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Evaluation of activities of antioxidants in rapeseed oil matrix by pressure differential scanning calorimetry

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Abstract

Samples of rapeseed oil and rapeseed oil inhibited with antioxidants were oxidized in the cell of a pressure differential scanning calorimeter (PDSC). The PDSC experiments were carried out under 1800 kPa oxygen pressure at temperatures in the range 131–156°C. The antioxidants used were 2-*t*-butyl-4-methoxyphenol (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT) and propyl gallate (PG). From resulting PDSC exotherms their extrapolated onset and peak maximum times were determined and used for the assessment of the oxidative stabilities of the samples. The efficiencies of antioxidants used for protection of rapeseed oil from oxidation can be ranked in the order BHT < BHA ≪ PG, with PG the most and BHT the least efficient of the three. As PDSC exotherms were obtained at different temperatures the activation energies for oxidation of inhibited and uninhibited rapeseed oil samples have been calculated.

INTRODUCTION

Deterioration of fats and fat-based foods by autoxidation is a well-known phenomenon. Accordingly, various aspects of lipid oxidation have been studied in the past, and many excellent books and review articles are now available in this field [1–4]. The reaction of oxygen with lipids involves free radical initiation, propagation and termination and its kinetics has been investigated for years. The experimental designs have been mainly based on analytical or volumetrical methods. Because the oxidation of lipids in an excess of oxygen can be treated as an apparent first-order reaction and is obviously exothermal, it can be quantified by thermal analysis techniques. Among these differential scanning calorimetry (DSC) and pressure differential scanning calorimetry (PDSC) seem to be the most useful. Cross [5] was the first to use DSC for determination of the oxidative stability of edible oils at normal pressures of oxygen, and

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he achieved a moderately successful correlation between measurements by DSC and the active oxygen method (AOM). Hassel [6] has used PDSC (120°C, 500 psig O_2) as an alternative method to AOM, obtaining an improved correlation for vegetable oils. Raemy et al. [7] have performed normal pressure isothermal DSC studies in an oxygen atmosphere of saturated and unsaturated C_{18} fatty acid methyl esters, vegetable oils and chicken fat inhibited with propyl gallate. Based on measured induction times for the oxidation of samples, they were able to rank the materials studied. They found that induction times of inhibited chicken fat samples increase as a function of propyl gallate content. The dynamic DSC studies under normal pressure of oxygen with the use of the more popular edible vegetable oils and of lard have been carried out by Kowalski [8–11]. The measurements have been performed at various heating rates and the kinetic parameters for the thermal oxidative decomposition of the fats studied have been calculated. When samples are heated quickly (>30°C min^{-1}) at elevated pressures of oxygen they ignite; the temperatures of their spontaneous ignition have been determined [12]. The oxidative stability and kinetic features of the oxidation of rapeseed, soybean, corn and sunflower oils have also been studied by PDSC [13].

Because the oxidation of fats is a radical promoted chain reaction it is frequently inhibited by the incorporation of certain organic additives into the system. A good antioxidant, if it passes health and nutritional tests, should protect oil or fat from autoxidation at low levels of treatment. Accordingly, the activity of antioxidants is also an important factor in their assessment. Several methods for such assessment have been developed. Generally, they are based on a comparison of the oxidative stabilities of fats with and without antioxidants. The traditionally used methods (AOM, oven or shelf tests) are time consuming and often imprecise, so there is a tendency to replace them by instrumental investigations. Recently, the influence of some phenolic-type antioxidants (chain breakers) on the thermal oxidative decomposition of rapeseed oil has been studied by dynamic DSC [11]. The present study was undertaken to investigate the potential of PDSC for evaluating the efficiency of these antioxidants in the protection of this oil against oxidation.

EXPERIMENTAL

Materials

The rapeseed oil (RSO) was obtained from a local factory; it was "pure rapeseed" without any addition of other oils. The oil was fresh (peroxide value = 0.8 and acid value = 0.1) and its fatty acids composition obtained by fatty acids methyl ester (FAME) GLC is listed in Table 1. The oil was investigated [11] by dynamic DSC in oxygen and displayed an

TABLE 1

Fatty acids composition of rapeseed oil

Fatty acid $C_{m:n}$ [a]	Content (%)
$C_{14:0}$	Trace
$C_{16:0}$	5.0
$C_{16:1}$	0.4
$C_{18:0}$	0.9
$C_{18:1}$	60.7
$C_{18:2}$	19.5
$C_{18:3}$	8.2
$C_{20:0}$	0.4
$C_{20:1}$	2.4
$C_{22:0}$	0.6
$C_{22:1}$	1.8

[a] Key: m is the number of carbon atoms; n is the number of double bonds.

onset thermal oxidative decomposition temperature of 179.1°C. The antioxidants 2-*t*-butyl-4-methoxyphenol (BHA), 2,6-di-*t*-butyl-4-methylphenol (BHT) and propyl gallate (PG) were the same as used in ref. 14, where their thermal properties and thermal and oxidative stabilities are reported.

A total of 17 samples (seven RSO + BHA, five RSO + BHT and five RSO + PG) containing different amounts of antioxidants were prepared by weight. In order to protect the samples from uncontrolled autoxidation they were kept at 5°C under nitrogen in darkness, packed in glass ampoules.

Apparatus and procedure

A Du Pont 1090 B thermal analyzer with 1091 Disc Memory, and a Du Pont 910 differential scanning calorimeter equipped with a pressure cell (PDSC, model No. 900830-902) were used. The instrument was calibrated using high purity indium as the standard. Weighed samples of pure or inhibited oil (3–4 mg) were placed in the open aluminium pan, the reference pan was left empty. Experiments were performed under 1800 kPa pressure of oxygen. After preliminary pressurization of the PDSC cell with oxygen to remove air, the oxygen pressure and temperature were adjusted and the experiment was started. Isothermal conditions were chosen to provide reasonable exotherm times (5–75 min) at four or five different temperatures. The run data were recorded on 8 in floppy discs. For each isoconcentrated solution six runs at given temperatures were performed; the two extreme values were rejected and the four others were analyzed. From the resulting PDSC heat flow curves the time for the extrapolated onset of oxidation τ_{ON} (obtained with the Du Pont

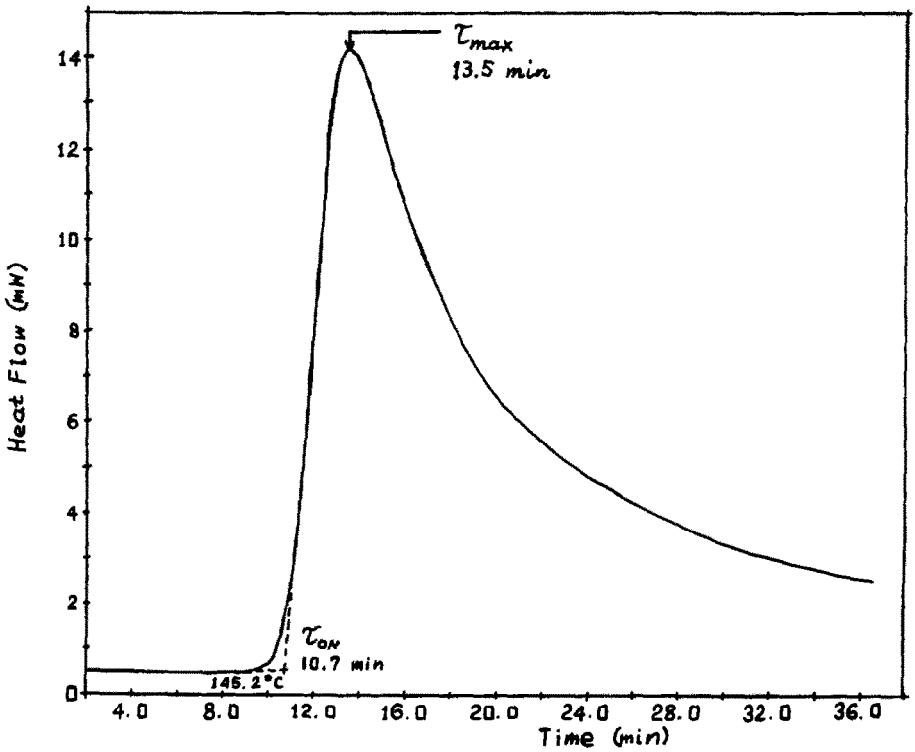

Fig. 1. PDSC exotherm for oxidation of rapeseed oil at 145.2°C: oxygen pressure, 1800 kPa; sample mass, 3.5 mg.

OXIDATIVE STABILITY V 2.0. program) and the maximum of the peak τ_{max} (obtained from plots) were determined, as shown in Fig. 1. The tabulated τ_{ON} and τ_{max} values are average from four measurements.

RESULTS AND DISCUSSION

Rapeseed oil

The times for the extrapolated onset of oxidation and the maximum of the PDSC peak for the samples of RSO are listed in Table 2. Both lg τ_{ON} and lg τ_{max} show a linear dependency (correlation coefficients > 0.99) on the reciprocal of exotherm temperatures (Fig. 2) and they can be described by the equations

$$\lg \tau_{ON} = aT^{-1} + b \quad (1)$$

and

$$\lg \tau_{max} = AT^{-1} + B \quad (2)$$

TABLE 2

Measured times τ (min) of PDSC exotherms obtained at 1800 kPa of oxygen for oxidation of RSO

Temperature (°C)	τ_{ON} [a]	τ_{max} [a]
131.0	26.3	34.2
135.0	21.5	26.0
138.6	16.2	19.8
140.0	14.9	18.5
145.2	10.7	13.5
150.3	6.9	9.4
	7.1	9.6
152.6	6.2	9.0
	6.3	9.1
155.0	5.1	7.0
	5.2	7.0

[a] See text.

where a, b, A and B are adjustable coefficients and T is the absolute temperature. The coefficients calculated by simple regression analysis were $a = 5245.9$, $b \doteq -11.535$, $A = 4816.5$ and $B = -10.389$.

The shapes of the PDSC exotherms obtained for oxidation of RSO fit the picture of an autocatalytic reaction, so τ_{ON} can be interpreted as an induction time (induction period) of reaction. The occurrence of the maximum in the PDSC exothermic curve can be utilized to obtain kinetic information by assuming that the extent of conversion at this maximum is constant. Accordingly, the values for the activation energy $E =$

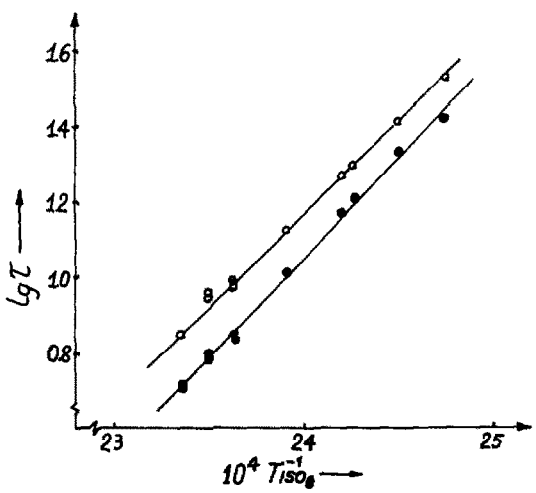

Fig. 2. lg τ_{max} (top line) and lg τ_{ON} (bottom line) versus reciprocal temperatures (K^{-1}) of PDSC exotherms of rapeseed oil.

92.2 kJ mol^{-1} and pre-exponential factor lg $Z = 10.39$ (min^{-1}) of the Arrhenius equation for the oil studied were calculated from the coefficients of eqn. (2). Similarly, as the threshold value in analysis leading to determination of the τ_{ON} data was kept constant the degree of conversion at these points for the systems studied do not differ substantially. Accordingly, from the coefficients of eqn. (1) the $E' = 100.4$ kJ mol^{-1} and lg $Z' = 11.54$ (min^{-1}) were also calculated. The clearly shown relatively large induction periods on the PDSC exotherms of RSO suggest that this oil possesses some defence system, probably formed by natural antioxidants, which enhanced its stability.

Inhibited rapeseed oil

The samples of RSO inhibited with different amounts of antioxidants were treated in the same way as uninhibited RSO. The results of τ_{ON} and

TABLE 3

Measured times τ (min) of PDSC exotherms for oxidation of RSO inhibited with BHA [a]

Temperature (°C)	τ_{ON} [b]	τ_{max} [b]	τ_{ON} [b]	τ_{max} [b]
	RSO + 0.011% BHA		RSO + 0.033% BHA	
135.6	23.6	29.5	29.3	33.5
140.1	17.3	21.3	19.8	23.7
145.5	12.0	14.9	14.2	16.9
150.4	8.6	10.9	10.3	12.6
	RSO + 0.089% BHA		RSO + 0.121% BHA	
135.6	39.5	43.8	42.4	47.2
140.1	28.5	32.2	32.5	36.4
145.5	19.4	22.2	22.5	25.5
150.4	14.5	17.0	15.2	18.0
	RSO + 0.233% BHA		RSO + 0.405% BHA	
135.6	55.8	61.3	58.1	62.5
140.1	39.0	43.5	44.1	47.3
145.5	25.9	29.8	30.4	32.9
150.4	19.6	22.0	21.1	23.4
	RSO + 0.806% BHA			
135.6	67.2	72.5		
140.1	50.1	53.8		
145.5	34.8	37.0		
150.4	25.1	27.5		

[a] Oxygen pressure $p = 1800$ kPa. [b] See text.

TABLE 4

Measured times τ (min) of PDSC exotherms for oxidation of RSO inhibited with BHT [a]

Temperature (°C)	τ_{ON} [b]	τ_{max} [b]	τ_{ON} [b]	τ_{max} [b]
	RSO + 0.017% BHT		RSO + 0.033% BHT	
135.5	20.5	25.8	27.0	32.9
140.2	15.0	19.6	19.0	24.1
145.5	9.7	13.7	12.3	17.1
150.4	7.3	9.8	10.1	13.0
155.2	5.6	7.3	7.1	9.0
	RSO + 0.056% BHT		RSO + 0.080% BHT	
135.5	30.3	36.6	35.5	41.2
140.2	22.1	26.7	25.1	29.7
145.5	16.0	19.4	17.5	21.0
150.4	11.1	13.9	12.1	14.6
155.2	8.2	10.6	9.1	11.0
	RSO + 0.112% BHT			
135.5	38.5	44.7		
140.2	25.8	30.8		
145.5	20.1	23.2		
150.4	13.7	16.4		
155.2	10.1	12.0		

[a] Oxygen pressure $p = 1800$ kPa. [b] See text.

τ_{max} for inhibited samples are listed in Tables 3–5. In Figs (3)–(5) the linear dependences of lg τ_{ON} and lg τ_{max} (correlation coefficients > 0.99) against the reciprocal of the absolute temperature of the PDSC exotherms are shown. The calculated values of E and Z for inhibited samples are listed in Table 6. The straight lines (Figs (3)–(5)) obtained from isothermal PDSC experiments performed in this work imply that the consumption of antioxidants follows the same mechanism over the temperature range studied. It is tempting to extrapolate these straight lines to higher or to lower temperatures, although this of course assumes no change in mechanism.

From the results obtained for inhibited RSO samples the activity (efficiency) of antioxidants used can be compared. In Figs 6–8 the plots of $\tau^{in}_{ON} - \tau^{o}_{ON}$ and $\tau^{in}_{max} - \tau^{o}_{max}$ vs. antioxidant concentration are shown; superscripts "in" and "o" denote inhibited and uninhibited RSO. As can be seen from Figs. 6–8, the activity of the antioxidants used can be ranked as follows: BHT < BHA ≪ PG. It should be noted that for stabilities of pure antioxidants [14] and for their protection of RSO from thermal

TABLE 5

Measured times τ (min) of PDSC exotherms for oxidation of RSO inhibited with PG [a]

Temperature (°C)	τ_{ON} [b]	τ_{max} [b]	τ_{ON} [b]	τ_{max} [b]
	RSO + 0.012% PG		RSO + 0.020% PG	
140.3	26.2	29.4	28.8	32.9
145.8	17.1	20.2	18.4	21.9
150.6	10.6	13.3	13.0	15.4
155.6	7.7	10.0	9.1	11.3
	RSO + 0.044% PG		RSO + 0.070% PG	
140.3	37.5	43.7	44.6	48.1
145.8	26.9	29.5	29.5	33.9
150.6	18.2	22.5	22.5	25.2
155.6	14.0	16.4	15.8	17.8
	RSO + 0.093% PG			
140.3	49.5	53.6		
145.8	34.7	37.2		
150.6	25.2	27.7		
155.6	18.5	21.0		

[a] Oxygen pressure $p = 1800$ kPa. [b] See text.

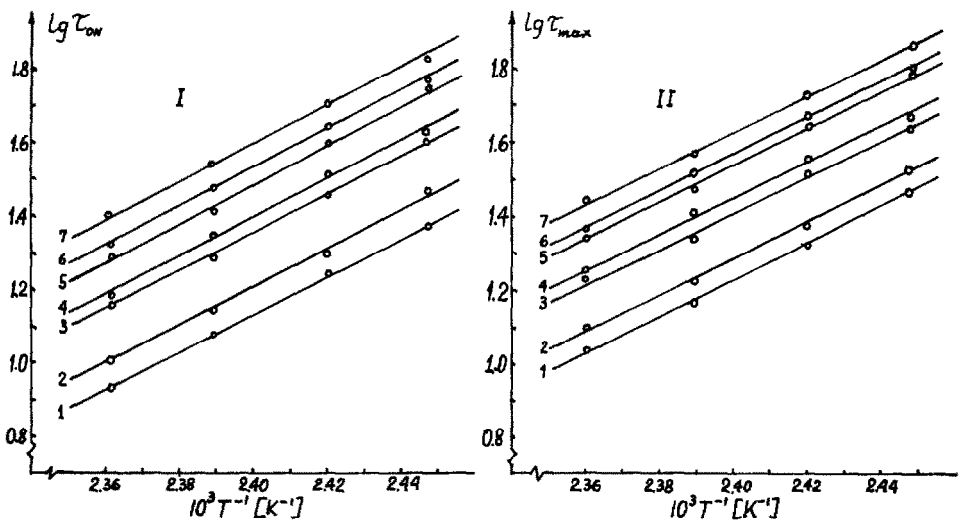

Fig. 3. lg τ_{ON} (I) and lg τ_{max} (II) versus reciprocal temperatures (K^{-1}) of PDSC exotherms obtained at 1800 kPa of oxygen for rapeseed oil inhibited with: 1, 0.011%; 2, 0.033%; 3, 0.089%; 4, 0.121% 5, 0.233%; 6, 0.405%; 7, 0.806% BHA.

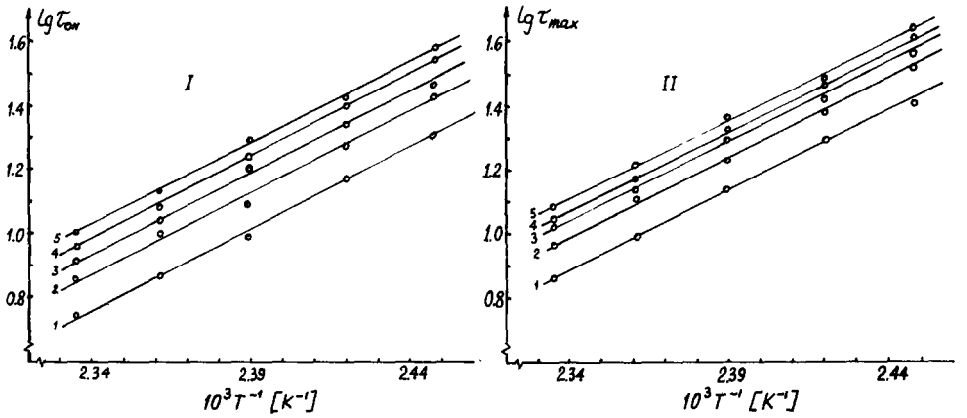

Fig. 4. Plot as in Fig. 3 for rapeseed oil containing: 1, 0.017%; 2, 0.033%; 3, 0.056%; 4, 0.080%; 5, 0.112% BHT.

oxidative decomposition [11], respectively, the following sequences have been obtained: BHA < BHT ≪ PG and BHA ≈ BHT ≪ PG. The BHA and BHT appear to be practically ineffective in protecting RSO against thermal oxidative decomposition, owing to their high volatility and susceptibility to oxidation [11, 14]. Whereas the experiments in this work were performed under elevated pressure, the evaporation of BHA and BHT from heated oil was suppressed and they have acted more efficiently, but not as well as PG. However, it appears that for the antioxidants studied, their efficiencies decrease if the temperature of inhibited RSO increases.

In this discussion one more point has to be considered. There are literature reports [15, 16] that at relatively high concentrations some

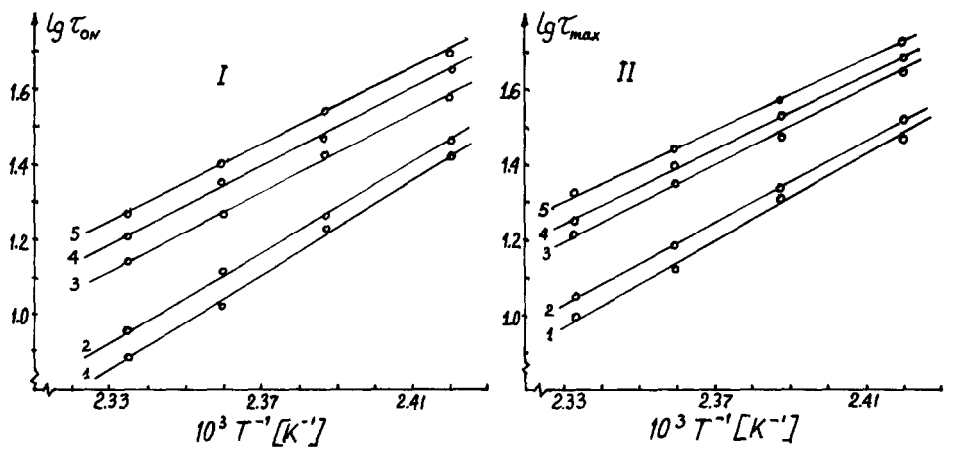

Fig. 5. Plot as in Fig. 3 for rapeseed oil containing: 1, 0.012%; 2, 0.020%; 3, 0.044%; 4, 0.070%; 5, 0.095% PG.

TABLE 6

Activation energies E (kJ mol^{-1}) and pre-exponential factors Z (min^{-1}) of the Arrhenius equation for oxidation of RSO inhibited with antioxidants

Sample	Calculated from eqn. (1)		Calculated from eqn. (2)	
	E	lg Z	E	lg Z
RSO + 0.011% BHA	97.8	11.12	96.3	10.84
RSO + 0.033% BHA	99.7	11.29	94.1	10.58
RSO + 0.089% BHA	97.7	10.90	92.4	10.18
RSO + 0.121% BHA	99.1	11.03	93.5	10.27
RSO + 0.233% BHA	102.2	11.33	99.3	10.91
RSO + 0.405% BHA	98.1	10.76	95.3	10.39
RSO + 0.806% BHA	95.4	10.37	94.5	10.22
RSO + 0.017% BHT	97.6	11.17	94.6	10.68
RSO + 0.033% BHT	96.7	10.95	94.2	10.52
RSO + 0.056% BHT	96.8	10.88	92.0	10.20
RSO + 0.080% BHT	101.4	11.42	98.5	10.98
RSO + 0.112% BHT	97.0	10.83	95.6	10.59
RSO + 0.012% PG	121.1	13.88	106.4	11.98
RSO + 0.020% PG	110.9	12.57	103.7	11.60
RSO + 0.044% PG	97.3	10.73	93.6	10.20
RSO + 0.070% PG	98.7	10.83	95.6	10.39
RSO + 0.095% PG	95.5	10.38	91.1	9.79

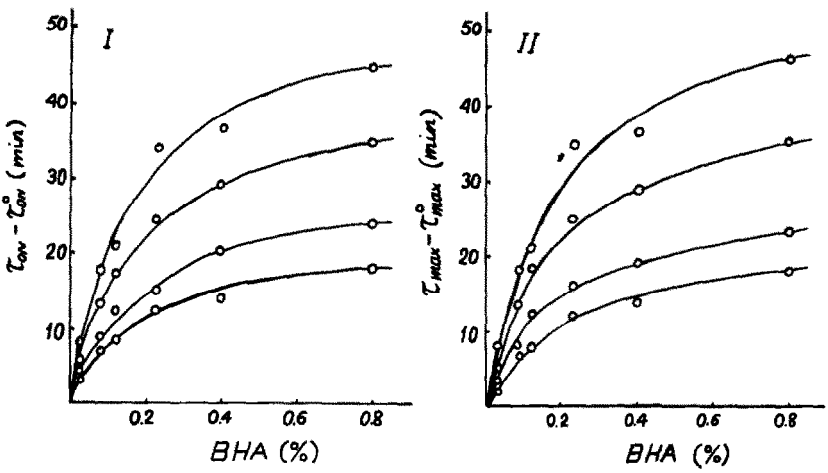

Fig. 6. Plots of $\tau^{in}_{ON} - \tau^{o}_{ON}$ (I) and $\tau^{in}_{max} - \tau^{o}_{max}$ (II) vs. antioxidant content for RSO inhibited with BHA; temperatures 135.6, 140.1, 145.5 and 150.4°C, respectively from top to bottom.

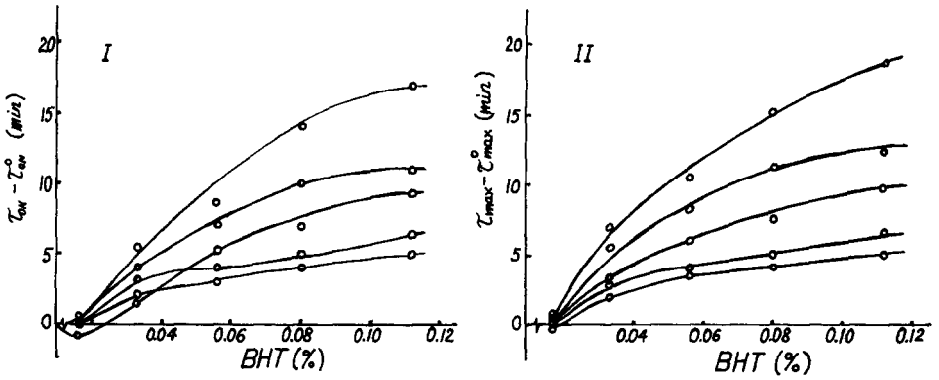

Fig. 7. Plots as in Fig. 6 for RSO + BHT; temperatures 135.5, 140.2, 145.5, 150.4 and 155.2°C, respectively from top to bottom.

phenolic-type antioxidants (e.g. BHA, tocopherols) can invert their action on lipids and act as the pro-oxidants. Therefore, in this work more concentrated model mixtures were deliberately also used. No inversion of the antioxidant action of BHA has been found up to 0.8% content, a concentration far above acceptable limits for inhibiting edible oils and fats.

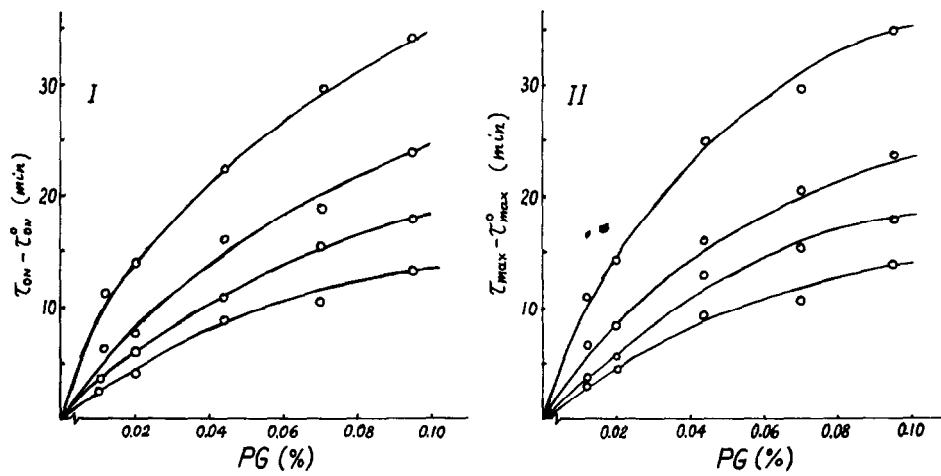

Fig. 8. Plots as in Fig. 6 for RSO + PG; temperatures 140.3, 145.8, 150.7 and 155.6°C, respectively from top to bottom.

CONCLUSION

PDSC was used successfully to investigate the oxidative stability and kinetic features of oxidation for uninhibited and inhibited samples of RSO. The method offers a simple and precise technique for such

investigations, with inherent advantages: milligram amounts of sample without its previous preparation and well defined measuring conditions. With the use of PDSC the assessment of antioxidant activities can also be directly performed. It has been mentioned on several occasions that the composition of vegetable oils can vary, in terms of both principal and trace components. Hence the results of any study cannot be generalized for a given kind of oil, but the assessment of oxidative stability by PDSC can be performed rapidly for any oil blend, with or without added antioxidants, providing data for kinetic analysis.

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