

THE ASSESMENT OF OXIDATIVE STABILITY AND MELTING CHARACTERISTIC OF PALM OIL AND COCOA BUTTER

Ewa Ostrowska-Ligeża[✉], Diana Mańko-Jurkowska, Sandra Brozio, Magdalena Wirkowska-Wojdyła, Joanna Bryś, Rita Głowacka, Agata Górską

WULS-SGGW, Faculty of Food Sciences

Summary. Two kinds of fats were investigated in this study: palm oil and cocoa butter. Cocoa butter is one of the most precious and useful vegetable fat obtained from the cocoa beans. Palm oil is an edible vegetable oil high in saturated fats and free of trans fats. A lot of methods for determining oxidative stability are known. Differential Scanning Calorimetry is in the group of thermoanalytical methods and allows the determination of oxidation parameters without the need for chemicals substances. With the use of DSC, polythermal and isothermal research can be carried out. The aim of this research was to analyse the oxidative stability of cocoa butter and palm oil. The kinetics parameters: activation energy (E_a), pre-exponential factor Z were investigated with used differential scanning calorimetry (DSC). The initial temperature (onset) and maximum temperature (final) of oxidation process, and melting characteristic for both lipids were determined by DSC. The kinetics parameters, temperatures (onset and maximum) were investigated at oxygen atmosphere. The melting characteristic was investigated at nitrogen atmosphere. Calibration was done with indium standards. The averages from measurements of T_{on} and T_{max} for each lipid at a given temperature were determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the recorded exotherm. The range of temperatures during determine melting characteristic were -80 to 80°C . If the heating rate of the system was constant for the test conditions, then the temperatures obtained: T_{on} and T_{max} were characteristic of the system and could be used as parameters differentiating the resistance (stability) of fats and oils to thermal decomposition. Palm oil was characterized by a lower activation energy at onset temperature than cocoa butter, it may be due to the fact that palm oil was unrefined

Ostrowska-Ligeża Ewa 0000-0002-8387-8462; Mańko-Jurkowska Diana 0000-0002-1773-6255; Wirkowska-Wojdyła Magdalena 0000-0002-5297-6844; Bryś Joanna 0000-0002-7852-3624; Głowacka Rita 0000-0002-5793-1776; Górską Agata 0000-0002-7134-3719

[✉] ewa_ostrowska_ligeza@sggw.pl

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and had a greater amount of pollutions such as e.g. free fatty acids and incomplete triacylglycerols. The course of melting characteristic for cocoa butter and palm oil were different. The differences in the melting characteristic for the lipids tested can be attributed to their different composition of triacylglycerols.

Key words: palm oil, cocoa butter, melting, DSC, oxidation

INTRODUCTION

Cocoa butter is the fat which derived from raw cocoa beans (*Theobroma cacao* L. – *Sterculiaceae*) [Niewiadomski 1984]. The fat content of cocoa bean is 40–50%. Cocoa butter is obtained by pressing cocoa mass at hydraulic presses or by extraction. Cocoa butter consists of 98% triacylglycerols, 1% free fatty acids, 0.3–0.5% diacylglycerols and 0.1% monoacylglycerols. It also contains about 0.13–5% phospholipids, 150–250 mg·kg⁻¹ tocopherols, many volatile compounds and 0.2% phytosterols such as β -sitosterol, stigmasterol and campesterol [Niewiadomski 1984]. In cocoa butter, over 60% of fatty acids are saturated fatty acids, which make it stable. The high stability of cocoa butter is also attributed to the presence of natural antioxidants and small amounts of polyunsaturated fatty acids in the cocoa butter particle.

The plant from which palm oil originates is oily palm (*Elaeis guineensis*). Due to the low content of linolenic and linoleic acids, this oil is considered stable, even at high temperatures. Triacylglycerols composed with a few short-chain fatty acids aren't easily hydrolysed, which protects the fat from the formation of an unpleasant smell. The high content of oleic acid makes this oil resistant to oxidation. Palm oil consists mostly of saturated, monounsaturated fatty acids and natural antioxidants, for example α -tocopherol or carotenoids [Kowalska et al. 2012].

The lipid oxidation at low and high temperatures may go through different steps or reaction pathways, depending on the reactivity of metal ions and antioxidants at different temperatures, light and/or pH [Farhoosh et al. 2008]. Both the reaction environment and its conditions are difficult and sometimes impossible to define because these transformations are extremely complex [Drozdowski 2000].

Differential Scanning Calorimetry is in the group of thermoanalytical methods and allows the determination of oxidation parameters without the need for chemicals substances. With the use of DSC, polythermal and isothermal research can be carried out. In the former, the sample placed in the DSC apparatus, surrounded by gas, is heated in a linearly programmed way of increasing temperature. The main parameters are the onset of oxidation or / and the maximum oxidation temperature. The record of flowing heat is created in a continuous way, which makes it possible to determine the line of thermal changes occurring in the tested sample [Ostrowska-Ligeza et al. 2009]. In addition, the advantages of the DSC method are the simplicity of measurement and the ability to quickly carry out the measurement without having to pre-treat the fat being tested [Micić et al. 2015].

The purpose of the present study was to determine oxidation stability of selected lipids using differential scanning calorimetry.

MATERIALS AND METHODS

Two fats were subjected to thermal analysis: palm oil and cocoa butter. Deodorized cocoa butter was purchased at the SweetGryf Wioletta Janicka from Szczecin. Unrefined palm oil was purchased at the Cuisines of the World store in Warsaw.

The calorimetric measurements were performed with a Q200 DSC (TA Instruments, New Castle, DE, USA). Oxygen was used as the purge gas at a rate of 50 ml/min. The instrument was calibrated for temperature and enthalpy with high purity indium according to the procedure for standard DSC. A normal-pressure DSC cell was used. Fat samples of 3–4 mg were placed in aluminium sample pans and inserted into the heating chamber of the DSC cell. The aluminium reference pan was left empty. Samples of compounds were heated in an open aluminium pan with linear heating rates of 2.5, 4, 6, 7.5, 10, 12.5 and 15°C·min⁻¹. For each programmed heating rate (β , °C·min⁻¹), at least triplicate determinations were carried out. Each run was recorded on the instrument's computer disc. When the run was completed, the onset and maximum oxidation temperature (T_{on} , T_{max} °C) were determined as the intersection of the extrapolated baseline and the tangent line (leading edge) and maximum temperature peak of the recorded exotherm.

The method DSC measurements of melting characteristics were carried out with the same equipment. Samples of 3–4 mg were placed into aluminium pans with a lid and were hermetically sealed. An empty sealed aluminium pan was used as a reference and the experiments were performed under a nitrogen flow rate of 50 ml·min⁻¹, normal pressure. Melted samples were heated to 80°C and held for 10 min, in order to melt all the crystals and to erase the thermal memory. The samples were then cooled to -80 °C at 10°C min⁻¹ and maintained at -80°C for 30 min. Then the melting (so-called second fusion) profiles were obtained by heating the samples to 80°C at a heating rate of 15°C·min⁻¹. Integration onset and maximum temperature measurements were performed using the functions of the Universal Analysis Software (TA Instruments) [Wirkowska et al. 2012].

Statistical Analysis

The data were reported as the means \pm standard deviation. Two-way ANOVA was performed using the Statgraphics Plus for Windows, version 4.1 (Statistical Graphics Corporation, Warrenton, VA, USA). Differences were considered to be significant at a p value of 0.05, according to Tukey's multiple range test. The experimental design was carried out with three replications.

RESULTS

A computer-coupled apparatus examined the DSC curves of each sample that was analyzed. The obtained T_{on} and T_{max} values for palm oil and cocoa butter, which were obtained for seven heating rates are presented in Table 1.

If the heating rate of the system was constant for the test conditions, then the temperatures obtained: T_{on} and T_{max} were characteristic for the system and could be used as

Table 1. DSC dates T_{on} and T_{max} obtained for palm oil and cocoa butterTabela 1. Dane DSC T_{on} i T_{max} otrzymane dla oleju palmowego i tłuszczu kakaowego

Heating rate [K·min ⁻¹] Szybkość ogrzewania	T_{on} [°C] palm oil olej palmowy	T_{max} [°C] palm oil olej palmowy	T_{on} [°C] cocoa butter tłuszcz kakaowy	T_{max} [°C] cocoa butter tłuszcz kakaowy
2.5	176.95 ±0.11	237.53 ±0.38	195.94 ±0.23	234.77 ±0.01
4	188.19 ±0.06	242.07 ±0.79	198.67 ±0.11	245.97 ±1.09
5	191.59 ±0.84	246.77 ±0.93	202.17 ±0.57	252.10 ±1.52
7.5	193.96 ±0.05	262.26 ±0.06	208.56 ±0.14	255.11 ±0.14
10	199.35 ±0.60	266.12 ±0.74	212.02 ±0.35	261.49 ±0.35
12.5	204.96 ±0.71	269.61 ±0.69	213.43 ±0.49	270.64 ±0.49
15	207.65 ±0.40	276.08 ±0.60	217.35 ±0.26	271.48 ±1.16

Values represent means ±standard deviations. Different letters indicate that the samples are considered significantly different at the 5% level ($p < 0.05$)

parameters differentiating the resistance (stability) of fats and oils to thermal decomposition. This stability increased with increasing onset temperature and maximum oxidation temperature. Determination of the T_{on} value can be an accelerated test, which can replace the determination of acid, anisidine or peroxide values [Górska et al. 2011].

In the thermal DSC analysis, results showing the influence of heating rate of the samples on the T_{on} and T_{max} values were obtained. Both the value of the start temperature of the oxidation process and the value of the maximum temperature of oxidation increased with increasing heating rate and were at a high level. The results obtained for both tested fats were similar. Cocoa butter was characterized by higher T_{on} values, which may be due to the composition of fatty acids, i.e. a higher proportion of saturated fatty acids and much lower polyunsaturated fatty acids. The susceptibility of fatty acids to oxidation depends mainly on their unsaturation degree [Sun et al. 2018]. The reduction of oxidative stability of palm oil may be affected by the presence of free fatty acids, monoacylglycerols and diacylglycerols released during hydrolysis [Wirkowska et al. 2008]. The oxidative stability of palm oil and cocoa butter could be positively affected by the presence of carotenes and tocopherols with antioxidant activity, and negatively by the presence of free fatty acids and incomplete triacylglycerols [Małecka 1995, Szajdek and Borowska 2004].

The experimental onset and maximum temperature (T_{on} and T_{max}) obtained at seven heating rates are shown in table 1. The T_{on} and T_{max} experimental values as a function of heating rates (β) were recalculated on onset and maximum temperatures (T_{on} and T_{max}). Putting values of T_{on} or T_{max} into the equation 1:

$$\text{Log } \beta = a(1 \cdot T_{on}^{-1} \text{ lub } T_{max}) + b \quad (1)$$

where: a and b are adjustable coefficients, β – heating rate.

The regression analyses of the data are listed in Table 2. The kinetic parameters of the oxidation process were calculated based on the assumption that the amount of heat released in the heat mode at a given time is proportional to the number of reagents present. For established initiation conditions, fat oxidation reactions occurring in excess of oxygen were first-order reactions [Flynn and Wall 1966, Ozawa 1970, Ostrowska-Ligęza et al. 2009]. If the maximum temperature increases linearly like the heat coefficient, then the Ozawa–Flynn–Wall method can be used to determine the activation energy E_a and the pre-exponential factor Z [Flynn and Wall 1966, Ozawa 1970, Ostrowska-Ligęza et al. 2009]. By using the transformation of constant temperatures (T_{\max} maximum, T_{on} onset), determined using the DSC method, the approximate value of the activation energy is calculated according to equation 2:

$$E_a = -2,19R \frac{d \log \beta}{d(1/T)} \quad (2)$$

where: R is the gas constant and T the temperature (K).

The value of the pre-exponential factor Z from the Arrhenius equation was calculated from the dependence 3:

$$Z = \frac{\beta E_a e^{\frac{E_a}{RT}}}{RT^2} \quad (3)$$

The kinetic parameters for the palm oil and cocoa butter are presented in Table 2.

Table 2. Regression analysis of the DSC data, activation energies (E_a), pre-exponential factors (Z) at different temperatures (T_{on} and T_{\max})

Tabela 2. Kinetyczne i statystyczne parametry charakteryzujące utleniający rozkład tłuszczu kakaowego i oleju palmowego

Parameter Parametr	Value calculated from T_{on} Wartość liczona z T_{on}		Value calculated from T_{\max}	
	Palm oil Olej palmowy	Cocoa butter Tłuszcz kakaowy	Palm oil Olej palmowy	Cocoa butter Tłuszcz kakaowy
a	-5737.2 ± 0.96	-7960.9 ± 0.59	-5204.9 ± 0.78	-5832.2 ± 0.74
b	13.104 ± 0.27	17.42 ± 0.19	10.656 ± 0.21	11.86 ± 0.11
R ²	0.97	0.98	0.97	0.97
E_a [kJ · mol ⁻¹]	104.45 ± 0.81 ^a	140.93 ± 0.66 ^b	94.76 ± 0.67 ^A	106.18 ± 0.72 ^B
Z	2.09×10^{11}	3.15×10^{15}	8.21×10^8	1.18×10^{10}

Values represent means ± standard deviations. Different letters indicate that the samples are considered significantly different at the 5% level ($p < 0.05$).

The values of activation energy (E_a) calculated for the onset (T_{on}) temperature of cocoa butter and palm oil are higher than for the maximum oxidation temperature (T_{max}). Pre-exponential factors (Z) also showed this regularity. This may be due to the auto-oxidation process, in which the first oxidation products begin to form at the initiation and propagation stages, which is associated with high energy expenditure. Significant differences were observed between the values of E_a for the palm oil and cocoa butter. Comparing the activation energy of cocoa fat: 140.93 kJ·mol⁻¹ (for T_{on}), 106.18 kJ·mol⁻¹ (for T_{max}) and palm oil: 104.45 kJ·mol⁻¹ and 94.76 kJ·mol⁻¹ with activation energy: for mustard oil – 90.6 kJ·mol⁻¹ and 88.5 kJ·mol⁻¹, respectively for T_{on} and T_{max} [Litwinienko and Kasprzyska-Guttman 1998], it was noticed that the values obtained in this article were much higher.

This was probably due to greater stability of cocoa butter and palm oil. The calculated activation energy values (E_a) when reaching the maximum temperature (T_{max}) of palm oil are lower than when obtaining the onset temperature (T_{on}). A similar tendency was shown for the calculated pre-exponential factor Z . The results can be due to the auto-oxidation process, where during the stages of reaction: initiation and propagation, the first oxidation products begin to form and a large energy input was needed. Qi et al. [2016] assessed the effect of vegetable oils composition on their oxidation process. They studied the following samples: palm, corn, sesame, soy, sunflower, grape, safflower and olive oils. They analyzed onset and maximum temperatures at the heating rate of 5, 7, 10, 12.5 and 15 K·min⁻¹. The oxidation onset temperatures at the used heating rates for palm oil in this study were 212.3°C, 216.4°C, 221.5°C, 224.1°C and 227.8°C, respectively, and the maximum temperatures of oxidation process: 337.1°C, 348.8°C, 354.1°C, 368.3°C and 372.7°C. The obtained onset and maximum temperature values for oxidation process were much higher than those obtained in this study (table 2). Qi et al. [2016] also determined activation energy values for tested vegetable oils. The activation energy calculated for the palm oil tested was for the onset temperature 134.7 kJ·mol⁻¹, and for the maximum temperature 91.88 kJ·mol⁻¹. In this study, the activation energy for onset temperature is lower than determined in the study of Qi et al. [2016]. For the maximum temperature of the oxidation process, the E_a value is higher than determined in Qi et al. [2016] analyzed. Differences in the E_a parameter values can be caused by the following factors: soil and climate conditions of the harvesting area, palm species, parts of the plant used to obtain oil (oil from the pulp or seeds of oil palm), as well as processing methods (refining) [Albuquerque et al. 2019]. The obtained results confirm that the activation energy value is not a sufficient measurement to assess the oxidative stability of fat.

Melting curve of palm oil is presented in Figure 1. The first, endothermic peak with the distinct course, was observed at maximum temperature about 3.46°C. It can be due to the presence of the significant content of long-chain fatty acids in palm oil. The second recorded melting temperature was about 7.47°C. The third highest melting temperature about 25.23°C indicated the share of long-chain saturated fatty acids in palm oil. The melting point of fats decreases with decreasing chain length and increasing the degree of unsaturation of fatty acids [Smiddy et al. 2012]. Pardaul et al. [2011] studied vegetable oils from the Amazon region. They found that there were correlations between the oil onset temperature values obtained and the values obtained from the Rancimat test. Arain et al. [2009] compared the oxidative stability of oil from *Bauhinia pupurea* seeds, oils from

rice and cotton seeds. The authors stated that the obtained onset temperature values are correlated with the values obtained by the OSI method (oxidative stability index).

Melting curve of deodorized cocoa butter is presented in Figure 2. The melting curve of cocoa butter was characterized by endothermic peaks. The first endothermic peak for deodorized cocoa butter occurred at temperature about 18.46°C, the second peak at temperature about 20.54°C. The appearance of the first peak may be due to the melting of the I crystalline form of cocoa butter (melting range is 16–18°C), and the second peak with distinct course indicated the melting of the II crystalline form (melting range 22–24°C) [Beckett 2000].

The presence of double or several peaks in the melting curves were a common result in the study of chocolate polymorphic structures [Roy et al. 2007]. The appearance of

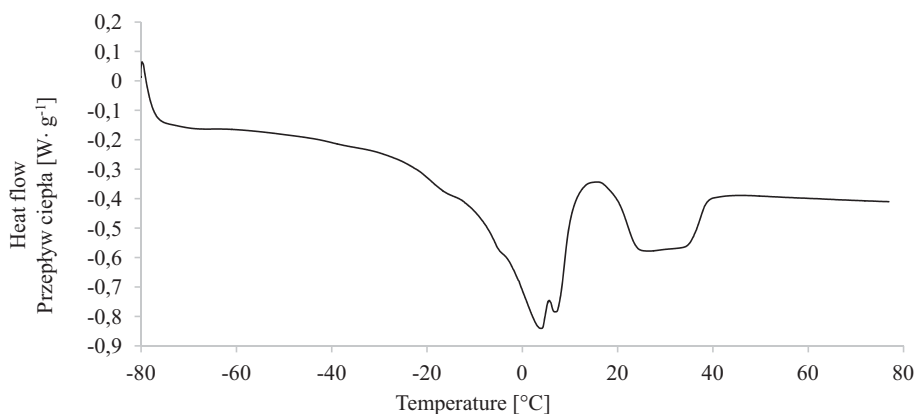


Fig. 1. Melting curve of palm oil

Rys. 1. Krzywa mięknięcia oleju palmowego

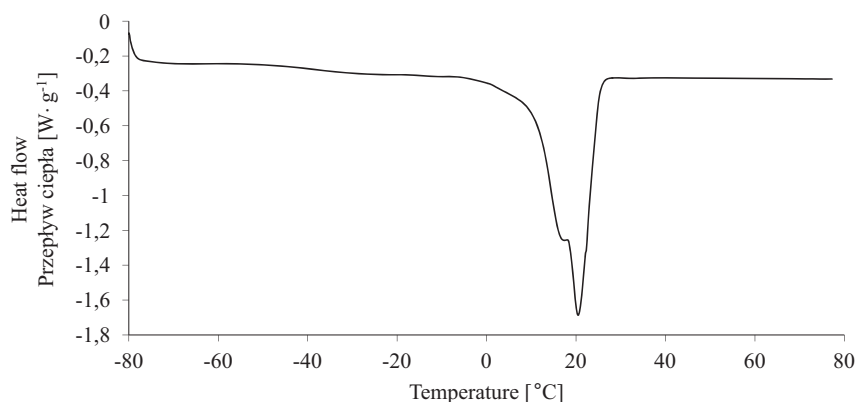


Fig. 2. Melting curve of cocoa butter

Rys. 2. Krzywa mięknięcia tłuszczu kakaowego

two or more peaks were attributed to the existence of different crystal structures within the same product, which indicated the polymorphism structures of fat [Pan et al. 2007]. A lot of researches confirm that determining the oxidative stability of fats by differential scanning calorimetry is effective. It was found that the differential scanning calorimetry test can be used in quality control as an analysis of vegetable oils and other fats [Arain et al. 2009, Pardauil et al. 2011].

CONCLUSION

Activation energy, pre-exponential factor Z for onset and maximum temperatures for cocoa butter were characterized by high values. Palm oil was characterized by a lower activation energy at onset temperature than cocoa butter, it may be due to the fact that palm oil was unrefined and had a greater amount of substances such as e.g. free fatty acids and incomplete triacylglycerols. Palm oil content polyunsaturated fatty acids, so the lower energy was needed for oxidation. Unstable polyunsaturated bonds oxidized at first step, followed by the oxidation of monounsaturated bonds, which required higher activation energy. The melting characteristics determined for both fats stated that the analysed palm oil was richer in long-chain fatty acids than cocoa butter. The differences in the melting profiles for the lipids tested can be attributed to their different composition of triacylglycerols. The DSC method allows to quickly characterize the oxidative stability of the research fat.

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OCENA STABILNOŚCI OKSYDACYJNEJ I CHARAKTERYSTYKI MIĘKNIĘCIA OLEJU PALMOWEGO ORAZ TŁUSZCZU KAKAOWEGO

Streszczenie. W pracy badaniom podano dwa rodzaje tłuszczów – tłuszcz kakaowy i olej palmowy. Technika różnicowej kalorymetrii skaningowej (DSC) należy do metod termooanalitycznych, które umożliwiają oznaczenie parametrów utleniania. Celem niniejszej pracy była analiza termiczna tłuszczu kakaowego i oleju palmowego, wyznaczenie parametrów termokinetycznych oraz określenie charakterystyk mięknienia. Korzystając z dynamicznej

opcji pracy aparatu DSC, przeprowadzono badania, stosując następujące szybkości ogrzewania próbek: 2,5; 4; 5; 7,5; 10; 12,5; 15 K·min⁻¹. Posługując się metodą Ozawy-Flynn-Wall'a, wyznaczono wartość energii aktywacji E_a oraz współczynnika przedpotęgowego Z . Olej palmowy i tłuszcz kakaowy charakteryzują się wysoką stabilnością oksydacyjną. Tłuszcz kakaowy charakteryzował się wyższymi wartościami energii aktywacji niż olej palmowy. Stabilność oksydacyjna tłuszczu kakaowego była wyższa niż oleju palmowego.

Słowa kluczowe: olej palmowy, masło kakaowe, mięknięcie, DSC, utlenianie