Journal of Thermal Analysis and Calorimetry, Vol. 65 (2001) 411–417

THERMOGRAVIMETRIC INVESTIGATION OF ANTIOXIDANT ACTIVITY OF SELECTED COMPOUNDS IN LIPID OXIDATION

G. Litwinienko[*] *and M. Dąbrowska*[**]

Warsaw University, Department of Chemistry, Pasteura 1, 02-093 Warsaw, Poland

Abstract

Uninhibited and inhibited autooxidation of ethyl linoleate (LnEt) in bulk phase initiated by 0.04 M azoisobutyronitrile (AIBN) was investigated by thermogravimetry in isothermal mode at 35, 40 and 50°C. LnEt oxidation was inhibited by: 2,6-di-*t*-butyl-4-methylphenol (BHT), 2-*t*-butyl-6-methylphenol (BMP), 2-hydroxyphenylacetic acid (PAA), 2-hydroxyacetophenone (HAP), α-tocopherol, 2,2'-methylene-*bis*-(4-methyl-6-*t*-butylphenol) (BIS), caffeic acid and β-carotene. All investigated compounds showed antioxidant activity measured by induction time, values of rate of oxidation during induction period (R_{inh}) and values of kinetic chain length ν. At lower temperatures the monohydroxyphenols are more efficient inhibitors than dihydroxyphenols while at 50°C dihydroxyphenols have better antioxidant efficiency.

Keywords: antioxidants, autooxidation, kinetics, lipids, thermogravimetry

Introduction

Lipid autooxidation is a free radical mediated process responsible for functional abnormality and pathological changes of polyunsaturated fatty acids of the biomembranes. Moreover, oxidation causes undesirable changes of oils, fats, and foods quality. In general scheme autooxidation is a free radical chain process:

initiation	Initiator	→	$L^{\bullet}$	R_i	(1)
propagation	$L^{\bullet}+O_2$	→	$LOO^{\bullet}$		(2)
	$LOO^{\bullet}+RH$	→	$L^{\bullet}+LOOH$	k_p	(3)
termination	$LOO^{\bullet}+ROO^{\bullet}$	→	products	k_t	(4)

where: LH, $L^{\bullet}$, $LOO^{\bullet}$ are lipid molecule, alkyl and peroxy radicals, respectively, R_i – rate of initiation; k_p, k_t – rate constants of propagation and termination steps.

Rate of uninhibited oxidation is given by dependence:

* Author for correspondence: E-mail: litwin@alfa.chem.uw.edu.pl; Fax: (+48 22) 822-59-96

** Present address: Polish Academy of Science, Institute of Physical Chemistry, Kasprzaka 44/52, 01-224 Warsaw, Poland

1418–2874/2001/ $ 5.00

Akadémiai Kiadó, Budapest

$$R = -\frac{\mathrm{d}[O_2]}{\mathrm{d}\tau} = \frac{k_p\sqrt{R_i}}{\sqrt{2k_t}}[LH] \tag{5}$$

Both the rates and pathways of lipid peroxidation are dramatically affected by other chemical species in the reaction medium. Some compounds, like phenols and amines are able to suppress the propagation step of oxidation by deactivation of peroxyl radicals:

$$\text{inhibition} \qquad LOO^{\bullet} + InH \rightarrow LOOH + In^{\bullet} \qquad k_{inh} \tag{6}$$

The rate of inhibited autooxidation (R_{inh}) can be described as:

$$R_{inh} = -\frac{\mathrm{d}[O_2]}{\mathrm{d}\tau} = \frac{k_p[LH]R_i}{nk_{inh}[InH]} \tag{7}$$

where: n – stoichiometric factor, [*InH*] – concentration of inhibitor, τ – time.

The activity of antioxidants can be demonstrated by several parameters like kinetic chain length (ν), stoichiometric factor (n), rate of inhibited autooxidation or ratio of rate constant of propagation to rate constant of inhibition k_p/k_{inh}. Accelerated tests as Rancimat method and Oxidative Stability Index are used to assess the oxidative stability at temperatures 60–120°C by determination of induction time defined as time of conductometric detection of volatile products of autooxidation like aldehydes and acids. On the other hand, more accurate studies of autooxidation kinetics based on monitoring of radicals recombination (chemiluminescence methods) or substrate and products analysis are expensive and complicated. The measurements of oxygen consumption in manometric-volumetric methods are difficult, periodic analysis of peroxide concentration is time consuming, therefore, we decided to investigate the possibility of thermogravimetric measurements to follow the oxidation course and to evaluate the kinetic parameters of lipid autooxidation. Periodic measurements of mass changes during isothermal oxidation were performed by Topallar *et al.* [1] but thermogravimetry can be a more useful tool for the continuous determination of the amount of oxygen absorbed during peroxidation. There are only a few papers concerning isothermal and non-isothermal TG measurements of oxidative stability of edible oils [2–5] but only qualitative parameters: the induction time or onset temperature were determined in these measurements.

In our work isothermal thermogravimetry was used to obtain the kinetic parameters such as oxidisability, kinetic chain length and rate of initiated autooxidation of pure and inhibited ethyl linoleate as simple lipid analogue. The investigated compounds were: (i) monohydroxyphenols: 2,6-di-*t*-butyl-4-methylphenol (BHT), 2-*t*-butyl-6-methylphenol (BMP), 2-hydroxyphenylacetic acid (PAA), 2-hydroxyacetophenone (HAP), α-tocopherol (Toc), (ii) dihydroxyphenols: 2,2'-methylene-*bis*-(4-methyl-6-*t*-butylphenol) (BIS), caffeic acid (Caf), and (iii) β-carotene (Car) as non-phenolic antioxidant.

Materials and methods

All measurements were carried out for ethyl linoleate 99% (Sigma Chemical Co.). The oxidation was initiated by 0.04 M azo-*bis*-isobutyronitrile (Merck, 98%) at temperatures 35, 40 and 50°C in oxygen flow 10 mL min^{-1}.

BHT and BMP were of 99% purity (Aldrich Chem. Co.), *p*-hydroxyphenylacetic acid 98%, caffeic acid 97%, α-tocopherol and β-carotene (both 95%) were purchased from Sigma Chemical Co. *p*-hydroxyacetophenone 98% was from Merck and BIS 97% was from K&K Laboratories. The compounds were stored under nitrogen at about 0°C.

Samples of ethyl linoleate (LnEt) with dissolved AIBN and inhibitors were prepared as follows: 1 mL of LnEt was placed in 5 mL flask and AIBN was added in the appropriate amount to obtain the concentration 0.04 mol dm^{-3}. Then, 100 μL of 0.01 M acetone solution of inhibitor was added and the acetone excess was removed on rotary vacuum evaporator at room temperature in the dark.

For thermogravimetric measurements a thermobalance DuPont 951 (DuPont Wilmington, DE) coupled with a thermal analyzer 9900 was employed. The precision of this instrument was ±0.4% and minimal mass 0.02 mg. Approximately 20 mg of sample was placed on a tared aluminum balance pan, then placed in furnace. When the desired temperature was reached, the nitrogen flow was changed to oxygen flow (10 mL min^{-1}).

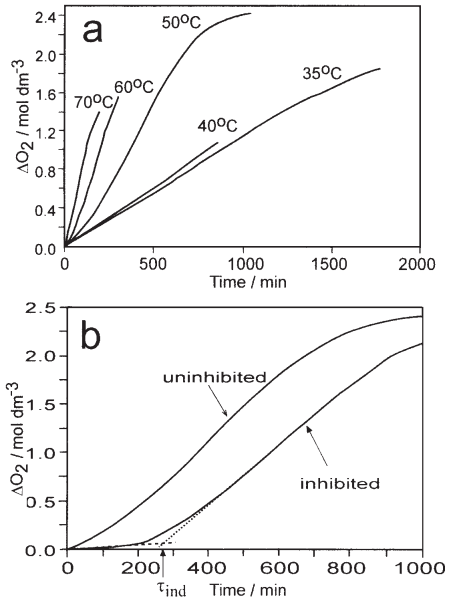

Fig. 1 Plots of non-inhibited oxidation of ethyl linoleate initiated by 0.04 M AIBN (a), comparison of autooxidation of LnEt+AIBN without inhibitor and inhibited by 0.001 M 2-*tert*-butyl-6-methylphenol at temperature 50°C (b)

Results and discussion

The concentration of absorbed oxygen at given time was calculated from equation: $\Delta[O_2]_\tau=(m_\tau-m_o)/(MV)$ where m_τ is mass of sample (mg) in time τ, m_o-mass for $\tau=0$, M-molecular mass of O_2 (32 mg mmol^{-1}), V-volume of lipid sample, $V=m_o/d_{LnEt}$ and density d_{LnEt}=903 mg cm^{-3}). Plot of oxygen concentration *vs.* time of oxidation for ethyl linoleate initiated AIBN in temperatures 35–70°C is shown in Fig. 1a. The oxidation course for a typical inhibited system is plotted in Fig. 1b. The induction period τ_{ind} was determined from the length of time from the start of the oxygen flow to the point of intersection of tangents to the inhibited and spontaneous oxidation curve as shown in Fig. 1b.

Table 1 presents kinetic parameters of uninhibited oxidation. Rates of initiation was determined by inhibitor method [6–10] from dependence $R_i=2[LH]/\tau_{ind}$ with BHT as model phenolic antioxidant. Rate of oxidation was calculated from equation: $R=\Delta[O_2]/\Delta\tau$. Kinetic chain lengths $\nu=R/R_i$ (in each case>10^3) for uninhibited autooxidation show that observed process is a chain reaction.

Table 1 Rate of initiation R_i, rate of uninhibited oxidation of ethyl linoleate R and kinetic chain length ν of uninhibited oxidation at temperatures 35, 40 and 50°C

Temperature/°C	R_i/mol dm^{-3} s^{-1}	R/mol dm^{-3} s^{-1}	ν
35	$4.80\cdot10^{-8}$	$1.76\cdot10^{-5}$	2727
40	$6.02\cdot10^{-8}$	$2.08\cdot10^{-5}$	2894
50	$16.10\cdot10^{-8}$	$4.18\cdot10^{-5}$	3852

Oxidisability $O_x=k_p/(2k_t)^{1/2}$ parameter of oxidative stability can be calculated from Eq. (5) transformed to the form:

$$O_x=\frac{k_p}{\sqrt{2k_t}}=\frac{R}{[LH]\sqrt{R_i}} \tag{8}$$

Values of O_x in units [dm$^{-3/2}$ mol$^{-1/2}$ s$^{-1/2}$] were $2.74\cdot10^{-2}$ (at 35°C), $2.90\cdot10^{-2}$ (at 40°C), and $3.56\cdot10^{-2}$ (at 50°C) and they are in good agreement with literature data obtained by other methods [11–14], thus TG measurements can be successfully used to follow oxidation course and to evaluate kinetic parameters of autooxidation.

The oxidation course of inhibited autooxidation at temperature 40°C is plotted in Fig. 2. In each case the oxidation rate was suppressed in comparison with uninhibited oxidation (τ not detectable). Values of induction time are presented in Table 2. Kinetic chain lengths collected also in Table 2 show that, for the investigated compounds, autooxidation was suppressed during induction period ($\tau<\tau_{ind}$) but inhibited oxidation was still a chain process ($\nu>1$). We noticed that ν increased when conversion of antioxidant increased, thus the presented values are the mean values from all induction period ranges.

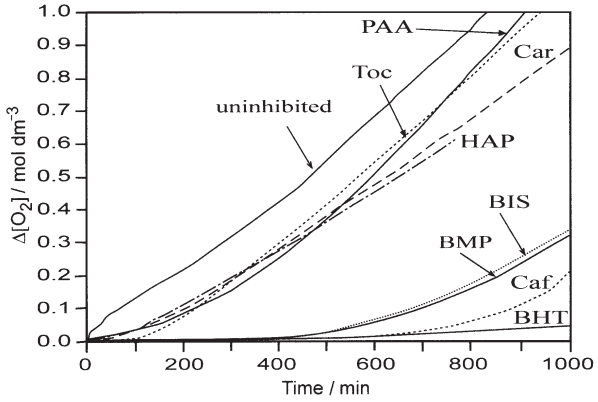

Fig. 2 Plots of $\Delta[O_2]$ during inhibition periods *vs.* time for antioxidant action of investigated compounds. Concentration of inhibitors 0.001 M, C_{AIBN}=0.04 M, temperature 40°C

Table 2 Values of induction time τ of LnET oxidation initiated by AIBN and inhibited by investigated compounds (n.d.-not determined)

Antioxidant	35°C		40°C		50°C	
	τ_{ind}/min	ν	τ_{ind}/min	ν	τ_{ind}/min	ν
BHT	694	7±3	555	35±13	207	12±4
BMP	704	10±3	678	24±7	384	95±13
Caf	n.d.		1021	28±7	320	8±3
PAA	406	114±25	327	116±10	50	60±24
HAP	406	119±9	95	106±25	87	103±8
BIS	724	12±5	587	24±3	244	5±3
Toc	300	9±3	130	20±8	96	21±7
Car	n.d.		166	86±24	38	98±10

All investigated compounds showed antioxidant activity measured as values of oxidation rate during induction period ($R_{inh}=\Delta[O_2]/\Delta\tau$) presented in Fig. 3. At temperatures 35, 40 and 50°C R_{inh} was three to sixty times lower than the rate of uninhibited autooxidation (R_O). The rate of inhibited autooxidation was greatly decreased by addition of compounds: BHT, BMP, caffeic acid and α-tocopherol. At lower temperatures the monohydroxyphenols are more efficient inhibitors than dihydroxyphenols. The results are in general agreement with reports showing the better antioxidant efficiency of dihydroxyphenols (BIS and Caf) at higher temperatures due to formation of further products which perform antioxidant activity [15, 16]. The values of R_{inh} for oxidation inhibited by *p*-hydroxyphenylacetic acid and *p*-hydroxyacetophenone were significantly higher but there was still an antioxidant effect. The measurements of antioxidant activity of non-phenolic β-carotene confirmed its moderate inhibitory effect. The ratio of *R* (Table 1) to R_{inh} was 3.9 at 40°C and 2.6 at 50°C.

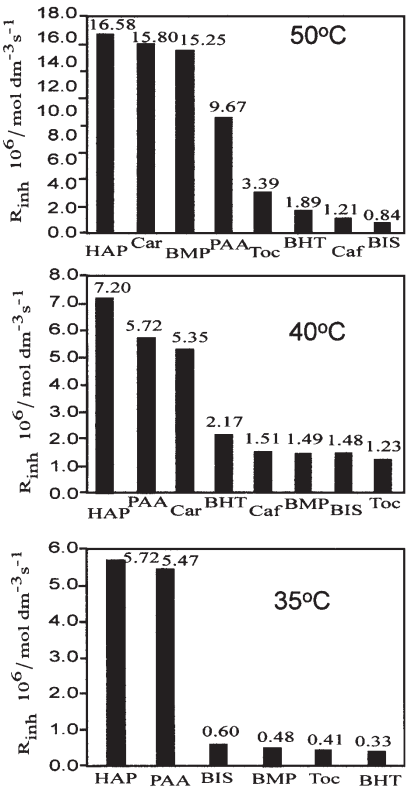

Fig. 3 Rates of inhibited autooxidation of ethyl linoleate at 35, 40 and 50°C. Rates of non-inhibited process and abbreviations are given in text

Conclusions

Concluding, we can state that uptake of oxygen by lipids during autooxidation can be monitored by thermogravimetry and kinetic parameters of oxidisability are in good agreement with parameters obtained by manometric and volumetric methods. All investigated compounds showed an antioxidant activity measured by induction time, values of rate of oxidation during induction period (R_{inh}) and values of kinetic chain length ν. At temperature 35, 40 and 50°C rate of oxidation R_{inh} was greatly decreased by addition of compounds: BHT, BMP, caffeic acid and α-tocopherol. At lower temperatures the monohydroxyphenols are more efficient inhibitors than dihydroxyphenols. The values R_{inh} for oxidation inhibited by *p*-hydroxyphenylacetic acid and *p*-hydroxyacetophenone were significantly higher but there was still an antioxidant effect.

* * *

This research was financially supported by grant number 120-501/68-BW-1483/20/2000.

References

1 H. Topallar, Y. Bayrak and M. Iscan, J. Am. Oil Chem. Soc., 74 (1997) 1323.
2 H. J. Nieschlag, J. W. Hagemann and J. Rothfus, Anal. Chem., 46 (1974) 2215.
3 I. Buzás, E. Kurucz-Lusztig and J. Hollo, J. Am. Oil Chem. Soc., 56 (1976) 685.
4 I. Buzás, J. Simon and J. Hollo, J. Thermal Anal., 12 (1977) 937.
5 L. Gennaro, A. Bocca, D. Modesti, R. Massella and E. Coni, J. Agric. Food Chem., 48 (1998) 4465.
6 G. W. Burton and K. U. Ingold, J. Am. Chem. Soc., 103 (1981) 6472.
7 V. A. Roginsky, T. K. Barsukova, A. A. Remorova and W. Bors, J. Am. Oil Chem. Soc., 73 (1996) 777.
8 T. Yamamura, K. Nishivaki, Y.Tanigaki, S. Terauchi, S. Tomiyama and T. Nishiyama, Bull. Chem. Soc. Jpn., 68 (1995) 2955.
9 L. R. C. Barclay, C. D. Edwards, K. Mukai, Y. Egawa and T. Nishi, J. Org. Chem, 60 (1995) 2739.
10 V. F. Tsepalow, V. A. Charitonowa, G. P. Gladyshev and N. M. Emanuel, Kinet. Catal., 18 (1977) 1261.
11 J. A. Howard and K. U. Ingold, Can. J. Chem., 45 (1967) 793.
12 W. A. Roginski, Kinet. Katal., 31 (1990) 546.
13 J. P. Cosgrove and D. F. Church, Lipids, 229 (1987) 299.
14 V. N. Ushalova, Kinet. Katal., 25 (1984) 283.
15 M. E. Cuvelier and W. Brand-Williams, Food Sci. Technol., London, 28 (1995) 25.
16 R. H. Stadler and F. L. Goillot, J. Agric. Food Chem., 44 (1996) 2503.