Radiat Environ Biophys (2006) 45: 73–77
DOI 10.1007/s00411-006-0041-8

ORIGINAL PAPER

Hongping Zhu · Hongwei Zhao · Zhaoxia Zhang
Wenfeng Wang · Side Yao

Laser flash photolysis study on antioxidant properties of hydroxycinnamic acid derivatives

Received: 16 January 2006 / Accepted: 6 March 2006 / Published online: 28 April 2006

Abstract The antioxidant properties of hydroxycinnamic acid derivatives (HCA) were studied by laser flash photolysis. The transient species with maximum absorption at 360 nm were assigned to the phenoxyl radical of HCA. The $SO_4^{\bullet -}$ induced oxidation of HCA was also investigated. It was shown that the interaction of $SO_4^{\bullet -}$ with HCA resulted in the formation of HCA phenoxyl radicals with rate constants of 2.0–3.9×10^9 M^{-1} s^{-1}. The reactions of HCA with triplet state of benzophenone were analyzed and quenching rate constants of 4.3–7.8×10^9 M^{-1} s^{-1} were determined.

Introduction

A deleterious effect of radiation is the production of reactive oxygen species (ROS). ROS are involved in aging, cancer, cardiovascular disease, and neurodegenerative diseases [1–4]. Investigators found evidence for increased oxidative damage and decreased antioxidant enzyme activity after space flight [5]. Thus, protection of astronauts from radiation injury has emerged as a crucial issue in human space exploration. It is well known that antioxidants can protect against ROS-induced oxidative damage to biomolecules by scavenging the ROS or repairing the oxidized substrate. A growing amount of evidence suggested that the incidence of cardiovascular diseases, Alzheimer and Parkinson diseases, can be diminished by diets rich in antioxidants [6, 7]. Much work has been carried out to study nutrition and antioxidants relevant to space flight [8]. It was shown that dietary supplementation of antioxidants decreases radiation-induced damage to tissues [9].

Hydroxycinnamic acid derivatives (HCA) are phenolic acids produced from L-phenylalanine and L-tyrosine via the shikimate pathway and are abundant in vegetable, fruits, tea, and herbs [10]. The intake of HCA has been estimated to be around 211 mg/day [11]. HCA have been shown to scavenge radicals [12, 13] and repair protein damage [14]. Other beneficial effects of HCA have been reported including anti-inflammatory and antimutagenic effects and protection of red blood cells from ROS [15, 16]. Recently, Hsieh et al. [17] reported that HCA exhibit strong inhibitory effects on UVB-induced oxidative damage in human erythrocytes and low-density lipoprotein (LDL).

Popular methods to evaluate the antioxidant activity of biological material include the steady analysis methods such as trolox equivalent antioxidant capacity (TEAC) assay, DPPH (2,2-diphenyl-1-picryhydrazyl radicals) assay, and Folin–Ciocalteu method [18]. However, the radioprotective mechanism of antioxidant is far from clear. Laser flash photolysis (LFP) technique can detect intermediates with short lifetime, thus it is a powerful tool for elucidating the mechanisms of actions of antioxidants. Considering the promising role of HCA in protection from radiation and photooxidation, a clear understanding of the antioxidative mechanism of HCA is needed. For this purpose, an effort has been made to study four HCA: caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), chlorogenic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-[3,4-dihydroxycinnamate]), and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) (Fig. 1) using nanosecond laser flash photolysis (LFP). Our aim is to elucidate their photophysical and photochemical properties and to shed light on the radioprotective mechanisms of HCA.

H. Zhu · H. Zhao · Z. Zhang · W. Wang (✉) · S. Yao
Shanghai Institute of Applied Physics,
Chinese Academy of Sciences,
Shanghai 201800, China
E-mail: wfwang@sinap.ac.cn
Fax: +86-21-59553021

H. Zhu · Z. Zhang
Graduate School of Chinese Academy of Sciences,
Beijing 100049, China

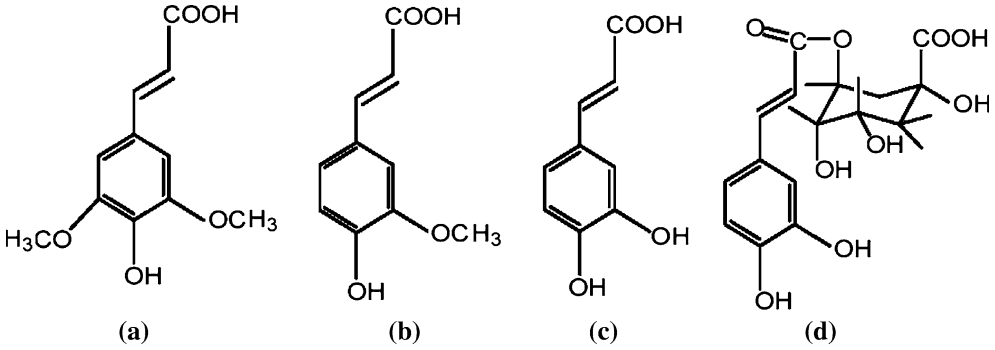

Fig. 1 Chemical structures of **a** sinapic acid, **b** ferulic acid, **c** caffeic acid, and **d** chlorogenic acid

Materials and methods

Chlorogenic acid, sinapic acid, ferulic acid, caffeic acid, and acetonitrile were from Sigma and used as received. Potassium persulfate, KI, fumaronitrile, and benzophenone (BP) were obtained from Acros and used without further purification.

All solutions were prepared freshly with triply distilled water provided by Millipore purification system. Prior to the irradiation, solutions were bubbled with the appropriate gas (high-purity nitrogen, O_2 or N_2O) for at least 20 min. All experiments were carried out at room temperature.

LFP experiments were carried out using a Nd:YAG laser, which provides 266 nm pulses with a duration of 5 ns and a maximum energy of 80 mJ per pulse. The laser and analyzing light beam passed perpendicularly through a quartz cell. The transmitted light entered a monochromator equipped with a R955 photomultiplier (Hamamatsu). The signals were collected using a HP54510B digital oscillograph and then transferred to a computer to be processed. A detailed technical description of the equipment and experimental conditions can be found elsewhere [19].

To determine the quantum yield of photoionization, a series of potassium iodide (KI) solution (the quantum yield of e^-_{aq}, $\Phi^-_e = 1$) was used as reference. Their absorbances at 266 nm were measured. HCA solutions were prepared whose concentrations were adjusted to make the absorption of the sample at 266 nm same to that of KI solutions. The yield of e^-_{aq} formed by ionization was measured from the absorbance at 650 nm immediately after the pulse. Dependence of absorbance measured at 650 nm on absorbance of HCA and KI solution is obtained. By comparing the slope of the two straight lines, the quantum yield of photoionization of HCA is determined.

Results and discussion

Photoionization of HCA

Figure 2 shows the transient absorption spectra recorded at 0.1 μs after the 266 nm laser irradiation of 0.1 mM ferulic acid aqueous solution saturated with N_2, N_2O, and O_2, respectively. After the laser pulse, a transient absorption band with maximum absorption at 360 nm and a broad one at wavelengths longer than 500 nm were observed when the solution was saturated with N_2. When the solution was saturated with N_2O or O_2, the broad band at >500 nm disappeared. Thus, it could be assigned to hydrated electrons (e^-_{aq}) as it can be scavenged by N_2O or O_2. Similar results were obtained with caffeic acid, sinapic acid, and chlorogenic acid. The information on e^-_{aq} indicates that HCA undergoes photoionization resulting in the formation of HCA cation radical.

It is known that O_2 can quench triplet states. If there is a triplet state of HCA, its decay will be accelerated by O_2. By comparing the decay trace of species with absorbance at 360 nm, obtained in N_2- saturated solution with that in O_2- saturated solution, no changes could be detected at all (see Fig. 3). When 0.01 M Mn^{2+} was added to the solution as a quencher of excited triplet states, the transient spectra and the

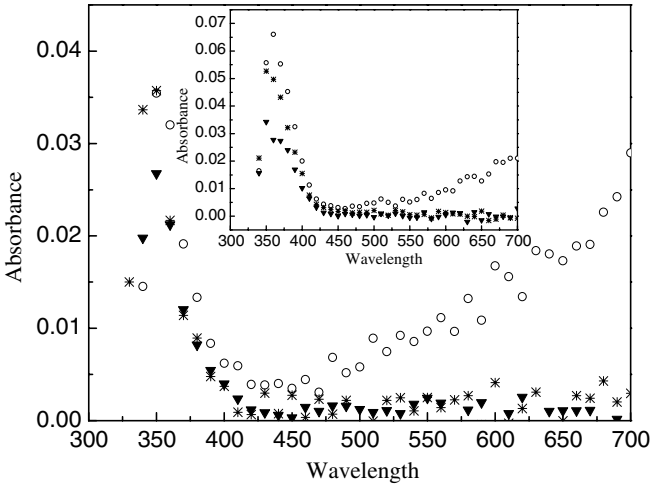

Fig. 2 Transient absorption spectra recorded at 0.1 μs after 266 nm LFP of 0.1 mM ferulic acid in N_2-saturated (*open circle*), N_2O-saturated (*asterisk*), and O_2-saturated (*filled inverted triangle*) aqueous solution. *Inset* transient absorption spectra measured 0.1 μs after 266 nm LFP of 0.1 mM chlorogenic acid in N_2-saturated (*open circle*), N_2O-saturated (*asterisk*), and O_2-saturated (*filled inverted triangle*) aqueous solution

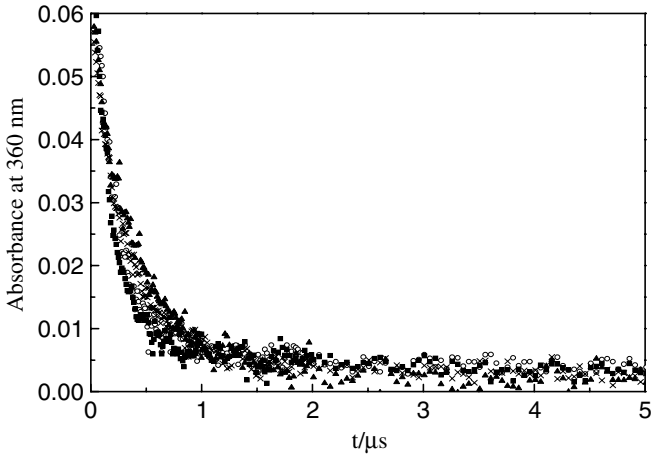

Fig. 3 Transient absorption decay monitored at 360 nm from 266 nm LFP of 0.1 mM chlorogenic acid in N_2-saturated (*open circle*), O_2-saturated (*filled square*), N_2-saturated containing 0.01 M fumaronitrile (*cross*), and N_2-saturated containing 0.01 M Mn^{2+} (*filled triangle*) aqueous solution

rate of decay at 360 nm showed no change compared with the solution without Mn^{2+} which indicates that the transient absorption cannot be attributed to contributions from an excited triplet state of HCA. Further confirmation came from experiments when fumaronitrile was added to the solution as a triplet scavenger. The fact that the decay of species with absorption at 360 nm are not affected by O_2, Mn^{2+}, or fumaronitrile shows that HCA are photoionized to generate radicals in neutral aqueous solution and no triplet states are formed in our experiment.

Thus, the following reaction occurs:

$$\mathrm{HCA} \xrightarrow[266\,\mathrm{nm}]{h\nu} \mathrm{HCA}^{\bullet+} + e^-_{aq}$$

Under our experimental conditions, the radical cations of HCA rapidly lose protons to form the neutral radicals.

$$\mathrm{HCA}^{\bullet+} \rightarrow \mathrm{HCA}^{\bullet} + \mathrm{H}^+$$

The quantum yield of photoionization is determined to be less than 0.02 at room temperature with KI as a reference.

Reaction with $SO_4^{\bullet-}$

As a one-electron oxidant, $SO_4^{\bullet-}$ can be generated easily with LFP (266 nm). Photolysis of 0.1 M $K_2S_2O_8$ aqueous solution showed the formation of $SO_4^{\bullet-}$ with maximum absorption spectra at about 320 and 460 nm (data not shown). Since the absorbance spectra of $SO_4^{\bullet-}$ and $HCA^{\bullet}$ overlap, the formation trace of $HCA^{\bullet}$ at 360 nm was obtained by subtracting the absorbance of $SO_4^{\bullet-}$ (Fig. 4). It is obvious that the growth trace of HCA radicals at 360 nm is synchronous with the decay trace of $SO_4^{\bullet-}$ at 460 nm. The photolysis of N_2-saturated aqueous solutions containing 0.1 M $K_2S_2O_8$ and different concentrations of HCA was studied. The kinetics analysis of the transient traces showed that $SO_4^{\bullet-}$ decays by a first order process and that its decay rate is proportional to the concentration of HCA. The reaction could be expressed as:

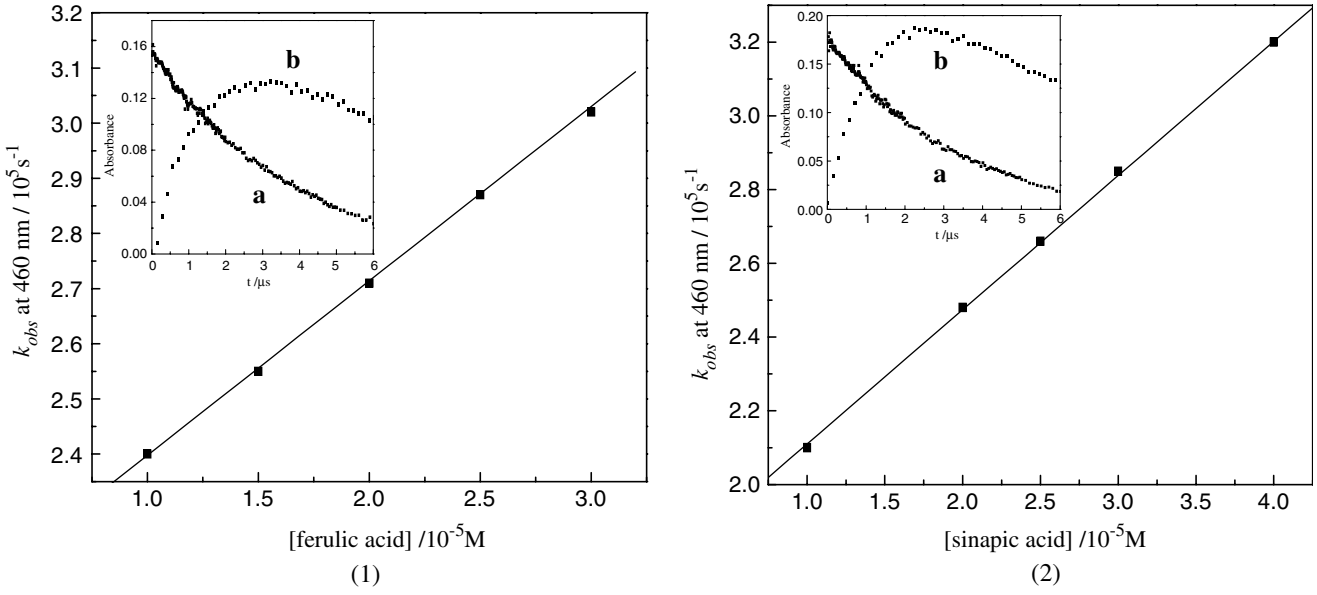

Fig. 4 Dependence of sulfate radical decay rate constant k_{obs} at 460 nm on concentration of HCA: *1* Ferulic acid and *2* Sinapic acid. *Inset* Kinetic traces showing growth and decay of HCA radical and sulfate radical. *a* Decay of sulfate radical monitored at 460 nm; *b* Growth of HCA radical

$$S_2O_8^{2-} \xrightarrow{hv} {}^2SO_4^{\bullet -}$$

$$SO_4^{\bullet -} + HCA \rightarrow SO_4^{2-} + HCA^{\bullet +} \rightarrow SO_4^{2-} + HCA^{\bullet} + H^+$$

The rate constants for the oxidation of HCA by $SO_4^{\bullet -}$ are given in Table 1. These results showed that HCA exhibit a second-order rate constant corresponding to a diffusion-controlled bimolecular reaction. By its high radical scavenging ability, HCA can eliminate oxidative stress and defense against radiation-induced radical damage.

Reaction with ^{3}BP*

It is well known that many excited ketones can result in biomolecule damage. The photosensitization of DNA damage by benzophenone (BP) has been reported via electron and energy transfer [20]. BP was used as a photosensitizer in this work to study the ability of HCA for quenching excited triplet state.

Table 1 Rate constants for reaction of HCA

HCA	$k(SO_4^{\bullet -} + HCA) \times 10^9$ $M^{-1} s^{-1}$	$k(^3BP^* + HCA) \times 10^9$ $M^{-1} s^{-1}$
Ferulic acid	3.2	7.8
Caffeic acid	3.9	4.5
Sinapic acid	3.6	5.3
Chlorogenic acid	2.0	4.3

Transient absorption spectra were recorded after the LFP of BP in N_2-saturated acetonitrile solution in the presence of HCA. They are similar to those obtained in the absence of HCA, which have absorption peaks at 310 and 520 nm. The transient species with maximum absorption at 520 nm is attributed to the triplet state of BP (^{3}BP*) [21]. The decay of the transient absorbance monitored at 520 nm was analyzed and are shown in Fig. 5. As shown in Fig. 5, decay of ^{3}BP* recorded at 520 nm is accelerated by the addition of HCA and follows a first-order kinetics, which means the reaction of HCA with ^{3}BP* occurred. We infer that HCA radicals are formed via electron transfer from HCA to ^{3}BP* according to previous results by Adam et al. [22].

$$BP \xrightarrow{hv} {}^1BP* \xrightarrow{ISC} {}^3BP*$$

$$^3BP* + HCA \rightarrow BP^{\bullet -} + HCA^{\bullet +}$$

Insets of Fig. 5 show the dependence of the decay rate of ^{3}BP* on the concentrations of HCA. From the slope of the straight line, the second-order rate constants for quenching ^{3}BP* by HCA have been evaluated and are given in Table 1.

The rate constants obtained for the quenching of ^{3}BP* by HCA are close to the diffusion-controlled limit. Above results indicated that HCA can protect against ^{3}BP*-induced damage to biomolecules by quenching ^{3}BP* efficiently.

The present investigation revealed that caffeic acid (two hydroxyl groups) is more effective than sinapic acid and ferulic acid (one hydroxyl group) in aqueous solution. Methoxy substitutions in positions *ortho* to the hydroxyl group result in an increase in the scavenging

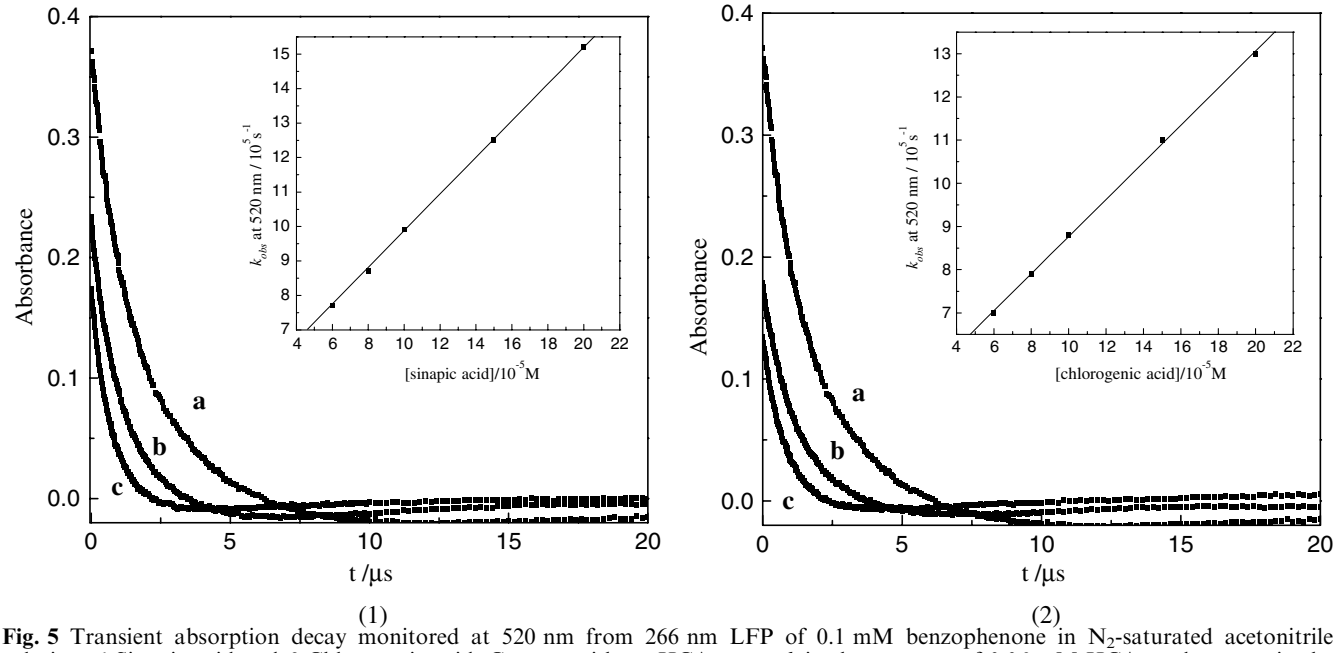

Fig. 5 Transient absorption decay monitored at 520 nm from 266 nm LFP of 0.1 mM benzophenone in N_2-saturated acetonitrile solution: *1* Sinapic acid and *2* Chlorogenic acid. Curve *a* without HCA, curve *b* in the presence of 0.06 mM HCA, and curve *c* in the presence of 0.15 mM HCA. *Inset* Dependence of ^{3}BP* decay rate constant k_{obs} at 520 nm on concentration of HCA

reaction: sinapic acid is more effective than ferulic acid in the aqueous phase. Minimum antioxidant capacity for chlorogenic acid is observed in both cases which shows that steric hindrance is another factor that might influence the efficacy. These observations indicate that the free radical scavenging effects of phenolic acids was influenced by the numbers and the sites of the hydroxyl group. Moreover, the existence of methoxyl groups also plays an important role. Thus, the antioxidant capacity is influenced by molecular structure, solvent, and other factors, which means that besides the antioxidant capacity of the compound itself, the environment in which the antioxidant has to execute its function is important.

As we know, the antioxidants form an intricate network. The synergistic interactions of antioxidants play an important role in the prevention of oxidative damage [23]. For example, vitamin E is the primary chain breaking antioxidant in the inhibition of lipid peroxidation. Vitamin C has the ability to reduce vitamin E radicals, thereby increasing the antioxidant capacity of vitamin E. HCA were found to recycle lipoic acids by donating electrons to lipoic acid radical cations [24]. The antagonistic effects or masking effects were also found [25]. The results presented here, along with those summarized above, indicate that the effect of antioxidants is often executed in complex biological mixtures where various interactions may take place.

Conclusions

In this study, we investigated the antioxidant properties of HCA using the LFP technique. HCA act as scavenger of radicals and quencher of triplet states via electron transfer mechanisms. Results show that there are two possible mechanisms by which HCA can inhibit radiation-mediated damage: (1) scavenging radicals and (2) quenching oxidative triplet states. Our present findings will serve as a guiding principle for the nutrition and disease therapy. Though the mechanism of the antioxidant action in biological environment is unclear, the advances in antioxidant research will hopefully turn more and more basic knowledge into practical use.

Acknowledgements This work is supported by the National Natural Science Foundation of China (NO. 20373086).

References

1. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247
2. Hawkins CL, Davies MJ (2001) Generation and propagation of radical reactions on proteins. Biochim Biophys Acta 1504: 96–219
3. Sohal RS (2002) Role of oxidative stress and protein oxidation in the aging process. Free Radic Biol Med 33:37–44
4. Atoui AK, Mansouri A, Boskou G, Kefalas P (2005) Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chem 89:27–36
5. Stein TP (2002) Space flight and oxidative stress. Nutrition 18:867–871
6. Ames BN, Shigenaga MK, Hagen TM (1993) Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922
7. Reiter RJ (1998) Oxidative damage in the central nervous system: protection by melatonin. Prog Neurobiol 56:359–384
8. Simonsen LC, Wilson JW, Kim MH, Cucinotta FA (2000) Radiation exposure for human Mars exploration. Health Phys 79:515–525
9. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants and nutrition. Nutrition 18:872–879
10. Pannala AS, Razaq R, Halliwell B, Singh S, Rice-Evans CA (1998) Inhibition of peroxynitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation?. Free Radic Biol Med 24:594–606
11. Lodovici M, Guglielmi F, Meoni M, Dolara P (2001) Effect of natural phenolic acids on DNA oxidation in vitro. Food Chem Toxicol 39:1205–1210
12. Foley S, Navaratnam S, McGarvey DJ, Land EJ, Truscott TG, Rice-Evans CA (1999) Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Radical Biol Med 26:1202–1208
13. Lin WZ, Navaratnam S, Yao SD, Lin NY (1998) Antioxidative properties of hydroxycinnamic acid derivatives and a phenylpropanoid glycoside. A pulse radiolysis study. Radiat Phys Chem 53:425–430
14. Lu CY, Yao SD, Lin NY (2001) Studies on reactions of oxidizing sulfur-sulfur three-electron-bond complexes and reducing α-amino radicals derived from OH reaction with methionine in aqueous solution. Biochim Biophys Acta 1525:89–96
15. Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbutya NA, Varfolomeev SD (1993) Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant Properties. FEBS Lett 329:21–24
16. Youdim KA, Shukitt-Hale B, MacKinnon S, Kalt W, Joseph JA (2000) Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. Biochim Biophys Acta 1523:117–122
17. Hsieh CL, Yen GC, Chen HY (2005) Antioxidant activities of phenolic acids on ultraviolet radiation-induced erythrocyte and low density lipoprotein oxidation. J Agric Food Chem 53:6151–6155
18. Klopotek Y, Otto K, Böhm V (2005) Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. J Agric Food Chem 53:5640–5646
19. Zuo ZH, Yao SD, Luo J, Wang WF, Zhang JS, Lin NY (1992) Laser photolysis of cytosine, cytidine and dCMP in aqueous solution. J Photochem Photobiol B, Biol 15:215–222
20. Lhiaubet V, Paillous N, Chouini-Lalanne N (2001) Comparison of DNA damage photoinduced by ketoprofen, fenofibric acid and benzophenone via electron and energy transfer. Photochem Photobiol 74:670–678
21. Stewart LC, Carlsson DJ, Wiles DW, Scaiano JC (1983) Triplet quenching by tert-butyl hydroperoxide. J Am Chem Soc 105:3605–3609
22. Adam W, Arnold MA, Nau WM, Pischel U, Saha-Mőller CR (2002) A comparative photomechanistic study (spin trapping, EPR spectroscopy, transient kinetics, photoproducts) of nucleoside oxidation (dG and 8-oxodG) by triplet-excited acetophenones and by the radicals generated from 8-oxy-substituted derivatives through Norrish-type I cleavage. J Am Chem Soc 124:3893–3904
23. Packer L, Witt EH, Tritschler HJ (1995) Alpha-lipoic acid as a biological antioxidant. Free Radic Biol Med 19:227–250
24. Lu CY, Liu YY (2002) Interactions of lipoic acid radical cations with vitamins C and E analogue and hydroxycinnamic acid derivatives. Arch Biochem Biophys 406:78–84
25. Arts MJTJ, Haenen GRMM, Voss HP, Bast A (2001) Masking of antioxidant capacity by the interaction of flavonoids with protein. Food Chem Toxicol 39:787–791