduced forms of ubiquinones Q_9 and Q_{10} was determined in commercial extra virgin olive and seed oils, where the amounts of α- and γ-tocopherols and β-carotene were also quantitated. Very high concentrations of ubiquinones were found in soybean and corn oils. Furthermore, the total antioxidant capability of each oil was evaluated by measuring total radical-trapping antioxidant parameters (TRAP) in *tert*-butyl alcohol and using egg lecithin as the oxidizable substrate. These values decreased in the order sunflower > corn > peanut > olive; the highest TRAP, which was found in sunflower oil, was related to the very high amount of α-tocopherol. Olive oil, because of the low content of α-tocopherol, exhibited a TRAP value approximately one-third that of sunflower oil. TRAP values of corn and soybean oils, in which low amounts of α-tocopherol but very high contents of γ-tocopherol and reduced ubiquinones were present, were intermediate. γ-Tocopherol exhibited a poor ability of trapping peroxyl radicals in *tert*-butyl alcohol. This behavior was probably due to the effects of the solvent on the rate of hydrogen abstraction from this phenol.

Keywords: *Antioxidants; oils; ubiquinones; tocopherols; phenolic compounds; TRAP*

INTRODUCTION

Epidemiological and biochemical evidence (*1*) indicates that the oxidative status of biological tissues can be influenced by dietary components. Natural antioxidants occurring in foods as lipophilic tocopherols, carotenes, and ubiquinones, as well as the hydrophilic ascorbic and phenolic compounds, are able, with different mechanisms and effects, to scavenge free radicals that lead to autoxidation of cellular lipids and to a variety of pathological conditions. It has been suggested that the intake of antioxidant vitamins, including α-tocopherol, β-carotene, and ascorbic acid, should be increased when the diet contains a high amount of polyunsaturated fatty acids (*2*, *3*) and that an optimal plasma status of the principal dietary antioxidants or perhaps the overall antioxidant potential is a prerequisite of optimal health (*4*). Although the biological significance of essential antioxidant nutrients cannot be neglected, numerous other non-essential antioxidants are consumed daily, often in amounts far exceeding nutrient antioxidants. It has been recently demonstrated that plant phenolic compounds such as flavonoids, simple phenols, and phenolic acids exhibit strong antioxidant activity in vitro (*5*, *6*), and suggested that they may also have a significant role in controlling oxidative reactions in vivo (*7*, *8*). Moreover, in many reports concerning the beneficial effects of ubiquinone-10 (Q_{10}) administration in a wide variety of pathological conditions (*9*), the role of ubiquinone as an antioxidant was considered and supporting evidence was produced (*10*). As far as the presence of ubiquinones in plant seeds is concerned, an extensive survey has not been undertaken so far. As known, the isoprenoid side-chain of these compounds can be of different lengths, varying between 6 and 10 isoprene units or more, depending on the organism (*11*). Data reported by the various authors concerning the Q content in oleaginous seeds and related oils are hardly comparable (*12*). Kamei et al. (*13*), investigating the distribution of ubiquinone homologues in food, measured the content of oxidized Q_9 and Q_{10} in various fuels. Q_9 was detected in cereals, some vegetables, and their products. In particular, corn oil and wheat germ have large amounts of Q_9.

In this paper we determined the contents of the oxidized and reduced forms of Q_9 and Q_{10}, and the amounts of α- and γ-tocopherols and β-carotene, in commercial extra virgin olive oil and different seed oils obtained from a local oil mill. Furthermore, the presence of phenolic compounds, such as tyrosol, hydroxytyrosol, and hydroxycinnamic and hydroxybenzoic acid derivatives, was quantitated in the polar fraction obtained from virgin olive oil. Finally, we evaluated the total antioxidant capability of the most-used edible oils by measuring the total radical-trapping antioxidant parameter (TRAP) (*14*, *15*).

MATERIALS AND METHODS

Chemicals. Extra virgin olive and seed (sunflower, soybean, peanut, and corn) oils, produced in the same month, were taken from a local oil mill and kept at 5−10 °C in the dark. Four samples of each oil from the same industrial preparation were analyzed and each determination was performed in

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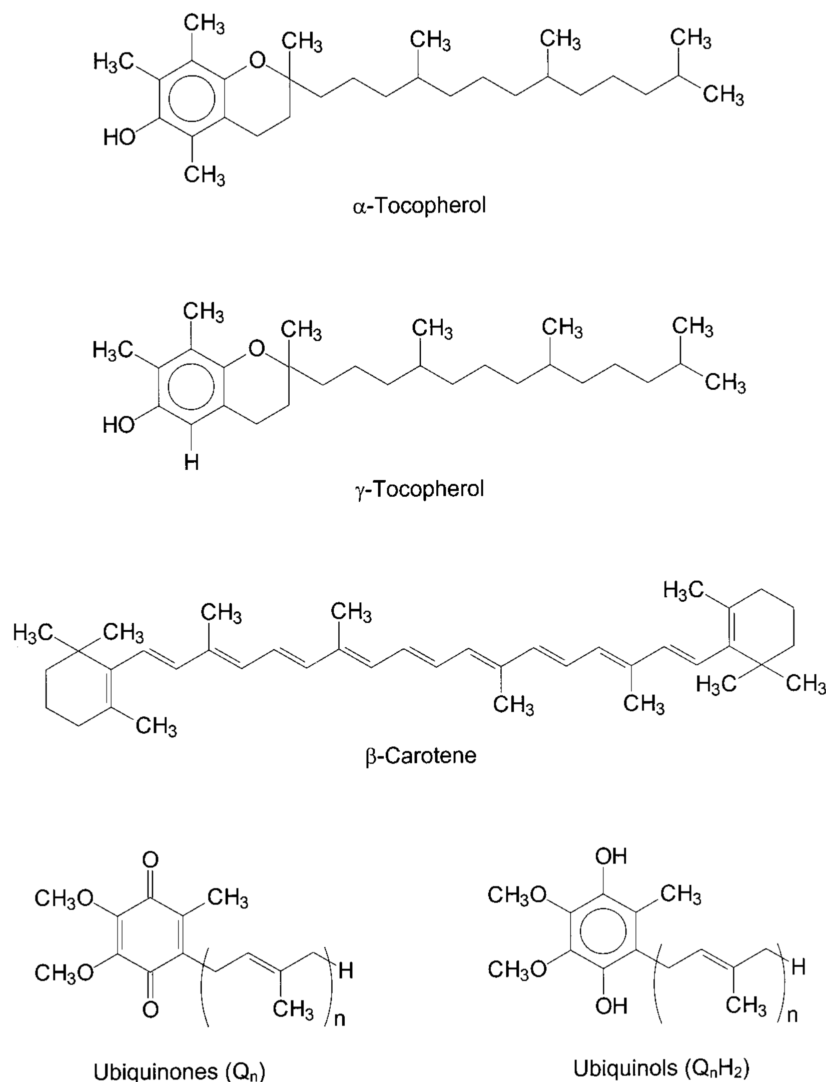
Scheme 1. Structures of α- and γ-Tocopherols, β-Carotene, and Oxidized and Reduced Form of Ubiquinones



duplicate. Egg yolk lecithin (PC) was purchased from Lipid Products (Redhill, UK). The thermolabile azo compound 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) was from Polyscience Inc. (Warrington, PA). α-Tocopherol (α-T), γ-tocopherol (γ-T), β-carotene, protocatechuic, vanillic, *p*-coumaric, and 3,4-dihydroxyphenylacetic acids were obtained from Sigma. Caffeic, 4-hydroxybenzoic, 4-hydroxyphenylacetic, and syringic acids were from Fluka, and tyrosol was obtained from Aldrich. Ubiquinone-9 (Q_9) and ubiquinone-10 (Q_{10}) were kindly supplied by Eisai Co. (Tokyo, Japan). Other reagents and solvents were of analytical or HPLC grade from Merck and J. T. Baker and used without further purification.

Fatty Acid Composition of Oils. The fatty acid composition of the oils tested was determined after transmethylation by gas chromatography using a Hewlett-Packard 5890 Plus Series II GC equipped with a capillary column (HP−FFAP 25 m × 0.32 mm i.d. × 0.52 μm film) at a programmed temperature (180−240 °C).

Reduction of Ubiquinones. Q_9 and Q_{10} were quantitatively reduced according to the methods of Rieske (*16*) and kept in absolute ethanol under slight acidic conditions at concentrations between 5 and 10 mM.

Determination of Oxidized and Reduced Ubiquinones and Tocopherols. Oil samples (0.1 mL) were dissolved in 9.9 mL of 2-propanol, and the mixture was centrifuged. α-Tocopherol, γ-tocopherol, and reduced and oxidized Q_9 and Q_{10} were determined by HPLC with electrochemical detection (detector Coulochem II with conditioning cell 5021 and analytical cell 5011), equipped with an Ultrasphere XL ODS column (75 mm × 4.6 mm i.d. × 3 μm particle size, Beckman). The elution was isocratic at a flow rate of 2 mL/min with glacial acetic acid/2-propanol/methanol (0.3:11.6:88.1, v/v/v) containing 10.3 mM sodium acetate. Conditioning cell was set at −600 mV, detector I at −150 mV, and detector II at +450 mV (*17*).

β-Carotene Determination. β-Carotene content of oil samples, previously subjected to saponification, was determined by normal-phase HPLC with a UV−Vis detector on a silica gel column (LiChrosorb Si-60; 250 mm × 4 mm i.d. × 5 μm particle size, Merck) equipped with a Guard-PAK Resolve Si precolumn (Waters Associates). The elution was isocratic at a flow rate of 1.0 mL/min with a mixture of 0.8% 2-propanol in *n*-hexane, according to the method developed by Vuilleumier et al. (*18*).

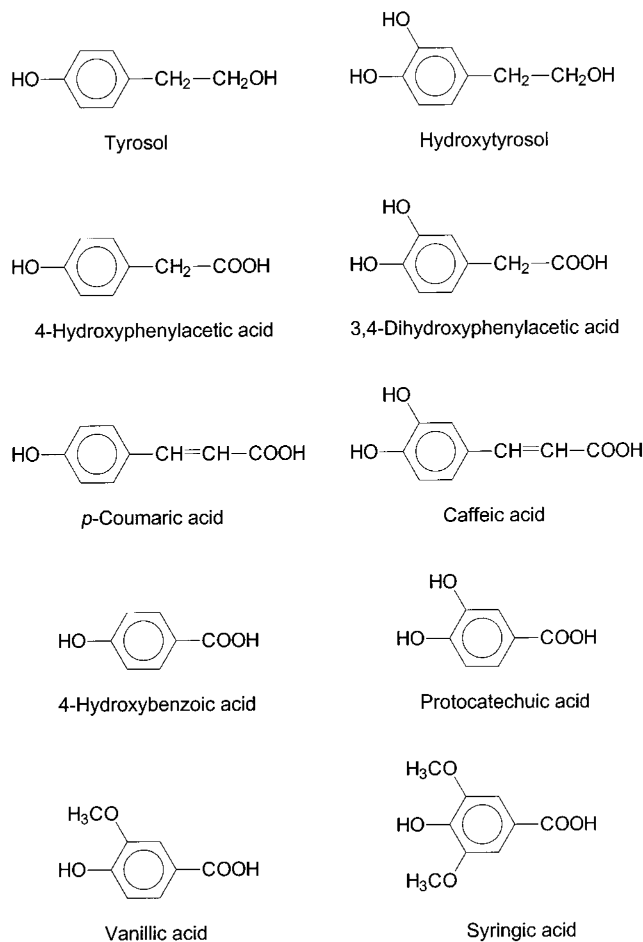
Determination of Phenolic Compounds. The polar fraction of virgin oil containing phenols was obtained by dissolving 50 g of oil in 50 mL of *n*-hexane and extracting three times with 30 mL of a methanol/water mixture (60:40, v/v) (*19*). The combined extracts were evaporated to dryness in a rotary evaporator and then added with 20 mL of *n*-hexane and 20 mL of acetonitrile. The acetonitrile layer was evaporated, and the remainder was dissolved in 1 mL of methanol containing 2 μL of glacial acetic acid. The phenolic compounds were determined by reversed-phase HPLC using a RP 18 LiChrospher 100 (Merck) column. The gradient elution used was a modification of a previously published method by Tsimidou et al. (*20*). The effluent was monitored using a

Table 1. Contents of Reduced (QH_2) and Oxidized Ubiquinones (Q) in Commercial Extra Virgin Olive and Seed Oils (mg/L)[a]

oil	Q_9H_2	$Q_{10}H_2$	total QH_2	Q_9	Q_{10}	total Q
extra virgin olive	−−	11 ± 1	11 ± 1	18 ± 2	94 ± 4	109 ± 4
peanut	4.0 ± 0.6	62 ± 8	67 ± 7	1.6 ± 0.8	9 ± 1	10 ± 2
soybean	12 ± 2	189 ± 34	201 ± 36	0.7 ± 0.1	68 ± 9	69 ± 9
corn	322 ± 31	96 ± 6	417 ± 29	51 ± 10	32 ± 2	83 ± 12
sunflower	41 ± 2	13 ± 1	55 ± 3	4.4 ± 0.7	1.4 ± 0.2	5.8 ± 1

[a] Data represent the means ± SD of four different samples.

Scheme 2. Structures of the Phenolic Compounds Quantitated in the Polar Fraction Obtained from Virgin Olive Oil



variable-wavelength UV detector set at 260 nm (procatechuic acid), 275 nm (tyrosol; 4-hydroxybenzoic, 4-hydroxyphenylacetic, vanillic, caffeic, and syringic acids), 280 nm (hydroxytyrosol and 3,4-dihydroxyphenylacetic acid), and 310 nm (*p*-coumaric acid). The concentrations of phenolics were determined from standard curves previously performed.

Total Radical-Trapping Antioxidant Parameter (TRAP). In oil samples, subjected to peroxyl radical attack by the lipophilic azo compound AMVN, the total antioxidant capacity of the oil was determined by oxygen consumption with a Clark-type electrode (Yellow Springs, OH). TRAP of a given sample was determined by measuring the length of time that oxygen uptake was inhibited (the induction period, τ) (*14*, *15*). The reaction mixture contained, in 3 mL of *tert*-butyl alcohol, 17 mM egg lecithin, 20−100 μL of oil or 15 μM α-T or 15 μM $Q_{10}H_2$, and 5 mM AMVN. The reaction vessel was stirred at 45 °C and maintained in the dark.

Statistical Analysis. All values are expressed as means ± standard deviations. Statistical differences were determined by one-way ANOVA analysis of variance and the Student−Newman−Keuls test, and were considered significantly different at $P < 0.05$.

Table 2. Contents of α- and γ-tocopherols and β-carotene in Commercial Extra Virgin Olive and Seed Oils (mg/L)[a]

oil	α-tocopherol	γ-tocopherol	β-carotene
extra virgin olive	188 ± 14	26 ± 2.5	6.7 ± 1.8
peanut	174 ± 25	337 ± 49	0.13 ± 0.02
soybean	89 ± 7.4	1630 ± 118	0.28 ± 0.09
corn	225 ± 19	1310 ± 110	1.2 ± 0.24
sunflower	578 ± 54	93 ± 10	2.9 ± 0.6

[a] Data represent the means ± SD of four different samples.

RESULTS

Our first aim was to update the knowledge about the content of natural lipophilic antioxidants, in particular Q_9 and Q_{10}, in widely used foods such as dietary oils. As shown in Table 1 the contents of reduced and oxidized Q_9 and Q_{10} in the edible oils examined are quite different. For the sake of concision and clarity we also report the amounts of both the reduced and oxidized two homologues considered as a sum. This simplification is based on the fact that the antioxidant effect of the reduced form of these molecules (Q_9H_2 and $Q_{10}H_2$) is independent of the length of the isoprenoid side-chain and has to be ascribed only to the substituted quinol moiety (*21*, *22*). Therefore, the antioxidant effectiveness of Q_9H_2 and $Q_{10}H_2$ can be considered almost the same. The maximum amount of ubiquinols was found in corn oil, and the lowest amount was in olive oil, where the highest concentration of ubiquinones was present. Ubiquinol concentrations in the oils tested decreased following this order: corn ≫ soybean ≫ peanut ≈ sunflower > olive ($P < 0.05$).

Data reported in Table 2 confirm that the contents of α- and γ-tocopherols and β-carotene in extra virgin olive and seed oils are very different. In sunflower oil α-T was present in the highest amount of all the oils, whereas the content of γ-T was very low with respect to that found in the other seed oils. On the other hand, very high concentrations of the γ-T were found in soybean and corn oils. In peanut and olive oils similar concentrations of α-T were present, but the former contained a higher amount of γ-T than the latter.

Extra virgin olive oil had the highest β-carotene content, whereas β-carotene concentration was particularly low in soybean and peanut oils.

In contrast to other vegetable oils, extra virgin olive oil is consumed unrefined. Thus, it contains polyphenols that are usually removed from other edible oils in the various refining stages. The concentration and relative proportion of this so-called “polar fraction” containing phenolic compounds differ widely among varieties and location. This fraction contributes to the stability of the olive oil and provides its typical taste and aroma (*23*). Among the phenols determined, tyrosol was present in the highest concentration, i.e., 40 mg/kg (Table 3) according to the data reported in the literature (*24*, *25*). However, this compound appears to contribute very little, if any, to the stability of olive oil (*26*, *27*).

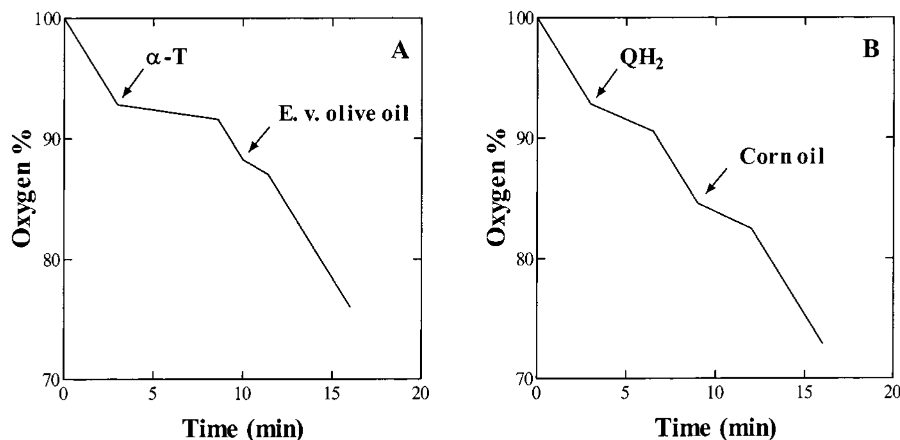


Figure 1. Oxygen uptake during autoxidation of 17 mM PC in *tert*-butyl alcohol induced by 5 mM AMVN at 45 °C. Panel A: effect of 15 μM α-T and 20 μL of extra virgin olive oil. Panel B: effect of 15 μM $Q_{10}H_2$ and 20 μL of corn oil.

Table 3. Phenolic Compounds Detected in Extra Virgin Olive Oil (mg/kg)[a]

tyrosol	40.15 ± 1.73
hydroxytyrosol	3.75 ± 0.09
protocatechuic acid	0.22 ± 0.02
3,4-dihydroxy-phenylacetic acid	4.37 ± 0.09
4-hydroxy-benzoic acid	1.16 ± 0.08
4-hydroxy-phenylacetic acid	1.56 ± 0.08
vanillic acid	0.88 ± 0.03
caffeic acid	trace
syringic acid	0.60 ± 0.04
p-coumaric acid	0.30 ± 0.03

[a] Data represent the means ± SD of four different samples.

The amounts of the other phenolic compounds tested were one order (hydroxytyrosol, 3,4-dihydroxyphenilacetic, 4-hydroxyphenilacetic, and 4-hydroxybenzoic acids) and two orders (protocatechuic, vanillic, syringic, and *p*-coumaric acids) of magnitude lower than tyrosol. Caffeic acid, known to exhibit a potent antioxidant effect (*28*), was present only in traces.

Finally, we determined the TRAP value of each oil. Egg yolk lecithin (PC), whose fatty acid composition is similar to that of membrane phospholipids, was used as the substrate to be oxidized. The total peroxyl radical-trapping capacity of oils was investigated by using a 17 mM *tert*-butanol solution of PC as a common target of free radicals generated by the lipid-soluble azo compound AMVN (5 mM), and adding an aliquot of each oil sample. The polarographic measure of the inhibited phase of the lipid peroxidation, the induction period (τ), in the presence of each oil was used as the basis for quantifying the results. This induction period was measured and compared to the τ in the presence of a known amount of α-T (15 μM). The lag-phase was taken to be directly related, under these experimental conditions, to the total antioxidant capacity of oils: in fact, by increasing the amounts of oil or α-T, the length of τ was proportional to the amount added (not shown).

Figure 1 shows the traces of oxygen uptake during PC oxidation in *tert*-butyl alcohol initiated by AMVN at 45 °C in the absence of antioxidants and in the presence of either 15 μM α-T and 15 μM $Q_{10}H_2$ or extra virgin olive and corn oils. As expected, when all α-T (Figure 1A) or $Q_{10}H_2$ (Figure 1B) was depleted, the induction time was over and a fast oxidation took place as in the absence of antioxidants. In Figure 1 it is also shown that the addition of 20 μL of extra virgin olive and corn oils protected PC from autoxidation for about

Table 4. TRAP Values (μM) in Olive and Seed Oils[a]

oil	$TRAP_{exp}$	$TRAP_{calc}$
extra virgin olive	1105 ± 115[a]	1024 ± 105
peanut	1952 ± 207[b]	2543 ± 261
soybean	2255 ± 250[b,c]	8552 ± 883
corn	2455 ± 257[c]	8135 ± 854
sunflower	2928 ± 331[d]	3139 ± 320

[a] Data represent the means ± SD of four different samples. Significant differences ($P < 0.05$) were determined by the Student–Newman–Keuls test after ANOVA. Numbers with different superscript letters are significantly different.

2 and 4 min, respectively. It is noteworthy that the rate of PC oxidation after the inhibition period produced by oil addition is similar to that in its absence. This means that the addition of a small aliquot of oil does not influence the rate of PC oxidation. The TRAP values of the tested oil experimentally obtained ($TRAP_{exp}$) were calculated according to the following equation (*14, 15*):

$$TRAP_{exp} = \frac{2[\alpha\text{-T}] \times \tau_{oil} \times V_{TOT}}{\tau_{\alpha-T} \times V_{oil}} \qquad (1)$$

where V_{TOT} is the volume of solution, V_{oil} is the volume of oil used, and [α-T] is the final concentration of α-T in the solution. The factor of 2 represents the number of peroxyl radicals trapped by each α-T molecule, i.e., the stoichiometric factor.

In Table 4 $TRAP_{exp}$ values are expressed as the μmoles of peroxyl radicals trapped per liter of oil. It can be seen that the antioxidant capacity varies considerably from one kind of oil to another, as reported by Wang et al. (*29*) for fruits and by Cao et al. (*30*) for vegetables. Sunflower oil had the highest antioxidant activity against peroxyl radicals among the oils examined.

$TRAP_{exp}$ increased in the order olive < peanut < corn < sunflower. The TRAP value of soybean was intermediate between that of peanut and corn oils. This pattern was approximately in accordance with the unsaturation index of fatty acid residues of oil triglycerides that increased approximately according to the same order (Table 5).

In addition to the direct measurement of $TRAP_{exp}$, a calculated TRAP ($TRAP_{calc}$) was also derived from the concentration of individual peroxyl radical-trapping antioxidants in the oil and the corresponding stoichiometric factors reported in the literature, i.e., 2 for α-T as well as for γ-T, and 1.5 for Q_9H_2 and $Q_{10}H_2$, previ-

Table 5. Major Fatty Acid Composition of Extra Virgin Olive and Seed Oils[a]

	fatty acid (mol %)					unsaturation index
	16:0	18:0	18:1	18:2n6	18:3n3	
extra virgin olive	12.56 ± 0.34	2.26 ± 0.06	78.02 ± 1.37	7.18 ± 0.30	0.45 ± 0.01	94.37 ± 1.77
peanut	12.22 ± 0.82	2.65 ± 0.05	51.55 ± 0.78	31.54 ± 1.35	0.27 ± 0.04	115.44 ± 2.17
soybean	11.72 ± 0.68	3.74 ± 0.05	21.33 ± 0.13	55.19 ± 0.90	7.50 ± 0.36	154.20 ± 1.39
corn	12.52 ± 0.40	1.45 ± 0.02	26.92 ± 0.25	58.63 ± 2.54	0.50 ± 0.02	145.68 ± 2.79
sunflower	6.63 ± 0.28	3.48 ± 0.04	30.07 ± 0.33	59.60 ± 1.40	0.32 ± 0.01	150.23 ± 1.73

[a] Data represent the means ± SD of four different samples. Unsaturation index is defined by $\Sigma m_i n_i$ where m_i is the percentage of and n_i is the number of double bonds, taking into account all fatty acids with two or more double bonds.

ously determined under similar experimental conditions (*22*, *31*).

$$TRAP_{calc} = 2[\alpha\text{-T}] + 2[\gamma\text{-T}] + 1.5[Q_9H_2] + 1.5[Q_{10}H_2] \quad (2)$$

In the case of soybean and corn oils a substantial difference was observed between the values of $TRAP_{exp}$ and $TRAP_{calc}$.

DISCUSSION

Edible oils that contain high amounts of lipophilic antioxidants are considered an important source of these compounds in human nutrition.

As far as we know, little information is available about the content of ubiquinones in edible oils. Data reported in the literature deal with the presence of the oxidized forms of these compounds (*11*, *13*). More recently, Pregnolato et al. (*17*) found substantial amounts of reduced Q_9 and Q_{10} in some edible oils.

As shown in Table 1 the highest amount of ubiquinols (Q_9H_2 + $Q_{10}H_2$) was found in corn oil, and the lowest amount was in olive oil, which, instead, contained the highest concentration of the oxidized quinones. With regard to the antioxidant activity of ubiquinones, initial results suggested that the oxidized form of ubiquinones also can behave as an antioxidant (*32*). However, subsequent experiments showed that the inhibition rate constant of oxidized ubiquinone was 2 orders of magnitude lower than that of α-T (*33*). Ubiquinols, instead, have been proved to perform as antioxidants in liposomes (*21*, *22*, *34*), submitochondrial particles (*35*), microsomes (*36*), and lipoproteins (*37*, *38*), suggesting that the direct antioxidant action can be one of its important physiological functions. Because ubiquinols are present in very low concentrations in extra virgin olive oil, they do not contribute to its antioxidant activity. On the other hand, oxidized Q, once absorbed, can be transformed into QH_2 by electron-transfer mechanisms present in mitochondria, microsomes, plasma membranes, and cytosol (*10*). Therefore, from a biochemical and nutritional point of view, the amount of oxidized Q in extra virgin olive oil has to be taken into consideration.

Table 2 shows that, as known, the contents of α- and γ-tocopherols in the edible oils examined are quite different. The highest amount of α-T was found in sunflower oil, whereas very high γ-T contents were present in soybean and corn oils. α-T is the most plentiful and the most biologically active of the four tocopherols, and one of the best chain-breaking phenolic antioxidants known. The results of Burton and Ingold (*39*) clearly showed that the order of antioxidant activity of the tocopherols is $\alpha > \beta \approx \gamma > \delta$. They found that α-T reacts more rapidly with peroxyl radicals than do the other tocopherols, i.e., the rate constants of inhibition are 3.2×10^6 M^{-1} s^{-1} and 1.4×10^6 M^{-1} s^{-1} in chlorobenzene for α-T and γ-T, respectively. This difference in the rate constants may in part explain the discrepancy between the $TRAP_{exp}$ values and the $TRAP_{calc}$ values of the soybean and corn oil samples containing very high amounts of γ-T (see later).

Contrasting results exist on the antioxidant effect of carotenes. The peroxyl radical-trapping activity of β-carotene and possibly other carotenoids is dependent on the partial pressure of oxygen applied (*40*). It is less efficient under conditions of air, but becomes a good peroxyl radical scavenger at the low pO_2 that prevails in biological tissues. For this reason, and because of its extremely low content in oils, the antioxidant activity of β-carotene has been neglected in this evaluation of $TRAP_{calc}$.

The biological properties of phenols extracted and purified from olive oils have been investigated (*27*, *41*). Oloeuropein and hydroxytyrosol, both sharing an *ortho*-diphenolic residue, but not tyrosol, present in the highest amount in the olive oil tested, were shown to exert potent antioxidant activity in LDL oxidation (*42*). Under the assay conditions used, the very small amounts of the diphenolic compounds present (Table 3) do not participate in the chain breaking antioxidant capacity of the oil; thus, they were not considered in the calculation of the TRAP value.

The TRAP method developed by Ingold and coworkers is based on the property of azo compounds to produce peroxyl radicals at a constant and temperature-dependent rate (*14*, *15*). Then, other methods, reviewed by Rice-Evans and Miller (*43*), have been developed for the measurement of the antioxidant activity of plasma and body fluids; they vary greatly as to the radical that is generated and the reproducibility of the generation process, but like the Ingold method are all essentially inhibition methods. However, it has to be pointed out that the measured antioxidant activity of a biological sample depends on which free radical or oxidant is used in the assay (*29*).

The choice of egg lecithin as the substrate to be oxidized implies that the information obtained from this model system may not be extrapolated directly to a food system, i.e., to the effects of oil antioxidants on the oxidative stability, but to the antioxidant capability of the oil once absorbed.

The $TRAP_{exp}$ values obtained can give information on the quantitative chain-breaking antioxidant capacity instead of measuring individual antioxidants separately. The antioxidant activity of sunflower oil ($TRAP_{exp}$ = 2928 μM), which was about 2.5 times the $TRAP_{exp}$ value of olive oil (1105 μM), can be related to the very high content of α-T (578 mg/L). The discrepancy observed in soybean and corn oils between the values of $TRAP_{exp}$ and $TRAP_{calc}$ can be ascribed to the very high amount of γ-T present in these oils.

γ-T reacts more slowly with peroxyl radicals than α-T does (*39*) and its ability of trapping peroxyl radicals can decrease in a hydrogen-bond-accepting solvent, such as *tert*-butyl alcohol. Actually, remarkable solvent effects on the rates of hydrogen abstraction from phenols have been reported in the literature (*44*, *45*). These very large kinetic solvent effects were attributed to hydrogen bond formation between the substrate (phenol) and a hydrogen-bond-accepting solvent. Intermolecular hydrogen bonding with the solvents prevents hydrogen atom abstraction, giving rise to a dramatic decrease in the observed rate constant. In particular, the rate constant for abstraction of the phenolic hydrogen atom of α-T by alkoxyl radicals decreases by a factor of about 60 upon changing the solvent from *n*-pentane to *tert*-butyl alcohol (*46*).

Under our experimental conditions, when 15 μM α-T is present in the reaction mixture, an induction period is still observed, indicating that the rate constant of inhibition is sufficiently high to completely prevent the oxidation of PC. On the other hand, γ-T, which reacts more slowly with peroxyl radicals than α-T does, does not completely prevent the oxidation of PC in *tert*-butanol. This possible explanation is confirmed by the observation that the addition of 15 μM γ-T to the reaction mixture does not give rise to an induction period but only to a decrease in the oxidation rate (data not shown).

ABBREVIATIONS USED

α-T, α-tocopherol; γ-T, γ-tocopherol; Q, ubiquinone; Q_9, ubiquinone-9; Q_{10}, ubiquinone-10; QH_2, ubiquinol; Q_9H_2, ubiquinol-9; $Q_{10}H_2$, ubiquinol-10; TRAP, total radical-trapping antioxidant parameter; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); PC, egg yolk lecithin; e. v. olive, extra virgin olive.

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