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Theoretical and Experimental Study of the Antioxidant Behaviors of 5-O-Caffeoylquinic, Quinic and Caffeic Acids Based on Electronic and Structural Properties

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The aim of this study was to elucidate the structural and electronic factors that determine the antioxidant capacity of 5-O-caffeoylquinic, caffeic and quinic acids under different experimental conditions. Antioxidant capacity was measured using different in vitro assays, involving diverse mechanisms of antioxidant action, namely, radical scavenging or reduction. The mechanisms of these reactions were analyzed by a theoretical study using the Density Functional Theory. Results allow relating in vitro antioxidant capacity of these three compounds with their chemical structures. The antioxidant capacity experimentally observed for these three acids was interpreted considering the reaction mechanism involved, the nature and stability of the intermediate formed, the formation and reaction of secondary compounds, and the effect of the reaction medium. The main goal of this report is presenting a theoretical analysis of reactions implicated in the antioxidant capacity, identifying the causes that increase or decrease such property. Thus, we present a quantum mechanical description of the antioxidant properties involved with these three compounds, considering equilibria and secondary oxidations involved.

Introduction

Hydroxycinnamic acids are present in a large variety of fruits and vegetables.[1] Some common hydroxycinnamic compounds are ferulic, *p*-coumaric and caffeic acids. Generally, these acids can be found esterified with sugars, lipids or organic acids.[2] For instance, chlorogenic acids, formed by esterification of diverse hydroxycinnamic compounds with quinic acid, are widely distributed in plant materials and have been reported as having beneficial health effects.[3–5] Coffee is one of the major sources of chlorogenic acids in the diet, mainly of 5-O-caffeoylquinic acid (5-CQA) (Figure 1).[6] This compound exhibits anti-obesity property, by improving the lipid metabolism in mice.[7]

Furthermore, 5-CQA demonstrates antiviral effects[3] and DNA-protective activities.[8] However, the biological properties of 5-CQA depend on both its absorption in the gut and its metabolism. It is known that 5-CQA is hydrolyzed by the intestinal microflora into various aromatic metabolites, including caffeic (CA) and quinic (QA) acids.[9] All of these compounds can act as antioxidants, being the metabolic form of 5-CQA determinant for the antioxidant property.

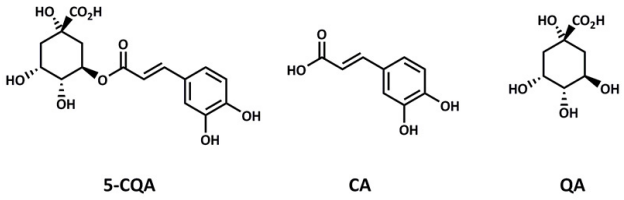

Figure 1. Chemical structures of 5-O-caffeoylquinic acid (5-CQA), caffeic acid (CA) and quinic acid (QA).

The antioxidant capacity of these compounds depends on both their chemical structure and the reaction medium. Consequently, diverse hydroxycinnamic derivatives can show significant differences in their effectiveness as antioxidants. Usually, the first evaluation of antioxidant capacity of dietary polyphenols is carried out by *in vitro* chemical methods, which assess either the redox capacity (ferric reducing ability of plasma method - FRAP) or the trapping capacity of free radicals (trolox equivalent antioxidant capacity - TEAC, 2,2-diphenyl-1-picrylhydrazyl - DPPH, etc.). However, sometimes rather controversial results are obtained, because the antioxidant capacity of compounds depends not only on their chemical structure but also on various other factors, such as pH, the solvent used, the concentration of the compound, the reaction time, among other factors. Therefore, it is necessary to perform theoretical studies linking the experimental antioxidant capacity to the structure and the physicochemical properties of compound having antioxidant activity. Theoretical results could help to understand the metabolic changes experienced by antioxidant

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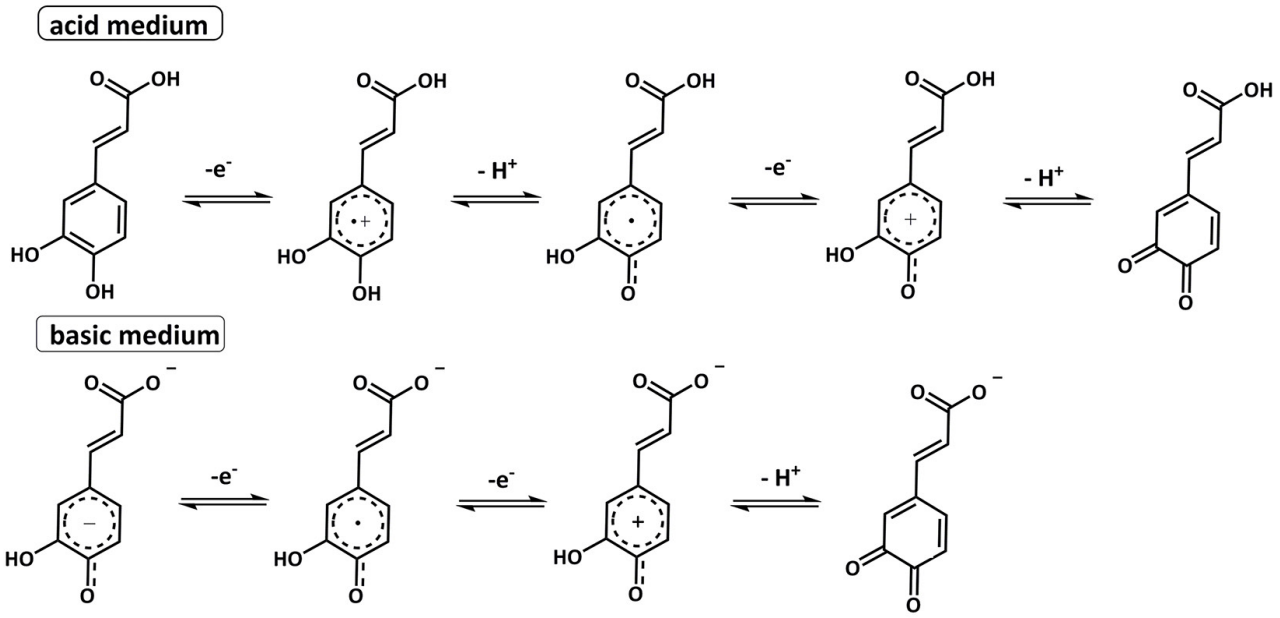

Figure 2. Mechanism for electro-oxidation of caffeic acid in aqueous acid and basic conditions.

compounds in living organisms and, thus, predicting the *in vitro*[10] antioxidant capacity of food products.

There are two main mechanisms by which phenolic antioxidants may eliminate or capture free radicals: hydrogen atom transfer (HAT) and single electron transfer (SET).[11] However, in acid species (AHn) other mechanism, such as Sequential Proton Loss Electron Transfer (SPLET), may be involved.[12]

Possible reaction mechanisms, which may involve different intermediate species are shown below (**Equations 1** to **3**).

Hydrogen Atom Transfer mechanism (HAT)

$$AH_n + Ox^{\bullet} \rightarrow AH^{\bullet}_{n-1} + OxH \quad (1)$$

Single Electron Transfer mechanism (SET)

$$AH_n + Ox^{\bullet} \rightarrow AH^{\bullet+}_{n} + Ox^{-} \rightarrow AH^{\bullet}_{n-1} + OxH \quad (2)$$

Sequential Proton Loss Electron Transfer (SPLET)

$$AH_n + Ox^{\bullet} \overset{-H^+}{\rightarrow} AH^{-}_{n-1} + Ox^{\bullet} \rightarrow AH^{\bullet}_{n-1} + Ox^{-} \overset{+H^+}{\rightarrow} AH^{\bullet}_{n-1} + OxH \quad (3)$$

Another possible mechanism is the Proton-Coupled Electron Transfer (PCET)[13] involving simultaneous proton and electron transfer, but it is only possible when the H-atom is transferred between two heteroatoms.[14]

The reaction mechanism responsible for the antioxidant capacity depends on the experimental technique used to measure the antioxidant capacity. In general, the DPPH assay can occur through a HAT or SET mechanism, according to the antioxidant employed.[11,15] Conversely, FRAP and TEAC methods follow a SET mechanism.[11,16,17] In some particular cases, such as cinnamic acid derivatives, it has also been reported that the DPPH assay occurs by a SPLET mechanism.[18] Additionally, the reaction of 5-CQA with DPPH$^{\bullet}$ also takes place by a SPLET mechanism.[19] In these cases, the hydrogen-atom abstraction from neutral ArOH by DPPH$^{\bullet}$ becomes a marginal reaction path due to strong hydrogen-bond-accepting solvents used, like methanol and ethanol.[20] This occurs very slowly and can be neglected.

Theoretical studies of related phenolic acids have also demonstrated that the HAT mechanism is the more favorable path in the gas phase and also by using a non-polar solvent such as benzene; whereas the SPLET mechanism is preferred when water and ethanol are the solvents.[21] Furthermore, the solvent can play a fundamental role in the reaction kinetics, especially when they are good electron acceptors.[22]

Considering the previous knowledge, and the nature of solvent and antioxidant compounds evaluated, the present study only considers SET and SPLET mechanisms.

In some particular cases, the reaction between peroxyl radicals and phenolic antioxidants bearing a vinyl group has been proposed through a mechanism involving Radical Adduct Formation (RAF).[23] However, RAF mechanisms cannot be proposed for bulky oxidizing species, such as those used in this work (TEAC, DPPH and FRAP). Hence, RAF was not considered in the present study.

Additionally, eventual secondary reactions, such as the oxidation of structures containing an antioxidant catechol ring (e.g. 5-CGA and CA) could be possible. These secondary reactions involve the transfer of two electrons and two protons to form the corresponding o-quinone. For instance, Giacomelli et al.[24] proposed a mechanism for the electrochemical oxidation of caffeic acid, which involves sequential transfers of electrons and protons. Figure 2 shows a scheme of caffeic acid oxidation in acid and basic mediums. The same behavior is expected for 5-CQA, due to structural similarities between both

compounds. Furthermore, there is no possibility of sequential oxidations leading to a stable structure using quinic acid. Considering this evidence, the secondary oxidation of 5-CQA and CA should be considered for a complete description of the antioxidant capacity.

There are several theoretical studies about antioxidants, studying the relationship between their chemical structure and antioxidant capacity.[25] However, most of these studies describe bond dissociation energy, ionization energy and proton dissociation enthalpy of the neutral compound as the main thermodynamic descriptors of the reactivity, without considering the contribution from anionic species or secondary oxidation products, which can be formed in some experimental conditions. To our knowledge, studies that include secondary reactions in the analysis of the antioxidant capacity of selected compounds are scarce.[26–28]

In view of these previous evidences, the main goal of this work was to study the matching between the antioxidant capacity, the structure and the physicochemical properties of 5-CQA and its metabolites (CA and QA). We seek to understand the mechanism by which these compounds produce their *in vitro* antioxidant capacity, considering the possible variables that can affect it. We are particularly interested in determining and studying both intermediate species and secondary products involved in such antioxidant reactions to fully understand the experimentally observed reactivity. Therefore, we look for an approximation to predict experimental results, using a theoretical prediction model, which should bring a new tool to predict the antioxidant capacity of other related compounds.

Results and Discussion

5-O-caffeoylquinic acid (5-CQA), caffeic acid (CA) and quinic acid (QA) are polyprotic acids (AH_3) that can dissociate depending on working conditions (**Equation 4**).

$$AH_3 \overset{Ka_1}{\rightleftharpoons} AH_2^- \overset{Ka_2}{\rightleftharpoons} AH^{2-} \overset{Ka_3}{\rightleftharpoons} A^{3-} \quad (4)$$

Considering that a significant change in the antioxidant capacity is expected when going from one acid-base specie to another, it is important to know the distribution of the different species at equilibrium as a function of pH (depending on the pH of the experimental assay). Figure 3 shows the speciation diagrams of the most relevant compounds, CA and 5-CQA. This plot was performed using tabulated pKa values for CA ($pKa_1 =$ 4.43 and $pKa_2 =$ 8.69)[29] and 5-CQA ($pKa_1 = 3.6$ and $pKa_2 =$ 8.5).[30]

From the speciation diagrams, we can observe that pKa_1 for 5-CQA is markedly lower than the CA; whereas the difference between pKa_2 is less among them, being 5-CQA slightly more acid than CA. For both compounds the pKa_3 is about 11.2,[31] which is outside the working pH for TEAC, FRAP and DPPH assays. The concentration of tri-anionic species (A^{3-}) is negligible in all assays; hence, our analysis does not consider A^{3-}. Considering the distribution of species at equilibrium, an analysis of the antioxidant capacity of 5-CQA, CA and QA was performed, based on both experimental and theoretical data.

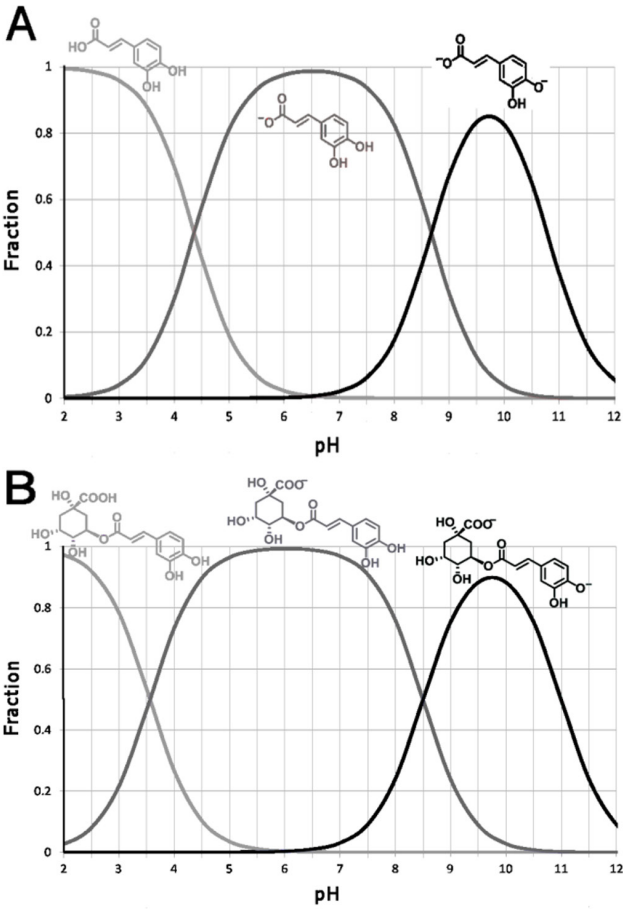

Figure 3. Distribution of acid-base species as a function of pH. **A)** Diagram obtained for CA: Totally protonated CA in light grey, anionic CA^- in grey and dianion (CA^{2-}) in black. **B)** Diagram corresponding to 5-CQA: Protonated 5-CQA in light grey, monoanion 5-CQA^- in grey and dianionic specie 5-CQA^{2-} in black.

In vitro antioxidant capacity.

TEAC, FRAP and DPPH assays were used to measure the antioxidant capacity of 5-CQA, CA and QA. The time used for these assays was 30 min, looking to achieve complete reactions (where the absorbance for each compound reaches a plateau).[32] Additionally, the antioxidant capacity (TEAC, DPPH and FRAP) at different concentrations of 5-CQA and CA were analyzed to determine the linear range of each compound in each assay. The linear range observed was from 0.1 to 6.0 μM. Thus, using 4μM for 5-CQA and CA allowed a better differentiation of their antioxidant capacity.

Results of TEAC assay show that 5-CQA presented a higher antioxidant capacity than CA (7.91 ± 0.21 μM Trolox equivalents (TE) and 6.8 ± 0.3 μM TE, respectively) (Figure 4). Conversely, QA did not show antioxidant capacity by TEAC at the concentration tested (4 μM), nor at higher concentration (830 μM).

In FRAP assays, a slight difference was found between the antioxidant capacities of 5-CQA (5.37 ± 0.10 μM TE) and CA

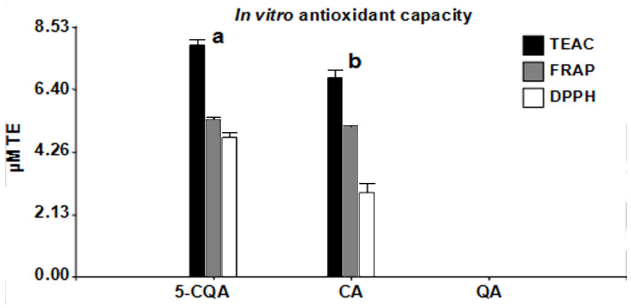

Figure 4. Antioxidant capacity of 5-CQA, CA and QA determinate by different assays (TEAC, DPPH and FRAP). Values are reported as mean ± S.D. corresponding to three independent experiments. Different letters indicate significant differences ($P < 0.05$).

Table 1. Ionization energies for SET reactions of different species in Kcal/mol.[a]

	ΔE	5-CQA	CA	QA
Single molecule	SET_1 ($AH_3 \rightarrow AH_3^{\bullet+}$)	133.9	133.9	151.7
	SET_2 ($AH_2^- \rightarrow AH_2^{\bullet}$)	124.0	115.8	126.9
	SET_3 ($AH^{2-} \rightarrow AH^{\bullet-}$)	103.1	94.6	–
	SET_{1B} ($AH_{2B}^{\bullet} \rightarrow AH_2^+$)	135.4	135.8	–
	SET_{3B} ($AH^{\bullet-} \rightarrow AH^{+-}$)	124.1	115.0	–
DPPH corrected	SET_1	26.0	26.1	43.8
	SET_2	16.0	7.8	19.0
	SET_3	-4.8	-13.4	–
TEAC corrected	SET_1	19.0	19.0	36.8
	SET_2	8.3	0.1	11.3
	SET_3	-12.5	-21.1	–

[a] Calculated energy difference zero point corrected using IEFPCM and MeOH as solvent model.

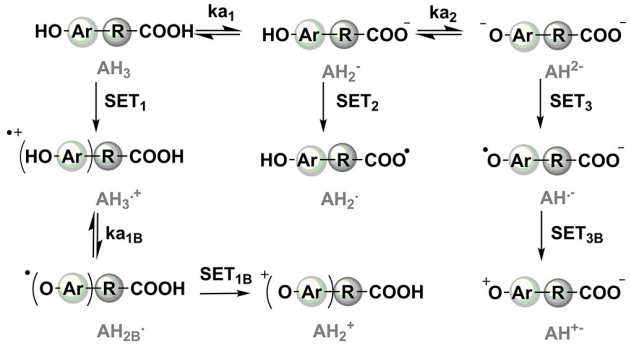

Figure 5. Schematic representation of SET processes for each species of antioxidant compounds.

(5.09 ± 0.08 μM TE) (Figure 4). Once more, QA did not show antioxidant capacity by FRAP, even at 830 μM.

DPPH results are also shown in Figure 4. 5-CQA showed the highest antioxidant capacity (4.76 ± 0.18 μM TE), followed by CA (2.9 ± 0.3 μM TE), with QA not showing detectable antioxidant capacity at 4 μM, nor at 830 μM.

From the experimental data it is evident that 5-CQA is generally the best antioxidant, although the difference between 5-CQA and CA is small evaluated by FRAP. These results tell us that the experimental evaluation of the antioxidant capacity depends on several variables, such as the oxidant used, the reaction media, the chemical structure and pH.

The overall antioxidant capacity of each compound can be described according to the contribution of the individual capacity of each species at equilibrium (AH_3, AH_2^- and AH^{2-}, shown in Figure 5), which depends on the experimental conditions used. Thus, the protonated specie (AH_3) and the anion (AH_2^-) can contribute in acidic conditions; whereas in neutral or basic conditions AH_2^- and the dianion (AH^{2-}) may be responsible for the antioxidant capacity observed.

Each acid-base species (AH_3, AH_2^- and AH^{2-}) has an associated SET reaction ($SET_{1\text{-}3}$), leading to other related specie as described in Figure 5. Considering the possible influence of each species on the antioxidant capacity, a theoretical analysis was conducted taking into consideration these three forms (AH_3, AH_2^- and AH^{2-}) for both 5-CQA and CA; whereas only AH_3 and AH_2^- were considered for QA. In some particular cases, such as electrochemical oxidation, secondary oxidation reactions could be observed.[33] Thus, primary oxidation products ($AH_3^{\bullet+}$ and $AH^{\bullet-}$) may undergo a second electron transfer to give a cation or a zwitterion respectively (AH_2^+ -SET_{1B} or AH^+–SET_{3B}, Figure 5).

Therefore, these secondary oxidations were considered to fully understand the overall reactivity.

Table 1 shows the thermochemistry associated to SET reactions (ionization energies for AH_3, AH_2^-, AH^{2-}, $AH_{2B}^{\bullet}$ and $AH^{\bullet-}$) for all isolated compounds (single molecules), including the correction that arises from considering the oxidant counterpart ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$).

Energetic analysis.

Theoretical results show that the SET reaction for neutral compounds (SET_1 in Table 1) is widely favored for 5-CQA and CA regarding to QA (133.9, 133.9, and 151.7 kcal/mol, respectively). Conversely, there are not differences between the reducing power of 5-CQA and CA. Furthermore, the SET reactions of mono-anionic species AH_2^- (SET_2) were more favorable than SET_1 reaction in all cases under study.

Regarding to the species AH_2^-, CA^- stands out for having a higher reducing power; whereas QA^- thermochemistry is the least favored. The same order is predicted for dianionic species AH^{2-} (CA^{2-} > $5\text{-}CQA^{2-}$). Finally, AH^{2-} species show the best re-

ducing capacity, establishing the following reactivity order $AH^{2-} > AH_2^- > AH_3$.

This last trend is in agreement with results observed for similar compounds, where a substantial decrease in the oxidation potential was observed when the pH increases from 4 to 8.[34]

This can be explained considering that the phenoxide anion is better reducing agent than the corresponding phenol. For instance, in the catechol moiety, the one-electron redox potential at pH = 7 (E_{pH7}) was 0.53 V, while at pH = 13.5 ($E_{pH13.5}$) was 0.04 V *vs* Normal Hydrogen Electrode (NHE), respectively.[35]

With regard to secondary oxidation reactions, it shows that SET_{1B} reduction power (Table 1) is slightly higher than SET_1 for both 5-CQA and CA, indicating that SET_{1B} reactions are less favorable than SET_1. On the other hand, SET_{3B} reactions may occur as a second stage of SET_3 (Figure 5), displaying energy values markedly less favorable than the initial oxidation (124.1 kcal/mol for 5-CQA, and 115.0 kcal/mol for CA). While, SET_2 reaction does not result in a second oxidation since it is known that such radical can undergo decarboxylation.[36]

In general, secondary oxidations exhibit the same order of reactivity that primary ones, but have less favorable ΔE values than the initial SET reactions.

Thermochemistry for SET reactions depends on the difference between the chemical stability of both reactants and products, which can be understood by analyzing the electronic properties. As shown in Table 1, the SET_1 does not exhibit difference between 5-CQA and CA, while the same process for QA proves to be less favorable. It is because the absence of an expanded π system prevents the stabilization of this unpaired spin intermediate for QA. Moreover, the SET_2 shows major differences between 5-CQA and CA, being CA the most favorable. Given the structural similarity between the AH_2^- anions, the observed order can be attributed to the difference in stability between formed radicals ($AH_2^{\bullet}$). This analysis was performed using the theoretical study of the reaction.

As it is shown in Figure 6, the anionic species (AH_2^-) lead to the formation of radicals of different nature. First, the caffeic acid anion (CA^-) produces a fully conjugated radical, which is delocalized throughout the π system. The resulting conjugation occurs due to the rotation of the carboxyl group outside the molecular plane, allowing its interaction with the rest of the molecule, giving high stability to the intermediary $AH_2^{\bullet}$. Thus, in this case, the electron is transferred from the entire molecule.

On the other hand, the anion arising from 5-CQA leads to the formation of a "localized" radical, due to the forbidden conjugation in $AH_2^{\bullet}$. The transferred electron comes from the isolated carboxylic group, which is not conjugated with the aromatic system. This difference is due to the presence of the aliphatic sp^3 cycle system in 5-CQA, which interrupts the interaction between the carboxylate group and the aromatic system. The large volume of the aliphatic substituent (quinic acid) determines the rotation of the ester group of cinnamic acid, inhibiting the conjugation of the unpaired electron and, consequently, decreasing the stability of the species 5-CQA$^{\bullet}$ with respect to CA$^{\bullet}$.

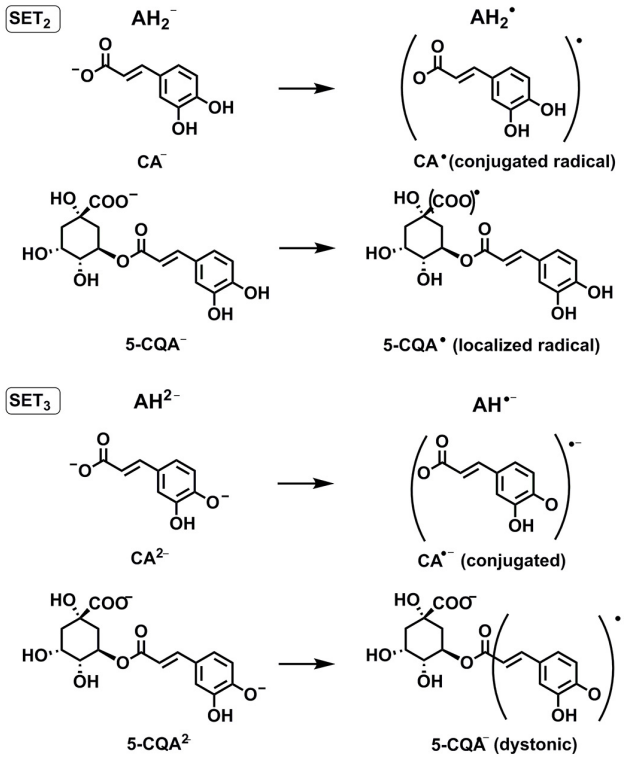

Figure 6. Proposed SET mechanisms for the oxidation of CA and 5-CQA.

A similar effect is observed for the SET_3 process, wherein the aliphatic system interrupts the interaction of aromatic (Ar-C=C-) and ester systems (O-C(O)-R). Consequently, it is observed that CA forms a conjugated anion radical, while 5-CQA results in the formation of a dystonic radical anion. The stabilization of these intermediates has an energetic consequence, making CA a better reducer with respect to 5-CQA in SET_2 and SET_3 processes (Table 1). The electronic properties of the radicals formed after the SET_2 and SET_3 are graphically described in Figure 7, where the spin density distribution through the system can be observed in each case.

From Figure 7 we can see that the calculated spin densities were consistent with the structures proposed in Figure 6. These two figures help to better visualize the stability of the oxidation products arising from the reaction SET_2, which heavily depends on the unpaired electron delocalization. However, this is not the case for SET_3, where charge and electronic delocalizations are similar for both CA^- and 5-CQA^-. The spin density in both species is located on the aromatic moiety, while the negative charge is mainly found in the carboxylate group (Figure 7B). In this case, the electrostatic properties of the anions, before and after SET, determine SET_3 thermochemical behavior. As shown from the maps of electrostatic potential (ESP maps) in Figure 7C, the dianion CA^{2-} has a strong electrostatic repulsion of the negative charges dispersed throughout the molecule; while the repulsion in the dianion of 5-CQA decreases due to charge separation, mediated by the aliphatic system. Consequently, SET_3 is more favorable for CA, since the electrostatic repulsion decreases significantly when going from CA^{2-} to CA^-. The en-

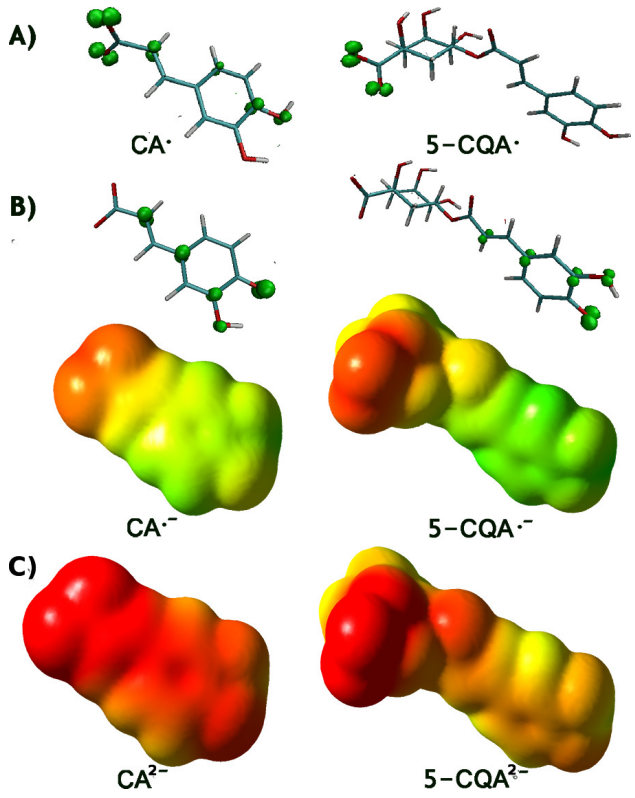

Figure 7. Optimized geometries, spin densities and ESP maps for oxidized species resulting for SET of studied antioxidants. **A)** Radicals formed after SET of anionic antioxidant (SET_2). Left conjugated radical corresponding to CA ($CA^{\bullet}$) and right localized radical produced for 5-CQA (5-$CQA^{\bullet}$). **B)** Spin densities (above) and ESP maps (bellow) for Radical Anions formed after SET from dianion (SET_3). Left conjugated RA for caffeic acid ($CA^{\bullet-}$) and right dystonic RA obtained for 5-O-caffeoylquinic acid (5-$CQA^{\bullet}$). **C)** ESP maps for dianions CA^{2-} (left) and 5-CQA^{2-} (right).

ergy change is less favored for 5-CQA due to the fact that the change in the repulsion is lower.

It is worth to remark that when comparing the same acid-base forms of 5-CQA and CA, CA shows a more favorable oxidation. These results are in agreement with those reported by Yardim,[37] who studied the electrochemical oxidation of these species.

Our theoretical results show that the CA is better reducer than 5-CQA; however, experimental results for the antioxidant capacity do not show the same behavior. That is why it is necessary to analyze the influence of the species in equilibrium, and its effect on the antioxidant capacity for each particular test.

TEAC assay

The absence of antioxidant capacity observed for quinic acid during the TEAC assay is well known, and it is explained in terms of its lower capacity to stabilize the intermediary after the reaction with the radical cation $ABTS^{\bullet+}$. This is due to the absence of an aromatic structure, allowing the stabilization by resonance of the unpaired electron. Furthermore, the better antioxidant capacity evidenced by the 5-O-caffeoylquinic and caffeic acids in the TEAC assay can be understood in terms of the effect of the reaction medium, where the most important component is the pH (pH = 7.4). Considering the pKa_1 of the studied compounds (whose values vary between 3 and 5), it is evident that under the working conditions used in the TEAC assay, antioxidants are found predominantly as anions (AH_2^-), in equilibrium with a minor amount of the dianion (AH^{2-}), when the compound has phenolic hydroxyls (i.e. CA and 5-CQA). In this context, the antioxidant capacity observed for each compound depends on the distribution of species in equilibrium and, ultimately, on the contribution of each acid-base species to the overall antioxidant capacity.

As can be seen in Figure 4, the 5-CQA evidences an antioxidant capacity higher than CA. Even considering that individual species derived from CA have a higher electron donor ability, the difference between pKa_2 shows that, under the experimental condition, there is a greater abundance of AH^{2-} for 5-CQA (7.36%) than the corresponding to CA (5.10%) (see speciation diagram in Figure 3). Considering these data, it can be concluded that the amount of dianion in the equilibrium determines the antioxidant capacity, although it is not the dominant species. The fast electron transfer of the dianion allows shifting the balance of acid-base species to their formation under experimental conditions. The higher concentration of AH^{2-}, for 5-CQA, is the main factor for the antioxidant capacity observed with these compounds. The importance of the concentration of dianions on the antioxidant capacity has been previously described.[38]

Under these conditions, the secondary oxidation is predicted to be negligible, given that ΔE values for SET_{3B} are markedly less favorable than SET_3, which is the dominating reaction.

DPPH assay

As it was the case with TEAC, 5-CQA has a higher antioxidant capacity than CA when evaluated by the DPPH assay. This result can also be understood in terms of the effects of pKa. Given that this assay is performed in pure methanol, which is less basic than water, it acts as a differentiating solvent. Therefore, it is expected that methanol allows further differentiation of pKa_2 between 5-CQA and CA. This effect leads to a greater difference in the population of AH^{2-}, which directly affects the antioxidant capacity. However, another important factor is the solvation, which also affects the pKa and can modify the acidity. In order to evaluate this last effect, $\Delta\Delta Gsolv$ were calculated in methanol, evidencing that 5-CQA^- is more acid than CA^- (see Supporting Information). Similar results were observed by Erdemgil et.al.[39] by measuring the values of pKa_2 of some hydroxylated benzoic acids in different mixtures of methanol/water, observing an increase of ΔpKa with the addition of methanol.[39]

Considering this evidence, it is possible to think that the difference in the population of the dianion should be higher in pure methanol, which is in agreement with the largest difference in the antioxidant capacity observed when changing from a partially aqueous medium (TEAC) to methanol (DPPH).

With respect to the influence of secondary oxidation, a similar conclusion to the above mentioned for the TEAC assay can be stablished.

There is an actual controversy about the meaning of DPPH results. Foti et al.[30] propose that results from this test allow determining only the stoichiometry of the reaction (*n*), but not the reactivity of the antioxidant. However, the *n* values for CA and 5-CQA have been determined to be the same,[41] due to their similar chemical structure (cathecol fragment). In this context, we propose that the different reactivity towards DPPH depends mainly on the above-mentioned difference in the quantity of dianions.

In summary, the SET process for the phenoxide anion (SET_3) is proposed to be the largest contributor to the overall ability of these compounds in neutral or slightly basic solution. These results imply that the anion AH^{2-} behaves as a better antioxidant than the parent species AH_2^-. A similar pH dependent effect has been observed by Mukai et al.[42] in the reaction of catechins at several pH values. The larger reactivity in the ionized form is attributed to the greater electron-donating capacity of the O- group.

FRAP assay

In FRAP, 5-CQA and CA show a smaller difference in their antioxidant capacity with respect to TEAC and DPPH assays. Under the acid conditions used in the experiment (buffer at pH = 3.6), the relevant species at equilibrium are AH_2^- and AH_3. The small difference determined in the antioxidant capacity may be due to two opposite effects. First, pKa_1 for 5-CQA is lower than the corresponding to CA, and close to the working pH (Figure 3), which translates into a significant population of the species 5-CQA^- (approximately 50%). Instead, the CA has a $pKa_1 = 4.43$ and only 14.80% of CA^- is present under these conditions (see speciation diagram in Figure 5). Considering that AH_2^- is more reactive than AH_3, these values should be reflected in a greater antioxidant capacity for 5-CQA. In contrast, ΔE values for SET_2 in both compounds are in opposite sense, showing that the reaction is more favorable for CA, mainly in the anionic form CA^- (SET_2). The latter effect would favor a greater antioxidant capacity for CA, as opposed to the speciation diagrams. As a result, both effects, the higher reactivity and low abundance of CA^-, are important in the overall antioxidant capacity, since they approach the antioxidant capacity between 5-CQA and CA. It is worth to mention that, under these conditions, the equilibrium shift is markedly lower than that observed under neutral conditions. This could be explained because the SET reactions are slower at the working pH (3.6) than in neutral conditions, as it was also observed by Amorati et al.[35]

On the other hand, the SET_{1B} oxidations have slightly higher values than SET_1. Thus, it is expected that, at equilibrium, these reactions do not determine the reactivity.

The secondary reactions in TEAC, DPPH and FRAP assays can take place but show thermochemical disadvantages regarding primary oxidation. Therefore, it is not expected a significant influence of these secondary reactions in the observed antioxidant capacity, which is directed by the initial SET reactions (primary oxidation).

Our current results confirm that quinic acid has no appreciable antioxidant capacity in any tests evaluated. Conversely, the inclusion of quinic acid as substituent in the structure of caffeic acid (5-CQA) generates an increase in antioxidant capacity compared to the CA, due to the change in the electronic properties of intermediaries that generates the separation between COOH and ArOH groups.

A difference in the antioxidant capacity could be observed using neutral or slightly basic conditions. Under these conditions (neutral or slightly basic), the value of the second pKa, or the relative population of the dianion, was predicted to be the key factor, since the electron transfer mainly involves the phenoxide anion (SET_3). On the contrary, under acidic conditions, differences in the antioxidant capacity were shortened due to the importance of two opposite effects: the relative population of the anion AH_2^-, and the thermochemistry for individual SET reaction.

Conclusions

The theoretical analysis of reactions involved in the antioxidant capacity allows identifying the causes that increase or decrease such capacity, based on differences in the electronic properties of the intermediates. The geometric and electronic factors affect appreciably the thermochemistry of the antioxidant reaction. This is a quantum mechanics description of the different nature in the intermediate radicals, considering the equilibria involved and including secondary oxidations, which attempts to interpret the changes in antioxidant capacity by modifying the reaction conditions.

Finally, further experiments using other assays, involving other food antioxidants; have to be performed in order to elaborate a rational basis to fully understand antioxidant capacity, enabling the construction of a predictive theoretical model based on structure-reactivity relationship which considers the influence of reaction medium.

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Keywords: DFT · mechanism · antioxidant capacity · secondary oxidations · intermediates

[1] C. Manach, A. Scalbert, C. Morand, C. Rémésy, L. Jiménez, *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
[2] M. N. Clifford, *J. Sci. Food Agric.* **2000**, *80*, 1033–1043.
[3] G.-F. Wang, L.-P. Shi, Y.-D. Ren, Q.-F. Liu, H.-F. Liu, R.-J. Zhang, Z. Li, F.-H. Zhu, P.-L. He, W. Tang, P.-Z. Tao, C. Li, W.-M. Zhao, J.-P. Zuo, *Antiviral Res.* **2009**, *83*, 186–190.
[4] Y. Sato, S. Itagaki, T. Kurokawa, J. Ogura, M. Kobayashi, T. Hirano, M. Sugawara, K. Iseki, *Int. J. Pharm.* **2011**, *403*, 136–138.
[5] M. H. Gordon, K. Wishart, *J. Agric. Food Chem.* **2010**, *58*, 5828–5833.
[6] N. S. Podio, R. López-Froilán, E. Ramirez-Moreno, L. Beltrand, M. V. Baroni, M. L. Pérez-Rodríguez, M.-C. Sánchez-Mata, D. A. Wundelin, *J. Agric. Food Chem.* **2015**, *63*, 9572–9582.

[7] A.-S. Cho, S.-M. Jeon, M.-J. Kim, J. Yeo, K.-I. Seo, M.-S. Choi, M.-K. Lee, *Food Chem. Toxicol.* **2010**, *48*, 937–943.
[8] J.-G. Xu, Q.-P. Hu, Y. Liu, *J. Agric. Food Chem.* **2012**, *60*, 11625–11630.
[9] M.-P. Gonthier, M.-A. Verny, C. Besson, C. Rémésy, A. Scalbert, *J. Nutr.* **2003**, *133*, 1853–1859.
[10] a) E. Klein, V. Lukes, *J. Phys. Chem. A* **2006**, *110*, 12312–12320. b) D. Mikulski, K. Eder, M. Molski, *J. Theor. Comp. Chem.* **2014**, *13*, 1450004-1–1450004-24.
[11] R. L. Prior, X. Wu, K. Schaich, *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.
[12] R. E. Galian, G. Litwinienko, J. Pérez-Prieto, K. U. Ingold, *J. Am. Chem. Soc.* **2007**, *129*, 9280–9281.
[13] S. Hammes-Schiffer, *Acc. Chem. Res.* **2001**, *34*, 273–281.
[14] K. U. Ingold, D. A. Pratt, *Chem. Rev.* **2014**, *114*, 9022–9046.
[15] G.; Litwinienko, K. U. Ingold, *Acc. Chem. Res.* **2007**, *40*, 222–230.
[16] M. J. T. J. Arts, J. S. Dallinga, H.-P. Voss, G. R. M. M. Haenen, A. Bast, *Food Chem.* **2003**, *80*, 409–414.
[17] R. Apak, S. Gorinstein, V. Böhm, K. M. Schaich, M. Özyürek, K. Güçlü, *Pure Appl. Chem.* **2013**, *85*, 957–998.
[18] M. C. Foti, C. Daquino, C. Geraci, *J. Org. Chem.* **2004**, *69*, 2309–2314.
[19] L. J. López-Giraldo, M. Laguerre, J. Lecomte, M.-C. Figueroa-Espinoza, B. Baréa, J. Weiss, E. A. Decker, P. Villenueve, *J. Agric. Food Chem.* **2009**, *57*, 863–870.
[20] M. H. Abraham, P. L. Grellier, D. V. Prior, J. J. Morris, P. J. Taylor, *J. Chem. Socienty Perkin Trans. 2* **1990**, 521–529.
[21] Y. Chen, H. Xiao, J. Zheng, G. Liang, *PLoS One* **2015**, *10*, 1–20.
[22] M. Foti, G. Ruberto, *J. Agric. Food Chem.* **2001**, *49*, 342–348.
[23] L. Kong, Z.-L. Sun, L.-F. Wang, H.-Y. Zhang, S.-D. Yao, *Helv. Chim. Acta* **2004**, *87*, 511–515.
[24] C. Giacomelli, K. Ckless, D. Galato, F. S. Miranda, A. Spinelli, *J.Braz. Chem. Soc.* **2002**, *13*, 332–338.
[25] N. Nenadis, M. Z. Tsimidou in *Density Functional Theory: Principles, Applications and Analysis* (Eds.: J. Morin, J. M. Pelletier), Nova publishers, New York, **2013**, pp. 121–146.
[26] E. Anouar, C. A. Calliste, P. Košinova, F. Di Meo, J.-L. Duroux, Y. Champavier, *J. Phys Chem. A* **2009**, *113*, 13881–13891.
[27] A. Amić, Z. Marković, J. M. Dimitrić Marković, V. Stepanić, B. Lučić, D. Amic, *Food Chemistry* **2014**, *152*, 578–585.
[28] a) D. Amić, V. Stepanić, B. Lučić, Z. Marković, J. M. Dimitrić Marković, *J. Mol. Model.* **2013**, *19*, 2593–2603. b) L. Valgimigli, R. Amorati, M. G. Fumo, G. A. DiLabio, G. F. Pedulli, K. U. Ingold, D. A. Pratt, *J. Org. Chem.*, **2008**, *73*, 1830–1841.
[29] E. P. Serjeant, D. Boyd in *Ionisation constants of organic acids in aqueous solution* (Eds.: IUPAC) Oxford; New York: Pergamon Press, **1979**.
[30] N. Sauerwald, M. Schwenk, J. Polster, E. Z. Bengsch, *Naturforsch* **1998**, *53*, 315–321.
[31] F. A. M. Silva, F. Borges, C. Guimaraes, J. L. F. C. Lima, C. Matos, S. Reis, *J. Agric Food Chem.* **2000**, *48*, 2122–2126.
[32] P. Stratil, B. Klejdus, V. Kubán, *J. Agric. Food Chem.* **2006**, *54*, 607–616.
[33] S. Seo, J. B. Taylor, M. F. Greaney, *Chem. Commun.* **2012**, *48*, 8270–8272.
[34] M. Namazian, H. R. Zare, *Electrochim. Acta* **2005**, *50*, 4350–4355.
[35] R. Amorati, G. F. Pedulli, L. Cabrini, L. Zambonin, L. Landi, *J. Agric. Food Chem.* **2006**, *54*, 2932–2937.
[36] S. Steenken, P. Neta, *J. Phys. Chem.* **1982**, *86*, 3661–3667.
[37] Y. Yardım, *J. Food Sci.* **2012**, *77*, C408–C413.
[38] M. Cordova-Gomez, A. Galano, J. R. Alvarez-Idaboy, *RSC Adv.* **2013**, *3*, 20209–20218.
[39] F. Z. Erdemgil, S. Şanli, N. Şanli, G. Özkan, J. Barbosa, J. Guiteras, J. L. Beltrán, *Talanta* **2007**, *72*, 489–496.
[40] M. Foti, *J. Agric. Food Chem.* **2015**, *63*, 8765–8776.
[41] P. Goupy, C. Dufour, M. Loonis, O. Dangles, *J. Agric. Food Chem.* **2003**, *51*, 615–622.
[42] K. Mukai, S. Mitani, K. Ohara, S.-I. Nagaoka, *Free Radic. Biol. Med.* **2005**, *38*, 1243–1256.

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