Journal of Thermal Analysis and Calorimetry, Vol. 88 (2007) 3, 781–785

DSC STUDY OF LINOLENIC ACID AUTOXIDATION INHIBITED BY BHT, DEHYDROZINGERONE AND OLIVETOL

Malgorzata Musialik and G. Litwinienko[*]

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

Non-isothermal oxidation of linolenic acid (LNA) in bulk phase was monitored by differential scanning calorimetry. The kinetic parameters E_a, Z and k (activation energies, pre-exponential factors, and rate constants, respectively) were calculated by Ozawa–Flynn–Wall method for the first detectable exothermic effect of uninhibited LNA oxidation. The kinetic parameters were also calculated for LNA oxidation inhibited by 2,6-di-*tert*-butyl-4-methylphenol (BHT), and two natural compounds, 1,3-dihydroxy-5-pentylbenzene (olivetol), and 4-(4'-hydroxy-3'-methoxyphenyl)-3-buten-2-one (DHZ, dehydrozingerone) at various concentrations.

For oxidation processes at 25, 90 and 180°C the plots of log$k$ values *vs*. concentration of phenolic compounds indicated that optimal concentration of inhibitor determined for one particular temperature cannot be extrapolated to other temperatures.

Keywords: *antioxidants, autoxidation, BHT, dehydrozingerone, DSC, kinetics, olivetol, thermal analysis*

Introduction

Autoxidation is a free radical mediated process which causes many unfavourable changes in lipid and food. In general, autoxidation processes can be distinguished into three groups of reactions. During the initiation small amount of primary free radicals is formed as an effect of thermolysis, photolysis or oxidation reduction reactions in the system. At presence of molecular oxygen the propagation step occurs, i.e. reactions of carbon centered lipid radicals $L^{\bullet}$ with oxygen (reaction (1)) followed by hydrogen atom abstraction from lipid molecules by peroxyl radicals (reaction (2)):

$$L^{\bullet}+O_2 \rightarrow LOO^{\bullet} \quad (1)$$

$$LOO^{\bullet}+LH \rightarrow LOOH+L^{\bullet} \quad (2)$$

These two reactions are repeated from several to thousands times, thus autoxidation is a chain reaction. During the third step, termination, the radicals are transformed into non-radical products in the recombination and disproportionation processes. At higher temperatures hydroperoxides and conjugated dienes (primary oxidation products) decompose rapidly to secondary autoxidation products like volatile aldehydes and ketones responsible for the odor of the frying fats.

Polyunsaturated fatty acids are important constituent of human diet. Unsaturated lipids are considerably more sensitive to oxidation than saturated fatty acids and during the last thirty years the studies of stability of fats and fat containing foods have gained great attention [1]. Thermal analysis as a valuable tool of lipid oxidation studies in bulk phase has been recently reviewed by one of us [2]. When the pressure of oxygen exceeds 13 kPa, the overall rate of lipid autoxidation (ν) obeys the first-order kinetics: $\nu=k_p(R_i/2k_t)^{1/2}[LH]=k[LH]$ where R_i is rate of initiation and k_p and k_t are the rate constants of reaction (2) and termination, respectively. Therefore, the overall rate constant (k) can be described by simple Arrhenius equation (3), where Z is pre-exponential factor, E_a is overall activation energy, R is gas constant and absolute temperature is denoted as T.

$$k=Z\exp(-E_a/RT) \quad (3)$$

In non-isothermal oxidation mode during a seria of measurements, temperature of DSC cell is programmed to increase with various linear heating rates β. For each β the oxidation starts at different initial temperature T_e (the higher β the higher T_e), thus the Ozawa–Flynn–Wall (OFW) method can be applied for determination of parameters E_a and Z from the relationship [3, 4]:

$$E_a = -2.19R(\mathrm{d}\log\beta / \mathrm{d}T_e^{-1}) \quad (4)$$

Temperatures of extrapolated start of the process are determined from several DSC curves and the plot of logβ values as a function of $1/T_e$ gives the straight line:

$$\log\beta=A/T_e+B \quad (5)$$

* Autor for correspondence: litwin@chem.uw.edu.pl

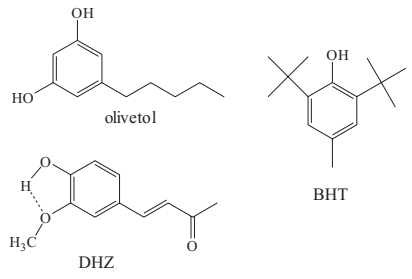
1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

with a slope $A = -0.4567E_a/R$ and reciprocal $B = -2.315 + \log(ZE_a/R)$. There are many advantages of the OFW method. Short time of measurement, simplicity of T_e determination, and the results in the form of kinetic parameters make DSC method competitive to other conventional methods of lipid oxidation analysis: oxidative stability index (OSI), Rancimat and Shaal oven method. These conventional methods are called accelerated tests where 'accelerated' means that the time of measurement is reduced from several months if the oxidation is carried out at room temperature to several days or hours for oils aged at temperatures higher than 100°C. It is worth to notify that both isothermal and non-isothermal thermoanalytical methods are also 'accelerated' because the measurements are performed at elevated temperatures, however in this article term 'accelerated' will be applied for conventional methods used in food and fats chemistry: Rancimat, OSI and Shaal tests.

The advantages and drawbacks of non-isothermal DSC methods have been widely described elsewhere [2, 5–8]. In this work we used OFW method to study the antioxidant activity of two natural compounds: 1,3-dihydroxy-5-pentylbenzene and 4-(4'-hydroxy-3'-methoxyphenyl)-3-buten-2-one. Scheme 1 shows the structures and abbreviations for these compounds as well as structure of synthetic antioxidant 2,6-di-*tert*-butyl-4-methylphenol, a model antioxidant used many times in our previous studies.

Olivetol is a resorcinoic phenol and can be found in olive oil [9, 10] and bran fractions of rye and other cereals [11]. Second natural compound, DHZ, is a constituent of ginger (*Zingiber officinale* rhizomes) [12] and as a metabolic product of curcumin shows antioxidant activity in solution [13–15].



Scheme 1 Structures of 1,3-dihydroxy-5-pentylbenzene (olivetol), 4-(4'-hydroxy-3'-methoxyphenyl)-3-buten-2-one (dehydrozingerone, DHZ) and 2,6-di-*tert*-butyl-4-methylphenol (BHT)

Experimental

Materials and methods

Linolenic acid (LNA,*cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid, 99%), 2,6-di-*tert*-butyl-4-methylphenol (BHT, 99%) and 1,3-dihydroxy-5-pentylbenzene (olivetol, 98%) were purchased from Sigma-Aldrich. Dehydrozingerone, i.e. 4-(4'-hydroxy-3'-methoxyphenyl)-3-buten-2-one) abbreviated as DHZ was prepared by condensation of 4-hydroxy-3-methoxybenzaldehyde with acetone according to method published by Elias and Rao [17]. The product was purified by recrystallization from water–ethanol mixture. Product purity was 99% (GC measurements).

All measurements were carried out using a DSC apparatus DuPont 910 differential scanning calorimeter with a DuPont 9900 thermal analyzer and normal pressure cell. An oxygen flow in calorimeter cell was 6 $dm^3\ h^{-1}$. Samples of compound (~5 mg) were heated from 50 to 350°C in an open pan at linear heating rates β=5–20 K min^{-1} and as a reference material an empty aluminum pan was applied. TA Instruments Software (General V4.01) was used for collecting the data and for the determination of temperatures of the extrapolated start of oxidation (T_e) from DSC oxidation curves. Typical DSC curves of LNA oxidation are presented on Fig. 1. The series of obtained T_e values (in K) were used to calculate the parameters A and B of Eq. (5), see inset in Fig. 2. Values of E_a and Z were calculated from A and B parameters, as described in 'Introduction'. The standard deviations of the slopes calculated for confidence level 90% have been used for error estimation ($\pm\Delta E_a$). The parameters E_a and Z were applied for calculation of rate constants of oxidation (k) at 25, 90 and 180°C from Eq. (3).

Results and discussion

As in our previous research focused on activity of phenolic chain-breaking antioxidants, we decided to use pure linolenic acid as a lipid matrix. Because of the presence of three double bonds in LNA, its oxidation starts at relatively low temperature in comparison with saturated fatty acids [18, 19] and clear exothermic effect of oxidation can be easily monitored by DSC. In our previous studies we carefully studied the LNA oxidation in these conditions in order to eliminate all possible misinterpretations of observed exothermic effects [2, 19, 20]. According to these studies, autoxidation processes are assigned to initial exothermal effects recorded on DSC curve (start of the process and first peak). Therefore, the parameter most sufficient for calculation of activation energy is temperature of extrapolated start of oxidation (T_e defined in Fig. 1), for which the constant conversion requirements are perfectly filled. Temperatures T_e and statistical parameters of Eq. (5) used for calculation of E_a and Z are collected in Table 1 (data shown for one series of measurements). Activation energy 68±8 kJ mol^{-1} obtained for LNA oxidation in

Table 1 Temperatures T_e determined from DSC curves of oxidation of LNA with various heating rates β. Parameters of Eq. (5), standard errors (σ), and errors calculated for confidence level 90% ($\sigma_{90\%}$). Activation energy (E_a in kJ mol^{-1}) and preexponential factor (Z in s^{-1}) were calculated from Eqs (4) and (5)

β/K min^{-1}	T_e/K	Statistical and kinetic parameters
5.0	374.3	A=–3.82
7.5	378.3	B=10.96
10.0	382.7	R^2=0.9829
12.0	388.0	σ=0.23
15.0	390.4	$\sigma_{90\%}$=0.43
17.0	393.4	E_a=69.6±7.8
20.0	396.6	log$Z$=9.35

this work (from three independent sets of measurements) is in excellent agreement with E_a's lying within the range 62–70 kJ mol^{-1} obtained in our previous works [5, 18, 19, 21], and is also in reasonable agreement with value 60±7 kJ mol^{-1} for isothermal LNA oxidation [6].

DSC curves obtained for oxidation of LNA inhibited by BHT, DHZ and olivetol are presented in Fig. 2 and, as for neat LNA oxidation, the increase of β causes the increase of T_e. Table 2 contains values of T_e obtained from measurements for various β and the same concentrations of olivetol and DHZ. Temperatures T_e were also dependent on phenolic compound concentration, however, as can be seen in Table 3 with parameters for LNA inhibited by BHT, for higher concentration the oxidative stability is decreased. Indeed, the kinetic parameters calculated in the same way as described for pure LNA (collected in Table 4) show the irregular tendencies for various concentrations of phenolic inhibitors. The increase of oxidative stability of the lipid with increasing

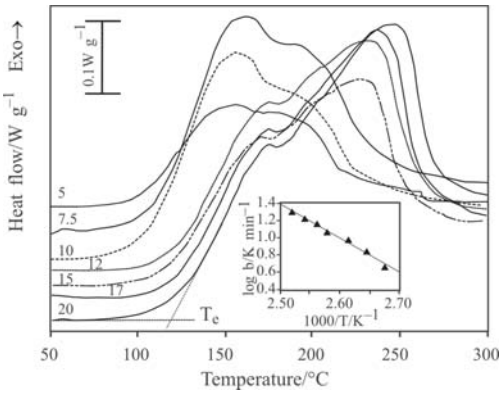


Fig. 1 DSC curves of non-isothermal oxidation pure linolenic acid obtained for various heating rates β (in K min^{-1}, indicated as numbers). For β=20 K min^{-1} the way of determination of extrapolated temperature of start of oxidation is shown. Inset presents the plot of logβ as a function of 1000/T for this seria of measurements. Parameters of the straight line are listed in Table 1

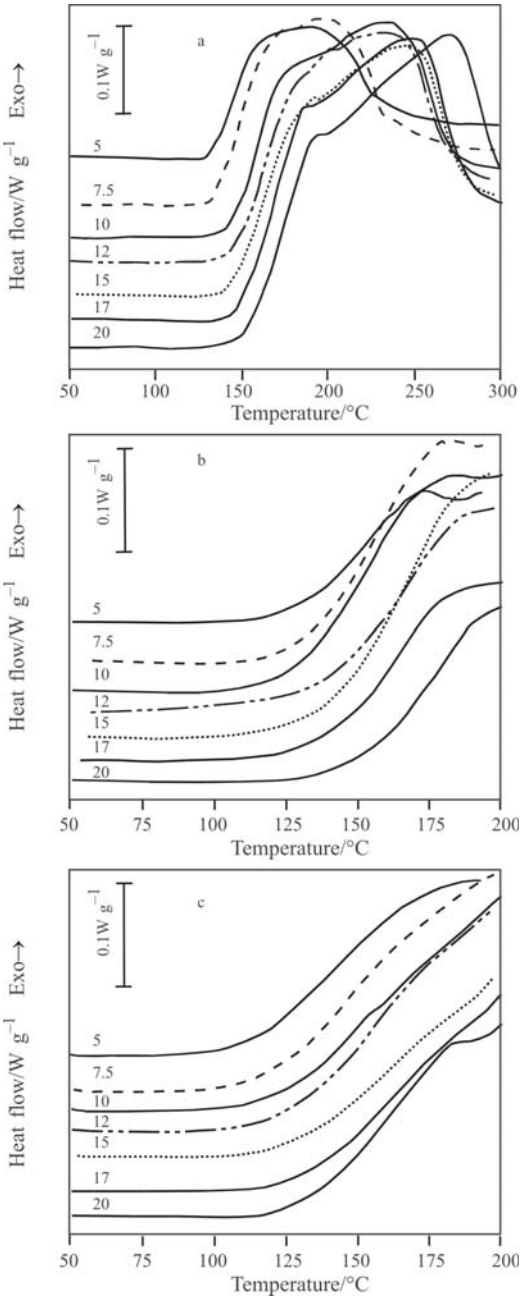


Fig. 2 DSC curves of non-isothermal oxidation of linolenic acid containing: a – 5.6 mmol BHT (mol of LNA)$^{-1}$; b – 5.4 mmol of DHZ (mol of LNA)$^{-1}$; c – 5.6 mmol OLIVETOL (mol of LNA)$^{-1}$. The numbers denote heating rates, β in K min^{-1}

concentration of inhibitor has been observed for all three compounds even for the smallest concentrations 1.1 mmol per mol of lipid. For model antioxidant, BHT the values of activation energy are in good agreement with previously reported ones [5]. The E_a values presented in Table 4 are the means of E_a's obtained from two or three measurements, for example, for E_a=82±11 kJ mol^{-1} for LNA oxidation inhibited by BHT (1.1 mmol (mol of lipid)$^{-1}$) is a mean of three values: 73.2, 92.9 and 79.0 kJ mol^{-1}.

Table 2 Temperatures T_e determined from DSC curves of oxidation of LNA containing DHZ (5.5 mol (mol of lipid)$^{-1}$) and olivetol (2.8 mol (mol of lipid)$^{-1}$)

β/K min^{-1}	T_e/K	
	DHZ	olivetol
5.0	399.8	379.5
7.5	401.2	384.9
10.0	405.2	387.9
12.0	407.4	388.8
15.0	408.8	390.1
17.0	411.5	393.9
20.0	414.5	395.2

Table 3 Comparison of T_e values for the linolenic acid oxidation inhibited by various concentrations of BHT (with the same β=5 K min^{-1})

C_{BHT}/mmol (mol of lipid)$^{-1}$	T_e/K
1.1	413.0
2.6	414.6
3.9	405.9
5.6	402.1
8.4	397.6
11.6	396.4

Among the three phenolic compounds used in the studies, the best antioxidative properties showed DHZ, in which hydroxyl group is internally hydrogen bonded to *ortho*-methoxyl group (Scheme 1). Such internal H-bond makes phenolic OH group not able to form intermolecular hydrogen bond with carboxyl group of lipid. The interactions between phenols and polar functional groups of lipids were observed even for strongly hindered phenols [22] and such interactions diminish the ability of phenols to scavenge the radicals [23]. However, the abstraction of hydrogen from internally H-bonded methoxyphenols occurs much easier than from intermolecular H-bonded phenols [24] and DHZ can be efficient radical scavenger. Moreover, the presence of double bond conjugated to aromatic ring brings additional stabilization of the radical formed after the H atom abstraction from DHZ molecule.

Olivetol, rezorcinoic compound found in olive oil exhibits good antioxidant activity at the concentrations 1–5.6 mmol (mol of lipid)$^{-1}$, depending on concentration. The maximal E_a value for LNA oxidation inhibited by that compound was 129 kJ mol^{-1}, being higher than E_a for LNA oxidation inhibited by resorcinol (107 kJ mol^{-1}) determined during our thermoanalytical study on antioxidant activity of dihydroxyphenols [25]. Since the only difference between these two compounds is pentyl group in position 5 of olivetol, the increase of that stability is assumed to be an effect of electron-donating feature of 5-alkyl chain. For concentration above 5 mmol (mol of lipid)$^{-1}$ the activation energy of autoxidation decreases from 126 to 86 kJ mol^{-1}. Though the radical formed from the resorcinoic compound cannot be stabilized as efficiently as in radicals formed from catechols [25], the good antioxidant activity of olivetol in comparison with BHT can be explained by the presence of two hydroxyl groups.

One of the main advantages of thermoanalytical methods is that the kinetic parameters E_a and Z resulting from at least five relatively short measurements (10–15 min each) can be used for calculation of overall rate constants of lipid autoxidation. Other conventional accelerated tests give induction time, τ_{ind}, i.e. time required to form volatile products of autoxidation during the heating of oil sample purged with oxygen during several hours. It is common practice that on the basis of this parameter the activity of studied compounds can be compared. Induction period length is relevant to kinetic parameters and it is possible to obtain Arrhenius like relationship [8, 26, 27] basing on several measurements done at various temperatures. However, such procedure is much more time consuming than OFW method.

Although the results of accelerated tests are comparable with results of thermal analysis [26, 27], the E_a and Z parameters can be easily used for calculation of k for extended range of temperatures, assuming the validity of the Arrhenius dependency. Despite the fact that extrapolation of the results is a crucial point of all accelerated tests and thermoanalytical methods [28], valuable information can be obtained.

Table 4 Values of activation energy (E_a, in kJ mol^{-1}) and pre-exponential factors (Z, in s^{-1}) obtained of linolenic acid oxidation inhibited by studied compounds at various concentrations (in mmol of phenol (mol of lipid)$^{-1}$)

BHT	E_a	log$Z$	DHZ	E_a	log$Z$	olivetol	E_a	log$Z$
1.2	82±11	10.32	1.1	88±15	11.60	1.1	129±17	17.49
2.6	80±13	10.09	2.8	187±34	24.50	2.8	107±15	14.39
3.9	70±23	8.75	3.8	180±42	21.30	3.9	84±18	10.91
5.6	85±20	10.54	4.6	118±6	15.24	4.5	129±30	16.93
8.4	141±93	17.64	5.5	118±25	15.13	5.6	86±20	11.43
11.6	99±22	12.13	8.2	132±28	16.54			

For example, such kind of calculation performed for LNA oxidation inhibited by BHT shows interesting phenomena. The plots of $k(T)$ or $\log k(T)$ *vs*. inhibitor concentration have different profiles for three temperatures: 25, 90 and 180°C (Fig. 3). The most striking observation is that for each from these three temperatures the different concentration of antioxidant can be chosen as optimal. At 25 and 90°C the maximal oxidative stability has LNA containing 3.9 mmol BHT (mol of lipid)$^{-1}$. That concentration of BHT is no longer optimal at temperature 180°C. It is clear evidence, that ranking of oxidative stabilities made on the basis of the induction times measured at high temperature measurements (accelerated tests are usually carried out at temperatures above 110°C) can be misleading and that the kinetic parameters will be more reliable.

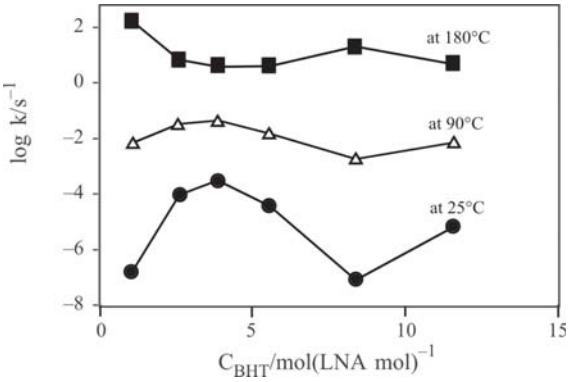


Fig. 3 Plot of the logarithm of the rate constants (log$k$) of autoxidation of linolenic acid *vs*. concentration of BHT at 25, 90 and 180°C

Conclusions

The Ozawa–Flynn–Wall method has been successfully used for calculations of Arrhenius parameters (E_a, Z and k) for non-isothermal autoxidation of linolenic acid inhibited by three phenols: BHT, olivetol and dehydrozingerone dissolved in the lipid at concentrations from 1 to 11 mmol (mol of lipid)$^{-1}$. Increase of phenol concentration caused increase of oxidative stability of the mixture, however, after optimal concentration of inhibitor a decrease of the antioxidant activity was observed. It has been demonstrated that the relative oxidative stabilities can be inverted when temperature is significantly increased or decreased, therefore the relative parameters like oxidation induction time cannot be extrapolated to temperatures other than temperature of accelerated test. In contrast to accelerated tests, DSC measurements of non-isothermal oxidation of lipids are useful tool for fast and simple determination of oxidative stability of fats and oils and for the measurements of antioxidant activity of chain-breaking antioxidants.

References

1 Food Lipids – Chemistry, Nutrition and Biotechnology, C. C. Akoh and D. B. Min, Eds, Marcel Dekker Inc., New York 1998.
2 G. Litwinienko, Analysis of lipid oxidation by Differential Scanning Calorimetry in Analysis of Lipid Oxidation, A. Kamal-Eldin and J. Pokorny, Eds, JAOCS Press, Champaign, IL, 2005, p. 152.
3 T. Ozawa, J. Thermal Anal., 2 (1970) 301.
4 J. H. Flynn and L. A. Wall, J. Polym. Sci. B, Polym. Lett., 4 (1966) 323.
5 M. Ulkowski, M. Musialik and G. Litwinienko, J. Agric. Food Chem., 53 (2005) 9073.
6 G. Litwinienko, J. Therm. Anal. Cal., 65 (2001) 639.
7 P. Šimon and L'. Kolman, J. Therm. Anal. Cal., 64 (2001) 813.
8 P. Šimon, J. Therm. Anal. Cal., 84 (2006) 263.
9 Y. Asahina and M. Yasue, Berichte Dtsch. Chem. Ges., 70 (1937) 206.
10 A. Kozubek and J. H. P. Tyman, Chem. Rev., 99 (1999) 1.
11 A. Kamal-Eldin, A. Pouru, C. Eliasson and P. Aman, J. Sci. Food Agric., 81 (2000) 353.
12 P.-C. Kuo, A. G. Damu, C.-Y. Cherng, Jye-Fu Jeng, C.-M. Teng, E.-J. Lee and T.-S. Wu, Arch. Pharm. Res., 28 (2005) 518.
13 D. V. Rajakumar and M. N. A. Rao, Biochem. Pharmacol., 46 (1993) 2067.
14 K. I. Priyadarsini, S. N. Guha and M. N. A. Rao, Free Rad. Biol. Med., 24 (1998) 933.
15 K. I. Priyadarsini, T. P. A. Devasagayam, M. N. A. Rao and S. N. Guha, Radiat. Phys. Chem., 54 (1999) 551.
16 G. Litwinienko and K. U. Ingold, J. Org. Chem., 69 (2004) 5888.
17 G. Elias and M.N.A. Rao, Eur. J. Med. Chem., 23 (1988) 379.
18 G. Litwinienko, A. Daniluk and T. Kasprzycka-Guttman, Ind. Eng. Chem. Res., 39 (2000) 7.
19 G. Litwinienko and T. Kasprzycka-Guttman, Ind. Eng. Chem. Res., 39 (2000) 13.
20 B. Kowalski, J. Thermal Anal., 34 (1988) 1321.
21 G. Litwinienko, J. Therm. Anal. Cal., 165 (2001) 639.
22 G. Litwinienko, E. Megiel and M. Wojnicz, Organic Lett., 4 (2002) 2425.
23 G. Litwinienko, T. Kasprzycka-Guttman and M. Studzinski, Thermochim. Acta, 307 (1997) 97.
24 M. I. de Heer, P. Mulder, H.-G. Korth, K. U. Ingold and J. Lusztyk, J. Am. Chem. Soc., 122 (2000) 2355.
25 G. Litwinienko, T. Kasprzycka-Guttman and D. Jamanek, Thermochim. Acta, 331 (1999) 79.
26 P. Šimon, L'. Kolman, I. Niklova and Š. Schmidt, J. Am. Oil Chem. Soc.,77 (2000) 639.
27 J. Polavka, J. Paligová, J. Cvengroš and P. Šimon, J. Am. Oil Chem. Soc., 82 (2005) 519.
28 L. Woo, A. R., Khare, C. L. Sandford, M. T. K. Ling and S. Y. Ding, J. Therm. Anal. Cal., 64 (2001) 539.

DOI: 10.1007/s10973-006-8507-0