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Antioxidants in coffee: a DFT mechanistic study of the free radical scavenging activity

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Coffee is the single biggest source of antioxidants in our diet. Theoretical calculations at B3LYP/6-31+G(d,p) level were used for the evaluation of five reaction descriptors (thermodynamic quantities) – BDE, IP, PA, PDE and ETE. Those values are related to three possible antioxidant mechanisms: hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) mechanisms. PCM implicit solvation model was used to simulate water environment for four coffee components - 5-O-caffeoylquinic acid, caffeic acid, cafestol and quinine. Both acids were the most potent antioxidants with similar activity for both mechanisms probable in water (SET-PT and SPLET), SPLET being the energetically favored one. Cafestol and quinine have weaker activity considering their aliphatic hydroxyl groups.

Keywords: Antioxidants, coffee, cafestol, caffeic acid, chlorogenic acid, quinine, DFT

INTRODUCTION

Coffee is one of the world's most popular beverages. We have estimated that worldwide people consume more than 2.9 billion cups of coffee every day [1, 2]. Drinking coffee stimulates us and provides an energy boost – that is why most people do it. Moreover, over the past few years, a series of studies have come out showing that drinking coffee has significant health benefits, such as a lower risk of: cancer (liver, colon and rectal cancer), type 2 diabetes, heart failure, premature ageing, cognitive decline, etc. [3-7]. Coffee is (potentially) identified as the biggest source of antioxidant power over the world: a new research indicates that taking 3-5 cups of coffee can serve up to 60 percent of daily phenolic antioxidant intake requirement. Coffee has more antioxidants than both green and black teas, red wine, dark chocolate…and even berry fruits [8]!

Although caffeine is not a particularly strong antioxidant [9], the beverage also contains a variety of other substances (phenolic and non-phenolic) with potential radical scavenging ability – caffeic acid (CA), chlorogenic acid (CGA), cafestol, trigonelline, hydroxycinnamic acid, quinine and melanoidins are only some of them. Some benefit the organism's natural antioxidant mechanisms [10]. The major phenolic compounds in coffee are chlorogenic acids [11]. Chlorogenic acids are a group of esters involving quinic acid (*(3R,5R)*-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid) and caffeic, ferulic or *p*-coumaric acid. There are a few isomers due to quinic acid having a few hydroxyl sites available. The 5-O-caffeoylquinic acid is part of melanoidins. Cafestol (a natural diterpenoid) and quinine (a natural cinchona alkaloid that has been used for centuries in the prevention and therapy of malaria) are non-phenolic compounds. Non-phenolic terpenoids from three hydrocarbon classes – monoterpenes, sesquiterpenes and diterpenes) have been found capable of acting as C–H scavengers (*via* HAT mechanism dominant in the gas phase and SPLET mechanism in polar medium) [12]. Sugar alcohols have been shown to have oxy-radical scavenging abilities dependent on the number of aliphatic hydroxyl groups [13].

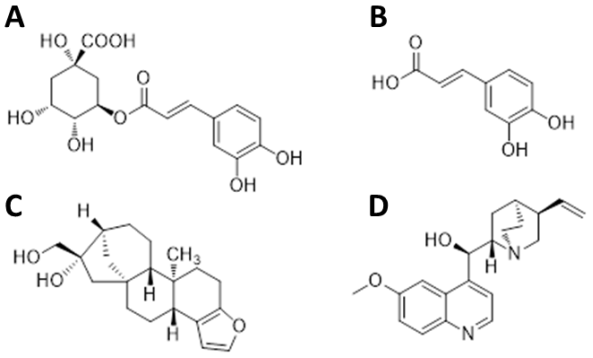

Figure 1. Chemical structures of chlorogenic acid (A), caffeic acid (B), cafestol (C) and quinine (D).

As there are many sorts of coffee beans and plenty of brewing methods, it is reasonable to mention the optimal variants for maximizing antioxidant concentrations. Current research shows that lightly roasted coffee beans combined with hot brew extraction methods show the best results [7, 14].

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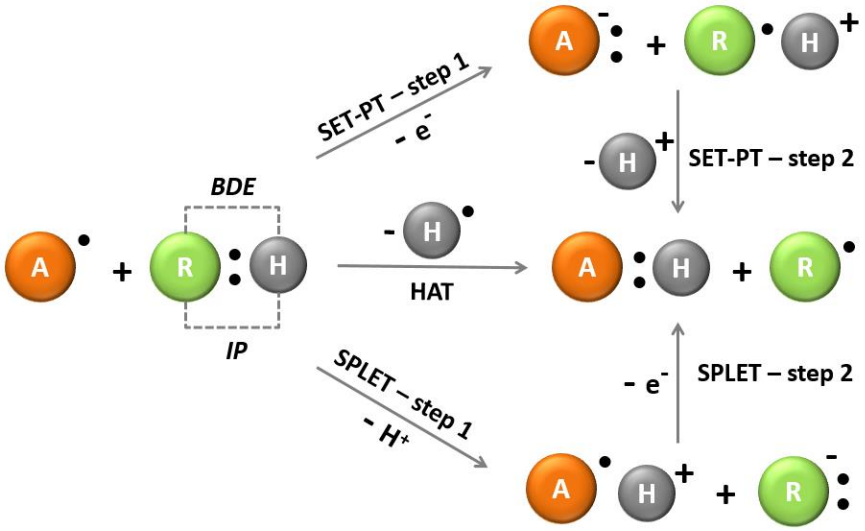
Roasting the beans has negative effect on subsequent caffeine and chlorogenic acid concentrations. Applying heat during the extraction process, however, is favorable. The quality of the coffee is also critical, as higher quality beans produce higher antioxidant concentrations [15].

The goal of our computational study is to shed light on the structure of some antioxidant active compounds in coffee and examine the mechanisms of their radical scavenging activity, including Hydrogen Atom Transfer (HAT), Sequential Proton Loss Electron Transfer (SPLET) and Sequential Electron Transfer – Proton Transfer (SET-PT) (shown in Scheme 1). The enthalpies of every step were calculated in water by means of Density Functional Theory (DFT) calculations.

Computational Details

Gaussian 16 [16] was used to perform quantum chemical calculations at the B3LYP/6-31+G(d,p) level of theory. B3LYP functional was chosen because it provides reliable geometries, frequencies, and bond lengths [17]. It has been reported that B3LYP used for evaluating the activity of phenolic hydroxyls complies well with experimental data [18, 19]. The 6-31+G(d,p) basis set was used by Koleva *et al.* to predict antioxidant activity for phenolic compounds [19]. Furthermore, increasing the basis set does not contribute to significant increase in accuracy [20]. An implicit solvation method (Polarizable Continuum Model, PCM [21]) was utilized to simulate solvation of the molecule species in water (ε=78). Geometry optimization was confirmed with frequency calculation at the same computational level to establish absence of negative frequencies. Possible intramolecular interactions (H-bonds) were taken into account in the initial geometries of the parent antioxidant structures. Radical scavenging activity was calculated as dissociation enthalpy for three distinct mechanisms, most often discussed in the literature [22, 23] and shown to occur in various conditions, illustrated below (Scheme 1).



Scheme 1. Possible mechanisms which describe antioxidant reactions (some mechanisms consist of two-step reactions). The free radical is designated with "A", the active molecule – with "R".

Radicals in non-singlet state are calculated using unrestricted B3LYP (UB3LYP). For every one of the studied molecules, enthalpies are calculated for the neutral molecule, radical, cation radical and anion states. Enthalpies for the hydrogen, proton and electron were calculated on the same level of theory. For the electron, a model proposed by Kumar *et al.* was used [24]. For the proton a standard solvation model was applied [25]. The obtained values are as follows: $H_{H^{\cdot}}$ = -312.45 kcal mol^{-1}, H_{H^+} = -236.00 kcal mol^{-1} and H_{e^-} = -48.70 kcal mol^{-1}.

Bond dissociation enthalpy (BDE) represents the reaction enthalpy of hydrogen atom abstraction or hydrogen atom transfer (HAT). Ionization potential (IP) is the enthalpy change from the loss of electron and formation of cation radical. Proton affinity (PA) is the enthalpy change for the dissociation of a proton and formation of an anion. Proton dissociation enthalpy (PDE) is the energy required for the cation radical to lose a proton during the SET-PT mechanism and lastly we have electron transfer enthalpy (ETE) when the anions become radicals in the SPLET mechanism.

The chlorogenic acid, modeled by us, is 5-O-caffeoylquinic acid. Cafestol and quinine were considered only as aliphatic O-H scavengers when discussing the SET-PT and SPLET mechanisms. BDEs of selected C-H bonds were calculated.

RESULTS AND DISCUSSION

The antioxidant active compounds selected for this study are chlorogenic acid, caffeic acid, cafestol and quinine (Fig. 1). The enthalpy of dissociation of their O−H bonds is calculated for three different mechanisms - HAT, SET-PT and SPLET (Scheme 1). The HAT mechanism only has a single step. For SET-PT and SPLET we have IP and PA as first step metrics, respectively.

An initial screening of the possible rotamers with H-bonds was performed for chlorogenic acid, caffeic acid and cafestol. Cafestol's rotamers are energetically identical in gas-phase calculations, so only one was considered. The energetically preferred rotamers of chlorogenic and caffeic acids and structures of cafestol and quinine are illustrated in Fig. 2.

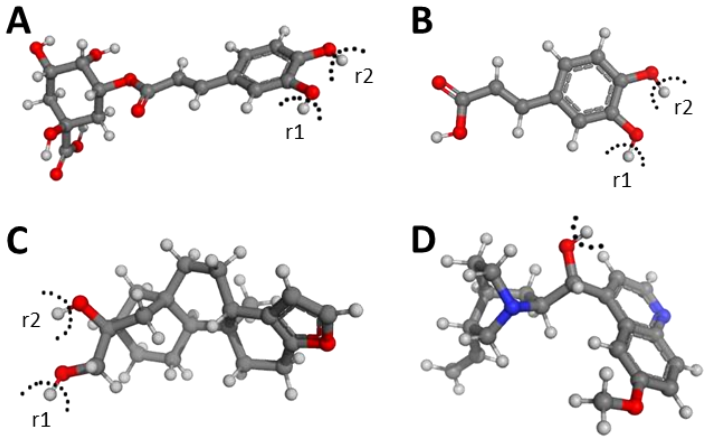


Figure 2. Optimized geometries of chlorogenic acid (A), caffeic acid (B), cafestol (C) and quinine (D). The dotted lines indicate the O-H bonds that participate most readily in antioxidant activity.

Table 1. Results obtained (in kcal mol^{-1}) for the BDE, the ionization potentials (IP), and the Proton Affinity (PA) of the neutral species, the Proton Dissociation Enthalpy (PDE) of the cation-radical and the Electron Transfer Enthalpy (ETE) of the anionic species.

	BDE	IP	PA	PDE	ETE
	ROH --> RO· + H·	ROH --> $ROH^{+\cdot}$ + e^-	ROH --> RO^- + H^+	$ROH^{+\cdot}$ --> RO· + H^+	RO^- --> RO· + e^-
CGA (r1)	76.77	86.98	48.68	17.55	48.93
CGA (r2)	78.71		50.79	19.48	48.75
Caffeic acid (r1)	76.90	87.34	48.55	17.31	49.19
Caffeic acid (r2)	78.82		50.59	19.23	51.11
Cafestol (r1)	100.09	83.17	68.74	44.67	52.19
Cafestol (r2)	99.15		69.86	43.73	50.13
Quinine	98.58	78.63	71.07	47.70	48.35

The calculated results for the different thermodynamic characteristics in water are collected in Table 1.

BDEs

CGA has the lowest BDE (76.77 kcal mol^{-1}) for r1 radical formation. It turns out that the BDEs calculated for CGA do not differ at all from those of parent caffeic acid (77.67 *vs.* 76.90 kcal mol^{-1} and 78.71 vs. 78.82 kcal mol^{-1}). The BDEs calculated for the hydroxyl groups of cafestol and quinine are high. According to the BDEs in water the ease by which OH-hydrogen atom abstraction from the compounds may be released approximately follows the order: CGA ~ caffeic acid > quinine > cafestol. When calculating C-H BDEs of tertiary carbon atoms of quinine and cafestol we get energies closer to the ones for hydroxyl groups of CA and CGA (Fig. 3). Despite this, when experimentally measuring antiradical activity, the values differ significantly. Caffeic acid (20 μg/ml) reduces 93.9% of DPPH [26] while the same amount of quinine reduces about 20% [27].

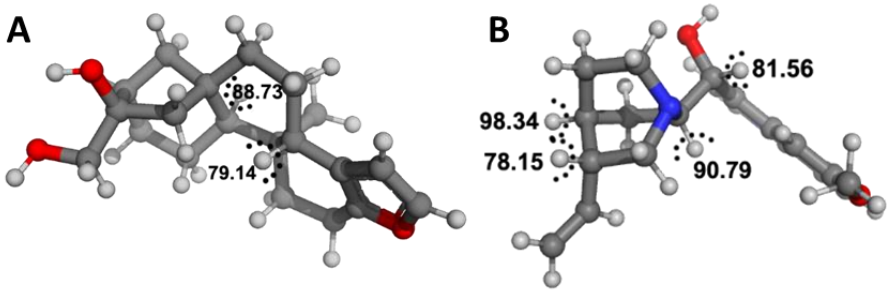


Figure 3. BDE of C-H bonds of tertiary carbon atoms (kcal/mol) of cafestol (a) and quinine (b).

A DFT study on the antioxidant activity of oleuropein (a phenylethanoid, a type of phenolic compound found in the olive leaf) suggests that since tertiary carbon atoms are usually tucked inside the molecule, the DPPH test is not a good method for measuring that activity [28].

IPs

The instability of cation-radicals is a reason for a higher IP value, and hence, for a lower reactivity. The most stable cation-radical is that of quinine. The IP of caffeic acid (87.34 kcal mol^{-1}) is the highest among all tested compounds, next is the IP of CGA. The order of decreasing the compounds' propensity to give up an electron is as follows: quinine > cafestol > CGA > caffeic acid. (The most powerful reducing agent in water is quinine (78.63 kcal mol^{-1}), and the most resistant to oxidation is caffeic acid (87.34 kcal mol^{-1})).

PDEs

The cation radicals lose their excess protons quite easily. The most stable once again prove to be quinine and cafestol. The caffeic and chlorogenic acids have similar values for both r1 and r2 radicals – very low at ~17 and ~19 kcal mol^{-1} respectively. This means that for the SET-PT mechanism the first step is the limiting one.

PAs

PA represents the reaction enthalpy of the protonic dissociation of the hydroxyl group from a neutral molecule, which is the first step in the SPLET mechanism. A reverse relation with IPs is observed with caffeic acid being the most reactive and quinine the most resistant. Caffeic acid and CGA have phenolic hydroxyl groups, so deprotonation is achieved more readily due to delocalization of the electron density.

ETEs

ETEs characterize the anions propensity for electron donation. With respect to ETE, the most active scavenger would be quinine (48.35 kcal mol^{-1}). Next come CGA (48.93 and 48.75 kcal mol^{-1}), followed by caffeic acid (49.19 and 51.11 kcal mol^{-1}). Least active toward radicals is cafestol (52.19 and 50.13 kcal mol^{-1}). The difference between all compounds is overall rather small.

CGA are a wide group of esters. The one we study is 5-O-caffeoylquinic acid. We focus on the phenolic hydroxyl groups, which are on the caffeic fragment of the ester (Fig. 2 A). Comparing all the studied values for CGA and CA, we see that they are very similar. The explanation is that the quinic acid fragment of CGA does not influence the radical scavenging properties of the caffeic fragment. Experiments show, however, that chlorogenic acids and caffeic acid have different antioxidant abilities [29]. We have to assume that those differences come from the quinic fragment of the ester.

The most readily occurring mechanism with similar values for both steps is SPLET, where CGA and caffeic acid are more reactive than cafestol and quinine. This is also valid when looking at the enthalpies of the HAT reaction. At physiological pH this mechanism would be even more favored. We are assuming therefore that out of the four compounds studied, those two are more important for direct radical scavenging abilities of coffee.

Quinine has an interesting reactivity. When looking at the steps that involve losing an electron – first step of SET-PT and second step of SPLET (IP and ETE values), we notice that it is energetically feasible, even when the other step of the mechanism might not be. In the case of SET-PT when the formation of the cation radical is the rate determining step, we cannot reject the possibility of the reaction going all the way.

CONCLUSIONS

Quantum chemical modelling was utilized to study 3 possible mechanisms (HAT, SPLET, SET-PT) of antioxidant activity for four compounds found in coffee (5-cafeoylquinic acid, caffeic acid, cafestol and quinine). Enthalpies for every step of the radical scavenging reaction were obtained (BDE, IP, PA, PDE, ETE). Of the two mechanisms most probable in water solution, SPLET is the more energetically feasible with 5-cafeoylquinic acid and caffeic acid being the best candidates with similar energy profiles, as also reported in literature [25, 30].

Cafestol's and quinine's O-H bonds are characterized with high BDEs in water (and the BDEs in non-polar environment are anticipated to be even higher), so the homolytic dissociation of the aliphatic OH group appears to be improbable. BDEs of C-H bonds of some of their tertiary carbons are comparable to those of caffeic acid. The O-H groups have ionization potentials with similar enthalpy, so SET-PT should still be considered a viable mechanism for them.

It is shown that even molecules in coffee without hydroxyl groups can positively influence the body's natural antioxidant abilities (trigonelline, caffeine) [4, 31]. While quinine and cafestol might not be as reactive as chlorogenic and caffeic acids, their effect as antioxidants cannot be overlooked. The wide variety of compounds with such properties in coffee and the variety of viable mechanisms they can exercise that activity with, makes the brew the best source of antioxidants accessible in everyday diet [8].

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