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Effects of selected phenol derivatives on the autoxidation of linolenic acid investigated by DSC non-isothermal methods

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Abstract

The kinetic features of the thermoxidation of linolenic acid (LNA) was investigated under non-isothermal conditions (50–300°C) in the presence of the phenolic compounds: *o*-cresol, *p*-cresol, 2,4-dimethylphenol, 2,5-dimethylphenol, 3,5-dimethylphenol, 2,4,6-trimethylphenol and 2,6-di-*t*-butyl-4-methylphenol at concentrations 0.26–20 mmol of compound per mol of LNA. Temperatures of reaction onset at different heating rates were used to evaluate kinetic parameters of linolenic acid thermoxidation by the Ozawa–Flynn–Wall method. Activation energy (E_a) of the pure linolenic acid thermoxidation was 70.4±0.5 kJ/mol and at the tested concentrations range of the phenolic compound, E_a increased maximally to 95.8±5.1 kJ/mol for 2,6-di-*t*-butyl-4-methylphenol, 101.1±3.0 kJ/mol for *o*-cresol, 90.9±1.5 kJ/mol for 2,4,6-trimethylphenol, 89.9±4.4 kJ/mol (*p*-cresol), 80.7±4.5 kJ/mol (2,5-dimethylphenol) 86.1±3.6 kJ/mol (3,5-dimethylphenol) and 75.1±3.0 kJ/mol for 2,4-dimethylphenol. At concentrations of the phenolic compound greater than 10 mmol per mol of LNA, a decrease of inhibitory effect was observed. © 1997 Elsevier Science B.V.

Keywords: Activation energy; Antioxidants; DSC; Phenolic compounds; Linolenic acid

1. Introduction

Atmospheric oxygen is the most accessible and economically important oxidising agent in many chemical processes in the petroleum and fat industries. The production of large quantities of organic chemicals, drying oils and modified polymers is based on the controlled oxidation of hydrocarbons. However, autoxidation is an undesirable process since rubber, plastics, fuel oil, fat and materials containing fat can undergo autoxidative deterioration or rancidification [1]. Unfavourable effects of oxidation are also observed in biological systems, where pathological events such as various diseases, cancer and ageing are possibly caused by free radical oxidation of polyunsaturated fatty acids, natural phospholipid membranes and nucleic acids. The autoxidation of lipids and other oxidizable substrates is a free-radical chain reaction and after initiation (catalysed by light, transition metal ions or water molecules), propagation of free hydroperoxy radicals occurs. This process can be represented by reaction:

$$ROO^{\bullet} + RH + O_2 \rightarrow ROOH + ROO^{\bullet} \quad (1)$$

An induction period is observed on the addition of certain phenols [2], aminophenols [3] or carotenoids [4], known as antioxidants, during the oxidation of

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triacylglycerols, phospholipids, diacylglycerols, sterols, steryl esters, cholesterol and other lipids. This means that the oxidation of the lipids is slow at the start of process and, after the end of the induction period, hydroperoxides and hydroperoxide products including aldehydes, ketones and hydrocarbons are produced rapidly.

The length of the induction period depends on the presence of antioxidants, pro-oxidants and the structure of the fatty acyl components of the lipids – polyunsaturated fatty acyl groups are oxidised much faster than monounsaturated or saturated fatty acid moieties.

Termination of the chain can occur by recombination of two peroxy radicals:

$$2ROO^{\bullet} \rightarrow \text{products of termination} \quad (2)$$

but in as inhibited system during the induction period, the phenolic antioxidant and further radicals are consumed in reactions:

$$PhOH + ROO^{\bullet} \rightarrow PhO^{\bullet} + ROOH \quad (3)$$

$$PhO^{\bullet} + ROO^{\bullet} \rightarrow \text{products} \quad (4)$$

$$PhO^{\bullet} + PhO^{\bullet} \rightarrow \text{products} \quad (5)$$

The rate of inhibited autoxidation during induction period is represented by Eq. (6):

$$-\frac{d[O_2]}{dt} = \frac{d[ROOH]}{dt} = \frac{k_1[RH]R_i}{nk_3[PhOH]} \quad (6)$$

where R_i is the rate of chain initiation, $n=2$ when $PhO^{\bullet}$ decays by reaction (4) or $n=1$ when it decays by reaction (5) [5]. The rate of oxidation and length of induction period as a criterion of antioxidant activity can be measured quantitatively by several methods based on Eq. (6). Measurements of peroxide concentration by iodometric or other classical analysis method [6], although rarely used [7], have a rather historical significance and there is a tendency to replace them by instrumental methods like reversed phase HPLC with UV or electrochemical detection of hydroperoxides [3], pulse radiolysis and laser photolysis studies [8]. These methods are examples of the approach to the problem by monitoring the formed product. On the other hand, the method following the substrate consumption or physicochemical parameters related to extension of the reaction may be used. The majority of studies on autoxidation and antioxidation are manometric measurements of oxygen uptake in Warburg apparatus and its modified versions. The studies on the antioxidant activity of vitamin E [9], polyalkylchromanols, naphtol derivatives and hydroquinones [2], flavonoids [5], aminophenols [3] and other phenolic derivatives as chain-breaking antioxidants are good examples of manometric methods. Some authors followed oxygen uptake by means of EPR detection [10,11].

Thermoanalytical methods are a different way of following the oxidation rate. Oxidation of fatty acids under excess of oxygen (pressure more than 13 kPa) can be assumed as an apparent first-order reaction and it is obviously exothermic. Cross [12] was the first to use DSC to determine the oxidative stability of edible oils and showed the correlation between the DSC measurements and the active oxygen method. Raemy et al. [13] studied the autoxidation of lipids by isothermal heat flux calorimetry at temperatures between 80 and 160°C and presented the role of unsaturation and antioxidants in the oxidation of C-18 fatty acids, their esters and several edible oils. These results obtained from calorimetric measurements were successfully compared with induction times determined by conventional method for highly unsaturated edible oil inhibited by propyl gallate. Antioxidant efficiencies of several plant flavonoids, propyl gallate, vitamins A, E, D3 and other antioxidants were examined by DSC methods during the oxidation of palm oil [14]. Pressure DSC was used to determine the oxidative stability of vegetable oils [15,16] and, also, to evaluate the antioxidant activities of 2-*t*-butyl-4-methoxyphenol, 2,6-di-*t*-butyl-4-methylphenol and propyl gallate in rapeseed oil matrix oxidation [17]. Communications in this field are rather limited and most of them concern isothermal calorimetry measurement.

Non-isothermal DSC methods are of practical analytical value. In contrast to the procedures described above, the methods are simple, non-time-consuming and may be applied in analysis of small samples (2–10 mg). All these advantages makes them more accessible than methods applying Warburg apparatus. Additionally, the evident advantage of thermoanalytical methods is continuous measurement. Therefore, we decided to investigate the potential of the non-isothermal method for evaluating the antioxidant efficiency of several simple phenolic compounds during the thermoxidation of linolenic acid. Due to the pre-

sence of three double bonds in the chain, the rate of oxidation of linolenic acid is high at relatively low temperature and the exothermic nature of reaction can be easily monitored by calorimetric measurements. Moreover, DSC experiments avoid solvent effects because the inhibitor-fatty acid system can be used without any other solvents. Recent papers [18,19] confirm the large effect of the solvent on the thermochemistry of PhO–H bond energies. Differences (~40 kJ/mol) between values of the PhO–H bond energy in polar and non-polar solvents were reported. In our experiments the interaction between phenols and hydrogen bond accepting solvent are eliminated.

2. Experimental

2.1. Materials

Linolenic acid (LNA) was obtained from Carl Roth, KG D75 Karlsruhe 21, Germany. The acid was stored in sealed vials (under nitrogen) in a refrigerator. The purity grade determined by GLC was >99%.

The commercial phenol derivatives: *o*- and *p*-cresol (Aldrich) of a 98% purity, were distilled under vacuum. All dimethylphenols and 2,4,6-trimethylphenol (TMP) were purchased from Fluka (purity grade of 98–99%). 2,4-dimethylphenol and 2,4,6-trimethylphenol were also purified by vacuum distillation; 2,5-dimethylphenol was distilled under nitrogen; 3,5-dimethylphenol was purified by sublimation under nitrogen. Their purity as determined by GLC was better than 99%. Commercial 2,6-di-*t*-butyl-4-methylphenol (BHT) were used without further purification. All phenolic derivatives were stored under nitrogen at a temperature about 0°C.

Chloroform was dried by calcium chloride and distilled in atmosphere of nitrogen.

2.2. Apparatus and methods

The DSC apparatus: A Du Pont model 910 differential scanning calorimeter with a Du Pont 9900 thermal analyser and a normal pressure cell were used in measurements. The study was carried out in an oxygen atmosphere. The oxygen flow rate was 15 l/h. The apparatus was calibrated with a high-purity indium standard.

In order to prepare the inhibited linolenic acid (concentration ranges from 0.26 to 20 mmol of phenolic compound per mol of LNA) the appropriate amounts (2–200 μl) of the chloroform solution of compound were added by variable volume pipettes to a weighed amount (~1 ml) of pure LNA. The mixture was gently stirred with 0.5 ml of chloroform and excess of solvent was removed under reduced pressure on a rotary vacuum evaporator. Prepared mixtures were immediately used in DSC experiments.

Determination of the kinetic parameters for the thermoxidation of LNA was described in previous papers concerned with thermoxidation of edible oils [20]. A 3–5 mg sample placed on an aluminium pan was heated at linear heating rates (β) of 5, 10, 20, 40 and 80 K/min in oxygen flow. Oxidation of the inhibited LNA was followed in the same mode.

3. Results

The antioxidant activities of alkylated phenolic derivatives were assessed in the oxidation of linolenic acid. It was necessary to evaluate the kinetic parameters of the pure LNA oxidation process. DSC curves obtained at various heating rates are shown in Fig. 1. The values of the temperature of the start of exothermic reaction measured as extrapolated onset temperature (T_e) and the values of maximum heat flow (T_{p1} and T_{p2} for first and second peak, respectively) are listed in Table 1. Every temperature is an average from at least three runs for the same β. An increase of the heating rate results in shift of the temperature ranges of the observed exothermic processes. These values were used for the determination of the activation energy of LNA thermoxidation for each concentration of phenolic compounds by means of the

Table 1
Temperatures of extrapolated onset of thermoxidation (T_e) and of the first and second peak (T_{p1}, T_{p2}) of DCS curves for different heating rates (β) for uninhibited LNA

β (K/min)	T_e (K)	T_{p1} (K)	T_{p2} (K)
5	359.3	423.4	472.3
10	369.4	431.9	480.5
20	380.0	445.8	486.5
40	391.9	469.9	510.4
80	404.5	477.2	532.6

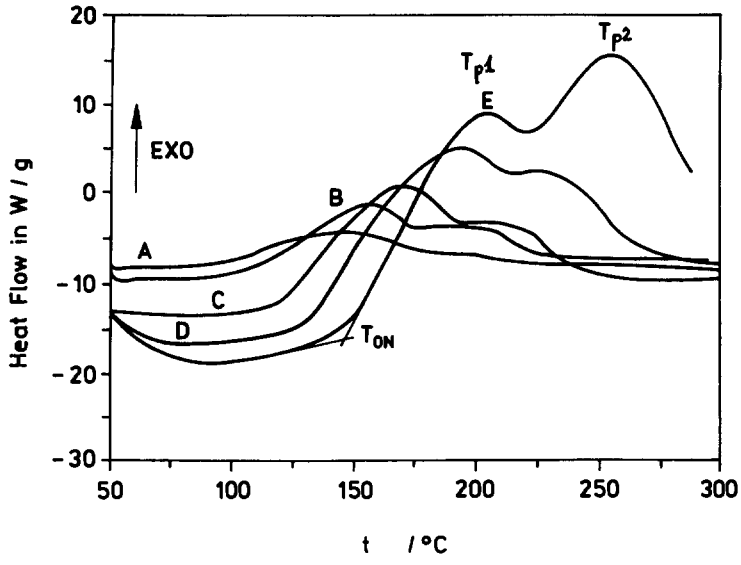


Fig. 1. DSC curves of linolenic acid oxidation for various heating rates: (A) 5 K/min; (B) 10 K/min; (C) 20 K/min; (D) 40 K/min; (E) 80 K/min

Ozawa–Flynn–Wall method, used in previous experiments [20].

The linearity of the basic dependencies:

$$\log\beta = aT^{-1} + b \tag{7}$$

for series of temperatures (in K) listed in Table 1 was evident. The slopes of these dependencies (=d $\log\beta$/d T^{-1}), calculated by means of the least-squares method, were utilised to calculate the apparent activation energies of autoxidation reaction by means of equation:

$$E_a = -2.19R\,\mathrm{d}\log\beta/\mathrm{d}T^{-1} \tag{8}$$

The pre-exponential factor (Z) and rate constant of reaction (k) were calculated from the basic Arrhenius kinetic equations. All details of applied procedures are previously described [20].

Statistical analysis of the presented data allowed the calculation of the standard deviations (σ_a), regression coefficients (R^2) and errors of estimation of activation energies. The activation energy of thermoxidation calculated from the extrapolated onset temperatures is 70.4±0.5 kJ mol^{-1}, calculated from the temperatures of the first peak is 80.1±4.8 kJ mol^{-1} and from the second peak E_a is equal 89.8±7.6 kJ mol^{-1}. The values of complete calculated kinetic parameters and statistic data for LNA are listed in Table 2.

Two examples of DSC curves obtained for inhibited LNA are shown in Fig. 2 (4-methylphenol) and Fig. 3 (BHT). Their shapes are similar to the uninhibited LNA oxidation curves but for the same heating rate the temperatures of the start of reaction are higher. The observed effect indicates the antioxidant activity of added compounds. The presented shapes of DSC curves are typical for all investigated phenolic derivatives. Values of T_e, T_{p1} and T_{p2} for LNA inhibited by 2,6-di-t-butyl-4-methylphenol at various concentrations are presented in Table 3. The addition of the inhibitor resulted in a shift of the onset temperature but

Table 2
Parameters of Eq. (7) and kinetic parameters of thermoxidation of pure LNA calculated from T_e, T_{p1} and T_{p2}

	T_e	T_{p1}	T_{p2}
Slope (a)	−3.865	−4.403	−4.933
Std. error of a estim.	0.034	0.264	0.415
Constant (b)	11.463	11.150	11.212
Std. error of b estim.	0.0079	0.0533	0.0796
R^2	0.9998	0.9928	0.9860
E_a (kJ/mol)	70.4	80.1	89.8
ΔE_a (kJ/mol)	0.5	4.8	7.6
ΔE_a (%)	0.8	6.0	8.4
Z (min^{-1})	5.65×10^9	2.05×10^9	2.07×10^9
k at 90°C (min^{-1})	0.792	0.013	0.6×10^{-3}

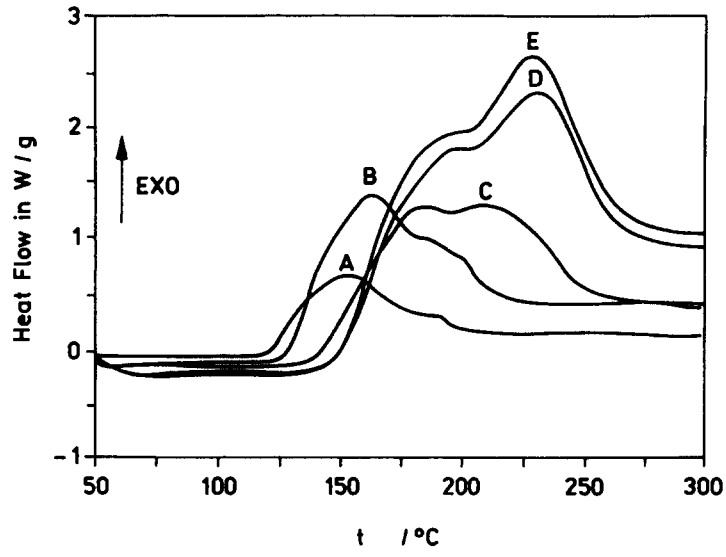


Fig. 2. DSC effects obtained during oxidation of LNA inhibited by 4.02 mmol BHT per mol LNA. (A) 5 K/min; (B) 10 K/min; (C) 20 K/min; (D) 40 K/min; (E) 80 K/min.

no significant changes in the temperature of the first and second peak were observed. In a few cases the values of T_{p1} and T_{p2}, listed in Table 2, fluctuated because observed peaks overlap and determination of those parameters with proper accuracy was difficult. This phenomena was observed for each investigated systems and for uninhibited linolenic acid, resulting in greater errors of calculated E_a (Table 2). Infrequent and rather stochastic fluctuations of T_{p1} and T_{p2} confirm that investigated compounds have an effect only at the first stage of oxidation and, in general, play no role in the further course of the process.

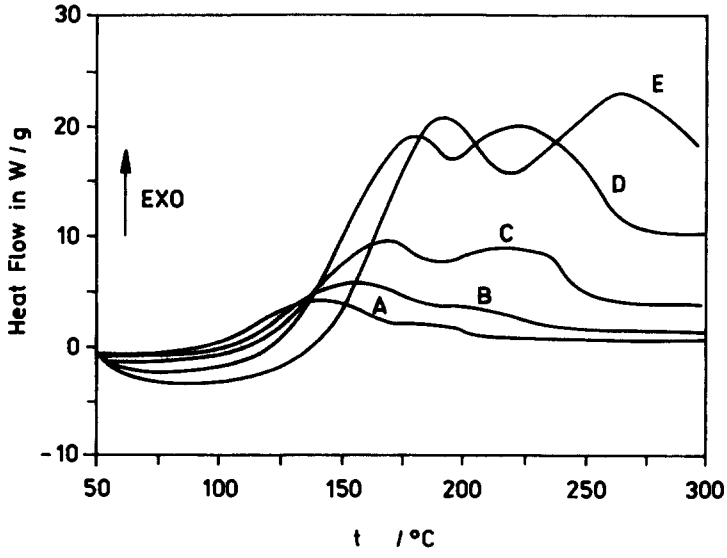


Fig. 3. DSC curve of oxidation of linolenic acid with addition of *o*-cresol for (A) 5 K/min, (B) 10 K/min, (C) 20 K/min, (D) 40 K/min and (E) 80 K/min.

Table 3
Measured temperatures of DCS curves for oxidation of LNA inhibited by BHT

β (K/min)	T_e (K)	T_{p1} (K)	T_{p2} (K)	β (K/min)	T_e (K)	T_{p1} (K)	T_{p2} (K)
	0.266 mmol BHT/mol LNA				3.08 mmol BHT/mol LNA		
5	365.3	421.0	472.7	5	391.4	423.1	467.7
10	376.4	435.8	487.5	10	401.6	441.6	475.5
20	384.8	450.2	490.7	20	409.7	457.0	487.4
40	400.1	467.1	508.9	40	418.3	466.2	509.0
80	408.4	476.2	538.6	80	432.5	474.4	542.6
		0.823 mmol BHT/mol LNA				4.02 mmol BHT/mol LNA	
5	382.3	424.5	464.0	5	392.8	427.3	464.3
10	386.6	430.0	476.5	10	400.2	437.6	463.2
20	399.3	448.6	512.7	20	410.9	459.5	486.9
40	407.0	450.3	508.5	40	420.4	470.1	505.2
80	425.2	488.5	531.6	80	430.2	461.6	546.0
	1.52 mmol BHT/mol LNA				9.86 mmol BHT/mol LNA		
5	386.4	425.3	465.2	5	399.0	429.8	463.4
10	395.8	441.0	486.4	10	407.1	443.3	482.2
20	403.9	453.5	486.9	20	415.9	456.5	483.3
40	412.8	452.8	490.2	40	424.7	467.5	506.9
80	428.6	483.1	527.4	80	439.0	478.5	529.9

The series of temperatures obtained for each investigated compound were correlated (Eq. (7)) to give Arrhenius plots. Typical Arrhenius plots calculated for the oxidation of LNA inhibited by BHT at various concentrations are presented in Fig. 4. The straight line relationships of the Arrhenius plots prove that a mechanism takes place at all used scanning rates. The calculated slopes of the plots, statistical analysis data, activation energies and other kinetic parameters for individual phenolic derivatives at various concentrations are listed in Tables 4–7. Among 36 values of activation energies, 23 were calculated with error of estimation less than 5%, in 7 values error was in 5–8% range and in 6 cases the error reached more than 8%. These errors are rather small in comparison with 10–20% or grater errors of the kinetic parameters estimation in the other, non-thermoanalytical methods of investigation.

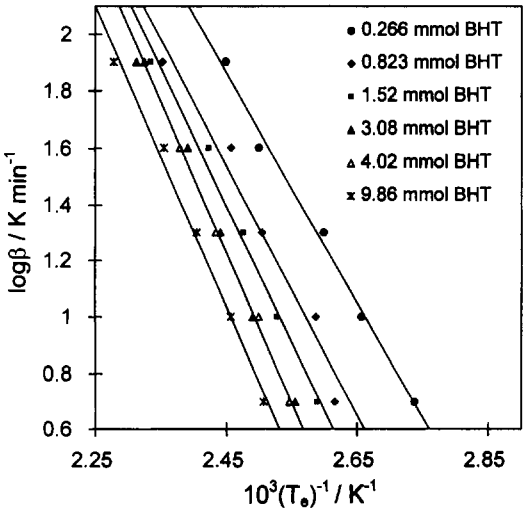


Fig. 4. Arrhenius plot calculated from temperatures of extrapolated start of linolenic acid oxidation for various concentration of BHT (mmol of BHT/mol LNA).

Figs. 5–7 show the dependencies of the activation energies for system with addition, respectively, dimethylphenols, cresols and trialkylated phenols for different concentrations of these compounds. The error bars are given to each value of E_a.

Dependencies of activation energy for two dimethylphenols (DMP) are plotted in Fig. 5. The errors of E_a estimation at lower concentrations are large, particularly in the case of 2,4-dimethylphenol, where the errors of the three initial values of E_a reach 12–16% and this compound is not shown in the plot. Plots of E_a show the maximum at concentrations about 3–8 mmol of DMP per mol of LNA and then decrease. The activation energies of LNA thermoxidation with *p*- and *o*-methylphenol are presented in Fig. 6. Both of

Table 4
Parameters of Eq. (7) and calculated kinetics parameters for thermoxidation of LNA inhibited by BHT

	Concentration of BHT (mmol of BHT/mol LNA)					
	0.266	0.823	1.519	3.080	4.020	9.860
Slope (a)	−4.0642	−4.4316	−4.8477	−5.116	−5.334	−5.3365
Std. error of (a) estim.	0.2087	0.4682	0.3071	0.2578	0.1362	0.2846
Constant (b)	11.8223	12.396	13.274	13.775	14.299	14.1116
Std. error of (b) estim.	0.0489	0.0989	0.0600	0.0467	0.0243	0.0506
R^2	0.99215	0.9676	0.9881	0.9928	0.9981	0.9915
E_a (kJ/mol)	73.0±3.7	79.6±8.4	87.1±5.5	91.9±4.5	95.8±2.4	95.8±5.1
ΔE_a (%)	5.1	10.6	6.3	4.9	2.6	5.3
Z (min^{-1})	8.98×10^{9}	2.45×10^{10}	2.06×10^{11}	6.57×10^{11}	2.04×10^{12}	1.28×10^{12}
k at 90°C (min^{-1})	0.5459	0.1778	0.1342	0.0908	0.0798	0.0492

them demonstrate better antioxidant activity than dimethylphenols. The decrease of the E_a values is observed for o-methylphenol but it exhibits enhanced activity at the low-concentration range.

4. Discussion

The shapes of DSC plots of pure linolenic acid oxidation for different heating rates indicates three kinds of behaviour of the LNA during single run. The analysis of these plots by Ozawa–Flynn–Wall method leads to obtain three different values of activation energies calculated from T_e, T_{p1} and T_{p2} values. We can confirm that the chemical nature of the oxidation of fatty acids and their esters differs at various temperatures. For a heating rate of 5 K/min these temperature ranges are generally delimited as follows: below 90°C, 90–160°C and above 160°C. According to data presented by other investigators [21,22], the first region is the most interesting one for the studies on antioxidation efficiency because the oxidation reaction is marked by an induction period, chemical changes at these temperatures are slight and no

Table 5
Parameters of Arrhenius plot and calculated kinetics parameters for thermoxidation of LNA inhibited by dimethylphenols. Concentration of inhibitors in (mmol/mol of LNA)

Concentration of inhibitor	a	σ_a	R^2	E_a (kJ/mol)	ΔE_a (%)	Z (min^{-1})	k (min^{-1})
2,4-dimethylphenol							
0.71	−3.9209	0.0975	0.9988	71.4±1.8	2.5	9.97×10^{9}	1.01
1.23	−3.9615	0.1688	0.9964	72.1±3.1	4.3	1.11×10^{10}	0.895
2.51	−3.9174	0.1477	0.9957	71.3±2.7	3.8	7.30×10^{9}	0.757
6.34	−4.1646	0.1671	0.9952	75.8±3.0	4.0	2.07×10^{10}	0.504
19.81	−4.1237	0.0281	0.9999	75.1±0.5	0.7	1.50×10^{10}	0.463
2,5-dimethylphenol							
0.39	−4.1311	0.5123	0.9702	75.2±9.3	12.4	1.55×10^{10}	0.459
0.87	−4.4338	0.7101	0.9750	80.7±12.9	16.0	8.03×10^{10}	0.403
1.89	−4.4391	0.6661	0.9569	80.8±12.1	15.0	1.59×10^{11}	0.771
7.02	−4.5830	0.2447	0.9943	83.4±4.5	5.3	2.36×10^{11}	0.492
15.45	−4.4769	0.1996	0.9940	81.5±3.6	4.5	9.28×10^{10}	0.362
3,5-dimethylphenol							
0.41	−4.3820	0.1790	0.9950	79.8±3.3	4.1	6.31×10^{10}	0.428
1.12	−4.5652	0.2230	0.9929	83.1±4.1	4.9	2.24×10^{11}	0.520
3.21	−4.7282	0.1987	0.9965	86.1±3.6	4.2	4.85×10^{11}	0.431
15.77	−3.9894	0.1260	0.9970	72.6±2.3	3.2	6.05×10^{9}	0.411

Table 6
Parameters of Arrhenius plot and calculated kinetics parameters for thermoxidation of LNA inhibited by *o*- and *p*-methylphenol. Concentration of inhibitors in (mmol/mol of LNA)

Concentration of inhibitor	a	σ_a	R^2	E_a (kJ/mol)	ΔE_a (%)	Z (min^{-1})	k (min^{-1})
o-methylphenol							
0.64	−5.5532	0.1620	0.9975	101.1±3.0	3.0	1.18×10^{14}	0.828
1.73	−5.7609	0.1255	0.9986	104.9±2.3	2.2	2.93×10^{14}	0.609
3.89	−4.7666	0.1568	0.9968	86.8±2.9	3.3	9.44×10^{11}	0.672
9.71	−5.0717	0.1111	0.9986	92.3±2.0	2.2	5.15×10^{12}	0.612
16.14	−5.0023	0.2613	0.9919	91.1±4.8	5.2	3.24×10^{12}	0.579
p-methylphenol							
1.18	−4.5945	0.3868	0.9860	83.7±7.0	8.4	5.79×10^{11}	1.131
4.07	−4.5884	0.1428	0.9858	83.5±2.6	3.1	5.73×10^{11}	1.162
8.01	−5.0538	0.3500	0.9858	92.0±6.4	6.9	8.47×10^{12}	1.118
15.53	−5.1091	0.3786	0.9838	93.0±6.9	7.4	1.34×10^{13}	1.282

Table 7
Parameters of Arrhenius plot and calculated kinetics parameters for thermoxidation of LNA inhibited by 2,4,6-trimethylphenol. Concentration of inhibitor in (mmol/mol of LNA)

Concentration of inhibitor	a	σ_a	R^2	E_a (kJ/mol)	ΔE_a (%)	Z (min^{-1})	k (min^{-1})
0.47	−3.8818	0.2953	0.9886	70.7±5.3	7.6	3.86×10^{9}	0.494
3.10	−4.8153	0.2010	0.9948	87.7±3.7	4.2	1.80×10^{12}	0.964
5.89	−4.5855	0.4456	0.9815	83.5±8.1	9.7	4.04×10^{11}	0.831
7.60	−4.8535	0.0732	0.9993	88.4±1.3	1.5	1.16×10^{12}	0.497
11.75	−4.9915	0.0799	0.9995	90.9±1.5	1.6	2.28×10^{12}	0.432

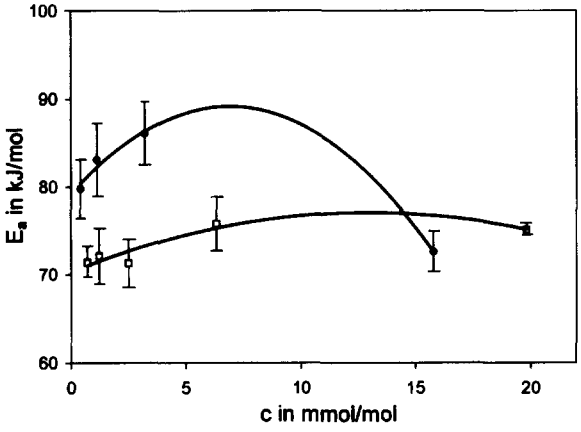


Fig. 5. Activation energies of LNA thermoxidation in presence of various concentrations of 2,4-dimethylphenol (□) and 3,5-dimethylphenol (○).

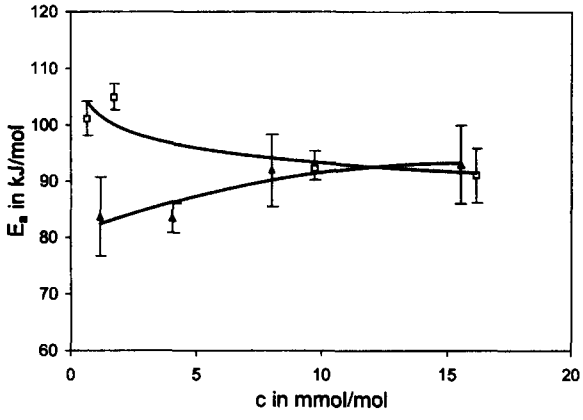


Fig. 6. Activation energy of thermoxidation of linolenic acid inhibited by *o*-methylphenol (□) and *p*-methylphenol (▲) at various concentrations.

exothermic process is observed on DSC curve. The end of the induction period manifests in acceleration of the exothermic oxidation of fatty acid. Consumption of all antioxidant molecules at the onset point is assumed and at second phase (90–160°C), the products containing a maximum of peroxides are formed by attack of the oxygen molecules at the double bonds of carbon chain. This point may be easily detected in

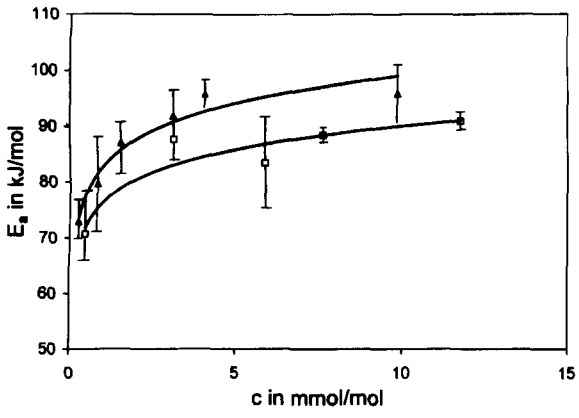


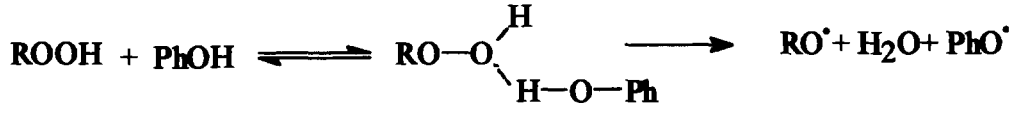
Fig. 7. Influence of concentration of BHT (▲) and TMP (□) in linolenic acid on activation energy of thermoxidation of LNA.

DSC measurements and the extrapolated temperatures of the start of exothermic process may be used to calculate the kinetic parameters of thermoxidation of inhibited LNA by isoconversional methods (the Ozawa–Flynn–Wall method in our study). At the third region (above 160°C), the oxidised product polymerise by conjugation and ring formation to dimmeric polymers linked through oxygen and the polymers are directly oxidised [23].These considerations lead to conclusion that from T_e, T_{p1} and T_{p2} only T_e may be used to indicate the antioxidant activity of the analysed inhibitors and antioxidant action takes place below 100°C. It should be noticed, that no exothermic or endothermic processes of decomposition, evaporation, polymerisation and isomerisation were observed in experiments described by Raemy et al. [13] when the samples of methyl linoleate and methyl linolenate were heated under argon flow at temperatures 80–140°C. Hence, these processes do not interfere with the observed oxidation under air or oxygen flow.

esterification of long chain fatty acids or transesterification of theirs esters at the temperature below 100°C and under oxygen flow seems to be extremely insignificant and has not been reported by other investigators. Moreover, even if esterification occurred it would be manifested by an evident decrease of antioxidant activity of reacting phenol at all used concentration ranges. In the current data, the temperature of the extrapolated start of oxidation for the best inhibited systems reached above 100°C but the possibility of the former esterification is eliminated because of radical nature of the anti oxidant action and the high antioxidant efficiency of phenol.

Dimethylphenols (DMP) exhibit poor antioxidant activity. In this case, the measurements have qualitative rather than quantitative significance because the activation energies of oxidation of LNA with 2,4- and 2-5-dimethylphenol are similar to the activation energies of oxidation of LNA without phenols. The values for all dimethyl derivatives did not exceed 85 kJ/mol and these compounds do not display inhibiting activity. This is in agreement with data obtained from investigations on autoxidation of tetralin given by manometric methods [24].

The low antioxidant aptitude of phenols with no *ortho*-substituent (*o*-methylphenol, 3,5-dimethylphenol) can be explained by the formation of the complex PhOH· · ·RH with hydrogen bond between carboxyl group of lipid and hydrogen atom from hydroxyl group of phenol. Reduction of antioxidant ability due to the absence of *ortho*-substituents was observed in other similar derivatives, for example in *p*-methoxyphenol [3,25]. Moreover, the absence of the *ortho*-substituents can lead to reaction of phenol with hydroperoxides:



(9)

We should also consider the possibility of the esterification. However, reaction of phenols proceeds with more difficulty than the esterification of primary and secondary aliphatic alkohols. Esterification of phenols is based on acid anhydrides or chlorides and occurs in the presence of some reagents which retain any acid that may be formed. Uncatalysed

We have no data about rates of this reaction but, for example, in the system: 4-methylphenol and cumyl hydroperoxide, the probability of an effective split of the intermediate complex presented in Eq. (9) is 0.27 at 120°C. This effect can be significant in the investigated system: LNA + *p*-methylphenol, although highly viscous fatty acid does multiply the cage effect.

The influence of *ortho*-substituents on the antioxidant activity of phenols was confirmed by experiments with the addition of tri-substituted compounds: TMP and BHT to linolenic acid matrix. The changes of activation energies of inhibited LNA oxidations shown in Fig. 7. Both curves demonstrate similar shapes and have increasing tendency at all concentration ranges. It is evident that the differences in the radical scavenging abilities of mono-, di- and trialkylated phenols can be explained by steric and stabilisation effect which is the highest when two *ortho*- and one *para*-alkyl group are present.

5. Conclusions

DSC is a valuable technique in obtaining the kinetic parameters of fatty acid oxidation. In the oxidation of linolenic acid the conditions of the experiments are such that the long chain fat molecules can be effectively inbibited and antioxidant activity can be elucidated by means of DSC. However, only the start of the process is shown to be useful for calculation of the kinetic parameters of the inhibited oxidation. The temperatures of extrapolated onset of thermoxidation of inhibited linolenic acid were used to determine the antioxidant activity of phenolic compounds by means the Ozawa–Flynn–Wall method and the statistical analysis of the results can confirm that the temperature dependencies obey the Arrhenius relationship. The calculated values of activation energies oxidation of the inhibited linolenic acid show that the antioxidant activity varies markedly with the compounds tested and with their concentration. 2,4-dimethylphenol, 2,5-dimethylphenol, 3,5-dimethylphenol and *p*-methylphenol exhibit small antioxidant activity while *o*-cresol, 2,4,6-trimethylphenol and 2,6-di-*t*-butyl-4-methylphenol demonstrate enhanced inhibiting ability due to presence of three alkyl substituents. The relative activities of investigated phenolic compounds are similar to those obtained by means of oxygen consumption methods, but in our experiments the activation energy of the first step of LNA thermoxidation is a measure of antioxidant activity of chain-breaking phenolic antioxidants. DSC used for the determination of the kinetic parameters by non-isothermal method is relatively simple, convenient and fast.

Acknowledgements

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