Peroxyl and Hydroxyl Radical Scavenging Activity of Some Natural Phenolic Antioxidants

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The autoxidation of linoleic acid dispersed in an aqueous media and the antioxidant effect of hydroxytyrosol, oleuropein, caffeic acid and tyrosol were studied. Linoleic acid autoxidation rate was estimated by the increase of conjugated diene level and by the decrease of linoleic acid content in the samples. The phenolic compounds exhibited an antioxidant activity which increased in the order: tyrosol < caffeic acid < oleuropein < hydroxytyrosol. The analysis of the hydroperoxide isomers pointed out that hydroxytyrosol, oleuropein and caffeic acid at a concentration of 10^{-4}M inhibited the formation of *trans-trans* isomers in the increasing order: caffeic acid < oleuropein < hydroxytyrosol. This inhibition could be related to the ability of phenolic compounds to scavenge peroxyl radical. Tyrosol did not inhibit the formation of *trans-trans* isomers. Phenolic compounds were degraded as a consequence of their antioxidant activity and their degradation rate was positively correlated to their antioxidant efficacy. These phenolic compounds, at a concentration of 6×10^{-3}M, also scavenged hydroxyl radical, with an efficiency which increased in the order: tyrosol < hydroxytyrosol < oleuropein < caffeic acid. Polar substituents at the para position, such as in caffeic acid and oleuropein, were correlated with higher hydroxyl radical quenching ability.

KEY WORDS: Free radical scavengers, hydroxyl radical, linoleic acid autoxidation, natural phenolic antioxidants, peroxyl radical.

Phenolic compounds are numerous and largely distributed within the plant kingdom. About twenty polyphenols were identified in virgin olive oils (1,2). The predominant phenolic compounds in virgin olive oils are tyrosol and hydroxytyrosol (3-14), followed by traces of substituted cinnamic acids such as caffeic (15,16), oleuropeilaglycone (6,17) and oleuropein (17).

Methods for isolation, characterization and analytical determination of phenolic compounds in olive oils have already been published (18-20). However, metabolic inter-relations between these phenolic compounds during the growth and development of the olive have been partially elucidated (21).

The exceptionally high content of *o*-diphenols in virgin olive oils is responsible for their good oxidative stability (5,22). Antioxidant activity of these *o*-diphenols has been attributed particularly to hydroxytyrosol (5,18,23-26). The influence of chemical structure on the antioxidant activity of phenolic compounds has been discussed in the literature (27-29) Chimi *et al.* (29) showed that at 50°C and in the dark the predominant phenolic compounds of virgin olive oil have an antioxidant efficacy which decreased in the order: hydroxytyrosol > caffeic acid > oleuropein > tyrosol.

Phenolic antioxidants inhibit autoxidation of lipids (RH) by trapping intermediate peroxyl radical in two ways (30,31):

$$ROO^{\bullet} + ArOH \text{ ------> } ROOH + ArO^{\bullet} \quad (I)$$
$$ROO^{\bullet} + ArO^{\bullet} \text{ ------> } ROO - ArO \quad (II)$$

First, the peroxyl radical abstracts an H proton from the phenolic antioxidant to yield hydroperoxide and aroxyl radical (Eq. I). Second, aroxyl radical undergo radical-radical coupling to give peroxide products (Eq. II).

The rate of oxidation of a lipid inhibited by a phenolic antioxidant requires consideration of other reactions as well as (27,28):

$$ArO^{\bullet} + ROOH \text{ ------> } ROO^{\bullet} + ArOH \quad (III)$$
$$2\ ArO^{\bullet} \text{ ------> Non radical products} \quad (IV)$$
$$ArO^{\bullet} + RH \text{ ------> } ArOH + R^{\bullet} \quad (V)$$

For sterically hindered phenols (hydroxytyrosol, oleuropein, caffeic acid), the rates of reactions (II) and (IV) greatly exceed the rates of reactions (III) and (V). As a result, peroxyl radical and alkoxyl radical are withdrawn from the chain reaction that consequently breaks down the autoxidation process; thus, hindered phenols are effective antioxidants. In tyrosol, the lack of hindrance favors reactions (III) and (V). Under these conditions, there are two chain-carrying free radicals; the peroxyl radical and the aroxyl radical, which explains the poor antioxidant activity of tyrosol.

This work aims to reconsider ranking of the four phenolic compounds according to their antioxidant activity in a micellar substrate composed of linoleic acid. Because oleuropein is more soluble in water than in olive oil, it might show higher antioxidant activity in a micellar medium. The second objective is to determine the reactivity of these phenolic compounds with peroxyl radical formed during the autoxidation of linoleic acid as a consequence of the distribution of hydroperoxide isomers. The last objective is to investigate the ability of these phenolic compounds to scavenge $OH^{\bullet}$ radical generated by photolysis of H_2O_2 in order to better understand their role in the initiation step of lipid peroxidation.

MATERIALS AND METHODS

Materials. Linoleic acid was purchased from Koch Light (England); tyrosol, caffeic acid and oleuropein were from Extrasynthese (France). Hydroxytyrosol was prepared in our laboratory from 3,4-dihydroxyphenyl acetic acid (29). Tween 20 was supplied by Merck (Darmstadt, Germany) 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and hydrogen peroxide were supplied by Aldrich Chemical Co. (Milwaukee, WI) and Merck, respectively. DMPO was purified by passing it through activated charcoal and

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stored according to the method described by Floyd *et al.* (32). All chemicals and solvents used were of analytical grade.

Autoxidation of micelles of linoleic acid. Linoleic acid and phenolic compounds were dispersed with 0.5% Tween 20 in a phosphate buffer solution at pH 6.9 (33). The samples contained linoleic acid at a concentration of 2.5×10^{-3}M with and without each phenolic compound (caffeic acid, oleuropein, hydroxytyrosol and tyrosol) at a concentration of 10^{-4}M. All the samples were left in the dark and under air at room temperature. Controls without linoleic acid were placed under the same conditions.

Measurement of the autoxidation rate of linoleic acid. The autoxidation rate of linoleic acid was estimated by the increase of conjugated diene level in the sample and by the decrease of linoleic acid content of the samples.

The autoxidation of linoleic acid was accompanied in the early stage by the formation of hydroperoxides with a conjugated diene system which exhibited an absorption at 234 nm (33,35). Measurement of an increase of this absorption was achieved by a Pye Unicam SP8-400 Spectrophotometer (Pye Unicam, Cambridge, U.K.).

Furthermore, the amount of unoxidixed linoleic acid left in the samples was evaluated by capillary gas chromatography as previously described (36). Linoleic acid was extracted from 1 mL of aqueous sample by addition of 1.5 mL $CHCl_3/CH_3OH$ (2:1, v/v). Palmitic acid (1 mg/mL ethanol) was added as internal standard. Fatty acids were reextracted twice by $CHCl_3$. The chloroform layers were collected and dried under nitrogen gas. The dry residue was dissolved in 1 mL of ethanol and 0.3 μL of this solution was injected into a gas chromatograph (United Technologies Packard, model 439), equipped with a flame ionization detector and a SGE column. The column (25m/0.25mm ID) was coated with FFAP (Rescom, Belgium) which allowed direct analysis of free fatty acids. These compounds were separated using a carrier gas (hydrogen) at a flow rate of 2 mL/min with a temperature gradient of 60°C to 260°C at 30°C/min during 5 min, the 10°C/min.

Determination of linoleic acid hydroperoxides. The hydroperoxide isomers of linoleic acid were extracted periodically from the samples. Then they were separated by high performance liquid chromatography (HPLC) as previously described (35). HPLC was achieved on a stainless steel column (25×0.47 cm) of Spherisorb Si 60 (particle size 3-4 μm). The solvent was composed of n-heptane and acetic acid (HOAC)(97:25:2.75) and the flow rate was 2 mL/min. The UV detection was at 234 nm.

Measurement of the degradation rate of phenolic compounds. As a consequence of their antioxidant activity, phenolic compounds showed different rates of degradation during the autoxidation of linoleic acid. The amount of unoxidized phenolic compound left in the samples was evaluated by HPLC using an LDC unit, equipped with a Constametric III Pump, a Valco 7000 psi injector and a Spectromonitor III UV detector set at 280 nm. HPLC was achieved on a Spherisorb S50DS2 (150×4.9 mm i.d.) (Sopares, Paris, France).

An aliquot of the sample containing phenolic compound was first diluted 10 times and then injected (50 μL) directly into the HPLC column. The eluting solvent was composed of water MeoH HOAC (60:40:0.2), and the flow rate was 1 mL/min.

Detection of DMPO-OH• radical adduct in presence of phenolic compounds. Hydroxyl radicals were generated by UV photolysis of H_2O_2 with and without phenolic compounds and allowed to spin trap with DMPO to produce DMPO-OH• adduct, which was then analyzed HPLC (34). Briefly, 40 μL of an aqueous solution of DMPO (800 mM), 140 μL of a solution of phenolic compound (6mM) in acetonitrile and 20 μL of an aqueous solution of H_2O_2 (20 mM) were added, in that order, in a small glass tube. After shaking, the mixture was transferred into a small tube of quartz (1 mm i.d., 10 cm height) and irradiated directly under UV light at 254 nm (Bioblock lamp, Paris) for 10 min. Controls in which the solution of phenolic compound was replaced by the same volume of acetonitrile were irradiated under the same conditions.

Immediately after irradiation, 30 μL of sample were analyzed by an HPLC procedure as previously described (37). The HPLC unit was identical to the one used for the analysis of phenolic compounds, but it was equipped in addition with a pulse damper. The column was the same as the one used for phenolic compounds and the eluting solvent was composed of citric acid (monohydrate), 0.03 M; dry sodium acetate, 0.05 M; NaOH, 0.05 M; and glacial acetic acid, 0.02 M; to obtain a final pH of 5.1. The solvent was filtered through a 0.45 μm pore size Millipore filter, and was run at a flow rate of 1.5 mL/min. The detection of DMPO-OH• adduct produced by UV photolysis of H_2O_2 was performed with an electrochemical detector (Esa-Coulochem, model 5100 A, Sopares, France) set at +0.4V.

Trapping of OH• radical by phenolic compounds led to a decline in the formation of DMPO-OH• adduct. The scavenging activity of the phenolic compounds was estimated by the percentage of decrease of the peak area of DMPO-OH• in reference to the control without phenolic compound. All the experiments were replicated three times.

RESULTS AND DISCUSSION

Linoleic acid autoxidation rate. In the early stage, the autoxidation of micelles of linoleic acid was accompanied by a rapid increase of conjugated diene level (A 234 nm) which reached a maximum value at seven days of autoxidation (Fig. 1). Addition of the phenolic compounds at a concentration of 10^{-4}M markedly slowed down the rate of conjugated diene formation. The inhibition of conjugated diene by phenolic compounds increased in the order: tyrosol < caffeic acid < oleuropein < hydroxytyrosol.

Assuming a molar extinction coefficient ($\epsilon M = 2.4\ 10^4$ mol^{-1} cm^{-1}) for diene absorption (38), the level of conjugated diene was calculated from the O.D. value and expressed in percentage of the initial concentration of linoleic acid. Thus, at 14 days of oxidation, the level of conjugated diene was ca. 25.7% in the control, while with phenolic compound the level of conjugated diene was very low — 1.9% with hydroxytyrosol, 3.0% with oleuropein, 4.1% with caffeic acid and 14.0% with tyrosol. These data are means of triplicate samples.

In the absence of phenolic compounds, the concentration of linoleic acid decreased dramatically, especially during the first three days, because it was rapidly oxidized (Fig. 2). The degradation rate of linoleic acid was greatly inhibited in presence of phenolic compounds at the concentration of 10^{-4} M. Hydroxytyrosol, oleuropein

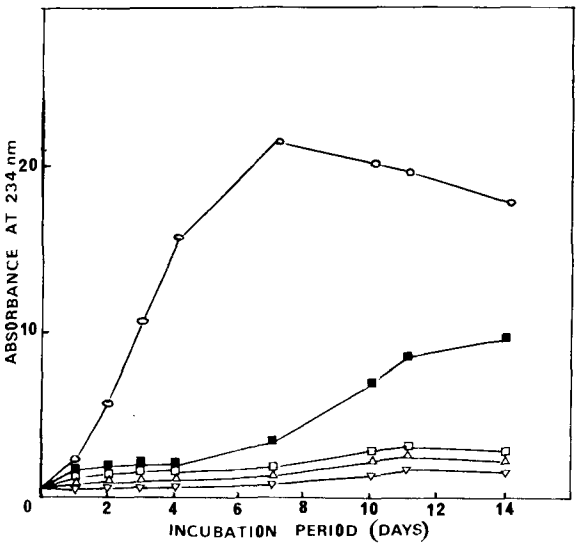

FIG. 1. Spectrophotometric measurement of conjugated diene during the autoxidation of micelles of linoleic acid with natural phenolic compounds.○——○, Linoleic acid (2.5×10^{-3}M); ■——■, linoleic acid = tyrosol (10^{-4}M); □——□, linoleic acid + caffeic acid (10^{-4}M); △——△, linoleic acid + oleuropein (10^{-4}M); ▽——▽, linoleic acid + hydroxytyrosol (10^{-4}M).

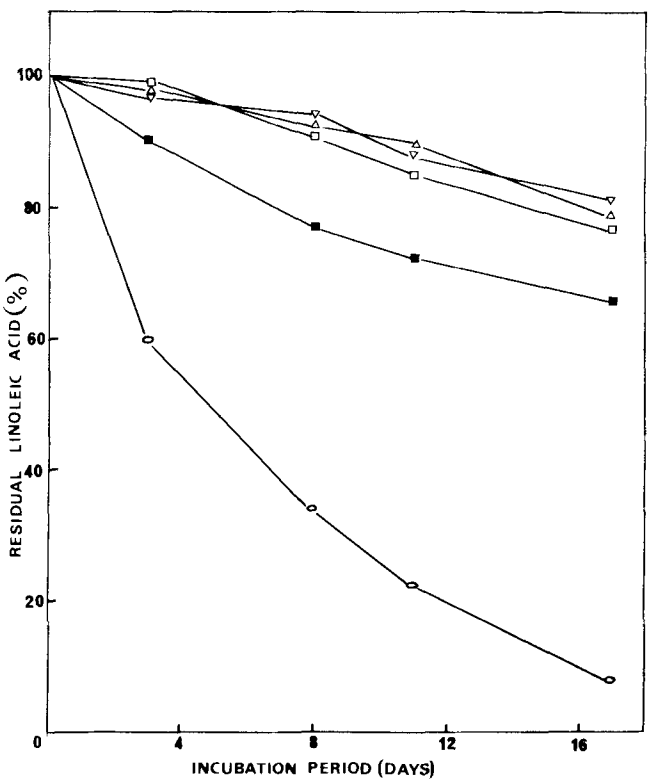

FIG. 2. Measurement of linoleic acid degradation by GLC during its autoxidation with phenolic compounds. ○——○, Linoleic acid (control); ■——■, linoleic acid + tyrosol (10^{-4}M); □——□, linoleic acid + caffeic acid (10^{-4}M); △——△, linoleic acid + oleuropein (10^{-4}M); ▽——▽, linoleic acid + hydroxytyrosol (10^{-4}M).

and caffeic acid afforded a good protection for linoleic acid, while tyrosol seemed less effective. After 16 days of autoxidation the loss of linoleic acid varied from ca. 20% with hydroxytyrosol, oleuropein, and caffeic acid to

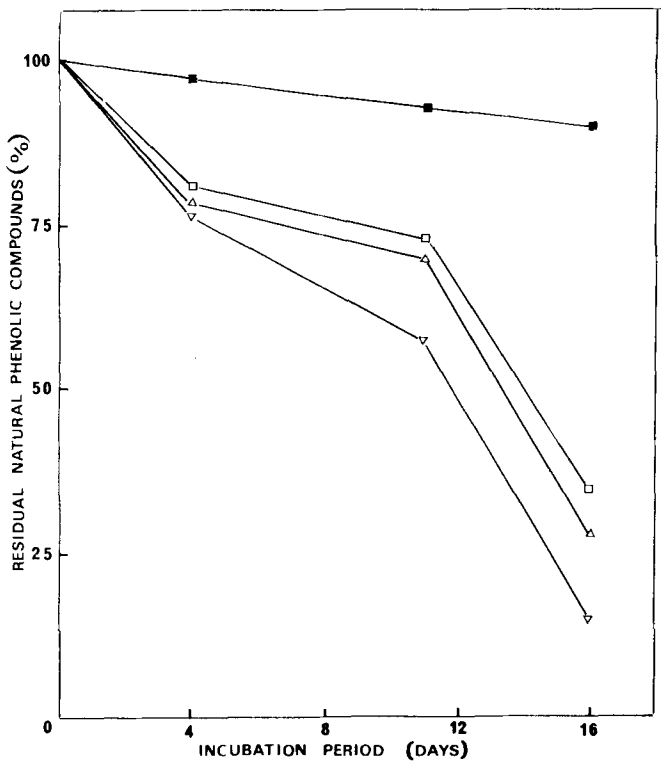

FIG. 3. Degradation of natural phenolic compounds during the autoxidation of micelles of linoleic acid. ■——■, linoleic acid + tyrosol (10^{-4}M); □——□, linoleic acid + caffeic acid (10^{-4}M); △——△, linoleic acid + oleuropein (10^{-4}M); ▽——▽, linoleic acid + hydroxytyrosol (10^{-4}M).

about 35% with tyrosol. For the same period of autoxidation, linoleic acid without phenolic compound showed a loss of ca. 90%. These results are in a good agreement with measurement of conjugated diene, and confirm the antioxidant activity of the tested phenolic compounds.

In a previous work (29) where refined olive oil was used as a substrate, we showed that oleuropein had a lower antioxidant efficiency than did caffeic acid. This discrepancy can be explained on the basis that oleuropein, being a polar compound, is more soluble in an aqueous emulsion (this study) than in a pure lipid system (refined olive oil).

Oxidation rate of phenolic compounds. The phenolic compounds were almost stable in aqueous solution, while in the presence of micelles of linoleic acid they degradaded rapidly (Fig. 3). In such conditions, hydroxytyrosol was the most oxidizable. Its concentration was reduced by ca. 50% after 12 days of experimentation. Oleuropein and caffeic acid exhibited nearly similar profiles of oxidation and degradaded to a lesser extent than hydroxytyrosol. Caffeic acid appeared to be little more stable than oleuropein. At 12 days of experimentation, these compounds exhibited ca. 35% degradation.

Tyrosol was the most stable among the phenolic compounds tested and its concentration remained almost constant during the test period. A loss of less than 10% of tyrosol was noted after 16 days.

Some authors have reported a similar oxidation rate for these phenolic compounds (5,12,25). Initial hydroxytrosol and peroxide concentration in virgin olive oils were found as a good indicator of their oxidative stability (12,25). Tyrosol is the predominant phenolic compound

in virgin olive oils (3,20). However, it exhibited a poor antioxidant activity, as shown in this study. This could explain the lack of correlation reported in the literature (1,3,14,20) between total content of phenolic compounds and oxidative stability of virgin olive oils.

Effect of phenolic compounds on the distribution of linoleic acid hydroperoxides isomers. The autoxidation of linoleic acid yields four hydroperoxide isomers: 13-hydroperoxy-9-*cis*, 11-*trans*-octadecadienoic, 13-hydroperoxy-9-*trans*, 11-*trans*-octadecadienoic, 9-hydroperoxy-10-*trans*, 12-*cis* octadecadienoic; 9-hydroperoxy-10-*trans*, 12-*trans* octadecadienoic acids (38,39). These hydroperoxides increased rapidly in the micelles.

The addition of phenolic compounds strongly inhibited the formation of these hydroperoxides; none of them could be detected before eight days of autoxidation. The first hydroperoxide isomers which were formed significantly are the 13-*cis-trans* and 9-*trans-cis* isomers while the 13-*trans-trans* and 9-*trans-trans isomers* were detected only as traces. This fact was clearly indicated by the ratio *cis-trans* isomers/*trans-trans* isomers which showed a high value, ca. 2.7, at 8 days with hydroxytyrosol and oleuropein and which increased regularly owing to the inhibition of *trans-trans* isomers (Fig. 4). The inhibition of *trans-trans* isomers increased in the order: caffeic acid < oleuropein < hydroxytyrosol. Tyrosol did not inhibit the formation of the *trans-trans* isomers since the ratio *cis-trans* isomers/*trans-trans* isomers decreased as in the control linoleic acid. Thus, the antioxidant properties of the phenolic compounds are directly related to their inhibitory effect on *trans-trans* isomers formation.

A mechanism has been proposed by Porter *et al.* (39) to account for the partial inhibition of *trans-trans* hydroperoxide isomers in presence of phenols. The same mechanism was applied by Peers *et al.* (40 and Torel *et al.* (41) to explain, the effect of α-tocopherol at high concentration and flavonoids on the inhibition of *trans-trans* hydroperoxide isomers, respectively. This inhibition was related to the H-atom donating ability of the antioxidant to the peroxyl radical, thus acting as chain radical terminator. Such a mechanism can also be applied to the phenolic compounds tested in this study.

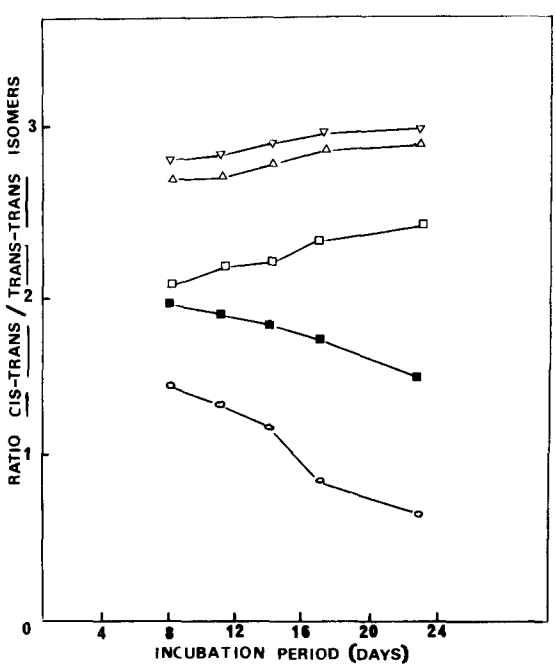

FIG. 4. Distribution of hydroperoxides of linoleic acid with and without natural phenolic compounds. O——O, Linoleic acid (control); ■——■, linoleic acid + tyrosol (10^{-4}M); □——□, linoleic acid + caffeic acid (10^{-4}M); △——△, linoleic acid + oleuropein (10^{-4}M); ▽——▽, linoleic acid = hydroxytyrosol (10^{-4}M).

Hydroxyl radical (OH•) scavenging activity of phenolic compounds. The UV photolysis of H_2O_2 yields predominantly DMPO-OH• in presence of high concentration of DMPO and low concentration of H_2O_2 (42). Such concentrations were used in our experiment, which assumes that OH• was the primary radical formed. Confirmation of OH• authenticity generated in our model was done by the ESR spectra of the fraction eluted at 11.4 min. This spectra was characteristic of DMPO-OH• adduct, as reported earlier by Pristos *et al.* (43). In addition, superoxide dismutase did not decrease the peak area of DMPO-OH• adduct produced by UV photolysis of H_2O_2, therefore superoxide anion, which can give also DMPO-OH•, was not generated in our system.

Addition of the phenolic compounds resulted in the reduction of the DMPO-OH• peak as depicted in Table 1. The OH• scavenging activity of phenolic compounds tested in this study depended upon their concentrations and structures. At a lower concentration (0.6 mM) of phenolic compound, only caffeic acid scavenged OH• radical (ca. 22%) whereas at 10 times higher concentration (6 mM), all phenolic compounds quenched OH • more or less efficiently. The OH• scavenging activity of phenolic compounds decreased in the order caffeic acid > oleuropein > hydroxytyrosol > tyrosol. Oleuropein was similar to ethanol (1 M) with regards to its OH• scavenging efficiency.

Some generalizations can be drawn from these results. The OH• quenching ability of the phenolic compounds tested seems directly correlated to the number of hydroxyl groups substituted at aromatic ring and to the nature of substituent at *p*-position. Diphenols such as caffeic acid, oleuropein and hydroxytyrosol had higher OH• scavenging capability than tyrosol, a monophenol. Similar results were reported by Bors *et al.* (44). These authors showed that methoxylation of hydroxyl group at the *o*-position, as in ferulic acid, resulted in a drastic decrease of the rate constant of phenolic antioxidant with OH• radicals. Polar substituents at *p*-position, as in caffeic acid and oleuropein, were correlated with higher OH• quenching ability.

Detailed studies of reaction rates between phenolic antioxidants and OH• radicals should help clarify individual mechanisms and make quantitative structure activity correlation possible.

Thus, the antioxidant activity of the natural phenolic compounds tested in this work could be ascribed to their free radical scavenging properties. They were able to quench OH• radical and peroxyl radical (excepted tyrosol in this later case) involved in the initiation and propagation steps of lipid peroxidation, respectively. The antioxidant effectiveness of these phenolic compounds seemed to be particularly related to their ability to quench peroxyl radicals.

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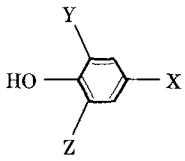
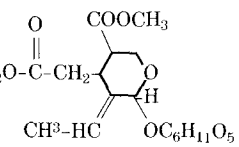
TABLE 1

Chemical Structure of Natural Phenolic Compounds and their Respective $OH^{\bullet}$ Radical Scavenging Activity[a]



Phenolic compound	Substituent			$OH^{\bullet}$ radical scavenged (%) by phenolic compounds at concentrations	
	Z	Y	X	0.6 mM	6 mM
Caffeic acid	-H	-OH	-CH=C-COOH	22	45
Oleuropein	-H	-OH		0	39
Hydroxytyrosol	-H	-OH	$-CH_2-CH_2OH$	0	22
Tyrosol	-H	-H	$-CH_2-CH_2OH$	0	16
Ethanol					40[b]

[a] Data are means of triplicate samples.

[b] Ethanol = 1 M.

REFERENCES

1. Montedoro, G., M. Bertuccioli and F. Anichini, *Flavor of Foods and Beverages in Chemistry and Technology,* edited by C. Charalambous and G. Inglett, Acad. Press Inc., 1978, p. 247.
2. Solinas, M., and A. Cichelli, *Riv. Soc. Ital. Sci. Aliment. 10*:159 (1981).
3. Ragazzi, E., and G. Veronese, *Riv. Ital. Sost. Grasse 50*:443 (1973).
4. Fedeli, E., *Prog. Chem. Fats 15*:57 (1977).
5. Vazquez Roncero, A., *Rev. Fran. Corps Gras. 25*:21 (1978).
6. Solinas, M., L. Digiovacchino and A. Mascolo, *Riv. Ital. Sost. Grasse 55*:19 (1978).
7. Janer Del Valle, C., and A. Vazquez Roncero, *Grasas y Aceites 31*:309 (1980).
8. Graciani Constante, E., and A. Vazquez Roncero, *Ibid. 31*:237 (1980).
9. Cortesi, N., A. Ponziani and E. Fedeli, *Riv. Ital. Sost. Grasse 58*:108 (1981).
10. Cortesi, N., and E. Fedeli, *Ibid. 60*:341 (1981).
11. Fedeli, E., *Rev. Fran. Corps Gras 30*:51 (1983).
12. Cichelli, A., and M. Solinas, *Riv. Merceol. 23*:55 (1984).
13. Solinas, M., *Riv. Ital. Sost. Grasse 64*:225 (1987).
14. Forcadell, M.L., M. Comas, X. Miquel and M.C. De Latorre, *Rev. Fran. Corps Gras 34*:547 (1987).
15. Montedoro, G. and C. Cantarelli, *Riv. Ital. Sost. Grasse 46*:115 (1969).
16. Montedoro, G., *Sci. Technol. Alimenti 2*:117 (1972).
17. Solinas, M., L. Digiovacchino and A. Cucurachi, *Ann. Ist. Sper. Eleiotecnica 5*:129 (1975).
18. Vazquez Roncero, A., C. Janer Del Valle and M.L. Janer Del Valle, *Grasas y Aceites 24*:350 (1973).
19. Vazquez Roncero, A., C. Janer Del Valle and M.L. Janer Del Valle, *Ibid. 27*:185 (1976).
20. Solinas, M., and A. Cichelli, *Riv. Soc. Ital. Sci Aliment 11*:223 (1982).
21. Pierpont, W.S., *Annual Proceedings of the Phytochemical Society of Europe,* Vol. 25, edited by C.F. Van Sumere and P.J. Lea, Clarendon Press, Oxford, 1985, p. 427.
22. Gutfinger, T., *J. Am. Oil Chem. Soc. 58*:966 (1981).
23. Vazquez Roncero, A., C. Janer Del Valle and M.L. Janer Del Valle, *Grasas y Aceites 26*:14 (1975).
24. Gutierrez Gonzalez Quijano, R., C. Janer Del Valle, M.L. Janer Del Valle, F. Guttierrez Rosales and A. Vazquez Roncero, *Ibid. 28*:101 (1977).
25. Solinas, M., L. Digiovacchino and A. Mascolo, *Ann. Ist. Sper. Elaiotecnica 8*:3 (1978).
26. Vazquez Roncero, A., C. Janer and E. Graciani, *E.F.C.E. Publi. 12*:195 (1980).
27. Mahoney, L.R., *Angew. Chem. Internat. Edit. 8*:547 (1969).
28. Denisov, E.T., and I.V. Khudyakov, *Chem. Rev. 87*:1313 (1987).
29. Chimi, H., A. Sadik, B. Letutour and M. Rahmani, *Rev. Fran. Corps Gras 35*:339 (1988).
30. Howard, J.A., *Adv. Free-Radical Chem. 4*:49 (1972).
31. Porter, N.A., *Acc. Chem. Res. 19*:262 (1986).
32. Floyd, R.A., C.A. Leuris and P.K. Wong, *Methods in Enzymology,* Vol. 105, edited by L. Porcker, Academic Press, New York, 1984, p. 231.
33. Cillard, J., P. Cillard, M. Cormier and L. Girre, *J. Am. Oil Chem. Soc. 57*:252 (1980).
34. Harbour, J.R., V. Chow and J.R. Bolton, *Can. J. Chem. 52*:3549 (1974).
35. Koskas, J.P., J. Cillard and P. Cillard, *J. Chromatogr. 258*:280 (1983).
36. Cillard, J., and P. Cillard, *J. Am. Oil Chem. Soc. 57*:39 (1980).
37. Husain, S.R., J. Cillard and P. Cillard, *Phytochemistry 26*:2489 (1987).
38. Chan, H.W.S., and G. Levett, *Lipids 12*:837 (1977).
39. Porter, N.A., B.A. Weber, H. Weenen and J.A. Khan, *J. Am. Chem. Soc. 102*:5597 (1980).
40. Peers, K.E., D.T. Coxon and H.W.S. Chan, *J. Sci. Food Agric. 32*:898 (1981).
41. Torel, J., J. Cillard and P. Cillard, *Phytochemistry 25*:383 (1986).

42. Finkelstein, E., G.M. Rosen and E.J. Rauckman, *Arch. Biochem. Biophys. 200*:1 (1980).
43. Pristos, C.A., P.P. Consantinides, T.R. Tritton, D.C. Heimbrook and A.C. Sartorelli, *Anal. Biochem. 150*:249 (1985).
44. Bors, W., C. Michel, M. Erben-Russ, B. Kreileder, D. Tait and M. Saran, *Oxygen Radicals in Chemistry and Biology*, Edited by W. Bors, M. Saran and D. Tait-Walter de Gruyter, Berlin, 1984.

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