Chapter 97

Structure–Activity Relationship of Phenolic Antioxidants and Olive Components

Juan C. Morales and Ricardo Lucas
Instituto de Investigaciones Químicas, CSIC, 49 Seville, Spain

97.1 INTRODUCTION

Oxygen is essential for the survival of the majority of living organisms but, at the same time, it is considered toxic since it generates free radicals and active oxygen species that have been increasingly related with cardiovascular and inflammatory diseases, and even with a role in cancer and aging (Halliwell and Gutteridge, 1989; Beckman and Ames, 1998). Therefore, antioxidants capable of counteracting the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and in the field of preventive medicine (Block, 1992; Rice-Evans et al., 1996).

A large part of the secondary metabolites of a huge number of plants are antioxidants. They are part of a defense mechanism that plants use to protect themselves from the oxygen free radicals that react with all type of biomolecules within their cells. Polyphenols are the largest family of antioxidants that include a wide variety of structures with a common motif, the phenol molecule. They expand from the simplest structures, such as phenolic acids and alcohols, to the most complex oligomeric ones such as procyanidins.

The phenolic antioxidants have raised the attention of the scientific community since they have revealed very interesting biological properties. For example, resveratrol (**1**) (Figure 97.1), an antioxidant with stilbene structure found in grapes and red wine, has shown anticancer and heart protecting effects (Jang et al., 1997), epigallocatechin (**2**), a flavonoid found in green tea and cocoa, has been described as a potent anti-inflammatory in cells (Selmi et al., 2006), or quercetin (**3**), another flavonoid molecule found in onions and several vegetables, possesses anti-atherosclerotic properties (Kawai et al., 2008).

Certainly, olives and olive oil contain several fascinating complex phenolic molecules such as oleuropein (**4**) or verbascoside (**5**) and other simpler molecules, such as tyrosol (**6**) or hydroxytyrosol (**7**) (Figure 97.2). In particular, hydroxytyrosol has been an important focus of research since its discovery (Ragazzi and Veronese, 1973). Hydroxytyrosol inhibits human LDL oxidation (Visioli et al., 1995), inhibits platelet aggregation (Petroni et al., 1995) and exhibits anti-inflammatory (de la Puerta et al., 1999) and anticancer properties (Owen et al., 2000).

It is important to note that these fruits and vegetables are part of our diet, and therefore, we could also take advantage of the antioxidant properties of these molecules for food preservation. In fact, antioxidants have been added to food for years to avoid oxidation processes and are widely used today. Traditionally, synthetic phenolic antioxidants such as BHT (2,6-di-*tert*-butyl-4-methylphenol, **8**), BHA (2, *tert*-butyl-4-methoxyphenol, **9**) or TBHQ (*tert*-butyl-hydroquinone, **10**) (Figure 97.3) have been widespread since they possess good antioxidant capacity although they have recently been questioned due to possible side effects for human health (Omura, 1995). Nowadays, several natural phenolic derivatives are

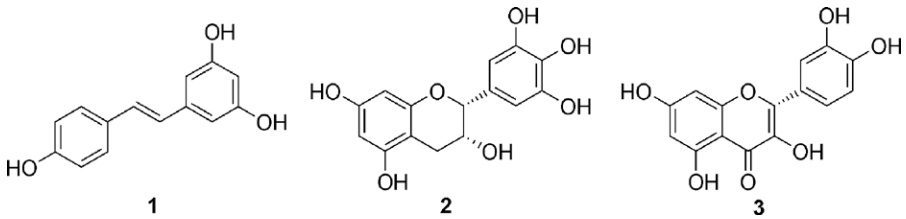


FIGURE 97.1 Structures of resveratrol (**1**), epigallocatechin (**2**), and quercetin (**3**).

Olives and Olive Oil in Health and Disease Prevention.
ISBN: 978-0-12-374420-3

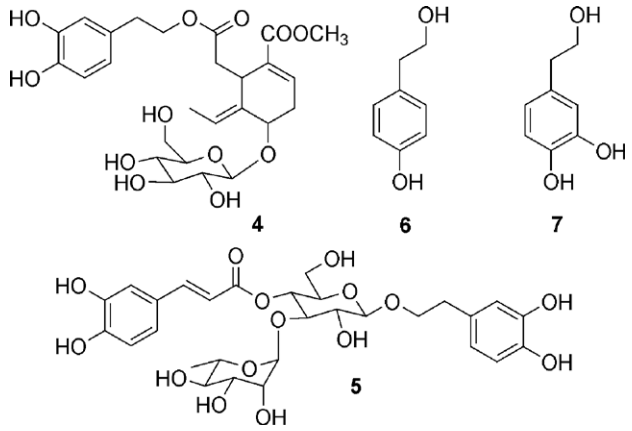


FIGURE 97.2 Structures of oleuropein (**4**), verbascoside (**5**), tyrosol (**6**) and hydroxytyrosol (**7**).

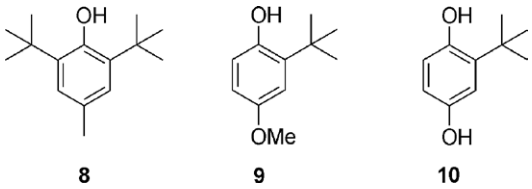


FIGURE 97.3 Structures of BHT, (2,6-di-*tert*-butyl-4-methylphenol, **8**), BHA, (2, *tert*-butyl-4-methoxyphenol, **9**) and TBHQ, (*tert*-butyl-hydroquinone, **10**).

being used as food antioxidants, for example, catechins in green tea extracts and rosmarinic acid in rosemary extracts.

Our group and others are carrying out research that could reveal which are the key structural features that make a compound a good antioxidant. Although there is a wealth of data on the importance of antioxidants in conferring stability towards or protection from oxidation, the correlation between antioxidant activity and chemical structure is far from clear.

We have decided to focus our attention on the most potent antioxidant in olive oil, hydroxytyrosol, so that it would be the starting point to explore the structural features of phenolic-based antioxidants and as a result, help us to understand how the different functionalities affect the antioxidant activity. The criteria that would be used to compare antioxidant capacity will be based on radical scavenging tests, especially the extensively used DPPH (Nenadis and Tsimidou, 2002) and ABTS methods (Re et al., 1999) and on other common experimental values used to study food antioxidants, such as the Rancimat test or spectrophotometric data used to follow oxidation in emulsion experiments, such as the conjugate diene formation.

This chapter encloses several sections where different structural aspects that affect the antioxidant capacity of phenolic derivatives will be described and discussed. As mentioned above, hydroxytyrosol will be our reference compound and the discussion will concentrate on the comparison of radical scavenging activity tests and food antioxidant capacity of different phenolic derivatives. The different aspects discussed are shown in Figure 97.4.

97.2 THE INFLUENCE OF THE NUMBER OF PHENOLIC HYDROXYL GROUPS

The main structural feature responsible for the antioxidative and free radical scavenging activity in the case 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. This effect is modulated by the ring substituents, so that electron-withdrawing groups increase the bond-dissociation enthalpy, due to the stabilization of the phenol by a polar structure that leaves a positive charge on the OH group. Consequently, electron-donating groups produce a reduction of the bond-dissociation enthalpy due to the stabilization of the phenoxyl radical by mesomeric structures bearing a positive charge on the substituent. This is the case in the presence of a second hydroxyl group at the ortho-position, yielding a catechol ring that also lowers the OH bond dissociation enthalpy and increases the rate of H-atom transfer to peroxyl radicals (Lucarini and Pedulli, 1994). In fact, early comparisons between tyrosol and hydroxytyrosol (Figure 97.2) (Chimi et al., 1991; Benavente-García et al., 2000) showed a much better radical scavenging capacity for hydroxytyrosol than for tyrosol. Similar results were obtained when comparing them as antioxidants in a refined olive oil matrix, where hydroxytyrosol showed up to five times higher induction time (IT) in the Rancimat test than tyrosol (Mateos et al., 2003; Artajo et al., 2006). A phenolic derivative of hydroxytyrosol containing three hydroxyl groups in the phenolic ring has not been isolated or prepared up to now, so there are no antioxidant data available. Nevertheless, comparisons between protocatechuic acid and gallic acid (Ranalli et al., 2003), or between protocatechuyl alcohol (3,4-dihydroxibenzylic alcohol, **11**) and galloyl alcohol (3,4,5-trihydroxybenzylic alcohol, **12**) (Torres de Pinedo et al., 2007a) (Figure 97.5) clearly show that the presence of a third hydroxyl group in the phenolic ring increases the antioxidant capacity, either in radical scavenging tests or with longer induction times obtained in the Rancimat test. In fact, galloyl alcohol is a better antioxidant than hydroxytyrosol as a radical scavenger and protecting an oil matrix against rancidity (Figure 97.6). The same trend has been previously observed for the antioxidant family of the flavonoids (Rice-Evans et al., 1996).

97.3 THE INFLUENCE OF THE NUMBER OF METHOXY GROUPS IN THE PHENOL RING

The methoxy groups in the phenol rings are common substituents in natural phenolic antioxidants, such as phenolic

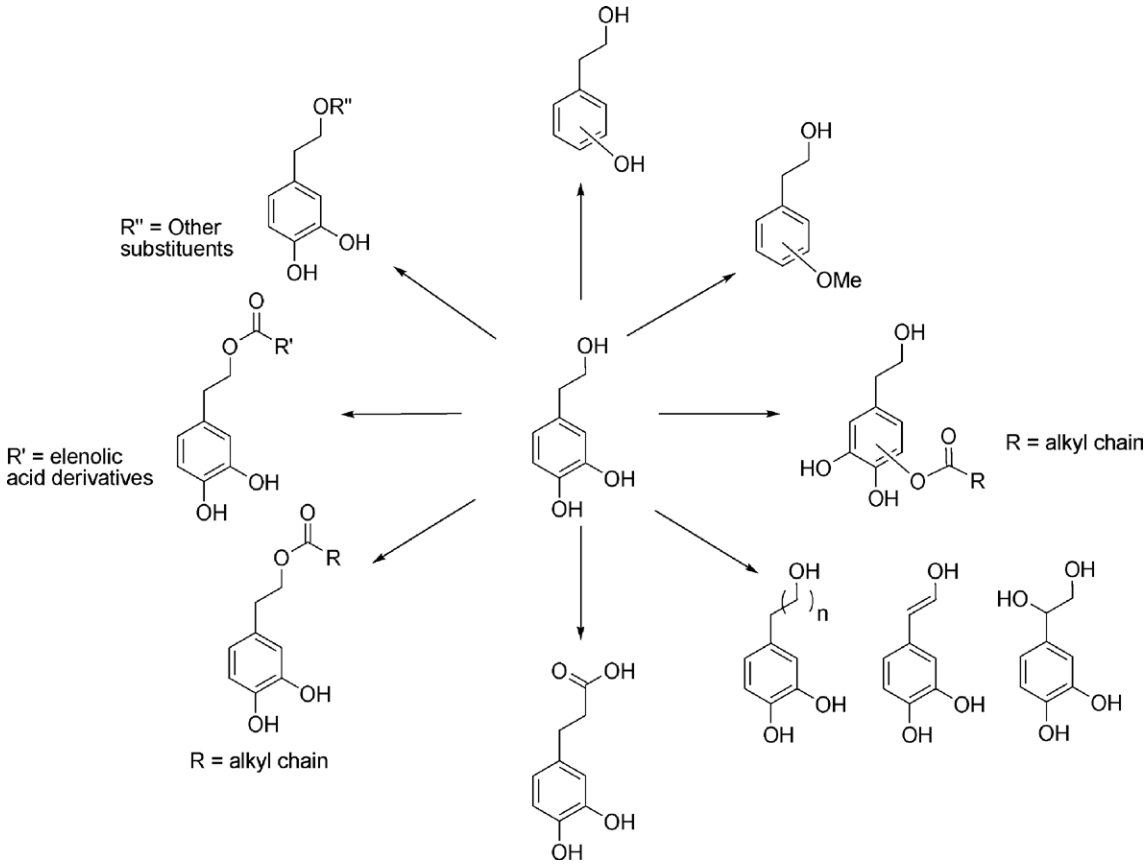
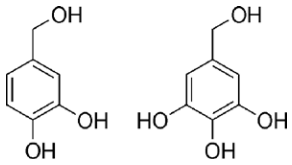


FIGURE 97.4 Phenolic structures that show the different aspects that will be discussed in comparison with hydroxytyrosol.



11 **12**

FIGURE 97.5 Structures of protocatechuyl alcohol (3,4-dihydroxibenzylic alcohol, **11**) and galloyl alcohol (3,4,5-trihydroxybenzylic alcohol, **12**).

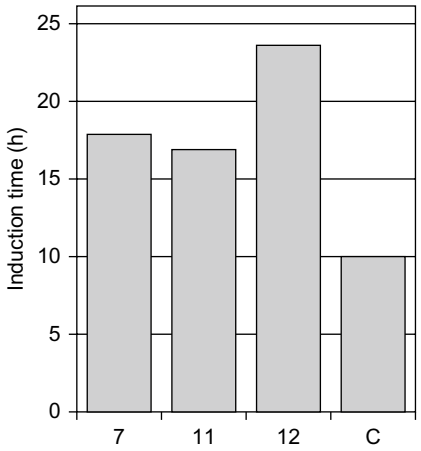


FIGURE 97.6 Average induction time values in the Rancimat test of hydroxytyrosol (**7**), protocatechuyl alcohol (3,4-dihydroxibenzylic alcohol, **11**) and galloyl alcohol (3,4,5-trihydroxybenzylic alcohol, **12**). C is the control olive oil with no added compounds.

alcohols, vanillic alcohol, homovanillic alcohol or veratryl alcohol and phenolic acids, vanillic acid, homovanillic acid, syringic acid, synapic acid, or ferulic acid. Preliminary data on radical scavenging efficiency comparing caffeic acid (**13**) and ferulic acid (**14**) were described by Son and Lewis (2002) (Figure 97.7). They observed that inhibition of DPPH radicals was almost double for caffeic acid containing a di-ortho phenolic motif than for ferulic acid where a methoxy group replaces the OH group at position meta. Again, this tendency is confirmed in a food matrix, where the Rancimat test shows that caffeic acid protects an oil matrix much better than ferulic acid, yielding three times longer induction time (Artajo et al., 2006).

Similar results were obtained in our group when comparing the radical scavenging capacity of phenolic alcohols that contain either a di-ortho phenolic motif, or a methoxyphenol motif or a dimethoxy phenyl ring (Torres de Pinedo et al., 2007a) (Figure 97.8). Their radical scavenging activity decreased in the following order: the most potent radical scavengers were the antioxidants with di-ortho phenolic

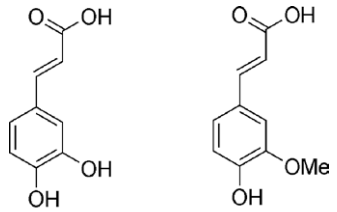


FIGURE 97.7 Structures of caffeic acid (**13**) and ferulic acid (**14**).

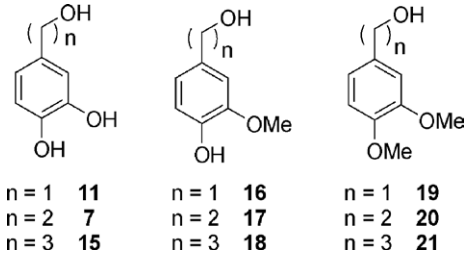


FIGURE 97.8 Structures of protocatechuyl alcohol (**11**), hydroxytyrosol (**7**), dihydrocaffeoyl alcohol (**15**), vanillyl alcohol (**16**), homovanillyl alcohol (**17**), dihydroconiferyl alcohol (**18**), veratryl alcohol (**19**), homoveratryl alcohol (**20**) and 3-(3,4-dimethoxyphenyl) propanol) (**21**).

structure (protocatechuyl alcohol **11**, hydroxytyrosol **7** and dihydrocaffeoyl alcohol **15**), then the mono-phenolic compounds (vanillyl alcohol **16**, homovanillyl alcohol **17** and dihydroconiferyl alcohol **18**), and finally, with very low radical inhibition capacity, the compounds with both hydroxyl groups methylated (veratryl alcohol **19**, homoveratryl alcohol **20** and 3-(3,4-dimethoxyphenyl) propanol) **21**) (Figure 97.9). When the antioxidant activity was tested in oils, we observed much longer induction times in the Rancimat test for the phenolic alcohols containing the di-ortho phenolic motif than either of the other two groups. Actually, the induction times for the phenolic alcohols containing a methoxy-phenol motif or a dimethoxy phenyl ring were quite similar, in contrast to the radical scavenging efficiency observed.

It is important to note that when a methoxy group is not replacing a hydroxyl group in the phenolic antioxidant structure it could also play a role (Torres de Pinedo et al., 2007a). Actually, the introduction of a methoxy group to a di-ortho phenolic structure, such as in 5-methoxy-protocatechuyl alcohol (**22**), increases the hydrogen-donating ability and therefore the radical scavenging capacity of the antioxidant when compared with the parent antioxidant, protocatechuyl alcohol (**11**) (Figure 97.10). A similar case is observed when syringyl alcohol (**23**) is compared with vanillyl alcohol (**16**), where the additional 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 radical. Curiously, this effect is not so clear when these antioxidants are compared in their capacity to stabilize an olive oil by the Rancimat test. Thus, 5-methoxy-protocatechuyl alcohol (**22**), that possesses an

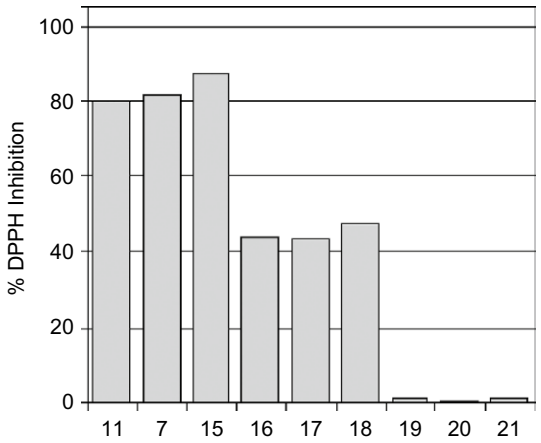


FIGURE 97.9 DPPH radical scavenging activity of protocatechuyl alcohol (**11**), hydroxytyrosol (**7**), dihydrocaffeoyl alcohol (**15**), vanillyl alcohol (**16**), homovanillyl alcohol (**17**), dihydroconiferyl alcohol (**18**), veratryl alcohol (**19**), homoveratryl alcohol (**20**) and 3-(3,4-dimethoxyphenyl) propanol) (**21**).

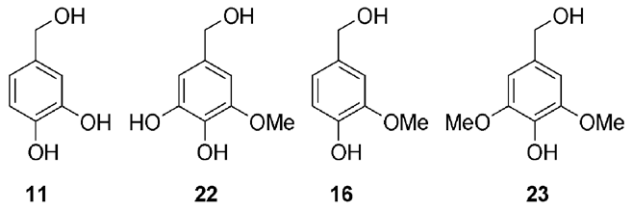


FIGURE 97.10 Structures of protocatechuyl alcohol (**11**), vanillyl alcohol (**16**), 5-methoxy-protocatechuyl alcohol (**22**) and syringyl alcohol (**23**).

extra methoxy group in its structure than protocatechuyl alcohol, shows lower stability than oils containing protocatechuyl alcohol (**11**). Whereas, syringyl alcohol (**23**) with an extra methoxy group than vanillyl alcohol (**16**), increases slightly the stability of the oil when compared to vanillyl alcohol, as shown by the Rancimat data.

97.4 THE INFLUENCE OF ACYL GROUPS IN THE PHENOL RING

Naturally occurring phenolic antioxidants are strongly hydrophilic, making difficult their use as fat and oil antioxidants since they do not incorporate easily in desired amounts in these food matrices. Several research groups have approached this problem by preparing lipophilic antioxidants from natural sources, for example, isoflavone fatty acid esters (Lewis et al., 2000), lipophilic clovamide derivatives or poly(lauroy(+)-catechins.

We and others reported recently the synthesis of a family of di-ortho phenolic fatty acid esters (Torres de Pinedo et al., 2005) that included several hydroxytyrosol fatty acid

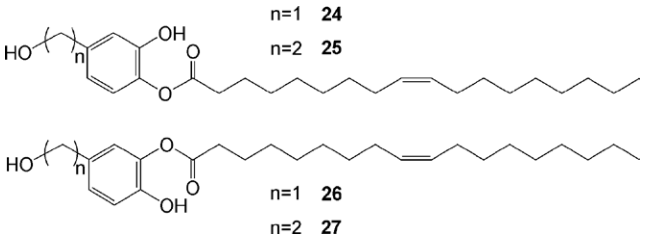


FIGURE 97.11 Structures of 4-hydroxy-3-oleoyloxibenzylic alcohol (**24**), 2-(4-hydroxy-3-oleoyloxiphenyl) ethanol (**25**), 3-hydroxy-4-oleoyloxibenzylic alcohol (**26**), 2-(3-hydroxy-4-oleoyloxiphenyl) ethanol (**27**), 3,4-dihydroxybenzyl oleate (**28**) and 2-(3,4-dihydroxyphenyl)ethyl oleate (**29**).

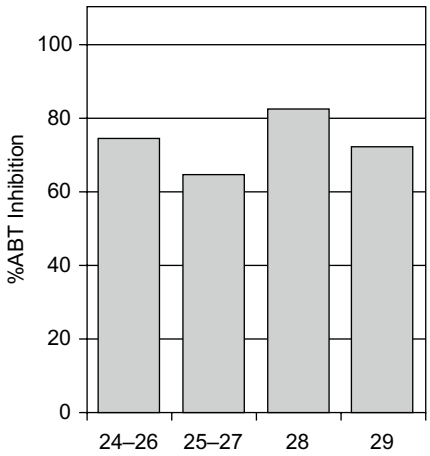


FIGURE 97.12 ABTS radical scavenging activity of 4-hydroxy-3-oleoyloxibenzylic alcohol (**24**), 2-(4-hydroxy-3-oleoyloxiphenyl) ethanol (**25**), 3-hydroxy-4-oleoyloxibenzylic alcohol (**26**), 2-(3-hydroxy-4-oleoyloxiphenyl) ethanol (**27**), 3,4-dihydroxybenzyl oleate (**28**) and 2-(3,4-dihydroxyphenyl)ethyl oleate (**29**).

esters (Trujillo et al., 2006; Grasso et al., 2007). All these fatty acid derivatives are substituted at the primary alcohol position of the alkyl chain and will be discussed in a section below. Nevertheless, a second family of fatty acid derivatives (compounds **24–27**) was prepared by our group where the ester bond is formed at one of the hydroxy phenol groups (Torres de Pinedo et al., 2007b) (Figure 97.11). The radical scavenging capacity of the phenolic fatty acid esters substituted at the phenolic group was less efficient than for the phenolic fatty acid esters substituted at the primary alcohol that conserved the di-orthophenolic motif in their structure (compounds **28** and **29**) (Figure 97.12). It seems that the existence of the extra primary alcohol in the phenolic fatty acid esters substituted at the phenolic group is not enough to equal the scavenging potency of the lipophilic antioxidants that contain the di-orthophenolic motif. In fact, this effect is much more dramatic when the antioxidant activity is compared in an oil matrix using the Rancimat test (Figure 97.13). The position of acylation of the phenolic alcohol seems to be an important factor in determining the effectiveness of lipid antioxidant activity. When oleic acid is attached to the primary alcohol of the

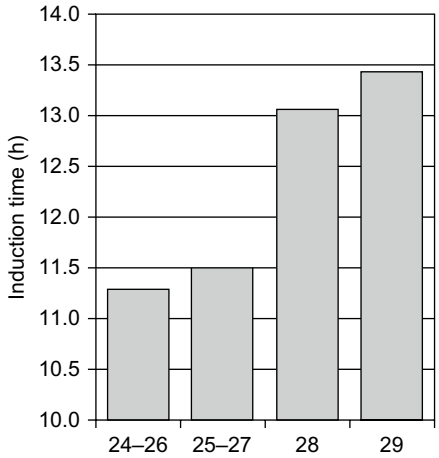


FIGURE 97.13 Average induction time values in the Rancimat test of 4-hydroxy-3-oleoyloxibenzylic alcohol (**24**), 2-(4-hydroxy-3-oleoyloxiphenyl) ethanol (**25**), 3-hydroxy-4-oleoyloxibenzylic alcohol (**26**), 2-(3-hydroxy-4-oleoyloxiphenyl) ethanol (**27**), 3,4-dihydroxybenzyl oleate (**28**) and 2-(3,4-dihydroxyphenyl)ethyl oleate (**29**).

phenolic alcohols, much longer ITs were observed than when oleic acid is attached to one of the phenolic groups.

97.5 THE INFLUENCE OF THE LENGTH AND NATURE OF THE ALKYL CHAIN

The effect of the length of the alkyl chain connecting the phenolic ring and the carboxylic or alcohol group in phenolic derivatives may play some role helping to stabilize the radical formed during the oxidation. Artajo et al. compared vanillic acid and homovanillic acid to find that their ITs measured at different concentrations in a refined olive oil were slightly longer for homovanillic acid that possesses a longer alkyl chain than vanillic acid (Artajo et al., 2006). We observed a similar trend when comparing phenolic alcohols which structures contain one, two or three methylene groups at the alkyl chain (protocatechuyl alcohol **11**, hydroxytyrosol **7** and dihydrocaffeoyl alcohol **15**, see Figure 97.8). An increase in the length of the alkyl chain increases the radical scavenging capacity of the antioxidant and the phenolic antioxidants also show longer ITs in the Rancimat test (Torres de Pinedo et al., 2007a) (Figure 97.14). This effect is probably due to the fact that as the alkyl chain increases its electron-donating activity also does, resulting in a better-stabilized phenoxy radical. Moreover, this small but clear effect is also observed when these phenolic alcohols are converted in the corresponding phenolic fatty acid esters with palmitic acid, stearic acid or oleic acid (Torres de Pinedo et al., 2007b).

The chain connecting the phenyl ring and the carboxylic or the alcohol group in these phenolic derivatives may contain an alkene moiety and its contribution remains controversial. It was first investigated by free radical scavenging

assays, by comparing caffeic acid (**13**) and dehydrocaffeic acid (**30**) (Figure 97.15). No difference was found in the DPPH test by Moon and Terao (1998), whereas several other authors found that dehydrocaffeic acid could scavenge radicals approximately 1.6 times better than caffeic acid (Chen et al., 1999; Silva et al., 2000; Nenadis et al., 2003a). It could be argued that the reduced activity of caffeic acid is due to the electron-withdrawing character of the double bond in the side chain, but the fact that caffeic acid is much faster scavenging the DPPH than dihydrocaffeic acid should also be considered (Nenadis et al., 2003a). Concerning their antioxidant activity in bulk oils, dihydrocaffeic acid was slightly better than caffeic acid, and it has been suggested that this could be due to the stability of the radicals, but also could be related to faster radical side reactions due to the presence of the alkene group.

In the case of the phenolic alcohols, radical scavenging experiments showed a different tendency than for the phenolic acids. DPPH data obtained by our research group revealed that caffeoyl alcohol (**31**) was a better radical scavenger than dihydrocaffeoyl alcohol (**15**) (Torres de Pinedo et al., 2007a) (Figure 97.15). It seems that the presence of the primary alcohol allows an extended conjugation and better radical stability when the alkene group is present. In contrast, this effect is reversed in the Rancimat test and caffeoyl alcohol shows shorter induction time than dihydrocaffeoyl alcohol, probably due to the fact that the unsaturated sidechain could also be oxidized and participate in the active degradation of the oil matrix. Once again, the radical scavenging and antioxidant results obtained for the phenolic alcohols get reproduced in the corresponding phenolic fatty acid esters with palmitic acid, stearic acid or oleic acid (Torres de Pinedo et al., 2007b) (Figure 97.10), showing that the fatty acid is far enough from the phenol moiety that it does not affect its antioxidant capacity, and only changes its lipophilicity.

Another modification found in the alkyl chain that connects the phenyl ring and the alcohol group is an additional hydroxyl group. 3,4-dihydroxyphenylglycol (DHPG, **32**) (Figure 97.15) was first described by Bianchi and Pozzi (1994) and its antioxidant properties have been recently investigated (Rodríguez et al., 2007). DHPG showed higher antiradical activity and better reducing power than hydroxytyrosol, most probably due to the possibility of the extra hydroxyl group to be oxidized to the corresponding ketone. We have observed spontaneous oxidation of hydroxytyrosol in aqueous solution at room temperature to the corresponding aldehyde and finally to the carboxylic acid.

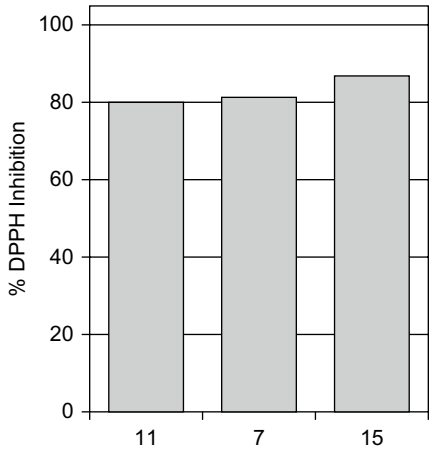


FIGURE 97.14 DPPH radical scavenging activity of protocatechuyl alcohol (**11**), hydroxytyrosol (**7**) and dihydrocaffeoyl alcohol (**15**).

97.6 THE INFLUENCE OF FUNCTIONAL GROUP AT THE END OF THE ALKYL CHAIN

Another structural feature that may be important to increase the antioxidant capacity of our reference compound, hydroxytyrosol, is the primary hydroxyl group in its alkyl chain. In fact, hydroxytyrosol (**7**) is a better antioxidant when added to olive oil than 3,4-dihydroxyphenyl acetic acid (**33**) or caffeic acid (**13**) (Figure 97.16), all of them containing the same diortophenolic structure. Longer induction times (Ranalli et al., 2003; Artajo et al., 2006), lower concentration needed to inhibit DPPH or ABTS radicals (Benavente-García et al., 2000; Bouaziz et al., 2005) and lower peroxide values (Papadopoulos and Boskou, 1991) confirmed the importance of the primary hydroxyl group. These data are in contrast to the reported values for 3,4-dihydroxyphenylacetic acid versus hydroxytyrosol (Figure 97.11) where peroxide and conjugated diene values in refined oils were slightly lower for the acid than for hydroxytyrosol (Papadopoulos and Boskou, 1991; Fki et al., 2005).

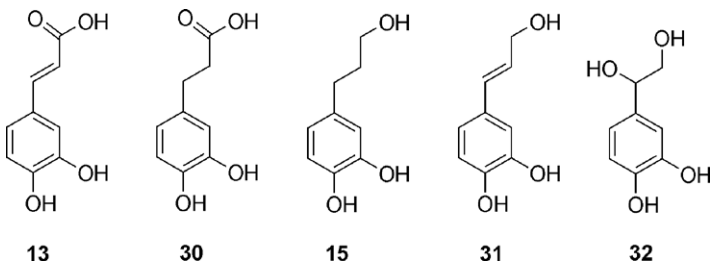


FIGURE 97.15 Structures of caffeic acid (**13**), dihydrocaffeoyl alcohol (**15**), dehydrocaffeic acid (**30**), caffeoyl alcohol (**31**) and 3,4-dihydroxyphenylglycol (DHPG, **32**).

Assays that compare the reducing ability of phenolic antioxidants that possess a hydroxyl group versus a carboxylic acid have not been reported so far. Definitely, this must be an important point since data from our laboratory support the spontaneous oxidation of hydroxytyrosol in aqueous solution at room temperature as described above, yielding the corresponding aldehyde and finally the corresponding carboxylic acid.

Tsimidou et al. reported a very interesting study on the structure–antioxidant activity relationship of ferulic acid derivatives comparing different functional groups at the side chain (Nenadis et al., 2003b). They observed that isoeugenol (**34**) and coniferyl alcohol (**35**), both containing electron-donating groups, were better radical scavengers and better antioxidants in bulk oil experiments than ferulic acid (**14**), coniferyl aldehyde (**36**) or ethyl ferulate (**37**) (Figure 97.17). In oil-in-water emulsion autoxidation experiments, lipophilicity of the phenols was the determining factor because the least polar compounds, isoeugenol (**34**) and ethyl ferulate (**37**), were the most effective ones.

97.7 THE INFLUENCE OF AN ACYL GROUP AT THE ALKYL CHAIN

Phenolic antioxidants have been modified with alkyl groups to increase their lipophilicity and make them more soluble in fats and oils, as was mentioned briefly above. In fact, hydroxytyrosol acetate, a natural component of olive oil, was the first one to be described (Brenes et al., 1999) and its antioxidant properties studied (Gordon et al., 2001; Mateos et al., 2003). Hydroxytyrosol acetate (**38**) (Figure 97.18) possesses a weaker DPPH radical scavenging activity than hydroxytyrosol, and Gordon et al. have suggested that the ester group could be hindering the scavenging effect of the hydroxyl groups by intra- or intermolecular hydrogen bonding. Since the radical scavenging assay was carried out in methanol it is difficult to imagine such an interpretation. The difference found in radical scavenging is better explained from the fact that the ester group is an electron-withdrawing group and therefore the phenoxy radical formed may be less stable than in the case of hydroxytyrosol, with a primary alcohol acting as an electron-donating group.

Oxidation experiments to determine the peroxide value, conjugated dienes, p-anisidine value, and induction time showed similar antioxidant activity for hydroxytyrosol acetate and hydroxytyrosol, with the ester being slightly less effective in oil, but slightly more effective in an oil-in-water emulsion (Gordon et al., 2001; Mateos et al., 2003).

Recently, our research group reported the synthesis of a family of di-ortho phenolic fatty acid esters (Torres de Pinedo et al., 2005) that included several hydroxytyrosol fatty acid esters (Figure 97.18) (Trujillo et al., 2006; Grasso et al., 2007). These fatty acid derivatives are substituted at the primary alcohol position of the alkyl chain and the following compounds have been prepared: hydroxytyrosol acetate (**38**), hydroxytyrosol propionate (**39**), hydroxytyrosol butanoate (**40**), hydroxytyrosol decanoate (**41**), hydroxytyrosol laurate (**42**), hydroxytyrosol palmitate (**43**), hydroxytyrosol stearate (**44**), hydroxytyrosol oleate (**45**), and hydroxytyrosol linoleate (**46**). The hydroxytyrosol lipophilic analogues showed similar antioxidant activity to hydroxytyrosol, both in the DPPH radical scavenging assay (Grasso et al., 2007) and in the Rancimat test carried out in a polyphenol purified virgin olive oil (Trujillo et al., 2006). These data seem to confirm that the di-ortho-phenolic moiety is one of the most important determinants in antioxidant capacity of phenolic antioxidants.

97.8 THE INFLUENCE OF OTHER ESTER GROUPS AT THE ALKYL CHAIN: OLEUROPEIN, SECOIRIDOIDS AND OTHER OLIVE OIL COMPONENTS

The major polyphenolic constituent in olives is oleuropein glycoside (**4**) that contains hydroxytyrosol in its structure

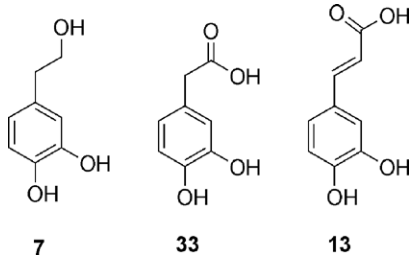


FIGURE 97.16 Structures of hydroxytyrosol (**7**), caffeic acid (**13**) and 3,4-dihydroxyphenyl acetic acid (**33**).

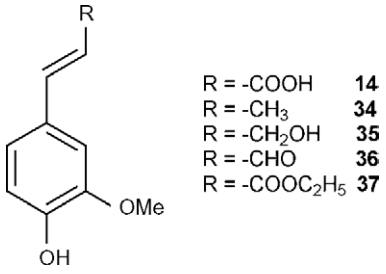


FIGURE 97.17 Structures of ferulic acid (**14**), isoeugenol (**34**) coniferyl alcohol (**35**), coniferyl aldehyde (**36**) and ethyl ferulate (**37**).

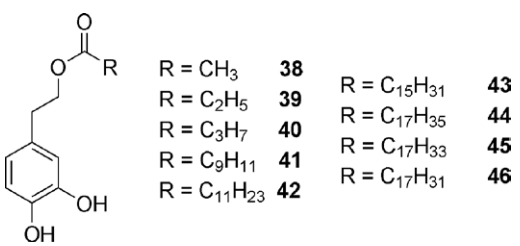


FIGURE 97.18 Structures of hydroxytyrosol fatty acid esters (**38–46**).

(Figure 97.2), but this compound is almost completely absent from olive oil because of its high water solubility. Apart from the phenolic acids and the phenolic alcohols, including hydroxytyrosol, the prevalent phenolic compounds found in virgin olive oil are secoiridoid derivatives of oleuropein such as the dialdehydic form of elenolic acid linked either to hydroxytyrosol (HTyr-EDA, **47**) or to tyrosol, and an isomer of oleuropein aglycon (hydroxytyrosol elenolic acid ester (HTyr-EA, **48**) (Figure 97.19). These compounds are the most concentrated of those with a phenolic structure in virgin olive oil, and the hydroxytyrosol derivatives are of particular significance because of their strong antioxidant activity.

Oleuropein has been reported to possess slightly weaker radical scavenging activity than hydroxytyrosol by the DPPH (Saija et al., 1998; Valavanidis et al., 2004; Bouaziz et al., 2005) and ABTS methods (Benavente-García et al., 2000), nevertheless, the opposite result was described when using the 5,5-dimethyl-1-pyrroline-N-oxide (Chimi et al., 1991). Comparison of hydroxytyrosol with oleuropein, HTyr-EDA and HTyr-EA showed that the radical scavenging capacity decreased as follows: hydroxytyrosol > HTyr-EDA > oleuropein > HTyr-EA (Carrasco-Pancorbo et al., 2005; Morello et al., 2005). In contrast, previous data that compared DPPH values indicated that oleuropein has a higher scavenging activity than HTyr-EDA (Paiva-Martins and Gordon, 2001).

Recently, two new secoiridoid derivatives have been isolated in olive mill waste extracts, olive leaves and olive fruits (Figure 97.20) (Karioti et al., 2006; Obied et al., 2007). Importantly, hydroxytyrosol acyclodihydroelenolate (**49**) showed higher efficiency as a radical scavenger than hydroxytyrosol in the DPPH assay, probably due to the fact that two extra hydroxyl groups appear in the structure. However, the second compound, comselogoside (6′-p-coumaroylsecologanoside, **50**) showed less efficiency as a radical scavenger than hydroxytyrosol or oleuropein.

Some of these secoiridoid derivatives have been tested in several lipid systems including oil. HTyr-EDA and HTyr-EA showed similar induction times at different molar concentrations in the Rancimat test when tested in purified olive oil (Baldioli et al., 1996) which seems to point to the fact that the di-ortho-phenolic moiety is once again a crucial structural factor of these phenolic antioxidants. However, recent data from two different groups have reported much lower induction times for HTyr-EA than for HTyr-EDA which brings controversy to this topic and is clearly demanding new experimental data for these olive components as antioxidants in oil matrices (Carrasco-Pancorbo et al., 2005; Artajo et al., 2006).

97.9 THE INFLUENCE OF OTHER MODIFICATIONS AT THE ALKYL CHAIN

Three other olive oil components have at least one phenolic ring in their structures: verbascoside (**5**) (Figure 97.2) and the lignans, pinoresinol (**51**) and acetoxypinoresinol (**52**) (Figure 97.21). The experimental data on the antioxidant capacity of these compounds are very scarce. Verbascoside, which contains two di-orthophenolic moieties in its structure, shows induction times in the Rancimat test slightly shorter than oleuropein and there are no data that compare it directly with hydroxytyrosol (Artajo et al., 2006).

Contradictory results have been reported for the antioxidant activity of the lignans. Artajo et al. have described a slight increase in the oxidative stability of a refined olive oil, whereas Carrasco-Pancorbo et al. showed a pro-oxidant effect in the OSI test (a method similar to the Rancimat test)

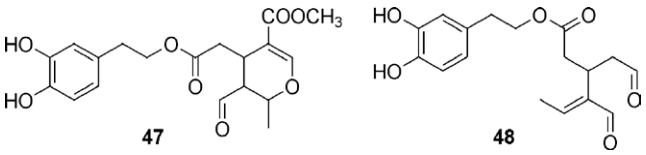


FIGURE 97.19 Structures of dialdehydic form of elenolic acid linked either to hydroxytyrosol (HTyr-EDA, **47**) and hydroxytyrosol elenolic acid ester (HTyr-EA, **48**).

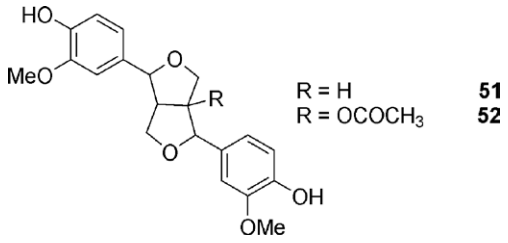


FIGURE 97.21 Structures of pinoresinol (**51**) and acetoxypinoresinol (**52**).

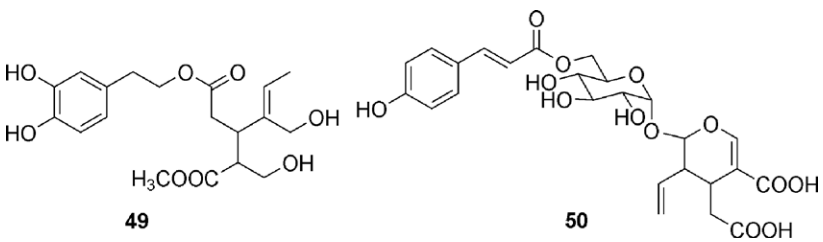


FIGURE 97.20 Structures of hydroxytyrosol acyclodihydroelenolate (**49**) and comselogoside (6'-*p*-coumaroylsecologanoside, **50**).

when triolein was enriched with acetoxypinoresinol and pinoresinol (Carrasco-Pancorbo et al., 2005). Artajo et al. argue that such a difference could be due to a synergetic effect since their antioxidant experiments were carried out in an oil matrix that contains some amounts of polyphenols (α-tocopherol and carotenoids).

SUMMARY POINTS

- The di-ortho phenolic structural motif seems to be crucial for antioxidant activity of these phenolic derivatives.
- A third hydroxyl group in the phenolic ring, as for gallic acid or galloyl alcohol, increases the antioxidant capacity with respect to hydroxytyrosol.
- Replacement of hydroxyl groups at the phenolic ring with methoxy groups decreases the antioxidant activity.
- Extra methoxy groups added to the phenolic moiety, in general, yield better radical scavengers and better antioxidants to stabilize fats and oils.
- Hydroxytyrosol fatty acid esters with the acyl group at the hydroxy phenol group possess lower antioxidant activity than the parent compound, hydroxytyrosol.
- Hydroxytyrosol fatty acid esters with the acyl group substituted at the primary alcohol show similar radical scavenging and antioxidant capacity in fats and oils to hydroxytyrosol.
- When the size of the alkyl chain in the phenolic structure increases, the antioxidant properties of the compounds also improve.
- The effect of the presence of an alkene moiety in the chain connected to the phenolic ring is still controversial.
- Contradictory results have been obtained when hydroxytyrosol and its analogue were compared with a carboxylic acid group at the end of the alkyl chain, 3,4-dihydroxyphenylacetic acid.
- Secoiridoid derivatives are potent antioxidants, although there are scarce data comparing them with hydroxytyrosol.

REFERENCES

Artajo, L.S., et al., 2006. Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index. J. Agric. Food Chem. 54, 6079–6088.

Baldioli, M., et al., 1996. Antioxidant activity of tocohopherols and phenolic compouns of virgin olive oil. J. Am. Oil Chem. Soc. 73, 1589–1593.

Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.

Benavente-García, O., et al., 2000. Antioxidant activity of phenolics extracted from *Olea europea* L. leaves. Food Chem. 68, 457–462.

Bianchi, G., Pozzi, N., 1994. 3,4-dihydroxyphenylglycol, a major C6-C2 phenolic in *Olea europea* fruits. Phytochemistry 35, 1335–1337.

Block, G., 1992. The data support a role for antioxidants in reducing cancer risk. Nutr. Rev. 50, 207–213.

Bouaziz, M., et al., 2005. Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar chemlali growing in Tunisia. J. Agric. Food Chem. 53, 236–241.

Brenes, M., et al., 1999. Phenolic compounds in Spanish olive oils. J. Agric. Food Chem. 47, 3535–3540.

Carrasco-Pancorbo, A., et al., 2005. Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J. Agric. Food Chem. 53, 8918–8925.

Chen, C.P., et al., 1999. Inhibitory effect of caffeic acid analogues isolated from Salviae Miltiorrhizae Radix against 1,1-diphenyl-2-picrylhydrazyl radical. Exp. Toxicol. Pathol. 51, 59–63.

Chimi, H., et al., 1991. Peroxyl and hydroxyl radical scavenging activity of some natural phenolic antioxidants. J. Am. Oil Chem. Soc. 68, 307–312.

de la Puerta, R., et al., 1999. Inhibition of leukocyte 5-lipoxygenase by phenolics from virgin olive oil. Biochem. Pharmacol. 57, 445–449.

Fki, I., et al., 2005. The use of polyphenolic extract, purified hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid from olive mill wastewater for the stabilization of refined oils: a potential alternative to synthetic antioxidants. Food Chem. 93, 197–204.

Gordon, M.H., et al., 2001. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. J. Agric. Food Chem. 49, 2480–2485.

Grasso, S., et al., 2007. Hydroxytyrosol lipophilic analogues: enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. Bioorg. Chem. 35, 137–152.

Halliwell, B., Gutteridge, J. M. C., 1989. In: Press, C. (Ed.), Free radicals in Biology and Medicine. Oxford, pp. 416–494.

Jang, M., et al., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275, 218–220.

Karioti, A., et al., 2006. Novel secoiridoid glucosides in Olea europea leaves suffering from boron defficiency. Biosci. Biotechnol. Biochem. 70, 1898–1903.

Kawai, Y., et al., 2008. Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries: implication in the anti-atherosclerotic mechanism of dietary flavonoids. J. Biol. Chem. 283, 9424–9434.

Lewis, P.T., et al., 2000. Synthesis of antioxidants isoflavone fatty acid ester. Tetrahedron 56, 7805–7810.

Lucarini, M., Pedulli, G.F., 1994. Bond dissociation enthalpy of α-tocopherol and other phenolic antioxidants. J. Org. Chem. 59, 5063–5070.

Mateos, R., et al., 2003. Antioxidant effect of phenolic compounds, alpha-tocopherol, and other minor components in virgin olive oil. J. Agric. Food Chem. 51, 7170–7175.

Moon, J.H., Terao, J., 1998. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. J. Agric. Food Chem. 46, 5062–5065.

Morello, J.R., et al., 2005. Antioxidant activity of olive pulp and olive oil phenolic compounds of the arbequina cultivar. J. Agric. Food Chem. 53, 2002–2008.

Nenadis, N., Tsimidou, M., 2002. Observations on the estimation of scavenging activity of phenolic compounds using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) tests. J. Am. Oil Chem. Soc. 79.

Nenadis, N., et al., 2003a. An experimental approach to structure-activity relationships of caffeic and dihydrocaffeic acids and related monophenols. J. Am. Oil Chem. Soc. 80, 451–458.

Nenadis, N., et al., 2003b. Structure-antioxidant activity relationship of ferulic acid derivatives: effect of carbon side chain characteristic groups. J. Agric. Food Chem. 51, 1874–1879.

Obied, H.K., et al., 2007. Novel secoiridoids with antioxidant activity from Australian olive mill waste. J. Agric. Food. Chem. 55, 2848–2853.

Omura, K., 1995. Antioxidant synergism between BHA and BHT. J. Am. Oil Chem. Soc. 72.

Owen, R.W., et al., 2000. Olive-oil consumption and health: the possible role of antioxidants. Lancet Oncol. 1, 107–112.

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

Papadopoulos, G., Boskou, D., 1991. Antioxidant effect of natural phenols on olive oil. J.Am. Oil Chem. Soc. 68, 669–671.

Petroni, A., et al., 1995. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb. Res. 78, 151–160.

Ragazzi, E., Veronese, G., 1973. Riv. Ital. Sost. Grasse. 50.

Ranalli, A., et al., 2003. Antioxidizing potency of phenol compounds in olive oil mill wastewater. J. Agric. Food Chem. 51, 7636–7641.

Re, R., et al., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.

Rice-Evans, C.A., et al., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Rodríguez, G., et al., 2007. Antioxidant activity of effluents during the purification of hydroxytyrosol and 3,4-dihydroxyphenyl glycol from olive oil waste. Eur. Food Res. Technol. 224, 733–741.

Saija, A., et al., 1998. 'In vitro' evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. Int. J. Pharm. 166, 123–133.

Selmi, C., et al., 2006. The anti-inflammatory properties of cocoa flavanols. J. Cardiovasc. Pharmacol. 47 (suppl 2), S163–S171 discussion S172–S176.

Silva, F.A., et al., 2000. Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. J. Agric. Food Chem. 48, 2122–2126.

Son, S., Lewis, B.A., 2002. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure-activity relationship. J. Agric. Food Chem. 50, 468–472.

Torres de Pinedo, A., et al., 2007a. Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. Food Chem. 103, 55–61.

Torres de Pinedo, A., et al., 2007b. Synthesis of new phenolic fatty acid esters and their evaluation as lipophilic antioxidants in an oil matrix. Food Chem. 105, 657–665.

Torres de Pinedo, A., et al., 2005. Efficient lipase-catalyzed synthesis of new antioxidants based on a catechol structure. Tetrahedron 61, 7654–7660.

Trujillo, M., et al., 2006. Lipophilic hydroxytyrosyl esters. Antioxidant activity in lipid matrices and biological systems. J. Agric. Food Chem. 54, 3779–3785.

Valavanidis, A., et al., 2004. Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. J. Agric. Food. Chem. 52, 2358–2365.

Visioli, F., et al., 1995. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis 117, 25–32.