

## Antioxidant properties of capsaicin analogues: A DFT study

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Capsaicin is the one of the primary components of fruits of genus *Capsicum* and is responsible of the pungent sensation known “spiciness”. In recent years, this compound has attracted special attention thanks to its beneficial biological effects, such as its antioxidant, anti-obesity and antidiabetic activity, among others. In the present studies, twelve capsaicin analogues with functional groups -SH, -NH<sub>2</sub>, -OCH<sub>3</sub> and -F in the aromatic ring, have been proposed and studied using DFT methodologies. The reactivity global indexes:  $\Delta E_{\text{HOMO-LUMO}}$ , Chemical hardness ( $\eta$ ), Electron donating power ( $\omega$ ) and Dipole Moment ( $\mu$ ) and primary antioxidant activities have been obtained. According to the results, most of the analogues with the amine (-NH<sub>2</sub>) and thiol (-SH) groups show better ability to donate and accept electrons, which translates into a better antioxidant activity.

**Keywords:** Capsaicin, Antioxidant activity, Electron transfer, Hydrogen transfer

Every living being that use oxygen to generate energy, creates free radicals as a result of ATP (adenosine triphosphate) production by the mitochondria. Reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) are the result of the cellular redox processes. These species have both toxic and beneficial properties. The subtle balance between their opposite effects play an important role in several important aspects of life. At low or moderate concentration levels, ROS and RNS have beneficial effects on cellular responses and immune function. At high concentrations levels, they could generate oxidative stress, a harmful process that can damage all cell structures<sup>1</sup>. Chronic exposition to ROS and RNS could lead to various life-threatening pathological conditions such as aging, heart diseases, diabetes, autoimmune diseases, cancer and neurological disorders<sup>2</sup>.

The free radicals are neutralized by enzymatic and non-enzymatic mechanisms. The living organisms have constructed an antioxidant defense system that includes enzymatic, metal chelating and free radical scavenging activities to destroy both ROS and RNS<sup>3</sup>. Additionally, the consumption of antioxidants in foods or beverages could help to maintain an adequate antioxidant status in the human body. Thus, utilization of antioxidants in the regular diet is a key strategy to prevent the progression of diseases caused by free radicals<sup>4</sup>.

Spices are traditionally defined as any part of a plant primarily used for flavoring, aromatizing, or coloring food with not or low nutritional value<sup>5</sup>. However, in recent years spices have been identified as sources of various phytochemicals with powerful antioxidant activities. Thus, spices may have a role in antioxidant defense and redox signaling<sup>6</sup>.

Chili peppers are fruits obtained from plants of the genus *Capsicum* and are widely used in several cuisines around the world as spice to add heat to the dishes. These fruits are a rich source of capsaicinoids, carotenoids (of which some of them have pro-vitaminic A activity), flavonoids, ascorbic acid (vit. C) and tocopherols (vit. E)<sup>7</sup>. Chilin peppers are used in a therapeutic diet due to the activities of unique components, capsaicin and capsaicinoids, that contribute to the pungent scent of hot chili pepper. Recent studies on chili peppers have demonstrated on their beneficial functions as potential anti-tumour, anti-cancer, antioxidant and anti-obesity agents<sup>8-10</sup>.

The antioxidant activity of capsaicin (Fig. 1)<sup>11</sup> is well documented, it is a crystalline, colourless, and lipophilic alkaloid. Various experimental and theoretical studies have been carried out in the design, synthesis, and testing of capsaicin analogues with the aim to obtain compounds with improved, antioxidant, antiobesity or anticancer activities<sup>12-15</sup>.

In this study twelve capsaicin analogues have been studied (Fig. 2), the proposed compounds were constructed by modifying the aromatic ring of capsaicin, -OH and -OCH<sub>3</sub> in the original molecule

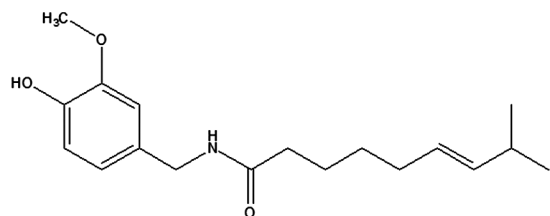


Fig. 1 — Capsaicin Structure

were replaced for different functional groups. The antioxidant activity has been calculated by means of electronic structure calculations within the DFT framework. The proposed analogues could help to obtain capsaicin analogues with improved antioxidant activity.

### Computational Details

Geometry optimizations and vibrational frequencies of the proposed molecules were carried out at B3LYP/cc-PVTZ level<sup>16</sup>. Solvent effects were taken into account with the implicit solvation model based on density (SMD)<sup>17</sup> with water as solvent.

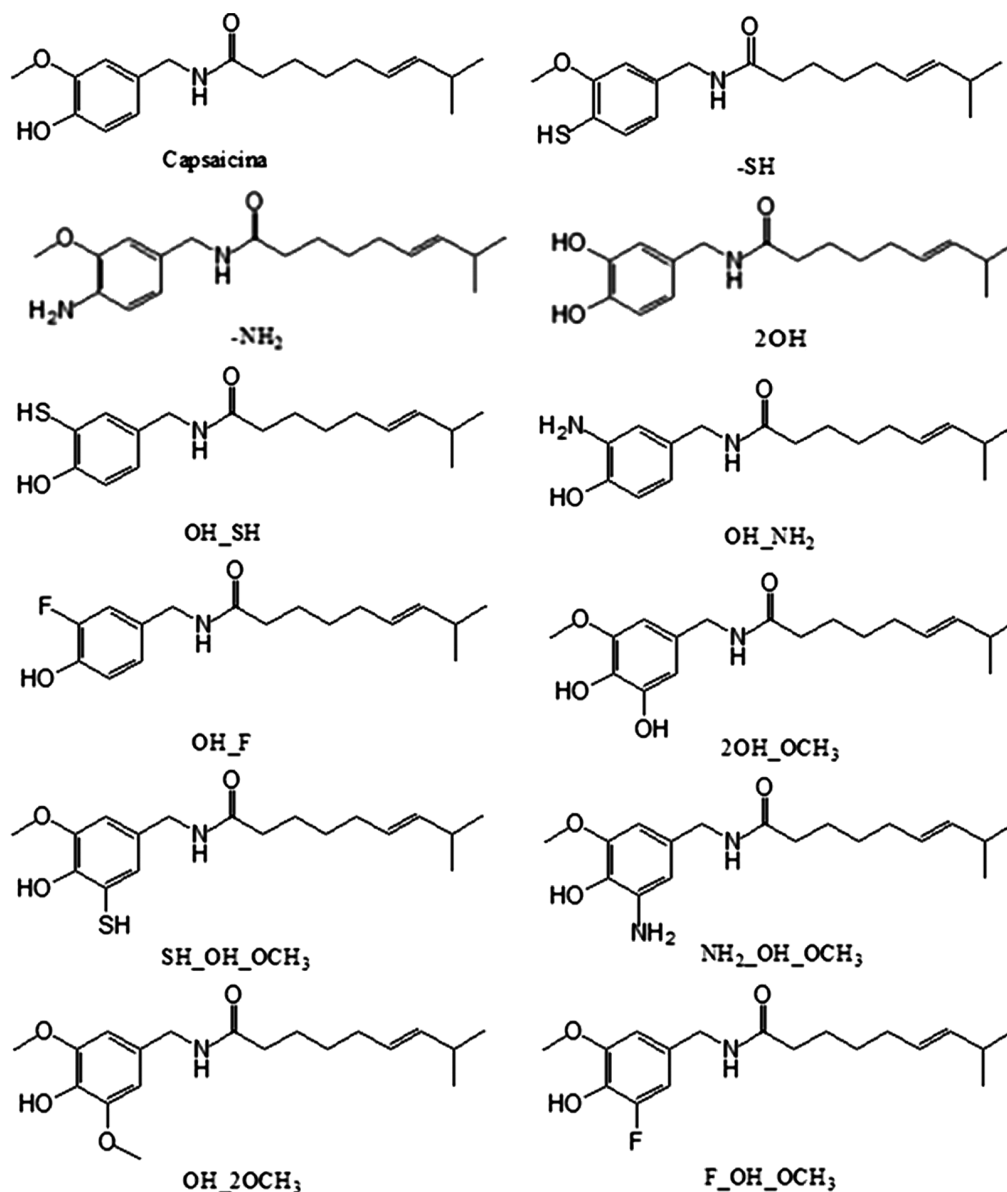


Fig. 2 — Proposed capsaicin analogues

Quantitative analysis of scavenging activity and preferred radical scavenging pathway was performed using these three routes<sup>13</sup>:

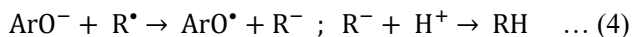
1) Hydrogen atom transfer (HA)



2) Single-electron transfer followed by proton transfer (SET-PT)



3) Sequential proton loss electron transfer (SPET)



The proposed routes were explored through selected local and global descriptors: molecular electrostatic potential (MEP), radical Fukui function ( $f^0$ ), ionization potential (IP), electron-affinity (EA), hardness ( $\eta$ )<sup>18</sup>, bond dissociation energy (BDE), phenol proton affinity (PPA), dipole moment ( $\vec{\mu}$ ) and electron transfer energy (ETE)<sup>13</sup>.

The HA pathway is defined by DBE descriptor (eq 1), SET-PT by IP (eq 2) and SPET that occurs in two stages, and is defined by PPA in the first stage (eq 3), and for ETE in the second stage (eq 4). In each case, the route with the lowest energy requirement will be the most favoured energetically, and therefore the one with the highest probability of occurrence. These energy requirements can be compared between antioxidant molecules as a measure of efficiency. All electronic density calculation were performed with

orca 4.2.0<sup>19</sup>, while MEP and were obtained with Multiwfn 2.1.2<sup>20</sup>.

## Results and Discussion

### Global Reactivity Descriptors

A practical approximation to analyse the chemical reactivity is described in the entire molecular system through mathematical expressions that take molecular information to express a certain property as a unique numerical value. These molecular descriptors are well-known as ‘‘Global molecular Descriptors’’. Of course, each one provides specific molecular properties that can be compared between molecular systems. Hence, a lot of them are reported in the literature. Values of global reactivity descriptors:  $\Delta E_{\text{HOMO-LUMO}}$ , Chemical hardness ( $\eta$ ), Electron donating power ( $\omega^{-}$ )<sup>21</sup> and Dipole Moment ( $\vec{\mu}$ ) are reported in Table 1. Chemical hardness and HOMO-LUMO gap are two parameters that are correlated to the molecular stability and polarizability capacity of the electronic system respectively. Low values of  $\eta$  means low molecular stability while low HOMO-LUMO gap means high polarizability, both properties that low values implies a better performance in HA and SET-PT pathways. With respect to Capsaicin, we found that OH\_NH2, SH, NH2, OH\_SH, SH\_OH\_OCH3 and SH\_OH\_OCH3 present low values of  $\eta$  and  $\Delta E_{\text{HOMO-LUMO}}$ , which implies a better performance in HA and SET-PT with respect to Capsaicin, while higher values of 2OH, OH\_2OCH3, 2OH\_OCH3, F\_OH\_OCH3 and OH\_F show their poor performance as electron scavenging molecules. Presence of thiol and amine groups, both with electron donating characteristics, there is a constant in the structures with better results. Electron donating

Table 1 — Global Reactivity Descriptors,  $\Delta E_{\text{HOMO-LUMO}}$ , Chemical hardness ( $\eta$ ) and Electrodonating power ( $\omega^{-}$ ) in a.u., and Dipole Moment ( $\vec{\mu}$ ) in Debyes at the B3LYP/cc-PVTZ level of theory

	$\Delta E_{\text{HOMO-LUMO}}$	$\eta$	$\omega^{-}$	$\vec{\mu}$
OH-NH <sub>2</sub>	0.1944	0.1764	0.1049	2.04
SH	0.1942	0.1800	0.1200	1.51
NH <sub>2</sub>	0.1959	0.1813	0.0986	1.87
OH-SH	0.1980	0.1853	0.1201	2.83
SH-OH-OCH <sub>3</sub>	0.2014	0.1863	0.1198	1.84
NH <sub>2</sub> -OH-OCH <sub>3</sub>	0.2031	0.1866	0.0999	1.28
Capsaicin	0.2050	0.1876	0.1142	2.37
2OH	0.2061	0.1887	0.1163	2.55
OH-2OCH <sub>3</sub>	0.2097	0.1964	0.1122	0.66
2OH-OCH <sub>3</sub>	0.2142	0.1981	0.1060	1.60
F-OH-OCH <sub>3</sub>	0.2132	0.2001	0.1153	1.31
OH-F	0.2143	0.2013	0.1246	3.58

power ( $w^-$ ) is a practical descriptor that measures the capacity of a system to donate electronic charge. Values reported in Table 1 are consistent with a better performance of SH, OH\_SH and SH\_OH\_OCH3 than Capsaicin, while OH\_NH2, NH2 and NH2\_OH\_OCH3 seem to have less capacity to donate charge. In practice, electron scavenging phenomena occur in aqueous media, which implies that the proposed structures must have large dipole moments to ensure their solubility. It is clear in Table 1, that all amine and thiol structures have large dipole moments, being OH\_SH the structure with better solubility capacities than capsaicin.

#### Molecular Electrostatic Map and Radical Fukui Function

The MEP is a local chemical reactivity index useful for the identification of electron rich and electron poor regions. Particularly in antioxidant molecules derived of phenolic compounds, the main reactivity sites are expected to be distributed over the phenyl ring. As can see in the MEP (Fig. 3), substitutions made in capsaicin base structure modify the distribution of positive and negative regions upon the phenyl ring, whereas amide and aliphatic

regions remain unchanged with respect to MEP behaviour. All substitutions cause electron rich regions in *para*- and *meta*- positions with respect to amide, for those are sites of interest for a scavenging reaction.

Although the information provided by MEP is useful, radical formation sites are a crucial step for HA and SET-PT pathways, whereas the same cannot be identified by MEP. For that reason, we explore  $f^0$ , the Fukui function that provides the radical susceptibility,  $f^0$  is a normalized local descriptor, that implies that unlike other local descriptors, it cannot be numerically compared between systems, but their spatial arrangement is still useful for getting information about internal reactivity. Graphical representation of  $f^0$  for all systems is presented in Fig. 4, where blue basins represent regions of radical susceptibility. According to Fig. 4, the main region of radical susceptibility in all systems is distributed over the phenolic ring and over its amine, thiol, and hydroxy substitutions, which allows the mechanisms of HA and SET-PT.

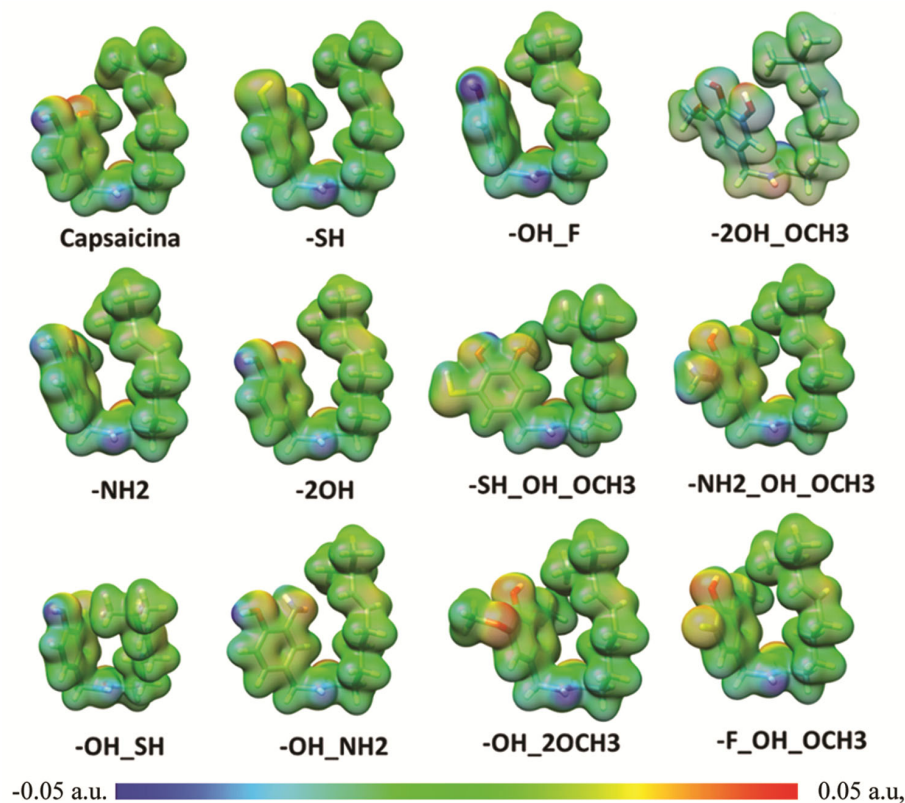


Fig. 3 — Molecular electrostatic potential mapped on the surface of the electron density at an isosurface of 0.01 a.u., calculated at the B3LYP/cc-PVTZ level. The red surface corresponds to negative regions of the electrostatic potential (-0.05 a.u.), whereas green and blue region correspond to neutral (0.00 a.u.) and positive (0.05 a.u.) regions of the electrostatic potential, respectively

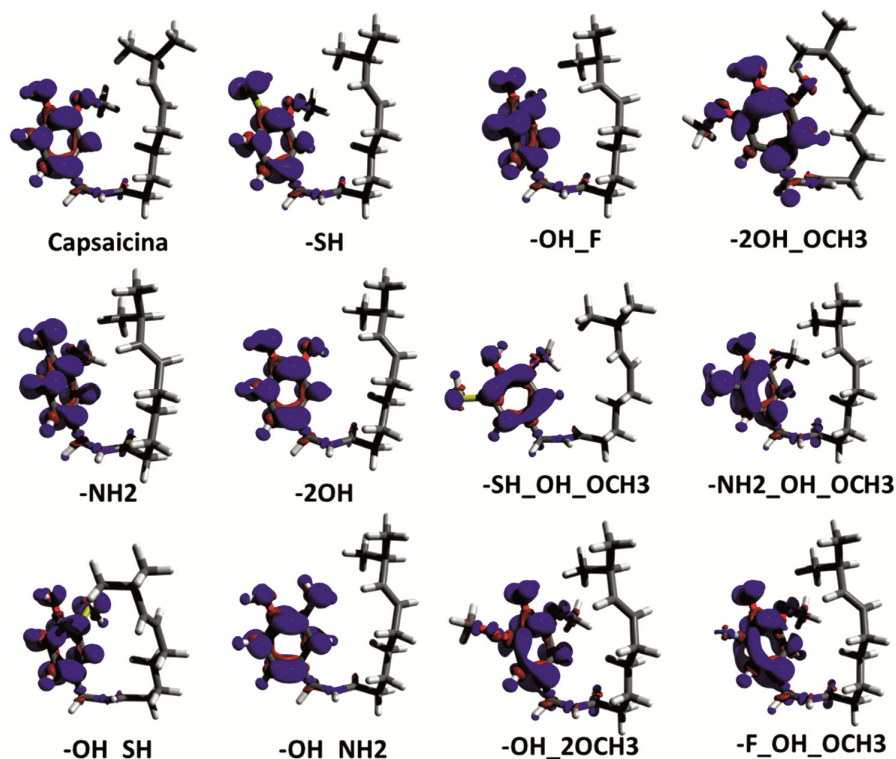


Fig. 4 — Radical Fukui function ( $f^0$ ) at isosurface value of 0.005 a.u. calculated at the B3LYP/cc-PVTZ level, blue basins indicated the position of radical susceptibility

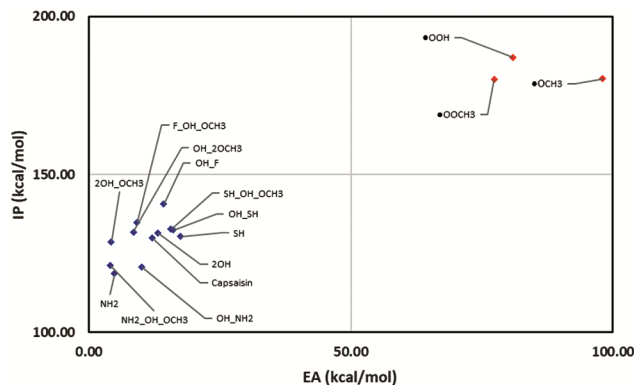


Fig. 5 — Full electron donor acceptor map (FEDAM) of Capsaicin and derivatives and radicals  $\bullet\text{OOH}$ ,  $\bullet\text{OOCH}_3$  and  $\bullet\text{OCH}_3$  as reference points

#### Full electron donor acceptor map

Reactivity indices estimated in this investigation were calculated to predict antioxidant behaviour *via* free radical scavenging activity. Global and local descriptors suggest that such activity involves single electron transfer and/or formal hydrogen atom transfer mechanisms. The full electron donor acceptor map (FEDAM)<sup>22</sup> is a graphical tool that allows to predict quickly and qualitatively, the direction of the flow of electrons on SET reactions (Fig. 5). This

useful tool is based on the precept that in SET reactions between two chemical species, that with the lower IE will be the electron donor, and that with the higher IE will be the electron acceptor, described graphically in Fig. 3. Some radicals ( $\bullet\text{OOH}$ ,  $\bullet\text{OOCH}_3$  and  $\bullet\text{OCH}_3$ ) were considered in the construction of FEDAM, as well as capsaicin itself to be compared with their analogues. As can be seen in Fig. 3, electron flow goes from the lower left corner, region in which all proposed structures are located to the upper right corner which corresponds to the region of free radicals used as reference point. Hence, it is expected that capsaicin analogues act as radical scavengers, at least *via* SET, of which  $\text{NH}_2$ ,  $\text{NH}_2\text{OH}_2\text{OCH}_3$ ,  $2\text{OH}_2\text{OCH}_3$  and  $\text{OH}_2\text{NH}_2$  are the structures with the best performance.

#### Preferred scavenging pathway

At this point, all global and local descriptors suggest that HA and SET-PT are the preferred scavenging pathways, but SPET pathway remains unexplored. Hence, BDE, IP, PPA and ETE parameters were analysed and compared between systems in aqueous media. As can see in Table 2, due to the lower energies of BDE, in all structures HA is

Table 2 — Bond Dissociation Energy (BDE), Ionization Potential (IP), Phenol Proton Affinity (PPA) and Electron Transfer Energy (ETE) in kcal/mol at the B3LYP/cc-PVTZ and SMD implicit solvent correction with water as solvent

	BDE	IP	PPA	ETE
SH	82.47	130.43	300.07	94.01
NH <sub>2</sub> -OH-OCH <sub>3</sub>	88.81	121.19	308.36	92.06
2OH	89.47	128.56	304.46	96.63
OH-NH <sub>2</sub>	90.15	120.75	306.12	95.65
OH-2OCH <sub>3</sub>	93.64	131.75	307.52	97.74
2OH-OCH <sub>3</sub>	94.07	129.88	305.26	100.42
F-OH-OCH <sub>3</sub>	94.34	134.79	304.97	100.98
Capsaicin	94.43	131.49	305.25	100.79
OH-SH	94.73	132.37	304.44	101.91
SH-OH-OCH <sub>3</sub>	95.12	132.61	305.14	101.60
OH-F	97.03	140.56	303.19	105.45
NH <sub>2</sub>	98.88	118.62	333.11	77.38

the preferred pathway in aqueous media, follow by SET-PT with energy differences of 19 – 48 kcal/mol, which reduces its probability of occurrence. Due to the large PPA values, SPET pathway is by and large the pathway with the low probability to take place for radical scavenging activity in all presented structures. Furthermore, the total energy requirement for SPET pathway can be estimated as the sum of PPA and ETE, value increases to 410 kcal/mol in the case of the higher barrier. In any case, SH is the structure with the lower energy requirements for HA pathway, 12 kcal/mol lower than Capsaicin.

**Conclusion**

Radical scavenging activity and preferred pathway has been analysed for several proposed structures, by means of global and local descriptors, as well as BDE, IP and PPA energies. Results indicate a better performance as radical scavenging structures for amine and thiol derivative structures, SH being the best structure of all those presented in this work. Furthermore, the preference for HA pathway is evidence due to its low energy barriers with respect to SET-PT and SPET mechanism.

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