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The Effect of Hydroxycinnamic Acids on Oxy-Radical Generating Iodide–Hydrogen Peroxide Reaction

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The influence of hydroxycinnamic acids (HCA) on the oxy-radical generated system, potassium iodide/hydrogen peroxide, was investigated through the enhancement of triiodide (I_3^-) yield. Caffeic acid, chlorogenic acid, and *p*-coumaric acid were used as typical representatives of HCA. A linear correlation, with positive slopes, was found between absorption maximum of I_3^- at 351 nm and HCA concentration in all cases. The magnitude of enhanced I_3^- production was found to increase in the following order: *p*-coumaric acid < chlorogenic acid ≤ caffeic acid. A reaction mechanism, which includes negative influence of oxygen-centered free radicals on the I_3^- yield, was proposed. The enhanced production of I_3^- by HCA is attributed to their radical scavenging activity. Supported by literature data, results obtained in this study have showed the correlation between radical scavenging activities of HCA and their ability to enhanced I_3^- generation.

The iodide–hydrogen peroxide (I^-–H_2O_2) reaction system is of great interest due to its importance in oscillatory chemistry (e.g. Bray–Liebhafsky or Brigss Rauscher reactions),[1–5] sonochemistry (e.g. KJ dosimetry),[6,7] atmospheric chemistry,[8,9] marine chemistry,[10–12] and biochemistry.[13,14] The chemical base of this complex reaction involves hydrogen peroxide (H_2O_2) decomposition to water and oxygen and the oxidation of iodide ions (I^-) to molecular iodine (I_2), which in the presence of I^- in excess, forms triiodide (I_3^-).[15]

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (1)$$

$$I^- + I_2 \rightarrow I_3^- \quad (2)$$

The minimal model which describes the reaction mechanism of I^- oxidation with H_2O_2 has been considered elsewhere.[16] Some recent investigations have confirmed that the I^-–H_2O_2 reaction system abounds in radical species. Namely, the EPR spin-trapping investigation of the I^-–H_2O_2 reaction system has provided clear evidence for the existence of hydroperoxyl radical ($HOO^\bullet$) and hydroxyl radical ($HO^\bullet$) as intermediary species.[17,18] Thus, the I^-–H_2O_2 reaction system can be considered as an easily available laboratory source of reactive oxygen species (ROS). It will be of great interest to investigate the effect of most famous antioxidant compounds on the oxy-radicals generated I^-–H_2O_2 system.

It is well-known that the function of antioxidants is to intercept and react with free radicals at a rate faster than substrate.[19] Therefore antioxidants can be considered as free radical scavengers. Phenolic compounds, widely present in the plant kingdom, stand out as the most efficient group of radical scevenger.[20] In order to investigate the effect of "scavenging" compounds on the I^-–H_2O_2 reaction system, caffeic (CAF), *p*-coumaric (*p*-CM), and chlorogenic (CGA) acids, typical hydroxycynnamic acids (HCAs) are used (Figure 1). Scavenging activity of this group of phenolic compounds is well-known.[20–23] Radical scavenging ability of HCA is ascribed to the hydroxy groups attached to the aromatic ring, acting as an electron acceptor or H-atom donor.[19,24]

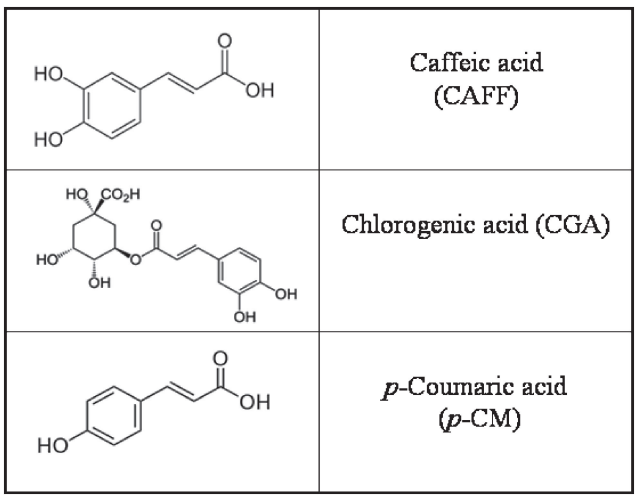

Figure 1. The structural formulas of the hydroxycinnamic acids HCA studied.

Scavenging activity of phenolic compounds is commonly tested by several radical scavenging assays which mostly involve artificial (nonbiological) radical species such as $DPPH^\bullet$ assay or $ABTS^{\bullet+}$.[25–27] It is important to point out, that the reaction between iodide and hydrogen peroxide is the source of highly reactive $HO^\bullet$ and $HOO^\bullet$ radicals which exist in biosystems.[13,28,29] The investigation of free radical scavenging

activity of phenolic compounds in a physiologically–real free radical generated systems is a demanding task. However, it is a poorly researched area. The iodide–hydrogen peroxide is a simple system, capable of generating free radical species. So far, this system has not been tested as a free radical scavenging assay, which is undoubtedly physiologically compatible, and inexpensive.

The aim of this paper is to investigate the influence of some typical hydroxycinnamic acids such as: caffeic, *p*-coumaric, and chlorogenic acid (as good antioxidants) on free-radical generated iodide–hydrogen peroxide reaction by measuring triiodide yield. Triiodide is one of the final products of the reaction between H_2O_2 and KI. The connection of HCA antiradical activity and triiodide yield is a key objective of this study.

Experimental

The chemicals used in this study were: potassium iodide, KI (p.a grade, Merck), hydrogen peroxide, H_2O_2 (30%, p.a. Merck) and caffeic acid, *p*-coumaric acid and chlorogenic acid (all used HCA were from Sigma-Aldrich, p.a. grade). The solutions were prepared with deionized water with resistivity of 18.2 MΩ cm (Milli-Q purification system, Millipore, Bedford, USA).

The stock solutions were: 0.06 mol dm^{-3} KI, 0.125 mol dm^{-3} H_2O_2, and the hydroxycinnamic acids 1.66×10^{-4} mol dm^{-3} caffeic, 1.97×10^{-4} mol dm^{-3} *p*-coumaric, and 1.47×10^{-4} mol dm^{-3} chlorogenic acid. The reaction mixture always contains the same concentration of KI and H_2O_2, while the concentration of particular HCA was varied, as presented in Table 1. Volume of the reaction mixture was 3 cm^3.

Constituents were added in the following order, 1) KI, 2) HCA acid, 3) deionized H_2O, and 4) H_2O_2. The reaction was initiated by addition of H_2O_2. The characteristic UV–vis absorption peak was recorded at 351 nm, the wavelength of I_3^- absorption maximum.[30] Spectrophotometric time-based measurement was performed on a UV–vis spectrophotometer (Agilent 8453), immediately after the reaction initiation. The reaction mixture was prepared directly in a quartz cell (the optical path is 1 cm). The iodide–peroxide–hydroxycinnamic acid system was monitored for 180 s with 10 s step size. All experiments were done at ambient temperature (23 °C). Measurements were repeated in triplicate and mean values are presented. For pH measurement a pH meter (Hanna Instruments, pH/°C Tester pHep4) was used.

Results and Discussion

The UV–vis time-based absorption spectrum of the KI–H_2O_2 reaction mixture is shown in the Figure 2. The absorption maximum centred at 351 nm is attributed to formation of I_3^-. This is to be expected taking into consideration that the oxidation of I^- by H_2O_2 leads to a certain amount of I_3^- formation. The temporal propagation of this maximum has revealed that equilibrium condition for I_3^- production is achieved between 120 and 180 s, after the reaction initiation, inserted in Figure 2.

The same was found concerning the time necessary for the system to achieve equilibrium production of I_3^- when particular a HCA like *p*-CM was added into the reaction mixture, shown in Figure 3. Therefore, measuring absorbance reading time is optimized at 180 s in all experiments. Evident increase of the absorption maxima at 351 nm, in the time-based absorption spectrum of the reaction mixture with the presence of *p*-CM, can be noticed in Figure 3. This implies that the presence of *p*-CM promotes I_3^- generation in the iodide–hydrogen peroxide system.

The UV–vis absorption spectra of the KI–H_2O_2 reaction system in the presence of caffeic acid in various concentrations are shown in Figure 4. The absorption spectra were recorded 180 s after the reaction initiation. The increase of I_3^- yield (increase of absorption at 351 nm) with the increasing concentration of caffeic acid can be noticed. The same trend is obtained when *p*-coumaric or chlorogenic acid is introduced to the iodide–peroxide system. Generally, the higher the concentration of HCA in the reaction system, the more I_3^- is generated.

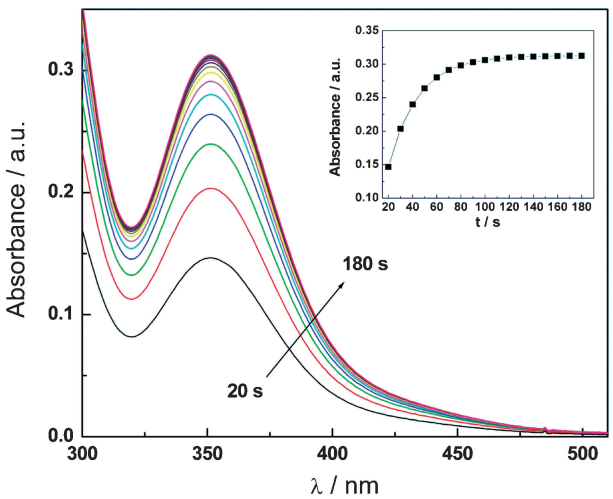

Figure 2. Time-based UV–vis absorption spectra of I^-–H_2O_2 reaction system recorded at every 10 s between 20th s to 180th s from the reaction initiation (arrow indicates increase of absorbance with time). Insert: time dependence of absorption maxima at 351 nm.

Table 1. The Initial Concentrations of Reaction Mixture

Trial	KI /mol dm^{-3}	H_2O_2 /10^{-3} mol dm^{-3}	*p*-CM[a)] /10^{-5} mol dm^{-3}	CAF[a)] /10^{-5} mol dm^{-3}	CGA[a)] /10^{-5} mol dm^{-3}
1	0.02	4.17	6.6	5.5	4.9
2	0.02	4.17	19.7	16.6	14.7
3	0.02	4.17	32.8	27.7	24.5
4	0.02	4.17	46.0	38.7	34.3
5	0.02	4.17	65.6	55.3	49.0

a) The reaction mixture contains only one HCA.

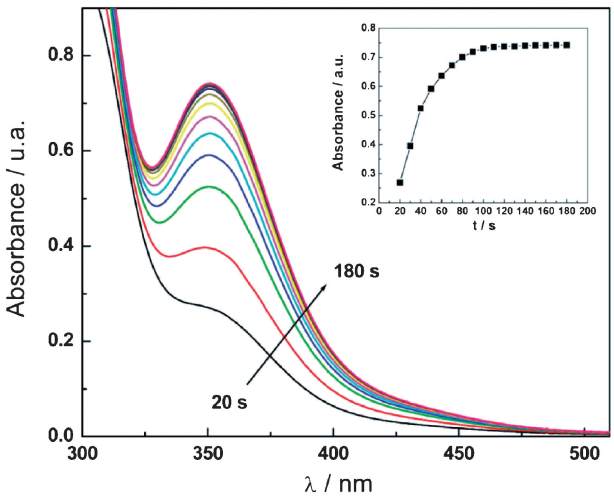

Figure 3. Time-based UV–vis absorption spectra of I^-–H_2O_2 reaction system in the presence of 65.6×10^{-5} mol dm^{-1} of *p*-CM, recorded at every 10 s between 20th s to 180th s from reaction initiation (arrow indicates increase of absorbance with time). Insert: time dependence of absorption maxima at 351 nm.

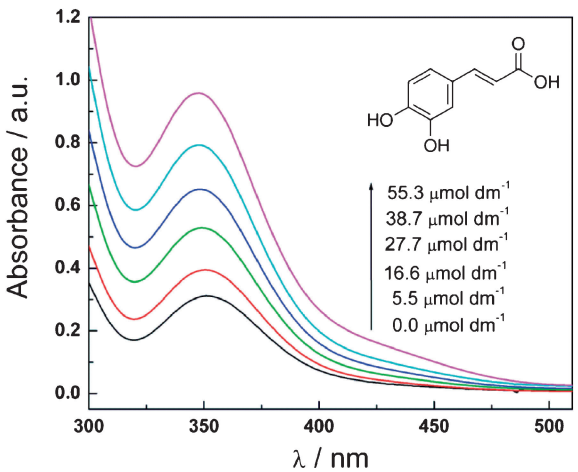

Figure 4. UV–vis absorption spectra of the KI–H_2O_2 reaction system, recorded after 180 s, in the presence of different concentrations of caffeic acid. The triiodide yield increases (the increase of absorption at 351 nm) with increasing initial concentration of caffeic acid (arrow indicates the increase of acid concentration). Concentration 0.0 μmol dm^{-3} denotes that deionized water added instead CAFF acid.

Since a certain amount of I_3^- will be generated even in the absence of HCA (Figure 2 or first spectra in Figure 4), the absorbance at 351 nm which originates from generated I_3^- in the absence of HCA (A_0^{351}) should be taken as background, and subtracted from the apparent measured absorbance value (A_{HCA}^{351}) in the presence of HCA. Therefore, the difference ΔA_{351}, $\Delta A_{351} = A_{HCA}^{351} - A_0^{351}$, will be used in this study. As shown in Figure 5, the linear correlation between ΔA_{351} and concentration of HCA was observed in all cases. It is noteworthy that the magnitude of enhanced I_3^- production in the system was found to increase in the following order *p*-coumaric acid < chlorogenic acid ≤ caffeic acid, Figure 5.

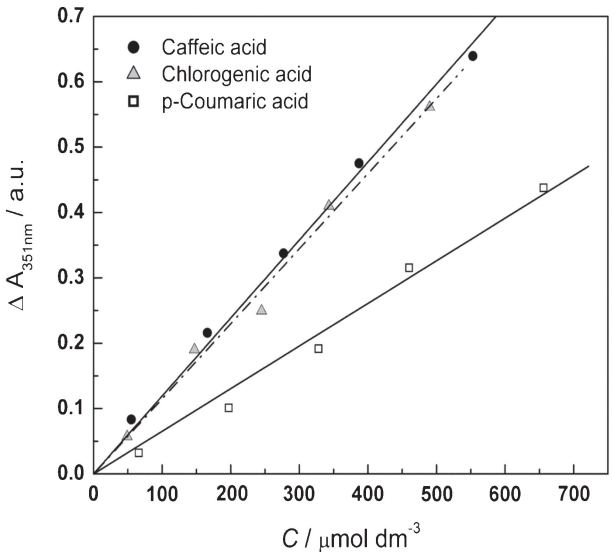

Figure 5. The linear correlation between ΔA_{352} and the concentration of hydroxycinnamic acids (CAF, CGA, and *p*-CM); Different HCA acids cause different increases of triiodide production.

The complex reaction between iodide and hydrogen peroxide is the result of different redox processes and it is pH dependent. The reference solution (absent HCA) contained H_2O_2 and excess KI. The net reaction of the whole process would be

$$3I^- + H_2O_2 \rightarrow I_3^- + 2OH^- \quad (3)$$

The reference solution becomes basic and after 180 s of hydrogen peroxide addition, the pH value of the investigated KI and H_2O_2 solution is 8.1 ± 0.2. The considered iodide–hydrogen peroxide–HCA system was not buffered and 180 s after H_2O_2 addition, in a system which contains a particular HCA acid, the pH value changed (reduced) for 0.4 ± 0.2 pH units. The examined HCA acids are week acids (having low dissociation constants).[31] Possible explanation for HCA influence on triiodide yield can be the system acidity change. More iodine occurs in more acidic medium,[2,32,33] and the reactions (eqs 2 and 3) result in further triidodide production.[15,16] Because all investigated HCA acids, after the 180 s of reaction initiation, changed pH for the same value 0.4 ± 0.2 within the uncertainty of the pH meter, the literature data for particular HCA dissociation constants are taken into consideration. Therefore, a surprising fact is that *p*-CM has slightly favorable dissociation (larger value pK_{a1} and pK_{a2}) than CAFF acid. In water at 298.2 K, dissociation constant for *p*-CM acid first dissociation is p$K_{a1} = 4.34$ and for second dissociation p$K_{a2} = 8.83$ and the same values for CAFF acid are p$K_{a1} = 4.30$ and p$K_{a2} = 8.51$.[31] Accordingly, it would be expected that more I_3^- is generated in the presence of *p*-CM acid, due to slightly higher acidity, but it was not. These findings further support the fact that acidity has a positive influence on triiodide yield, but it is not responsible for a given order of HCA (*p*-coumaric acid < chorogenic acid ≤ caffeic acid, Figure 5).

Accordingly, it seems to be possible to connect the antiradical (radical scavenging) activity of investigated acids with their ability to enhance production of I_3^-. Further, the

predictions of antioxidant activity of HCA acids using theoretical method and analysis of substituent effects are made. We tried to do some rough calculations of bond dissociation enthalpies (BDE). This procedure is based on density functional theory (DFT), used for the calculation of O–H bond dissociation enthalpy. Bond dissociation enthalpy (BDE) in ArOH is an important factor in determining the efficacy of antioxidants, since the weaker the OH bond the faster the reaction with free radical. Procedures for estimating O–H BDE is based on group additivity rules, proposed by Wright et al.[19] As the reaction between free radicals and generic radical scavenger (ArOH) becomes more exothermic, the energy barrier necessary for the reaction occurring should decrease, and the radical scavenger will react faster with the radicals, thus preventing reaction with substrate. Phenolic compounds which contain only one hydroxy group attached to their aromatic ring are less effective antioxidants compared to ones that posses a second hydroxy in the ortho or para position.[19,20,23] Therefore, caffeic acid and chlorogenic acid, with two OH groups are better free-radical scavengers than *p*-coumaric acid (one OH group). Due to BDE theory CAFF and CGA acids have a similar antiradical activity relative to phenol, 87.05 kcal mol^{-1}, $\Delta BDE = (-9.2 - 4.7)\,\text{kcal mol}^{-1} = -13.9\,\text{kcal mol}^{-1}$, while *p*-CM is weaker antioxidant $\Delta BDE = -4.7\,\text{kcal mol}^{-1}$.[19] The value $\Delta BDE = -9.2\,\text{kcal mol}^{-1}$ is taken due to both, CAFF and CGA acids having a OH group at the ortho phenol position (Figure 1). In the calculations, for all investigated HCA acids, we approximate the side chain on the para position of phenol, so that it has the same effect as a –CH=CH$_2$ group on para position ($\Delta BDE_{(\text{para } -CH=CH_2 \text{ group})} = -4.7\,\text{kcal mol}^{-1}$), since Wright claims that groups three bonds from an O–H group have little effect on BDE.[19] This is a severe approximation, and in our opinion, the steric hindrance (pronounced in CGA) should also be taken into account in these calculations. The results which suggest that CAF is slightly better free radical scavenger than CGA, obtained in this study, are supported by the work of Chen et al.[20]

The reaction mechanism between a generic radical scavenger like HCA (assigned as Ar–OH), and OH$^\bullet$, HOO$^\bullet$ radicals in the aqueous iodide–peroxide system may precede via these reaction Schemes:[19,22]

with OH$^\bullet$ radical

$$OH^\bullet + ArOH \rightarrow HOH + ArO^\bullet \quad (4)$$

and with HOO$^\bullet$ radical

$$ArOH + HOO^\bullet \rightarrow ArOH^{\bullet+} + HOO^- \quad (5)$$

Scheme 5 is followed by a rapid and reversible deprotonation in solution[22]

$$ArOH^{\bullet+} \rightleftarrows ArO^\bullet + H^+ \quad (6)$$

The proton reacts immediately with the hydroperoxyl anion to form hydrogen peroxide[22]

$$H^+ + HOO^- \rightarrow H_2O_2 \quad (7)$$

Reaction between hydroxycinnamic acid and hydroxyl radical (OH$^\bullet$) is slightly favored, due to a very high BDE of the H–OH bond in water (119 kcal mol^{-1}), thereby making all reactions with ArOH very exothermic. HOO$^\bullet$ radical has a much lower BDE on the formation of the parent, H_2O_2, it is typically about 88 kcal mol^{-1}.[19] It is also an additional factor of selectivity, and it plays an important role in chemical kinetics.

The reaction scheme of the I^-–H_2O_2 system is a complex one still under theoretical and experimental investigation.[15–18,22,34–37] It is presumed that the reaction mechanism starts with formation of iodine radical (I$^\bullet$) and OH$^\bullet$[16]

$$I^- + H_2O_2 \rightarrow I^\bullet + OH^\bullet + OH^- \quad (8)$$

Iodine radicals can mutually interact to form iodine (I_2), which will further react with I^- to form I_3^-, as considered by Torii et al.[34] However, it is more likely that when I^- is present in excess, I$^\bullet$ will consequently react with I^-, which will result in generation of molecular iodine radical ($I_2^{\bullet-}$)[35–37]

$$I^\bullet + I^- \rightleftarrows I_2^{\bullet-} \quad (9)$$

Molecular iodine radical can undergo disproportional reaction, generated triiodide[34–36]

$$2I_2^{\bullet-} \rightleftarrows I_3^- + I^- \quad (10)$$

As proposed by McDermott et al.[36] and adapted by Olexová et al.,[37] $I_2^{\bullet-}$ can be removed from the system through the reaction with HOO$^\bullet$

$$I_2^{\bullet-} + HOO^\bullet \rightarrow H^+ + 2I^- + O_2 \quad (11)$$

The existence of HOO$^\bullet$ in the I^-–H_2O_2 reaction system was experimentally proved by Stanisavljev et al.[18] Namely, characteristic signal of the DEPMPO–HOO adduct was obtained by EPR spin trapping measurements of a I^-–H_2O_2 reaction system. It is reasonable to assume that if present in reaction system, phenolic compounds (e.g. hydroxycinnamic acid, HCA) can scavenge HOO$^\bullet$, as proposed in eq 5. Thus, elimination of $I_2^{\bullet-}$ by HOO$^\bullet$ will be suppressed (eq 11), which will consequently lead to enhanced production of I_3^- (eq 10).

The oxy-radical generating iodide–peroxide reaction is a very interesting system for the potentially measured antiradical activity of some compounds. It should be better investigated as a new inexpensive radical assay. Results are found to be in good agreement with the BDE theory and literature data for the free radical scavenging activity of examined hydroxycinnamic acids. Although preliminary, these results make a good starting point for further research.

Conclusion

The hydrocynnamic acids HCA influence on oxy-radical generating iodide–peroxide reaction is investigated in this paper. For this purpose, three characteristic hydroxycynnamic acid compounds: caffeic (CAF), chlorogenic (CGA), and *p*-coumaric (*p*-CM) acids are used. This influence is considered by monitoring the formation of I_3^- as the final product of the iodide–peroxide reaction. The linear correlation between triiodide absorption (ΔA_{352}) and the hydroxycinnamic acids concentration was found. The different HCA cause different increases of triiodide production (*p*-CM < CGA ≤ CAF). Our results show that it should be possible to connect the antiradical activity of these acids with their ability to produce more triiodide.

The oxy-radical generating iodide–peroxide reaction is a new system with the potency to be employed as a novel

antioxidant activity assay. It should be better investigated, as a possible innovative, inexpensive, physiologically compatible free oxy-radical scavenging assay. Results presented demonstrate that the potassium iodide–hydrogen peroxide system has potency toward free radical scavenging assessment.

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