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Hydroperoxyl Radical ($HOO^\bullet$) as a reducing agent: unexpected synergy with antioxidants. A review.

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Keywords

Superoxide, lipid peroxidation, nitroxide, polydopamine, ferroptosis, γ-terpinene

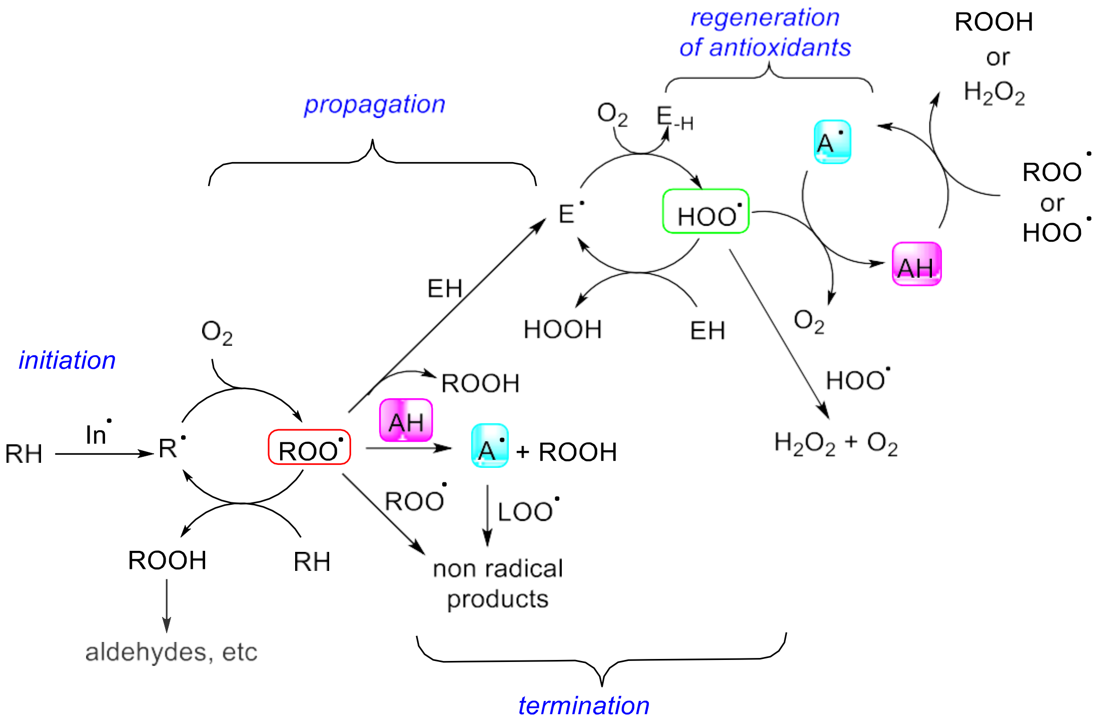
Abstract

This review highlights the progress made in recent years in harnessing the peculiar chemistry of the hydroperoxyl, or perhydroxyl, radical ($HOO^•$) during lipid peroxidation, particularly with regard to its interaction with antioxidants. The $HOO^•$ radical, the protonated form of superoxide, plays an important role in the propagation and termination of lipid peroxidation in nonaqueous systems. However, differently from alkylperoxyl ($ROO^•$) radicals that have only oxidizing ability, $HOO^•$ has a two-faced oxidizing and reducing activity. The $HOO^•$ radical can reduce the radical of the antioxidant (phenols and aromatic amines) by H-atom transfer ($A^• + HOO^• \longrightarrow AH + O_2$) thus increasing the length of the inhibition period and the effectiveness of the antioxidant. The simultaneous presence of $HOO^•$ and $ROO^•$ radicals triggers the catalytic antioxidant activity of quinones and nitroxides, and explains the antioxidant activity of melanin-like polymers. The $HOO^•$ radical can be formed by fragmentation of $ROO^•$ radicals deriving from amines, alcohols, substituted alkenes and may be present at low concentrations in many oxidizing systems. Pro-aromatic compounds, like the natural essential oil component γ-terpinene, are the most effective sources of $HOO^•$, and behave as co-antioxidants in the presence of nitroxides or quinones. The future developments and applications of the $HOO^•$ chemistry in the context of the inhibition of autoxidation are also discussed.

1. Introduction

Autoxidation is a reaction of organic compounds under mild conditions with atmospheric O_2, leading to their deterioration by producing hydroperoxides and other oxygenated compounds. Autoxidation affects not only man-made materials like plastics, cosmetics, pharmaceuticals and food, but also biomolecules in living organism, especially those rich in polyunsaturated lipids; thus, it is of extreme importance in food, polymers and biomedical applications. Strikingly, the base mechanism of the reaction with O_2 of all these materials shares the same mechanistic features, consisting of a radical-chain reaction with the classical initiation, propagation, and termination steps [1-3]. The most effective and convenient way to protect organic substrates from oxygen reactivity, both in nature, as in man-made materials, is to use antioxidants. Depending on the mechanism of inhibition of the autoxidation reaction, antioxidants can be classified between preventive and chain-breaking (or radical-trapping antioxidants, RTA) if they reduce the initiation or the propagation step, respectively [1].

The peroxidation mechanism is well established, and is based on a radical chain mechanism propagated by carbon-centred (alkyl, $R^•$) and oxygen-centred (alkylperoxyl, $ROO^•$) radicals, as shown in Scheme 1 [1].



Scheme 1. Co-oxidation of an organic substrate (RH) with a compound known to oxidize via hydroperoxyl radical (EH), initiated by In$^{\bullet}$ radicals, in the presence of antioxidants (AH), showing the role of hydroperoxyl (HOO$^{\bullet}$) radicals in the regeneration mechanism.

In such system, the autoxidation rate depends on the reactivity of the substrate (RH) with ROO$^{\bullet}$ radicals (propagation step, in Scheme 1 exemplified by a H-atom abstraction), and on the steady state concentration of ROO$^{\bullet}$ radicals. This, in turn, depends on the amount of ROO$^{\bullet}$ generated by the initiation process, and on the decay rate of ROO$^{\bullet}$ radical by self-termination or by reaction with chain-breaking antioxidants (AH). Effective antioxidants must be able to compete with RH for ROO$^{\bullet}$ radicals, thus must have a high inhibition constant (k_{inh}). Moreover, the radical of the antioxidant (A$^{\bullet}$) must not propagate the oxidative chain, and possibly should trap further radicals [4]. This last aspect is very important in real conditions because antioxidants are expected to protect oxidizable materials for a long time. The length of the protection by chain-breaking antioxidants can be increased by agents able to regenerate the active antioxidant by reducing the $A^\bullet$ radical back to AH. For example, it is well known that the antioxidant activity of α-tocopherol in liposomes is prolonged by the presence of water soluble co-antioxidants (like vitamin C) in the water phase [5].

Besides the well-established chemistry of $R^\bullet$ and $ROO^\bullet$ radicals, the hydroperoxyl radical ($HOO^\bullet$) has been recently discovered as an important player in biological systems, in atmospheric pollutant degradation [6,7], in photocatalysis [8], and in organic synthesis [9]. Although the formation of $HOO^\bullet$ during the autoxidation of certain organic substrates was recognized long time ago [10,11], the role of $HOO^\bullet$ radical has been appreciated in its full importance only after the pioneering work by Foti and Ingold on the antioxidant effect of γ-terpinene [12].

$HOO^\bullet$ is known as a source of oxidative damage in biological systems and is essentially similar to alkylperoxyl radicals in H-atom abstraction. On the other hand, the knowledge about the effects of the reducing properties of $HOO^\bullet$ and of its conjugate base $O_2^{\bullet-}$, is still very limited [13-15]. The reducing ability of superoxide has been previously recognized as an important pathway for the repairing of oxidative damage of tyrosyl residues in proteins subject to oxidative stress. For example, by using mass spectrometry it was found that superoxide prevented myoglobin dimer formation by reducing the protein tyrosyl radical to the parent phenol. However, this reaction is also accompanied by the addition of superoxide at Tyr151, fully demonstrating the two-faced reducing and oxidizing character of the $HOO^\bullet/O_2^{\bullet-}$ couple [16].

In the context of the autoxidation reaction, $HOO^\bullet$ is formed typically by the fragmentation of $ROO^•$ radicals of specific oxidizable substrates (EH), which therefore represent a possible source of this radical. As summarized in Scheme 1, and explained in this review, the $HOO^•$ radical can potentially impact on both propagation and termination steps of the autoxidation reaction, as effect of its peculiar chemical characteristics. Furthermore, the $HOO^•$ reactivity with several phenolic and nonphenolic antioxidants (AH) has been somewhat neglected and, only recently, its enhancing effect on their antioxidant activity has been observed.

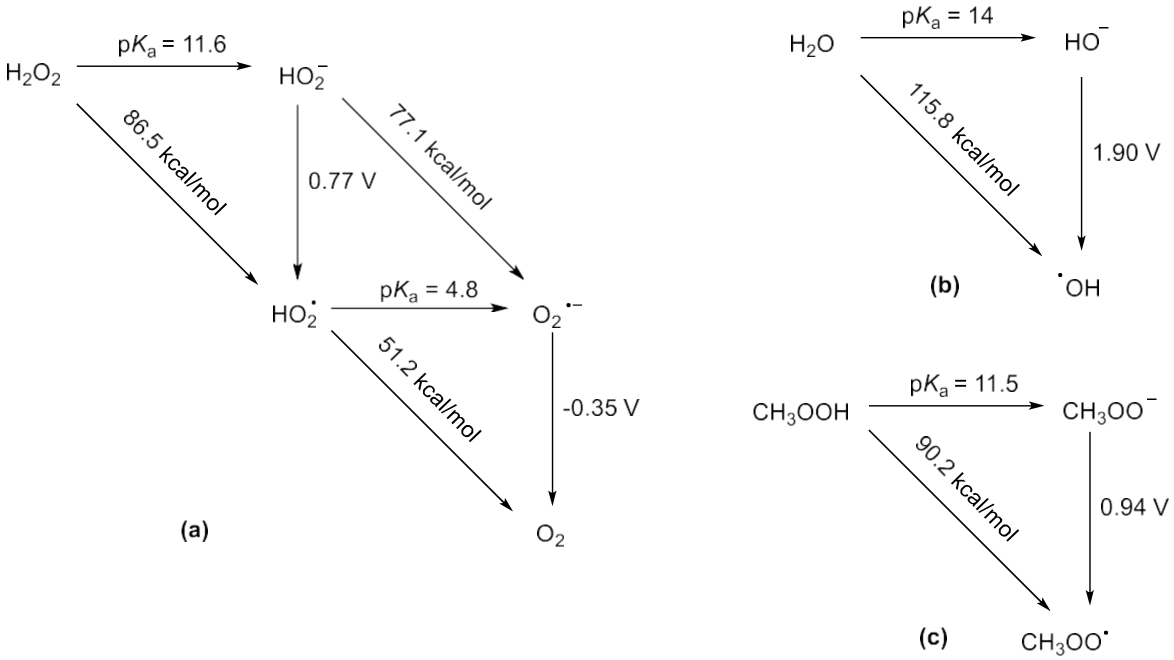
2. Chemical characteristics of hydroperoxyl radical

$HOO^•$ (hydroperoxyl radical), also known as perhydroxyl radical, is the protonated form of superoxide $O_2^{•-}$. In an aqueous environment at physiological pH, $HOO^•$ is mostly in the deprotonated form ($pK_a = 4.8$) [17]. The role of $HOO^•$ / $O_2^{•-}$ in autoxidation reactions is dependent on its ability to abstract or donate a H-atom, and this property in turn is a function of the strength of the bonds being formed (or broken). In order to have a quantitative description of the oxidizing and reducing properties and to compare them with other radicals in solution, we use the Bond Dissociation Free Energy (BDFE) and redox potentials ($E°$) values critically reviewed by Mayer and co-workers, as they represent the most complete source of chemical data for proton-coupled electron transfer (PCET) reagents [17].

2.1 H-atom abstraction by $HOO^•$

The BDFE of the HOO-H bond is 86.5 kcal/mol, that is superimposable to that of the ROO-H bond in alkylperoxyl radicals, that ranges from 90.2 to 86.6 kcal/mol for MeOO-H and

^{t}BuOO-H, respectively (see Scheme 2) [17]. Regarding H-atom abstraction, $HOO^{\bullet}$ behaves similarly to $ROO^{\bullet}$ radicals. The hydroperoxyl radical propagates the oxidative chain in the autoxidation of 1,4-cyclohexadienes and other easily oxidizable substrates [10]. Moreover, $HOO^{\bullet}$ abstracts the phenolic H-atom from α-tocopherol with a $k_H = 1.6\times10^6$ $M^{-1}s^{-1}$ in PhCl [18], that is very similar to that reported for $ROO^{\bullet}$ under identical conditions ($k_H = 2.7\times10^6$ $M^{-1}s^{-1}$) [18].



Scheme 2: Aqueous proton-coupled electron transfer thermochemistry of (a) hydrogen peroxide, (b) H_2O and (c) CH_3OOH, with pK_a values above horizontal arrows, redox potentials (in V *vs* NHE) beside vertical arrows, and BDFE values on diagonal lines. Data from ref. [17].

However, differently from $ROO^{\bullet}$, the H-atom abstracting ability of $HOO^{\bullet}$ in organic solvents is influenced by the formation H-bonds with the solvent. We have shown that the formation of S---$HOO^{\bullet}$ interactions between $HOO^{\bullet}$ and the solvent (**S**) reduces the rate constant of H-atom abstraction from C-H and ArO-H bonds because the H-bond becomes weaker going from the reactants (**S**---$HOO^{\bullet}$ + ArOH) to the products (**S**---HOOH + $ArO^{\bullet}$) [18]. Superoxide, $O_2^{\bullet-}$, is much less active as H-atom abstracting radical than $HOO^{\bullet}$[19], due to the relatively small BDFE of $^{-}$OO-H that is reported as 77.1 kcal/mol in water [17].

2.2. H-atom donation by $HOO^{\bullet}$

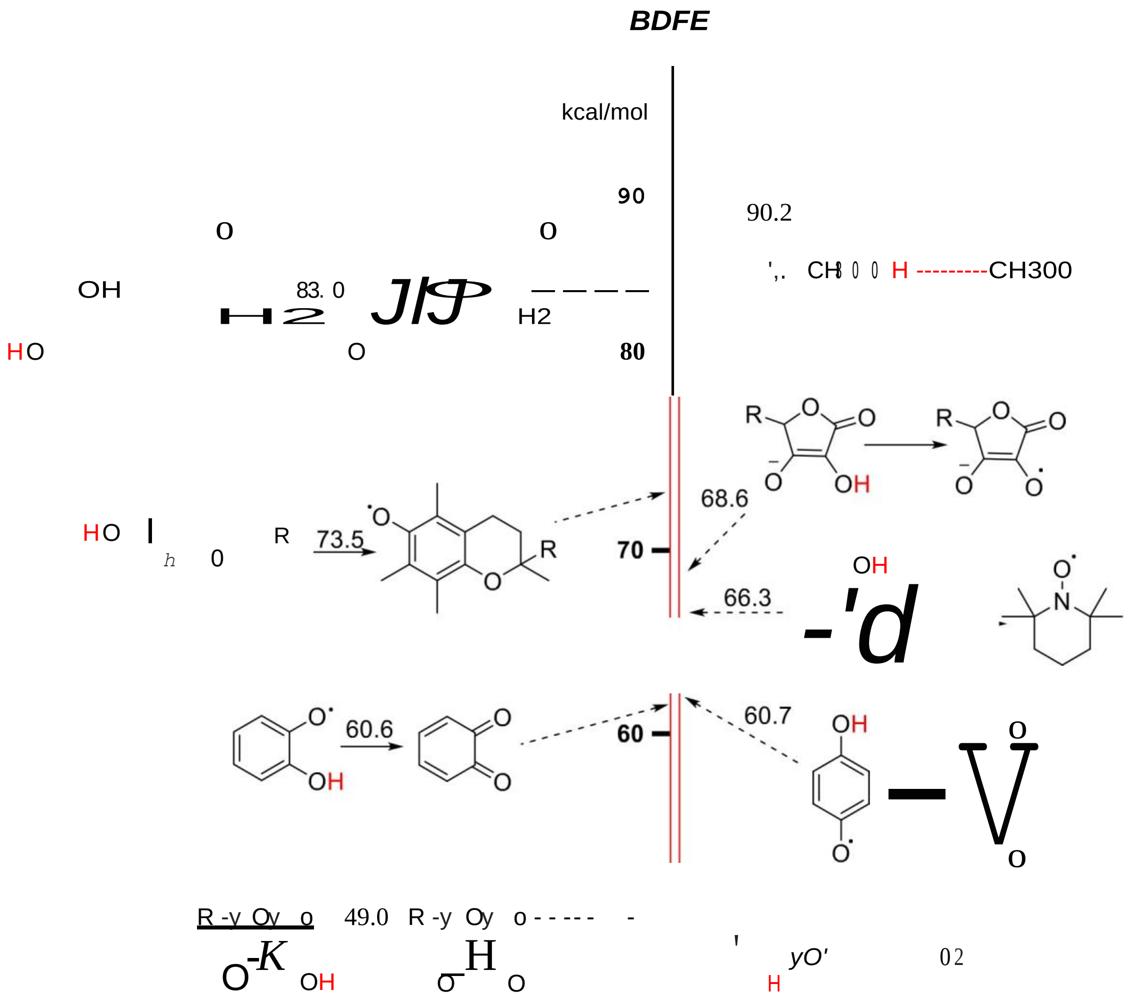
The unusual chemical property of the hydroperoxyl radicals is rooted in its hydrogen-donating ability. The BDFE ($^{\bullet}$OO-H) has been estimated as 51.2 kcal/mol in water and 45.1 kcal/mol in the gas phase [17]. Likewise, $O_2^{\bullet-}$ is a strong one-electron reducing agent, since the $E°(O_2)$ is –0.35 V (*vs* NHE). The H-$OO^{\bullet}$ BDFE is much smaller than that of phenolic and non-phenolic antioxidants (typically from 74 to 84 kcal/mol). This demonstrates from a thermodynamic point of view the possibility of regenerating antioxidants from their radicals [17].

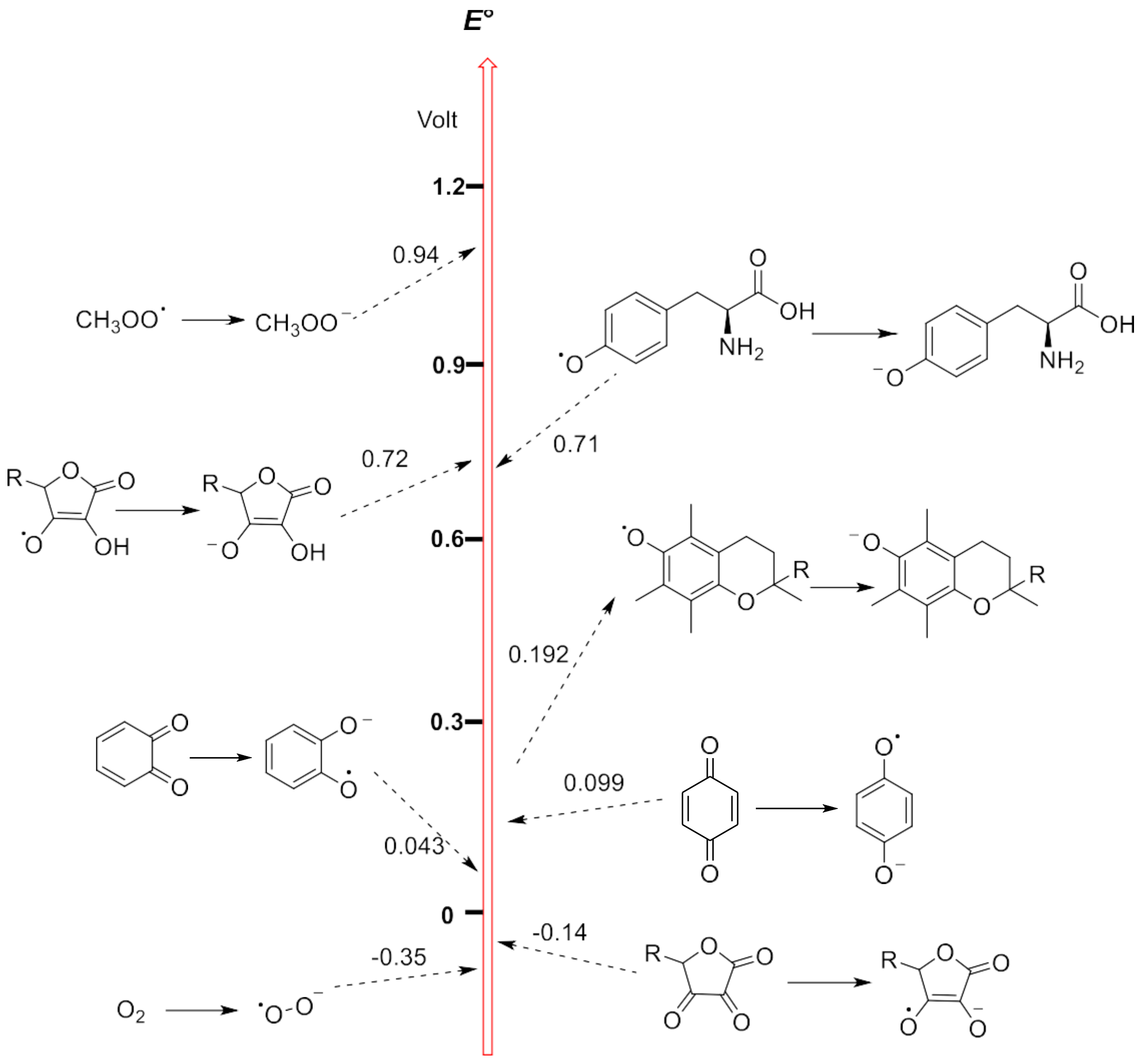
2.3. Comparison with $H_2O/HO^{\bullet}$

Despite the chemical similitude (only H and O atoms!) radicals derived from H_2O have a completely different reactivity. The BDFE of HO-H is extremely high (115.8 kcal/mol), due to the absence of any resonance or hyperconjugative stabilization in $HO^{\bullet}$. The hydroxyl radical is also a strong one-electron oxidizing agent, $E°(OH^{\bullet})$ is 1.90 V (Scheme 2b). Moreover, the H-O BDFE of the H-$O^{\bullet}$ radical is also very high (102 kcal/mol) due to the instability of the O (atomic oxygen) specie, therefore $HO^{\bullet}$ has no reducing ability [17].

2.4 Thermodynamic basis of antioxidant regeneration

In Scheme 3, a BDFE and potential ladder showing the comparison between the BDFE($^{\bullet}$OO-H) and that of relevant species for the chemistry of peroxidation and antioxidant activity are reported. In this scheme, BDFE of all compounds that can form a bond stronger than 51.2 kcal/mol with a H-atom can potentially be reduced by $HOO^•$, although the reaction will be more efficient if the strength of the newly formed bond is much higher than this value. An analogous ladder can be built by considering redox potentials, and species with a reduction potential bigger than –0.35 V *vs* HNE are amenable to be reduced by $O_2^{•-}$. For example, $HOO^•$ reduces alkylperoxyl radicals ($CH_3OO^•$ in Scheme 3) at a nearly diffusion rate, as reported by Ingold and Foti [12], while we estimated that the H-atom donation from $HOO^•$ to the α-tocopheroxyl radical (in PhCl at 30 °C) is as high as 10^9 $M^{-1}s^{-1}$ [18]. As most autoxidation reactions occur in nonaqueous media, where deprotonation of acids is relatively unimportant, the actual interaction between $HOO^•$ and antioxidants is better described by the BDFE ladder. It should also be kept in mind that BDFE values are expected to change considerably as effect of H-bond with the solvent, so the relative order of the species reported in the ladder may change when considering the reactivity in solvents less able to form H-bonds. Besides PCET reactivity, $HOO^•$ / $O_2^{•-}$ radicals can also easily add to phenoxyl radicals forming hydroperoxides which are no longer able to trap radicals or to be reduced to the parent phenols (see section 4.1) [16,18]. This reaction is in competition with ET or PCET from $HOO^•$ / $O_2^{•-}$ to the radical of the antioxidant and reduces the regeneration. In summary, the data reported show that the $HOO^•$ radical has a two-faced oxidizing and reducing ability, that render it very different from the alkylperoxyl radical ($ROO^•$) that is the main chain-carrying specie in autoxidation. While $HOO^•$ can potentially propagate the oxidative chain, indeed it is able to react, in some cases with high-rate constants, with a series of redox active substrates that would be otherwise inert under oxidative-only conditions.

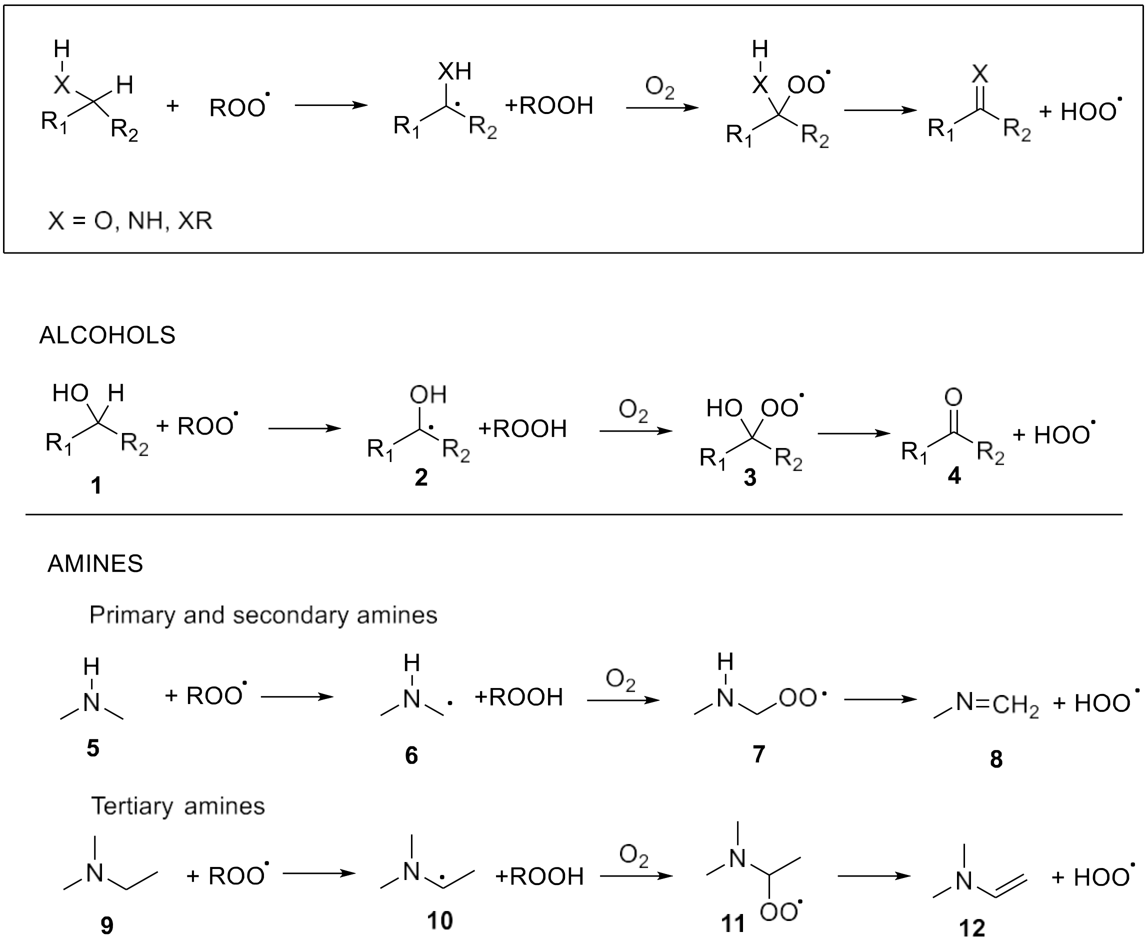

BDFE
kcal/mol
90
80
70
60
90.2
83.0
73.5
68.6
66.3
60.6
60.7
49.0
OH
HO
R
H2
CH300
O
N



Scheme 3: BDFE (upper) and reduction potential (*vs* NHE) (lower) ladders showing the thermodynamics of the donation of a H-atom from HOO$^\bullet$ or the electron donation from $O_2^{\bullet-}$ to various acceptors. Data from [17].

3. Formation of hydroperoxyl radicals during autoxidation

Hydroperoxyl radicals can be present along with alkylperoxyl radicals during autoxidation reaction by fragmentation of ROO$^\bullet$. The oxidizable substrate must have specific structural characteristics to allow an efficient generation of HOO$^\bullet$ radicals. The presence of a H-atom in 4 position respect to the terminal oxygen of the peroxyl group is essential, in order to allow an intramolecular 1,4-hydrogen atom transfer (intra-1,4-HAT), that causes the production of $HOO^{\bullet}$. However, 1,4-HAT will be more or less effective depending on structural features of the molecule. Some examples of $HOO^{\bullet}$ production by 1,4-HAT, subdivided by type of functional group, are summarized below (Scheme 4).



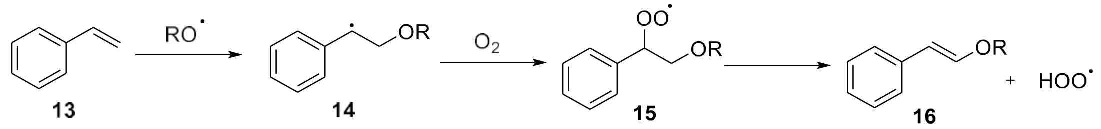
Scheme 4: Box: general mechanism of $HOO^{\bullet}$ elimination by 1,4-HAT from alkyl peroxyl radicals, and typical reaction pathway in alcohols and amines.

3.1 Alcohols: The α-C-H group in primary and secondary alcohols (**1**), is preferentially attacked by radicals, owning to the adjacent hydroxyl group which stabilizes the alkyl radical [20]. The α-hydroxy alkyl radical, also named ketyl radical (**2**) reacts with O_2 at diffusion rate to afford the α-hydroxy-peroxyl radical **3** which decomposes to a carbonyl compound (**4**) and HOO• [21].

3.2 Amines Like the oxygen atom in alcohols, nitrogen in amines stabilizes the α alkyl radical by resonance with the unpaired electron. Thus, the α-position of alkyl-substituted amines (**5**, **9**) is strongly activated toward H-atom abstraction, forming amino alkyl radicals (**6**, **10**) that, in the presence of O_2, form the corresponding α-amino peroxyl radicals (**7**, **11**). In the case of primary and secondary amines, the aminoperoxyl radical decomposes to HOO• and an imine **8** [22]. In the case of tertiary alkylamines having at least one $-CH_2CH_2-$ substituent, such in the case of compound **9**, HOO• is generated with great efficiency clearly indicating that the aminoalkene **12** is the product of the reaction (see Scheme 4) [23].

3.3. Alkenes and pro-aromatic compounds

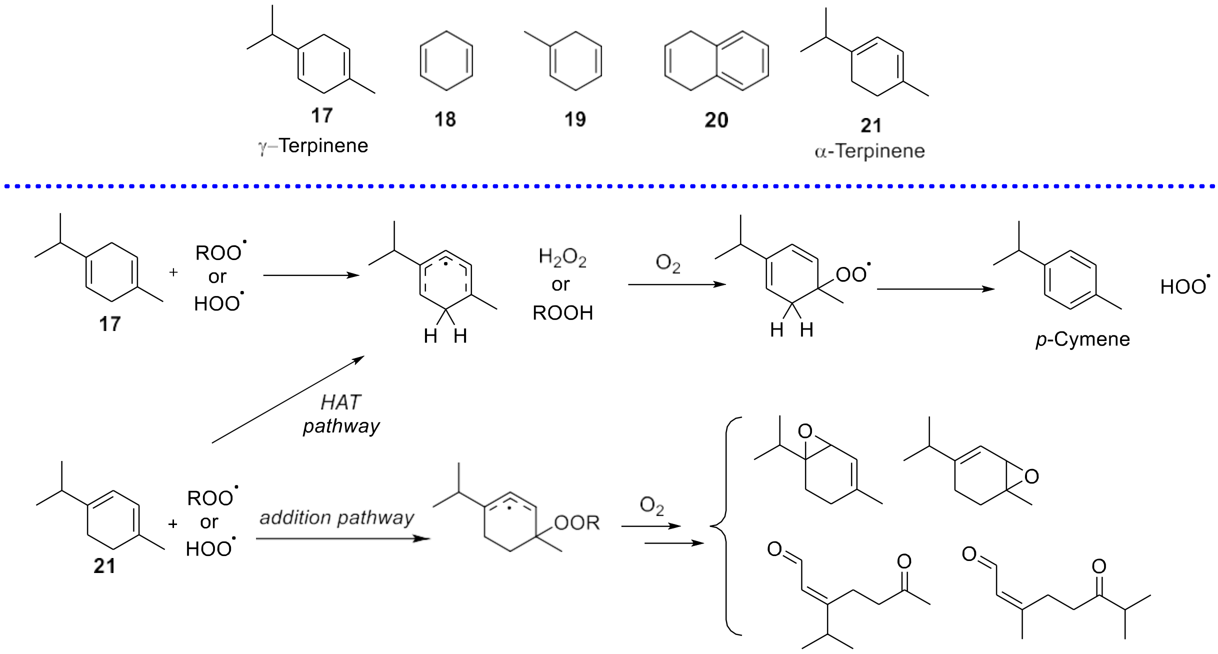
Recently has it been reported that the HOO• radical is formed during the autoxidation of styrene or cyclooctene at 70 °C and for hexadecene at 100 °C [24]. According to Scheme 5, based on theoretical calculations, HOO• production could be explained on the basis of a chain reaction mechanism involving alkoxyl radicals. In the case of styrene, that undergoes autoxidation only by radical addition, the alkoxyl radicals (generated together with epoxides by an addition-fragmentation pathway) combine with the double bond providing the alkoxyl-alkyl radical **14**. The alkyl radical then reacts with oxygen to yield a peroxyl radical, which in turn produces HOO• and the alkoxy-substituted alkene **16** via intra-1,4-HAT. However, at low temperature and at high concentration of oxidizable substrate, the autoxidation of styrene affords a very limited amount of HOO• [25].



Scheme 5: Mechanism proposed for the formation of HOO• during the autoxidation of alkenes.

Some reports, based on the antioxidant effect of superoxide dismutase (SOD) on autoxidation rates, suggest that during the autoxidation of methyl linoleate in micelles a small fraction of the propagating peroxyl radicals is represented by HOO•/$O_2^{\bullet-}$ radicals [26,27]. Considering the biological relevance of peroxidation of polyunsaturated lipids, these preliminary results call for further investigations about the mechanism of HOO• production.

1,4-Cyclohexadiene derivatives such as γ-terpinene **17**, a common terpene found in the essential oils (Scheme 6), represent a unique class of alkenes as they produce high yields of HOO• during their autoxidation [12]. γ-Terpinene reacts with radicals exclusively by H-atom transfer from the bis-allylic CH_2 groups forming a highly stabilized alkyl radical which reacts with O_2 to yield a peroxyl radical with conjugated double bonds. Then, HOO• is released by intra-1,4-HAT mechanism, whose driving force is the formation of an aromatic product, *p*-cymene [28]. Other pro-aromatic compounds such as 1,4-cyclohexadiene **18** itself, methyl-1,4-cylohexadiene **19**, 1,4-dihydronaphthalene **20** quantitatively generate HOO• during their autoxidation [9,10].

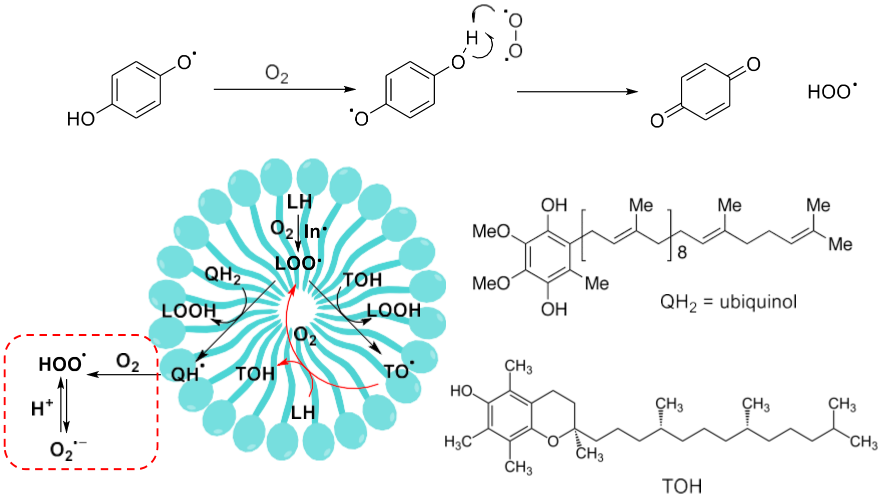


Scheme 6: Mechanism proposed for the formation of HOO• during the autoxidation of alkenes.

Contrary to γ–terpinene, the 1,3-cyclohexadiene isomer α-terpinene **21**, which has conjugated double bonds, only partially propagates the chain by HOO• [29]. The main products of α-terpinene's autoxidation are epoxides and diketones, with the latter likely resulting from the breakdown of alkyl hydroperoxides. Only about 20% of the products of the autoxidation are *p*-cymene, which is produced by an intra-1,4-HAT from the peroxyl radical of α-terpinene, suggesting that HOO• is only marginally involved in chain propagation [30]. Recent kinetic studies support the view that the different behavior between γ- and α-terpinene is due to the preference of α-terpinene for ROO• addition rather than HAT [31]. Therefore, while potentially all pro-aromatic hydrocarbons may generate HOO• during their autoxidation, the efficiency of the process depends on the nature of the alkyl radical formed after their reaction with the chain-propagating peroxyl radicals.

3.3. Semiquinones and phenolates

Hydroquinones are an important class of natural and synthetic redox-active organic molecules, able to switch between the oxidized (quinone), the radical (semiquinone) and the reduced (hydroquinone) forms. Many hydroquinones have antioxidant properties, and are used as additive to counteract lipid peroxidation [32]. After the reaction with $ROO^\bullet$, hydroquinones generate the corresponding semiquinones. The reaction with O_2 to produce $HOO^\bullet$ or $O_2^{\bullet-}$ is a well-known process that is usually related to quinone toxicity [33]. From a mechanistic point of view, (protonated) semiquinones can produce hydroperoxyl radical by H-atom abstraction, reacting directly with molecular oxygen, or by an addition-fragmentation pathway [21,34]. However, the reaction of semiquinones with O_2 may have also unexpected antioxidant effect. Ingold and co-workers demonstrated that ubiquinol (QH_2) is a much more effective chain-breaking antioxidant than α-tocopherol (α-TOH) in low-density lipoproteins (LDL). Unexpectedly, after the reaction of α-TOH with a $ROO^\bullet$ radical, the α-tocopheroxyl (α-$TO^\bullet$) radical remains trapped into the LDL. Being unable to decay, α-$TO^\bullet$ eventually propagates the oxidative chain by reacting with polyunsatured lipid. On the other hand, the semiquinone radical formed after the reaction of QH_2 reacts with oxygen to form the corresponding quinone and $HOO^\bullet$. The hydrophilic $HOO^\bullet$ radical migrates from LDL to the aqueous phase, where forms after deprotonation the low-oxidizing $O_2^{\bullet-}$ radical, thus stopping the radical reaction inside the lipid core (Scheme 7) [35,36].



Scheme 7: Formation of HOO$^\bullet$ from 1,4-semiquinone and protection of LDL autoxidation by HOO$^\bullet$ production by the ubiquinol radical.

Superoxide is also produced by electron transfer to O_2 from deprotonated phenols [37]. This reaction is the basis for the autoxidation of aqueous solutions of polyphenols under O_2, that for example is responsible for degradation of polyphenolic antioxidants in cell culture medium [38] or to the formation of melanine-like polymers [39,40].

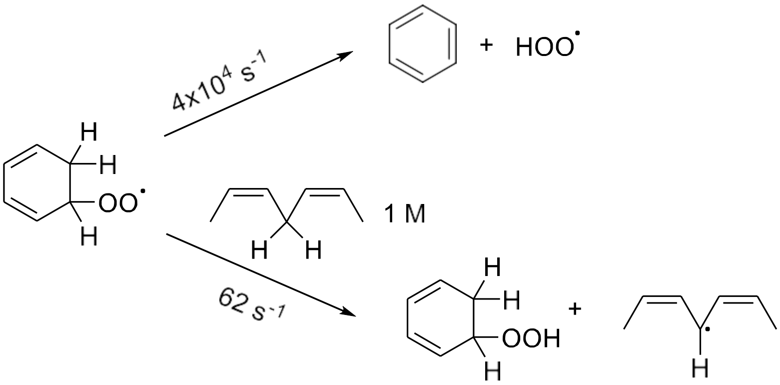
3.4. Kinetics aspects of Intra-1,4-HAT

The autoxidation of pro-aromatic 1,4-cyclohexadienes can be considered as an interesting strategy to have HOO$^\bullet$ as chain-carrying radicals, in order to study their reactivity. For quantitative chemico-physical determinations, well-characterized oxidizable substrates must be chosen. Some examples of typical oxidizable substrates with known propagation and termination rate constants are given in Table 1, together with their respective k_p, $2k_t$, and oxidizability Ox = $k_p/(2k_t)^{1/2}$ values [15]. Interestingly, 1,4 cyclohexadiene displays a very large propagation constant, due to the bis-allylic nature of the reactive C-H groups, but at the same time it has a high termination constant, due to the self-decay of $HOO^{\bullet}$ radicals.

Table 1. Rate constants for the autoxidation of selected organic substrates in chlorobenzene at 30 °C.

Substrate	**k_p [$M^{-1}s^{-1}$]**	**2k_t [$M^{-1}s^{-1}$]**	**Ox**	**Ref**
Cumene	0.32	4.6×10^{4}	0.0015	[41]
p-Cymene	0.83	2.9×10^{6}	0.0005	[42]
Styrene	41	4.2×10^{7}	0.0063	[43]
Methyl linoleate	62	8.8×10^{6}	0.0209	[43]
1,4-Cyclohexadiene	1400	1.3×10^{9}	0.0388	[10]

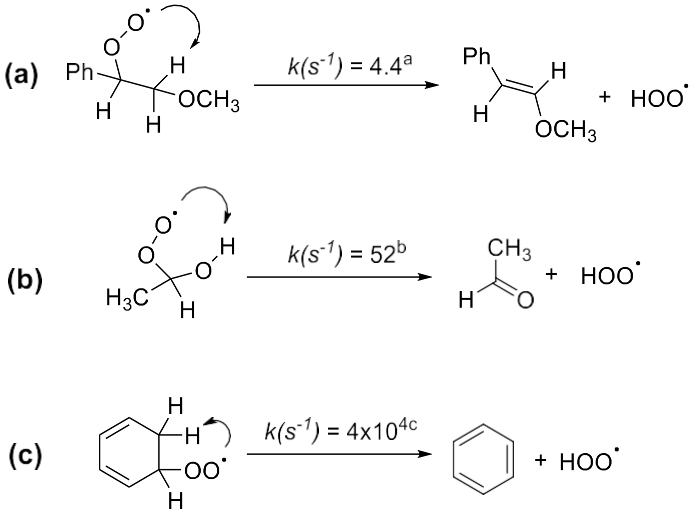
The generation of $HOO^{\bullet}$ from the cleavage of $ROO^{\bullet}$ radicals requires that the intra-1,4-HAT is faster than other reaction involving $ROO^{\bullet}$, like addition to double bonds or H-atom abstraction from the substrate, as shown in Scheme 8.



Scheme 8. Competition between fragmentation and propagation of the cyclohexadienyl peroxyl radical. The rate of propagation is calculated for a hypothetical concentration of

methyl linoleate of 1 M from its propagation constant (see Table 1) [28,43].

In Scheme 9, selected experimental and theoretical values of intra-1,4-HAT are reported, including the HOO$^•$ production from the putative alkoxyperoxyl radical (see Scheme 5) formed during the autoxidation of styrene (a), of ethanol (b) and 1,4-cyclohexadiene (c). It is evident that the fragmentation is very effective for peroxyl radicals deriving from pro-aromatic derivatives, while it is much slower for those deriving from alcohols and simple alkenes [24,28,44]. This clearly demonstrate the importance of aromatization in determining the production of HOO$^•$ [45].



Scheme 9: Rate constant for the elimination of hydroperoxyl from peroxyl radicals by intra-1,4-HAT. [a] Calculated, at 70 °C [24], [b] Experimental, in water at 22 °C [15],[c] experimental, in acetonitrile at 20 °C [28].

From a quantitative point of view, considering that the propagation occurs with a pseudo-first order given by k_p [substrate], the HOO$^•$ generation in the case of styrene autoxidation will be efficient only when [styrene] × 41 $M^{-1}s^{-1}$ < 4.4 s^{-1}, that is [styrene] < 0.1 M. In case of the autoxidation of pure 1,4-cyclohexadiene (k_p = 1400 $M^{-1}s^{-1}$), $HOO^{\bullet}$ generation occurs at concentrations of the substrate < 2.8 M. This line of reasoning can also be applied to mixtures of 1,4-cyclohexadiene and other oxidizable lipids. For example, with methyl linoleate in the 1 M concentration range (like in case of an oil rich in polyunsaturated lipid), $HOO^{\bullet}$ generation by co-oxidizing 1,4-cyclohexadiene will be feasible, as the 1,4-intra-HAT (4×10^4 s^{-1}) is faster than the hypothetical propagation of the cyclohexadienyl radical (1 M × 62 $M^{-1}s^{-1}$ = 62 s^{-1}) (see Scheme 8). The fast fragmentation of cyclohexadienyl radical is the basis for the use of 1,4-cyclohexadiene derivatives as additives to generate $HOO^{\bullet}$ during the autoxidation of different substrates. We used this method to investigate the interaction of $HOO^{\bullet}$ with antioxidants, as it is shown in the next sections of this review.

4. Synergy of $HOO^{\bullet}$ with Antioxidants

4.1. Synergy of $HOO^{\bullet}$ with Phenols and Aromatic Amines

The ability of $HOO^{\bullet}$ to increase the length of the inhibition period, and thus the duration of the antioxidant effect, of an analogue of α-tocopherol, was demonstrated by us by studying the autoxidation of 1,4-cyclohexadiene [18] and was then confirmed in further studies by employing different phenols and pro-aromatic derivatives. For example, when studying the autoxidation of sunflower oil at 130 °C, the addition of small amount of γ-terpinene was able to increase the inhibition period of the α-tocopherol naturally present in the oil. Experiments performed with stripped sunflower oil (that is, containing only triglycerides and no endogenous tocopherols or carotenes) [46], showed that γ-terpinene itself had no retarding effect on the autoxidation, but it only acted in synergy with α-tocopherol [47] (see Figure 1).

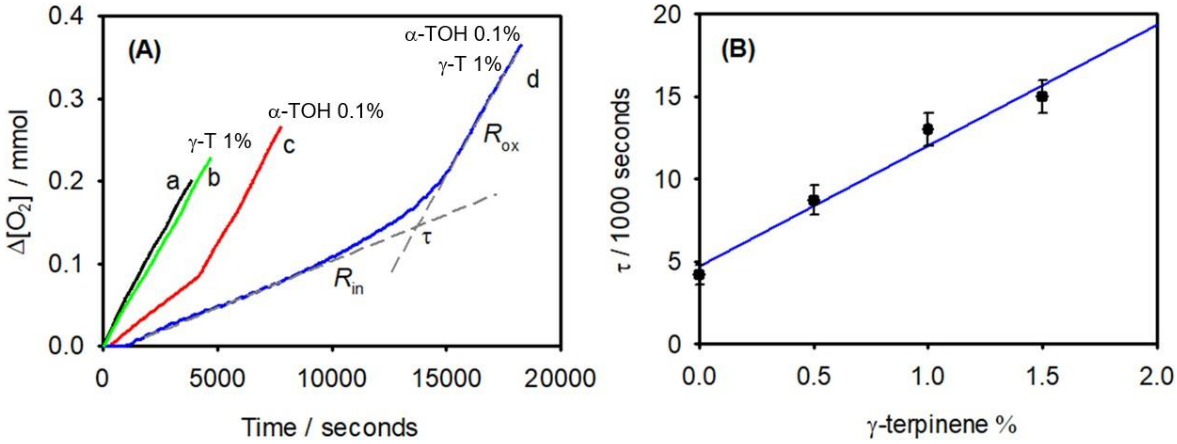
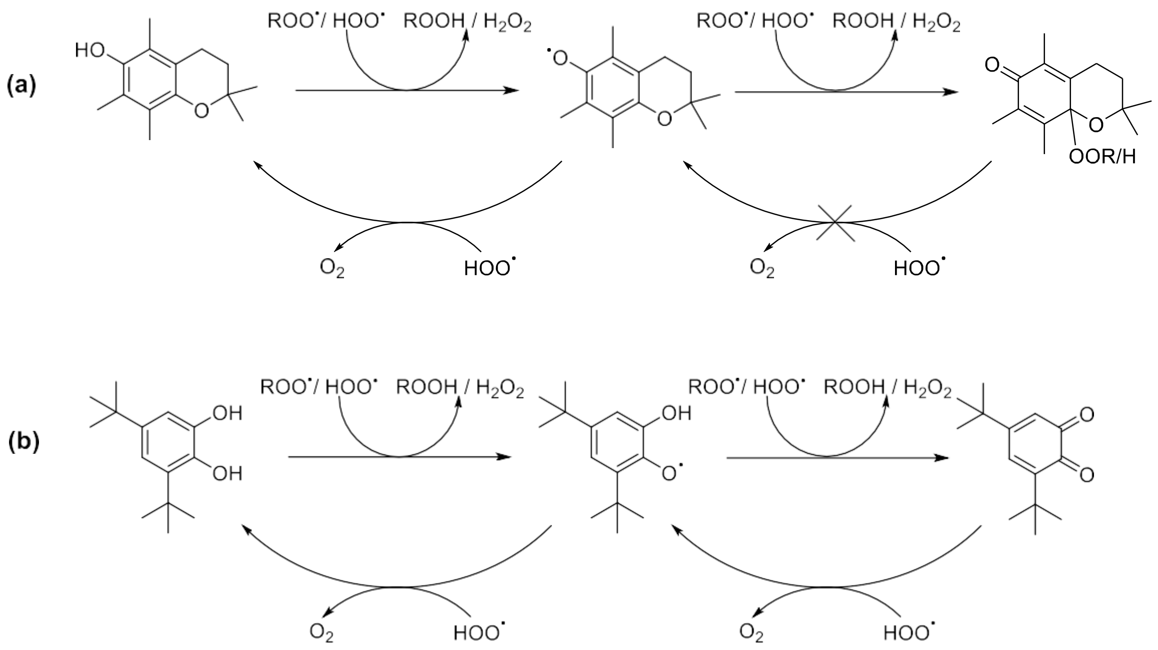


Figure 1. A) Inhibition activity on the autoxidation of sunflower oil triglycerides (130 °C) of α-tocopherol (α-TOH) and γ-terpinene (γ-T), alone or in mixture. B) Inhibition period length due to α-TOH 0.1% and variable amounts of γ-T. Reprinted with permission from 47. Copyright 2023 American Chemical Society.

This effect was also visible using γ-terpinene in association with other oxidizable substrates, like styrene and squalene, and with different antioxidants like caffeic acid [48]. The mechanism of antioxidant regeneration is exemplified in Scheme 10.

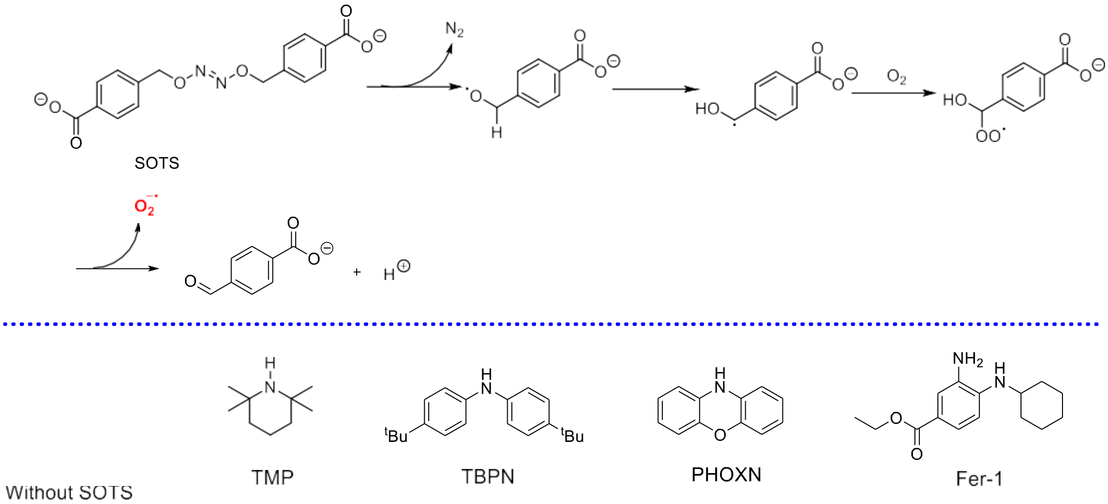


Scheme 10. Reaction pathways for the reaction of HOO$^\bullet$ and ROO$^\bullet$ with (a) the analog of α-tocopherol PMHC, (b) 3,5-di-*tert*-butylcatechol.

Due to its double-faced oxidizing and reducing ability, HOO$^\bullet$ can reduce the radical of the antioxidant and regenerate the parent phenol. However, especially in the case of phenols, this reaction does not occur indefinitely because HOO$^\bullet$ is also capable to add to the phenoxyl ring, forming oxidized products unable to be regenerated. For catechols this regenerating effect is effectively observed even when the semiquinone is already fully oxidized to the corresponding *ortho*-quinone [48] (Scheme 10b).

Aromatic amines are also important radical-trapping antioxidants, used to protect petroleum-based products from autoxidation and limiting their deterioration. Some of them are able to preserve phospholipid membranes from peroxidation, preventing cell death by ferroptosis (see for instance ferrostatin-1, Fer-1 in Scheme 11) [49]. As reported by Ingold, superoxide thermal source (SOTS), can be used as $O_2^{\bullet-}$ source [50], see Scheme

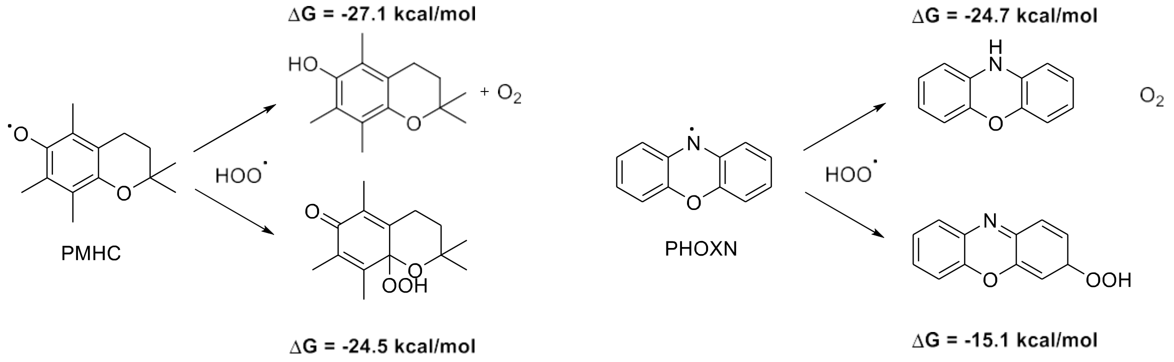
11. Recently, Pratt and co-workers used this knowledge, to address whether $HOO^•/O_2^{•-}$ could regenerate aromatic amines when used as antioxidants to protect unsaturated phospholipids in liposomes. The kinetic data, including inhibition constant (k_{inh}) and stoichiometric factor (n) for different amines in the absence or presence of SOTS were calculated as shown in Scheme 11. As expected, the aliphatic amine 2,2,6,6-tetramethylpiperidine (TMP) is inactive both in the presence and absence of SOTS. On the contrary, the other aromatic amines show interesting results. For instance, the most active amine phenoxazine (PHOXN), had a rate constant of reaction with $ROO^•$ radicals (k_{inh}) approximately identical in both conditions, but showed an increase in the duration of its antioxidant activity (inhibited period) of about 8-times by adding SOTS into the system. This effect, although less evident, is also present for the other compounds reported in Scheme11.



	TMP	TBPN	PHOXN	Fer-1
Without SOTS				
k_{inh}(10^4M/s)	slow	3.9 ± 0.6	14 ± 0.4	8.2 ± 0.3
n	/	3.0 ± 0.4	2.2 ± 0.2	3.5 ± 0.2
With SOTS				
k_{inh}(10^4M/s)	slow	3.9 ± 0.3	20 ± 2	8.0 ± 0.1
n	/	7.7 ± 0.9	16 ± 1	7.0 ± 0.4

Scheme 11. Superoxide generation by SOTS, and kinetic data for the antioxidant activity of amines in the presence and absence of SOTS, autoxidation of liposomes at 37 °C [49].

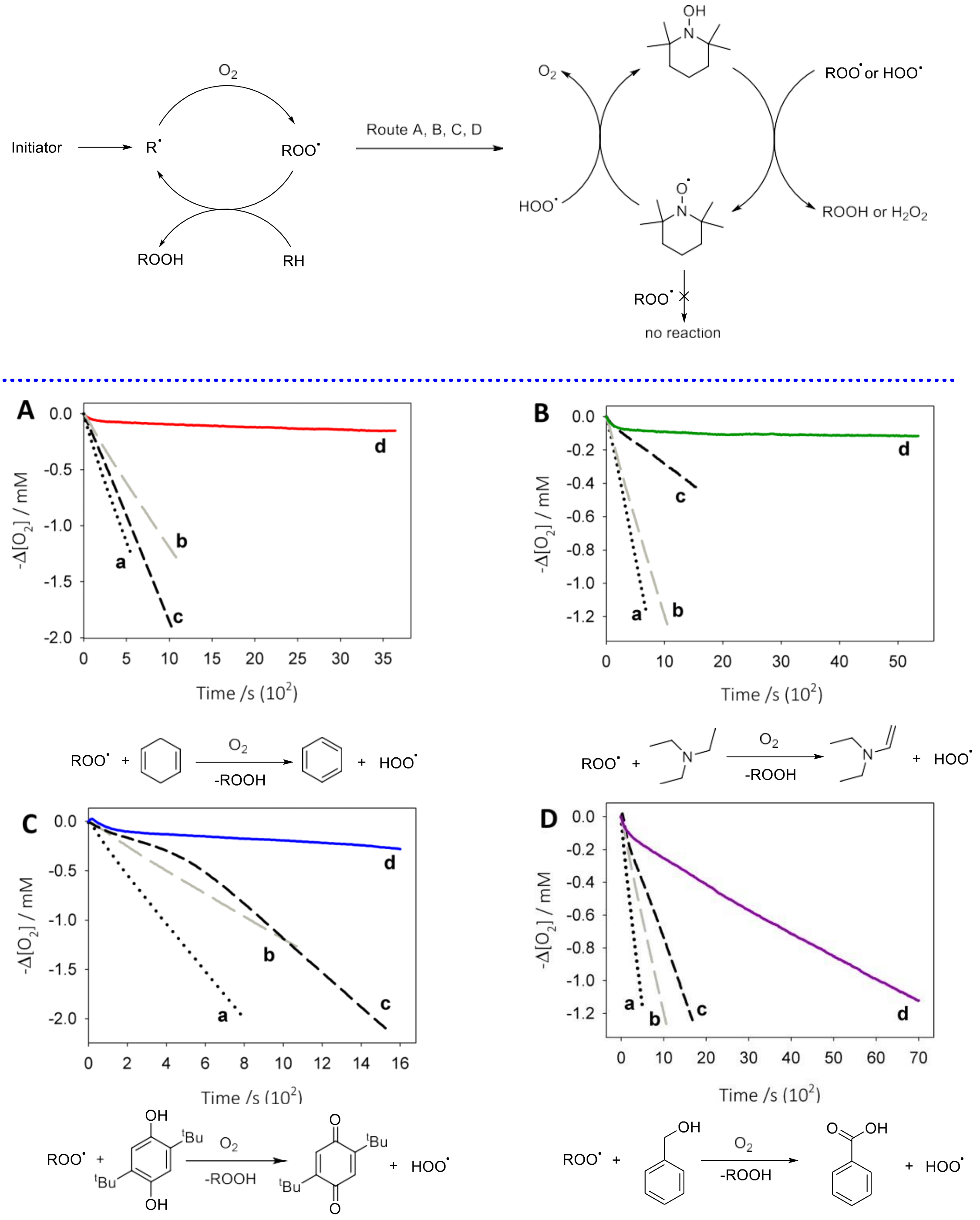
Interestingly, under identical conditions, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) and α-TOH, were not regenerated by $HOO^{\bullet}/O_2^{\bullet-}$, and conversely an erosion in the radical trapping stoichiometry was observed in the presence of SOTS [49]. This suggests that the H-atom transfer from $HOO^{\bullet}$ to the phenoxyl radicals derived from α-TOH or PMHC is slow if compared with the addition of $HOO^{\bullet}$ to form a stable peroxidic adduct. As already discussed, there are two parallel competing pathways when the phenoxyl radical of the phenolic antioxidant is generated: the regeneration of the starting antioxidant compound or the formation of the addition product which is no longer regenerable. For this reason, phenoxyl and aminyl radicals derived from α-TOH/PMHC and PHOXN respectively, perform differently. By CBS-QB3 calculations it was observed that the H-atom transfer from $HOO^{\bullet}$ to the aminyl radical derived from PHOXN is similarly exergonic to the H-atom transfer from $HOO^{\bullet}$ to the phenoxyl radical derived from PMHC (or α-TOH). On the other hand, the radical addition of $HOO^{\bullet}$ with PHOXN-derived radical is 9.4 kcal/mol less advantageous than with the PMHC-derived radical (shown in Scheme 12), presumably because the C=N bond is much weaker than the C=O bond [49].



Scheme 12. Competing Reactions of $HOO^{\bullet}$ with phenoxyl and aminyl radicals derived from α-TOH/PMHC and PHOXN [49].

4.2 Synergy of $HOO^{•}$ with Nitroxides and Quinones

When $HOO^{•}$ radicals are present, molecules that are devoid of antioxidant activity can act as autoxidation inhibitors. For example, the nitroxide 2,2,6,6-tetramethylpiperidinoxyl (TEMPO, Scheme 13a) doesn't react with alkylperoxyl radicals in organic solvents, but the $HOO^{•}$ radical assists the reduction of the stable radical TEMPO to the corresponding unstable hydroxylamine, that instead can react with $ROO^{•}$. TEMPO is a strong antioxidant in water [51] and in organic solvents but only when weak acids are present [52,53]. In these circumstances, nitroxides trap $ROO^{•}$ by giving an electron to the $ROO^{•}$ radical, which is subsequently protonated by the solvent or by the acid added [54]. Instead, in non-protic systems, nitroxides are ineffective against organic substrate autoxidation. The addition of 1,4-cyclohexadiene (CHD) to the oxidizable substrate significantly boosts the antioxidant activity of the nitroxide TEMPO (Scheme 13) in non-protic solvents. The co-oxidation of 1,4-cyclohexadiene with the substrate causes the production of hydroperoxyl radicals, that can be effectively quenched by TEMPO. In this system a catalytic cycle is generated, resulting in a strong antioxidant effect, and an extremely long duration. The end is not visible in common reaction times, because peroxyl radicals don't add to nitroxides. The cyclic mechanism can be interrupted only by the recombination of alkyl radicals with the nitroxide, but their concentration is low in the presence of O_2 [55]. This mechanism demonstrates why nitroxides are effective as antioxidants during the autoxidation of alkenes at high temperature where the chain-carrying radicals are both $ROO^{•}$ and $HOO^{•}$ [49]. Moreover, the antioxidant effect of TEMPO is visible in the presence of three more model compounds, 2,5-di-tert-butylhydroquinone (QH_2), trimethylamine, and benzyl alcohol, which are known to generate $HOO^•$ upon interaction with free radicals (Scheme 13 b).

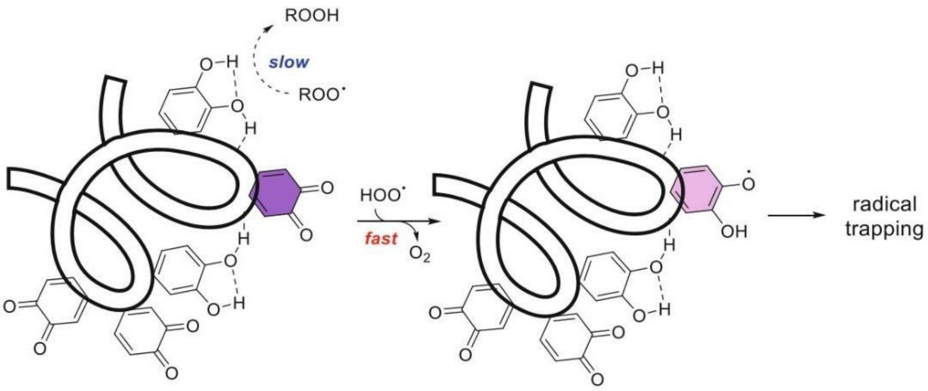


Scheme 13. Top: mechanism of the synergic antioxidant activity of TEMPO with $HOO^•$-

generating molecules. Lower panels: O_2 consumption during the autoxidation of styrene (4.3 M) in chlorobenzene initiated by AIBN at 30° without antioxidants (a in panels A, B, C and D), or in the presence of TEMPO 5 µM (b in panels A, B, C and D), CHD 0.27 M or CHD 0.27 M and TEMPO 5 µM (c and d in panel A), Et_3N 0.018 M or Et_3N 0.018 M and TEMPO 5 µM (c and d in panel B), QH_2 5 µM or QH_2 5 µM and TEMPO 5 µM (c and d in panel C), $PhCH_2OH$ 0.24 M or $PhCH_2OH$ 0.24 M and TEMPO 5 µM (c and d in panel D). Adapted with permission from reference 55. Copyright 2023 American Chemical Society.

Lastly, it has been observed that also quinones show antioxidant activity in the presence of hydroperoxyl radicals [56-58]. This observation is very counterintuitive considering that quinones are the oxidized form of catechols and hydroquinones. It is interesting to note that *ortho*-benzoquinones have a greater antioxidant impact than *para*-benzoquinones in organic solvents, since the *ortho* isomer's HAT from $HOO^\bullet$ to the quinone is more exothermic [59]. However, unlike nitroxides, the semiquinone radicals also react via addition with $ROO^\bullet$/ $HOO^\bullet$ radicals, shortening the duration of inhibition.

These unexpected properties can help us explain the antioxidant effect of polymeric nanoparticles based on melanin and polydopamine. These phenol-based compounds are rich in *ortho*-quinone moieties, and therefore in the presence of $ROO^\bullet$/$HOO^\bullet$ systems, shows good radical trapping activity [59] (Scheme 14).



Scheme 14. Antioxidation mechanism of polydopamine nanoparticle [59].

In nonaqueous systems, the *ortho*-quinone moiety is better reduced by HOO• than the *para*-quinone one, and thus the former behaves as better antioxidant than the latter [59], in agreement with recent findings about the lack of superoxide-scavenging activity of thymoquinone in neural oxidative stress models [60]. Recently, we have demonstrated that honokiol-related neolignans, after the reaction with alkylperoxyl radicals, form quinonic species that are able to trap HOO• radicals similarly to polydopamine *ortho*-quinones [61]. This suggests that polyphenols with a catechol group (like quercetin or catechins) may also display a prolongation of their antioxidant effects when a source of HOO• is present in the system.

5. Conclusions

With this review, we have provided some recent examples of the importance of HOO• in the inhibition of autoxidation reactions by chain-breaking antioxidants. Despite the simplicity of this radical, many aspects of its chemistry are still unexplored. For example, the influence of HOO•/$O_2^{\bullet-}$ on the autoxidation of emulsions and LDL, and the capability of natural lipids like fatty acids, phospholipids and phytosterols, to produce $HOO^\bullet$ has not been investigated in detail. Due to the $HOO^\bullet$ acidity, the relative importance of $HOO^\bullet$ and $O_2^{\bullet-}$ may change in the different compartments of the cells, as at physiological pH deprotonated $O_2^{\bullet-}$ would be dominant, while at acidic pH (such as in lysosomes) [62,63] the main specie would be $HOO^\bullet$. It can be expected that the hydrophilicity of $HOO^\bullet$ and its deprotonation to the not-oxidizing superoxide radical ($O_2^{\bullet-}$), explaining the reduction of radical chain propagation in LDL, may play a role also in other systems such as micelles, liposomes and nano / micro emulsions. At the same time, $O_2^{\bullet-}$ and $HOO^\bullet$ are able to regenerate the antioxidants present at the interface, thus providing an increase of the radical trapping stoichiometry. In the case of aromatic amines, the regeneration by $HOO^\bullet$ has been related to the efficacy to inhibit ferroptosis. The catalytic antioxidant behavior of redox-active nanomaterials (also called “nanozymes”) could also be explained by the interplay between $HOO^\bullet$ and $ROO^\bullet$ radicals. In conclusion, a better understanding of the $HOO^\bullet$ role in oxidative radical chain processes is expected to increase our comprehension of the mechanisms of lipid peroxidation, and to foster the rational development of novel antioxidant systems.

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Author contributions

R.A. planned the content, R. A., Z. J. and A.B. wrote and R.A and A.B. revised the manuscript.

Disclosure statement

The authors report no conflict of interest.

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