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## Article

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*Reference:* Shamilova, Maltam/Hajiyeva, Sevinj (2021). Analysis of DSC (differential scanning calorimetry) thermograms of milk fat. In: Technology audit and production reserves 5 (3/61), S. 36 - 39.

<http://journals.uran.ua/tarp/article/download/242804/240849/557454>.

doi:10.15587/2706-5448.2021.242804.

This Version is available at:

<http://hdl.handle.net/11159/7210>

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## ANALYSIS OF DSC (DIFFERENTIAL SCANNING CALORIMETRY) THERMOGRAMS OF MILK FAT

The object of current research is the oxidation and melting properties of milk fat samples in different heating rates. One of the most problematic issues is the evaluation dependence of temperature and oxidation time regarding to heat flow, and the estimation of attitude of enthalpy values to heating rates. In order to gain a comprehensive assessment of oxidation and melting properties of milk fat samples on differential scanning calorimeter in various heating rates, it is necessary to conduct experimental studies.

The analysis was performed using the dynamic option of the differential scanning calorimetry (DSC) with the following sample heating rates: 2.5, 4, 5, 7.5, 10, 12.5, 15 °C·min<sup>-1</sup>. Analyses were performed on 14 samples of milk fat, thus, for each heating rate were intended to two milk fat samples.

As a result of the analysis, in the proper heating rates increased, it was found, that the oxidation properties of milk fat depend on the heating rates on DSC examination. In the thermal DSC analysis, the start temperature ( $T_s$ ) (inlet), the onset temperature ( $T_{on}$ ), and the maximum heat flow-peak temperatures ( $T_p$ ) of oxidation were rising gradually. All the value of oxidation increased gradually with increasing heating rate, only in the  $T_{end}$  values were chainable among all heating rates. However, the oxidation time of milk fat is inversely proportional to the various heating rates in DSC. The oxidation enthalpy was calculated according to the heating rates too. The masses of the samples differ from each other, albeit slightly, which the individuality in the value of enthalpy could be explained through this ratio and duration of exothermic. The melting point considers the important indicator to explain the purity of samples. Melting curves of extracted milk fat samples on DSC were characterized by endothermic behavior and observed with the mild peaks, the first and the second distinct peaks due to the low-melting triacylglycerols (with high unsaturated fatty acids content) and high-melting fats, which present in milk fat.

In concluded results, the characteristics of DSC oxidation curves are melting point due to the chemical structure of the fatty acids which milk fat samples contain.

**Keywords:** milk fat, heating rates, oxidation properties, melting point, differential scanning calorimetry (DSC).

Received date: 12.05.2021

Accepted date: 19.06.2021

Published date: 20.10.2021

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### How to cite

Shamilowa, M., Hajiyeva, S. (2021). Analysis of DSC (differential scanning calorimetry) thermograms of milk fat. *Technology Audit and Production Reserves*, 5 (3 (61)), 36–39. doi: <http://doi.org/10.15587/2706-5448.2021.242804>

### 1. Introduction

Milk fat contains over 400 different fatty acids, including saturated with a wide range of carbon chain length, unsaturated, trans, branched, and even cyclic fatty acids [1, 2]. Milk fat is one of the most complex fats in nature and varies from animal to animal [3–5]. These 3 groups of milk fat triacylglycerols (TG) are called the low melting point (LMP), medium melting point (MMP), and high melting point (HMP) fractions [6]. One of the thermos analytical method is Differential Scanning Calorimetry, which allows the determination of oxidation parameters without the need for analyzing chemical substance content [7].

The thermal properties of milk fat are usually studied by differential scanning calorimetry (DSC). Differential scanning calorimetry is a useful tool for determining the temperature of final melting and initial crystallization of fat, as well as for following polymorphic evolutions with

these 3 groups of milk fat triacylglycerols (TG), which are called the low melting point (LMP), medium melting point (MMP), and high melting point (HMP) fractions. Milk fat is considered polymorphic due to its structure and may crystallize taking three different polymorphic forms:  $\gamma$ ,  $\alpha$ , and  $\beta'$ . The most stable form of the milk fat is a form of  $\beta'$  and the least form  $\gamma$  [8–10].

As well as the use of DSC to analyze lipid oxidation is a reliable, simple and convenient technique. It provides qualitative and quantitative information and offers unique advantages, such as the small amount of sample use, short test time, and good reproducibility. DSC is widely used as an analytical, diagnostic and research tool, from which relevant information, such as the start temperature ( $T_s$ ) (inlet), the onset temperature ( $T_{on}$ ), maximum heat flow-peak maximum temperatures ( $T_p$ ) and  $T_{end}$  of oxidation, height, shape and position of peaks are obtained and used for subsequent kinetic calculations [11].

Moreover, the oxidation induction time, obtained from DSC measurements, can be used as parameters for the assessment of the resistance of tested fats to its thermal oxidative decomposition [12].

Thus, *the object of research* is the oxidation and melting properties of milk fat samples in different heating rates.

*The aim of current research* is to evaluate the dependence of temperature and oxidation time with regard to heat flow, and the attitude of enthalpy values to heating rates.

## 2. Methods of research

The research material consisted of 14 milk fat samples, which were provided by Department of Chemistry of Warsaw University of Life Sciences. The samples were stored at  $-20\text{ }^{\circ}\text{C}$  until they were analyzed.

The methodology, described in paper [7], was used to conduct the study. 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. Samples of compounds were heated in an aluminum pan with linear heating rates of 2.5, 4, 5, 7.5, 10, 12.5 and  $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ .

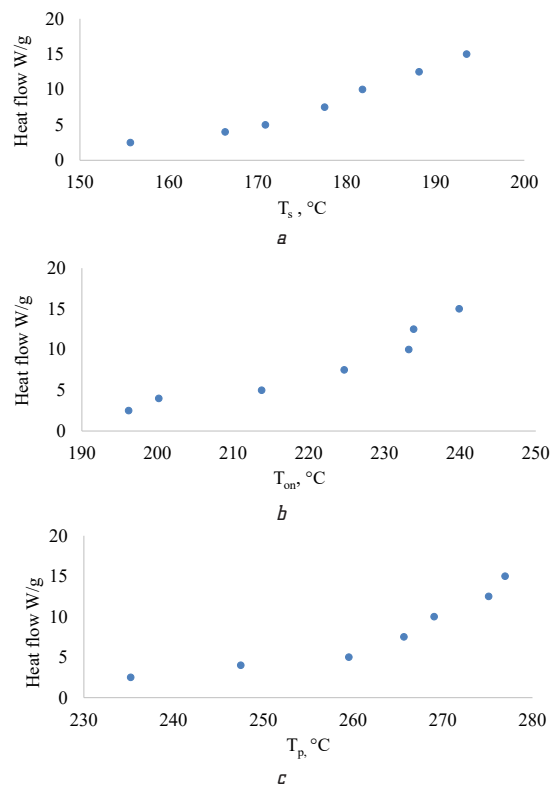
Milk chocolate samples were ground separately before extraction. Fats were extracted according to the procedure, described by [13, 14]. The experiments were performed under a nitrogen flow rate of  $50\text{ ml}\cdot\text{min}^{-1}$ . Integration, the start temperature ( $T_s$ ) (inlet), the onset temperature ( $T_{on}$ ), the maximum heat flow- peak maximum temperatures ( $T_p$ ) and  $T_{end}$  of oxidation temperature measurements were performed using the functions of the Universal Analysis Software (TA Instruments) [15].

There were used the values of the DSC curves of 7 samples among 14 samples, and discussion, carried out according to the oxidation temperatures, enthalpies, the dependence of the oxidation continuity on the heat flux and melting characteristics.

## 3. Research results and discussion

A computer-coupled apparatus examined the DSC curves of each sample that was analyzed. The obtained  $T_s$ ,  $T_{on}$ , and  $T_p$  values for milk fat samples, which were measured for seven heating rates, are presented in Fig. 1.

If the heating rate of the system was constant for the test conditions, then the temperatures obtained:  $T_s$ ,  $T_{on}$ , and  $T_p$  were characteristic for the system and could be used as parameters differentiating the resistance (stability) of fats and oils to thermal decomposition [7]. In the thermal DSC analysis, the influence of heating rate of the samples on the  $T_s$ ,  $T_{on}$ , and  $T_p$  values were observed. All the values of oxidation increased with increasing heating rate, only in the  $T_{end}$  values were chainable among all heating rates. In the value  $10\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  and  $12.5\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ , the linear motion was observed with deviation on the dependence of  $T_s$  (Fig. 1, a),  $T_{on}$  (Fig. 1, b), and  $T_p$  (Fig. 1, c) values of DSC curves of milk fat samples to the heating rates. According to [7] due to the composition of fatty acids, a higher proportion of saturated fatty acids and much lower polyunsaturated fatty acids cocoa butter was characterized by higher  $T_s$  and  $T_{on}$  values in respect of palm oil and were expressed equal  $T_s$  and  $T_{on}$  with regard to milk fat.  $T_p$  temperature were relative to each other with close values. The susceptibility of fatty acids to oxidation depends mainly on their unsaturation degree [16].



**Fig. 1.** The dependence of temperature values of DSC curves of milk fat samples from the heating rates of 2.5, 4, 5, 7.5, 10, 12.5 and  $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ : a –  $T_s$ ; b –  $T_{on}$ ; c –  $T_p$

In general, the  $T_s$  was determined between  $\sim 155\text{--}193\text{ }^{\circ}\text{C}$ , the  $T_{on}$  was considered between  $\sim 196\text{--}239\text{ }^{\circ}\text{C}$ , the  $T_p$  was mentioned  $\sim 235\text{--}246\text{ }^{\circ}\text{C}$  at 2.5, 4, 5, 7.5, 10, 12.5 and  $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ , respectively. According to [15], the reduction of oxidative stability of palm oil may be affected by the presence of free fatty acids, monoacylglycerols and diacylglycerols, released during hydrolysis. Thus, as proper heating rates increased,  $T_s$ ,  $T_{on}$ , and  $T_p$  of oxidation were rising gradually.

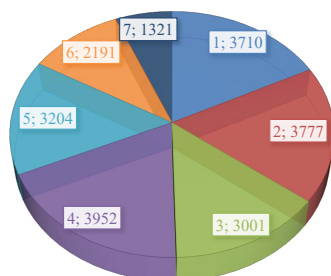
The enthalpy of the exothermic processes was determined in the current study. The oxidation enthalpy was calculated according to the heating rates (Fig. 2). The masses of the samples differ from each other, albeit slightly, which the individuality in the value of enthalpy could be explained through this ratio. Taken into account that the weights of the tested samples are not in the stabile, it could be considerate the current dependence are conditionally related to the weights of the samples studied and duration of exothermic.

In same studies, the higher heating rate, such as 5, 10 and  $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ , should not necessarily cause an increase in enthalpy [17], the right value for the heating rate must be chosen by considering the enthalpy of reaction [18].

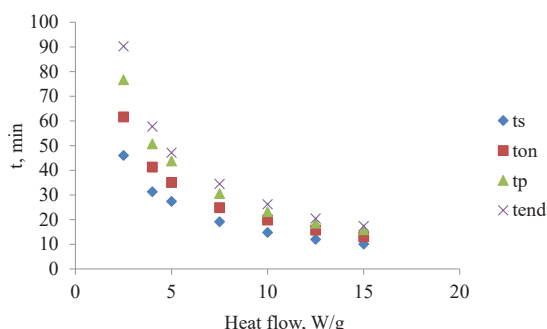
Moreover, according to the terms between heat and temperature, there are related concepts, but their units are completely different. In the current system the average kinetic energy of the atoms or molecules is estimated by temperature. The heating rate is slowed down during endothermic reactions, accelerated during exothermic reactions [19].

The dependence of heating rate to time was directly proportional in all values (Fig. 3). The curves explain the dependence on the start/onset/peak/end of oxidation time and duration of oxidation to the heat flow, which oxidation begins and ends quickly at high heat rate. Thus, on the low value of heating rate, the start oxidation time was

observed at 46<sup>th</sup> minute, the onset oxidation time was accepted at 61<sup>th</sup> minute, the peak oxidation time was culminated at 76<sup>th</sup> minute, and the end oxidation time was considered at 90<sup>th</sup> minute. However, on the high value of heating rate, the start/onset/peak/end of oxidation time determined at 10<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>, 17<sup>th</sup> minutes, respectively. These regularities were observed for the rest heating rates.



**Fig. 2.** The enthalpy values of DSC curves of milk fat samples heating rates of: 1 – 2.5 °C·min<sup>-1</sup>; 2 – 4 °C·min<sup>-1</sup>; 3 – 5 °C·min<sup>-1</sup>; 4 – 7.5 °C·min<sup>-1</sup>; 5 – 10 °C·min<sup>-1</sup>; 6 – 12.5 °C·min<sup>-1</sup>; 7 – 15 °C·min<sup>-1</sup>



**Fig. 3.** The dependence of heating rate to  $t_{start}$ ,  $t_{onset}$ ,  $t_{peak}$ ,  $t_{end}$  time oxidation time of DSC curves of milk fat samples in 2.5, 4, 5, 7.5, 10, 12.5 and 15 °C·min<sup>-1</sup>

A lot of researches confirm that determining the oxidative stability of fats by differential scanning calorimetry is the effective method, used to in quality control as an analysis of vegetable oils and other fats [20–22].

Although oxidation temperature is an intensive property, the melting point considers the important indicator to explain the purity of samples. Melting curves of extracted milk fat samples on DSC were characterized by endothermic behavior. The first, mild peak was observed at temperature nearly – 11.66 °C, due to the low-melting triacylglycerols (with high unsaturated fatty acids content), the second distinct peak was observed at temperature ~16.5 °C, related to the presence of the low-melting triacylglycerols content of milk fat, which has a lower melting point than cocoa butter and the third at ~30.99 °C, expected of high-melting fats, which present in milk fat. The appearance of two or more peaks were attributed to the existence of different crystal structures within the same product, which indicated the polymorphism structures of fat [23], and the presence of double or several peaks in the melting curves were a common result in the study of chocolate polymorphic structures [24].

According to [21], the saturated and unsaturated fatty acids content in fat affects its melting characteristics. Thus, the DSC curves of milk fat and fats, extracted from milk chocolates, were characterized by endothermic peaks, and the mild peaks were observed at –76.13 to –63.99 °C,

and –9.35 °C, the second endothermic peaks at 26.05 °C and 17.83 °C, the third endothermic peaks at 39.41 °C and 31.40 °C, respectively [22]. Moreover, the first distinct endothermic peak of milk fat was at 11.48 °C. According to [25], who studied the properties of milk fat, the distinct peak on melting curves was present at temperature of 14 °C and due to its polymorphism [10].

It should be noted, that this study has certain limitations, those, the weights of the tested samples are not specified within the same quantity, due to this fact, the current dependences are conditionally related to the weights of the samples studied. The development of this work is a mathematical calculation the dependence of heat flow regard to temperature, oxidation time and enthalpy with the application of a theoretical approach under conditions of minimal impact of the masses of research samples for other types of chocolate.

## 4. Conclusions

In the concluded results ( $T_s$ ) (inlet), the onset temperature ( $T_{on}$ ), the maximum heat flow – peak maximum temperatures ( $T_p$ ) and  $T_{end}$  of oxidation were characterized by heating rates values. The  $T_s$ ,  $T_{on}$ , and  $T_p$  were ~155–193 °C, ~196–239 °C, ~235–246 °C at 2.5, 4, 5, 7.5, 10, 12.5 and 15 °C·min<sup>-1</sup>, respectively. The linear motion was observed with deviation on the dependence of  $T_s$  in the value 10 °C·min<sup>-1</sup> and 12.5 °C·min<sup>-1</sup>. At high values of heating rate, oxidation is characterized by an increasing temperature, and the oxidation time is determined by a shorter time. The start/onset/peak/end of oxidation time was observed at 46<sup>th</sup>, 61<sup>th</sup>, 76<sup>th</sup>, 90<sup>th</sup> minutes on the low value of heating rate and the start/onset/peak/end of oxidation time determined at 10<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>, 17<sup>th</sup> minutes on the high value of heating rate, respectively. The reaction enthalpy was assessed individually for all heating rates, taking into account the duration of an exothermic process and the weight of tested samples.

The melting properties of the extracted milk fats depend on the content of triacylglycerols, the qualitative and quantitative concepts of the tested samples. The first mild peak was observed at temperature nearly – 11.66 °C, the secondly ~16.5 °C, and the third at ~30.99 °C related to the presence of the low-melting triacylglycerols (with high unsaturated fatty acids content) and high-melting fats, which present in extracted milk fat.

## Acknowledgements

To Prof. Dr. Ewa Ostrowska-Ligęza for instructions at the Warsaw University of Life Sciences – SGGW.

The study was carried out under the support of Erasmus Mundus Action 2 Projects.

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