

# Spectrophotometric studies of the reaction of quercetin with peroxynitrite at different pH

Research Article

Lidia Gebicka\*, Katarzyna Stawowska

Institute of Applied Radiation Chemistry,  
Technical University of Łódź, Wroblewskiego 15, 93-590 Łódź, Poland

Received 19 April 2011; Accepted 29 September 2011

**Abstract:** Peroxynitrite (ONOOH/ONOO<sup>-</sup>) which is formed *in vivo* under oxidative stress is a strong oxidizing and nitrating agent. It has been reported that several flavonoids, including quercetin, inhibit the peroxynitrite-induced oxidation and/or nitration of several molecules tested; however, the mechanism of their protective action against peroxynitrite is not univocally resolved. The kinetics of the reaction of quercetin with peroxynitrite was studied by stopped-flow as well as by conventional spectrophotometry under acidic, neutral and alkaline pH. The obtained results show that the protective mechanism of quercetin against peroxynitrite toxicity cannot be explained by direct scavenging of peroxynitrite. We propose that quercetin acts via scavenging intermediate radical products of peroxynitrite decomposition (it is an excellent scavenger of •NO<sub>2</sub>) and/or via reduction of target radicals formed in the reaction with peroxynitrite.

**Keywords:** Autoxidation • Bicarbonate • Flavonoids • Peroxynitrite • Quercetin

© Versita Sp. z o.o.

## 1. Introduction

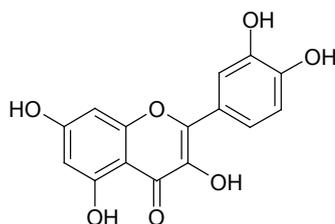
Peroxynitrite is the product of the diffusion controlled reaction between nitric oxide (•NO) and the superoxide anion (O<sub>2</sub><sup>-</sup>). The term peroxynitrite is used to refer to both peroxynitrite anion (ONOO<sup>-</sup>) and peroxynitrous acid (ONOOH). The peroxynitrite anion is stable, but ONOOH (pK<sub>a</sub> = 6.8) rapidly (k = 1.2 s<sup>-1</sup> at 25°C [1]) isomerizes to nitrate, partially (about 30%) via intermediate radical products, •NO<sub>2</sub> and •OH [2,3]. Peroxynitrite may oxidize biologically important molecules either directly or via intermediate radicals. It is also a strong nitrating agent. The participation of peroxynitrite in many pathological events has been reported [4 and references therein]. On the other hand, peroxynitrite has been identified as a key cytotoxic effector of immune system cells towards invading bacteria and parasites [5]. One of the major targets of peroxynitrite *in vivo* seems to be CO<sub>2</sub>, present in biological media at millimolar concentration. This reaction leads to the formation of an adduct, ONOOCO<sub>2</sub><sup>-</sup> (k = 3×10<sup>4</sup> M<sup>-1</sup> s<sup>-1</sup> at 24°C [6]), which rapidly undergoes homolysis to form CO<sub>3</sub><sup>-</sup> and •NO<sub>2</sub> with the yield of about 30% [7 and references therein]. The CO<sub>3</sub><sup>-</sup>/•NO<sub>2</sub>

radical pair possesses a stronger nitration potential than the •OH/•NO<sub>2</sub> radical pair formed from the isomerization of peroxynitrous acid.

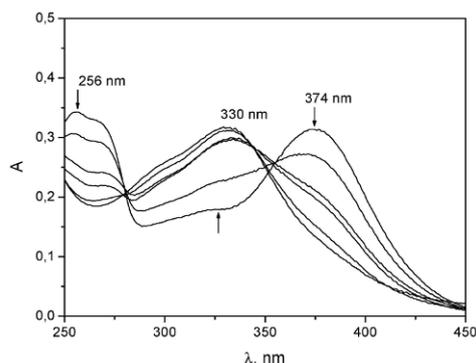
Flavonoids are plant polyphenols and are present as common components in human diet. Their antioxidant properties has been extensively studied [8 and references therein]. It has been reported that, among others, several flavonoids inhibit different peroxynitrite-mediated injuries [9 and references therein]. However, their protective mechanism of action against peroxynitrite is not univocally resolved. It has been suggested that flavonoids do not react directly with peroxynitrite but rather with reactive intermediates from peroxynitrite decomposition [10] or with radicals formed in the reaction of peroxynitrite with several molecules previously investigated (e.g. tyrosyl radicals) [11]. On the other hand, Kono *et al.* have concluded from a competitive kinetic analysis that peroxynitrite reacts directly with chlorogenic acid, a polyphenolic non-flavonoid compound [12].

In the present study the kinetics of the reaction of peroxynitrite with quercetin at neutral and alkaline pH is investigated by stopped-flow as well as by conventional spectroscopy. We have selected quercetin (3,3',4', 5,7 –

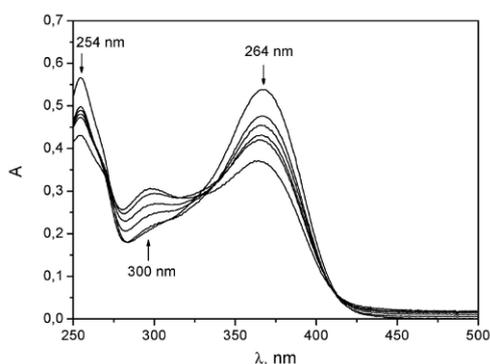
\* E-mail: lgebicka@mitr.p.lodz.pl



**Scheme 1.** Structure of quercetin (3,3',4',5,7-pentahydroxyflavone).



**Figure 1.** Absorption spectra of 17  $\mu\text{M}$  quercetin taken after 1 min incubation with peroxynitrite at concentrations: 0, 25, 50, 75, 100 and 150  $\mu\text{M}$ , at pH 7.2.



**Figure 2.** Absorption spectra of 20  $\mu\text{M}$  quercetin taken after 0.5 min incubation with peroxynitrite at concentrations: 0, 50, 100, 150, 200 and 250  $\mu\text{M}$ , at pH 5.0.

pentahydroxyflavone, Scheme 1) as a model flavonoid, because it is one of the most efficient flavonoids for inhibiting peroxynitrite-induced oxidation and/or nitration of previously tested molecules [13-16].

## 2. Experimental procedure

A stock solution of quercetin (Sigma) was prepared in ethanol. The concentration of quercetin was determined spectrophotometrically using the extinction coefficient  $\epsilon_{374} = 2.84 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  [17]. Peroxynitrite was synthesized by the reaction of nitrite with hydrogen peroxide under acidic conditions [18]. Unreacted

hydrogen peroxide was removed from peroxynitrite solution by treatment with solid manganese dioxide whereas nitrite remained as a contaminant. The final concentration of peroxynitrite was equal to 25-50 mM as determined spectrophotometrically using the extinction coefficient  $\epsilon_{302} = 1670 \text{ M}^{-1} \text{ cm}^{-1}$  [19]. The stock solutions of  $\text{ONOO}^-$  at pH 12 were stored at  $-25^\circ \text{C}$  and used within 3-4 weeks of synthesis. Quercetin, nitrite and hydrogen peroxide (30%) were obtained from Sigma. All others chemicals were of analytical grade. Nano-pure water from MilliQ (Millipore) was used throughout.

The reactions of peroxynitrite with quercetin were studied at pH 5.0, 6.0, 7.2 and 11. Peroxynitrite solutions were brought to the desired concentrations by diluting the stock solution with 0.01 M NaOH. Quercetin solutions were prepared in 0.2 M acetate or phosphate buffers of a desired pH and were bubbled with nitrogen in order to remove traces of  $\text{CO}_2$ . In some experiments 40 mM bicarbonate was added to quercetin solution. Solutions used in experiments taken at pH 11.0 were saturated with argon for 2 h in a glove box.

Kinetic measurements on millisecond- and second-time scales were performed using the SX-20 MV Applied Photophysics stopped-flow spectrophotometer with 1-cm cell and with a mixing time  $< 1$  ms. In some experiments, the photodiode-array accessory (PDA1, covering the spectral range 325-700 nm) attached to the stopped flow machine was used.

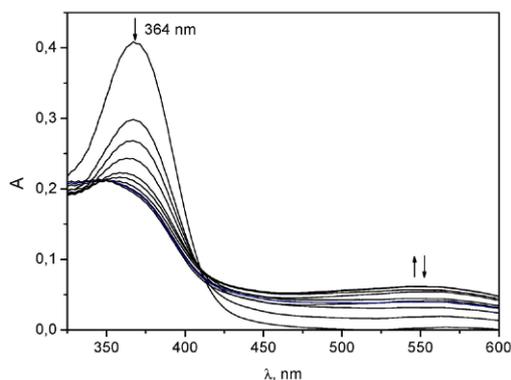
Kinetic measurements in the long-time regime were recorded using a Specord S600 diode-array spectrophotometer.

All kinetic measurements were carried out at  $23 \pm 0.5^\circ \text{C}$  and were repeated at least three times.

Kinetic simulations were performed with the use of Chemical Kinetic Simulator (CKS) 1.01 (IBM Almaden Research Center).

## 3. Results and discussion

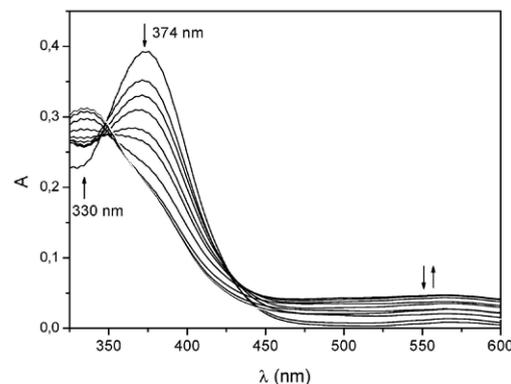
The absorption spectra obtained after mixing quercetin with peroxynitrite at pH 7.2 and 5.0 are shown in Figs. 1 and 2, respectively. The addition of peroxynitrite to a solution of quercetin at pH 7.2 causes a decrease in the quercetin absorption bands at 256 and 374 nm and build up of a new band at 330 nm. The latter is probably connected with the formation of a quercetin quinone-water adduct [20]. Similar absorption spectra have been reported for enzymatically oxidized quercetin [21,22]. The addition of peroxynitrite to a solution of quercetin at pH 5.0 and 6.0 (data not shown) causes a decrease in the quercetin absorption bands at 254 and 364 nm and a build up of a new band around 300 nm. It should be



**Figure 3.** Time-resolved spectra of 15  $\mu\text{M}$  quercetin taken 0, 0.2, 0.6, 1, 1.6, 2, 3, 5, 7, and 9 seconds after mixing with 100  $\mu\text{M}$  peroxyntirite at pH 6.0.

stressed that at pH below 7, quercetin is fully protonated [23], which affects its absorption spectrum. The pH variations can also modify the nature of quercetin oxidation products. For example, under acidic conditions the absorption spectrum of enzymatically oxidized quercetin exhibits a maximum at 293 nm [24,25].

The kinetics of the reaction of quercetin with peroxyntirite at different pH was studied by a stopped-flow method. We did not observe measurable changes in the absorption spectrum of quercetin during the reaction with peroxyntirite under conditions when quercetin (15–20  $\mu\text{M}$ ) was in excess or at comparable concentration with that of peroxyntirite. Thus, we studied the kinetics of this reaction under conditions at which peroxyntirite was in excess. The rates and yields of peroxyntirite-induced absorption changes in quercetin were dependent on peroxyntirite concentration. The time-resolved absorption spectra taken after mixing quercetin with a 5-fold excess of peroxyntirite at pH 6.0 and 7.2 are shown in Figs. 3 and 4, respectively. Characteristic absorption peaks of quercetin at 364 nm (pH 6.0) and 374 nm (pH 7.2) disappear and new bands in the UV (with a maximum dependent on pH) and visible regions (very broad one, with a maximum around 550 nm) are formed. The latter band disappears in a matter of seconds (Fig. 5). The kinetics of the decay of 364 or 374 nm absorption bands is complex and we are not able to resolve it. The higher peroxyntirite concentration, the faster and the higher were the observed absorption changes of quercetin. Surprisingly, the fastest absorption changes were detected at pH 6.0. The obtained results may be explained by the known facts that a variety of products are formed as a result of quercetin oxidation depending on pH [22,25,26]. On the other hand, both stages of kinetic traces (an absorption increase was followed by an absorption decrease) recorded at 550 nm could be fitted with a single exponential equation and the obtained rate constants ( $k_i$  and  $k_d$ )



**Figure 4.** Time-resolved spectra of 15  $\mu\text{M}$  quercetin taken 0, 0.2, 0.6, 1, 1.6, 2, 3, 5, 7, and 9 seconds after mixing with 100  $\mu\text{M}$  peroxyntirite at pH 7.0.

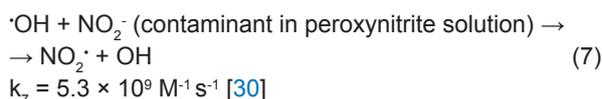
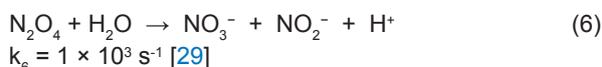
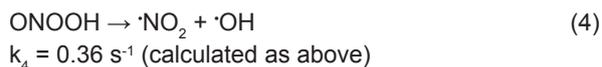
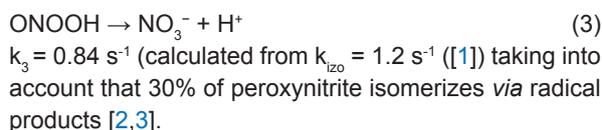
depend on peroxyntirite concentration. The values of these rate constants determined at different pH are summarized in Table 1. These absorption changes are probably connected with the formation and decay of an unstable free quinone [20].

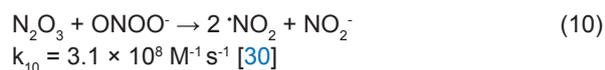
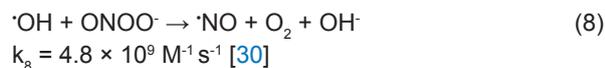
To simulate the kinetic traces obtained from stopped-flow measurements, the following processes were included:

pH-dependent peroxyntirous acid/peroxyntirite anion ratio:

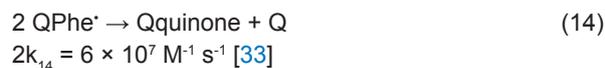
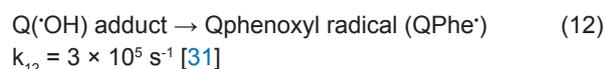
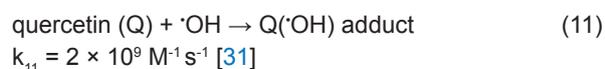


isomerization of peroxyntirite including radical formation and decay:





reactions of quercetin with  $\cdot\text{OH}$  and  $\cdot\text{NO}_2$  and disproportionation of quercetin radicals:

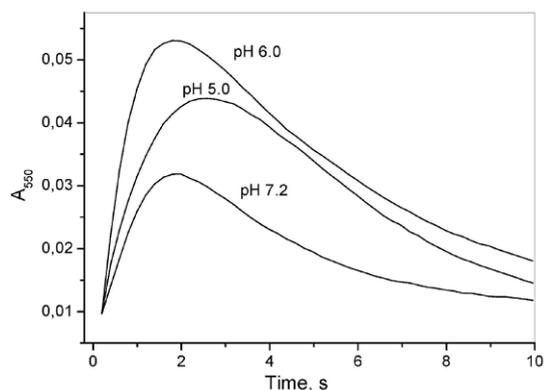


and reaction of quercetin with peroxyxynitrous acid:

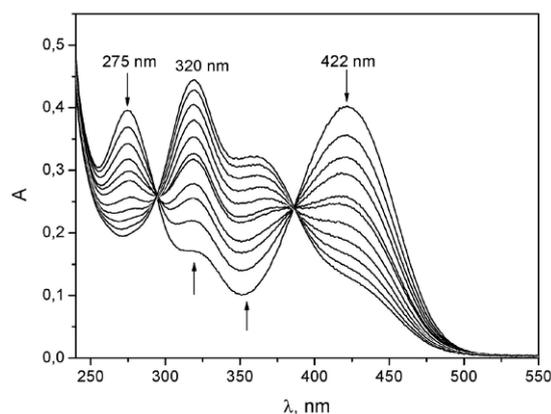


The simulations showed that introducing the rate constant of the reaction of 20  $\mu\text{M}$  quercetin with equimolar amounts of peroxyxynitrite at pH 7.2 equal to  $10^3 \text{ M}^{-1} \text{ s}^{-1}$  yielded a concentration of the product for this reaction of about 0.3  $\mu\text{M}$ , which corresponds to absorption changes at 374 nm of < 0.01. Recalling the fact that we did not detect measurable changes in the absorption spectrum of 20  $\mu\text{M}$  quercetin after mixing it with peroxyxynitrite at comparable concentration, it seems that the value of  $10^3 \text{ M}^{-1} \text{ s}^{-1}$  represents the upper limit for the rate constant of this reaction, if it takes place at all.

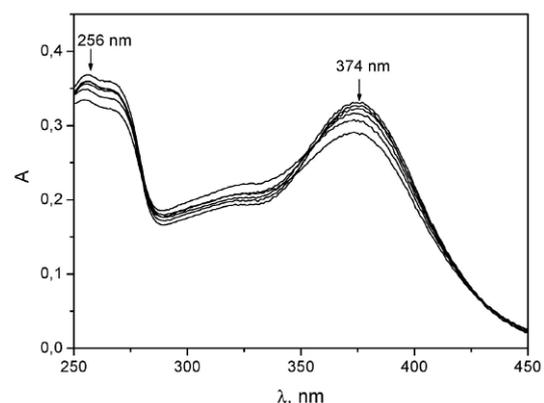
In order to check whether peroxyxynitrite anion is involved in the reaction with quercetin, we studied this reaction at pH 11.0, where peroxyxynitrite exists exclusively in its anionic form. As quercetin in alkaline solution, in the presence of air, is very unstable due to autoxidation process [20,34], all reagents were deoxygenated prior to mixing. An absorption spectrum taken immediately after alkalization of quercetin solution up to pH 11.0 shows two absorption peaks, at 275 and 422 nm, characteristic for deprotonated quercetin [34]. In spite of deoxygenation, this spectrum decays in tens of minutes and a new spectrum of oxidized



**Figure 5.** Kinetic traces observed at 550 nm after mixing 15  $\mu\text{M}$  quercetin with 100  $\mu\text{M}$  peroxyxynitrite at pH 5.0, 6.0 and 7.2.



**Figure 6.** Absorption spectra of 20  $\mu\text{M}$  quercetin at pH 11.0 taken every 2 min after alkalization of quercetin solution.



**Figure 7.** Absorption spectra of 15  $\mu\text{M}$  quercetin taken after 1 min incubation with 40 mM bicarbonate and peroxyxynitrite at concentrations: 0, 25, 50, 75, 100 and 150  $\mu\text{M}$ , at pH 7.2.

quercetin, with absorption maximum at 320 nm and a shoulder around 365 nm is formed. Two isosbestic points at 294 and 386 nm are observed (Fig. 6). Most probably, the concentration of residual oxygen was enough to initiate the oxidation of quercetin. When peroxyxynitrite

**Table 1.** The observed first-order rate constants ( $s^{-1}$ ) of quercetin absorbance changes at 550 nm induced by peroxyntirite at different pH as a function of peroxyntirite concentration. [quercetin] = 20  $\mu$ M.

[peroxyntirite], mM	pH 5.0		pH 6.0		pH 7.2	
	$k_i$	$k_d$	$k_i$	$k_d$	$k_i$	$k_d$
<b>0.05</b>	0.90	0.19	1.32	0.15	0.84	0.07
<b>0.1</b>	1.22	0.25	1.72	0.20	1.12	0.08
<b>0.15</b>	1.25	0.30	3.20	2.16	1.63	0.43
<b>0.2</b>	1.32	0.34	7.20	2.97	2.67	1.12

anion was added to an alkaline quercetin solution, the rate of quercetin oxidation increased with an increase of the concentration of peroxyntirite. In order to verify whether an increased rate of quercetin oxidation is the consequence of the quercetin reaction with peroxyntirite anion or results only from the increased ionic strength [20], we obtained time-resolved spectra of quercetin at pH 11.0 in the presence of  $\text{NaNO}_2$  (peroxyntirite contaminant) or NaCl at the same ionic strength as that of peroxyntirite solution. The rate of quercetin oxidation measured at 422 nm did depend on ionic strength in an identical manner as for  $\text{NaNO}_2$ , NaCl and peroxyntirite. Isosbestic points (one near the maximum of the absorption spectrum of peroxyntirite anion) were detected in all cases. The above observations suggest that the peroxyntirite anion does not react with quercetin.

The results obtained in these studies do not allow for a definitive conclusion regarding whether quercetin reacts directly with peroxyntirous acid or not. We can only speculate, based on the kinetic simulation (see above), that even if peroxyntirite reacts directly with quercetin, the rate constant of this reaction is not higher than  $10^3 \text{ M}^{-1} \text{ s}^{-1}$ . If this is the case, the combination of such a small rate constant with the fact that the concentration of quercetin is relatively low *in vivo* [35], indicates that this reaction is practically insignificant.

Studies *in vitro* have shown that quercetin is very effective in protecting against peroxyntirite-induced tyrosine nitration and oxidation of dihydrorhodamine 123 ( $\text{RhH}_2$ ) [13–16]. For example, the yield of oxidation of 25  $\mu$ M  $\text{RhH}_2$  by 1.2  $\mu$ M peroxyntirite at pH 7.4 is lowered by almost a factor of two in the presence of 1  $\mu$ M quercetin [15]. It is quite likely that  $\text{RhH}_2$  is oxidized by peroxyntirite indirectly, *i.e.*, *via* the radical products [36]. Under such conditions, the protective mechanism of quercetin against peroxyntirite toxicity cannot be explained by the direct scavenging of peroxyntirite (see discussion above). Both  $\text{RhH}_2$  and quercetin react with hydroxyl radicals with rate constants close to the diffusion-controlled limit [31,37]. The rate constant of the reaction of quercetin with  $\cdot\text{NO}_2$

(reaction 15) is, however, significantly higher than that reported for  $\text{RhH}_2$  ( $\sim 4\text{--}7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  [38]). Thus quercetin may protect dihydrorhodamine against peroxyntirite via scavenging  $\cdot\text{NO}_2$  radicals. It should be noted that the rate constants of the reactions of some other flavonoids with  $\cdot\text{NO}_2$  are also significantly higher than those reported for other molecules [31,32,39,40]. In an aerated solution, dihydrorhodamine radicals ( $\text{RhH}\cdot$ ) react with oxygen with a rate constant of  $\sim 7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  [38] to form unstable peroxy radicals ( $\text{RhHOO}\cdot$ ). It has been suggested that these radicals eliminate  $\text{O}_2\cdot^-$  which reacts with  $\cdot\text{NO}_2$  to form peroxyntirite at a diffusion controlled rate [38]. Most likely, under conditions described by Santos and Mira [15], quercetin also scavenges  $\text{RhHOO}\cdot$  radicals before they eliminate  $\text{O}_2\cdot^-$ . Quercetin and some others flavonoids are very efficient scavengers of peroxy radicals [41].

Considering the physiological relevance of the reaction between carbon dioxide and peroxyntirite we also studied the absorption spectra of quercetin incubated with bicarbonate and peroxyntirite. Under such reaction conditions peroxyntirite was scavenged by  $\text{CO}_2$  and  $\cdot\text{NO}_2$  and  $\text{CO}_3\cdot^-$  radicals were formed as intermediate products. These radicals could decay via biradical reactions or via reaction with quercetin. Taking into account all the possible reaction routes, their rate constants [32,39,42] and concentrations of reagents, we concluded that some of  $\cdot\text{NO}_2$  and  $\text{CO}_3\cdot^-$  radicals could react with quercetin. Fig. 7 shows the absorption spectra of quercetin incubated with peroxyntirite and bicarbonate. The characteristic quercetin bands at 256 and 374 nm decrease with an increase of peroxyntirite concentration, but the yield of quinone formation is very low, if any. This could mean that other stable products of quercetin oxidation are formed under such conditions (e.g. nitrated quercetin) [16]. It has been shown that the protective effect of quercetin and some other flavonoids against the peroxyntirite-mediated nitration of tyrosine and oxidation of dihydrorhodamine diminishes in the presence of physiological concentrations of bicarbonate [15,43].

## 4. Conclusions

The results presented above indicate that the protective mechanism of quercetin against peroxynitrite toxicity cannot be explained by the direct scavenging of peroxynitrite. We propose that quercetin acts via scavenging the intermediate radical products of peroxynitrite decomposition (it is an excellent scavenger of  $\cdot\text{NO}_2$ ) and/or *via* the reduction of target radicals formed in the reaction with peroxynitrite.

## Acknowledgement

This work was supported by the Ministry of Science and Higher Education, Grant NN 312 158 034 and by the COST Action CM 0603.

## References

- [1] W.H. Koppenol, R. Kissner, *Chem. Res. Toxicol.* 11, 87 (1998)
- [2] O.V. Gerasimov, S.V. Lyamar, *Inorg. Chem.* 38, 4317 (1999)
- [3] G.R. Hodges, K.U. Ingold, *J. Am. Chem. Soc.* 121, 10695 (1999)
- [4] P. Pacher, J.S. Beckman, L. Liaudet, *Physiol. Rev.* 87, 315 (2007)
- [5] G. Ferrer-Sueta, R. Radi, *ACS Chem. Biol.* 4, 161 (2009)
- [6] S.V. Lyamar, J.K. Hurst, *J. Am. Chem. Soc.* 117, 8867 (1995)
- [7] O. Augusto, M.G. Bonini, A.M. Amanso, E. Linares, C.C.X. Santos, S.L. de Menezes, *Free Radic. Biol. Med.* 32, 841 (2002)
- [8] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, *J. Nutr. Biochem.* 13, 572 (2002)
- [9] M. Trujillo, G. Ferrer-Sueta, R. Radi, *Antiox. Redox. Sign.* 10, 1 (2008)
- [10] G.E. Arteel, P. Schroeder, H. Sies, *J. Nutr.* 130, 2100S (2000)
- [11] P. Schroeder, H. Zhang, L.-O. Klotz, B. Kalyanaraman, H. Sies, *Biochim. Biophys. Res. Commun.* 289, 1334 (2001)
- [12] Y. Kono, K. Kobayashi, S. Tagawa, K. Adachi, A. Ueda, Y. Sawa, H. Shibata, *Biochim. Biophys. Acta* 1335, 335 (1997)
- [13] G.R.M.M. Haenen, J.B.G. Paquay, R.E.M. Korthouwer, A. Bast, *Biochem. Biophys. Res. Commun.* 236, 591 (1997)
- [14] J.S. Choi, H.Y. Chung, S.S. Kang, M.J. Jung, J.W. Kim, J.K. No, H.A. Jung, *Phytother. Res.* 16, 232 (2002)
- [15] M.R. Santos, L. Mira, *Free Rad. Res.* 38, 1011 (2004)
- [16] M. Sadeghipour, R. Terreux, J. Phipps, *Toxicol. in Vitro* 19, 155 (2005)
- [17] X. Chen, D.U. Ahn, *J. Am. Oil Chem. Soc.* 75, 1717 (1998)
- [18] J.S. Beckman, J. Chen, H. Ischiropoulos, J.P. Crow, *Methods Enzymol.* 233, 229 (1994)
- [19] M.N. Huges, H.G. Nicklin, *J. Chem. Soc.* 450 (1968)
- [20] O. Dangles, C. Dufour, S. Bret, *J. Chem. Soc. PerkinTrans 2*, 737 (1999)
- [21] D. Metodiewa, A.K. Jaiswal, N. Cenas, E. Dickancaite, J. Segura-Aguilar, *Free Radic. Biol. Med.* 26, 107 (1999)
- [22] A. Zhou, O.A. Sadik, *J. Agric. Food Chem.* 56, 12081 (2008)
- [23] J.M. Herrero-Martinez, M. Sanmartin, M. Roses, E. Bosch, C. Rafols, *Electrophoresis* 26, 1886 (2005)
- [24] U. Takahama, T. Oniki, S. Sirota, *J. Agric. Food Chem.* 50, 4317 (2002)
- [25] S. Hirota, U. Takahama, T.N. Ly, R. Yamauchi, *J. Agric. Food Chem.* 53, 3265 (2005)
- [26] H.M. Awad, M.G. Boersma, J. Vervoort, I.M.C.M. Rietjens, *Arch. Biochem. Biophys.* 32, 224 (2000)
- [27] R. Kissner, T. Nauser, C. Kurz, W.H. Koppenol, *IUMB Life* 55, 567 (2003)
- [28] R.K. Broszkiewicz, *Bull. Acad. Sci. Sedr. Sci. Chim.* 24, 221 (1976)
- [29] S.E. Schwartz, W.H. White, *Adv. Environ. Sci. Technol.* 12, 1 (1983)
- [30] S. Goldstein, J. Lind, G. Merenyi, *Chem. Rev.* 105, 2457 (2005)
- [31] A. Torreggiani, A. Trincherro, M. Tamba, P. Taddei, *J. Raman Spectrosc.* 36, 380 (2005)
- [32] C.Y. Zhao, Y.M. Shi, S.D. Yao, Z.J. Jia, B.T. Fan, W.F. Wang, W.Z. Lin, N.Y. Lin, R.L. Zheng, *Pharmazie* 58, 742 (2003)
- [33] W. Bors, C. Michel, S. Schikora, *Free Rad. Biol. Med.* 19, 45 (1995)
- [34] L.-M. Lin, H.-Y. Wu, W.-S. Li, W.-L. Chen, Y.-J. Lee, D.C. Wu, P. Li, A. Yeh, *Inorg. Chem. Commun.* 13, 633 (2010)

- [35] S.K. Nicholson, G.A. Tucker, J.M. Brameld, Br. J. Nutr. 103, 1398 (2010)
- [36] J. Glebska, W.H. Koppenol, Free Rad. Biol. Med. 35, 676 (2003)
- [37] M. Wrona, K. Patel, P. Wardman, Free Rad. Biol. Med. 38, 262 (2005)
- [38] L.K. Folkes, K.B. Patel, P. Wardman, M. Wrona, Arch. Biochem. Biophys. 484, 122 (2009)
- [39] P. Neta, R.E. Huie, A.B. Ross, J. Phys. Chem. Ref. Data 17, 1027 (1988)
- [40] J.-L. Miao, W.-F. Wang, J.-X. Pan, C.-Y. Liu, R.-Q. Li, S.-D. Yao, Radiat. Phys. Chem. 60, 163 (2001)
- [41] W. Bors, C. Michel, M. Saran, Methods Enzymol. 234, 420 (1994)
- [42] S. Herold, M. Exner, F. Boccini, Chem. Res. Toxicol. 16, 390 (2003)
- [43] U. Ketsawatsakul, M. Whiteman, B. Halliwell, Biochem. Biophys. Res. Commun. 279, 692 (2000)