Structural Chemistry
https://doi.org/10.1007/s11224-021-01780-4

ORIGINAL RESEARCH

DFT study of the radical scavenging activity of isoxanthohumol, humulones (α-acids), and iso-α-acids from beer

Ilija Cvijetić[1] · Miljan Bigović[2] · Petar Ristivojević[1] · Maja Vitorović-Todorović[3] · Mire Zloh[4,5] · Dušanka Milojković-Opsenica[1]

Received: 11 February 2021 / Accepted: 8 April 2021

Abstract

Humulones and iso-humulones are potent natural antioxidants found in beer. In this study, density functional theory (DFT) method was applied for elucidating the structure-antioxidant activity relationship and molecular mechanism of antioxidant activity of eight bioactive humulones previously identified in different beer samples: isoxanthohumol, (*R*)- and (*S*)-adhumulone, *cis*- and *trans*-iso-adhumulone, *cis*- and *trans*-iso-n-humulone, and desdimethyl-octahydro-iso-cohumulone. The calculated bond dissociation enthalpies (BDEs) suggest that desdimethyl-octahydro-iso-cohumulone was the most potent compound with BDEs 5.1 and 23.9 kJ/mol lower compared to the values for resveratrol in gas phase and water, respectively. The enolic –OH is the most reactive site for hydrogen atom transfer (HAT). The presence of β-keto group with respect to enolic –OH diminishes the HAT potency via the formation of a strong intramolecular hydrogen bond. Another common antioxidant mechanism, single electron transfer followed by proton transfer (SET-PT), is only feasible for isoxanthohumol. The results of this study indicate a strong correlation between the increased antioxidant activity of beer products and the higher content of reduced iso-α-acids.

Keywords Structure-antioxidant activity relationship · Density functional theory · Prenylated flavonoids · Bond dissociation enthalpy · Hop

Introduction

Beer is the second most-consumed low alcoholic beverage in Europe, accounting for 37% of the total EU alcohol consumption [1]. Beer contains a wide range of compounds such as proteins, carbohydrates, B vitamins (niacin, riboflavin, folate, cobalamin, and pyridoxine), amino acids, phenolic compounds, as well as minerals (mainly potassium and magnesium). The pharmaceutical properties of beer mainly depend on the chemical composition of hops and malt, as well as parameters involved in brewing, e.g., the variety of barley and hops, temperature, and pH during mashing, sparing, boiling, as well as yeast fermentation. Although the harmful effects associated with the increased alcohol intake are well-known, the effects of moderate consumption of beer (one glass for woman and two for a man per day) require further study. These effects mainly vary for diverse alcoholic beverages due to their heterogeneous content of non-alcoholic components.

Amongst those, polyphenolic compounds from beer are potent radical scavengers and could stabilize beer products [2] and potentially be responsible for various beneficial effects in moderate consumption. About 70–80% of beer polyphenols originate from malt, and the remaining fraction belongs to bitter acids from hops. Hops are flowers of the plant *Humulus lupulus* added into the beer wort as a natural preservative and a source of bitter taste. Hops contains about 14.4% of polyphenols such as phenolic acids, prenylated chalcones, flavonoids, catechins, and proanthocyanidins [3]. Bitter acids

Ilija Cvijetić and Miljan Bigović contributed equally to this work.

✉ Ilija Cvijetić
ilija@chem.bg.ac.rs

[1] University of Belgrade - Faculty of Chemistry, Studentski trg 12-16, Belgrade, Serbia

[2] Faculty of Natural Sciences and Mathematics, University of Montenegro, Dzordza Vasingtona bb, Podgorica, Montenegro

[3] Military Technical Institute, Ratka Resanovića 1, Belgrade, Serbia

[4] Faculty of Pharmacy, University Business Academy, Trg Mladenaca 5, Novi Sad, Serbia

[5] Nanopuzzle Medicines Design Ltd, Bessemer Road, Stevenage, UK

Published online: 21 April 2021

are prenylated polyketides divided into two classes: α-acids (humulones) and β-acids (lupulones). During the wort boiling, tasteless humulones (α-acids) isomerize into bitter-taste iso-humulones (iso-α-acids). Iso-α-acids are significantly more abundant in beers compared to non-isomerized humulones [1], and its content highly influences the flavor [4] and foam stability of beer [5]. Beer is one of the primary dietary sources of prenylflavonoids. Isoxanthohumol and 6-prenylnaringenin were recognized as major prenylflavonoids in various beer samples, accompanied by minor amounts of 8-prenylnaringenin [6]. However, xanthohumol is generally a minor prenylflavonoid in beer due to the thermal isomerization into isoxanthohumol during the brewing process.

Various biological activities of humulones and their isomers have been confirmed. For example, unisomerized bitter acids such as adhumulone, cohumulone, and n-humulone selectively inhibit enzyme aldo-keto reductase AKR1B10, an enzyme upregulated in various cancer types [7]. Xanthohumol degraded BCR-ABL oncoprotein, a significant factor for the development of chronic myelogenous leukemia [8]. Additionally, beer ingredients have significant effects on skin health [9]. Isoxanthohumol from hops can be activated into the potent phytoestrogen in the human intestine [10].

Antioxidant activity of hops components has been confirmed using various in vitro assays [11–13]. The radical scavenging activity of natural polyphenols is determined by the number and position of phenolic –OH groups and their tendency to donate hydrogen atom and quench free radicals. However, a quantitative study on the pharmacophoric features and the mechanism of antioxidant activity of potent hops ingredients is not reported in the literature. The antioxidant activity of isoxanthohumol and humulones might originate from the inactivation of highly reactive free radicals ($R^{\bullet}$) through several mechanisms. In the hydrogen atom transfer (HAT), the antioxidant compound (HuOH) transfers hydrogen atom ($H^{\bullet}$) to $R^{\bullet}$ via homolytic cleavage of O–H bond:

$$HuOH + R^{\cdot} \rightarrow HuO^{\cdot} + RH \quad (1)$$

This reaction generates another free radical species ($HuO^{\bullet}$), stabilized via electronic and resonance effects and therefore less reactive than $R^{\bullet}$.

Single electron transfer (SET) mechanism inactivates the free radicals ($R^{\bullet}$) through electron transfer from the antioxidant (HuOH) to $R^{\bullet}$:

$$HuOH + R^{\cdot} \rightarrow HuOH^{\cdot +} + R^{-} \quad (2)$$

The anion R^{-} is a stable, closed-shell structure, while radical cation $HuOH^{\bullet+}$ is more stabilized compared to reactive $R^{\bullet}$ species. This step is usually followed by proton transfer (PT) from the radical cation.

Sequential proton-loss electron transfer (SPLET) is a three-step process, where initial proton dissociation from a molecule (Eq. 3) is followed by the electron transfer, forming a radical species (Eq. 4). The last step is the protonation of anion:

$$HuOH \rightarrow HuO^{-} + H^{+} \quad (3)$$

$$HuO^{-} + R^{\cdot} \rightarrow HuO^{\cdot} + R^{-} \quad (4)$$

$$R^{-} + H^{+} \rightarrow RH \quad (5)$$

The net result of HAT, SET, and SPLET is the same, i.e., transfer of H atom (which can be represented as $H^{+} + e^{-}$) from antioxidant to the free radical. All these processes can be quantitatively examined using quantum chemical calculations, where DFT methodology proved to be particularly useful for an accurate prediction of thermodynamic data at a moderate computational cost. This methodology proved to be a useful tool for studying the antioxidant properties of many polyphenols and the design of novel antioxidants [14–18].

In a previous study, Ristivojević et. al. [19] identified several hops phenyl flavonoids (isoxanthohumol, (*R*)- and (*S*)-adhumulone, *cis*- and *trans*-iso-adhumulone, *cis*- and *trans*-iso-n-humulone, and desdimethyl-octahydro-iso-cohumulone) as potent antioxidant compounds from beer extracts. The main aim of the present study was to investigate pharmacophoric features and antioxidant mechanisms of these hops phenyl flavonoids using DFT calculations. The HAT and SET mechanisms were predicted in the gas phase and water. All results were systematically compared to those of resveratrol, a highly potent phenolic antioxidant. The stability of corresponding radical species was studied and visualized using spin-density distribution maps. The strength of intramolecular hydrogen bonds was calculated for several isomers to aid the rationalization of structure-antioxidant relationships. The most important structural features are highlighted, and structural modifications leading to more potent humulone antioxidants are suggested. The obtained results could be used in the brewing practice to increase the content of bioactives in beer and develop novel super beers enriched with antioxidant prenylated chalcones.

Methods

Initial 3D structures of compounds were generated using Vega ZZ 3.2.0 [20] and MMFF94 force field [21] and afterward submitted to conformational analysis in AMMP program [22] implemented in Vega ZZ. The lowest energy conformer was located through the systematic search by rotating all single bonds by 30° and setting the dielectric constant of the environment to 78.4 (water). All other parameters were at default settings. Several possible isomers of desdimethyl-octahydro-iso-cohumulone were modeled using MMFF94

force field, and all-*R* isomer appeared as the lowest energy isomer. The geometry of the most stable conformer of each compound was further optimized at the DFT level of theory using B3LYP functional and 6-311g++(2d,2p) basis set. Starting from the optimized ground state, the geometries of corresponding radicals, radical cations, and anions were optimized at the same level of theory. For radicals and radical cations, the unrestricted calculations were performed to assure the correct treatment of species with unpaired electrons. The non-specific solvent effects were simulated using the IEF-PCM model of water [23]. Frequency calculations were performed after each optimization to confirm that geometries correspond to the minimum (absence of imaginary vibrational frequencies) and to obtain thermodynamic data. All DFT calculations were performed in Gaussian 16, version B.01 [24]. For the analysis of intramolecular non-covalent interactions (NCI) [25], bond critical points (BCPs) were calculated in Multiwfn 3.7 [26] using Quantum Theory of Atoms in Molecules (QTAIM) [27]. The NCI were visualized in VMD [28].

Results and discussion

The structures of potent beer antioxidants are shown in Fig. 1. To study the structure-antioxidant activity relationship of these compounds, DFT descriptors of two dominant antioxidant mechanisms, HAT and SET-PT, were computed and discussed herein.

Hydrogen atom transfer mechanism

Bond dissociation enthalpy (BDE) is a descriptor of HAT mechanism of antioxidants. It can be computed as:

$$\mathrm{BDE} = H(\mathrm{H}^{\cdot}) + H(\mathrm{HuO}^{\cdot}) - H(\mathrm{HuOH}), \quad (6)$$

where $H(\mathrm{H}^{\cdot})$, $H(\mathrm{HuO}^{\cdot})$, and $H(\mathrm{HuOH})$ denote the enthalpies of hydrogen atom, radical, and molecule, respectively. The lower BDE indicates higher antioxidant ability to transfer hydrogen atom and neutralize the harmful radical species.

The BDEs of isoxanthohumol and seven humulones/iso-humulones (compounds **1**-**8**, Fig. 1) were calculated. In order to screen for the most probable site where hydrogen atom abstraction occurs, BDE of each –OH bond of compounds **1**-**8** was computed and compared to those of phenol and resveratrol (Table 1).

The results depict an excellent HAT ability of desdimethyl-octahydro-iso-cohumulone (compound **8**), with the BDE 5.1 and 23.9 kJ/mol lower than the value for resveratrol in water and gas phase, respectively. The exceptionally low BDE for 3–OH bond originates from the conjugation of enolic 3–O˙ via with C=C bond, providing the additional spin density (SD) delocalization compared to allylic –OH groups at positions 1 and 4. As can be seen from Fig. 2, only 29% of 3-O˙ SD remains on this atom, compared to 63.9% for SD of 1-O˙.

The *trans*-isomers of iso-adhumulone (compound **4**) and iso-n-humulone (compound **6**) have 48 kJ/mol lower BDEs of 4-OH group, indicating higher antioxidant activity of *trans*-isomers. Compared to *cis*-counterparts, 4-O radicals of **4** and **6** are additionally stabilized through the intramolecular addition

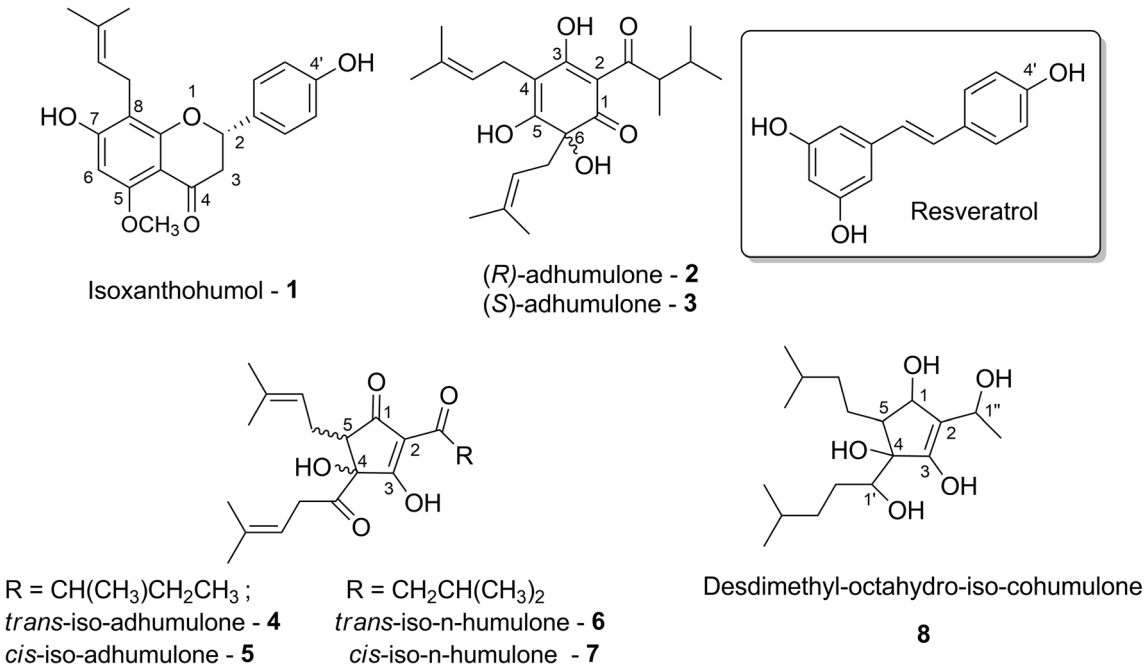

Fig. 1 Structure of isoxanthohumol (**1**), humulones (**2**-**8**), and resveratrol. The position of each –OH group evaluated as HAT site is labeled

Table 1 Bond dissociation enthalpies (BDE) and ionization potentials (IP) of compounds **1**-**8**, phenol, and resveratrol computed on B3LYP/6-311g++(2d,2p) level in the gas phase and using PCM model of water

Comp.	BDE (water)			IP (water)	BDE (gas)			IP (gas)
1	354.6 (7-)	344.8 (4'-)		566.1	346.6 (7-)	340.0 (4'-)		666.3
2	406.7 (3-)	331.2 (5-)	422.5 (6-)	595.6	398.7 (3-)	322.8 (5-)	416.6 (6-)	701.9
3	405.7 (3-)	331.1 (5-)	420.3 (6-)	594.7	398.4 (3-)	323.2 (5-)	413.0 (6-)	701.4
4	399.1 (3-)	352.4 (4-)		605.5	395.4 (3-)	336.1 (4-)		739.7
5	391.7 (3-)	400.6 (4-)		605.4	390.2 (3-)	392.3 (4-)		727.7
6	400.6 (3-)	352.9 (4-)		605.6	400.7 (3-)	342.4 (4-)		731.1
7	397.0 (3-)	404.0 (4-)		598.2	400.5 (3-)	396.6 (4-)		716.2
8	409.2 (1-)	313.1 (3-)	408.9 (4-)*	599.9	409.3 (1-)	296.5 (3-)	399.5 (4-)**	733.2
Phenol	345.5			601.7	368.2[b]			820.9[b]
Resveratrol	318.2 (4'-)			524.6	320.4 (4'-)			642.9

The position of –OH group is indicated in brackets, see Fig. 1. All values are given in kJ/mol

* BDEs for 1'- and 1"- OH groups were 411.5 and 406.6 kJ/mol, respectively

** BDE for 1'- and 1"- OH groups were 393.6 and 396.6 kJ/mol, respectively

[b] Experimentally determined values

of O radical to prenyl group attached to C_4-atom, forming the 5-membered ring (Fig. 3). This results in the transfer of the entire SD from 4-O radical to the prenyl group.

Negligible differences have been found between BDEs of (*R*)- and (*S*)-isomers of adhumulone (**2** and **3**). The most susceptible OH group is the enolic 5-OH group, which confirms the importance of enolic –OH moiety for the antioxidant activity of humulones. On the other hand, the enolic 3–OH group is significantly less reactive than 5–OH as BDE is nearly 80 kJ/mol higher. The possible explanation lies in the presence of carbonyl group in the β-position to enolic –OH, which interacts with –OH group via intramolecular hydrogen bonding (IHB) forming a pseudo-6-membered ring. This interaction might stabilize the ground state of a molecule and increase the BDE according to Eq. 6. This trend is also observed in compounds **4**-**7**, where enolic 3–OH have large BDEs (~400 kJ/mol) due to IHB with β-keto groups. Therefore, enolic –OH groups that lack the β-keto group are the most important pharmacophoric features for the antioxidant activity of humulones.

According to literature, *trans*-iso-α-acids are more sensitive to the presence of reactive oxygen species (ROS) compared to *cis*-isomers, while reduced forms of iso-α-acids are remarkably stable [29]. Therefore, compound **8** as a fully reduced isomer is the most promising natural antioxidant from hops due to exceptionally low BDE and excellent stability.

Single electron transfer

Ionization potential (IP) reflects the ability of a molecule to donate one electron to a free radical, creating a closed-shell

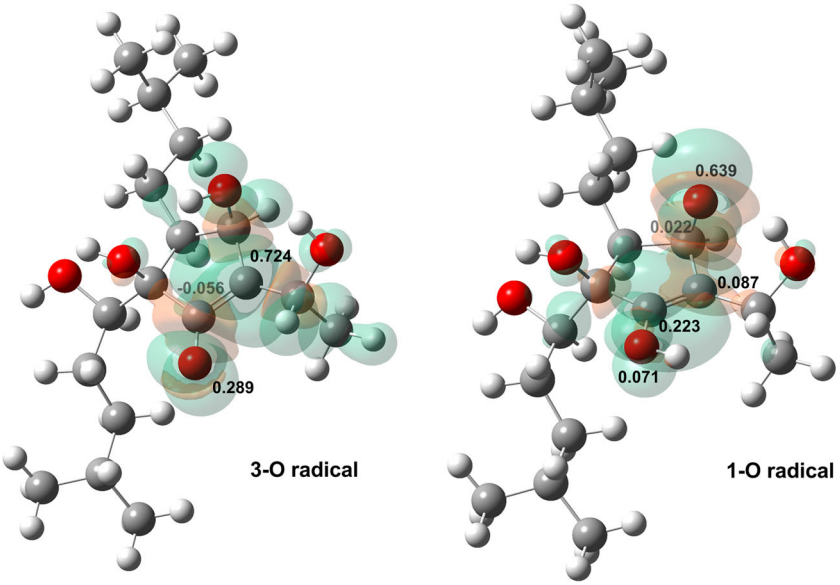

Fig. 2 Spin density distribution maps for 3-O (enolic) and 1-O (allylic) radicals of compound **8**. The spin density distribution is shown as a transparent surface, where labels indicate Mulliken spin density on corresponding atoms

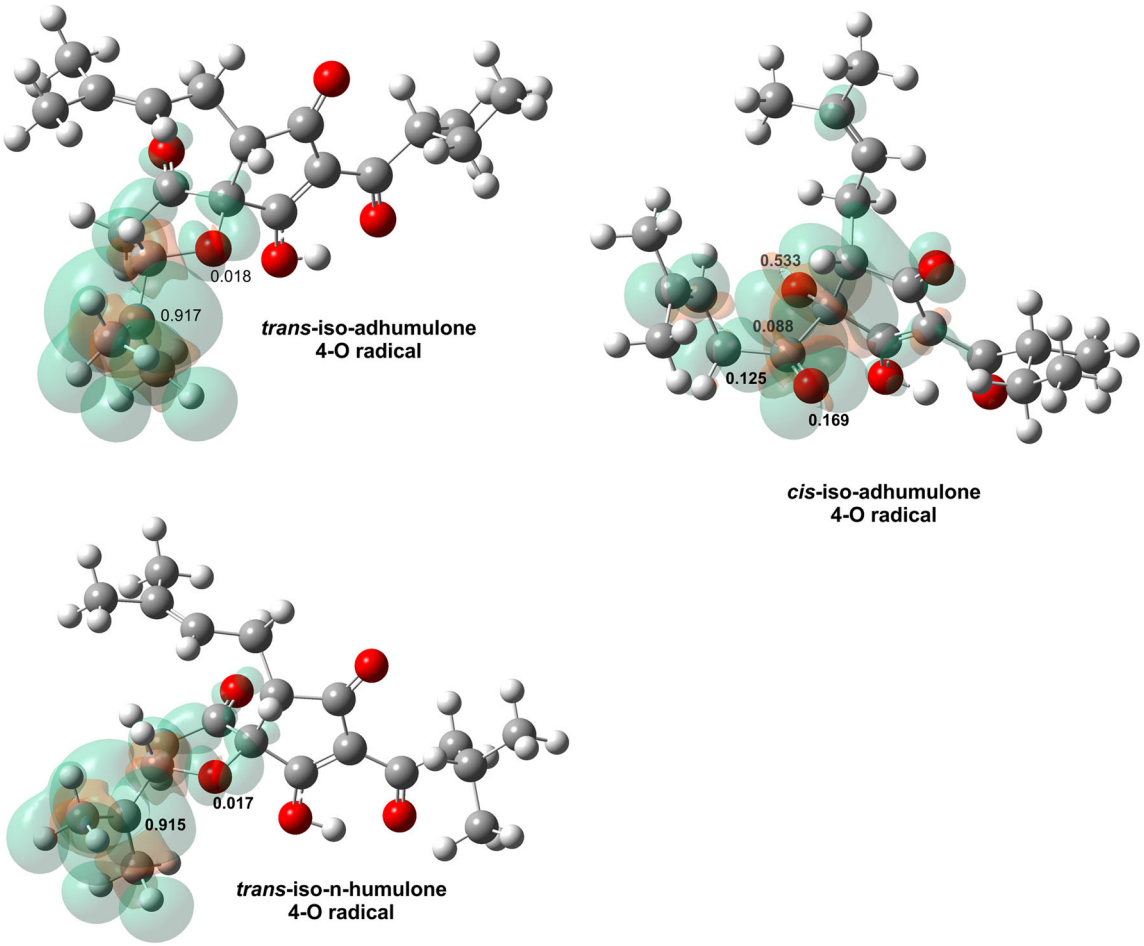

Fig. 3 Spin-density distribution maps for 4-O radical of *trans*-and *cis*-iso-adhumulone (**4** and **5**) and *trans*-iso-n-humulone (**6**). The Mulliken SD values on the corresponding atom are labeled

species with lower reactivity. This is another mechanism of how antioxidants exert their activity, as anion formed upon electron transfer from antioxidant to the free radical generally stabilizes the harmful, reactive radical species. Upon the electron transfer, the antioxidant molecule turns into radical cation, where stabilization through the resonance and electronic effects decreases the IP and increases the feasibility of the SET process.

IP can be calculated from the following equation:

$$\mathrm{IP} = H(\mathrm{HuO}^{\cdot+}) + H(\mathrm{e}^{-}) - H(\mathrm{HuOH}), \tag{7}$$

where $H(\mathrm{HuO}^{\cdot+})$ is the enthalpy of radical cation, and $H(\mathrm{e}^{-})$ is the enthalpy of an electron [30]. The IPs of all studied compounds, computed in the gas phase and water, are listed in Table 1.

Wright et al. reported that the dominant antioxidant mechanism of a compound can be elucidated through the comparison between the compound's IP and BDE with the corresponding values for phenol in the gas phase (ΔIP and ΔBDE) [31]. They suggested that two mechanisms, HAT and SET, occur simultaneously but with different rates. For the antioxidants having ΔIP ≥ –150 kJ/mol and ΔBDE around –40 kJ/mol, the main working mechanism is HAT, while SET is a principal antioxidant mechanism when ΔIP ≤ –188 kJ/mol.

The low ΔIP values for humulones **2**–**8** indicate that SET is not a favorable mechanism of their radical scavenging activity. The SET mechanism is the most probable for isoxanthohumol (**1**), with an IP value higher than resveratrol by 23.4 kJ/mol. On the other hand, low BDE values of compounds **2** and **3** and particularly 3–OH group of compound **8** along with relatively high ΔIP suggest that the main radical scavenging mechanism of humulones is HAT.

The results obtained using the water model show that the solvation has a more pronounced influence on IP compared to BDE. This is expected, as polar solvents better stabilize radical cations formed upon ionization (Eq. 7) compared to the products of homolytic bond cleavage (Eq. 6). Compound **8** appears to be more potent toward HAT

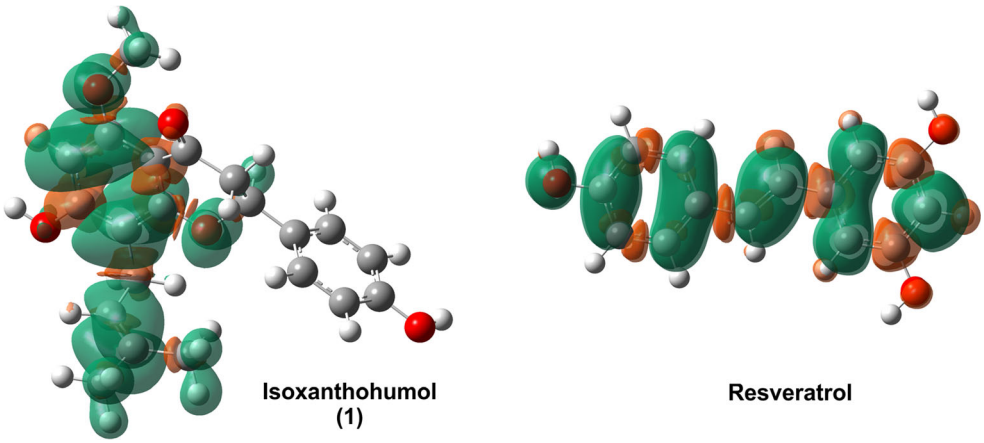

Fig. 4 Spin density distribution of radical cations of **1** and resveratrol.

compared to resveratrol, while SET is more feasible for resveratrol than for **1**–**8**.

The A ring of isoxanthohumol stabilizes the radical cation formed in the first step of SET mechanism, although to a lesser extent than resveratrol (Fig. 4). Compounds **2**–**8** do not possess this structural feature, which is reflected in higher IP values and lower probability for SET process.

The analysis of intramolecular non-covalent interactions

The NCI analysis [25] of compounds **1**, **4**, and **8** was performed to quantify the strength of IHB and give more details on the structure-antioxidant activity relationship of humulones. NCI analysis provided several parameters correlated with the hydrogen bond energy such as electron density (ρ), Laplacian of electron density ($\nabla^2\rho$), and potential energy density (V(r)). According to Espinosa et al. [32], the strength of hydrogen bonding (E_{HB}) can be approximated by 1/2 of V(r). The results of NCI analysis are given in Table 2.

The results reveal exceptionally strong IHB for between enolic 3–OH group and side-chain keto group of compound **4** (73.3 kJ/mol). On the other hand, three CPs of compound **8** are located far from the enolic 3–OH group (Fig. 5), and the corresponding strength of intramolecular interaction is much lower compared to **4**. These findings confirmed the hypothesis that the presence of keto group in β-position to enolic –OH increases the BDE and decreases the antioxidant activity of compounds.

Suggested structural modifications for the improvement of humulone antioxidants

The summary of BDE calculations and NCI analysis suggests several structural motifs important for the antioxidant activity of humulones. The hydrogen atom transfer (HAT) is the most probable antioxidant mechanism of compounds **2**–**8**, while SET is only feasible for isoxanthohumol (**1**). The –OH group in the vinylic (enolic) position of cyclopentene ring represents a highly active site toward HAT. However, no β-keto groups next to this moiety should be present in the structure of humulone antioxidant, as strong IHB increases the BDE of vinylic –OH by ~80 kJ/mol (see Table 1, BDEs for 3–OH of **8** (β-keto group) and **4**–**7**). In the case of *cis*- and *trans*-isomers of iso-α-acids (compounds **4**–**7**), the most potent HAT site is the allylic –OH group (4–OH). The results suggest a higher antioxidant activity of *trans*-iso-α-acids. On the other hand, higher reactivity of *trans*-isomers favors the reaction with ROS in beer and confirms the instability of beers with a high content of *trans*-isomers [29].

The reduced form of iso-α-acids (compound **8**) appears to be a more potent antioxidant compared to iso-α-acids (**4**–**7**) and unisomerized α-acids (**2** and **3**). It is established that the use of reduced hop products in the beer production results in

Table 2 Electron density (ρ), Laplacian of electron density ($\nabla^2\rho$), potential energy density (V(r)), H-bond binding energy (E_{HB}), and donor-acceptor (D-A) distance for bond critical points (CP) representing non-covalent interactions of compounds **1**, **4**, and **8**

Comp.	ρ, a.u.	$\nabla^2\rho$, a.u.	D-A distance, Å	V(r), a.u.	E_{HB}, kJ/mol
1 (CP 1)	0.01723	0.06256	N.A	−0.01163	15.3
1 (CP 2)	0.01246	0.05109	N.A	−0.00995	13.1
4 (CP 1)	0.05991	0.13170	1.612	−0.05580	73.3
8 (CP 1)	0.02234	0.09620	2.070	−0.01849	24.3
8 (CP 2)	0.01013	0.03543	2.413	−0.00685	9.0
8 (CP 3)	0.02452	0.08201	1.992	−0.01850	24.3

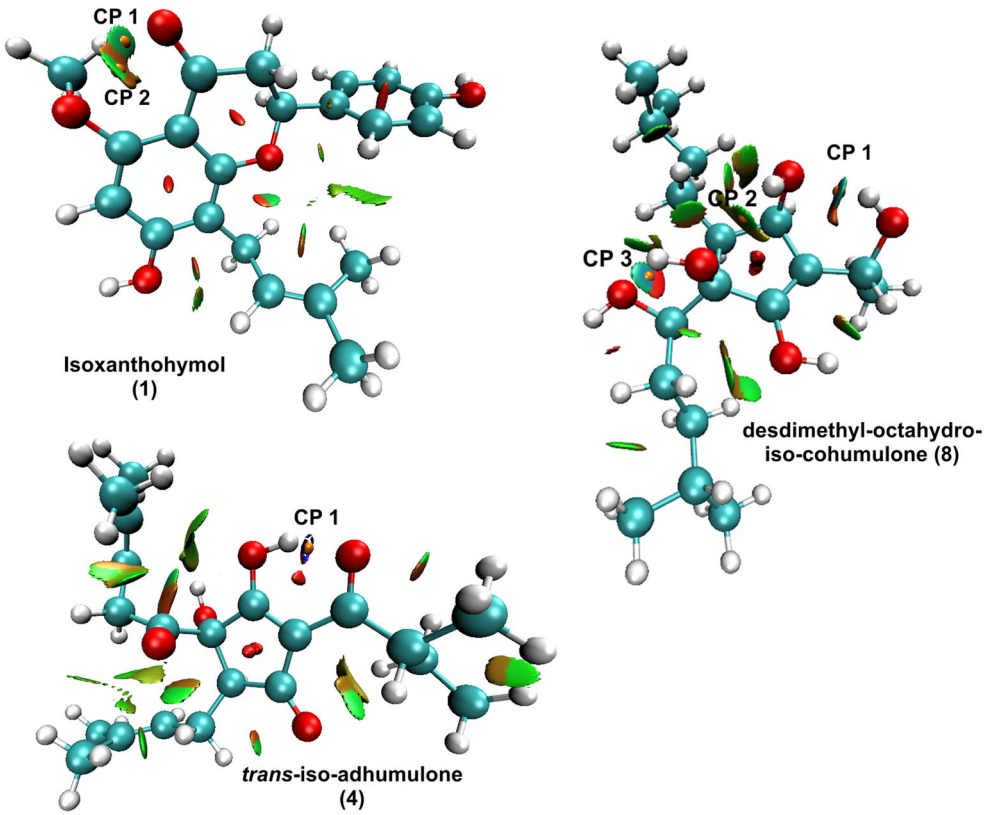

Fig. 5 The BCPs and NCI surfaces. The surface is color coded according to the strength of intramolecular interactions: blue, highly attractive regions (hydrogen bonding); green, weak attractions (VdW); and red, steric repulsions. Orange spheres represent bond critical points

beverages with an increased shelf-life due to the higher stability of the reduced forms of iso-α-acid (including compound **8**) toward degradation. Moreover, such beers have different bitterness profiles and enhanced foam stability. The methods for the hop pretreatment to increase the content of the reduced iso-α-acids, before their addition into brewing mixture, are continuously developed and patented [33]. For instance, the content of the dihydro-iso-α-acids can be increased by the treatment of the hop with sodium-borohydride [34], while the content of the tetrahydro-iso-α-acids and hexahydro-iso-α-acids can be increased by heterogeneous hydrogenation of the hop using Pd/C and Ru/Cu catalysts, respectively [35, 36]. Besides chemical pretreatment of hops pellets, the use of industrial yeast strains with high activity of certain reductases is a different route to optimize of the beer production with higher content of reduced iso-α-acids [37, 38]. Therefore, the continuous development of chemical and biochemical methods to ensure higher content of the different reduced forms of iso-α-acids is of the utmost importance in the brewing industry and would yield products with higher antioxidant capacity and an increased shelf-life.

The results of DFT calculations corroborate with our previous studies using DPPH-high-performance thin-layer chromatography (HPTLC) assay, where isoxanthohumol and desdimethyl-octahydro-iso-cohumulone were recognized as prenylflavonoids with the highest radical scavenging activity [19, 39].

Conclusion

In conclusion, the DFT study on structure-antioxidant relationship indicated a fully reduced iso-α-acid, desdimethyl-octahydro-iso-cohumulone, as a compound particularly prone to HAT mechanism, with BDEs 5.1 and 23.9 kJ/mol lower than the values for resveratrol in the gas phase and water, respectively. The enolic –OH is the most reactive site toward HAT. The presence of the β-keto group next to enolic –OH diminishes the HAT potency via the formation of a strong intramolecular hydrogen bond. The SET mechanism is most pronounced in isoxanthohumol. The results of this study strongly suggest the increased antioxidant activity of beer products with the higher content of reduced iso-α-acids. This study deepens the understanding of antioxidant activity of compounds in beer products and could contribute to unraveling potential beneficial effects of this popular beverage.

Acknowledgements I.C. acknowledges the National Institute of Chemistry in Ljubljana, Slovenia, and Ažman Supercomputing Centre for providing software and computational resources for this work.

Availability of data and material Not applicable.

Code availability Not applicable.

Author contribution Conceptualization, P.R. and I.C.; methodology, M.B. and I.C.; software, I.C. and M.B.; validation, M.Z. and D.M-O.; formal analysis, D.M-O.; investigation, M.B., P.R., and I.C.; resources, I.C. and M.Z.; data curation, M.V-T.; writing (original draft preparation), I.C. and M.B.; writing—P.R. and D.M-O.; visualization, I.C.; supervision, M.Z.; project administration, M.V-T.; funding acquisition, M.B., D.M-O., and M.Z.

Funding This research was funded by the Ministry of Education, Science and Technological Development of Serbia, Contract No 451-03-68/2020-14/200168.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors approved this submission.

Conflict of interest The authors declare no competing interests. M.Z. is founder and director of Nanopuzzle Medicines Design Ltd, that will derive no financial gain from this work.

References

1. De Keukeleire D (2000) Fundamentals of beer and hop chemistry. Quim Nova 23:108–112
2. Zhao H, Chen W, Lu J, Zhao M (2010) Phenolic profiles and antioxidant activities of commercial beers. Food Chem 119:1150–1158. https://doi.org/10.1016/j.foodchem.2009.08.028
3. Magalhães PJ, Vieira JS, Gonçalves LM et al (2010) Isolation of phenolic compounds from hop extracts using polyvinylpolypyrrolidone: characterization by high-performance liquid chromatography–diode array detection–electrospray tandem mass spectrometry. J Chromatogr A 1217:3258–3268. https://doi.org/10.1016/j.chroma.2009.10.068
4. Huvaere K, Sinnaeve B, Van Bocxlaer J, De Keukeleire D (2004) Photooxidative degradation of beer bittering principles: product analysis with respect to lightstruck flavour formation. Photochem Photobiol Sci 3:854–858. https://doi.org/10.1039/B403666B
5. Kunimune T, Shellhammer TH (2008) Foam-stabilizing effects and cling formation patterns of iso-α-acids and reduced iso-α-acids in lager beer. J Agric Food Chem 56:8629–8634. https://doi.org/10.1021/jf8011079
6. Miranda CL, Stevens JF, Ivanov V et al (2000) Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. J Agric Food Chem 48:3876–3884. https://doi.org/10.1021/jf0002995
7. Seliger JM, Cicek SS, Witt LT et al (2018) Selective inhibition of human AKR1B10 by n-humulone, adhumulone and cohumulone isolated from Humulus lupulus extract. Molecules 23:3041. https://doi.org/10.3390/molecules23113041
8. Lu X, Geng J, Zhang J et al (2019) Xanthohumol, a prenylated flavonoid from hops, induces caspase-dependent degradation of oncoprotein BCR-ABL in K562 cells. Antioxidants 8:402. https://doi.org/10.3390/antiox8090402
9. Chen W, Becker T, Qian F, Ring J (2014) Beer and beer compounds: physiological effects on skin health. J Eur Acad Dermatol Venereol 28:142–150. https://doi.org/10.1111/jdv.12204
10. Possemiers S, Bolca S, Grootaert C et al (2006) The prenylflavonoid isoxanthohumol from hops (Humulus lupulus L.) is activated into the potent phytoestrogen 8-prenylnaringenin in vitro and in the human intestine. J Nutr 136:1862–1867. https://doi.org/10.1093/jn/136.7.1862
11. Yamaguchi N, Satoh-Yamaguchi K, Ono M (2009) In vitro evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (Humulus lupulus) addressing acne vulgaris. Phytomedicine 16:369–376. https://doi.org/10.1016/j.phymed.2008.12.021
12. Tagashira M, Watanabe M, Uemitsu N (1995) Antioxidative activity of hop bitter acids and their analogues. Biosci Biotechnol Biochem 59:740–742. https://doi.org/10.1271/bbb.59.740
13. Gorjanović S, Pastor FT, Vasić R et al (2013) Electrochemical versus spectrophotometric assessment of antioxidant activity of hop (Humulus lupulus L.) products and individual compounds. J Agric Food Chem 61:9089–9096. https://doi.org/10.1021/jf401718z
14. Wang G, Liu Y, Zhang L et al (2020) Computational study on the antioxidant property of coumarin-fused coumarins. Food Chem 304:125446. https://doi.org/10.1016/j.foodchem.2019.125446
15. Wang L, Yang F, Zhao X, Li Y (2019) Effects of nitro- and amino-group on the antioxidant activity of genistein: a theoretical study. Food Chem 275:339–345. https://doi.org/10.1016/j.foodchem.2018.09.108
16. Dung NT, Thanh DM, Huong NT et al (2020) Quinolone and isoquinolone alkaloids: the structural-electronic effects and the antioxidant mechanisms. Struct Chem 31:2435–2450. https://doi.org/10.1007/s11224-020-01602-z
17. Horton W, Peerannawar S, Török B, Török M (2019) Theoretical and experimental analysis of the antioxidant features of substituted phenol and aniline model compounds. Struct Chem 30:23–35. https://doi.org/10.1007/s11224-018-1183-4
18. Assaleh MH, Božić AR, Bjelogrlić S et al (2019) Water-induced isomerism of salicylaldehyde and 2-acetylpyridine mono- and bis-(thiocarbohydrazones) improves the antioxidant activity: spectroscopic and DFT study. Struct Chem. https://doi.org/10.1007/s11224-019-01371-4
19. Ristivojević PM, Morlock GE (2018) Effect-directed classification of biological, biochemical and chemical profiles of 50 German beers. Food Chem 260:344–353. https://doi.org/10.1016/j.foodchem.2018.03.127
20. Pedretti A, Villa L, Vistoli G (2004) VEGA – An open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. J Comput Aided Mol Des 18:167–173. https://doi.org/10.1023/B:JCAM.0000035186.90683.f2
21. Halgren TA (1999) MMFF VI. MMFF94s option for energy minimization studies. J Comput Chem 20:720–729
22. Harrison RW (1993) Stiffness and energy conservation in molecular dynamics: an improved integrator. J Comput Chem 14:1112–1122
23. Mennucci B, Tomasi J, Cammi R et al (2002) Polarizable continuum model (PCM) calculations of solvent effects on optical rotations of chiral molecules. J Phys Chem A 106:6102–6113. https://doi.org/10.1021/jp020124t
24. Gaussian 16, Revision B.01, Frisch MJ, Trucks GW, Schlegel, HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Petersson GA, Nakatsuji H, Li X, Caricato M, Marenich AV, Bloino J, Janesko BG, Gomperts R, Mennucci B, Hratchian HP, Ortiz JV, Izmaylov AF, Sonnenberg JL, Williams-Young D, Ding F, Lipparini F, Egidi F, Goings J, Peng B, Petrone A, Henderson T, Ranasinghe D, Zakrzewski VG, Gao J, Rega N, Zheng G, Liang W, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Throssell K, Montgomery JA Jr, Peralta JE, Ogliaro F, Bearpark MJ, Heyd JJ, Brothers EN, Kudin KN, Staroverov VN, Keith TA, Kobayashi R, Normand J, Raghavachari K, Rendell AP, Burant JC, Iyengar SS, Tomasi J, Cossi M, Millam JM, Klene M, Adamo C, Cammi R,

Ochterski JW, Martin RL, Morokuma K, Farkas O, Foresman JB, Fox DJ (2016) Gaussian, Inc., Wallingford CT
25. Johnson ER, Keinan S, Mori-Sánchez P et al (2010) Revealing noncovalent interactions. J Am Chem Soc 132:6498–6506. https://doi.org/10.1021/ja100936w
26. Lu T, Chen F (2012) Multiwfn: A multifunctional wavefunction analyzer. J Comput Chem 33:580–592. https://doi.org/10.1002/jcc.22885
27. Matta C, Boyd RJ (eds) (2007) Quantum theory of atoms in molecules: recent progress in theory and application. Wiley-VCH, New York
28. Humphrey W, Dalke A, Schulten K (1996) VMD: Visual molecular dynamics. J Mol Graph 14:33–38. https://doi.org/10.1016/0263-7855(96)00018-5
29. Cooman L, Aerts G, Overmeire H, Keukeleire D (2000) Alterations of the profiles of iso-α-acids during beer ageing, marked instability of trans-iso-α-acids and implications for beer bitterness consistency in relation to tetrahydroiso-α-acids. J Inst Brew 106:169–178. https://doi.org/10.1002/j.2050-0416.2000.tb00054.x
30. Marković Z, Tošović J, Milenković D, Marković S (2016) Revisiting the solvation enthalpies and free energies of the proton and electron in various solvents. Comput Theor Chem 1077:11–17. https://doi.org/10.1016/j.comptc.2015.09.007
31. Wright JS, Johnson ER, DiLabio GA (2001) Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. J Am Chem Soc 123:1173–1183. https://doi.org/10.1021/ja002455u
32. Espinosa E, Molins E, Lecomte C (1998) Hydrogen bond strengths revealed by topological analyses of experimentally observed electron densities. Chem Phys Lett 285:170–173. https://doi.org/10.1016/S0009-2614(98)00036-0
33. Steenackers B, De Cooman L, De Vos D (2015) Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. Food Chem 172:742–756. https://doi.org/10.1016/j.foodchem.2014.09.139
34. Goldstein H, Fly W, Ting P, Chicoye E (1982) US 4324810 (A). Patent and Trademark Office, Washington, DC
35. Hay BA (1991) US 5013571 (A). Patent and Trademark Office, Washington, DC
36. Mertens P, De Vos D, Steenackers B (2011) WO 2011/157692 (A1). World Intellectual Property Organization, Geneva
37. Steensels J, Snoek T, Meersman E et al (2014) Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbiol Rev 38:947–995. https://doi.org/10.1111/1574-6976.12073
38. Olaniran AO, Hiralal L, Mokoena MP, Pillay B (2017) Flavour-active volatile compounds in beer: production, regulation and control. J Inst Brew 123:13–23. https://doi.org/10.1002/jib.389
39. Ristivojević PM, Morlock GE (2019) Phenolic fingerprints and quality assessment of three types of beer. JPC-Journal Planar Chromatogr TLC 32:191–196