A DENSITY FUNCTIONAL THEORY STUDY OF ANTIOXIDANT ACTIVITY OF ISOTHIOCYANATES IN BROCCOLI SPROUTS (*BRASSICA OLERACEA* L.)

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ABSTRACT

Antioxidant activity of 9 isothiocyanate derivatives ($-N=C=S$) extracted from Broccoli sprouts (*Brassica oleracea* L.) has been investigated using density functional theory (DFT) – based computational methods. Through the hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, three thermodynamic parameters including bond dissociation enthalpy (BDE), vertical ionization energy (IE), and vertical electron affinity (EA) were calculated in the gas phase using B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry. As a result, the isothiocyanate (ITC) shows potential antioxidant activity via HAT mechanism. The most potential antioxidant is 3-isothiocyanato pro-1-en (3ITCP) with BDE(C$-$H) of 72.9 kcal/mol. The SET mechanism is not dominant in case of the studied ITCs. Moreover, the radicals formed H• removal had more reactive and less stable than the intial neutral compounds with lower IE, higher EA and ω.

1. INTRODUCTION

Broccoli sprouts (*Brassica oleracea* L.) is one of nutrient vegetables which is widely consumed in Vietnam. Several studies have demonstrated that Broccoli sprouts contains several chemical compound families that represent chemopreventive properties such as alkyl-hydrocarbon, -carbonyls, -alcohols, esters, aromatic compounds, nitriles, sulfides, etc. [1, 2, 3]. Among them, isothiocyanates (ITCs) represent as one of the most massive components identified in Broccoli sprouts. These organosulfur compounds are commonly constituted of one isothiocyanate ($-N=C=S$) moiety. ITCs have particularly received great attention because they show health promoting properties such as anticancer, antioxidant as well as induction of phase 2 detoxifying enzymes [4]. Several works in literature dedicated to identify and evaluate experimentally the antioxidant potential of ITCs available in different natural products such as Emeraude cauliflower [5]. The recent studies have found that the antioxidant potency of ITCs is

depending on either the type of functional groups or number of methylene ($-CH_2$) groups on the side chain [6]. Although the antioxidant capacity of ITCs is reported as a key factor for their health-promoting role, very few studies have dealt with a direct assessment of their antioxidant behavior in vivo as well as in vitro. To the best of our knowledge, no computational investigation has performed in literature to give insights into the possible antioxidant mechanism of ITCs.

Thus, the purpose of this work is to evaluate the antioxidant capacity of 9 ITCs identified in Broccoli sprouts [1] by comparing ability of H-donation and electron transfer to free radicals. The 9 studied compounds includes 3-isothiocyanato pro-1-en (3ITCP), Isothiocyanato-2-methyl propane (ITC-2MP), 1-isothiocyanato-3-methyl butane (1ITC-3MB), 1-isothiocyanato-3methylsulfanyl propane (1ITC-3MSP), 1-isothiocyanato-4-methylsulfinyl butane (1ITC-4MSOB), 1-isothiocyanato-4-methyl pentane (1ITC-4MP), 1-isothiocyanato-4-methylsulfany butane (1ITC-4MSB), 1-isothiocyanato-4-methyl benzene (1ITC-4MB), 2-isothiocyanato ethylbenzene (2ITCEB) (Fig 1). These ITCs were firstly extracted from Brocoli sprouts (*Brassica oleracea L.*) by water distillation. These compounds in deionized water were then extracted with dichloromethane during 6 hours and dried over anhydrous sodium sulfate. Lastly volatile sample was collected and analyzed by GC-MS [1]. Three thermodynamic parameters characterizing the hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms including bond dissociation enthalpy (BDE), vertical ionization energy (IE), and affinity electrons (EA) were calculated in the gas phase using density-functional theory (DFT) at B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry. In addition, quantum descriptors like chemical potential (μ), chemical hardness (η) and global electrophilicity (ω) of the neutral compounds and the corresponding radicals were also calculated to evaluate their reactivity and stability.
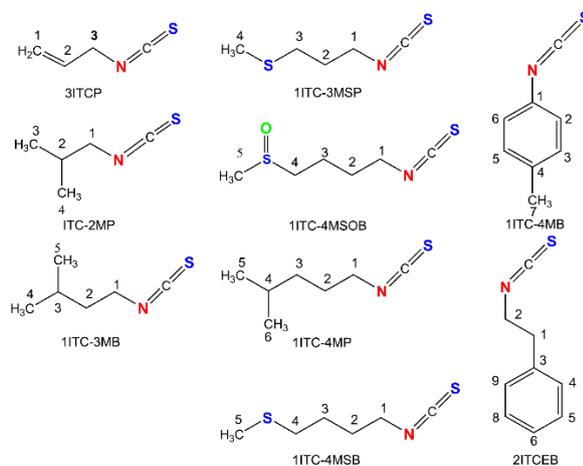

Figure 1. Molecular structures of the studied compounds.

2. COMPUTATIONAL METHODS

The geometry and the vibrational frequency of all investigated ITCs and related radicals, radical anions and cations were calculated using the Gaussian 09 (Revision E.01) suite of program [7]. Two common antioxidant mechanisms including hydrogen atom transfer (HAT) and single electron transfer (SET) which are characterized by bond dissociation enthalpy (BDE)

and ionization energy (IE) respectively, were evaluated in this study. Firstly, the semi-empirical PM6 method was used to determine the BDE of all possible C–H bonds dissociation in ITCs. The BDE(C–H) of the weakest C–H bond is then calculated using ROB3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry. Via SET mechanism, the higher antioxidant capacity of ITCs is corresponding to the easier electron donating capacity (lower IE value), or the easier electron accepting capacity (higher EA). The EA reflects the capability of accepting only one electron from the environment [8]. Vertical IE and EA values of each ITC were computed in this study using ROB3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry. The three thermo-parameters describing above were calculated via the following equations [9]:

$$BDE(R–H) = H(R^{\bullet}) + H(H^{\bullet}) - H(R–H) \tag{1}$$

$$IE = H(R–H^{\bullet+}) - H(R–H) \tag{2}$$

$$EA = H(R–H^{\bullet-}) - H(R–H) \tag{3}$$

In addition, based on the calculated IEs and additional electron affinity (EA), the global reactivity indicators including chemical potential (μ), chemical hardness (η) and global electrophilicity (ω) of corresponding radicals which are required to analyze the trend of stability and chemical reactivity of each compound and its radical, were also calculated as follows [9]:

$$\mu = -\frac{1}{2}(IE + EA) \tag{4}$$

$$\eta = \frac{1}{2}(IE + EA) \tag{5}$$

$$\omega = \frac{\mu^2}{2\eta} \tag{6}$$

The chemical hardness is defined as resistance of cloud polarization or deformation of chemical species. In addition, global electrophilicity ω measures the energy lowering of a ligand due to maximal electron flows between free radical and potential antioxidant [8]. The electron flows may be either less or more than one. The compound with the lowest values of μ, η, and highest value of ω is predicted to have the highest reactivity.

3. RESULTS AND DISCUSSION

3.1. Finding weakest C-H bonds of studied compounds

In order to find out the weakest bond positions, we primarily used semi-empirical PM6 method to compute the BDEs of all available C–H bonds in the ITCs which are listed in Table SI1 of the Supporting Information. As a result, it is generally observed that all the weakest C–H bonds are found nearby isothiocyanate moiety. In 9 studied ITCs, there are 5 compounds (3ITCP, 1ITC-3MB, 1ITC-4MP, 2ITC-EB, ITC-2MP) which have the lowest BDE C–H located at C atom nearby ITC moiety. And other 4 ITCs do not have same phenomenon because their structure have S atom in the side chain (i.e. 1ITC-3MSP, 1ITC-4MSB, 1ITC-4MSOB) or benzyl ring (i.e. 1ITC-4MB). Indeed, ITCs including 1ITC-3MSP, 1ITC-4MSB, 1ITC-4MSOB have lowest BDE C–H located at C atom nearby S atom instead of nearby the ITC functional group.

3.2. Bond dissociation enthalpies (BDEs)

In this study, we computed BDE(C–H) corresponding to the weakest C–H bond of each ITC the gas phase at B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) level of theory (Table 1). As a result, the highest H• donating ability of the studied compounds follows decreasing trend: 3ITCP > 1ITC-3MB > 1ITC-4MP > 2ITC-EB. The corresponding BDE (C–H) are 72.9, 84.6, 85.1 and 85.6 kcal/mol, respectively. In comparison with BDE of phenol (89.5 kcal/mol) [10] and terpinene (74.4 kcal/mol) [9], these four compounds are the potential antioxidants via HAT mechanism.

Table 1. Calculated thermo-parameters for ITCs in the gas phase by
B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry.

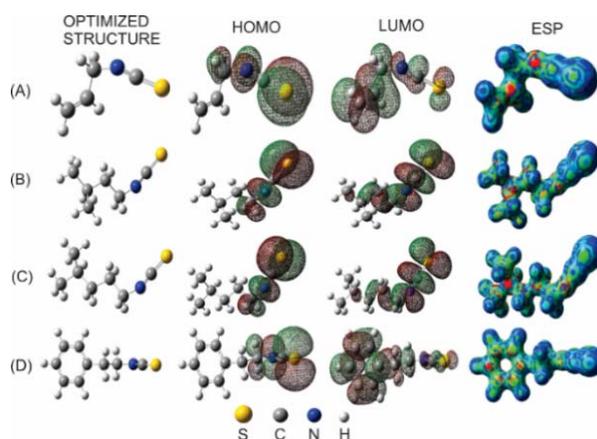| Compounds	3ITCP	1ITC-3MB	1ITC-4MP	2ITC-EB	ITC-2MP	1ITC-4MB	1ITC-3MSP	1ITC-4MSB	1ITC-4MSOB
Weakest C–H	C3–H	C1–H	C1–H	C2–H	C1–H	C4–H	C3–H	C4–H	C3–H
BDE (kcal/mol)	72.9	84.6	85.1	85.6	86.1	87.6	88.7	91.5	95.1
IE (eV)	9.06	8.94	8.96	8.63	8.99	8.42	8.24	8.17	8.37
EA (eV)	-0.40	-0.58	-0.33	-0.78	-0.30	-0.39	-0.30	-0.38	-0.37

Figure 2. Optimized structures, HOMO, LUMO and electrostatic potential (ESP) structures of (**A**) 3ITCP, (**B**) 1ITC-3MB, (**C**) 1ITC-4MP, (**D**) 2ITC-EB. (ESP are displayed at an isovalue of 0.1 and mapped in range from 0.085 to 0.1)

Figure 2 shows the structural geometries, highest-occupied molecular orbital (HOMO), lowest-unoccupied molecular orbital (LUMO) and electrostatic potential (ESP) of four neutral compounds which have the lowest BDE (C–H) including: 3ITCP, 1ITC-3MB, 1ITC-4MP, 2ITC-EB. The HOMO and LUMO structures show that ITC moiety plays as electron donating as well as accepting centers of the compounds. For the cases of 3ITCP and 2ITC-EB where a π-bond and an aromatic ring are available, the electron accepting sites are also located in site chain and benzyl ring, respectively. Finally, ESP map shows molecular parts where electron density is

higher than other part. And it is found that –N–, C=C bonds and benzyl ring have high electron density (i.e. red color parts) than the other parts of the molecule.

3.3. Ionization energy (IE) and electron affinity (EA)

The ionization energy (IE) and electron affinity (EA) are important parameters characterizing the single electron transfer (SET). So we evaluated systematically the IE and EA of all the investigated ITCs compounds. The lower IE value is, the easier electron transfer and higher antioxidant activity is. The computed IEs of all studied ITCs are reported in Table 1. In comparison with IE of phenol (8.49 eV), α-pinene (8.07 eV), limonene (8.3 eV) [11], some ITCs like 1ITC-4MSB, 1ITC-3MSP, 1ITC-4MSOB, 1ITC-4MB with IE value of 8.2, 8.24, 8.37 and 8.42 eV, respectively, show similar antioxidant potential via SET mechanism. Moreover, the electron affinity (EA) represents the the amount of energy released when an electron is added to a neutral molecule in the gaseous state to form a negative ion. The higher EA value is, the easier electron acceptor and higher antioxidant activity is. The four compounds that have the highest EA value are following: 1ITC-3MSP = ITC-2MP (-0.3 eV) > 1ITC-4MP (-0.33 eV) > 1ITC-4MSOB (-0.37 eV).

Furthermore, the three global quantum indicators including: chemical potential (μ), hardness (η) and electrophilicity (ω) were also calculated to evaluated the reactivity and stability of radical formed from H removal at the lowest C-H bond of ITCs. Table 2 compares the values of the neutral compounds as well as the corresponding radicals.

Table 2. Calculated quantum indicators of neutral ITC and corresponding radical at B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p) model chemistry.

Compounds	3ITCP	1ITC-3MB	1ITC-4MP	2ITC-EB	ITC-2MP	1ITC-4MB	1ITC-3MSP	1ITC-4MSB	1ITC-4MSOB
μ (neutral)	-4.33	-4.18	-4.31	-3.92	-4.34	-4.01	-3.97	-3.90	-4.00
(neutral)	4.73	4.76	4.65	4.7	4.65	4.4	4.27	4.27	4.37
ω (neutral)	1.98	1.84	2.00	1.64	2.03	1.83	1.85	1.78	1.83

Generally, it is observed that the formed radicals show lower values of μ, η and higher values of ω than the ones of the neutral compounds. These indices show the high reactivity and low stability of the formed radicals of ITC compounds. This may allow explaining the pro-oxidant potential of ITCs as reported in literature [12].

4. CONCLUSIONS

In this study, the antioxidant activities of 9 ITC compounds extracted from Broccoli sprouts (Brassica oleracea L.) have been studied chemical properties via their thermochemical parameters including BDE, IE, EA, μ, and ω. The obtained results show that the 3ITCP, 1ITC-3MB, 1ITC-4MP and 2ITC-EB represent as potential antioxidants via HAT mechanism. The easiest C−H breaking bond is usually found at the C atoms located nearby −N=C=S. The SET mechanism is not dominant in case of the studied ITCs. Moreover, the radicals formed H• removal had more reactive and less stable than the parent neutral compounds with lower IE,

higher EA and ω. This results demonstrates that the formed radical may act as antioxidant or pro-oxidant by donating or accepting electrons from free radicals.

REFERENCES

1. Jang H. W., et al. - Analysis and antioxidant activity of extracts from broccoli (Brassica oleracea L.) sprouts, J. Agric. Food Chem. **63** (2015) 1169–1174.

2. Li Y., et al. - Sulforaphane, a dietary component of broccoli/broccoli sprouts, inhibits breast cancer stem cells, Clin. Cancer Res. **16** (2010) 2580–2590.

3. Mbithi S., et al. - Effects of sprouting on nutrient and antinutrient composition of kidney beans (Phaseolus vulgaris var. Rose coco), Eur. Food Res. Technol. **212** (2001) 188–191.

4. Xie S. H., et al. - DNA damage and oxidative stress in human liver cell L-02 caused by surface water extracts during drinking water treatment in a waterworks in China, Environ. Mol. Mutagen. **51** (2010) 229–235.

5. Podsedek A. - Natural antioxidants and antioxidant capacity of Brassica vegetables: A review, Food Sci. Technol. **40** (2007) 1–11.

6. Prawan A., et al. - Structural influence of isothiocyanates on the antioxidant response element (ARE)-mediated heme oxygenase-1 (HO-1) expression, Pharm. Res. **25** (2008) 836–844.

7. Frisch M. J., et al. Gaussian 09, Revision E.01, Gaussian, Inc., Wallingford CT, 2013.

8. Parr R. G., et al. - Electrophilicity index, J. Am. Chem. Soc. **121** (1999) 1922–1924.

9. Ngo T. C., et al. - Insights into the antioxidant properties of non-phenolic terpenoids contained in essential oils extracted from the buds of Cleistocalyx operculatus: a DFT study, RSC Adv. **6** (2016) 30824–30834.

10. Yu-Ran L. - Bond dissociation energies in organic compound, CRC Press LLC, 2003.

11. NIST Webbook, http://webbook.nist.gov/chemistry/2016.

12. Valgimigli L., Iori R. - Antioxidant and pro-oxidant capacities of ITCs, Environmental and Molecular Mutagenesis **50** (2009) 222-237.

TÓM TẮT

NGHIÊN CỨU HOẠT TÍNH CHỐNG OXY HÓA CỦA CÁC HỢP CHẤT ISOTHIOCYANATE CÓ TRONG RAU CẢI MẦM (BRASSICA OLERACEA L.)

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Hoạt tính chống oxy hóa của 9 hợp chất isothiocyanate (−N=C=S) được chiết xuất từ rau cải mầm (Brassica oleracea L.) đã được nghiên cứu bằng phương pháp phiếm hàm mật độ (DFT). Thông qua cơ chế chuyển nguyên tử hydro (HAT) và chuyển electron (SET), ba thông số nhiệt động đặc trưng gồm năng lượng phân li liên kết (BDE), năng lượng ion hóa (IE) và ái lực electron (EA) của các hợp chất nghiên cứu đã được tính toán trong pha khí tại mức lí thuyết B3LYP/6-311++G(3df,3p)//B3LYP/6-311G(d,p). Kết quả cho thấy hợp chất isothiocyante (ITC) là chất chống oxi hóa tiềm năng theo cơ chế HAT. Chất có khả năng chống oxy hóa cao nhất là 3-isothiocyanato pro-1-en (3ITCP) với BDE(C−H) of 72,9 kcal/mol. Cơ chế SET chưa giải thích rõ hoạt tính chống oxi hóa của các hợp chất ITCs. Ngoài ra, các gốc tự do hình thành từ quá trình chuyển nguyên tử hydro có hoạt tính cao hơn so với chất ban đầu với giá trị IE thấp hơn, EA và ω cao hơn.

Từ khóa: Brassica oleracea L., rau cải mầm, isothiocyanate, thiocyanate, antioxidant, HAT, SET.