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Review article

Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review

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ABSTRACT

Hindered phenols find a wide variety of applications across many different industry sectors. Butylated hydroxytoluene (BHT) is a most commonly used antioxidant recognized as safe for use in foods containing fats, pharmaceuticals, petroleum products, rubber and oil industries. In the past two decades, there has been growing interest in finding novel antioxidants to meet the requirements of these industries. To accelerate the antioxidant discovery process, researchers have designed and synthesized a series of BHT derivatives targeting to improve its antioxidant properties to be having a wide range of antioxidant activities markedly enhanced radical scavenging ability and other physical properties. Accordingly, some structure–activity relationships and rational design strategies for antioxidants based on BHT structure have been suggested and applied in practice. We have identified 14 very sensitive parameters, which may play a major role on the antioxidant performance of BHT. In this review, we attempt to summarize the current knowledge on this topic, which is of significance in selecting and designing novel antioxidants using a well-known antioxidant BHT as a building-block molecule. Our strategy involved investigation on understanding the chemistry behind the antioxidant activities of BHT, whether through hydrogen or electron transfer mechanism to enable promising anti-oxidant candidates to be synthesized.

1. Introduction

Oxidation is a process whereby electrons are transferred from one atom to another, with the molecule losing an electron being oxidized. Free radicals are generated in normal metabolism in the human body when oxidation occurs during aerobic respiration [1]. Reactive oxygen species (ROS) and related species acting as free radicals play an important physiological role but, at the same time, they may also exert toxic effects throughout the body. Free radicals are often derived from external sources such as cigarette smoke, air pollution, ultra-violet light and ionizing radiation. These free radicals not only cause premature aging and wrinkles, but also are the primary causes of cancer and other chronic diseases [2,3].

ROS have strongly disruptive effect on products industry such as oil, plastics, rubbers and pharmaceuticals. ROS are the oxygen-centered free radicals. ROS exist in different forms such as hydroxyls (HO•), superoxides ($O2^{\bullet -}$), peroxyls (ROO•), alkoxyls (RO•), and nitric oxides (NO•) [4]. ROS can potentially cause oxidation in foodstuffs [5], fats and oils deteriorate through several degradation reactions both on heating and on long term storage.

Oxidation reactions and the decomposition of oxidation products are the main deterioration processes which result in decreased nutritional value and sensory qualities in food products. The prevention or retardation of these oxidation processes is essential for the food producer, and for all involved in the value chain. Various methods can be used to inhibit oxidation, including prevention of oxygen access, use of lower temperatures, inactivation of enzymes catalyzing oxidation, reduction of oxygen pressure, and the use of suitable packaging [6]. Another method for protection against oxidation is the use of specific additives, which inhibit or delay the

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reaction.

Our bodies have adapted to the changes required over time to develop defence systems to reduce the damages done by ROS. Damaged cells can cause disease [7]. Antioxidants are the main defence mechanism of the body acting as free radical scavengers [8]. They are produced within the body and include dismutase, peroxidase, and catalase enzymes, as well as glutathione and cytochrome [9]. Other main sources of antioxidants are fruits and vegetables, (all containing phenolic compounds). Synthetic antioxidants particularly phenols also used as food, polymer and oil additives include butylated hydroxyanisole (BHA), BHT and *tert*-butylhydroquinone [10]. Whatever the source of the antioxidants, all antioxidants function similarly, which is to prevent damage done by free radicals.

2. Antioxidant classification

The human body has various mechanisms to act against oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally provided through foods (exogenous antioxidants). Endogenous antioxidants in cells can be classified as enzymatic antioxidants and non-enzymatic antioxidants [11]. The major antioxidant enzymes instantaneously involved in the neutralization of initial free radical production [12] are: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. The respiratory burst is a major source of superoxide, hydrogen peroxide and hydroxyl radicals (•OH) [13]. Catalase and glutathione peroxidase serve to decrease the risk of •OH formation [14].

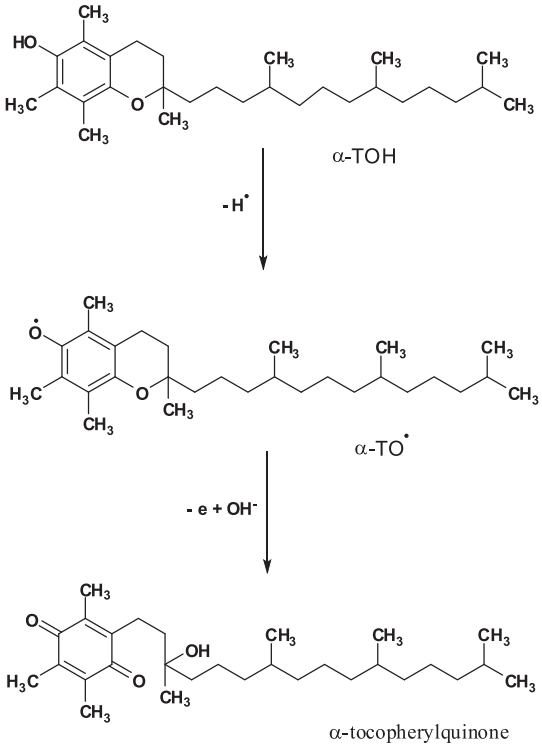
DNA is the repository of genetic information, its integrity and stability is essential to life. DNA subject to assault from the environment, and any resulting damage, if not repaired, will lead to mutation and possibly disease such as cancer. Beyond environmental agents, DNA is also subject to oxidative damage from byproducts of metabolism, such as free radicals. In fact, it has been estimated that an individual cell can suffer up to one million DNA changes per day [15]. DNA damage is repaired by effective repair mechanisms, nucleotide excision repair, photoreactivation and base excision repair [15]. Repair of DNA damage can be accomplished by glutathione, and its related enzymes before such damage becomes a genetic mutation. This is seen in the case when antioxidant donates protons to mildly damaged DNA [16]. On the other hand, the repair of lipids is done by phospholipase enzymes, which catalyzes the cleavage of peroxidized fatty acid side chains from the membrane, and replaces them with new, undamaged fatty acids [17]. In cases where free radical attack is uncontrollable and cell damage cannot be repaired; the result will be the growth of cancerous cells [8]. The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants, are produced by metabolism in the body, such as lipoid acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc.

Exogenous antioxidants are antioxidants, which cannot be produced in the body and must be provided through diet or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids, etc [11]. In general, a diet rich in vegetables and fruits has been associated with various beneficial effects on human health, such as reducing the risk of cardiovascular disease [18], hypertension [19], cancer [20], diabetes [21], and inflammatory processes [22]. The constituents responsible for these protective effects (or antioxidant activity) include some vitamins (such as A, C, and E), minerals (such as potassium, zinc and selenium), dietary fiber and phenolic compounds [23–26]. Although serious negative side-effects of antioxidant supplements have not been reported, some phenolic compounds can be harmful when consumed in large amounts [27]. The best known negative side-effect properties attributed to ability of various phenolic compounds to bind and precipitate proteins [28].

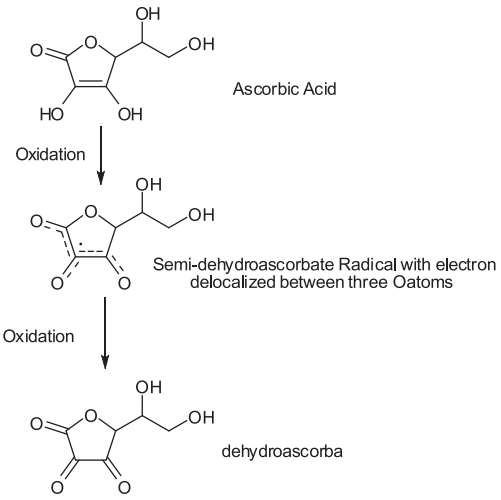
Vitamin E is a lipophilic antioxidant and plays an important role in a wide spectrum of biochemical and physiological processes [29]. Vitamin E can donate one or two protons (Scheme 1). The first proton is donated from a hydroxyl group. However, this donator does not affect the structure of vitamin E [30]. Due to the resonance ability of Vitamin E, its free radical is unreactive. Vitamin E activity is lost when it neutralizes a free radical, but it can be regenerated by another antioxidant such as Vitamin C [31,32]. The second proton can be donated without the first proton having been replenished leading to the irreversible breaking of the chromanol ring [30,33].

Ascorbic acid is a hydrophilic antioxidant [35]. It is able to donate one or two protons (Scheme 2) [36]. A donation of one electron by ascorbate gives a semi-dehydroascorbate radical, and donation of a second proton resulted in dehydroascorbate, which is unstable and can be broken down to oxalic and threonic acid. Dehydroascorbate can be reconverted to ascorbic acid with the addition of glutathione [37,38].

Primary antioxidants such as Vitamin C and vitamin E are susceptible to breaking down if their donated protons are not replenished. The source of protons comes from other compounds that can donate a proton easily remaining stable so as not to become a free radical. Examples of replenishers include carotenoids, flavonoids, coenzyme Q and glutathione. Both β-carotene and coenzyme Q work in lipid and have a synergistic relationship with vitamin E [39]. Glutathione and flavonoids are synergistic in the aqueous environment where they can work as both a proton donor to free radicals, and act to replenish ascorbic acid. These synergistic effects between antioxidants provide further supporting evidence that the intracellular antioxidant defense machinery is organized as



Scheme 1. Oxidation states of α-TOH [34].



Scheme 2. Oxidation states of ascorbic acid [38].

a network and functions as an integrated system [40].

3. Synthetic antioxidants

Although the use of herbs and spices as antioxidants for food preservation dates back to ancient times, modern antioxidant technology is only about 60 years old [10]. Since free radicals were found to be responsible for causing cellular damage, hundreds of natural and synthetic antioxidants have been evaluated for their efficacy as radical scavengers. Synthetic antioxidants are widely used in the food and feed industry, and also in the confectionery and edible oil industry. BHA, BHT, propyl gallate and *tert*-butylhydroquinone [41,42] are commonly used antioxidant, and much work has been directed towards designing novel synthetic antioxidants aimed at retarding the effects of free-radical-induced damage in various fields, particularly, food, biomedical, rubber, plastic, oil, and petroleum industries [10,43,44].

The activity of antioxidants is not enough to be used in the food and other industries as direct or indirect additives because all antioxidants have points of strength and weakness. Certain factors, such as, effective concentration, thermal stability and synergism, should be taken into consideration when selecting antioxidants [10]. Some antioxidants have been reported to show potential adverse health effects [45]. Due to this, synthetic antioxidants are tested for safety and must be approved for use in food. Allowable limits for the use of antioxidants vary greatly from country to country [46]. Synthetic antioxidants are classified based on their functional properties into two major groups, primary and secondary antioxidants.

4. General mechanisms of action of antioxidants

Antioxidants are compounds or systems that can delay or inhibit autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms: (1) scavenging species that initiate peroxidation [47], (2) chelating metal ions, Fe^{2+} in particular, can catalyze oxidative processes, leading to formation of hydroxyl radicals, and can decompose hydroperoxides via Fenton reactions, chelating these metals can effectively reduce oxidation [48,49], (3) quenching $\bullet O2^{-}$ preventing formation of peroxides [49], (4) breaking the autoxidative chain reaction, and/or (5) reducing localized O_2 concentrations [50]. The most effective antioxidants are those that interrupt the free radical chain reaction. Usually containing aromatic or phenolic rings, these antioxidants donate H to the free radicals formed during oxidation becoming a radical themselves. There are two pathways for oxidation in which antioxidants can play a preventive role [51]. The first is the H-atom transfer, described below for the important case of lipid peroxidation (Scheme 3). Once a free radical R• has been initiated step (1), then steps (2) and (3) form a chain reaction, yielding many lipid molecules (R—H) which are converted into lipid hydroperoxide (ROOH). Reaction (2) is very fast, ca. 109 M^{-1} s^{-1}, whereas (3) is much slower at 101 M^{-1} s^{-1}. The role of the antioxidant ArOH is to interrupt the chain reaction according to step (4). To be effective, the ArO• must be a relatively stable free radical, so that it reacts slowly with the substrate, RH but rapidly with ROO•, hence the term "chain-breaking antioxidant" (Scheme 4).

Another possible mechanism by which an antioxidant can deactivate a free radical is electron transfer [52]. Here, the radical cation is first formed followed by rapid and reversible deprotonation in solution, as shown in (Scheme 4).

5. Hindered phenol antioxidants

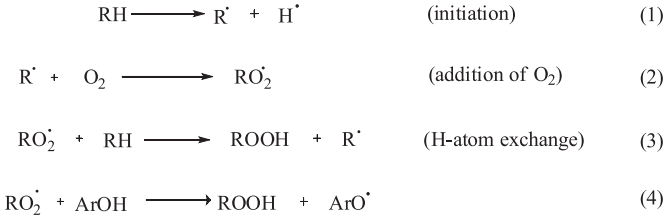
Phenolic antioxidants form a significant class of compounds which serve to inhibit oxidation of both commercial and biological importance. Antioxidants represent a wide class of additives in food [53], lubricants [54], polymers [55], paints [56], etc. They are used to contrast the autoxidation reaction of hydrocarbons, unsaturated fatty acids or esters and other substances [57]. The function of antioxidants is to intercept and react with free radicals at a rate faster than the substrate. Since free radicals are able to attack a variety of targets, including lipids, fats and proteins, they have been implicated in a number of important degenerative diseases [58–62].

The structural variation of phenolic antioxidants can play an important role in their physical properties, resulting in differences of their antioxidant activity. BHA and BHT are hindered phenols in which the phenolic ring contains di-*tert*-butyl groups, which are extremely effective as primary antioxidants [46] (Fig. 1).

BHA has two isomers: 3-*tert*-butyl-4-methoxy phenol and 2-*tert*-butyl-4-methoxy phenol (Fig. 2 **I** and Fig. 2 **II**, respectively). The isomer (**II**) is generally considered to be a better antioxidant than (**I**) [63].

BHA is a highly fat-soluble antioxidant that is used extensively in bulk oils as well as oil-in-water emulsions [64]. BHA is commonly used for the preservation of soybean [65] and palm oil in cereal and confectionery products [66]. It is also good in baking because of its stability in the presence of heat and its mild alkaline conditions, although its application in frying is limited due to its volatility. Furthermore, BHA has been reported to possess antimicrobial activity [67] and is known to be co-antioxidant (CoAH) by the regeneration of other antioxidants such as BHT and α-tocopherol (α-TOH) [68–70].

BHT (Fig. 3) is a white crystalline solid with properties similar to



Scheme 3. Oxidation and H-atom transfer mechanism of antioxidant.

$RO_2^{\bullet} + ArOH \longrightarrow RO_2^{-} + ArOH^{+}$ (electron transfer)

$ArOH^{+} + H_2O \rightleftharpoons ArO^{\bullet} + H_3O^{+}$ (deprotonation equilibrium)

$RO_2^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O$ (hydroperoxide formation)

Scheme 4. Electron transfer mechanism of an antioxidant.

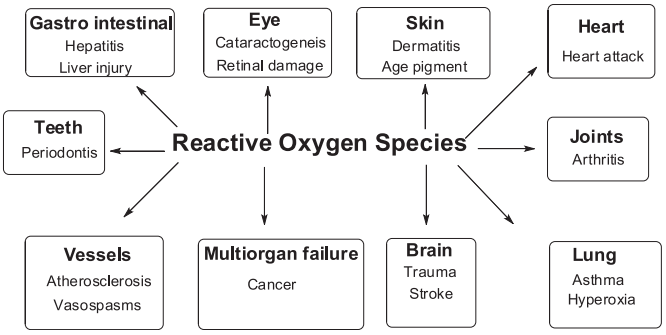


Fig. 1. Clinical conditions involving ROS [4].

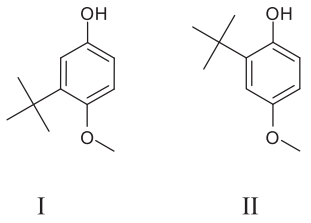


Fig. 2. I and II BHA isomers.

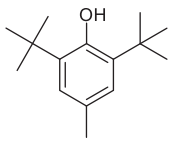


Fig. 3. BHT structure.

BHA [63]. It is appropriate for thermal treatment but is not as stable as BHA [46]. BHT has a low molecular weight, and is a non-staining hindered-phenol antioxidant. Hindered phenols find a wide variety of applications, including inhibitors of free radical-chain reactions [71].

The phenolic antioxidant BHT (CAS 128-37-0; NCI C03598) was patented in 1947 [72]. Thus far, there are more than fifteen thousand publications on BHT and its applications. It has been documented in thousands of scientific journals, patents, general reviews and conferences which mainly discussed on the role of BHT as key substrate in food and feed chemistry, pharmaceuticals and pharmacology. It has been broadly used in plastic manufacturing and processing industries, fermentation and bio industrial chemistry. Moreover, BHT can be used as additive in essential oils and cosmetics. BHT has been approved for use in foods and food packaging in low concentrations by the U.S. FDA since 1954 [10]. BHT is recognized as safe for use in foods (Federal Register, 1977) and is one of the most commonly used antioxidants in foods containing fats [73], petroleum products and rubber [74]. Due to its widespread use as a food preservative, the biochemical properties of BHT have been studied extensively [75]. Large doses of BHT produce centrilobular necrosis, increased serum transaminase activities, and hemorrhage in the liver. BHT has also been found to increase the mitotic activity of hepatocytes in rats and cats as a promoter for hepatocarcinogenesis [76]. Following a two generation study in rats, BHT was described as a potential rat liver carcinogen, yet there was no significant evidence to indicate a hepatocarcinogenic potential for BHT when it was originally tested in either rats or mice. In addition, BHT also inhibits chemical carcinogenesis in various organs when fed before or concurrently with a carcinogen [76], and is not carcinogenic for F344 rats or B6C3F1 mice [77]. It also possesses a potent inactivator of lipid-enveloped viruses [78]. The effects of BHT on experimental animals are still controversial [79] Thus, BHT remains the most popular antioxidant for use as additive in industrial applications. For instance, BHT functions as an antioxidant in cosmetic formulations as determined by the U.S. FDA in 1998 based on industry reports, which indicated that BHT is used in 1709 formulations. Historical data from 1984 indicated that BHT was used at concentrations of up to 1% (FDA 1984), while the usage in industry in 1999 was between 0.0002%–0.5% [80].

Apart from all advantages and benefits of BHT and its derivatives, this interesting family of aromatic compounds is very well known as promising antioxidants. There are more nearly six thousand publications discussing exclusively on antioxidant properties of BHT and its derivatives. Currently, BHT is one of the antioxidants used extensively in the food industry. It is used in low-fat foods, fish products, packaging materials, paraffin, and mineral oils [74]. BHT is also widely used in combination with other antioxidants such as BHA, propyl gallate, and citric acid for the stabilization of oils and high-fat foods [81]. In addition, BHT and BHA can be used as co-antioxidants by the regeneration of α-TOH. Due to these widespread applications, BHT and its derivatives have become attractive antioxidant or CoAH groups [82].

It is therefore, no surprise that BHT has been modified to prepare a series of new antioxidants having new properties in both polymer and pharmaceutical industries [83–85].

6. Multipotent antioxidants (MPAOs)

MPAOs are defined as compounds with other pharmacological effects, as well as different types of antioxidants [86,87]. In recent years a great deal of effort has been devoted to finding MPAOs in an attempt to combine radical-scavenging (and/or radical-generation-preventing) activity and enzyme-inhibiting potential into a single structure [88]. Recently, qualitative structure activity relationships for antioxidants and rational design strategies for radical-scavenging antioxidants have been used to combine multiple functions which include various antioxidant properties such as a radical-scavenging ability and diversified pharmacological activities to offer hybrid compounds, which can exert multiple pharmacological functions through one structure [86,89]. This type of MPAO is of great interest for the treatment of complex diseases. For instance, Kato et al. [90] designed and synthesized thiazolidinones bearing butylated hydroxyphenyl (BHP) moiety to have Ca^{2+} antagonistic activity as well as both Ca^{2+} overload prevention and antioxidant activity in one molecule. In addition to the two major mechanisms mentioned earlier, H-atom transfer, and electron transfer mechanisms are not mutually exclusive. In some cases, other factors may also play a role in determining the antioxidant effectiveness, as the presence of bulky groups near the OH group [91] hydrogen bonding characteristics [92] or in a biological context, solubility and transport to specific tissues [35,93].

7. Parameters affecting the performance of bulky phenols

To accelerate the discovery of novel antioxidants, considerable effort has been devoted to investigate the structure–activity relationships (SARs) for antioxidants. Furthermore, rational design strategies for antioxidants have been proposed and applied in practice with two strategies. The first reasonable strategy is the good features of two or more antioxidants into one structure. This does not need the aid of theoretical computation. The other is to find novel structures through computer-aided methodologies [89]. From the literature review, we have found that antioxidant influence is enhanced by some parameters that play an important role in selecting and designing novel antioxidants. In this review, we attempt to summarize the current knowledge on this topic, which is of significance in selecting and designing novel antioxidants using BHT as a building block to enhance their antioxidant power with a wide range of antioxidant activities with markedly enhanced radical scavenging ability and other physical properties of the well-known antioxidant, BHT. Our strategy involved an investigation on the BHT structural effects on efficiencies of antioxidants, whether through hydrogen or electron transfer mechanism has provided some indicators on the features that would play important roles in antioxidant properties. Therefore, it is important that designs of antioxidants based on BHT employ the indicated features to enable promising anti-oxidant candidates to be synthesized.

7.1. Effect of BDE of O–H, N–H and S–H bonds

Bond dissociation energy (enthalpy change) (BDE) is the energy needed to break one mole of the bond to give separated atoms. BDE value is one of the most important physical parameters used for evaluating antioxidant activity in chemical compounds that were used as inhibitors of free radical reactions. In this case, the BDE of the O–H, N–H and S–H plays a central role in determining antioxidant efficacy. In general, compounds having lower BDEs have been reported to have better antioxidant properties. These compounds exert their action initially via hydrogen transfer whose rate constant depends on the strength of the BDEs. Indeed, knowledge of substituent's contributions to the BDE has been the key to rational design and development of novel and more effective antioxidants and radical scavengers [94]. For instance, BDEs of six phenols have been determined by equilibration studies on the stable galvinoxyl radical and found to be in the increasing order of BDE of ArO–H as shown in Fig. 4 below [95].

The order of BDE value of bisphenol IV is, to some extent, surprising. This could be attributed to the stabilization of phenoxyl radicals by the alkylthio group which may only occur when the donating substituent is co-planar to the aromatic ring (stereo-electronic effect) [50,96]. This is highly unlikely in compound IV since the -$SCMe_2S$- group is a very bulky group making alkylthio group at *p*-position to be less co-planar with the aromatic ring. Thus, lowering the conjugative electron delocalization between the heteroatoms (S) of the bisphenol IV and two aromatic rings.

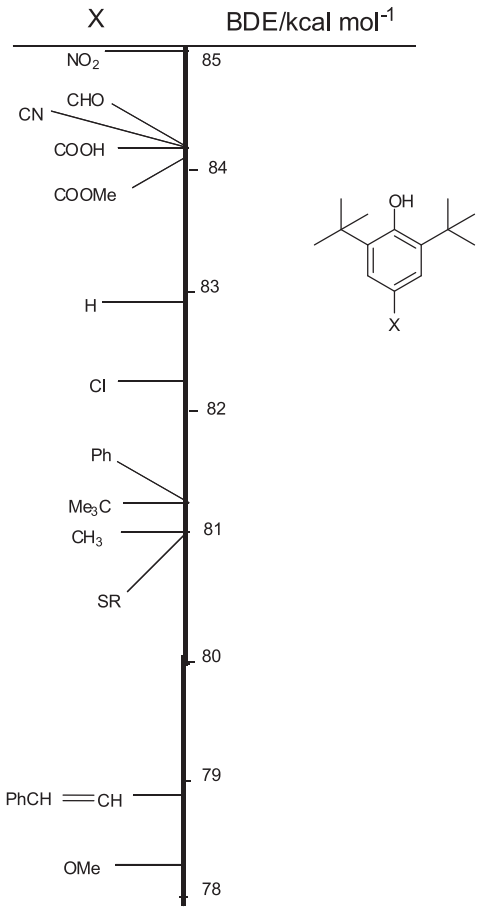


Fig. 5. BDE values (kcal/mol) of the O–H bond of *p*-substituted BHT in benzene [97].

BDE values in *p*-substituted phenols have been found to depend strongly on the nature of the substituents being linearly correlated with the σ^+ values. This suggests electron-withdrawing substituents such as CN, NO_2, CHO, COOR and COOH increase BDE of the O–H bond (Fig. 5) [97]. For example, the stabilization of the

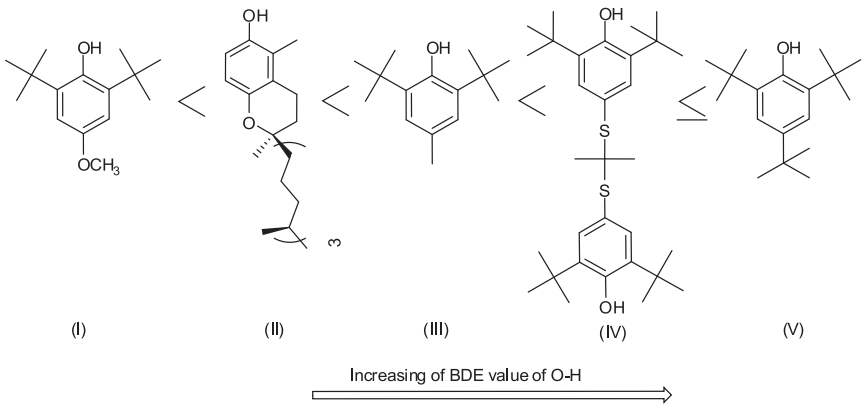


Fig. 4. Increasing of BDE of some substituted phenols.

Fig. 6. Stabilization of *p*-cyano phenol.

phenol by a polar substituent for a cyano substituent is shown below (Fig. 6).

On the other hand, electron-donating substituents decrease the BDE of the O–H bond due to the stabilization of the phenoxyl radical through mesomeric effect of hybrid structures bearing a positive charge on the substituent (Fig. 7) [95,97].

Similarly, BDE values of the N–H bond of some secondary aromatic amines have been determined by an equilibration method. Here, the reaction of aromatic amines with alkoxyl radicals in aprotic solvent led to hydrogen abstraction from the N–H bond forming a stable aminyl radical which is a key step in the power of antioxidant hypothesis (Fig. 8) [98].

Recently, a computational analysis of the radical stabilities and BDE based on DFT (uB3LYP/6-31G (d, p)) calculations enable to rationalize the antioxidant experimental results. By calculating the spin density on the radical intermediate, they could predict which intermediate would be expected to be more stable [99].

As observed with phenols, electron-withdrawing groups make hydrogen abstraction more energetically demanding, while electron-donating substituents significantly decrease the BDE value [98]. This is attributed to the change in the ground-state energies caused by the polar–polar interaction of the substituents with the N–H groups [100]. An examination of tricyclic compounds (Fig. 8) showed that phenothiazine (I), phenoxazine (II) and to a lesser extent, phenoselenazine (III) have low N–H BDE values even when compared with the most active radical trapping phenol such as 2,4,6-trimethoxyphenol (IV), 2,6-di-*tert*-butyl phenol and α-tocopherol (VI) (Fig. 9) [98,101].

BDE values for some thiophenols (Table 1) were found to be in the range of 292–341 kJ/mol. This is about 30–80 kJ/mol lower than the BDE values of peroxides (377 kJ/mol) and hydroperoxide (368 kJ/mol), suggesting thiophenols to be good radical scavengers and antioxidants.

7.2. Antioxidant activities of phenols in steric hindrance

Some important features that deserve consideration in answering why hindered phenols are antioxidants were identified in this section. It has been reported extensively that electron-donating substituents, such as methyl and *tert*-butyl on 2,4 and 6-positions, increase the antioxidant activity of phenols [91]. This is due to the lowering of the phenolic O–H BDE [95] and the stabilization of the phenoxyl radical by inductive and hyperconjugative effects. In addition, substituents at the *o*-position would cause steric hindrance to minimize undesirable reactions such as pro-oxidation (Fig. 10) [102,103]. This parameter has been successfully applied to rationalize experimentally observed antioxidant activities of some thiosemicarbazides and corresponding 1,2,4-triazolethiones [99].

The relevance of the BDE value of hindered and non-hindered phenols can be understood by considering the inhibition of the autoxidation reaction of phenol and BHA [51]. In phenol, the BDE value is practically identical to that of the hydroperoxide (BDE,

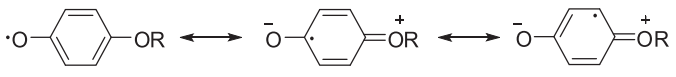


Fig. 7. Mesomeric structures of *p*-alkoxy phenol.

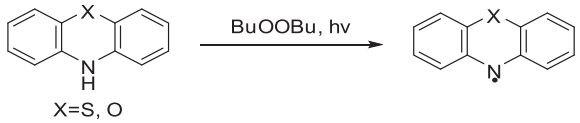


Fig. 8. Hydrogen abstraction from the N–H of phenothiazine and phenoxazine.

ROO–H, ca.88 kcal/mol) [104] in hydrogen transfer propagation. This reaction is thermo neutral and highly reversible. BHA having a weaker O–H bond strength (78 kcal/mol), which is exothermic by 10 kcal/mol compering with phenol, and is totally irreversible (Fig. 11) [105].

Thermodynamically, BHA is a good antioxidant while unsubstituted phenol is a bad antioxidant since it is not able to subtract the chain propagating peroxyl radicals from the reaction medium. Therefore, BHP group is chosen as chain-breaking antioxidant unit in the designs of an antioxidant (Fig. 11). This should make newly designed antioxidant have very similar properties of BHT or BHA as mentioned earlier.

7.3. Effect of hydrogen bonding

The antioxidant capabilities of phenols are strongly reduced by protic solvents since the hydrogen-bonded molecules ArO···S are virtually unreactive toward ROO• radicals. For instance, antioxidant activities of four inhibitor molecules in protic solvent have been studied and found to possibly exist in the free (InH) or bound (InH···Y) form (Fig. 12). The peroxyl radicals preferentially attack the free In–H bonds that are not involved in a complex formation [106]. This can be rationalized by the fact that the H-bond can stabilize the starting phenol. This stabilization is lost in the phenoxyl radical. The energy needed to abstract the hydrogen atom from H-bonded phenol is larger than non H-bonded phenol [82]. Thus, order of decreasing activities of tested compounds against peroxyl radicals is as shown below (Fig. 12).

7.4. Effect of the p-substituent

p-Methoxy substituents stabilize aryloxyl or arylaminiyl radicals stereoelectronically by conjugative electron delocalization with the heteroatoms. For stabilization, the oxygen *p*-type lone-pair orbital must overlap with the semi-occupied orbital (SOMO) of the free radical species. The extent of the overlap depends on the dihedral angle, θ, between the oxygen lone pair and the SOMO which is perpendicular to the atoms of the aromatic plane. This angle, θ, should be the same as the angle θ' between the $O_1–C_2$ bond and the aromatic plane as illustrated in (Fig. 13). Accordingly, the stabilization of the radical will be at a maximum when $\theta = 0^\circ$ and at a minimum when $\theta = 90^\circ$ [50,96,107,108]. This fact can be correctly observed by the x-ray diffraction analysis of a single crystal.

Other types of *p*-substituted phenols have also been studied. For instance, it has been found that the BDE of *p*-SMe and *p*-OMe phenol are 78.3 and 81.6 kcal/mol, respectively. Sulfur atom decreases the BDE (O–H) value, resulting in an increase in the antioxidant properties of *p*-SMe phenol [109]. Thus, in designing of antioxidant structure, the target compounds were designed to comprise sulfur rather than oxygen atom [110].

7.5. Effect of the o-substituent

It has been reported that the radical scavenging activity of phenol (or aniline) decreased when its ortho position is substituted with group which can form intramolecular hydrogen bond [91]. It is also reported that the electron-donating substituents on the 2,4

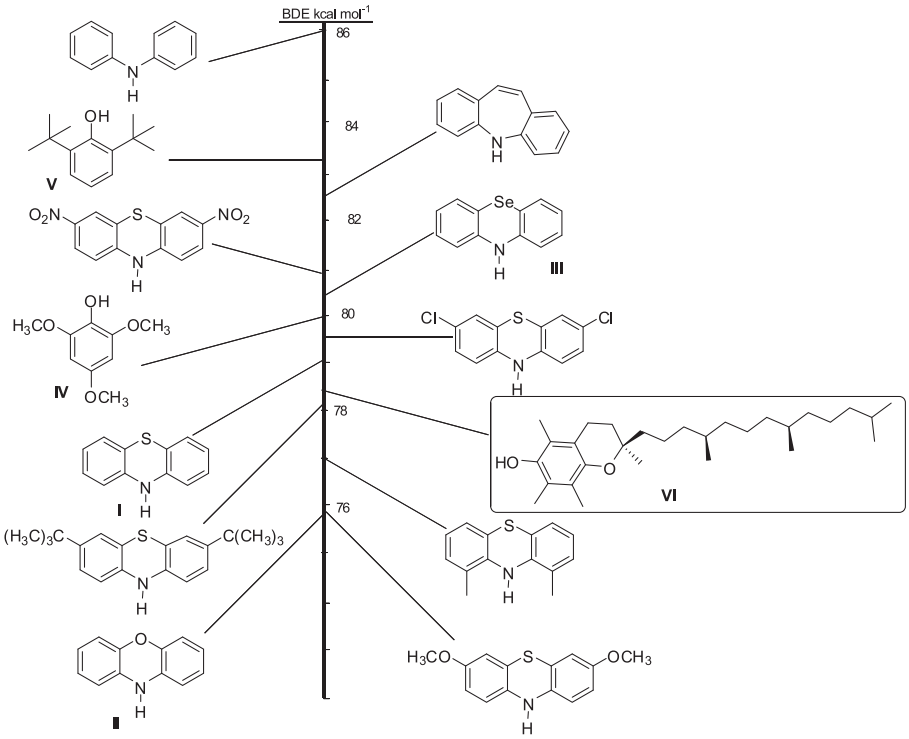


Fig. 9. BDE values (kcal/mol) of the ArN–H and ArO–H bonds in benzene [98,110].

Table 1
BDE values of thiophenols.

Thiophenol	BDE_{N-H} kJ/mol
p-NO_2	341
p-Br	332
p-Cl	331
Thiophenol	331
p-OMe	329
p-NH_2	292

and 6-positions of phenol can increase antioxidant activity [91], increasing the reaction rate with peroxyl radical as well as stabilizing the phenoxyl or aminyl radicals through inductive and hyperconjugative effects. It was also found that antioxidant activities are dependent on the position of the substituents where para > ortho > meta [111]. This can be attributed to an intramolecular hydrogen bond [91].

BDE has also been used to study the electronic and hydrogen bonding effects on the chain-breaking activity of sulfur-containing phenolic antioxidants. For instance, the BDE value of BHT is 80.1 kcal/mol [101] while the BDE of *p*-SMe is 78.3 kal/mol [109] (Fig. 5). This result is in agreement with the established fact that the O–H bond in phenol becomes weak in the presence of para electron donor [97]. Thus, the effect of *p*-SMe on the antioxidant activity is increased with decreasing the BDE of the O–H bond and increasing rate constant for the reaction with peroxyl radicals. The BDE of *o*-SMe is 82.22 kcal/mol [109]. Comparing this BDE with *p*-SMe (78.3 kal/mol) indicates *p*-SMe to be much better oxidation inhibitor than *o*-SMe. This difference can be attributed to the intramolecular hydrogen bonding of *o*-SMe forming bridge between the O–H and the sulfur atom (O–H···S) (Fig. 14). This bridge increases the BDE by stabilizing the phenol form and makes it more difficult for the approach of peroxyl radicals towards O–H group [82,109].

7.6. Effect of the m-substituent

As discussed earlier, an electron-donating substituent in the *p*-position of phenol not only increases the electron density of phenolic oxygen, it also increases the activity of the phenol by localization of a radical electron of the phenoxy radical [91]. Lawandy et al. [111] investigated the antioxidant activities of *o*-, *m*- and *p*-hydroxyphenylacrylamides, and reported their activities to be dependent on the position of the substituent (para > ortho > meta). The effect of *m*-substituents, however, has not been sufficiently investigated so far. Presumably, due to the fact that *m*-substituent showed only small resonance effect. Tetsuto et al. [112] have evaluated the antioxidant activity of different *m*-substituted phenol such as, *tert*-butyl, NH_2, OCH_3, CH_2CH_3, $OCH_2C_6H_5$ and that *m*-substituents do not influence the antioxidant activity of a phenol at all.

7.7. The concept of CoAHs

In 2002, Amorati et al. [113] studied a mixture of a α-TOH and a secondary aromatic amine (CoAH) and found that CoAH reversibly recycled α-TOH. Regeneration of α-TOH can only be observed if the BDE value of the CoAH is lower or at least close to that of the O–H bond of α-TOH. Amorati and co-workers [68] reported that the strong antioxidant activity of resveratrol (phenol) and some of its derivatives in micelles arise from their ability to reinforce α-TOH. Similarly, CoAH has been shown to effectively recycle α-TOH (AH) when the O–H BDE for CoAH is lower, or at least comparable to that of AH, and the rate constant for regeneration, K_r, is at least 10^3 M^{-1} s^{-1} while the rate constants for the reactions of peroxyl radicals, ROO•, with A• and CoA• are similar (Scheme 5).

O–H BDE value of BHT (81.0 kcal/mol) which is 2.7 kcal/mol less than that estimated for resveratrol. This would imply that BHT, although significantly less reactive than resveratrol toward peroxyl radicals but it could quantitatively regenerate resveratrol from the

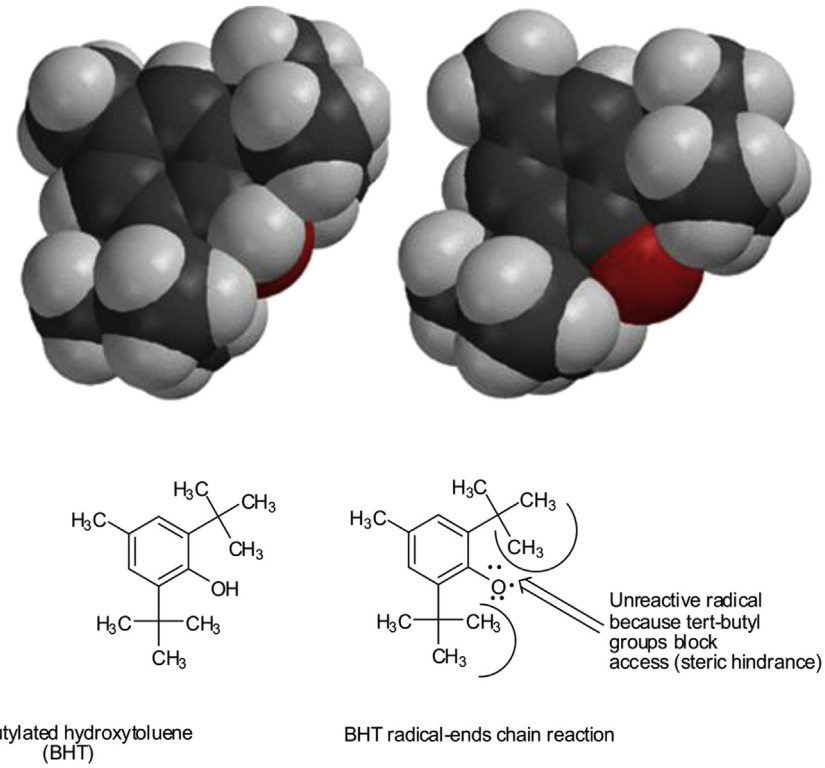


Fig. 10. Steric hindrance effects on stabilization of phenolic antioxidants.

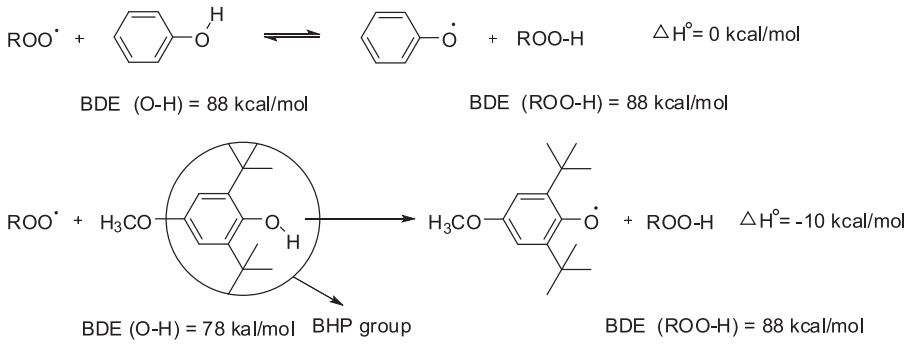


Fig. 11. BDE values of phenol and BHA.

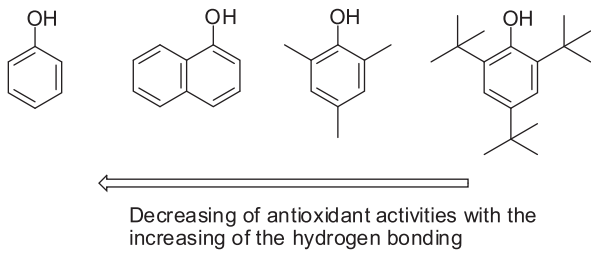


Fig. 12. The effect of the hydrogen bonding on antioxidant activities.

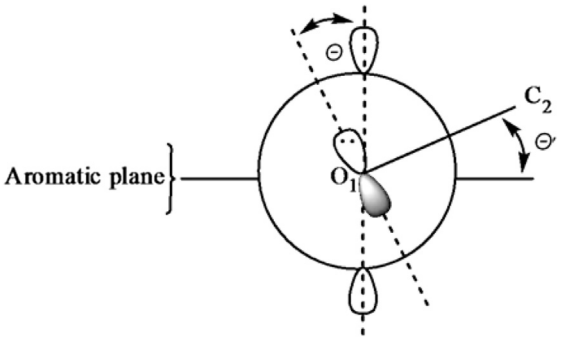


Fig. 13. Stereoelectronic effects of heteroatoms on stabilization of free radical.

corresponding phenoxyl radical (Fig. 15) [68].

7.8. Antioxidant behavior of the thioether group

Thioethers are classified as secondary antioxidants, and can be used in combination with primary antioxidants to improve the performance of the primary antioxidant [103]. Thioethers or peroxide decomposers however, do not act as radical scavengers but undergo redox reactions with hydroperoxidants to form non-radical stable products (Scheme 6) [114,115].

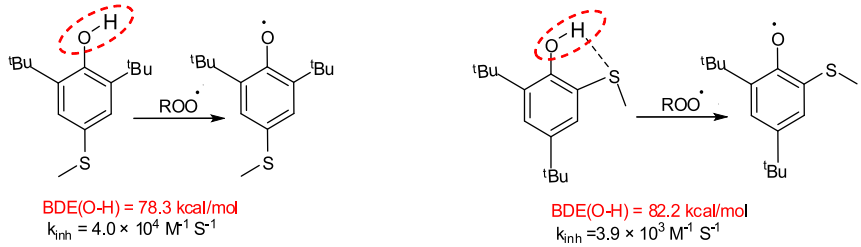
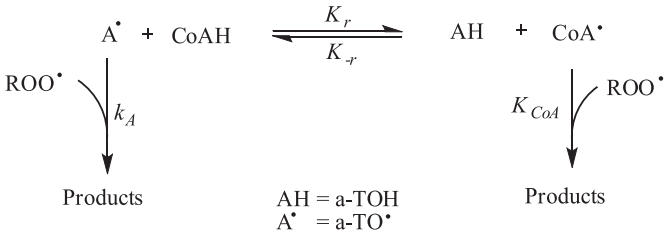
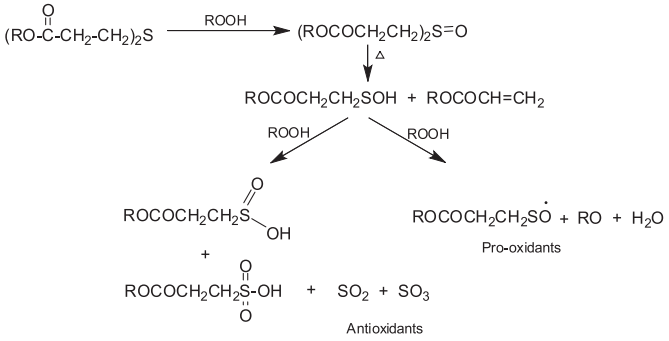


Fig. 14. BDE and effect of k_{inh} of *o*- and *p*-methylthio group on antioxidant activity.



Scheme 5. CoAH effectively recycles α-TOH.

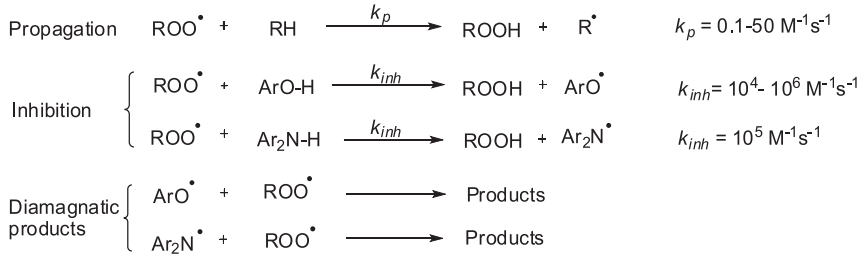


Scheme 6. Mechanism of sulphide antioxidants.

Among inhibitors of free radical oxidation of hydrocarbon substrates, sulfur-containing phenols are of particular interest since they can be have synergistically: i) as chain-breaking antioxidants by scavenging peroxyl radicals through hydrogen atom transfer from the phenol group (Scheme 3 step 4) and ii) as preventive antioxidants by decomposing hydroperoxides to the corresponding alcohols through nucleophilic attack of sulfur on oxygen [106]. Moreover, sulfur is considered to be more effective than oxygen at stabilizing a neighboring radical center due to better lone pair interaction with the carbon *p*-orbital [116]. This sulfur containing phenols might be better antioxidants than phenols containing oxygen substituents. Therefore, the designed compounds are preferable to contain thioether bridge link BHP with other antioxidants as well as its functionalities.

7.9. The role of phenol and secondary aromatic amine antioxidants

Similar to phenolic derivatives, aromatic amines and their derivatives can easily transfer their amine hydrogen to peroxyl radicals [98]. Scheme 7 shows the inhibition rate constant (k_{inh}) of phenolic and aromatic amines. The rate constants of the hydrogen transfer reaction of phenolic and aromatic amines are about 4 or 5 orders of magnitude larger than the propagation rate constant, k_p. Therefore, the antioxidants are present as inhibitors even at low concentrations, and the phenolic or amine antioxidant is able to suppress two oxidative chains [51].



Scheme 7. k_p and K_{inh} of phenol and aromatic amine.

7.10. Solubility of antioxidants

Antioxidants by their solubility can be physically classified into two groups; i) hydrophilic antioxidants (water loving/polar), such as ascorbic acid and most of polyphenolic compounds, and ii) lipophilic antioxidants (oil loving/non-polar), mainly vitamin E and carotenoids. The difference in the behavior of these two types of free radical scavengers (FRS) in food is referred to as the antioxidant polar contradiction (Fig. 16). This is based on the observation that, in emulsified oils, non-polar FRS are more effective than polar FRS, while polar FRS are more effective than non-polar FRS in bulk oils

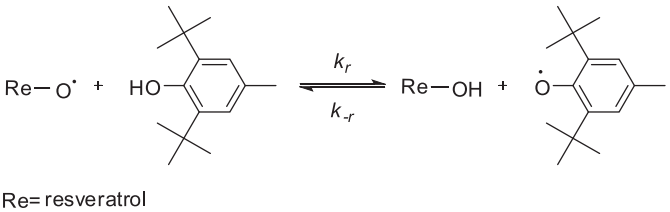


Fig. 15. BHT effectively recycles resveratrol.

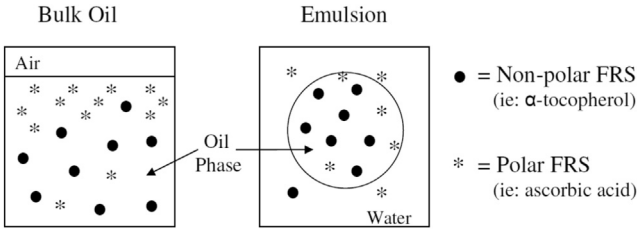


Fig. 16. Physical location of hydrophillic and hydrophobic free radical scavengers in bulk oils and oil-in-water emulsions.

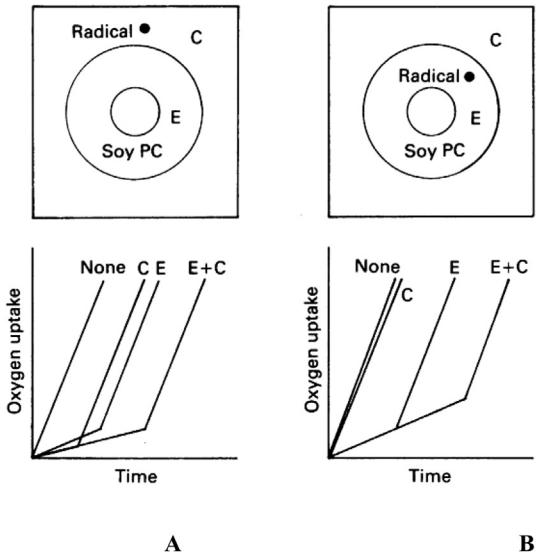


Fig. 17. Inhibition of oxidation of soybean phosphatidylcholine liposomes in aqueous dispersions by vitamin E, ascorbic acid, and their mixture. A-watersoluble radical initiator. B- lipid-soluble radical initiator.

[117,118]. The key to this fact is the ability of the FRS to accumulate in a medium where lipid oxidation is most common. Polar FRS concentrate at oil-air or oil-water interfaces in bulk oils, where most oxidation occurs due to the high concentrations of oxygen and prooxidants. In emulsions, non-polar FRS accumulate in the lipid phase and at the oil-water interface where interactions between hydroperoxides at the droplet surface and prooxidants in the aqueous phase occur [93].

The synergistic inhibition of the oxidation of soybean phosphatidylcholine liposomes in aqueous dispersions by vitamin E, ascorbic acid and their mixture in the membrane systems has been studied. It was found that when free radicals are generated in the aqueous phase from a water-soluble initiator, soybean PC is oxidized gradually. Both ascorbic acid, which is added to the aqueous phase and vitamin E that is incorporated into liposomal membranes are able to suppress the oxidation. In the presence of both vitamin E and ascorbic acid, the induction period is prolonged to the sum of their individual induction periods (Fig. 17A). On the other hand, when the oxidation is initiated with a lipid-soluble initiator, vitamin E suppressed the oxidation, while ascorbic acid does not (Fig. 17B). However, the ascorbic acid acts as a synergist and decreased the rate of consumption of vitamin E [35].

Many di-*tert*-butylphenol bicyclic derivatives are highly lipophilic, resulting in generally poor aqueous solubility and associated bioavailability. On the other hand, addition of ionizable functionality to the heterocyclic ring (hydrophilic) links to the phenol ring would reduce their lipophilicity and aid in absorption and elimination of these compounds. Thus, with this parameter, compounds

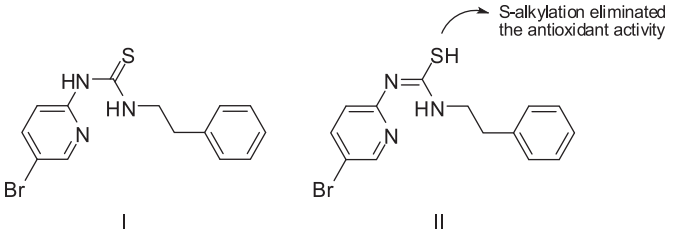


Fig. 18. Phenethyl-5-bromo-pyridyl thiourea (thione I and thiol II).

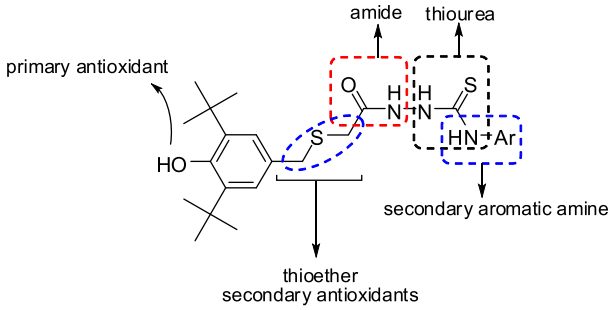
could be designed to have a wide range of these phenomena obtainable in one molecule, which could increase the role of the synergistic systems of antioxidants.

7.11. Effect of the thiourea system on antioxidant activity

Thiourea [119], propylthiouracil [120], 1,3-dimethyl-2-thiourea [121,122] and hydroxyphenylurea derivatives [123] are effective scavengers of reactive oxygen intermediates and free radicals. The antioxidant activities of thione and thiol forms in a series of phenethyl-5-bromo-pyridyl thiourea (Fig. 18 **I** and **II**, respectively) and the S-alkylated derivatives (Fig. 18, **II**) have been evaluated [124]. It has been found that all phenethyl-5-bromopyridyl thiourea compounds exhibited antioxidant activity and also showed potent anti-HIV activity. While S-alkylation eliminated the antioxidant activity and anti-HIV activity which indicated that the unalkylated thiourea group is critical to both antioxidant activity and HIV activity. This result suggests that the thiol group (Fig. 18, **II**) is responsible for the antioxidant activity due to its favorable electron-donating characteristics.

The analysis described above led to a proposed model of action of MPAOs based solely on SAR. Acylthiosemicarbazides (Fig. 19) were designed to form an MPAO in a single structure by linking the well-known antioxidant BHT at the 4-position via a thioether bridge, which provides a linkage between an amide, thiourea (which is classified as a free radical scavenger [125] and secondary antioxidant [126]) and a secondary amine.

Recently, we noted a few papers in which thiosemicarbazides and related compounds have been evaluated for their ability to scavenge free radicals and found no evidence for their antioxidant activities or mechanisms [128–131]. Canan and coworkers [132] have reported that 1-acylthiosemicarbazides are more effective as free radical scavengers than triazoles and thiadiazoles. Consequently, the higher antioxidant activity of acylthiosemicarbazides



Ar= o-, p-OMe and *m*-F to increase antioxidant activity

Fig. 19. SAR analysis of MPAO 1-acylthiosemicarbazide as an MPAO [110,127].

could be attributed to the aryl radicals generated by thermal decomposition react with S=C-NHR group to give S-substituted isothiosemicarbazides (Scheme 8); and second, when R=Ar, better yield was obtained due to hydrogen abstraction by the aryl radical from the substituted thioamide group [132,133].

Thus, the scavenging potential of the DPPH free radical reaction could occur as proposed in Scheme 9(A and B) [110].

7.12. Effect of a five membered heterocyclic ring on antioxidants

Further, heterocycles bearing a symmetrical triazole ring have been reported to show a broad spectrum of biological activities [134,135]. It was also reported that 1,2,4-triazoles are an important class of five membered heterocyclic compounds [136,137]. 1,2,4-triazole-5-thiones are known for their anti-inflammatory [138], selective COX-2 inhibitor [139] and antimycotic [140] activities.

A series of Schiff bases of 3-substituted-1,2,4-triazo-5-thione were synthesized and evaluated for their antioxidant activity using the hydrogen peroxide scavenging method. They were found to have good bioavailability and have significant antioxidant activity with IC_{50} value range from 20 to 60 μg/ml. This activity may be due to the presence of the −SH group in the 5th position of triazole ring (Fig. 20) [141]. This clearly shows the electron-donating and electron-withdrawing effects.

DPPH free radical scavenging measurements were used to determine the antioxidant power with 4-benzylidenamino-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives (Fig. 21). Most compounds tested were found to be more active or close to the activity of the well-known standard antioxidant, BHT [142].

Similarly, derivatives of thiadiazoles (Fig. 22) exhibit good antioxidant activity. The activity of thiol derivatives supports the hypothesis of a direct link between the thiol group and an aromatic ring. The thiol captured the free radical while the aromatic ring permits the trapping of this free radical [143].

In 2010 Imtiaz et al. [144] found the triazole-5-thione derivatives to be relatively more active than the derivatives of thiadiazole (Fig. 23). This due to the presence of thiourea system in triazole ring. For example, the experimentally determined antioxidant activities of hydroxyphenylurea derivatives exhibited 10 times higher antioxidant activity than α-TOH [89].

Considering these results, thiadizoles structures (Fig. 24) were designed to contain secondary aromatic amines, which act as inhibitors of the radical-chain oxidation. Triazole structures (Fig. 24), which have N*H* ionizable protons and a thiourea system in the triazole ring, may have enhanced antioxidant activity as well as solubility and biological properties.

7.13. Effects of hindered phenol in a biological system

The design of the BHT derivatives is based on their potential antioxidant and antiradical activity [74]. Evidence is accumulating to show that most diseases are connected with harmful ROS reactions [145]. These diseases include inflammation, atherosclerosis, cancer, diabetes, asthma, alzheimer's disease, aging, degenerative eye disease and other chronic diseases [146–148]. The conversion of arachidonic acid to prostaglandin endoperoxide PGG2 [149,150] occurs through a series of free radical reactions [151] (Scheme 10). Thus, derivatives bearing the BHT moiety were designed to be inhibitors of COX-2 pathway [152].

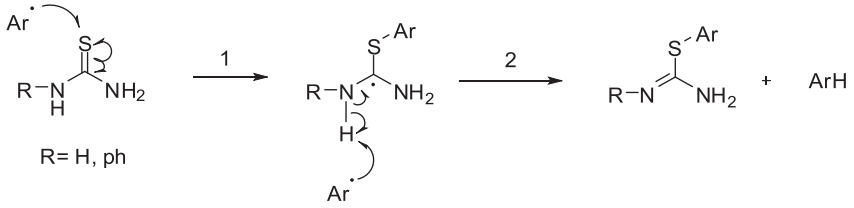
Following the same route, researchers of Parke-Davis have disclosed a new class of potent, selective and orally active COX-2 inhibitors, comprising 2,6-di-*tert*-butyl phenol such as PD 164387 and PD 138387 (Fig. 25) [153,154]. It was found that two *tert*-butyl groups flanking the OH group are required to retain *in vivo* anti-inflammatory potency [83].

Based on the Parke-Davis inhibitors, researchers have carried out SAR to improve activities of COX-2 inhibitors. Thus, various 1,2-isothiazolidine-1,1-dioxide (γ-sultam) derivatives containing an antioxidant moiety, 2,6-di-*tert*-butyl phenol substituent, have been prepared (Fig. 26). Some compounds showed potent inhibitory effects on COX-2 as well as the production of interleukin-1 *in vitro* assays. They also proved to be effective in several animal arthritic models without any ulcerogenic activities. This result suggested that the activities might be related to their free radical scavenging potency [155]. On the other hand, sterically hindered phenols linked with heterocyclic rings have also been extensively studied as COX inhibitor [152,156–158].

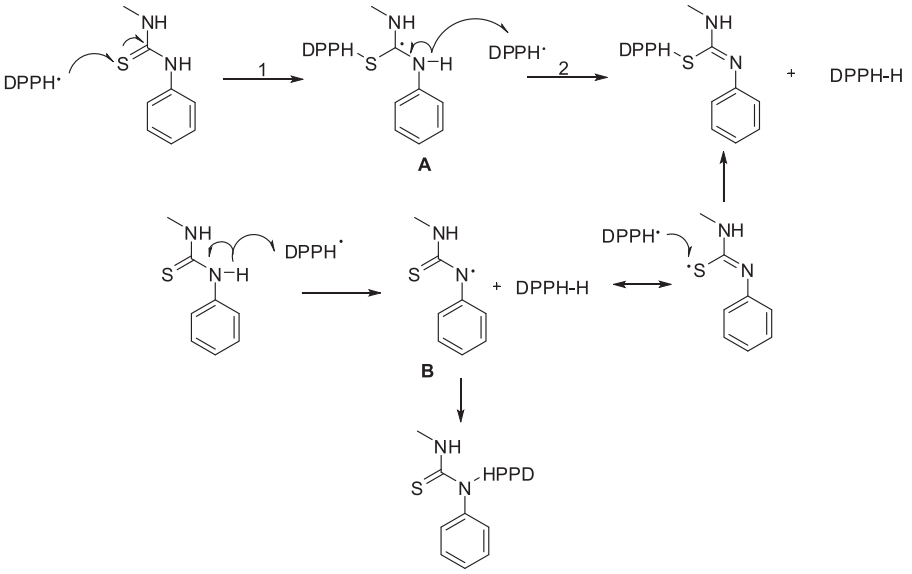
8. Assay methods for antioxidant activity

Several methods are used to evaluate antioxidant activities of natural and synthetic compounds in foods or biological systems with varying results [80,159,160]. Depending upon the reactions involved; antioxidant capacity and activity assays can be generally classified into two types: assays based on H-atom transfer reactions and assays based on electron transfers [161].

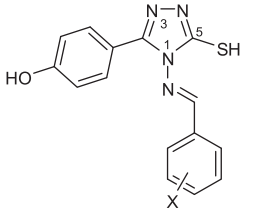
H-atom transfer assays include oxygen radical absorbance capacity. This analysis measures the ability of antioxidants to protect a fluorescent molecule from damage by free radicals [162]. In this assay, beta-phycoerythrin is used as an indicator protein, 2,2′-azobis(2-amidinopropane) dihydrochloride as a peroxyl radical generator, and Trolox (water-soluble derivative of vitamin E) as a control standard [162] with a total radical trapping antioxidant parameter. This creates a competitive reaction where antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds [163,164]. Electron transfer assays involve two components in the reaction mixture, antioxidants and the oxidant (which is the probe). The probe has ability to extract an electron from the antioxidant causing a color change in the reaction. The color change is used as an indicator to track the end point of the reaction [165]. Free radical scavenging assays that are commonly used to evaluate antioxidant activity *in vitro* are: TEAC assay [166], FRAP assay [167] and DPPH assay [168]. FRAP is an electron transfer-based antioxidant capacity assay. The method measured the ferric reducing ability of plasma (FRAP) at a low pH, when a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ) form [18]. Once the antioxidant reacted with the probe, an intense blue color



Scheme 8. Proposed mechanism of S-arylisothiouronium base formation [133].

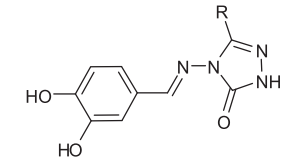


Scheme 9. Proposed scavenging of DPPH• by aryl thiourea.



X	IC_{50} μg/ml
H	24
3-OH	20
2-Cl	60
2-NO_2	32
3-NO_2	38

Fig. 20. Antioxidant activity of Schiff's bases of 3-substituted 1,2,4-triazo-5 thione.



R	IC_{50} μg/ml
CH_3	0.025
$CH_2C_6H_5$	0.026
$CH_2C_6H_4Cl(-4)$	0.026
$CH_2C_6H_4OCH_3(-4)$	0.050
C_6H_5	0.500
	0.050
BHT	0.040

Fig. 21. DPPH free radical scavenging of 4-benzylidenamino-4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives.

with an absorption maximum at 593 nm is observed, and reported in terms of FRAP value.

8.1. DPPH method

The DPPH reagent (Fig. 27) is a stable organic nitrogen radical, and has a deep purple color with maximum absorption at 515 nm [169]. When the radical is trapped by antioxidants, the color of the solution changes from a deep purple to a light yellow, and the absorbance at 515 nm decreases [170]. Compounds with high antioxidant will then result in a rapid decline in the absorbance of the DPPH• [171,172].

Brand-Williams et al. [12] (1985) first reported the DPPH assay. It has widespread use in antioxidant capacity screening, probably due to the simplicity of the equipment required. The general reaction of DPPH is shown in Scheme 11 below where **AH** is the antioxidant and **R•** is the free radical species.

The DPPH method allows a direct investigation of the ability of the antioxidant to donate hydrogen and/or electrons to quench the DPPH radical. The DPPH method is widely used to determine

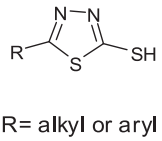


Fig. 22. Derivatives of 2-substituted−thiadiazole-5-thiols.

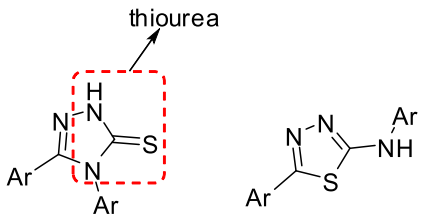


Fig. 23. 1,2,4-triazole and 1,3,4-thiadiazole derivatives.

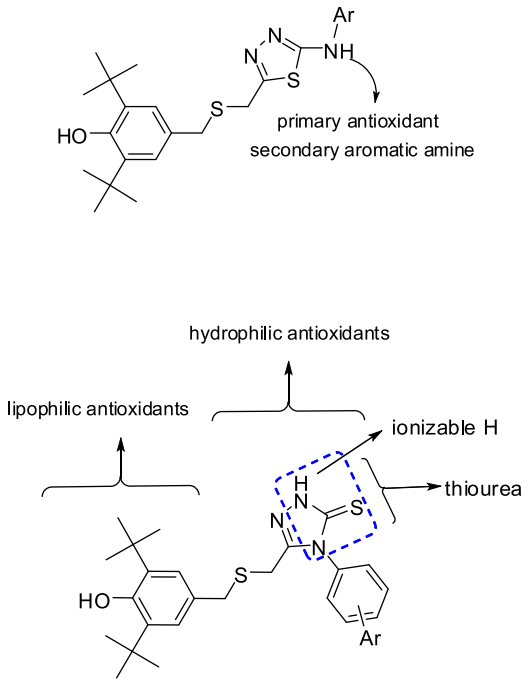


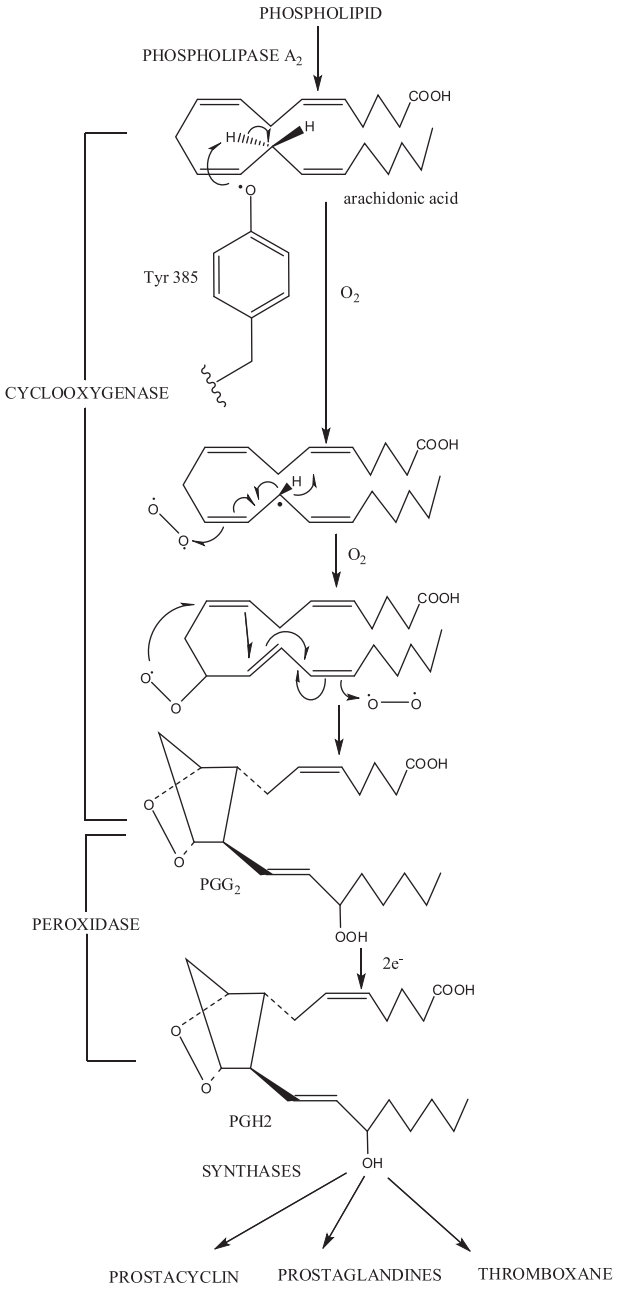
Fig. 24. SAR analysis of MPAOs, 1,3,4-thiadiazoles and 1,2,4-triazoles [110,127].

antiradical activity of phenolic compounds as well as natural plant extracts [173,174]. However, the DPPH method also has its limitations, particularly since the N-radical portion at the center of the DPPH structure is more accessible only to small molecules. Larger molecules may have limited access due to steric hindrances [175]. Steric accessibility of an antioxidant compound determines the type of reaction mechanism [176]. Small molecules that have better contact with the N-radical centre site show higher antioxidant activity. Since DPPH• is a stable nitrogen radical, unlike the highly reactive and transient peroxyl radicals involved in lipid peroxidation, many phenolic antioxidants that react quickly with peroxyl radicals may react slowly with, or may even inert to DPPH•, due to steric inaccessibility effects [176].

Bondet et al. [177] reported that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1–6 h or longer. For example, BHT and protocatechuic acid did not reach steady state, or the reaction end point, until three and two hours, respectively [169] due to certain antioxidant compounds have different reaction kinetics with DPPH• [169]. Additional to the above limitations, it was also reported that the reaction of DPPH• with eugenol to be reversible. This would result in falsely low readings for antioxidant capacity of samples containing eugenol and other phenols [177]. Furthermore, a color interference of DPPH with samples that contain anthocyanins led to underestimation of the antioxidant activity [178].

8.2. Measurement of lipid oxidation

Several methods have been used to evaluate the oxidative stability of different types of substances. Primary lipid oxidation compounds are the first oxidation products produced by initiation and propagation steps of lipid oxidation. They can appear early in the oxidative deterioration of protein lipids. At later stages of the oxidation, the concentrations of primary compounds decrease since their rates of formation become slower than their rates of



Scheme 10. Diagram for the conversion of arachidonic acid to prostaglandins and other eicosanoids by the COX enzymes [150].

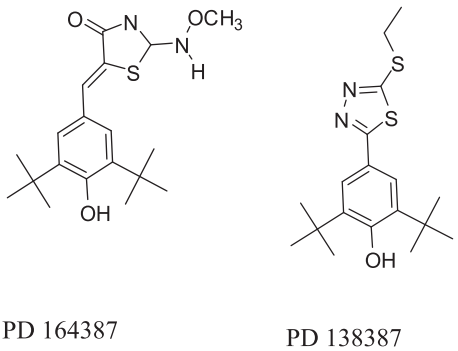


Fig. 25. Parke-Davis COX-2 inhibitors.

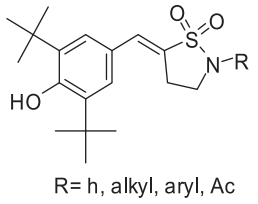


Fig. 26. Structure of 1,2-isothiazolidine-1,1-dioxide (γ-sultam).

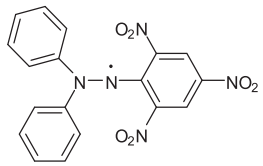


Fig. 27. The structure of 2,2-diphenyl-1-picrahydrazyl (DPPH).

$$DPPH^{\bullet} + AH \longrightarrow DPPH\text{-}H + A^{\bullet}$$

$$DPPH^{\bullet} + R^{\bullet} \longrightarrow DPPH\text{-}R$$

Scheme 11. DPPH reactions with antioxidant (AH) or/and free radical species (R•).

decomposition. Lipid hydroperoxide measurements are typically used to determine primary oxidation products. Secondary lipid oxidation products are compounds that are formed from the decomposition of fatty acid hydroperoxides by means of β-scission reactions. Since these reactions can generate hundreds of volatile and non-volatile compounds, which would be impossible to measure simultaneously. Since the formation of secondary products relies on the decomposition of lipid hydroperoxides, the presence of antioxidants can cause the concentrations of secondary products to be low while concentrations of primary products are high [179–181].

8.3. Lipid hydroperoxides

Determining the peroxide value is one of the most commonly used method for measuring the extent of oxidation in oils. It is expressed as millimoles of hydroperoxide per kg of lipid (mmol/kg) [179,182]. The ferric thiocyanate method requires a smaller sample size is more sensitive than other methods. This method is based on the oxidation of ferrous to ferric ions, which is determined calorimetrically as ferric thiocyanate [183]. In bulk oils, the peroxide value can be analyzed directly. In food, such as emulsions and muscle tissues, the lipid must first be extracted by mixing with suitable solvents [179]. The peroxide value is an empirical measure of oxidation, which is useful for samples that are oxidized to relatively low levels under mild conditions so that the hydroperoxides are not appreciably decomposed. During oxidation, the peroxide value reaches a maximum peak and then begins to decrease at more advanced stages of oxidation [182]. The maximum peroxide value can occur at early or late, depending upon the fatty acid composition of the oil and the conditions of the oxidation. For instance, for fish oil which is polyunsaturated oil, the peroxide value maximum takes place at an early stage because its hydroperoxide which decomposes more rapidly. Hydroperoxide also rapidly decomposes during oxidation conditions involving exposure to UV light, presence of metals and exceeding temperatures more than 100 °C [179].

8.4. TBARS method

The thiobarbituric acid-reacting substances (TBARS) method is used to measure the extent of secondary lipid oxidation products. TBARS is a rapid and easy assay procedure. It has been modified by researchers for use with several types of samples, including, food, drugs, animal and human tissues [184–188]. The basis of this test is the absorbance of a pink color complex spectrophotometrically at 532–535 nm which is formed between thiobarbituric acid and the oxidation products of unsaturated lipids. This pink color is formed by the condensation of 2:1 mol of thiobarbituric acid and malonaldehyde, under thermal acidic environments [179,182]. The measurement of the thiobarbituric acid value is expressed as the mg of malonaldehyde per kg of sample.

Similar to the DPPH assay, there are many factors that can affect the production of the pink color complex such as duration of heating, temperature, pH, metal ions and antioxidant structures. Variations to the thiobarbituric acid test were designed to increase the sensitivity, including heating in acids and adding ferric ion, or reducing production of decomposition materials during the assay by adding antioxidants [179,189].

9. Conclusion

Considerable theoretical effort has been devoted to both elucidating the SARs for antioxidants and designing novel antioxidants based on BHT molecule. The theoretical strategy involves appropriate parameters have to be identified and understood to reveal which structural factor is beneficial to enhance their activity and which one is not. This will not only facilitate the screening of antioxidants from thousands of candidates, but lead to a theoretical explanation on the SARs providing clues to rational design of novel antioxidants, which can be designed and synthesized according to the specific demand, by tuning the theoretical parameters. We have identified 14 very sensitive parameters, which may play a major role in the antioxidant performance of bulky phenols. Antioxidant influence is enhanced by a relatively low BDE of relevant O–H in phenols and amines, respectively. Electron-donating substituents on a phenol are usually lower the value of BDE. It is likely that changes in BDE will be strongly correlated and indeed this correlation will play an important role in the design of antioxidant. The antioxidant capabilities of phenols are strongly reduced by protic solvents since the intermolecular hydrogen-bonded is virtually unreactive toward free radicals. Electron-donating substituents, such as methyl and tert-butyl on 2,4 and 6-positions increase the antioxidant activity of phenols. The effect of m-substituents do not influence the antioxidant activity of a phenol at all due to the fact that m-substituent showed only small resonance effect. p-Methoxy substituents stabilize aryloxyl or arylaminiyl radicals stereoelectronically by conjugative electron delocalization with the heteroatoms. The extent of the overlap depends on the dihedral angle, θ, between the oxygen lone pair and the SOMO which is perpendicular to the atoms of the aromatic plane. Accordingly, the stabilization of the radical will be at a maximum when $\theta = 0^{\circ}$ and at a minimum when $\theta = 90^{\circ}$. CoAH has been shown to effectively recycle AH when the O–H BDE for CoAH is lower, or at least comparable to that of AH. Secondary antioxidants thioethers or peroxide decomposers do not act as radical scavengers but undergo redox reactions with hydroperoxidants to form non-radical stable products. Sulfur-containing phenols are of particular interest since they can be have synergistically as chain-breaking and as preventive antioxidants. Sulfur containing phenols might be better antioxidants than phenols containing oxygen substituents. Aromatic amines and their derivatives can easily transfer their amine hydrogen to peroxyl radicals. In term of solubility of antioxidants,

with this parameter, compounds could be designed to have a wide range of phenomena obtainable in one molecule, hydrophilic antioxidants and lipophilic, which could increase the role of the synergistic systems of antioxidants. Thiol group in thiourea system is responsible for the antioxidant activity due to its favorable electron-donating characteristics. Triazole-5-thione derivatives turned out to be relatively more active than the derivatives of thiadiazole due to the presence of thiourea system in triazole ring. However, a new trend in this area is to combine multiple functions, including various antioxidant properties and BHT moeity in one structure. It can be expected that more and more novel antioxidants drived from BHT with desirable functions will appear in not very far future.

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