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REGULAR ARTICLE

A quantum chemical study on the free radical scavenging activity of tyrosol and hydroxytyrosol

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Abstract The free radical scavenging activity of hydroxytyrosol (HTyr) and tyrosol (Tyr) has been studied in aqueous and lipid solutions, using the density functional theory. Four mechanisms of reaction have been considered: single electron transfer (SET), sequential electron proton transfer (SEPT), hydrogen transfer (HT), and radical adduct formation. It was found that while SET and SEPT do not contribute to the overall reactivity of HTyr and Tyr toward $^{\cdot}OOH$ and $^{\cdot}OCH_3$ radicals, they can be important for their reactions with $^{\cdot}OH$, $^{\cdot}OCCl_3$, and $^{\cdot}OOCCl_3$. The $^{\cdot}OOH$-scavenging activity of HTyr and Tyr was found to take place exclusively by HT, and it is also predicted to be the main mechanism for their reactions with $^{\cdot}OCH_3$. HT is proposed as the main mechanism for the scavenging activity of HTyr and Tyr when reacting with other $^{\cdot}OR$ and $^{\cdot}OOR$ radicals, provided that R is an alkyl or an alkenyl group. The major products of reaction are predicted to be the phenoxyl radicals. In addition, Tyr was found to be less efficient than HTyr as free radical scavenger. Moreover, while HTyr is predicted to be a good peroxyl scavenger, Tyr is predicted to be only moderately for that purpose.

Keywords Rate constant · Mechanism of reaction · Branching ratios · UV-vis spectra

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1 Introduction

Free radicals are present in living organisms, where they can be either harmful or beneficial depending on their concentration. High concentrations are caused by an imbalance between their production and consumption, which is commonly referred to as oxidative stress (OS). Under such conditions, free radicals can be very dangerous. OS is recognized as a major health problem involved in the development of several diseases such as cancer [1–7], cardiovascular disorders [8–12], atherosclerosis [13–16], and several neurological disorders including Parkinson's and Alzheimer's diseases [17–21]. As a result searching for efficient ways of preventing, or reducing OS, and therefore the above-mentioned diseases are of great interest.

To that effect, there is an increasing evidence supporting the health benefits of the Mediterranean diet, which has been proven to lower the incidence of coronary heart diseases [22–24], cancer [25–30], and inflammatory processes [31]. Many of these benefits have been attributed to the phenolic components of olive oil, the prime fat source in the Mediterranean diet. The major phenolic compounds in this oil are tyrosol (Tyr, 4-(2-hydroxyethyl) phenol) and hydroxytyrosol (HTyr, 4-(2-Hydroxyethyl)-1,2-benzenediol) [32, 33]. These compounds are also present in white wines [34]. Moreover, it has been proposed that the beneficial effects of consuming white wines are derived from the presence of Tyr and HTyr [35].

HTyr has been reported to have anti-cancer [36–38] and anti-inflammatory [39] activities to inhibit low-density lipoprotein (LDL) [40–43] and lipid [44] oxidation, platelet aggregation [45, 46], atherosclerosis [47], hyperglycemia and OS induced by diabetes [48], and endothelial cell activation [49]. It has also been reported that HTyr is able to prevent protein damage induced by ultraviolet radiation [50] as well as the oxidation of olive oil [51, 52] and to protect DNA from damages caused by hydrogen peroxide [53] and peroxynitrite [54]. Many of these benefits can be associated with its well-recognized antioxidant activity [55–63], which has been reported to be higher than that of α-tocopherol [64–66]. HTyr has been proven to efficiently scavenge peroxyl [67–69], NO [33], tert-butylhydroperoxide [70], and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [71] radicals. It is also a potent scavenger of hydroxyl radical [33, 43], which was attributed to its catechol moiety [33]. HTyr has also been described as an excellent scavenger of peroxynitrite and superoxide radicals [43]. In the particular case of the NO radical, the related compound, Tyr, showed no protective effects [33]. This is in agreement with other reports describing Tyr as a less potent scavenger than HTyr when reacting with hydroxyl, peroxynitrite, and superoxide radicals [43]. However, Tyr still offers protection against OS. It has been found that Tyr inhibits LDL oxidation [72] and presents cardioprotective effects [34].

According to the data gathered so far, there are no doubts about the antioxidant activity of HTyr, and it seems likely that its related compound, Tyr, is also able to present this protective activity. However, the mechanism of action of HTyr, and related compounds, is not clear yet [69, 73]. There is also a lack of information on the kinetics of the reactions involved in their chemical protection. The only rate constants reported so far corresponds to the Tyr+ peroxide radicals (in chlorobenzene solution) [74], which was found to be 9.4×10^3 M^{-1} s^{-1}, about 350 times slower than that of α-tocopherol [74]. In addition, from a theoretical point of view, there is only one previous study on these compounds. It provided structures and electronic properties of HTyr, and three of its radical isomers, obtained from semiempirical calculations with AM1 [75].

Therefore, the main goal of the present work is to perform a detailed study on the mechanisms of reaction involved in the free radical scavenging activity of HTyr and Tyr and to provide kinetic data on such processes. To that purpose, we have modeled their reactions with $^{\cdot}OOH$ and $^{\cdot}OCH_3$ radicals, in polar and non-polar environments. Three different mechanisms of reaction have been considered: (1) single electron transfer (SET), (2) hydrogen transfer (HT), and (3) radical adduct formation (RAF). In addition, SET and sequential electron proton transfer (SEPT) processes have been investigated for a larger set of free radicals. Thermodynamic and kinetic data are provided, as well as the contributions of the different mechanisms to the $^{\cdot}OOH$- and $^{\cdot}OCH_3$-scavenging activity of HTyr and Tyr. In addition, the UV-vis spectra of the formed products are also provided. All this new information is expected to contribute to the better understanding of the protective role of HTyr and Tyr against OS.

2 Computational details

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional [76] and the 6-311+G(d,p) basis set, in conjunction with the SMD continuum model [77], using pentylethanoate and water as solvents to mimic lipidic and aqueous environments. The M05-2X functional has been recommended for kinetic calculations by their developers [76], and it has been also successfully used by independent authors to that purpose [78–83]. All the electronic calculations were performed with Gaussian 09 package of programs [84]. Thermodynamic corrections at 298 K were included in the calculation of relative energies.

Unrestricted calculations were used for open shell systems and local minima and transition states (TS) were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). In the case of the TS, it was verified that the imaginary frequency corresponds to the expected motion along the reaction coordinate, by Intrinsic Reaction Coordinate calculations (IRC). Spin contamination was checked for all the studied radical species. In all the cases, the deviations from the correct value ($\langle S^2 \rangle = 0.75$) were lower than 6 and 0.5% before and after annihilation of the first spin contaminant. It has been established that for differences within 10% error the obtained results can be trusted [85]. Therefore, the spin contamination can be considered negligible for all the radicals species studied in this work and their energy values are reliable.

The solvent cage effects have been included according to the corrections proposed by Okuno [86], taking into account the free volume theory [87]. These corrections are in good agreement with those independently obtained by Ardura et al. [88] and have been successfully used by other authors [89–91].

The rate constants (k) were calculated using Conventional Transition State Theory (TST) [92–94] and 1 M standard state as:

$$k = \sigma\kappa \frac{k_B T}{h} e^{-(\Delta G^{\neq})/RT} \quad (1)$$

where k_B and h are the Boltzmann and Planck constants, $\Delta G^{\neq}$ is the Gibbs free energy of activation, σ represents

the reaction path degeneracy, accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections. The tunneling corrections, defined as the Boltzmann average of the ratio of the quantum and the classical probabilities, were calculated using the Zero Curvature Tunneling corrections (ZCT) [95].

For the mechanisms involving electron transfers (ET), the Marcus theory [96–98] was used. It relies on the transition state formalism and defines the ET activation barrier $\left(\Delta G_{\mathrm{ET}}^{\neq}\right)$ as:

$$\Delta G_{\mathrm{ET}}^{\neq} = \frac{\lambda}{4}\left(1 + \frac{\Delta G_{\mathrm{ET}}^{0}}{\lambda}\right)^{2} \tag{2}$$

where $\Delta G_{\mathrm{ET}}^{0}$ is the free energy of reaction and λ is a reorganization term.

Some of the calculated rate constants (k) are close to the diffusion limit. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work, the Collins–Kimball theory is used to that purpose [99]:

$$k_{\mathrm{app}} = \frac{k_D k_{\mathrm{act}}}{k_D + k_{\mathrm{act}}} \tag{3}$$

where k_{act} is the thermal rate constant, obtained from TST calculations (Eq. 1), and k_D is the steady-state Smoluchowski [100] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R\, D_{AB} N_A \tag{4}$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of the reactants A (free radical) and B (antioxidant). D_{AB} has been calculated from D_A and D_B according to Ref. [101], D_A and D_B have been estimated from the Stokes–Einstein approach [102, 103]:

$$D = \frac{k_B T}{6\pi\eta a} \tag{5}$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent, in our case water ($\eta = 8.91 \times 10^{-4}$ Pa s) and pentylethanoate ($\eta = 8.62 \times 10^{-4}$ Pa s); and a is the radius of the solute.

The electronic spectra have been computed using the time-dependent density functional theory (TD-DFT) [104–110], based on vertical excitations involving the three lowest lying excited states. TD-DFT efficiently and rapidly provides transition energies for mono-determinental systems in both gas and condensed phases [111]. Moreover, it has been demonstrated that he UV-vis spectra obtained from this approach frequently agree with experiments [112–116].

3 Results and discussion

The structures of HTyr and Tyr as well as the numbers assigned to each site of reaction are shown in Fig. 1. The antioxidant activity of these compounds can take place through different mechanisms, as for many other compounds [117–123]. Those considered in this work are the following:

Single electron transfer (SET):

$$\mathrm{HTyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{HTyr}^{\cdot+} + \mathrm{R}^{-}$$

$$\mathrm{Tyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{Tyr}^{\cdot+} + \mathrm{R}^{-}$$

Sequential electron proton transfer (SEPT):

$$\mathrm{HTyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{HTyr}^{\cdot+} + \mathrm{R}^{-} \rightarrow \mathrm{HTyr}^{\cdot} + \mathrm{H}^{+} + \mathrm{R}^{-}$$

$$\mathrm{Tyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{Tyr}^{\cdot+} + \mathrm{R}^{-} \rightarrow \mathrm{Tyr}^{\cdot} + \mathrm{H}^{+} + \mathrm{R}^{-}$$

Hydrogen transfer (HT):

$$\mathrm{HTyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{HTyr}(-\mathrm{H})^{\cdot} + \mathrm{HR}$$

$$\mathrm{Tyr} + {}^{\cdot}\mathrm{R} \rightarrow \mathrm{Tyr}(-\mathrm{H})^{\cdot} + \mathrm{HR}$$

Radical Adduct formation (RAF):

$$\mathrm{HTyr} + {}^{\cdot}\mathrm{R} \rightarrow [\mathrm{HTyr} - \mathrm{R}]^{\cdot}$$

$$\mathrm{Tyr} + {}^{\cdot}\mathrm{R} \rightarrow [\mathrm{Tyr} - \mathrm{R}]^{\cdot}$$

The Gibbs free energies of reaction (ΔG), at 298.15 K, for the different mechanisms and reaction sites are provided in Table 1. As these values show, the SET mechanism was found to be endergonic for all the studied cases. As expected the ΔG values of this process are lower in aqueous solution than in non-polar media. All these values are large enough to overcome any uncertainty related to the calculations. Therefore, the SET mechanism does not contribute to the overall reactivity of HTyr and Tyr toward ${}^{\cdot}$OOH and ${}^{\cdot}$OCH$_3$ radicals. However, it can be viable for the reactions of these compounds with other free radicals. We will address this point latter, when discussing the kinetic data.

Regarding the RAF mechanism, for the HTyr + ${}^{\cdot}$OCH$_3$ reaction all the studied paths are predicted to be thermochemically viable in non-polar environments, and all but path 3 in aqueous solution. For the reaction of this radical with Tyr, in non-polar media, paths 2, 3, and 5 are

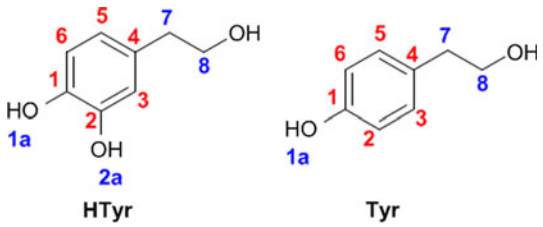

Fig. 1 Hydroxytyrosol (HTyr) and tyrosol (Tyr). Structures and site numbers

Table 1 Gibbs free energies of reaction (ΔG),at 298.15 K, in kcal/mol

	HTyr				Tyr			
	$^{\cdot}OCH_3$		$^{\cdot}OOH$		$^{\cdot}OCH3$		$^{\cdot}OOH$	
	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous
SET	61.97	19.49	70.76	25.80	66.09	25.48	74.88	31.80
HT								
p1a	−24.79	−24.78	−6.67	−6.97	−17.87	−19.11	0.25	−1.30
p2a	−23.38	−23.50	−5.26	−5.69				
p7	−14.86	−16.91	3.26	0.90	−14.47	−16.97	3.65	0.84
p8	−9.83	−10.60	8.29	7.21	−8.79	−10.18	9.33	7.63
RAF								
p1	−8.20	−6.61	10.34	9.74	−2.75	−3.86	12.95	12.04
p2	−3.25	−4.77	13.69	11.79	1.58	−0.74	19.00	15.96
p3	−0.45	0.44	14.76	14.50	1.74	1.54	16.02	15.93
p4	−3.52	−2.12	14.88	14.17	−2.07	−1.25	15.92	15.43
p5	−1.24	−1.57	14.51	13.33	1.23	0.91	17.56	16.35
p6	−1.49	−0.78	15.24	12.69	−1.97	−0.98	17.27	14.80

endergonic. In aqueous solution, the $^{\cdot}OCH_3$ additions to sites 3 and 5 are also predicted to be thermochemically unfeasible. Therefore, the number of active channels of reaction through the RAF mechanism is smaller for Tyr compared with HTyr. In general, the thermochemical viability of the RAF processes seem to be favored by polar environments, and the radical additions to HTyr more favored than those involving Tyr. For the reactions of both HTyr and Tyr with $^{\cdot}OOH$, all the RAF channels were found to be endergonic. This indicates that this radical, and probably other peroxyl radicals, does not react with the studied compounds by this mechanism.

It was found that the largest exergonicities are systematically those arising from H transfer processes. Moreover, for the reactions involving $^{\cdot}OOH$, the only reaction paths predicted to be thermochemically viable are those corresponding to the HT mechanism. The H atoms susceptible to be transferred from HTyr and Tyr to $^{\cdot}OOH$ are only the phenolic ones. The ΔG values of the $^{\cdot}OOH$ reactions with HTyr are systematically more negative than those corresponding to those with Tyr. In the particular case of the reaction Tyr + $^{\cdot}OOH$, in non-polar solution, all channels of reaction were found to be endergonic. Therefore, Tyr is not expected to be reactive toward $^{\cdot}OOH$, under such conditions. All the HT channels for the reactions of both HTyr and Tyr with $^{\cdot}OCH_3$ are predicted to be exergonic. However, the exergonicity is systematically larger for the reactions involving HTyr. In addition, the energy release associated with the HT from the phenolic sites (1a and 2a) is larger than those of the alkyl sites (7 and 8).

According to the above discussion, relevant information can be obtained from the thermochemical analysis. The main findings are the following: (1) the $^{\cdot}OOH$-scavenging activity of HTyr and Tyr takes place exclusively by H transfer. This is predicted to be valid also for other peroxyl radicals, in particular for alkyl and alkenyl peroxyl radicals. (2) It was found that the ΔG values are systematically lower for HTyr, compared with those of Tyr, supporting the higher reactivity of the first one. (3) The number of thermochemically viable channels is larger for the reactions with $^{\cdot}OCH_3$ than for the reactions with $^{\cdot}OOH$, in line with their relative reactivity.

For the kinetic study, we have not include the channels of reaction described above as endergonic because even if they take place at a significant rate, they would be reversible and therefore, the formed products will not be observed. However, it should be noticed that they might still represent significant channels if their products rapidly react further. This would be particularly important if these later stages are sufficiently exergonic to provide a driving force, and if their barriers of reactions are low. That is the case for the SET mechanism, since the formed radical cation can spontaneously deprotonate.

The fully optimized geometries of the TS are shown in Figs. 2, 3, 4. TS for the phenolic abstractions (sites 1a and 2a) from HTyr were located and characterize in penthylethanoate solution; however, in aqueous solution, it was not possible to locate them using full optimizations. Using partial optimizations with frozen O–H and H–OH bond distances, we were able to obtain structures that present a single imaginary frequency. Unfreezing these two distances, during a saddle point optimization, invariably lead to an increase in the H–OH distance, and the corresponding decrease in the imaginary frequency and gradient, yielding the separated

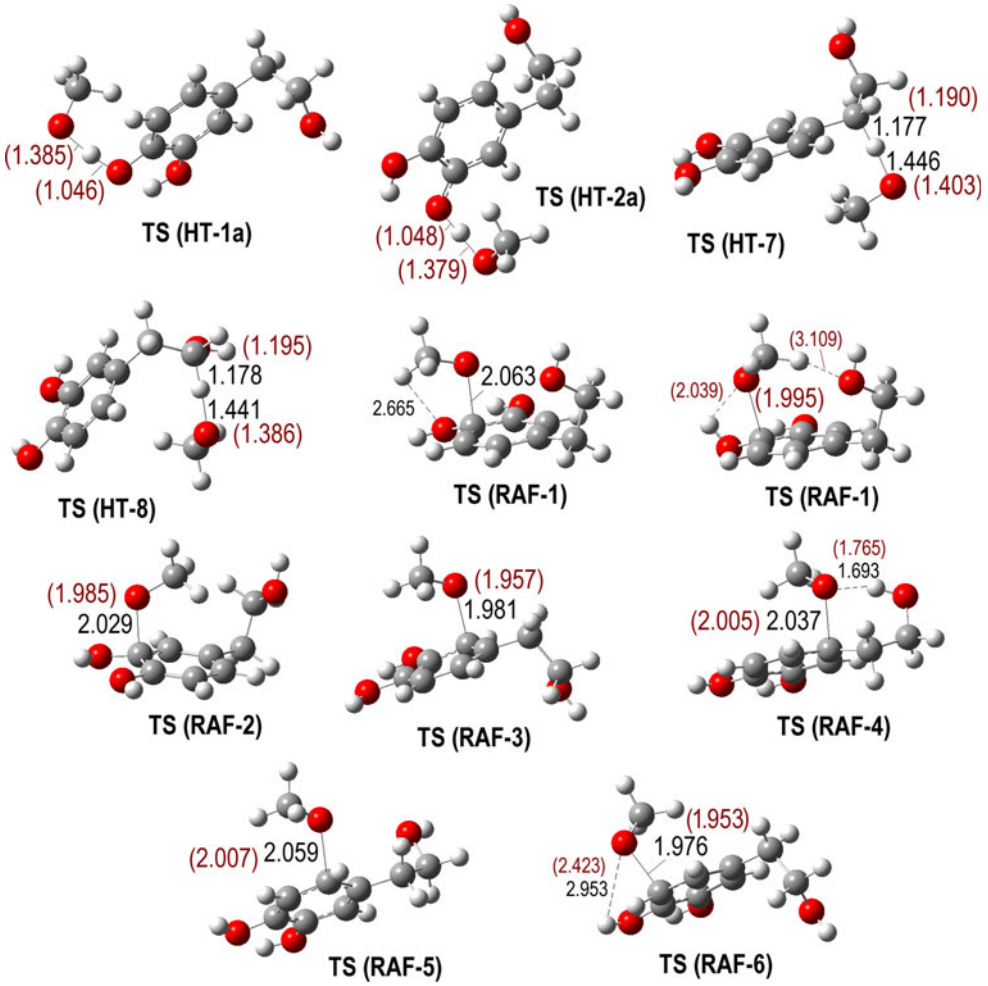

Fig. 2 Optimized geometries of transition states involved in the reactions of HTyr with $^{\cdot}OCH_3$, in water (penthyletanoate) solution. The distances are reported in Å

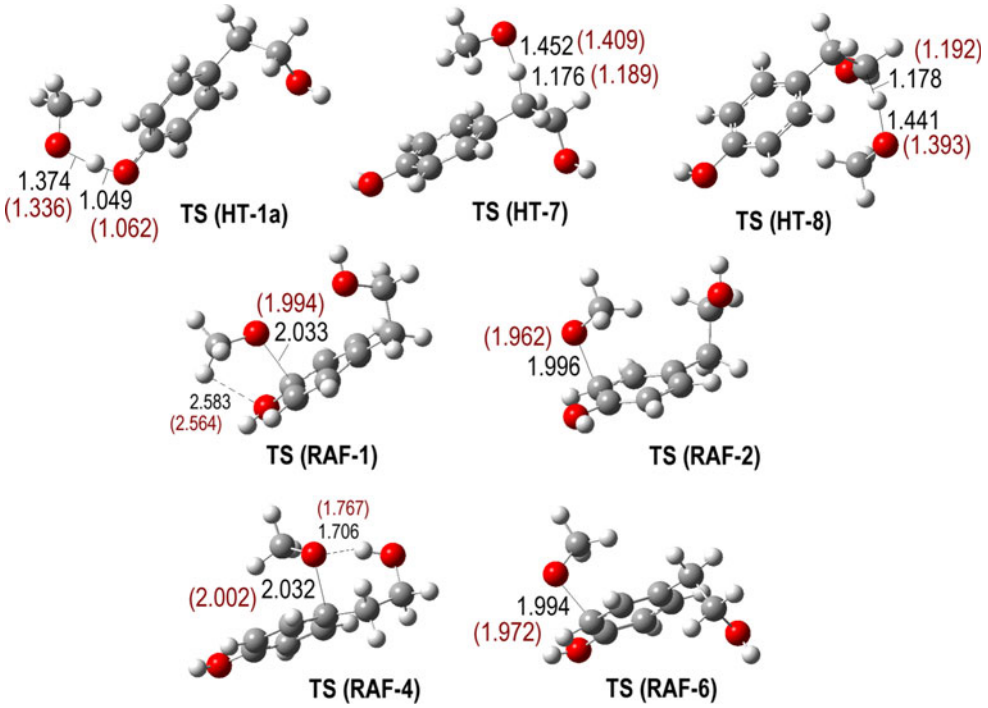

Fig. 3 Optimized geometries of transition states involved in the reactions of Tyr with $^{\cdot}OCH_3$, in water (penthyletanoate) solution. The distances are reported in Å

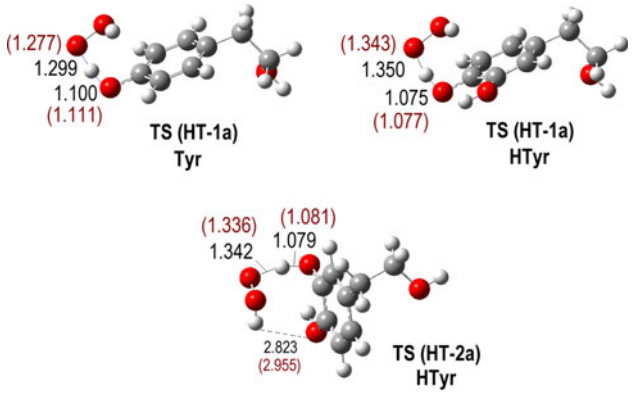

Fig. 4 Optimized geometries of transition states involved in the reactions of HTyr and Tyr with ˙OOH, in water (penthyletanoate) solution. The distances are reported in Å

reactants. A relaxed scan, obtained by decreasing the H–OH distance, produces a similar result, i.e., the energy decreases until the H atom is completely transferred. This means that the reaction is barrier-less and strictly diffusion-controlled. In other words, every encounter is effective producing the conversion of reactants into products.

The geometries optimized in water and in penthyletanoate are very similar. The only exception is the TS of the RAF reaction between HTyr and $^{\cdot}OCH_3$, on site 1. In this case, the geometries are quite different. Therefore, they are both explicitly shown in Fig. 3. Some of the TSs present H-bond like interactions, which in general are weaker in water solution compared to lipid media. The exceptions are the TSs corresponding to the addition of $^{\cdot}OCH_3$ on site 4 of Tyr, and to the HT from site 2a in Tyr to ˙OOH. In these two cases, the interaction distances are slightly shorter for the geometries optimized in aqueous solution. Among those TSs presenting H-bond like interactions, the shortest distances correspond to RAF processes on site 4, thus they are the strongest ones. This kind of interactions is expected to lower the energy of the TS, contributing to increase the reactivity.

The Gibbs free energies of activation ($\Delta G^{\neq}$), at 298.15 k, are reported in Table 2. The barriers corresponding to the reactions with HTyr are systematically lower than those involving Tyr. The only exception is the addition of $^{\cdot}OCH_3$ to site 6. The lowest barriers are those of the HT from the phenolic sites, for both HTyr and Tyr, regardless of the reacting radical and of the environments' polarity. Among the RAF paths, the lowest barrier corresponds to radical additions to site 1 in HTyr and to site 4 in Tyr. The effect of the solvent's polarity on the barriers' height was found to be almost negligible for the HT paths. However, for the RAF channels, the effect is larger. In particular, the barriers of the $^{\cdot}OCH_3$ addition to sites 2 and 3 in HTyr become about 2.5 and 1.7 kcal/mol lower, respectively, in aqueous solution. For the reactions of Tyr, the largest effect also corresponds to RAF on site 2. In this case, the barrier is about 5 kcal/mol lower in aqueous solution than in non-polar media.

The rate constants for the different channels of reaction, in aqueous and lipid solutions, are reported in Table 3, together with the overall rate coefficients, which have been calculated as the sum of the rate constants of each path. For example, for the HTyr + $^{\cdot}OCH_3$ reaction in aqueous solution:

$$k_{\text{overall}} = k_{\text{app}}^{\text{HT}} + k_{\text{app}}^{\text{RAF}}$$

where

$$k_{\text{app}}^{\text{HT}} = k_{\text{app}}^{\text{p1a}} + k_{\text{app}}^{\text{p2a}} + k_{\text{app}}^{\text{p7}} + k_{\text{app}}^{\text{p8}}$$

$$k_{\text{app}}^{\text{RAF}} = k_{\text{app}}^{\text{p1}} + k_{\text{app}}^{\text{p2}} + k_{\text{app}}^{\text{p4}} + k_{\text{app}}^{\text{p5}} + k_{\text{app}}^{\text{p6}}$$

According to the overall rate coefficients, both HTyr and Tyr are predicted to react faster in aqueous solution than in

Table 2 Gibbs free energies of activation ($\Delta G^{\neq}$), at 298.15 K, in kcal/mol

	HTyr				Tyr			
	$^{\cdot}OCH_3$		˙OOH		˙OCH3		˙OOH	
	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous
HT								
p1a	4.26	~0.00	11.70	12.55	8.26	8.13	16.52	16.69
p2a	5.39	~0.00	12.64	14.18				
p7	10.77	10.85			11.70	11.00		
p8	10.31	10.25			11.65	11.08		
RAF								
p1	8.74	9.13			13.01	12.18		
p2	12.00	9.52			16.92	11.89		
p3	13.18	11.44						
p4	10.65	9.44			10.88	10.42		
p5	12.59	11.16						
p6	12.96	12.31			12.88	12.00		

Table 3 Rate constants (k) of the different channels of reaction, and overall rate coefficient (M^{-1} s^{-1}), at 298.15 K

	HTyr				Tyr			
	$^{\cdot}OCH_3$		$^{\cdot}OOH$		$^{\cdot}OCH3$		$^{\cdot}OOH$	
	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous
HT								
p1a	2.18E+09	2.49E+09	4.77E+05	6.33E+05	4.35E+07	4.25E+08	7.13E+02	4.24E+03
p2a	1.34E+09	2.49E+09	1.66E+05	1.15E+05				
p7	2.06E+06	1.60E+06			4.44E+05	1.32E+06		
p8	5.03E+06	4.91E+06			5.54E+05	1.27E+06		
RAF								
p1	1.94E+07	9.99E+06			1.43E+04	5.86E+04		
p2	8.85E+04	5.87E+06			2.19E+01	1.08E+05		
p3	1.36E+04	2.56E+05						
p4	1.07E+06	8.13E+06			7.27E+05	1.57E+06		
p5	4.38E+04	4.94E+05						
p6	2.56E+04	7.66E+04			2.91E+04	1.30E+05		
Overall	3.54E+09	5.02E+09	6.42E+05	7.49E+05	4.53E+07	4.30E+08	7.13E+02	4.24E+03

non-polar media with oxygenated free radicals. The overall rate coefficients in aqueous solution were found to be about 1.4, 1.2, 9.5, and 6.0 times higher than in non-polar media for the reactions HTyr + $^{\cdot}OCH_3$, HTyr + $^{\cdot}OOH$, Tyr + $^{\cdot}OCH_3$, and Tyr + $^{\cdot}OOH$, respectively (Table 3).

The overall reactivity of HTyr toward $^{\cdot}OCH_3$ was found to be diffusion-controlled in both lipid and aqueous solutions (3.54×10^9 and 5.02×10^9 M^{-1} s^{-1}, respectively), supporting the excellent scavenging activity of this compound toward this radical, and probably toward other alkoxyl radicals. The efficiency of HTyr to scavenge $^{\cdot}OOH$ is predicted to be significantly lower in agreement with the relative reactivity of these free radicals. According to the overall rate coefficients (6.42×10^5 and 7.49×10^5 M^{-1} s^{-1}, in lipid and aqueous solutions, respectively), HTyr is predicted to be a good $^{\cdot}OOH$ scavenger. Moreover, it is also expected to be efficient for deactivating other peroxyl radicals. It is important to notice that the reactivity of $^{\cdot}OOH$ is lower compared with those of other free radicals. Therefore, rate constants in the order of 10^5 M^{-1} s^{-1}, or higher, indicate a good scavenging activity.

Tyr was found to be less efficient than HTyr for scavenging $^{\cdot}OCH_3$ and $^{\cdot}OOH$, in agreement with previous reports on their relative reactivity with other free radicals [43]. The Tyr + $^{\cdot}OCH_3$ reaction, in aqueous solution, was found to be very fast, but about 11 times slower than that of HTyr. For all the other rate coefficients, the difference with respect to HTyr is even larger. When the same reaction takes place in a non-polar environment, it is predicted to be about 78 times slower than that of HTyr. For the reactions with $^{\cdot}OOH$, the lower reactivity of Tyr is even more noticeable. These reactions are 901 and 176 times slower than those of HTyr, in non-polar and polar environments, respectively. Moreover, while HTyr is predicted to be a good peroxyl scavenger, Tyr is predicted to be only moderately efficient. Therefore, regarding the antioxidant protective effect of olive oil, and white wine, the role of HTyr is more important than that of Tyr.

A very important aspect of studying the antioxidant activity of chemical compounds is to identify those with higher activity. To that purpose, it is recommended to compare different scavengers using their reactions with free radicals that are not particularly reactive. For example, if we use their reactions with $^{\cdot}OH$, we might conclude that they have similar reactivity, since the reactions with this radical often are diffusion-controlled [124, 125]. Since such comparison might be misleading, we prefer and recommend to use the less reactive of the studied radicals ($^{\cdot}OOH$).

In non-polar environments, the peroxyl-scavenging activity of HTyr was found to be similar to that of carotenes ($\sim 10^5$–10^6 M^{-1} s^{-1}) [126] and canolol (6.8×10^5 M^{-1} s^{-1}) [127]; higher than that of sinapinic acid (1.7×10^4 M^{-1} s^{-1}) [128]; and much higher than that of melatonin (3.1×10^2 M^{-1} s^{-1}) [129], caffeine (3.2×10^1 M^{-1} s^{-1}) [123], and Tyr (7.1×10^2 M^{-1} s^{-1}, this work). Moreover, taking into account that the rate constants corresponding to the $^{\cdot}OOH$ damage to unsaturated fatty acids are in the range 1.18–3.05 $\times 10^3$ M^{-1} s^{-1} [130], HTyr is predicted to protect against peroxyl oxidation of lipids, while the protective effects of Tyr are not expected to be significant.

In aqueous solution, the peroxyl-scavenging activity of HTyr was found to be similar to that of carotenes

($\sim 10^4$–10^6 M^{-1} s^{-1}) [126] and sinapinic acid (5.4×10^5 M^{-1} s^{-1}) [128]; higher than that of allicin (7.4×10^3 M^{-1} s^{-1}) and thioacrolein (2.9×10^4 M^{-1} s^{-1}) [131]; much higher than that of melatonin (2.0×10^1 M^{-1} s^{-1}) [129], caffeine (3.3×10^{-1} M^{-1} s^{-1}) [123], and Tyr (4.2×10^3 M^{-1} s^{-1}, this work); and significantly lower than that of 2-propenesulfenic acid (2.6×10^7 M^{-1} s^{-1}) [131] and glutathione (2.7×10^7 M^{-1} s^{-1}) [132], which are excellent for scavenging $^{\cdot}$OOH. According to these comparisons, HTyr is among the best peroxyl radical scavengers in lipid media, while in aqueous solution there are other compounds with higher activity.

The branching ratios of the different reaction paths are reported in Table 4. They represent the percent contribution of the different channels to the overall reaction and have been calculated as:

$$\Gamma_i = \frac{k_i}{k_{\text{overall}}} \times 100. \quad (6)$$

According to the calculated branching ratios, the main mechanism involved in the $^{\cdot}OCH_3$- and $^{\cdot}$OOH-scavenging activity of both HTyr and Tyr is the H transfer. The contributions of this mechanism to the overall reactivity of these compounds toward the studied radicals were found to be higher than 98%, regardless of the polarity of the environment. For the HTyr + $^{\cdot}OCH_3$ reaction, in non-polar environments, path 1a was found to be the most important one; while in aqueous solution paths 1a and 2a are equally important. This is caused by the diffusion-limited regime. For the reaction of Tyr with $^{\cdot}OCH_3$, the contributions of reaction channels other than path 1a are almost negligible, both in polar and in non-polar media. For the HTyr + $^{\cdot}$OOH reaction, the contributions of path 1a to the overall reactivity are systematically higher than those of path 2a, regardless of the polarity of the environment. Accordingly, it can be stated the H transfer mechanism, from the phenolic sites in HTyr and Tyr, is responsible for most of the free radical scavenging activity of these compounds. HT is also predicted to be the main scavenging mechanism of HTyr and Tyr when reacting with other $^{\cdot}$OR and $^{\cdot}$OOR radicals, provided that R is an alkyl or an alkenyl group.

As mentioned before the SET mechanism, and therefore the SEPT mechanism, was ruled out for the $^{\cdot}OCH_3$- and $^{\cdot}$OOH-scavenging activity of HTyr and Tyr, based on thermochemical considerations. However, when they react with other free radicals, particularly with those that are more electrophilic than $^{\cdot}OCH_3$ and $^{\cdot}$OOH, the outcome can be different. Therefore, we have included a larger set of free radicals in the study of the SET mechanism. To that purpose, we have considered only aqueous solution since non-polar environments do not promote the necessary solvation of the intermediate ionic species yielded by the SET mechanism. We have included in the set of free radicals the hydroxyl radical ($^{\cdot}$OH) because it is the most electrophilic [133], and reactive, of the oxygen-centered radicals, with a half-life of $\sim 10^{-9}$ s [134]. We have also included a series of peroxyl radicals, which are relative low-reactive species capable of diffusing to remote cellular locations [135], with half-lives of seconds [136]. The $OOCCl_3$ radical is the most electrophilic among the chosen peroxyl radicals, and $^{\cdot}$OOR1 ($^{\cdot}$OO–CH_2–CH=CH_2) and $^{\cdot}$OOR2 (CH_3–CH($^{\cdot}$OO)–CH=CH–CH_3) have been used to mimic lipid peroxyl radicals. An equivalent set of alkoxyl radicals has also been included, since their reactivity is expected to be in between of those of $^{\cdot}$OH and peroxyl radicals. DPPH has also been taken into account since it is frequently used in experiments related to antioxidant behavior.

Table 4 Branching ratios (Γ) of the different channels of reaction, at 298.15 K

	HTyr				Tyr			
	$^{\cdot}OCH_3$		$^{\cdot}$OOH		$^{\cdot}$OCH3		$^{\cdot}$OOH	
	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous	Lipid	Aqueous
HT								
p1a	61.53	49.69	74.23	84.61	96.10	98.96	100.00	100.00
p2a	37.69	49.69	25.77	15.39				
p7	0.06	0.03			0.98	0.31		
p8	0.14	0.10			1.22	0.30		
RAF								
p1	0.55	0.20			0.03	0.01		
p2	0.00	0.12			0.00	0.03		
p3	0.00	0.01						
p4	0.03	0.16			1.60	0.37		
p5	0.00	0.01						
p6	0.00	0.00			0.06	0.03		

Table 5 Gibbs free energies of reaction (ΔG, kcal/mol), reorganization energies (λ, kcal/mol), Gibbs free energies of activation ($\Delta G^{\neq}$, kcal/mol), diffusion-limited rate constants (k_D, M^{-1} s^{-1}), and apparent rate constants (k_{app}, M^{-1} s^{-1}) of the SET reactions of HTyr and Tyr with different free radicals, in aqueous solution

Radical	ΔG	λ	$\Delta G^{\neq}$	k_D	k_{app}
HTyr					
·OH	2.13	8.03	3.22	1.31E+09	**1.25E+09**
·OCH3	19.52	9.39	22.25	1.19E+09	3.05E−04
·OCCl3	−35.35	25.52	0.95	1.09E+09	**1.08E+09**
·OR1	18.15	9.20	20.32	1.11E+09	7.89E−03
·OOH	25.78	18.65	26.47	1.24E+09	2.46E−07
·OOCH3	27.71	18.95	28.72	1.16E+09	5.46E−09
·OOCCl3	5.44	20.26	8.15	1.07E+09	**6.54E+06**
·OOR1	26.91	19.57	27.60	1.09E+09	3.65E−08
·OOR2	28.96	19.78	30.02	1.03E+09	6.10E−10
·DPPH	19.84	8.13	24.05	9.09E+08	1.47E−05
Tyr					
·OH	8.13	5.66	8.40	1.31E+09	**4.30E+06**
·OCH3	25.51	7.02	37.69	1.20E+09	1.45E−15
·OCCl3	−29.35	23.15	0.42	1.09E+09	**1.09E+09**
·OR1	24.14	6.83	35.11	1.11E+09	1.14E−13
·OOH	31.78	16.28	35.47	1.24E+09	6.15E−14
·OOCH3	33.71	16.58	38.13	1.16E+09	6.91E−16
·OOCCl3	11.44	17.89	12.02	1.08E+09	**9.59E+03**
·OOR1	32.91	17.20	36.49	1.09E+09	1.10E−14
·OOR2	34.95	17.41	39.37	1.04E+09	8.49E−17
·DPPH	25.83	5.77	43.30	9.15E+08	1.13E−19

The Gibbs free energies of reaction (ΔG), reorganization energies (λ), Gibbs free energies of activation ($\Delta G^{\neq}$), diffusion-limited rate constants (k_D), and the apparent rate constants (k_{app}) of the SET reactions between HTyr and Tyr and the extended set of free radicals are reported in Table 5. The k_{app} values of the reactions that are predicted to contribute to the overall scavenging activity have been highlighted in bold letters. As the values in this table show the SET reactions of HTyr with OH and $OCCl_3$ radicals, as well as that between Tyr and $^{\cdot}OCCl_3$ are predicted to be diffusion-controlled. The electron transfer from HTyr to $^{\cdot}OOCCl_3$ and from Tyr to ·OH are slower but still very fast ($\sim 10^6$ M^{-1} s^{-1}). Therefore, they are expected to be important channels of reactions. The SET reactions with the rest of the studied radicals were found to be highly endergonic and with high barriers of reaction. Moreover, their values of k_{app} indicate that the contributions of this process to the overall free radical scavenging activity of HTyr and Tyr are negligible.

It is important to call attention on the fact that the following SET reactions: HTyr + ·OH, HTyr + $^{\cdot}OOCCl_3$, and Tyr + ·OH, are described as endergonic or slightly exergonic. However, they still represent significant channels of reaction since the formed radical cations spontaneously deprotonate, through a barrier-less process that is exergonic enough to provide a driving force. The Gibbs free energies of the deprotonations of $HTyr^{\cdot+}$ and $Tyr^{\cdot+}$ were found to be −9.95 and −10.27 kcal/mol, respectively. The deprotonation site is the phenolic group in site 1a. For $HTyr^{\cdot+}$, this Gibbs free energy of the deprotonation from the other phenolic site (2a) is 1.3 kcal/mol higher. Accordingly, these processes actually correspond to the SEPT mechanism. The possible participation of other mechanisms would change the products distribution but cannot decrease this reactivity, which in fact represents a lower limit since no other mechanisms have been considered.

3.1 UV-vis spectra

The UV-vis spectra of HTyr, Tyr, their radical cations, and their main products of reaction have been computed and are reported in Fig. 5. While HTyr and Tyr absorb in the 200–240 nm region, their main products present one absorption band significantly red-shifted which appear in the 330–350 nm region. This means that the evolution of the reactions of HTyr and Tyr with $^{\cdot}OOH$, $^{\cdot}OCH_3$, and probably other oxygenated free radicals, can be followed using this technique, provided that the experiments are conducted fast enough to capture the intermediate formed in the first step of the oxidation, i.e, the radicals formed by HT from the phenolic sites. This information is expected to be useful to further experimental investigations on the title reaction.

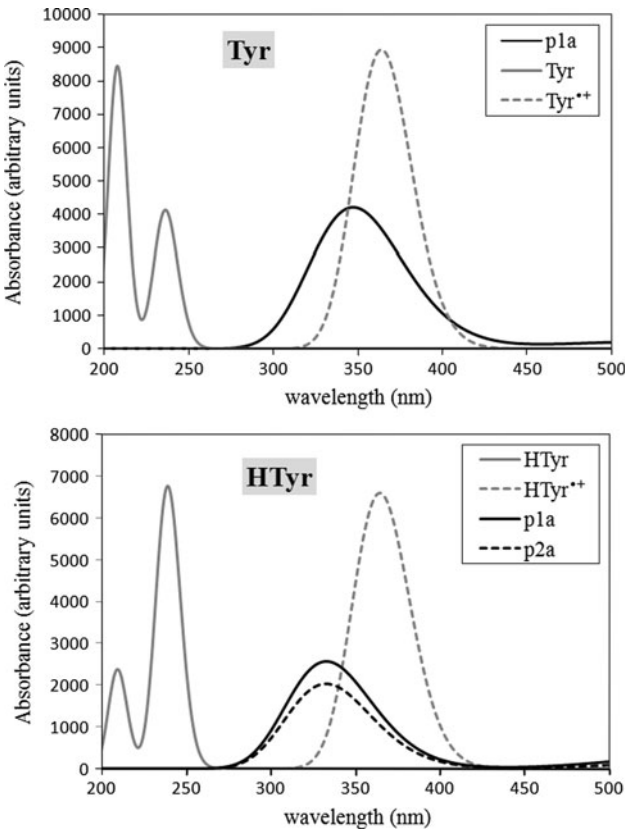

Fig. 5 Computed UV-vis spectra of HTyr, Tyr, their radical cations, and their main products of reaction, in water solution

In addition, the radical cations of HTyr and Tyr also present one absorption band, red-shifted with respect to the neutral molecules, with λ_{max} at approximately 365 nm. Based on the calculated data for the deprotonation of the radical cations, this band is not expected to be experimentally observed, since these species are predicted to spontaneously and rapidly deprotonate yielding the p1a phenoxyl radicals. We are providing their spectra nonetheless to facilitate possible future comparisons that allow confirming or rejecting this hypothesis.

4 Conclusions

The reactions of HTyr and Tyr with OCH_3 and OOH free radicals, in lipid and aqueous media, have been studied. Four mechanisms of reaction have been considered: SET, SEPT, HT, and RAF.

It was found that the SET mechanism does not contribute to the overall reactivity of HTyr and Tyr toward $^{\cdot}OOH$ and $^{\cdot}OCH_3$ radicals. However, it can be important for the reactions of these two compounds with other free radicals. In particular, the SEPT processes from HTyr to OH and $OCCl_3$ radicals, as well as that from Tyr to $^{\cdot}OCCl_3$ are predicted to be diffusion-controlled. The electron transfer from HTyr to $^{\cdot}OOCCl_3$ and from Tyr to $^{\cdot}OH$ are predicted to be slower but still very fast ($\sim 10^6\ M^{-1}\ s^{-1}$). The deprotonation of the formed radical cations ($HTyr^{\cdot+}$ and $Tyr^{\cdot+}$) was found to occur spontaneously from site 1a, and thus, the SEPT mechanism would yield the p1a phenoxyl radicals.

The $^{\cdot}OOH$-scavenging activity of HTyr and Tyr was found to take place exclusively by H transfer, and it is also predicted to be the main mechanism for their reactions with $^{\cdot}OCH_3$. The contributions of the HT mechanism to the overall reactivity of HTyr and Tyr toward $^{\cdot}OOH$ and $^{\cdot}OCH_3$ were found to be >98%. This mechanism is also predicted to be the main one for the scavenging activity of HTyr and Tyr when reacting with other $^{\cdot}OR$ and $^{\cdot}OOR$ radicals, provided that R is an alkyl or an alkenyl group.

Accordingly, it is predicted that the major products of reaction yield from the oxygenated radicals scavenging activity of HTyr and Tyr are the phenoxyl radicals. For the particular case of HTyr, depending on the nature of the reacting radical, the product can be mainly the p1a phenoxyl radical or a mixture of p1a and p2a phenoxyl radicals.

Tyr was found to be less efficient than HTyr for scavenging $^{\cdot}OCH_3$ and $^{\cdot}OOH$, in agreement with previous reports on their relative reactivity with other free radicals. Moreover, while HTyr is predicted to be a good peroxyl scavenger, Tyr is predicted to be only moderately efficient. Therefore, regarding the antioxidant protective effect of olive oil, and white wine, the role of HTyr is more important than that of Tyr.

In addition, both HTyr and Tyr are predicted to react faster in aqueous solution than in non-polar media with oxygenated free radicals.

HTyr and Tyr differ in the number of OH groups in the phenyl group. The higher reactivity of HTyr then can be rationalized based on the additional hydroxyl group, located in ortho position. Therefore, it can be safely concluded that this second OH group dramatically changes the reactivity of the phenol moiety, i.e., catechols are much more efficient as radical scavengers than single phenols. This can be extended to other phenolic compounds; for example, piceatannol is expected to be more efficient as free radical scavenger than resveratrol.

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