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Relationship structure-antioxidant activity of hindered phenolic compounds

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SUMMARY: The relationship between the structure and the antioxidant activity of 21 hindered phenolic compounds was investigated by Rancimat and DPPH· tests. 3-*Tert*-butyl-5-methylbenzene-1,2-diol is the strongest antioxidant in the Rancimat test but not in the DPPH· test because its two hydroxyl groups have very strong steric synergy. 2,6-Di*tert*-butyl-4-hydroxy-methylphenol exhibits a strong antioxidant activity as 2,6-di*tert*-butyl-4-methoxyphenol does in lard. 2,6-Di*tert*-butyl-4- hydroxy-methylphenol also exhibits stronger activity than 2-*tert*-butyl-4- methoxyphenol. The methylene of 2,6-di*tert*-butyl-4-hydroxy-methylphenol can provide a hydrogen atom to active free radicals like a phenolic hydroxyl group does because it is greatly activated by both the aromatic ring and hydroxyl group. Five factors affect the antioxidant activities of the phenolic compounds: how stable the phenolic compound free radicals are after providing hydrogen atoms; how many hydrogen atoms each of the phenolic compounds can provide; how fast the phenolic compounds provide hydrogen atoms; how easily the phenolic compound free radicals can combine with more active free radicals, and whether or not a new antioxidant can form after the phenolic compound provides hydrogen atoms.

KEYWORDS: *DPPH; Hindered phenolic; Rancimat test; Steric synergist effect; Structure activity relationship*

RESUMEN: ***Relación estructura-actividad antioxidante de compuestos fenólicos impedidos estéricamente***. La relación entre estructura y la actividad antioxidante de 21 compuestos fenólicos con impedimentos estéricos fue investigado mediante ensayos con Rancimat y DPPH·. El 3-*terc*-butil-5-metilbenceno-1,2-diol es el antioxidante más potente en los ensayos mediante Rancimat pero no mediante ensayos con DPPH·, porque sus dos grupos hidroxilo tienen una fuerte sinergia estérica. El 2,6-Di-*terc*-butil-4-hidroxi-metil-fenol mostró una actividad antioxidante tan fuerte como el 2,6-di-ter-butil-4-metoxifenol en ensayos con manteca de cerdo. El 2,6-di-*terc*-butil-4-hidroxi-metilfenol también mostró una actividad más fuerte que el 2-*terc*-butil-4-metoxifenol. El grupo metileno del 2,6-di-ter-butil-4-hidroxi-metilfenol puede suministrar átomos de hidrógeno y activar radicales libres como lo hace un grupo hidroxilo fenólico porque se activa en gran medida tanto por anillo aromático como por el grupo hidroxilo. Cinco factores afectan a la actividad antioxidante de los compuestos fenólicos: cómo de estable son los radicales libres de los compuestos fenólicos después de suministrar átomos de hidrógeno; cuántos átomos de hidrógeno pueden proporcionar cada uno de los compuestos fenólicos; la rapidez con la que los compuestos fenólicos donen átomos de hidrógeno; la facilidad con la que los radicales libres de los compuestos fenólicos pueden combinarse con los radicales libres más activos, y si es o no un nuevo antioxidante el que se puede formar después de que el compuesto fenólico done los átomos de hidrógeno.

PALABRAS CLAVE: *DPPH; Efecto sinérgico estérico; Fenoles impedidos estéricamente; Rancimat; Relación estructura-actividad*

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1. INTRODUCTION

Autoxidation is the main process of fat, oil and lipid-based food deterioration, which results in a loss in nutrition, and the production of an off-flavor and other harmful substances (Pokorny *et al.*, 2001). Many hindered phenolic antioxidants such as 2,6-ditert-butyl-4-methylphenol (BHT), 4-hydroxy-3-*tert*-butylanisole (BHA), *tert*-butylated hydroquinone (TBHQ) are widely used as antioxidants in the food industry, especially for bulky oils and fatty foods. These synthetic antioxidants are highly active and cheap. They are colorless, odorless, tasteless and non-toxic (Zhang *et al*., 2004). The addition of antioxidants is the most effective, convenient and economical way to retard lipid autoxidation (Li *et al.*, 2006). Therefore, the antioxidant activity of natural and synthetic phenolic compounds attracts the attention many researchers.

Determination of the oxidative stability of oils and fats can be used to measure the activity of antioxidants in oil. Various chemical tests and accelerated methods have been reported for the determination of the oxidative stability of oils and fats. The active oxygen method (AOM) has traditionally been used for such determinations (Weng and Wu, 2000). This method is tedious and involves the use of large quantities of toxic chemicals and laborious titration (Läubli and Bruttel, 1986; Läubli *et al*., 1988).

The Metrohm Rancimat is a rapid automated method (Gordon and Mursi, 1994) which is fairly consistent with the AOM method (Läubli and Bruttel, 1986; Läubli *et al*., 1988) and can avoid the disadvantages mentioned above. These methods allow for the determination of the induction period of oils and fats.

The ability of phenolic compounds to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Bonde *et al*., 1997; Mensor *et al*., 2001; Philip 2004), superoxide anion radical, and the hydroxyl radical (Lebeau *et al*., 2000) is commonly used to study antioxidant activity. A new parameter can be used to characterize the activity of phenolic antioxidants in eliminating DPPH·. The parameter is $1/ (EC_{50}\ t_{1/2})$, in which EC_{50} stands for the concentration of antioxidants and $t_{1/2}$ is the time taken to eliminate half the amount of DPPH·. This parameter can be used to compare activities of antioxidants better than EC_{50}.

The antioxidant structure-activity relationship of phenolic compounds was previously investigated by some researchers. Zhang 1998, 1999a, 1999b, 2000; Zhang *et al.*, 1999, 2000) used several models to calculated the parameters, such as the difference in heat of formation between an antioxidant and its free radical (ΔHOF) and the highest occupied molecular orbit (HOMO) energy level to express the free radical scavenging activity of phenolic compounds, which are mainly flavonoids. Their results were highly consistent with the experiment results. They studied the phenolic hydroxyl, alkoxyl and carbonyl group and conjugated systems affecting the antioxidant activity of phenolic compounds. Burton and Ingold 1981, 1986; Burton *et al*., 1983, Burton *et al*., 1985), Lucarini (1996) and Wright *et al*., (2001) studied how various groups (mainly alkyl, hydroxyl and alkoxyl groups) were effective on the H-O bond dissociation energy of phenolic compounds. Burton (1980) investigated how the stereoelectronic factors of substituents affect the antioxidant activity of phenols. Weng (1993) discussed how the conjugated systems, *ortho*- and *para*-groups affect the antioxidant activity of phenolic compounds. Duan *et al.* (1998) found that some phenolic compounds can form new compounds which have strong antioxidant activity after they have acted as antioxidants.

In this paper, our study focused on the relationship between antioxidant structure and activity of these phenolic compounds by using the Rancimat test in lard and the DPPH assay.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals

Lard was rendered in the laboratory and stored in a deep freezer for use. 2-Methylbenzene-1,4-diol (compound 18) and 4-methylbenzene-1,2-diol (compound 19) were purchased from Sigma-Aldrich Trading Co, Ltd. 2-*Tert*-butyl-5-methyl- benzene-1,4-diol (compound 20) and 3-*tert*-butyl-5-methyl-benzene-1,2-diol (compound 21) were synthesized in our laboratory (Huang *et al*., in press). Other hindered phenolic antioxidants were purchased from Tokyo Kase Kogyo Co, Ltd. 2-*tert*-butyl-benzene-1,4-diol (compound 12, TBHQ) was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Other chemicals used in this experiment were all AR grade and from this company.

2.2. Antioxidant activity evaluated by Rancimat test

The antioxidant activity of hindered phenolic antioxidants at different concentrations in lard was determined by Rancimat (Metrohm AG, Herisau, Switzerland) based on the method by Guo *et al.* (2005). The air flow rate was controlled at 20 L/h, the temperature was controlled at 100 °C and lard was used as a substrate. 0.02% Antioxidant (w/w) was added to each lard sample (3 ± 0.02g) separately. Each sample was treated in duplicate.

2.3. Antioxidant activity evaluated by DPPH· test

The antioxidant activities of the hindered phenolic compounds were measured in terms of hydrogen-donating or radical-scavenging ability (Lebeau

et al., 2000; Li *et al*., 2006) using the DPPH· method. All spectrophotometric measurements were performed with a UV-2012 PC spectrophotometer (UNICO Corp, Shanghai, China). In terms of hydrogen-donating or radical-scavenging ability, the EC_{50} value is defined as the concentration of substrate that causes a 50% loss in DPPH· activity. The EC_{50} of phenolic compounds were calculated by linear regression of plots, where the abscissa represented the concentration of tested samples and the ordinate represented the average percentage of scavenging capacity from triplicates. A new parameter, $1/(EC_{50}\ t_{1/2})$, is used to characterize the activity of phenolic antioxidants for eliminating DPPH· by Qiu *et al*. (2005).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity by Rancimat test

The antioxidant activities of the hindered phenolic compounds were tested on the Rancimat at 100 °C. To explain the effect of the compound structures on the antioxidant activities, the antioxidant protection factors (Pf) have been calculated according to equation 1.

$$Pf = IP\ (induction\ periods\ of\ lard\ added\ antioxidant)\ /\ IP\ (induction\ period\ of\ lard) \quad \text{Eq 1}$$

The compounds used to study antioxidant activities in the experiment are shown in Figure 1. The Pf values of the compounds are shown in Table 1. A higher value of Pf means a stronger antioxidant activity of a compound. If $Pf < 1$, the compound had pro-oxidant activity; if $Pf = 1$, the compound had no antioxidant activity at all; if $2 > Pf > 1$, the compound had some (weak) antioxidant activity; if $3 > Pf > 2$, the compound had an obvious antioxidant activity and if $Pf > 3$, the compound had a strong antioxidant activity (Wang *et al*., 2000).

When lard is used as the substrate and Rancimat as assaying instrument, the temperature is controlled at 100 °C, and the air flow rate is controlled at 20 $L \cdot min^{-1}$, the antioxidant activities of the compounds shown in figure 1 are reduced in the following order (Table 1):

Compound 21 >> Compound 12 >> Compound 6 > Compound 4 > Compound 13 > Compound 18 > Compound 14 ≈ Compound 19 > Compound 7 > Compound 15 ≈ Compound 3 > Compound 20 ≈ Compound 9 > Compound 10 ≈ Compound 2 ≈ Compound 8 ≈ Compound 5 = Compound 17 > Compound 1 > Compound 11 ≈ Compound 16.

The results in Table 1 show that all 21 compounds in Figure 1 have very different levels of antioxidant activity. It is interesting that compound 21 (3-*tert*-butyl-5-methylbenzene-1,2-diol) presents extremely powerful antioxidant activity (Pf = 16.55). Compound 12 (TBHQ) also demonstrates super-strong antioxidant activity (Pf = 10.85), but much weaker than compound 21. Compounds 6, 4 and 13 show very strong antioxidant activities (Pf = 6.54, 6.17 and 5.97 respectively). Compounds 18, 14, 19, 7, 15 and 3 demonstrate strong antioxidant activities (Pf = 5.09, 4.52, 4.39, 3.86, 3.26 and 3.21 separately). Compounds 20, 9, 10, 2, 8, 5 and 17 have obvious antioxidant activities (Pf = 2.86, 2.73, 2.25, 2.20, 2.11, 2.03 and 2.02). Compounds 1, 11 and 16 show weak antioxidant activities (Pf = 1.82, 1.40, 1.38).

3.2. Antioxidant activity evaluated by the DPPH· test

The determination of the radical scavenging activities of hindered phenolic antioxidants was carried out at 517 nm and the results are shown in Table 1.

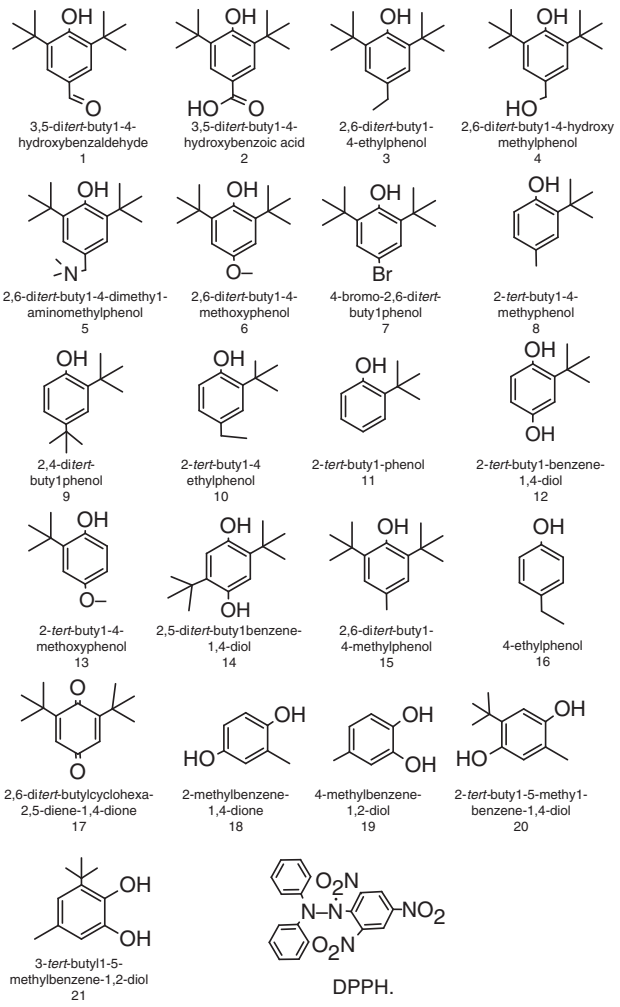

FIGURE 1. The chemical structure of hindered phenolic compounds whose antioxidant activities are investigated and free radical source DPPH·.

TABLE 1. The protection factors (Pf) of the lard containing different compounds at 0.02% concentration by the Rancimat test and the EC_{50}, half elimination time ($t_{1/2}$), the new parameter $1/(EC_{50}\ t_{1/2})$ in the DPPH· assay.

No	Compounds	Pf	$1/(EC_{50}\ t_{1/2})$ $(mol{\cdot}L^{-1})^{-1}{\cdot}min^{-1}$	$t_{1/2}$ (min)	EC_{50} $(mol{\cdot}L^{-1})$
1	3,5-di*tert*-butyl-4-hydroxybenzaldehyde	1.82 ± 0.02	31.53 ± 0.01	158.60 ± 0.09	2.00×10^{-4} ± 0.01
2	3,5-di*tert*-butyl-4-hydroxybenzoic acid	2.20 ± 0.08	242.13 ± 0.05	196.67 ± 0.05	2.10×10^{-5} ± 0.01
3	2,6-di*tert*-butyl-4-ethylphenol	3.21 ± 0.03	306.59 ± 0.03	171.67 ± 0.1	1.92×10^{-5} ± 0.03
4	2,6-di*tert*-butyl-4-hydroxy-methylphenol	6.17 ± 0.03	636.62 ± 0.08	108.33 ± 0.07	1.45×10^{-5} ± 0.05
5	2,6-di*tert*-butyl-4-dimethylamino-methylphenol	2.03 ± 0.00	226.41 ± 0.01	119.37 ± 0.08	3.70×10^{-5} ± 0.03
6	2,6-di*tert*-butyl-4-methoxyphenol	6.54 ± 0.06	505.39 ± 0.05	99.43 ± 0.00	1.99×10^{-5} ± 0.00
7	4-Bromo-2,6-di*tert*-butylphenol	3.86 ± 0.08	188.46 ± 0.03	113.64 ± 0.04	4.67×10^{-5} ± 0.01
8	2-*tert*-butyl-4-methylphenol	2.11 ± 0.01	47.99 ± 0.09	473.56 ± 0.07	4.40×10^{-5} ± 0.02
9	2,4-di*tert*-butylphenol	2.73 ± 0.02	31.80 ± 0.07	361.42 ± 0.09	8.70×10^{-5} ± 0.09
10	2-*tert*-butyl-4-ethylphenol	2.25 ± 0.01	73.64 ± 0.03	234.12 ± 0.08	5.80×10^{-5} ± 0.07
11	2-*tert*-butylphenol	1.40 ± 0.02	5.82 ± 0.02	324.10 ± 0.05	5.30×10^{-4} ± 0.08
12	2-*tert*-butyl-benzene-1,4-diol (TBHQ)	10.85 ± 0.07	1754.85 ± 0.04	19.65 ± 0.08	2.90×10^{-5} ± 0.03
13	2-*tert*-butyl-4-methoxyphenol (BHA)	5.97 ± 0.01	191.60 ± 0.05	326.20 ± 0.04	1.60×10^{-5} ± 0.06
14	2,5-di*tert*-butylbenzene-1,4-diol (DTBHQ)	4.52 ± 0.01	181.23 ± 0.05	149.13 ± 0.06	3.70×10^{-5} ± 0.05
15	2,6-di*tert*-butyl-4-methylphenol (BHT)	3.26 ± 0.08	56.31 ± 0.09	569.22 ± 0.01	3.12×10^{-5} ± 0.07
16	4-ethylphenol	1.38 ± 0.03	1.38 ± 0.01	460.03 ± 0.08	1.57×10^{-3} ± 0.01
17	2,6-di*tert*-butylcyclohexa-2,5-diene-1,4-dione	2.02 ± 0.03	3.13 ± 0.05	60.21 ± 0.01	5.30×10^{-3} ± 0.03
18	2-methylbenzene-1,4-diol(MHQ)	5.09 ± 0.02	702.40 ± 0.00	58.83 ± 0.00	2.42×10^{-5} ± 0.04
19	4-methylbenzene-1,2-diol (HPC)	4.39 ± 0.03	241.32 ± 0.00	180.17 ± 0.00	2.30×10^{-5} ± 0.04
20	2-*tert*-butyl-5-methylbenzene-1,4-diol(TBMHQ)	2.86 ± 0.02	955.46 ± 0.07	17.83 ± 0.08	5.87×10^{-5} ± 0.06
21	3-*tert*-butyl-5-methylbenzene-1,2-diol (TBHPC)	16.55 ± 0.01	359.02 ± 0.08	54.83 ± 0.01	5.08×10^{-5} ± 0.07

3.00 ± 0.02g Lard was added, the induction period (IP) of blank lard is 4.09 ± 0.05 h under 100 °C, air flow rate was 20L/h, Pf = The IP of lard with antioxidant/the IP of control lard, Values were expressed as mean ± relative deviation.
EC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%, A value obtained from the regression line with 95% confidence level, Values were expressed as mean ± SD, n = 3.
$t_{1/2}$ is the time taken to eliminate half the amount of DPPH·, Values were expressed as mean ± relative deviation, n = 2.

Compound 12 (TBHQ) ($EC_{50} = 2.90\times10^{-5}$ mol·L^{-1}) shows strong activity. Similarly, compounds 4 and 13, 3 show very strong radical scavenging ability (EC_{50} = 1.45, 1.60, and 1.92×10^{-5} mol·L^{-1}), even stronger than TBHQ. The other results are almost similar to the results from the Rancimat test except for compounds 21 and 2. The EC_{50} mainly depends on how many molecular active free radicals can be scavenged by one molecular phenolic compound, or whether a phenolic radical can combine with another active radical, and then form a new antioxidant or not (Duan *et al*., 1998), but the radical-scavenging speed does not affect EC_{50} as much. The lower the EC_{50} of an antioxidant is, the stronger its antioxidant activity is. According to EC_{50}, the antioxidant activities of the 21 compounds listed in Table 1 reduce in the following order:

Compound 4 ≈ Compound 13 > Compound 3 ≈ Compound 6 ≈ Compound 2 ≈ Compound 19 ≈ Compound 18 > Compound 12 ≈ Compound 15 > Compound 14 = Compound 5 ≈ Compound 8 > Compound 7 > Compound 21 > Compound 10 ≈ Compound 20 > Compound 9 > Compound 1 > Compound 11 > Compound 16 > Compound 17.

It is interesting to note that the two strongest antioxidants, compounds 21 and 12 exhibited much weaker antioxidant activity when they were evaluated by Rancimat in oil. Similarly, when they were evaluated by the EC_{50} of DPPH·-scavenging, they were ranked as numbers 14 and 8. Compounds 20 and 21 are much weaker antioxidants than their mother compounds 18 and 19, because the free radicals of compounds 20 and 21 cannot combine bulky free radical DPPH· while their mother compounds can. This is explained in our previous research work (Huang *et al*., 2013).

It has been proven theoretically that the new parameter, $1/(EC_{50}\ t_{1/2})$, is related to the reaction velocity constant k, which is equal to [ln (1/2)]/$(EC_5\ t_{1/2})$, which can correctly characterize the ability

of the antioxidants to eliminate DPPH· in quantity (Qiu *et al*., 2005). This means that the bigger the reciprocal [1/(EC_{50} $t_{1/2}$)] of the arithmetic product EC_{50} and $t_{1/2,}$ the larger the k value is, and the stronger the phenolic compound is (Table 1).

According to the 1/(EC_{50} $t_{1/2}$) in the DPPH test, the antioxidant activities of the 21 compounds listed in Table 1 reduce in the following order, and these result are somewhat similar to the Rancimat test.

Compound 12 >> Compound 20 > Compound 18 > Compound 4 > Compound 6 > Compound 21 > Compound 3 > Compound 2 ≈ Compound 19 > Compound 5 > Compound 13 ≈ Compound 7 > Compound 14 >> Compound 10 > Compound 15 > Compound 8 ≈ Compound 9 ≈ Compound 1 >> Compound 11 > Compound 17 >> Compound 16.

Here compound 12 exhibits the highest activity in both test parameters: Pf and 1/(EC_{50} $t_{1/2}$).

It is interesting to note that the antioxidant activities of compound 3 and compound 15 (BHT) in table 1 (Pf = 3.21 and 3.26) are almost equal, but compound 4 (Pf = 6.17) is much stronger than these two compounds. Figure 2a and Figure 2b can clearly explain why compound 4 can act as a much stronger antioxidant than compounds 3 and 15. However, the antioxidant activity of compound 5 should be close to compound 4 according to their chemical structures. The fact is that compound 5 is a much weaker antioxidant (Pf = 2.03) than compound 4 (Pf = 6.17). The reason is that the amino group is an alkaline group, it can firmly combine with a proton to form R-$N^{+}H(CH_3)_2$, so compound 5 cannot behave as compound 4 does and the lone pair electrons of the amine group cannot conjugate with the *ortho*-carbon free radical like the hydroxyl group in compound 4. Figs. 2a, b and c illustrate this very well. All the Pf, EC_{50} and 1/(EC_{50} $t_{1/2}$) of compounds 5 and 4 give highly consistent results (Table 1).

1/(EC_{50} $t_{1/2}$) is a much better parameter than EC_{50} because it is not only considered how many active free radicals one molecular phenolic compound can scavenge, but also how fast the phenolic compounds can scavenge active free radicals.

Comparing compound 7, compounds 15 (BHT) and 6, compound 6 shows the strongest antioxidant activity by Pf, EC_{50} and [1/(EC_{50} $t_{1/2}$)] (6.54, 1.99×10^{-5}, 505.39), and is much stronger than compounds 15 (3.26, 3.12×10^{-5}, 56.31) and 7 (3.86, 4.67×10^{-5}, 188.46). Theoretically, both the methoxyl group and bromine at the 4-position of the phenols have strong electronic pushing effects, because their unshared lone pair electrons can conjugate with the aromatic ring by p-π form. However, the oxygen atom and carbon atom have a close atomic radius (Slater, 1964), so the p-electrons of oxygen can conjugate with the π-electrons of the benzene ring very well, much better than the conjugation of bromine and benzene, because bromine is located in period 4 and its atomic radius (94 pm) is much bigger than carbon (70 pm) and oxygen (60 pm) (Slater, 1964). Therefore, the unshared lone pair electrons in oxygen (Fig. 3a) can distribute the benzene ring much better than bromine (Fig. 3b). It has also been found that compound 7 (Pf = 3.86) shows nearly double the strength of antioxidant activity as compound 2 (Pf = 2.20) in the Ranciamt test, maybe the stronger electronic pushing effect of bromine brings about this phenomenon. However, a reverse result was obtained in the DPPH· test [EC_{50} (compound 7) = 4.67×10^{-5} mol·L^{-1}; EC_{50} (compound 2) = 2.10×10^{-5} mol·L^{-1}]. Bromine has a large diameter. However, the carboxyl group is smaller and in the same plane with the aromatic ring. So the former shows more steric hindrance than the later; while in the Rancimat test, ROO· is relatively small. Steric hindrance does not affect the same phenolic free radical to combine with ROO·, when DPPH· is a very large free radical in the DPPH test, the steric hindrance of the phenolic compound will remarkably affect DPPH· to combine with the phenolic free radical (Fig. 4). So it is quite clear that the characteristics of the substituent on the 4-position of the 2,6-ditert-phenol greatly affect the antioxidant activity in two ways. One is the ability of this constituent to push electrons to the aromatic ring. Here, the order of the ability to push electrons to the aromatic ring is as follows:

$-OH > -OCH_3 > -Br > -CO_2H > -CH_2\,CH_3 > -CH_3 > -CHO.$

The authors have not found any previous research reports about how the halogens on the *para*-position of phenolic compounds affect their own antioxidant activity.

When the substituent is -CHO (compound 1), its antioxidant activity in lard (Pf = 1.82) is even weaker than compound 17 (Pf = 2.02), because -CHO is a strong electron pulling group (Duan *et al*., 1998).

Another factor is whether the substituent can enhance the phenolic compound to form a new compound or not. Sometimes, this new compound formed after its mother compound behaved as antioxidant, still has strong antioxidant activity (Duan *et al*., 1998). Some substituents on the 4-position of the 2,6-ditert-butylphenol can even directly provide a hydrogen atom to more active free radicals like the phenolic hydroxyl group does. Compound 4 shows stronger activity than compounds 3 and 15, because the Bond Dissociation Energy (BDE) of HOArCH(OH)-H is much lower than HOArCH(CH_3)-H, which is, in turn, lower than HOArCH_2-H (Fossey *et al*., 1995). Since the methylene group at the 4-position of compound 4 is highly activated by both the aromatic ring and the hydroxyl group at same time. Figure 2a and figure 2b can provide a good interpretation.

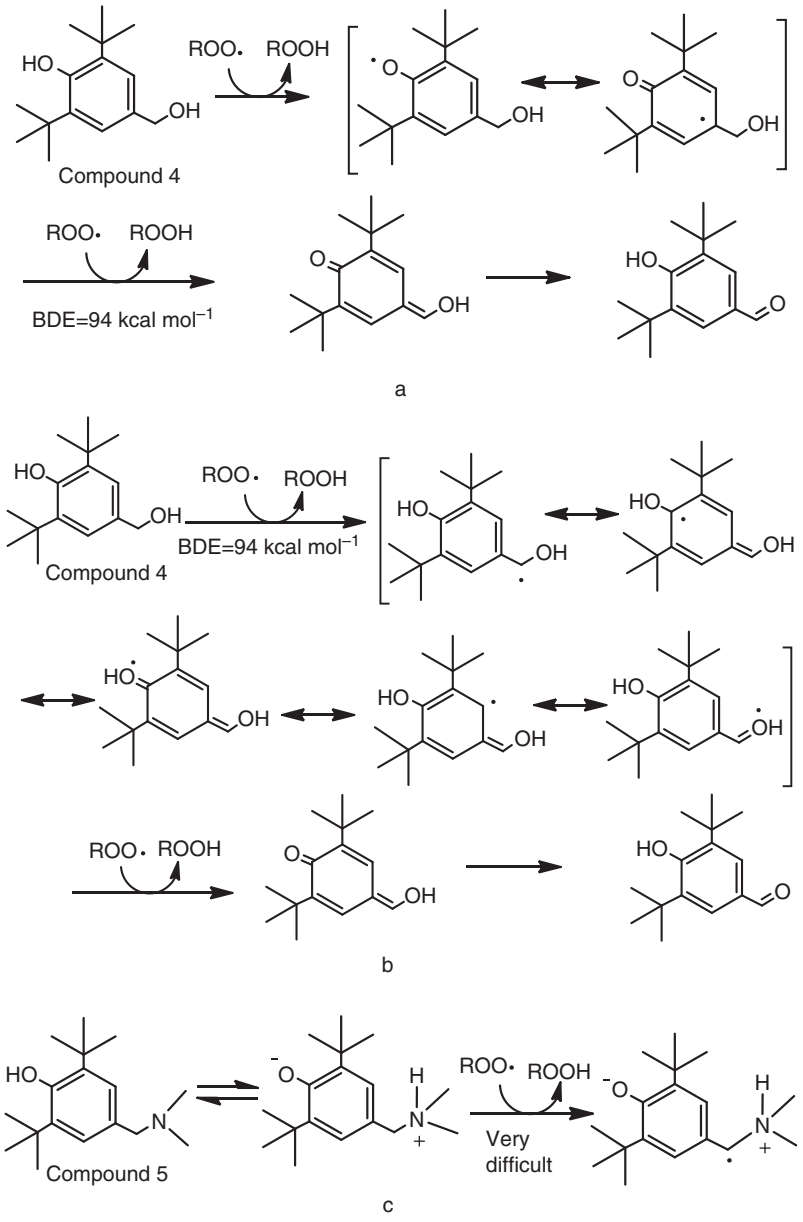

FIGURE 2. Illustration of the fact that compound 4 demonstrates very strong antioxidant activity and much stronger than compound 5.

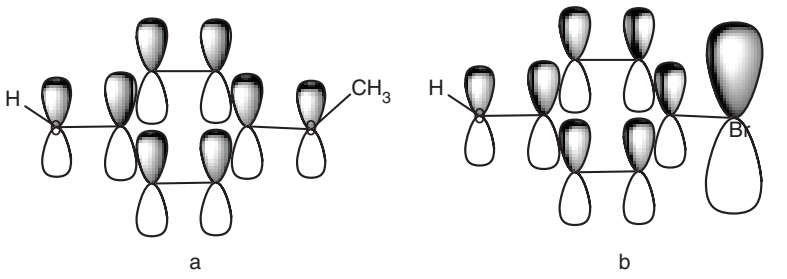

FIGURE 3. Illustration of how unshared lone pair electrons of different substituents conjugated with the aromatic ring at the 4-position: a-Methoxyl group; b-Bromine.

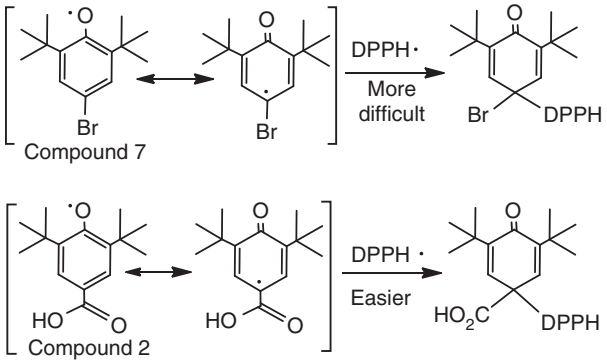

FIGURE 4. Illustration of the fat that compound 2 exhibits stronger antioxidant activity than compound 7 in the DPPH test (according to the EC50 value).

In conclusion, there are five factors which affect the antioxidant activities of the phenolic compounds:

1. After the phenolic compound provides hydrogen atoms, it depends how stable the phenolic compound free radicals are. This is especially true in the case of the autoxidation of bulky relative saturated oils and fats, such as lard and palm oil because the free radicals produce very slowly and the concentration of free radicals is relatively low.
2. It also depends on how many hydrogen atoms each of the phenolic compounds can provide. Of course, it is true both in the cases of Rancimat and DPPH· test.
3. Another factor is how fast the phenolic compounds provide hydrogen atoms. In the DPPH· test and autoxidation of highly unsaturated oils such as concentrated eicosapentaeoic acid and docosahexaeonic acid (Cao and Weng 1995), the speed of phenolic compounds providing hydrogen atoms to relatively more active free radicals is particularly important.
4. How easily the phenolic compound free radicals can combine with more active free radicals.
5. And whether a new antioxidant can form or not after the phenolic compound provides hydrogen atoms.

Factors (4) and (5) are both important for the evaluation of the phenolic compounds by the Racimat test and DPPH· test.

It has also been concluded that the DPPH·-scavenging method is more sensitive to stereohindrance than the Rancimat method because firstly free radicals are produced much more slowly in the Rancimat test and their concentration is much lower in comparison to the DPPH· scavenging test, and secondly, DPPH· is much more bulky than ROO·.

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