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Thermodynamics of primary antioxidant action of flavonols in polar solvents

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Abstract: Very recently, a report on the antioxidant activity of flavonoids has appeared, where authors concluded that Hydrogen Atom Transfer mechanism represents the thermodynamically preferred mechanism in polar media (https://doi.org/10.1016/j.foodres.2018.11.018). Unfortunately, serious errors in the theoretical part of the paper led to incorrect conclusions. For six flavonols (galangin, kaempferol, quercetin, morin, myricetin, and fisetin), reaction enthalpies related to three mechanisms of the primary antioxidant action were computed. Based on the obtained results, the role of intramolecular hydrogen bonds (IHB) in the thermodynamics of the antioxidant effect is presented. Calculations and the role of solvation enthalpies of proton and electron in the determination of thermodynamically preferred mechanism is also briefly explained and discussed. The obtained results are in accordance with published works considering the Sequential Proton-Loss Electron-Transfer thermodynamically preferred reaction pathway.

Keywords: phenolic antioxidant; intramolecular hydrogen bond; DFT calculation; sequential proton-loss electron-transfer; solvent effect

Introduction

Phenolic compounds are naturally present in almost all plant materials and represent an integral part of human diet. Tocopherols, flavonoids and (poly)phenolic acids are considered the most important groups of naturally occurring phenolic antioxidants.

Flavonoids were identified in almost all parts of plants, such as leaves, stems, roots, fruits or seeds. Besides the antioxidant action, they exhibit other biological effects: antiviral, antibacterial, anti-inflammatory, anticancer, vasodilatory and anti-ischemic (Procházková et al., 2011). From the structural point of view, they consist of benzene ring, A, condensed with heterocyclic ring, C, and phenyl ring, B, attached on C2 carbon atom (Fig. 1). Experimental reports on flavonoids radical scavenging activity (primary antioxidant effect) have shown that the following structural features are required (Bors, et al., 1990; Burda and Oleszek, 2001; Croft, 1998; Procházková et al., 2011; Rice-Evans et al., 1996):

(*i*) catechol (*ortho*-dihydroxy) structure of the B ring enabling electron delocalization in the formed phenoxy radical,
(*ii*) C2═C3 double bond conjugated with C4═O keto group in the C ring providing electron delocalization from the B ring,
(*iii*) hydroxy OH groups at positions 3 and 5 providing hydrogen bonds to C4═O group.

In general it is expected that radical scavenging activity of flavonoids is mainly related to the B ring. The number and positions of OH groups in this ring play an important role. However, the total number of OH groups in flavonoids also affects their antioxidant activity, because OH groups present in the C ring (3-OH neighboring with C2═C3 double bond) and A ring also contribute to the overall observed effect (Musialik et al., 2009; Rice-Evans et al., 1996; Trouillas et al., 2006).

Flavonoids as primary (chain-breaking) antioxidants scavenge free radicals via three mechanisms (Foti et al., 2004; Galano et al., 2016; Ingold and Litwinienko, 2005; Litwinienko and Ingold, 2003, 2004; Musialik et al., 2009):

1. Hydrogen atom transfer (HAT) — one step mechanism where homolytic cleavage of phenolic O—H bond takes place; O—H bond dissociation enthalpy, BDE, represents the reaction enthalpy of this process.
2. Single Electron Transfer–Proton Transfer (SET-PT), also known as Sequential Electron Proton Transfer (SEPT) — two-step mechanism, where electron abstraction from antioxidant is followed by proton transfer.
3. Sequential Proton-Loss Electron-Transfer (SPLET) — two-step mechanism, where OH group deprotonation is followed by electron transfer.

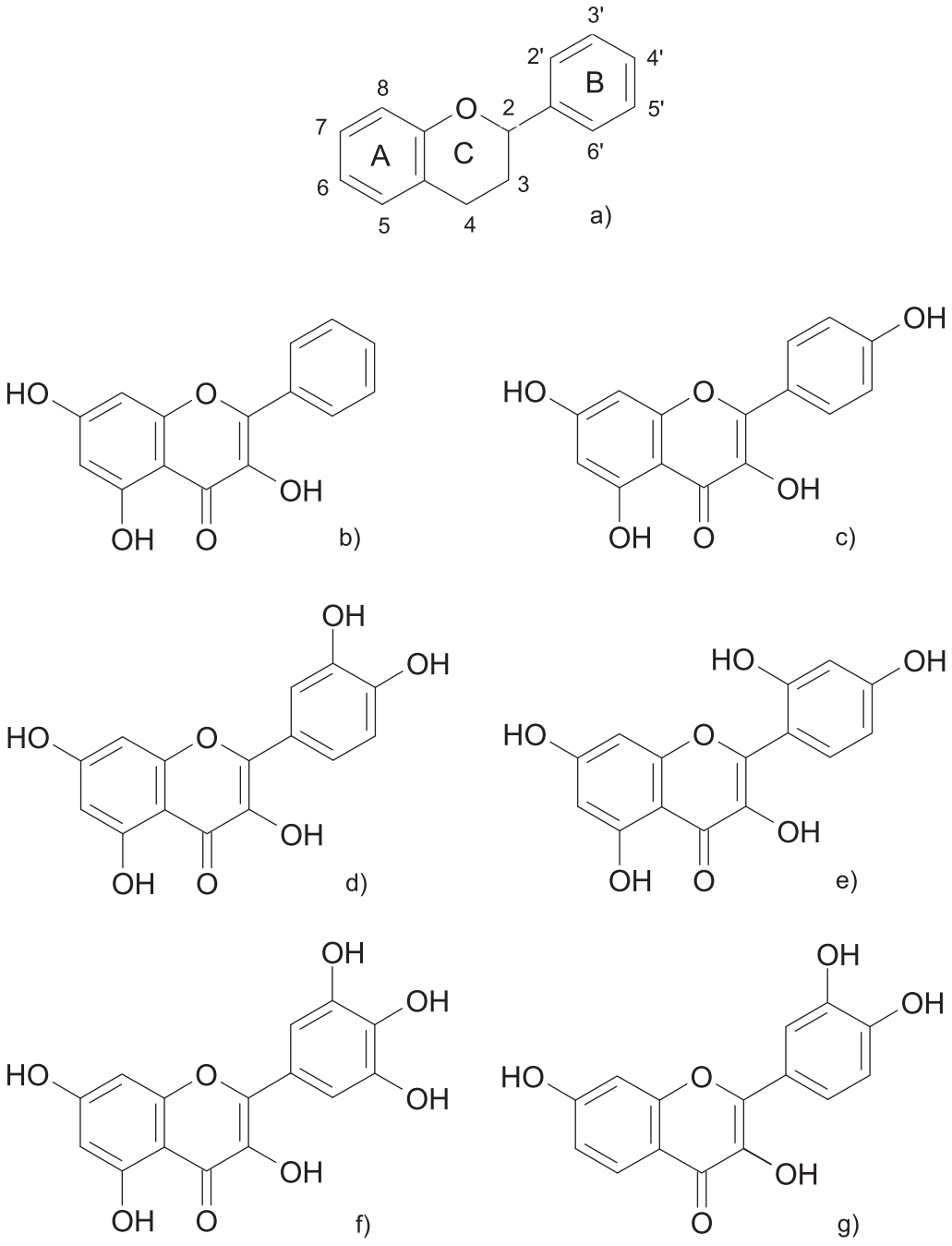


Fig. 1. Atom numbering and ring denotation in flavonoids (a) and studied flavonols: galangin (b), kaempferol (c), quercetin (d), morin (e), myricetin (f), and fisetin (g).

Thermodynamics of the Single Electron Transfer–Proton Transfer (SET-PT) mechanism is described by Ionization Potential (IP) and Proton Dissociation Enthalpy (PDE). Proton Affinity (PA) of the phenoxide anion and Electron Transfer Enthalpy (ETE) represent reaction enthalpies related to the Sequential Proton-Loss Electron-Transfer (SPLET) mechanism.

Very recently, Chen et al. (2018) published a paper focused on the theoretical and experimental study of antioxidant activity of flavonoids. Unfortunately, theoretical calculations show serious inaccuracies. Erroneous thermodynamic data for HAT, SET-PT and SPLET mechanisms enabled the authors to draw incorrect conclusions which disagree with available literature. Therefore, the aims of this work are: (*i*) to explain all details and general rules of a reliable theoretical study of primary antioxidant action thermodynamics, (*ii*) to present re-calculated reaction enthalpies for six flavonols (galangin, kaempferol, quercetin, morin, myricetin, and fisetin) in ethanol and acetone, and (*iii*) to confirm that found values allow drawing conclusions in agreement with available experimental and theoretical reports.

Computational details

All calculations were performed using the Gaussian 09 program package (Frisch et al., 2013). The geometry of each flavonoid molecule (Fl—OH), phenoxy radical (Fl—O$^{\bullet}$), radical cation (Fl—OH$^{\bullet+}$) or phenoxide anion (Fl—O^{-}) were optimized using the Density Functional Theory (DFT) method with M06-2X (Zhao and Truhlar, 2008) functional without any constraints (energy cut-off of 10^{-5} kJ mol^{-1}, final RMS energy gradient under 0.01 kJ mol^{-1} Å^{-1}). Calculations were performed using the 6-311+G(d,p) basis set (Binkley et al., 1980; Rassolov et al., 2001). Solvent (ethanol and acetone) contribution to the total enthalpies was computed employing SMD polarized continuum model (Solvation Model based on the quantum mechanical charge density of a solute molecule interacting with a continuum) developed by Marenich et al. (2009). Optimized structures were confirmed to be real minima by frequency analysis. O—H bond dissociation enthalpies, BDE, ionization potentials, IP, proton dissociation enthalpy PDE, phenoxide anion proton affinity, PA, and electron transfer enthalpies, ETE, values were calculated

$$\mathrm{BDE} = H(\mathrm{Fl{-}O^{\bullet}}) + H(\mathrm{H^{\bullet}}) - H(\mathrm{Fl{-}OH}) \quad (1)$$

$$\mathrm{IP} = H(\mathrm{Fl{-}OH^{\bullet+}}) + H(\mathrm{e^{-}}) - H(\mathrm{Fl{-}OH}) \quad (2)$$

$$\mathrm{PDE} = H(\mathrm{Fl{-}O^{\bullet}}) + H(\mathrm{H^{+}}) - H(\mathrm{Fl{-}OH^{\bullet+}}) \quad (3)$$

$$\mathrm{PA} = H(\mathrm{Fl{-}O^{-}}) + H(\mathrm{H^{+}}) - H(\mathrm{Fl{-}OH}) \quad (4)$$

$$\mathrm{ETE} = H(\mathrm{Fl{-}O^{\bullet}}) + H(\mathrm{e^{-}}) - H(\mathrm{Fl{-}O^{-}}) \quad (5)$$

where H(Fl—OH) represents total enthalpy of the flavonoid, H(Fl—OH$^{\bullet+}$) is total enthalpy of the flavonoid radical cation, H(Fl—O$^{\bullet}$) and H(Fl—O^{-}) are total enthalpies of the phenoxy radical and phenoxide anion, respectively.

Proton and electron solvation enthalpies were also computed using the (SMD) M06-2X/6-311+G(d,p) approach to obtain values compatible with total enthalpies of the studied species. For hydrogen atom solvation enthalpy, $\Delta_{sol}H(\mathrm{H^{\bullet}})$, published values of 3.7 kJ mol^{-1} and 4.5 kJ mol^{-1} (Parker, 1992) for acetone and ethanol were employed, respectively.

Results and Discussion

For the studied flavonols, calculated solution-phase reaction enthalpies are compiled in Tables 1 (ethanol) and 2 (acetone) which also show results published by Chen et al. (2018). Table 1 summarizes also data for quercetin found using identical computational approach, i.e. (SMD) M06-2X/6-311+G(d,p) (Zheng et al., 2017a). In the tables, the lowest values of individual reaction enthalpies for each flavonol are set in italic.

Structure of flavonoids and the role of intramolecular hydrogen bonds

Optimum geometries of the studied flavonoids must preserve all possible intramolecular hydrogen bonds (IHB). Geometries used for the calculations significantly affect the electronic structure of the studied species and consequently the energetics of homolytic or heterolytic O—H bonds cleavage. If the hydrogen atom of OH group participates in IHB, HAT or proton loss requires also the disruption of the hydrogen bond. As a result, an increase in O—H BDE, PDE or PA values can be observed. Therefore, 5-OH BDE, PDE and PA are usually the highest ones in flavonols, as it is evident from our results in Tables 1 and 2. Analogously, in case of the *ortho*-dihydroxy structure of the B ring, values for the 3´-OH group are higher than those obtained for the 4´-OH group (see results for quercetin and fisetin in Tables 1 and 2).

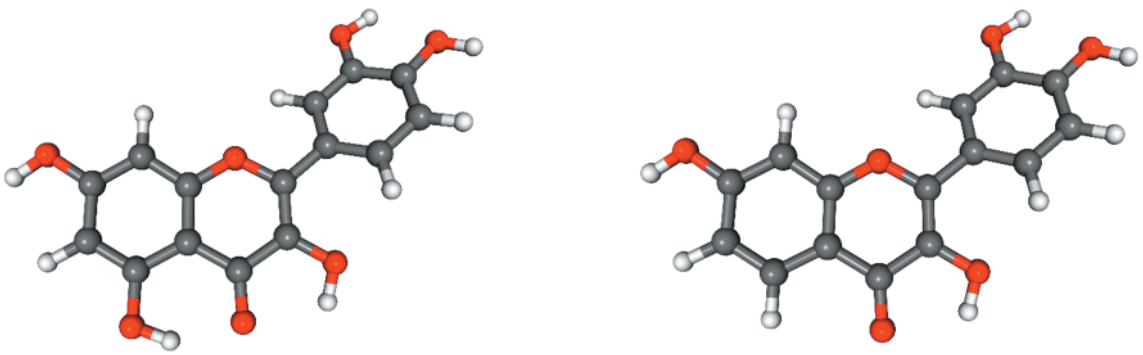
In galangin, kaempferol, quercetin, morin, myricetin, chrysin, apigenin, and luteolin, Chen et al. (2018) neglected the C4═O···H—O5 intramolecular hydrogen bond (IHB). Besides, the proposed optimum structure of fisetin assumes no IHB between 3´-OH and 4´-OH groups, while in other molecules, the mutual orientation of the two OH groups on the B ring is correct. In Fig. 2, correct geometries of quercetin and fisetin obtained in this work are depicted.

For quercetin, Galano et al. (2016) showed that IHB between 3´-OH and 4´-OH groups leads to a more stable conformation, by 18.0 kJ mol^{-1}, compared to the conformation lacking IHB. This IHB also stabilizes the 4´-O$^{\bullet}$ phenoxy radical and results in a 12.5 kJ mol^{-1} decrease in 4´-OH BDE. In case of C4═O···H—O5, even stronger IHB is expected (Nazarparvar et al., 2012; Zheng et al., 2017b, 2018). In the solution-phase, the effect of IHB on phenolic O—H groups cleavage is lower compared to that in the gas-phase, but 5-OH BDE, PDE and PA values still belong to the highest ones obtained in flavonoids (Lengyel et al., 2013; Lucarini et al., 2002; Marković et al., 2012, 2013; Vagánek et al., 2012, 2014; Zheng et al., 2019, 2018, 2017a, 2017b). Using model compounds catechol, pyrogalol, and resorcinol, Thavasi et al. (2006) confirmed the importance of IHBs for the antioxidant effect of polyphenols.

In literature, various reports on flavonoids with correct geometries, which are in agreement with

Tab. 1. (SMD) M06-2X/6-311+G(d,p) reaction enthalpies in kJ mol^{-1} for selected flavonoids in ethanol: Chen et al. (2018) / this work, or Zheng et al. (2017a[a]). The lowest values for a molecule are in italic.

Flavonoid	BDE	IP	PDE	PA	ETE
Galangin		675/549			
3-OH	*313/349*		*488/9*	630/149	*533/409*
5-OH	335/395		510/55	*603*/154	582/450
7-OH	355/396		530/55	604/ *134*	601/471
Kaempferol		656/531			
4´-OH	325/367		519/45	618/147	557/429
3-OH	*306/343*		*500/21*	630/153	*526/399*
5-OH	335/395		528/73	604/155	581/449
7-OH	352/393		546/71	*603/135*	598/468
Quercetin		652/530			
3´-OH	314/349		512/29	616/143	547/416
4´-OH	314/ *343*		512/ *22*	622/ *133*	541/418
3-OH	*307/343*		*504*/23	631/152	*526/400*
5-OH	336/395		534/74	603/155	583/449
7-OH	352/393		549/73	*601*/135	599/468
Morin		655/529			
2´-OH	333/375		527/55	619/146	564/438
4´-OH	335/374		529/54	620/146	564/437
3-OH	*308/343*		*502/23*	631/151	*526/401*
5-OH	336/397		530/76	602/152	583/453
7-OH	355/395		549/75	*601/132*	603/472
Myricetin		654/528			
3´-OH	*298*/354		*494*/36	611/142	534/422
4´-OH	320/ *326*		516/ *7*	615/ *124*	555/410
5´-OH	326/354		522/35	633/142	542/420
3-OH	307/344		503/25	630/149	*527/404*
5-OH	336/395		532/76	*604*/154	582/449
7-OH	352/394		548/75	*604*/134	598/469
Fisetin		656/528			
3´-OH	310/349		504/30	*617*/143	543/414
4´-OH	318/ *341*		512/ *22*	618/ *134*	550/415
3-OH	*308*/345		*502*/27	626/158	*532/396*
7-OH	353/390		546/72	607/136	595/463
Quercetin[a]		652/536			
3´-OH	314/361		512/7	616/133	547/424
4´-OH	314/344		512/-10	622/ *108*	541/430
3-OH	*307/343*		*504/–11*	631/123	*526/404*
5-OH	336/397		534/43	603/131	583/449
7-OH	352/395		549/50	*601*/111	599/468



a) b)

Fig. 2. Optimum geometries of quercetin (a) and fisetin (b).

Tab. 2. (SMD) M06-2X/6-311+G(d,p) reaction enthalpies in kJ mol^{-1} for selected flavonoids in acetone: Chen et al. (2018)/this work. The lowest values for a molecule are in italic.

Flavonoid	BDE	IP	PDE	PA	ETE
Galangin		700/483			
3-OH	*312/349*		*466/8*	681/181	*486/310*
5-OH	331/402		485/62	637/193	549/351
7-OH	349/391		503/51	*636/159*	568/375
Kaempferol		682/462			
4´-OH	320/363		492/43	651/173	524/332
3-OH	*305/344*		*477/24*	683/187	*477/299*
5-OH	331/402		503/82	638/195	548/350
7-OH	346/389		518/69	*635/160*	566/372
Quercetin		675/463			
3´-OH	*306*/343		486/23	637/168	524/318
4´-OH	313/*336*		493/*15*	664/*154*	504/324
3-OH	*306*/345		*485*/24	665/186	*495/301*
5-OH	332/402		512/81	*627*/195	560/349
7-OH	348/390		528/70	635/160	567/372
Morin		686/463			
2´-OH	330/374		498/53	655/178	529/338
4´-OH	329/371		498/50	652/173	532/341
3-OH	*308/345*		*476/24*	685/186	*477/302*
5-OH	332/404		501/83	633/190	554/356
7-OH	348/392		517/71	*631/155*	572/379
Myricetin		659/463			
3´-OH	*292*/349		*487*/29	636/167	511/325
4´-OH	310/*318*		505/*-3*	636/*142*	529/318
5´-OH	324/349		519/29	681/168	498/324
3-OH	306/344		501/24	678/181	*482/305*
5-OH	331/402		527/81	625/193	561/351
7-OH	347/379		542/58	*622*/159	579/362
Fisetin		673/463			
3´-OH	*304*/342		*486*/27	652/169	508/315
4´-OH	314/*334*		516/*18*	*635/157*	533/319
3-OH	306/346		487/31	656/197	*504/291*
7-OH	335/385		516/70	*635*/163	554/364

Fig. 2, can be found, see for example Ajitha et al. (2012), Álvarez-Diduk et al. (2013), Galano et al. (2016), Leopoldini et al. (2006), Marković et al. (2012, 2013), Mendoza-Wilson et al. (2011), Osorio et al. (2013), Sadasivam and Kumaresan (2011), Zheng et al. (2018, 2017). Leopoldini et al. (2011) and Trouillas et al. (2006) also presented optimum geometries of phenoxy radicals and phenoxide anions of various flavonoids.

Thermodynamically preferred mechanism vs solvation enthalpies of proton and electron

Solution phase calculations of reaction enthalpies related to SET-PT and SPLET require the application of total enthalpies of electron, $H(e^-)$, and proton, $H(H^+)$, in the studied solvent (eqs. 2—5). Using implicit solvation models, such as variants of the Polarized Continuum Model (PCM), solvation enthalpies of proton, $\Delta_{sol}H(H^+)$, and electron, $\Delta_{sol}H(e^-)$, can be simply calculated from the definition of the solvation enthalpy as follows (Marković et al., 2016; Rimarčík et al., 2010)

$$\Delta_{sol}H(H^+) = H[(S—H)^+, sol] - H(S, sol) - H(H^+, g) \quad (6)$$

$$\Delta_{sol}H(e^-) = H[(S—e)^{\bullet -}, sol] - H(S, sol) - H(e^-, g) \quad (7)$$

using the computed total enthalpies of solvent molecule S, solvated by the solvent itself $H(S, sol)$ and total enthalpies of $(S—H)^+$ and $(S—e)^{\bullet -}$ species that represent proton and electron added to the single solvent molecule, respectively.

Marković et al. (2016) performed calculations of H^+ and e^- solvation enthalpies using the SMD solvation model and the 6-311++G(d,p) basis set. The calculations were performed using *ab initio* (MP2) method and the Density Functional Theory (DFT) approach. Results for ten functionals, including the most frequently employed ones, i.e. B3LYP, M05-2X, and M06-2X, were reported. For ethanol, the obtained DFT proton solvation

enthalpies lie in the range from –1063 kJ mol^{-1} to –1076 kJ mol^{-1} and electron solvation enthalpies are in the range from –44 to –76 kJ mol^{-1}. In acetone, the DFT proton solvation enthalpies are similar, from –1049 to –1067 kJ mol^{-1}. Electron solvation enthalpy values obtained in acetone are more negative in comparison to those obtained in ethanol: from –112 kJ mol^{-1} to –133 kJ mol^{-1}. Hydration enthalpies of proton and electron were found in the range from –1052 kJ mol^{-1} to –1064 kJ mol^{-1} and from –66 kJ mol^{-1} to –101 kJ mol^{-1}, respectively. Rimarčík et al. (2010) performed analogous (IEF-PCM) B3LYP/6-311++G(d,p) calculations. Although different solvation models, SMD (Marenich et al., 2009) and IEF-PCM (Cances and Mennucci, 1998; Cances et al., 1997), were used in the two works, B3LYP results in the two papers can be considered similar (Marković et al., 2016). Table 3 summarizes B3LYP, M05-2X, and M06-2X solvation enthalpies for common solvents (ordered by their increasing polarity) published in the two papers, as well as (SMD) M06-2X/6-311+G(d,p) solvation enthalpies corresponding to the computational method employed in this work.

Chen et al. (2018) did not specify the employed total or solvation enthalpies of proton and electron. However, the IPs, PDEs, PAs, and ETEs determined indicate the use of incorrect values. Due to BDEs being lower than IP and PA values, the authors anticipate that HAT represents the thermodynamically preferred reaction mechanism of antioxidant activity of flavonoids in ethanol and acetone. Comparing our results with data obtained by Chen et al. (2018), discrepancies in PAs and PDEs reached roughly 500 kJ mol^{-1} in ethanol (Table 1) and more than 400 kJ mol^{-1} in acetone (Table 2). Differences in IP and ETE values exceed 120 kJ mol^{-1} and 200 kJ mol^{-1} in ethanol and acetone, respectively.

Correct conformations with all intramolecular hydrogen bonds induce considerable changes in the results obtained for the 5-OH group. In some cases, different conformations cause a change in the thermodynamically preferred OH group of the flavonoid (see for example PA values for quercetin in Tables 1 and 2). For quercetin, available calculated reaction enthalpies in aqueous solution are provided in Table 4. Although shifts in IP, PDE, PA, and ETE values are apparent, all methods provide practically identical general trends. The observed shifts stem from the application of different computational approaches (functionals, basis sets, and solvation models) employed in the published works. Also, the used solvation enthalpies of electron and proton may contribute to the uniform shifts considerably and therefore total or solvation enthalpies of proton and electron used in all calculations of reaction enthalpies have to be specified.

From the thermodynamics point of view, the tendency to enter a reaction mechanism is driven by BDE, IP and PA because in the two-step SET-PT and SPLET mechanisms, the energetics of the first step is determining. Despite the differences induced by various computational methods, data in Tables 1, 2, and 4 unambiguously show that SPLET is the thermodynamically preferred mechanism in polar solvents such as ethanol, acetone and water.

It is also worth to point out that confrontation of solution-phase reaction enthalpies summarized in Tables 1, 2, and 4, as well as other available computational results for the polar organic solvents

Tab. 3. Solvation enthalpies of H^+ and e^- in common solvents for widely used functionals.[a]

Solvent	$\Delta_{solv}H(H^+)$/kJ mol^{-1}			$\Delta_{solv}H(e^-)$/kJ mol^{-1}		
	B3LYP	M05-2X	M06-2X	B3LYP	M05-2X	M06-2X
benzene	–903.9 (–894)	–879.3	–877.4	–16.5 (–7)	–8.7	–10.5
toluene	–937.9 (–925)	–913.7	–911.7	–21.7 (–13)	–13.9	–15.2
aniline	–1108.5 (–1092)	–1101.7	–1100.5	–78.9 (–51)	–77.2	–77.4
acetone	–1060.2 (–1070)	–1056.1	–1053.6 –1054[b]	–132.9 (–119)	–119.8	–116.6 –116[b]
ethanol	–1068.4 (–1045)	–1064.5	–1064.0 –1065[b]	–73.6 (–76)	–43.6	–56.3 –39[b]
methanol	–1067.9 (–1038)	–1065.2	–1065.4	–80.0 (–86)	–48.7	–61.4
acetonitrile	–1043.8 (–1031)	–1031.7	–1032.4	–132.2 (–95)	–116.7	–113.0
DMSO	–1119.6 (–1115)	–1120.3	–1119.7	–53.0 (–84)	–25.6	–42.9
water	–1055.4 (–1022)	–1052.0	–1055.7	–101.1 (–105)	–66.8	–77.5

[a]Calculations in 6-311++G(d,p) basis set and SMD solvation method (Marković et al., 2016). B3LYP data in parentheses were calculated using IEF-PCM method (Rimarčík et al., 2010).

[b](SMD) M06-2X/6-311+G(d,p) – this work.

Tab. 4. Published DFT and semi-empirical PM6 reaction enthalpies in kJ mol^{-1} for quercetin in aqueous solution. The lowest values for a molecule are in italic.

Method	BDE	IP	PDE	PA	ETE
(SMD) M05-2X/6-311G+(d,p) (Marković et al., 2013)					
		334			
3´-OH	349		14	116	232
4´-OH	*333*		*-1*	*93*	240
3-OH	334		0	108	*226*
5-OH	383		48	112	270
7-OH	383		49	94	289
(SMD) M06-2X/6-311+G(d,p) (Zheng et al., 2017a)					
		535			
3´-OH	351		15	126	424
4´-OH	336		1	*106*	430
3-OH	*333*		*-2*	116	*416*
5-OH	385		49	124	460
7-OH	386		50	107	478
(IEF-PCM) B3LYP/6-311++G(d,p) (Vagánek et al., 2014)					
		447			
3´-OH	316		68	174	341
4´-OH	*305*		*56*	*159*	344
3-OH	317		68	192	*324*
5-OH	369		120	205	363
7-OH	351		103	170	380
(COSMO) PM6 (Amić et al., 2013)					
		361			
3´-OH	311		–51	51	*260*
4´-OH	*298*		*–63*	31	267
3-OH	305		–57	35	269
5-OH	373		11	22	350
7-OH	383		21	*14*	368

and water (Amić et al., 2013; Marković et al., 2013; Vagánek et al., 2014; Zheng et al., 2019, 2018, 2017a, 2017b) indicate that the three polar solvents exert similar effect on the reaction enthalpies for processes involving charged species, where solvation plays an important role. On the contrary, solvent induces only minute changes in BDEs because they are calculated from the total enthalpies of neutral species only.

Our values compiled in Tables 1 and 2 are in accordance with previously published experimental (Foti et al., 2004; Ingold and Litwinienko, 2005; Litwinienko and Ingold, 2003, 2004; Musialik et al., 2009; Staško et al., 2007) and theoretical reports (Amić et al., 2013; Lengyel et al., 2013; Marković et al., 2013; Toscano and Russo, 2016; Vagánek et al., 2012, 2014; Vakarelska-Popovska and Velkov, 2016; Zheng et al., 2019, 2018, 2017a, 2017b) concluding that SPLET is the favored reaction pathway in polar solvents.

Preferred reaction sites in studied flavonols

For the HAT mechanism, data in Tables 1 and 2 indicate that the lowest BDE values were found for 4´-OH group on the B ring or for the 3-OH group on the C ring. Comparing the results for kaempferol, quercetin and myricetin possessing one, two and three OH groups on the B ring, BDE values reveal that 4´-OH BDE in myricetin with pyrogallol (trihydroxy) structure of the B ring is the lowest one, while in kaempferol with only one OH group on the B ring, 4´-OH BDE is the highest one in this series. In kaempferol, the lowest BDE was found for 3-OH group analogously to galangin that has no OH group on the B ring. For morin with two OH groups on the B ring, the corresponding BDEs are significantly higher because the two groups are mutually in *meta* position, where OH group shows electron-withdrawing effect resulting in the increased BDE (Klein and Lukeš, 2006). In morin, the 3-OH group shows the lowest BDE. For quercetin in ethanol, 3-OH and 4´-OH BDE reached identical values. 5-OH and 7-OH groups of the A ring have the lowest tendency to homolytic O—H bond cleavage. From the thermodynamic cycle it follows that identical trends hold also for proton dissociation enthalpies in the SET-PT mechanism as the second step of the SET-PT mechanism also results in phenoxy radical formation.

Another key factor of radical species stability, spin density, is shown for quercetin in ethanol in Fig. 3. The 7-OH radical has the highest spin density on the O-atom and BDE compared to the 3-OH and 4´-OH radicals having the lowest spin density on the O-atom and BDEs. This means that the formation of the latter radicals is more favorable as the spin density is more delocalized over the whole molecule and the species are more stabilized. In case of 5-OH BDE, the higher value is caused by hydrogen bond cleavage during hydrogen atom transfer and not by the spin density distribution. These results are comparable with those obtained by Zheng et al. (2017a).

For SPLET, data in Tables 1 and 2 indicate that in galangin, kaempferol, and morin, the 7-OH group is most prone to deprotonation. In quercetin, myricetin and fisetin, the 4´-OH group is the preferred one. However, for quercetin and fisetin, 4´-OH and 7-OH PA values in ethanol can be considered practically identical, the difference is only 2 kJ mol^{-1}. In acetone, 4´-OH and 7-OH PA values of the two flavonols are again close and the differences do not exceed 6 kJ mol^{-1}. These data, as well as the published reaction enthalpies for quercetin in aqueous solution in Table 4, unambiguously indicate the significant role of the 7-OH group (ring A) in the overall antioxidant activity. For quercetin, Musialik et al. (2009) experimentally confirmed that the anion formed at position 7 in ionization-supporting solvents is responsible for very fast kinetics of the quercetin/dpph• reaction because of the participation of both mechanisms: HAT (from catechol moiety in ring B) and SPLET (from ionized 7-hydroxyl in ring A). For isoflavones, Lengyel et al. (2013) found that deprotonation of the 7-OH group is thermodynamically favored in aqueous solution as well as in non-polar environment (benzene).

Thermodynamics of primary antioxidant action vs antioxidant activity

Activity of individual flavonoids does not depend solely on their structure and the polarity of the environment. The type of scavenged radical and the kinetics related to the individual reaction pathways also play important roles (Galano et al., 2016;

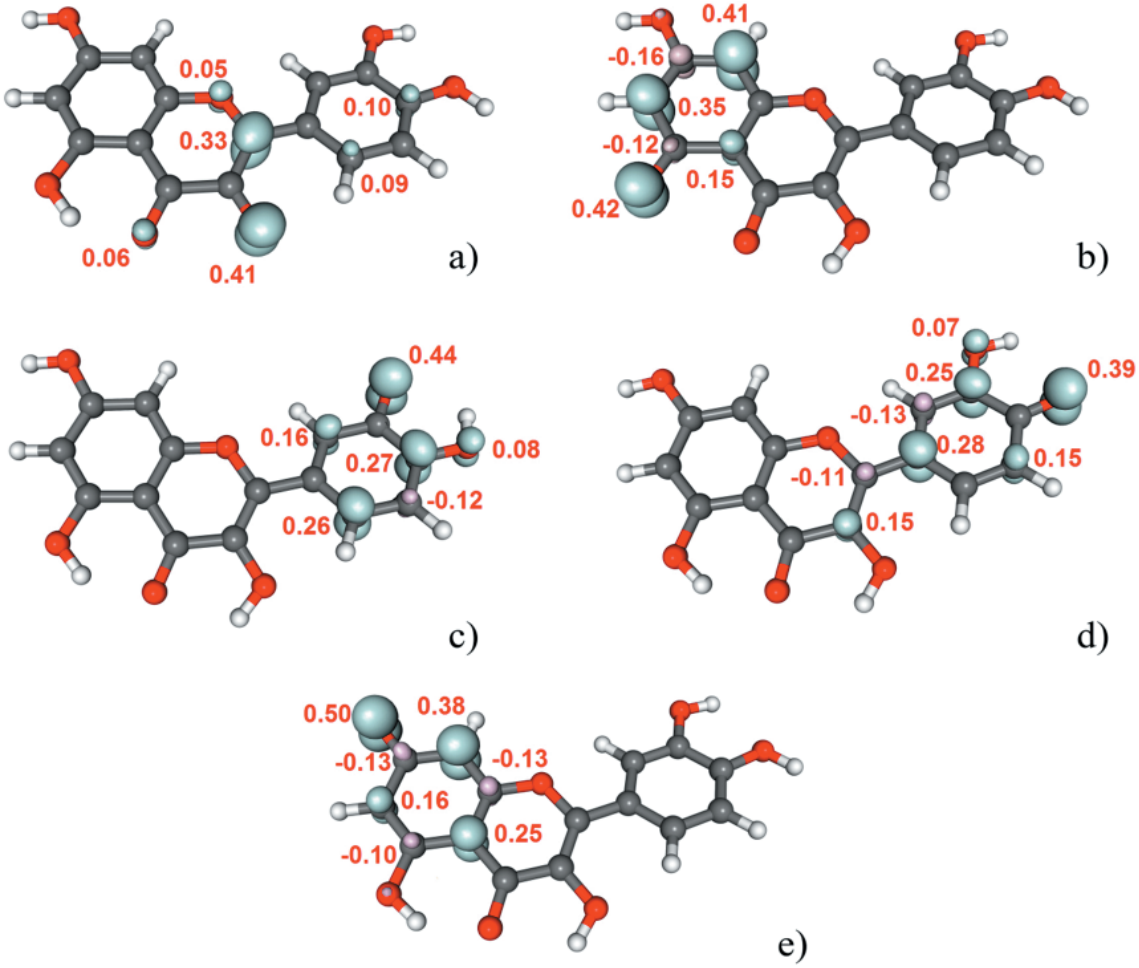


Fig. 3. Spin densities (values are in a.u.) of quercetin radicals in ethanol: a) 3-OH, b) 5-OH, c) 3´-OH, d) 4´-OH, e) 7-OH (M06-2X/6-311G, isosurface value 0.01).

Galano and Raúl Alvarez-Idaboy, 2019; Marković et al., 2013; Procházková et al., 2011; Rice-Evans et al., 1996).

In general, other processes can also affect the experimentally observed radical scavenging activity of flavonoids in polar environment. Recently, it has been shown that HAT from deprotonated flavonoids is more thermodynamically feasible in comparison to parent (non-dissociated) flavonoids; see for example Galano et al. (2016), Galano and Raúl Alvarez-Idaboy (2019), Klein et al. (2016). This mechanism has been denoted as SPLHAT (Galano et al., 2016; Galano and Raúl Alvarez-Idaboy, 2019) because it represents the combination of the first step of SPLET (deprotonation of phenolic OH group) followed by HAT from a different OH group of the formed anion. Relevance of both, SPLET and SPLHAT mechanisms for phenolic antioxidants in the polar ionization supporting solvents was indicated by many experiments and rationalized also by theoretical studies (Álvarez-Diduk et al., 2013; Dueñas et al., 2010; Foti et al., 2004; Galano et al., 2011; Ingold and Litwinienko, 2005; Klein et al., 2016; Lemańska et al., 2001, 2004; León-Carmona et al., 2012; Litwinienko and Ingold, 2003, 2004; Musialik et al., 2009; Staško et al., 2007). Experimentally observed increase of free radical scavenging activity of polyphenols with the increase in pH can be related to lower O—H BDEs in deprotonated species. It can be assumed that the SPLHAT mechanism considerably contributes to the observed antioxidant action of flavonoids in aqueous solutions.

Conclusion

In this report, a theoretical study of the thermodynamics of three mechanisms of primary antioxidant action of six flavonols in two polar solvents is presented. Another important aim of this work was to provide an explanation of all issues to be aware of in order to perform reliable theoretical calculations providing correct values of the investigated reaction enthalpies.

In general, computational chemistry offers reasonable predictions of antioxidant action thermodynamics for solution-phase reactions where no experimental data are available. Published theoretical papers allow deeper insight and explanation of the experimentally observed antioxidant effect. However, to obtain reliable theoretical results, certain general rules have to be obeyed:

(*i*) It is necessary to use a suitable computational method. In antioxidant research, DFT methods are dominant. Previous studies have confirmed the applicability of B3LYP (probably still the most popular one) (Becke, 1993; Lee et al., 1988) and newer M05-2X (Zhao et al., 2006) and M06-2X (Zhao and Truhlar, 2008) functionals. For solvent effect description, implicit solvent models (polarized continuum models — PCM) are usually employed. Sufficiently large basis sets including both, diffuse and polarization, functions are recommended for balanced description of molecules, radicals and charged species. Pople's basis sets, such as 6-311++G(d,p) or 6-311+G(d,p), are widely used in this field.

(*ii*) Conformational analysis of the studied molecules is necessary to identify conformation with the lowest energy, i.e. to identify the most stable conformation. In this step, it is inevitable to consider possible intramolecular hydrogen bonds. Their presence often affects the obtained results significantly.

(*iii*) In solution-phase thermochemistry of proton and electron transfer, proton and electron solvation enthalpies represent important quantities. On the other hand, solvation does not significantly alter the thermodynamics of homolytic bond dissociation in neutral molecules. Hydrogen atom $H^{\bullet}$ solvation enthalpy values in various solvents are within several units of kJ mol^{-1} (Parker, 1992; Wilhelm and Battino, 1973). The authors should always specify the employed total or solvation enthalpies of $H^{\bullet}$, H^{+}, and e^{-} used in the calculations. Application of different available values of solvation enthalpies can shift the obtained results by tens of kJ mol^{-1}. If the employed solvation enthalpies are not specified, mutual comparison of various published values is questionable.

It should also be noted that the Gibbs free energy represents the general criterion of the thermodynamically favored mechanism. However, in case of the studied reactions, the absolute values of the entropic term, $-T\Delta_r S$, are only a few units or tens of kJ mol^{-1} and the reaction Gibbs free energies, $\Delta_r G = \Delta_r H - T\Delta_r S$, are just slightly shifted compared to the reaction enthalpies (Dewar, 1990; Klein and Lukeš, 2006; Rimarčík et al. 2010). Thus, BDE, IP and PA values indicate the thermodynamically preferred mechanism. In polar solvents, the differences between them are in hundreds of kJ mol^{-1}.

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