Solvent Effects on the Structure and The Antioxidant Activity of Tyrosol, Hydroxytyrosol and Hydroxytyrosol Acetate

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Received: 17/05/2023; Accepted: 23/10/2023

Abstract

Hydroxytyrosol [(3,4-dihydroxyphenyl)ethanol] is one of the major natural phenolic compounds present in olive fruits, virgin olive oil, table olives, and waste streams generated during olive processing [1]. This compound has shown antimicrobial, hypoglycemic, hypolipidemic, antioxidant, and hypocholesterol properties of particular interest with regard to food and human health [2].

The molecular structure and radical scavenging activity of Hydroxytyrosol have been explored by using density functional theory (DFT). The homolytic O–H bond dissociation enthalpy (BDE), the ionization potential (IP), the heterolytic O–H bond dissociation enthalpy proton dissociation enthalpy (PDE), proton affinity (PA) and electron transfer enthalpy (ETE), were determined in gas phase, water and pentylethanoate the hydrogen atom transfer (HAT) appears as a major mechanism in antioxidant action.

Keywords: Hydroxytyrosol, Tyrosol,Hydroxytyrosol acetate,Antioxidant, Density Functional Theory (DFT), Antimicrobial, Hypoglycemic, Hypolipidemic.

***TobRegul Sci.™ 2023;9(2):*1448 -1465**

DOI: doi.org/10.18001/TRS.9.2.90

I. Introduction:

The beneficial effects of a Mediterranean diet rich in olive oil have been widely reported [1–4]. Olive products are rich in natural antioxidants that may inhibit oxidative stress during the development of major diseases such as coronary heart disease [5, 6], cancer [7] or neurodegenerative diseases [8, 9] and inflammatory processes [10]. The antioxidant properties of olive oil products have been attributed to the presence of phenolic compounds [11, 12], which act by scavenging free radicals and chelating metal ions to lower hydroxyl radical formation [1]. The major phenolic compounds in this oil, are tyrosol (Tyr, 4-(2-hydroxyethyl)

phenol),hydroxytyrosol (HTyr, 4-(2-Hydroxyethyl)-1,2-benzenediol) and hydroxytyrosolacetate (HTyr-ac, 2-(3,4-Dihydroxyphenyl)ethyl acetate)[13-15].

HTyr has been shown to have anti-atherogenic, anti-thrombotic and anti-inflammatory[16,17]. Numerous studies have also shown that HTyr may be a potential anti-cancer agent[18-21]. Moreover, HTyr has been shown to be a potent antioxidant which can scavenge the superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radicals [14,22,23].

Tyr seems to be effective in inhibiting the oxidation of cholesterol in LDL and preventing the modification of the apoproteic moiety [24]. Tyr has also been effective in inhibiting leukocyte 5-lipooxygenase [25] and protecting the Caco-2 intestinal mucosa cells against the cytostatic and cytotoxic effects produced by oxidized LDL.

HTyr-ac was the first one to be described [26] and its antioxidant properties studied[27,28]

The main aim of this study is to calculate reaction enthalpies related to the three antioxidant mechanisms: the hydrogen atom transfer (HAT), single-electron transfer–proton transfer (SET–PT) and sequential proton loss electron transfer (SPLET) for the conformation of Tyr, HTyr and HTyr-ac(Fig. 1), using the DFT functional M062X [32]associated to the Popletriple-zeta basis set 6-311++G(d,p)[a-c]. The various reaction enthalpies BDE, IP, PDE, PA and ETE values were used as molecular descriptors to elucidate the radical scavenging activity of compounds under investigation.Solvent effects on these parameters has been also examined.

II. Computational Method

Geometry optimizations and frequency calculations have been carried out using the M06-2X functional [32] and the Pople triple-zeta basis set 6-311++G(d,p),[a-c] in conjunction with the SMD continuum model [33], using pentylethanoate and water as solvents to mimic lipidic and aqueous environments. The M06-2X functional has been recommended for thermochemistry calculations by their developers [31]. All the electronic calculations were performed with Gaussian 09 package of programs [34].

A fully relaxed potential energy scan was carried out against one or two dihedral anglesat the M062X/6-31+G(d) level of theory.A further geometry optimization was performed at the same level of theory. Vibrational frequencies of the optimized structures were computed using the same level of theory.

For thermochemical computations, we used the revised versionof our home made FORTRAN code named Tempo[d,e] forquickly accessing thermodynamic parameters of the various clustersinvestigated at any temperature. Tempo was also used to derivetemperature effects on the relative stability (relative population)of the various isomers of a given structure. For a givingtemperature, their relative Stabilities are evaluated through theircanonical probabilities defined as;

$$P_k(T) = \frac{e^{-\beta G_k(T)}}{\sum_i e^{-\beta G_i(T)}} \qquad (1)$$

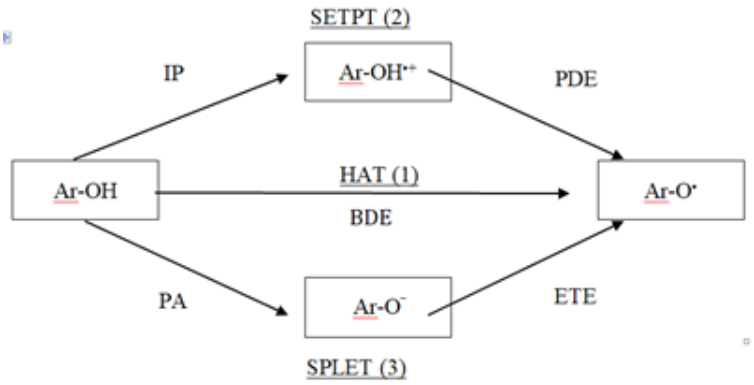
Where, $\beta = k_B(T)$ is the Boltzmann constant, $G_k(T)$is the free energy of the k^{th} isomer of the considered molecule at the temperature T as calculated by the program Tempo. This program has been used successfullyto study the temperature-dependence relative population of various clusters.[e-i].

In the literature [35–38],at least three mechanisms are involved in the antioxidant activitiesof phenolic antioxidants (ArOH):

(1) The mechanism of direct hydrogen atom transfer (HAT)[39]: bond dissociation enthalpy (BDE) of theO–H of the phenolic antioxidants (ArOH) is a good parameter to evaluate this mechanism.

(2) The mechanism of single electron transfer followed by a proton transfer (SETPT): ionizationpotential (IP) and the proton dissociation enthalpy (PDE) are suitable parameters to qualify and quantify such a mechanism.

(3) The mechanism of sequential proton loss and electron transfer (SPLET): proton affinity (PA)and electron transfer enthalpy (ETE) are appropriate parameters for this mechanism.



Scheme 1: Mechanisms of the antioxidant activity.

The BDE, IP, PA, PDE and ETE are calculated by the following expressions:

$$BDE=\Delta H(ArO^{\bullet})+\Delta H(H^{\bullet})-\Delta H(ArOH) \qquad (1)$$

$$IP=\Delta H(ArOH^{\bullet+})+\Delta H(e^{-})-\Delta H(ArOH) \qquad (2)$$

$$PDE=\Delta H(ArO^{\bullet})+\Delta H(H^{+})-\Delta H(ArOH^{\bullet+}) \qquad (3)$$

$$PA=\Delta H(ArO^{-})+\Delta H(H^{+})-\Delta H(ArOH) \qquad (4)$$

$$ETE=\Delta H(ArO^{\bullet})+\Delta H(e^{-})-\Delta H(ArO^{-}) \qquad (5)$$

III. Results and discussion

III-1 Conformational study Tyrosol (Tyr) could present two conformers Tyr1 and Tyr2 (Fig.1).The Tyr2 structure is obtained from the latter after turning the dihedral angle Θ_1=C_3-C_4-O_4-H_4 from 180° to 0° by a step size of 10° (Fig.1).It is worth mentioning that the difference in zero-point corrected electronic energy (ZEE) between both conformers isonly0.4 kJ/mol, indicating that the global minimum ZEEof Tyrosol is twofold degenerated. In addition, the temperature dependent relative stability indicates that both isomers strongly compete irrespective the temperature (Fig.2).

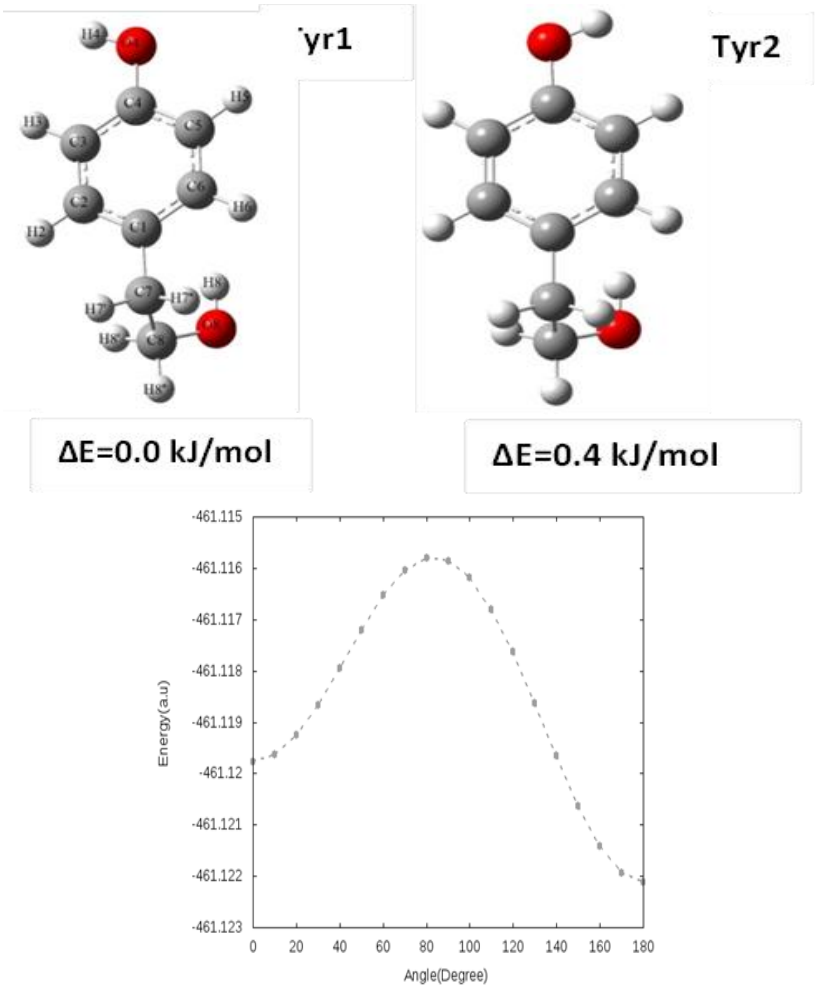


Fig. 1. Optimized geometries of isomers Tyr1 and Tyr2 of Tyrosol and relaxed scan applied leading to Tyr1 and Tyr2, obtainedat the M06-2X/6-311++G(d,p) level of theory.

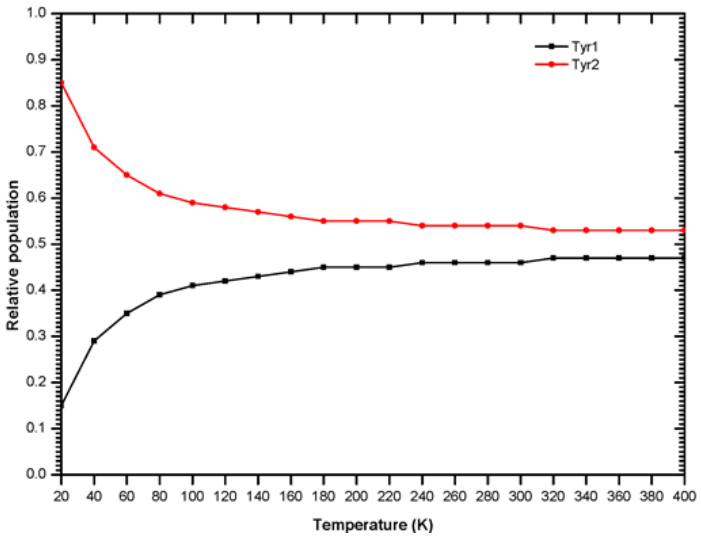
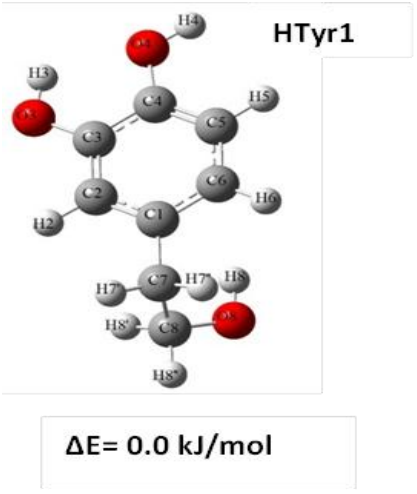
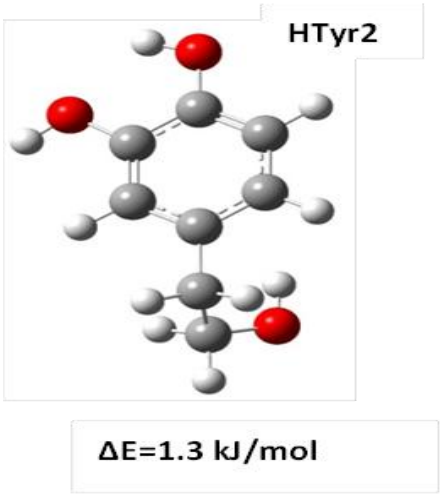


Fig. 2.Temperature dependence of the relative stability of different conformers of Tyrosol.

Hydroxytyrosol (HTyr), present three conformers HTyr1, HTyr2 and HTyr3, obtained by rotating dihedral angles Θ_1=C_3-C_4-O_4-H_4 and Θ_2=C_2-C_3-O_3-H_3 (Fig.3). HTyr1 is the most stable isomer of HTyr at 0 K. HTyr2 lies 1.3kJ/mol above HTyr1. This slight difference indicates that HTyr1 and HTyr2 are isoenergetic. However, HTyr3 lies 18.8kJ/mol above HTyr1. This high difference indicates that this isomer would be difficult to be observed experimentally. The calculated canonical probabilities of the various isomers of HTyr show and confirm that HTyr3 does not participate at all to the population of the isomers of HTyr(Fig.4). Moreover, both isenergetic structures compete strongly for temperatures higher than 100K.





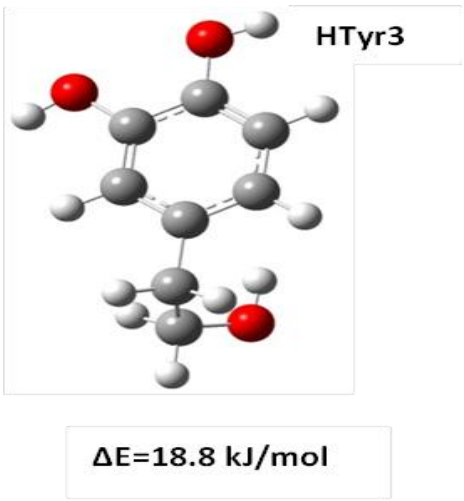


Fig. 3. Optimized geometries of isomers HTyr1, HTyr2 and HTyr3 of Hydroxytyrosolobtainedat the M06-2X/6-311++G(d,p) level of theory.

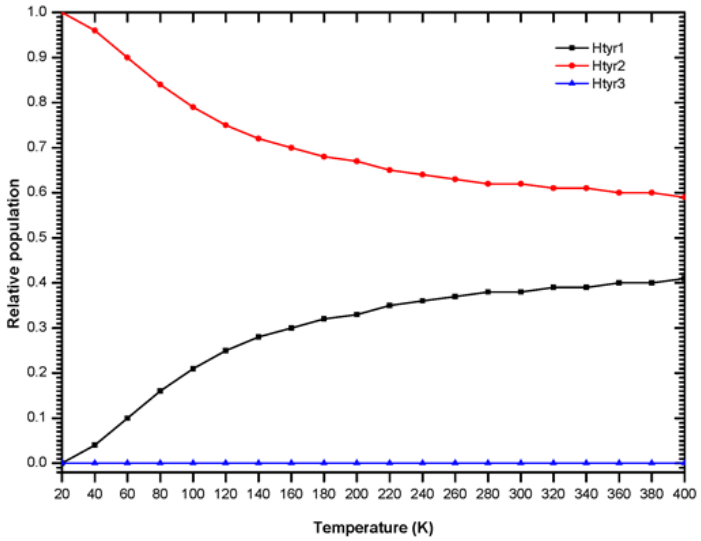


Fig. 4.Temperature dependence of the relative stability of different conformers of hydroxytyrosol.

As far as Hydroxytyrosol acetate (HTyr-ac) is concerned, three conformers have been also located HTyr1-ac, HTyr2-ac and HTyr3-ac (Fig. 5). HTyr1-ac is the most stable isomer followed by HTyr2-aclying 1.3kJ/mol above. The less stable isomer is HTyr3-ac lying 18.8kJ/mol above HTyr1-ac. These relative ZEE indicate that the population of the isomers of Hydroxytyrosol acetate would be constituted only of HTyr1-ac and HTyr2-ac. This anticipation is nicely corroborated by the calculated temperature dependent relative stability (Fig.6). In fact, HTyr3-ac has no chance to participate in the population of isomers of Hydroxytyrosol acetate, while HTyr1-ac and HTyr2-ac compete strongly and the competition is stronger as the temperature increases.

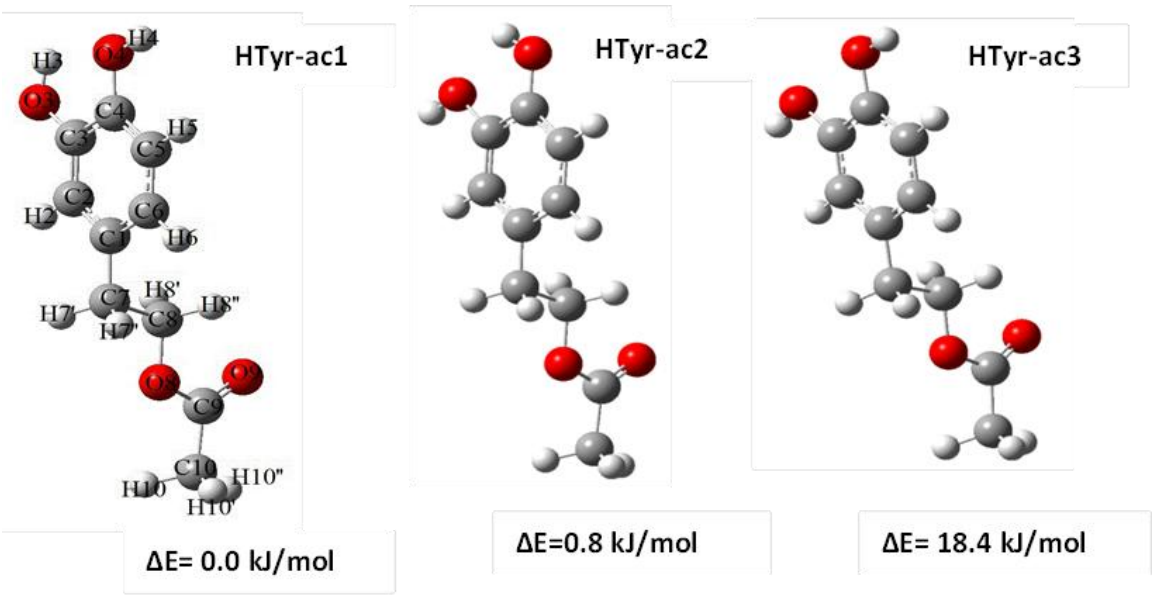


Fig. 5. Optimized geometries of isomers HTyr-ac1, HTyr-ac2 and HTyr-ac3 of Hydroxytyrosolacetate obtained at the M06-2X/6-311++G(d,p) level of theory.

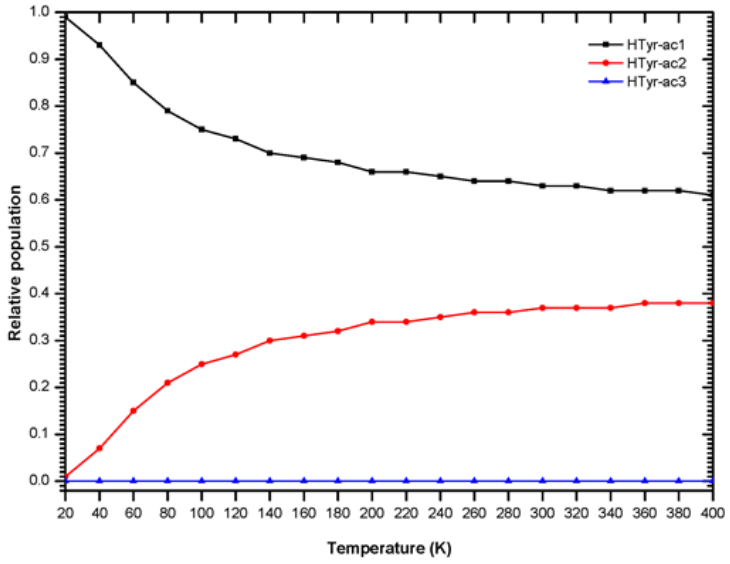


Fig. 6.Temperature dependence of the relative stability of different conformers of hydroxytyrosol.

We then focalize our study in the global minimum ZEE structures of Tyrosol, Hydroxytyrosol and Hydroxytyrosol acetate. Some selected structural parameters (bond lengths, bond angles and dihedral angles) of the Tyr1, HTyr1 and HTyr-ac1 calculated in vacuum, pentylethanoate andwater are reported in Table 1.

The overall results show that solvation of each molecular system,does not seriously modify its skeleton. The main differences in bond lengths and bond angles are generally equal or less than 0.01 Å and 2° respectively. Similar results were reported by Fifen et al.[42] in the solvent effects

on the structure of 3,4-dihydroxyphenylpyruvic acid.Theyalso noted that solvation has the additional effect of leading to slightly larger phenolic O-H bond lengths.[42]We can also note that the bond lengths O_3-H_3 and O_4-H_4increases with the dielectric constant of the solvant. The longer the bond length is, the weaker the bond is. Thus, the solvent weaken the O_3-H_3 and O_4-

Parameters	Tyr1			HTyr1			HTyr-ac1		
	vac.	pent.	wat.	vac.	pent.	wat.	vac.	pent.	wat.
Bond lengths									
C_1-C_2	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
C_2-C_3	1.38	1.39	1.39	1.38	1.38	1.38	1.38	1.39	1.38
C_3-C_4	1.39	1.39	1.39	1.40	1.40	1.40	1.40	1.40	1.40
C_4-C_5	1.39	1.39	1.39	1.38	1.38	1.38	1.38	1.38	1.39
C_5-C_6	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39
C_1-C_7	1.51	1.51	1.51	1.51	1.52	1.51	1.50	1.51	1.51
C_7-C_8	1.53	1.53	1.53	1.53	1.53	1.53	1.51	1.51	1.52
C_9-C_{10}	-	-	-	-	-	-	1.50	1.50	1.49
C_3-O_3	-	-	-	1.35	1.36	1.37	1.35	1.36	1.37
C_4-O_4	1.36	1.36	1.37	1.37	1.37	1.37	1.37	1.37	1.37
C_8-O_8	1.41	1.42	1.43	1.41	1.42	1.43	1.43	1.44	1.44
C_9=O_9	-	-	-	-	-	-	1.20	1.20	1.21
O_3-H_3	-	-	-	0.964	0.966	0.967	0.964	0.966	0.967
O_4-H_4	0.960	0.964	0.965	0.960	0.964	0.966	0.960	0.963	0.965
O_3-H_3 …O_4	-	-	-	2.133	2.135	2.178	2.133	2.136	2.180
Angles									
C_1-C_2-C_3	121.9	121.5	121.4	120.9	120.8	120.8	120.8	120.7	120.6
C_2-C_3-C_4	119.6	119.6	119.5	119.6	119.7	120.0	119.5	119.7	120.0

C_3-C_4-C_5	119.8	119.9	120.2	120.2	120.1	119.7	120.2	120.1	119.8
C_4-C_5-C_6	119.8	119.7	119.5	119.9	119.8	120.0	119.8	119.8	119.9
C_5-C_6-C_1	121.3	121.4	121.3	120.6	120.7	120.7	120.6	120.7	120.7
C_6-C_1-C_2	117.8	117.9	118.0	118.3	118.8	118.7	119.0	119.0	118.9
C_6-C_1-C_7	121.3	121.3	121.3	121.0	121.3	121.4	120.7	120.7	120.8
C_1-C_7-C_8	111.3	111.5	111.6	111.2	111.5	111.5	110.4	107.5	109.7
C_3-O_3-H_3	-	-	-	108.5	108.7	109.0	108.5	108.6	109.0
C_4-O_4-H_4	109.8	109.7	109.5	110.6	110.6	110.3	110.5	110.6	109.9
C_8-O_8-H_8	108.0	107.8	108.0	108.0	107.8	108.0	-	-	-
C_3-C_4-O_4	117.4	117.5	117.6	115.0	115.2	1159	115.0	115.2	116.0
C_7-C_8-O_8	112.1	112.4	112.1	112.2	112.3	112.1	107.4	107.5	107.6
O_8-C_9=O_9	-	-	-	-	-	-	123.1	123.0	122.5
O_8-C_9-C_{10}	-	-	-	-	-	-	111.2	111.5	112.3
Dihedrals									
C_3-C_4-O_4-H_4	179.2	179.8	179.7	175.2	179.3	177.5	178.8	179.5	176.7
C_2-C_3-O_3-H_3	-	-	-	179.1	180.0	179.4	179.4	179.4	179.8

H_4bond lengths and thereafter would enhance the antioxidant activity of the studied molecules.

Note that in the most stable structure of Hydroxytyrosoland Hydroxytyrosol-acetate (HTyr1 and HTyr-ac1), there is one internal hydrogen bond (IHB): O_3-H_3.....O4. This IHB has a stabilizing effect on the molecular structure as has been proven in several previous works[42-45].HTyrhas a Tyr structure with an extra OH group forming a catechol group, which is revealed to be responsible of its higher antioxidant activity. This catechol group is clever to stabilize free radicals leading to the creation of intermolecular hydrogen bonds.

Table 1. Some selected bond lengths (Å), angles (o) and dihedrals (o) of Tyr1, HTyr1and HTyr-ac1 (vac., wat. and pent. are for vacuum, water and pentylethanoate, respectively).

III-2 Solvation enthalpies of the proton andthe electron

Enthalpies are evaluated here at 298K and 1atm.

Solvation enthalpies of the proton,electron and hydrogen atom are respectively the enthalpy change of thereactions below:

$$Solvent(solv) + H^{+}_{(g)} \rightarrow solvent - H^{+}_{(solv)} \quad (6)$$

$$Solvent(solv) + e^{-}_{(g)} \rightarrow solvent^{-}_{(solv)} \quad (7)$$

$$Solvent(solv) + H_{(g)} \rightarrow solvent - H_{.(solv)} \quad (8)$$

In this work, we used the model wherea proton or an electron or an hydrogen atom is attached to one molecule of solvent,solvent(solv) representing a molecule of solvent in its cavity(this means that the molecule of solvent is solvated in the two solvents: water and pentylethanoate). Enthalpyof solvent(solv) is obtained from a geometry optimization followed by a frequency calculation. As a matter of fact, the reaction (6)with water as solventcould be written as:

$$H_2O_{(water)} + H^{+}_{(g)} \rightarrow H_3O^{+}_{(water)} \quad (8)$$

For gas phase,we used published values of6.1398 KJ/mol and 3.1351 KJ/mol[43,44] respectively for the proton enthalpy and the electron enthalpy at 298K and 1atm.The calculated solvation enthalpies of the proton and the electron in pentylethanoate andwater are reported in Table 2.

The only available experimental valuesfor the proton and electron solvation enthalpiesare hydration enthalpies, $\Delta_{hydr}H(H^{+})$ = –1090 kJ/ mol [45] and$\Delta_{hydr}{}^{H}(e^{-})$ = -153.1 kJ/mol [46]).Note that some calculated solvation enthalpies of the proton and the electron have been reported by several researchers[47-49].

Table 2.Solvation enthalpies of the proton ($\Delta_f H°_{solv}(H^{+})$) and the electron ($\Delta_f H°_{solv}(e^{-})$) inkJ/mol at 298K and 1atm

		Solvent	
		Water	Pentylethanoate
ε		78.4	4.73
$\Delta_f H°_{solv}(H^{+})$	This work	-1055.7	-1004.8
	a	-1052	-996
	b	-1024.3	
	c	-1022	
	d		

$\Delta_f H°_{solv}(e^-)$	This work	-77.4	-18.1
	a	-66	-43
	b	-104.4	
	c	-105	
	d		

[a] from Ref. [46]

[b] from Ref.[48]

[c]from Ref.[47]

[c]Experiment from Ref.[49]

III-3 Antioxidant Activities of Tyr, HTyr and HTyr-ac

In this work, the enthalpy is used instead of the free energy as a criterion of the thermodynamically preferred mechanism since in the investigated reactions, the entropy term does not exceed 9 kJ/mol.

Computational results of BDE, IP,PDE, PA and ETE in vacuum, water and pentylethanoate are presented in Table 3.

Table 3:DFT calculated parameters (in kJ/mol) of antioxidant mechanisms for Tyr1, HTyr1 and HTyr-ac1.

		HAT	SETPT		SPLET	
Systems	solvents	BDE	IP	PDE	PA	ETE
4-OH						
	Vacuum	366	782	894	1434	243
Tyr1	Pentylethanoate	359	609	30	278	361
	Water	368	502	30	156	375
	Vacuum	331	758	884	1396	246
HTyr1	Pentylethanoate	329	588	22	251	359
	Water	343	483	23	141	366

	Vacuum	330	777	864	1401	240
HTyr-ac1	Pentylethanoate	328	594	15	254	355
	Water	344	483	24	142	365
3-OH						
	Vacuum	335	758	887	1394	252
HTyr1	Pentylethanoate	333	588	26	249	365
	Water	345	483	25	139	369
	Vacuum	369	777	903	1456	224
HTyr-ac1	Pentylethanoate	355	594	42	290	346
	Water	355	483	36	153	366

III.3.1. HAT mechanism

A high rate of hydrogen atom transfer is expected to be related to a low O–H bond dissociation enthalpy (BDE).Geometry optimizations of the radicals were performed by the UM062X/6-311++G** method in vacuum, water and pentylethanoate, starting from the optimized structure of the parent molecule after the H-atom was removed from the positions 3 and 4at the aromatic ringofHTyr and HTyr-ac, and from the position 4 for Tyr . Calculated BDE, IP, PDE, PA and ETE are summarized in Table 4. In this table,we noticed clearly that learnHTyr-ac1and HTyr1 clearly have the lowest BDE in4-OH. In the vacuum, BDEs on the preferential sites of hydrogen atom cleavage are ordered as follows: 4-OH (Htyr-ac1)≈ 4-OH (Htyr1)<3-OH (Htyr1)<4-OH (Tyr1)<3-OH (Htyr-ac1).

This ordering confirms that H-transfer from the 4-OH,ismucheasier than from the 3-OH.This behavior f can beexplained by the fact that,the resonance structures have an unpaired electron on thetertiary carbon C1 in the solely case of radical 4-OH.

This electron deficiency can be immediately supplied by the presence of the electron-donors-CH_2CH_2OH group (for Tyr and HTyr) and -$CH_2CH_2OCOCH_3$ group (for HTyr-ac),with a consequent stabilization of the conformer.

Our results are found to be in good agreementwith previous theoretical and experimentalworks [28,50-52]

In their study, Lucarini et al. [51] have measured by EPR spectroscopy, equilibrium constants for several substitued phenolic antioxidants. Then, the BDE of the corresponding ArO-H bonds were calculated by comparison with the value of the 2,4,6-tri-tert-butylphenol. They concluded that, the presence of a second hydroxyl group at the ortho-position,increases the rate of H-atom transfer to peroxyl radicals,yielding a catechol ring that also lowers the O–H bond dissociation enthalpy (BDE).

Chimi et al. [53] and Benavente-Gracia et al. [54] showed a much better radical scavenging capacity for HTyr, relatively toTyr.

Gordon et al. [27] have suggested that ester group of HTyr-ac could be hindering the scavenging effect of the hydroxyl groups by intra- or intermolecular hydrogen bonding.

The difference found in radical scavenging is better explained from the fact that the ester group is an electron-withdrawing group and therefore the formed phenoxy radical formed may be less stable than in the case of HTyr.

peroxide (PV) and p-anisidine (AV) values to determine the primary and secondary oxidation products respectively. They found that HTyr and HTyr-ac have similar antioxidant activity with HTyr-ac being more effective in an emulsion.

III.3.2. SET-PT and SPLET Mechanisms

According to SET-PT mechanism, appropriate thermodynamically values are ionization potential (IP) and proton dissociation enthalpy (PDE). SET-PT mechanism is not favorable in vacuum

Molecules with low IP values are more susceptible to ionization and have stronger antioxidant properties. The calculated IPs of Tyr, Htyr and Htyr-ac have been presented in Table 4.Our calculations show that IPs are lower in water solvent then in pentylethanoate. IP values for Htyr and Htyr-ac is similarfrom 3 and 4 positions on the aromatic ring.

PDE values are also presented in Table 4.

PA and ETE parameters are related with the SPLET mechanism (Table 4). The lower PAs obtained in the solvent than that obtained in the vacuum.ETEs in solvents are higher than those in vacuum.

Conclusion

In the present article, Structure-antioxidant relationships of the tyrosol, hydroxytyrosol and hydroxytyrosol acetate have been investigated employing the DFT/M062X method together with the 6-311++G(d,p) basis set in vacuum , water and pentylethanoate media.

Each phenolic acid (PhA) studied in this work exhibits two significant isomers competing strongly in the population of the PhA at all temperatures. Apart from O-H bond lengths, solvent has practically no effect on the orther geometrical parameters of the phenolic acids studied. As far as the antioxidant activity of these PhAs is considered, hydroxytyrosolhas a higher antioxidant activity than tyrosol, due to the presence of the catechol group in hydroxytyrosol. Although theacetate group strengthens the O_3---H_3HBinhydroxytyrosol acetate, it has practically no effect on the O4---H4 HB. Thus, although the hydroxytyrosol acetate has a longer backbone than the hydroxytyrosol, it has a lower antioxidant activity than the latter.Elsewhere, andhydroxytyrosol have the same.

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