

Why is quercetin a better antioxidant than taxifolin? Theoretical study of mechanisms involving activated forms

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Abstract The stronger antioxidant capacity of the flavonoid quercetin (Q) compared with taxifolin (dihydroquercetin, T) has been the subject of previous experimental and theoretical studies. Theoretical work has focused on the analysis of hydrogen bond dissociation energies (BDE) of the OH phenolic groups, but consider mechanisms that only involve the transfer of one hydrogen atom. In the present work we consider other mechanisms

involving a second hydrogen transfer in reactions with free radicals. The relative stability of the radicals formed after the first hydrogen transfer reaction is considered in discussing the antioxidant activity of Q and T. In terms of global and local theoretical reactivity descriptors, we propose that the radical arising from Q should be more persistent in the environment and with the capability to react with a second radical by hydrogen transfer, proton transfer and electron transfer mechanisms. These mechanisms could be responsible of the stronger antioxidant capacity of Q.

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Introduction

Quercetin (Q) and taxifolin (T) are flavonol- and dihydroflavonol-type flavonoids, which are widespread secondary metabolites in plants [1–4]. In flavonols it has been clearly proved that the B-ring (see Scheme 1) is largely responsible for their antioxidant capacity, especially when it includes a catechol moiety, as in both Q and T. The structural difference between Q and T is the presence of a 2,3 double bond in Q which is believed to contribute to its stronger antioxidant activity [4]. It is generally accepted that polyphenols (ArOH) such as Q and T scavenge free radicals (R[•]) following four different mechanisms:

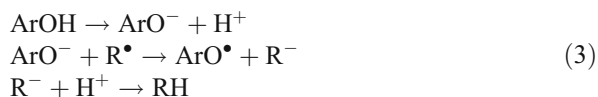
1. Hydrogen atom transfer (HAT) from the molecule to the radical [5, 6].



2. Electron transfer-proton transfer (ET-PT). The electron transfer is followed by release of a proton [7, 8].



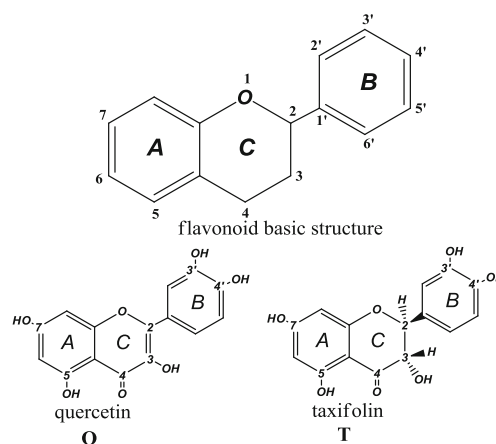
3. Sequential proton loss-electron transfer (SPLET), in which a proton is lost first [9–11].
4. Adduct formation; this mechanism may lead to stable products by bond formation between a free radical and the antioxidant molecule, thus stabilizing the radical [12].



The final products of reactions 1 to 3 are the harmless RH species and the oxygen-centered radical ArO^\bullet , which is less reactive than R^\bullet due to the stabilizing effect of the aryl group [13–17]; the latter mechanism is relatively specific and is mainly observed in solutions rich in reactive species, e.g., radiolytic solutions [12]. The HAT mechanism is driven by the O-H bond dissociation enthalpy (BDE), while the ET-PT mechanism is directly dependent on the ionization potential. The energetics of the SPLET mechanism are strongly solvent- and pH-dependent, and in the gas phase can be related to the proton affinity [9–11]. The main proposed mechanisms through which antioxidants may play their protective role have been analyzed and discussed in detail in a recent review [18].

Theoretical calculations of the O-H BDE have been used to explain the antioxidant capacity of flavonols because of its relationship to the HAT mechanism [14, 15, 17, 19–27]. In this context, the stronger antioxidant activity of Q compared to T has been attributed to the fact that Q has lower O-H BDE than T [28].

The contribution (or not) of the 3-OH group (on ring C, see Scheme 1) to the antioxidant potential of Q has been the subject of experimental [4] and theoretical [25] studies. It has been proposed that the effectiveness of this group depends of the presence on the 2,3 double bond in conjugation with the 4-carbonyl group [25]. Trouillas et al. [25] have carried out a theoretical evaluation of the importance of the 2,3 double bond in a comparative study of the antioxidant activity of Q and T. Based on calculated BDE, the authors showed that H transfer is energetically more favorable from the OH groups on the ring B in both flavonols and that Q has a lower BDE than T (71.1 vs 72.7 kcal mol⁻¹ at the B3LYP/6-311+G(d,p) level of theory). In the same work the enol-keto tautomerism in Q between the 3-OH and C-2 centers was evaluated. Although the keto form is not the thermodynamically preferred form in the gas

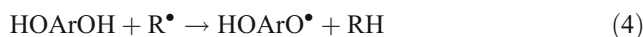


Scheme 1 Basic structure of flavonoids (top), quercetin and taxifolin (bottom)

phase, the authors suggested that in some enzymatic environments it could exist to a significant extent, and the hydrogen positioned on C-2 should be easily removed based on its lower BDE (61.6 kcal mol⁻¹ at the B3LYP/6-311+G(d,p) level of theory). The experimental evidence shows that the antioxidant activity of T is about half of that of Q [4]. Considering that experiments quantify the comparison of a reference radical system with the antioxidant compound under investigation [29, 30], how much of this difference in antioxidant power could be due to the difference between the first H-BDE? In the present work we present a comparative theoretical analysis of Q and T in the gas phase, including in the study some activated forms that could be involved in free radical quenching mechanisms. Our analysis involves only the reactivity of the antioxidant molecules and does not consider the nature of the oxidant species.

Theoretical models and computational methods

The antioxidant mechanisms of Q and T involving hydrogen transfer were evaluated considering the following reactions:



To measure the reactivity or instability of the studied systems we used global and local theoretical descriptors that have been defined within the context of density functional theory [31, 32]. The global electrophilicity measures the capability of a system to acquire electronic charge from the environment, and is calculated by the following simple

expression [33], $\omega = \frac{\mu^2}{2\eta}$, where μ and η are the electronic chemical potential and chemical hardness of the ground state of atoms and molecules, respectively. While μ describes the charge transfer pattern in the system in its ground state geometry, η describes the resistance to this charge transfer [34–37]. Both have been calculated using the finite difference approximation [37, 38].

$$\mu = \left[\frac{\partial E}{\partial N} \right]_v \approx - \left(\frac{I + A}{2} \right) \quad (6)$$

$$\eta = \frac{1}{2} \left[\frac{\partial \mu}{\partial N} \right]_v \approx - \left(\frac{I - A}{2} \right), \quad (7)$$

where I is the first ionization energy and A is the electron affinity.

As local descriptors of reactivity we have used the spin density (SD) and the radical Fukui function ($f^0(\vec{r})$). The SD and its importance in describing radical stabilization has been discussed by Parkinson [39]. It is defined as the difference between α and β electron densities. Greater spin density delocalization should contribute to the stabilization of the respective radical and therefore to its persistence in the medium. The radical Fukui function used in our analysis was obtained using the following approximation:

$$f^0(\vec{r}) = (\rho_{N+1}(\vec{r}) - \rho_{N-1}(\vec{r}))/2, \quad (8)$$

where $\rho_N(\vec{r})$ is the electron density of the N -electron species. It is common to use condensed values of these local functions to obtain numbers associated with regions or atoms that can be interpreted chemically. To do this, it is necessary to partition the molecular space with subsequent integration (condensation) of the Fukui function on those separate regions. Tiznado et al. proposed a new methodology to condense the Fukui functions as the expression [40, 41]

$$f_k^0 = \int_{\Omega_k} f_k^0(\vec{r}) d\vec{r}, \quad (9)$$

where the Ω_k region is obtained from the topological analysis of the Fukui function, and f_k^0 is the condensed value of the Fukui function in that region. This methodology has been used to correctly describe the reactivity of different organic and inorganic molecules [40–48]. Additionally, the local electrophilicity is described by the following expression: $\omega_k = \omega f_k^0$ [49]. The chemical interpretation of f_k^0 and ω_k is that higher values of these two descriptors represent the regions in a molecule that are most susceptible to free-radical attack. All calculations were performed at the B3LYP [50, 51] 6-311+G(d,p) [52, 53] level of theory with

the Gaussian 03 package of programs [54]. All the structures were optimized and it was verified that imaginary frequencies are absent, which ensures that the structures correspond to true minima at this level of theory. The energy values used in the BDE calculations were corrected taking into account the zero point energy (ZPE). We have been careful to consider the stabilizing effect of the H bonds between the two adjacent OH groups in ring B of the molecules and in the radicals formed. The topological analysis of the scalar functions and the calculation of the condensed Fukui function were done with the *DGrid 4.4* set of programs [55].

Results and discussion

Table 1 shows the enthalpy and free energy changes (ΔH and ΔG) when a hydrogen atom is removed from the different OH centers in the studied molecules. These parameters can be compared with the BDE previously reported. We identified them according to the position of the OH from which the H atoms are removed (Scheme 1), i.e., the ΔH and/or ΔG corresponding to the reaction where a H is removed from the OH on carbon 3' is designated as 3'-Q or 3'-T, depending on whether the reaction corresponds to Q or T, respectively. As can be seen in Table 1, our results for the ΔH and ΔG involved in the removal of the first H are in good agreement with those previously reported at the same level of theory for the BDE [25], the 4'-OH hydrogen transfer is slightly favored in Q compared with T, with reaction enthalpies (ΔH) of 72.9 and 74.2 kcal mol⁻¹, respectively, while for the 3'-OH dissociation the opposite is true, with ΔH values of 75.5 and 74.4 kcal mol⁻¹, respectively, .

The free radicals formed by H removal from the 4'-OH groups of Q and T are called Q-4' and T-4', and the radicals formed by H removal from the 3'-OH groups are called Q-3' and T-3', respectively. After the loss of the first hydrogen atom, these radicals are the most favored ones thermodynamically (see Table 1), and it is expected that they will be the major products initially formed in HAT reactions. The ΔH and ΔG values are identified in ascending order, labeled in the Table by numbers in parentheses. In the same table are shown the ΔH and ΔG corresponding to H removal from these four radicals (from the semiquinones in Table 1). Interestingly, the smallest ΔH and ΔG values are quite similar for radicals coming from Q and T, and they are even slightly lower than the energies associated with the hydrogen transfer of Q and T. Moreover, in aqueous solution the semiquinone radical (Q-4', T-4', Q-3' and T-3') is acidic and could ionize to the semiquinone radical anion, which could then undergo an electron transfer reaction with another radical, forming the quinone and deactivating the radical (see Fig. 1). This possibility has now been considered and

Table 1 Reaction energies (ΔH and ΔG) computed for quercetin (Q), taxifolin (T) and activated forms. All values are in kcal mol⁻¹. The numbers in parentheses represent the relative stabilities of the species formed

Reactions involving first hydrogen transfer									
$Q \rightarrow Q - sq_x + H \bullet$									
		Q-4'	Q-3'	Q-3	Q-7	Q-5			
		(1)	(2)	(3)	(4)	(5)			
	ΔH	72.9	75.5	80.9	86.5	95.2			
	ΔG	65.3	67.2	72.5	78.1	86.2			
$T \rightarrow T - sq_x + H \bullet$									
		T-4'	T-3'	T-7	T-5	T-3			
		(1)	(2)	(3)	(4)	(5)			
	ΔH	74.2	74.4	89.6	96.1	104.5			
	ΔG	66.6	66.9	81.0	86.7	95.7			
Reactions involving semiquinone forms $Q - sq_x \rightarrow Q - q_x + H \bullet$									
		Q-4'-3	Q-4'-3'	Q-4'-7	Q-4'-5	Q-3'-4'	Q-3'-3	Q-3'-7	Q-3'-5
		(1)	(3)	(5)	(7)	(2)	(4)	(6)	(8)
ΔH		71.0	74.9	90.6	99.5	72.4	83.4	98.6	106.7
ΔG		62.5	66.1	82.8	91.4	64.2	76.3	91.2	99.2
$Q - sq_x \rightarrow Q - q_x + H^+$									
		Q-4'-7	Q-4'-3	Q-4'-3'	Q-4'-5	Q-3'-7	Q-3'-3	Q-3'-4'	Q-3'-5
		(1)	(2)	(6)	(7)	(3)	(4)	(5)	(8)
PA		312.1	313.1	321.9	325.8	315.2	318.4	319.4	328.4
ΔG		312.4	313.3	321.6	325.6	316.5	319.1	319.7	329.3
$T - sq_x \rightarrow T - q_x + H \bullet$									
		T-4'-3'	T-4'-3	T-4'-7	T-4'-5	T-3'-4'	T-3'-3	T-3'-7	T-3'-5
		(2)	(3)	(5)	(7)	(1)	(4)	(6)	(8)
ΔH		71.8	74.3	107.0	114.4	71.6	79.0	108.5	115.2
ΔG		63.6	64.0	99.3	106.2	63.4	73.1	100.8	106.9
$T - sq_x \rightarrow T - sq_x + H^+$									
		T-4'-7	T-4'-3'	T-4'-5	T-4'-3	T-3'-7	T-3'-4'	T-3'-5	T-3'-3
		(1)	(4)	(5)	(7)	(2)	(3)	(6)	(8)
PA		316.7	327.9	329.7	341.4	317.2	327.7	330.7	342.2
ΔG		317.2	327.4	329.9	341.2	318.2	327.1	330.9	342.5

proton affinities (PA) were calculated for the semiquinone forms. The PA values are clearly different, being lower for the semiquinone derived from Q compared with T: 312.1 and 316.7 kcal mol⁻¹, respectively.

Based on these results, we propose that the reactions shown in Fig. 1 are possible and energetically competitive. The radicals formed after removal of the 4'-OH and 3'-OH hydrogen atoms (Q-4' and Q-3', T-4' and T-3') are in equilibrium due to migration of the respective hydrogens between the two neighboring oxygens. Q-4' is thermodynamically favored over Q-3'. Thus, Q-4' is expected to be the major product in this equilibrium. It is important to remark that Q-4' presents lower ΔH and ΔG associated with mechanisms involving a second HAT and a smaller PA that might be associated with PT mechanisms.

At this point of our discussion there are small differences favoring Q in terms of the ΔH and ΔG of the reactions involving the first hydrogen transfer of Q and T. Both

systems could transfer the same number of phenolic hydrogen atoms and almost as easily energetically. A 2 kcal mol⁻¹ difference is not conclusive at the level of calculation used in our estimate. Considering the possibility of a second hydrogen transfer in the antioxidant mechanisms, the Q derivatives seem to have a thermodynamic advantage compared to the products obtained from T. Complementarily, we propose that the ability of the Q-4', Q-3', T-4' and T-3' radicals to act as antioxidants through hydrogen transfer reactions depends on their relative stability versus other possible reactions such as polymerization, reactions with O₂ [56] or different degradation processes. We have used the global reactivity descriptors, hardness η , and electrophilicity index ω , to evaluate the relative stabilities of the radicals. The most stable radicals will be the most persistent ones in the medium, allowing them to react with other potentially harmful radicals through specific hydrogen transfer mechanisms.

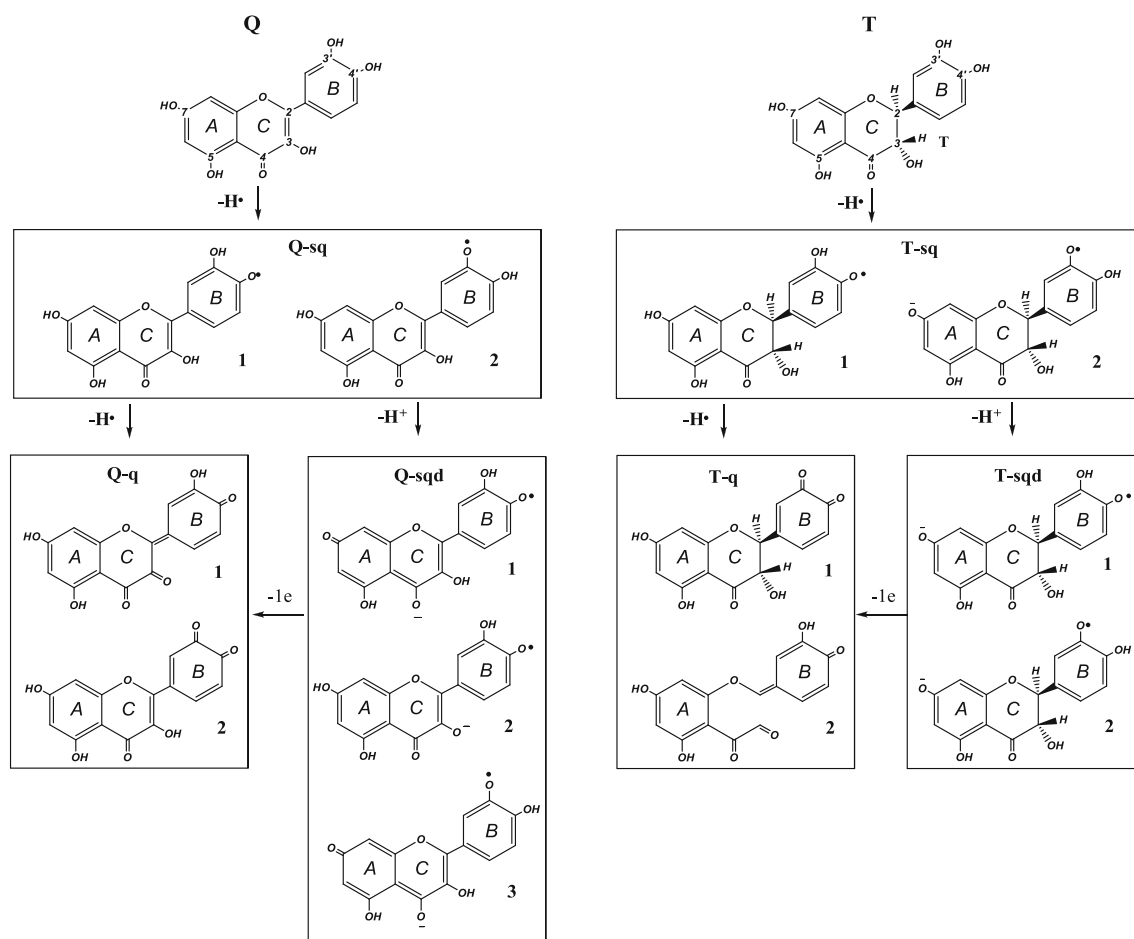


Fig. 1 Schematic reaction sequences proposed based on the reaction energy analysis (ΔH and ΔG). A represents the quercetin (Q-related) reactions, and B represents the taxifolin (T-related) reactions. The

subscripts sq, sqd, and q correspond to semiquinone, deprotonated-semiquinone and quinone forms, respectively

The estimated values of these global descriptors are shown in Table 2.

As can be seen in Table 2, Q-4' and Q-3' are the hardest systems, but the differences are very small ($2.0 \text{ kcal mol}^{-1}$) compared with T-4' and T-3' to assign them as the most stable systems in terms of this descriptor. Also, Q-4' and Q-3' are the least reactive in terms of their electrophilicity indexes, Q-4' having the lowest value. Therefore, the stability analysis based on these descriptors suggests that the two radicals obtained as Q products after the first hydrogen removal are relatively more stable compared with those from T, and radical Q-4' is the most stable among them. We have included the ionization potentials (IPs) in the results shown in Table 2 as a simple descriptor to indicate the feasibility of possible reactions that involve electron transfer prior to the proton transfer (Eq. 2) to a potentially free radical-derived base. Considering this simple model, the values of the IPs between the analyzed radicals are comparable with the Q and T (170.3 and $183.8 \text{ kcal mol}^{-1}$ respectively) values calculated at the same level of theory (B3LYP/6-311+G(d,p)). In terms of this descriptor, Q-4'

should be the radical system most favored when the reaction described by Eq. 3 occurs, considering the electron transfer as the limiting step of the reaction.

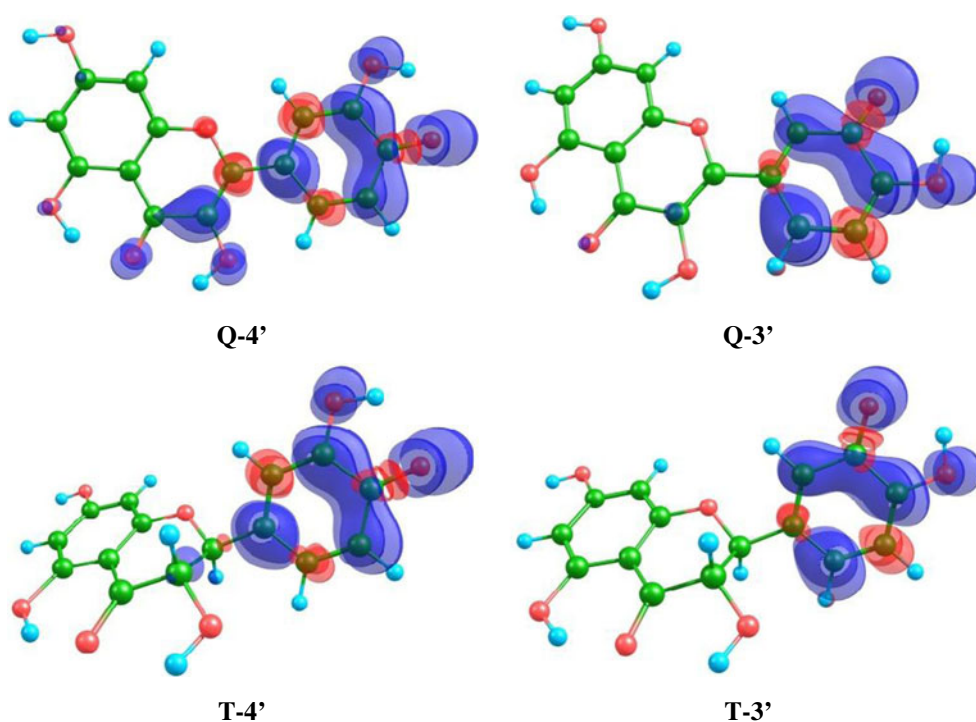
The spin density isosurfaces of the most stable radicals are shown in Fig. 2. It can be clearly seen that the spin density of Q-4' is delocalized over rings B and C, whereas in the other radicals it is principally delocalized over ring B. Therefore, in terms of the spin density delocalization analysis, Q-4' is the most stable radical.

As can be seen in Fig. 3, the local reactivity of the four radicals evaluated here is more widely distributed in Q-4'

Table 2 Global electronic properties in kcal mol^{-1}

	IP	η	ω
Q-4'	172.8	119.0	12.2
Q-3'	178.7	119.5	14.6
T-4'	182.7	117.1	18.3
T-3'	183.5	117.0	18.9

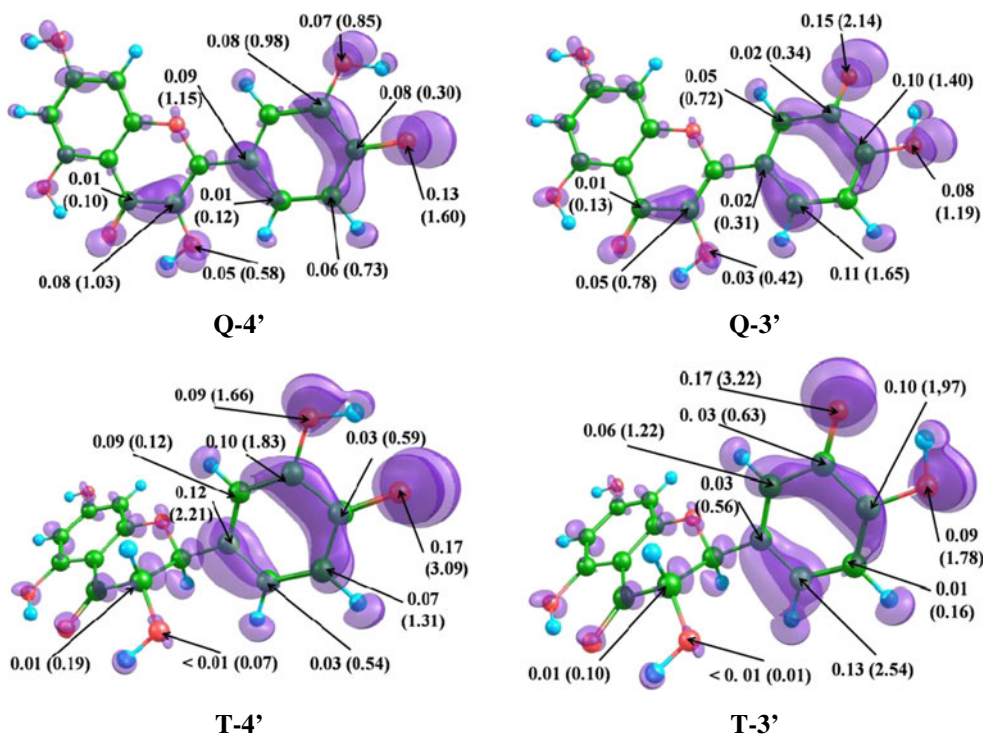
Fig. 2 Spin density isosurfaces of the most stable radicals: in blue the α spin density and in red the β spin density



and Q-3'. The Fukui function and local electrophilicity index identify the oxygen from which the first hydrogen is removed as the most reactive center in the four radicals, but the local values on T-4' and T-3' are the highest. Additionally, these local reactivity descriptors clearly show that the 3-OH group is more reactive in Q-4' than

in T-3', in agreement with the energy analysis which predicts that the second hydrogen will be transferred from the 3-OH in Q-4'. It is important to note that both local reactivity descriptors agree completely in their assignment of local reactivities in these four evaluated radicals.

Fig. 3 Isosurfaces of the radical Fukui function. The numbers represent condensed Fukui functions using Eq. 9 and in parentheses are the corresponding ω_k values



Conclusions

Finally, we can summarize the results as follows. In terms of the energy analysis the transfer of a second hydrogen atom to quench a second free radical through a HAT reaction (Eq.5) is a possible route. In both Q and T, the most thermodynamically probable reactions lead to two radicals from each molecule (Q-4', Q-3' and T-4', T-3'). From the global and local reactivity descriptor analysis, Q-4' is the most stable of all four possible radicals. It is important to remark that although global hardness values are in agreement with the reactivity trends, they are very close to each other and it is not possible to rank the reactivities this descriptor. In the light of these results we can hypothesize that only Q-4' is sufficiently stable to remain in the environment long enough to react through a HAT mechanism with a second free radical. Additionally the semiquinone radicals arising from the Q reaction (Q-4') are energetically favored to react via proton and/or electron transfer to another anion or radical, forming the quinone and deactivating the radical. Considering this model, each Q molecule could react with two free radicals whereas T could do so only with one. We believe that this mechanism deserves to be considered in the explanation of why the antioxidant activity of Q is almost twice that of T.

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