



Myricetin, rosmarinic and carnosic acids as superior natural antioxidant alternatives to α -tocopherol for the preservation of omega-3 oils



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ABSTRACT

22 natural polyphenols are compared to 7 synthetic antioxidants including BHT, BHA, TBHQ and PG with regard to their ability to protect omega-3 oils from autoxidation. The antioxidant efficiency of phenols is assessed using the DPPH $\cdot$ test and the measurement of oxygen consumption during the autoxidation of oils rich in omega-3 fatty acids. Also, the bond dissociation enthalpies (BDE) of the Ar-OH bonds are calculated and excellent correlations between thermodynamic, kinetic and oxidation data are obtained. It is shown that kinetic rates of hydrogen transfer, number of radicals scavenged per antioxidant molecule, BDE and formation of antioxidant dimers from the primary radicals play an important role regarding the antioxidant activity of phenols. Based on this, it is finally shown that myricetin, rosmarinic and carnosic acids are more efficient than α -tocopherol and synthetic antioxidants for the preservation of omega-3 oils.

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1. Introduction

Omega-3 essential fatty acids have drawn the attention of scientists for many years and studies have multiplied in recent decades, highlighting their virtues and indispensable character to the proper functioning of human bodies. The discovery of the positive effects of omega-3 fatty acids has been the result of contributions from several scientists all over the world who have made some unexplained observations (Dyerberg, Bang, & Hjoerne, 1975). The main omega-3 fatty acids found in animal and plant food are α -linolenic acid (ALA, 18:3), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). Several studies have shown that rebalancing the input of fatty acids by eating foods containing omega-3 improves many biological parameters, such as triglyceride level, blood pressure and cancer prevention (Burr, 2000; Kris-Etherton, Harris, Appel, & Committee, 2002; Kryzhanovskii & Vititnova, 2009).

However, highly unsaturated lipids (LH) are prone to oxidation, which takes place in three main steps. The first one is the initiation step and consists of the loss of a hydrogen atom under the action of trace metals, light or heat (Eq. (1)). The resulting lipid radical (L $\cdot$) reacts with oxygen in a second step to form a peroxy radical LOO $\cdot$ (Eq. (2)). During the propagation, LOO $\cdot$ radicals react with LH to form fatty acid hydroperoxides (LOOH), which are the primary

products of autoxidation (Eqs. (3–5)). In a third step, *i.e.* the termination step, two radicals react together to form non-radical products (Eqs. (6–8)) (Kanner & Rosenthal, 1992). Because of their large number of double bonds, omega-3 fatty acids undergo oxidative degradation (Frankel, 1984; Shahidi & Zhong, 2010) leading to the modification of organoleptic properties (Choe & Min, 2006) and the formation of lipid peroxides in food.



To prevent omega-3 fatty acid from this oxidative degradation, phenols are widely used as antioxidants. Phenolic compounds are the most frequently encountered antioxidants in food. They significantly delay the oxidation of easily oxidizable materials, such as fats (Becker, Nissen, & Skibsted, 2004). Different types of

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antioxidants are commonly used: the primary or chain-breaking antioxidants, such as the well-known butylated hydroxytoluene (BHT), which convert PUFA (polyunsaturated fatty acids) radicals ($\text{ROO}^\bullet$) into hydroperoxides (ROOH) and act as chelators of metal ions (Marteau, Nardello-Rataj, Favier, & Aubry, 2013; Reische, Lillard, & Eitenmiller, 2008). Nevertheless, due to an increase in safety concerns and a strong push to replace them by natural alternatives, synthetic antioxidants are increasingly disputed. Recent research has focused on the isolation and identification of effective naturally occurring antioxidants, such as ascorbic acid (vitamin C) and α -tocopherol (vitamin E).

We report in this paper the study of 22 natural phenolic antioxidants in comparison to 7 synthetic ones using experimental and theoretical methods. First of all, bond dissociation enthalpies (BDE) of the phenolic bond (O–H) have been calculated using the density functional theory (DFT) method, providing a predictive scale of the antioxidant activity of the phenols. These results have been correlated with the rate constants of hydrogen transfer from phenolic antioxidants to the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^\bullet$). Furthermore, measurements of oxygen consumption during the autoxidation reactions of FAMES linseed oil were carried out under oxygen pressure. This method allows the determination of the induction periods (IP) and oxidation rates (R_{ox}) of unsaturated oils in the presence of phenolic antioxidants, reflecting their efficiency towards the preservation of omega-3 oils. Very good correlations were obtained between the different approaches thus leading to highlighting myricetin, rosmarinic and carnolic acids as efficient natural alternative antioxidants to α -tocopherol.

2. Materials and methods

2.1. Materials

Solvents were of the purest grade commercially available from Sigma-Aldrich. The 2,2-diphenyl-1-picrylhydrazyl (DPPH $^\bullet$) radical was purchased from Sigma-Aldrich and kept at a temperature lower than 5 °C. Aluminum oxide, basic, Brockmann I, for chromatography, 50–200 μm , 60 Å was from Acros Organics. Refined linseed oil was from the Vandeputte Group, Belgium. FAME mix GLC-10 containing palmitic acid methyl ester (C16:0), stearic acid methyl ester (C18:0), oleic acid methyl ester (C18:1), linoleic acid methyl ester (C18:2) and linolenic acid methyl ester (C18:3) was from Supelco. Phenols **1–29** were purchased at the purest grade from Sigma-Aldrich, Alfa Aesar, Cayman Chemical Company, ACROS, Merck, TCI and Chromadex. For further details, see the [Supporting information](#).

2.2. Calculation of bond dissociation enthalpies BDEs (O–H)

Bond dissociation enthalpy (BDE) is given by the difference between the enthalpy of the phenoxyl radical (plus that of the hydrogen atom) and that of the starting phenol as described by Eqs. (9) and (10). All of the calculations were performed using Gaussian 03 packages (Szymbus & Zielinski, 2003) as described in our previous work (Marteau et al., 2016; Marteau, Nardello-Rataj, et al., 2013). The method is described as B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p). Details are given in the [Supporting information](#).

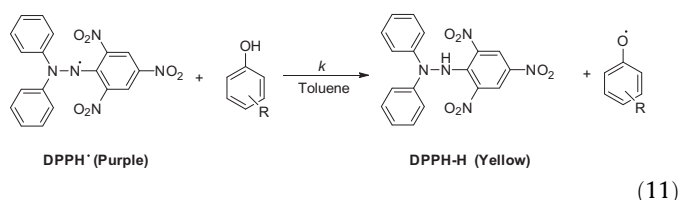


$$\text{BDE (ArO-H)} = H_f^\circ(\text{ArO}^\bullet) + H_f^\circ(\text{H}^\bullet) - H_f^\circ(\text{ArO-H}) \quad (10)$$

2.3. Determination of the rate constants for hydrogen transfer from phenols to DPPH $^\bullet$ radical

The analytic method to determine the kinetic rates of hydrogen transfer from phenols to DPPH $^\bullet$ radical (Eq. (11)) has already been

described in our previous works (Marteau et al., 2016; Marteau, Nardello-Rataj, et al., 2013).



The rate constants of hydrogen transfer k were determined by mixing the most reactive phenols with DPPH $^\bullet$ in stoichiometric amount. Under these conditions, second order kinetics (SOK) can be described by Eqs. (12–14). On the other hand, phenols with low reactivity, i.e. 4, 5, 10, 11 and 18, were introduced in excess at different concentrations. Under these conditions, $[\text{ArOH}]_0 \approx [\text{ArOH}]$ and pseudo first order kinetics (FOK) is described by Eq. (15).

$$\frac{d[\text{DPPH}^\bullet]}{dt} = -k [\text{DPPH}^\bullet] [\text{ArOH}] \quad (12)$$

$$\frac{d[\text{DPPH}^\bullet]}{dt} = -k [\text{DPPH}^\bullet]^2 \quad (13)$$

$$\frac{1}{[\text{DPPH}^\bullet]} - \frac{1}{[\text{DPPH}^\bullet]_0} = -k t \quad (14)$$

$$[\text{DPPH}^\bullet] = [\text{DPPH}^\bullet]_0 \exp\{-k[\text{ArOH}]_0 t\} \quad (15)$$

At 515 nm, DPPH $^\bullet$ and DPPH-H exhibit a strong (ϵ) and weak (ϵ') molar absorption coefficient, respectively, which allows the monitoring of the disappearance of DPPH $^\bullet$ (Eq. (11)). Eqs. (14) and (15) can be expressed as a function of the absorbances A , A_0 and A_f (Eqs. (16) and (17)), which provide the rate constants k and $k'[\text{ArOH}]_0$ as the slope of the linearized curve obtained by plotting $1/(A - A_f)$ and $\ln(A - A_f)/(A_0 - A_f)$ respectively versus time. In the latter case, the experiment was performed at different concentrations of ArOH in order to determine k .

$$\frac{1}{(A - A_f)} = \frac{1}{(A_0 - A_f)} - \frac{k}{(\epsilon - \epsilon')} t \quad (16)$$

$$\ln \frac{(A - A_f)}{(A_0 - A_f)} = -k [\text{ArOH}]_0 t \quad (17)$$

Fig. S2 shows an example of SOK reaction with α -tocopherol **1** in toluene. The rate constant of the reaction was determined during the first 15 s of the reaction (Eq. (13)) when the reaction of the phenolic hydrogen with DPPH $^\bullet$ occurs. The kinetic analysis holds as long as aryloxyl radicals recombine instead of reacting with another DPPH $^\bullet$ radical leading to *ortho*-quinone derivatives in the case of catechol compounds. Fig. S3 shows an example of FOK reaction with BHT **4** in toluene. To confirm that pseudo first order kinetic (FOK) and second order kinetic (SOK) give equivalent results for the same phenolic antioxidants, vanillic acid **13** and eugenol **18** were studied under both conditions leading to similar values. Rate constants k measured for phenols **1–29** are summarized in Table 2. For more information, see the [Supporting information](#). Values of the kinetic rates of hydrogen transfer are given in the [Supporting information](#) (see Table S3).

2.4. Determination of the stoichiometric number

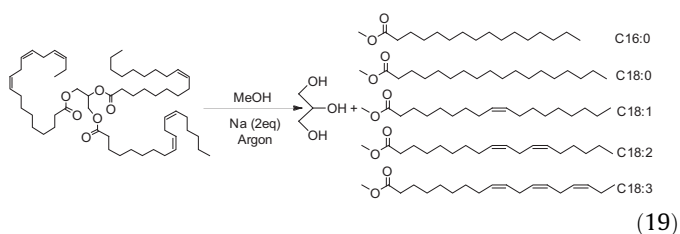
The stoichiometric number σ_{exp} is determined according to Eq. (18). The experiment consists in measuring the final A_f and initial A_0 absorbances of a solution containing a large excess of DPPH $^\bullet$ (1.5×10^{-4} M) compared to phenols (2.0×10^{-3} M). Values of

σ_{exp} are summarized in Table 2. For more details, see the Supporting information (Table S5).

$$\sigma_{\text{exp}} = \frac{[\text{DPPH}^{\bullet}]_0 - [\text{DPPH}^{\bullet}]_f}{[\text{ArOH}]_0} = \frac{A_0 - A_f}{(\varepsilon - \varepsilon')[\text{ArOH}]_0} \quad (18)$$

2.5. Synthesis of antioxidant-free fatty acid methyl esters (FAMES) by transesterification

Linseed oil was purified 3 times beforehand by alumina column chromatography to eliminate the antioxidants naturally present in neat oil. The transesterification reaction of triglycerides of purified linseed oil with methanol into fatty acid methyl esters (FAMES) is given by Eq. (19). 1 L of methanol was introduced into a 2 L three-necked flask, equipped with a condenser and a gas bubbler. Sodium (10 g, 2 equiv) was introduced piece by piece under argon followed by purified linseed oil (200 g, 1 equiv). The reaction was performed during 12 h under magnetic stirring. FAMES were extracted with 3×300 ml of petroleum ether. The combined organic phases were evaporated under pressure. Isolated FAMES were stored at -20 °C. The FAMES were composed of 5.3% of methyl palmitate (C16:0), 33.1% of methyl oleate (C18:1), 11.2% of methyl linoleate (C18:2), 45.1% of methyl linolenate (C18:3, ω -3) and 5.3% of methyl stearate (C18:0) as determined by GC/MS analyses.



2.6. Analysis

A Thermofisher GC Trace equipped with an AI 3000 injector connected to a Thermofisher DSQ II simple quadrupole detector was used for the GC–MS analysis of FAMES. Compound separation was achieved on a 30 m, DB5MS with 0.25 mm i.d. and 0.25 μm film thickness gas chromatographic column (J&W Scientific, Folsom, CA, USA). Carrier gas (ultra-pure helium) flow rate was 1.0 ml/min and the injector, the transfer line and the ion source were maintained at 250, 270 and 220 °C, respectively. The MS detector was used in the EI mode with an ionization voltage of 70 eV. The column was held at 130 °C for 0.5 min and then programmed at 0.3 °C min^{-1} to 180 °C and maintained for 5 min. Then, the column was heated to 250 °C at 3 °C min^{-1} and maintained for 10 min. The compounds were injected in the Split mode at a ratio of 20. FAME mix GLC-10 was used to analyze and quantify the FAMES composition.

2.7. Autoxidation of FAMES linseed oil in the presence of the phenolic antioxidants 1–29

The autoxidation of FAMES in the presence of the phenolic antioxidants was investigated with the RapidOxy apparatus (Scheme 1) (Marteau, Ruyffelaere, et al., 2013). 2 ml of FAMES linseed oil were introduced into the RapidOxy cell and 100 μl of phenol (1–29) solutions (10^{-2} mol l^{-1}) were then added to reach a final antioxidant concentration of 5×10^{-4} mol l^{-1} . Antioxidants were solubilized in ethyl acetate and an ultrasound bath was used to homogenize solutions. Small amounts of ethanol could be used to pre-solubilize antioxidants.

As reported previously, the RapidOxy[®] is particularly suitable to follow the inhibition of oils autoxidation (Marteau, Ruyffelaere, et al., 2013). As shown in Fig. 1, the curves corresponding to the oxygen consumption as a function of time exhibit three stages: 1) after the set pressure (450 kPa) is reached, the cell is heated to 90 °C and the pressure increases, 2) the maximum pressure stabilizes and then slowly decreases during the induction period in which the phenolic antioxidant is effective and the oxidation of FAMES is prevented and 3) the phenol is completely consumed and the oxidation of the oil takes place.

Moreover, the oxygen consumed as a function of time, i.e. $-d[\text{O}_2]/dt$, can be used as an indicator of the oxidability (Marteau, Ruyffelaere, et al., 2013). Experiments were stopped when the pressure of oxygen had decreased to half of the starting value after heating ($P_f = P_{\text{max}}/2$). The evolution of the pressure was then converted to a concentration of oxygen consumed in the liquid phase $\Delta[\text{O}_2]_t$ defined by Eq. (20) where V_{tot} and V_{liq} are the volumes of the cell and of the FAMES respectively, P_{max} is the maximum pressure obtained a few minutes after heating the cell and P_t is the pressure measured at a given time. The oxidation rate R_{ox} is the rate of oxygen consumption and corresponds to the slope of the trend curve of $[\text{O}_2]$ consumed during the second stage. Values of induction periods (IP) and oxidation rates (R_{ox}) are summarized in Table 2 and detailed in the Supporting information (Table S4).

$$\Delta[\text{O}_2]_t = \frac{(P_{\text{max}} - P_t)}{RT} \times \frac{(V_{\text{tot}} - V_{\text{liq}})}{V_{\text{liq}}} \quad (20)$$

3. Results and discussion

Antioxidants are of interest to the food industry because they prevent rancidity (Halliwell, Aeschbach, Löliger, & Aruoma, 1995). To have a large overview of the different classes of phenolic antioxidants, 29 phenols have been investigated (Scheme 2). α -Tocopherol 1, chosen as the reference, is a very effective natural antioxidant widely used in food. The ubiquitous synthetic antioxidants BHA 2, propyl gallate (PG) 3, BHT 4, *o*-*tert*-butyl-*p*-cresol 5, TBHQ 6, 5-*tert*-butyl-pyrogallol 7 and 4-*tert*-butyl-catechol 8 have also been studied for comparison.

Gallic acid 9, protocatechuic acid 10, *p*-hydroxybenzoic acid (PHBA) 11, syringic acid 12 and vanillic acid 13 were selected as derivatives of hydroxybenzoic acid. In the same way, caffeic acid 14, ferulic acid 15, chlorogenic acid 16 and sinapic acid 17 were retained because they derive from hydroxycinnamic acid. Eugenol 18 and isoeugenol 19 are considered as classical monophenols, with both fragrant and antioxidant properties, and were included to investigate the effect of double bond position. Hydroxytyrosol 20 and catechol 21 are polyphenols found in olive oil (García Aranzazu, Ruiz-Mendez, Romero, & Brenes, 2006). The other antioxidants represent the different classes of phenolic compounds: quercetin 22 and myricetin 23 for flavonoids (Pietta, 2000), resveratrol 24, aesculetin 25 and sesamol 26 are categorized as stilbenes, coumarins and lignans respectively. Finally, rosmarinic acid 27, carnolic acid 28 and carnolol 29 are the major components of rosemary extract (*Rosmarinus officinalis* L.) and are authorized in food in the form of extract (Erkan, Ayranci, & Ayranci, 2008).

3.1. Phenolic antioxidants and bond dissociation enthalpy BDE (O–H)

The activity of phenolic antioxidants is related to their ability to quench peroxy radicals by abstracting the phenolic hydrogen in a first step. An important parameter for estimating antioxidant activity of phenol is the bond dissociation enthalpy (BDE) of the ArO–H bond. A number of theoretical investigations with various basis

Table 1

Contributions to the BDE values of the ArO–H bond of phenols bearing different *ortho* (*o*), *meta* (*m*) or *para* (*p*) substituents calculated with the B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p) DFT method in toluene. Δ BDE are calculated from the BDE of phenol (82.2 kcal mol^{−1}). Electronic effects which destabilize (−) and stabilize (+) ArOH and ArO[•] are indicated, H-bonding which stabilize (+) ArOH and ArO[•] are illustrated.

Entry	Substituent	Position	Electronic effect		H-bonding		BDE (kcal mol ^{−1})	ΔBDE (kcal mol ^{−1})	
			ArOH	ArO [•]	ArOH	ArO [•]		Calc.	Literature
Alkyl group (−R), EDG									
1	−H	—					82.2	—	—
2	−CH ₃	<i>o</i>	— —	++			80.2	−2.0	−2.1 ^a , −1.9 ^b , −2.0 ^g , −2.1 ^h
3	−CH ₃	<i>m</i>	—	+			82.2	0	−0.5 ^a , −0.4 ^b , −0.4 ^d , −0.5 ^j
4	−CH ₃	<i>p</i>	— —	++			80.1	−2.1	−2.1 ^{a,f} , −2.1 ^b , −2.0 ^c , −2.5 ^d
5	−CH ₂ CH ₃	<i>o</i>	— —	++			80.1	−2.1	nd
6	−CH ₂ CH ₃	<i>m</i>	—	+			81.4	−0.8	nd
7	−CH ₂ CH ₃	<i>p</i>	— —	++			80.0	−2.2	nd
8	−C(CH ₃) ₃	<i>o</i>	— — —	+++			79.3	−2.9	−3.2 ^d , −3.3 ^d
9	−C(CH ₃) ₃	<i>m</i>	—	+			82.0	−0.2	−0.9 ^a , −0.6 ^d
10	−C(CH ₃) ₃	<i>p</i>	— —	++			80.5	−1.7	−1.9 ^a , −2.0 ^d , −1.7 ^h , −1.9 ⁱ
11	−CH ₂ −CH=CH ₂	<i>o</i>	— —	++			80.2	−2.0	−2.4 ^g , −1.6 ⁱ
12	−CH=CH−CH ₃	<i>o</i>	— —	++ +			79.2	−3.0	nd
13	−CH=CH−CH ₃	<i>m</i>	+	—			83.5	+1.3	nd
14	−CH=CH−CH ₃	<i>p</i>	— — —	++ +			77.3	−4.9	nd
Methoxy group (−OR), EDG									
15	−OCH ₃	<i>o</i>	— —	++	++		80.4	−1.8	−1.4 ^d , −0.9 ^m , −2.3 ⁿ
16	−OCH ₃	<i>m</i>	+	—			83.1	+0.9	−1.2 ^a , −0.5 ^b , −1.2 ^d , −0.7 ^h
17	−OCH ₃	<i>p</i>	— —	++			77.6	−4.6	−5.7 ^{a,f} , −5.9 ^b , −4.4 ^j , −4.1 ⁱ
Hydroxyl group (−OH), EDG									
18	−OH	<i>o</i>	— —	++	++	+++	73.4	−8.8	−9.1 ^d , −7.2 ^h , −8.9 ^k , −6.8 ^m
19	−OH	<i>m</i>	—	+			81.2	−1.0	−0.2 ^a , −0.3 ^b , −0.3 ^d
20	−OH	<i>p</i>	— —	++			77.2	−5.0	−5.4 ^e , −5.0 ^e , −5.8 ^d , −5.8 ^b
Carboxyl group (−CO ₂ R), EWG									
21	−CO ₂ H	<i>o</i>	++	— —	+++		95.2	+13.0	+7.7 ^d , +11.4 ^g
22	−CO ₂ H	<i>m</i>	+	—			84.5	+2.3	+2.7 ^d
23	−CO ₂ H	<i>p</i>	+	— —			84.7	+2.5	+2.8 ^d , +2.5 ^l
24	−CO ₂ CH ₃	<i>o</i>	++	— —	+++		94.9	+12.7	+12.2 ^g
25	−CO ₂ CH ₃	<i>m</i>	+	—			84.1	+1.9	nd
26	−CO ₂ CH ₃	<i>p</i>	+	—			84.1	+1.9	+1.7 ^h

nd: not determined.

Data from:

^a Klein and Lukes (2006).

^b Chandra and Uchimarui (2002).

^c Lucarini, Mugnaini, Pedulli, and Guerra (2003).

^d Wright et al. (2001).

^e Lucarini et al. (2003).

^f Klein, Lukes, Cibulkova, and Polovkova (2006).

^g Korth et al. (2002).

^h Bosque and Sales (2003).

ⁱ Lucarini et al. (1996).

^j Zhang (2003).

^k Bakalbassis et al. (2003).

^l Szymusiak and Zielinski (2003).

^m Amorati and Valgimigli (2012).

ⁿ Foti et al. (2008).

sets (Bakalbassis, Lithoxidou, & Vafiadis, 2003; Klein & Lukes, 2006; Leopoldini, Russo, & Toscano, 2010; Li, Liu, Fu, & Guo, 2007; Perez-Gonzalez, Rebollar-Zepeda, Leon-Carmona, & Galano, 2012) as well as experimental studies (Amorati & Valgimigli, 2012; Lucarini, Pedrielli, Pedulli, Cabiddu, & Fattuoni, 1996; Lucarini & Pedulli, 2010) have been reported for the determination of the BDE of phenols. It is noteworthy that theoretical results are basis set and solvent dependent (Marteau et al., 2016). Moreover, a systematic underestimation of about 5 kcal mol^{−1} can arise from the DFT calculation. Hence, Δ BDE values compared to the theoretical BDE of phenol (82.2 kcal mol^{−1}) should be considered (Tables 1 and 2 and S1). The absolute BDE of phenols have been calculated using the DFT method. The values concerning the group contribution of BDE are consistent with published theoretical values as reported in Table 1 except for the methoxy substituent (Table 1, entry 16) which is overestimated but can be discussed and explained. Moreover, Δ BDE values of alkyl additive effect are consistent with the literature (Table S1). All the calculated BDE values

(Table 2) are in agreement with theoretical and experimental values found in the literature.

The effect of electron-donating and electron-withdrawing substituents has been studied in numerous experimental and theoretical works from the early 90's (Lucarini & Pedulli, 2010; Wright, Johnson, & DiLabio, 2001). In previous work, we also showed that electron-donating groups decrease BDE values, in particular at the *ortho*- and *para*-positions (Marteau, Nardello-Rataj, et al., 2013). Here, we have extended the study by calculating using the same DFT method (described as B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p)), the BDE of the ArO–H bond of 25 phenols bearing different substituents at the *ortho*-, *meta*- or *para*-positions frequently encountered in natural phenols. Table 1 summarized the effect of substituents on hydrogen bonding and BDEs. The BDE values were strongly dependent on the nature of the substituents, which were classified into four groups: alkyl (−R), carboxyl (−CO₂R), alkoxy (−OR) and hydroxyl (−OH) groups. The additive effect of alkyl (CH₃ and *tert*-butyl) groups has also been investigated (Table S1).

Table 2

Bond dissociations enthalpies (BDE) of ArO-H computed in toluene and Δ BDE are calculated compared to the BDE of phenol (82.2 kcal mol⁻¹), rate constants k of hydrogen transfer from ArOH to DPPH[•] in toluene at 20 °C, stoichiometric numbers of H atoms σ_{exp} determined with an excess of DPPH[•] in toluene at 20 °C. Induction period (IP) and oxidation rates (R_{ox}) are determined with the RapidOxy test.

Phenols	BDE (kcal mol ⁻¹)	Δ BDE ^c (kcal mol ⁻¹) literature		k (M ⁻¹ s ⁻¹)		Induction period (min)	Oxidation rate (mM min ⁻¹)	σ_{exp}
		Calc.	Literature	SOK ^a	FOK ^b			
1	69.1	-13.1	-11.3 ^d , -11.2 ^{e,g} , -12.11 ⁱ	2690		177	0.17	2.0
2	72.3	-9.9	-8.9 ^d , -8.2 ^j	184		167	0.35	2.0
3	69.6	-12.6	-11.2 ^d , -11.8 ^j	1240		162	0.26	3.9
4	72.4	-9.8	-7.3 ⁿ		0.18	131	0.44	2.0
5	77.4	-4.8	nd		0.36	56	0.77	2.5
6	74.3	-7.9	nd	600		45	0.53	2.0
7	66.6	-15.6	nd	9480		234	0.06	2.1
8	72.3	-9.9	-7.7 ^m	776		220	0.37	2.5
9	70.2	-12	-10.6 ^{e,g}	ns		178	0.32	5.0 ^q
10	75.5	-6.7	-8.2 ⁱ , -4.7 ^k , -6.9 ^o	ns		50	0.62	1.9 ^q
11	84.7	+2.5	+2.4 ⁱ , +3.9 ^k , +2.7 ^o	ns		6	1.20	0 ^q
12	78.1	-4.1	-3.3 ^k	10.6		37	0.76	1.1
13	83.1	+0.9	+1.2 ^k	1.4	1.0	5	1.03	0 ^q
14	72.1	-10.1	-9.3 ^{e,g} , -11.5 ⁱ , -10.0 ^j , -11.6 ^o	ns		148	0.36	2.0 ^q
15	79.7	-2.5	-2.0 ^h , -3.1 ^p	8.4		28	0.82	1.8
16	73.4	-8.8	-7.8 ⁱ	ns		138	0.48	1.9 ^q
17	75.4	-6.8	-5.8 ⁱ , -7.1 ^p	165		54	0.57	1.4 ^q
18	80.2	-2.0	-3.7 ⁿ	3.9	2.7	27	0.93	2.1
19	76.6	-5.6		37.8		49	0.72	0.9
20	72.1	-10.1	-9.40 ^e , -10.5 ^j	1070		172	0.30	2.0
21	73.4	-8.8	-6.9 ^{e,g} , -8.9 ^h , -10.1 ^o	400		147	0.46	1.9
22	71.8	-10.4	-10.6 ^e , -10.2 ^j	ns		135	0.34	1.9 ^q
23	67.4	-14.8	-15.1 ^j	ns		262	0.11	3.4 ^q
24	76.7	-5.5	-5.6 ^{e,g}	ns		67	0.68	0.9 ^q
25	72.5	-9.7	nd	ns		112	0.50	2.1 ^q
26	75.1	-7.1	-7.2 ^p	250		161	0.55	2.1 ^q
27	69.2	-13.0	nd	ns		262	0.27	4.1 ^q
28	70.8	-11.4	nd	640		230	0.29	2.0
29	70.7	-11.5	nd	1680		166	0.35	1.9

nd: not determined, ns: not soluble.

^a SOK: Second Order Kinetics ([DPPH[•]]₀ = [ArOH]₀).

^b FOK: pseudo-First Order Kinetics ([ArOH]₀ ≫ [DPPH[•]]₀).

^c Δ BDE are calculated compared to the BDE of phenol (82.2 kcal mol⁻¹), Data from.

^d Wright et al. (2001).

^e Leopoldini, Marino, Russo, and Toscano (2004).

^f Zhang (2003).

^g Leopoldini et al. (2010).

^h Bakalbassis et al. (2003).

ⁱ Mohajeri and Asemani (2009).

^j Li et al. (2007).

^k Nenadis and Tsimidou (2012).

^l Hussain et al. (2003).

^m Amorati et al. (2006).

ⁿ Lucarini and Pedulli (2010).

^o Hoelz et al. (2010).

^p Foti et al. (2008).

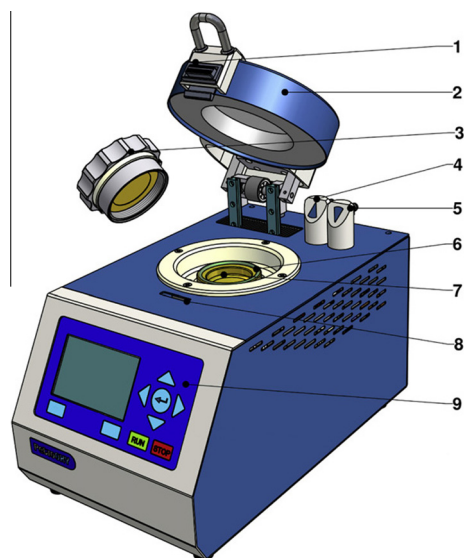
^q Ethyl acetate used as solvent.

Generally speaking, lower BDE values are obtained for electron-donating groups (EDG) because they destabilize the phenol and/or stabilize the phenoxyl radical through electronic effects and intramolecular hydrogen bonding (Lucarini et al., 1996). As expected, alkyl substituents decrease the BDE via electronic effects (Table 1). *ortho*-methyl (entry 2), *para*-*tert*-butyl (entry 10), *ortho*-ethyl (entry 5) and *ortho*-propenyl (entry 12) substituents decrease the BDE values of -2.0, -1.7, -2.1 and -3.0 kcal mol⁻¹ respectively. *ortho*-substitution by an OH group leads to a stronger decrease of the BDE compared to a OCH₃ group (entry 18 versus entry 15) (respectively -8.8 and -1.8 kcal mol⁻¹). As reported by Wright and co-workers, this results from the disappearance of the intramolecular hydrogen bonding in the phenoxyl radical in the case of OCH₃ while with the OH group, the radical stabilization becomes more important, besides the electronic effects of the *o*-OH group, leading to a dramatic decrease of BDE (Wright et al., 2001). Compared to the *o*-allyl group (entry 11), the *o*-propenyl substituent (entry 12) allows further electron delocalization, which increases the stability of the phenoxyl radical and leads to a stronger BDE decrease from -3.0

and -2.0 kcal mol⁻¹ respectively. However, *meta*-alkyl, alkoxy and hydroxyl groups have low or no impact compared to *ortho*- and *para*-substitutions (Chandra & Uchamaru, 2002; Lucarini & Pedulli, 2010; Zhang, Sun, & Chen, 2001).

On the contrary, electron withdrawing groups (EWG) induce a stabilization of the phenol and/or a stabilization of the phenoxyl radical (Lucarini et al., 1996) leading thus to higher BDEs. As an example, a COOH group in the *ortho*-position allows intramolecular hydrogen bonding, which increases the stabilization of the phenol (Korth, de Heer, & Mulder, 2002; Wright et al., 2001). Nevertheless, it disappears when the phenoxyl radical is formed. Indeed, both effects are unfavorable for an easy transfer of the hydrogen involving a huge increase of +13.0 kcal mol⁻¹ (entry 21).

Using ReqEPR, Pedulli and co-workers have demonstrated the additive effect of some alkyl substituents and supported the results with some additivity rules (Lucarini & Pedulli, 2010). According to theoretical calculation using the B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p) DFT method, additive effects are also found for methyl and *tert*-butyl substituents (Table S1).



Scheme 1. RapidOxy® apparatus for the measurement of oxygen consumption during the autoxidation process.

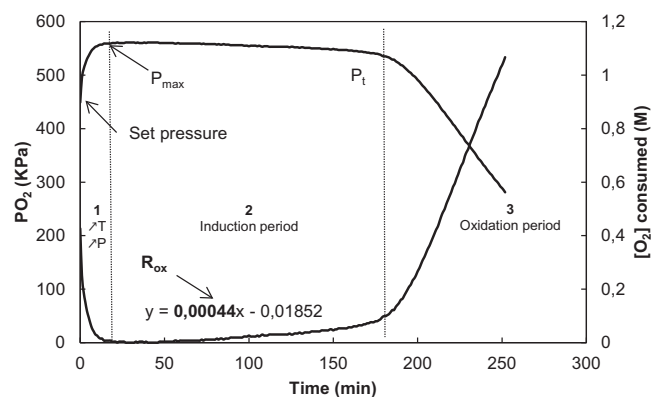
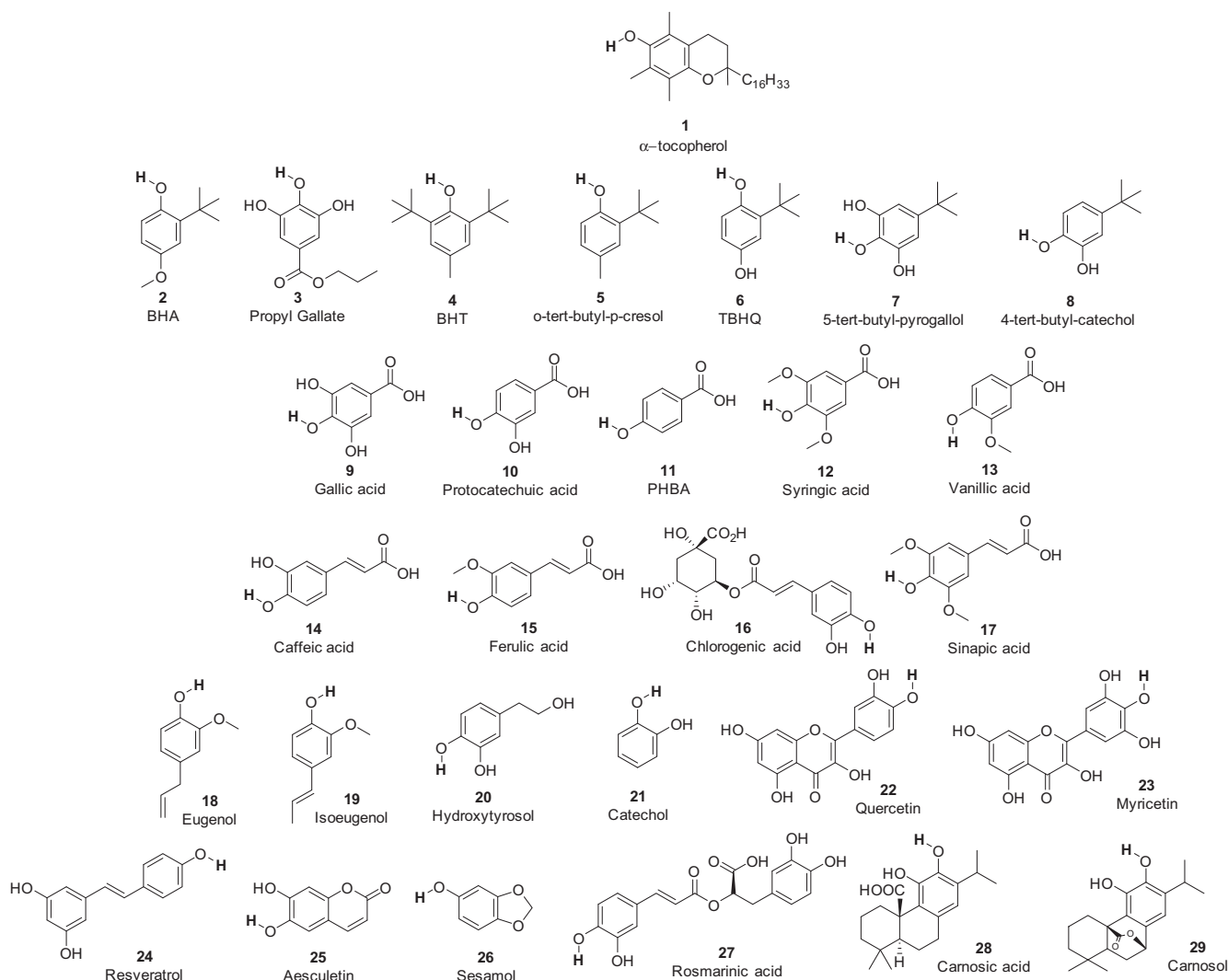


Fig. 1. Evolution of the oxygen pressure (left axis) and concentration of oxygen (right axis) during the oxidation of FAMES in the presence of $[BHT] = 5 \times 10^{-4} \text{ mol/l}^{-1}$ at 90°C .

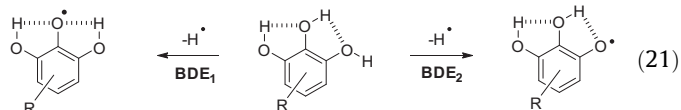
In order to establish a scale of the antioxidant activity, the B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p) DFT method has been applied to the 29 phenolic antioxidants (Scheme 3). As mentioned previously, the values of the BDEs are strongly influenced by the number, nature and position of the substituents



Scheme 2. Chemical structures of phenolic antioxidants studied, hydrogen atoms with the lowest OH BDE are indicated in bold.

linked to the phenol ring. According to the BDE values and the chemical structures of the phenols, three main classes of antioxidants can be distinguished: 1) those with very low BDE (from 66 to 72 kcal mol⁻¹), 2) those with medium BDE (from 72 to 79 kcal mol⁻¹) and 3) those with high BDE (from 79 to 86 kcal mol⁻¹). This division is arbitrary but allows the rationalization of the structural effects in each set.

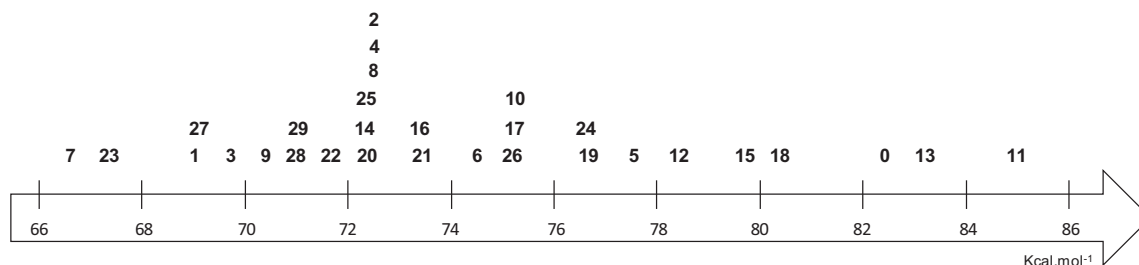
Very low BDEs have been found for 5-*tert*-butylpyrogallol **7**, myricetin **23**, propyl gallate **3** and gallic acid **9**. Wright and co-workers have reported that the central phenoxyl radical stabilized by two hydrogen bonds would be the more stable conformer compared to the phenol stabilized by only one intramolecular hydrogen bonding (Eq. (21)) (Wright et al., 2001). As the phenol parent is the same in both cases, BDE is lower when the phenoxyl radical is formed at the central hydroxyl group (see Table S2). This class also contains α -tocopherol **1** with a BDE of 69.1 kcal mol⁻¹. Such a low BDE results from different factors: 1) the alkoxy group in the *p*-position, 2) the four alkyl substituents on the phenolic ring and 3) the molecular rigidity imposed by the pyran structure (Marteau, Nardello-Rataj, et al., 2013). Finally, quercetin **22**, rosmarinic acid **27**, carnosic acid **28** and carnosol **29** also exhibit very low BDEs. Indeed, they bear a catechol-type ring moiety, conjugated double bonds and alkyl substituents on the phenol rings which strongly contribute to lower the BDE.



On the other hand, catechol **21** itself and catechol-based derivatives with COOH groups (*i.e.* protocatechuic acid **10**, caffeic acid **14**), OCH₃ groups (*i.e.* BHA **2**, syringic acid **12**, sinapic acid **19**) and conjugated double bonds (*i.e.* chlorogenic acid **16**, aesculetin **25**) have medium BDEs as well as those with different alkyl substituents (*i.e.* BHT **4**, *o*-*tert*-butyl-*p*-cresol **5**, 4-*tert*-butyl-catechol **8**) and substituted hydroquinone (*i.e.* TBHQ **6**).

Finally, ferulic acid **15**, eugenol **18**, vanillic acid **13** and PHBA **11** have high BDEs. Eugenol **18** has a higher BDE than isoeugenol **19** because of the non-conjugated double bond. Vanillic acid **13** and PHBA **11** have higher BDEs than phenol itself. This is due to the effect of the electron withdrawing carboxyl group in the *para* position (Table 1, entry 23), which results in an increase of +2.5 kcal mol⁻¹. This effect is not fully balanced by the *ortho*-OCH₃ group (Table 1, entry 15), which allows a decrease of -1.8 kcal mol⁻¹.

Based on these theoretical calculations, gallic acid **9** as a derivative of hydroxybenzoic acid, myricetin **23** as a flavonoid and rosmarinic acid **27**, carnosic acid **28** and carnosol **29** as phenols present in rosemary extracts are expected to be very effective antioxidants towards omega-3 oils autooxidation as they have a very low BDE.



Scheme 3. Scale of reactivity of phenolic antioxidants based on bond dissociation enthalpies (BDE) calculated by a B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p) DFT method in toluene.

3.2. Phenolic antioxidants and kinetic rates of hydrogen abstraction (DPPH[•] test)

To confirm the antioxidant activity of phenols highlighted above on the basis of thermodynamic data, rate constants of hydrogen transfer have then been determined using the DPPH[•] (2,2-diphenyl-1-picrylhydrazyl) test (Sharma & Bhat, 2009). The DPPH[•] radical is stable at room temperature and its disappearance can be readily followed by UV-visible spectroscopy. Thus, rate constants of hydrogen transfer from the phenols to DPPH[•] were obtained using pseudo-first order (FOK) and second order (SOK) kinetics. Toluene was chosen as an inert solvent. The mechanism involved in this apolar and aprotic solvent is called hydrogen atom transfer (HAT) in contrast to the sequential proton loss electron transfer (SPLET) mechanism which takes place in polar solvents (Amorati, Pedulli, Cabrini, Zamboni, & Landi, 2006; Litwinienko & Ingold, 2005; Lucarini & Pedulli, 2010; Marteau, Nardello-Rataj, et al., 2013).

As expected, the logarithm of the rate constants for the reaction of hydrogen transfer is well correlated with the calculated BDEs of phenols ($R^2 = 0.95$) confirming that the HAT mechanism occurs in toluene (Fig. 2) (Foti, Daquino, Mackie, DiLabio, & Ingold, 2008; Foti et al., 2002; Marteau, Nardello-Rataj, et al., 2013). Nevertheless, there are some exceptions. Indeed, due to steric hindrance of *ortho*- and *di-ortho-tert*butyl groups such as in BHA **2**, *o*-*tert*-butyl-*p*-cresol **5** and BHT **4**, the kinetic transfer of the phenolic hydrogen is sharply slowed down as explained by Marteau, Nardello-Rataj, et al. (2013). Concerning the natural phenolic antioxidants, α -tocopherol **1** exhibits the higher rates (2670 M⁻¹ s⁻¹) followed by carnosol **29** (1680 M⁻¹ s⁻¹), hydroxytyrosol **20** (1070 M⁻¹ s⁻¹) and carnosic acid **28** (640 M⁻¹ s⁻¹). Log *k* decreases when the BDE values increase as also demonstrated by Foti et al. in heptane (Foti et al., 2008) and Marteau et al. in *m*-xylene (Marteau, Nardello-Rataj, et al., 2013). Foti and co-workers have also proven that the kinetics obtained with the DPPH[•] method are correlated with the reaction of phenols with peroxy radicals (LOO[•]). Indeed, potent chain-breaking antioxidants react with LOO[•] and not with L[•] because its reaction with O₂ is too fast. Accordingly, this test can mimic the behaviour of phenolic antioxidants during the inhibition of oils oxidation thanks to hydrogen transfer with a radical mechanism (Foti et al., 2002).

3.3. Stoichiometric number (σ) of phenols 1–29

Phenolic antioxidants are also characterized by the maximum number of radicals trapped by one antioxidant molecule, named the stoichiometric number σ_{exp} . It is determined with the DPPH[•] test from the final absorbance reached by DPPH[•] in the presence of a large excess of DPPH[•] with respect to the antioxidant concentration (Eq. (18)) (Goupy, Dufour, Loonis, & Dangles, 2003). All of the stoichiometric numbers σ_{exp} determined for phenols (see Table S5 in the Supporting information) are summarized in Table 2.

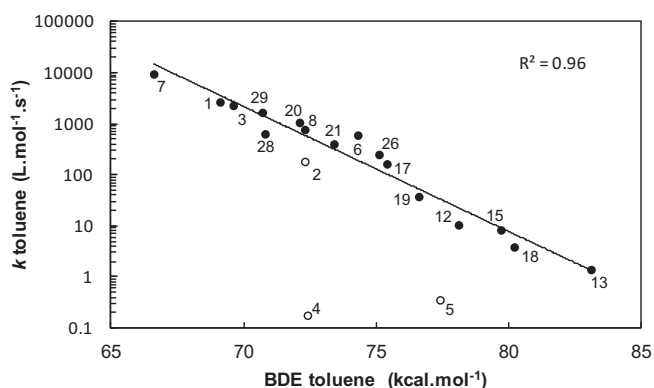
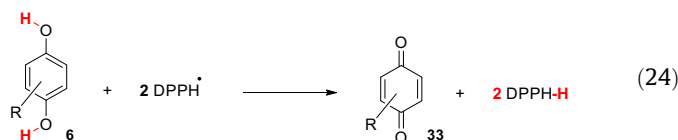
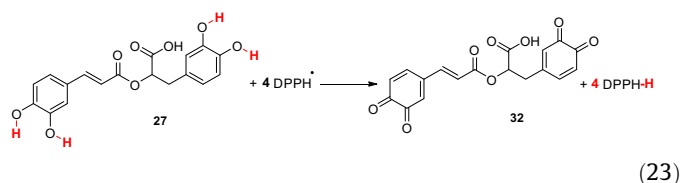
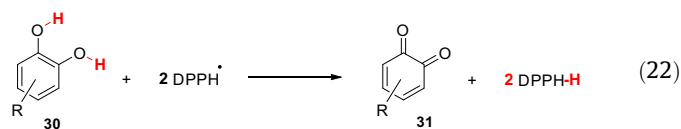


Fig. 2. Logarithm of the rate constants ($\log k$) for the reaction of phenolic antioxidants with $\text{DPPH}^\bullet$ (○ hindered phenols and ● other phenols) in toluene as a function of the BDEs calculated with the B3LYP/6-311++G(2d,2p)//B3LYP/6-311G (d,p) DFT method in toluene.

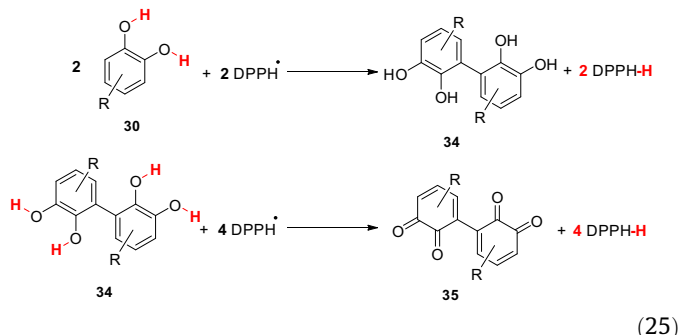
Toluene was replaced by ethyl acetate when antioxidants were not soluble. Fig. S4 reports the results for α -tocopherol **1** at various concentrations.

The stoichiometric number depends on the nature of the substituents at the *ortho* and *para* positions. The determination of the number of hydrogen transfer provides some information on the oxidation products, but, usually, several competitive pathways are simultaneously involved, complicating the interpretation. The mechanism of interaction between the $\text{DPPH}^\bullet$ radical and the phenol takes place in two steps: 1) abstraction of the phenolic hydrogen and 2) transfer of a second hydrogen or formation of reactive or non-reactive dimers from the phenoxyl radical $\text{ArO}^\bullet$.

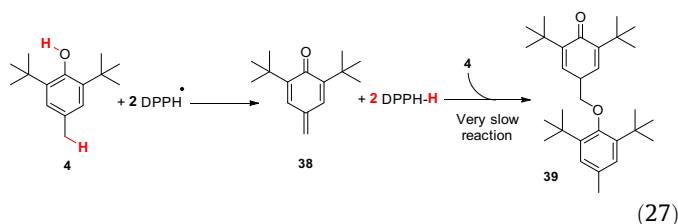
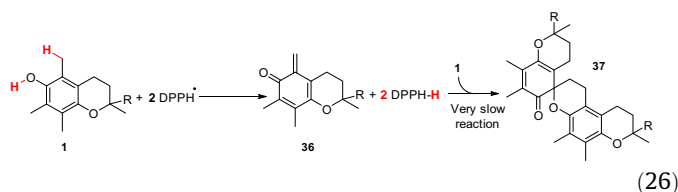
Phenolic antioxidants with catechol structure **30** are known to transfer 2 hydrogens to $\text{DPPH}^\bullet$. Indeed, the phenolic hydrogen from the *ortho*-OH substituent releases its second hydrogen and the *ortho*-quinone compound **31** is formed (Eq. (22)). The global stoichiometric numbers of 2.0 are in agreement with the reported oxidized forms of catechol derivatives **7** (Tkachev et al., 2007), **10** (Saito & Kawabata, 2006), **14** (Arakawa, Yamaguchi, Hotta, Osakai, & Kimoto, 2004), **16** (Pierpoint, 1966), **20** (De Lucia, Panzella, Pezzella, Napolitano, & D'Ischia, 2006), **21** (Balla, Kiss, & Jameson, 1992), **22** (McPhail, Hartley, Gardner, & Duthie, 2003), **28** (Masuda, Inaba, & Takeda, 2001) and **29** (Masuda, Kirikihira, & Takeda, 2005). With 2 active catechol moieties (BDEs of 69.2, 72.4, 75.2 and 75.9 kcal mol⁻¹), rosmarinic acid **27** traps 4 radicals per molecule and forms 2 *ortho*-quinone substructures **32** in the same molecule (Fujimoto & Masuda, 2012), which correlates with a stoichiometric number of 4.1 (Eq. (23)). Antioxidants with hydroquinone structure such as **6** give a stoichiometric number of 2.0 due to the formation of *p*-quinone compound **33** (Eq. (24)) (Hamama & Nawar, 1991).



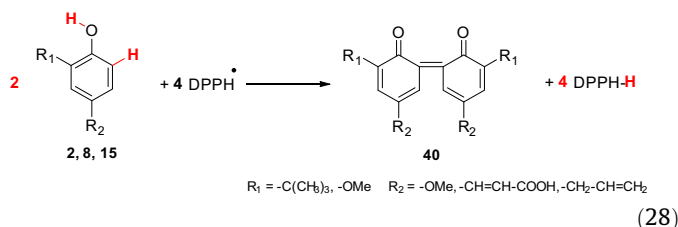
Surprisingly, gallic acid **9**, propyl gallate **3**, myricetin **23** and 4-*tert*-butylcatechol **8** trap, respectively, 5.0, 3.9, 3.4 and 2.5 radicals per molecule. Given the number of phenolic hydrogens in their chemical structure, it is assumed that they form active dimers which react again with a $\text{DPPH}^\bullet$ radical leading to an increase of the stoichiometric number and the formation of oxidized antioxidant dimers (Eq. (25)) as already observed. (Arab Chamjangali, Bakherad, & Ameri, 2015; Caregnato et al., 2008; Imagawa & Takino, 1962).



Monophenols, which can apparently trap only one hydrogen per molecule, are in fact able to transfer more than one. As already published in our previous work, α -tocopherol **1** (Eq. (26)) and BHT **4** (Eq. (27)) firstly transfer the phenolic hydrogen and then a second hydrogen from the *o*- or *p*-methyl group leading to a *o*- or *p*-quinone-methide derivative, which is consistent with a stoichiometric number σ_{exp} of 2. These *p*- and *o*-quinone-methide derivatives **36** and **38** react with starting phenols **1** and **4** to form dimers **37** and **39**. Nevertheless, quinone-methides react very slowly with starting phenols and dimers are not obtained (Marteau et al., 2016).



Moreover, *o*-*tert*-butyl-*p*-cresol **5** traps 2.5 radicals per molecule via 2 competitive pathways: 1) formation of *p*-quinone methide **41** (Eq. (29)) and 2) formation of dimer **42** via an *ortho*-dimerization (Eq. (30)) (Marteau et al., 2016).



antioxidants. The trend is clear: highly effective antioxidants are polyphenols with high stoichiometric numbers. Indeed, the number of hydrogens released by one molecule of antioxidant has an important impact on the inhibition of oxidation and influences the induction period. Thus, myricetin **23**, rosmarinic acid **27** and 4-*tert*-butylcatechol **8** have stoichiometric numbers higher than 2.0 and are more efficient than α -tocopherol **1**. However, 5-*tert*-butylpyrogallol **7** and carnosic acid **28** are also more efficient than α -tocopherol **1** while they trap the same number of radicals. Moderately effective antioxidants such as gallic acid **9**, propyl gallate **3**, hydroxytyrosol **20**, carnosol **29** and caffeic acid **14** exhibit interesting capacity against FAMES oxidation and have almost the same effectiveness as α -tocopherol **1**. Basically, they trap 2 radicals except for gallic acid **9** and propyl gallate **3** which have higher stoichiometric numbers. It is noteworthy that carnosol **29** is less efficient than carnosic acid **28** while they have a similar chemical structure and equal stoichiometric number ($\sigma_{\text{exp}} = 2.0$). This is probably due to the fact that carnosic acid **28** is the starting phenol and carnosol is obtained during its degradation pathway as shown by Zhang et al. (2012). Finally, poorly effective antioxidants such as sinapic acid **17**, isoeugenol **19** and resveratrol **24** exhibit the lowest induction periods and stoichiometric numbers. They do not transfer enough hydrogen to be efficient at delaying action of the oxidation process. Nevertheless, *o*-*tert*-butyl-*p*-cresol **5**, protocatechuic acid **10** and eugenol **18** trap 2 or more hydrogens without impeding the oxidation of FAMES.

Accordingly, the number of radicals trapped by a molecule of phenol (σ_{exp}) is a crucial parameter for the protection of FAMES against oxidation but the exceptions above-mentioned point out other essential factors than just the BDE. Indeed, there is a very good correlation between oxidation rates (R_{ox}) and BDE values as shown in Fig. 4 ($R^2 = 0.97$). We have previously shown a correlation between BDEs and rate constants k obtained with the DPPH $\cdot$ test in toluene involving a transfer of hydrogen according to the HAT mechanism. Thereby, we can assume here that the mechanism involved during the inhibition of oxidation by phenolic antioxidants is a radical mechanism. During the oxidation of the FAMES linseed oil, the lower the oxygen consumption is, the more efficient the antioxidant is. Myricetin **23** with a BDE of 67.4 kcal mol $^{-1}$ provides an oxidation rates of 0.11 mM $^{-1}$ s $^{-1}$ whereas vanillic acid **13** with a BDE of 83.14 kcal mol $^{-1}$ does not reduce the oxidation rate (1.03 mM $^{-1}$ s $^{-1}$). Sterically hindered phenols (*i.e.* BHA **2**, *o*-*tert*-butyl-*p*-cresol **5** and BHT **4**) which did not follow the trend curve with the DPPH $\cdot$ test are now included in the tendency. Gallic acid **9** and propyl gallate **3**, identified as exceptions by their higher stoichiometric number (5 and 3.9 respectively), do not transfer their hydrogen fast enough and the oxidation takes place at a rate of

0.32 and 0.26 mM min $^{-1}$. Therefore, even if antioxidants could transfer more than 2 hydrogens, they would not be efficient enough to avoid the autoxidation of omega-3 oils.

Consequently, the number of radicals trapped by the phenolic antioxidant (σ_{exp}) and the BDE value are the two essential parameters depicting the antioxidant efficiency of phenols towards oils autoxidation. Indeed, phenols with the highest stoichiometric number and the lowest BDE are the more efficient antioxidants. Thus, myricetin **23**, rosmarinic acid **27** and carnosic acid **28** are more effective than α -tocopherol **1**.

The better antioxidant capacity of myricetin **23** compared to that of α -tocopherol **1** has already been reported with regards to other targets: triacylglycerols of sunflower oil (Marinova, Toneva, & Yanishlieva, 2007), methyl esters of soybean oil (Moser, 2008) and unilamellar liposomes (Gordon & Roedig-Penman, 1998). The better effectiveness of carnosic acid **28** has been shown by the improvement of the stability of squalene under heating and UV irradiation (Wang et al., 2011). Finally, rosmarinic acid **27** has been found to be more efficient than α -tocopherol **1** during the inhibition of lipid oxidation in cooked ground beef (Alamed, Chaayasit, McClements, & Decker, 2009).

Carnosol **29**, quercetin **22**, hydroxytyrosol **20** and caffeic acid **14** are as effective as α -tocopherol **1**. They all have a BDE lower than 72.1 kcal mol $^{-1}$ and a stoichiometric number equal to 2. Apart from α -tocopherol **1**, which is well known as an effective phenolic antioxidant, myricetin **23**, rosmarinic acid **27** and carnosic acid **28** are relevant alternatives to synthetic antioxidants, such as propyl gallate **3**, BHA **2** and BHT **4**, which have similar or higher oxidation rates.

4. Conclusion

Edible oils rich in omega-3 are very sensitive to autoxidation because of the large number of double bonds in their molecular structure. Following a radical mechanism of degradation with steps of initiation, propagation and termination, fatty acids are converted into hydroperoxides as primary oxidation products. The preservation of these edible oils against oxidation is essential to enable a longer product lifetime and maintain their organoleptic properties. Many publications are related to the inhibition of triglycerides oxidation by antioxidants but few of them are focused on omega-3 oils.

Bond dissociation enthalpies (BDEs) calculated for 29 phenols have confirmed the important role of electron-donating groups on the phenol ring, providing a theoretical classification of these phenols regarding their antioxidant potential. Thus, low BDE values were found for myricetin **23**, rosmarinic acid **27** and gallic acid **9**, highlighting their capacity to act as efficient antioxidants. The pyrogallol and bis-catechol moieties are key structural parameters to reach very low BDE values. On the other hand, the DPPH $\cdot$ test, which mimics well the reaction of peroxy radicals LOO $\cdot$ with phenolic antioxidants, has provided the rate constants of hydrogen transfer from phenols to radicals. In non-polar solvents, the HAT mechanism prevails and α -tocopherol **1**, carnosic acid **28** and carnosol **29** are the best natural phenolic antioxidants. The DPPH $\cdot$ test also provides useful information through the determination of the stoichiometric number. The formation of dimers and trimers, via C-C and C-O couplings, with an antioxidant activity higher than that of the starting phenol, improves the number of hydrogens released by a molecule. With a stoichiometric number higher than 2, gallic acid **9**, myricetin **23** and rosmarinic acid **27**, respectively, trap 5.0, 3.4 and 4.1 radicals per molecule. This key parameter reflects the antioxidant effectiveness of phenols. Finally, monitoring the oxygen consumption during the autoxidation of FAMES rich in omega-3 has been shown to be well suited to study both the oxidative degradation of such oils and their preservation.

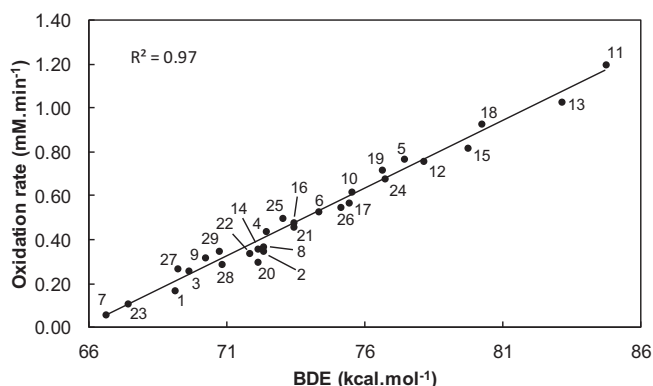


Fig. 4. Oxidation rates as a function of the BDEs calculated with the B3LYP/6-311++G(2d,2p)//B3LYP/6-311G(d,p) DFT method in toluene.

Remarkably, very good correlations between the three approaches were found. The correlation between oxidation rates and BDEs clearly shows that the mechanism involved during the inhibition of oils oxidation by phenolic antioxidants is a radical mechanism. Furthermore, the number of radicals trapped by molecules of phenols influence their induction periods. Consequently, stoichiometric number and BDE govern the efficiency of phenols. Thereby, the longer the induction period is, the better the effectiveness of phenolic antioxidant against oxidation of FAMES. Based on these findings, requirements for efficient antioxidants for the preservation of omega-3 oils against oxidation are: 1) presence in the structure of catechol or pyrogallol moieties, 2) low bond dissociation enthalpy (BDE), 3) formation of active dimers during the oxidation process and 4) high number of hydrogens released. Thus, with the best capacities to trap radicals per molecule combined with the lowest BDE, myricetin **23**, rosmarinic acid **27** and carnosic acid **28** are the best natural alternatives to α -tocopherol **1** and synthetic antioxidants to avoid or at least slow down the autoxidation of edible oils rich in omega-3 and sustain their physiological benefits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.06.038>.

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