Copper or free radical scavenger?

Ana Martínez *, Miguel Reina

Departamento de Materiales de Baja Dimensionalidad, Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior S.N., Ciudad Universitaria, P.O. Box 70-360, Coyoacán C.P. 04510, Ciudad de México, México

ARTICLE INFO

ABSTRACT

Free radicals are very reactive molecules that produce so-called oxidative stress. To prevent oxidative stress there are two types of molecules: primary and secondary antioxidants. The chelation of copper is an example of a secondary antioxidant process. Therefore, these secondary antioxidants can be named *copper scavengers*. The main purpose of this investigation is to compare the capacity of four molecules: silybin (SIL); astaxanthin (ASTA); the hormone melatonin (MELA) and one of the metabolites of MELA named cyclic 3-hydroxymelatonin (3OHM) to scavenge copper, instead of a free radical. In order to decide whether these molecules are predominantly free radical scavengers rather than *copper scavengers*, we studied their primary antioxidant capacity analyzing their interaction with ·OH and two mechanisms: Radical Adduct Formation (RAF) and Hydrogen Atom Transfer (HAT). In order to study *copper scavenger* capacity, we investigated the formation of chelate complexes with Cu(II) by these four molecules and their interaction with Cu(I). In the presence of Cu ions and free radicals, SIL, ASTA, MELA and 3OHM preferentially react with ·OH. These molecules are able to act as *copper scavengers* but not in the presence of ·OH and therefore play a limited role as chelating agents.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Reactive oxygen species (ROS) are very well-known molecules that are formed during metabolism and play important roles in cell signaling [1]. These species are very reactive free radicals that can bond to biomolecules and cause a wide variety of diseases [2–13]. ROS levels in the human body can increase dramatically due to stress, UV radiation, the presence of metal ions or many others factors, such as heat exposure [14–16]. Excess of ROS produces so-called oxidative stress; a harmful phenomenon caused by an imbalance between the production and consumption of free radicals, which correlates with numerous illnesses [10–24].

Molecules that scavenge free radicals prevent oxidative stress. These molecules are also known as antioxidants. They have been studied extensively, because they show several positive effects on human health [17–24]. Two processes are involved in scavenging free radicals to prevent oxidative stress [25]: direct reaction with ROS (primary free radical scavengers) or reaction with species that generate free radicals preventing the production of ROS (secondary antioxidants). Chelation of metal atoms (such as copper) is one of the processes undergone by secondary antioxidants and the

importance of this mechanism for human health has been demonstrated [26–33].

Copper is one of the most important trace metals in the human body. On average, the adult liver contains from 18 to 45 µg Cu/g dry weight, while blood contains about 6 mg of Cu [34,35]. As is the case with almost all substances, it can be either beneficial or toxic depending on its concentration [36]. Copper is commonly found as Cu(II) or Cu(I) and the ionic form can be interconverted by redox reactions.

Copper produces hydroxyl radicals in the Haber-Weiss reaction, as shown in the following [37,38]:

$$Cu(II) + O_2^{\cdot-} \rightarrow Cu(I) + O_2$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + OH^- + \cdot OH$$
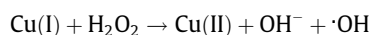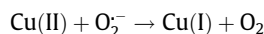
A small amount of copper is capable of producing a large amount of ·OH free radicals as Cu(II) and Cu(I) act as catalysts in the reaction. Secondary antioxidants react with copper forming chelates, thus preventing the formation of free radicals. In this sense, these molecules can be labeled as *copper scavengers*. In fact, the same molecule can act either as a antioxidant and/or a *copper scavenger*. Many studies analyze one or other of these roles and reach conclusions concerning primary or secondary antioxidant capacity [27,28,39–42]. Copper and free radicals are both present in the human body and may compete to react with the scavenger.

* Corresponding author.
  E-mail address: martina@unam.mx (A. Martínez).

A single scavenger may trap free radicals or copper ions, depending on thermodynamics and kinetics. It is thus very important to compare these two mechanisms, although few investigations attempt this [28,39,43].

The main purpose of this investigation is to compare the capacity for scavenging copper with that of scavenging free radicals, on the part of four molecules: silybin (SIL); astaxanthin (ASTA); the hormone melatonin (MELA) and one of the metabolites of MELA named cyclic 3-hydroxymelatonin (3OHM), also reported as a good scavenger [43]. Schematic representation of these molecules is included in Fig. 1. The idea is to compare four different substances: SIL is a dietary supplement; ASTA is present in human food; MELA and 3OHM are human hormones.

SIL is the most abundant component of silymarin, the active substance of milk thistle plant (*Silybum marianum*) [44,45]. Protective action on the part of SIL is thought to be derived from its antioxidant properties and theoretical and experimental evidence suggests that SIL is a primary antioxidant by different mechanisms, such as the sequential electron transfer (SET) or the single proton loss electron transfer (SPLET) [44–66]. Likewise experimental reports point to the secondary antioxidant capacity of SIL by chelating Cu(II) but theoretical investigations are scarce [41]. ASTA is found in food (fruits, vegetables and salmon for example) and it forms M-O bonds with metal ions, including Cu(II) [27]. It is a carotenoid that was previously reported as a good free radical scavenger molecule [27,39,67–69]. MELA in humans is produced by the pineal gland. It helps to regulate sleep and wake cycles. 3OHM is one of its metabolites. Both molecules were reported as good *copper scavengers* and also as good free radical scavengers [28,43,70].

In order to decide whether these molecules have greater tendency to act as free radical scavengers rather than *copper scavengers*, we studied their primary free radical scavenger capacity, analyzing the interaction with ·OH and employing two mechanisms: the Radical Adduct Formation (RAF) and the Hydrogen Atom Transfer (HAT). ·OH was selected because it is one of the most reactive free radicals and because it is produced by Cu(II) in the Haber-Weiss reaction. *Copper scavenger* capacity was assessed by investigating the formation of chelate complexes by these four molecules (SIL, ASTA, MELA and 3OHM) with Cu(II) and also the interaction with Cu(I). Some of these results have been reported previously [27,28,43], however for consistency and for comparative purposes, we recalculated the most stable structures. Our most important conclusion indicates that in the presence of Cu ions and free radicals, SIL, ASTA, MELA and 3OHM preferentially react with ·OH. Consequently it was impossible for these molecules to act as *copper scavengers* in the presence of ·OH, thus limiting their role as chelating agents. A single scavenger may trap free radicals or copper ions, depending on thermodynamics. In this sense, it is imperative in future works to compare these two mechanisms because free radicals and copper ions are both present.

2. Computational details

Gaussian 09 code [71] was implemented to optimize all structures. Initial geometries were fully optimized at M06/6-31+G(d,p) level of theory [72–76] in conjunction with SMD continuum model using water to mimic a polar environment [77]. This methodology has been successfully used to study small organic molecules interacting with metal atoms [28,30]. Harmonic analyses were calculated to verify local minima (zero imaginary frequencies). In Fig. 1 we present a schematic representation of SIL with the conventional numbering of —OH and —OCH$_3$ groups and labeling the aromatic and heterocyclic rings for future reference. To investigate the chelation of Cu with the four molecules under study, two possible mechanisms reported before [28] are analyzed: the direct chelation and the coupled deprotonation-chelation. In the first one, SIL, ASTA, MELA and 3OHM form chelates with Cu(II) and bonded with Cu(I). In the second one, all the molecules are first deprotonated and then chelate to Cu(II) or bonded with Cu(I). For the direct chelation mechanism, the Gibbs free energies (ΔG) are calculated as follows:
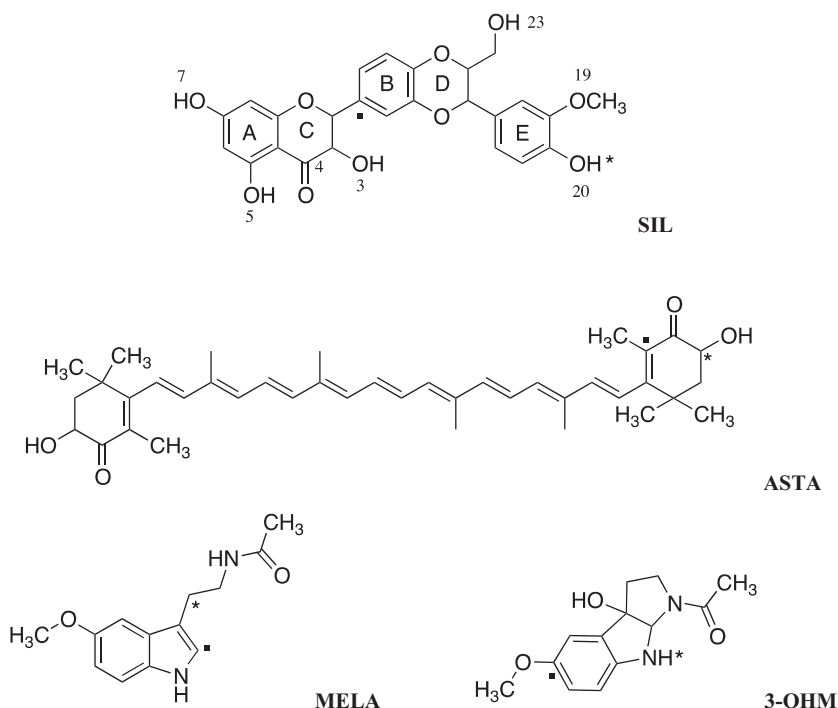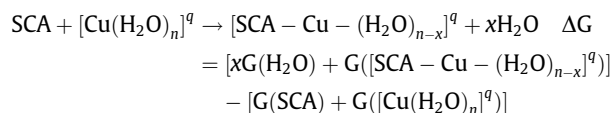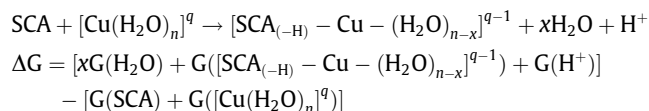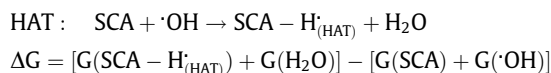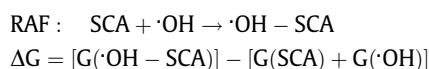

Fig. 1. Schematic representation of silybin (SIL), astaxanthin (ASTA), melatonine (MELA) and cyclic 3-hydroxymelatonin (3OHM). Bullet (■) indicates the preferred reaction site for RAF mechanism. Star (*) indicates the hydrogen atom that was transferred in the case of the HAT mechanism.

$$SCA + [Cu(H_2O)_n]^q \rightarrow [SCA - Cu - (H_2O)_{n-x}]^q + xH_2O \quad \Delta G$$
$$= [xG(H_2O) + G([SCA - Cu - (H_2O)_{n-x})^q)]$$
$$- [G(SCA) + G([Cu(H_2O)_n]^q)]$$

SCA is the scavenger, i.e. SIL, ASTA, MELA or 3OHM; $[Cu(H_2O)_n]^q$ represents copper ions coordinated to water molecules. For Cu(II), we modeled the complex with $n = 4$ (four water molecules since four-coordinate configuration is favored for Cu(II)) and for Cu(I) we employed $n = 2$ (two water molecules since two-coordinate configuration is favored for Cu(I)); q represents the charge of the system and it is +2 and +1, for Cu(II) and Cu(I), respectively. For coupled deprotonation-chelation mechanism, the Gibbs free energies (ΔG) are obtained with the next reaction scheme:

$$SCA + [Cu(H_2O)_n]^q \rightarrow [SCA_{(-H)} - Cu - (H_2O)_{n-x}]^{q-1} + xH_2O + H^+$$
$$\Delta G = [xG(H_2O) + G([SCA_{(-H)} - Cu - (H_2O)_{n-x}]^{q-1}) + G(H^+)]$$
$$- [G(SCA) + G([Cu(H_2O)_n]^q)]$$

SCA is the scavenger, i.e. SIL, ASTA, MELA or 3OHM; $SCA_{(-H)}$ is de-protonated SCA. In this equation q is equal to +1 or 0, for Cu(II) and Cu(I) respectively. As suggest by Camaioni et al. [78], the proton Gibbs free energy in aqueous solution used in this work is $G(H^+) = -270.28$ kcal/mol. In order to investigate RAF and HAT mechanisms, the following equations were used:

$$RAF: \quad SCA + {}^\cdot OH \rightarrow {}^\cdot OH - SCA$$
$$\Delta G = [G({}^\cdot OH - SCA)] - [G(SCA) + G({}^\cdot OH)]$$

$$HAT: \quad SCA + {}^\cdot OH \rightarrow SCA - H^\cdot_{(HAT)} + H_2O$$
$$\Delta G = [G(SCA - H^\cdot_{(HAT)}) + G(H_2O)] - [G(SCA) + G({}^\cdot OH)]$$

SCA is the scavenger, i.e. SIL, ASTA, MELA or 3OHM; $SCA-H^\cdot_{(HAT)}$ is de-hydrogenated SCA.

3. Results and discussion

3.1. Geometry optimization

Initial geometries used for SIL-Cu(II) are those that can form chelates. Cu(II) is tetra-coordinate and for this reason SIL-Cu(II) complex has two water molecules. In SIL there are three possible positions for the chelation process: O4-O3, O4-O5 and O19-O20 (see Fig. 1). Fig. 2 presents the optimized structures of SIL-Cu(II) and the relative energy with respect to the most stable. In this case, we are analyzing direct chelation mechanism. Evidently, the most stable structure presents Cu(II) bonded to O3 and O4. The least stable structure is 6.6 kcal/mol higher in energy, and shows Cu(II) bonded to O19-O20. This latter structure has one hydrogen
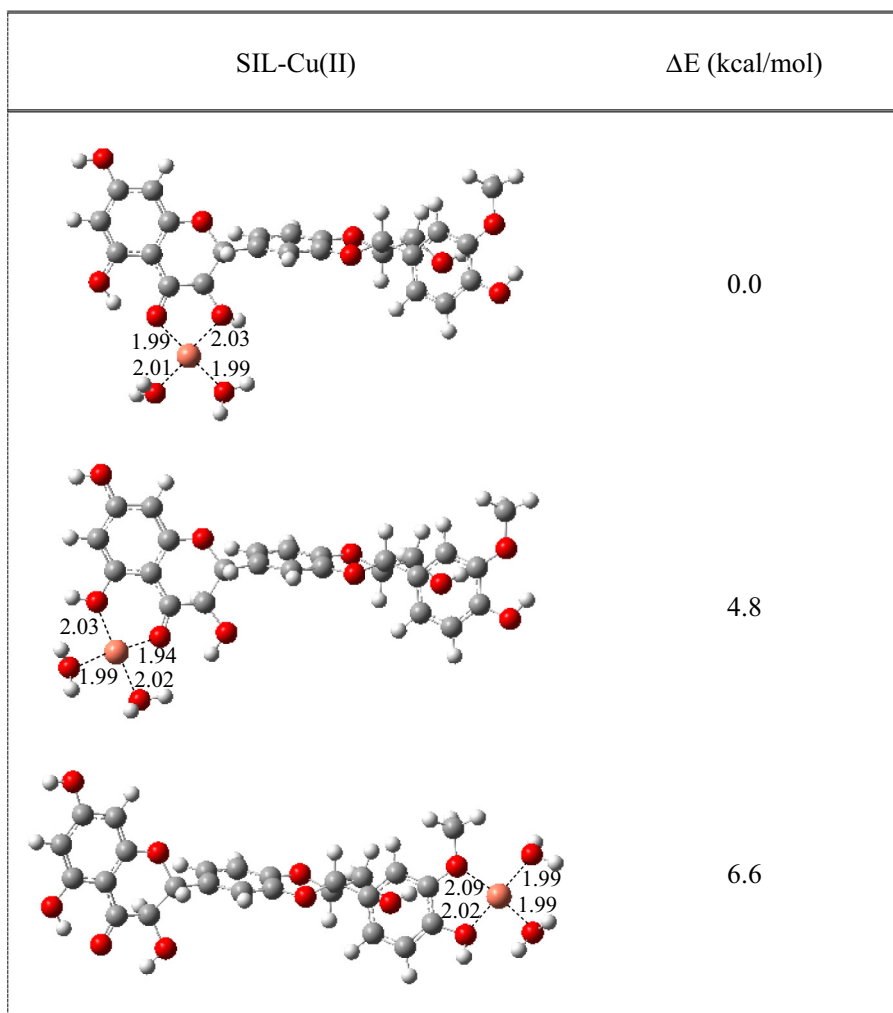

SIL-Cu(II)	ΔE (kcal/mol)
	0.0
	4.8
	6.6

Fig. 2. Optimized structures of SIL interacting with Cu(II). The global charge of the molecules is +2. There are two water molecules bonded to the copper atom. Relative energies with respect to the most stable structure are included.

bond less than the other two in Fig. 2, a factor that contributes to destabilization. As reported previously, this may be due to the importance of the different intramolecular hydrogen bonds present in the molecule [56,57]. Because the energy difference is not very large, we expect these to form under experimental conditions. Fig. 3 presents the most stable structures for $SIL_{(-H)}$-Cu(II) resulting from the coupled-deprotonation-chelation mechanism. The stability of the structures is similar to previous results shown in Fig. 2. The most stable structure is where Cu(II) forms a chelate with O4-O5. This structure has been experimentally reported as the most stable example of a silybin derivative [42]. The structure interacting with O3-O4 is the least stable. The preferred chelation site is pH dependent because deprotonated SIL is present at high pH values and therefore, the chelation site changes from O3-O4 to O4-O5. This is related to the stabilization of the different intramolecular hydrogen bonds in SIL. Distances between the metal atom and SIL are slightly shorter employing the coupled deprotonation-chelation mechanism, than they are employing the direct mechanism.

The optimized structures presented in Fig. 4 represent the direct chelation mechanism with Cu(I). We obtained four stable geometries for structures corresponding to SIL-Cu(I). In all cases, Cu(I) was simulated in a linear two-coordination geometry. The ground state presents a water molecule formed from one hydrogen bond with one oxygen atom of SIL, with Cu forming a bridge between two oxygen atoms. All the structures present similar stability and may be present under experimental conditions. In Fig. 5, the optimized structures for the coupled deprotonation-chelation process with Cu(I) are shown. The coupled deprotonation-chelation mechanism suggests that one proton is dissociated and one Cu atom is bonded at the same position where this dissociation takes place. The most stable geometry presents Cu(I) bonded to O7. The hydrogen at position O7 is the most acid and therefore the dissociation of this proton is more favorable than the dissociation of other protons. The structures in Fig. 5 show minimal variation in terms of stability and therefore it is no surprise to find all these in an experiment. Notably, in the case of Cu(II), we did not report the structure derived from the deprotonation of a proton bonded to O7, although this is the most acid, as it results in a less stable structure for Cu(II). It shows a greater tendency to form bidentate compounds than to bond with a single oxygen atom that is de-protonated.

The optimized structures of ASTA interacting with Cu ions are reported in Fig. 6. The structures for ASTA-Cu(II) forming a chelate with two oxygen atoms have been reported previously [27]. For consistency, we recalculated these geometries, applying methodology from this investigation. Cu(II) is tetra-coordinate because it has two water molecules. For Cu(I), we consider a linear two-coordination geometry. For the coupled deprotonation-chelation process, one of the OH groups is deprotonated and Cu(II) or Cu(I)
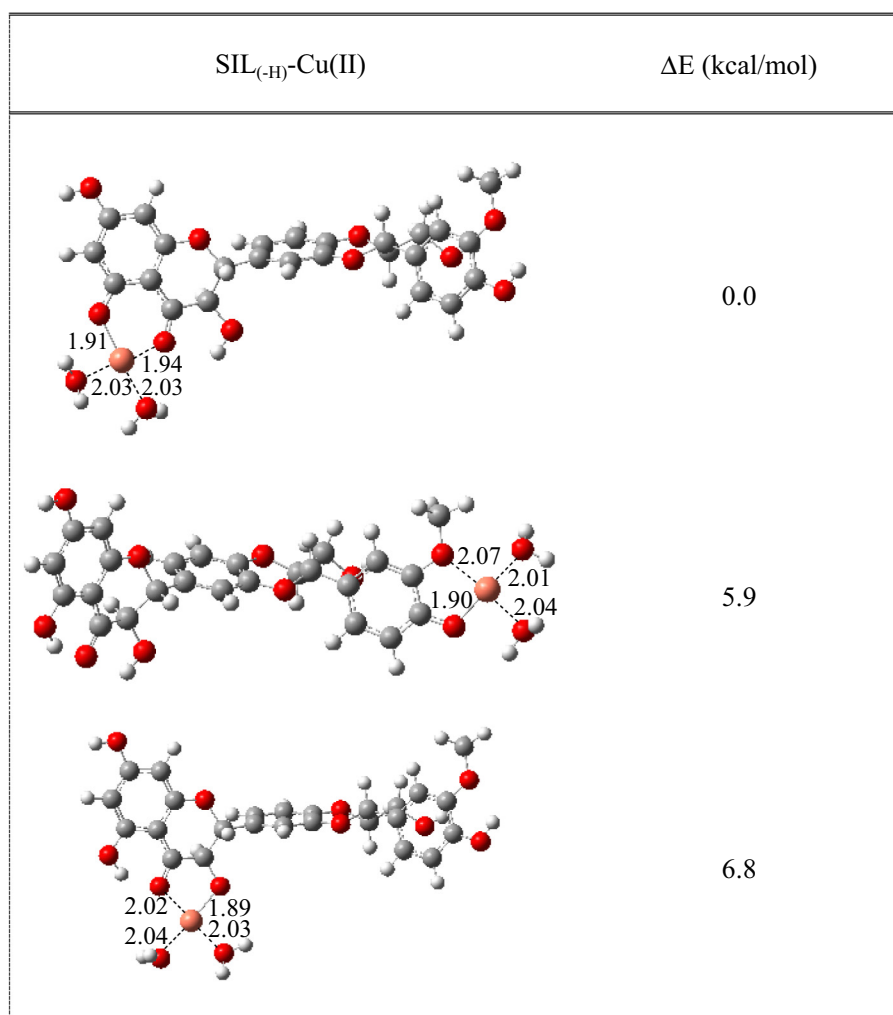

Fig. 3. Optimized structures of deprotonated SIL ($SIL_{(-H)}$) interacting with Cu(II). The global charge of the molecules is +1. There are two water molecules bonded to the copper atom. Relative energies with respect to the most stable structure are included.
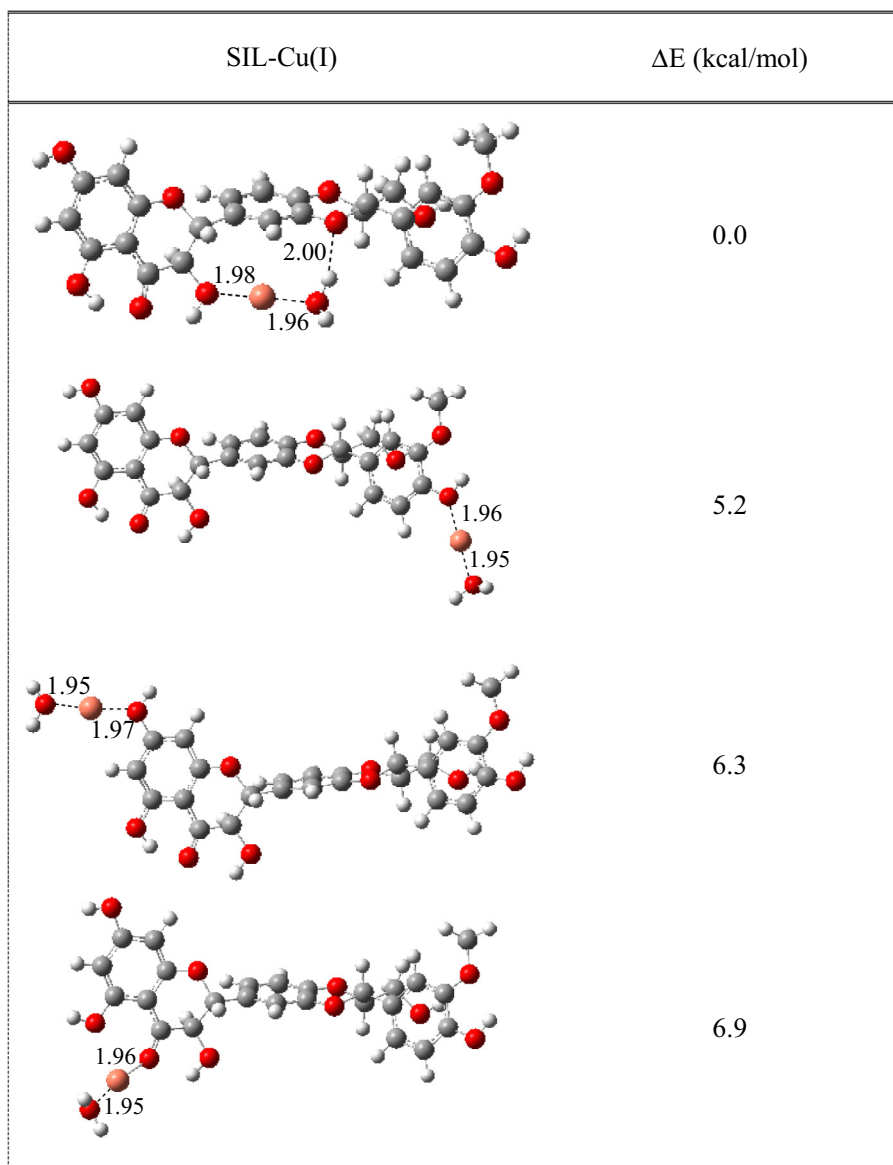
Fig. 4. Optimized structures of SIL interacting with Cu(I). The global charge of the molecules is +1. There is one water molecule bonded to the copper atom. Relative energies with respect to the most stable structure are included.

is bonded to that position. ASTA may simultaneously chelate two Cu atoms, as it has two oxygen atoms; one at each extreme of the molecule. However, in this investigation we will consider only one metal atom in order to facilitate direct comparison with the other molecules.

Figs. 7 and 8 present the optimized structures of MELA and 3OHM, respectively. Molecules are interacting with Cu(II) and Cu(I). These structures were obtained previously, using another methodology [28] and were deemed to be the most stable. For consistency, we have recalculated these geometries applying methodology from this investigation. Our results are similar to those reported previously.

Optimized structures of the RAF and HAT mechanisms are included as Supplementary Material.

3.2. Copper scavengers

Table 1 presents the Gibbs free energies (ΔG) associated with the direct chelation process involving Cu(II) and Cu(I). Only the

most stable structures are considered. Some previously reported values are also included. Evidently, results are similar to those obtained before, using other methodology. With Cu(II) all values are negative and therefore the reaction is exergonic. The reaction of ASTA and 3OHM with Cu(II) is more exergonic than the corresponding reaction with SIL and MELA. Therefore, ASTA and 3OHM are the best Cu(II) scavengers. In the case of Cu(I), the reaction with SIL has a very small negative value of ΔG and with ASTA it is small and positive. MELA and 3OHM present negative ΔG values with Cu(I). According to these results, SIL and ASTA are good Cu(II) scavengers but are not good secondary antioxidants, if the reaction with Cu(I) is considered. This is an important finding because Cu(II) is the most abundant copper ion in the human body. MELA and 3OHM are good *copper scavengers* for both, Cu(II) and Cu(I) but the reactions are not very exergonic. Apparently, MELA and 3OHM are better able to sequester Cu(I) than SIL and ASTA.

The Gibbs free energies for the coupled deprotonation-chelation process are reported in Table 2. This mechanism depends indirectly on the dissociation energy of H+. Some previously reported values
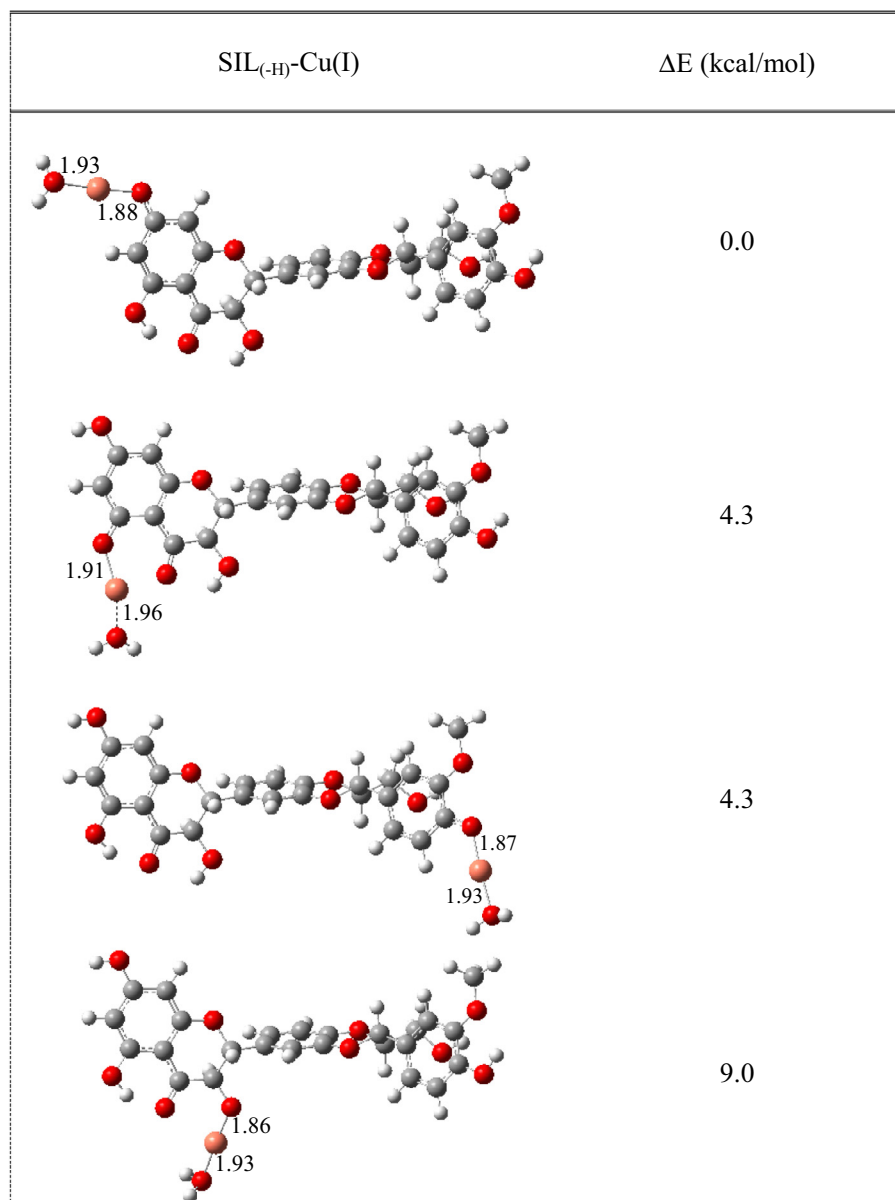
Fig. 5. Optimized structures of de-protonated SIL (SIL$_{(-H)}$) interacting with Cu(I). The global charge of the molecules is 0. There is one water molecule bonded to the copper atom. Relative energies with respect to the most stable structure are included.

are also included. It is apparent that results are similar to those obtained before, using other methodology. Firstly, it is notable that all reactions with Cu(I) are endergonic and therefore not thermodynamically feasible. However, reactions with Cu(II) are all exergonic with the exception of MELA$_{(-H)}$. Values reported in Table 2 indicate that 3OHM$_{(-H)}$ and SIL$_{(-H)}$ are better Cu(II) scavengers than ASTA$_{(-H)}$ with respect to the coupled-deprotonated-chelation mechanism, and MELA$_{(-H)}$ will not scavenge Cu(II) because this reaction is endergonic.

If we compare values from Table 1 with those from Table 2, it is apparent that the coupled deprotonated-chelation mechanism is the main reaction pathway involved in the copper sequestering action of SIL, but it is not the case for 3OHM, ASTA and MELA. For ASTA, the direct chelation mechanism is more exergonic than the coupled deprotonated-chelation mechanism. For 3OHM, both mechanisms have almost the same Gibbs free energy values, and for MELA the direct chelation mechanism is exergonic, whereas the coupled deprotonated-chelating mechanism is not.

In summary and considering both chelating mechanisms (direct and coupled deprotonation), SIL, ASTA and 3OHM are better scavengers for Cu(II) than MELA. Concerning Cu(I), reactions with SIL and ASTA are not favorable, and although possible with MELA and 3OHM, this only occurs by means of the direct chelation mechanism.

3.3. Free radial scavengers. RAF and HAT mechanisms

Tables 3 and 4 report the ΔG values for the RAF and the HAT processes, respectively. Some previously reported results are also included in brackets. Our results are similar in the sense that the reactions are exergonic or endergonic, irrespective of method. Results of ASTA show the biggest difference. In this molecule, the difference in optimized geometries is also the largest. As expected, all reactions with ˙OH are exergonic. The Gibbs free energies for both mechanisms indicate that the reactions of MELA and 3OHM
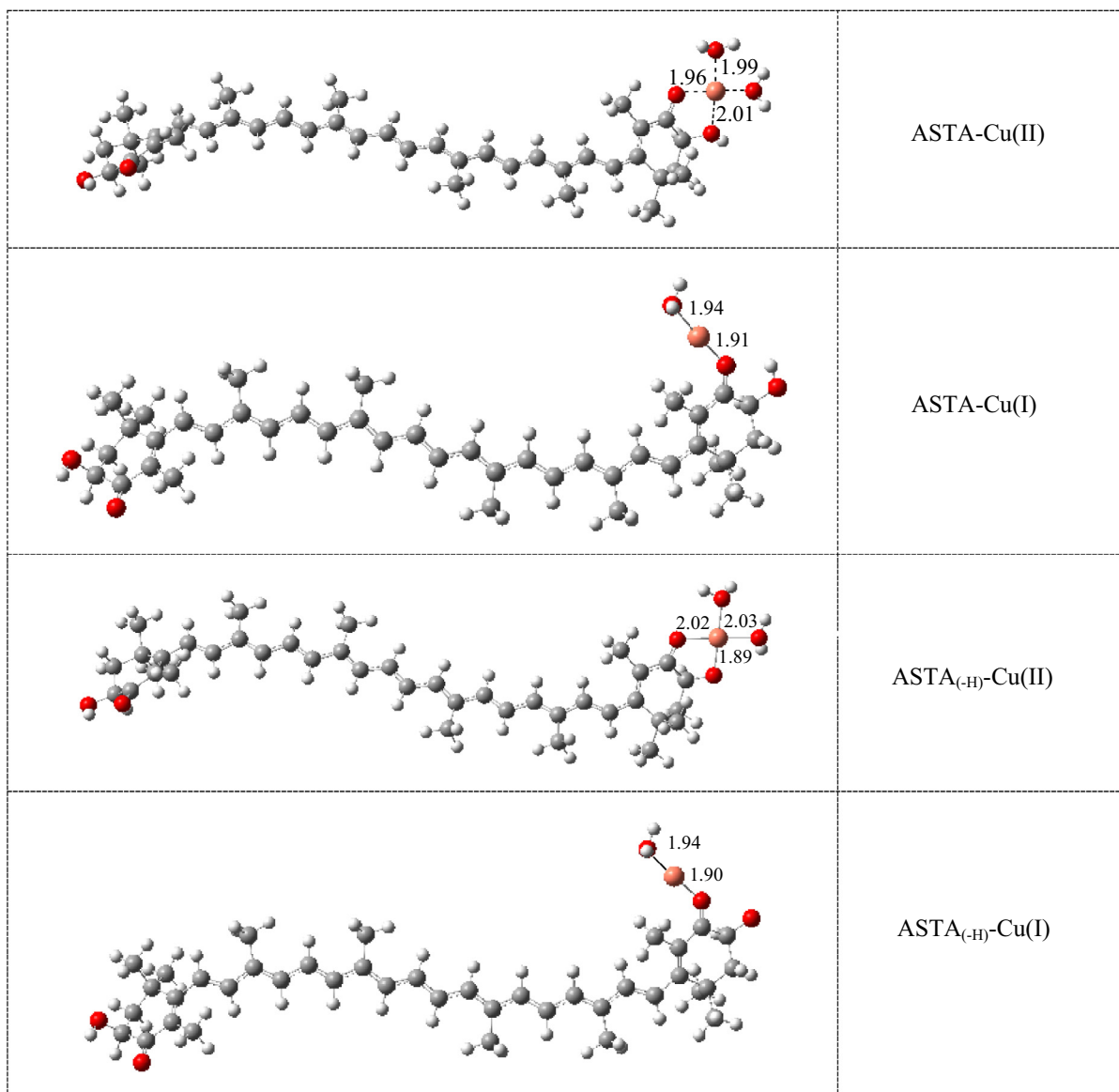
Fig. 6. Optimized structures of ASTA and de-protonated ASTA (ASTA$_{(-H)}$) interacting with Cu(II) and Cu(I).

are more exergonic than the reactions with SIL and ASTA. The preferred mechanism for all systems is HAT.

3.4. Copper or free radical scavengers?

This investigation's main objective is to decide whether chelating molecules effectively diminish oxidative stress by sequestering copper in the presence of free radicals. Molecules may either chelate copper ions or scavenge free radicals. To assess which mechanism prevails according to thermodynamics, we compare the results in Tables 1–4. In each case, we selected the most exergonic reactions, assuming that the scavenger will be in contact with both, copper and ·OH free radical.

For SIL as *copper scavenger*, the main mechanism is the coupled deprotonation-chelation (ΔG value is −13.61 kcal/mol). The preferred *free radical scavenger* mechanism is HAT with ·OH. The latter is more exergonic (ΔG value is −22.14 kcal/mol) than the former. Therefore, in the presence of both (Cu(II) and ·OH), SIL will preferably act as a *free radical scavenger*. Similar results are found for ASTA. To sequester Cu(II), the reaction of the direct chelation mechanism is exergonic (ΔG value is −10.35 kcal/mol), but the

reaction to scavenge ·OH is more exergonic (ΔG equal to −27.77 kcal/mol). For MELA and 3OHM the comparison is even clearer. MELA as *copper scavenger* reacts with Cu(II) through the direct chelation mechanism but ΔG value is nine nine times smaller than the ΔG value of the HAT reaction with ·OH (−4.04 *versus* −36.22 kcal/mol). Finally, the same conclusion is reached with 3OHM. The ΔG value for the chelating mechanism is almost four times smaller than the ΔG value of the HAT reaction with ·OH (−12.52 *versus* −45.26 kcal/mol). Moreover, one of the most reactive free radicals is OH·. It can be expected that studied compounds will react faster with this free radical than with copper cation. Therefore, it will be difficult to scavenge copper in the presence of OH· [81]. In summary, none of the studied molecules will be a good *copper scavenger* in the presence of ·OH. Scavengers can work on Cu before ·OH is formed.

4. Concluding remarks

The reactions of SIL and ASTA with Cu(II) are exergonic but they are endergonic if the reaction with Cu(I) is considered. MELA and 3OHM are good *copper scavengers* for both, Cu(II) and Cu(I) but
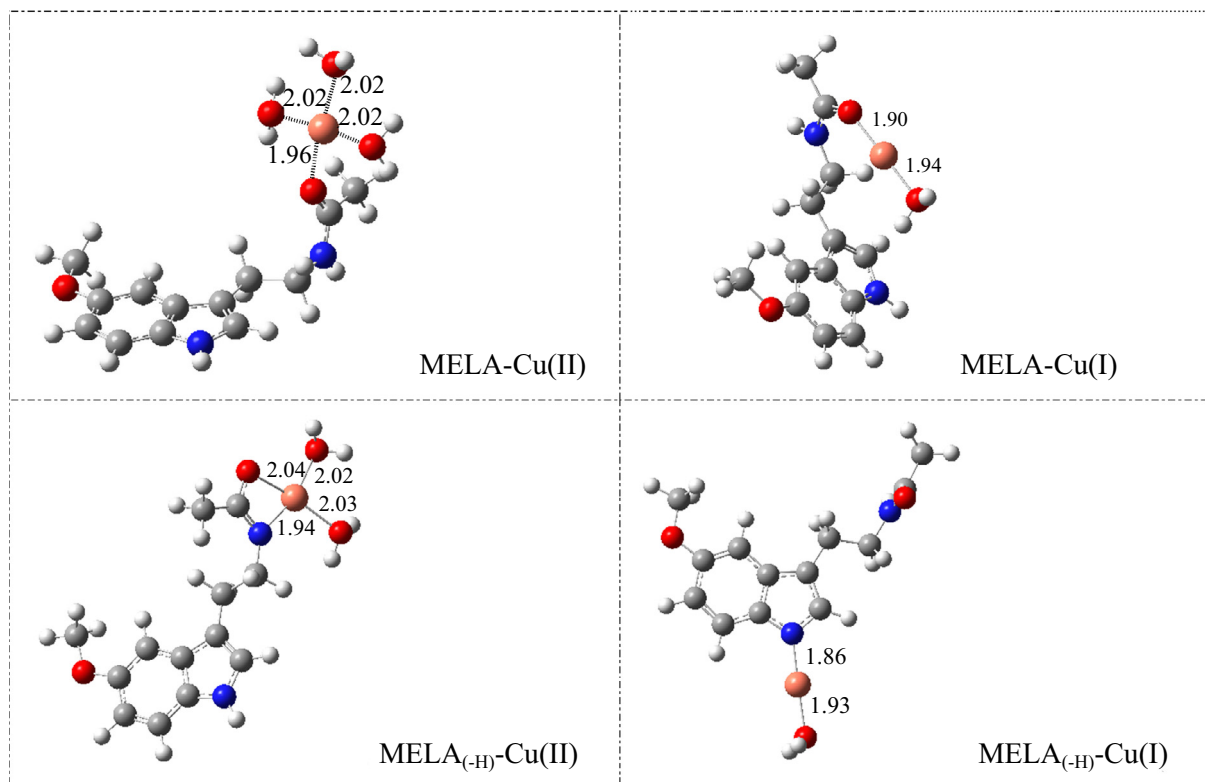
Fig. 7. Optimized structures of MELA and de-protonated MELA (MELA$_{(-H)}$) interacting with Cu(II) and Cu(I).
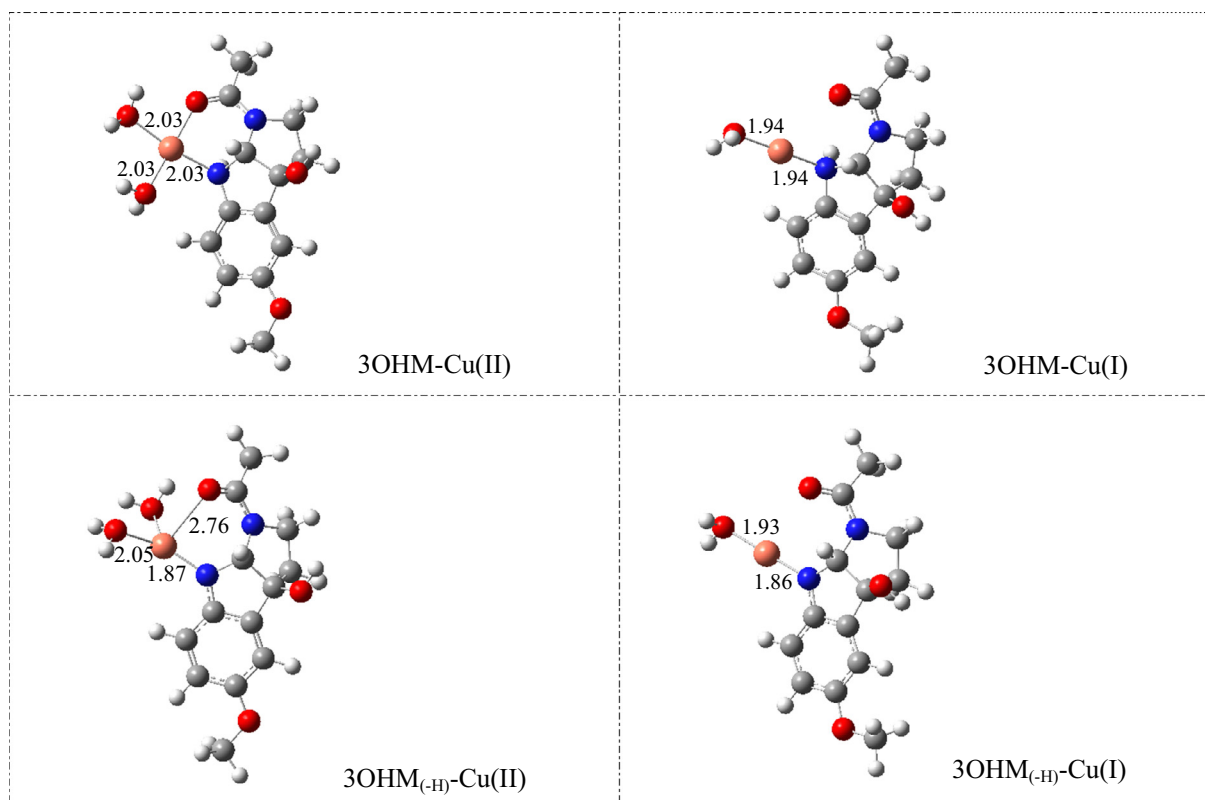

Fig. 8. Optimized structures of 3OHM and de-protonated 3OHM (3OH$_{(-H)}$) interacting with Cu(II) and Cu(I).

Table 1
Values are ΔG (Gibbs free energies of reaction) for the direct chelation mechanism (in kcal/mol at 298.15 K). Values in brackets form Ref. [28].

SIL-Cu(II)	SIL-Cu(I)
−8.15	−0.59
ASTA-Cu(II)	ASTA-Cu(I)
−10.35	0.51
MELA-Cu(II)	MELA-Cu(I)
−4.04 (−2.49)	−2.31 (−3.64)
3OHM-Cu(II)	3OHM-Cu(I)
−12.52 (−7.06)	−6.41 (−5.18)

Table 2
Values are ΔG (Gibbs free energies of reaction) for the coupled deprotonation-chelation process (in kcal/mol at 298.15 K). Values in brackets form Ref. [28].

SIL$_{(-H)}$-Cu(II)	SIL$_{(-H)}$-Cu(I)
−13.61	3.71
ASTA$_{(-H)}$-Cu(II)	ASTA$_{(-H)}$-Cu(I)
−8.06	29.56
MELA$_{(-H)}$-Cu(II)	MELA$_{(-H)}$-Cu(I)
2.76 (8.22)	10.31 (11.28)
3OHM$_{(-H)}$-Cu(II)	3OHM$_{(-H)}$-Cu(I)
−12.03 (−13.39)	12.24 (12.59)

Table 3
Values are ΔG (Gibbs free energies of reaction) for the RAF process (in kcal/mol at 298.15 K). Values in brackets form Refs. [43,79,80].

	SIL	ASTA	MELA	3OHM
·OH	−10.56	−15.16 (−32.43)	−23.97 (−25.7)	−20.73 (−19.11)

Table 4
Values are ΔG (Gibbs free energies of reaction) for the HAT process (in kcal/mol at 298.15 K). Values in brackets form Refs. [43,80].

	SIL	ASTA	MELA	3OHM
·OH	−22.14	−27.77	−36.22 (−31.40)	−45.26 (−35.76)

the reactions are not very exergonic. Apparently, MELA and 3OHM are better able to react with Cu(I) than SIL and ASTA.

As expected, all reactions with ·OH are exergonic. The Gibbs free energies for RAF and HAT mechanisms indicate that the reactions of MELA and 3OHM are more exergonic than the reactions with SIL and ASTA. The preferred mechanism for all systems is HAT.

SIL, ASTA, MELA and 3OHM can be either *copper scavengers* or *free radical scavengers* according to thermodynamics. In the human body, both copper and free radicals compete to react with the scavenger. Thermodynamics will determine whether a particular antioxidant will trap free radicals or copper ions. In this sense, the comparison of these two mechanisms is desirable because free radicals and copper ions are both present. For the molecules in this investigation and based on Gibbs free energies, it is possible to conclude that SIL, ASTA, MELA and 3OHM are better *scavengers of free radical* than they are of copper.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.comptc.2017.01.033.

References

[1] M.E. Sosa-Torres, J.P. Saucedo-Vázquez, P.M. Kroneck, The dark side of dioxygen, in: P.M. Kroneck, M.E. Torres (Eds.), Sustaining Life on Planet Earth: Metalloenzymes Mastering Dioxygen and Other Chewy Gases, Metal Ions in Life Sciences, vol. 15, Springer, 2015, pp. 1–12, http://dx.doi.org/10.1007/978-3-319-12415-5_1.

[2] S.K. Yang, L. Xiao, J. Li, F. Liu, L. Sun, Oxidative stress, a common molecular pathway for kidney disease: role of the redox enzyme p66Shc, Ren. Fail. 36 (2014) 313–320.

[3] G. Aruna, K. Ambika Devi, Oxidative stress in chronic renal failure, Int. J. Pharma Bio Sci. 5 (2014) B127–B133.

[4] M. Shadab, D.K. Agrawal, M. Aslam, N. Islam, Z. Ahmad, Occupational health hazards among sewage workers: oxidative stress and deranged lung functions, J. Clin. Diagn. Res. 8 (2014) BC11–BC13.

[5] N. Ghasemzadeh, R.S. Patel, D.J. Eapen, E. Veledar, H.A. Kassem, P. Manocha, M. Khayata, A.M. Zafari, L. Sperling, D.P. Jones, A.A. Quyyumi, Oxidative stress is associated with increased pulmonary artery systolic pressure in humans, Hypertension 63 (2014) 1270–1275.

[6] M. Veselinovic, N. Barudzic, M. Vuletic, V. Zivkovic, A. Tomic-Lucic, D. Djuric, V. Jakovljevic, Oxidative stress in rheumatoid arthritis patients: relationship to siseases activity, Mol. Cell. Biochem. 391 (2014) 225–232.

[7] S. Kundu, P. Ghosh, S. Datta, A. Ghosh, S. Chattopadhyay, M. Chatterjee, Oxidative stress as a potential biomarker for determining disease activity in patients with rheumatoid arthritis, Free Radical Res. 46 (2012) 1482–1489.

[8] J. Wang, J.Z. Li, A.X. Lu, K.F. Zhang, B.J. Li, Anticancer effect of salidroside on A549 lung cancer cells through inhibition of oxidative stress and Phospho-p38 expression, Oncol. Lett. 7 (2014) 1159–1164.

[9] S. Granados-Principal, N. El-Azem, R. Pamplona, C. Ramirez-Tortosa, M. Pulido-Moran, L. Vera-Ramirez, J.L. Quiles, P. Sanchez-Rovira, A. Naudí, M. Portero-Otin, P. Perez-Lopez, M. Ramirez-Tortosa, Hydroxytyrosol ameliorates oxidative stress and mitochondrial dysfunction in doxorubicin-induced cardiotoxicity in rats with breast cancer, Biochem. Pharmacol. 90 (2014) 25–33.

[10] M. Pohanka, Alzheimer's disease and oxidative stress: a review, Curr. Med. Chem. 21 (2014) 356–364.

[11] M.H. Yan, X. Wang, X. Zhu, Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease, Free Radical Biol. Med. 62 (2013) 90–101.

[12] B. Halliwell, Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment, Drugs Aging 18 (2001) 685–716.

[13] L.M. Sayre, G. Perry, M.A. Smith, Oxidative stress and neurotoxicity, Chem. Res. Toxicol. 21 (2008) 172–188.

[14] K. Michail, A. Baghdasarian, M. Narwaley, N. Aljuhani, A.G. Siraki, Scavenging of free-radical metabolites of aniline xenobiotics and drugs by amino acid derivatives: toxicological implications of radical-transfer reactions, Chem. Res. Toxicol. 26 (2013) 1872–1883.

[15] E. Vejerano, S. Lomnicki, B. Dellinger, Lifetime of combustion-generated environmentally persistent free radicals on Zn(II)O and other transition metal oxides, J. Environ. Monit. 14 (2012) 2803–2806.

[16] N. Valencia-Islas, A. Zambrano, J.L. Rojas, Ozone reactivity and free radical scavenging behavior of phenolic secondary metabolites in lichens exposed to chronic oxidant air pollution from Mexico City, J. Chem. Ecol. 33 (2007) 1619–1634.

[17] H. Parhiz, A. Roohbakhsh, F. Soltani, R. Rezaee, M. Iranshashi, Antioxidant and anti-inflammatory properties of citrus flavonoids hesperidin and hesperetin: an update review of their molecular mechanism and experimental models, Phytother. Res. 29 (2015) 323–331.

[18] P. Widsten, C.D. Cruz, G.C. Fletcher, M.A. Pajak, T.K. McGhie, Tannins and extracts of fruit byproducts: antibacterial activity against foodborne bacteria and antioxidant capacity, J. Agric. Food Chem. 62 (2014) 11146–11156.

[19] R.G. Panchal, S.P. Reid, J.P. Tran, A.A. Bergeron, J. Wells, K.P. Kota, J. Aman, S. Bavari, Identification of an antioxidant small molecule with broad-spectrum antiviral activity, Antivir. Res. 93 (2012) 23–29.

[20] C.C. Danta, P. Piplani, The discovery and development of new potential antioxidant agents for the treatment of neurodegenerative diseases, Expert Opin. Drug Discov. 9 (2014) 1205–1222.

[21] J. Tinkel, H. Hassanain, S.J. Khouri, Cardiovascular antioxidant therapy: a review of supplements, pharmacotherapies, and mechanisms, Cardiol. Rev. 20 (2012) 77–83.

[22] F.M.F. Roleira, E.J. Tavares-Da-Silva, C.L. Varela, S.C. Costa, T. Silva, J. Garrido, F. Borges, Plant derived and dietary phenolic antioxidants: anticancer properties, Food Chem. 83 (2015) 235–2358.

[23] G. Marrazzo, I. Barbagallo, F. Galvano, M. Malaguarnera, D. Gazzolo, A. Frigiola, N. D'Orazio, G. Li Volti, Role of dietary and endogenous antioxidants in diabetes, Crit. Rev. Food Sci. Nutr. 54 (2014) 1599–1616.

[24] A. Vysakh, M. Ratheesh, T.P. Rajmohanan, C. Pramod, S. Premlal, E. Girish kumar, P.I. Sibi, Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action, Int. Immunopharmacol. 20 (2014) 124–130.

[25] E.A. Decker, Antioxidant mechanisms, in: C.C. Akoh, D.B. Min (Eds.), Food Lipids: Chemistry, Nutrition, and Biotechnology, Marcel Dekker, New York, 2002.

[26] A. Martínez, Y. Romero, T. Castillo, M. Mascaró, I. López-Rull, N. Simões, F. Arcega-Cabrera, G. Gaxiola, A. Barbosa, The effect of copper on the color of shrimps:redder is not always healthier, PLoS ONE 9 (2014) e107673.

[27] E. Hernández-Marín, A. Barbosa, A. Martínez, The metal cation chelating capacity of astaxanthin. Does this have any influence on antiradical capacity?, Molecules 17 (2012) 1039–1054

[28] A. Galano, M.E. Medina, D.X. Tan, R.J. Reiter, Melatonin and its metabolites as copper chelating agents and their role in inhibiting oxidative stress: a physicochemical analysis, J. Pineal. Res. 58 (2015) 107–116.

[29] M. Leopoldini, N. Russo, S. Chiodo, M. Toscano, Iron chelation by the powerful antioxidant flavonoid quercetin, J. Agric. Food Chem. 54 (2006) 6343–6351.

[30] M. Reina, A. Martínez, How the presence of metal atoms and clusters can modify the properties of Silybin? A computational prediction, Comput. Theor. Chem. 1099 (2017) 174–184.

[31] A. Primikyri, G. Mazzone, C. Lekka, A.G. Tzakos, N. Russo, I.P. Gerothanassis, Understanding Zinc(II) chelation with quercetin and luteolin: a combined NMR and theoretical study, J. Phys. Chem. B 119 (2015) 83–95.

[32] J.C. García-Ramos, R. Galindo-Murillo, F. Cortés-Guzman, L. Ruiz-Azuara, Metal-based drug-DNA interactions, J. Mex. Chem. Soc. 57 (3) (2013) 245–259.

[33] M. Avelar, A. Martínez, Do Casiopeinas® prevent cancer disease by acting as antiradicals? A chemical reactivity study applying density functional theory, J. Mex. Chem. Soc. 56 (3) (2012) 250–256.

[34] D.G. Barceloux, Copper, J. Toxicol. Clin. Toxicol. 37 (1999) 217–237.

[35] M.C. Linder, L. Wooten, P. Cerveza, S. Cotton, R. Shulze, N. Lomeli, Copper transport, Am. J. Clin. Nutr. 67 (1998) 965S–971S.

[36] L.M. Gaetke, C.K. Chow, Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology 189 (2003) 147–163.

[37] J. Weinstein, B.H.J. Bielski, Kinetics of the interaction of H_2O and O_2^- radicals with hydrogen peroxide. The Haber-Weiss reaction, J. Am. Chem. Soc. 101 (1) (1979) 58–62.

[38] F. Haber, J. Weiss, The catalytic decomposition of hydrogen peroxide by iron salts, Proc. Roy. Lond. Ser. A 147 (1934) 332.

[39] A. Martínez, Astaxanthin interacting with metal clusters: free radical scavenger and photovoltaic materials, Theo. Chem. Acc. 135 (2016) 130.

[40] L. Mira, M.T. Fernandez, M. Santos, R. Rocha, M.H. Florencio, K.R. Jennings, Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity, Free Rad. Res. 36 (2002) 1199–1208.

[41] M. Zatloukalová, V. Křen, R. Gažák, M. Kubala, P. Trouillas, J. Ulrichová, J. Vacek, Electrochemical investigation of flavonolignans and study of their interactions with DNA in the presence of Cu(II), Bioelectrochemistry 82 (2011) 117–1124.

[42] J. Vacek, M. Zatloukalová, T. Desmier, V. Nezhodová, J. Hrbac, M. Kubala, V. Kren, J. Ulrichová, P. Trouillas, Antioxidant, metal-binding and DNA-damaging properties of flavonolignans: a joint experimental and computational highlight base don 7-galloylsilybin, Chem. Biol. Interact. 205 (2013) 173–180.

[43] A. Galano, D.X. Tan, R.J. Reiter, Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin, RSC Adv. 4 (2014) 5220–5227.

[44] H. Wagner, L. Hörhammer, R. Münster, On the chemistry of silymarin (silybin), the active principle of the fruits of *Silybum marianumv*(L) Gaertn. (*Carduus marianus* L.), Arzneimittelforschung 18 (1968) 688–696.

[45] D. Schuppan, J.D. Jia, B. Brinkhaus, E.G. Hahn, Herbal products for liver diseases: a therapeutic challenge for the new millennium, Hepatology 30 (1999) 1099–1104.

[46] M. Reina, A. Martínez, Is silybin the best free radical scavenger compound in silymarin?, J Phys. Chem. B 120 (2016) 4568–4579.

[47] P.F. Surai, Silymarin as a natural antioxidant: an overview of the current evidence and perspectives, Antioxidants 4 (2015) 204–247.

[48] L. Mira, M. Silva, C.F. Manso, Scavenging of reactive oxygen species by silybin dihemisuccinate, Biochem. Pharmacol. 48 (1994) 753–759.

[49] R. Gazák, A. Svobodová, J. Psotová, P. Sedmera, V. Prikrylová, D. Walterová, V. Kren, Oxidised derivatives of silybin and their antiradical and antioxidant activity, Bioorg. Med. Chem. 12 (2004) 5677–5687.

[50] A. Jain, N. Dwivedi, R. Bhargava, S.J.S. Flora, Silymarin and naringenin protects nicotine induced oxidative stress in young rats, Oxidants Antioxidants Med. Sci. 1 (2012) 41–49.

[51] C.C. Winterbourn, Reconciling the chemistry and biology of reactive oxygen species, Nat. Chem. Biol. 4 (2008) 278–286.

[52] T.C. Bai, J.J. Zhu, J. Hu, H.L. Zhang, C.G. Huang, Solubility of silybin in aqueous hydrochloric acid solution, Fluid Phase Equilib. 254 (2007) 204–210.

[53] I. György, S. Antus, A. Blázovics, G. Földiák, Substituent effects in the free radical reactions of silybin: radiation-induced oxidation of the flavonoid at neutral pH, Int. J. Rad. Biol. 61 (1992) 603–609.

[54] E. van Wenum, R. Jurczakowski, G. Litwinienki, Media effects on the mechanism of antioxidant action of silybin and 2,3-dehydrosilybin: role of the enol groups, J. Org. Chem. 78 (2013) 9102–9112.

[55] K. Lemańska, H. Szymusiak, B. Tyrakowska, T. Zieliski, A.E.M. Soffers, I.M.C. Rietjens, The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxylflavones, Free Rad. Biol. Med. 31 (2001) 869–881.

[56] P. Trouillas, P. Marsal, A. Svobodová, J. Vostálová, R. Gazak, J. Hrbác, P. Sedmera, V. Kren, R. Lazzaroni, J.L. Duroux, D. Walterová, Mechanism of the antioxidant action of silybin and 2,3-dehydrosilybin flavonolignans: a joint experimental and theoretical study, J. Phys. Chem. A 112 (2008) 1054–1063.

[57] M. Reina, A. Martínez, Silybin and 2,3-dehydrosilybin flavonolignans as free radical scavengers, J. Phys. Chem. B 119 (2015) 11597–11606.

[58] S. Fan, M. Qi, Y. Yu, L. Li, G. Yao, S. Tashiro, S. Onodera, T. Ikejima, P53 activation plays a crucial role in silibinin induced ROS generation via PUMA and JNK, Free Radical Res. 46 (2012) 310–319.

[59] A. Rajnochová, A. Svobodová, B. Zálešák, D. Biedermann, J. Ulrichová, J. Vostálová, Phototoxic potential of silymarin and its bioactive components, J. Photochem. Photobiol. B: Biol. 156 (2016) 61–68.

[60] R. Agarwal, C. Agarwal, H. Ichikawa, R.P. Singh, B.H. Aggarwal, Anticancer potential of silymarin: from bench to bed side, Antican. Res. 26 (2006) 4457–4498.

[61] D. Gallo, S. Giacomelli, C. Ferlini, G. Raspaglio, P. Apollonio, S. Prislei, A. Riva, P. Morazzoni, E. Bombardelli, G. Scambia, Antitumour activity of the silybin-phosphatidylcholine complex idB 1016, against human ovarian cancer, Eur. J. Cancer 39 (2003) 2403–2410.

[62] M.C. Comelli, U. Mengs, C. Schneider, M. Prosdocimi, Toward the definition of the mechanism of action of silymarin: activities related to cellular protection from toxic damage induced by chemotherapy, Integr. Cancer Ther. 6 (2007) 120–129.

[63] G. Deep, R. Agarwal, Chemopreventive efficacy of silymarin in skin and prostate cáncer, Integr. Cancer Ther. 6 (2007) 130–145.

[64] S.K. Katiyar, Silymarin and skin cancer prevention: anti-inflammatory, antioxidant and immunomodulatory effects, Int. J. Oncol. 26 (2005) 169.

[65] M.G. Hertog, E.J. Feskens, P.C. Hollman, M.B. Katan, D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study, Lancet 342 (1993) 1007–1011.

[66] M.J. Wang, W.W. Lin, H.L. Chen, Y.H. Chang, H.C. Ou, J.S. Kuo, J.S. Hong, K.C.G. Jeng, Silymarin protects dopaminergic neutrons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation, Eur. J. Neurosci. 16 (2002) 2103–2112.

[67] A. Martínez, A. Barbosa, Antiradical power of carotenoids and vitamin E: testing the hydrogen atom mechanism, J. Phys. Chem. B 112 (2008) 16945–16951.

[68] A. Galano, R. Vargas, A. Martínez, Carotenoids can act as antioxidants by oxidizing the superoxide radical anion, Phys. Chem. Chem. Phys. 12 (2010) 193–200.

[69] M. Zeeshan, H.-R. Sliwka, V. Partali, A. Martínez, Electron uptake by classical electron donators: astaxanthin and carotenoids aldehydes, Tetrahedron Lett. 53 (2012) 4522–4525.

[70] M. Najafi, D. Farmanzadeh, E. Klein, M. Zahedi, A theoretical study on the enthalpies of homolytic and heterolytic N-H bond cleavage in substituted melatonins in the gas-phase and aqueous solution, Acta Chim. Slov. 60 (2013) 43–55.

[71] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.J.A. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople Gaussian 09, Revision A.08 Inc., Wallingford, CT, 2009.

[72] Y. Zhao, D.G. Truhlar, The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals, Theor. Chem. Acc. 120 (2008) 215.

[73] G.A. Petersson, A. Bennett, T.G. Tensfeldt, M.A. Al-Laham, W.A. Shirley, J.A. Mantzaris, A complete basis set model chemistry. I. The total energies of closed-shell atoms and hydrides of the first-row atoms, J. Chem. Phys. 89 (1988) 2193–2198.

[74] G.A. Petersson, M.A. Al-Laham, A complete basis set model chemistry. II. Open-shell systems and the total energies of the first-row atoms, J. Chem. Phys. 94 (1991) 6081–6090.

[75] A.D. McLean, G.S. Chandler, Contracted Gaussian-basis sets for molecular calculations. 1. 2nd row atoms, Z = 11-18, J. Chem. Phys. 72 (1980) 5639–5648.

[76] K. Raghavachari, J.S. Binkley, R. Seeger, J.A. Pople, Self-consistent molecular orbital methods. 20. Basis set for correlated wave-functions, J. Chem. Phys. 72 (1980) 650–654.

[77] A.V. Marenich, C.J. Cramer, D.G. Truhlar, Universal solvation model base don solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions, J. Phys. Chem. B 113 (2009) 6378.

[78] D.M. Camaioni, C.A. Schwerdtfeger, Comment on accurate experimental values for the free energies of hydration of H+, OH-, and H_3O+, J. Phys. Chem. A 109 (2005) 10795–10797.

[79] A. Galano, On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals, Phys. Chem. Chem. Phys. 13 (2011) 7178–7188.

[80] A. Martínez, A. Galano, R. Vargas, Theoretical study on the chemical fate of adducts formed through free radical addition reactions to carotenoids, Theor. Chem. Acc. 127 (2010) 595–603.

[81] L.M. Dorfman, G.E. Adams, Reactivity of the Hydroxyl Radical in Aqueous Solutions, National Bureau of Standards, Washington D. C., 1973, pp. 32–37, NSRDS-NBS 46.