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THE THERMAL ANALYSIS OF LIPIDS ISOLATED FROM VARIOUS TISSUES OF DEERS AND DOES

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Abstract

TG analysis of lipids is a suitable analytical method that offers the possibility to correlate kinetic parameters of thermal degradation (activation energy) and lipid composition. For this purpose, an inert (nitrogen) or oxidising atmosphere (oxygen) was applied during the thermal treatment (30–220°C) of lipids isolated from intramuscular and fatty tissues (L-IMT and L-FT) of deers and does. Prior to investigation, the extracted samples of lipids were kept at –24°C or +4°C for nine months, thus enabling detection of the influence of storage temperature on the thermal behaviour of the lipids.

Keywords: fatty tissue lipids, intramuscular lipids, lipids thermal degradation, TG analysis

Introduction

Nutritional culture in many European countries involves returning to natural "healthy" food [1]. Thus, meat from game has become an important source of raw materials of animal origin. It differs from meat of domestic animal, as deer freely choose their food, move extensively, live freely in nature, and there is natural selection. This deer is a type of game, whose meat is considered as dietary food because of the high protein content and low levels of fats and cholesterol [2, 3].

Thermal analysis can be applied as a standard method for controlling lipid stability in meat, i. e. in raw materials as well as in the final meat products. By studying the composition of the intramuscular lipids of the *M. Semimembranosus* of white meaty hogs, it was concluded that thermogravimetric analysis (TG) could be used for the fast detection of differences in the origin of total lipids, and to establish a correlation between the kinetic parameters of oxidation and total lipids composition [4]. TG may also assist in the quality evaluation of meat products, which has been confirmed by investigation of the total lipids of smoked Zlatibor bacon, indicating its good stability against oxidation [5].

As there are not enough data in the literature dealing with the quality of game meat, as well as its stability during thermal treatment and storage, the need for

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analysis of this type of product arises. The goal of this study was to establish a method for the detection of changes in the lipids of muscle and fatty tissue of different game tissues. The lipids were stored and thermally treated under various conditions by applying TG. The observed TG effects could be better explained utilizing previous results of the analytical determination (GC-MS) of the fatty acid composition of the total lipids of deer (*Cervus Dama dama L*).

Experimental

Sample preparation

Samples of intramuscular tissue and fatty tissue-kidney fat of deer and doe (*Cervus Dama dama L*) were used for lipids extraction. The game originated from the Military Establishment "Karadjordjevo"-Serbia (deers 2.5, and does 4–5 years old). After shooting, separation of warm "polutke" and their detoning was performed in the butchering line, and finally samples of muscle and fatty tissue were collected. All the samples of different tissues were packed under vacuum in polyvinyl chloride bags and stored at 2±2°C. The total lipids were extracted according to the Folch method [6]. The hydrolysis of total lipids was performed in sodium methanoate solution, while the separated fatty acids were extracted by ethyl ether. The fatty acids were converted to the methyl esters by reaction with diazomethane. Gas chromatography was employed to determine qualitatively and quantitatively the fatty acid composition. All the analyses were performed on a Varian 3400 GC with a DB-5, 30 m, fused silica capillary column (4°C min^{-1}, 150–300°C).

The extracted lipids were packed into jars and kept at –24°C, and at +4°C for nine months. After this period, TG of the samples was performed under a constant gas flow (oxygen or nitrogen) of 25 cm^3 min^{-1} using a Perkin Elmer TGS-2 thermobalance. The heating rates for the lipids of muscle tissue (L-IMT) were 2.5, 5 and 10°C min^{-1}, while the lipids of fatty tissue (L-FT) was heated at 2.5°C min^{-1}. The initial sample masses ranged from 10–14 mg. Mass changes of the total lipids were monitored in the temperature interval 30–220°C.

Results and discussion

Kinetic parameter calculation

The non-isothermal TG curves of the fatty tissue samples showed similar trends to the TG curves of smoked Zlatibor bacon obtained under the same conditions [5]. According to previous information from the thermal behaviour of lipids extracted from hog and bacon [4, 5], the rate of thermal degradation in N_2 or O_2 may be presented by:

$$r_{TD} = k(T)\varphi(m) \tag{1}$$

where: k is the rate constant of thermal degradation defined by the Arrhenius equation [$k=A\exp(-E_a/RT)$], while $\varphi(m)$ is a function which depends on the concentra-

tion of the component susceptible to thermal degradation. The most commonly used form of this function is the equation valid for irreversible first order reactions:

$$\varphi(m) = m \tag{2}$$

If the rate of thermal degradation (r_{TD}) is expressed as the amount of volatile products (dm), formed when the lipids are heated from T to $T+dT$, Eq. (1) may be presented in its simplest form:

$$r_{TD} = -\frac{dm}{dT} = k(T)m \tag{3}$$

Determination of the kinetic parameters

Doyle-Gorbachev integral method

In order to determine the kinetic parameters (A, E_a and k) on the basis of the results of TG of the total lipids of the fatty tissue samples of male and female specimens of fallow deer (*Cervus Dama dama L*), the Doyle-Gorbachev integral method as a solution of Eq. (3) was used [7, 8].

However, the application of this method, with appropriate assumptions, yielded results which could not satisfactorily explain the thermal degradation of the total lipids of the muscle tissue (*M. Semimembranosus*) of fallow deer performed in an inert (N_2) or an oxidising (O_2) atmosphere. In this case, a linear dependence between the characteristic function (ordinate on Fig. 1) was not obtained.

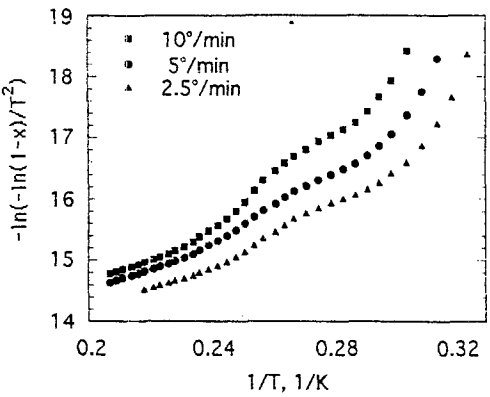

Fig. 1 Application of the Doyle and Gorbachev method for analysing the thermal behaviour of intramuscular lipids. Heating rate: ▲ – 2.5; ● – 5.0; ■ – 10°C min^{-1} in nitrogen (25 cm^3 min^{-1})

Flynn-Wall method

The Flynn-Wall (F-W) method was applied to determine the activation energy of the thermal degradation of the total lipids of fallow deer muscle tissue on the basis of non-isothermal TG curves obtained at various heating rates. Among others, this method was chosen because of its simplicity and easiness of obtaining satisfactory

results. The F-W method assumes that mass changes (Δm) during thermal degradation defines the rate of mass degradation:

$$\frac{dm}{dt} = \frac{A}{\beta} f(\Delta m)\exp\left(-\frac{E_a}{RT}\right) \tag{4}$$

where: A is the Arrhenius pre-exponential factor, β a constant heating rate, $f(\Delta m)$ an unknown function of degree of conversion.

By means of series of operations with the assumption that A, $f(\Delta m)$ and E_a are independent of temperature and that A and E_a are independent of mass change, the following expression for calculating the activation energy, at a constant degree of conversion, is obtained:

$$E_a = -\frac{R}{0.457}\frac{d(\log\beta)}{d(1/T)} \tag{5}$$

The value of E_a calculated by Eq. (5) is only the first approximation. The correct value is obtained by further adjusting of coefficient (0.457) in Eq. (5). This means that it is possible to calculate the true E_a using the relation:

$$E_a = -\frac{R}{X}\frac{d(\log\beta)}{d(1/T)} \tag{6}$$

where X, i.e. the corrected coefficient, is defined by:

$$X = \frac{\log[p(E_a/RT)] + 2.315}{E_a/RT} \tag{7}$$

In Eq. (7), E_a/RT is calculated on the basis of the first approximation of E_a. The value of the function $\log[p(E_a/RT)]$ is determined by an interpolation procedure applied for the chosen value of E_a/RT [9] .

Fatty acids composition of the lipid samples

Analysis of the fatty acids composition of the total lipids of the fatty and muscle tissue of fallow deer revealed the presence of: C^{0}_{14}–C^{0}_{20}, $C^{1=}_{14}$, $C^{1=}_{15}$, $C^{1=}_{16}$, $C^{1=}_{17}$, $C^{1=}_{18trans}$, $C^{1=}_{18cis}$, $C^{4=}_{20}$. The results of the investigation of the fatty acid composition of the total lipids are presented as percent fractions of the total peak area in the gas chromatograms.

Table 1 The ratio of saturated (S), monounsaturated (MU) and polyunsaturated (PU) fatty acids of the fatty tissue of fallow deer

Sample	MU/S	PU/S	PU/MU
Deers	0.257	0.021	0.081
Does	0.360	0.010	0.024

Table 2 The ratio of saturated (S), monounsaturated (MU) and polyunsaturated (PU) fatty acids of the intramuscular tissue

Sample	MU/S	PU/S	PU/MU
Deers	0.930	0.079	0.089
Does	0.820	0.049	0.059

Tables 1 and 2 show the ratios of monounsaturated/saturated and polyunsaturated/saturated and polyunsaturated/monounsaturated fatty acids of the total lipids of the fatty and muscle tissue of fallow deer. It may be seen that the ratio of monounsaturated/saturated and polyunsaturated/saturated acids is much higher in the case of muscle tissue, which obviously causes greater oxidative changes in the lipid samples during their storage.

TG results - qualitative analysis

The TG results (Fig. 2) indicate that the mass of the lipids extracted from the muscle tissue (L-IMT), decreases much faster with increasing temperature compared to the lipids from the fatty tissue samples (L-FT). The observed differences between the mass change of the lipids from the two different tissues are higher also at lower temperatures. The shapes of the TG curves obtained by analysing the lipids extracted from various tissues obviously indicate very well their origin (Fig. 2).

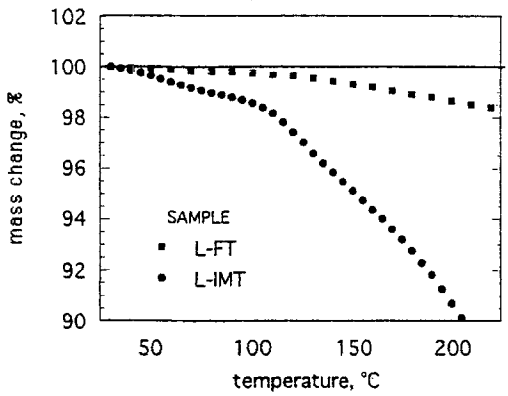

Fig. 2 Characteristic TG curves of samples of total lipids of intramuscular tissue (L-IMT) (•) and fatty tissue (L-FT) (■). Heating rate: 2.5°C min^{-1}, nitrogen (25 cm^3 min^{-1}) as the carrier gas

Qualitative analysis of TG curves

The mass changes for a given temperature interval (30–220°C) are different for the various lipid samples. For the lipids from fatty tissue (L-FT) they range from 0.25 to 1.65% when non-isothermal TG analysis were performed in a nitrogen atmosphere. The analysis of TG results of intramuscular lipids in an oxygen or nitro-

gen atmosphere exhibit very small differences. This means that L-IMR shows the same thermal behaviour in an inert or oxidising atmosphere. This was not observed in the case for the thermal degradation of L-FT performed in an inert and oxidising atmosphere. The analysis of L-FT in oxygen indicates a very slight mass increase (TG curves greater than 100%) in the interval 30–170°C. After 170°C, which is a "critical" temperature, there is a sudden mass loss of up to 10%. Such behaviour of L-FT is probably caused by oxygen links to specific unsaturated bonds resulting in peroxide formation even at temperatures below 170°C. At temperatures higher than 170°C, thermal degradation starts accompanied by a larger mass loss due to the decomposition of the formed peroxide bridges. On the basis of the lipid compositions, i. e. the ratio of specific types of fatty acids in L-FT or L-IMT, it may be concluded that L-IMT is more susceptible to oxidation and autooxidation. Moreover, in the case of L-IMT, of both does and deer, none of the samples have a mass increase in an oxygen atmosphere as observed in the case of L-FT. One of the explanations is, that regarding to the higher content of mono- and polyunsaturated fatty acids than in L-FT, autooxidation already started during its storage. It caused the higher mass degradation of lipids even in an inert atmosphere.

Activation energy calculation

Lipids of fatty tissue (L-FT)

The kinetic parameters of lipids thermal degradation were obtained on the basis of TG data. The activation energy, pre-exponential factor in the Arrhenius equation, as well as the rate constant of the thermal degradation of the total lipids of deer and does fatty tissue were determined using the Doyle-Gorbachev method [7, 8]. These values are presented in Table 3.

Table 3 The kinetic parameters (E_a, A) of thermal degradation of total lipids extracted from fatty tissue and stored at different temperature for nine months (–24°C or +4°C)

	Oxygen				Nitrogen			
	–24		+4		–24		+4	
	E/ kJ mol^{-1}	*A*/ min^{-1}	*E*/ kJ mol^{-1}	*A*/ min^{-1}	*E*/ kJ mol^{-1}	*A*/ min^{-1}	*E*/ kJ mol^{-1}	*A*/ min^{-1}
Deers	167.5	$1.47 \cdot 10^{16}$	130.1	$1.27 \cdot 10^{12}$	25.1	0.046	33.8	2.790
Does	190.2	$3.70 \cdot 10^{18}$	125.7	$4.10 \cdot 10^{11}$	37.7	1.400	40.8	18.40

Lipids of intramuscular tissue (L-IMT)

The shape of characteristic TG curves of intramuscular lipid samples stored at +4°C and –24°C in nitrogen atmosphere, indicate the complexity of the thermal degradation process. The TG curve of L-IMT (stored at +4°C) is shown in Fig. 3.

The corresponding DTG curve, obtained at a 10°C min^{-1} heating rate in a nitrogen atmosphere, indicates that the main process of thermal degradation is fol-

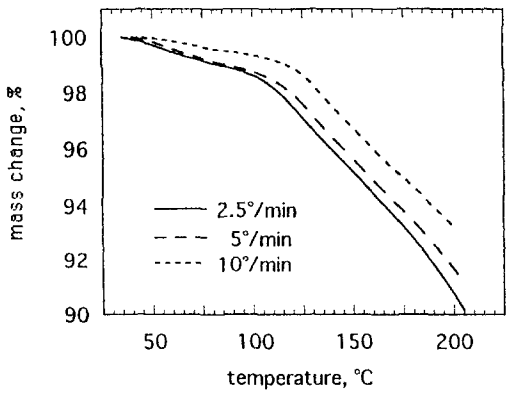

Fig. 3 Characteristic non-oxidative TG curves of L-IMT (stored at +4°C), nitrogen flow rate 25 cm^3 min^{-1}

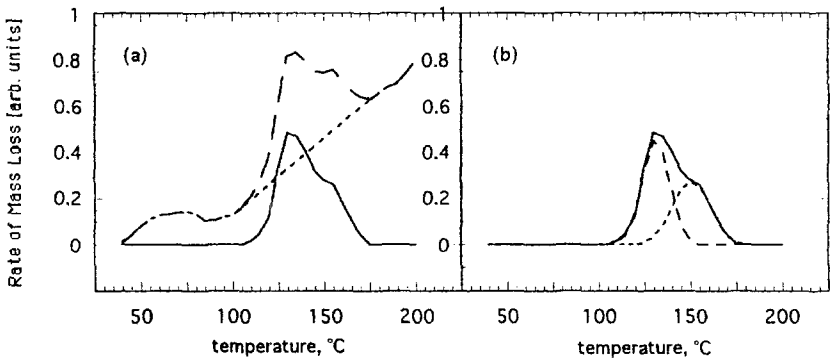

Fig. 4 (a) Total non-oxidative DTG curve of L-IMT (– – –), the main process (——) and of the minor processes; b) Deconvolution of the DTG curve of the two minor processes; Heating rate 10°C min^{-1}, nitrogen flow rate 25 cm^3 min^{-1}

lowed by two smaller effects expressed as peaks P1 and P2 (Fig. 4a and 4b). The main process of thermal degradation is determined as a base line (Fig. 4a), while the two peaks, separated using a non-commercial peak separation program, are

Table 4 Activation energy (E_a) of the thermal degradation of the total lipids of the intramuscular tissue of fallow deer calculated using the Flynn-Wall method

Mass loss/	E_a/kJ mol^{-1}	
%	+4°C	-24°C
1.0	21.08	26.93
2.0	37.38	23.34
3.0	44.56	24.44
4.0	49.23	26.60
5.0	52.75	30.44

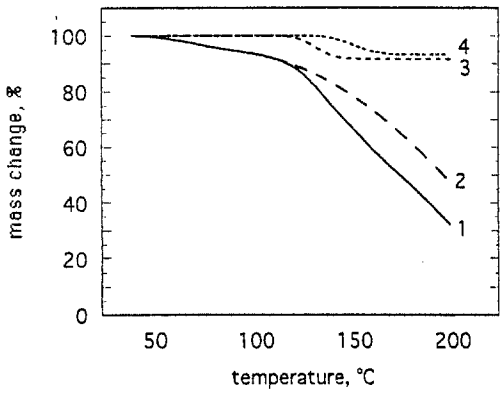

Fig 5 Non-oxidative TG curve of L-IMT; 1) the total process (—), 2) the main process (– – –) after subtraction of the minor processes, 3) and 4) the two minor processes (· · · ·); Heating rate 10°C min^{-1}, nitrogen flow rate 25 cm^3 min^{-1}

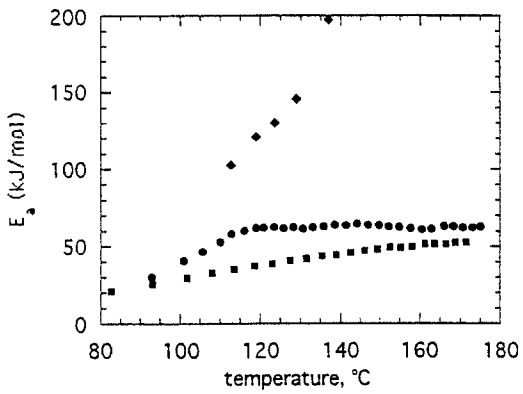

Fig. 6 Activation energy of thermal degradation of L-IMT (4°C storage temp.). The main process (●), the complete process (■) and the two minor processes (◆)

shown in Fig. 4b. The above procedure for the separation of the two overlaping peaks from the main process of thermal degradation, enabled the subsequent recalculation of the TG curves. Such a procedure of the separation of the TG curve into three steps (main process of degradation followed by two smaller effects) enables the determination of the dependence of the mass loss on temperature for each step. Of course, most interest is paid to the major process of thermal degradation (Fig. 5).

The same procedure was used for the TG analysis of lipid samples stored at the temperature of –24°C. In this case the second peak was not so distinctly separated from the first one.

Now, the values of the activation energy of the major degradation process for the samples of intramuscular lipids of fallow deer were determined by the Flynn-Wall method. The obtained results are presented in Table 4, for the mass changes of 1, 2,

3, 4 and 5%. The determined activation energies of the other steps of the complex thermal degradation process are shown in Fig. 6.

In the case of the samples stored at –24°C, the activation energy of the main thermal degradation process, calculated using the Flynn-Wall procedure up to 4% mass loss, are almost constant (23–27 kJ mol^{-1}). Further mass loss (4–5.2%, Table 4) leads to a slight increase in the activation energy (to 32 kJ mol^{-1}). These results agree very well with the activation energy of the L-FT thermal degradation process, in an inert atmosphere (25.1 kJ mol^{-1}, Table 3).

Conclusion

The values of the activation energy (E_a) of the thermal degradation process of samples of the total lipids of fatty tissue analysed in an oxidative atmosphere are up to five times greater than the corresponding values of the E_a of samples analysed in nitrogen. This is a consequence of the reaction between the unsaturated fatty acids and oxygen which leads to peroxide formation and a mass increase at the beginning of thermal treatment [10]. Such compounds are unstable at temperatures above 170°C and are easily susceptible to thermal degradation accompanied by a decrease in the mass of the fat mass of up to 10%.

Comparing the values of the E_a obtained by TG of the total lipids of the fatty muscle tissue of fallow deer in nitrogen it may be concluded that the fatty tissue, regardless of the storage conditions, has higher oxidative stability than muscle tissue. In the case of the intramuscular lipids stored at +4°C, the E_a increases with mass loss, which is an indication of the increased instability of these samples during storage as compared to the same samples stored in the deep freeze. The therrmal behaviour of L-IMT analysed in an inert or an oxidising atmosphere is very similar. The degradation of the lipids could be explained by one main process followed by two independent steps of smaller intensity.

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