Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Food Chemistry 103 (2007) 55–61
Food Chemistry
www.elsevier.com/locate/foodchem

Synthesis and evaluation of new phenolic-based antioxidants: Structure–activity relationship

A. Torres de Pinedo, P. Peñalver, J.C. Morales *

Department of Chemistry, Puleva Biotech, Camino de Purchil 66, 18004 Granada, Spain

Received 21 April 2005; accepted 7 July 2006

Abstract

The structure–activity relationships of synthetic phenolic-based derivatives as potential antioxidants have been investigated. Their efficiency as radical scavengers was evaluated by their activity towards the stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and their potency as antioxidants in food was evaluated in refined olive oil using the Rancimat method. Four of the new antioxidants possessed better radical-scavenging capacity than the natural antioxidant hydroxytyrosol (**2**). They were galloyl alcohol (**12**), containing three phenolic hydroxyl groups and a primary alcohol, and the *ortho*-diphenolic derivatives dihydrocaffeoyl alcohol (**3**), caffeoyl alcohol (**4**) and 5-methoxy-protocatechuyl alcohol (**13**). Two of these antioxidants, **3** and **12**, also showed higher antioxidant capacity than **2** in stabilising olive oil. The contribution of the length of the alkyl chain linking the phenyl ring to the primary alcohol, with regard to its antioxidising potency was studied and contradictory results were obtained. The polar paradox fails to explain the antioxidant capacity found for these new phenolic antioxidants, except from a very general perspective.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Antioxidants; Phenols; Hydroxytyrosol; Free radical-scavenging activity; Antioxidant potency; Structure–activity relationship

1. Introduction

Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases, and even with a role in cancer and ageing (Beckman & Ames, 1998; Halliwell & Gutteridge, 1989). Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants (Block, 1992; Rice-Evans, Miller, & Paganga, 1996). Food oxidation is one of the main causes of food deterioration, specially in food items with a high lipid fraction. Antioxidants have been added to food for years to prevent this process and are widely used today for better food preservation. Development of new antioxidants with better antioxidant capacity and less toxicity, which could be used in the prevention of certain diseases or in better food preservation, is very desirable.

Phenolic derivatives are one of the groups of antioxidants that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogues, which are known to have antiviral, antiinflammatory and antiatherosclerotic properties (Nardini et al., 1995), resveratrol with known anticancer and heart protecting effects (Jang et al., 1997) and olive oil phenols, particularly hydroxytyrosol, which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis) (Visioli, Bellomo, Montedoro, & Galli, 1995), inhibits platelet aggregation (Petroni et al., 1995) and exhibits antiinflammatory (De la Puerta, Ruiz-Gutierrez, & Hoult, 1999) and anticancer properties (Owen et al., 2000).

Phenols have been utilised extensively for food preservation. Synthetic phenolic antioxidants, such as BHT, BHA

* Corresponding author. Present address: Instituto de Investigaciones Químicas, CSIC, 49 Avda Americo Vespucio, 41092 Seville, Spain. Tel.: +34 954 489568; fax: +34 954 460565.
E-mail address: jcmorales@iiq.csic.es (J.C. Morales).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.07.026

or TBHQ posses good antioxidant capacity but have been questioned due to possible side effects for human health (Omura, 1993). On the other hand, phenols are a family of natural compounds present in a wide variety of plants with very different structures and some of them are known to act as antioxidants. For example, caffeic acid and its analogues found in coffee beans, fruit and vegetables; isoflavones, such as genistein and daidzein, in soy; flavones, such as luteolin, apigenin or naringenin, in fruit; complex phenolic molecules, such as oleuropein in olives or simpler molecules, such as tyrosol and hydroxytyrosol, also in olives and olive oil. Moreover, some of these natural phenolic derivatives are being used as antioxidants for food preservation, for example, catechins in green tea extracts and rosmarinic acid and its derivatives in rosemary extracts.

The main structural feature responsible for the antioxidative and free radical-scavenging activity of phenolic derivatives is the phenolic hydroxyl group. Phenols are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus stopping the propagation chain during the oxidation process. The presence of a second hydroxyl group at the *ortho*-position, to give a catechol ring, also lowers the O–H bond dissociation enthalpy and increases the rate of H-atom transfer to peroxyl radicals (Lucarini & Pedulli, 1994; Shahidi & Wanasundara, 1992). A third hydroxyl group in the phenolic ring increases the antioxidant capacity further (Ranalli, Lucera, & Contento, 2003).

Another structural feature that may increase the antioxidant capacity is the primary hydroxyl group on the alkyl chain of antioxidants such as tyrosol and hydroxytyrosol. In fact, hydroxytyrosol is a better antioxidant when added to olive oil than caffeic acid and homoprotocatechuic acid (Benavente-García, Castillo, Lorente, Ortuño, & Del Rio, 2000; Ranalli et al., 2003) all of them containing the same *ortho*-diphenolic structure. In addition, the alkyl chain connecting the phenolic ring and the carboxylic or alcohol group in phenolic derivatives may stabilize the radical formed during oxidation, and its contribution remains uncertain (Moon & Terao, 1998; Nenadis, Boyle, Bakalbassis, & Tsimidou, 2003; Silva et al., 2000). Actually, some phenolic antioxidants contain an alkenyl chain for this connection, such as in caffeic acid or sinapic acid, and this functionality may also be important to stabilize the radical formed (Nenadis et al., 2003; Rice-Evans et al., 1996).

In order to better explore the structural features of phenolic-based antioxidants and understand how the different functionalities affect the antioxidant activity, we prepared a series of phenolic-based analogues, varying the number of phenolic hydroxyl groups, the number of methyl-protected phenolic hydroxyl groups and the length and nature of the alkyl chain. All of the compounds in the series possess a primary alcohol instead of a carboxylic acid group in an attempt to increase their antioxidant capacity. Some of the compounds in this study are natural compounds and others have been chemically synthesised. We have carried out a structure–activity relationship study with this series of compounds. Their efficiency as radical scavengers was evaluated by their activity towards a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\cdot$) (Blois, 1958; Brand-Williams, Cuvelier, & Berset, 1995; Nenadis & Tsimidou, 2002). Their potency as antioxidants in food was evaluated in refined olive oil using the Rancimat method (Läubly & Bruttel, 1986). Ascorbic acid and α-tocopherol served as reference compounds.

2. Materials and methods

2.1. General

Synthesised compounds were purified on a silica gel 60 (200–400 mesh) (Sigma–Aldrich Chemical Co., St. Louis, MO) column and identified by TLC, mass spectrometry and NMR analysis. TLC was performed on precoated silica gel 60 Alugram SIL/UV_{254} from Macherey-Nagel. Fast atom bombardment (FAB) mass spectra were collected on a Hewlett–Packard 5988 mass spectrometer, using a Fisons VG platform or a Fisons VG Autospec-Q. NMR experiments were performed on a Bruker AMX-400, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. $CDCl_3$ was used as solvent. Chemical shifts are expressed in δ (parts per million), using the solvent as internal reference. A Cary 100 UV–vis spectophotometer from Varian Co. was used in the DPPH· and log P assays. A Rancimat 743 apparatus from Metrohm A.G. (Hensan, Switzerland) was used to measure the induction time in oils containing antioxidants.

2.2. Chemicals

Protocatechuic acid, homoprotocatechuic acid, dihydrocaffeic acid, caffeic acid, vanillyl alcohol (**5**), homovanillyl alcohol (**6**), dihydroferulic acid, ferulic acid, 3,4-dimethoxybenzyl alcohol (veratryl alcohol) (**9**), 2-(3,4-dimethoxyphenyl)ethanol (homoveratryl alcohol) (**10**), 3-(3,4-dimethoxyphenyl)propanoic acid, gallic acid, 3,4-dihydroxy-5-methoxy benzoic acid, syringic acid, ascorbic acid, α-tocopherol and 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\cdot$) were purchased from Sigma-Aldrich Chemical Co. All other reagents and solvents were of analytical, spectrometric or HPLC grade.

2.3. Synthesis

The following alcohols, protocatechuyl alcohol (**1**) (3,4-dihydroxybenzylic alcohol), hydroxytyrosol (**2**) (2-(3,4-dihydroxyphenyl)ethanol), dihydrocaffeoyl alcohol (**3**) (3-(3,4-dihydroxyphenyl)-1-propanol), caffeoyl alcohol (**4**) (3-(3,4-dihydroxyphenyl)-1-propanol), dihydroconiferyl alcohol (**7**) (3-(4-hydroxy-3-methoxyphenyl)-1-propanol), coniferyl alcohol (**8**) (4-hydroxy-3-methoxycinnamylic alcohol), 3-(3,4-dimethoxyphenyl)-1-propanol (**11**) and syringyl alcohol (**14**) (4-hydroxy-3,5-dimethoxybenzylic alcohol) were prepared from their corresponding carboxylic acids, by reduction with lithium aluminum hydride in

anhydrous tetrahydrofurane (THF). Briefly, a solution of 5 g of the corresponding carboxylic acid in dry THF (50 ml) was added slowly to a stirred dispersion of $LiAlH_4$ (2.5 equivalents) in dry THF (350 ml) at 0 °C. Once the addition was finished, the reaction was allowed to reach room temperature and then heated under reflux for 2–4 h until the starting material had disappeared, as monitored by TLC. The reaction mixture was cooled to room temperature and then placed in an ice-water bath. Water (100 ml) was carefully added, to destroy excess $LiAlH_4$ and finally a 5 N HCl solution (100 ml) was added. The crude mixture was extracted with ethyl acetate (3×250 ml), dried over anhydrous Na_2SO_4 and the solvent was evaporated. The residue was purified by silica gel column chromatography, using a mixture of diethyl ether/*n*-hexane (10:1, except for **1** and **2**, where pure ether and ether/hexane 2:1, were used respectively) as eluents. Yields were between 60% and 81%.

The compounds galloyl alcohol (**12**) (3,4,5-trihydroxybenzylic alcohol) and 5-methoxy-protocatechuyl alcohol (**13**) (3,4-dihydroxy-5-methoxy-benzylic alcohol) were prepared from their corresponding carboxylic acids by acetylation of the phenolic hydroxyl groups followed by reduction with lithium aluminum hydride in anhydrous THF. Briefly, to a solution of 5 g of the corresponding carboxylic acid in dry pyridine (50 ml) was added acetic anhydride (25 ml), and the reaction was stirred for 15 h at room temperature. Then, the mixture was evaporated to dryness, toluene (30 ml) added and evaporated again. The acetylated crude product was dissolved in THF and reduction of the carboxylic acid group was carried out following the procedure described above. Yields were between 43% and 45%.

3,4,5-trihydroxybenzylic alcohol (12). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.33 (s, 2H, Ar), 4.35 (s, 2H, –CH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.9, 133.6, 108.4, 107.4 (Ar), 65.4 (–CH_2OH); HRFABMS$^+$ calcd for $C_7H_8O_4$ *m/z* 156.0467 *m/z* found 156.0465.

3,4-dihydroxy-5-methoxy-benzylic alcohol (13). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 (s, 2H, Ar), 4.42 (s, 2H, –CH_2OH), 3.77 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 146.3, 134.3, 133.4, 108.9, 103.6 (Ar), 65.4 (–CH_2OH), 56.5 (–OCH_3); HRFABMS$^+$ calcd for $C_8H_{10}O_4$ *m/z* 170.0624, *m/z* found 170.0619.

4-hydroxy-3,5-dimethoxy-benzylic alcohol (14). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.61 (s, 2H, Ar), 4.48 (s, 2H, –CH_2OH), 3.80 (s, 6H, –OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.9, 133.6, 128.4, 107.4 (Ar), 65.4 (–CH_2OH), 56.6 (–OCH_3); HRFABMS$^+$ calcd for $C_9H_{12}O_4$ *m/z* 184.0781, *m/z* found 184.0773.

2.4. DPPH radical-scavenging assay

Measurement of DPPH$^\cdot$ radical-scavenging activity was performed according to recommendations by Nenadis and Tsimidou (2002). Conditions consisted of an approximately 20 min reaction period and a molar ratio between DPPH$^\cdot$ and antioxidant that permits 60–80% radical-scavenging activity for the most potent antioxidant. Briefly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol (250 μM, 2 ml) was added to 2 ml of the test compounds at different concentrations in ethanol. The final concentrations of the test compounds in the reaction mixtures were 0.5, 5, 10, 25 and 50 μM. Each mixture was then shaken vigorously and held for 30 min at room temperature in the dark. The decrease in absorbance of DPPH at 517 nM was measured. Ethanol was used as a blank solution. DPPH solution (2 ml) in ethanol (2 ml) served as the control. All tests were performed in triplicate. The radical-scavenging activity of the samples was expressed as % inhibition of DPPH absorbance: $\%\text{Inhibition} = [(A_{control} - A_{test})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus compound). Ascorbic acid (**15**) and α-tocopherol (**16**) were used as reference compounds.

2.5. Rancimat test

Measurement of the antioxidant potency of the new compounds in a food matrix (oil) was performed using the well-established Rancimat method (Ranalli et al., 2003). Moreover, the Rancimat method correlates well with the active oxygen method (Capasso, Evidente, Avolio, & Solla, 1999). The Rancimat apparatus was operated at 120 °C. A dry air flow of 20 l/h was passed through the oil sample (5 ± 0.001 g) containing the antioxidant. The volatile oxidation products coming from the oxidation of the oil dissolved in cold milli-Q water (60 ml), causing an increase in the electrical conductivity. All tests were performed in triplicate. The time (in h) taken to reach a specific conductivity value, corresponding to the flex point of the peroxidation curve, was considered as the induction time (IT). The higher the induction time was, the higher was the antioxidant potency of the compounds.

2.6. Log P solubility measurement

Dispersions of each compound were prepared in 1-octanol (0.3 mM) and were heated up to 60 °C for 1 h, to solubilise the antioxidant. UV spectra for each solution were obtained and the maximum absorbance was determined (A_0). Equal volumes of the organic solution (2 ml) and a phosphate buffer (0.1 M, pH = 7.4) were vigorously mixed using a vortex mixer for 1 min. The mixture was allowed to stabilize and separate for 30 min; then the organic phase was obtained and its absorbance determined (A_x). Partition coefficient (log *P*) was determined from the equation:

$$P = \frac{A_x}{(A_0 - A_x)}.$$

All tests were performed in triplicate. Vitamin C and α-tocopherol were used as reference compounds.

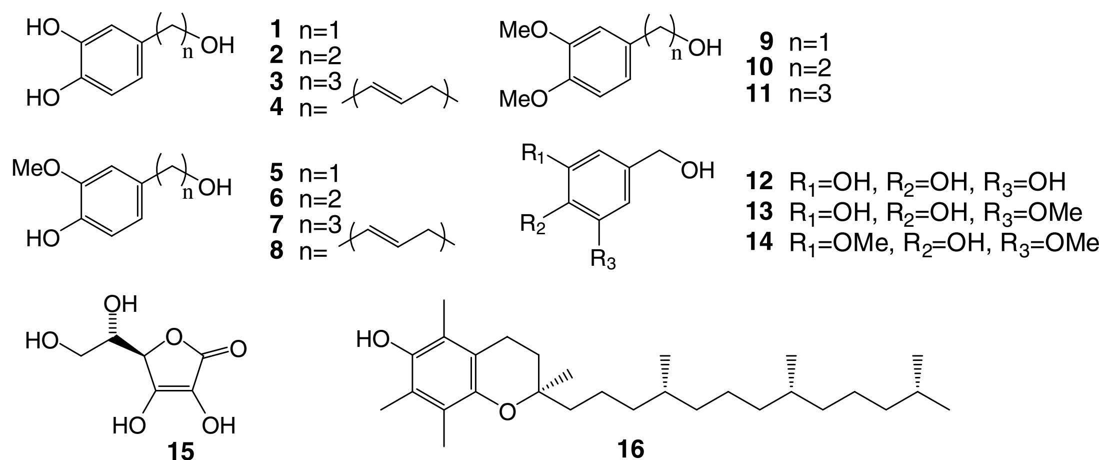

Fig. 1. Chemical structures of phenolic-based antioxidants under study.

3. Results and discussion

3.1. Synthesis

Several of the compounds used in this study were commercially available (**5**, **6**, **9** and **10**) (see Fig. 1). Other compounds, such as protocatechuyl alcohol (**1**), hydroxytyrosol (**2**), dihydrocaffeoyl alcohol (**3**) and coniferyl alcohol (**8**), were previously synthesised by others from their corresponding carboxylic acids, using lithium aluminum hydride as the reducing agent (Atkinson, Brown, & Gilby, 1973; Capasso et al., 1999; Shibata, Kubota, & Kamisaka, 1975). Synthesis of caffeoyl alcohol (**4**), dihydroconiferyl alcohol (**7**) and 3-(3,4-dimethoxyphenyl)-1-propanol (**11**) have been previously described using other procedures and starting materials (Arfmann & Abraham, 1993; Shibata et al., 1975). In our work, we prepared all the compounds under study by lithium aluminum hydride reduction of their corresponding carboxylic acids. This simple methodology gave very poor yields for galloyl alcohol (**12**) and 5-methoxy-protocatechuyl alcohol (**13**), probably because of the high water content of the corresponding carboxylic acids. Finally, we prepared them by protection of the phenolic hydroxyl groups by acetylation, then reduction of the carboxylic acid, and final deprotection of the acetyl groups during work-up of the reduction reaction. Compounds **12**, **13** and **14** are fully characterised above as, to our knowledge, they have not been previously described in the literature.

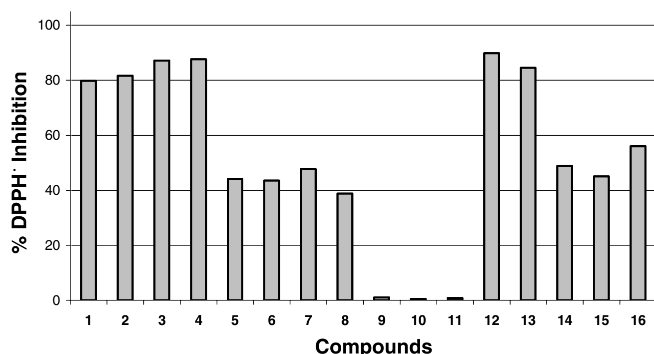

Fig. 2. DPPH· radical-scavenging activity. The concentration of DPPH ethanolic solution was 250 μM and the concentration of compounds was 50 μM. All standard deviations were below 3%.

3.2. Radical-scavenging activity

The DPPH· assay is a simple method to measure the ability of antioxidants to trap free radicals. The scavenging effects of the phenolic-based antioxidants in the study are shown in Fig. 2. Two controls, traditional food antioxidants ascorbic acid (**15**) and α-tocopherol (**16**), were also included. The most potent radical scavenger was galloyl alcohol (**12**), containing three phenolic hydroxyl groups, closely followed by all the antioxidants with di-ortho phenolic structure, compounds **1–4** and **13**, then the mono-phenolic compounds **5–8** and **14**, and finally, with very low radical inhibition capacity, the compounds **9–11**, with both hydroxyl groups methylated. Notably, four of the new antioxidants possessed better radical-scavenging capacity than the natural antioxidant hydroxytyrosol (**2**): galloyl alcohol (**12**), 5-methoxy-protocatechuyl alcohol (**13**), dihydrocaffeoyl alcohol (**3**) and caffeoyl alcohol (**4**). Hydroxytyrosol was shown to be a very good radical scavenger, similar to oleuropein (Gordon, Paiva-Martins, & Almeida, 2001). At the same time, it is important to note that all antioxidants in the study with two or three phenolic hydroxyl groups showed a higher scavenging capacity than the two control antioxidants, ascorbic acid (**15**) and α-tocopherol (**16**).

An increase in the number of hydroxyl groups in the phenyl ring increases the radical-scavenging activity, due to the fact that more hydrogen atoms of the phenolic hydroxyl groups can be donated to stabilize the free radicals. (Rice-Evans et al., 1996; Shahidi & Wanasundara, 1992). In the series of the di-ortho phenolic derivatives **1–3** and mono-phenolic derivatives **5–7**, it can also be observed that the increase in the length of the alkyl chain increases the radical-scavenging capacity. This effect is

probably due to the fact that as the alkyl chain increases, its electron-donating activity also increases, resulting in a well-stabilized phenoxy radical.

Extension of the conjugation *via* the side chain can be studied by comparing radical-scavenging activity of compounds possessing an alkene chain instead of an alkyl chain linking the primary alcohol with the phenyl ring. This effect seemed to be inconsistent. In the case of the series of the di-ortho phenolic antioxidants, caffeoyl alcohol (**4**), which possessed an alkene in its structure, showed a higher scavenging capacity than antioxidants **1**–**3** that possessed an alkyl chain. However in the mono-phenolic series (**5**–**8**) the opposite effect is observed. Actually, Nenadis et al. (2003) found that caffeic acid had a lower radical-scavenging activity than dihydrocaffeic acid, in contrast to the extended conjugation and better radical stability expected when the alkene group is present.

The use of methoxy groups in these antioxidants was not just to check the effect of the elimination of a possible phenolic OH, but to check if they could also play a role in radical-scavenging. Actually, the introduction of a methoxy group to a di-ortho phenolic structure such as in 5-methoxy-protocatechuyl alcohol (**13**), increases the hydrogen-donating ability and therefore increases the radical-scavenging capacity of the antioxidant, when compared with its analogue, protocatechuyl alcohol (**1**). A similar example is observed when syringyl alcohol (**14**) is compared with vanillyl alcohol (**5**), where the extra methoxy group also increases the radical-scavenging activity. This effect may be due to the fact that methoxy groups are electron-donating groups, which help to stabilize the phenoxy radicals.

3.3. Antioxidant activity in oils

The Rancimat test is a commonly used method to measure the antioxidant capacity of antioxidants in oils and fats. Each antioxidant was added to refined olive oil to a final concentration of 100 mg/l. Several controls were also carried out, such as traditional food antioxidants **15** and **16**, and blank oil with no antioxidants added. The results for stability time in refined olive oil are shown in Fig. 3. The highest induction time was obtained for galloyl alcohol (**12**), followed by the *ortho*-diphenolic antioxidants **1**–**4** and, slightly less stable, 5-methoxy-protocatechuyl alcohol (**13**). All the rest of the compounds showed stability times much lower than these, slightly lower than ascorbic acid and α-tocopherol, and all of them higher than the blank oil. Remarkably, galloyl alcohol shows an induction time 5 h higher than hydroxytyrosol (**2**), and is 10–13 h more stable than any of the mono-phenolic and di-methoxyphenyl antioxidants. Hydroxytyrosol had been shown to be the most potent antioxidant in olive oil, with higher induction time in the Rancimat test than gallic acid or caffeic acid (Ranalli et al., 2003) and also possessed the best peroxide and *p*-anisidine values in an 80 days study carried out by Gordon et al. (2001). Actually, the presence of the diorthophenolic structure and the primary alcohol in hydroxytyrosol seems even better than containing three phenolic hydroxy groups, since hydroxytyrosol is a better antioxidant in olive oil than gallic acid. The primary alcohol of hydroxytyrosol must play an important role in the antioxidant potency of this molecule. In fact, we have observed by HPLC-MS that, the first step in oxidation of hydroxytyrosol in aqueous solution is the oxidation of the primary alcohol to the corresponding aldehyde and later on to the corresponding carboxylic acid. A similar mechanism must be acting in all the compounds under study since they possess such a primary alcohol.

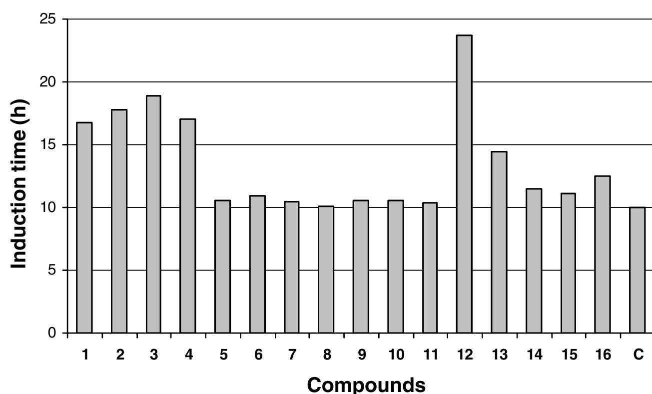

Fig. 3. Average induction time values. C is the control olive oil with no added compounds. Standard deviations values were between 0.3 and 1.3 h.

The tendency found in the Rancimat test for the series of phenolic antioxidants under study is very similar to the one found in the DPPH$^{\cdot}$ test, although galloyl alcohol (**12**) shows a bigger difference compared to the *ortho*-diphenolic antioxidants, and the fact that, surprisingly, the mono-phenolic antioxidants show low stability times and are similar to the dimethoxylated antioxidants, in contrast to data from the DPPH$^{\cdot}$ test.

The effect of the alkyl chain in the di-ortho phenolic series **1**–**3** in the Rancimat test is similar to the effect observed for the DPPH$^{\cdot}$ experiments, and therefore, the stability of the oils increases when the length of the alkyl chain of the antioxidant is increased. This effect is not observed in the mono-phenolic series **5**–**7** or the dimethoxylated-phenolic series **9**–**11**, where almost no differences in induction time are found. Also, in contrast with the DPPH$^{\cdot}$ results, the unsaturation in caffeoyl alcohol (**4**) seems to show a distabilizing effect in the Rancimat experiment when compared with dihydrocaffeoyl alcohol (**3**), which contains a saturated sidechain, probably due to the fact that the oxidation of an alkene chain than that of an alkyl chain, and this effect must be more important than the fact that **4** is better than **3** as a radical scavenger.

The effect of the methoxy groups in the antioxidants in stabilising the olive oil in the Rancimat test was quite contradictory and no clear tendency was observed. In contrast to the DPPH$^{\cdot}$ test, 5-methoxy-protocatechuyl alcohol (**13**), with an extra methoxy group than protocatechuyl alcohol (**1**), showed lower stability than **1** in the Rancimat test.

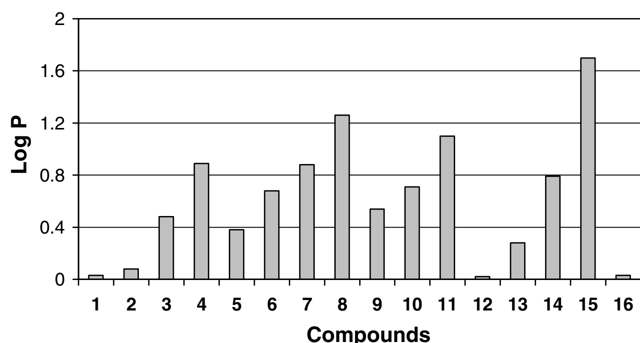

Fig. 4. Log *P* values. Standard deviations were below 5%.

Whereas, in the case of comparison of syringyl alcohol (**14**) with vanillyl alcohol (**5**), the extra methoxy group increased slightly the stability of the oil, as shown by the Rancimat data.

3.4. Solubility of the antioxidants

The partition coefficient (log *P*) was measured for all the compounds in the study, in order to try to correlate them with the Rancimat values (see Fig. 4). According to the polar paradox, more polar antioxidants are more effective in less polar media (Frankel, Huang, Kanner, & German, 1994; Porter, 1993). In general terms, this assumption seems to be true, since the more polar compounds protocatechuyl alcohol (**1**), hydroxytyrosol (**2**) and galloyl alcohol(**12**) are potent antioxidants in oils, and the less polar coniferyl alcohol (**8**) and 3-(3,4-dimethoxyphenyl)-1-propanol (**11**) are less active antioxidants in oils. But, there is not a clear correlation between polarity of the compounds and their radical-scavenging activity or stability in oils shown in the Rancimat experiments. For example, when in the **1**–**3** antioxidant series the alkyl chain length increases and therefore, log *P* also increases, surprisingly an increase in antioxidant capacity is observed. At the same time, caffeoyl alcohol (**4**) is less polar than vanillyl alcohol (**5**), but **4** is better antioxidant than **5** in both the DPPH· assay and the Rancimat test. Moreover, the two controls, ascorbic acid and α-tocopherol, with very different polarity, showed very similar induction times in the Rancimat test. Other authors have found similar problems with the polar paradox. For example, Gordon et al. (2001) found that, although the polar paradox would suggest marked differences in antioxidant activity for hydroxytyrosol and hydroxytyrosol acetate in oil, the effect is rather small.

4. Conclusion

In conclusion, we have found four new compounds with higher radical-scavenging activity than the natural antioxidant hydroxytyrosol (**2**), and two of those, galloyl alcohol (**12**) and dihydrocaffeoyl alcohol (**3**), show higher antioxidant capacity than **2** to stabilize olive oil. Clearly, an increase in the number of phenolic hydroxy groups and the presence of a primary alcohol are two of the main structural factors that help to increase antioxidant capacity. A small effect is observed when a longer alkyl chain is attached to the phenyl ring, which seems to help to stabilize the radicals formed in the oxidation process. Nevertheless, the length effect of the alkyl chain is not totally clear, since in the series **5**–**7** very small differences are observed in the Rancimat test. The polar paradox does not explain the antioxidant capacity found for these new phenolic antioxidants, except from a very general perspective. It is important to note that the biological activity of hydroxytyrosol, usually explained by its antioxidant potency, could be higher for dihydrocaffeoyl alcohol (**3**), caffeoyl alcohol (**4**), galloyl alcohol (**12**) and 5-methoxyprotocatechuyl alcohol (**13**), since all of them are better radical scavengers than hydroxytyrosol (**2**).

References

Arfmann, H. A., & Abraham, W. R. (1993). Microbial reduction of aromatic carboxylic acids. *Zeitschrift fur Naturforschung, 48c*, 52–57.

Atkinson, P. W., Brown, W. V., & Gilby, A. R. (1973). Phenolic compounds from insect cuticle, identification of some lipid antioxidant. *Insect Biochemistry, 3*, 309–315.

Beckman, K. B., & Ames, B. N. (1998). The free radical theory of ageing. *Physiological Reviews, 78*, 447–581.

Benavente-García, O., Castillo, J., Lorente, J., Ortuño, A., & Del Rio, J. A. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chemistry, 68*, 457–462.

Block, G. (1992). A role for antioxidants in reducing cancer risk. *Nutrition Reviews, 50*, 207–213.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199–1200.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie, 28*, 25–30.

Capasso, R., Evidente, A., Avolio, S., & Solla, F. (1999). A highly convenient synthesis of hydroxytyrosol and its recovery from agricultural waste waters. *Journal of Agricultural and Food Chemistry, 47*, 1745–1748.

De la Puerta, R., Ruiz-Gutierrez, V., & Hoult, J. R. (1999). Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. *Biochemical Pharmacology, 57*, 445–449.

Frankel, E. N., Huang, S.-W., Kanner, J., & German, J. B. (1994). Interfacial phenomena in the evaluation of antioxidants: bulk oils vs emulsions. *Journal of Agricultural and Food Chemistry, 42*, 1054–1059.

Gordon, M. H., Paiva-Martins, F., & Almeida, M. (2001). Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *Journal of Agricultural and Food Chemistry, 49*, 2480–2485.

Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine*. Oxford: Clarendon Press, pp. 416–494.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., et al. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science, 275*, 218–220.

Läubly, M. W., & Bruttel, P. A. (1986). Determination of oxidative stability of fats and oil: comparison between the active oxygen method (AOCS CD 12-57) and the "Rancimat" method. *Journal of the American Oil Chemists' Society, 63*, 792–795.

Lucarini, M., & Pedulli, G. F. (1994). Bond dissociation enthalpy of α-tocopherol and other phenolic antioxidants. *Journal of Organic Chemistry, 59*, 5063–5070.

Moon, J. H., & Terao, J. (1998). Antioxidant activity of caffeic and dihydrocaffeic acid inlard and human low-density lipoprotein. *Journal of Agricultural and Food Chemistry, 46*, 5062–5065.

Nardini, M., D'Aquino, M., Tomassi, G., Gentill, V., Di Felice, M., & Scaccini, C. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic derivatives. *Free Radical Biology and Medicine, 19*, 541–552.

Nenadis, N., Boyle, S., Bakalbassis, E. G., & Tsimidou, M. (2003). An experimental approach to structure–activity relationships of caffeic and dihydrocaffeic acids and related monophenols. *Journal of the American Oil Chemists' Society, 80*, 451–458.

Nenadis, N., & Tsimidou, M. (2002). Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-pycrylhydrazyl (DPPH$^{\bullet}$) tests. *Journal of the American Oil Chemists' Society, 79*, 1191–1195.

Omura, K. (1993). Antioxidant synergism between BHA and BHT. *Journal of the American Oil Chemists' Society, 72*, 1505.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Spiegel-halder, B., & Bartsch, H. (2000). The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer, 36*, 1235–1247.

Petroni, A., Blasevich, M., Salami, M., Papini, M., Montedoro, G. F., & Galli, C. (1995). Inhibition of platelet aggregation and eicosanoid production by phenolic component of olive oil. *Thrombosis Research, 78*, 151–160.

Porter, W. L. (1993). Paradoxical behaviour of antioxidants in food and biological systems. *Toxicology and Industrial Health, 9*, 93–122.

Ranalli, A., Lucera, A., & Contento, S. (2003). Antioxidizing potency of phenol compounds in olive oil mill wastewater. *Journal of Agricultural and Food Chemistry, 51*, 7636–7641.

Rice-Evans, A. C., Miller, N. J., & Paganga, G. (1996). Structure–antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*, 933–956.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Shibata, K., Kubota, T., & Kamisaka, S. (1975). *Plant and Cell Physiology, 16*, 871–877.

Silva, F. M., Borges, F., Guimaraes, J. J., Lima, F. C., Matos, C., & Reis, C. (2000). Phenolic acid derivatives: studies on the relationship among structure, radical scavenging and physicochemical parameters. *Journal of Agricultural and Food Chemistry, 48*, 211–2126.

Visioli, F., Bellomo, G., Montedoro, G., & Galli, C. (1995). Low-density lipoprotein oxidation is inhibited in vitro by olive oil constituents. *Atherosclerosis, 117*, 25–32.