THERMAL BEHAVIOUR OF HOG INTRAMUSCULAR LIPIDS BY DSC ANALYSIS

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Lately, thermal analysis is being used not only in investigations in the field of lipids /1/, but also as a routine analysis for controlling their quality. Two techniques of thermal analysis are, therefore, interesting: differential scanning calorimetry (DSC) and thermogravimetry (TG). DSC analysis was applied in this study to characterize changes occurring during the storage of intramuscular lipids extracted castrate (C) and boar (B) M.Semimembranosus. The endothermal effects during sample heating at 5 K/min in the interval 240-330 K were measured in order to determine the temperatures and corresponding heat effects of characteristic phase transformations.

THEORETICAL

Determination of the polymorphous forms of intramuscular lipids by DSC

Many substrances exis in two or more different solid forms which differ in regard to physical and physico-chemical properties. Various crystalline forms have different solubility, melting rate, melting point and stability range. In the case of metastable polymorphous forms, some are rapidly transformed to more stable forms, while others tend to stay indefinitely in the state of frozen stopped transformation. It is known for polymorphous forms that even in the case of simple compounds at least two kinds of structural arrangement exist.

Analysis of the composition of castrate /2/ and boar intramuscular lipids shows that the major fraction are triglycerides, thus special attention was paid to the phenomenon of polymorphous triglycerides.

Phenomena of the "double" of "triple" melting point of triglycerides have been known for a long time /3/ and have been ascribed to isomeric forms. Malkin et al /3/ studied glyceride polymorphism by X-ray diffraction in order to solve this phenomenon. Glyceride polymorphism is relatively complex and even the simplest triglycerides exhibit 3 or 4 polymorphous modifications, while mixed glycerides, especially those containing acid chaiš of considerably different length, can crystallize in various modifications, most commonly in α, β' and β structural forms. It has been determined that α and β' are unstable forms which can transform to the β form upon heating. For some triglycerides, however, the β' form can be very stable. Triglyceride polymorphism is also complicated due to the possibility of different longitudinal distribution of the molecules, which is determined on the basis of X-ray diffraction.

When the melted monoacid-triglyceride is cooled rapidly, it solidifies at the lowest melting point, i.e. in the α-form. If this melted form is slowly heated and kept almost at the melting temperature, it solidifies in the β' form. In a similar way, the stable β form can be obtained from the β' form. Characteristic melting points of various structural forms of some pure triglycerides (with C_{12}-C_{22} saturated acids) are presented in paper /4/.

It is not always possible to determine all three polymorphous forms during triglyceride heating. Some data indicate, for example, that the β form appears during crystallization from solvent /4/. The same form in the case of mixed saturated triglycerides (16:0, 18:0, 16:0 - palmitin-stearin-palmitin) is difficult to achieve and these glycerides appear mostly in the β' form.

Many papers have dealt with the study of triglyceride polymorphism, most of them involving the structure of various crystalline forms /3/, the influence of chain length and unsaturation /5,6/. Little literature data exists on the melting enthalpy of triglycerides /7,8,9/. On the basis of the works of King and Garner /7/ on saturated homogeneous triglycerides, Bailey /7/ has suggested that the melting enthalpy be correlated with the number N of total carbon atoms of the acylated chains. Peron, in his paper, discussed the various equations proposed in the literature for determining melting enthalpies of polymorphous forms of various homogeneous and mixed (saturated--unsaturated) triglycerides /7/. He has also determined that there is an exponential type law for the change in the value of the melting enthalpy as a function of the number of double bonds, no matter what the number of C atoms of the acid residue and the crystalline form. The same author confirmed the fictituous value of the number of C atoms suggested by Tims /10/ for a mixture of saturated-unsaturated triglycerides, which connects the melting enthalpy of the β-form with the number of C-atoms.

By connecting the chemical composition with the thermal behaviour of hog lipids, M. LeMeste /9/ studied hog fat, its fractions that solidify at 36°C and 15°C and intramuscular lipids. It was shown that the DSC curves of fats and intramuscular lipids (glyceride fraction) are very similar, while a negligible influence of phospholipids was determined. Three main melting zones can be distinguished in the DSC curves: around 0°C, around 30°C and between 40 and 50°C. They refer to the melting of the following groups of glycerides: di-unsaturated triglycerides, mono-unstaureted and trisaturated triglycerides (considerably less). By tempering the sample the stability of various phases constituting the fat was estimated by analysing the registered changes (latent heats and melting points). M. Le Meste /9/ indicates that in hog fat, as well as in its fractions, most of the glycerides are arranged in the β'-form. The β' to β transition demands, in the opinion of the authors, considerably more time than that used in the experimental part.

EXPERIMENTAL

The thermal behaviour of total lipids was followed in the temperature interval of 240 to 330 K. The head of a Perkin-Elmer DSC-2 instrument was cooled with methanol and solid CO_2. In this way the temperature of the sample and the reference pan was maintained at or around 240 K, while the temperature of the cooled methanol was about 15-20° lower. In order to prevent humidity from the air condensing on the inner surface of the DSC head its temperature was maintained at the moment of inserting the sample at about +25°C, while the complete head of the instrument was isolated from the surroundings by a plexiglass housing in which an overpressure of dry nitrogen was maintained. Before each determination calibration of the endothermal effect (by n-decane, $t_m = -9.4^{o}C$) was performed. For analysis of the thermal effects sample masses of about 50 mg were used. All analyses were performed only at one heating rate, 5 K/min, with a nitrogen flow of 15 cm^3/min. Upon analysis, the sample was measured on a Perkin-Elmer microbalance with an accuracy of 10^{-5} g. There was practically no difference in the mass of the sample before and after analysis. Treatment of the corresponding DSC diagrams demands determination of the characteristic, in this case, endothermal effects. Upon setting the base line, the total area between the DSC curve and base line was determined by planimetry, and then recalculated according to the standard to the corresponding values of the thermal effects. The characteristic temperatures at which maxima of the DSC curves appear were also registered.

Effects of tempering the sample before DSC analysis

Hagemann and Tallent /5/ have shown that depending on the duration of triolein tempering various β' forms appear. It was determined that the complete structuring of a triglyceride in the β form is a temporal process which was also shown by M.Le Meste /9/ in his paper. After a certain time, depending on the tempering conditions, the less stable structures almost completely disappear. This is very important and in this study this was kept in mind during the DSC analysis of intramuscular lipids extracted from castrate and boar M. Semimembranosus. Analyses were always performed the same way, with samples that were kept at a temperature of $+4^{o}$ (conditions in the refrigerator) for a shorter or longer period (0 to 44 weeks). Detailed investigations of the tempering of fect (partial or complete heating, storage at low temperatures) indicate that:

- The structural arrangement of total lipids upon transforming from completely liguid to the solid state is a more or less longlasting process;
- Repeated analyses upon reheating and cooling yield practically the same total thermal effect of endothermal processes with shifted endothermal peaks toward lower temperatures;
- There is a greater difference between the repeated DSC and initial analyses (comparison for the same sample) if the sample of total lipids has been stored longer at $+4^{o}C$; in the case of fresh sample the change in characteristic endothermal peaks is negligible;
- Investigation of the influence of aging and therfere, possible chemical changes should be performed only once with a sample of exactly defined age that was stored always at the same temperature. In the applied investigations in this study those were samples the age of which increased up to 44 weeks at a temperature of $+4^{o}C$. By age the time that passes from total lipids extraction to the moment of analysis is meant.

RESULTS AND DISCUSSION

DSC analysis of fresh total lipids samples of castrate M.Semimembranosus

DSC curves of fresh castrate and boar total intramuscular lipids samples are presented in Figure 1. Characteristic endothermal peaks for both samples are at about 275 K and in a broader range around 300 K. A considerably higher melting temperature of castrate intramuscular lipids indicates a higher content of the solid phase in regard to boar lipids.

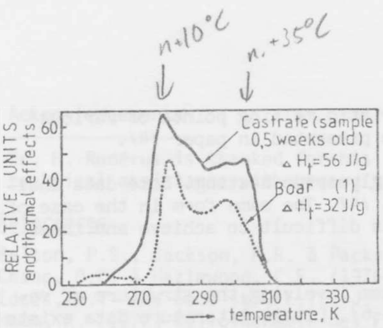

Fig.1.-

Besides, data concerning the composition of both samples confirm that sample C contains a higher fraction of the triglyceride fraction which solidifies at these temperatures. Data concerning the composition of both samples enabled the interpretation of DSC diagrams as is presented in detail in the example of sample C.

On the basis of the mean composition of the total lipids isolated from castrate M.Semimembranosus (4.00% in regard to the muscle) in which there are 87.2% neutral lipids, 11.4% phospholipids and 1.4% glucolipids, it may be concluded that the greatest influence on the appearance of the solid phase in the total lipids will be the neutral lipids fraction, i.e. the triglyceride content /2/.

According to M.Le Meste /9/ the thermal effects during the heating of pure phospholipids is negligible, and as there is no available information in the literature about the behaviour of glucolipids and their fraction being the smallest in the total lipids composition, the melting enthalpy may be expressed as:

$$\Delta H_{t,c} = 0.666 \cdot \Delta H_{t,T} \qquad (a)$$

where 0.666 is the mass fraction of the triglycerides (T) in intramuscular lipids and $\Delta H_{t,T}$ the triglyceride melting enthalpy.

In order to calculate the value of ΔH_t it is necessary to derive the approximative triglyceride composition on the basis of the determined composition of fatty acids (GC-MS, /2/) as basic triglyceride components. Only then on the basis of literature data can individual characteristic melting temperatures, i.e. melting enthalpies be adopted. A statistical calculation of the composition of triglycerides yields that in each triglyceride molecule there are on the average two acyl groups originating from oleic acid. The general formula of triglycerides can, therefore, be expressed by a symmetric of asymmetric triglyceride:

O X O   O O X

in which X is most probably palmitic or stearic acid. For a more detailed calculation of the composition of the triglyceride fraction, the following fatty acids were adopted: $C_{16}o$, $C_{16}1{=}$, $C_{18}1{=}$, $C_{18}2{=}$, $C_{18}o$, and $C_{20}2{=}$ of which there is more than 1% according to GC-MS analysis /2/.

Of the seven denoted fatty acids 196 combinations of mixed or pure triglycerides can be formed. Adopting that the probability with which individual pure triglycerides will appear in the triglyceride fraction is equal to the product of the relative ratios of fatty acid molecules involved in the composition of triglycerides, i.e. the composition of the triglyceride fraction is more precisely defined.

In Table 1, on the basis of literature data, the possible polymorphous forms and melting enthalpies are given. For some of these triglycerides it was not possible to find these data in the literature, so approximations were made using empirical equations /7/.

TABLE 1.- Structural and thermodynamic characteristics of some pure and mixed triglycerides classified according to the order of descending mass fraction[x]

Type	mass %	Characteristic structural arrangements					
		α T,K	α H_t,J/g	β' T,K	β' H_t J/g	β T,K	β H_t J/g
O-O-O	40.8	236	47.7	261	108.0	278	125.6
O-P-O	13.1						
O-O-P	13.1	261	-	274.5	110.1	292	125.6-146.5
O-S-O	6.5						
O-O-S	6.5	271.5	-	281.5	110.1	296	123.8
O-P-S	4.21						
P-O-S	2.10	297	-	313	146.5	314	192.8
P-O-P	4.20						
P-P-O	4.20	291.5	86.2	302.8	139.8	307.5	183.9

[x] The combinations O-M-M, P-P-P, O-M-O, O-O-L were disregarded as were ones with lower probability.

According to the data in Table 1 and by analysing the DSC curve for castrate intramuscular lipids it may be concluded that the most important endothermal effects during melting are a consequence of upsetting β and β' structural forms (around 275 and around 300 K). There are very little or practically none of the α structural forms.

For the theoretical calculation of the thermal effects during the heating of castrate total intramuscular lipids the following observations regarding the thermal behaviour of pure triglycerides were used:

- Mixed unsaturated triglycerides exist in the β form only if they are symmetrical;
- Asymmetrical isomers have the stable β' form.

The calculated lower boundary of the thermal effect is

$$\Delta H_t = 0.666 \;.\; (0.67)_{min} \;.\; 121.5 = 54.2 \text{ J/g}$$

While the measured value (by DSC anylysis) was 56.0 J/g. The contribution of individual triglycerides (121.5 J/g) is presented in Table 2.

TABLE 2.- Melting enthalpies of characteristic triglucerides made up of oleic, palmitic and stearic acid

No	Structural form	Triglyceride	Melting enthalpy in triglyceride mixture Wi . ΔHi
1			
2	β	O-O-O	0.408 . 125.6 = 51.2
3	β	O-P-O	0.131 . 136.0 = 17.8
4	β'	O-O-P	0.131 . 110.1 = 14.4
5	β	O-S-O	0.065 . 123.8 = 8.0
6	β'	O-O-S	0.065 . 110.1 = 7.2
7	β	P-O-P	0.042 . 183.9 = 7.7
8	β'	P-P-O	0.042 . 139.8 = 5.9
9	β'	O-P-S	0.042 . 146.5 = 6.2
	β'	P-O-S	0.021 . 146.5 = 3.1
			$\sum$= 121.5 J/g

The calculation has deficiencies because other possible triglycerides were neglected and everything was based on the fraction of the three major fatty acids (0.67) in the triglyceride composition (of which there are 66.6%). A detailed calculation which would include not only the other tri- but also di- and monoglycerides would certainly yield a somewhat higher value of the melting enthalpy. However, it must be noted that, already for the simplest binary mixtures such as triolein + tristearin or triolein + tripalmitin, a decrease in the melting enthalpy of saturated triglycerides of up to 20% takes place as stated by Norton et al in their paper /11/.

On the basis of data from Table 2 a DSC curve is presented in Fig. 2 by means of a histogram and compared to the corresponding experimental DSC curve. It was adopted that the enthalpy melting of each characteristic triglyceride is proportional to the area on the DSC curve presented by means of a rectangle of width ΔT = 12 K with a vertical axis at the chracteristic temperature T_β or $T_{\beta'}$. Fig. 3 shows the summed values of the melting enthalpy of each individual triglyceride, by the additive rule, (shaded area). On the basis of the presented analysis it was noted that:

a) The maxima of the endothermal effects at around 280 K originate from the triolein, while the maximum at around 302 K represents the superposed melting effect of several mixed (unsaturated-saturated) triglycerides in which 1 or 2 acyl residues of oleic acid are represented;

b) There is disagreement in the characteristic melting temperatures of mixed triglycerides in regard to experimental values. According to the analyses of Norton /11/ there is truly a decrease in the characteristic melting point in regard to theoretical values when the melting of tristearin in triolein or tripalmitin also in triolein are regarded. This is the explanation for the denoted differences betweenn the theoretical and DSC curves in the higher temperature region (> 300 K). This data was used in simulating the experimental DSC curve. The characteristic melting temperatures of β and β' structural arrangements for the following triglycerides were reduced: O-S-O by 2K, P-O-P and P-P-O by 6K, O-P-S and P-O-S by 10K. By representing the thermal melting curve of pure triglycerides by log-normal distributions, satisfactory agreement between the theoretical and experimental DSC curve was obtained (Fig. 3-b).

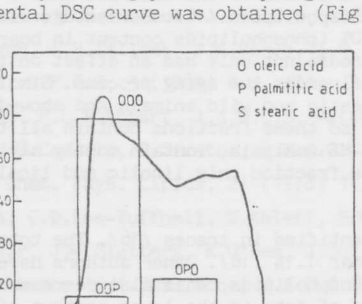

Fig. 2.- DSC-castrate

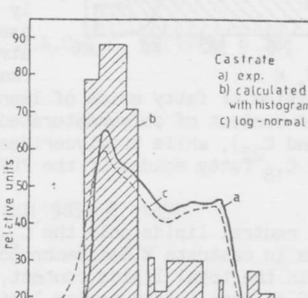

Fig.3.- DSC-castrate

Theoretical interpretation of the endothermal effects of boar intramuscular lipids - DSC curves

According to the mean composition data, the total intramuscular lipids of boar M.Semimembranosus (1.5% in regard to muscle) contain about 70% neutral, and a considerably higher percent of polar lipids: phospholipids about 24%

and glucolipids 6%. The data about the higher content of polar lipids is important because in this fraction there is a higher percentage of acyl residues of polyunsaturated fatty acids, which is the basis for greater oxidative changes during sample storage. In the theoretical interpretation of the DSC curve it was adopted that, as in the case of castrate total lipids, the solid phase of total lipids is defined by the triglyceride content. The most important fatty acids in the triglyceride composition are: $C_{16}o$, = 11.7%, $C_{18}1=$ = 40.6% and $C_{18}o$ = 4.9%, which makes up 57.2% of the triglyceride fraction. By applying the same method of calculation as for castrate total lipids the most probable composition of the triglyceride fraction was derived and presented in Table 3 on the basis of which the interpretation of the experimental DSC curve by histograms was performed (Fig.4).

TABLE 3.- Most probable composition of the boar triglyceride fraction

No	Structural form	Triglyceride	mass% (Wi.100)	Melting enthalpy in the triglyceride mixture
1	β	O-O-O	46,6	58,5
2	β	O-P-O	14,0	19,0
3	β'	O-O-P	14,0	15,4
4	β	O-S-O	6,2	6,8
5	β'	O-O-S	6,2	7,7
6	β	P-O-P	4,1	7,5
7	β'	P-P-O	4,1	5,7
8	β'	O-P-S	3,2	4,7
9	β'	P-O-S	1,6	2,3
				Σ= 127.6 J/g

Everything that was mentioned in the theoretical calculation of the thermal effects of castrate total lipids also stands in this case.

According to the fraction of the major represented fatty acids (57.2%), the neutral lipids fraction (70%) and triglycerides in neutral lipids (75%), the following value of the melting enthalpy of boar total intramuscular lipids was calculated.

$$\Delta H_t, \text{ theoretical value} = 0.572 \cdot 0.75 \cdot 0.70 \cdot 127.6 = 38.3 \text{ J/g}$$

By comparision to the experimentally determined total melting enthalpy (32 J/g) satisfactory agreement is obtained. From the above it may be seen that the major effects which originate from trioleate as well as di-oleopalmitate, i.e. dioleostearate (at obout 280 K), i.e. the same mixed but symmetric triglycerides (O-P-O, O-S-O at 290-300 K).

Chemical and physical changes during total lipids storage at +4°C

Castrate (C) intramuscular lipids

Fig.5 presents the results of DSC analysis of samples aged 0.5, 16 and 29 weeks. In the last case two analysed were performed at different times, but with the same age of the sample. Very good agreement was obtained in repeated investigations (Fig. 5c). Results indicate a change not only of the character of the DSC curve, but also a change in the total thermal effect, i.e. the melting enthalpy of the sample solid phase. This value during the first weeks of storage at +4°C, which is the temperature at which the greatest endothermal peak appears, decreases and then later increases by more than 5% in regard to the initial value. The endothermal effects at 275-280 K and 300-310 K indicate the β and β' structural forms of unsaturated or mixed saturated-unsaturated triglycerides.

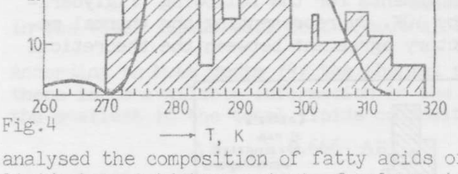

Fig.4

By carefully analysing the data it seems that the denoted changes connot be explained to occur in the autooxidation of lipids without additional information . The decrease in the total thermal effect can be a consequence of a gradual upset of the initially formed structural arrangement and later only after a longer period would the establishment of the most favorable thermodynamic state begin.

In all of this a considerable role is played by the other constituents of the total lipids - basically the phospholipids fraction for which it is known that it is oxidation prone.

Boar (B) intramuscular lipids

The results of determining the composition of boar total intramuscular lipids in regard to the same of the castrate show a considerably higher fraction of phospholipids /16/. Percentage-wise this increase is greater than 100% (phospholipids content in boar 24%, in castrate 11.7%). This increase not only has an effect on the total thermal effect, it also influences the aging process. Sinclair et al /12/ analysed the composition of fatty acids of lean meat of various domestic and wild animals and showed that phospholipids have a higher content of polyunsaturated fatty acids (PUFA), so these fractions contain all the long chain PUFA (C_{18}, C_{20} and C_{22}), while triglycerides, on the basis of GC-MS analysis, contain mostly acyl residues of C_{14}, C_{16}, $C_{18}1=$ and C_{18} fatty acids. Of the PUFA in the triglyceride fraction only linolic and linolenic acid are present.

Of the PUFA in boar neutral lipids only the $C_{20}2=$ fatty acid was identified in traces /16/. The total content of intramuscular lipids in castrate M.Semimembranosus is 4.0% and in boar 1.1% /16/. Other authors have also shown that with decrease in the total lipids content, the triglyceride /phospholipids ratio also decreases /13,14/. For interpreting the DSC of boar intramuscular lipids an important piece of data is the lower content of intramuscular lipids in boar M.Semimembranosus in regard to castrate, and a higher fraction of unsaturated fatty acids and a higher fraction of PUFA responsible for the oxidation processes. This is also confirmed by the work of F.L.Luddy et al /15/ who investigated the color and composition of lipids of various hog muscles.

Samples aged 1 week (from extraction according to Folch) were investigated. The next stages involved investigation of the same sample that was stored at +4°C for 4 weeks (Fig. 6b), 7 (c), 13 (d) and 33 weeks (Fig. 6.e). Changes are evident not only in the total thermal effect but also in the character of the DSC curve. For this sample, as opposed to castrate samples, the change in melting enthalpy cannot be by analysed by means of structural arrangements. In this case mostly tri-unsaturated glycerides are activated with the occurrence of the following oxidation processes:

$$\underset{\text{Trioleate}}{(O\text{-}O\text{-}O)} \longrightarrow \underset{\text{Dioleate}}{(O\text{-}O\text{-}X_1)} \longrightarrow \underset{\text{Monooleate}}{(O\text{-}X_1\text{-}X_2)}$$

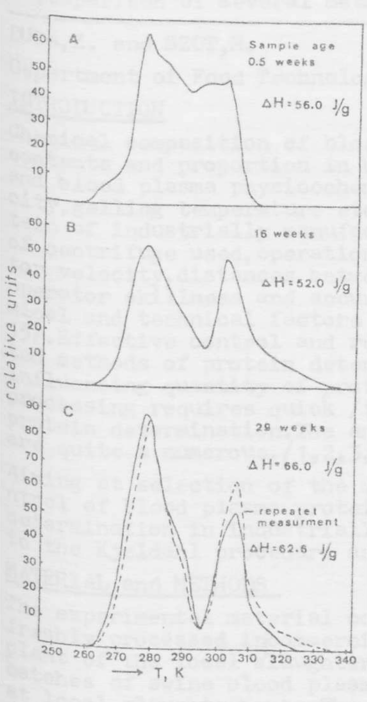

Fig.5.- DSC curves of sample C of different age

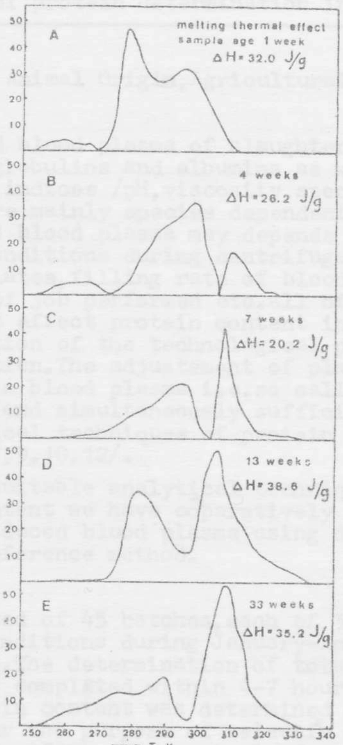

Fig.6.- DSC curves of boar intramuscular total lipids of different age

This, in the first phase, leads to a decrease in the characteristic peak at 278 K (βform of O-O-O) with a tendency of a more expressed effect below 270 K (β'form of O-O-X_1). At the same time the endothermal peak between 300-320 K increases which means that there are more and more β and β'structural forms of monosaturated triglycerides (of the O-X_1-X_2 type, where X_1 and X_2 are oxidized acyl residues of oleic acid)and a smaller content of di-unstaurated triglycerides (of the O-O-X_1 type).

The decrease in the total thermal effect in the initial phase indicates considerable upset of the arranged structures due to oxidation with rearrangement later on, which leads to an increase in the thermal effect of the melting of the total lipids solid phase.

Thus, it should be noted that on the basis of the work of M.Le Mestre /9/ in all DSC analyses the influence and thermal effect of phospholipids have been neglected. For completely fresh samples this assumption is correct, but it probably is not during storage when primarily PUFA from phospholipids react, by the oxidation of which products are obtained for which thermal effects during heating are unknown. The obtained results should, therefore, be considered also as a basis for detailed investigations of thermal effects during storage of every fraction of total lipids, especially of triglycerides and phospholipids.

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